FRIEDRICH-ALEXANDER
UNIVERSITÄT
ERLANGEN-NÜRNBERG
MEDIZINISCHE FAKULTÄT

Universitätsklinikum
Erlangen

IZKF Erlangen
Annual Report 2016

IZKF Annual Report 2016

interdisciplinary
Center for
Clinical Research

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IZKF Erlangen

Annual Report 2016

Editorial

Welcome to the annual report of the Interdisciplinary Center for Clinical Research Erlangen (IZKF), the central intramural funding platform of the Faculty of Medicine. This year's annual report has a new design, the same layout as used by the Faculty. This change in appearance reflects important modifications in our structures and bylaws that became effective 2016. The most important is the merging of the two intramural funding programmes IZKF and ELAN-Fonds under the roof of the IZKF. This consolidation into a single programme was a longstanding recommendation of our Scientific Advisory Board (SAB). Previously, funding limitations of the ELAN-programme restricted access to start-up projects to those parts of the Faculty belonging to the University Hospital. A combination of political will and additional funding provided by the FAU within its *Emerging Talent Initiative* programme allowed the creation of a unified intramural funding structure. Its funding lines aiming at the different career steps of physicians and scientists are now open to all parts of the Faculty. We retain the positive elements of both entities, ELAN-Fonds and IZKF, and ensure an equal access and participation of all departments and institutes of the Faculty.

These changes required adaptation of our governance structures and operations and thus modifications to our bylaws. Within the Board we created the positions of spokesman for promotion of young researchers and spokesman for start-up support (ELAN), reflecting the incorporation of the start-up funding (ELAN) and the growing importance of supporting young scientists and developing additional programmes for this target group. Another important change was the election of all Board members including the chairman, not by the General Assembly but by the Faculty directly. The General Assembly remains an important forum for all members to contribute their thoughts and ideas to the further development of IZKF. A further change is that we have now a standing board member from the Department of Biology in the FAU Faculty of Natural Sciences. We hope that this new structure will ensure an even better promotion of research in the entire Faculty of Medicine.

It is now 20 years since the foundation of our IZKF and we marked this anniversary with various celebrations. The team of the Administrative Office under the leadership of Dr. Faber and myself were deeply involved in the preparation of an advertorial in the Journal DEUTSCHE UNIVERSITÄTSZEITUNG (DUZ), which was published in June 2016. Together with the other IZKFs still in operation (Würzburg, Münster and Aachen) we commemorated in this *DUZ Spezial* this special anniversary by highlighting developments and successes and how the IZKFs with their commonalities but also their local particularities have contributed to shaping the research infrastructure at the different Faculties. Over all these years, IZKF Erlangen has funded around 250 projects in 8 funding periods, 9 junior research groups, 4 core units, 61 junior projects and more than 80 laboratory rotations for clinicians. More facts & figures on this impressive track record can be found in the back of this book.

We also held a dedicated symposium on 12th October in Erlangen to commemorate this anniversary. In their introductory presentations, FAU Vice-President Prof. Günther Leugering and the Dean of the Faculty of Medicine, Prof. Jürgen Schüttler, recognised the impact of IZKF on the development of the FAU and the Faculty and I followed with a review of 20 years success story of our IZKF. For this occasion we also welcomed Prof. Esther von Stebut-Borschitz from Mainz and the head of our SAB, Prof. Dieter Häussinger from Düsseldorf, who spoke on the role of the Clinician Scientist and on the future of clinical research in University Medicine, respectively. Altogether, these activities were an important boost for our outreach activities.

Last year we also held our biannual international IZKF-Symposium in Kloster Banz, the beautiful and serene baroque monastery in the upper Main valley. We chose again the topic of *Translational Medicine* but structured the programme not by research focus areas as in previous occasions but rather by interdisciplinary research fields with important recent developments. As in previous meetings we had a programme consisting of selected members form our Faculty and external scientists. This year we welcomed 12 outside speakers, half of them from overseas. The meeting was again very well received and the venue was at full capacity. Besides the formal scientific presentations, there was plenty of time to discuss science and also some football (we coincided with the Euro 2016 championship). I am very grateful to the sponsors who supported the meeting financially, but especially to all colleagues from the Programme Committee who shape an attractive scientific programme and making this a memorable event.

A strong and renowned Scientific Advisory Board (SAB) is an important governance element of any intramural funding organization such as IZKF. We are very grateful to all the colleagues who for so many years have offered their time and expertise to our cause. Our bylaws call for a maximum of 12 years duration for all members, so that a regular renewal of its membership occurs. Last year a major renewal was necessary due to cessations and end of regular term with a total of 8 new members elected and appointed by FAU president Prof. Joachim Hornegger. This renewal brings our SAB back to its full strength ready for our next evaluation.

We regularly review all funding programmes to monitor their success. Application for extramural funding is a key output parameter for all project funding programmes, be it at the junior or advanced levels. The latter enjoy an additional 6 months prolongation of their funding when applying for extramural funding. This has proven very popular and this year we have reached for the first time the mark of 100% of projects applying for extramural funding. More importantly, this is accompanied by an extremely high percentage of successful projects, 77% of them being granted. Junior projects are as successful as the advanced with a 76% funding rate and both programmes obtained more extramural research funding than was spent intramurally. For further details I recommend the figures at the end of this annual report. This enormous success was not anticipated and together with the unexpected high number of projects entering funding in the period 2016-2019 following the last evaluation has put a severe strain on the finances of IZKF. This left the Board no other option than to postpone the next funding period for one year. The next call for projects will therefore occur only in 2018.

The support of young scientists continues to be a main goal of IZKF. As in previous years the call for applications in the Junior Projects programme jointly carried out with the ELAN-Fonds was very well received. This programme supports young scientists with first research experience in pursuing interesting ideas and concepts that will hopefully lead to extramural funding after a 2.5 year period. In 2016, proposals for 18 projects from 13 institutions were reviewed from which 6 were selected for funding. Also the programme for laboratory rotations was again very successful with a rate of utilisation close to 100%, confirming a continued interest in a physician-scientist career.

On a more experienced level, IZKF supports junior research groups for up to 6 years. The funding level is comparable to that of ERC starting grants with an international candidate search and competitive allocation. Last year we appointed Dr. David Dulin from Oxford/UK as the new leader of Junior Group 1 with the title “Physics and Medicine”. The research focus is in the area of the new “Max-Planck-Center Physics and Medicine”, a joint venture between Max-Planck-Institute, FAU, the Faculty of Medicine and the University Hospital. Dr. Dulin’s group focuses on single-molecule replication dynamics of human RNA viruses, a topic combining physical methods and approaches including high-end microscopy with fundamental biological questions of nucleic acid metabolism. The group started September 1st and is housed in the Opitical Imaging Centre at the Kussmaul Campus where it has access to sophisticated microscopic methods. We wish him and his team success.

Also last year the junior group of Prof. Beate Winner with the topic modeling neurodegenerative diseases using stem cells reached its end of term. Prof. Winner’s group was very successful and she had two outside offers for professorships but it was possible to keep her at FAU where she became head of the Department of Stem Cell Biology. Thus we were able to maintain the important future-oriented stem-cell technology at the Faculty of Medicine, which was introduced with the help of IZKF.

Further activities include the development of a joint graduate school with the Department of Biology and the creation of a Physician Scientist Programme. Both activities are still ongoing under the leadership of Prof. Christoph Becker. The concepts are currently being discussed and will hopefully be implemented later this year. The aim is to join forces with other graduate programmes to create synergies and to better tailor programmes to the individual need of every graduate student and also to physician scientists. We hope that these developments will materialise during 2017.

Finally, at the annual meeting in November, we bid farewell to Prof. Dr. Beate Winner, Prof. Dr. Markus Neurath and Prof. Dr. Alexander Steinkasserer after the end of their respective terms in the Management Board. We warmly thank them for their dedication and support of IZKF.

Prof. Dr. André Reis

Contents

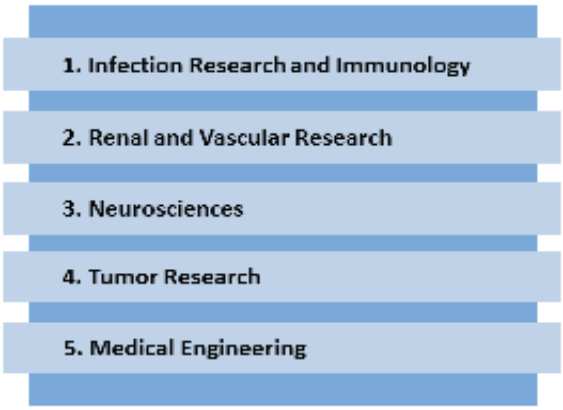
About us

History

The Interdisciplinary Center for Clinical Research (IZKF) was founded in 1996 under the leadership of Prof. Joachim Kalden with the focus "Inflammatory Processes: Aetiopathogenesis, Diagnostics and Therapy". It was established as an interactive research network of the Faculty of Medicine with scientific projects, several core units and two junior research groups. Aims were to foster clinical research, to promote young scientists and to increase transparency and competitiveness of fund allocation through peer review procedures. During the first 8 years (1996-2004) it received regressive funding from the Federal Ministry of Research and Technology within the programme "Health related research 2000". Since 2004 it has been fully funded by the Faculty of Medicine and the University.

Under the leadership of Prof. André Reis, the initial scientific focus on inflammation research has been further developed to accommodate other focal research areas and interdisciplinary fields of the Faculty as well. This allows nearly all institutions of the Faculty of Medicine to file applications with IZKF.

The IZKF offers research grants in all focal research areas of the Faculty of Medicine.



Main research areas of the Faculty of Medicine

Over the years funding of junior scientists has become ever more important and is now a par with project funding. In an attempt to better provide a uniform platform for career development the two separate funding instruments of the Faculty of Medicine - IZKF and ELAN-Fonds - were consolidated in 2016 under the umbrella of IZKF.

Mission Statement

The IZKF is the central structure of research development of the Faculty of Medicine. Its mission is to improve the overall quality of clinical research, to stimulate interdisciplinary research, to advance the careers of young scientists and to foster the acquisition of extramural funds.

Improvement of quality

Clinical research has to meet the challenge of transferring the enormous advances of biomedical research to patient care in a situation of limited human and financial resources. IZKF especially supports clinical research through efficient structures supporting research, protected time for clinicians, interdisciplinary research projects and an intensive career development of young scientists.

Stimulation of interdisciplinarity

Important scientific and medical advances are often achieved at the interface of disciplines. Thus fostering interdisciplinarity is an important goal of IZKF. To that end, IZKF Erlangen especially encourages interdisciplinary projects from all areas of the Faculty but also with co-applicants from other faculties.

Support for young scientists

Supporting young scientists is a major aim of the IZKF. Targeted promotion of young scientists is achieved by various career development programmes, workshops, seminars and a mentoring-programme.

Acquisition of extramural funding

In recent years greater emphasis has been put on the goal of enabling research projects to acquire extramural funding. Success is closely monitored and selection criteria now include past performance. A special programme for young researchers was established to help them start an independent scientific career and successfully acquiring external funding.

Project funding is allocated after a stringent peer-review process based solely on scientific criteria. Research grants applications are assessed in a two-stage review process. Pilot projects (ELAN) are also predominantly reviewed in a two-stage review process. Junior projects are subject to a one-stage internal review only.

Governance

IZKF is a self-organized structure within the Faculty of Medicine. The IZKF has a set of written rules and regulations approved by the Faculty of Medicine. All rules and regulations are continuously reviewed and revised if necessary. Governing bodies include the General Assembly, the Management Board, the Junior Scientist Committee, the ELAN Commission and the External Scientific Advisory Board (SAB). The Management Board is the general steering commission of the IZKF. It is responsible for developing the scientific programme, controlling the financial framework and allocating resources to projects as well as ensuring that results are reported. It is composed of 13 members with voting right, 11 elected by the Faculty of Medicine for a three year period and two ex-officio members from the Faculty of Medicine as well as four advisory members from the University Hospital and the University. Five annual meetings are held and decisions are taken by simple qualified majority. Elected members include the Chairman who is responsible for daily operations with the support of the Administrative Office.

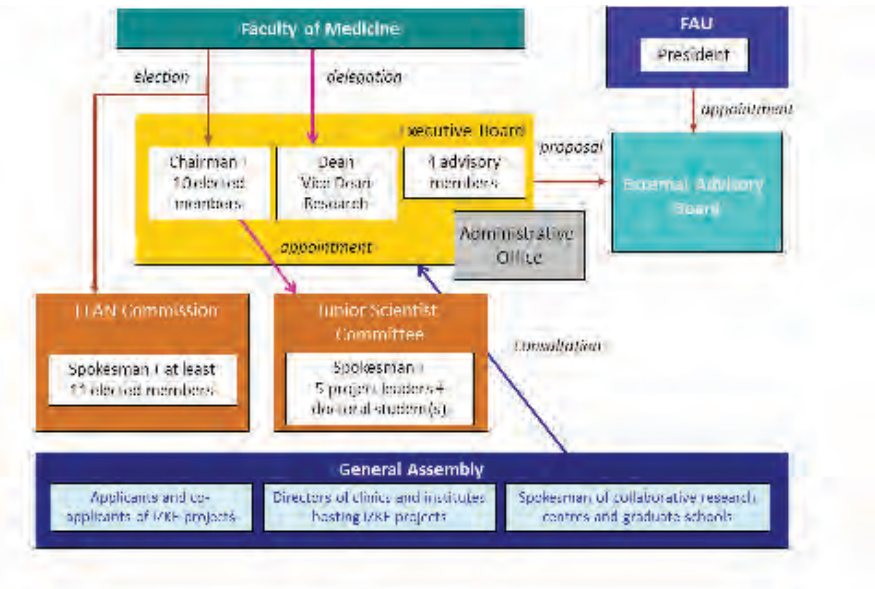
Programmes and the financial framework are reviewed and approved by the External Scientific Advisory Board. This body meets on site every two or three years to oversee the general development of the IZKF and the proposed projects. The board consists of at least 10 internationally recognized scientists (currently 16) from universities and research institutes led by an elected chairperson.

Members are appointed by the University president, upon the proposal of the Management Board for a period of six years.

The Junior Scientist Committee supports the Management Board in establishing and supervising career development programmes for young scientists. It assigns the MD-thesis scholarships and organizes the IZKF Graduate School. In addition, it participates in the internal review process for project funding and for laboratory rotations. It is composed of the spokesman for promotion of young researchers elected by the Faculty of Medicine, five project leaders, three from advanced projects, one from junior projects and one of the junior research group leaders and at least one representative from the doctoral students.

The ELAN-Commission consists of the spokesman for start-up support (ELAN) and at least 11 further members all elected by the Faculty of Medicine for a period of three years. This commission is responsible for reviewing pilot projects and assists in the selection of advanced and junior projects.

The General Assembly convenes once a year to discuss the annual report of the chairman and to contribute proposals for the further development of the IZKF. The members are all project leaders, the directors of clinics and institutes receiving funding, and the speakers of all local collaborative research centers and graduate schools.



Governance of the IZKF

About us

Statutary Bodies

Management Board

Chairman

Prof. Dr. André Reis, Institute of Human Genetics

Deputy Chairman

Prof. Dr. Michael Wegner, Institute of Biochemistry

Members

Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I
Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene
Prof. Dr. Andreas Mackensen, Department of Medicine 5
Prof. Dr. Dr. Jürgen Schüttler, Dean of the Faculty of Medicine, Department of Anaesthesiology
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

Prof. Dr. Christoph Becker, Department of Medicine 1 (since 01.10.2016)
Prof. Dr. Anja Bosserhoff, Institute of Biochemistry (since 01.10.2016)
Prof. Dr. Johann Helmut Brandstätter, Division of Animal Physiology (since 01.10.2016)
Prof. Dr. Raymund Horch, Department of Plastic and Hand Surgery (since 01.10.2016)

Prof. Dr. Kai-Uwe Eckardt, Department of Medicine 4 (till 31.03.2017)
Prof. Dr. Markus F. Neurath, Department of Medicine 1 (till 30.09.2016)
Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation (till 30.09.2016)
Prof. Dr. Beate Winner, IZKF Junior Research Group 3 (till 30.09.2016)

Consultative Members

Prof. Dr. Joachim Hornegger, President of the FAU
Christian Zens, Head of Administration of the FAU (since 01.02.2017)
Dr. Sybille Reichert, Head of Administration of the FAU (till 15.06.2016)
Prof. Dr. Heinrich Iro, Medical Director of the University Hospital Erlangen
Dr. Albrecht Bender, Head of Administration of the University Hospital Erlangen

Prof. Dr. Reis

Prof. Dr. Wegner

Prof. Dr. Becker

Prof. Dr. Bogdan

Prof. Dr. Bosserhoff

Prof. Dr. Brabletz

Prof. Dr. Brandstätter

Prof. Dr. Eckardt

Prof. Dr. Horch

Prof. Dr. Mackensen

Prof. Dr. Dr. Schüttler

Prof. Dr. Winkler

Prof. Dr. Hornegger

Prof. Dr. Iro

Dr. Bender

Members of the Management Board (31.12.2016)

About us

ELAN-Commission

Spokesman for start-up support (ELAN)

Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I

Members

Prof. Dr. Tobias Bäuerle, Institute of Radiology

Prof. Dr. Jürgen Behrens, Chair of Experimental Medicine II

Prof. Dr. Robert Cesnjevar, Department of Paediatric Cardiac Surgery

Prof. Dr. Yesim Erim, Department of Psychosomatic Medicine and Psychotherapy

Prof. Dr. Peter A. Fasching, Department of Obstetrics and Gynaecology

Prof. Dr. Martin Fromm, Chair of Clinical Pharmacology and Clinical Toxicology

Prof. Dr. Gerhard Krönke, Department of Medicine 3

Prof. Dr. Ralf Linker, Department of Neurology

Prof. Dr. Christian Pilarsky, Department of Surgery

Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation

Prof. Dr. Regina Trollmann, Department of Paediatrics and Adolescent Medicine

Prof. Dr. Klaus Überla, Institute of Clinical and Molecular Virology

Prof. Dr. Beate Winner, IZKF Junior Research Group 3 (till 30.09.2016), Institute of Human Genetics (since 22.03.2016)

Prof. Dr. Brabletz

Prof. Dr. Bäuerle

Prof. Dr. Behrens

Prof. Dr. Cesnjevar

Prof. Dr. Erim

Prof. Dr. Fasching

Prof. Dr. Fromm

Prof. Dr. Krönke

Prof. Dr. Linker

Prof. Dr. Pilarsky

Prof. Dr. Steinkasserer

Prof. Dr. Trollmann

Prof. Dr. Überla

Prof. Dr. Winner

Current Members of the ELAN Commission

About us

Junior Scientist Committee


Prof. Dr. Becker


Dr. Bosch-Voskens


Prof. Dr. Bozec


Dr. Ceppi


Prof. Dr. Engel


Häberle


Schöpe


Prof. Dr. Schulze

Current Members of the Junior Scientist Committee

Spokesman

Prof. Dr. Christoph Becker, Department of Medicine 1

Members

Dr. Caroline Bosch-Voskens, Department of Dermatology
Prof. Dr. Felix Engel, Department of Nephropathology
Prof. Dr. Schulze, Department of Oto-Rhino-Laryngology - Head and Neck Surgery

Prof. Dr. Aline Bozec, Department of Medicine 3 (since 01.11.2016)
Dr. Paolo Ceppi, IZKF Junior Research Group 1 (since 01.11.2016)
Benjamin Häberle, Institute of Biochemistry (since 01.11.2016)
Isabelle Schöpe, Department of Surgery (since 01.11.2016)

Tobias Bormann, Department of Medicine 1 (till 31.10.2016)
Prof. Dr. Beate Winner, IZKF Junior Research Group 3 (till 30.09.2016)

Administrative Office


Reichel


Dr. Faber


Meyerhöfer-Klee

Current staff of the Administrative Office

Manager

Dr. Katrin Faber

IZKF Administration

Anne Reichel

Bianca Meyerhöfer-Klee (part-time)

About us

General Assembly

Surname	Name
Alzheimer	Christian
Amann	Kerstin
Bach	Christian
Becker	Christoph
Beckmann	Matthias W.
Bernkopf	Dominic
Bogdan	Christian
Bosch-Voskens	Caroline
Boßerhoff	Anja
Bozec	Aline
Brabletz	Thomas
Brandstätter	Johann Helmut
Buchholz	Björn
Ceppi	Paolo
Dietrich	Peter
Distler	Jörg
Dörfler	Arnd
Dudziak	Diana
Dulin	David
Eckardt	Kai-Uwe
Engel	Felix
Ensser	Armin
Enz	Ralf
Fasching	Peter

Surname	Name
Full	Florian
Gefeller	Olaf
Gerlach	Katharina
Gramberg	Thomas
Grützmann	Robert
Günther	Claudia
Häberle	Benjamin
Hashemolhosseini	Said
Horch	Raymund
Iro	Heinrich
Jäck	Hans-Martin
Klucken	Jochen
Kornhuber	Johannes
Krappmann	Sven
Kremer	Andreas
Krönke	Gerhard
Kruse	Friedrich E.
Küspert	Melanie
Lang	Roland
Lee	De-Hyung
Lehmann	Christian
Lie	Dieter Chichung
Linker	Ralf
Mackensen	Andreas

Surname	Name
Marxreiter	Franz
Mayr	Andreas
Mielenz	Dirk
Mougiakakos	Dimitrios
Müller	Christian
Naschberger	Elisabeth
Neurath	Markus
Nimmerjahn	Falk
Nitschke	Lars
Palumbo-Zerr	Katrin
Regensburger	Martin
Reichel	Martin
Reis	André
Reuter	Nina
Schett	Georg
Schleicher	Ulrike
Schlötzer-Schrehardt	Ursula
Schmidt	Manuel
Scholtysek	Carina
Schöpe	Isabella
Schubert	Ulrich
Schuler	Gerold

Surname	Name
Schulze	Holger
Schüttler	Jürgen
Schwab	Stefan
Sonnewald	Uwe
Spriewald	Bernd
Stamminger	Thomas
Stürzl	Michael
Überla	Klaus
Veelken	Roland
Vöhringer	David
Waldmann	Elisabeth
Waldner	Maximilian
Warnecke	Christina
Wegner	Michael
Winkler	Jürgen
Winner	Beate
Wirtz	Stefan
Zimmermann	Katharina
Zweier	Christiane

General Assembly of the IZKF (09.11.2016)

About us

External Scientific Advisory Board

Prof. Dr. Häussinger

Chairman

Prof. Dr. Dieter Häussinger,
Düsseldorf University Hospital - Department of Gastroenterology, Hepatology and Infectiology

Prof. Dr. Sendtner

Vice-Chair

Prof. Dr. Michael Sendtner,
University Hospital Würzburg - Institute for Clinical Neurobiology

Members

Prof. Dr. Reinhard Büttner (till 31.03.2017),
Cologne University Hospital - Institute of Pathology

Prof. Dr. Hartmut Hengel,
Freiburg University Hospital - Department of Virology

Prof. Dr. Dörthe Katschinski,
Göttingen University Medical Center - Department of Cardiovascular Physiology

Prof. Dr. Malte Kelm (till 31.03.2017),
Düsseldorf University Hospital - Department of Cardiology, Pneumology and Angiology

Prof. Dr. Christian Kurts (till 31.03.2017),
Bonn University Hospital - Institute of Molecular Medicine and Experimental Immunology

Prof. Dr. Hermann Pavenstädt,
Münster University Hospital - Internal Medicine, Department of Nephrology and Rheumatology

Prof. Dr. Klaus Pfeffer,
Düsseldorf University Hospital - Institute of Medical Microbiology

Prof. Dr. Olaf Rieß,
University of Tübingen - Institute of Human Genetics

Prof. Dr. Wolff Schmiegel,
Bochum University Hospital - Department of Medicine

Prof. Dr. Jörg B. Schulz,
University Hospital Aachen - Department of Neurology

Prof. Dr. Thomas Seufferlein,
University Hospital Ulm - Internal Medicine I

Prof. Dr. Gisa Tiegs,
Hamburg-Eppendorf University Medical Center - Institute of Experimental Immunology and Hepatology

Prof. Dr. Thomas Wirth,
University of Ulm - Institute of Physiological Chemistry

Prof. Dr. Dirk Busch (since 01.01.2017),
Technical University of Munich, Institute for Medical Microbiology, Immunology and Hygiene

Prof. Dr. Ulrich Kalinke (since 01.01.2017),
TWINCORE, Centre for Experimental and Clinical Infection Research

Prof. Dr. Thomas Kamradt (since 01.01.2017),
Jena University Hospital, Institute of Immunology

Prof. Dr. Tanja Kuhlmann (since 01.01.2017),
University Hospital Münster, Institute of Neuropathology

Prof. Dr. Holger Moch (since 01.01.2017),
University Hospital Zurich, Institute of Pathology and Molecular Pathology

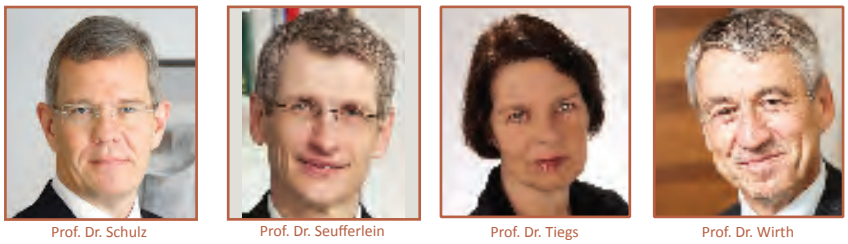
Prof. Dr. Jörg Prinz (since 01.01.2017),
LMU München, Department of Dermatology and Allergology

Prof. Dr. Reiner Siebert (since 01.01.2017),
University Hospital Ulm, Institute of Human Genetics

Prof. Dr. Lydia Sorokin (since 01.01.2017),
University of Münster, Institute of Physiological Chemistry and Pathobiochemistry


Prof. Dr. Büttner | Prof. Dr. Hengel | Prof. Dr. Katschinski | Prof. Dr. Kelm


Prof. Dr. Kurts | Prof. Dr. Pavenstädt | Prof. Dr. Pfeffer | Prof. Dr. Rieß | Prof. Dr. Schmiegel


Prof. Dr. Schulz | Prof. Dr. Seufferlein | Prof. Dr. Tiegs | Prof. Dr. Wirth

External Scientific Advisory Board (31.12.2016)

Programmes

Programmes

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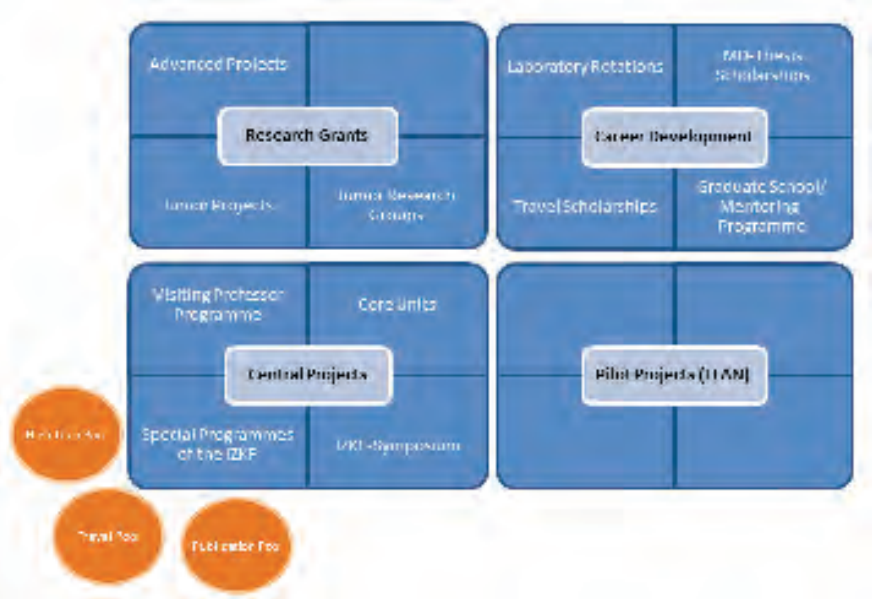
Programmes

Overview

Advanced and junior projects, pilot projects (ELAN), junior research groups, core facilities, MD-thesis scholarships and laboratory rotations are periodically requested for proposal within the Faculty of Medicine.

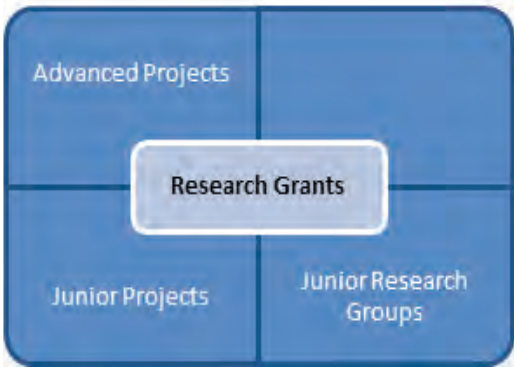


Programmes of the IZKF

Research Grants

Advanced Projects

The IZKF offers research grants in all focal research areas of the Faculty of Medicine, i.e. immunology and infection research, renal and vascular research, neurosciences, tumor research and medical engineering. The project duration is 30 months. After a single funding period projects should be transferred to extramural funding. If the application for extramural funding was filed within the duration of the IZKF project, the duration of the projects will be extended for another 6 months.



IZKF projects ordinarily include two personnel positions (postgraduate and technical assistant or two postgraduates, or in exceptional cases a post doctoral scientist). Applicants are expected to have an active publication record and own external funding. Preliminary results should yield the promise of a successful transfer of the project into external funding after the 30-months term. Innovative and original ideas and concepts are especially valued as well as the clinical relevance and interdisciplinary approaches. Applicants from all clinics, departments and institutes of the Faculty of Medicine and co-applicants from other faculties are entitled with no age limit.

Project funding is allocated after a stringent peer-review process based solely on scientific criteria. Research grants are approved after a two-stage review process. In an initial step, draft proposals are subject to an internal review by the Management Board, the Junior Scientist Committee, members of the ELAN Commission and other recognized scientists of the Faculty of Medicine based on a written proposal and public presentation. Decisions are reached after internal deliberation and are communicated immediately afterwards. Successful proposals are presented in full to the Scientific Advisory Board during their peer-review site visits. Negative funding decisions of the board are binding. Projects must start within six months after acceptance. Over the years funding rates were about 30 - 40%. Proposals are accepted every two or three years.

Staff	Postgraduate scientist Technical assistant	Two postgraduate scientists
Consumables	T€ 20 p.a.	T€ 20 p.a. / scientist and institution
Others	Participation in Travel, Publication and High Tech Pool	
Duration	30 + 6 months	

Junior Projects

For scientists starting their independent career, obtaining their first extramural research funding is an important step. To aid in this process, the IZKF offers starting grants to young postdoctoral physicians and scientists up to 35 years of age without previous significant external funding. Candidates should have a visible publication record and projects should be based on an original idea with first tangible results. Projects include a position for a technician or a postgraduate and consumables for 30 months. After this time it is expected that successful candidates submit an external grant application. If the application is filed within duration of the junior project, the spending period will be extended by another 6 months.

Staff	Technical assistant or Postgraduate scientist
Consumables	T€ 15 p.a.
Others	Participation in Travel, Publication and High Tech Pool; IZKF laboratory rotations for physicians
Duration	30 months

Junior projects are subject to a one-stage internal review only. Full proposals are reviewed by the Management Board, the Junior Scientist Committee and the ELAN Commission based on a written proposal and public presentation. Decisions are reached after internal deliberation and communicated immediately afterwards to the proponents. Proposals are accepted every year.

Programmes

Pilot Projects (ELAN)

The aim of ELAN is to support scientific projects at a very early stage and help prepare them for successful application for external funding (start-up projects), to support newly established working groups, to develop new innovative ideas (pilot projects) or as interim funding if temporary gaps arise between individual extramural funding periods. ELAN supports young scientists up to 38 years of age from the entire Faculty of Medicine with maximum € 50,000 euros for a period of up to 12 months.

Staff	One position
Consumables	max. T€ 50
Others	Participation in Publication Pool
Duration	max. 12 months

Junior Research Groups

Junior research groups offer an attractive career development opportunity for outstanding young scientists with a training in medicine or natural sciences and a strong background and reputation in one of the Faculties' main research fields. Over a period of 6 years each junior research group receives funding for the group leader, one postdoctoral and one postgraduate scientist, one technical assistant and consumables. From this position several previous junior research group leaders have been appointed to a professorship or have achieved other attractive positions. The groups operate independently but may be associated to individual clinics or institutes. For physicians a part time involvement in clinical activities is possible. Groups also have access to research funds allocated by the Faculty based on scientific performance criteria. At the end of 2016 there are two junior research groups. One group is housed in the Nikolaus Fiebiger Center for Molecular Medicine with its attractive scientific environment and diverse activities; the other is located at the Kußmaul-Campus at the Optical Imaging Center Erlangen (OICE).

Staff	Group leader Postdoctoral scientist Postgraduate scientist Technical assistant
Consumables	T€ 50 p.a.
Others	Participation in the allocation of funds based on performance criteria (LOM) Laboratory space Investment funds
Duration	6 years

Career Development Programmes

Support for and development of young scientists has been a central goal of the IZKF since its inception. In addition to the junior research groups, advanced and junior projects, the IZKF also offers other specific programmes for young scientists such as MD-thesis scholarships and laboratory rotations.

Laboratory Rotations

Access to protected research time is essential for young clinicians developing their projects. The laboratory rotation positions enable young scientists to fully devote themselves to a research project. This rotation can be for 6-12 months in full time or 12-24 months in part time. This programme is open to all young clinicians of the Faculty. Junior project leaders can also access this programme in addition to their project funding. The IZKF can allocate 8 full-time positions; this equates to 96 months, which can be used flexibly. The initial grant always consists of 6 months in full time or 12 months in part time. Extensions are conditional on successful evaluation based on oral presentation of work progress and updated work programme.

MD-Thesis Scholarships

This programme was initiated to arouse interest for science in motivated medical students early on in their career. Medical students are supported in performing an experimental thesis in association with the IZKF or externally funded projects. It is expected that they spend a significant time in a laboratory. The IZKF offers 7 months grants and the supervision of a tutorial committee consisting of 3 experts. Up to 18 grants are available for medical students with good study degrees and a demonstrated scientific interest. The programme provides the participation in specific programmes, events and workshops of the IZKF. In accordance with the recommendations of the Scientific Advisory Board of the IZKF, medical doctoral students have been integrated in the IZKF Graduate School. Medical students are required to participate in the structured seminar programme of the Graduate School and to present their projects.

Travel Scholarships

Travel scholarships allow IZKF's young researchers to spend time at other laboratories in Germany or abroad to conduct important experiments or learn the latest techniques and methods. The programme also allows doctoral candidates to intensify existing collaborations or establish new ones. Travel grants include transportation and accommodation for up to 3 months. An extension of the travel scholarship for another 3 months is possible.

Programmes

Graduate School

The IZKF established its own Graduate School for all PhD students of the IZKF. Participation is mandatory for all doctoral candidates in sciences who are not involved in an alternative structured training programme run by the Faculty/ University and also for doctoral candidates who receive funding as part of an IZKF MD-thesis scholarship. Other students may associate with the Graduate School.

The Graduate School is divided into the two areas neuroscience and immunology/infection/oncology/renal and vascular research. Initially all topics were covered in one Graduate School. The neuroscience part was later integrated into the ICN (Interdisciplinary Center of Neuroscience).

Aims include fostering networking and scientific self-organization, methodological competence and soft skills as well as offering insights into other scientific fields and career opportunities. A structured seminar programme, courses in basic methods, in scientific writing and presentation as well as site visits to other laboratories in academia and industry are organized by the Junior Scientist Committee.

In 2016, the IZKF started to organize a post graduate programme which will comprise all doctoral students within the Faculty of Medicine and the Department of Biology which are interested in a structured doctoral training.

Mentoring-Programme

IZKF established a mentoring programme for all doctoral students in IZKF projects. Each doctoral student announces two mentors from among the IZKF project leaders. In some instances it is possible to determine an external mentor.

At least one annual meeting between the supervisor, the mentors and the doctoral student is expected. A participation in the IZKF Graduate School and the Postgraduate Workshop is mandatory.

Postgraduate Workshop

Every two years, the Junior Scientist Committee organizes the IZKF Postgraduate Workshop. The Postgraduate Workshop alternates with the International Symposium at Kloster Banz.

At the IZKF Postgraduate Workshop, lectures are held by internationally recognized speakers on a timely topic. The focus of the workshop is on a poster session in which all members of the Graduate School are requested to present their projects. Two poster prizes are awarded.

Central Projects

Core Facilities

Modern molecular technologies, such as genomics, proteomics and advanced molecular imaging, require very expensive and complicated instrumentation and are methodologically very demanding. Thus it is often not scientifically worthwhile or cost-effective to establish and maintain these techniques in parallel in different groups. Core facilities or units are centralized methodological platforms that offer access to these modern methods and technologies to a broad user spectrum. This enables access to modern technologies to smaller groups and also to those with other main methodological interests as well as allows students to be directly exposed to these modern developments.

Core facilities are operated under the leadership of a scientific group with demonstrated excellence and interest in developing the methodology. In return for institutional support, it is expected that the operating group assists other groups with their know-how in accessing this technology. The support provided by the IZKF and the Faculty usually includes the initial investment for the instrumentation of the platform, the cost for setting up the operation as well as its continued technological development. IZKF pioneers the development of core facilities in Erlangen and usually supports them for an initial start-up phase of up to 6 years. Once established and successfully working, long-term support is provided directly by the Faculty.

Services and costs are to be made transparent and equal access has to be ensured. Core facilities are regularly evaluated for their effective operation, scientific excellence and timeliness.

The IZKF offers a platform for developing new core units. Important core units of the Faculty of Medicine are based on a start-up funding by the IZKF.

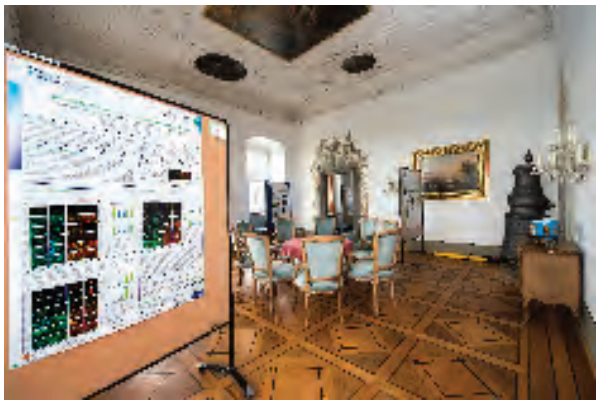
Core units of the Faculty of Medicine currently in operation:

- Ultra deep sequencing
- Cell sorting unit with immune monitoring
- Preclinical animal unit
- Small animal imaging – PIPE

Programmes

International IZKF Symposium

IZKF regularly organizes international scientific symposia which are held at the conference center at the baroque monastery of Kloster Banz in the upper Main valley. This venue offers a unique stimulating and interactive environment. An attractive programme with many speakers from Germany and abroad is developed by a Programme Committee. In addition, the participants of the symposium present their concepts and results in poster sessions. All interested scientists are welcome to join the symposium.



Historical seminar room at the conference venue

Intensive poster discussion

Conference hall at the IZKF Symposium

Visiting Professor Programme

To encourage cooperation and to foster the exchange of ideas, IZKF promotes visits of external scientists. Currently it administrates and supports two complementary programmes.

IZKF Visiting Professor Programme

The IZKF Visiting Professor Programme is running successfully for many years. Every year approx. 10 scientists from abroad but also from other places in Germany can be invited for a stay of between 2 days and 4 months. The programme covers travel and accommodation costs for visiting researchers in the amount of up to € 3,000. Application is restricted to IZKF members and the invited researcher's subject must be related to IZKF. Since the existence of the FAU Visiting Professor Programme the IZKF Programme is focused on promoting younger scientists.

FAU Visiting Professor Programme

IZKF manages the FAU Visiting Professor Programme according to the FAU bylaws. A maximum of € 3,000 of funding is available to cover travel and accommodation costs for visiting professors from abroad with high international reputation. At least one presentation must be given in Erlangen, with members of the Faculty and IZKF being invited. All appointed professors of the Faculty of Medicine can apply for this programme.

Special Programmes

Special programmes provide additional funding for IZKF projects.

High Tech Pool

IZKF actively encourages the use of modern "omics" technologies in the projects, such as those provided by the Core Unit Ultra Deep Sequencing. Since these experiments are generally expensive and consumables within IZKF advanced and junior projects are restricted to € 20,000 or less, additional support is necessary. Costs for consumables can therefore be supported upon request with up to € 10,000 per project, provided that the project itself contributes at least 30% of the total.

Travel Funding

To enable IZKF members to present their results to the academic community, IZKF supports their participation in international conferences. All applicants are expected to give a lecture or present a poster. The subject matter of the event must be related to the IZKF project in order to receive funding. The financial contribution of the IZKF is limited to € 500 for conferences in Germany, € 1,000 in Europe, and up to € 1,500 for conferences outside Europe.

This programme is also available for successful applicants for MD-thesis scholarships and laboratory rotations, but not for pilot projects.

Publication Funding

The publication of results obtained in IZKF projects in scientific journals is actively supported. It is expected that the IZKF funding of the project is acknowledged. The IZKF covers publication costs up to € 1,500. If the total costs are more then € 3,000 a financial contribution of € 2,000 is given by the IZKF. This programme is also available for successful applicants for MD-thesis scholarships, laboratory rotations and pilot projects.

Advanced Research Grants

Advanced Research Grants

Progress and Final Reports

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Advanced Grants

Immunology and Infection

Project No.	Project title	Term	Applicant(s)	Institute
A52	cFlip isoforms in the intestinal epithelium	01.11.2013-31.10.2016	Dr. Günther, Prof. Becker	Department of Medicine 1
A53	Th17/piTreg differentiation in vivo	01.10.2013-30.09.2016	Prof. Hildner	Department of Medicine 1
A54	Fam180a in inflammatory diseases	01.11.2013-31.10.2016	PD Dr. Dr. Wirtz, Prof. Waldner	Department of Medicine 1
A55	NR4a1 during immunologic tolerance	01.01.2014-31.12.2016	Prof. Krönke	Department of Medicine 3
A56	Role of HIG2 in atherosclerosis	01.03.2014-28.02.2017	PD Dr. Warnecke	Department of Medicine 4
A57	Nr4a1 as a novel target for the treatment of scleroderma-tous chronic graft-versus-host disease	01.01.2014-31.12.2016	Prof. Distler, Prof. Spriewald	Department of Medicine 3, Department of Medicine 5
A58	Characterization of DN T cells from ALPS patients	01.10.2013-30.09.2016	Prof. Mackensen, Dr. Völkl	Department of Medicine 5
A59	IL-10 and lung cancer	01.10.2013-30.09.2016	Prof. Finotto	Department of Molecular Pneumology
A60	Monocyte derived Dendritic cells (Mo-DC) by DC Exosomes	01.10.2013-30.09.2016	Prof. Baur, Dr. Schierer	Department of Dermatology
A61	Leishmania, iNOS and iron	01.02.2014-31.01.2017	Prof. Bogdan, PD Dr. Schleicher	Institute of Clinical Microbiology, Immunology and Hygiene
A62	ND10 and interferon-induced gene expression	01.01.2014-31.12.2016	Prof. Stamminger	Institute of Clinical and Molecular Virology
A63	Mechanisms of TNF-Mediated Control of Intracellular Pathogens in Mice and Man	01.07.2016-31.12.2018	Prof. Bogdan	Institute of Clinical Microbiology, Immunology and Hygiene
A64	The tyrosine-protein phosphatase SHP2 regulates TGFβ -dependent activation of JAK2/STAT3 in fibrotic diseases	01.02.2016-31.07.2018	Prof. Distler, Prof. Schett	Department of Medicine 3
A65	Tolerizing potential of human dendritic cell subpopu-lations	01.04.2016-30.09.2018	Prof. Dudziak	Department of Dermatology
A66	Genome wide CRISPR/Cas9 knockout for the identifica-tion of antiviral cellular restriction factors	01.07.2016-31.12.2018	Prof. Ensser	Institute of Clinical and Molecular Virology
A67	Analysis of the TRIM5alpha-mediated block to LINE-1 retroelements	01.02.2016-31.07.2018	Prof. Gramberg	Institute of Clinical and Molecular Virology
A68	Analysis of the role of the IL-23/Th17 axis during the control of antibody activity in rheumatoid arthritis	16.06.2016-15.12.2018	Prof. Krönke, Prof. Nimmerjahn	Department of Medicine 3, Division of Genetics
A69	Contribution of ATM kinase and the DNA-damage response in the innate response to infection	01.07.2016-31.12.2018	Prof. Lang	Institute of Clinical Microbiology, Immunology and Hygiene

Project No.	Project title	Term	Applicant(s)	Institute
A70	Novel targets for antiretroviral therapy - deubiquitinating enzymes regulate HIV-1 replication	01.07.2016-31.12.2018	Prof. Schubert	Institute of Clinical and Molecular Virology
A71	Viral modulation of the protein kinase ULK1	01.07.2016-31.12.2018	Prof. Stamminger	Institute of Clinical and Molecular Virology
A72	Targeted modulation of regulatory T cells and analyses of the underlying mechanisms	01.07.2016-31.12.2018	Prof. Steinkasserer	Department of Immune Modulation
A73	Checkpoint inhibitors as adjuvants for viral vaccines	01.07.2016-30.06.2017	Prof. Überla	Institute of Clinical and Molecular Virology
A74	The Role of Eosinophils in Allergic Bronchopulmonary Aspergillosis	01.06.2016-30.11.2018	Prof. Vöhringer, Prof. Krappmann	Department of Infection Biology, Institute of Clinical Microbiology, Immunology and Hygiene
A75	Role of MLKL-dependent programmed necrotic cell death in the pathogenesis of hepatitis	01.07.2016-31.12.2018	Dr. Günther, PD Dr. Dr. Wirtz	Department of Medicine 1

Oncology

Project No.	Project title	Term	Applicant(s)	Institute
D19	Role of intestinal epithelial SMAD7 for tumor development	01.11.2013-31.10.2016	Prof. Becker	Department of Medicine 1
D20	Collagen 10 and metastasis in CRC	01.11.2013-31.10.2016	Prof. Stürzl, Prof. Croner, PD Dr. Naschberger	Department of Surgery
D21	DAPK and colon cancer	16.10.2013-15.10.2016	Prof. Schneider-Stock, PD Dr. Neufert	Institute of Pathology, Department of Medicine 1
D22	Identification and functional characterisation of novel components of the Wnt/β-catenin signal transduction pathway	01.11.2013-31.10.2016	Prof. Behrens	Chair of Experimental Medicine II
D23	Influence of bone marrow adipocytes on the metastatic niche in experimental bone metastasis	01.01.2016-30.06.2018	Prof. Bozec	Department of Medicine 3
D24	Differentiation-associated Schwann cell transcription factors in melanoma– learning from embryogenesis	01.06.2016-30.11.2018	Prof. Bosserhoff, Prof. Wegner	Institute of Biochemistry

Advanced Grants

Project No.	Project title	Term	Applicant(s)	Institute
D25	Interaction of the EGFR- and the ZEB1-pathway in aggressive cancer types	01.05.2016-31.10.2018	Prof. Brabletz	Chair of Experimental Medicine I
D26	Identification of antigen specificity of tumor-infiltrating lymphocytes in triple-negative breast cancer	01.01.2016-30.06.2018	Prof. Mackensen, Prof. Fasching	Department of Medicine 5, Department of Obstetrics and Gynecology
D27	2-Hydroxyglutarate in Acute Myeloid Leukaemia: Novel Molecular Targets and Impact on Immune Escape	01.07.2016-31.12.2018	Prof. Mougiakakos	Department of Medicine 5
D28	SPARCL1 function in vessel maturation and metastasis of colorectal carcinoma	01.02.2016-31.07.2018	Prof. Stürzl, PD Dr. Naschberger	Department of Surgery
D29	Aging and senescence of the adaptive immune system in colorectal cancer	01.01.2016-30.06.2018	Prof. Waldner	Department of Medicine 1

Neurosciences

Project No.	Project title	Term	Applicant(s)	Institute
E11	H50Q aSyn mutation in PD	01.12.2013-30.11.2016	Prof. Klucken, PD Dr. Xiang	Department of Molecular Neurology, Institute of Biochemistry
E12	Adult hippocampal neurogenesis in synucleinopathies	01.04.2014-31.03.2017	Prof. Winkler, Prof. Lie	Department of Molecular Neurology, Institute of Biochemistry
E13	The role of acid sphingomyelinase in depression/anxiety-induced alcohol addiction	01.04.2014-31.03.2017	Prof. Müller, PD Dr. Reichel, Prof. Kornhuber	Department of Psychiatry and Psychotherapy
E14	Role of TRPC5 in trigeminal nociception	01.04.2014-31.03.2017	Prof. Zimmermann	Department of Anaesthesiology
E15	GlyT1 and neuropathic pain	01.11.2013-31.10.2016	PD Dr. Eulenburg, Prof. Schulze	Institute of Biochemistry, Department of Otorhinolaryngology – Head and Neck Surgery
E16	Regulatory networks in neurogenesis and neurodevelopmental disorders	01.04.2014-31.03.2017	Prof. Lie, Prof. Reis	Institute of Biochemistry, Institute of Human Genetics
E17	The neuromuscular role of Wnt signaling pathways	01.04.2014-31.03.2017	Prof. Hashemolhosseini	Institute of Biochemistry
E18	Assessing developmental potential and differentiation capabilities of NG2-glia in the healthy and diseased central nervous system	01.12.2013-30.11.2016	Prof. Wegner, Prof. Winkler	Institute of Biochemistry, Department of Molecular Neurology
E19	Inhibitory neurotransmission in the cochlea: Glutamate and endocannabinoids	15.02.2016-14.08.2018	Prof. Enz	Institute of Biochemistry
E20	Identification of molecules, receptors and genes involved in chronic pruritus	01.05.2016-31.10.2018	Dr. Dr. Kremer, Prof. Zimmermann	Department of Medicine 1, Department of Anesthesiology

Project No.	Project title	Term	Applicant(s)	Institute
E21	Modulation of alpha-Synuclein pathology by FoxO-dependent pathways	01.05.2016-31.10.2018	Prof. Lie, Prof. Dr. Klucken	Institute of Biochemistry Department of Molecular Neurology
E22	The role of Swiprosin-1/EFhd2 in resilience to drug addiction	01.03.2016-31.08.2018	Prof. Müller, Prof. Alzheimer, PD Dr. Mielenz	Department of Psychiatry and Psychotherapy, Institute of Physiology and Pathophysiology, Department of Molecular Immunology
E23	Identification and characterization of LOXL1 risk variants for pseudoexfoliation syndrome and glaucoma	01.01.2016-30.06.2018	Prof. Schlötzer-Schrehardt, Prof. Reis	Department of Ophtalmology, Institute of Human Genetics
E24	The role of alpha-synuclein during inflammatory demyelination and degeneration in the central nervous system	01.01.2016-30.06.2018	Prof. Winkler, Prof. Linker	Department of Molecular Neurology, Department of Neurology
E25	Modeling pain syndromes using human induced pluripotent stem cell-derived nociceptors	01.07.2016-31.12.2018	Prof. Winner, Prof. Schüttler	IZKF Junior Research Group 3/ Institute of Human Genetics, Department of Anesthesiology
E26	Genetics and pathomechanisms of intellectual disability with microcephaly	01.03.2016-31.08.2018	PD Dr. Zweier	Institute of Human Genetics
E27	Lysophosphatidic acid-induced pruritus of cholestasis	01.03.2016-31.08.2018	Dr. Dr. Kremer, Prof. Fischer	Department of Medicine 1, Institute of Physiology and Pathophysiology

Renal and Vascular Research

Project No.	Project title	Term	Applicant(s)	Institute
F3	Fam60a in heart and brain development	01.03.2014-28.02.2017	Prof. Engel	Department of Nephropathology
F4	Pathogenesis of the short rib-polydactyly syndrome	01.10.2013-30.09.2016	PD Dr. Thiel	Institute of Human Genetics
F5	The Role of ANO1 in Polycystic Kidney Disease	01.07.2016-31.12.2018	Dr. Buchholz	Department of Medicine 4
F6	Renal afferent nerve activity - sympathoinhibitory or sympathoexcitatory?	01.07.2016-31.12.2018	Prof. Veelken, Prof. Amann	Department of Medicine 4, Department of Nephropathology

A52 - Final Report

01.11.2013 - 31.10.2016

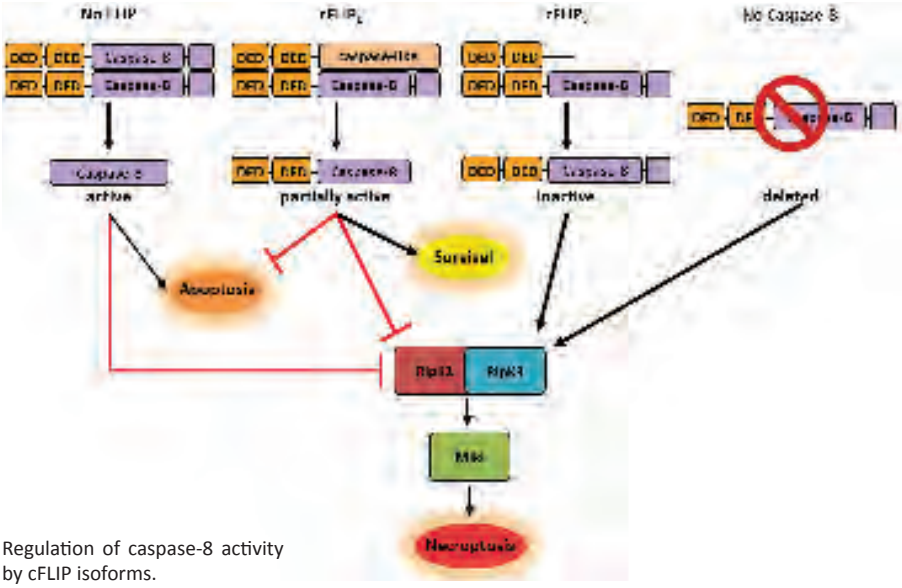
cFlip isoforms in the intestinal epithelium

Dr. Claudia Günther, Prof. Dr. Christoph Becker,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

cFLIP, an important regulator of cell death and survival, tightly controls the activity of caspase-8. It decides if cell death is initiated and which cell death program is triggered. The pathogenesis of chronic IBD results from this regulatory network. Caspase-8 also has a role in infectious colitis. We investigated the role of caspase-8 in the S. Typhimurium infection model. Our data demonstrate a crucial role for Caspase-8, which is regulated by cFLIP, in infectious colitis.

Excessive epithelial cell death has been associated with inflammation and has been identified as a major factor in the pathogenesis of inflammatory bowel diseases (IBD) and other chronic inflammatory disorders of the gastrointestinal tract. Accordingly previous studies have identified increased levels of epithelial cell death in patients suffering from Crohn's disease as well as ulcerative colitis. The current concept predicts that increased levels of proinflammatory cytokines in these patients trigger the activation of cell death in the intestinal epithelium. An important mediator for cell death that is activated by extracellular signals is caspase-8. Recent data have demonstrated, that the activation status of caspase-8 decides which form of cell death is initiated. Caspase-8 is activated via cell death receptors and consequently initiates the caspase-cascade, which leads to classical apoptosis. Thus blocking of caspases appeared to be a valid potential therapeutic option for patients with IBD. Surprisingly, our recent studies have shown, that inhibition of caspases does not protect from cell death. On the contrary, a novel form of regulated cell death, denoted as necroptosis, is initiated, mediated by the effector molecules such as Ripk1, Ripk3 and Mlkl.

In summary our data provide evidence that caspase-8 activity has to be tightly controlled in order to maintain intestinal homeostasis. Accordingly the host expresses the cellular FLICE-inhibitory protein, cFLIPs, which tightly regulates the activity of caspase-8. Two isoforms of cFLIP can interact with the inactive pro-caspase-8: short ($cFLIP_S$) and long ($cFLIP_L$). Binding of $cFLIP_L$ to pro-caspase-8 blocks autocatalytic cleavage and therefore the production of a fully active caspase-8 molecule, which can initiate apoptosis. Instead a partially active form of caspase-8 is produced, which is able to block necroptosis by inactivation of Ripk3 through its residual catalytic activity. In contrast to this, inac-



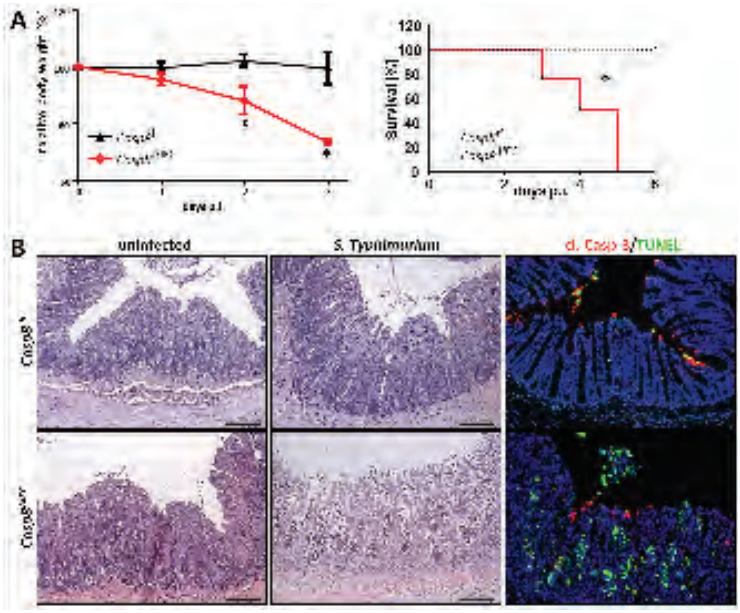
Regulation of caspase-8 activity by cFLIP isoforms.

Dr. Günther Prof. Dr. Becker

tivation of pro-caspase-8 by cFLIPS completely prevents the initial activation of caspase-8. As a result the activity of Rip kinases cannot be blocked resulting in the activation of necroptosis. This was mimicked by genetic deletion of caspase-8.

Due to the important role for caspase-8 and its regulation in inflammatory conditions, we further aimed to address its role during infectious colitis caused by enteric pathogens.

Therefore, we orally infected mice with 10^9 CFU of *Salmonella* Typhimurium. As a result of the infection, mice with a conditional deletion of caspase-8 in the intestinal epithelium ($Casp8^{\Delta IEC}$) displayed severe weight loss in the first days after infection, as compared to control animals. Consequently, these mice showed high lethality, while controls survived the infection. Histological analyses revealed severe destruction of the colon, namely an excessive amount of epithelial cell death. Additional deletion of Ripk3 or Mlkl could rescue epithelial cell death and lethality of S. Typhimurium infected $Casp8^{\Delta IEC}$ mice. In summary, our results show a crucial role for caspase-8 to maintain intestinal immune homeostasis and barrier function during pathogenic infection.



$Casp8^{\Delta IEC}$ mice are highly susceptible to infection with *Salmonella* Typhimurium. A) Weight curve and Kaplan-Meier survival curve. B) Colon cross sections stained with H&E, β-Catenin or cleaved Caspase-3 and TUNEL of infected $Casp8^{\Delta IEC}$ and control animals.

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Invited lectures

BTCure Workshop Animal Models of Disease, 18.09.-20.09.2016, Athens, Greece, "Pathways to Necrosis in Inflammatory Liver Injury"

9th Swiss Apoptosis Meeting, 08.-09.09.2016, Bern, Switzerland, „Cell death pathways driving inflammation in the liver and gut „

MIAMI-Symposium, 13.-15.04.2016, Münster, Germany, „Innate Immune Pathways in the Gut"

Publications during funding period

Martini E, Wittkopf N, Günther C, Okada H, Watson A, Podstawa E, Backert I, Neurath MF, Becker C (2016) Loss of Survivin in intestinal epithelial progenitor cells leads to mitotic catastrophe and breakdown of gut immune homeostasis. Cell Reports 14(5): 1062-7

Gunther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Amann K, Vandenabeele P, Linkermann A, Poremba C, Schleicher U, Dewitz C, Krautwald S, Neurath MF, Becker C, Wirtz S (2016) The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. J Clin Invest 126(11): 4346-4360

He GW*, Gunther C*, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S Becker C (2016) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut. [Epub ahead of print]

* Shared first authorship

Gunther C, Josenhans C, Wehkamp J (2016) Crosstalk between microbiota, pathogens and the innate immune responses. Int J Med Microbiol 306(5): 257-265

A53 - Final Report

01.10.2013 - 30.09.2016

Th17/piTreg differentiation in vivo

Prof. Dr. Kai Hildner, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

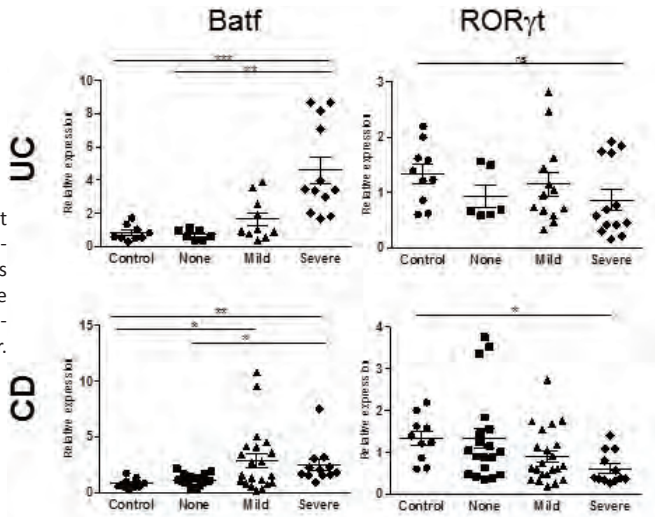
The AP1 transcription factor Batf is strongly upregulated within IBD-affected tissues. Functionally, upon experimental disruption of the mucosal barrier in mice Batf deficiency abrogated intestinal epithelial cell homeostasis in a T cell intrinsic manner suggesting that in the gut Batf-dependent immune-regulatory prevail over pro-inflammatory properties. Overall, our studies revealed that Batf represents a central regulator of both Th17 and induced Treg development in vivo.

Human Batf gene expression levels are strongly upregulated during intestinal inflammation

Given the established key role of Batf during Th17 cell differentiation and murine T cell driven colitis, we asked whether human Batf gene expression is regulated within inflammatory bowel disease (IBD)-affected intestinal tissues. Strikingly, Batf expression was strongly upregulated within colitic tissues of both Crohn's disease (CD) and Ulcerative colitis (UC) patients in an inflammation-dependent manner while Th17 cell fate regulating transcription factor RORγt showed reduced expression levels as colitis activity increased. Given our experimental results that Batf also exerts important immune-regulatory functions in T cells limiting tissue-destruction, differentially regulated expression levels might indicate non-overlapping functions of Batf and RORγt in the course of the colitis-mediating immune response.

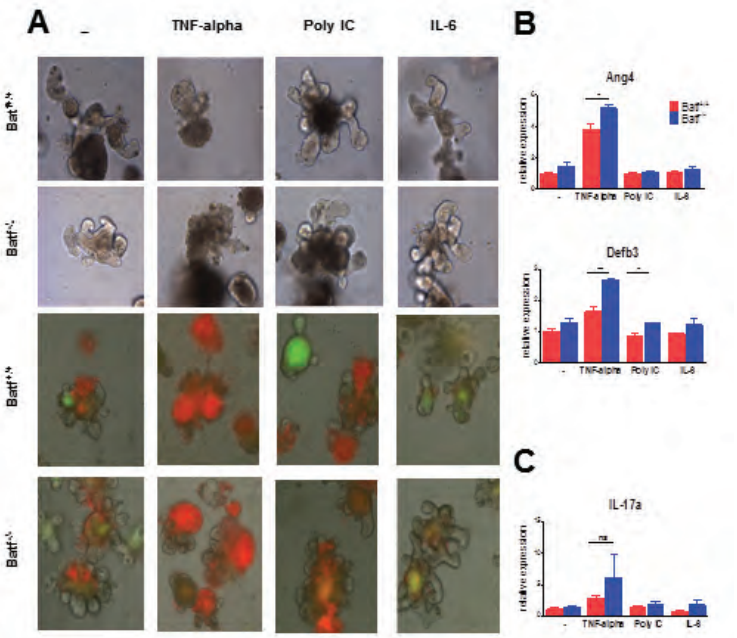
Batf is dispensable for intestinal epithelial cell differentiation and function

Transcription factors like signal transducer and activator of transcription 3 (Stat3) or Hypoxia-inducible factor 1-alpha (HIF-1α) were shown to cooperate with Batf during Th17 cell differentiation but are also known to exert direct functions within intestinal epithelial cells (IEC) hampering the interpretation of gene expression data obtained by whole gut tissue analyses. To further explore whether Batf is involved in intestinal epithelial cell homeostasis in an



Human Batf but not RORγt gene expression is upregulated in Ulcerative colitis (UC) and Crohn's disease (CD) tissues in an inflammation-dependent manner.

Prof. Dr. Hildner



Loss of Batf within murine intestinal epithelial progenitor cells is dispensable for IEC homeostasis and functionality ex vivo; (A) organoid morphology; (B-C) Ang4 (Paneth cells), Defb3 and IL17a (IEC) gene expression profiles within organoids.

IEC-intrinsic manner, we performed intestinal crypt culture experiments adopting a well-established ex vivo culture system. Here, intestinal crypts are cultured in the presence of a defined cocktail of growth and differentiation inducing factors resulting in the formation of so called organoids that originate from intestinal stem cells residing at the bottom of the crypts and display all cell types normally present in the IEC compartment including goblet and Paneth cells. Importantly, kinetics and dynamics of organoid development occurred in a Batf-independent manner. In addition, morphologic features as well as the rate of apoptotic cell death observed in these cultures over time were indistinguishable between Batf-sufficient and Batf-deficient organoid cultures. Furthermore, spontaneous expression levels of anti-microbial molecules like angiogenin (Ang4) and defensing beta 3 (Defb3) representing signature molecules of specialized intestinal epithelial cells (e.g. Paneth cells) were also independent of Batf expression. Finally, IL-17a expression within organoids was uncompromised in the absence of Batf overall indicating that development, morphology and functionality of intestinal organoids is not regulated by Batf in the steady state. Altogether, Batf appears to exclusively regulate T cell functionality and does not modulate IECs directly. Hence in contrast to e.g. attempts to inhibit Stat3, therapeutic targeting of Batf might emerge as a T cell centered approach modulating intestinal inflammation.

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Invited lectures

GvH/ GvL 2016, 13th International Symposium, March 17th, Regensburg, „The Impact of Dendritic Cells on Intestinal Microbiota, Dysbiosis and Colitis phenotypes“

Publications during funding period

Punkenburg E, Vogler T, Büttner M, Amann K, Waldner M, Atreya R, Abendroth B, Mudter J, Merkel S, Gallmeier E, Rose-John S, Neurath MF, Hildner K (2016) Batf-dependent Th17 cells critically regulate IL-23 driven colitis-associated colon cancer. Gut 65(7): 1139-1150

Hildner K, Punkenburg E, Abendroth B, Neurath MF (2016) Immunopathogenesis of IBD: Batf as a Key Driver of Disease Activity. Dig Dis. 34, Suppl 1:40-7

A54 - Final Report

01.11.2013 - 31.10.2016

Fam180a in inflammatory diseases

PD Dr. Dr. Stefan Wirtz, Prof. Dr. Maximilian Waldner,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

The characterization of so far unknown signal molecules in the coordination of the immune reaction in infections, chronic inflammatory diseases or the anti-cancer immune response represents a central approach in identifying novel drug targeting strategies. Fam180a, a putative cytokine and possible member of the IL-12 family of cytokines, is a highly conserved, yet so far in the literature undescribed polypeptide. We characterized Fam180a in terms of expression, localization and secretion, as well as its functional role in different models of acute inflammation and observed significant effects in its absence as well as in its abundance in the murine system in a context of acute colitis.

Composition as a homomultimer

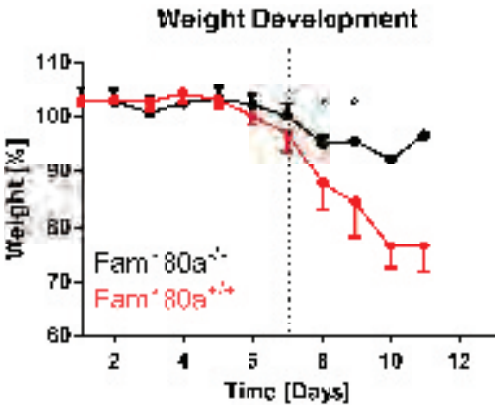
Various cytokines including members of the IL-12 cytokine family are known to combine different subunits to a functional heterodimeric cytokine prior to the release from the cell. According to our data, Fam180a can be released from cells as a monomer, and also multimeric forms. So far, it remains to be revealed, if these multimers are composed of Fam180a alone or in combination with other cytokine subunits.

Highest expression in stromal cell populations of the lymph nodes

While basic expression levels of Fam180a were observed in several evaluated tissues and cell types, high expression levels were found in stromal cell populations of the lymph nodes, spleen and gut-associated lymphoid tissues (GALT). In particular, the population of gp38+ fibroblastic reticular cells (FRCs) and blood endothelial cells showed the most abundant steady state presence of Fam180a transcripts.

Fam180a is secreted from cells

The Fam180a peptide carries a N-terminal secretion tag. By Western-Blot and mass spectrometry we could show that Fam180a is secreted into the culture supernatant following overexpression in various cell lines. Furthermore, after overexpression of constructs lacking the signal peptide, we could not identify FAM180A in the supernatant indicating that Fam180a acts as a secreted protein.



Analysis of weight development as an indicator of colitis severity in wildtype and Fam180A$^{-/-}$ mice subjected to oral treatment with 2% DSS for 7 days.

Fam180a drives acute intestinal inflammation in DSS induced colitis

To functionally address the role of FAM180A in the context of inflammation, we next took advantage of Fam180A deficient mice. Moreover, we generated mice conditionally overexpressing Fam180a controlled by an ubiquitously active (β-actin) and an more restricted (Tie2) promoter. Both systemic Fam180a deficiency and its overexpression in mice leads to no spontaneous phenotype indicating that this protein does not play a substantial


PD Dr. Dr. Wirtz


Prof. Dr. Waldner

immune regulatory role in the steady state. By contrast, we were able to establish an important functional role of Fam180A in a colitis model induced by oral administration of dextran sodium sulfate (DSS). For example, animals lacking Fam180a showed significantly reduced loss of weight, immune cell infiltrations and colon wall thickening in the context of DSS induced colitis. Interestingly, a next generation sequencing based strategy to compare the intestinal microbiome revealed profound compositional shifts in the Fam180A$^{-/-}$ mice after colitis induction. Interestingly, in most of the mice Fam180A deficiency was associated with a strong prevalence of *Akkermansia mucinphilia*, a mucin-degrading bacterium belonging to the phylum *Verrumicrobia* implicated in the pathophysiology of human diseases.

Concluding remarks

While the secreted protein Fam180a does not seem to have an important physiological function in the steady state, our data clearly indicate a significant role in the context of acute intestinal inflammation. The future identification of the cellular receptor of Fam180a and its relevant downstream signaling pathways in target cells will ascertain, whether FAM180A represents a novel target for the therapy of inflammatory diseases such as inflammatory bowel disease.

Stool samples of mice subjected to acute DSS induced colitis were collected and genomic DNA was isolated. The bacterial microbiome was analyzed by 16S-based next generation sequencing using Illumina technology. Phylum level analysis is shown.

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Invited lectures

Seminar des Instituts für Medzinische Mikrobiologie und Hygiene, Universität Regensburg, 14.04.2016, Regensburg, M. Waldner, „Hypoxia-dependent signaling pathways in colitis-associated cancer“

MSOT Symposium, 06.09.2016, Memorial Sloan Kettering Cancer Center, New York, M. Waldner, „MSOT for the non-invasive evaluation of intestinal inflammation in Crohn's disease“

Publications during funding period

Mattner J, Wirtz S. (2016) Friend or Foe? The Ambiguous Role of Innate Lymphoid Cells in Cancer Development. Trends in Immunology. 38: 29-38

Waldner, M. J., et al. (2016). Multispectral Optoacoustic Tomography in Crohn's Disease: Noninvasive Imaging of Disease Activity. Gastroenterology 151: 238-240

A55 - Final Report

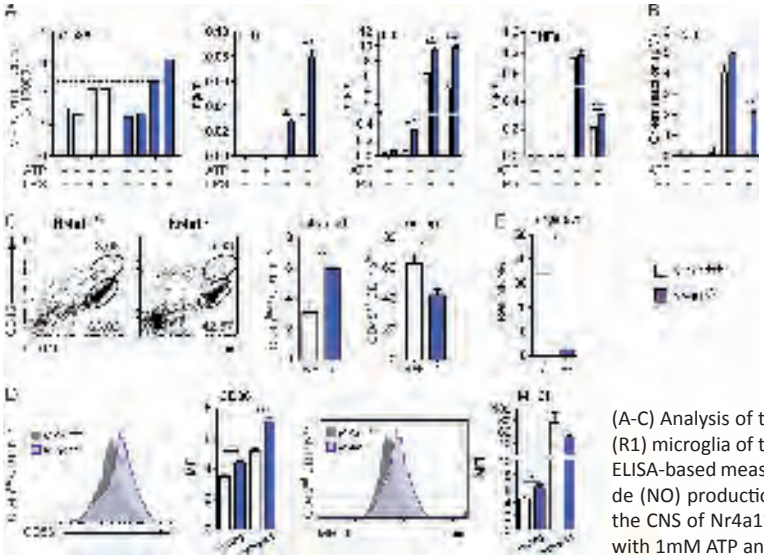
01.01.2014 - 31.12.2016

NR4a1 during immunologic tolerance

Prof. Dr. Gerhard Krönke, Department of Medicine 3 – Rheumatology and Immunology

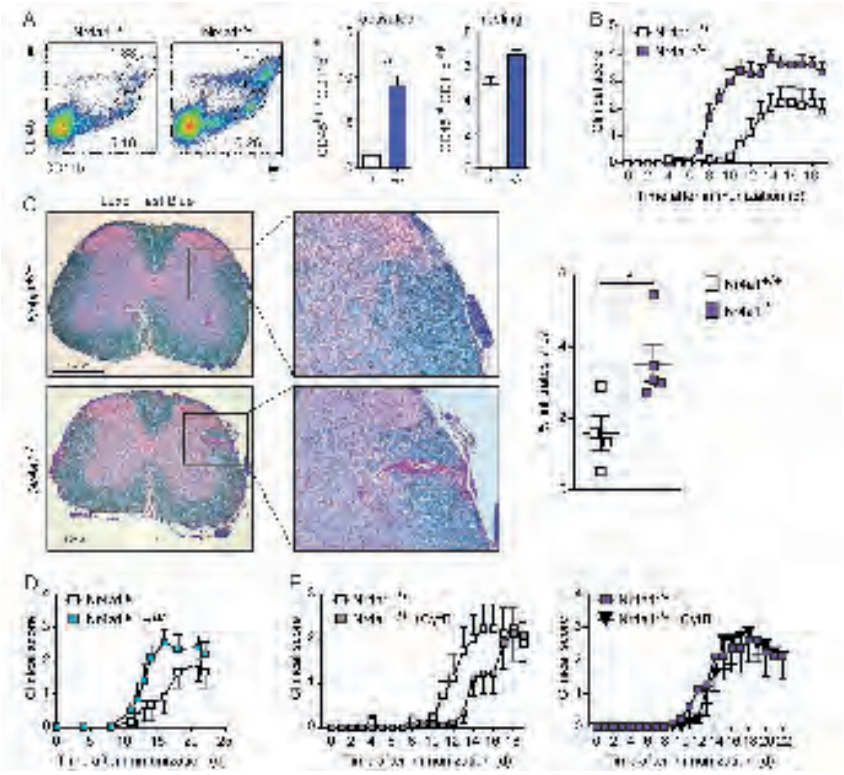
During the last 3 years, we were able to dissect the role of the nuclear receptor (NR) Nr4a1 in macrophages and microglia, where Nr4a1 negatively controlled the activation status and polarization of these cells. Deletion of Nr4a1 resulted in the exacerbation of murine models of autoimmune diseases such as systemic lupus or multiple sclerosis; whereas ligand induced activation of this NR protected mice from such inflammatory diseases.

Our data show that the nuclear receptor (NR) Nr4a1 acts as key rheostat controlling the macrophage and microglia activation threshold and protecting from autoimmune-driven CNS inflammation. In steady state microglia, ubiquitous neuronal-derived stress signals such as ATP induced expression of this NR, which contributed to the maintenance of a resting and non-inflammatory microglia phenotype. Global and myeloid-specific deletion of Nr4a1 triggered the spontaneous and overwhelming activation of microglia and resulted in an accelerated and exacerbated form of experimental autoimmune encephalomyelitis (EAE). Ligand-induced activation of Nr4a1, in turn, strongly ameliorated the course of disease. Furthermore, Nr4a1 controlled the non-inflammatory phagocytosis of apoptotic cells (ACs) resulting in an increased production of inflammatory mediators upon phagocytosis of AC by Nr4a1-deficient macrophages. This in turn interfered with the non-immunogenic removal of AC-derived autoantigens and an exacerbation of autoimmune diseases such as systemic lupus. On a molecular level, Nr4a1 interfered with the phosphorylation and activation of the NfkB subunit p65 and thus blocked the expression of pro-inflammatory genes in microglia and macrophages. Our current data thus identify Nr4a1 as regulator of microglia and macrophage activation and potentially new target for the treatment of inflammatory diseases such as multiple sclerosis and systemic lupus.



(A-C) Analysis of the activation state of resting (R2) and activated (R1) microglia of the brain of Nr4a1$^{+/+}$ and Nr4a1$^{-/-}$ mice. (D and E) ELISA-based measurement of indicated cytokines and of nitric oxide (NO) production of in vitro-cultivated microglia isolated from the CNS of Nr4a1$^{+/+}$ and Nr4a1$^{-/-}$ mice. Microglia were stimulated with 1mM ATP and/or LPS (100ng/ml).

Prof. Dr. Krönke



(A) Analysis of resting (R2) and activated (R1) microglia in the brain of $Nr4a1^{+/+}$ and $Nr4a1^{-/-}$ mice after induction of EAE. (B) Clinical disease course and (C) histological analysis of the spinal cord of $Nr4a1^{+/+}$ and $Nr4a1^{-/-}$ mice after induction of EAE. (D) EAE disease course in Nr4a1fl/fl-LysM mice and their Nr4a1fl/fl littermate controls as well as (E) EAE disease course of $Nr4a1^{+/+}$ and $Nr4a1^{-/-}$ mice that received a daily i.p. dose of the Nr4a1 ligand cytosporone-B (CytB).

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Invited lectures

10th International Congress on Spondyloarthritides, 2016, Gent, Belgium,

Annual Meeting of the German Society of Immunology (DGfI), 2016, Hamburg, German

Joint Meeting on Vascular Biology, Inflammation and Thrombosis, 2016, Vienna, Austria

Keynote Lecture at the Meeting of Infectious Immunology of the German Society of Immunology (DGfI), 2016, Burg Rothenfels, Germany

Conference on Macrophages in vertebrates, 2016, Faculté des Sciences de Montpellier, Montpellier, France

Publications during funding period

Pfeifle R, Rothe T, Ipseiz N, Scherer HU, Culemann S, Harre U, Ackermann JA, Seefried M, Kleyer A, Uderhardt S, Haugg B, Hueber AJ, Daum P, Heidkamp GF, Ge C, Böhm S, Lux A, Schuh W, Magorivska I, Nandakumar KS, Lönnblom E, Becker C, Dudziak D, Wuhrer M, Rombouts Y, Koeleman CA, Toes R, Winkler TH, Holmdahl R, Herrmann M, Blüml S, Nimmerjahn F, Schett G, Krönke G (2017) Regulation of autoantibody activity by the IL-23-TH17 axis determines the onset of autoimmune disease. Nat Immunol. 18(1): 104-113

Rothe T, Gruber F, Uderhardt S, Ipseiz N, Rössner S, Oskolkova O, Blüml S, Leitinger N, Bicker W, Bochkov VN, Yamamoto M, Steinkasserer A, Schett G, Zinser E, Krönke G (2015) 12/15-Lipoxygenase-mediated enzymatic lipid oxidation regulates DC maturation and function. J Clin Invest.125(5): 1944-54

A56 - Progress Report

01.03.2014 - 28.02.2017

Role of HIG2 in atherosclerosis

PD Dr. Christina Warnecke, Department of Medicine 4 – Nephrology and Hypertension

The hypoxia-inducible lipid droplet protein Hig2/Hilpda is highly expressed in atherosclerotic foam cells, but its role in atherogenesis has not been shown. We found that Hilpda is crucial to neutral lipid accumulation, resistance against lipid overload and regulated PGE2 production in macrophages. Tie2-cre-mediated Hilpda depletion in apolipoprotein E-deficient mice reduced plaque size and macrophage content under normal diet, whereas the differences were blurred after high fat diet.

Hig2/hilpda is a Hif-1 and Ppar target in macrophages and mediates lipid droplet (LD) formation.

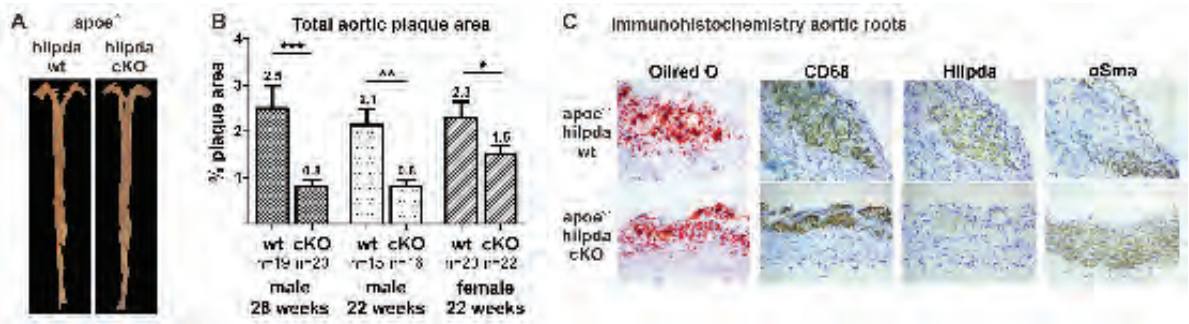
Bone marrow-derived macrophages (BMDMs) from tie2-cre hilpda knockout (cKO) mice could not form LDs after Hif-1 activation or loading with fatty acids or cholesterol. Hilpda was upregulated by Hif-1 under M1 and M2 conditions, as well as by Ppar ligands and fatty acids. In contrast, adrp/plin2, which has been implicated in hypoxic lipid accumulation in human tumor cells, was a Ppar target, but hardly induced by Hif-1 in BMDMs, presumably due to the different promoter structure of the mouse gene. Hif-1α cKO and adrp knockdown reduced, but did not abolish lipid accumulation. Localization, induction kinetics and stability of Hilpda and Adrp/Plin2 indicated that Hilpda mediates LD formation, whereas Adrp stabilizes LDs.

Mechanisms and functional consequences of Hilpda-mediated neutral lipid storage.

Hilpda-mediated lipid storage did not require increased uptake of lipoproteins or fatty acids. Tracing of "click"-fatty acids confirmed that esterification to di- and triacylglycerol is impaired in hilpda cKO BMDMs, but not uptake and integration into membrane phospholipids.

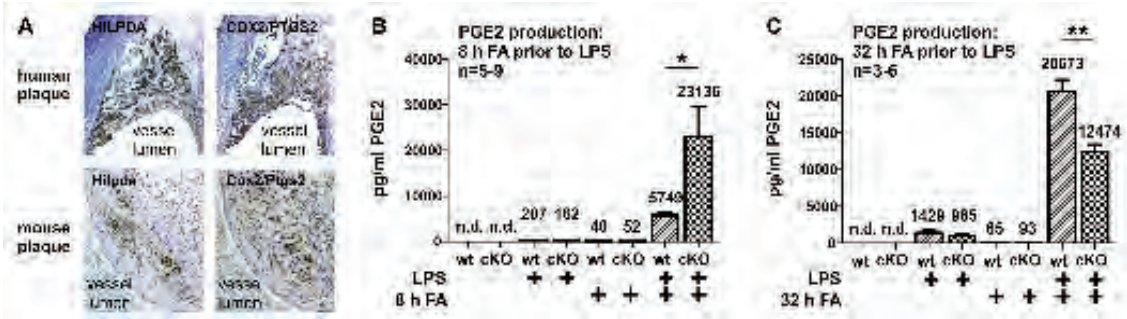
Hilpda-mediated LD formation reduced ROS levels after reoxygenation and protected against membrane damage after fatty acid overload, but did not increase ATP levels under various experimental conditions.

After fatty acid loading for 8 h, which markedly enhanced subsequent LPS-induced PGE2 formation, hilpda cKO BMDMs produced more PGE2, whereas after fatty acid loading for 32 h LPS-stimulated PGE2 production was reduced in hilpda cKO BMDMs, indicating that LDs can serve both as buffer and as reservoir for the fatty acid substrates of PGE2 production. Thus, Hilpda-mediated LD formation is crucial to a controlled and inducible production of PGE2.



In apoe$^{-/-}$ tie2-cre hilpda cKO mice total aortic plaque area in *En face* preparations is reduced (A and B). Aortic root lipid deposition (Oil red O) and macrophage content (CD68) are decreased, and α-smooth muscle actin (αSma) expression increased (C).

PD Dr. Warnecke



Hilpda co-localizes with Cox2 in murine and human plaques (A). Hilpda-mediated LDs restrict PGE2 production during acute fatty acid overload (B), but serve as fatty acid substrate reservoirs for PGE2 production 32 h after fatty acid loading (C).

Hilpda contributes to atherogenesis in Apolipoprotein E-deficient mice.

In human and murine plaques HILPDA was highly expressed in foam cells surrounding the lipid core and in the vulnerable plaque shoulder. Co-localization with COX2 suggested that not only foam cell formation, but also PGE2 production depend on Hilpda and contribute to destabilization and rupture of advanced human plaques.

We analyzed 22- and 28-week-old apolipoprotein E-deficient (apoe$^{-/-}$) mice with lysM-cre- and tie2-cre-driven hilpda cKO. In tie2-cre cKO, but not in lysM-cre cKO mice, Hilpda depletion reduced macrophage content and lipid deposition in aortic roots, and total lesion areas of whole aortas. The effect was more pronounced in male than in female mice. We then investigated male apoe$^{-/-}$ tie2-cre hilpda cKO mice after 8 weeks of high fat diet (HFD), expecting to accentuate differences between hilpda cKO and wt mice. However, although atherogenesis was enhanced, HFD did not increase, but blurred the differences between Hilpda cKO and wt mice. Thus, similar to the role of Hilpda in murine models of hepatosteatosis, lipid overflow can mask the effect of Hilpda on atherogenesis.

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Publications during funding period

none

A57 - Final Report

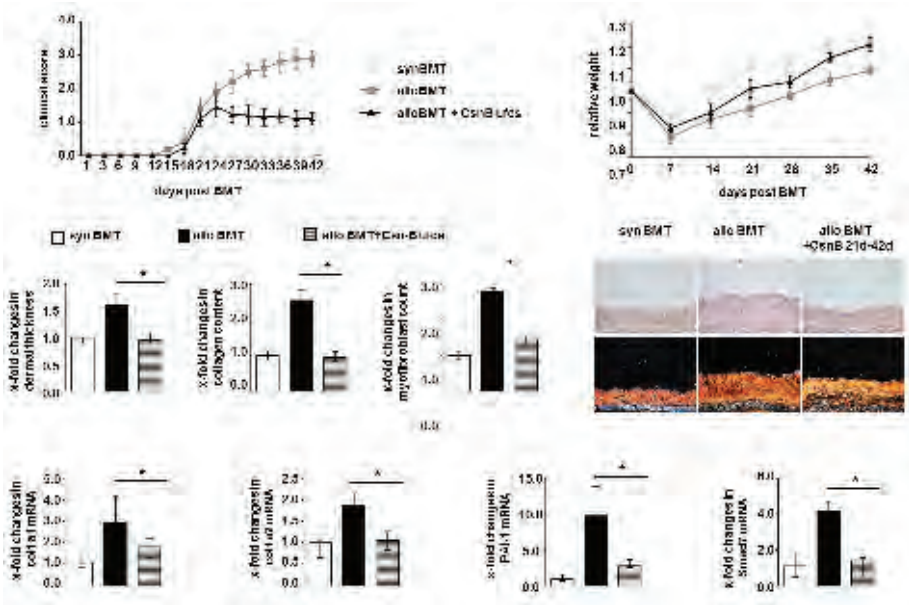
01.01.2014 - 31.12.2016

Nr4a1 as a novel target for the treatment of sclerodermatous chronic graft-versus-host disease

Prof. Dr. Jörg Distler, Department of Medicine 3 – Rheumatology and Immunology
Prof. Dr. Bernd Spriewald, Department of Medicine 5 – Haematology and Oncology

The orphan nuclear receptor Nr4a1 is an endogenous antagonist of TGF-β, which is inactivated in sclerodermatous cGvHD by phosphorylation. Pharmacologic agonists of Nr4a1 inhibit the phosphorylation-induced inactivation of Nr4a1 in cGvHD, thereby preventing the aberrant activation of fibroblasts and inhibiting tissue fibrosis in experimental cGvHD.In addition to cGvHD, Nr4a1 may also be a target for therapeutic intervention in other fibrotic diseases as shown be the efficacy of Nr4a1 agonists in preclinical models of IPF, SSc, renal fibrosis and liver cirrhosis.

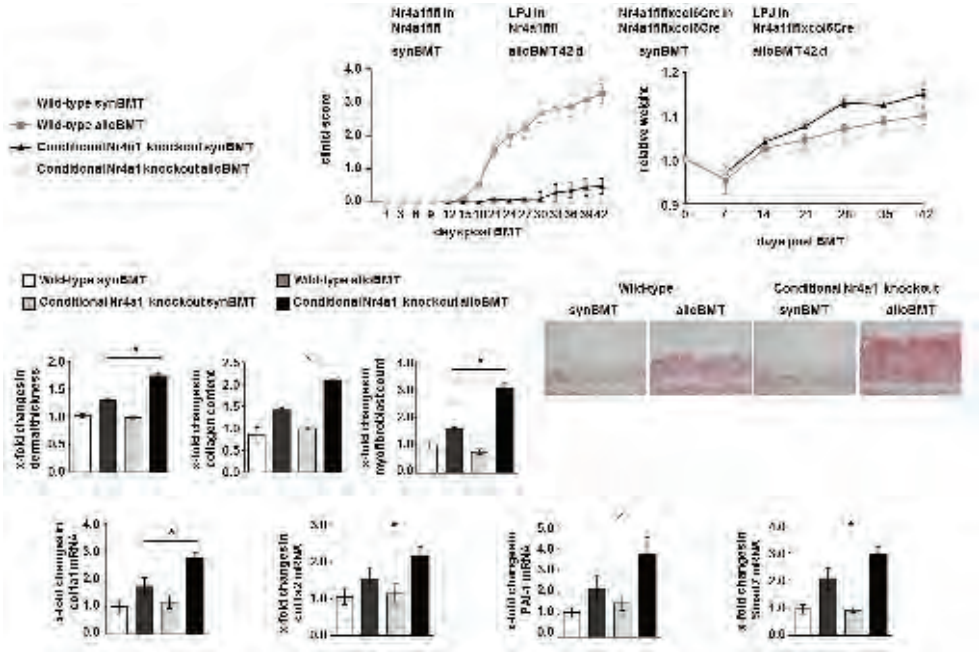
Nr4a1 inhibits TGF-β signaling via SP1-dependent transrepression with recruitment of cofactors such as Sin3A, CoREST, LSD1 and HDAC1 and after elucidating GSK3-dependent phosphorylation and HDAC-dependent epigenetic silencing as underlying mechanisms for the inactivation of Nr4a1 upon prolonged TGF-β stimulation, we further evaluated the effects of Nr4a1 in murine sclerodermatous cGvHD and on the graft-versus-leukemia reaction (GvL). We demonstrated that the Nr4a1 agonist Csn-B also ameliorated clinical features of murine cGvHD such as weight loss and the cutaneous cGvHD score, when applied not in preventive, but in therapeutic dosing schedules with dosing initiated only after the onset of first clinical signs of Scl cGvHD on d21 post bone marrow transplantation. Therapeutic dosing of Csn-B also effectively inhibited enhanced TGF-β signaling with decreased mRNA levels of target genes such as Smad7 and PAI-1, reduced myofibroblast differentiation and ameliorated collagen accumulation and skin thickening. In contrast, mice with fibroblast-specific knockout of Nr4a1 (Col1a2 CreER; $Nr4a1^{fl/fl}$ mice with tamoxifen) were more sensitive to cGvHD induced fibrosis than control mice with normal levels of Nr4a1 (Col1a2 CreER; $Nr4a1^{fl/fl}$ mice with corn oil; further referred to as wildtype phenotype). While fibrosis was more severe and the upregulation of TGF-β target genes was enhanced in mice with fibroblast-spe-



Therapeutic dosing of CsnB, initiated after the first onset of clinical signs of cGvHD, inhibits TGF-β signaling and ameliorates clinical as well as histological features of murine cGvHD.

Prof. Dr. Distler

Prof. Dr. Spriewald



Fibroblast-specific, inducible knockout of Nr4a1 (Col1a2 CreER; Nr4a1 fl/fl mice) enhances TGF-β signaling and exacerbates cGvHD-associated fibrosis, but not inflammation-dependent features such as weight loss, skin ulcers and hair loss.

cific knockout of Nr4a1, inflammation-dependent features such as weight loss, skin ulcers and hair loss were comparable between both groups. We further analyzed the effects of Nr4a1 activation on GvL and demonstrated that treatment with Csn-B may only slightly impair the GvL reaction as mice treated with Csn-B died slightly earlier than vehicle-treated, allogeneically transplanted control mice. Similar results were obtained by mixed lymphocyte reactions. The antifibrotic effects of the Nr4a1 agonist Csn-B were not restricted to experimental cGvHD, but were also observed in murine models of other fibrotic diseases such as IPF, SSc, renal fibrosis and liver cirrhosis. Together, these data highlight a central role of Nr4a1 in TGF-β signaling and indicate that Nr4a1 agonists may have potential as novel treatment approaches for fibrotic diseases.

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Invited lectures

Keystone Meeting “Fibrosis: From Basic Mechanisms to Targeted Therapies”, February 2016, Keystone/CO/USA, Targeting TGFbeta signaling for therapeutic intervention

Publications during funding period

Liang R, Šumová B, Cordazzo C, Mallano T, Zhang Y, Wohlfahrt T, Dees C, Ramming A, Krasowska D, Michalska-Jakubus M, Distler O, Schett G, Šenolt L, Distler JH (2016) The transcription factor GLI2 as a downstream mediator of Transforming Growth Factor-β induced fibroblast activation in SSc. Ann Rheum Dis [Epub ahead of print]

Palumbo-Zerr K, Soare A, Zerr P, Liebl A, Mancuso R, Tomcik M, Sumova B, Dees C, Chen CW, Wohlfahrt T, Mallano T, Distler A, Ramming A, Gelse K, Mihai C, Distler O, Schett G, Distler JH (2017) Composition of TWIST1 dimers regulates fibroblast activation and tissue fibrosis. Ann Rheum Dis. 76(1): 244-251

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A58 - Final Report

01.10.2013 - 30.09.2016

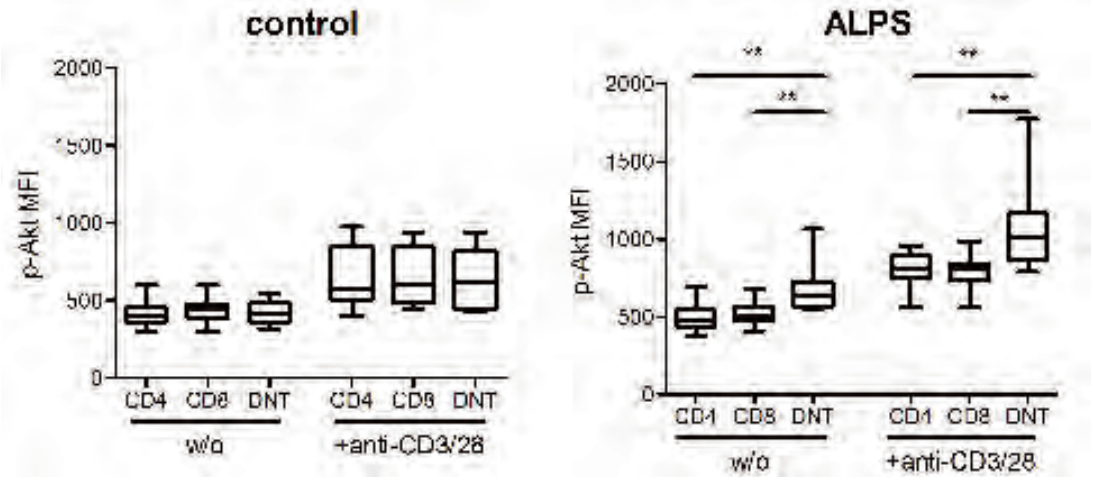
Characterization of DN T cells from ALPS patients

Prof. Dr. Andreas Mackensen, Dr. Simon Völkl, Department of Medicine 5 – Haematology and Oncology

Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of lymphocyte homeostasis associated with mutations in genes involved in the Fas apoptosis pathway. Defective Fas signaling results in chronic benign lymphoproliferation with clinically relevant splenomegaly, lymphadenopathy and autoimmune manifestations. A prominent feature of ALPS is the accumulation of CD3+ TCRαβ+ CD4-/CD8- (double negative, DN) T cells. Despite being a hallmark of this disease, the origin and function of DNT cells in ALPS is widely unknown.

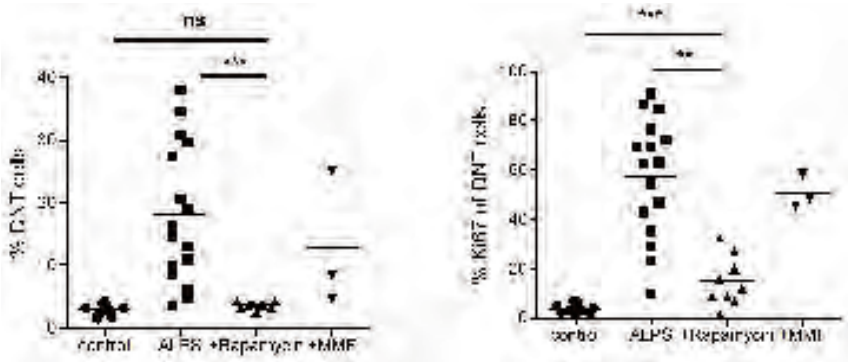
Abnormally differentiated DNT cells of ALPS patients are highly proliferative in vivo

Most characteristic indication of ALPS is the chronic benign lymphoproliferation with massive, often visible lymphadenopathy and splenomegaly. However, it has been unclear whether proliferative capacity affects all lymphocytes or is restricted to defined cell subsets. To examine in detail the mitotic activity of lymphocyte subsets, we analyzed expression of the nuclear protein Ki67, which is present during active phases of cell cycle (G1 to M) but is absent in resting cells (G0). We observed low but significantly increased frequencies of Ki67+ CD4+ and CD8+ T cells in ALPS patients compared to healthy controls. In contrast, DNT cells from ALPS patients highly expressed Ki67, indicating that these cells progress through cell cycle. The majority of ALPS DNT cells are CCR7-, CD45RA+, and CD57+, an expression pattern of terminally differentiated effector memory cells. Further analysis of T cell differentiation markers revealed a linear relationship between the percentage of DNT cells showing a CCR7-/CD45RA+ differentiation state and the fraction of DNT cells with mitotic activity. Thus, in contrast to CCR7-/CD45RA+/CD57+ terminally differentiated T cells of healthy individuals, which are generally associated with poor proliferative potential and low Ki67 expression, ALPS DNT cells expressing the same markers are highly proliferative *in vivo*.



ALPS DNT cells exhibit hyperactive mTOR signaling. Cells from ALPS patients and healthy controls were left unstimulated (w/o) or stimulated with anti-CD3/CD28 mAbs and analyzed for phosphorylation of protein kinase Akt. Graphs show mean fluorescence intensity (MFI).

Prof. Dr. Mackensen Dr. Völkl



Rapamycin therapy controls accumulation and mitotic activity in vivo. Frequency of DNT cells among all T cells and percentage of Ki67+ cells among DNT cells in healthy controls (circle), ALPS patients (square), and ALPS patients treated with rapamycin (up-pointing triangle) or mycophenolate mofetil (MMF, down-pointing triangle) is shown.

ALPS DNT cells show hyperactive mTOR signaling

As ALPS DNT cells vigorously proliferate *in vivo* but paradoxically express costimulatory receptors CD27 and CD28, we considered that signal transduction downstream of costimulatory receptors might be crucial for accumulation of DNT cells in ALPS. An important molecule, activated downstream of CD28, is the serine/threonine kinase mTOR, which plays a key role in regulating cell proliferation and effector differentiation. We therefore analyzed the phosphorylation status of protein kinase Akt(S473), mTOR(S2448) and its downstream target ribosomal protein S6 at both phosphorylation sites (S235/6 and S240) in T cells from ALPS patients *ex vivo.* Intriguingly, ALPS DNT cells showed enhanced basal and activation-induced phosphorylation of Akt, mTOR and S6 as compared to ALPS bulk CD4+ and CD8+ T cells or the respective cell populations from healthy controls. The mTOR inhibitor rapamycin has successfully been used to treat autoimmune cytopenias and lymphoproliferation in ALPS patients. Given that enhanced mTOR signaling is present in ALPS DNT cells but not in CD4+ or CD8+ T cells, we hypothesized that rapamycin predominantly affect this cell subset. We first investigated how rapamycin affects proliferation and survival of ALPS DNT cells *in vitro.* Notably, even low concentrations of rapamycin induced a substantial inhibition of the mitotic activity of ALPS DNT cells. In contrast, ALPS CD4+ and CD8+ T cells demonstrated a vast insensibility to rapamycin treatment. We next monitored the effect of rapamycin on ALPS DNT cells *in vivo.* The percentage and mitotic activity of DNT cells was dramatically reduced in ALPS patients under rapamycin therapy. Of interest, a more detailed analysis revealed that the reduction specifically affected ALPS DNT cells displaying the abnormal differentiation phenotype. Taken together, our data indicate that absence of Fas signaling leads to abnormal differentiation and high mitotic activity of DNT cells due to hyperactive mTOR signaling, which can be pharmacologically reversed by rapamycin therapy.

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Publications during funding period

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A59 - Final Report

01.10.2013 - 30.09.2016

IL-10 and lung cancer

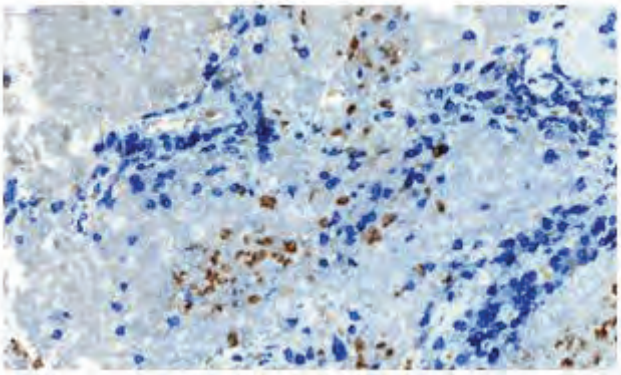
Prof. Dr. Dr. Susetta Finotto, Department of Molecular Pneumology

Here we investigated the impact of Interleukin (IL)-10, an immunosuppressive cytokine, on lung tumour in a cohort of 77 patients with Non small cell lung cancer (NSCLC). IL-10 was found induced in the cytoplasm of cells around the tumour in the lung of these patients. IL-10R was found upregulated in the tumour region in Foxp-3+ T regulatory cells. In the human A549- adenocarcinoma lung tumour cell line, IL-10R was found to be induced under metabolic restrictions present during tumour growth.

IL-10 is a cytokine with pleiotropic effects, mainly known for its strong immunosuppressive function. Moreover, it also takes part in cell survival, proliferation and differentation, apopotosis and angiogenesis

Here we investigated the impact of IL-10 on lung tumour by looking at its distribution in lung tissue in our cohort of 77 patients affected by Non small cell lung cancer (NSCLC). In this tissues, Interleukin (IL)-10 was found induced in cells around the tumour, and rarely expressed in the ,,tumour zone" by immunohistochemistry. Moreover, few cancer cells and CD3+ T cells releasing IL-10 were observed. Further, IL-10 release was found induced in the adenocarcinoma as compared to squamous cell carcinoma cells. Finally, in freshly isolated cells obtained and sorted out immediately after surgery, we found increased numbers of IL-10R producing CD4+ T-cells and CD4+CD25+Foxp3+ T-regulatory cells in the tumoural area of NSCLC patients.

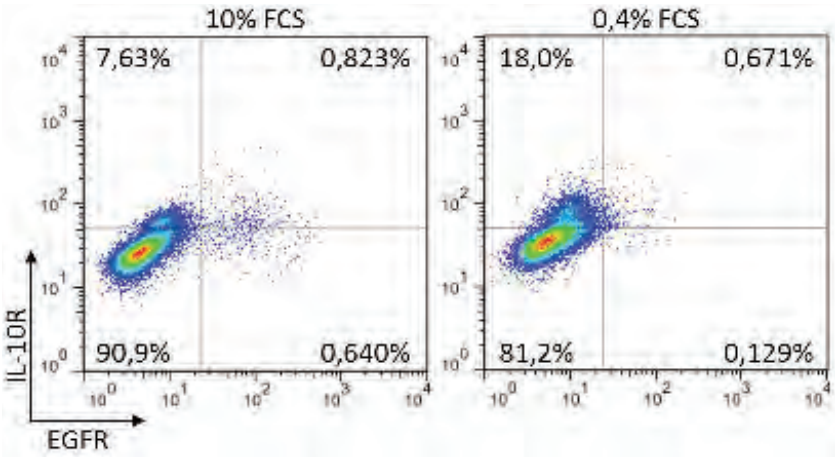
We next analyzed the human A549- adenocarcinoma cell line cultured with 10% serum and with low level of nutrients like 0.4% FCS and found that under metabolic limiting conditions, IL-10R was induced in the lung tumour cell line A549. We thus discovered some new pro-tumoural effects of IL-10 in NSCLC, supporting tumour immune escape via T regulatory cell induction and tumour cell survival under metabolic limiting conditions. Finally, we discovered that the main sources of IL-10 in the lung of patients with NSCLC are neither T nor tumour cells but other cells surrounding the tumour cells, probably macrophages like tumour associated macrophages (TAM).



Immunohistochemistry for IL-10+ (brown) cells and Tumour infiltrating lymphocytes (TIL) immun-reacting to anti CD3 antibodies (blue) in the tumour region of a patient with NSCLC shown at 40 magnification.

Prof. Dr. Dr. Finotto

To further analyse the role of IL-10 and the IL-10R in an experimental model of lung cancer, we injected mice with a lung tumour cell line intravenously (L1C2 or LL2-luc-M38 cells). A previously performed flow cytometry analysis revealed that about 30-40% of the murine L1C2 lung tumour cells express the receptor for IL-10. In our murine model for lung cancer we found that total lung cells from mice bearing tumour release significantly more IL-10 as compared to lung cells, isolated from tumour-free mice. In accordance with our human data, also CD4+CD25+Foxp3+T regulatory cells from mice bearing tumour were found to express increased IL-10R on their surface indicating an IL-10 autocrine loop on the surface of T regulatory cells in mice bearing tumour that needs to be further investigated. Thus IL-10 is an important regulator of lung tumour and involved in the regulation of cellular key check-point discovered to be important for the cure of NSCLC like tumour growth associated with nutrient deprivation and enhanced T regulatory cell mediated immunosuppression in lung cancer.



Human lung adenocarcinoma cell line A549 were cultured in the presence of 10% fetal bovine serum (FCS) or in 0.4% FCS and analyzed by FACS. Here we saw an induction of IL-10R upon metabolic deprivation in cell culture with 0.4% FCS.

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Publications during funding period

Eisenhut F, Heim L, Trump S, Mittler S, Sopel N , Andreev K, Ferrazzi F, Ekici AB, Rieker R, Springel R, Assmann VL, Lechmann M, Koch S, Engelhardt M, Warnecke C, Trufa DI, Sirbu H, Hartmann A Finotto S (2016) FAM13A is associated with non-small cell lung cancer (NSCLC) progression and controls tumour cell proliferation and survival. Oncoimmunology. [Epub ahead of print]

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A60 - Final Report

01.10.2013 - 30.09.2016

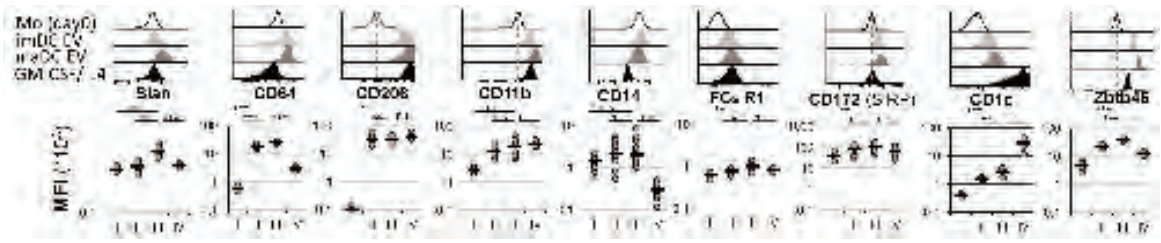
Monocyte derived Dendritic cells (Mo-DC) by DC Exosomes

Prof. Dr. Andreas Baur, Dr. Stephan Schierer, Department of Dermatology

Monocyte-derived Dendritic cells (Mo-DC) can be generated in-vitro using cytokines. By which mechanisms Mo-DC are naturally induced is unclear. We show here that Exosomes (Exo) derived from mature DC (Exo maDC) are sufficient to differentiate Mo to functional inflammatory Slan⁺DC. Furthermore, intradermally injected Exo maDC led to infiltration of mainly activated Mo in skin and enter the DC/T-cell area in the draining lymph node.

Exosomes (Exo) are secreted membrane vesicles from endosomal origin, with the size of 40-100nm, which contain active biologic cytosolic- (e.g. miRNA and cytokines) and surface-derived molecules. So far exosomes derived from Dendritic cells (Exo DC) were mainly applied for therapeutic cell free vaccination, but the biological purpose of DC Exo remains to be elucidated.

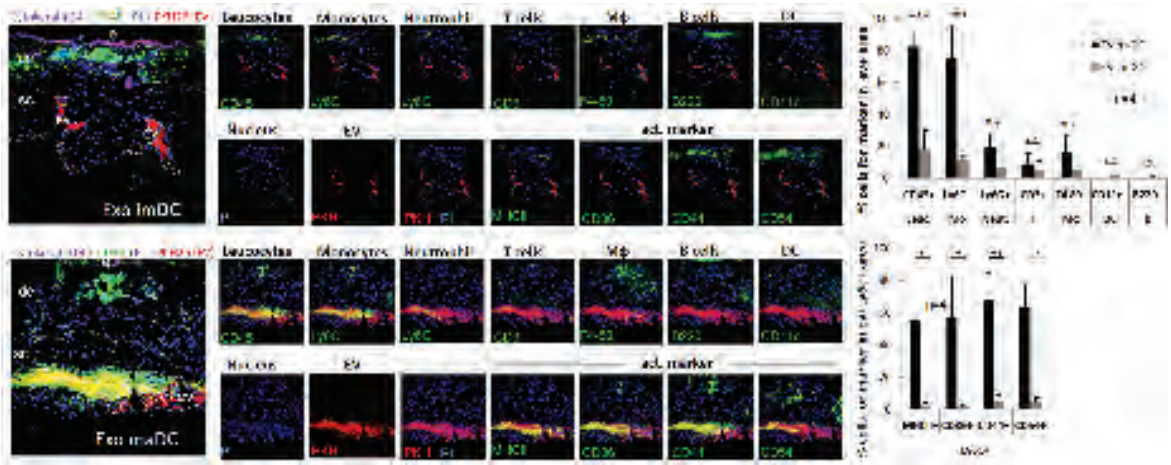
Exo maDC derived Slan⁺DCs showed an allogeneic stimulatory capacity similar to conventional generated DC. Regarding additional functions of Exo DC, long term incubation (10-13 days) of PBMC with Exo DC lead to prolonged survival and proliferation of DC similar to PBMC treated with rec. GM-CSF. Western blot analysis confirmed that both Exo imDC and Exo maDC contain large amounts of GM-CSF. Blocking



Mo were used on day0 (I) or incubated with Exo either derived from imDC (II) or maDC (III) or with GM-CSF/IL4 (IV) for 5 days.Surface marker expression were determined with flow cytometry. Data are representative for N=6-27 independent experiments (grey dots), mean MFI of each marker is depicted as black line. (*** p<.001, ** p<.01, * p<.05)

To understand the role of DC Exo we started to analyze effects on resting human PBMC. First experiments of labeled DC Exo incubated with PBMC revealed that mainly monocytes (Mo), and to a minor degree other leukocytes take up DC Exo. Immediately (3h) after uptake of Exo DC exclusively Mo showed activation via phosphorylation of Stat5. After 6 days Mo incubated with Exo derived from immature DC (Exo imDC) showed a heterogonous macrophage and imDC morphology, whereas Exo derived from mature DC (Exo mDC) lead to homogenous veiled maDCs. In agreement with that Exo maDC, but not Exo imDC generated DC showed complete phenotype (e.g. Slan, CD64, Zbtb46, CD14, and CD206) described for Slan+ DCs. Consequently, only matured the essential DC growth factor GM-CSF abrogated the Exo induced survival and differentiation of Mo. Elucidating differences we found that Exo maDC include active TNF-α together with its corresponding active protease Adam17 in contrast to Exo imDC that harbor only the inactive precursors, pro-TNF-α and pro-Adam17. Antibody array analysis of chemokine and cytokine levels supported that especially Exo maDC include inflammatory cytokines (e.g. Il1β, IL-6, TNF-α) needed for maturation and survival of DC. Furthermore, we found additional chemokines (e.g. IL-8, MIP1-α and -β) and cytokines (e.g. IL3, MIF) in Exo maDC capable to attract and activate a broad range of immune cells. To confirm these predicted effects *in-vivo* we generated Exo from Bone marrow

Prof. Dr. Baur Dr. Schierer



Skin cryosections of intradermally injected labeled (PKH26) Exo imDC or Exo maDC were analyzed with MELC and number of cell marker co-stained with labeled Exo were determined by software based counting (Strataquest, Tissue Gnostic).

derived DC (Exo BMDC) that were either not matured (Exo imBMDC) or matured with Poly I:C (Exo maBMDC). Array analysis revealed that both Exo BMDC contain GM-CSF, but only Exo maBMDC showed elevated levels of cytokines and chemokines. In cooperation with AG Zinser (dermatology) labeled Exo BMDC were injected intradermally in autologous C57/Bl6 mice. After one-day skin cryosections analyzed with MELC (Multi-Epitope-Ligand-Cartography) technology and software based cell counting (Strataquest, Tissue Gnostic) revealed that different leukocytes (Mo, Neutrophil, Mφ, T cells) infiltrated the area with Exo, but mainly activated Mo (Ly6c+, MHCII+, CD86+) ingested Exo maBMDC, but not Exo imBMDC. At the same time both Exo BMDC appear mainly in medullary macrophages, but in addition only Exo maBMDC reached the DC/T cell area of the draining lymph node.

In summary Exo maDC seem to have a multifunctional capability to recruit, activate and differentiate Mo.

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Invited lectures

Meeting „Optics, Algos & Ice“ - Symposium on Image Analysis of Cells in Tissue“, 17-20.Nov.2016, Obergurgl (Österreich),Titel: „Multi Fluorescence Tissue Analysis reveals that Extracellular Vesicles (EV) from Mature Dendritic Cells (DC) are sufficient to activate and differentiate Monocytes to DC“

Publications during funding period

Lee J-H, Schierer S, Blume K, Dindorf J, Wittki S, Xiang W, Ostalecki C, Koliha N, Wild S, Schuler G, Fackler OT, Saksela K, Harrer T and Baur AS (2016) HIV-Nef and ADAM17-Containing Plasma Extracellular Vesicles Induce and Correlate with Immune Pathogenesis in Chronic HIV Infection. EBioMedicine Apr 6: 103-13

A61 - Progress Report

01.02.2014 - 31.01.2017

Leishmania, iNOS and iron

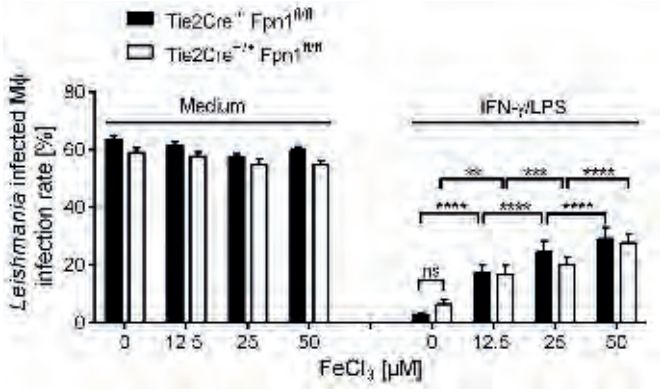
Prof. Dr. Christian Bogdan, PD Dr. Ulrike Schleicher,
Institute of Clinical Microbiology, Immunology and Hygiene

Leishmania are infectious pathogens whose intracellular, iron-dependent survival and proliferation is counteracted by the activity of inducible nitric oxide synthase (NOS2). This project aims to test whether and by which mechanism the iron metabolism and the expression of NOS2 cross-regulate each other during cutaneous and visceral leishmaniasis and thereby affect the antileishmanial activity of macrophages.

Replication and intracellular survival of *Leishmania* parasites depends on iron, but is limited by the activity of inducible nitric oxide (NO) synthase (iNOS or NOS2). NOS2 is expressed in phagocytes upon stimulation by cytokines (e.g. IFNγ, TNF) and/or microbial ligands (e.g. lipopolysaccharide [LPS]) and converts L-arginine into citrulline and NO. Based on studies with extracellular (promastigote) *Leishmania,* NO is capable to exert direct leishmanicidal effects. However, whether this is also true for intracellular (amastigote) *Leishmania,* has never been demonstrated. Considering the capacities of NO as a signaling molecule, possible indirect antimicrobial effects of NO have to be considered.

In order to test the hypothesis that the anti-leishmanial effect of NO is also a result of the withdrawal of iron from the microenvironment of amastigotes, we performed killing assays with infected bone marrow-derived macrophages (BMM) that were stimulated for endogenous NO production or incubated with an exogenous NO donor in the presence or absence of exogenous Fe^{2+} ($FeSO_4$) or Fe^{3+} ($FeCl_3$). In both settings the Fe compounds were able to reverse the killing of intracellular *Leishmania.* To elucidate the underlying mechanism(s), we tested whether the expression of ferroportin-1 (Fpn1), the only known cellular export system for Fe^{2+}/Fe^{3+}, is regulated by NOS2/NO. We found that NO did not increase Fpn1 mRNA or protein expression. To definitively rule out a role for Fpn1, we analyzed BMM from Tie2cre $Fpn1^{fl/fl}$ mice. Killing of *Leishmania* remained unimpaired in IFN-γ/LPS-stimulated BMM lacking Fpn1.

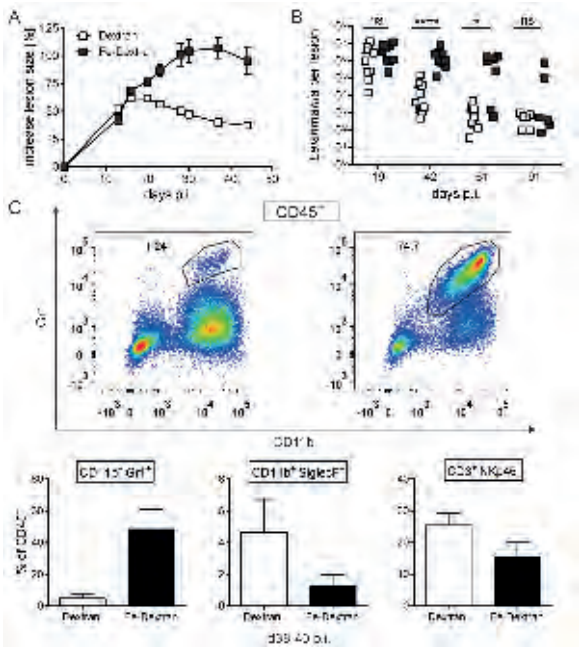
In a reverse approach we could demonstrate that iron-overloading of *L. major*-infected mice caused an exacerbation of cutaneous leishmaniasis (CL), which was paralleled by higher parasite loads, an increased influx of $CD11b^+Ly6C^+Ly6G^+$ neutrophil-like cells into skin lesions and a decreased accumulation of $CD3^+$ T cells and $CD11b^+SiglecF^+$ eosinophils. $CD11b^+$ cells purified from the lesions of infected iron-loaded or control mice were equally potent in suppressing anti-CD3/CD28-induced T cell proliferation, indicating that the disease-promoting effect of iron is not related to the activity of these myeloid suppressor cells. In addition, iron overload caused enhanced expression of arginase 1 and transforming growth factor-β mRNA, which both might contribute to the reduced parasite control in the presence of iron. Interestingly, unlike to *L. major*-infected mice iron loading ameliorated the course of visceral leishmaniasis (VL, *L. infantum*).



Killing of *Leishmania* in IFNγ/LPS-stimulated Fpn1-deficient or wildtype control BMM in absence or presence of $FeCl_3$

Prof. Dr. Bogdan

PD Dr. Schleicher



Course of infection (A), parasite burden (B) and cellular composition in the skin lesion in *L. major*-infected iron-loaded versus control-treated (dextran) C57BL/6 mice

We conclude that (a) NOS2-derived NO does not upregulate iron export, but affects intracellular *Leishmania* survival by another iron-dependent mechanism (e.g. by destruction of Fe-S clusters) and that (b) iron differentially affects the course of CL and VL by mechanisms which remain to be further clarified. Ongoing studies investigate whether iron-dependent regulation of NOS2, NADPH oxidase, metabolism and/or ferroptosis might be involved.

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Invited lectures

Retreat of the HOROS International Doctoral Programme (University of Innsbruck) together with the University of Copenhagen, March 6 to March 10, 2016, Innsbruck-Obergurgl, Austria. C. Bogdan: Arginase 1 and inducible nitric oxide synthase: two key players in antimicrobial defence (invited plenary lecture)

Guest lecture series of the SFB815 Redox-Regulation, February 4, 2016, University of Frankfurt, Frankfurt. C. Bogdan. Regulation of arginase 1 by tumor necrosis factor: mechanism and functional implications

Publications during funding period

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A62 - Final Report

01.01.2014 - 31.12.2016

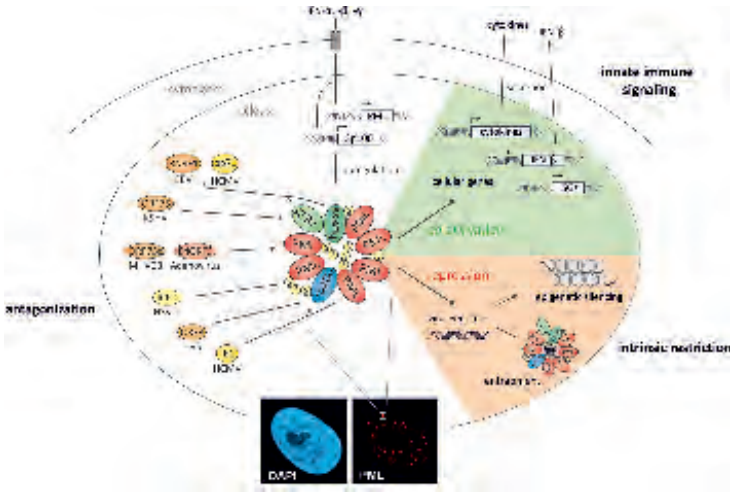
ND10 and interferon-induced gene expression

Prof. Dr. Thomas Stamminger, Institute of Clinical and Molecular Virology

Research of the last years revealed that a specific structure of the cell nucleus, termed nuclear domain ND10 or PML nuclear bodies (PML-NBs), is frequently modified during viral infection. Our data demonstrate that PML plays a novel co-regulatory role in type I as well as type-II interferon-induced gene expression. This finding supports the view that targeting of PML-NBs by viral regulatory proteins has evolved as a strategy to inhibit both intrinsic and innate immune defense mechanisms.

Viral targeting of PML bodies perturbs both intrinsic and innate immune responses during HCMV infection

PML is the organizer of cellular structures termed PML nuclear bodies or nuclear domain 10 (ND10). We have shown that PML, and other ND10 components like hDaxx and Sp100 act as cellular restriction factors against human cytomegalovirus (HCMV) and a variety of other viruses. Interestingly, PML is an interferon-stimulated gene (ISG) and its expression is strongly increased by interferons (IFNs). Furthermore, it is known that the antiviral function of ND10 is antagonized by viral regulatory proteins such as the immediate-early protein IE1 of HCMV. IE1 binds to the coiled-coil domain of the TRIM protein PML through its globular core domain ($IE1_{CORE}$) and induces ND10 disruption in order to initiate lytic HCMV infection. During this project, we were able to demonstrate a co-regulatory role of PML in both type-I and type-II IFN-induced gene expression: we detected that primary human fibroblasts with an siRNA-mediated depletion of PML exhibit a severe reduction of IFN-induced ISG expression. Furthermore, we show that upregulation of interferon-induced genes is abrogated by $IE1_{CORE}$. In conclusion, our data suggest that targeting PML by viral regulatory proteins represents a strategy to antagonize both intrinsic and innate immune mechanisms.

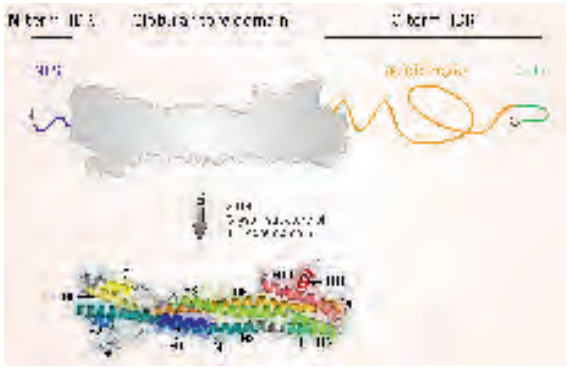


PML-NBs: a cellular structure that mediates intrinsic immunity against viruses and acts as a coactivator of the interferon response. Viral effector proteins that are known to antagonize PML-NBs are shown in the left part of the figure

Prof. Dr. Stamminger

Mechanism of IE1-mediated ND10 disruption

So far it was known that the IE1 protein induces a loss of PML SUMOylation which results in a dispersal of the subnuclear structure ND10. In order to unravel the mechanism of IE1-mediated PML deSUMOylation we utilized in vitro SUMOylation reactions together with inducible cellular expression systems. We show that tight binding of IE1 to PML interferes with the de novo SUMOylation of a distinct lysine residue that is also the target of stress-mediated hyperSUMOylation of PML. This is of importance since it represents a novel mechanism used by a viral effector protein to antagonize the antiviral functions of ND10.



Structural architecture of the IE1 protein of human cytomegalovirus. The recent solution of the crystal structure of the IE1 core domain provided novel insight into the molecular basis of interaction with the PML protein

Contribution of the ND10 proteins to the regulation of HCMV latency and the interferon response in monocytic cells

Since the contribution of ND10 proteins to the regulation of HCMV latency is still controversial, we utilized the monocytic cell line THP-1 to establish a stable knockdown of PML, hDaxx or Sp100. Importantly, depletion of the major ND10 proteins did not prevent the terminal cellular differentiation of THP-1 monocytes nor did it affect the IFN-β response in undifferentiated cells. While during differentiation-induced reactivation from latency an increase in the number of IE-expressing cells was readily detectable in the absence of the major ND10 proteins, no effect was observed in non-differentiated monocytes. We conclude that PML, hDaxx and Sp100 primarily act as cellular restriction factors during the dynamic process of reactivation but do not serve as key determinants for the establishment of HCMV latency.

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Invited lectures

European Congenital Cytomegalovirus Initiative ECCI, 24.-26.4.2016, Venice, Italy: Characterization of cytomegalovirus IE1 mutants reveals that viral targeting of PML bodies perturbs both intrinsic and innate immune responses.

Seminar Talk, Institute of Virology, University of Ulm, 30.5.2016, Innate antiviral defense by PML nuclear bodies.

Medical Faculty, University of Ulm, 24.10.2016, Ulm: Das humane Cytomegalovirus – von molekularen Mechanismen zu neuen Therapien.

Publications during funding period

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A63 - Progress Report

01.07.2016 - 31.12.2018

Mechanisms of TNF-Mediated Control of Intracellular Pathogens in Mice and Man

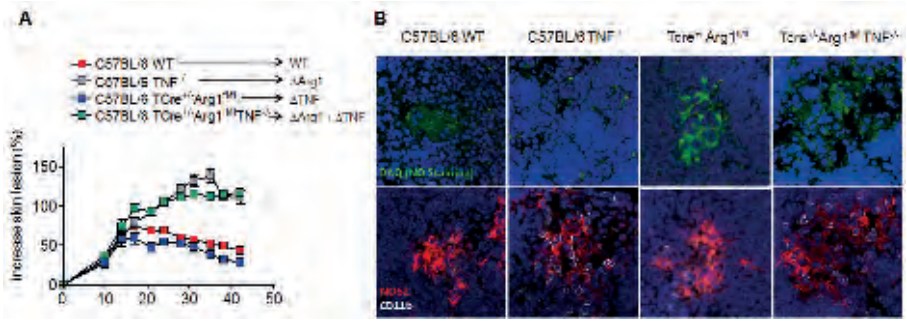
Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene

Neutralization or deletion of tumor necrosis factor (TNF) causes loss of control of intracellular pathogens in mice and humans, but the underlying mechanisms are incompletely understood. This project aims to identify by which mechanisms TNF protects from progressive cutaneous leishmaniasis. In parallel, TNF-regulated protective versus disease-mediating pathways will be evaluated in immune cells of patients with rheumatoid arthritis (RA) before and after treatment with TNF-antagonists.

In parasite infections caused by the intracellular pathogen *Leishmania (L.) major* effective control is mediated by the expression of type 2 nitric oxide (NO) synthase (NOS2). The activity of NOS2, which metabolizes L-arginine to citrulline and antimicrobial NO, can be influenced by host cell-derived arginase 1 (Arg1). Arg1 is a cytosolic enzyme hydrolyzing L-arginine into urea and ornithine. Previous studies of our group revealed that TNF-mediated restriction of Arg1 expression in infected skin and draining lymph nodes is required to allow efficient production of NO by NOS2 and represents one of the TNF-specific protective mechanisms preventing progressive disease in C57BL/6 mice upon *L. major* infection.

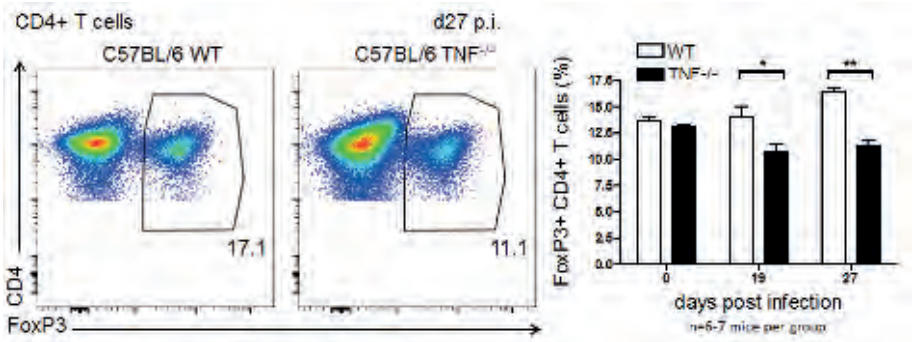
In an attempt to further demonstrate that hyper-expression of Arg1 is decisive for the non-healing cutaneous disease in *L. major*-infected $TNF^{-/-}$ mice, C57BL/6 mice deficient for TNF and Arg1 ($Tie2cre^{+}Arg1^{fl/fl}$ $TNF^{-/-}$) were generated. Surprisingly, C57BL/6 mice lacking both TNF and Arg1 developed progressive cutaneous leishmaniasis comparable to TNF single knockout mice, although NO production in the infected skin and lymph node tissue was restored. These data indicate that additional effector pathways other than upregulated Arg1 expression are involved in the non-healing outcome in *L. major*-infected $TNF^{-/-}$ mice.

L. major-infected $TNF^{-/-}$ mice also suffered from visceral disease with high parasite loads in the spleen, although Arg1 was not detectable in this organ. As inhibition of *Leishmania* growth in the spleen rather depends on reactive oxygen species (ROS) production by the phagocyte NADPH-oxidase than on NO release by NOS2, we began to investigate whether TNF regulates ROS release during cutaneous leishmaniasis. Besides its effect on myeloid cells TNF may also act on T cells. Since TNF was found to modulate the compartment of regulatory T cells (Tregs), Tregs



(A) Course of infection and (B) nitric oxide/NOS2 staining in draining lymph nodes at day 25-35 after *L. major* infection of TNF-deficient, Arg1-deficient ($Tie2Cre^{+/-}Arg1^{flox/flox}$), TNF- and Arg1-double deficient mice or C57BL/6 WT controls

Prof. Dr. Bogdan



Percentage of Foxp3^{+} Tregs within CD4^{+} T cells in *L. major*-infected TNF$^{-/-}$ mice versus C57BL/6 WT controls

in *L. major*-infected TNF$^{-/-}$ versus wildtype (WT) controls were analyzed. Interestingly, the percentage of Foxp^{3+} Tregs was significantly reduced following infection when TNF was absent. Further studies are required to elucidate the relevance of this finding. In order to discover „novel" TNF-regulated antimicrobial effector pathways, we prepared RNA samples of the spleens of *L. major*-infected TNF$^{-/-}$ mice versus WT controls at two different time points of infection, which will be analyzed by RNAseq technology.

Finally, we have started to collect blood samples from RA patients prior to and during the treatment with TNF-antagonists and established all techniques for RNA and cell preparation in order to study TNF-mediated regulatory effects in human samples and to compare them to the mouse data.

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Publications during funding period

none

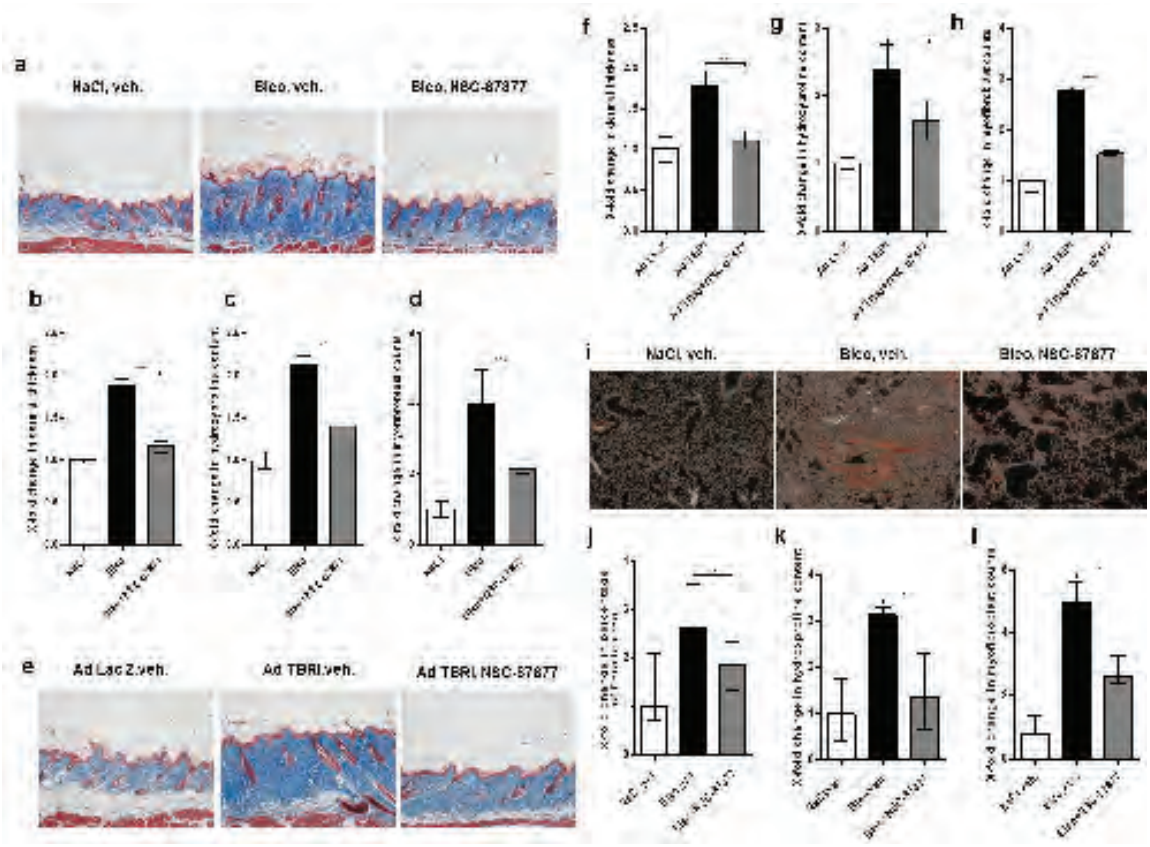
A64 - Progress Report

01.02.2016 - 31.07.2018

The tyrosine-protein phosphatase SHP2 regulates TGFβ-dependent activation of JAK2/STAT3 in fibrotic diseases

Prof. Dr. Jörg Distler, Prof. Dr. Georg Schett, Department of Medicine 3 – Rheumatology and Immunology

SHP2 is a ubiquitously expressed non-receptor tyrosine phosphatase with important regulatory effects on receptor tyrosine kinase-, cytokine- and G-protein coupled receptor signaling. Altered SHP2 activity due to mutations of the PTPN11 gene have been found in Noonan syndrome, juvenile myelomonocytic leukemia, and several types of human malignancies. We provide first evidence that SHP2 might also play a central role in the pathogenesis of fibrotic diseases such as Systemic Sclerosis (SSc). Inactivation of SHP2 signaling prevents the TGF-β mediated activation of JAK2 / STAT3 signaling to prevent myofibroblast differentiation and collagen release. Inactivation of SHP2 by genetic or pharmacologic approaches inhibits TGF-β-dependent fibroblast activation and ameliorates experimental fibrosis. Given the availability of SHP2 inhibitors, SHP2 might thus be a novel target for the treatment of fibrotic diseases.



Treatment with the SHP2 inhibitor NSC-87877 ameliorates experimental skin and lung fibrosis. a-d: Bleomycin-induced skin fibrosis. e-h: Skin fibrosis induced by overexpression of a constitutively active TGFβ-receptor type 1. i-l: Bleomycin-induced pulmonary fibrosis.


Prof. Dr. Distler


Prof. Dr. Schett

After demonstrating anti-fibrotic effects of SHP2 knockout in TBRI- and bleomycin-induced skin fibrosis in our preliminary work, we showed that fibroblast-specific knockout of SHP2 also ameliorates fibrosis in Tsk1 mice, which resemble later, less inflammatory stages of SSc with endogenous activation of fibroblasts. Pharmacologic inactivation of SHP2 by treatment with NSC-87877 reduces dermal and pulmonary fibrosis induced by subcutaneous and intratracheal application of bleomycin. Moreover, treatment with NSC-87877 in well-tolerated doses also ameliorated fibrosis induced by overexpression of a constitutively active TGFβ-receptor type 1.

We demonstrated that the stimulating effects of SHP2 on TGFβ-induced fibroblast activation require the phosphatase-function of SHP2: Overexpression of a phosphatase-dead SHP2 mutant inhibited fibroblast activation in a dominant-negative manner. Moreover, we identified Y-570 of JAK2 as the critical residue of the stimulating effects of SHP2 on TGFβ-induced JAK2 / STAT3 signaling. Phosphorylation of Y-570 was regulated in cultured fibroblasts in a SHP2 dependent manner and overexpression of a Y-570 phosphomimetic JAK2 mutant strongly inhibited the regulatory effects of SHP2 on JAK2 / STAT3 signaling and on TGFβ-induced fibroblast activation.

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Invited lectures

Keystone Meeting “Fibrosis: From Basic Mechanisms to Targeted Therapies”, February 2016, Keystone/CO/USA, Targeting TGFbeta signaling for therapeutic intervention.

Publications during funding period

Liang R, Šumová B, Cordazzo C, Mallano T, Zhang Y, Wohlfahrt T, Dees C, Ramming A, Krasowska D, Michalska-Jakubus M, Distler O, Schett G, Šenolt L, Distler JH (2016) The transcription factor GLI2 as a downstream mediator of Transforming Growth Factor-β induced fibroblast activation in SSc. Ann Rheum Dis [Epub ahead of print]

Palumbo-Zerr K, Soare A, Zerr P, Liebl A, Mancuso R, Tomcik M, Sumova B, Dees C, Chen CW, Wohlfahrt T, Mallano T, Distler A, Ramming A, Gelse K, Mihai C, Distler O, Schett G, Distler JH (2016) Composition of TWIST1 dimers regulates fibroblast activation and tissue fibrosis. Ann Rheum Dis [Epub ahead of print]

Lin NY, Distler A, Beyer C, Philipi-Schöbinger A, Breda S, Dees C, Stock M, Tomcik M, Niemeier A, Dell‘Accio F, Gelse K, Mattson MP, Schett G, Distler JH (2016) Inhibition of Notch1 promotes hedgehog signalling in a HES1-dependent manner in chondrocytes and exacerbates experimental osteoarthritis. Ann Rheum Dis.75: 2037-2044

A65 - Progress Report

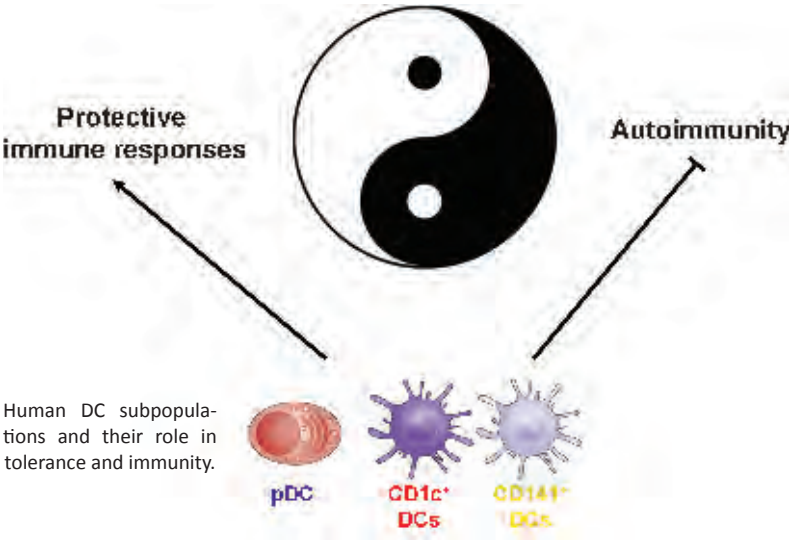
01.04.2016 - 30.09.2018

Tolerizing potential of human dendritic cell subpopulations

Prof. Dr. Diana Dudziak, Department of Dermatology

Dendritic cells (DCs) play a major role in the maintenance of tolerance. Expression profiling of DC subsets revealed that lympho-hematopoietic organs have only a minor influence on DC ontogeny and the resulting DC subtype signature in the steady state. Strikingly, isolated thymic DCs displayed a tolerogenic phenotype upon pathogenic stimulation. We are aiming to investigate this tolerizing potential in thymic DC subsets on functional and epigenetic level.

In our preliminary data we sorted DC subsets from blood, spleen, and thymus and stimulated the isolated DC subsets with a variety of Toll like receptor (TLR) ligands. After culture we measured the concentration of secreted cytokines and chemokines by FACS based cytokine bead array (CBA) assay from collected supernatants. Strikingly, in those experiments we found that the cellular surface expression profile did not reflect the secretion profile of cytokines and chemokines as thymic DCs expressed all costimulatory molecules expected to be there upon pathogenic stimulation. However, the amount of several cytokines was strongly reduced in the supernatants of thymic DCs compared to blood or splenic DCs. Most importantly, we found that the production of the TH1 polarizing cytokine IL-12 was almost completely blocked in the thymus, whereas blood and splenic DCs produced comparable amounts of it. As IL-12 is a critical cytokine in the regulation of immune responses it is interesting why thymic DCs are unable to secrete this cytokine although the transcriptional profile compared to the other DC family members in blood and spleen did not indicate such regulatory mechanisms. We are hypothesizing that DCs in the thymus have a tolerogenic potential, inhibiting the pre-activation of thymocytes even in the case of a potential infection. We are interested to better understand this tolerizing potential of human thymic DC subsets as this immunoprivileged site might harbor important aspects also for understanding of tumor development. In the next paragraphs we will outline our ongoing work from two out of the three planned aims:

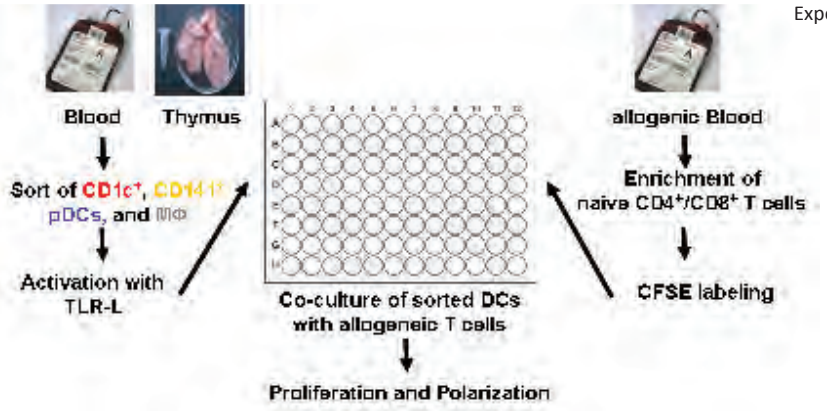


Human DC subpopulations and their role in tolerance and immunity.

Results in Aim 1: Determination of functional differences between blood and thymic DCs

To strengthen our preliminary findings we started to perform side-by-side polarization and proliferation assays in a co-culture of isolated and activated thymic, splenic, and blood DCs with HLA-mismatched naïve CD4+ peripheral blood T cells. We cell-sorted CD1c+, CD141+ DCs, and pDCs from these lympho-hematopoietic tissues and stimulated the DCs with a variety of TLR ligands. After 24 hrs, the cells were co-cultured with purified allogeneic blood CD4+ T cells. FACS analyses indicate that thymic DCs display a differential polarizing potential. Next, we will investigate other CD4+ and CD8+ driven responses.

Prof. Dr. Dudziak



Experimental outline of DC/T cell culture.

Results in Aim 2: Identification of epigenetic modifications in steady-state and activated thymic and blood DC subpopulations.

Thymic DCs might be influenced by either tissue factors or differential activity of regulatory components (e.g. transcriptions factors). Investigating the expression of NFκB and NFκB regulating factors we found only slight differences between thymic and blood DC subsets suggesting other mechanisms of activation. Therefore, we measured the phosphorylation of signaling molecules by FACS based Phosflow staining upon stimulation with TLR ligands of sorted blood and thymic DCs. Notably, we found a differential DC specific phosphorylation profile in dependency of the investigated organ. Those first data are of specific importance as we needed more evidence about potential regulatory mechanisms in thymic DCs. With our new findings we will be conducting the planned epigenetic profiling including ATAC sequencing combined with RNA-Seq.

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Invited lectures

46th Annual Meeting of the German Society of Immunology, 26.09.2016, Hamburg, Understanding human dendritic cell identity in lymphoid tissues

30th Annual Meeting of the European and macrophage society, 23.09.2016, Human lymphoid tissue dendritic cells are less influenced by their microenvironment than their counterparts in non-lymphoid tissues

Annual Meeting of the NK group of Frankfurt University, 20.03.2016, Kleinwalsertal, Austria, Identity of human dendritic cells is mainly dictated by origin, not tissue microenvironment

ImmunoGambia, 20.11.2016, Banjul, The Gambia, Dendritic cells: Master switches of immunity

Annual Meeting of the SFB643, 16.07.2016, Erlangen, Identity of human lymphoid dendritic cells is mainly dictated by origin, not tissue microenvironment

Annual Meeting of the Autumn School of Immunology, 10.10.2016, Merseburg, How dendritic cells (interact and) activate T cells

Awards

Election to the faculty member board of the German Society of Immunology, Diana Dudziak, December 2016, Berlin

Initiator and organizer of Dendritic cell working group (AKDC) in the German Society of Immunology, 27.09.2016, Hamburg

Publications during funding period

Heidkamp GF*, Sander J*, Lehmann CHK, Heger L, Eissing N, Baranska A, Lühr JJ, Hoffmann A, Reimer KC, Lux A, Söder S, Hartmann A, Zenk J, Ulas T, McGovern N, Alexiou C, Spriewald B, Mackensen A, Schuler G, Schauf B, Forster A, Repp R, Fasching PA, Purbojo A, Cesnjevar R, Ullrich E, Ginhoux F, Schlitzer A, Nimmerjahn F, Schultze JL*, Dudziak D* (2016) Human lymphoid organ dendritic cell identity is predominantly dictated by ontogeny, not tissue microenvironment. Science Immunology 1, eaai7677; 1-17

Lehmann CHK, Heger L, Heidkamp GF, Baranska A, Lühr JJ, Hoffmann A, Dudziak D (2016) Direct delivery of antigens to Dendritic cells via antibodies specific for endocytic receptors as a promising strategy for future therapies. Vaccines 4, 8

A66 - Progress Report

01.07.2016 - 31.12.2018

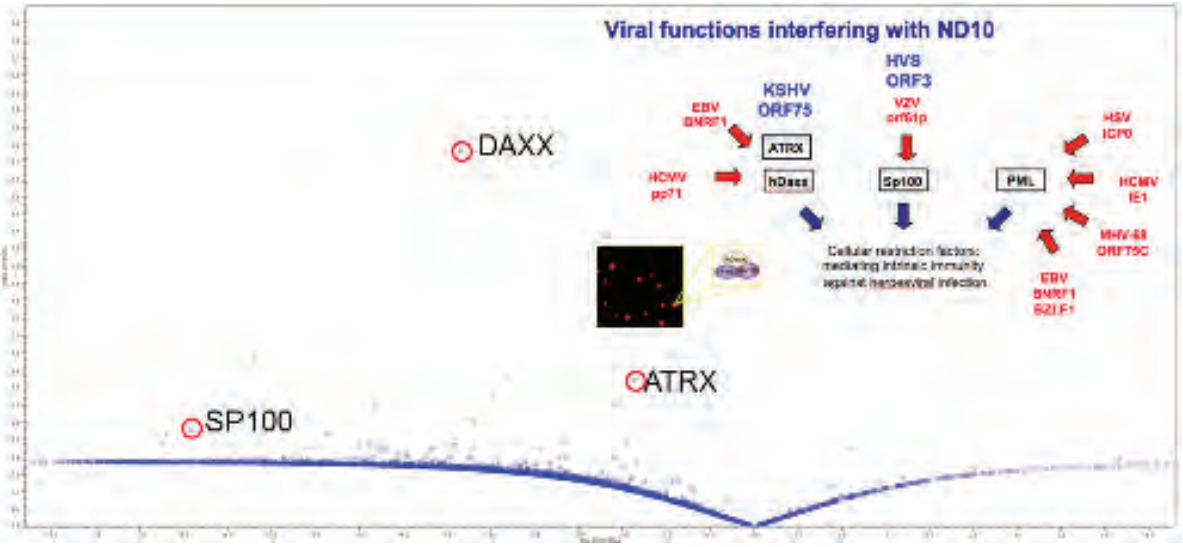
Genome wide CRISPR/Cas9 knockout for the identification of antiviral cellular restriction factors

Prof. Dr. Armin Ensser, Institute of Clinical and Molecular Virology

The project focuses on cellular factors that restrict herpesviruses and/or limit the growth of tumor cells transformed by human gammaherpesviruses. These factors represent primary therapeutic targets. We employ a two-pronged, unbiased approach at identifying such restriction factors using the powerful CRISPR/Cas9 knockout technology. One system targets each human gene with several independent constructs for knockout, the other system is capable of activating the promoter of each human gene.

Viruses, like other intracellular parasites, must evade the actions of the host cell's innate immune response, and often devote a substantial portion of their coding capacity to counteract these cellular restriction factors. The systematic and unbiased approach at identifying cellular restriction factors of DNA viruses uses the powerful CRISPR/Cas9 knockout and SAM technology. The projects major objectives are (1) performing complementary, unbiased CRISPR/Cas9 based screens for the identification of novel candidate cellular factors restricting DNA viruses and in particular Gammaherpesviruses, and factors restricting growth of Gammaherpesvirus-transformed cells; this data from objective (1) will also represent a valuable resource on their own that can be tapped into for future research projects. Objective (2) is the verification of a subset of these cellular candidate restriction factors, that are selected based on novelty and effect strength, which is followed by the (3) identification of the viral proteins that are the targets of the cellular restriction factors and (4) the elucidation of the mechanism.

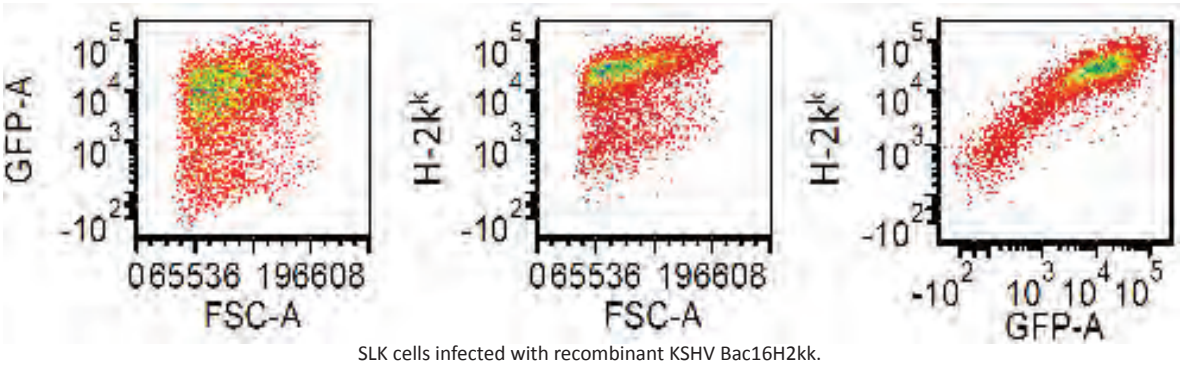
Within the first months the project is running according to plan and we have performed first genome wide screening experiments on KSHV infected SLK target cells, with several ND10 associated proteins identified as hits that enhance infection (as measured by GFP expression from the viral genome). However, the initial screens were limited by the FACS-sorting rate of large epithelial cells, such as



Hits identified from rKSHV.219 virus infected SLK cells that were sorted for high GFP expression

Prof. Dr. Ensser

SLK(Caki) or HeLa, which allowed to separate ~1500 cells per second, and would have resulted in sort times exceeding 20 hours for a typical sample of 50-100 million cells. Therefore, we have now constructed recombinant KSHV Bac16, Bac16RGB and HVS, expressing a murine H-2Kk surface marker that allows magnetic (pre-)sorting of virus infected cells. This can then be followed by FACS sorting of GFP and GFP/BFP populations. Another screen tested for KSHV reactivation from BJAB B-cells harboring a recombinant rKSHV.219 (GFP/RFP) genome, and experiments in collaboration with IZKF project leaders Stürzl (D20/D28) and Überla (A73) yielded interesting hits currently under evaluation. Furthermore, the synergistic activation mediator (SAM) system is almost established as a complementary approach to validate the sgRNA ko-screen. The genome-wide sgRNA2.0 library of >70.000 plasmids was obtained (Addgene), successfully amplified in E. coli and then verified by NGS. The components of the SAM system, NLS-dCas9-VP64 and helper transactivator MS2-p65-HSF1 are selected in the model cell lines (e.g. SLK(Caki), HeLa, BJAB and Jurkat). Two complementary screen help us to identify targets with increased confidence via the respective opposite ranking in knockout vs. SAM screens, ensuring that we can focus on relevant genes.



SLK cells infected with recombinant KSHV Bac16H2kk.

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Publications during funding period

none

A67 - Progress Report

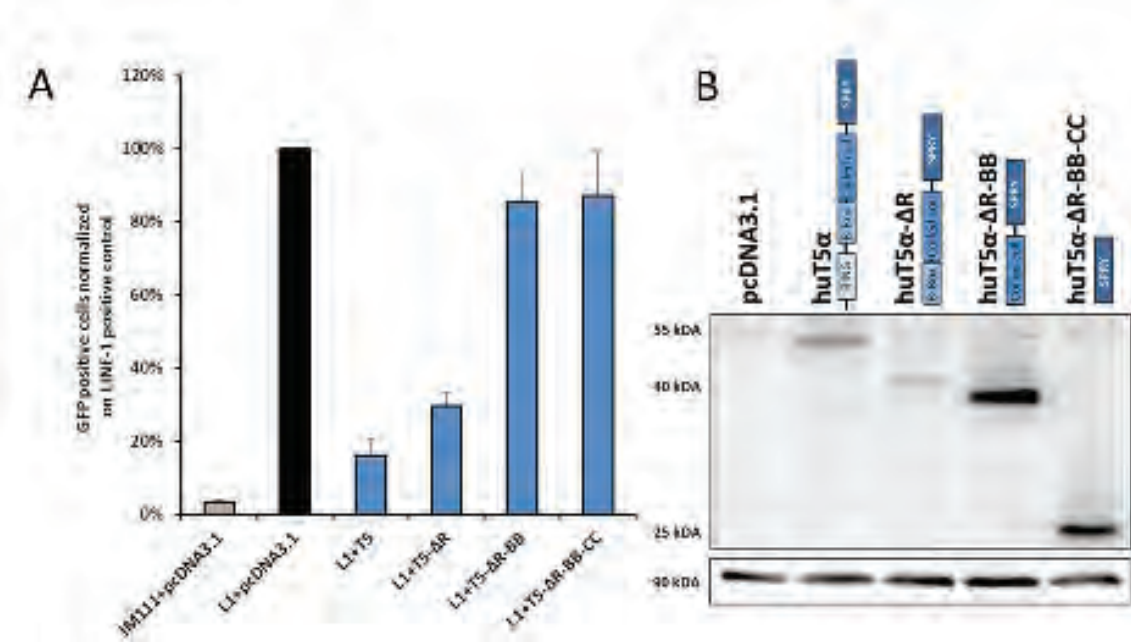
01.02.2016 - 31.07.2018

Analysis of the TRIM5alpha-mediated block to LINE-1 retroelements

Prof. Dr. Thomas Gramberg, Institute of Clinical and Molecular Virology

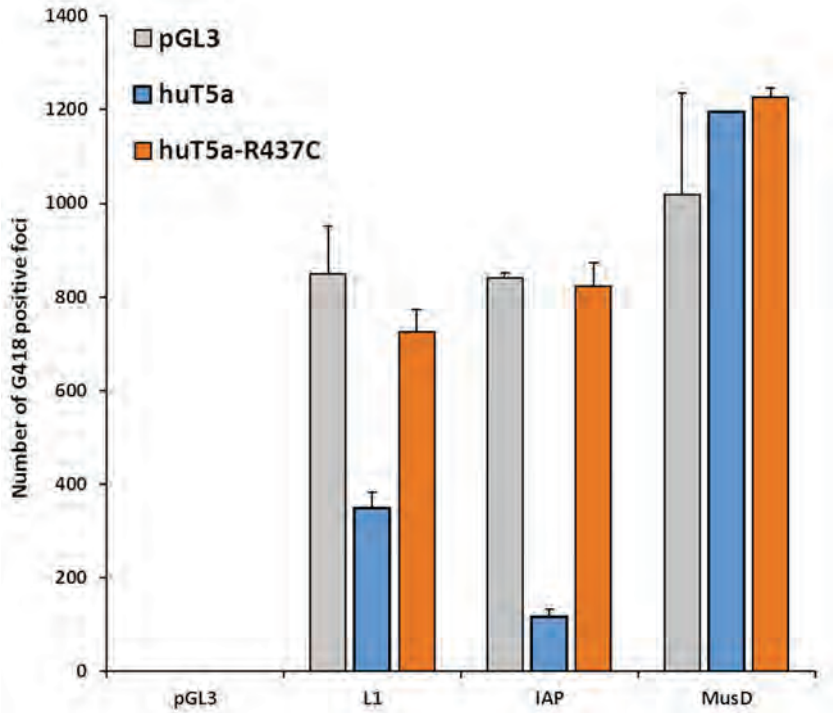
LINE-1 is the only autonomously active retrotransposon in humans and it is therefore essential to control the replication of LINE-1 to maintain genome integrity. We found that the retroviral restriction factor TRIM5α also inhibits LINE-1 elements. Within this study, we determine the domains within TRIM5α important for LINE-1 inhibition. We will also analyze the mechanism of LINE-1 inhibition by TRIM5α and ask whether this activity is specific for LINE-1 or whether other mobile genetic elements are restricted as well.

Within in this study, we are analyzing the TRIM5α-mediated inhibition of the mobile endogenous retroelement LINE-1. In order to identify regions of human TRIM5α important for LINE-1 restriction, we analyzed naturally occurring TRIM5α variants (single nucleotide polymorphisms, SNPs), as wells as TRIM5alpha deletion mutants in LINE-GFP reporter assays. First, we cotransfected wt TRIM5a and TRIM5a deletion mutants lacking the RING domain, the RING domain and the B-Box, or RING, B-Box, and the coiled-coil region, together with LINE1-GFP reporter plasmids in 293T cells. Four days posttransfection, we analyzed the cells by flow cytometry and found the RING domain of TRIM5a, which contains an E3 ligase function and which is important for retroviral restriction, to be dispensable for restriction of LINE-1 retrotransposition. This is indicating that the mechanism of restriction might differ between both agents. In addition, we found a single SNP located in the PRYSPRY domain of rare humanTRIM5α alleles to cause a complete loss of restriction of LINE-1. This finding suggests that, similar to retroviral restriction,



TRIM5α RING Domain is dispensable for LINE-1 restriction. (A) 293T cells were cotransfected with wt TRIM5a or a truncated mutant and an L1-GFP reporter plasmid. Retrotransposition events were quantified by flow cytometry five days post transfection. The average of three independent experiments is shown. (B) Expression level of HA-tagged- TRIM5a proteins were compared via immunoblot in 293T cells.

Prof. Dr. Gramberg



TRIM5α restricts LINE-1 and IAP, but not MusD retroelements. Reporter elements based on LINE-1, IAP, and MusD were cotransfected with plasmids encoding TRIM5, TRIM5-R437C, or empty vector. Cells were transfected and analyzed in triplicates 14 days post transfection. One of two independent experiments is shown.

the PRYSPRY domain of the protein is essential for restriction and might directly interact with target sequences with LINE-1. In addition to LINE-1, we found that TRIM5α also reduces the retrotransposition frequency of the murine LTR-containing retrotransposon Intracisternal A Particle (IAP) in HeLa cells using a Neomycin-based reporter assay. Interestingly and using a similar assay, we found that the replication of another murine LTR-retroelement, MusD, was not blocked by TRIM5a. This suggests that, similar to retroviruses, the interaction of TRIM5a with endogenous retroelements is highly specific and might therefore indicate a direct protein-protein interaction. Together, our data suggest that TRIM5a contributes to LINE-1 regulation and maintaining genome integrity. TRIM5a blocks certain endogenous retroelements by a mechanism similar but not identical to the retroviral block. Our findings will therefore contribute to a better understanding of the biology of the host factor TRIM5α and its role in counteracting endogenous and exogenous viruses.

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Publications during funding period

none

A68 - Progress Report

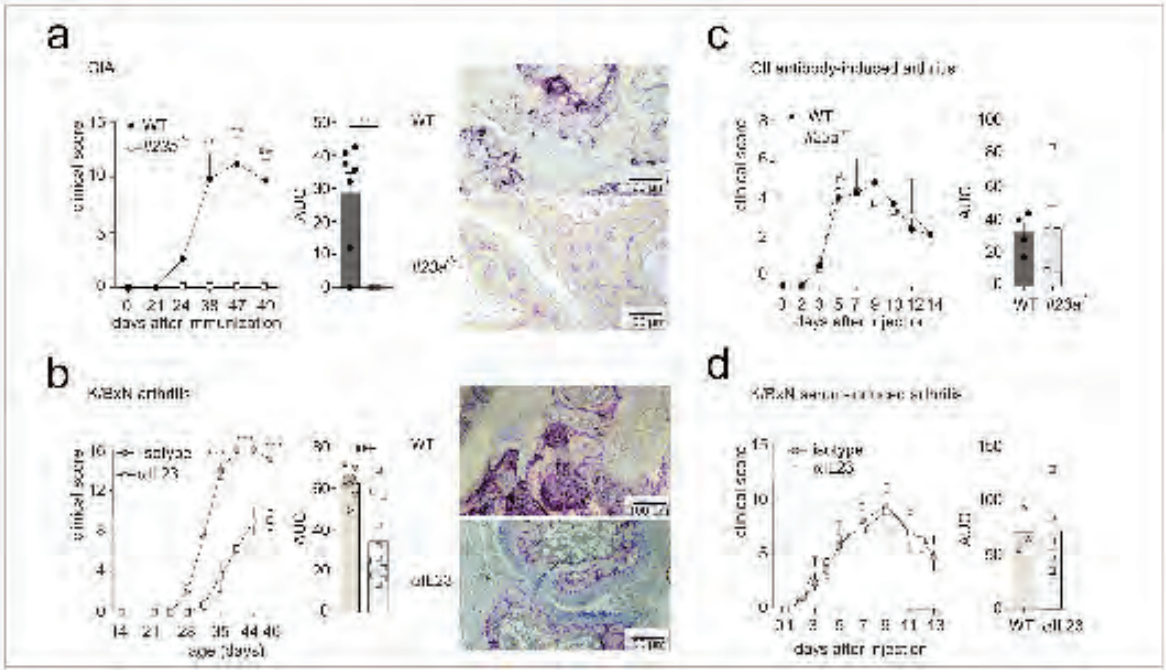
16.06.2016 - 15.12.2018

Analysis of the role of the IL-23/Th17 axis during the control of antibody activity in rheumatoid arthritis

Prof. Dr. Gerhard Krönke, Department of Medicine 3 – Rheumatology and Immunology
Prof. Dr. Falk Nimmerjahn, Division of Genetics

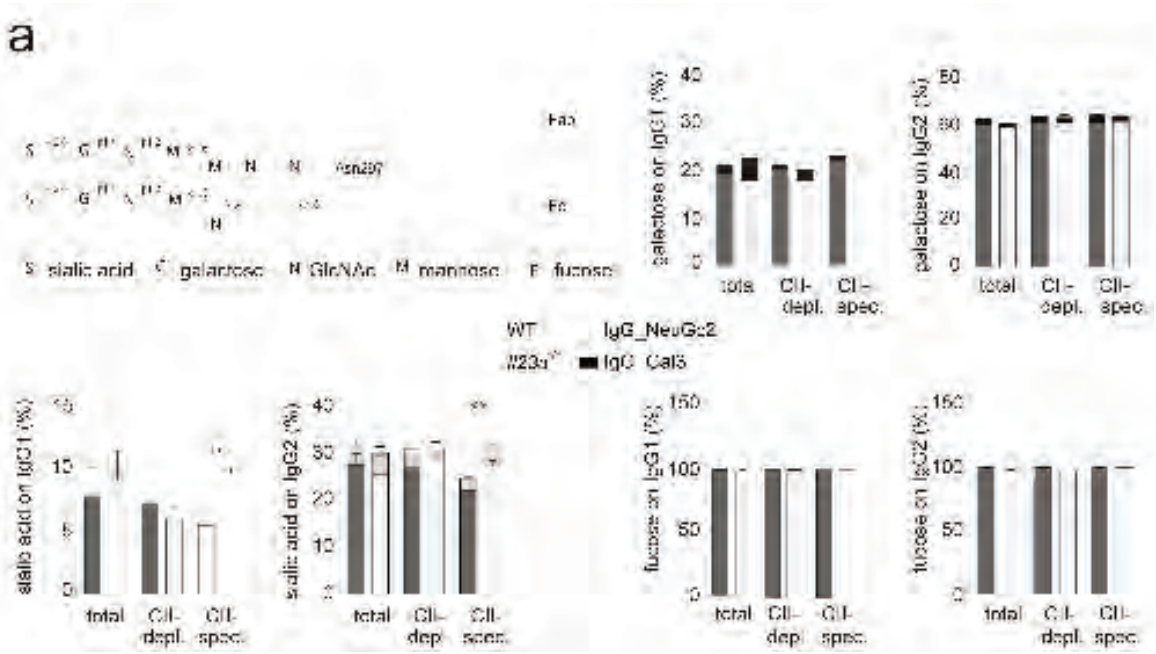
We currently determine the role of the IL-23/TH17 axis during the control of the intrinsic inflammatory activity of autoantibodies during onset of autoimmune arthritis. We have identified a pathway where TH17 cells regulate expression of glycosyltransferases in newly differentiating antibody-producing cells and thereby determine the glycosylation profile and activity of consecutively produced immunoglobulin G.

During the last year, we were able to dissect the role of the IL-23/TH17 axis during rheumatoid arthritis (RA). Our results that were derived from different active and passive arthritis models in wild-type and IL-23-deficinent mouse strains show that TH17 cells are critical players during autoimmune arthritis. However, these cells were dispensable during the autoantibody-mediated effector phase of this inflammatory disease as transfer of autoantibodies into IL-23-deficient mice induced a regular arthritis. In contrast, TH17 cells critically contributed to the initial production of pro-inflammatory and arthritogenic IgG before onset of inflammation. Here, we identified TH17 cells in secondary lymphatic organs



IL-23 contributes to the initiation of arthritis. (a) Il23a-/- mice after induction of CIA. (b) K/BxN mice, treated with an IL23 antibody. (c) Il23a-/- mice that received CII-specific antibodies or (d) WT mice that received serum from K/BxN mice together with an IL23 antibody.

Prof. Dr. Krönke Prof. Dr. Nimmerjahn



IL-23 regulates the glycosylation of autoantibodies. (a) Schematic structure of sugar moieties on the IgG molecule and mass spectrometry-based analysis of the glycosylation profile of total and CII-specific from WT and Il23a-/- mice after induction CIA.

that displayed a T follicular helper cell phenotype and entered germinal centers where they regulated the glycosyltransferase expression in newly differentiating plasma cells. This IL-23-dependent pathway determined the glycosylation profile of newly-produced autoantibodies. These changes in autoantibody glycosylation in turn dramatically increased the pro-inflammatory activity of these immunoglobulins and were essential for the autoantibody triggered onset of autoimmune arthritis in serum-transfer arthritis models.

Additional data show that humans that develop arthritis undergo similar changes in the glycosylation profile and activity of their autoantibodies before the initiation of clinical arthritis, suggesting that onset of human RA relies on related mechanisms.

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Publications during funding period

none

A69 - Progress Report

01.07.2016 - 31.12.2018

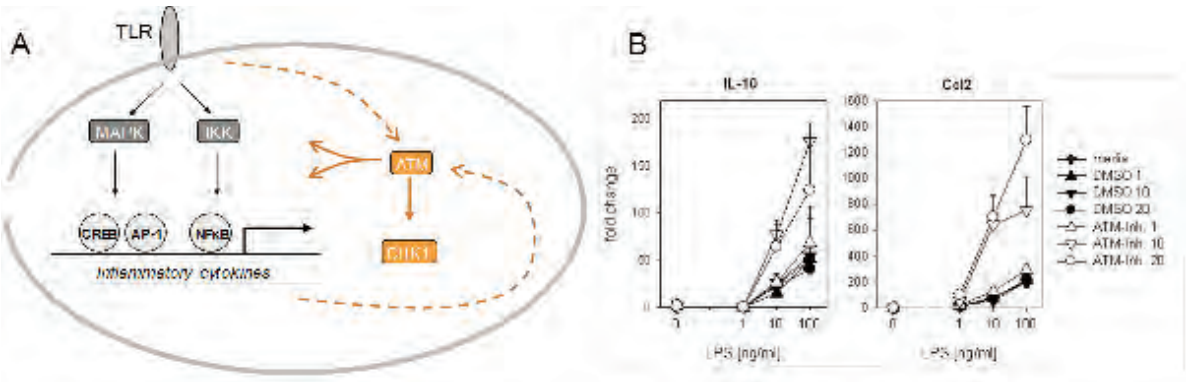
Contribution of ATM kinase and the DNA-damage response in innate response to infection

Prof. Dr. Roland Lang, Institute of Clinical Microbiology, Immunology and Hygiene

The DNA-damage response (DDR) requires the kinase ATM and is essential for the integrity of the host genome. We observed activation of the ATM kinase pathway in Toll-like receptor (TLR)-stimulated macrophages and a modulation of the inflammatory response by ATM-inhibition. Here, we carry out detailed studies to elucidate the molecular mechanisms and the consequences of ATM/DDR activation for the host response, protection and immunopathology during infection.

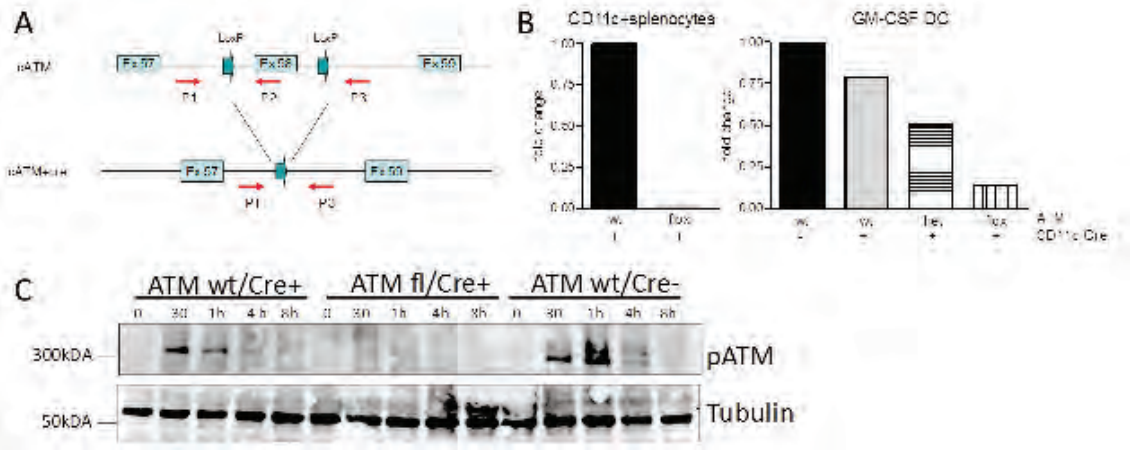
The DNA-damage response (DDR) is classically triggered by radiation-induced double strand breaks, requires the kinase ATM, and is essential for the integrity of the host genome. We have obtained evidence in phosphoproteomic studies for activation of the ATM kinase path-way in Toll-like receptor (TLR)-stimulated macrophages and observed that inhibition of ATM kinase alters the inflammatory response. Thus, it becomes evident that DDR and TLR pathways intersect during infections. This emerging theme requires now detailed studies to elucidate both fundamental aspects of the molecular mechanisms and signaling requirements in macrophages, as well as the consequences of DDR-activation for the host response, protec-tion and immunopathology during infection.

The goal of this proposal is to unravel the contribution of the DDR to innate immune responses to infection. We have obtained cell-type specific conditional ATMflox mice and crossed them with LysM-Cre and CD11c-Cre delete mice to dissect the role of the DDR in macrophages and dendritic cells (DC). We will also ask how IR regulates host responses to infection via ATM, a question of high relevance for patients undergoing chemotherapy or radiotherapy.



TLR activation co-opts ATM signalling to modulate inflammatory gene expression. (A) Enrichment of substrate motifs for ATM/ATR and CHK1 among LPS-regulated phosphopeptides. (B) Inhibition of ATM kinase activity increases LPS-induced expression of several chemokines and cytokines.

Prof. Dr. Lang



Efficient deletion of ATM in DC from conditional knockout mice. (A) Position of loxP sites around exon 58 (Lee et al. 2012. EMBO J: 31, 1177-1189). (B) Relative expression of Atm mRNA in sorted CD11c+ DC (left panel) from spleens and in vitro generated BMDM from CD11c-Cre+ mice with the indicated ATM genotype. (C) Lack of phosphorylated ATM in irradiated BMDC from ATMfl/fl;CD11c-Cre+.

The specific aims are therefore:

1. To determine cross-regulation of DNA-damage response and TLR signaling in macrophages. Transcriptomics of WT and ATM-deficient macrophages after stimulation with LPS and ionizing radiation (IR) will be used to define overlapping, synergistic and antagonistic gene expression responses. Mechanistically, the contribution of MAPK and ROS/RNI to ATM-dependent macrophage responses will be determined.

2. To investigate the role of ATM kinase during acute and chronic intracellular infection in vivo: Inflammation and tissue damage, pathogen load and adaptive immune response will be determined following infection of conditional ATM-deficient mice with *Listeria monocytogenes and Mycobacterium bovis* BCG. Dependent on the different intracellular life-styles of these pathogens, the mechanisms and consequences of ATM-DDR triggering are expected to differ substantially.

3. To determine the impact of radiation-induced DDR on inflammation and infection: is ATM activation protective or deleterious? Whole body IR of mice and its effects on LPS-induced and Listeria monocytogenes-triggered cytokine response and tissue damage will be studied, followed by dissecting the cell type-specific role of ATM. These studies are of particular relevance for understanding infection and inflammation in patients undergoing DNA-damaging treatments for malignant diseases.

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Publications during funding period

none

A70 - Progress Report

01.07.2016 - 31.12.2018

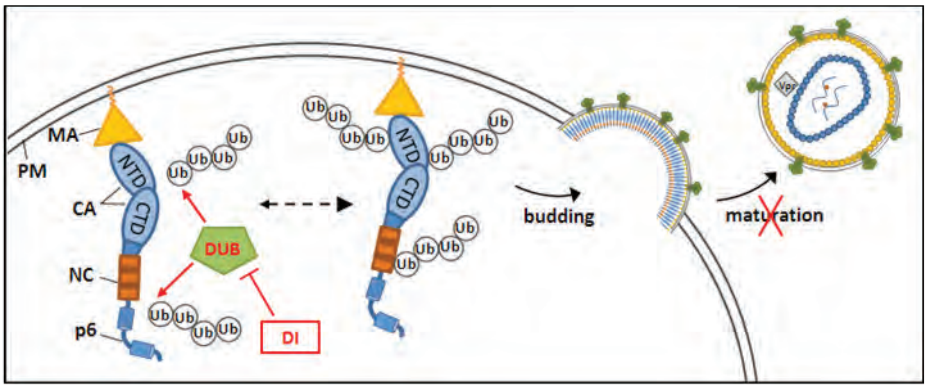
Novel targets for antiretroviral therapy – deubiquitinating enzymes regulate HIV-1 replication

Prof. Dr. Ulrich Schubert, Institute of Clinical and Molecular Virology

We have been investigating the role of two small regulatory HIV-1 proteins, Vpu and p6, in the interaction with the ubiquitin proteasome system (UPS). While Vpu directs the polyubiquitination of the host cell-receptors CD4 and tetherin, p6 regulates the polyubiquitination of Gag. In addition, we found that certain deubiquitinating enzymes (DUBs), especially USP47, play an essential role in HIV-1 replication. We will investigate the role of USP47 in Vpu-mediated degradation of CD4 and tetherin.

To further unravel the role of the UPS in HIV-1 replication, we have been investigating for years the interaction of small regulatory HIV-1 proteins with the UPS. In particular, we have been involved in the observation that the HIV-1 specific accessory protein Vpu directs certain host cell glycoproteins into the 26S proteasome. Currently, it is not clear of whether the ion channel activity of the Vpu transmembrane domain is involved in this activity. However, we were able to demonstrate that this activity is conserved throughout the evolution of HIV-1 and its ancestor SIV_{cpz}. Another small regulatory protein, the C-terminal domain of HIV-1 Gag, the p6 protein, does not only regulate the late steps in virus replication but also the polyubiquitination of Gag. Recently, we were able to demonstrate that the interaction of Gag with the plasma membrane and its subsequent polyubiquitination and access to DUBs, as well as the 26S proteasome, is regulated by the charge distribution in p6.

Furthermore, we were able to demonstrate that DUBs are involved in HIV-1 replication by regulating the processing of Gag proteins and virus infectivity. Inasmuch as this antiretroviral effect appears to be specific to certain DUB-inhibitors (DIs), which specifically inhibit USP47, we hypothesized that USP47 was one of the major DUB candidates that play a significant role in HIV-1 replication. By performing loss of function analysis we could confirm that USP47 is crucial for the maintenance of the infectivity of HIV-1.

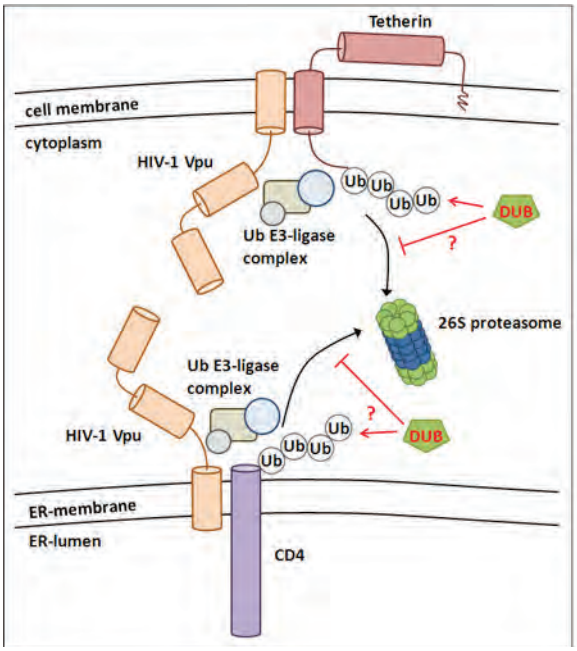


Hypothetical model: influence of DIs on HIV-1 replication.

Prof. Dr. Schubert

Furthermore, we also investigated the effect of DIs on HIV-1 replication in human lymphoid tissues (HLT) derived from tonsillary tissue, following permanent (up to 15 days post infection (pi)) or structured treatment (only day 1 and 3 p.i.). The statistical evaluation of replication profiles from 5 different donors revealed a dose- dependent decrease of HIV-1 replication during permanent and even structured application of USP47 specific DIs, resulting in a complete block of virus replication. This antiviral effect occurs independent of cell tropism (T-cell (X4)- or macrophage (R5)-tropic HIV-1). In contrast, a USP7 specific DI had no effect on virus replication, further supporting our notion that only USP47 plays a crucial role in HIV-1 replication. Most strikingly, virtually no toxicity was detected even at the highest concentrations of the DIs, indicating a broad therapeutic window for any application of DIs in lymphatic tissues.

As a further follow-up, we would like to investigate the impact of DIs on the function of accessory proteins in the interaction of HIV-1 with the UPS, particularly on the Vpu-mediated down-regulation of CD4 and tetherin.



DUBs interfere with the Vpu-mediated degradation of tetherin and CD4.

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Publications during funding period

Greiner T, Bolduan S, Hertel B, Groß C, Hamacher K, Schubert U, Moroni A & Thiel G (2016) Ion Channel Activity of Vpu Proteins Is Conserved throughout Evolution of HIV-1 and SIV. Viruses 8(12), 325

Friedrich M, Setz C, Hahn F, Matthaei A, Fraedrich K, Rauch P, Henklein P, Traxdorf M, Fossen T & Schubert U (2016) Glutamic Acid Residues In HIV-1 p6 Regulate Virus Budding and Membrane Association of Gag. Viruses 8(4), 117

A71 - Progress Report

01.07.2016 - 31.12.2018

Viral modulation of the protein kinase ULK1

Prof. Dr. Thomas Stamminger, Institute of Clinical and Molecular Virology

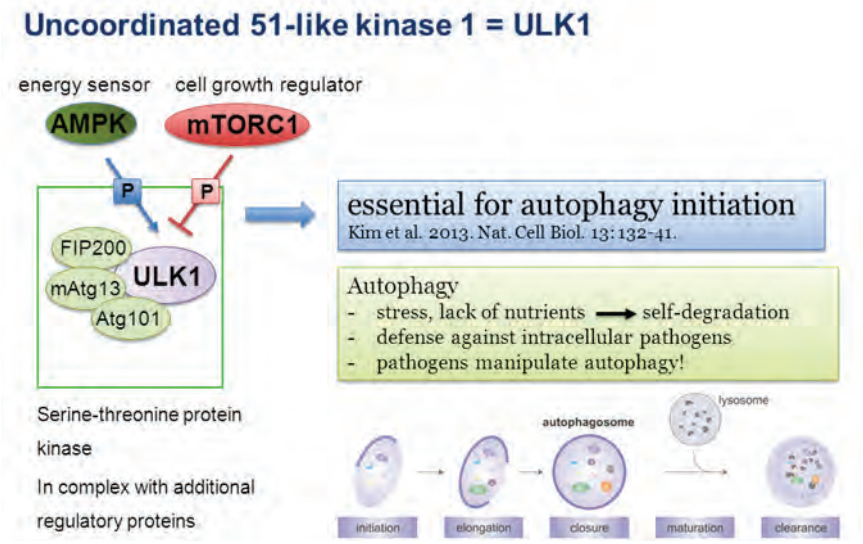
Research of the last years revealed that the cellular protein kinase ULK1 exerts critical functions at the intersection of autophagy, innate immunity and inflammatory disorders. We observed that ULK1 is strongly upregulated after infection with human cytomegalovirus. Consequently, this project aims at a detailed characterization of the role of ULK1 for HCMV infection and the viral mechanisms that mediate ULK1 induction. This may reveal novel strategies to avoid HCMV induced hyperinflammation.

Strong upregulation of ULK1 after HCMV infection of primary human fibroblasts

During a yeast two-hybrid screen we detected that the viral protein pUL26 is able to interact with the cellular protein kinase ULK1. Since ULK1 (uncoordinated 51-like kinase 1) has recently been identified as a serine-threonine protein kinase which exerts an essential role during the initiation of autophagy under control of the key energy sensor AMPK and the cell-growth regulator mTOR, we wanted to analyze the protein expression of ULK1 during the HCMV replication cycle. This revealed a strong upregulation of ULK1 protein levels that was most pronounced at late times after infection. Additionally, we observed a shift in the electrophoretic mobility of ULK1 suggesting an HCMV-induced modulation of the ULK1 phosphorylation pattern. By using phosphosite-specific antibodies we detected that HCMV stimulated both the phosphorylation of AMPK- as well as mTOR-specific sites thus revealing a rather unusual hyperphosphorylation of ULK1.

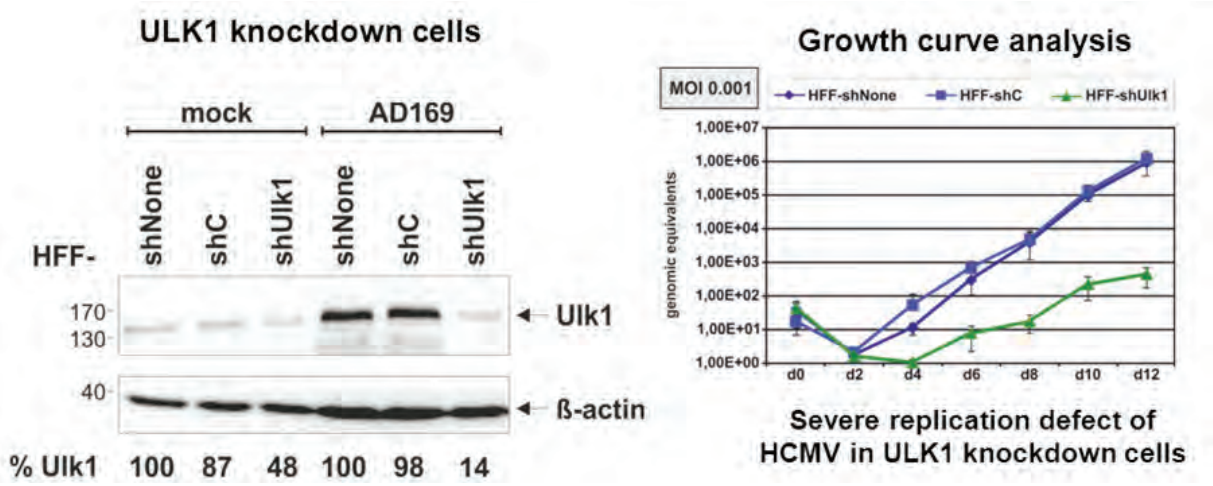
An siRNA-mediated knockdown of ULK1 correlates with reduced viral replication

In order to investigate the contribution of ULK1 to HCMV replication, primary human fibroblasts with a stable knockdown of ULK1 were generated. This was done by transducing fibroblasts with lentiviral vectors that express a shRNA specific for ULK1. Although the achieved knockdown was not complete, it efficiently prevented the upregulation of ULK1 upon exposure to HCMV. Infection of these knockdown cells revealed a severely reduced viral release of viral particles indicating that ULK1 is of critical importance for efficient HCMV multiplication.



ULK1 is regulated via AMPK and mTOR mediated phosphorylation and plays an important role during the initiation of autophagy

Prof. Dr. Stamminger



Knockdown of ULK1 in primary human fibroblast cells and replication of HCMV in ULK1 knockdown cells. Left panel: Western blot analysis of ULK1 in ULK1 knockdown cells (shULK1). Right panel: Growth curve analysis in shULK1

Identification of viral target proteins that are phosphorylated by ULK1

Since ULK1 is a protein kinase, we asked whether viral proteins may serve as phosphorylation targets. In order to investigate this, we performed in vitro kinase assays using a panel of viral proteins that was available in the laboratory. In these assays we observed a phosphorylation of the viral proteins pUL26, pp28 and IE1 by ULK1 indicating that the activity of these proteins may be modulated in a phosphorylation-dependent manner. As mentioned above, ULK1 has been identified as a direct binding partner of pUL26 by yeast two-hybrid analysis. This protein-protein interaction was further confirmed by co-immunoprecipitation experiments. Furthermore, we could show that ULK1 binds to pp28 and we detected a slight shift in the electrophoretic mobility of pp28 after co-expression of ULK1. This indicates that pp28 which exerts an essential function during the secondary envelopment of HCMV particles may be affected by ULK1 phosphorylation. Experiments to narrow down the phosphorylation sites of pp28 are currently underway.

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Invited lectures

Medical Faculty, University of Ulm, 24.10.2016, Ulm: Das humane Cytomegalovirus – von molekularen Mechanismen zu neuen Therapien

Seminar Talk Department Virologie, 06.12.2016, Medizinische Universität Wien, Österreich: Human cytomegalovirus encoded G-protein coupled receptors – news and views

Publications during funding period

none

A72 - Progress Report

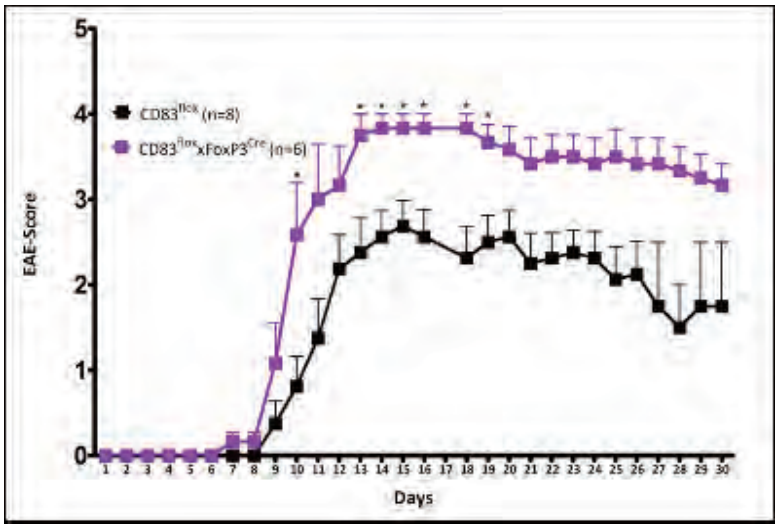
01.07.2016 - 31.12.2018

Targeted modulation of regulatory T cells and analyses of the underlying mechanisms

Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation

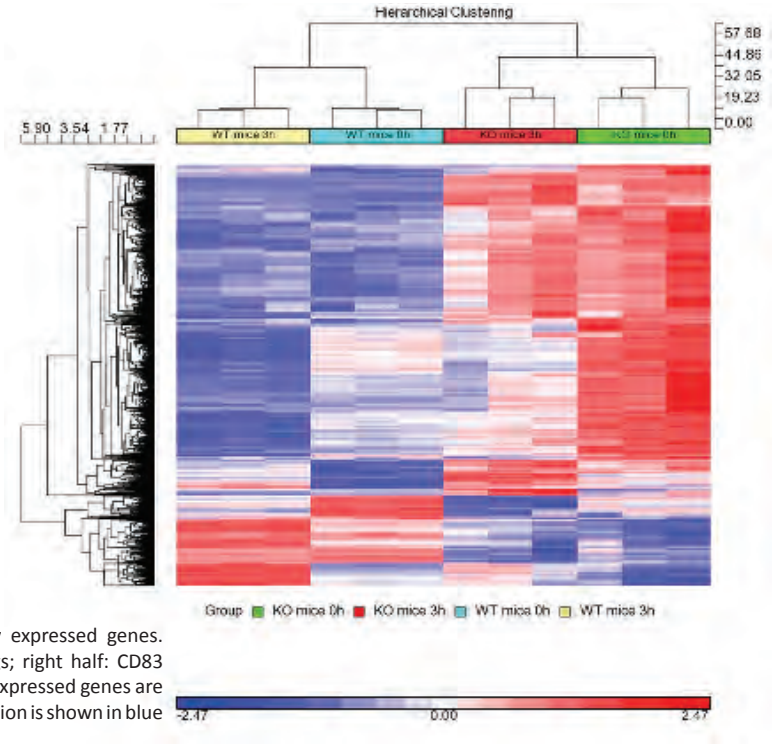
Regulatory T cells (Tregs) are crucial players to maintain immune homeostasis, to establish tolerance mechanisms and to prevent autoimmunity. Recently we showed that activated murine as well as of human Tregs express the cell surface molecule CD83. This indicates that this molecule is functionally important and to elucidate the biological function of CD83 expression on Tregs we generated Treg-specific CD83 conditional knockout (CKO) animals, which are now under investigation in our laboratory.

Previously we could show that transduction of naive $CD4^+$ $CD25^-$ T cells with CD83 encoding retroviruses induces a regulatory phenotype *in vitro,* which is accompanied by the induction of Foxp3. Functional analyses of CD83-transduced T cells *in vivo* demonstrated that these cells are able to interfere with the effector phase of severe contact hypersensitivity reaction of the skin. Moreover, adoptive transfer of these CD83 transduced cells prevented the paralysis associated with experimental-autoimmune-encephalomyelitis (EAE) in an antigen specific manner. These data provided the first evidence that CD83 expression can contribute to the immunosuppressive function of $CD4^+$ T cells *in vivo.* In addition, upon activation of murine and human Tregs, CD83 expression is highly upregulated, thereby providing a new phenotypic marker for activated Tregs. However, the precise functional role of CD83 expression on Tregs was still completely unknown and represents therefore the major aim of this project. In order to investigate the specific role of CD83 exclusively on Tregs we generated conditional KO mice (Foxp3Cre $CD83^{flox/flox}$) using the Cre-loxP system. In addition to these CD83 conditional knockout (CKO) animals, also CD83 complete knockout mice (KO) were generated using EIIa-Cre mice. After having verified the CD83 CKO-status we performed *in vivo* experiments using the EAE model which is the best animal model to study the early inflammatory phase of multiple sclerosis (MS). These data revealed that animals, which do not express CD83 on their Tregs, show (i) an earlier and highly increased disease onset and (ii) a prolonged paralyses, indicating that the resolution of inflammation is critically impaired in CD83 CKO mice. These first findings demonstrate that Treg specific CD83 CKO animals have a functional phenotype, further supporting our hypothesis that CD83 is of critical importance for regulatory T cells. Encouraged by these very interesting *in vivo* data, gene expression



EAE experiment: CD83 conditional knockout animals (i.e. Foxp3Cre $CD83^{flox}$; magenta) show (i) an earlier and highly increased disease onset and (ii) a prolonged disease progression in comparison to controls i.e. $CD83^{flox}$ (black).

Prof. Dr. Steinkasserer



Heat map of differentially expressed genes. Left half: WT derived Tregs; right half: CD83 CKO derived Tregs. Highly expressed genes are shown in red, lower expression is shown in blue

analyses were performed in order to identify possible differences regarding the transcriptome of Tregs derived from CD83-CKO in comparison those derived from WT animals. Thus, Treg cells from both animals groups (resting or activated for 3 hours) were then analyzed using transcriptome analyses. Interestingly, there are clear differences between stimulated and unstimulated cells which can be observed in both cell types, i.e. in WT and CD83 CKO derived Tregs. However, very striking and to this extend completely unexpected is the difference between the expression patterns of Tregs derived from CKO and WT animals, indicating that CD83 expression highly modulates gene expression patterns of Tregs *in vivo.* We are currently further investigating the specific phenotype and function on CD83 CKO derived Tregs *in vitro* as well as *in vivo* and to decipher the role of CD83 for the differentiation and survival of Tregs.

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Invited lectures

Research Seminar at the “DFG - Research Training Group 1949” - Meeting, 31.05.2016, in Schermbeck, Title: ”CD83 modulates the immune system by the induction of regulatory T cells“

Research Seminar at the “ForBIMed” Symposium, 10.11.2016, in Regensburg, Title: “Development of next generation vaccine strategies for the transcriptional targeting of DC directly in patients”

Research Seminar at the “DFG – FOR 2240”, 17.11.2016, in Cologne, Title: ”Modulation of the immune system using the CD83 molecule”

Research Seminar at the “Medical University of Innsbruck”, 22.11.2016, in Innsbruck, Title: ”The CD83 molecule modulates autoimmunity and transplantation via DC and the induction of Tregs”

Publications during funding period

none

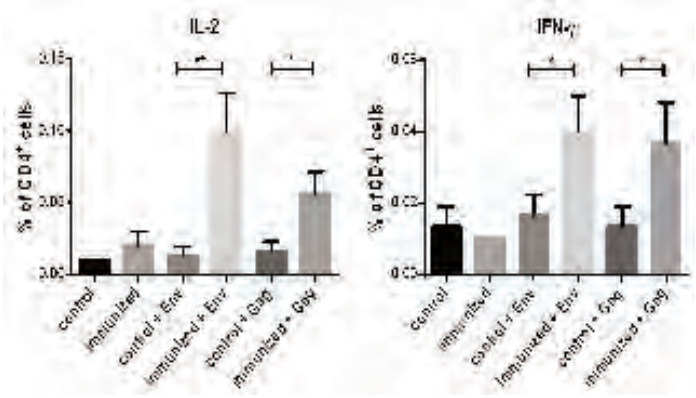
A73 - Progress Report

01.07.2016 - 30.06.2017

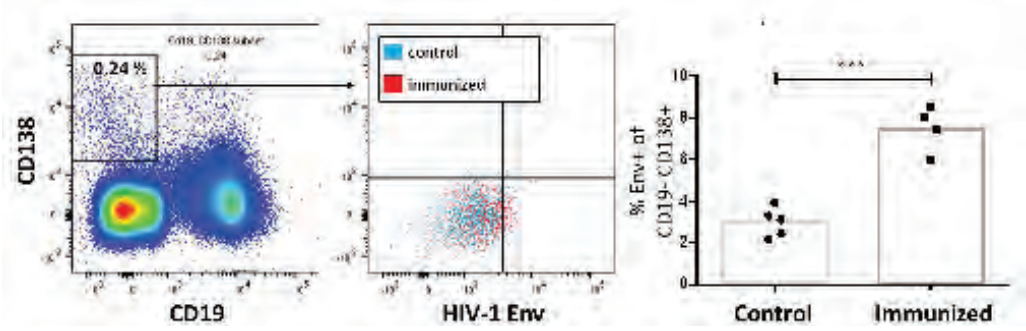
Checkpoint inhibitors as adjuvants for viral vaccines

Prof. Dr. Klaus Überla, Institute of Clinical and Molecular Virology

Checkpoint inhibitors (CPI) show great promise in improving immune control of cancer and some of them are already approved for clinical use. How these antibodies affect the immunogenicity of vaccines is largely unknown. Using a mouse model, the project therefore aims i) to better understand the consequences of CPI therapy on vaccine-induced adaptive immune responses and ii) to explore the potential of CPIs as a systemic or local immunomodulatory vaccine adjuvant.



Detection of Env- and Gag-specific CD4+ T cell responses two weeks after intramuscular DNA immunization as determined by intracellular cytokine staining. Shown are mean values with SEM (*P < 0.05, **P < 0.005) of three animals per group.



Gating strategy to detect CD19-CD138+Env+ B cells and comparison of their frequencies in the bone marrow of BALB/c mice two weeks after an HIV DNA prime virus-like particle booster immunization. Shown are mean values with SEM (***P < 0.0001).

Prof. Dr. Überla

During the first six months of the project, we established assays to analyze antigen-specific T and B cell immune responses in mice after priming with DNA immunization and virus-like particle (VLP) boosting. For that purpose, we performed initial immunization experiments. BALB/c mice were immunized by intramuscular electroporation of DNA vaccines encoding the HIV envelope (Env) and Gag-Pol proteins. After two weeks, cytokine expression was monitored after stimulation of splenocytes with immunodominant peptides using intracellular cytokine staining. As expected, CD4+ T cells producing IL-2, IFNγ and TNFα could be detected in immunized animals, but not in controls.

In order to detect HIV-Env-specific B and plasma cells, we optimized the labelling of these cells by fluorochrome-conjugated antigen. HIV-Env gp120 protein from consensus clade B HIV-1 was produced in 293F cells, purified by lectin affinity chromatography, and covalently coupled with fluorochrome Alexa 488. To prove specificity of binding between the antigen and antigen-specific B-cells we used b12 BCR-transgenic donor mice expressing the broadly neutralizing HIV-Env antibody b12.

To quantitatively assess the B cell response after immunization, mice primed by the DNA vaccines were boosted twice with VLPs containing Env and Gag-Pol in four week intervals. Two weeks after the last immunization, we analyzed the frequencies of Env-specific long-lived plasma cells in the bone marrow of immunized animals and compared it with control animals. To avoid unspecific binding, possible surface binding sites were blocked with an excess of non-conjugated Env prior to permeabilization. Intracellular staining of plasma cells with fluorochrome-conjugated Env revealed induction of Env-specific CD19-, CD138+ cells in the bone marrow.

Taken together, we established assays and optimized tools for qualitative and quantitative analysis of HIV-specific immune cells after different immunization regimens.

We now aim to analyze the effect of CPI administration during HIV DNA and VLP immunization on T helper and B cell responses. Furthermore, the impact of CPIs on differentiation and affinity maturation in BCR transgenic B cells will be elucidated. The overall goal is to evaluate the potential applicability of CPIs as adjuvants in viral vaccine platforms.

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Invited lectures

ForBiMed Symposium, 10.11.2016, Regensburg, Optimierung der Antikörperantwort nach Impfung mit partikulären HIV Impfstoffen durch intrastrukturelle Hilfe

EAVI 2020 Annual Meeting, 11.11.2016, Barcelona, Modulation of the HIV Env antibody response by intrastructural help

Publications during funding period

none

A74 - Progress Report

01.06.2016 - 30.11.2018

The Role of Eosinophils in Allergic Bronchopulmonary Aspergillosis

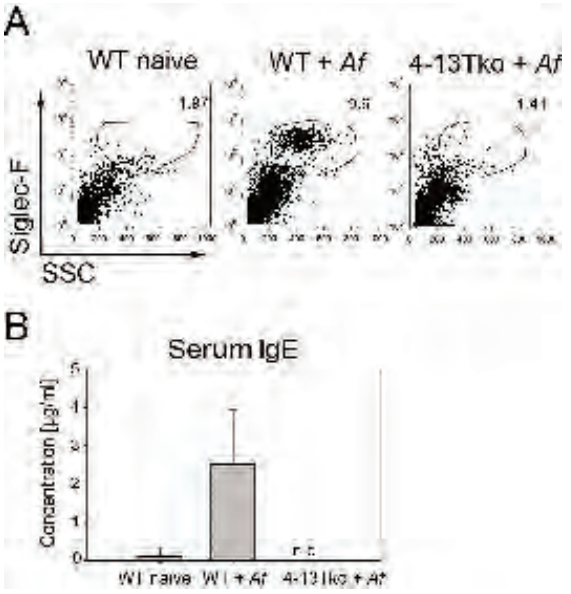
Prof. Dr. David Vöhringer, Department of Infection Biology
Prof. Dr. Sven Krappmann, Institute of Clinical Microbiology, Immunology and Hygiene

This interdisciplinary project targets the intimate interplay of eosinophilic granulocytes with cells and cellular components of the fungal pathogen Aspergillus fumigatus in the context of allergic pulmonary aspergillosis (ABPA) which mainly affects asthma and cystic fibrosis patients. The main research questions to be answered relate to the contribution of eosinophils to the immunopathology of ABPA and the activation of eosinophils by A. fumigatus. Infections in an established murine model of ABPA using recombinant mouse strains together with co-culturing experiments will shed light on the main host and fungal determinants triggering this complex allergic disease.

We report the establishment of an *in vivo* mouse model of ABPA, in which repetitive intranasal application of *A. fumigatus* conidia simulates chronic exposure to fungal elements. Infected mice reproducibly develop strong lung eosinophilia, accompanied by accumulation of PD-L2 positive alternatively activated macrophages. Activation of goblet cells was evident by elevated expression of mucin and the chloride channel *gob5.* Further, repetitive application of conidia results in a high amount of IL-13 in the supernatant of re-stimulated lymph node cells, which indicates Th2 polarization of lymph node T cells. Finally our infection model is also characterized by a boost of total IgE levels in the mouse serum.

One of our main goals is to determine the cellular source of the potentially disease driving type 2 cytokines IL-4 and IL-13 during ABPA. We found, that mice deficient for IL-4/IL-13 in T cells fail to develop lung eosinophilia, AAM accumulation, goblet cell activation and elevated serum IgE levels, after infection with *A. fumigatus*. This further correlates with a lack of *IL-5* expression, representing the most potent eosinophil survival factor. Hence $CD4^+$-T cells represent a critical effector cell type contributing to eosinophilia and ABPA severity.

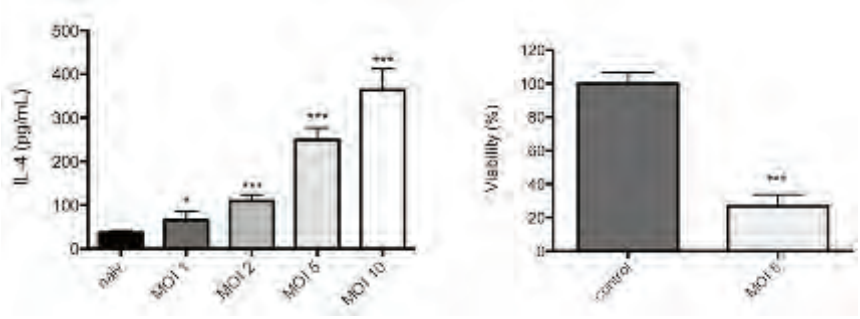
To investigate the activation pattern of eosinophils as well as the fungal response to the activated immune cells, preliminary co-culturing experiments were set up to explore the optimal *in vitro* conditions of confrontation. Accordingly, mouse (C57BL/6) bone marrow-derived eosinophils were incubated in the presence of live *A. fumigatus* conidia at various multiplicities of infection. The release of the signature cytokines IL-4 and IL-13 served as activation marker and was quantified by measuring their levels in the culture medium. In comparison to unstimulated eosinophils, a significant increase of cytokine levels could be monitored, indicating the rapid and strong response of these immune cells to the fungal elements. In co-cultures with dead conidia, however a



T cell-derived IL-4/IL-13 is required for *A. fumigatus*-induced lung eosinophilia and increased serum IgE levels. A) Wild-type (WT) or T cell-specific IL-4/IL-13-deficient mice (4-13Tko) were administered *Aspergillus fumigatus* (Af) conidia intranasally. The gate indicates the eosinophil population in the lung. B) Total serum IgE levels.

Prof. Dr. Vöhringer

Prof. Dr. Krappmann



Monitoring the interplay of eosinophils and *A. fumigatus* in vitro. A) IL-4 release in co-culture of murine bone marrow-derived eosinophils and *A. fumigatus* after culturing for six hours and in multiplicities of infection (MOI) ranging from 1 to 10. B) Viability of *A. fumigatus* conidia after six hours of incubation with C57BL/6 bone marrow-derived eosinophils.

prominent cytokine release response was not triggered (data not shown), which indicates that fungal epitopes formed in the course of germination might be recognized by eosinophils. In line with these *in vitro* data, we do not observe lung accumulation of eosinophils or AAMs in the mouse model in response to repetitive application of killed conidia. Thus, we figure out that viability and germination are a prerequisite for the fungus to cause symptoms of ABPA.

With respect to the response of *A. fumigatus* upon confrontation with mouse eosinophils, a viability assay based on XTT reduction by the fungus was established, that allows quantification of killing mechanisms that might be exerted by the immune effector cells towards the fungal target.

In essence, our preliminary experimental data set the stage for an in-depth characterization of the *A. fumigatus*-eosinophil interplay on various levels to allow inspection of the pathomechanisms that underlie ABPA.

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Publications during funding period

none

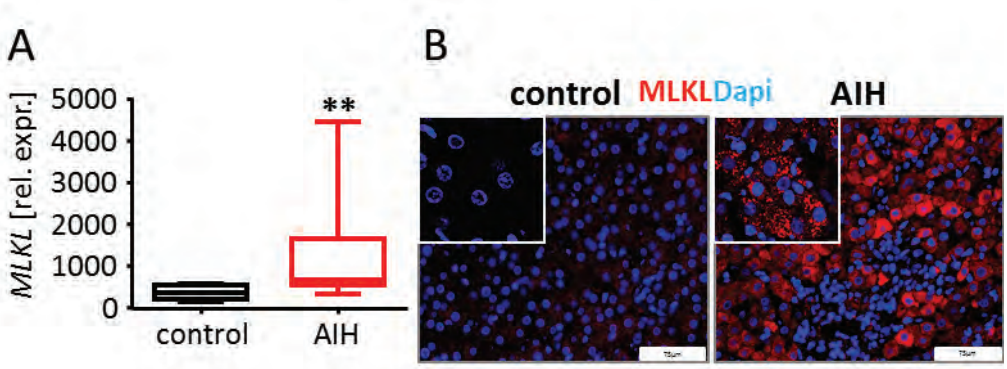
A75 - Progress Report

01.07.2016 - 31.12.2018

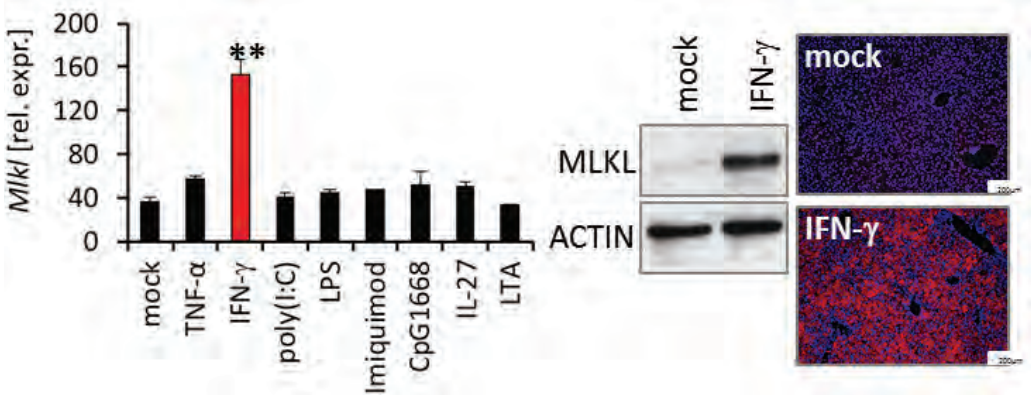
Role of MLKL-dependent programmed necrotic cell death in the pathogenesis of hepatitis

Dr. Claudia Günther, PD Dr. Dr. Stefan Wirtz,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

In this project, we aim to analyze role of the pseudokinase MLKL in liver diseases. We found that MLKL is upregulated and activated in human autoimmune hepatitis and in a model of inflammation-dependent hepatitis. Using genetic and pharmacologic approaches, we determined that hepatocellular necrosis in experimental hepatitis is driven by an MLKL-dependent pathway that occurs independently of RIPK3 and is connected to induction of MLKL expression via activation of the transcription factor STAT1.



Hepatic MLKL transcripts (A) and protein (B) are increased in patients with autoimmune hepatitis (AIH)



IFN-γ stimulation drives upregulation of MLKL *in vitro* in cultivated murine primary hepatocytes (left panel) or *in vivo* in the liver


Dr. Günther


PD Dr. Dr. Wirtz

With regard to the vital contribution of hepatocellular death to virtually all hepatic diseases, precise mechanistic knowledge of cell death regulation is essential to understand the pathophysiology of liver diseases. While for a long time apoptosis and necrosis were the most widely recognized forms of cell death, this concept was recently challenged by the discovery of necroptosis. Necroptosis has been described as a form of cell death mediated by the receptor-interacting protein kinase RIPK3 and MLKL that is sensitized under certain conditions, such as caspase-8 inhibition.

Given that MLKL has been identified as a key mediator and potential biomarker of regulated necrosis, we determined hepatic expression of MLKL in patient cohorts with diverse liver diseases of viral, toxic, or autoimmune origin. While the abundance of *MLKL* transcripts was very low in healthy controls, patients with steatosis, and patients with primary biliary cirrhosis, we observed highly increased hepatic *MLKL* expression in patients with AIH. Remarkably, MLKL immunostaining in these samples was particularly restricted to hepatocytes in areas of severe inflammation and hepatocellular death. Moreover, we also found elevated levels of MLKL mRNA and protein in hepatocytes relate in mice during experimentally induced hepatitis. Importantly, its upregulation in hepatitis clearly correlated to strong MLKL translocation to the plasma membrane and other membranous compartments, indicating that MLKL-dependent disruption of cellular integrity contributes to inflammation-dependent death of hepatocytes. In order to provide direct functional evidence for a role of the MLKL protein in experimental hepatitis, we subjected *Mlkl*$^{-/-}$ mice to experimental hepatitis. Interestingly, *Mlkl*$^{-/-}$ animals had largely diminished plasma aminotransferases and histological signs of liver damage compared with those in wild-type controls suggesting that MLKL drives inflammation-dependent hepatitis. Further experiments demonstrated that the kinase activity of RIPK1 is required for MLKL-dependent liver injury as a pharmacological approach to block RIPK1 kinase function was sufficient to markedly attenuate ConA-induced liver injury and to block translocation of MLKL to the plasma membrane.

Having shown that MLKL is strongly upregulated in experimental hepatitis and AIH, our next objective was to identify potential extracellular activators of *Mlkl* gene transcription. We therefore exposed primary hepatocytes ex situ to well-known triggers for necroptosis or cytokines involved in the pathogenesis of inflammatory liver disease. Interestingly, IFN-γ, a well-known driver of liver pathology, profoundly increased *Mlkl* transcription and protein abundance via activation of the transcription factor STAT1 and transactivation of the Mlkl promoter.

In summary, our results suggest a physiological relevance for IFN-triggered MLKL-dependent death in the pathogenesis of inflammation-dependent liver injury.

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Publications during funding period

Günther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Amann K, Vandenabeele P, Linkermann A, Poremba C, Schleicher U, Dewitz C, Krautwald S, Neurath MF, Becker C, Wirtz S (2016) The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. J Clin Invest. 1;126(11): 4346-4360

D19 - Final Report

01.11.2013 - 31.10.2016

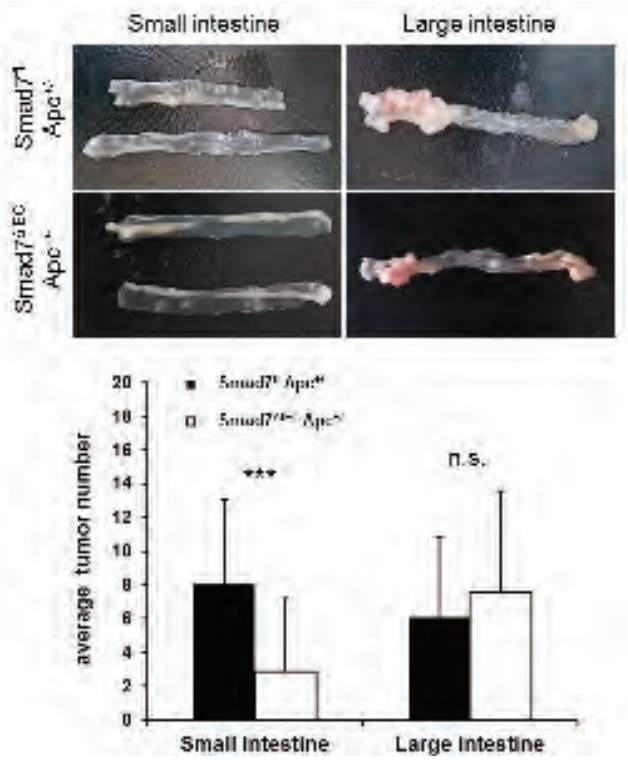
Role of intestinal epithelial SMAD7 for tumor development

Prof. Dr. Christoph Becker, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

SMAD7 is an inhibitor of the transforming growth factor beta (TGF-β) pathway, which regulates numerous cellular processes. Since polymorphisms in the SMAD7 gene have been associated with an increased risk for developing colorectal cancer, we investigated the role of SMAD7 in the intestinal epithelium for gut homeostasis and development of intestinal cancer. We studied the molecular mechanisms influenced by SMAD7 in the intestinal epithelium using mice with an intestinal epithelial cell (IEC) specific deletion of SMAD7 and aimed to create a basis for the development of innovative therapeutic approaches.

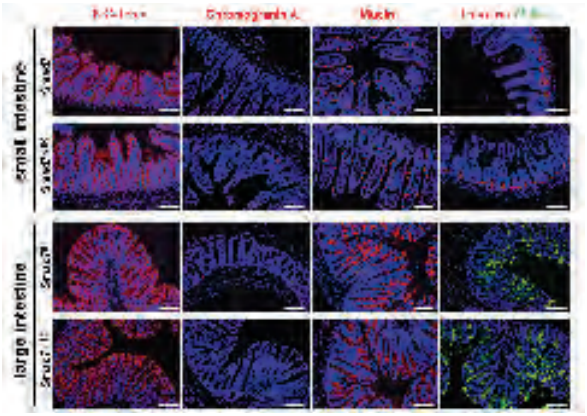
Mice with a conditional knockout for *SMAD7* in the intestinal epithelium (Smad7$^{\Delta IEC}$) were generated to evaluate the function of *SMAD7* in IECs. Analyses indicated no postnatal developmental gut defects in Smad7$^{\Delta IEC}$ mice and furthermore, no macroscopic intestinal lesions could be detected in control (Smad7^{fl}) or knockout animals. Moreover, histological and morphometric evaluations revealed no differences in epithelial structure of small and large intestine. An additional analysis of differentiated intestinal epithelial cell types like Paneth cells, goblet cells, enteroendocrine cells or enterocytes showed no overt differences between control and Smad7$^{\Delta IEC}$ animals. Furthermore there was no difference in the presence of immune cells in the small and large intestine between control and Smad7$^{\Delta IEC}$ animals.

In previous experiments we could show, that in a colitis associated tumor model (AOM/DSS) mice with *SMAD7* deficiency developed less tumors in comparison to the control group. To further investigate the role of *SMAD7* during tumor development independent of colitis, we used a sporadic tumor mouse model (APCmin). Therefore, we analyzed the tumor formation in the small intestine and colon at different ages. Mice with *APCmin* alleles and an additional deletion of *SMAD7* in IECs (Smad7$^{\Delta IEC}$ APC$^{+/-}$) showed a reduced tumor number in the small intestine when compared to the control group (Smad7^{fl} APC$^{+/-}$). These data support the hypothesis that Smad7 influences the process of tumor development in the small intestine.



Representative pictures and statistical analysis of the tumor number in the small and large intestine of Smad7^{fl} APC$^{+/-}$ and Smad7$^{\Delta IEC}$ APC$^{+/-}$ mice. Smad7$^{\Delta IEC}$ APC$^{+/-}$ mice developed significantly less tumors in the small intestine. ($p < 0.001$ = ***; n.s. = not significant).

Prof. Dr. Becker



Representative fluorescent pictures of small and large intestine of Smad7^{fl} and Smad7$^{\Delta IEC}$ mice stained for β-Catenin, Chromogranin A, Mucin or ulex europaeus agglutinin-1 (Ulex) and Lysozyme. Nuclei were counterstained with Hoechst. Scale bars, 100 µm.

We further performed *in vitro* analyses by generating small intestinal organoids from Smad7^{fl} and Smad7$^{\Delta IEC}$ mice. Stimulation with TGF-β for 36 hours led to increased cell death in Smad7$^{\Delta IEC}$ mice compared to controls.

In summary, our data show that a deletion of *SMAD7* in IECs does not disturb the overall gut homeostasis in the steady state but leads to reduced tumor burden in an sporadic tumor model in mice. The detailed mechanism on how *SMAD7* influences the TGF-β signaling pathway or if also TGF-β independent pathways like NF-κB or Wnt are involved, has still to be elucidated. Further experiments are needed to investigate the role of *SMAD7* on these side pathways to understand its role during colon tumorigenesis. The project will be continued within the DFG funded Research Unit FOR 2438.

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Invited lectures

9th Swiss Apoptosis Meeting, Bern, Schweiz, 08. - 09.09.2016, „Cell death pathways driving inflammation in the liver and gut“

MIAMI-Symposium, Münster, Deutschland 13. - 15.04.2016, „Innate Immune Pathways in the Gut“

Publications during funding period

Martini E, Schneider E, Neufert C, Neurath MF, Becker C (2016) Survivin is a guardian of the intestinal stem cell niche and its expression is regulated by TGF-β. Cell Cycle 7: 1-7

Martini E, Wittkopf N, Günther C, Okada H, Watson A, Podstawa E, Backert I, Neurath MF, Becker C (2016) Loss of Survivin in intestinal epithelial progenitor cells leads to mitotic catastrophe and breakdown of gut immune homeostasis. Cell Rep 14(5): 1062-73

D20 - Final Report

01.11.2013 - 31.10.2016

Collagen 10 and metastasis in CRC

Prof. Dr. Dr. Michael Stürzl, Prof. Dr. Roland Croner, PD Dr. Elisabeth Naschberger, Department of Surgery

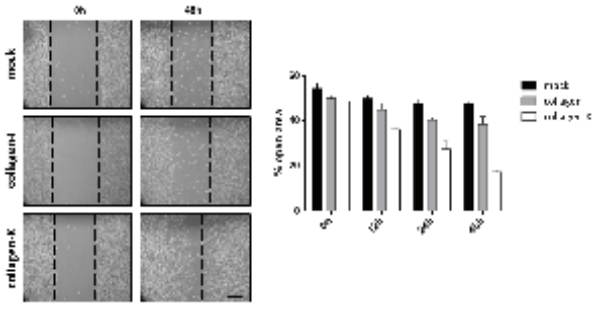
In the present project we found that expression of the collagen 10 mRNA is increased in primary lesions of metastasizing colorectal carcinomas. Collagen 10 was found to be expressed in mesenchymal, fibroblast-like cells. Purified collagen 10 increased migratory activity of colorectal carcinoma tumor cells. A collagen 10 gene knock-out mouse model to analyze the function of collagen 10 in metastasis has been successfully established.

Collagen 10 RNA and protein are increased in colorectal carcinoma in a stage-dependent manner and are expressed by vimentin/PDGF-Rβ/α-SMA-positive cells

Immunohistochemical staining with a specific monoclonal antibody (provided by Prof. von der Mark, Inst. of Experimental Medicine 1) revealed that the collagen 10 protein is present in colorectal carcinoma (CRC) tissues (n=122) and significantly increases with progression of the disease. Protein expression was categorized and correlated significantly with collagen 10 mRNA (TaqMan-qPCR) expression in consecutive tissue sections of these patients. Moreover, immunofluorescent co-localization studies suggested that collagen 10 is expressed by vimentin-, PDGF-β-receptor- or α-SMA- but not CD31-positive stromal cells in CRC tissues.

Recombinant collagen 10 fosters migration of colorectal cancer cells

Wounding assays using plates coated with recombinant collagen 10 precipitated from cell culture supernatants showed that collagen 10 fosters migration of colorectal cancer cells (DLD-1) as compared to the same cells growing on uncoated or collagen 1 coated cell culture plates. By the observed increase of tumor cell mobility collagen 10 may contribute to a tumor microenvironment fostering metastatic growth of CRC.



Collagen 10 fosters migration of colorectal cancer cells (DLD-1). Multiwell plates were coated with collagen 10, collagen I or left uncoated. DLD-1 cells were seeded onto the wells, scratched and monitored for 48 hrs. The relative open area of the wound is given percent.

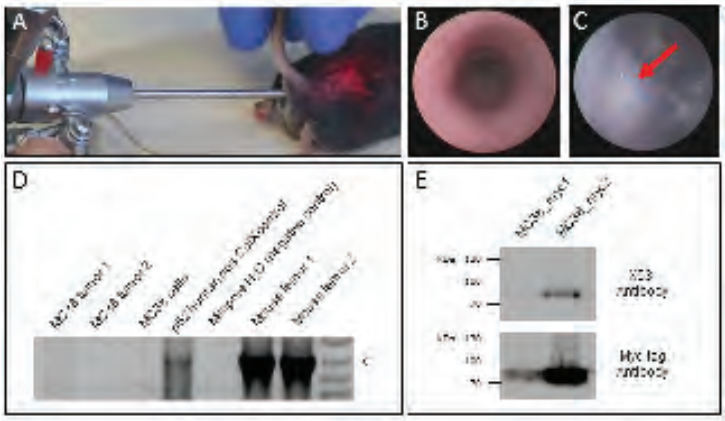
Establishing of collagen 10 knockout mice

Collagen 10 knockout mice were obtained from Dr. Brachvogel, University of Cologne (Center for Biochemistry). The animals were sent to our institution as shock-frozen embryos. Embryos were implanted into pseudo-pregnant female mice. These gave birth to heterozygous collagen 10 knockout mice. Currently, these mice are breeding and homozygous collagen 10 knockout mice have been born. For genotyping of these animals a PCR protocol has been successfully established. As a tumor model endoscopic injection of MC38 B/6 cells in the mouse colon mucosa of the respective animals will be used. The respective technique has already been established in cooperation with Prof. Becker (Medical Clinic 1, University Erlangen). The animal experimentation has been approved (Az: 54-2532.1-53/13). *In vitro* analyses showed that untreated MC38 B/6 cells do not express collagen 10. Therefore, stable cell lines of MC38 B/6 cells overexpressing recombinant collagen 10 have been successfully established. These cells will be used to compare tumor growth with/without collagen 10 expression after endoscopic injection into wildtype and collagen 10 knockout mice colon.


Prof. Dr. Dr. Stürzl


Prof. Dr. Croner


PD Dr. Naschberger



Tumors after submucous tumor cell (MC38) injection. (A) Mouse colonoscopy, (B) regular colon mucosa, (C) tumor growth (red arrow). (D) Expression of collagen 10-RNA (arrow) in MC38-cells before and after tumors growth in mice (MC38 tumor) and (E) recombinant over-expression (X53 antibody).

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Publications during funding period

Feiersinger F, Nolte E, Wach S, Rau TT, Vassos N, Geppert C, Konrad A, Merkel S, Taubert H, Stürzl M, Croner R (2016) MiRNA-21 Expression Decreases from Primary Tumors to Liver Metastases in Colorectal Carcinoma. PLoS One 11(2): e0148580

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D21 - Final Report

16.10.2013 - 15.10.2016

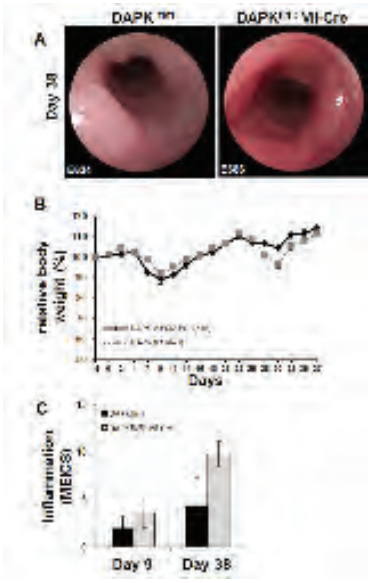
DAPK and colon cancer

Prof. Dr. Regine Schneider-Stock, Institute of Pathology
PD Dr. Clemens Neufert, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

DAPK1 is a serine/threonine kinase with dual functions dependent on cellular context and experimental stimuli. Recently, we described that inactivation of DAPK1 at the invasion front of colorectal cancer is associated with gain in migratory abilities. To examine the DAPK1 driven molecular mechanisms for colorectal cancer initiation and progression we aimed to investigate the consequences of DAPK1 loss in in vitro and in vivo models. We designed several CRISPR-ko clones of three different colorectal cancer cell lines and developed a DAPK1$^{fl/fl}$;Vil-Cre conditional knockout mouse. These approaches will allow evaluating the impact of its kinase activity or scaffold function in more detail.

Role of DAPK in AOM/DSS model of colorectal carcinogenesis

We aimed to analyze DAPK dependent colonic tumor development using i) *Dapk1$^{tm1a(EUCOMM)Hmgu}$/H*, ii) *Dapk1$^{fl/fl}$;Vil-Cre* and iii) DAPK1-inhibitor (kinase inhibitor) treated C57BL6/J mice. The animals were monitored after one initial intraperitoneal injection of AOM (10 mg/kg body weight) and 3 cycles of DSS (7 days of 1.5-2.0 % DSS in drinking water followed by 2 weeks of recovery). Gut inflammation and tumor development was evaluated by different readouts including mini-endoscopy and MEICS scoring. Here, we found that the control groups *C57BL6/J, Dapk1$^{fl/fl}$* and *Dapk1$^{tm1a(EUCOMM)Hmgu}$/H* knockout mice showed expected disease activities (slight differences were likely caused by different genetic backgrounds *C57BL6/J* vs. "mixed") for inflammation and increasing tumor loads over time. Preliminary experiments showed that the DAPK1-inhibitor is well tolerated by the animals. Mouse experiments with *Dapk1$^{fl/fl}$;Vil-Cre* having conditional DAPK1 loss only in the intestinal epithelial cells are still in progress. They were delayed in particular by challenging breeding conditions, complemented by comprehensive molecular / biological / histopathological assessment and interpretation of disease pathology in mouse tissues. Preliminary data suggest a higher vessel density in DAPK1$^{fl/fl}$;Vil-Cre tumors compared to DAPK1$^{fl/fl}$.



CRC tumorigenesis in DAPK1$^{fl/fl}$ and DAPK1$^{fl/fl}$; Vil-Cre mice (A) induced by AOM and three cycles of DSS. Representative endoscopic pictures are shown. (B) Colitis-severity is represented by weight curves and (C) MEICS scoring.

Establishment of stable HCT116-DAPK1$^{-/-}$ cell lines as in vitro model

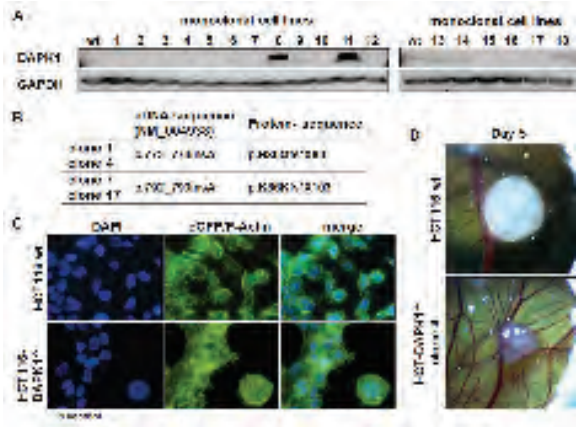
Using CRISPR/Cas9 genome editing technology, we successfully generated DAPK1$^{-/-}$ clones of three different colorectal cancer cell lines. For molecular cloning, we fabricated two Dapk1 gene-specific all-in-one CRISPR/Cas9 vectors. After co-transfection with a puromycin^{R} vector for antibiotic selection, CRISPR/Cas9-mediated double strand breaks efficiently introduced homozygeous single nucleotide insertion (adenine) mutations p.H80Gfs*X83 or p.K86Kfs*X103 in tumor cells resulting in a total loss of DAPK1 protein expression. The stable kinase knockout was validated by Sanger sequencing, Western Blot analysis, and immunofluorescence microscopy. Microscopic examination of actin-filaments of DAPK$^{-/-}$ cells indicate a remarkable reorganization of F-actin fibers whereas DAPK wt cells show well-organized stress fibres and lamellipodia and a higher F-actin net-density in cell periphery.

Characterization of growth in an in vivo graft model

The chick chorioallantoic membrane (CAM) assay was used to study DAPK1-dependent tumor forming capacity, growth histology, and vascularization of DAPK1$^{-/-}$ cells. Briefly,


Prof. Dr. Schneider-Stock


PD Dr. Neufert



CRISPR DAPK1-knockout in HCT116-derived clones. DAPK1-knockout was validated in 11/18 clones using (A) Western Blot and (B) Sanger sequencing. (C) F-actin organization in wt versus DAPK1$^{-/-}$ cells (100x) (D) Microxenografts on the chorioallantoic membrane (CAM, day 5).

one million tumor cells mixed in a 50% matrigel (1:1 mixture with culture medium) were put onto the CAM at day 9 where they formed adherent plaques. On chick embryonic development day 14 the CAM tumors were harvested according to the protocol of Sys et al. (2013). Tumor size did not differ between both genotypes. Histological evaluation on formalin-fixed in paraffin embedded tissues showed a higher vessel density and less necrotic areas in DAPK1$^{-/-}$ cell clones. We will further examine if DAPK1 has an anti-angiogenic effect in colorectal tumor cells. Contact:

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Awards

Award of the Japanese Society of Gastroenterology, Clemens Neufert, 22.04.2016

Publications during funding period

Martini E, Schneider E, Neufert C, Neurath MF, Becker C (2016) Survivin is a guardian of the intestinal stem cell niche and its expression is regulated by TGF-β. Cell Cycle 15(21): 2875-2881

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D22 - Final Report

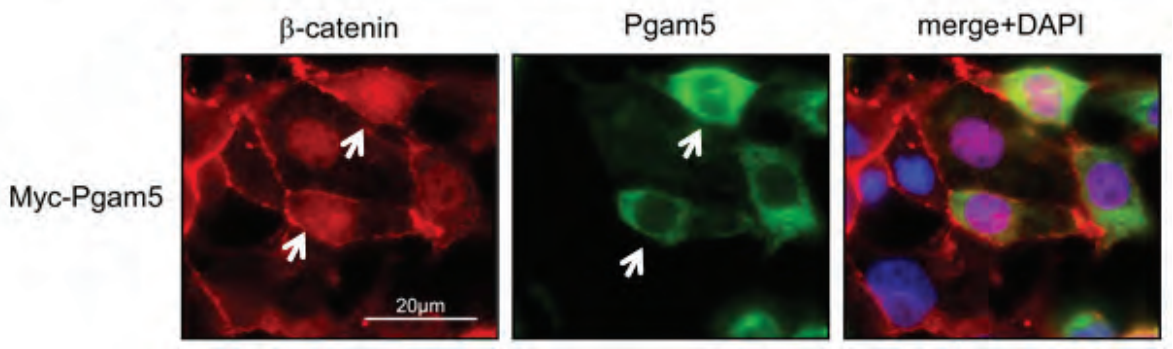
01.11.2013 - 31.10.2016

Identification and functional characterisation of novel components of the Wnt/β-catenin signal transduction pathway

Prof. Dr. Jürgen Behrens, Chair of Experimental Medicine II – Molecular Oncology

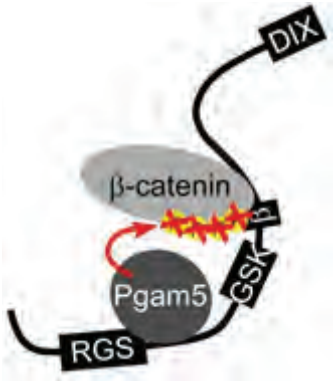
We searched for novel components of the Wnt signal transduction pathway at the level of Wnt receptors and the β-catenin destruction complex using yeast two hybrid screens and proteomic analyses. We found that the mitochondrial phosphatase Pgam5 represses Wnt signalling in vitro and in vivo through an unknown mechanism but that cytosolic Pgam5 activates Wnt signalling by dephosphorylating β-catenin. The LIM domain protein Ajuba was shown to interact with E3 ligases downregulating Wnt receptors.

β-Catenin is degraded after its phosphorylation by GSK3 in the axin-based β-catenin destruction complex. After binding of Wnt ligands to co-receptor pairs consisting of frizzled and LRP5/6, β-catenin phosphorylation is inhibited. As a consequence, β-catenin is stabilized and activates transcription of Wnt target genes. In our previous work we found that Axin interacts with the mitochondrial protein phosphatase Pgam5 and that Pgam5 antagonizes Wnt signaling both in vitro and in vivo (Xenopus). Surprisingly, Pgam5 lacking the first 24 amino acids, which is no longer associated with mitochondria and locates in the cytoplasm has an activating role in Wnt signaling, as revealed by increased β-catenin levels and reporter activity following its overexpression. This form is generated by mitochondrial damage induced by exposure of mitochondria to membrane depolarization agents such as the substance CCCP. We found that Pgam5 dephosphorylates β-catenin in vitro which is stimulated by axin. In line, CCCP treatment of cells diminished β-catenin phosphorylation which was abolished by Crispr/CAS mediated mutation of Pgam5. Thus, Pgam5 might stabilize β-catenin directly through dephosphorylation. Moreover, axin appears to act as a scaffold to promote β-catenin dephosphorylation by Pgam5 very much as it acts as a scaffold to promote phosphorylation of β-catenin by GSK3. Wnt signaling was previously shown to stimulate mitochondrial biogenesis, i.e. increase the numbers of mitochondria per cell. We suggest that soluble Pgam5 generated as a consequence of mitochondrial damage might activate Wnt signaling and thereby stimulate mitochondrial biogenesis and maintain homeostasis.



Expression of cytoplasmic Pgam5 (Myc-Pgam5, green) induces stabilization of endogenous β-catenin (red) which is present in the nucleus (blue).

Prof. Dr. Behrens



Model showing that axin functions as a scaffold to promote dephosphorylation of β-catenin by Pgam5.

E3 ligases RNF43 and ZNRF3 are known to downregulate Wnt recptors Frizzled and LRP. We found that Ajuba and LimD1 which are both Lim domain containing adaptor proteins interact with specific cytoplasmic domains of these E3 ligases. We are currently analyzing the function of Ajuba and LimD1 in Wnt signaling.

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Invited lectures

Symposium „Cell Adhesion and Communication“, 13.10.2016 Ghent, Belgium. “Regulation of Axin Proteins in Wnt signaling”.

Publications during funding period

none

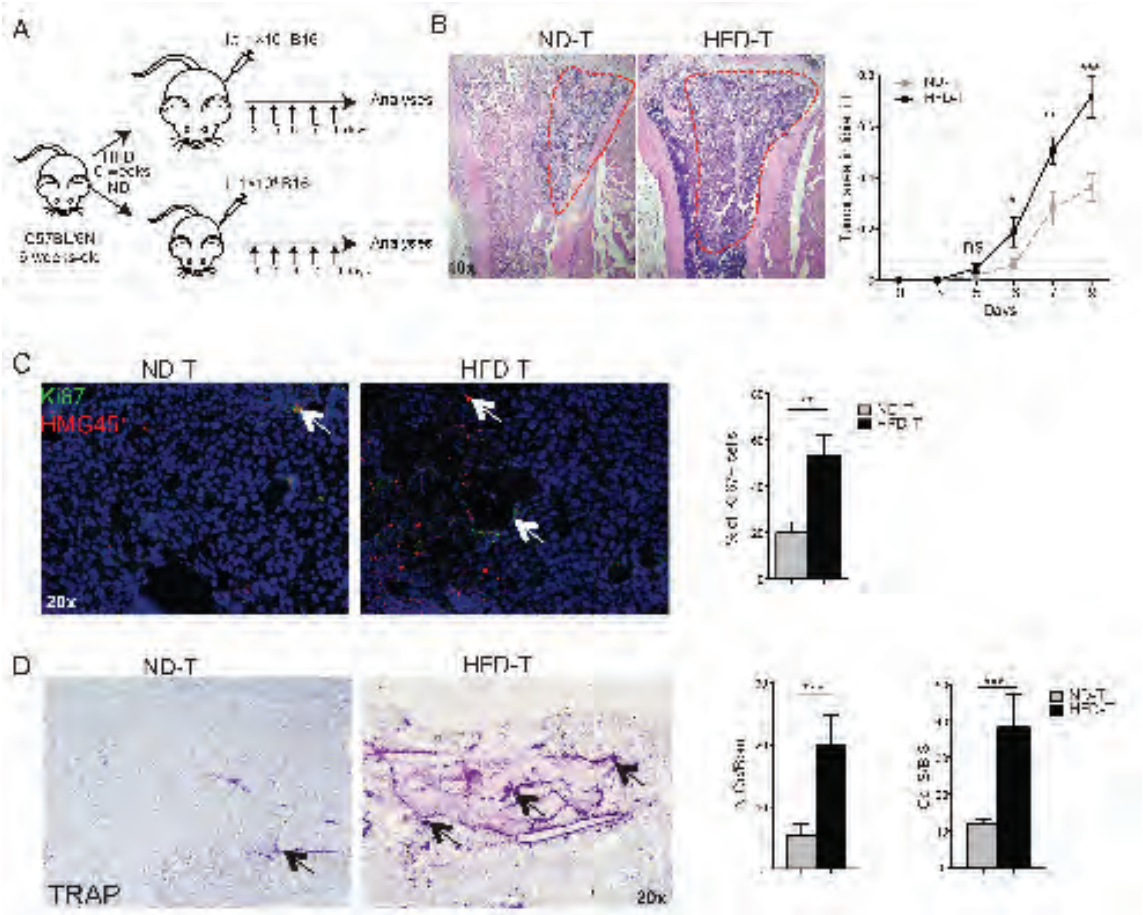
D23 - Progress Report

01.01.2016 - 30.06.2018

Influence of bone marrow adipocytes on the metastatic niche in experimental bone metastasis

Prof. Dr. Aline Bozec, Department of Medicine 3 – Rheumatology and Immunology

The project aims to determine the effect of bone marrow adipocyte in melanoma bone metastatic niche using a high fat diet model. During the first year of our funding, we delineate that metabolic stress by HFD promotes melanoma growth in the bone marrow by an increase in bone marrow adipocytes and IL-6-JAK2-osteopontin mediated activation of tumor cells and osteoclast differentiation (Chen G et al. 2016).



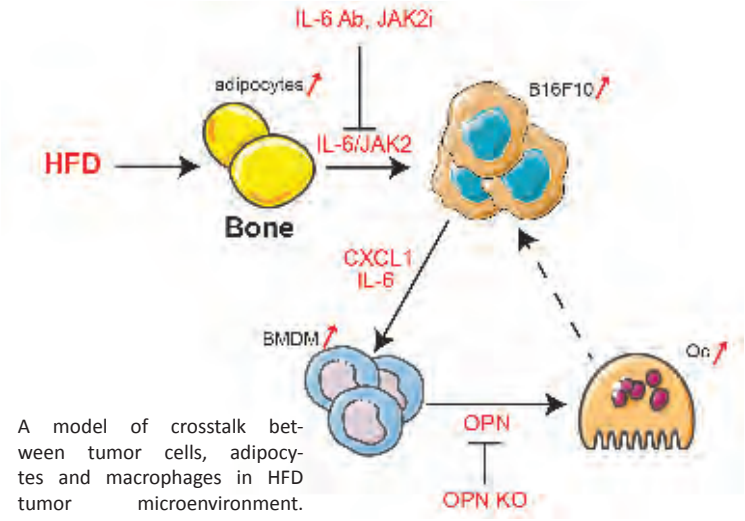
High fat diet mice have an increased bone tumor growth correlated with increased osteoclasts. A-B. Experimental scheme (A) Hematoxilin & Eosin pictures and tumor growth quantification in ND and HFD tibia at day 7 post i.t. B16F10 cell injection (B- ×10). C. Ki67 staining and quantication in ND and HFD bone tumor (×20). D. osteoclast staining and quantication in ND and HFD bone tumor (×20). N.Oc/B.Pm, Number of osteoclasts per bone perimeter; Oc.S/BS, osteoclast surface/bone surface.

Prof. Dr. Bozec

In vivo:

- High fat diet increases melanoma growth in the bone and increases bone-resorbing osteoclasts

By histological analyses of tibias injected with B16-F10 melanoma cells, we showed an increased tumor volumes in high-fat diet mice compare to lean mice correlated with an increased osteoclast number.



A model of crosstalk between tumor cells, adipocytes and macrophages in HFD tumor microenvironment.

- Metabolic stress induced by high fat diet leads to increased osteopontin level

ELISA assays and flow cytometry analyses showed an increased level of osteopontin in serum from high-fat diet fed mice after B16-F10 melanoma cells injection.

- Increase in osteopontin-producing macrophages in the bone marrow tumor area of high fat diet mice

By histological analyses, we found an increased of osteopontin producing cells in the bone marrow of high-fat diet fed mice after B16-F10 injection, correlated with an upregulation of pro inflammatory macrophage number.

- Enhanced tumor growth in obese mice is mediated by IL-6-JAK2-osteopontin axis

Knocking out OPN or inhibiting JAK2 or IL-6 rescued the increased bone tumor volume phenotype of high-fat diet fed mice.

In vitro:

- High fat diet serum increases melanoma cell proliferation and osteoclastogenesis

B16-F10 melanoma cells grown *in vitro* in the presence of serum isolated from high fat diet mice showed upregulation of genes associated with proliferation. Moreover, serum isolated from high fat diet mice could increase osteoclastogenesis *in vitro.*

- B16F10 cells become more “aggressive” in the presence of adipocytes

In vitro co-culture of B16F10 cells with adipocytes showed an increase pro-inflammatory cytokine secretion by B16-F10 melanoma cells.

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Invited lectures

German Stem Cell Network (GSCN) meeting 2016, Title: Gut microbiome from obese mice regulates the hematopoietic stem cell differentiation by altering the bone niche.

6th International Conference on Osteoimmunology (2016), Title: Obesity alters the bone niches

Publications during funding period

Chen GL, Luo Y, Eriksson D, Meng X, Qian C, Bauerle T, Chen X X, Schett G, Bozec A (2016) High fat diet increases melanoma cell growth in the bone marrow by inducing osteopontin and interleukin 6. Oncotarget 7(18): 26653-69

D24 - Progress Report

01.06.2016 - 30.11.2018

Differentiation-associated Schwann cell transcription factors in melanoma - learning from embryogenesis

Prof. Dr. Anja Bosserhoff, Prof. Dr. Michael Wegner, Institute of Biochemistry

Melanoma is an aggressively disseminating cancer with continuously rising incidence. Melanoma cells derive from melanocytes, which originate from the neural crest and display characteristics of cells of the nervous system. Interestingly, Schwann cells, nervous system cells derived from the neural crest, can transdifferentiate into melanocytes and vice versa. Based on the expertise of both PIs, we are focussing on central transcription factors of Schwann cell differentiation and their role in melanoma.

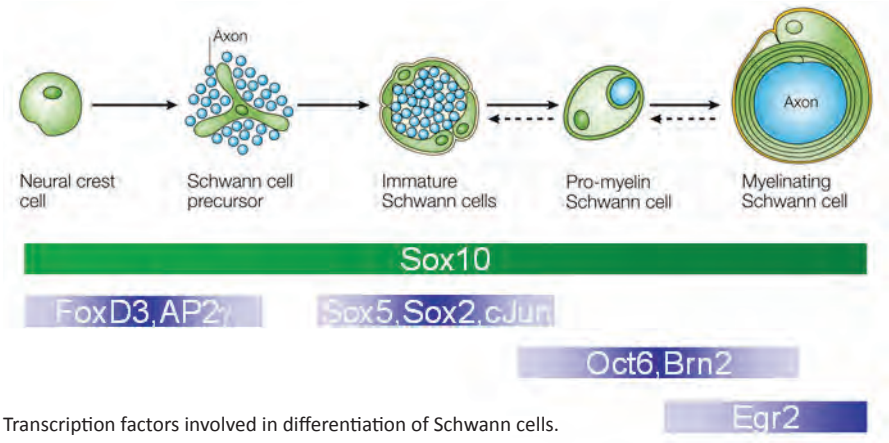
Our aims in this project are the following:

1. Definition of differentiation-associated Schwann cell transcription factors that play a role in melanoma

2. Determination of molecular differences and similarities between schwannomas and melanomas

At the beginning of the project in June 2016 we started to determine, which of the transcription factors that are expressed and important in Schwann cells during development and in the adult differentiated cells, are deregulated in melanoma development or progression. Until now, we were able to define several Schwann cell transcription factors as strongly deregulated in melanoma cell lines compared to melanocytes (e.g. TFAP2C, EGR2). After analysing expression in melanoma cell lines, a confirmation of the data in tissue material is ongoing.

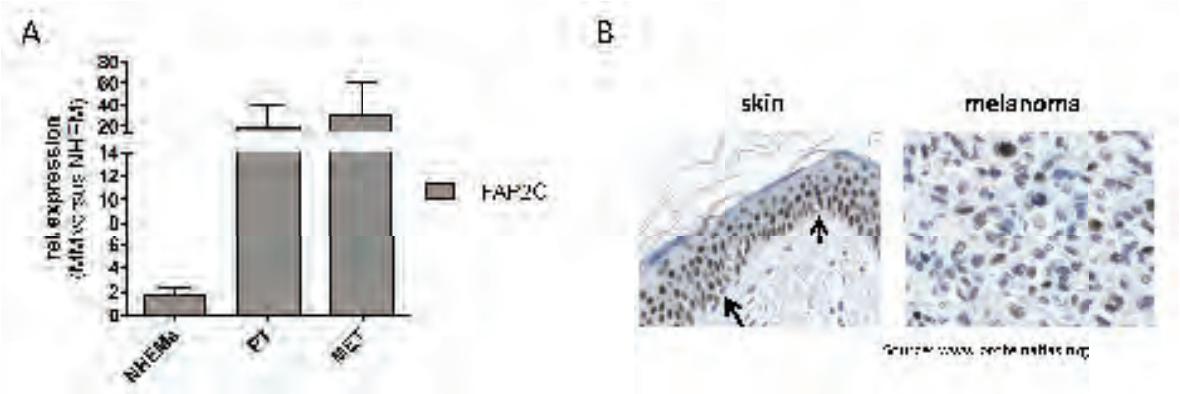
Next, we will focus in this collaborative project on the transcription factors most strongly deregulated and analyse their specific impact on melanoma in detail. We will determine the functional role of these factors in tumour development. An important task will be the definition of target genes of these transcription factors in both melanoma and Schwann cell lines. For that purpose, we are planning to generate melanoma and Schwann cell lines in which the transcription factors are deleted by CRISPR/Cas9. A proof-of-principle experiment has already been successfully conducted for the Sox10 transcription factor in the S16 Schwann cell line. The resulting transcription factor-deficient cell lines will be compared in their expression profile to the original ones and between melanoma and Schwann cell line. In case of the Sox10-deficient S16 Schwann cell line, RNA-seq studies showed a loss of Schwann cell identity and differentiation markers and an up- or downregulation of several signalling pathways and regulatory microRNAs.



Transcription factors involved in differentiation of Schwann cells.


Prof. Dr. Bosserhoff


Prof. Dr. Wegner



(A) Analysis of AP2gamma *(TFAP2C)* transcript levels in melanoma cell lines derived from primary tumors (PT) and metastasis (MET) versus melanocytes (NHEM) by qRT-PCR (B) Immunohistochemical analysis of AP2gamma proteins in melanoma confirming the strong induction of expression observed on mRNA level (proteinatlas.org; arrows mark melanocytes in the basal layer of the epidermis).

Schwann cells also give rise to tumours. The resulting schwannomas are mostly benign and slow growing and thus very different from melanoma. In a second part of the project we will therefore compare the mRNA expression pattern of schwannomas and melanomas to set the basis for a characterisation of genes that promote or repress the metastatic process in melanoma.

In summary, we will use knowledge from Schwann cell differentiation to define central transcriptional regulators for melanoma development and progression, which have not been associated with pathogenesis before, and thereby obtain a better molecular and cellular understanding of this tumour entity.

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Invited lectures

Seminar of the Dept. of Dermatology, University Hospital Mannheim (3.2.2016, AB)

Hinterzartener Kreis meeting, Cadenabbia, Italy (28.4.2016, AB)

ADO Kongress, Dresden (22.9.2016, AB)

Symposium on Eukaryotic Regulatory Biology, San Diego, USA (5/6.8.2016, MW)

Publications during funding period

none

D25 - Progress Report

01.05.2016 - 31.10.2018

Interaction of the EGFR- and the ZEB1-pathway in aggressive cancer types

Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I – Molecular Pathogenesis Research

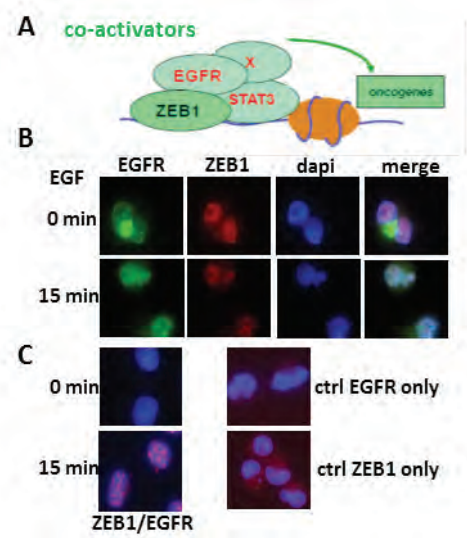
The program of epithelial to mesenchymal transition (EMT) provides cancer cells with motility, invasiveness and stem cell features. One main EMT inducer is the transcriptional repressor ZEB1. However, many of the underlying molecular mechanisms of its tumor promoting effects are unknown. To clarify the versatile functions of ZEB1, we validate, verify and map interactions with novel candidates identified by MassSpec, and further investigate their functional relevance for cancer progression.

We could show that under certain conditions - e.g. in an oncogenic context of cancer cells – the oncogenic factor ZEB1 can switch from a transcriptional repressor to a transcriptional activator of tumor promoting genes. We proposed novel nuclear interaction partners and previously exemplified this by showing interaction with YAP1, a main effector of Hippo signalling, to activate a specific common target gene set. By analysing own ChIP-Seq-data we now revealed that AP-1 is one additional candidate to interact and cooperate with ZEB1/YAP1 and validated this by Co-Immunoprecipitations (Co-IPs). In order to identify additional coactivators of ZEB1, we had performed Co-IPs from nuclear extracts of aggressive cancer cells, coupled to mass-spec and proteomic analyses and had detected about 20 unknown nuclear co-factors of ZEB1. Among the top 5 identified putative co-factors of ZEB1 were the nuclear EGFR and STAT3. Since a nuclear cooperation of EGFR and STAT3 in transcriptional activation was already reported, our working model is, that recruitment of EGFR, STAT3 and other candidate factors (x) shifts the function of ZEB1 from a transcriptional repressor to an activator of a tumor promoting gene set.

During the first 6 months of our funding, we started to confirm the nuclear interactions of these two candidates.

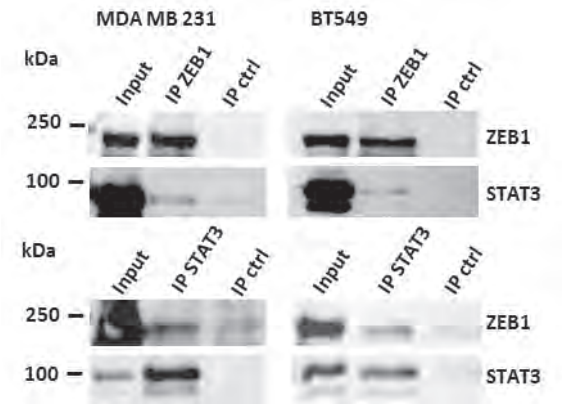
Stimulation with EGF leads to nuclear accumulation of EGFR and co-localization with ZEB1

To verify the interaction found in the MassSpec analysis, we performed Immunofluorescence (IF) and found that the bulk of EGFR protein in MDA MB 231 breast cancer cells localized in a cytoplasmic compartment next to the nucleus. Thus, we stimulated the cells with EGF and observed already after 15 minutes of treatment most of the protein in the nucleus co-localizing with ZEB1. Positive signals in *in situ* proximity ligation assays (PLAs) after EGF treatment also support the putative interaction of ZEB1 and nuclear EGFR. At the moment, we optimize Co-IPs and



(A) Model: EGFR, STAT3 and others (x) act as ZEB1 co-activator of tumor promoting genes. (B) IF showing cytoplasmic EGFR but nuclear co-localization with ZEB1 after EGF treatment. (C) PLA showing complex formation of ZEB1 and EGFR upon EGF stimulation.

Prof. Dr. Brabletz



Co-IPs of endogenous ZEB1 and STAT3 in nuclear lysates of MDA MB 231 and BT549 cells showing that STAT3 interacts with ZEB1 in the nucleus.

have started to produce constructs for the expression of partial proteins *in vitro* as well as *in vivo* in cancer cell lines to map the interaction domains.

STAT3 interacts with ZEB1 in the nucleus

STAT3 was shown to form a complex with nuclear EGFR and both oncoproteins behave as transcriptional co-regulators. Strikingly, we found not only EGFR, but also STAT3 as a prominent candidate for interaction with ZEB1 in the nucleus. For STAT3 we could already confirm the interaction of the endogenous proteins via Co-IPs from nuclear extracts in two different aggressive breast cancer cell lines. To further document the interaction we are currently optimizing Co-IF and PLA for STAT3 and ZEB1. Additionally, we started the cloning of constructs for the expression of partial proteins *in vitro* as well as *in vivo* in cancer cell lines to map the interaction domains.

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Invited lectures

International Symposium Translational Medicine of the IZKF Erlangen Jun. 16. 2016, Kloster Banz, Germany, Cellular plasticity: a driving force in cancer and other diseases

Seminarreihe “De Facto Onkologie”, Jun. 08. 2016, Universitätsklinikum Schleswig-Holstein, Kiel, Germany, Cellular plasticity in cancer: driving force and therapeutic target

Seminarreihe FOR2127, Jul. 07. 2016, Universität Regensburg, Germany, Cellular plasticity in cancer: driving force and therapeutic target

Seminar Klinikum Rechts der Isar TU München, Jul. 19. 2016, TU München, Germany, Cellular plasticity in cancer: driving force and therapeutic target

Joint Congress DGTI & DGI, Sep. 9. 2016, Nürnberg, Germany, Cellular plasticity in cancer: driving force and therapeutic target

9th International Heinrich F.C. Behr-Symposium on Stem Cells and Cancer, Sep. 20. 2016, DKFZ Heidelberg, Germany, Cellular plasticity in cancer: driving force and therapeutic target

26. Deutscher Hautkrebskongress, Sep. 22. 2016, Dresden, Germany, Cellular plasticity in cancer: driving force and therapeutic target

3. DGP Nachwuchsakademie Kloster Johannisberg, Oct. 18. 2016, Geisenheim, Germany, Cellular plasticity in cancer: driving force and therapeutic target

Conference on EMT, stemness and metastasis, Oct. 25. 2016, Cancer Research Center, School of Medicine, Zhejiang University, Hangzhou, China, Cellular plasticity in cancer: driving force and therapeutic target

11th UK Cancer Stem Cell Symposium, Nov. 17. 2016, Barts and the London School of Medicine and Dentistry, London, United Kingdom, Cellular plasticity in cancer: driving force and therapeutic target

DKTK/WTZ Onkologisches Seminar, Dec. 12. 2016, Universitätsklinikum Essen, Germany, Cellular plasticity in cancer: driving force and therapeutic target

Publications during funding period

none

D26 - Progress Report

01.01.2016 - 30.06.2018

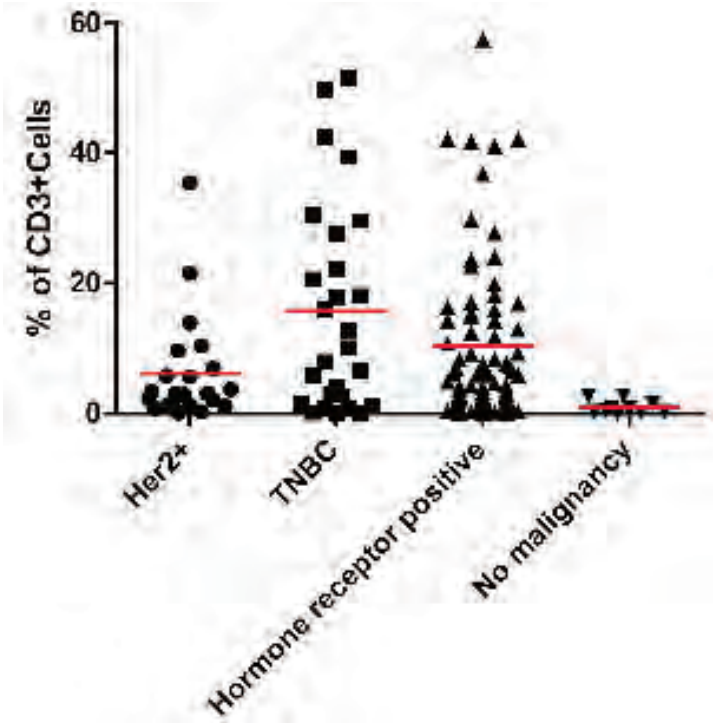
Identification of antigen specificity of tumor-infiltrating lymphocytes in triple-negative breast cancer

Prof. Dr. Andreas Mackensen, Department of Medicine 5 – Haematology and Oncology,
Prof. Dr. Peter A. Fasching, Department of Obstetrics and Gynecology

Breast cancer is the most common malignancy in women. About 15-20% of breast cancers do not express hormone receptors or HER2 [triple-negative breast cancer; TNBC]. TNBC mainly affects younger women and is difficult to treat. The density of immune cell infiltrates in the tumor correlates with clinical outcome. However, it is so far unknown which antigens are targeted by the tumor infiltrating T-lymphocytes. The aim of this project is to identify the targets of tumor infiltrating T-cells in TNBC.

Breast cancer is the most common tumor in women with an annual incidence of 75,000 women in Germany. The tumor is classified based on expression of hormone receptors (estrogen or progesteron-receptor) and HER2. About 15-20% of breast cancers do neither express HER2 nor hormone receptors [triple-negative breast cancer (TNBC)]. This entity is biologically more aggressive and is predominantly diagnosed in young women. The lack of surface expression of hormone receptors or HER2 has an additional negative impact on therapeutic options. Interestingly, several studies have shown that the density of the T-lymphocyte infiltrate in the primary tumor has a strong positive prognostic value in TNBC. These data indicate that TNBC is an immunogenic tumor and that T-cell based immunotherapy could be a promising therapeutic approach. To allow highly potent cellular immunotherapy, it would be desirable to identify tumor-specific antigens as e.g. in tumor specific mutations.

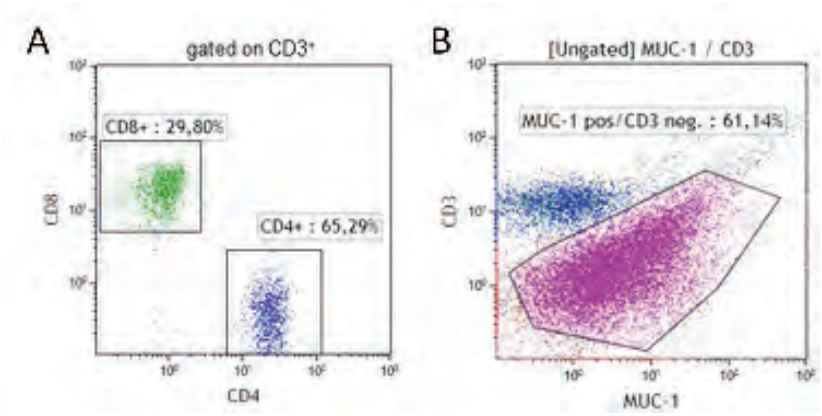
We therefore aimed to identify the targets of tumor-infiltrating T-cells in TNBC with special emphasis being placed on tumor-specific mutations.



Percentage of infiltrating T-lymphocytes in biopsies of TNBC, Her2+ tumors and hormone receptor positive tumors as well as from no malignant breast tissues.


Prof. Dr. Mackensen


Prof. Dr. Fasching



Ex vivo flowcytometric analysis of infiltrating lymphocytes. (A) Percentage of CD4 and CD8 T-cells within the CD3+ fraction. (B) CD3+ T-cells and MUC-1 positive epithelial cells.

Characterization of tumor-infiltrating immune cells

We so far collected and analyzed the infiltrating immune cells of 196 breast biopsies. 30 (15%) were derived from patients with initial diagnosis of TNBC, while 22 (11%) were Her2+ tumors, 102 (52%) hormone-receptor positive and 42 (21%) were derived from non-malignant lesions. Patients diagnosed with TNBC had an average age of 55.7 years as compared to 61.4y for Her2+ tumors and 60.7y for patients with hormone receptor positive tumors. Biopsies from TNBC contained an average of 17% T-lymphocytes as compared to 12% in hormone-receptor positive tumors and 5% in Her2+ tumors.

Expansion of tumor-infiltrating T-lymphocytes

T-lymphocytes in biopsies derived from TNBC were expanded in vitro to average cell number of around 50-60 million T-lymphocytes within 2-3 weeks.

Whole genome sequencing of tumor and reference DNA

So far whole genome sequencing has been performed for reference and tumor DNA of 2 patients. Bioinformatics revealed 32 and 78 coding, missense mutations in these two patients, respectively.

To link these tumor specific mutations to the infiltrating immune cells, we are currently analyzing whether the tumor-derived T-cells are directed against one or more of these neoantigens.

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Publications during funding period

none

D27 - Progress Report

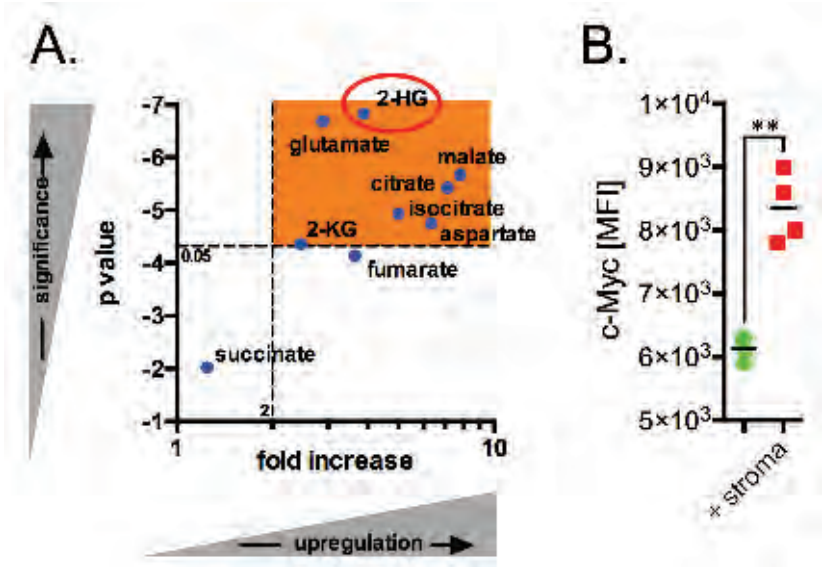
01.07.2016 - 31.12.2018

2-Hydroxyglutarate in Acute Myeloid Leukaemia: Novel Molecular Targets and Impact on Immune Escape

Prof. Dr. Dimitrios Mougiakakos, Department of Medicine 5 – Haematology and Oncology

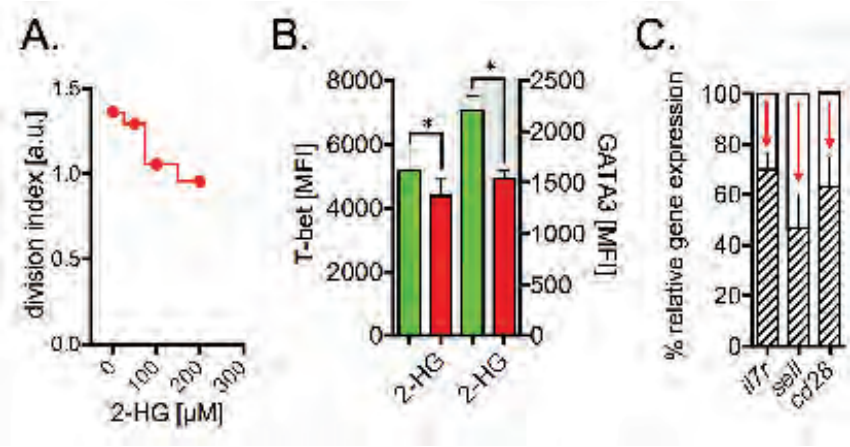
Increased 2-hydroxyglutarate (2-HG) levels are found in 15% of acute myeloid leukaemia (AML) patients. 2-HG overproduction is attributed to mutations in isocitrate dehydrogenase 1/2 (IDH1/2). Our data indicates a link between increased 2-HG levels and c-Myc pathway. AML patients display substantial immune defects. Several tumor-derived metabolites hamper immune responses. The impact of 2-HG remains unexplored. Our aim is to investigate the impact of 2-HG on immune responses and to identify targetable pathways contributing to its production.

Metabolic alterations in the tumor microenvironment can impact the malignant potential of cancer cells by promoting amongst others invasiveness and chemo-resistance. At the same time increasing evidence suggests a negative effect on the anti-tumor immune response. Our main goal is to specify those metabolic processes in order to (A) achieve cancer cell eradication by so-called metabolic targeting and to (B) bolster anti-tumor immune responses by metabolic reprogramming of cancer and immune cells. Increasing evidence suggests that 2-HG might act as an "onco-metabolite" by driving the cancer cells' malignant phenotype, which would be in concordance with its negative prognostic impact. 2-HG competitively inhibits α-KG binding to histone demethylases thereby leading to changes of the epigenetic profile. Furthermore, 2-HG might also promote a HIF-1α-orchestrated "pseudohypoxic" response, which could at least in part be attributed to allosteric inhibition of HIF prolyl hydroxylases. Such increased HIF-1α stability could explain the recently observed glycolytic switch in cells carrying mutated IDH. Metabolic alterations found in malignant cells can provide growth advantages or even therapeutic resistance. This makes them promising therapeutic targets as highlighted by a novel class of drugs against mutated IDH2 (AG-221, Agios Pharmaceuticals). However, metabolic reprogramming can render cells more susceptible towards interferences within their metabolic framework. In this context a current study shows how accumulating 2-HG impacts the mitochondrial metabolism and thereby leads to increased BCL-2 dependency, which could be therapeutically exploited in IDH1/2 mutated AML cells.



HG production linked to c-Myc expression. AML-cells were FACS-sorted and cultured alone or on a stromal cell monolayer for 72 hours. (A) A semi-quantitative metabolite analysis was performed in cell lysates (n=6) using a GC/MS and LC/MS approach. Cells cultured alone are set as 1. (B) The c-Myc MFI was evaluated by FACS. Abbreviations: *, p>0.05; **, p>0.01; ***, p>0.01.

Prof. Dr. Mougiakakos



2-HG suppresses T cell responses. T cells were stimulated +/- 2-HG for 72 hours. (A) Proliferation was measured by FACS (n=3). (B) Transcription factors T-bet (T_H1) and GATA3 (T_H2) was assessed in $CD4^+$ T cells by FACS (n=3). (C) Relative expression of genes linked to memory formation in T cells was measured using quantitative PCR (n=3). Expression in untreated cells is set as 100%. Abbreviations: *, p>0.05.

Increasing evidence shows that "metabolic communication" between tumor cells and the immune system contributes to tumor immune escape further harnessing malignant capabilities. Metabolic derangement in cancer cells can lead to metabolic antagonism (by e.g. tryptophan, depletion) and to production of immune modulating metabolites (such as lactic acid). To date, various immune defects have been identified in AML involving effector (T and NK cells) as well as suppressive (regulatory T cells/T_{Regs} and myeloid derived suppressor cells/MDSCs) cell subsets. The role of abundantly produced 2-HG on shaping immune responses remains to be investigated.

Our preliminary data on AML cells indicates that increased 2-HG production can be linked to c-Myc activation, which is novel for AML. This data is further corroborated by an increased HIF-1α stability that has been previously associated with elevated 2-HG levels harnessing "pseudohypoxic" responses by driving amongst others aerobic glycolysis. Furthermore, we notice a previously unknown 2-HG-mediated suppression of T cell activation, of T_H1/T_H2 differentiation, and of memory formation. At the same suppressive T_{Reg}-subsets accumulate by as yet not elucidated mechanism in presence of 2-HG.

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Invited lectures

GvH/GvL Meeting, 16.03.2016, Regensburg, Influence of oxidative stress on GvL.

Immunotherapy of Cancer Conference 3, 21.03.2016, Munich, Modulating cancer cell metabolism as a strategy to treat hematologic malignancies.

European Hematology Association Congress, 10.06.2016, Copenhagen, The importance of translational research for progress in hematology.

International Symposium on the Clinical Advances of Gene Therapy, 23.09.2016, Thessaloniki, Immune Regulatory Cells in GvHD.

Awards

Else Kröner Exzellenzstipendium 2016

Publications during funding period

Qorraj M, Bruns H, Böttcher M, Weigand L, Saul D, Mackensen A, Jitschin R, Mougiakakos D (2016) The PD-1/PD-L1 axis contributes to immune metabolic dysfunctions of monocytes in chronic lymphocytic leukemia. Leukemia [Epub ahead of print]

Braun M, Qorraj M, Büttner M, Klein FA, Saul D, Aigner M, Huber W, Mackensen A, Jitschin R, Mougiakakos D (2016) CXCL12 promotes glycolytic reprogramming in acute myeloid leukemia cells via the CXCR4/mTOR Axis. Leukemia 30: 1788-92

Böttcher M, Hofmann AD, Bruns H, Haibach M, Loschinski R, Saul D, Mackensen A, Le Blanc K, Jitschin R, Mougiakakos D (2016) Mesenchymal Stromal Cells Disrupt mTOR-Signaling and Aerobic Glycolysis During T-cell Activation. Stem Cells 34: 516-21

D28 - Progress Report

01.02.2016 - 31.07.2018

SPARCL1 function in vessel maturation and metastasis of colorectal carcinoma

Prof. Dr. Michael Stürzl, PD Dr. Elisabeth Naschberger, Department of Surgery

We demonstrated tumor microenvironment (TME)-dependent heterogeneity of tumor endothelial cells in colorectal carcinoma (CRC) and obtained evidence that SPARCL1 acts as a TME-dependent endothelial cell-secreted antagonist of tumor growth and regulatory molecule of blood vessel homeostasis. The project investigates function and underlying mechanisms of SPARCL1 in both processes. Long-term objective is the development of a SPARCL1-based treatment approach to suppress metastases in CRC patients.

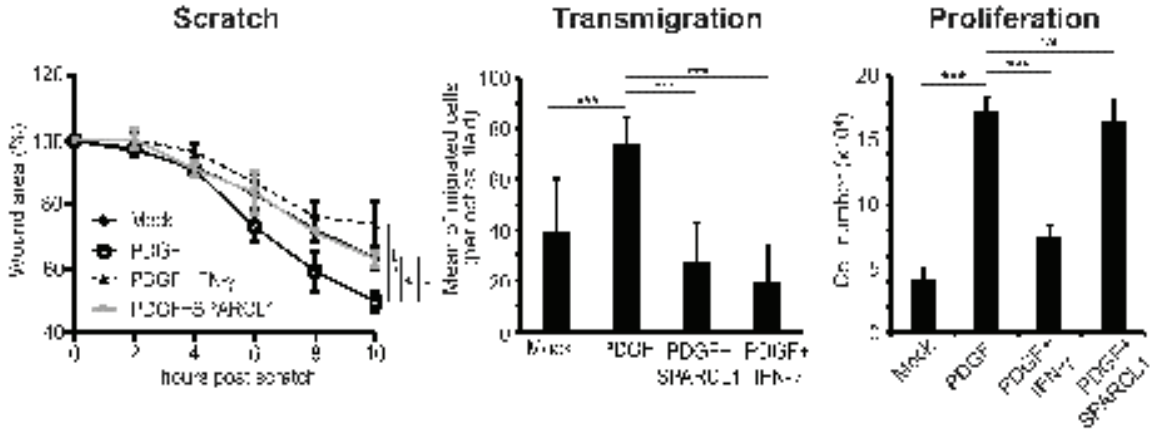
Specific aims of the project are to determine the impact of SPARCL1 on: (1) vessel maturation, (2) physiological and pathological angiogenesis in mouse models and (3) clinical prognosis of CRC patients.

Ad aim 1: SPARCL1 and vessel maturation

In order to investigate the impact of SPARCL1 on vessel maturation, smooth muscle cells (SMCs) as a model for mural cells were treated with recombinant human SPARCL1. We could demonstrate that SPARCL1 inhibited PDGF-induced migration and transmigration of SMCs but not their proliferation. In contrast, SPARCL1 inhibited bFGF-induced migration, transmigration and proliferation of endothelial cells (previous results). These results indicated that SPARCL1 does not directly activate vessel maturation but instead blocks the activity of resident mural cells which leads to vessel stabilization.

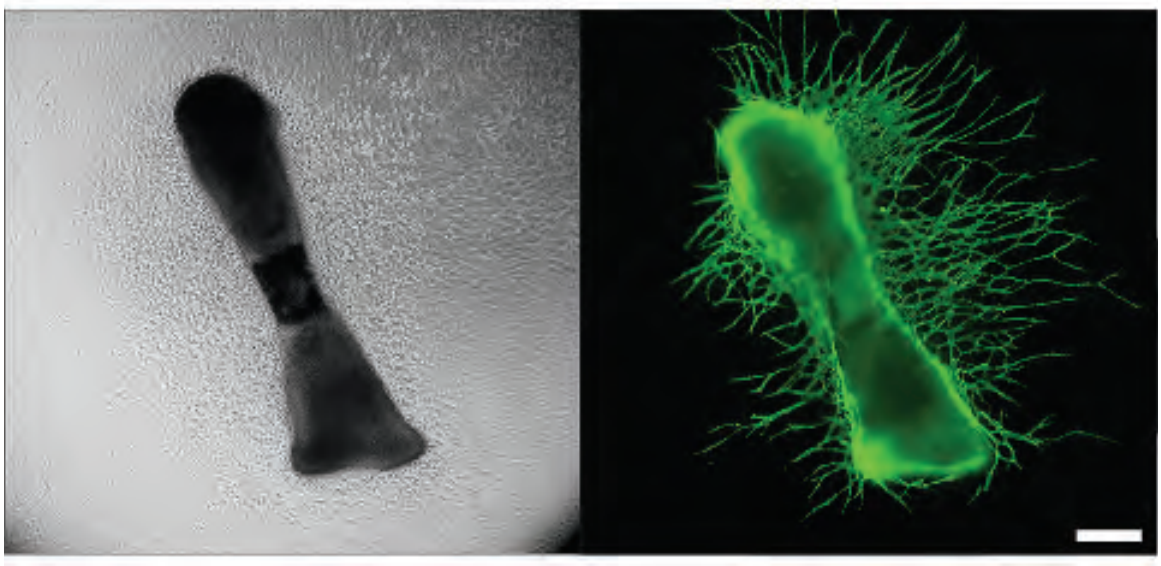
Ad aim 2: Establishment of metatarsal angiogenesis assay in mice

In order to analyze the impact of SPARCL1 on angiogenesis/vessel maturation *in vivo* the metatarsal angiogenesis assay (cooperation Ramming/Wohlfahrt, Med3) has been successfully established. In brief, metatarsal bones from embryos of wild type SPARCL1 animals were explanted at E18.5 and cultivated under conditions allowing outgrowth of endothelial sprouts with a supporting feeder layer. The sprouts were demonstrated to be of endothelial origin by CD31 staining. At present, the quantification of sprouting activity is established. This test will be used in order to comparatively analyze the angiogenic activity of wild type and SPARCL1 knockout mice.



Recombinant SPARCL1 inhibits migration and transmigration but not proliferation of smooth muscle cells (SMCs). Migration, transmigration and proliferation of primary human SMCs after treatment with human recombinant SPARCL1 (1.5 µg/ml). Naschberger et al., JCI 2016

Prof. Dr. Stürzl PD Dr. Naschberger



Endothelial sprouts outgrowth from metatarsal bones of wild type SPARCL1 mice. Metatarsal bones were explanted from embryos (E18.5) of wild type SPARCL1 mice and cultivated in order to foster sprouting outgrowth. Endothelial sprouts were stained using CD31 immunofluorescence.

Ad aim 3: SPARCL1 and clinical prognosis

SPARCL1 expression was determined in FFPE-extracted RNA of CRC patient samples (n=614) recruited in the Polyprobe study. At present, implementation of the corresponding clinical data in adequate software tools (TranSMART, cooperation MIK) in order to analyze potential clinical correlations is carried out. Preliminary analyses showed a trend of improved disease-free survival of patients with high SPARCL1 expression. However, as yet the follow-up time of the patients is still limited.

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Publications during funding period

Naschberger E, Liebl A, Schellerer VS, Schütz M, Britzen-Laurent N, Kölbel P, Schaal U, Haep L, Regensburger D, Wittmann T, Klein-Hitpass L, Rau TT, Dietel B, Méniel VS, Clarke AR, Merkel S, Croner RS, Hohenberger W, Stürzl M (2016) Matricellular protein SPARCL1 regulates tumor microenvironment-dependent endothelial cell heterogeneity in colorectal carcinoma. J Clin Invest. 126(11): 4187-4204*

*highlighted in: Thomas, H.: CRC endothelial regulation. Nature Reviews Gastroenterology & Hepatology (2016) doi:10.1038/nrgastro.2016.180

Britzen-Laurent N, Herrmann C, Naschberger E, Croner RS, Stürzl M (2016) Pathophysiological role of guanylate-binding proteins in gastrointestinal diseases. World J Gastroenterol 22(28): 6434-43

Croner RS, Sevim M, Metodiev MV, Jo P, Ghadimi M, Schellerer V, Brunner M, Geppert C, Rau T, Stürzl M, Naschberger E, Matzel KE, Hohenberger W, Lottspeich F, Kellermann J (2016) Identification of Predictive Markers for Response to Neoadjuvant Chemoradiation in Rectal Carcinomas by Proteomic Isotope Coded Protein Label (ICPL) Analysis. Int J Mol Sci 17(2).pii: E209

D29 - Progress Report

01.01.2016 - 30.06.2018

Aging and senescence of the adaptive immune system in colorectal cancer

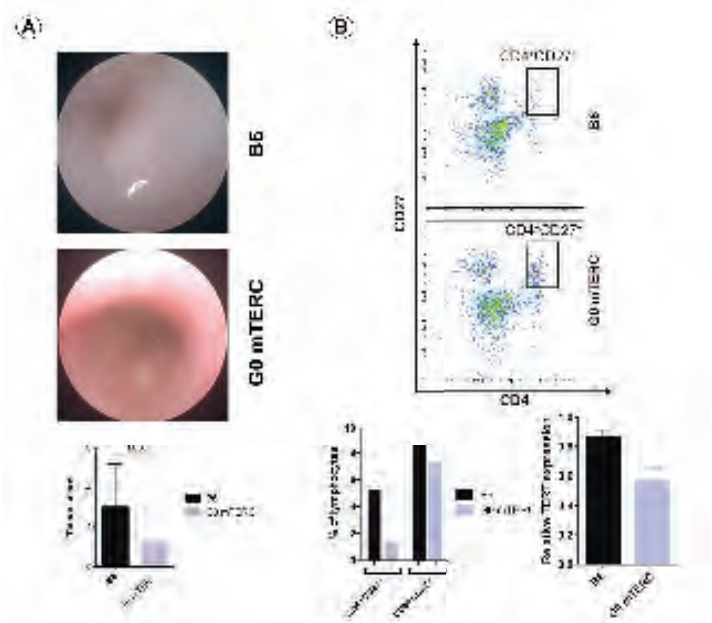
Prof. Dr. Maximilian Waldner, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

There is an increasing incidence of colorectal cancer (CRC) during lifetime. This has been attributed to an accumulation of oncogenic mutations. Recent data also propose a role for an aged-immune system for CRC development. For instance, a short leukocyte telomere length has been associated with poor prognosis in CRC. However, the functional role of an aged immune system in cancer development has not been evaluated. In this project, we analyze the role of an aged immune-phenotype in CRC.

The process of aging is widely described as progressive functional loss at the cellular level that occurs during time. Pathologies such as osteoporosis or hearth failure were described as age-related degenerations, mainly due to an age-related loss of tissue function and maintenance. Furthermore, the incidence of many cancer types, such as colorectal cancer (CRC), is known to increase with age. Besides well-known effects of aging on tumor cell precursors such as the accumulation of mutations, recent data also propose a role for the immune system in the pathology of age-related diseases. For instance, indirect signs of an aging phenotype such as short telomeres in immune cells have been correlated with a worse prognosis in various types of cancer such as CRC or hepatocellular cancer.

What are the characteristics of an aged immune system?

Several studies show a decline of immune function such as low vaccination efficiency and decreased resistance to infections in elderly. This has been attributed to age-related alterations of the adaptive immune system. For instance, aged T cells have short telomeres (protective (TTAGGG)n repeats at the end of chromosomes), lack of telomerase activity (an enzyme defined by telomerase reverse transcriptase TERT and a telomerase RNA component TERC that elongates telomeres) and loss of important costimulatory molecules such as CD27 and CD28. These changes of the T cell phenotype could have dramatic effects on the anti-tumor immune response. However, there are no functional data in the literature that show the role of an aged immune system in cancer development.



(A) Endoscopic images and tumor scores 4 weeks after MC38 injection. (B) Expression of CD27 and TERT in CD4+ T cells isolated from MC38-tumors grown in wt and mTERC-/- mice (top and lower left panel: flow cytometry; lower right panel: qPCR).

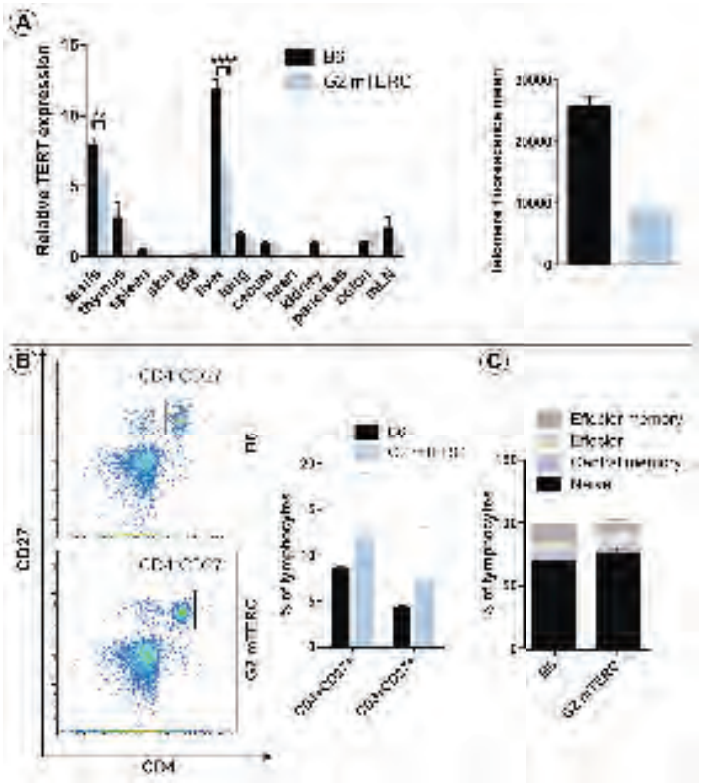
Prof. Dr. Waldner

Evaluation of an aging-phenotype with the mTERC-/- mouse model in CRC

To evaluate the role of an aged immune system on CRC development, we utilize mTERC-/- mice. These mice are lacking the telomerase RNA component and therefore cannot maintain telomere length. However, since the telomeres are not getting short enough for age-related effects during the life span of a single mouse (G0), an aging-phenotype will be evident only after crossing several generations of mTERC-/- mice (G2-G4). Accordingly, G0 mTERC-/- in comparison to wild type mice did not show any difference regarding tumor development in the orthotopic MC38 model of CRC. However, fewer tumor infiltrating CD4+CD27+ and CD8+CD27+ T cells were observed in G0 mTERC-/- mice.

Effect of shortened telomeres in G2 mTERC-/- mice

In comparison to wild type mice, G2 mTERC-/- mice had dramatically shortened telomere lengths in lymphocytes from blood, bone marrow, mesenteric lymph nodes, spleen and thymus under physiological conditions, as shown by telomere fluorescence mean. Moreover, TERT gene expression was downregulated in most organs such as liver, testis etc. When we compared several subsets of T cells, such as naïve, effector, central memory, and effector memory or CD4+CD27+ and CD8+CD27+ T cells from spleen, no statistically relevant differences between G2 mTERC-/- and wild type mice were observed under physiological conditions.



TERT gene expression in different organs and telomere fluorescence mean of splenocytes from B6 and G2 mTERC-/- mice. (B) Expression of CD27 in CD4+ and CD8+ T cells and (C) percentage of CD4+ T cells subsets in G2 mTERC-/- and wt mice.

Next steps will involve the evaluation of G2 mTERC-/- mice in the orthotopic MC38 CRC model in comparison to G0 mTERC-/- and wild type mice. This will allow us the evaluation of an effect of progressive telomere shorting on the immune response against CRC.

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Invited lectures

Seminar des Instituts für Medizinische Mikrobiologie und Hygiene, Universität Regensburg, 14.04.2016, Regensburg, „Hypoxia-dependent signaling pathways in colitis-associated cancer“

MSOT Symposium, 06.09.2016, Memorial Sloan Kettering Cancer Center, New York, „MSOT for the non-invasive evaluation of intestinal inflammation in Crohn's disease“

Publications during funding period

Waldner MJ, Knieling F, Egger C, Morscher S, Claussen J, Vetter M, Kielisch C, Fischer S, Pfeifer L, Hagel A, Goertz RS, Wildner D, Atreya R, Strobel D, Neurath MF (2016) Multispectral Optoacoustic Tomography in Crohn's Disease: Noninvasive Imaging of Disease Activity. Gastroenterology 151: 238-240

E11 - Final Report

01.12.2013 - 30.11.2016

H50Q aSyn mutation in PD

Prof. Dr. Jochen Klucken, Department of Molecular Neurology
PD Dr. Wei Xiang, Institute of Biochemistry

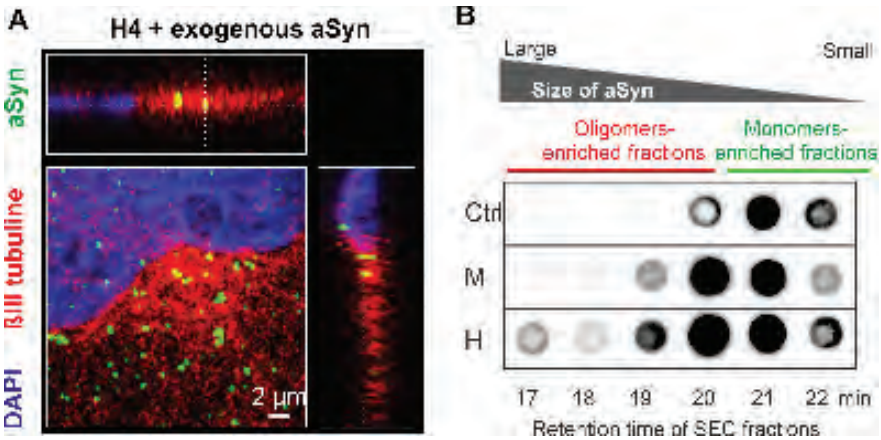
The novel Parkinson's disease (PD)-linked H50Q-mutation of alpha-synuclein (aSyn) supports the pivotal role of histidine 50 (H50) of aSyn in PD-related neurodegeneration. While mutations of aSyn are linked to familial PD, oxidative stress (OS)-mediated posttranslational modifications (PTM) of aSyn may contribute to sporadic PD. Here, we addressed the pathological relevance of H50 by investigating the impact of both H50 mutation and PTM on aggregation, toxicity, and propagation of aSyn.

H50 alterations increase the aggregation and cytotoxicity of aSyn

To understand the role of H50, we modified H50 of aSyn by genetic and posttranslational modifications and analyzed the effects induced by these alterations in a neural cell culture model. We generated aSyn H50Q mutant, because of its relevance to familial PD patients. In addition, we produced modified aSyn using 4-Hydroxy-2-Nonenal (HNE), a reactive lipid peroxidation product. We focused on HNE-modification of aSyn, because HNE levels in brains of sporadic PD patients are elevated and H50 is the major target of HNE in aSyn. We revealed that both genetic mutation and HNE-PTM of H50 promote the aggregation of aSyn. Specifically, HNE modification of H50 increases oligomerization, whereas the H50Q mutation triggers oligomerization and fibrillization of aSyn. In line with these *in vitro* findings, overexpressing aSyn H50Q mutant led to an increase in aSyn oligomerization within cells. Consistently, overexpression of H50Q mutant promoted apoptotic cell death, which was even more pronounced under increased OS levels. Substitution of H50 attenuated HNE-induced cell death, indicating that HNE modification of aSyn-H50 is crucial for HNE-mediated cytotoxicity. Thus, both mutation and HNE-modification of H50 trigger aSyn pathology, supporting an essential role of H50 alterations in aSyn-mediated neurodegeneration in PD.

HNE modification of H50 promotes the propagation of aSyn pathology

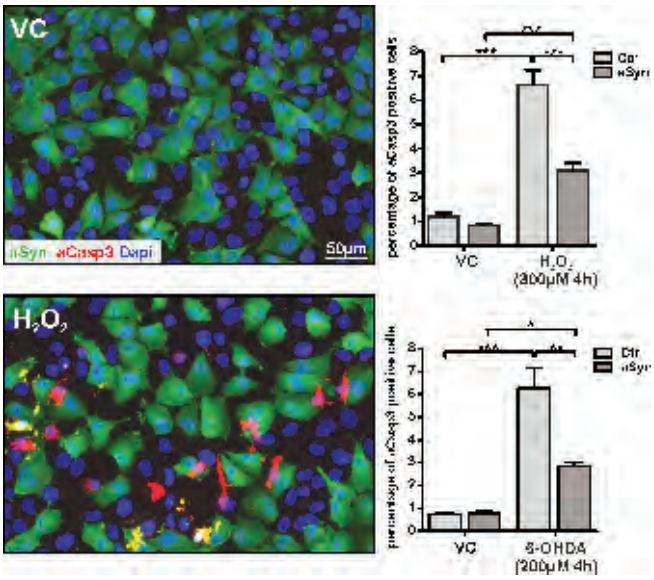
Recently, cell-to-cell transmission of aSyn pathology was suggested to be an important mechanism underlying the spread of PD-related pathology. Thus, we studied the impact of H50 modification on the propagation of aSyn pathology. We revealed that HNE, which preferentially modifies H50 of aSyn, increases the secretion of aSyn. When compared to unmodified aSyn, modified aSyn can be more efficiently taken up by recipient cells, is more resistant to degradation by impairing lysosomal activity, and is capable of inducing the aggregation of endogenous aSyn in recipient cells. Moreover, modified aSyn trig-



Uptake of extracellular aSyn by H4 neuroglioma cells (A). Size exclusion chromatographic (SEC) analysis of H4 cells exposed to monomeric (M) and HNE-modified (H) aSyn. Modified aSyn increases aggregation of intracellular aSyn (B).

Prof. Dr. Klucken

PD Dr. Xiang



Mixed cultures of control and aSyn overexpressing H4 neuroglioma cells were exposed to H_2O_2 or 6-OHDA and the activation of Caspase3 (aCasp3) was analyzed by immunofluorescence staining and flow cytometry.

gers a selective loss of dopaminergic neurons. Our data supports a role of aSyn H50 in the propagation of aSyn pathology under elevated levels of HNE, a PD-associated condition.

aSyn plays a role in OS-induced mitochondrial dysfunction

The findings that the toxic effect of the H50Q mutation is reinforced under OS and that mitochondria are major targets of OS motivated us to assess the impact of aSyn on the mitochondrial response to OS. We revealed a dichotomic role of aSyn in mitochondrial biology, which is linked to distinct types of stress-induced mitochondrial fragmentation. Specifically, aSyn appears to be part of a cellular defense mechanism preserving mitochondrial homeostasis in the presence of increased OS levels, while not protecting against stressors directly affecting mitochondrial function. In future studies, we will study the relevance of H50 for the function of aSyn in mitochondrial homeostasis.

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Invited lectures

Heat-Net Symposium, 20.05.16, Barcelona – “alpha-Synuclein Pathology”

Neuroscience Winterschool, 03.04.16, Sölden – “Extra- intracellular alpha Synuclein Pathology”

Publications during funding period

Chen YJ, Xiang W, Klucken J, Vollmer F (2016) Tracking micro-optical resonances for identifying and sensing novel procaspase-3 protein marker released from cellcultures in response to toxins. Nanotechnology. 27(16): 164001

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Hoffmann A, Ettle B, Bruno A, Kulinich A, Hoffmann AC, von Wittgenstein J, Winkler J, Xiang W, Schlachetzki JC (2016) Alpha-synuclein activates BV2 microglia dependent on its aggregation state. Biochem Biophys Res Commun. 28;479(4): 881-886

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Ettle B, Reiprich S, Deusser J, Schlachetzki JCM, Xiang W, Prots I, Winner B, Wegner M, Winkler J (2014) Link between intracellular alpha-synuclein level and maturation potential of primary oligodendrocyte progenitor cells. Mol Cell Neurosci, 62: 68-78

Casadei N, Poehler AM, Tomas-Zapico C, Torres-Peraza J, Schwedhelm I, Witz A, Zamolo I, De Heer R, Spruijt B, Noldus LP, Klucken J, Lucas JJ, Kahle PJ, Krüger R, Riess O and Nuber S (2014) Overexpression of synphilin-1 promotes clearance of soluble and misfolded alpha-synuclein without restoring the motor phenotype in aged A30P transgenic mice. Hum Mol Genet, 23: 767-781

Poehler AM, Xiang W, Spitzer P, May VE, Meixner H, Rockenstein E, Chutna O, Outeiro TF, Winkler J, Masliah E and Klucken J (2014) Autophagy modulates SNCA/alpha-synuclein release, thereby generating a hostile microenvironment. Autophagy, 10: 2171-2192

E12 - Progress Report

01.04.2014 - 31.03.2017

Adult hippocampal neurogenesis in synucleinopathies

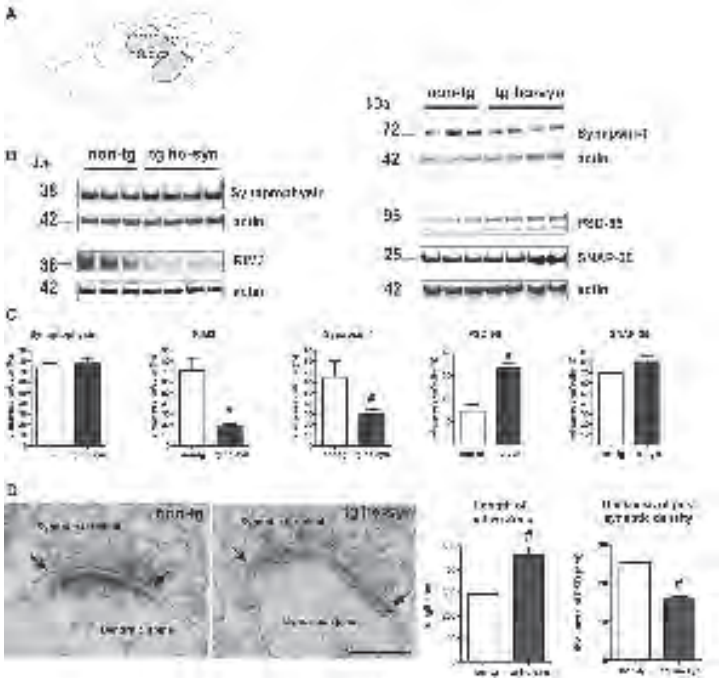
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology
Prof. Dr. Dieter Chichung Lie, Institute of Biochemistry

Non-motor symptoms (NMS) like anxiety and depression play an important role in Parkinson's disease (PD), frequently occurring prior to the onset of motor symptoms. Using a transgenic alpha-synuclein (α-syn) rat model our findings indicate that α-syn severely impairs the hippocampal serotonergic system prior to the onset of motor symptoms resulting in a compromised axonal and synaptic hippocampal circuitry and reduction of hippocampal neurogenesis, which might contribute to early NMS in PD.

The aim of the project is to analyze non-motor neuropsychiatric symptoms (NMS) related to the hippocampus and the generation of new hippocampal neurons in various animal models of synucleinpathies, in particular Parkinson's disease (PD). Moreover, we aim to decipher the molecular pathophysiology of these symptoms to contribute to the identification of new molecular targets for treating these highly debilitating NMS, in particular anxiety and depression.

Analysis of the hippocampal serotonergic system in BAC transgenic α-syn rats and consequences on hippocampal neurogenesis

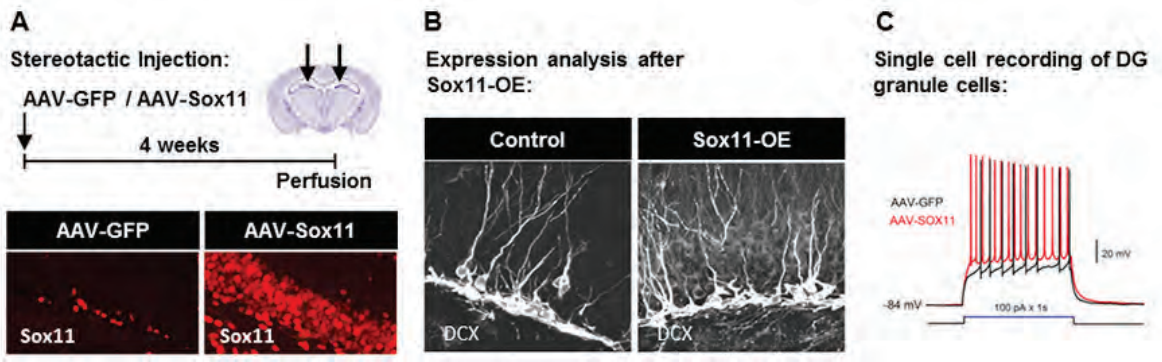
To characterize the early interplay between the hippocampus and the serotonin (5-HT) system, we used a α-syn transgenic rat model which develops key features of PD such as pathological α-syn accumulation and motor deficits at the age of 12 months. Prior to the onset of this phenotype, we observed a severe 5-HT dysfunction in the hippocampus of 4-month-old animals, as detected by reduced input of 5-HT transporter expressing neurites, low 5-HT levels, and altered 5-HT receptor expression in the dentate gyrus (DG)/CA3 subfield of the hippocampus. As a consequence, this model shows a severe impairment of hippocampal neurogenesis, namely a profound reduction of neuroblasts and new-born neurons. We further detected a reduced expression of presynaptic proteins in the DG/CA3 subfield of the hippocampus in α-syn overexpressing rats together with an altered structure of synapses at the ultrastructural level, further stressing the findings of a compromised intrahippocampal circuitry in this PD model. Importantly, BAC α-synuclein rats showed an early anxiety-like phenotype consisting of reduced exploratory behavior and feeding.



Reduced expression levels of pre-synaptic proteins in the hippocampal DG/CA3 subfield (A) of α-syn transgenic rats (tg hα-syn; B, C). Significant ultrastructural alterations of hippocampal CA3 synaptic terminals (D). Adapted from Kohl et al., 2016.

Prof. Dr. Winkler

Prof. Dr. Lie



Sox11 overexpression (OE) by stereotactic injection of adeno-associated viral vectors (AAV; A) increases expression of the immature neuronal marker DCX (B) and excitability in DG granule cells, detected by single cell recordings in fresh hippocampal slices from AAV-injected animals.

Function and regulation of the putative antidepressant target and plasticity regulator Sox11

Plasticity of the DG critically modulates mood and anxiety behavior. Data from our laboratory and others revealed that antidepressant treatments such as electroconvulsive shock (ECS) and antidepressants of the Selective Serotonin Reuptake Inhibitor Class (SSRI) strongly increase the expression of the transcription factor Sox11 in hippocampal dentate granule (DG) neurons. We have now firmly established that the transcription factor Sox11 is expressed in DG neurons in a neuronal activity-dependent manner. Moreover, our new electrophysiological and behavioral analyses revealed that Sox11 expression substantially alters DG neuron plasticity and hippocampus-dependent memory consolidation. RNA-Sequencing analysis showed that the expression of neuronal cytoskeleton-associated genes, ion channels, and regulators of pre- and postsynaptic development were changed in DG neurons by Sox11 activity. We are presently analyzing if and how Sox11-dependent plasticity of the DG is altered in ageing and preclinical models for neurodegenerative diseases.

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Invited lectures

Fusion Conference “Neurogenesis”, February 2-6, 2016, Cancun, Mexico

Eurogenesis Conference, July 11-13, 2016, Bordeaux, France

Developmental Biology Seminar Series, University of Basel, July 26, Basel, Switzerland

Annual Symposium of the Zurich Neuroscience Center (ZNZ), September 15, Zurich, Switzerland

Publications during funding period

Kohl Z, Ben Abdallah N, Vogelgsang J, Tischer L, Deußer J, Amato D, Anderson S, Müller CP, Riess O, Masliah E, Nuber S, Winkler J (2016) Severely impaired hippocampal neurogenesis associates with an early serotonergic deficit in a BAC α-synuclein transgenic rat model of Parkinson’s disease. Neurobiology of Disease 85: 206–217

Salvi R, Steigleder T, Schlachetzki JCM, Waldmann E, Winner B, Schwab S, Winkler J, Kohl Z (2016) Distinct effects of chronic dopaminergic stimulation on hippocampal and striatal neurogenesis in adult mice. Front Neurosci 10:77

E13 - Progress Report

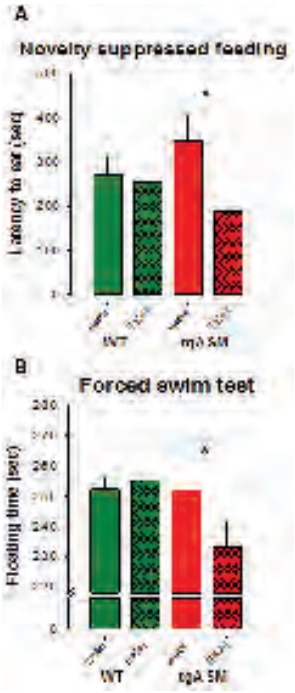
01.04.2014 - 31.03.2017

The role of acid sphingomyelinase in depression/anxiety-induced alcohol addiction

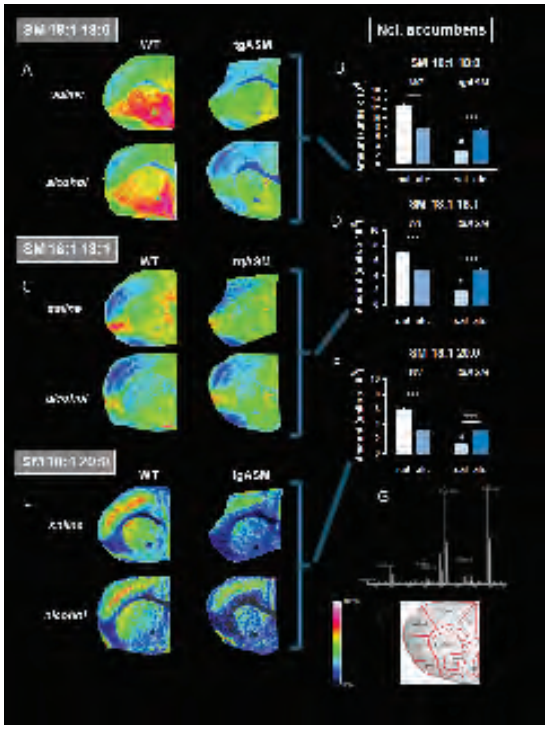
Prof. Dr. Christian P. Müller, Department of Psychiatry and Psychotherapy
PD Dr. Martin Reichel, Department of Psychiatry and Psychotherapy (till 30.09.2016),
Department of Medicine 4 – Nephrology and Hypertension (since 01.10.2016)
Prof. Dr. Johannes Kornhuber, Department of Psychiatry and Psychotherapy

Depression and anxiety are common causes for the establishment of alcohol addiction, a devastating psychiatric disorder. Based on a dysfunction of the acid sphingomyelinase/ceramide pathway, which is associated with depression/anxiety, we will investigate in a translational approach how alcohol addiction and related neuronal adaptations are established. The identified mechanism may then provide a new target for a personalized treatment of alcohol addiction comorbid with depression/anxiety.

In a previous study we found that mice with a transgenic over-expression of acid sphingomyelinase (tgASM) show enhanced ceramide levels in the hippocampus and anxiety and depression-related behaviour. They also drink more alcohol than wild type (WT) controls. Now we asked whether the enhanced alcohol consumption is motivated by drug-instrumentalization in that the drug is consumed to reverse an aversive emotional state, i.e. do animals drink more to reduce their anxiety/depression levels? We tested tgASM and WT mice in a two-bottle free choice alcohol drinking paradigm and found that free-choice alcohol drinking reduced depression-like behaviour in tgASM animals in a series of depression tests. Free-choice drinking reversed the genetically-induced increase in brain ASM activity selectively in the depressed tgASM mice, but not in WT animals. In a second study we asked whether the depressive phenotype can be reversed by the pharmacological effects of the alcohol alone, or whether the free-choice and, thus, self-titration, was a crucial element in this action. In this study, animals had no free-choice, but received repeated alcohol-injections (i.p.). When depression/anxiety behaviour was tested afterwards, we found rather opposite effects compared to a free-choice administration. Alcohol enhanced depression-like beha-



Free-choice alcohol drinking has antidepressant effects in mice over-expressing ASM (tgASM), but not in wild type (WT) mice in A. the novelty suppressed feeding test and B. the forced swim test (p<0.05; EtOH-ethanol).



Slice mass spectrograms for most abundant sphingomyelin (SM) species in the brain of water or alcohol drinking tgASM or WT mice. Free-choice alcohol drinking reduces SM levels in WT mice. This effect is partially reversed in tgASM mice. ($^{\#}$p<0.05, vs. WT; ***p<0.001).

Prof. Dr. Müller PD Dr. Reichel Prof. Dr. Kornhuber

viour. Forced alcohol-exposure did not affect ASM activity, neither in tgASM nor in WT mice. We then searched for a brain mechanism that could mediate the potential therapeutic effects of alcohol in the brain of depressed animals within the sphingolipid system. Mass spectrometric analysis of brain slices showed in tgASM mice, several sphingomyelin species largely reduced in the nucleus accumbens (Nac) and hippocampus. Free-choice alcohol drinking reduces the content of sphingomyelin species in the Nac and hippocampus in WT animals. However in tgASM mice, alcohol partially reversed the decline in sphingolipids, which suggests an action towards sphingolipid homeostasis. This effect was Nac specific, and not observed in the dorsal hippocampus. Post mortem neurochemical analysis showed that a similar effect of the free-choice alcohol was found at the level of serotonin and dopamine tissue levels. Furthermore, in-vivo microdialysis showed that response dynamic of dopaminergic transmission was largely enhanced in tgASM mice with a stronger response to an alcohol challenge. These findings suggest the ASM-sphingomyelin/ceramide pathway as a potential mediator of depression-induced alcohol preference, and possibly, addiction, by controlling sphingolipid and monoaminergic homeostasis in specific parts of the brain reward system.

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Invited lectures

Christian P. Müller

23rd Multidisciplinary International Neuroscience and Biological Psychiatry Conference “Stress and Behavior”, 16.05.2016, St Petersburg, Russland, Sphingolipids in stress, memory extinction and depression

2nd Central European Biomedical Congress, 15.06.2016, Krakau, Polen, Sphingolipids and the transition from depression to alcoholism

Johannes Kornhuber

Wissenschaftliches Seminar, Psychiatrische Klinik Freiburg, Universitätsklinikum, 20.04.2016, Freiburg, Sphingolipide bei Depression und Alkoholkrankheit

Seminarreihe des SFB 1039: Lipid Signalling, 21.06.2016, Frankfurt, Sphingolipids in psychiatric disorders

3rd International Conference on the Molecular Medicine of Sphingolipids, 21.09.2016, French Lick Resort, USA, Sphingolipids in major depressive disorder

Publications during funding period

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Müller CP, Quednow BB, Lourdusamy A, Kornhuber J, Schumann G, Giese KP (2016) CaM kinases - From memories to addiction. Trends in Pharmacological Sciences 37(2): 153-166

Schöpf I, Easton AC, Solati J, Golub Y, Kornhuber J, Giese KP, Müller CP (2015) αCaMKII autophosphorylation mediates neuronal activation in the hippocampal dentate gyrus after alcohol and cocaine in mice. Neuroscience Letters 591C: 675-68

Süß P, Kalinichenko L, Baum W, Reichel M, Kornhuber J, Ettle B, Distler JHW, Schett G, Winkler J, Müller CP, Schlachetzki JCM (2015) Hippocampal structure and function are maintained despite severe innate peripheral inflammation. Brain Behavior and Immunity 49: 156-170

Gulbins E, Walter S, Becker KA, Halmer R, Liu Y, Müller CP, Fassbender K, Kornhuber J (2015) Ceramide in neurogenesis and major depression. Journal of Neurochemistry 134(2): 183-92

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Müller CP, Reichel M, Mühle C, Rhein C, Gulbins E, Kornhuber J (2015) Brain membrane lipids in major depression and anxiety disorder. Biochimica et Biophysica Acta 1851: 1052-1065

Kornhuber J, Müller CP, Becker KA, Reichel M, Gulbins E (2014) The ceramide system as a novel antidepressant target. Trends in Pharmacological Sciences 35(6): 293-304

E14 - Progress Report

01.04.2014 - 31.03.2017

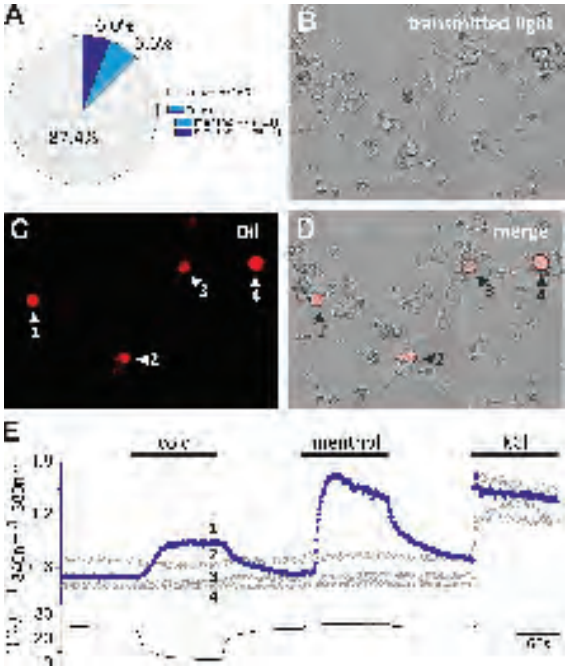
Role of TRPC5 in trigeminal nociception

Prof. Dr. Katharina Zimmermann, Department of Anaesthesiology

Cold hyperalgesia and cold hypersensitivity are common dental problems. To investigate the so far unresolved cold transduction mechanism in teeth we used a retrograde labeling model of dental primary afferent neurons (DPAN) and developed a new ex vivo mouse jaw-nerve preparation. We analyzed cold responses in cultured DPAN and in the pulpal nerve endings and are the first to find a remarkably uniform population of cold-sensitive mouse tooth pulp nociceptors that partly depends on TRPC5.

Cold sensitivity of cultured mouse DPAN.

We previously established a retrograde labeling technique of DPAN innervating mouse maxillary molars located in the trigeminal ganglion with NeuroTrace® DiI. We obtained cultured DiI-labeled DPAN from C57BL/6J mice and subjected them to cold stimulation. Cold sensitivity occurs in 13% (n=23 of 183) and 45% of the cold responses coincide with sensitivity to the TRPM8 agonist menthol (n=10 of 22). Only one cold-sensitive DPAN was menthol-insensitive and sensitive to the TRPA1 agonist carvacrol. In accordance with this result, DPANs of TRPM8/A1-deficient mice were reduced to half (n=8 of 132) and menthol- and carvacrol-insensitive. These cold responses are presumably mediated by TRPC5 and we found them to share the response pattern with the cold responses obtained from HEK cells heterologously expressing TRPC5. To test for TRPC5-induced responses we utilized ML204 as recently identified potent and selective antagonist, but in contrast to the recordings from jaw nerve preparations (see below) we found no clear blocking effect of ML204. We are currently identifying the amount of cold-sensitive DPANs in TRPC5-/-, TRPA1-/-, TRPC5/A1-/-, TRPM8/C5-/- and triple knockouts.

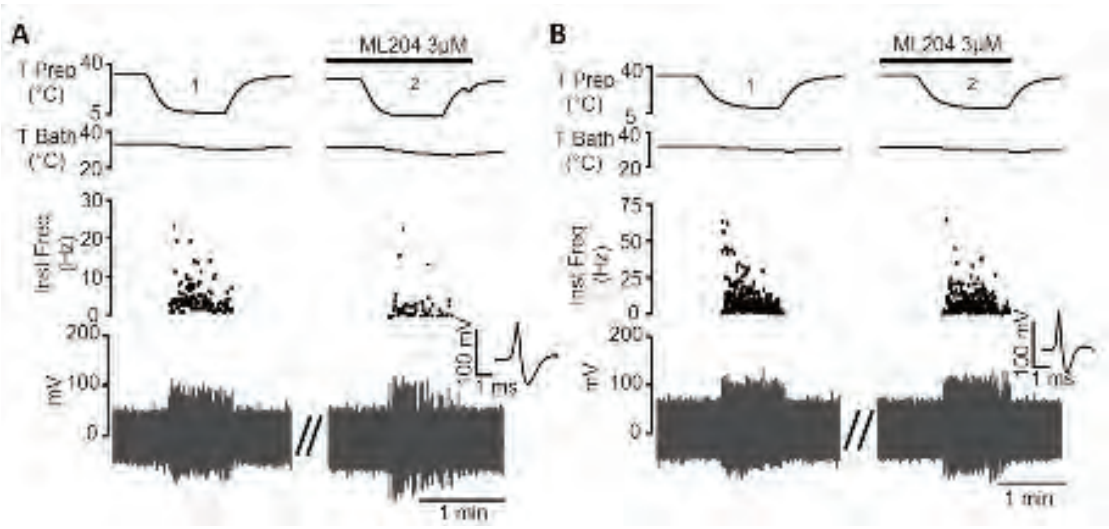


Cold sensitivity of mouse DPAN. (A) DPANs specified according to cold and menthol-sensitivity. B-D. Cultured mouse TG neurons with labeled DPANs. E. depicts individual calcium transients of DPANs measured with the ratiometric calcium dye FURA 2AM.

Prof. Dr. Zimmermann

Cold sensitivity of mouse tooth pulp nerve endings

We transferred the jaw-inferior alveolar nerve preparation from the rat to the mouse. Because the mouse preparation is too small to use the split fiber technique, we established suction electrodes. Cold responses can be recorded from afferents innervating molars and incisor only if the pulp is superfused and exposed to oxygen (incisor needs to be cut and molars to be cracked *post mortem*). Either approach yields the same amount and similar cold responses. Cold stimulation requires placement of the entire preparation in a small tube and the recording nerve to be outside the tube to prevent (reversible) cold block. Although there are only 10% cold-sensitive fibers in the mouse alveolar nerve, the thresholds of activation and response properties are comparable to published properties of cat pulp nociceptors of which 80% are cold sensitive. We quantified the cold responses in knockouts lacking TRPC5, TRPM8, TRPA1, TRPC5/A1 and TRPA1/M8. Briefly, the number of cold sensitive fibers are strongly reduced in TRPC5-, but also in TRPM8/A1- and TRPC5/A1-deficient mice. Reduced number of action potentials in the remaining cold sensitive fibers and reduced thresholds of activation are observed in TRPC5A1-deficient mice. Pharmacological evidence for an involvement of TRPC5 is provided by the TRPC5 antagonist ML204 which blocks cold response in WT and lacks effect in TRPC5-deficient mice. Our results provide a strong argument for a function of TRPC5 as cold transducer in the tooth pulp nociceptors. Our next experiments investigate if TRPC5 is functional as component of a heteromultimer with TRPM8 or TRPA1, if cold responses are altered in TRPM8/C5-/- and if they are absent in triple knockouts.



TRPC5 antagonist ML204 reduces cold responses in C57BL6J (A) but not TRPC5$^{-/-}$ (B) tooth pulp nociceptors. From bottom to top: voltage signal, instantaneous frequency, bath and stimulus temperature. Insets: action potential shape, // 5 min interval.

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Publications during funding period

none

E15 - Final Report

01.11.2013 - 31.10.2016

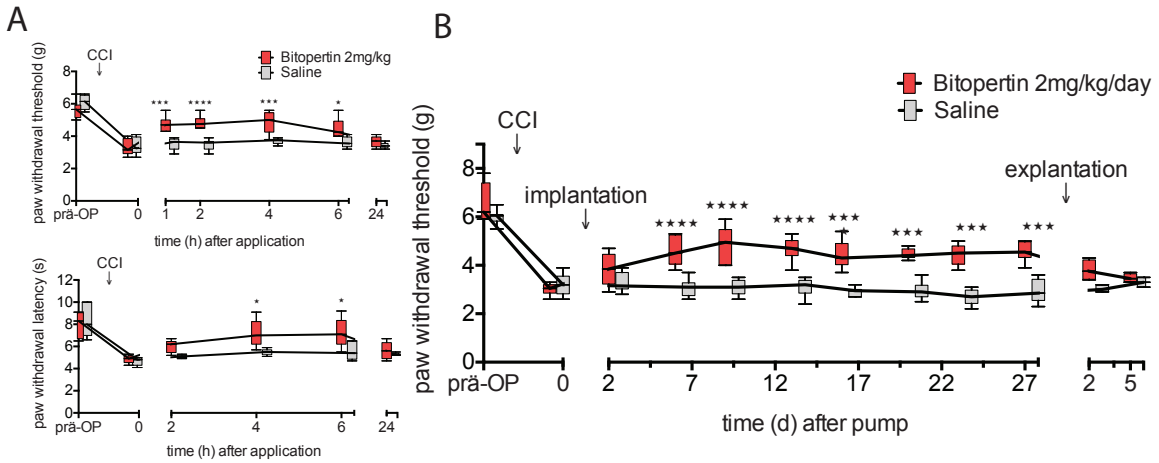
GlyT1 and neuropathic pain

PD Dr. Volker Eulenburg, Institute of Biochemistry
Prof. Dr. Holger Schulze, Department of Otorhinolaryngology – Head and Neck Surgery

Chronic pain conditions are despite intense research clinically challenging and the therapeutic results are in many cases not satisfactory. In this project we have shown that in animal models for chronic pain, artificial substrates and/or inhibitors of GlyT1 are beneficial and ameliorate transiently the facilitated pain response. Electrophysiological recordings in somatosensory cortex and spinal cord revealed that this effect is at least in part elicited at the level of the dorsal horn or below.

Chronic pain causes enormous economic loss and is one of the most debilitating conditions for affected patients. Despite intense research in the last decades, the treatment of chronic pain conditions is still difficult and the results in many cases not satisfactory. In addition to peripheral causes for chronic pain, it was suggested that a dysbalance in the excitation/inhibition ratio within the dorsal horn of spinal cord is at least partially responsible for the maintenance of chronic pain. Therefore new treatment strategies aimed at the reestablishment of a normal inhibitory tone within the dorsal horn.

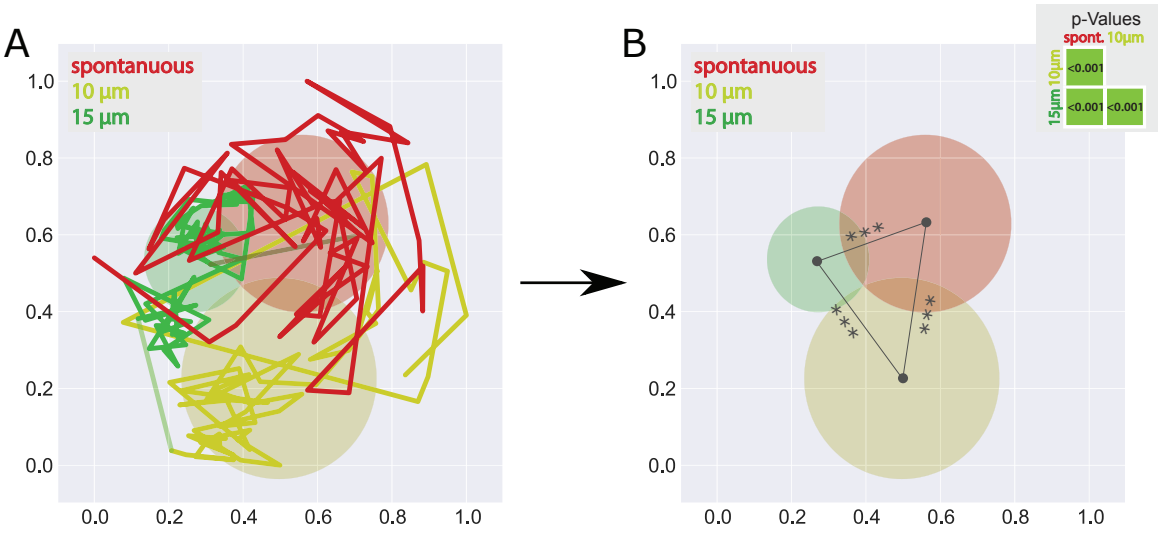
We have shown previously that the lidocaine metabolite N-Ethyl-Glycine (NEG) acts as a substrate for the glycine transporter 1. In the current project we now demonstrate that NEG acts specifically on GlyT1 whereas no NEG effects were observed on other glycine responsive proteins like Glycine receptors or NMDA receptors. We could show that the NEG treatment of rodents causes an increase in the cerebrospinal fluid glycine concentration whereas serum glycine concentrations remained unchanged. Treatment of animals suffering from chronic inflammatory and/or neuropathic pain with NEG resulted in an efficient but transient amelioration of the facilitated pain responses observed in these animals whereas acute pain responses were unaltered. Using in vivo electrophysiological recordings from the fibers projecting from the dorsal horn to supra-spinal areas we could show that the effect observed after NEG treatment is caused at least in part at the level of the dorsal horn or before. Taken together these data suggest that inhibition of the glycine transport activity is a promising strategy for the treatment of chro-



Single injection (A) or continuous application (B) of Bitopertin ameliorates the facilitated pain response induced by chronic constriction of the nervous ishiadicus elicited by mechanical (A, upper panel and B) or thermal stimulation (A lower panel).


PD Dr. Eulenburg


Prof. Dr. Schulze



A: Cortical activation patterns recorded with 16-channel electrode arrays. Visualization by multidimensional scaling (projection onto 2D).
B: Patterns recorded during different stimulation conditions differ significantly as demonstrated by a newly developed permutation test.

nic pain states. These data might provide a novel use for GlyT1 inhibitors like e.g. Bitopertin. Consistently, Bitopertin ameliorated the facilitated pain response in animal models for both inflammatory and neuropathic pain in a dose dependent manner.

Support that GlyT1 function is conserved between humans and rodents and thus might also be a suitable target for the treatment of chronic pain conditions, here, comes from the identification of GlyT1 deficient humans. GlyT1 deficient patients postnatally develop a complex neurological disease phenotype called GlyT1-encephalopathy that includes hypotonia progressing to respiratory depression and premature death in many cases. Thus, major disease aspects of GlyT1 encephalopathy are consistent with rodents treated with GlyT1 inhibitors or with mice carrying GlyT1 deficiency.

To describe the cortical representation of chronic pain, a statistical method for analysing spatio-temporal patterns was developed for in vivo neuronal recordings using a 16 electrode array. The resulting 16 dimensional activation pattern vector can be projected onto 2D by multidimensional scaling for visualization. For statistical evaluation of cortical activation patterns, a novel permutation test has been developed.

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Invited lectures

Pain mechanisms and therapeutics, 3-8.6.2016, Taormina, Italy, V. Eulenburg, „Glycine transport inhibition“

Publications during funding period

Tziridis K, Buerbank S, Eulenburg V, Dlugaiczyk J, Schulze H (2016) Deficit in acoustic signal-in-noise detection in glycine receptor α3 subunit knockout mice. Eur J Neurosci [Epub ahead of print]

Kurolap A*, Armbruster A*, Hershkovitz T, Hauf K, Mory A, Paperna T, Hannappel E, Tal G, Nijem Y, Sella E, Mahajnah M, Ilivitzki A, Hershkovitz D, Ekhilevitch N, Mandel H, Eulenburg V$*, Baris H$* (2016) Loss of glycine transporter 1 causes a subtype of glycine encephalopathy with arthrogryposis and mildly elevated cerebrospinal fluid glycine. Am J Hum Gen 99: 1172-1180

Werdehausen R, Mittnacht S, Bee LA, Minett MS, Armbruster A, Bauer I, Wood JN, Hermanns H, Eulenburg V (2015) The lidocaine metabolite N-ethylglycine has antinociceptive effects in experimental inflammatory and neuropathic pain. Pain 156: 1647-59

* contributed equially; $ shared corresponding author

E16 - Progress Report

01.04.2014 - 31.03.2017

Regulatory networks in neurogenesis and neurodevelopmental disorders

Prof. Dr. Dieter Chichung Lie, Institute of Biochemistry
Prof. Dr. André Reis, Institute of Human Genetics

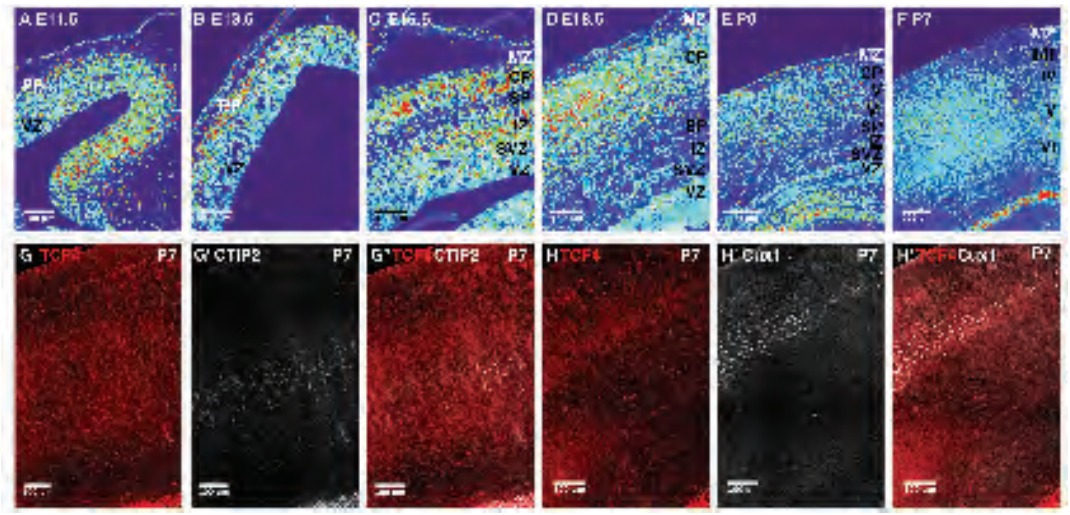
Genetic defects are responsible for the vast majority of intellectual disability (ID) cases in countries with high standard of living. There is evidence that ID-gene encoded proteins are connected in pathways that regulate neurodevelopment and –plasticity. This project aims to identify common pathophysiological pathways in ID and to probe components of such pathways as novel etiological genes in ID.

Functional Studies of Chromatin-Remodeling-Factor ARID1B

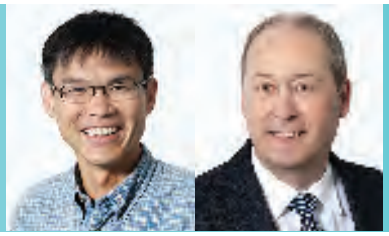
ARID1B encodes the DNA-binding component of the BAF-complex and is the gene mutated in the majority of patients with Coffin-Siris Sydrome (CSS). ARID1B mutations are also observed in ID patients with a more unspecific, broader clinical presentation. Our studies of patients' material and of cellular knock-down models revealed that genes encoding components of migration pathways were consistently deregulated and that cell migration is impaired by ARID1B mutation and loss-of-function. Currently we are establishing patient-derived induced pluripotent stem cells (iPSC) and cellular models with a CRISPR/Cas9-mediated knock-out of ARID1B, to gain deeper insight into the ARID1B-dependent regulation of migration of neural cells.

Search for further ARID1B interacting ID genes

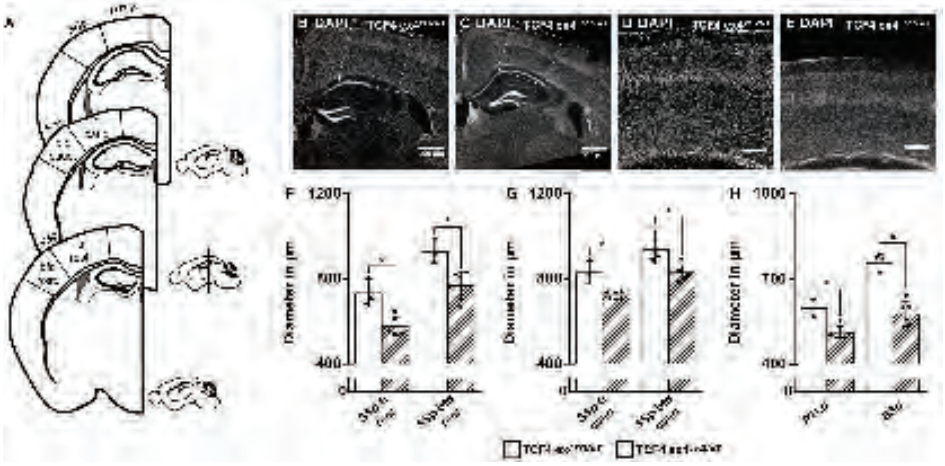
In exome sequencing studies of ID patients with symptoms overlapping with those seen in CSS but lacking a mutation in BAF-complex members we identified mutations in candidate genes. In a series of female ID-patients from laboratories worldwide we identified missense variants of the X-linked gene NAA10, a main component of the complex catalyzing N-terminal acetylation of proteins. In collaboration with a laboratory in Bergen, Norway, we confirmed variable effect of the variants on protein stability and enzymatic activity. These impacted the phenotype in girls, although the situation is further complicated by the variable degree of X-inactivation. Our findings support the concept that X-linked recessive genes can also manifest in girls.



TCF4 expression during corticogenesis
(A-F) Heatmap of TCF4 expression during development. (G-H'') TCF4 co-stainings with layer specific markers. CP, cortical plate. IZ, intermediate zone. MZ, marginal zone. PP, preplate. SP, subplate. SVZ, subventricular zone. VZ, ventricular zone.



Prof. Dr. Lie Prof. Dr. Reis



Cortical thickness is reduced in TCF4$^{+/-}$ mice
(A) Analyzed regions. (B-H) The cortical diameter is decreased in TCF4$^{+/-}$ mice compared to TCF4$^{+/+}$ mice. PTLp, Posterior parietal association area. SSp-bfd, Primary somatosensory area-barrel field. SSp-tr, Primary somatosensory area-trunk.

Functional analysis of the ID-linked transcription factor Sox11

Mutations in the transcription factor Sox11 were identified as a cause for a subset of CSS cases. We found that Sox11 regulates stem cell differentiation and synaptic integration. In present studies we use CRISPR/Cas9-mediated genome editing to generate iPSCs with deletions of Sox11 or CSS-associated Sox11-mutations for studying the pathophysiological mechanisms underlying Sox11-mutation associated CSS.

Functional analysis of the SOX11 interactor and ID factor TCF4

Using proteomic analysis, we identified the transcription factor TCF4 as novel SOX11 interactor. TCF4 mutations cause Pitt-Hopkins syndrome, a disorder characterized by developmental delay and ID. We found that the highest levels of TCF4 expression are found in the developing and adult cortex and hippocampus. In ongoing analyses of TCF4-heterozygote knockout mice, we observed hypoplasia of cortical and hippocampal structures. Moreover, we found that TCF4 gene dosage is critical for neuronal differentiation of stem cells. These data suggest a role for TCF4 in the regulation of cortical and hippocampal neurogenesis.

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Invited lectures

Fusion Conference “Neurogenesis”, February 2-6, 2016, Cancun, Mexico, (DCL), Control of neurogenesis by FoxO Transcription factors

Eurogenesis Conference, July 11-13, 2016, Bordeaux, France, (DCL), Molecular regulation of adult hippocampal neurogenesis

Developmental Biology Seminar Series, University of Basel, July 26, Basel, Switzerland, (DCL), Molecular regulation of adult hippocampal neurogenesis

Annual Symposium of the Zurich Neuroscience Center (ZNZ), September 15, Zurich, Switzerland, (DCL), Metabolic control of adult hippocampal neurogenesis

Publications during funding period

Saunier C, Støve SI, Popp B, Gérard B, Blenski M, AhMew N, de Bie C, Goldenberg P, Isidor B, Keren B, Leheup B, Lampert L, Mignot C, Tezcan K, Mancini GM, Nava C, Wasserstein M, Bruel AL, Thevenon J, Masurel A, Duffourd Y, Kuentz P, Huet F, Rivière JB, van Slegtenhorst M, Faivre L, Piton A, Reis A, Arnesen T, Thauvin-Robinet C, Zweier C (2016) Expanding the Phenotype Associated with NAA10-Related N-Terminal Acetylation Deficiency. Hum Mutat 37: 755-64

E17 - Progress Report

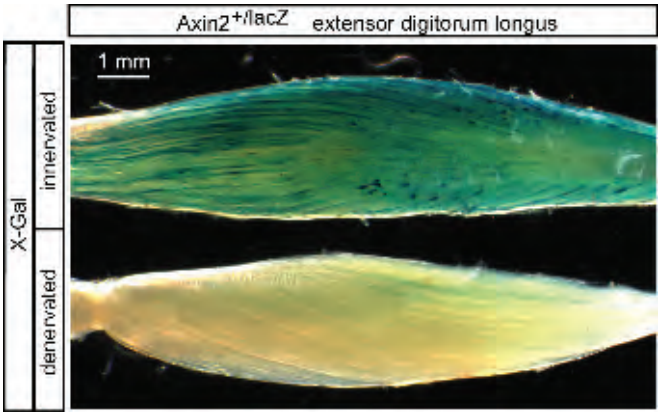
01.04.2014 - 31.03.2017

The neuromuscular role of Wnt signaling pathways

Prof. Dr. Said Hashemolhosseini, Institute of Biochemistry

The Wnt family of proteins encodes 19 secreted glycoproteins, which bind to the Frizzled transmembrane receptors on target cells. Wnt proteins regulate processes such as development and differentiation and are fundamental during embryonic myogenesis. Previously, canonical Wnt signaling activity was detected and investigated in skeletal muscles mostly during development. However, the role of canonical Wnt signaling in resting adult muscle fibers remained fully unknown. We recently reported canonical Wnt signaling activity in adult muscle fibers belonging to fiber type IIa and IIx, and at neuromuscular junctions.

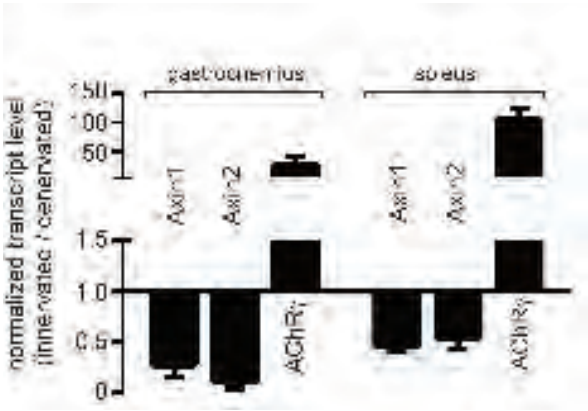
We started to elucidate the role of canonical Wnt activity in adult muscle fibers using a well-established Axin2-lacZ reporter mouse. In these mice, canonical Wnt signaling is reflected by lacZ expression under control of the endogenous Axin2 promoter. We detected active canonical Wnt signaling (1) in myotubes derived from cultured C2C12 cells or murine primary myoblasts, (2) in muscle fibers with small fiber diameter of type IIa and, most likely type IIx, (3) at neuromuscular synapses, as well as (4) during regeneration of skeletal muscle after injury. Interestingly, YAP/Taz/Tead1-mediated signaling accompanied canonical Wnt signaling in adult muscle fibers. X-Gal- positive muscle fibers (reflecting canonical Wnt activity) were also found to be positive for nuclear β-catenin, YAP/Taz and Tead1. In cultured muscle cells, (1) absence of Axin1 interfered with proliferation, (2) absence of Axin2 slowed down differentiation into myotubes; interestingly, treatment with Wnt3a had a similar effect, and (3) after knockdown of either β-catenin or Tead1 myogenesis was increased. Moreover, canonical Wnt3a induced TOPflash and Tead1 reporters, and importantly both inductions did not occur in the presence of Dickkopf-1, an inhibitor of canonical Wnt signaling. All these data have been recently published (Huraskin et al., 2016).



Extensor digitorum longus of heterozygous Axin2-lacZ mice were dissected, fixed and stained by X-Gal. Note, if the muscle was denervated 5 days before no Axin2-lacZ reporter expression was observed.

Prof. Dr. Hashemolhosseini

We also started to address the question, where canonical Wnt proteins come from (unpublished data). Recently, we decided to approach the influence of the nerve ending in providing Wnts by applying sciatic nerve lesion to heterozygous Axin2-lacZ reporter mice. Impressively, after denervation reporter gene expression is completely halted; this observation is in agreement with previous findings that Wnt signaling might be reduced in denervated murine skeletal muscle shown by global gene expression profiling. Excitingly, even Axin1 expression is significantly down-regulated by sciatic nerve lesion in muscles. This is even more striking as Axin1 is believed to be constitutively expressed and not regulated, like Axin2 (Frank Costantini, Columbio University, NY, USA; personal communication). A simultaneous downregulation of both Axins has been described in a different context (chondrocyte maturation) and related to TGF-β signaling activity and its crosstalk with the canonical Wnt pathway. Up to now, there is no evidence for a similar mechanism in skeletal muscle cells and in particular at the NMJ, but increased TGF-β signaling has been associated with muscle denervation.



Transcript amounts of Axin1 and Axin2 were significantly reduced upon denervation in muscles gastrocnemius and soleus. Functional denervation was confirmed by an increase of AChRγ mRNA level.

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Invited lectures

5th International Congress of Myology, AFM Telethon, Lyon Convention Center, France, 14.-18.03.2016, CK2-dependent phosphorylation in skeletal muscle regulates neuromuscular junction stability and mitochondrial homeostasis

Muscle Decline in Aging and Neuromuscular Disorders, Padua Muscle Days, Montegrotto, Italy, 13.-16.04.2016, CK2-dependent phosphorylation in skeletal muscle regulates neuromuscular junction stability and mitochondrial homeostasis

Inaugurations-Symposium des Interdisziplinären Zentrums, MURCE, FAU Erlangen-Nürnberg, 21.-22.06.2016, CK2-dependent phosphorylation in skeletal muscle regulates neuromuscular junction stability and mitochondrial homeostasis

8th International Conference on Protein Kinase CK2, Bad Homburg, 06.-09.09.2016, CK2-dependent phosphorylation in skeletal muscle regulates neuromuscular junction stability and mitochondrial homeostasis

Molecular and Cell Biology of the Neuromuscular System, Guarda, Switzerland, 18.-23.09.2016, CK2-dependent phosphorylation in skeletal muscle regulates neuromuscular junction stability and mitochondrial homeostasis

Max-Planck-Institute Bad Nauheim, 07.10.2016, The neuromuscular role of canonical Wnt and/or YAP/Taz/Tead signaling

Awards

IZKF Posterpreis, Danyil Huraskin, Kloster Banz 2016

Publications during funding period

Huraskin D, Eiber N, Reichel M, Zidek L, Kravic B, Behrens J, von Maltzahn J, Hashemolhosseini, S (2016) Wnt/β-catenin signaling via Axin2 is needed for myogenesis and active in IIa/IIx fibers with Hippo pathway members. Development. 143: 3128-42

Rudolf R, Khan M, Wild F, Hashemolhosseini S (2016) The impact of autophagy on peripheral synapses in health and disease. Frontiers in bioscience (Landmark edition) 21: 1474

Kravic B, Frick A, Jung J, Mei L, Borg JP, Hashemolhosseini S (2016) The role of Erbin, Lano and Scribble at the neuromuscular junction of skeletal mouse muscles. J Neurochem. 139: 381-395

Durmus H, Ayhan O, Cirak S, Deymeer F, Parman Y, Franke A, Eiber N, Chevessier F, Schlötzer-Schrehardt U, Clemen CS, Hashemolhosseini S, Schröder R, Hemmrich-Stanisak G, Tolun A, Serdaroglu-Oflazer P (2016) Neuromuscular endplate pathology in recessive desminopathies: Lessons from man and mice. Neurology. 87: 799-805

E18 - Final Report

01.12.2013 - 30.11.2016

Assessing developmental potential and differentiation capabilities of NG2-glia in the healthy and diseased central nervous system

Prof. Dr. Michael Wegner, Institute of Biochemistry
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

In the healthy central nervous system, NG2-glia differentiate mostly to oligodendrocytes. In this project it is planned to alter Sox gene expression in these cells to improve their differentiation in mice and to increase their capacity to give rise to a large spectrum of different cell types for cell replacement therapy. Altered NG2-glia will be analyzed for their impact on disease in cell and mouse models of multiple system atrophy (MSA), a fast progressing atypical parkinsonian disorder.

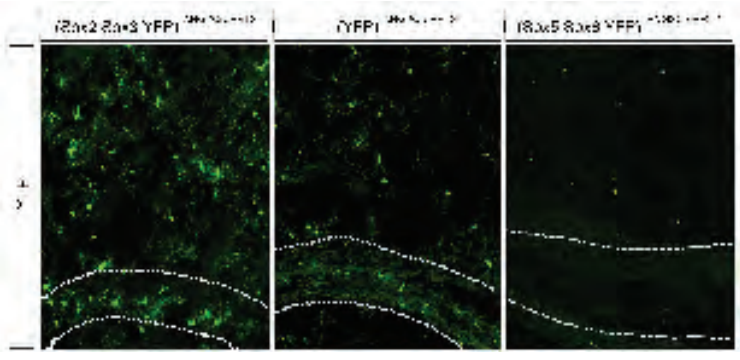
We analyzed the consequences of altered Sox gene expression in adult NG2-glia with the aim to exploit resulting changes in developmental potential and differentiation capacity of these cells in disease models. The disease that we primarily focussed on is MSA, a fast progressing neurodegenerative disease characterized by alpha-synuclein (aSyn)-positive glial cytoplasmic inclusions (GCIs) within mature oligodendrocytes, and widespread myelin loss as a neuropathological hallmark. In this project we obtained a better understanding of adult NG2-glia and their role in MSA pathology.

Analysis of mouse mutants

To study the consequences of altered Sox gene expression in adult NG2-glia, we generated compound mouse mutants that allow adult-onset, tamoxifen-induced and NG2-CreERT-mediated Sox gene deletion or overexpression. While adult NG2-glia exhibited transiently increased numbers and impaired differentiation capacities in gray and white matter of mice with combined Sox2 and Sox3 gene deletions, joint loss of the closely related Sox5 and Sox6 led to a dramatic loss of affected NG2-glia. Most of the NG2-glia disappeared shortly after the Cre-dependent recombination event in Sox5/Sox6-deficient NG2-glia, most likely by apoptosis. The few remaining cells failed to keep their identity as NG2-glia in the absence of Sox5 and Sox6 and overwhelmingly differentiated into oligodendrocytes. Overexpression of Sox10 in NG2-glia also promoted differentiation into oligodendrocytes, but did not affect viability. It can be concluded that Sox5 and Sox6 are required for survival of NG2 glia and maintenance of the undifferentiated state, whereas increased Sox10 levels promote differentiation with more subtle and transient effects of Sox2 and Sox3. Sox10 overexpression may represent a valid strategy for increasing differentiation potential of NG2-glia in the diseased central nervous system.

Defining the pathophysiological events in MSA

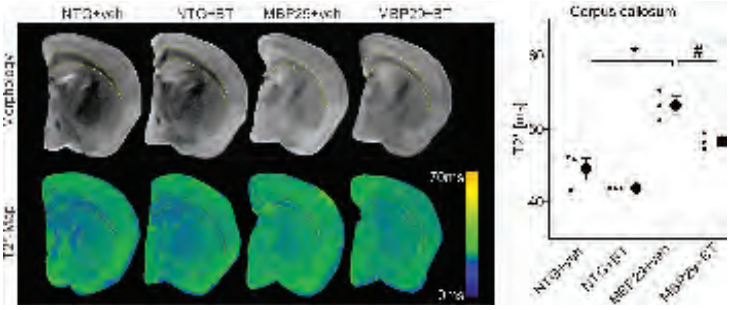
In MSA, comprehensive studies investigating the behavior of NG2-glia and their remyelination capacities are lacking. Thus, we generated a cellular system that gave us important insights into these processes by studying the effect of human aSyn accumulation on primary rat NG2-glia and their maturation in culture. Both upon lentiviral overexpression of aSyn and uptake of recombinant aSyn from the culture medium, the differentiation potential of NG2-glia was severely impaired. We further extended these



Detection of NG2:CreERT2-recombined NG2-glia in mouse forebrain by YFP. In YFP-positive glia, Sox2 and Sox3 (left panel) or Sox5 and Sox6 (right panel) were deleted. The middle panel shows the control. Cortical gray matter is in the upper part; corpus callosum is marked by stippled lines.


Prof. Dr. Wegner


Prof. Dr. Winkler



aSyn transgenic mice (MBP29) showed myelin loss detected by increased T2*-relaxation times. Benztropine attenuated the myelin deficit in MBP29 confirmed by a reduction of T2* time. MRI study was performed by Dr.Gillmann and Prof.Dr.Bäuerle, Dep. of Radiology.

findings and showed that an intervention in a transgenic MSA model using the differentiation-promoting small molecule benztropine is able to overcome the aSyn-induced differentiation defect in vitro and in vivo.

These findings define the α-syn-induced myelin deficit as a novel and crucial pathomechanism in MSA. Importantly, the reversible nature of this oligodendroglial dysfunction opens a novel avenue for an intervention in MSA.

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Invited lectures

M. Wegner:

Stockholm Nobel Forum Conference on “Developing Brains” in Stockholm, Sweden, 31.8.2016: “Genetic And Epigenetic Control Of Glial Development & Myelination”

J. Winkler:

Annual Meeting of the Society for Neuroscience in San Diego, 12.11.-16.11.2016: “α-Synuclein impairs myelin formation: a novel pathomechanism and interventional target in MSA”

Awards

Ben Ettle:

Young Researcher Award of the German Parkinson Associationt (€ 25,000), 2016

Poster Prize at the IZKF Symposium 2016 „Translational Medicine“, 2016

MSA Prize of the Dr.Mähler-Linke-Foundation (€ 2,000), 2016

Publications during funding period

Weider M, Wegner M (2017) SoxE factors: Transcriptional regulators of neural differentiation and nervous system development. Semin Cell Dev Biol. doi: 10.1016/j.semcdb.2016.08.013 [Epub ahead of print]

Ettle B, Kerman BE, Valera E, Gillmann C, Schlachetzki JC, Reiprich S, Büttner C, Ekici AB, Reis A, Wegner M, Bäuerle T, Riemenschneider MJ, Masliah E, Gage FH, Winkler J (2016) α-Synuclein-induced myelination deficit defines a novel interventional target for multiple system atrophy. Acta Neuropathologica 132: 59-75

Küspert M, Wegner M (2016) SomethiNG 2 talk about-Transcriptional regulation in embryonic and adult oligodendrocyte precursors. Brain Res. 1638: 167-182

Stolt CC, Wegner M (2015) Schwann cells and their transcriptional network: Evolution of key regulators of peripheral myelination. Brain Res 1641: 101-110

Reiprich S, Wegner M (2015) From CNS stem cells to neurons and glia: Sox for everyone. Cell Tissue Res.359: 111-124

Ettle, B., Schlachetzki, J.C., Winkler, J. (2015) Oligodendroglia and Myelin in Neurodegenerative Diseases: More Than Just Bystanders? Mol Neurobiol. 53:3046-62.

Ettle B, Reiprich S, Deusser J, Schlachetzki JC, Xiang W, Prots I, Masliah E, Winner B, Wegner M, Winkler J (2014) Intracellular alpha-synuclein affects early maturation of primary oligodendrocyte progenitor cells. Mol Cell Neurosci 62: 68-78

E19 - Progress Report

15.02.2016 - 14.08.2018

Inhibitory neurotransmission in the cochlea: Glutamate and endocannabinoids

Prof. Dr. Ralf Enz, Institute of Biochemistry

Sensory organs need tailor-made signal transduction pathways. Pre-synaptic glutamate- and endocannabinoid receptors regulate activity and survival of sensory neurons via inhibitory feedback loops. While pre-synaptic inhibition in photoreceptors of the retina is described in detail, corresponding protective mechanisms in hair-cells of the cochlea are largely unknown. This project investigates pre-synaptic receptor expression in hair-cells and elucidates their regulation by interacting proteins.

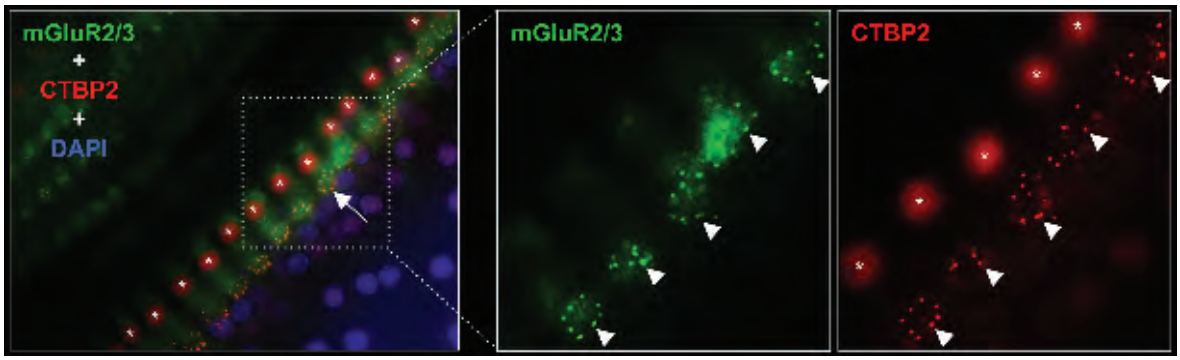
Introduction

Neuronal signal transduction is largely guided by the expression of neurotransmitter receptors at synaptic sites. These receptors contain specific intracellular sequences for binding regulatory proteins, such as enzymes and scaffolds that regulate their trafficking, localization, ligand affinity, desensitization behaviour and surface concentration. In this way, receptors and regulatory proteins assemble into synaptic signal complexes.

Inhibitory feedback loops are important factors for the activity and survival of sensory neurons, as well as for the protection against noxious stimuli. G-protein coupled metabotropic glutamate receptors (mGluRs) expressed at pre-synaptic sites can invert the activity of the excitatory neurotransmitter glutamate into neuronal inhibition and thus are well suited to build inhibitory feedback loops in glutamatergic neurons. The same holds true for pre-synaptically localized endocannabinoid (CB) receptors.

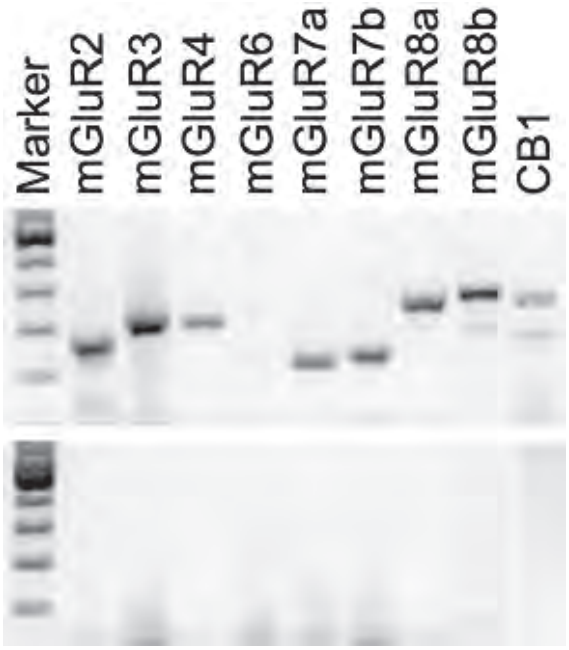
While molecular mechanisms of pre-synaptic inhibition have been analysed in detail in the retina, the identity of inhibitory protective circuits in the cochlea is not well understood. Based on previous findings in our laboratory, we hypothesize that different sensory organs, e.g. the retina and the cochlea, need a tailor-made regulation of these signal complexes. In this project, we therefore analyse receptors and regulatory binding partners in hair-cells of the cochlea.

The identification and characterization of receptors and regulatory proteins involved in pre-synaptic protective mechanisms can define new players specific of the signal transduction in the inner ear that might represent key proteins for the design of new drugs.



CTBP2 labels pre-synaptic ribbons of inner hair-cells in cochlear wholemounts (e.g. arrow). These synapses also express mGluR2/3, evident by a perfect co-localization of both proteins (yellow, arrowheads). Nuclei were counterstained with DAPI and cross-react with CTBP2 (asterisks).

Prof. Dr. Enz



Detection of pre-synaptically expressed group II (mGluR2, 3) and group III (mGluR4, 7a, 7b, 8a, 8b) metabotropic glutamate receptors and of the endocannabinoid receptor CB1 in the cochlea by PCR-techniques. MGluR6 is present in the retina, only. Negative controls are shown in the lower panel.

Results

Which pre-synaptic mGluR and CB receptors are expressed in the cochlea, and where are they localized? Expression and localization of the mostly pre- synaptically localized mGluR2, 3, 4, 7a, 7b, 8a, 8b and CB1 in the inner ear is largely unknown. RT-PCR studies showed expression of all these receptor types in the mouse cochlea. Receptor specific antibodies confirmed the expression of mGluR2/3, mGluR4, mGluR8, CB1 and CB2 in this tissue on the protein level. Furthermore, we could co-localize mGluR2/3, mGluR4, mGluR8 and CB2 with ribbon synapses in cochlear wholemounts using CTBP2 as a marker for the pre-synaptic ribbon.

How are cochlear mGluR and CB receptors regulated by interacting proteins? Based on our expression data, we searched for intracellular proteins that bind to and thereby regulate receptor efficacy. On-going yeast 2-hybrid screens were performed using intracellular C-terminal domains of mGluR2, mGluR4, mGluR7a, mGluR8a, CB1 and CB2 as baits for a cochlear cDNA-library. Currently, obtained sequences are analysed and encoding proteins are verified for receptor interactions.

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Publications during funding period

none

E20 - Progress Report

01.05.2016 - 31.10.2018

Identification of molecules, receptors and genes involved in chronic pruritus

Dr. Dr. Andreas Kremer, Department of Medicine 1 - Gastroenterology, Pneumology and Endocrinology
Prof. Dr. Katharina Zimmermann, Department of Anesthesiology

Chronic pruritus is an agonizing symptom accompanying many dermatological and systemic disorders. Aim of this project is to identify pruritogens in plasma of patients suffering from chronic pruritus, to characterize the specific NaV channel subtypes that generate and propagate the action potentials in itch pathways, and to identify and characterize novel gene products that predispose to or protect from itch by quantifying the phenotypic differences in scratch behavior in inbred mouse strains.

Identification of pruritogens in plasma of patients suffering from chronic pruritus

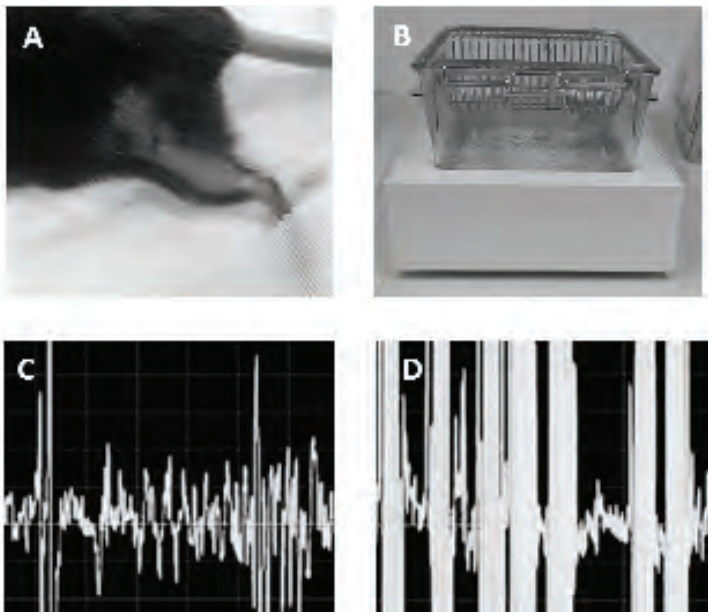
Several GPCRs of the Mas-gene related G protein-coupled receptor (Mrg) family are selectively activated by certain pruritogens. We cloned relevant members of the human Mrg receptors (X1-4, D, E, F, G) and expressed the constructs stably in HepG2 cells. We confirmed function by testing known ligands (e.g. BAM8-22 for X1, compound 48/80 for X2, β-alanine for D) and we tested a selection of potential ligands. We identified plant extracts of an itch-causing legume as novel Mrg agonist and we will characterize this compound in detail by physical and chemical analysis.

Identification of specific NaV channel subtypes required for itch signalling

To identify the specific NaV subtypes functional in itch signaling pathways, we measured activation of cultured sensory neurons and scratching behavior in NaV1.7-, NaV1.8- and NaV1.9- knock-out mice and the underlying wildtype strain. Using calcium imaging we found that all pruritogens, including histamine, chloroquine, BAM 8-22, 5-Hydroxy-tryptamine (5HT) and β-alanine showed comparable activation of primary sensory neurons isolated from DRGs of both, knock-out and wild-type mice. In preliminary in vivo experiments where we injected the pruritogens histamine, chloroquine and 5HT in the skin of the neck, we observed a significantly reduced scratching activity in NaV1.7 ko mice.

Quantification of itch in various inbred mouse strains to identify heritable differences.

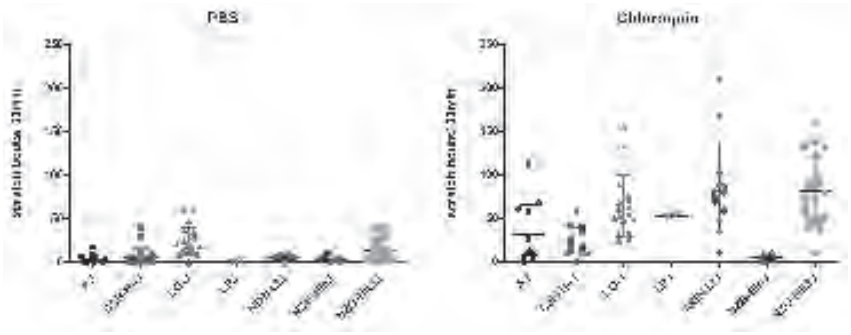
Scratching activity is quantified without experimenter bias; tiny teflon-coated magnets are implanted into both hind paws and mice placed in a cage surrounded by a magnet coil. Movement of magnets induces an electric current in the magnet field which is registered by an oscillograph. A software counts the movements and filters scratch-like movements based on a low cutoff frequency of 10 Hz, a high cutoff frequency of 20 Hz, a threshold level of 300 mV,



Software-based method to record scratch activity. A) Teflon-coated magnets implanted in both hind paws induce electric currents through B) magnetic coils around cages. C/D) Software can distinguish between body movement (C) and scratching (D).

Dr. Dr. Kremer

Prof. Dr. Zimmermann



Scratch activity following intradermal injection of phosphate-buffered saline (PBS; (A)) and (B) chloroquine; n: 2-13 per group; black lines: mean value, error bars: SD

a minimum of 4 beats per bout, and a maximal coefficient of variation of 40% between the beats of a scratching bout. The analytical procedure has been validated with intradermal compound 48/80, showing a positive predictive value of 95% at a sensitivity of 50%.

To uncover novel itch-related pathways based on heritable differences we quantifed scratching behaviour in a body of inbred strains. So far, we phenotyped seven inbred mouse strains (NZB/BinJ, NON/ShiLtJ, NZO/HlLtJ, A/J, LG/J, C3H/HeJ, BTBR/T^{+}Itpr3^{tf}/J) and observed strong differences between number and time of scratch bouts following the intradermal injection of PBS, histamine and chloroquine. As control parameters we measured several parameters in the phenomaster cage system including in-cage activity and indirect calorimetry. The experiments will be expanded to include additional pruritogens to include at least 20 inbred mouse strains to allow a powerful prediction of genes with high correlation to the phenotypic differences with genetic mapping.

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Invited lectures

Hepatologie-Update, Hamburger Lebertage, May 27-28, Hamburg, “Juckreiz bei Lebererkrankungen – was kann man therapeutisch tun“

DGVS-Satellitensymposium, DGVS, September 21-24, Hamburg, „Neue Therapieoptionen für die PBC“

National PBC Day, October 19, Paris, “Prurit et CBP: des mécanisme pathophysiologique aux perspective thérapeutique“

48. Tagung Aktuelle Gastroenterologie, November 4-5, Frankfurt, “Diagnostik und Therapie der autoimmunen Pankreatitis und Cholangitis“

11. Kursus Klinische Hepatologie, November 24-26, Hamburg, „IgG4-assoziierte Cholangitis: neue Tests und Therapie“

Awards

Best PhD thesis Award of the year 2015 to A.E. Kremer (Academic Medical Center, University of Amsterdam, 2016)

Publications during funding period

Günther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Amann K, Vandenabeele P, Linkermann A, Poremba C, Schleicher U, Dewitz C, Krautwald S, Neurath MF, Becker C, Wirtz S (2016) The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. J Clin Invest. 126(11): 4346-4360

He GW, Günther C, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S, Becker C (2016) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut. [Epub ahead of print]

Wunsch E, Krawczyk M, Milkiewicz M, Trottier J, Barbier O, Neurath MF, Lammert F, Kremer AE*, Milkiewicz P* (2016) Serum Autotaxin is a Marker of the Severity of Liver Injury and Overall Survival in Patients with Cholestatic Liver Diseases. Sci Rep. 6: 30847
*contributed equally

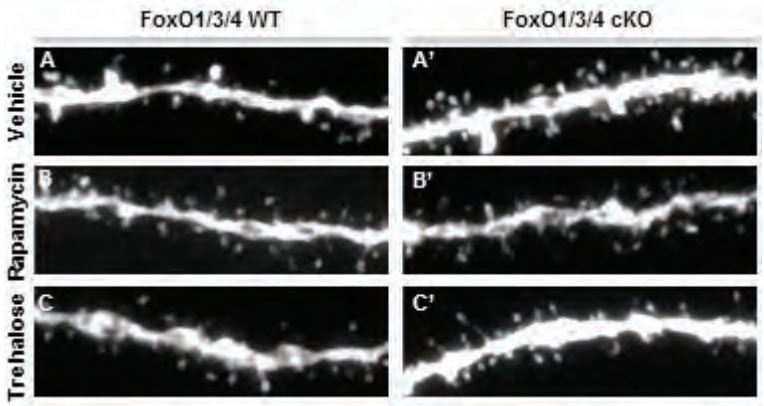
E21 - Progress Report

01.05.2016 - 31.10.2018

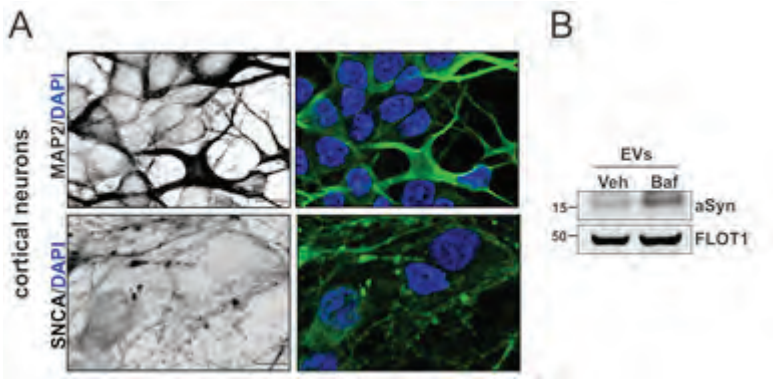
Modulation of alpha-Synuclein pathology by FoxO-dependent pathways

Prof. Dr. Dieter Chichung Lie, Institute of Biochemistry
Prof. Dr. Jochen Klucken, Department of Molecular Neurology

Synucleinopathies are a group of age-associated neurodegenerative disorders, including Parkinson´s disease, characterized by the intracellular accumulation of the cytosolic protein alpha-synuclein (aSyn) in aberrantly folded inclusions/aggregates. Dysregulation of autophagy, the central cellular self-clearance mechanism, is impaired in synucleinopathies and has been implicated in the cell-to-cell transfer of aSyn potentially leading to disease progression. This project addresses the currently unresolved question of how ageing accelerates aSyn-related toxicity and cerebral spreading. Specifically, it will investigate whether modulation of autophagy by the ageing-associated FoxO-pathway affects exosomal release of aSyn, transcellular spread and acceleration of toxic aSyn-related responses.



(A, A') FoxO-deficient neurons display excessive spine density. (B-C') Treatment with the autophagy inducers Rapamycin and Trehalose ameliorate the aberrant spine phenotype of FoxO-deficient neurons.



(A) E18 rat primary neurons express aSyn in the cell soma and along the axons. (B) aSyn is released in exosome fractions, positive for lipid raft marker Flotillin-1 (FLOT1). Upon autophagy inhibition using Bafilomycin A1 (Baf), aSyn levels are significantly increased.


Prof. Dr. Lie


Prof. Dr. Klucken

FoxO transcription factors potently modulate autophagy in neural cells

FoxO-deficiency was found to profoundly impact the function and homeostasis of neural precursor cells and neurons. Cultured FoxO-deficient neural precursor cells showed excessive proliferation and premature differentiation. Conditional knockout mouse analysis showed that FoxO1/3/4-deficient neurons display cell biological hallmarks of impaired autophagy, including excessive synaptogenesis, enlarged mitochondria, and enhanced sensitivity to degeneration. Moreover, we observed increased activity of the mTOR-pathway –a major inhibitory pathway of autophagy – in FoxO-deficient neurons and adult neural precursor cells. Molecular and biochemical analyses in FoxO-deficient adult neural precursor cells and neurons revealed a substantial decrease in the expression of key autophagy genes and strong impairment of autophagic flux, respectively.

Intriguingly, our first experiments show that treatment with the compounds rapamycin and trehalose increases autophagic flux in FoxO-deficient adult neural precursor cells and neurons and this is sufficient for reverting the proliferation and differentiation phenotype and the synaptogenesis and mitochondria phenotype in FoxO-deficient neural precursor cells and neurons, respectively. Collectively, these results demonstrate that FoxO transcription factors are transcriptional modulators of autophagy and that the FoxO-autophagy pathway is critical for homeostasis of neural cells.

Impairment of autophagic flux in neural cells stimulates aSyn release in exosomes

We found that in human neuroglioma cells, inhibition of autophagy leads to toxicity and notably stimulates the cellular release of aSyn. It was further deciphered that aSyn release is partly via the multivesicular body-to-exosome release pathway and extracellular aSyn was identified in fractions with distinct morphological and biochemical characteristics of exosomes. Exosomes are important mediators of intercellular communication throughout the central nervous system. We therefore addressed whether in neurons, which primarily rely on autophagy for maintaining protein homeostasis during aging, inhibition of autophagy may also lead to a similar effect. Using non-transgenic E18 rat primary cortical neuron cultures, we found that aSyn is released via exosomes under basal conditions. After treatment with the lysosomal ATPase inhibitor Bafilomycin A1 aSyn was found significantly up-regulated in exosomes. Moreover, exosomes were found to associate with recipient cells upon release. Intriguingly, in FoxO1/3/4 cKO neuronal precursor cell cultures, in which autophagy is impaired, exosome release was found to be altered as compared to non-transgenic controls. This data collectively supports that exosomes are likely contributors to the cell-to-cell transfer of aSyn under basal conditions and that autophagy impairing factors may accelerate this process.

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Invited lectures

Fusion Conference “Neurogenesis”, February 2-6, 2016, Cancun, Mexico

Eurogenesis Conference, July 11-13, 2016, Bordeaux, France

Developmental Biology Seminar Series, University of Basel, July 26, Basel, Switzerland

Annual Symposium of the Zurich Neuroscience Center (ZNZ), September 15, Zurich, Switzerland

NSAS Course on “Autophagy and Neuroprotection”, May 7-14th 2016 in Cortona, Italy

Publications during funding period

none

E22 - Progress Report

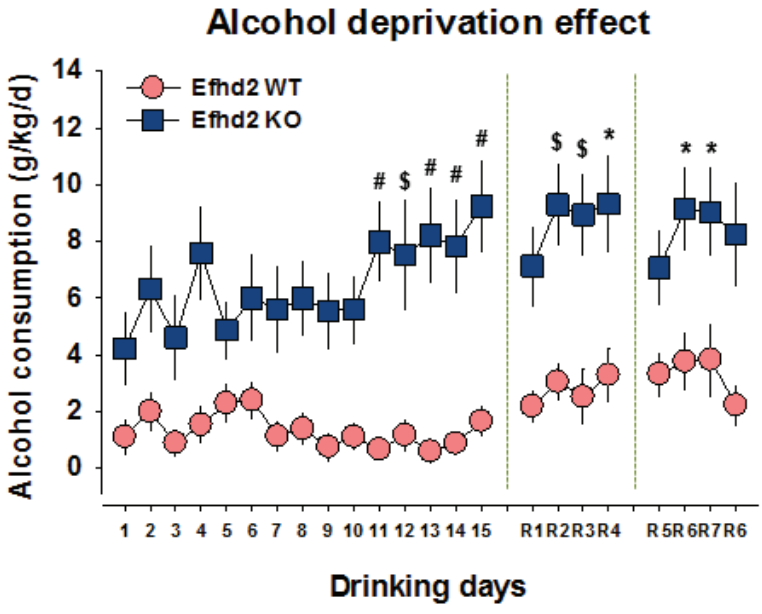
01.03.2016 - 31.08.2018

The role of Swiprosin-1/EFhd2 in resilience to drug addiction

Prof. Dr. Christian Müller, Department of Psychiatry and Psychotherapy
Prof. Dr. Christian Alzheimer, Institute of Physiology and Pathophysiology
PD Dr. Dirk Mielenz, Department of Molecular Immunology

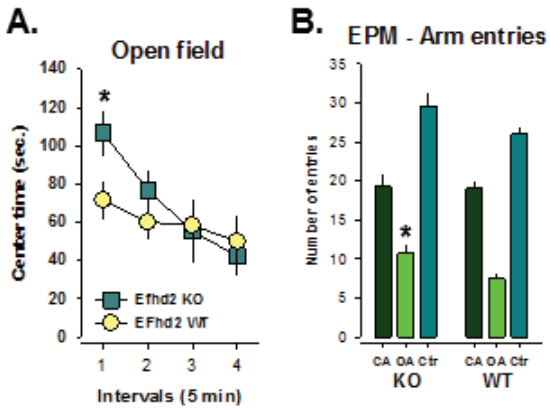
Drug addiction is a prevalent psychiatric disorder which develops from controlled consumption of psychoactive drugs. Normal behavioural traits, such as sensation seeking and/or low anxiety render an organism more or less susceptible to the addictive effects of alcohol. Present findings suggest that Swiprosin-1/ EFhd2 may be a resilience factor against the establishment of alcohol addiction working via a reduction of sensation seeking and enhancement of trait anxiety.

In many societies, the majority of adults regularly consume alcohol. However, only a small proportion develops alcohol addiction. Individuals at risk often show a high sensation-seeking/ low anxiety behavioural phenotype. Here we asked which role EFhd2 (Swiprosin-1) plays in the control of alcohol addiction-associated behaviours. EFhd2 knock out (KO) mice drink more alcohol than controls and spontaneously escalate their consumption. This coincided with a sensation-seeking and low anxiety phenotype. A reversal of the behavioural phenotype with β-carboline, an anxiogenic inverse benzodiazepine receptor agonist, normalized alcohol preference in EFhd2 KO mice, demonstrating an EFHd2-driven relationship between personality traits and alcohol preference. These findings were confirmed in a human sample where we observed a positive association of the EFHD2 SNP rs112146896 with lifetime drinking and a negative association with anxiety in healthy adolescents. The lack of EFhd2 reduced extracellular dopamine levels in the brain, but enhanced responses to alcohol. In confirmation, gene expression analysis revealed reduced tyrosine hydroxylase expression and the regulation of genes involved in



The lack of Swirprosin-1/ EFhd2 in mice leads to enhanced consumption of alcohol in a free-choice drinking paradigm and spontaneous escalation of consumption. Withdrawal from alcohol for three weeks (dotted green lines) increases alcohol consumption in wild type mice (alcohol deprivation effect), but does not further increase consumption in EFhd2 KO mice (*p<0.05, $p<0.01; #p<0.001 vs. WT).

Prof. Dr. Müller | Prof. Dr. Alzheimer | PD Dr. Mielenz



EFhd2 knock out (KO) mice display a sensation seeking/ low anxiety behavioural phenotype which is frequently associated with an enhanced risk for alcohol addiction. (A) In the open field test EFhd2 KO mice show higher locomotor activity in a novel environment than wild type (WT) mice. (B) The elevated plus maze (EPM) test suggests reduced levels of anxiety in EFhd2 KO compared to WT mice (*p<0.05).

cortex development, Eomes and Pax6, in EFhd2 KO cortices. These findings were corroborated in Xenopus tadpoles by EFhd2 knock-down. Magnetic resonance imaging (MRI) in mice showed that a lack of EFhd2 reduces cortical volume in adults. Moreover, human MRI confirmed the negative association between lifetime alcohol drinking and superior frontal gyrus volume. We propose that EFhd2 is a conserved resilience factor against alcohol consumption and its escalation, working through Pax6/Eomes. Reduced EFhd2 function induces high-risk personality traits of sensation seeking/ low anxiety associated with enhanced alcohol consumption which may be related to cortex function.

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Invited lectures

Host: Prof. Martin Hof, J. Heyrovský Institute of Physical Chemistry of the ASCR, v.v.i. Dolejškova 2155/3, Prague 8, 182 23, Czech Republic, 25.05.2016. PD Dr. Dirk Mielenz. "The role of Swiprosin-1/EFhd2 in drug addiction and neurodegeneration"

Immunogambia course at the MRC Unit in Banjul, The Gambia, 21.11.2016. PD Dr. Dirk Mielenz, "B cell activation and differentiation."

Publications during funding period

Mielenz D, Gunn-Moore F (2016) Physiological and pathophysiological functions of EFhd2 in the nervous system. Biochem J. 15 473(16): 2429-37

E23 - Progress Report

01.01.2016 - 30.06.2018

Identification and characterization of LOXL1 risk variants for pseudoexfoliation syndrome and glaucoma

Prof. Dr. Ursula Schlötzer-Schrehardt, Department of Ophtalmology
Prof. Dr. André Reis, Institute of Human Genetics

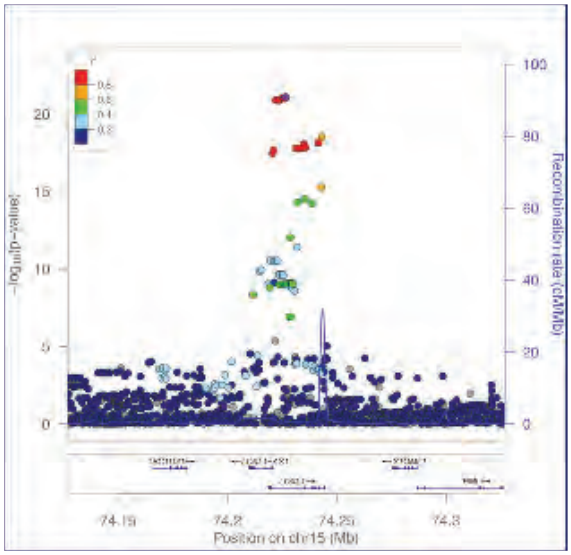
Pseudoexfoliation (PEX) syndrome represents an age-related systemic connective tissue disorder and a major cause of glaucoma and cardiovascular complications. Although LOXL1 (lysyl oxidase-like 1), coding for a cross-linking matrix enzyme, is known as the principal genetic risk factor for PEX syndrome/glaucoma, no functional variants have been identified to date. The aim of this project is to describe mechanisms of LOXL1 gene regulation and to identify functional LOXL1 variants and analyze how they confer susceptibility to PEX syndrome/glaucoma.

Functional variants impact on LOXL1 expression via differential transcription factor binding and alternative splicing

We conducted a genome-wide association scan (GWAS) on German PEX patients and controls, followed by independent testing of associated variants in Italian and Japanese datasets. We focused on a 3.5-kb four component polymorphic locus positioned in intron 1 and 2 of *LOXL1* within genomic regions with enhancer-like chromatin features. Among those four common variants, rs11638944:C>G was found to exert a cis-acting effect on the expression levels of *LOXL1* by differential binding of the transcription factor RXRα and by enhancing splicing of an alternative *LOXL1* transcript *(LOXL1-002)* associated with nonsense-mediated decay (NMD), eventually reducing the final steady state levels of *LOXL1* mRNA in specific cells and tissues of risk allele carriers. These findings provide an explanation for how *LOXL1* non-coding variants influencing *LOXL1* gene expression confer susceptibility to disease.

Alternative splicing and nonsense-mediated mRNA decay contribute to regulation of LOXL1 expression in response to cellular stress

Transcript *LOXL1-002*, characterized by inclusion of an additional exon introducing a premature termination codon in exon 2, was detected in all ocular tissues of PEX and control eyes. Treatment of various cell types with NMD inhibitors puromycin, emetine and caffeine significantly increased *LOXL1-002* mRNA levels, but reduced levels of wild-type *LOXL1* mRNA. Significantly enhanced *LOXL1-002* expression upon knockdown of UPF1, a key regulator of NMD pathway, further confirmed that *LOXL1-002* is a direct target of NMD. Exposure of cells to various PEX-associated (stress) factors, including TGF-ß1, retinoic acid, UV light, oxidative and mechanical stress, resulted in upregulated *LOXL1-002* expression in a cell type-specific manner and altered steady state levels of wild-type *LOXL1* expression. These findings indicate that alternative splicing coupled to NMD is dynamically modulated by cellular stress and contributes to post-transcriptional regulation of *LOXL1* expression.



Regional association plot for *LOXL1* in a German cohort with 100 kb upstream and downstream regions shows the association peak region for SNPs located on the *LOXL1* gene locus.

Prof. Dr. Schlötzer-Schrehardt Prof. Dr. Reis

A worldwide genetic association study identifies common genetic variants at five new loci and highly protective rare mutations at LOXL1

As members of the International Glaucoma Genetics Consortium (IGGC), we participated in a GWAS of >10.000 PEX cases and >100.000 controls, performed at the Genome Institute of Singapore. We observed consistent enrichment of rare *LOXL1* nonsynonymous variants predicted to affect protein function in controls compared to PEX cases. Functional assays showed a protective p.407F allele to result in increased elastin and fibrillin 1 levels compared to the wild-type p.407Y allele. Significant association was further observed at five new loci, underlined by *POMP, TMEM136, AGPAT1, RBMS3* and *SEMA6A*. Protein and mRNA expression levels of POMP and TMEM136 were significantly reduced in ocular tissues of PEX patients compared to age-matched controls and associated with abnormal accumulations of PEX material. These findings provide new biological insights into the pathology of PEX and highlight a role for rare *LOXL1* gain-of-function variants protecting against PEX by likely stabilization of the extracellular matrix.

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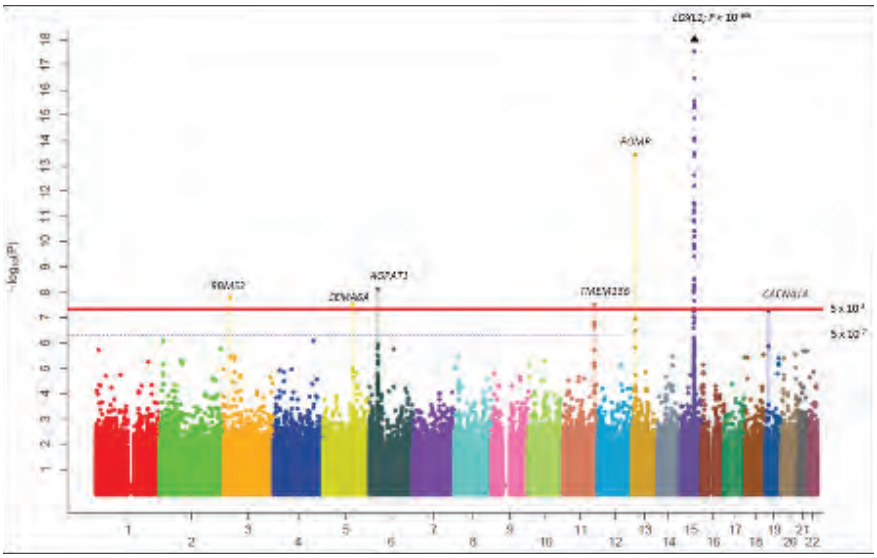


Manhattan plot of the results from the GWAS comprising 13,620 PEX cases and 109,837 controls. SNP markers at seven loci *(LOXL1, CACNA1A, POMP, TMEM136, AGPAT1, SEMA6A and RBMS3)* surpass genome-wide significance.

Invited lectures

31st Asia-Pacific Academy of Ophthalmology (APAO) congress. 24.-27.3.2016, Taipei: "Molecular pathology of exfoliation syndrome"

23rd Annual Glaucoma Foundation Think Tank, 10.-11.06.2016, New York: "The role of LOXL1 regulatory genetic variants in exfoliation syndrome"

European Elastin Meeting, 17.-19.6.2016, Stuttgart: „Functional implications of LOXL1 risk variants in exfoliation syndrome/glaucoma"

Awards

The Dr. Robert Ritch Award for Excellence and Innovation in Glaucoma, Ursula Schlötzer-Schrehardt, 09.06.2016, New York

Publications during funding period

none

E24 - Progress Report

01.01.2016 - 30.06.2018

The role of alpha-synuclein during inflammatory demyelination and degeneration in the central nervous system

Prof. Dr. Jürgen Winkler, Department of Molecular Neurology
Prof. Dr. Ralf Linker, Department of Neurology

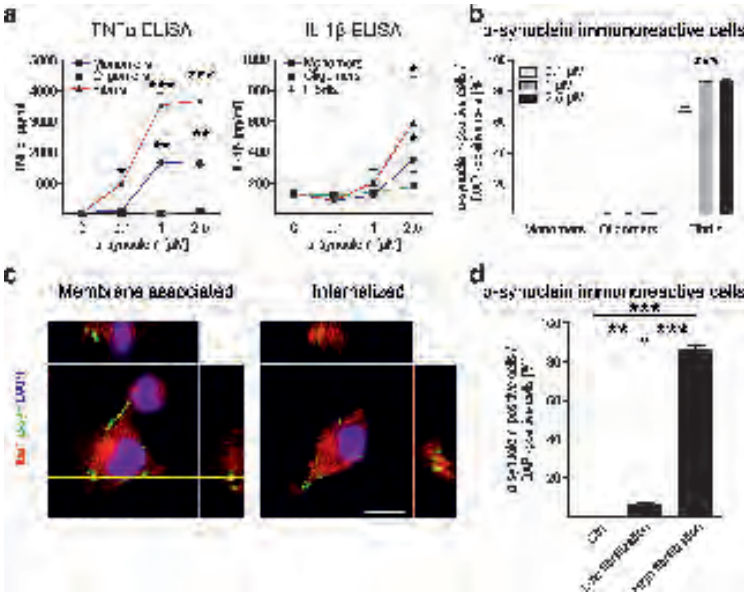
Interaction of alpha-synuclein (aSyn) and inflammation plays an important role in neurodegenerative and -inflammatory diseases like multiple system atrophy and multiple sclerosis. aSyn aggregation is accompanied by activation of immune responses in central and peripheral tissues. Therefore, this project aims first to investigate the role of aSyn in modulating immunological processes.

Neurodegenerative and –inflammatory disorders share certain features, such as inflammation and accumulation of alpha-synuclein (aSyn) as it is described for multiple system atrophy and multiple sclerosis (MS). However, the exact connection of aSyn and inflammatory processes is still incompletely understood. Thus, the aim of our project is to study the influence of aSyn on central and peripheral immunological processes in the context of myelin deficit and demyelinating diseases.

First, we focused on the effect of aSyn on inflammatory processes. As microglial activation plays a major role in neurodegenerative diseases, we investigated the impact of aSyn on microglia immune responses. Furthermore, we studied the role of aSyn in experimental autoimmune encephalomyelitis (EAE), a prototypical inflammatory demyelinating model of MS.

Role of aSyn in microglial activation

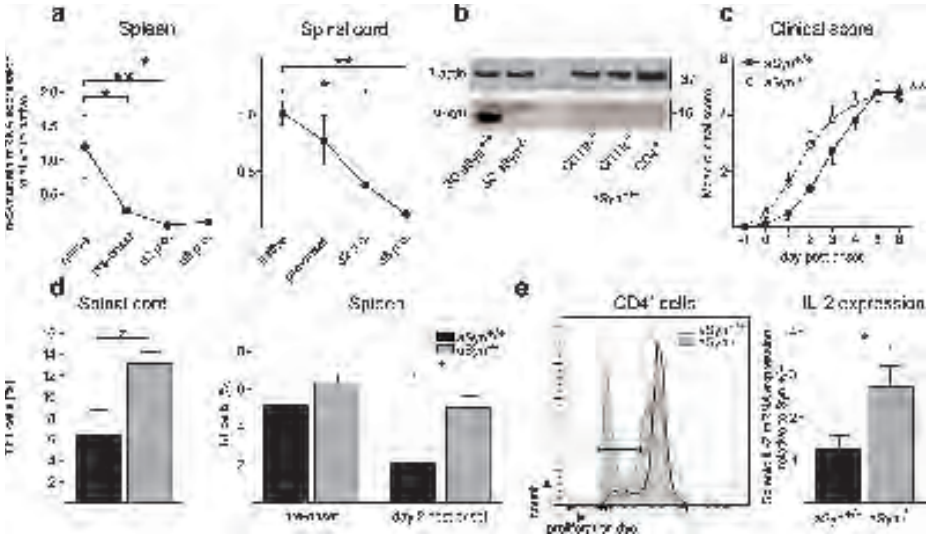
In order to investigate the crosstalk of aSyn and neuroinflammation, we analyzed the influence of extracellular pathophysiological relevant aSyn aggregation states on BV2 cell immune responses. Using human aSyn monomers, we generated aSyn fibrils and oligomers and exposed all three species to BV2 cells, a microglial cell line. aSyn fibrils induced increased production and secretion of the pro-inflammatory cytokines, TNFα and IL1β, compared to monomers and oligomers. Moreover, BV2 cells preferentially phagocytosed aSyn fibrils, a process that was tightly linked to the degree of fibrilization. Both, uptake and pro-inflammatory activation of BV2 cells were concentration- and time-dependent processes. Summarizing, our results emphasize the potential of distinct aSyn aggregation states to modulate microglial immune responses.



(a) Relative dose-dependent release of TNF-α and IL-1β by BV2 cells. (b) Quantification of aSyn+ BV2 cells. (c) Co-localization analysis of aSyn and Iba1 in BV2 cells. (d) Fibrilization-dependent uptake after treatment with low/high fibrilized aSyn species.


Prof. Dr. Winkler


Prof. Dr. Linker



(a) aSyn mRNA expression in spleen and spinal cord during EAE. (b) Western blot of aSyn in peripheral immune cells. (c) Clinical score of early EAE phase. (d) Th1 cell infiltrates in spinal cord and spleen and (e) proliferation of CD4+ cells and IL-2 expression in the spleen.

Role of aSyn in early EAE

To investigate the functional role of endogenous aSyn in neuroinflammation, we employed EAE in wildtype (aSyn+/+) and aSyn-knockout (aSyn-/-) mice. In the spleen and spinal cord of aSyn+/+ mice, we observed a gradual reduction of aSyn expression during EAE, starting already in the presymptomatic disease phase. Interestingly, analysis of different immune cell subsets revealed aSyn expression in CD4+ as well as CD11b/c+cells. Compared to aSyn+/+ mice, aSyn-/- mice showed an earlier onset of EAE symptoms, accompanied by increased spinal cord infiltration of T helper 1 (Th1) cells. Pre-symptomatically, aSyn-/- mice exhibited hyperproliferative CD4+ splenocytes consistent with increased splenic interleukin-2 mRNA expression, resulting in increased numbers of Th1 cells in the spleen at the onset of symptoms. Overall, our data identify endogenous aSyn as a new regulator of Th1 responses in neuroinflammation during early EAE.

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Awards

Pette Prize, German Neurology Society, 23.09.2016 to Prof. Ralf Linker.

Publications during funding period

Hoffmann A*, Ettle B*, Bruno A, Kulinich A, Hoffmann AC, von Wittgenstein J, Winkler J, Xiang W#, Schlachetzki JC# (2016) Alpha-synuclein activates BV2 microglia dependent on its aggregation state. Biochemical and Biophysical Research Communications 479(4): 881-886

*authors contributed equally

#shared last author

Ettle B*, Kuhbandner K*, Jörg S, Hoffmann A, Winkler J, Linker RA (2016) α-Synuclein deficiency promotes neuroinflammation by increasing Th1 cell-mediated immune responses. Journal of Neuroinflammation 13(1): 201

*authors contributed equally

E25 - Progress Report

01.07.2016 - 31.12.2018

Modeling pain syndromes using human induced pluripotent stem cell-derived nociceptors

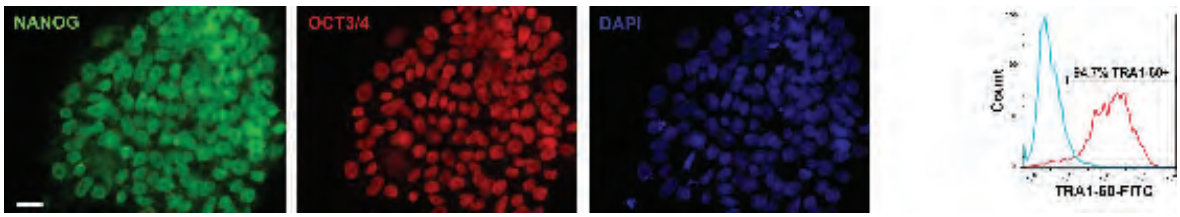
Prof. Dr. Beate Winner, IZKF Junior Research Group 3 (till 30.09.2016),
Institute of Human Genetics (since 22.03.2016)
Prof. Dr. Jürgen Schüttler, Department of Anesthesiology

Our aim is to understand the role of the sodium channel subtype Nav1.9 in pain syndroms. Fibroblasts from patients with hereditary pain syndromes due to Nav1.9 mutations were reprogrammed into IPSC and differentiated to nociceptors. Using electrophysiological and molecular methods, we monitored the development of excitability in these neurons in order to understand mechanisms of nociception and the role of Nav1.9 in the development of human pain.

Human monogenic pain disorders can be caused by mutations in peripheral voltage-gated sodium channels. Cellular expression systems are lacking the patients´ individual genetic background. To understand the impact of *SCN11A* on human nociceptors, we generated IPSC-derived nociceptors from pain patients who carry rare genetic variants of *SCN11A* (Nav1.9). We currently study two patients with painful peripheral neuropathy with erythromelalgia-like features with continuous, severe burning pain, late onset of symptoms and signs of SFN (referred to as SFN1 and SFN2).

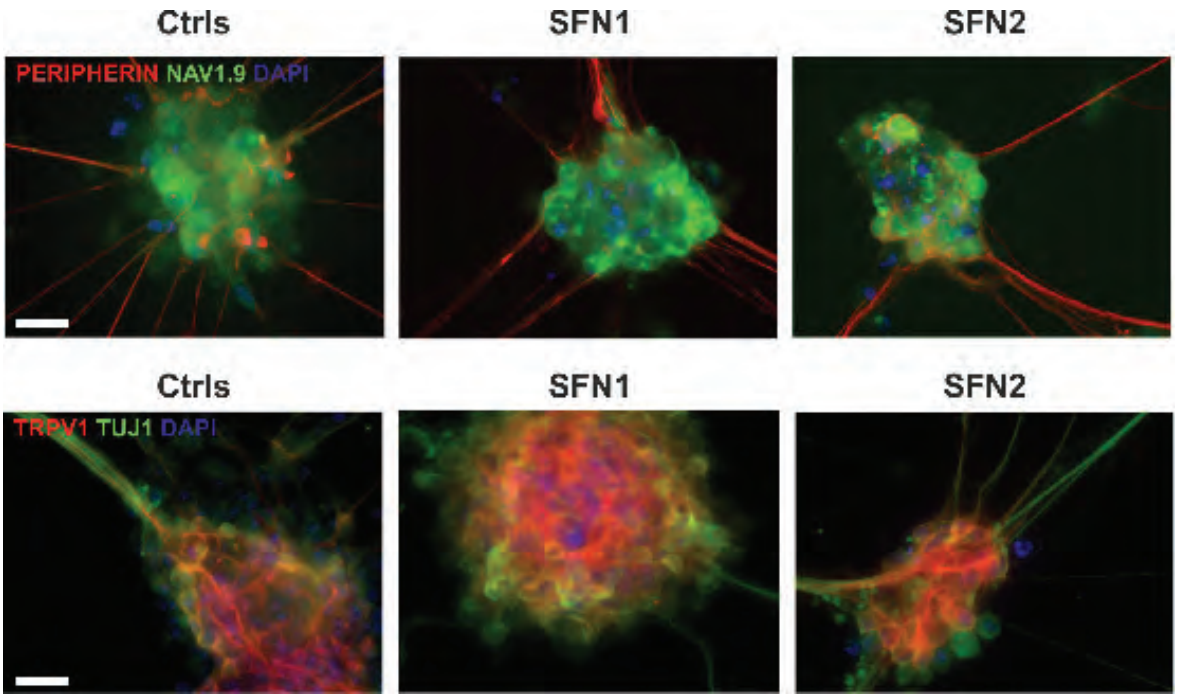
Generation of patient-specific IPSCs and differentiation into human nociceptors.

In order to investigate the functional relevance of *SCN11A* variants in a human system, we received skin biopsies from both patients and from two healthy controls upon informed consent. We isolated and reprogrammed fibroblasts into IPSCs by applying the retroviral Yamanaka-protocol. All IPSC lines expressed the pluripotency markers NANOG and OCT3/4 and exhibited 92.6 – 99.0% TRA1-60-positive cells by FACS-analysis. IPSCs were differentiated into human nociceptors by applying our previously described protocol. Nociceptors generated from both, SFN- and Ctrl-IPSCs, had ganglion-like cluster morphology and exhibited a comparable expression pattern of the peripheral neuron marker Peripherin. Almost all Peripherin-positive nociceptors also expressed Nav1.9 in IEM- and Ctrl-nociceptor clusters. mRNA expression of the canonical peripheral Navs including Nav1.9 in nociceptors was not different between patients and controls. Furthermore, the TUJ1-positive cells within clusters were positive for the nociceptor-specific marker TRPV1, consistent with comparable expression of TRPV1 mRNA levels.



Exemplary immunostaining for pluripotency markers and exemplary FACS analysis for pluripotency marker TRA1-60.

Prof. Dr. Winner Prof. Dr. Schüttler



Expression of peripheral neuronal markers shown by Immunostaining for nociceptor specific markers PERIPHERIN, Nav1.9, TRPV1 and TUJ1.

SFN1- and SFN2-nociceptors show substantial differences in excitability.

We performed whole-cell current clamp experiments in order to assess the nociceptors' excitability. The intrinsic resting membrane potential was not changed in nociceptors of SFN1 and SFN2 compared to controls. 19.4% of the SFN1-nociceptors displayed temporary or sustained spontaneous action potential (AP) firing, whereas only 1.8% of the Ctrl-nociceptors did so. Unevoked APs were not present in SFN2-nociceptors. The significant hyperactivity of SFN1-nociceptors was further supported by exclusive tonic firing in response to stepwise injections of 500 ms-current pulses.

Clinical phenotypes and single C-fiber activity in microneurography parallel findings in patient-specific IPSC-derived nociceptors. In microelectrode array analysis lacosamide showed potential therapeutic effects on firing frequencies. Thus, patients' IPSC-derived nociceptors are a valuable tool to study sodium channel mutations in a personalized setting.

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Publications during funding period

none

E26 - Progress Report

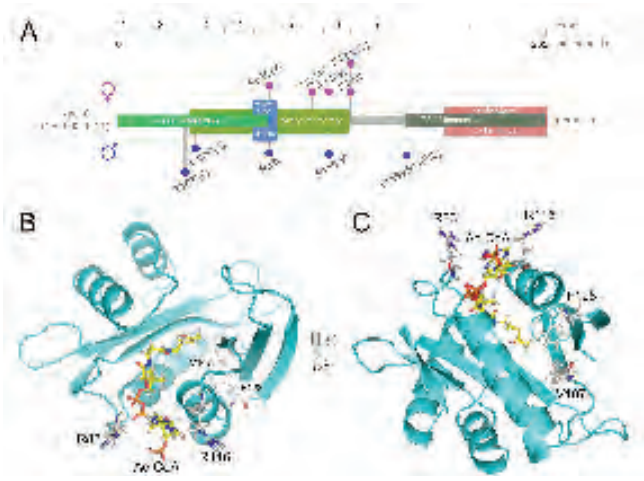
01.03.2016 - 31.08.2018

Genetics and pathomechanisms of intellectual disability with microcephaly

PD Dr. Christiane Zweier, Institute of Human Genetics

Mutations in genes from the same pathway often result in overlapping clinical phenotypes. Thus, co-morbidity of postnatal microcephaly with intellectual disability (ID) can indicate a genetic defect affecting neuronal migration, apoptosis or dendrite and synapse formation. We aim at the identification of novel, underlying genes in a group of patients with postnatal microcephaly and ID and to characterize their roles and interactions within common pathways and biological processes.

Intellectual disability (ID) occurs with a frequency of 2-3% and is clinically and genetically extremely heterogeneous with an unsolved etiology in many cases. Microcephaly is frequently noted in patients with developmental disorders, thus suggesting overlapping pathomechanisms. The aims of this project include the identification of novel ID-microcephaly genes, characterization of the clinical and mutational spectrum, functional characterization of the underlying gene/protein and investigation of shared pathomechanisms with other genes/proteins.

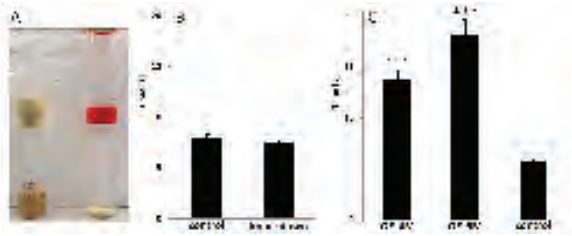


A: The NAA10 protein and localization of mutations in affected males (blue) and females (red). B, C: NAA10 homology model highlighting recurrently mutated Arginine 83 and 116 in the Ac-CoA binding pocket and Phe128 with its side chain pointing towards the hydrophobic core. (figure from Saunier et al., Hum Mutat, 2016)

De novo NAA10 mutations in females

N-terminal acetylation is a common protein modification in eukaryotes associated with numerous cellular processes. Inherited mutations in NAA10, encoding the catalytic subunit of the major N-terminal acetylation complex NatA have been associated with diverse, syndromic X-linked recessive disorders, whereas *de novo* missense mutations have been reported in one male and one female individual with severe intellectual disability but otherwise unspecific phenotypes. Thus, the full genetic and clinical spectrum of NAA10 deficiency is yet to be delineated. We identified three different novel and one known missense mutation in *NAA10, de novo* in 11 females, and due to maternal germ line mosaicism in another girl and her more severely affected and deceased brother. *In vitro* enzymatic assays for the novel, recurrent mutations p.(Arg83Cys) and p.(Phe128Leu) revealed reduced catalytic activity. X-inactivation was random in five females. The core phenotype of X-linked NAA10-related N-terminal-acetyltransferase deficiency in both males and females includes developmental delay, severe intellectual disability, postnatal growth failure with severe microcephaly, and skeletal or cardiac anomalies. Genotype-phenotype correlations within and between both genders are complex and may include various factors such as location and nature of mutations, enzymatic stability and activity, and X-inactivation in females.

PD Dr. Zweier



A: Negative Geotaxis: innate behaviour to crawl up after being tapped down. Measuring time until 7/10 flies reach the red line allows assessment of gross neurological function. B: Knockdown of the Rho GTPase in motoneurons does not show an effect compared to control, while C: overexpression with two different lines results in severe locomotor defects.

Identification and characterization of a new candidate gene

By in-house trio exome sequencing and world-wide collaboration we identified and collected several patients with *de novo* missense mutations in a gene encoding a member of the Rho GTPase family, which has not yet been linked to ID. Patients consistently display severe ID, sometimes developmental regression, epilepsy and microcephaly. The mutations cluster in a domain region of the protein, and we hypothesize that they might result in accumulation of the protein due to reduced ubiquitination. We established an expression vector of the gene plus three different mutant vectors and are currently following up our hypothesis by cell culture based assays such as transfections with and without proteasome inhibitor, immunofluorescence and co-immunoprecipitation assays with a known binding partner. In accordance with our hypothesis we observed that fruitflies have severe defects in gross neurological function upon overexpression but not upon knockdown of the gene.

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Invited lectures

International Scientific Symposium on Syndromic Autism, 27.11.2016, Kehl-Kork, Genetic aspects of syndromic autism and developmental disorders

Publications during funding period

Smogavec M, Cleall A, Hoyer J, Lederer D, Nassogne M-C, Palmer EE, Deprez M, Benoit V, Maystadt I, Noakes C, Leal A, Shaw M, Gecz J, Raymond L, Reis A, Shears D, Brockmann K, Zweier C (2016) Eight further individuals with intellectual disability and epilepsy carrying bi-allelic CNTNAP2 aberrations allow delineation of the mutational and phenotypic spectrum. J Med Genet 53: 820-27

Saunier C, Støve SI, Popp B, Gérard B, Blenski M, AhMew N, de Bie C, Goldenberg P, Isidor B, Keren B, Leheup B, Lampert L, Mignot C, Tezcan K, Mancini GM, Nava C, Wasserstein M, Bruel AL, Thevenon J, Masurel A, Duffourd Y, Kuentz P, Huet F, Rivière JB, van Slegtenhorst M, Faivre L, Piton A, Reis A, Arnesen T, Thauvin-Robinet C, Zweier C (2016) Expanding the Phenotype Associated with NAA10-Related N-Terminal Acetylation Deficiency. Hum Mutat 37: 755-64

E27 - Progress Report

01.03.2016 - 31.08.2018

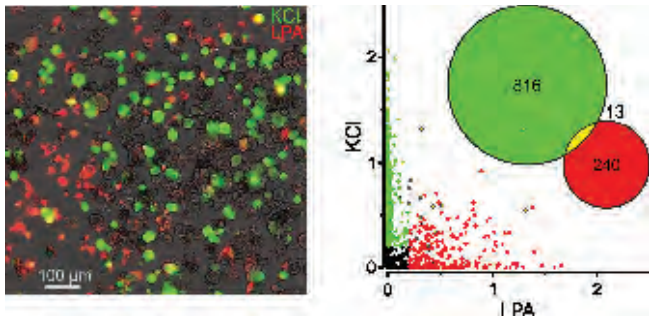
Lysophosphatidic acid-induced pruritus of cholestasis

Dr. Dr. Andreas Kremer, Department of Medicine 1 - Gastroenterology, Pneumology and Endocrinology
Prof. Dr. Michael Fischer, Institute of Physiology and Pathophysiology (till 31.08.2016)

In cholestatic patients with chronic pruritus we previously found elevated serum levels of lysophosphatidic acid (LPA). The aim of this translational project is to unravel the molecular mechanisms of LPA in cellular assays and to understand the interaction with substances known to cause itch. This will be validated in an animal model and tested in preclinical human studies. Unravelling this pathway could open new avenues for causal anti-pruritic treatment strategies.

Unravelling the LPA-signalling axis between glia cells and sensory neurons

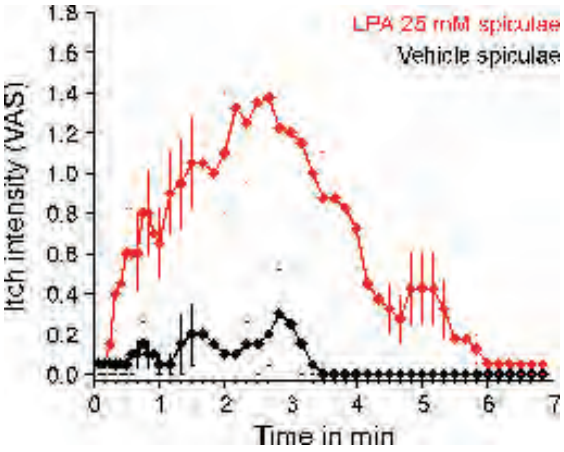
In cultures of dissociated sensory ganglia, responses to LPA and other widely used agonists were investigated. In pilot experiments, cells responsive to both LPA and capsaicin were rare, as shown by comparing the calcium time course of cells responding to either LPA or capsaicin and the inverse correlation between these responses ($R = –0.63$, $p < 0.001$, product-momentum correlation). Subsequently, this was investigated more thoroughly exposing cultures of dissociated sensory ganglia to LPA 1 µM, GSK1016790A 100 nM (TRPV4 agonist), CIM0216 3 µM (TRPM3 agonist), Carvacrol 100 µM (TRPA1 agonist), Capsaicin 200 nM (TRPV1 agonist) and KCL 60 mM. The time course of intracellular calcium indicated two distinct response patterns which also matched the cell phenotype. Only 1.4% (13 of 816) of the cells reacting to LPA were considered neurons based on their phenotype and response to KCl. The percentage of cells being classified as neurons and responding to both LPA and one of the signature ion channel agonists GSK1016790A, CIM0216, Carvacrol or Capsaicin was minimal. Responsiveness to LPA and potassium was inversely correlated ($r = –0.37$, $p < 0.001$, $n = 1237$, product-momentum correlation). Responses to potassium were smaller in neurons compared to SGCs ($p < 0.001$, t-test independent samples), but sufficient to not easily distinguish based on this criterion. In contrast, LPA differentiates the two populations more clearly.



LPA activates satellite glia cells but only 1.4% of neurons. Left panel: Overlay of the transmission image (greyscaled) and the response intensity to LPA (red) and to KCl (green). Right panel: Scatterplots of the ratio increase for every cell to LPA and Capsaicin.


Dr. Dr. Kremer


Prof. Dr. Fischer



LPA but not vehicle induced a mild itch sensation upon focal application via cowage spicules to the skin of healthy volunteers. Itch intensity was rated 0–10 on a numerical scale.

LPA-mediated activation of sensory neurons in healthy volunteers and cholestatic patients.

Oleoyl-LPA (18:1) was applied intradermally by insertion of LPA-loaded heat-inactivated cowhage spicules in healthy volunteers (N=18). In addition, we analyzed for the effect of intradermal injections of 50 µL LPA. Control applications were performed using histamine, capsaicin and the vehicle solution. Pain and itch intensities were quantified using a numeric rating scale with the range 0–10. LPA applied into the skin using cowhage spicules induced a mild itch sensation compared to vehicle control (mean ± SEM; 1.4 ± 0.4 vs. 0.3 ± 0.2; $p<0.001$) lasting for several minutes. Associated sensations such as burning or stinging were reported by a few volunteers. In contrast, intradermal injection of LPA caused a dose-dependent burning pain. In contrast to capsaicin, burning pain sensation occurred delayed. LPA hardly induced any flare reaction in comparison to histamine. LPA caused a sensitization to heat, whereas responses to cold, mechanical and electrical stimuli remained unaltered.

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Invited lectures

Hepatologie-Update, Hamburger Lebertage, Mai, Hamburg, “Juckreiz bei Lebererkrankungen – was kann man therapeutisch tun“

DGVS-Satellitensymposium, DGVS, September, Hamburg, „Neue Therapieoptionen für die PBC“

National PBC Day, Paris, “Prurit et CBP: des mécanismes pathophysiologiques aux perspective thérapeutique“

48. Tagung Aktuelle Gastroenterologie, Frankfurt, “Diagnostik und Therapie der autoimmunen Pankreatitis und Cholangitis“

Viszeralmed. Arbeitskreis, Universität Essen, „Hepatogener Juckreiz: Pathophysiol. und Therapie“

11. Kursus klin. Hepatologie, Hamburg, „IgG4-assoziierte Cholangitis: neue Tests und Therapie“

Österreichische Physiologische Gesellschaft, Graz, „Tissue acidosis-induced pain“

Physiologisches Kolloquium, RWTH Aachen, „Human sensors for tissue acidosis”

Awards

Best PhD thesis Award of the year 2015 to A.E. Kremer (Academic Medical Center, University of Amsterdam, 2016)

Publications during funding period

Günther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Amann K, Vandenabeele P, Linkermann A, Poremba C, Schleicher U, Dewitz C, Krautwald S, Neurath MF, Becker C, Wirtz S (2016) The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. J Clin Invest. 126(11): 4346-4360

He GW, Günther C, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S, Becker C (2016) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut. [Epub ahead of print]

Wunsch E, Krawczyk M, Milkiewicz M, Trottier J, Barbier O, Neurath MF, Lammert F, Kremer AE*, Milkiewicz P* (2016) *contributed equally; Serum Autotaxin is a Marker of the Severity of Liver Injury and Overall Survival in Patients with Cholestatic Liver Diseases. Sci Rep. 6: 30847

F3 - Progress Report

01.03.2014 - 28.02.2017

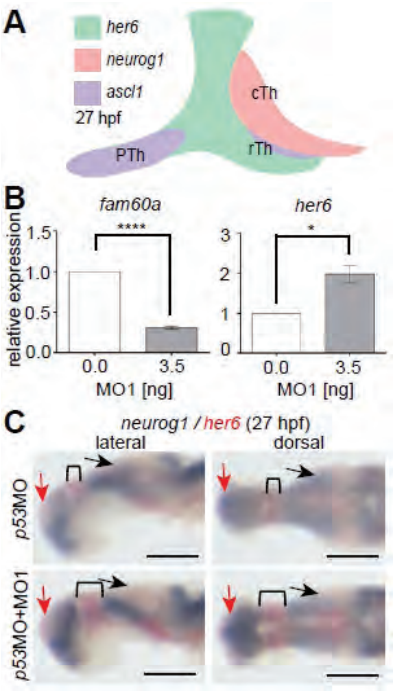
Fam60a in heart and brain development

Prof. Dr. Felix Engel, Department of Nephropathology

Neurodevelopmental disorders are the most common and disabling long-term complication of congenital heart diseases and thus the NHLBI stated "one of the most important challenges in the 21st century for CHD patients is to improve neurological deficits." The goal of this project is to better understand the function of genes that are co-expressed in brain and heart to contribute to the elucidation of this heart-brain connection. The main focus lies on fam60a, a member of the SIN3-HDAC complex.

In silico analyses combined with the analysis of mutants indicate that Fam60a contains a bipartite NLS.

Immunofluorescence analyses confirmed that FAM60A is a nuclear protein expressed in the neural tube, in dorsal root ganglia, and in the mouse embryonic heart. Whole mount in situ hybridization (WISH) in zebrafish detected fam60a mRNA expression in the brain primordium, otic vesicle, heart ventricle, and anterior part of the atrium.



fam60a knockdown leads to an expansion of her6 expression. Th: thalamus, P: pre, r: rostral, c: caudal. p53MO: control for unspecific MO-effects. Brackets: her6 expression. Red (increased), black (reduced) neurog1 expression. Scale bars: 200 µm.

fam60a knockdown disrupts brain development altering her6, neurog1, and ascl1b expression

Injection of two different morpholinos (MOs) targeting fam60a caused a severe brain phenotype with the formation of a hydrocephalus. Injection of fam60a mRNA rescued the hydrocephalus phenotype indicating that the MO-mediated brain phenotype is due to Fam60A depletion. qPCR experiments indicated that her6 expression is increased in morphants. WISH showed an expansion of her6-positive cells in the thalamus and a significantly reduced ascl1b expression in the midbrain and the prethalamus and no expression in the rostral thalamus. neurog1 expression was reduced in the caudal thalamus and increased in the telencephalon. In contrast, shh expression was not affected suggesting that the mid-diencephalic organizer itself is not perturbed. Importantly, injection of fam60a mRNA, but not fam60a mRNAΔNLS, along with MO was able to restore her6 expression in the thalamus. Taken together, our data indicate that nuclear Fam60A is required for the correct spatial expression of her6 to control the expression pattern of the pro-neural genes neurog1 and ascl1b in the mid-diencephalic organizer to drive formation of the rostral thalamus, the prethalamus and the caudal thalamus.

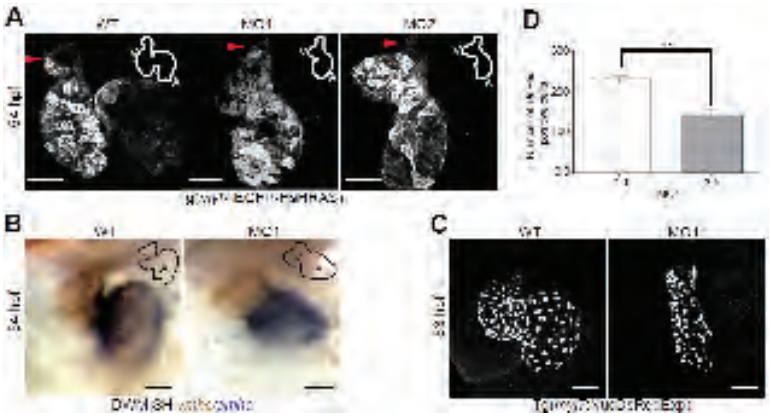
fam60a knockdown disrupts heart development

Morphant hearts were dysmorphic and not correctly looped. Hearts consisted of significantly fewer cardiomyocytes, ventricles were round-shaped and their arterial part towards the outflow tract (OFT) was missing. The OFT was mal-formed and the atrioventricular canal was less constricted and not any longer perpendicular to the blood flow.

Prof. Dr. Engel

fam60a TALEN-mediated mutants do not recapitulate the fam60a morphant phenotype

It has been shown that MOs can induce non-specific phenotypes. Thus, we have generated a fam60a mutant line utilizing TALENs introducing a 10 bp (69-78) deletion. Surprisingly, our fam60a mutant does not recapitulate the fam60a morphant phenotype. This might be due to compensatory mechanisms induced by the mutation (note, morphants do not induce compensatory mechanisms) or by alternative start sites downstream of the mutation. Deep sequencing experiments have not revealed compensatory mechanisms. Surprisingly, injections of MOs in fam60a mutant embryos still exhibited similar phenotypes. Currently, we analyze adult fam60a mutants for phenotypes.



fam60a morphant hearts were dysmorphic and not correctly looped. The ventricle (V) was significantly smaller and consisted of fewer cardiomyocytes and the outflow tract (red arrowhead) was mal-formed.

The actin binding protein Flightless I is essential for cardiac chamber morphogenesis and trabeculation

(collaboration with Didier Stainier, MPI for Heart and Lung Research)

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Invited lectures

International Symposium: “Erlangen Institute of Biomaterials: Inauguration of new building and facilities” and 3rd Erlangen Symposium on Biomaterials: “Challenges for the 21st Century”, 28.11.2016, Erlangen, Germany; “Cardiac tissue engineering”

Inauguration-Symposium of MURCE (Muscle Research Center Erlangen), 21.-22.07.2016, Erlangen, Germany; “Heart Regeneration”

100. Annual Meeting of the German Pathology Society, 19.-21.05.2016, Berlin, Germany; “Recombinant spider silk proteins for cardiac tissue engineering”

82nd Annual Meeting of the German Cardiac Society, 30.03.-02.04.2016, Mannheim, Germany; “Activation of PPAR-delta induces cardiomyocyte proliferation and rescues cardiac function after myocardial infarction”

20th German-American Frontiers of Science Symposium, 10.-13.03.2016, Potsdam, Germany; “Heart regeneration: from zebrafish to mammals”

3rd international symposium on “New Frontiers in Cardiovascular Research”, 07.-09.03.2016; “Challenges in cardiac regeneration based on cardiomyocyte proliferation”

The Faculty of Informatics and Center for Computational Medicine in Cardiology (CCMC) Seminar, 10.02.2016, Lugano, Switzerland; “From heart development to cardiac regeneration”

Publications during funding period

Cabrera-Fuentes HA, Aragones J, Bernhagen J, Boening A, Boisvert WA, Bøtker HE, Bulluck H, Cook S, Di Lisa F, Engel FB, Engelmann B, Ferrazzi F, Ferdinandy P, Fong A, Fleming I, Gnaiger E, Hernández-Reséndiz S, Kalkhoran SB, Kim MH, Lecour S, Liehn EA, Marber MS, Mayr M, Miura T, Ong SB, Peter K, Sedding D, Singh MK, Suleiman MS, Schnittler HJ, Schulz R, Shim W, Tello D, Vogel CW, Walker M, Li QO, Yellon DM, Hausenloy DJ, Preissner KT (2016) From basic mechanisms to clinical applications in heart protection, new players in cardiovascular diseases and cardiac theranostics: meeting report from the third international symposium on „New frontiers in cardiovascular research“. Basic Res Cardiol. 111(6): 69

Ferrazzi F, Bellazzi R, Engel FB (2015) Gene network analysis: from heart development to cardiac therapy. Thromb Haemost. 113(3): 522-31

F4 - Final Report

01.10.2013 - 30.09.2016

Pathogenesis of the short rib-polydactyly syndrome

PD Dr. Christian T. Thiel, Institute of Human Genetics

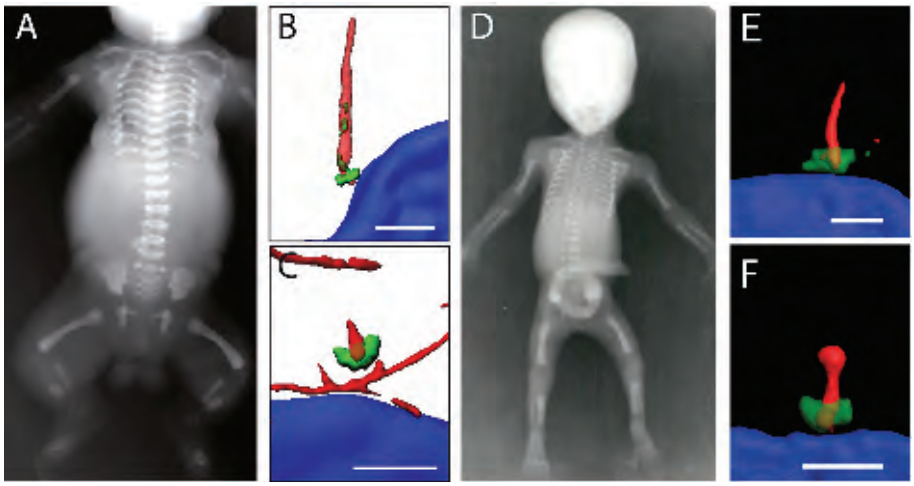
Defects of ciliogenesis have been implicated in a wide range of human phenotypes and play a crucial role in signal transduction and cell cycle coordination. We broadened the clinical spectrum of ciliary defects by identification and functional characterization of mutations in the genes NEK1, MAP4 and DYNC2LI1 associated with growth defects in human. By applying CRISPR/Cas9 and RNASeq we identified 257 genes involved in cilia maintenance and function in a NEK1 deficient cell line.

Expanding the phenotypic spectrum of NEK1 associated defects of ciliogenesis

The primary cilium is a nearly ubiquitous organelle of vertebrate cells. It detects extracellular stimuli to initiate intracellular transduction cascades (Hedgehog, Wnt, planar cell polarity, FGF, Notch, mTor, PDGF and the Hippo signaling pathways) playing an important role in differentiation, migration and proliferation during development. Several disorders have been associated with defects of a variety of proteins involved in cilia formation, maintenance and function. One entity, the short-rib polydactyly syndromes (SRPS), belong to the group of autosomal recessive osteochondrodysplasias, where we identified mutations in NEK1. NEK1 localizes to the basal body and absence of NEK1 leads to severely reduced cilia number and alters cilia morphology in vivo. The pleiotropic phenotype spectrum includes polydactyly, kidney cysts, skeletal abnormalities and further cilia specific morphologic alterations. Using exome sequencing in further *NEK1* mutation negative patients we identified and functionally characterized MAP4 and PIK3C2A as novel growth related genes.

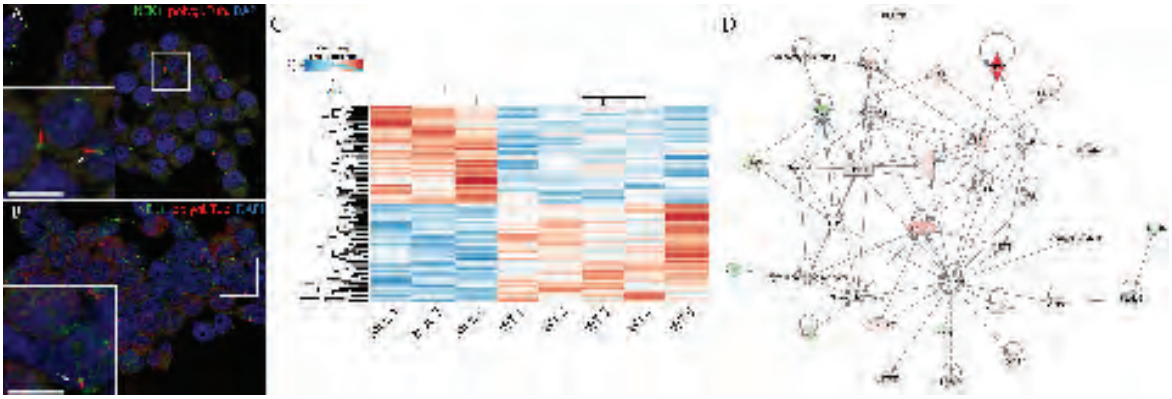
Identification and characterization of the NEK1 interaction partner DYNC2LI1

To explore the role of NEK1 in the context of the primary cilium we used a combined approach of Y2H with a mouse retinal cDNA library and exome sequencing in further patients. We identified 82 NEK1 interacting proteins of which 66 have yet not been associated with the primary cilium before. Further evidence came from the identification of mutations *DYNC2LI1* in 1 family with a SRPS phenotype. In immunofluorescence analysis and co-immunoprecipitation experiments with our custom made antibody we confirmed DYNC2LI1, a member of the retrograde flagellar transport complex dynein-2, as interaction partner of NEK1. DYNC2LI1 depleted cells showed a to NEK1 deficient cells comparable cilia morphology.



Radiographic and ciliary features of NEK1 and DYNC2LI1 patients. (A-C) Short-rib polydactyly type Majewski patient with *NEK1* mutation. (D-F) SRPS-like patient with *DYNC2LI1* mutation. (B, E) Normal cilium compared to (C, F) shortened patient cilia.

PD Dr. Thiel



Pathway analysis of CRISPR/Cas9 NEK1 depleted cells. Immunofluorescence presentation of (A) non target and (B) NEK1 targeted cells (C) Heat plot of differential expressed genes. (D) Enrichment in the Growth Hormone Signalling pathway (green: lower, red: higher expression).

In addition, we observed an accumulation of proteins at the ciliary tip reported in other osteochondrodysplasias. Thus, we expect to identify mutations in our novel ciliary genes in further patients.

NEK1 effect on ciliary signaling pathways

Expression experiments of genes encoding key members of the hedgehog, Wnt, and PDGF pathways proposed a compensatory up-regulation after starvation induced ciliogenesis. To further understand how NEK1 defects effect known signal transduction pathways and to explore effects on further pathways we established an NEK1 deficient cell line using the CRISPR/Cas9 system. Genome wide expression analysis under normal and starvation conditions revealed 257 significant differentially expressed genes. Computer assisted analyses showed an enrichment of genes in growth related pathways involved in *Growth Hormone, PDGF,* MAPK, Thrombopoietin and und EGF signaling. Here, further functional studies and analysis of patients might provide a global characterization of the roles of ciliary proteins in the manifestation of phenotypic features.

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Publications during funding period

Wheway G, Schmidts M, Mans DA, Szymanska K, Nguyen TM, Racher H, Phelps IG5, Toedt G, Kennedy J, Wunderlich KA, Sorusch N, Abdelhamed ZA, Natarajan S, Herridge W, van Reeuwijk J, Horn N, Boldt K, Parry DA, Letteboer SJ, Roosing S, Adams M, Bell SM, Bond J, Higgins J, Morrison EE, Tomlinson DC, Slaats GG, van Dam TJ, Huang L, Kessler K, Giessl A, Logan CV, Boyle EA, Shendure J, Anazi S, Aldahmesh M, Al Hazzaa S, Hegele RA, Ober C, Frosk P, Mhanni AA, Chodirker BN, Chudley AE, Lamont R, Bernier FP, Beaulieu CL, Gordon P, Pon RT, Donahue C, Barkovich AJ, Wolf L, Toomes C, Thiel CT, Boycott KM, McKibbin M, Inglehearn CF, UK10K Consortium, University of Washington Center for Mendelian Genomics, Stewart F, Omran H, Huynen MA, Sergouniotis PI, Alkuraya FS, Parboosingh JS, Innes AM, Willoughby CE, Giles RH, Webster AR, Ueffing M, Blacque O, Gleeson JG, Wolfrum U, Beales PL, Gibson T, Doherty D, Mitchison HM, Roepman R, Johnson CA (2015) An siRNA-based functional genomics screen for the identification of regulators of ciliogenesis and ciliopathy genes. Nature Cell Biology 17(8): 1074-87

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Zahnleiter D, Hauer NH, Kessler K, Uebe S, Sugano Y, Neuhauss SCF, Giessl A, Ekici AB, Blessing H, Sticht H, Dörr HG, Reis A, Thiel CT (2015) MAP4 dependent regulation of microtubule formation affects centrosome, cilia and Golgi architecture as a central mechanism in growth regulation. Human Mutation 36(1): 87-97

F5 - Progress Report

01.07.2016 - 31.12.2018

The Role of ANO1 in Polycystic Kidney Disease

Dr. Björn Buchholz, Department of Medicine 4

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a highly prevalent renal disorder which is characterized by continuous growth of multiple cysts in both kidneys often leading to end stage renal disease. We have shown that in vitro cyst growth depends on calcium-activated chloride secretion which is mediated by anoctamin 1 (ANO1). In this project we want to characterize the role of ANO1 in a PKD1 orthologous mouse model and its impact on ciliary calcium homeostasis of renal tubular cells.

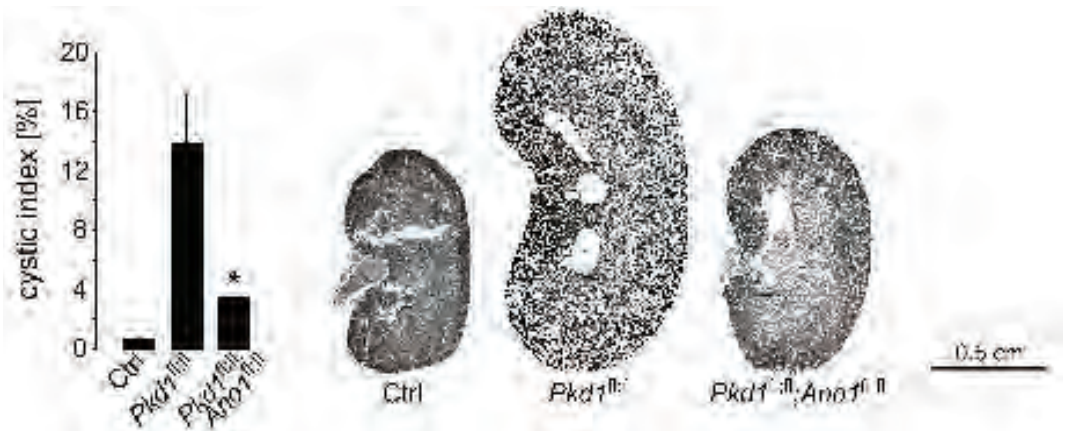
ANO1 promotes cyst growth in an orthologous PKD1 mouse model

Within the first 6 months of the funding period we were able to establish the PKD1 orthologous mouse model KSPCreER^{T2};PKD1$^{lox;lox}$ (kind gift from Prof. Peters, Dept. of Human Genetics, Leiden) and to cross them with floxed ANO1 mice (KSPCreER^{T2};PKD1$^{lox;lox}$;ANO1$^{lox;lox}$).

Tubule-specific deletion of PKD1 by application of tamoxifen at postnatal day 20 in KSPCreER^{T2};PKD1$^{lox;lox}$ mice, resulted in polycystic kidneys 8 weeks after induction which was represented by a cystic index (cystic area divided by whole kidney area) of ~14%. Double knockout of PKD1 and ANO1 in a preliminary set of experiments led to a significantly ameliorated phenotype represented by a cystic index of ~3.5%. However, since animal numbers are still low, this needs further investigation.

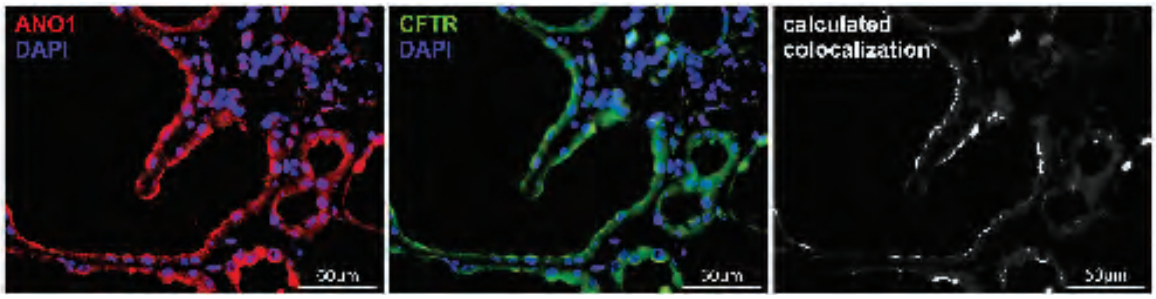
P2Y2R mediates ATP-dependent chloride secretion and cyst expansion

ANO1 gets activated by cytosolic increase of calcium. Accumulation of intraluminal ATP suggested that calcium increase might be mediated by apically localized purinergic receptors. We have shown that the purinergic receptor P2Y2R is localized in the apical cyst membrane of mouse and human polycystic kidneys. Stable knockdown of P2Y2R significantly reduced calcium-activated chloride conductance of cyst-forming epithelial cells in Ussing Chamber experiments and prevented *in vitro* cysts from secretion-dependent growth.



Tubule-specific deletion of PKD1 (*Pkd1$^{fl;fl}$*) causes polycystic kidneys. Deletion of ANO1 in addition to PKD1 (*Pkd1$^{fl;fl}$;Ano1$^{fl;fl}$*) results in a significantly milder cystic phenotype. Ctrl = control kidney.

Dr. Buchholz



ANO1 (red) is localized in the apical membrane of renal cysts and colocalizes with CFTR (green). White signals represent significant colocalization by the use of the algorithm by Laummonerie et al. and ImageJ (V1.50; NIH). DAPI = nuclei (blue).

Does ANO1 affect ciliary calcium homeostasis?

ANO1 is not only expressed in the apical membrane of renal tubular cells but also in the primary cilium. Since ciliary calcium concentration is elevated in comparison to the cytoplasm and this has been shown to be critical for cell differentiation, we wanted to test for an effect of ANO1 on ciliary calcium concentration. In collaboration with Prof. Kunzelmann and Prof. Schreiber (Dept. of Physiology, Regensburg) ciliary calcium could be reproducibly measured by the use of the calcium sensor 5HT6–mcherry–G-GECO1.0 in epithelial cells. Next, we want to test the impact of ANO1 deletion on ciliary calcium concentration.

ANO1 colocalizes with CFTR

Next to calcium-activated chloride secretion, cAMP-dependent chloride secretion via the cystic fibrosis transmembrane regulator (CFTR) chloride channel has been shown to be involved in cyst growth. Recent data by Prof. Kunzelmann revealed that CFTR conductance depends on co-expression of ANO1 on cellular level. In line with these findings, we found a strong colocalization of ANO1 and CFTR in the PKD1 mouse model.

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Invited lectures

"Cyst Secretion" at TRENAL Summer School, 2016/07/07, Erlangen, "From Physiology To Clinical Application"

TranCYST Mini Symposium, 2016/09/09, Brussels, Belgium, "The impact of hypoxia on cyst progression in PKD"

Publications during funding period

Kraus A, Grampp S, Goppelt-Struebe M, Schreiber R, Kunzelmann K, Peters DJ, Leipziger J, Schley G, Schodel J, Eckardt KU, and Buchholz B (2016) P2Y2R is a direct target of HIF-1α and mediates secretion-dependent cyst growth of renal cyst-forming epithelial cells. Purinergic Signalling. 12(4): 687-695

F6 - Progress Report

01.07.2016 - 31.12.2018

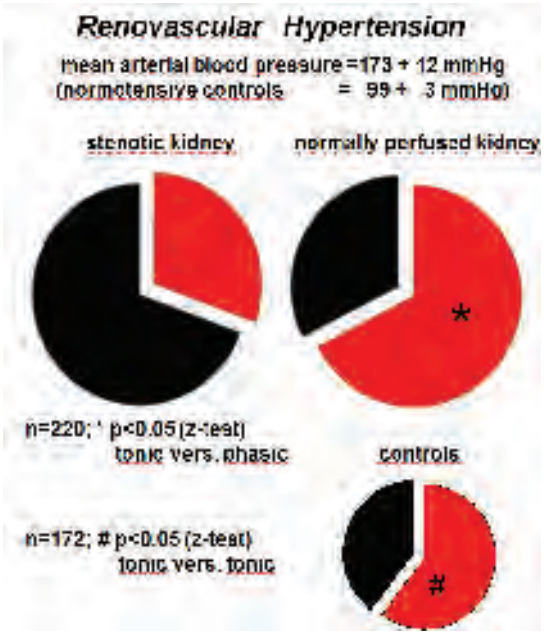
Renal afferent nerve activity - sympathoinhibitory or sympathoexcitatory?

Prof. Dr. Roland Veelken, Department of Medicine 4
Prof. Dr. Kerstin Amann, Department of Nephropathology

The renal afferent innervation is likely involved in the control of sympathetic nerve activity in hypertension and cardiovascular disease. Since afferent nerves from the kidney are difficult to investigate in vivo a cell culture model for respective neurons was developed. The main hypothesis of the project suggests that these afferent nerves exert a sympathoinhibitory effect on central sympathetic outflow in the healthy organism that is lost under pathophysiological conditions.

Confirmation of preliminary results

Preliminary results had suggested that in the model of renovascular hypertension, in which one renal artery is stenotic, cultured neurons from dorsal root ganglia with axons from the kidney exhibited a significant lower amount of highly active so-called tonic axons in neuronal samples with connections to the stenotic kidney (the number of less active so called phasic neurons increased respectively). However, in cultured neurons with axons from the normally perfused kidney the amount of highly active tonic neurons was not different from normal controls without renovascular hypertension. This is a first piece supporting the main hypothesis of the project in that afferent renal nerves exert a sympathoinhibitory effect on efferent sympathetic outflow. In this respect these results support furthermore the idea that neurogenic signals from the stenotic kidney are involved in the development of renovascular hypertension.



Proportion of tonic and phasic neurons in samples from stenotic and normally perfused kidneys in renovascular hypertension and controls. Proportion of tonic neurons from kidneys without stenosis and controls not different from one another: hence high blood pressure did not alter response patterns.

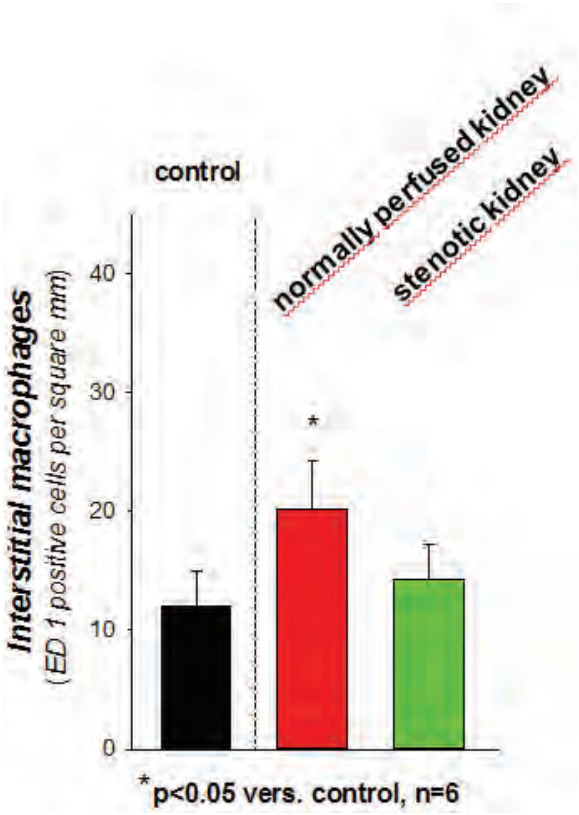

Prof. Dr. Veelken


Prof. Dr. Amann

Renal Afferent Nerves - What are Primary Stimuli?

The conclusion that neurons with renal projections from stenotic kidneys are less active in the production of action potentials upon respective stimulation does not provide clues why these investigated neurons related to the kidney with a renal artery stenosis were less active. In a further series of comparable experiments in rats suffering from experimental nephritis, cultured neurons with axons from the kidneys *in vivo* exhibited also a significantly decreased number of highly active so-called tonic axons. At the same time, the nephritic kidneys exhibited signs of inflammation e.g. an increased number of interstitial and glomerular macrophages. Hence, a first subsequent hypothesis was that the activity of renal afferent neuronal units is primarily altered in the presence of inflammatory processes.

However, when investigations were conducted to determine signs of inflammation in kidneys suffering from renovascular hypertension, the results surprisingly showed increased interstitial and glomerular macrophages in both kidneys, but macrophage infiltration was significantly higher in the non-stenotic kidney exposed to high blood pressure as compared to the stenotic kidney. Since the non-stenotic kidney was the one, whose corresponding neurons linked to renal tissue via respective axons were not responding differently to neurons from normal animals upon stimulation, this can only mean that - in contrast to our hypothesis - inflammation *per se* may be less important for the decreased activity pattern of afferent renal neurons seen in our experiments. High blood pressure alone was also not able to alter activity of neurons with renal axons. Rather altered perfusion occurring in a clipped kidney with renal stenosis and likely also present in nephritis could be of pivotal importance.



Rats with renovascular hypertension exhibited mainly in the interstitium of kidneys with normal perfusion signs of renal inflammation. In stenotic kidneys infiltration of ED1 positive cells was not even different from controls in this area. Comparable pattern seen in glomeruli.

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Awards

1st Price, Moderated Poster Discussion, PD Dr. Tilmann Ditting, 26th Scientific Meeting of the International Society of Hypertension, September 24-29, 2016, Seoul, Korea

Publications during funding period

none

Junior Groups / Projects

Junior Groups / Projects

Progress and Final Reports

146

Junior Research Group 1

Dr. Paolo Ceppi

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Biographical Sketch

The Junior Group Leader started his appointment at the Interdisciplinary Center for Clinical Research (IZKF), Friedrich-Alexander University Erlangen-Nürnberg in Erlangen on August 1st, 2015. Below is a list of the previous reseach employements:

Mar 2011 – Jun 2015 Postdoctoral fellow at the Division of Hematology/Oncology, Feinberg School of Medicine, Robert H. Lurie Comprehensive Cancer Center Northwestern University, Chicago, USA (Prof. M. Peter).

Feb 2009 – Dec 2009 Visiting PhD student at the Department of Experimental Surgery and Molecular Oncology of Solid Tumors, Medical Faculty Mannheim, University of Heidelberg and DKFZ Heidelberg, Germany (Prof. H. Allgayer).

Jan 2007 – Dec 2010 PhD student in the Pathology Division of the Department of Clinical and Biological Sciences, University of Turin, Italy (Prof. M. Papotti).

Jul 2004 – Dec 2006 Research assistant at Thoracic Oncology Unit and the Pathology Division of the Department of Clinical and Biological Sciences, University of Turin, Italy (Prof. G. Scagliotti and Prof. M. Papotti).

Dec 2004 – Jun 2005 Visiting Research scholar at Department of Biochemistry and Molecular Biology, Norris Cancer Center, University of Southern California, Los Angeles, USA (Prof. P. Danenberg). Training at ResponseGenetics Inc. Los Angeles, USA (Dr. K. Danenberg).

Mar 2002 – Jul 2004 Research student at the Department of Genetics, Biology and Biochemistry, University of Turin, Italy (Prof. F. Malavasi).

From the left: Maria Eleni Vazakidou, Aarif Siddiqui, Annemarie Schwab, Paolo Ceppi

Research Focus

The focus of the Junior Group 1 is „Understanding the plasticity of cancer cells“.

Background and Rationale: Despite the progresses made in the last years with the development of novel molecularly targeted agents, cancer is still a very deadly disease. This could be attributable to several aspects, including the fact that only a minority of selected patients benefit from the novel compounds (such as those targeting oncogenic drivers like EGFR, BRAF, HER2 and many others), while poor therepeutcal options are available for the vast majority of the patients in which a targetable driving oncogenic mutation is undetermined. Moreover, the pathway redundancy and the very frequent occurrence of mutations limit the efficacy of these drugs even in potentially responding patients. There is therefore an urgent need for the identification of novel fundamental mechanisms of cancer biology and of relevant determinants of chemoresistance in order to develop more effective drugs and therapeutic strategies. The recent discovery of epithelial-to-mesenchymal transition (EMT), cancer stem cells (CSCs) and of their functional association and interdependence represent some of the most promising advances in the last two decades of cancer research. CSCs are defined as a subpopulation of undifferentiated cancer cells with stem-like features responsible for tumors' heterogeneity and for some of the most lethal features of cancers: tumorigenicity, metastatic spread, relapse and chemoresistance. The interconversion between CSCs and non-CSCs has been recently reported and the EMT clearly functionally involved. The EMT is a de-differentiation process frequently observed in cancers with increased invasive potential and drug resistance. A recently emerging concept is that the plasticity of cancers is greater than what initially hypothesized, and therefore a better understanding of the mechanisms behind the inter-conversion of cancer cells between differentiation stages may have many therapeutic implications. Moreover, cancers, and the CSC population in particular, are highly dependent on aerobic glycolysis, which they use as a major pathway for biosynthesis. The enhanced rate of glycolysis occurs largely because of the increased demand of a transformed cell for macromolecule components (the so-called Warburg effect). The connection between increased glycolytic rate, EMT and CSCs has recently started to emerge in the literature, but the molecular determinants involved are still undefined. Understanding the metabolic pathways associated with EMT and CSCs could provide new important insights in the biology of cancer, leading to the identifications of novel targets for therapeutic intervention.

Aim of the research: The Junior Group aims at identifying novel fundamental mechanisms and molecular determinants that regulate the plasticity and the aggressiveness of cancer cells and at studying the association between cancer differentiation and sensitivity to chemotherapy. We are particularly interested in studying the metabolic changes associated with EMT and CSCs and in understanding how to possibly interfere with these pathways to reduce the aggressiveness and the lethality of cancer cells (in terms of growth, metastasis development and intrinsic and acquired chemoresistance). The Group uses several cell and molecular biology techniques, mouse models, and the analysis of human samples as well as by –omics and high-throughput approaches.

Third-party funding

Dr. Paolo Ceppi, Deutsche Krebshilfe, Determination of the role of aldose reductase AKR1B1 and associated pathways in epithelial-to-mesenchymal transition and cancer stem cells, 07/2017 – 06/2020

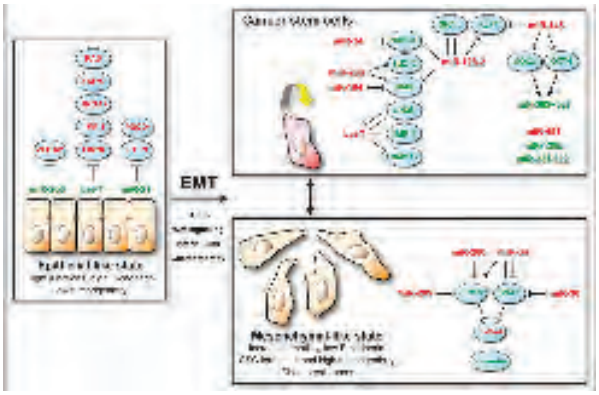
N1 - Progress Report

01.08.2015 - 31.07.2021

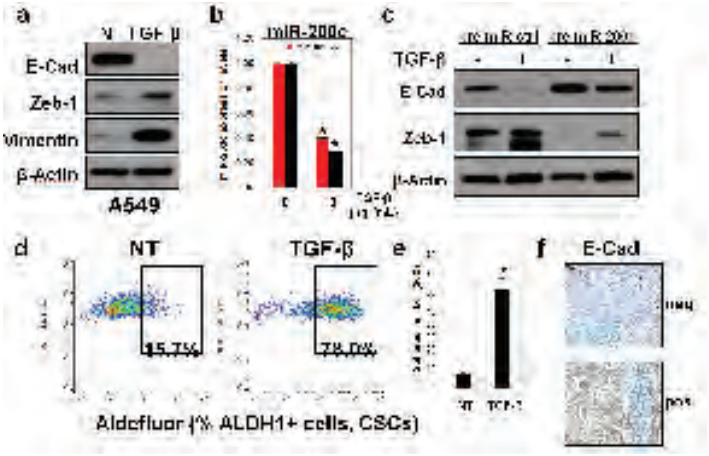
Understanding the plasticity of cancer cells

Dr. Paolo Ceppi, IZKF - Junior Research Group 1

The group focuses the identification of novel fundamental mechanisms of cancer biology using several cell and molecular biology techniques, mouse models, high-throughput approaches and the analysis of human samples. We aim at discovering novel genes and molecular pathways that regulate the plasticity and the aggressiveness of cancer cells and at studying the association between cancer differentiation and sensitivity to chemotherapy, with a special attention on metabolism genes. This may lead to the development of more effective drugs and therapeutic strategies.



Schematic illustrating the connection between epithelial-to-mesenchymal transition (EMT) induction and cancer stem cells (CSCs) formation. miRNAs can contribute to these alterations by targeting key-components of differentiation pathways (from Ceppi P. Oncogene, 2013).



Impact of miRNA-200 in an *in vitro* model of EMT/CSCs. a-c) EMT induction by TGF-beta in A549 lung cancer cells is accompanied by loss of miR-200c. d-e) TGF-beta induces stemness and the activity of the CSC marker ALDH1. f) Different E-Cadherin expression patterns are found in lung cancer patients.

Dr. Ceppi

During the first year of activity, the Junior Group N1 has completed the establishment in in the location of the ground floor of the Nikolaus-Fiebiger-Zentrum in Erlangen. All the major equipment required for the development of the projects has been acquired during the year. The Junior Group is composed of two PhD students: Mr. Mohammed Aarif Siddiqui and Ms. Annemarie Schwab. Moreover, a postdoctoral fellow, Dr. Maria Eleni Vazakidou, has joined the group in February 2016. During the course of the year, two additional master students have temporarily joined the group and the activities of the lab as research assistants: Ms. Francesca Napoli from University of Torino, Italy (from January to November) and Ms. Cristina Fernandez Molina, from the University of Granada, Spain (from July to September).

Current work of the group: As outlined above, the main area of research of the Junior Group is the metabolism of cancer cells and we are currently working on the identification of metabolic genes with a role in cancer plasticity, EMT and CSCs. By the use of bioinformatic approaches on large data sets, we have identified a list of candidate metabolic genes highly correlating (positively and negatively) with the expression of CSC and EMT markers in cancer cells. These candidate molecules have also been screened for their correlation with CSCs and EMT markers in tissues from patients with cancer of different origin (all solid malignancies) using molecular signatures available in the literature and very recently published by others. The group is currently working on the validation and assessing the functional relevance of some of the most interesting candidate genes identified (for instance those related to the metabolic energy pathways) in a panel of cancer cell lines *in vitro*. The plan for the next months will also include the test of the expression and the role of these candidate metabolic genes in tissues of cancer patients and in mouse cancer models.

During the first year, we have also worked on the establishment of internal and external collaborations. Internal collaborations have been established, among others, with the groups at the Experimental Medicine I, at the Department of Internal Medicine 5, at the Department of Medicine 1, and with researchers at the Molecular Pneumology Department. External collaborations have been established during the course of the year, among others, with researchers at the Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt am Main, with the University of Turin, Italy and with the University of Leeds, United Kingdom.

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Publications during funding period

none

Junior Research Group 2

Dr. David Dulin

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Biographical Sketch

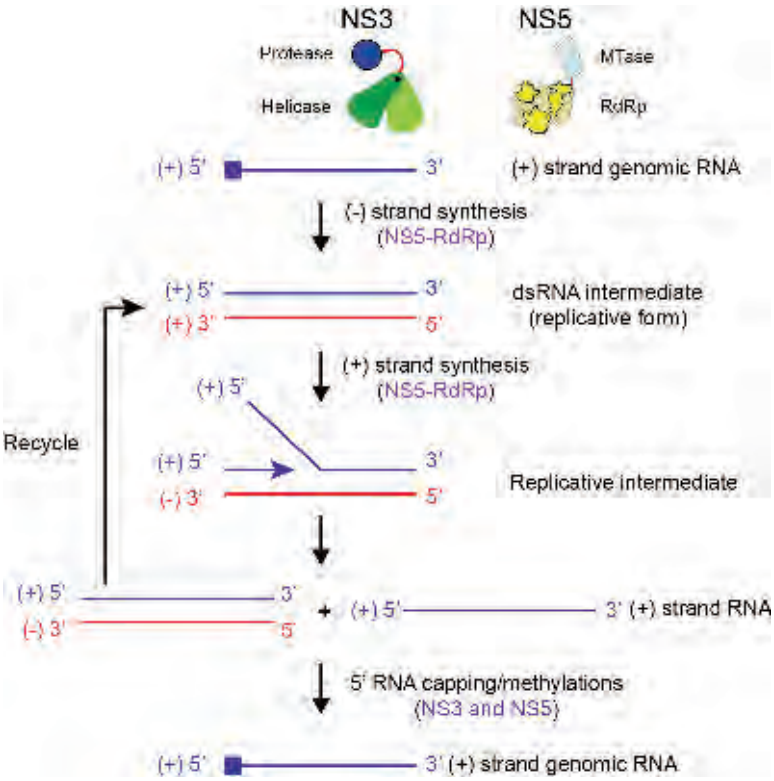
Since September 2016, Dr. Dulin has started the "Physics and Medicine" IZKF Junior Research group N2 at Erlangen, aiming at studying human virus replication dynamics with biophysical techniques.

Before starting his lab, Dr. Dulin graduated his Bachelor in physics and mathematics at the Université de Bordeaux (France) in 2004 and the interdisciplinary Master "Laser, Matter and Nanoscience" in 2006. During his master, he got his first introduction to biophysics by studying transmembrane neuroreceptor diffusion dynamics using single-particle tracking in the laboratory of Prof. B. Lounis.

Between 2006 and 2009, he did his PhD thesis in the Laboratoire Charles Fabry de l'Institut d'Optique in the group of Prof. A. Aspect and under the supervision of Prof. N. Westbrook. There, he worked at establishing a new biophysics lab, with a focus on bacterial ribosome translation kinetics using single-molecule fluorescence microscopy.

He moved then to his first postdoctoral position in the lab of Prof. N. Dekker at TU Delft (The Netherlands) until August 2014. There, he developed new magnetic tweezers approaches for high throughput and high spatiotemporal resolution study of polymerases and helicases. In particular, he studied the mechanism of error incorporation in RNA virus replication, essential in virus evolution.

From August 2014 to June 2016, he performed a second postdoc in the lab of Prof. A. Kapanidis at the University of Oxford (UK), where he studied bacterial transcription initiation dynamics using single-molecule FRET.



Viral RNA replication of Flavivirus by NS3 and NS5 proteins.

Research Focus

RNA viruses are amongst the most deadly human pathogens, including the Ebola virus, Influenza virus, and Hepatitis C virus. Within the RNA virus family, the flavivirus genus is considered the most emerging or resurging group of viruses, capturing headlines as West Nile virus (WNV), Zika virus (ZV) and Dengue virus, to cite only a few. The prevalence of flaviviruses has increased dramatically in the last twenty years, with millions of infection cases every year across the world. However, no antiviral drug treatment, and a limited number of vaccines are available against flavivirus infection. The most promising approach as therapeutics comes from the development of drugs that target specifically the flavivirus replicase complex. Flaviviruses have a ~10 kilobases (kb) positive (+) single stranded (ss) RNA genome that is replicated by two proteins forming a complex: the protease-helicase NS3 and the methyltransferase (MTase)-RNA dependent RNA polymerase (RdRp) NS5, both highly conserved enzymes within the flavivirus genus. The flavivirus replicase is the key element for virus survival, being in charge of synthesizing all the various forms of viral RNA during the virus life cycle and, simultaneously, adding diversity to the viral population, allowing the virus to adapt to the host immune system by controlling the rate of mutations in the genome. The NS3-NS5 complex also performs RNA capping on the 5'-end, a process essential for host immune defense evasion and mRNA translation. All this makes replication and capping important drug targets. The knowledge gap in flavivirus NS3 and NS5 enzymatic activities originates on the one hand from the lack of studies on the full-length proteins, either individually or in association, although NS5-MTase stimulates NS5-RdRp, and preliminary data show that NS3 stimulates NS5 and NS5 stimulates NS3. Overall, a clear understanding of how this multi-subunits replicase complex coordinates the replication and the capping of the viral genome is still lacking. On the other hand, assays that can perform high-resolution measurement on ~kb long templates are necessary to complement the current arsenal of biochemical technique that uses short RNA templates (~10 nt), given the length of the flavivirus genome (~10 kb). New approaches are thus necessary to tackle this multidimensional problem.

Here, the Junior Research Group N2 will use single-molecule approaches, e.g. magnetic tweezers and Fluorescence Resonance Energy Transfer (FRET), to investigate the formation of the flavivirus replicase complex and its dynamics in replication. We will unravel the replication dynamics of the replicase of WNV and ZV using the high spatiotemporal resolution and unprecedented statistical power of magnetic tweezers using ~kb long RNA templates. We will also aim at defining the mechanism of assembly and the conformational dynamics of the complex formed by NS3 and NS5 during early RNA synthesis.

Since September 1st 2016, the Junior Research Group N2 has been settling the activity on the Kussmaul Campus at the OICE building. Dr. Flavia Stal-Papini has been hired as a research assistant in October 2016 and we are currently hiring PhD students to assist the lab in its research effort. So far, we have installed a molecular biology laboratory, where we can prepare the nucleic acid scaffolds used in our experiments, and a microscopy laboratory, where we have built up two custom magnetic tweezers apparatuses.

Third-party funding

No third-party funding.

Molecular biology lab and microscopy lab.

Junior Research Group 3

Prof. Dr. Beate Winner

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Biographical Sketch

Dr. Winner studied Medicine in Regensburg, Würzburg and Toronto from 1992 to 1999. Her MD thesis was carried out at the Department of Medicine, University of Würzburg. From 1999 to 2007 she worked at the Department of Neurology, University of Regensburg (board examined neurologist 2005). The clinical focus was neurodegenerative diseases. Her postdoctoral training was performed in the Neuroregeneration Laboratory of the Department of Neurology with Profs. G. Kuhn, J. Winkler, and L. Aigner. After completing the postdoctoral lecturer qualification in neurology in 2007, she joined the Laboratory of Genetics (Prof. FH Gage) at the Salk Institute, La Jolla as a Feodor-Lynen fellow. Dr. Winner started her own laboratory in Erlangen as head of the IZKF junior research group 3: *Modeling neurodegenerative diseases using stem cells* in October 2010. In addition, she was awarded a BMBF research group neuroscience in 2011 and an associated junior group within the BioSysNet consortium in 2012. She was the first laboratory at the FAU Erlangen-Nürnberg to introduce human induced pluripotent stem cell technology. Prof. Winner was appointed W2 professor for *Stem Cell based Models of rare neural Diseases* in March 2016 at the Institute of Human Genetics (Head Prof. Reis), Universitätsklinikum Erlangen, FAU Erlangen-Nürnberg.

From the left: Dr. Francesc Perez-Branguli, Daniela Gräf, Annika Sommer, Marius Brazdis (at the back), Tania Rizo (in the middle), Sandra Loskarn (in the front), Katrin Simmnacher, Himanshu Mishra, Dr. Iryna Prots, Prof. Beate Winner, Holger Wend

Research Focus

NEURODEGENERATION IN STEM CELL-BASED MODELS

Neurons in the central nervous system (CNS) are only taken for biopsy under rare conditions and previously our understanding about disease-related neuronal phenotypes in humans was mainly derived from analyzing *postmortem* brain tissues. This inability to sample live brain cells limited our knowledge of human neuropathological abnormalities during the course of neurodegenerative diseases. Therefore, stem cell derived human neurons represent a means of exploring patient-specific pathological mechanisms and test individualized therapeutic interventions. The aim is to use these individualized induced pluripotent stem cell derived models as read-out systems for testing of small compounds and the reversibility of cellular phenotypes and eventually go back to the patients. Within the Universitätsklinikum Erlangen, the human induced pluripotent stem cell technology is able to bridge basic and translational research. We receive patients' somatic cells (e.g. blood, fibroblasts) from clinicians and then turn these into induced pluripotent stem cells and from there into the cell type of interest (mostly neural cells). The research focus is to define disease phenotypes of neurodegenerative diseases using stem cell based in vitro neuronal models. We started by comparing controls and patients with monogenic motor neuron diseases called hereditary spastic paraplegias and synucleinopathies. More recently we started to target monogenic forms of cognitive dysfunction disorders, pain, and multifactorial diseases. We are specifically interested in understanding connectivity of neurons, at the level of synaptic function, cellular excitability, and axonal transport.

Third-party funding

Steven Havlicek, Bayerische Forschungsstiftung, Modeling familial motor-neuron disease by the use of human induced pluripotent stem cells (hiPSCs), 2010-2011.

Beate Winner, BioSysNet, Transcriptome analysis to delineate genes involved in synaptic dysfunction in synucleinopathies. 2012-2017.

Beate Winner, BMBF, Disease modeling and target identification of motor neuron disease using induced pluripotent stem cells. 2011-2018.

Iryna Prots, ELAN, Distinct alpha-synuclein species interfere with neuronal transport mechanisms. 2011-2012.

Martin Regensburger, ELAN, Neuroprotective role of EFhd2 (swiprosin-1) in neuronal development and neurodegeneration. 2014.

Beate Winner, Zacharias Kohl, Jürgen Winkler, ForIPS, Forschungsverbund Induzierte Pluripotente Stammzellen.

TP1: Zentralprojekt ForIPS: humane Induzierte pluripotente Stammzellen. 2013-2017.

Iryna Prots, Beate Winner, ForIPS, Forschungsverbund Induzierte Pluripotente Stammzellen.

TP11: Humanes in vitro Modell für Neuroinflammation. 2013-2017.

Angelika Lampert, Beate Winner, Johannes und Frieda Marohn-Stiftung, Neuronale Differenzierung von peripheren Neuronen aus humanen induzierten pluripotenten Stammzellen (hiPSC). 2013-2015.

Beate Winner, Zacharias Kohl, Jürgen Winkler, Tom-Wahlig Stiftung, Individualized human in vitro model for hereditary spastic paraplegia. 2009-2016.

Beate Winner, Jürgen Schüttler, IZKF N25, Modeling pain syndromes using human induced pluripotent stem cell-derived nociceptors. 2016-2019.

Beate Winner, DFG, Subproject GRK2162, Examining the impact of the hereditary spastic paraplegia gene SPG11 on neuronal development and maintenance. 2016-2020.

Beate Winner, Florian Krach, Stefan Aigner, BaCaTec, TDP-43 pathology and cellular phenotypes in genome-engineered and iPSC-derived neurons of patients with ALS. 2016-2017.

Iryna Prots gemeinsam mit Wei Xiang, Marohn Stiftung Alpha-Synuclein aggregation in dopaminergic neurons 2016-2017.

Martin Regensburger, Initiative Therapieforschung ALS Wuppertal, Reprogrammierung humaner induzierter pluripotenter Stammzellen (iPS) von isogenen Zwillingen mit Mutationen in ALS5/SPG11/KIAA18402016, 2016-2017.

N3 - Final Report

01.10.2010 - 30.09.2016

Modeling neurodegenerative diseases using stem cells

Prof. Dr. Beate Winner, IZKF - Junior Research Group 3

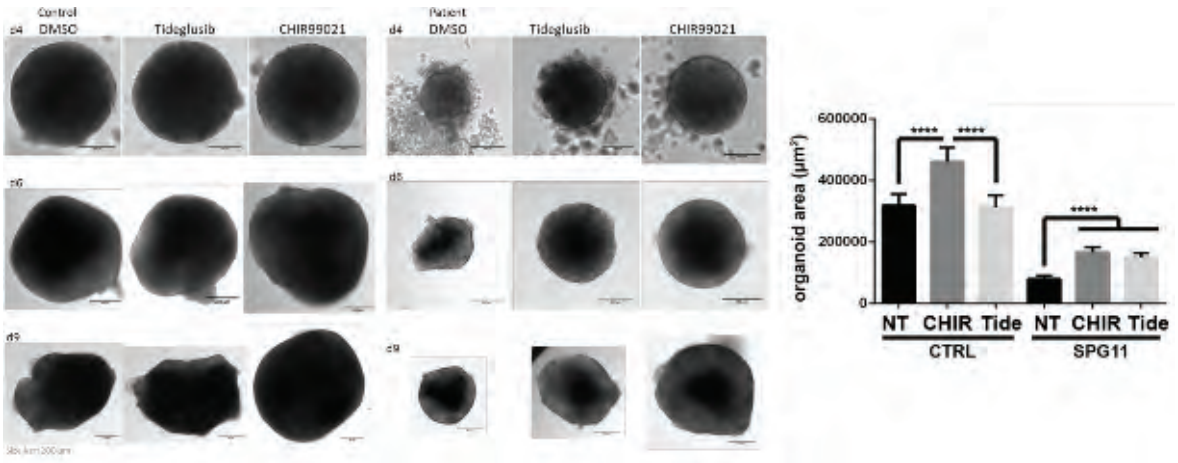
The overall goal in our laboratory is to investigate neurodegeneration using human stem cell derived models. In this respect, we investigated neural phenotypes. In the most frequent complicated autosomal recessive (SPG11) form of hereditary spastic paraplegia (HSP) our major findings were (1) a dual role for spatacsin in neurodevelopment and neurodegeneration, (2) a specific premature neurogenesis defect in SPG11 derived neural precursor cells, which could be revealed in 3-D organoid systems. Studies to understand the role of alpha-synuclein for axonal transport were able to show that increased dosage of α-Syn causes synaptic dysfunction due to axonal transport deficits and mitochondria dysfunction.

Dysfunction of spatacsin leads to axonal pathology and GSK3ß-dependent dysregulation of neurodevelopment

One of our main research interests is to understand the role of spatacsin (mutated in SPG11 linked hereditary spastic paraplegia) in induced pluripotent stem cell derived neurons from patients. In these neurons we recently reported an accumulation of vesicle-like structures and inclusions within the neurites from SPG11 patients indicating axonal pathology and impaired axonal function (Perez-Branguli, Mishra et al., HMG 2014).

The specific transcriptome signature showed that roughly half of the differentially expressed genes in SPG11-neural progenitor cells (NPCs) were related to neural development and included transcriptional changes in the regulation of neurogenesis, neuronal differentiation, and nervous system development. Our study revealed an impaired GSK3ß/ß-Catenin signaling in SPG11-NPCs that could be rescued by a antagonists of GSK3 (Mishra et al., Annals of Neurology 2016).

A novel link between a GSK3 dependent pathway and a cortical developmental phenotype in SPG11 patients was shown by taking advantage of life-cell imaging and three-dimensional organoid models.



Size measurements of organoids derived from induced pluripotent stem cells indicate that the reduced size in SPG11 can be rescued by GSK3 inhibition.

Prof. Dr. Winner

SPG11-NPCs undergo an excessively prolonged cell cycle and an increased rate of asymmetric division, which led to altered ventricular architecture and premature neurogenesis resulting in less cortical neurons. This process is modulated by GSK3ß via the interaction of spatacsin (the protein encoded by SPG11) and DISC1, as demonstrated by pharmacological and genetic strategies that rescue of the premature neurogenesis in NPC-SPG11.

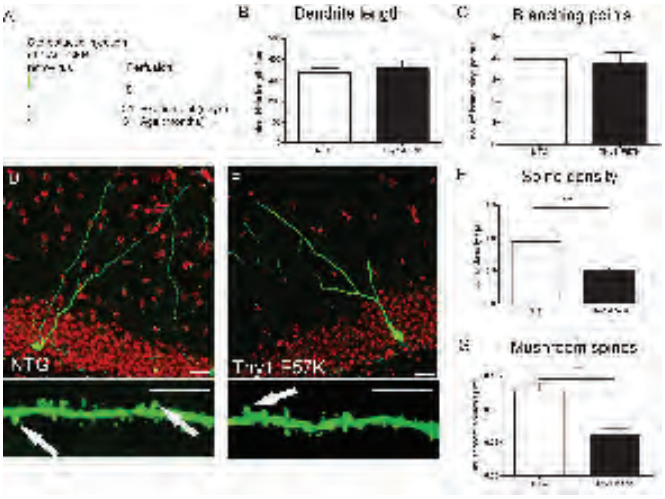
Model of how alpha-synuclein oligomers impact axonal function.

Alpha-Synuclein oligomers impair axonal function in human neurons

Another important aspect of understanding neurodegeneration using human stem cell based models was to decipher, how alpha-Synuclein species impact the axonal compartment in human neurons. We report a correlation of impaired mitochondria axonal transport with increased alpha-Synuclein aggregation in neurons derived from induced pluripotent stem cells of a patient with Parkinson's disease caused by an alpha-Synuclein gene duplication. We further analyzed an impact of alpha-Synuclein oligomers on axonal integrity and mitochondrial transport in human iPSC-derived neurons expressing alpha-Synuclein oligomer-forming mutants or wild-type alpha-Synuclein. An increase of alpha-Synuclein oligomers specifically impaired anterograde mitochondrial transport accompanied by intercellular redistribution/reorganization of kinesin adaptor proteins Miro1 and KLC1 and by elevated syntaphilin neurite concentration and Tau pathology. Furthermore, morphological changes of mitochondria, reduced ATP levels, and degenerated synapses correlated with high levels of alpha-Synuclein oligomers. Thus, increased dosage of alpha-Synuclein causes axonal transport deficits and mitochondria dysfunction leading to a synapse loss in human neurons.

Role of alpha-synuclein oligomers in adult neurogenesis

In vivo studies in alpha-synuclein transgenic animal models (overexpressing oligomer-prone alpha-synuclein species) revealed an additional pathogenic function of extracellular oligomers on the postsynapse. In collaboration with Prof. Eliezer Masliah (UC San Diego), we analyzed the morphology of newborn neurons in adult transgenic mice and observe significant reductions of the density of dendritic spines including mushroom spines. These data indicate impaired postsynaptic integration of adult stem cell derived neurons in the presence of oligomeric alpha-synuclein *in vivo*.



Impaired mushroom spine density in Thy1-WTS mice. (A) Newborn neurons retrovirally labeled (B), numbers of branching points (C), density of spines (F) and density of mushroom spines (F). (D, E) Representative micrographs in NTG and Thy1-WTS mice. * P<0.05.

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N3 - Progress Report

01.10.2010 - 30.09.2016

Invited lectures

9th Meeting of the German Neuroscience Society, 25.03.2011, Göttingen, Adult neurogenesis in transgenic animal models of PD. (BW)

3rd ÖGMBT annual meeting, 29.09.2011, Salzburg, Induced pluripotent stem cells: new developments and future applications in neuroscience. (BW)

ISSCR annual meeting, 15.06.2012, Yokohama, Japan, Cell autonomous versus non-autonomous effects of human alpha-synuclein on neurite development of new neurons in the adult DG. (BW)

Route 28 stem cell meeting, 05.08.2012, Frauenchiemsee, Stem cells in neurodegenerative disease. (BW)

Seminar BioSysNet, 07.03.2013, Munich, Contribution of a-synuclein oligomers to axonal dysfunction. (IP)

12th Tom Wahlig Symposium, 22.03.13, Dresden, SPG11: A new member of synaptic family?. (FPB)

Young Investigators Symposium of the Stem Cell Society Singapore, 31.05.2013, Biopolis, Singapore, Modeling Autosomal Dominant Hereditary Spastic Paraplegia (SPG4) Using Human Induced Pluripotent Stem Cells. (SH)

2nd Eurogenesis meeting: Adult neurogenesis in physiology and disease, 26.06.2013, Bordeaux, France, Modeling synucleinopathies using stem cells. (BW)

Gage lab symposium, 09.11.2013, La Jolla, USA, Modeling hereditary spastic paraplegia using stem cells. (BW)

Seminar Series, Institute of Physiology, 21.01.2014, Uniklinik RHTW Aachen, Modeling motor neuron disease using human iPSC derived neurons. (BW)

Keystone symposia, Adult Neurogenesis, 15.05.2014, Stockholm, Adult Neurogenesis in Parkinson's Disease. (BW)

Symposium: Sensory Neuropathies – Peripheral Neurodegeneration, 08.- 09.01.2015, Vaals, NL, Examining the impact of the hereditary spastic paraplegia gene SPG11 on neuronal development and maintenance in the peripheral nervous system. (BW)

Tom Wahlig Symposium, HSP, 17.04.2015, Graz, Human in vitro modeling of HSP. (BW)

11th meeting of the German Neuroscience Society, Symposium 34: Modeling evolution, neuronal development and neurodegenerative disorders using mammalian induced pluripotent stem cells, 21.03.2015, Göttingen, A tale of traffic jams and bumpy roads and more? (BW)

ForIPS Symposium, Bavarian Research network induced pluripotent stem cells, 03.07.2015, Munich, Human in vitro modeling of Hereditary Spastic Paraplegia. (BW)

DGPPN Kongress, 26.11.2015, Berlin, Modellierung der Neuroentwicklung und -degeneration anhand von Patienten-abgeleiteten induzierten pluripotenten Stammzellen. (BW)

„Science meets School" of the BioSysNet Concortium, 12.10.2015, FAU Erlangen-Nürnberg, Nutzung der induzierten pluripotenten Stammzellen für die Untersuchung der Neurodegeneration bei Menschen. (IP)

Awards

Junior Faculty Award for AD/PD 2011, 09.03.2011, Barcelona. (BW)

BioSysNet Associated Junior Research Group, 19.03.2012, Munich. (BW)

13th IZKF PhD workshop, IZKF Erlangen poster prize, 07.10.2013, Erlangen, Gene-dosage dependent neurite defects in a human induced pluripotent stem cell model of SPG4 related hereditary spastic paraplegia. (SH)

Neurowind e.V. Travelgrant for German Stem Cell Network Conference, 04.11.2014, Bonn. (AS)

Travel grant for Early Career Researchers in Overseas for 37th Annual Meeting of the Molecular Biology Society of Japan, 25.-27.11.2014, RIKEN Center for Integrative Medical Sciences, Yokohama City (Japan). (HK)

8th Route 28 Summit in Neurobiology, 05.-11.09.2014, Frauenchiemsee, Best project proposal: The nature of stem cells in adult neurogenesis.(MR)

Paper of the Month der Deutschen Physiologischen Gesellschaft (Eberhardt, Havlicek, and Schmidt et al. 2015, Stem Cell Reports)

Selected publications during funding period

Mishra HK, Prots I, Havlicek S, Kohl Z, Perez-Branguli F, Boerstler T, Anneser L, Minakaki G, Wend H, Hampl M, Leone M, Brückner M, Klucken J, Reis A, Boyer L, Schuierer G, Behrens J, Lampert A, Engel FB, Gage FH, Winkler J, Winner B. Rescue of GSK3/ß-Catenin-dependent human neuronal precursor proliferation defects in spatacsin-linked motor neuron disease. Annals of Neurology, Mar 11. doi: 10.1002/ana.24633. [Epub ahead of print]

Sommer A, Fadler T, Dorfmeister E, Hoffmann AC, Xiang W, Winner B, Prots I. Infiltrating T lymphocytes reduce myeloid phagocytosis activity in synucleinopathy model. J Neuroinflammation. 2016 Jun 30;13(1):174. doi: 10.1186/s12974-016-0632-5

Salvi R, Steigleder T, Schlachetzki JC, Waldmann E, Schwab S, Winner B, Winkler J, Kohl Z. Distinct effects of chronic dopaminergic stimulation on hippocampal neurogenesis and striatal doublecortin expression in adult mice. Front Neurosci. 2016 Mar 11;10:77. doi: 10.3389/fnins.2016.00077

Schulze M, Hoja S, Winner B, Winkler J, Edenhofer F, Riemenschneider MJ. Model testing of PluriTest with Next-Generation Sequencing Data. Stem Cells Dev. 2016 Apr 1;25(7):569-71. doi: 10.1089/sc

Eberhardt E*, Havlicek S*, Schmidt D*, Link AS, Neacsu C, Kohl Z, Hampl M, Kist AM, Klinger A, Nau C, Schüttler J, Alzheimer C, Winkler J, Namer B, Winner B#, Lampert A#. Pattern of functional TTX-resistant sodium channels reveals developmental stage of human iPS- and ES cell-derived nociceptors. Stem Cell Reports, 2015 5(3): 305-13 *# contributed equally

Schreglmann S, Regensburger M, Rockenstein E, Masliah E, Xiang W, Winkler J, Winner B. The temporal expression pattern of alpha-Synuclein modulates olfactory neurogenesis in transgenic mice. PlosOne, 2015 11;10(5): e0126261

Link AS, Kurinna S, Havlicek S, Lehnert S, Reichel M, Kornhuber J, Winner B, Huth T, Zheng F, Werner S, Alzheimer C. Kdm6b and Pmepa1 as targets of bioelectrically and behaviorally induced Activin A signaling. Mol Neurobiol. 2015 Jul 28. [Epub ahead of print]

Pérez-Brangulí F, Mishra HK, Prots I, Havlicek S, Kohl Z, Saul D, Rummel C, Dorca-Arevalo J, Regensburger M, Graef D, Sock E, Blasi J, Groemer TW, Schlötzer- Schrehardt U, Winkler J, Winner B. Dysfunction of spatacsin leads to axonal pathology in SPG11 linked hereditary spastic paraplegia. HMG, 2014 23(18): 4859-74

Havlicek S, Kohl Z, Mishra HK, Prots I, Eberhardt E, Denguir N, Wend H, Plötz S, Boyer S, Marchetto MCN, Aigner S, Sticht H, Groemer TW, Hehr U, Lampert A, Schlötzer-Schrehardt U, Winkler J, Gage FH, Winner B. Gene dosage dependent rescue of HSP neurite defects in SPG4 patients' neurons. HMG, 2014; 23(10): 2527-41

Purohit P*, Perez-Branguli F*, Prots I*, Borger E, Gunn-Moore F, Welzel O, Loy K, Wenzel EM, Grömer TW, Brachs S, Holzer M, Buslei R, Fritsch K, Regensburger M, Böhm KJ, Winner B, Mielenz D. The Ca2+ sensor protein Swiprosin-1/EFhd2 is present in neurites and involved in kinesin-mediated transport in neurons. Plos One, 2014; 9(8): e103976 *contributed equally

Ettle B, Reiprich S, Deusser J, Schlachetzki JC, Xiang W, Prots I, Masliah E; Winner B, Wegner M, Winkler J. Intracellular alpha-synuclein affects early maturation of primary oligodendrocyte progenitor cells. Molecular and Cellular Neuroscience. 2014;62: 68-78

May VE, Ettle B, Poehler AM, Nuber S, Ubhi K, Rockenstein E, Winner B, Wegner M, Masliah E, Winkler J. Alpha-synuclein impairs oligodendrocyte progenitor maturation in multiple system atrophy. Neurobiology of Aging, 2014;35(10): 2357-68

Rockenstein E, Nuber S, Overk CR, Ubhi K, Mante M, Patrick C, Adame A, Trejo-Morales M, Riek R, Winklder J, Gage FH, Winner B, Masliah E. Synaptic accumulation of oligomer prone a synuclein exacerbates synatpci degeneration and neuronal loss in a transgenic mouse model. Brain, 2014;137(5): 1496-513

Winner B, Marchetto MC, Winkler J, Gage FH. Human-induced pluripotent stem cells pave the road for a better understanding of motor neuron disease. HMG, 2014; 23(R1): R27-34

Marxreiter F, Ettle B, May VE, Esmer H, Patrick C, Kragh CL, Klucken J, Winner B, Riess O, Winkler J, Masliah E, Nuber S. Glial A30P alpha-synuclein pathology segregates neurogenesis from anxiety-related behavior in conditional transgenic mice. Neurobiol Dis. 2013;59: 38-51

Prots I, Veber V, Brey S, Campioni S, Buder K, Riek R, Böhm KJ, and Winner B (2013) Alpha-synuclein oligomers impair neuronal microtubule-kinesin interplay. J. Biol. Chem. 288: 21742-21754

Winner B, Regensburger M, Schreglmann S. Boyer L, Prots I, Rockenstein E, ManteM, Zhao C, Winkler J, Masliah E, Gage FH (2012). Role of α-synuclein in adult neurogenesis and neuronal maturation in the dentate gyrus. J Neurosci, 32(47): 16906-16916

May VE, Nuber S, Marxreiter F, Riess O, Winner B, Winkler J (2012). Impaired olfactory bulb neurogenesis depends on the presence of human wild-type alpha-synuclein. Neurosci, 11;222: 343-55

Hinkle KM, Yue M, Behrouz B, Dächsel JC, Lincoln SJ, Bowles EE, Beevers JE, Dugger B, Winner B, Prots I, Kent CB, Nishioka K, Lin WL, Dickson DW, Janus CJ, Farrer MJ, Melrose HL (2012). LRRK2 knockout mice have an intact dopaminergic system but display alterations in exploratory and motor coordination behaviors. Mol Neurodegen. 30;7: 25

Kohl Z, Winner B, Ubhi K, Rockenstein E, Mante M, Münch M, Barlow C, Carter T, Masliah E, Winkler J (2012). Fluoxetine rescues impaired hippocampal neurogenesis in a transgenic A53T synuclein mouse model. Eur J Neurosci, 35(1): 10-9

Winner B, Kohl Z, Gage FH. (2011) Neurodegenerative disease and adult neurogenesis. Eur J Neurosci. 33(6): 1139-51

Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, Hetzer C, Loher T, Vilar M, Campioni S, Tzitzilonis C, Soragni A, Jessberger S, Mira H, Consiglio A, Pham E, Masliah E, Gage FH, Riek R. (2011) In vivo demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A. 108(10): 4194-9

Junior Projects

Immunology and Infection

Project No.	Project title	Term	Applicant	Institute
J37	Adoptive cell therapy with ex-vivo expanded NK and γδT cells in metastatic melanoma	01.07.2013-30.04.2016	Dr. Bosch-Voskens	Department of Dermatology
J38	MCS-18 for the treatment of atherosclerosis	01.02.2014-31.01.2016	Dr. Dietel	Department of Medicine 2
J41	Resolution of inflammation in gout	01.12.2013-30.11.2016	Dr. Schauer	Department of Medicine 3
J43	The role of IL-33/ST2 signaling in the development of infectious colitis	01.02.2015-31.10.2016	Dr. Mchedlidze	Department of Medicine 1
J45	Modulation of PRC2 activity by HCMV IE2	01.01.2015-30.06.2017	Dr. Reuter	Institute of Clinical and Molecular Virology
J50	Analysis of the role of IL-9 in the induction of Colitis-associated cancer (CAC)	16.10.2015-15.04.2018	Dr. Gerlach	Department of Medicine 1
J56	Epigenec reprogramming of macrophages	01.01.2017-30.06.2019	Dr. Palumbo-Zerr	Department of Medicine 3
J57	Herpesviruses and DUX4	01.01.2017-30.06.2019	Dr. Full	Institute of Clinical and Molecular Virology

Oncology

Project No.	Project title	Term	Applicant	Institute
J54	Analysis of alternative mechanisms of tumor rejection	01.11.2015 - 30.04.2018	Dr. Lehmann	Department of Dermatology
J55	The role of microRNA-188-5p dysregulation in hepato-cellular carcinoma development and progression	01.01.2016 - 30.06.2018	Dr. Dietrich	Institute of Biochemistry
J58	Counteracting Wnt signaling	01.09.2016-28.02.2019	Dr. Bernkopf	Chair of Experimental Medicine II
J59	Immunotoxin induced anti-tumor immunity	30 months	Dr. Müller	Department of Medicine 5

Neurosciences

Project No.	Project title	Term	Applicant	Institute
J46	The role of zinc finger protein Zfp276 in glial development of the mouse nervous system	01.04.2015-30.09.2017	Dr. Küspert	Institute of Biochemistry
J51	Inflammatory signature in Parkinson's disease	01.10.2015-31.03.2018	Dr. Marxreiter	Department of Neurology
J52	Modeling cortical dysfunction of SPG11 spastic paraplegia using patient-derived pluripotent stem cells	01.11.2015-30.04.2018	Dr. Regensburger	Department of Neurology
J53	Diffusion tensor imaging of the visual pathway in pseudoexfoliation glaucoma	03.08.2015-02.02.2018	Dr. Schmidt	Department of Neuroradiology

Renal and Vascular Research

Project No.	Project title	Term	Applicant	Institute
J47	Post-transcriptional regulation by Hoxa9	01.03.2015-31.08.2017	Dr. Bach	Department of Medicine 5

Molecular Medicine

Project No.	Project title	Term	Applicant	Institute
J42	Bayesian reverse engineering of developmental networks	01.04.2014-31.03.2016	Dr. Ferrazzi	Institute of Human Genetics
J48	PPARβ/δ in the crosstalk of bone and glucose metabolism	01.01.2015-30.06.2017	Dr. Scholtysek	Department of Medicine 3
J60	The role of Hck/Lyn in Vesicles secretion	01.10.2016-31.03.2019	Dr. Lee	Department of Dermatology

Other methodologically oriented projects, informatics, statistics

Project No.	Project title	Term	Applicant	Institute
J49	Extending statistical boosting algorithms for biomedical research	01.04.2015-30.09.2017	Dr. Mayr	Department of Medical Informatics, Biometry and Epidemiology
J61	Extending joint models in biomedical outcomes	01.01.2017-30.06.2019	Dr. Waldmann	Department of Medical Informatics, Biometry and Epidemiology

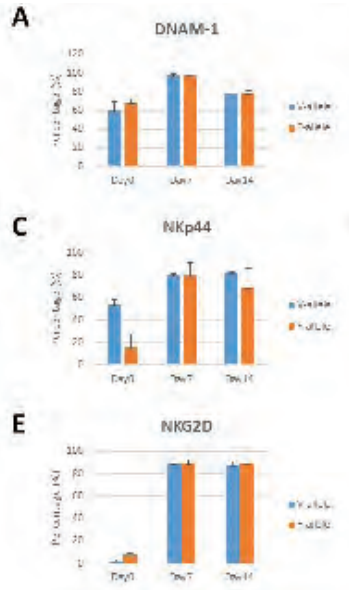
J37 - Final Report

01.07.2013 - 30.04.2016

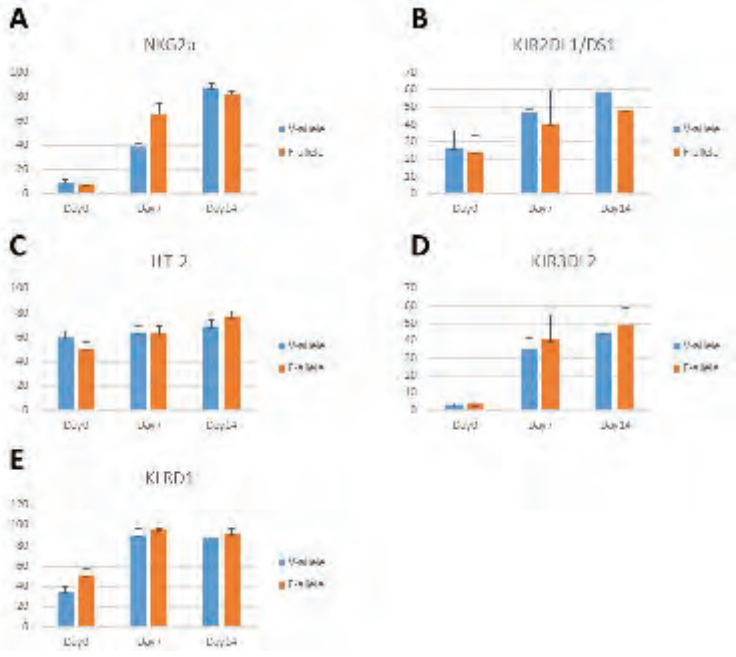
Adoptive cell therapy with ex-vivo expanded NK and γδT cells in metastatic melanoma

Dr. Caroline Bosch-Voskens, Department of Dermatology

Tumor cells can escape a T cell attack in many ways, including by down-regulation of HLA class I molecules. Innate immune cells kill tumor cells in a HLA-unrestricted fashion and as such, the adoptive transfer of natural killer (NK) cells and γδT cells is an attractive strategy to boost T cell immunity. This project aims to develop a GMP-compliant protocol to expand NK and γδT cells from melanoma patients and simultaneously tests the significance of FcγRIIIa polymorphisms on NK and γδT cell activation.



Activating receptors on NK cells from healthy donors expressing one *V*-allele or two *F*-alleles. Expression levels of DNAM-1 (A), NKp30 (B), NKp44 (C), NKp46 (D) and NKG2D (E) were determined before (day 0), during (day 7) and after (day 14) NK cell expansion.



Inhibitory receptors on NK cells from healthy donors expressing one *V*-allele or two *F*-alleles. Expression levels of NKG2a (A), KIR2DL1/DS1 (B), ILT-2 (C), KIR2DL2 (D) and KLRD1 (E) were determined before (day 0), during (day 7) and after (day 14) NK cell expansion.

Dr. Bosch-Voskens

Successful cancer immunotherapy does not solely depend on the effective activation or transfer of cytotoxic T cells. It requires the design of therapeutic combinations which augment anti-tumor responses and simultaneously overcome tumor-specific escape mechanisms. Tumor cells can escape an T cell attack in many ways, including by down-regulation of HLA class I molecules. Innate immune cells kill tumor cells in a HLA-unrestricted fashion and as such, the adoptive transfer of natural killer (NK) and γδT cells is an attractive strategy to boost T cell immunity.

One means to augment the therapeutic benefit of adoptively transferred NK and γδT cells is to define the patients most likely to respond. Growing experience with antibody therapy shows that select patients experience superior clinical outcomes based upon FcγRIIIa polymorphisms. The most relevant polymorphism depends on the presence of a phenylalanine (*F*) or valine (*V*) at amino acid position 158 within the FcγRIIIa receptor. In general, NK cells derived from individuals expressing the FcγRIIIa polymorphism with higher affinity for IgG1 (*V/V* genotype) show superior natural cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC). In the current funding period a close collaboration was established with the research group of Prof. B. Sriewald (Department of internal medicine 5, Hematology and Oncology, Friedrich-Alexander Universität Erlangen). Within this collaboration, by conventional PCR predefined F- and V-allele frequencies were re-evaluated by a highly sensitive TaqMan® SNP Genotyping assay. Using this assay, 8,7% of patients were homozygous for the V-allele, 42,0% of patients were homozygous for the F-allele and 49,3% of patients were heterozygous and expressed both an *V*- and an *F*-allele. These frequencies are similar to those published in prior studies with predominantly Caucasian populations. Unfortunately, due to the limited number of patients solely expressing an *V*-allele, no analysis could be performed with this subgroup of patients. In the next funding period, a second set of melanoma patients will be genotyped for FcγRIIIa polymorphisms in order to perform comparison studies between patients who are homozygous for the *V*- and *F*-allele, respectively.

Alternatively, as a first step, we analyzed the expression of receptors associated with NK cell activation and inhibition in healthy donors bearing an *V/V-, V/F-* or *F/F* polymorphism before and after *ex vivo* NK cell expansion. While initial differences in expression levels of the receptors NKp44, NKp46 and KLRD1 were observed, these differences were overcome after NK cell expansion. These data may suggest that resting NK cells derived from *V/V* individuals are better natural killers compared to NK cells from *F/F* individuals. Additional studies are ongoing to define the impact of FcγRIIIa genotype on NK cell degranulation and cytolytic activity against NK cell sensitive tumor cells.

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Publications during funding period

Fischer A, Zundler S, Atreya R, Rath T, Voskens C, Hirschmann S, López-Posadas R, Watson A, Becker C, Schuler G, Neufert C, Atreya I, Neurath MF (2015) Differential effects of α4β7 and GPR15 on homing of effector and regulatory T cells from patients with UC to the inflamed gut in vivo. Gut 65(10): 1642-64

Dietel B, Cicha I, Voskens CJ, Verhoeven E, Achenbach S, Garlichs CD (2013) Decreased numbers of regulatory T cells are associated with human atherosclerotic lesion vulnerability and inversely correlate with infiltrated mature dendritic cells. Atherosclerosis Sep;230(1): 92-9

J38 - Final Report

01.02.2014 - 31.01.2016

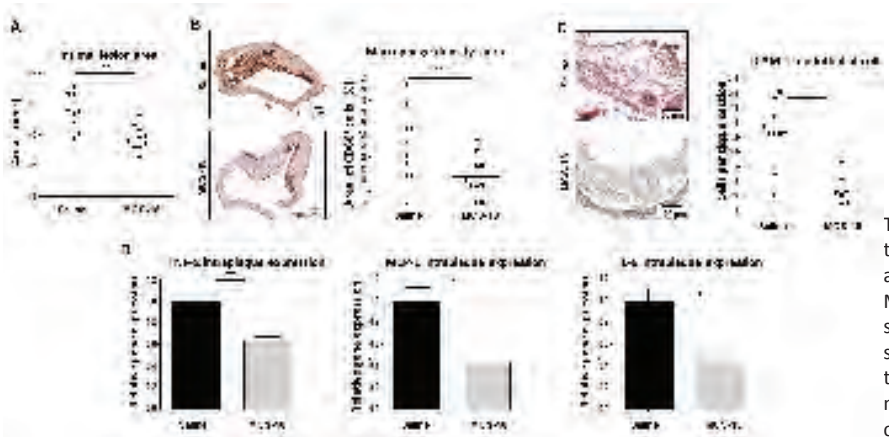
MCS-18 for the treatment of atherosclerosis

Dr. Barbara Dietel, Department of Medicine 2 – Cardiology and Angiology

Progression of atherosclerosis is associated with pronounced inflammatory processes, such as the recruitment of leukocytes and their adhesion to the endothelium. The aim of this project was, to investigate the impact of the herbal substance MCS-18, an anti-inflammatory root compound of helleborus purpurascens which has been shown to exhibit protective effects in murine atherosclerosis onset, on plaque progression in a mouse model of advanced atherosclerosis and on proatherogenic processes in vitro.

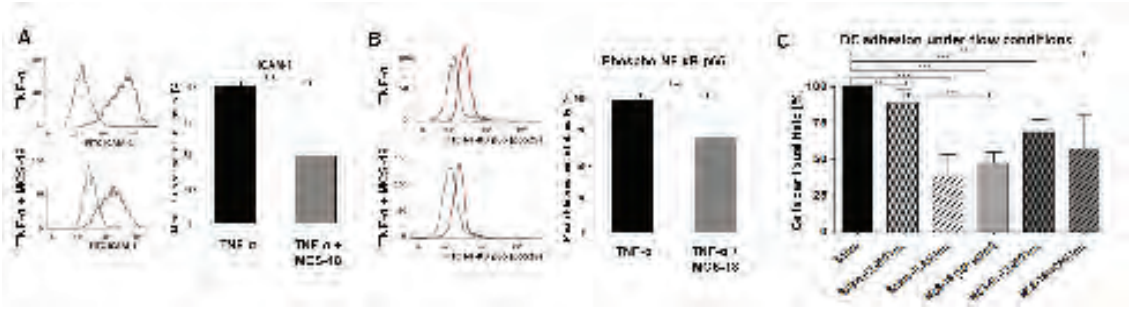
Therapeutic impact of MCS-18 on advanced atherosclerosis

Atherosclerosis is associated with chronic inflammatory responses of the arterial blood vessels. Here, we investigated the impact of the anti-inflammatory compound MCS-18 on murine plaque progression in advanced atherosclerosis and on proatherogenic processes. ApoE-deficient mice were fed a high-fat diet for 12 weeks to induce atherosclerosis, followed by normal chow and intraperitoneal injections of either MCS-18 or saline for another 12 weeks. Plaque size was reduced in MCS-18 treated mice compared to controls, which was associated with a reduced size of the lipid core, indicating an increased plaque stability. In addition, MCS-18 led to significantly lower counts of apoptotic cells and an increased collagen content in atherosclerotic lesions, which also suggests a more stable plaque phenotype. While no changes were detectable with regard to DC maturation in lymphoid organs and numbers of plaque infiltrated mature DCs, immunohistochemical analyses showed that macrophage density and number of ICAM-1 expressing endothelial cells was considerably decreased in plaques of MCS-18-treated mice. Apart from that, quantitative real-time PCR was performed from atherosclerotic lesions of the carotid arteries, which demonstrated reduced transcription levels of intraplaque proinflammatory cytokines (TNF-α, MCP-1 and IL-6) following MCS-18 treatment, confirming a suppressed inflammation in plaques of those mice compared to control treatment.



Therapeutic impact of MCS-18 on the size and inflammatory state of advanced atherosclerotic lesions. MCS-18 reduces intimal lesion size (A), lesional macrophage density (B), ICAM-1 expressing endothelial cells (C) and intraplaque mRNA levels of proatherogenic cytokines and chemokines (D).

Dr. Dietel



Antiatherogenic impact of MCS-18 on HUVECs under flow conditions. MCS-18 decreases levels of ICAM-1 (A) and of intracellular phosphorylated NF-κB-p65 (B) in perfused HUVECs. DC adhesion to a HUVEC monolayer is reduced to the same extent as observed following blocking of ICAM-1 (C).

Impact of MCS-18 on proatherogenic processes *in vitro*

In addition, human and murine dendritic cells (DCs) and human umbilical vein endothelial cells (HUVECs) were treated with MCS-18 to analyze cell migration and adhesion under flow conditions. In human DCs, flow cytometric analyses showed that MCS-18 reduces the expression of CD209, which is involved in cell rolling along the endothelium. However, blocking of CD209 did not lead to a pronounced reduction of DC migration and adhesion. In addition, MCS-18 also showed a pronounced impact on endothelial cells *in vitro*. Accordingly, it reduced levels of ICAM-1 and of phospho-NF-κB-p65 in HUVECs under flow conditions, which might depict an essential mechanism for a hampered transmigration of leukocytes into the intima. In the performed *in vitro* dynamic flow experiments, MCS-18 reduced DC adhesion to the endothelial cell layer in regions of laminar and non-uniform shear stress. While blocking of CD209 in DCs only slightly reduced their adhesion rate, blocking of ICAM-1 in HUVECs led to a significant reduction of DC adhesion. As the co-incubation with MCS-18 and the ICAM-1 inhibitor did not cause any additional reduction of DC adhesion, we speculate that the MCS-18-induced suppression of ICAM-1 in endothelial cells is an important mechanism underlying its antiatherogenic impact.

In summary, our data show that MCS-18 exhibits interesting therapeutic effects in advanced murine atherosclerosis, in which a suppressed adhesion to the endothelium due to downregulation of endothelial ICAM-1 expression might be involved.

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Publications during funding period

Kuehn C, Tauchi M, Stumpf C, Daniel C, Bäuerle T, Schwarz M, Kerek F, Steinkasserer A, Zinser E, Achenbach S, Dietel B (2015) Suppression of Proatherogenic Leukocyte Interactions by MCS-18 - Impact on Advanced Atherosclerosis in ApoE-Deficient Mice. Atherosclerosis. 245: 101-110

Dietel B, Muench R, Kuehn C, Kerek F, Steinkasserer A, Achenbach S, Garlichs CD, Zinser E (2014) MCS-18, a natural product isolated from helleborus purpurascens, inhibits maturation on of dendritic cells in apoe-deficient mice and prevents early atherosclerosis progression. Atherosclerosis. 235: 263-272

J41 - Final Report

01.12.2013 - 30.11.2016

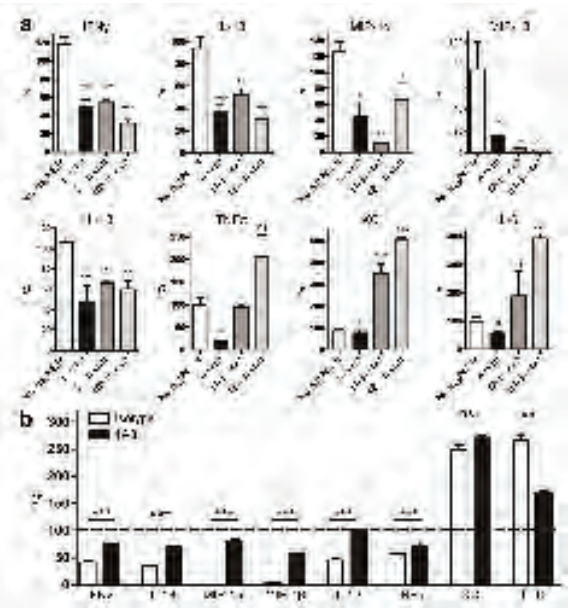
Resolution of inflammation in gout

Dr. Christine Schauer (née Schorn), Department of Medicine 3 – Rheumatology and Immunology

Acute gouty arthritis is a self-limiting process despite persistent monosodium urate (MSU) crystals. For this big enigma of gouty arthritis we propose the following model: In the early phase, MSU crystals induce the formation of solitaire neutrophil extracellular traps (NETs) and huge amounts of pro-inflammatory cytokines. In the late phase in the presence of a high neutrophil density, NETs aggregate and form dense gouty tophi. The latter immobilize MSU and degrade pro-inflammatory mediators.

MSU crystals trigger NETosis and aggregation of NETs in cultured human neutrophils

In low-density cultures (5 × 10^6 neutrophils ml^{-1}), typically for the early phase of gouty arthritis, we observed NETosis but no aggregation of NETs after incubating with MSU crystals *in vitro*. To mimic the situation during acute inflammation in vivo, we increased the density of neutrophils in our *in vitro* NETosis assays to values typically encountered in densely infiltrated tissue (10^8 neutrophils ml^{-1}). Under these conditions, MSU induced aggregation of NETs (aggNETs). In cryosections of these aggregates, we found extracellular DNA colocalized with granule proteins that resembled gouty tophi.



Degradation of cytokines and chemokines by crystal aggregates

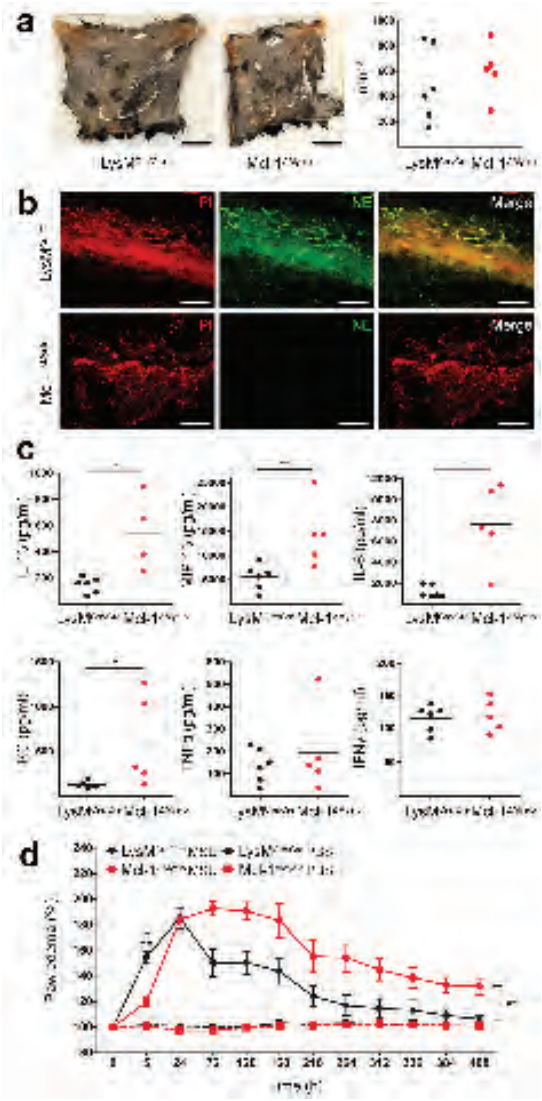
We analyzed the inflammatory cytokines and chemokines released during MSU-induced single NETosis and aggregation of NETs. Whereas mediators were detected at high concentrations in supernatants from low-density cultures (5 × 10^6 neutrophils ml^{-1}), their concentrations were substantially reduced in supernatants from high-density neutrophil cultures (10^8 cells ml^{-1}). Next we used *in vitro* and in vivo generated MSU crystal aggregates and incubated them with recombinant mouse cytokines and chemokines. Both, *in vitro* produced aggNETs generated from MSU crystals and murine bone marrow and in vivo formed peritoneal aggregates, significantly lowered concentrations of most inflammatory mediators in the supernatants.

MSU-induced NETosis and aggNET formation depend on ROS

Reactive oxygen species (ROS) inhibitors (N-acetylcysteine, butylated hydroxyanisole, diphenylene iodonium) strongly decreased the formation of NETs and aggNETs in *in vitro* analyses. Furthermore, NETosis was also reduced after incubation with MSU in blood ex vivo from human and murine individuals with mutations in the Ncf1 subunit encoding the NADPH oxidase. To analyze NET formation *in vivo*, we injected MSU crystals into air pouches of wild-type (WT) and Ncf1** mice. The NET aggregation was strongly reduced in air pouches of Ncf1** mice.

Degradation of cytokine/chemokines by murine aggNETs (a) Concentrations of inflammatory mediators after incubation with aggNETs. (b) Proteolytic activity of aggregates of mice pretreated with neutrophil-depleting antibody 1A8 or isotype antibody

Dr. Schauer



Role of neutrophils in formation of aggNETs and MSU-induced arthritis (a) aggNETs of control and Mcl-$1^{f/f}$ mice (b) Tissue sections from control and Mcl-$1^{f/f}$ mice (c) Mediators in lavages of control and Mcl-$1^{f/f}$ mice (d) paw edema of control and Mcl-$1^{f/f}$ mice

Role of neutrophils in formation of MSU crystal aggregates and MSU induced arthritis

We also employed mice with a myeloid-specific conditional deletion of the anti-apoptotic Mcl-1 protein Mcl-$1^{\Delta Myelo}$ (Mcl-$1^{f/f}$) which exhibit a selective neutrophil deficiency caused by the requirement of Mcl-1 for the survival of neutrophils, whereas other myeloid-lineage cells are not affected. These mice also formed MSU aggregates with comparable size to control mice, which, however, did not contain NETs or neutrophils. Nevertheless, the concentrations of most inflammatory mediators were elevated in the MSU-injected air pouches of Mcl-$1^{f/f}$ mice as compared to controls. Importantly, arthritis induced by injection of MSU crystals in the foot pads of Mcl-$1^{f/f}$ mice exhibited both delayed onset and resolution. These results indicate that NETosis and the aggregation of NETs (tophus formation) promote resolution of MSU-induced inflammation in gouty arthritis.

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Awards

IZKF Publikationspreis für Nachwuchswissenschaftler, Preisträgerin: Dr. Christine Schauer, Verleihung: 17.06.2016, Kloster Banz

Publications during funding period

Reinwald C*, Schauer C*, Csepregi JZ, Kienhöfer D, Weidner D, Malissen M, Mocsai A, Schett G, Herrmann M, Hoffmann M (2016) Reply to "Neutrophils are not required for resolution of acute gouty arthritis in mice". Nat Med. 6;22(12): 1384-1386 *equally contributed

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Muñoz LE, Bilyy R, Biermann MH, Kienhöfer D, Maueröder C, Hahn J, Brauner JM, Weidner D, Chen J, Scharin-Mehlmann M, Janko C, Friedrich RP, Mielenz D, Dumych T, Lootsik MD, Schauer C, Schett G, Hoffmann M, Zhao Y, Herrmann M (2016) Nanoparticles size-dependently initiate self-limiting NETosis-driven inflammation. Proc Natl Acad Sci U S A. 4;113(40): E5856-E5865

Schauer C, Janko C, Munoz LE, Zhao Y, Kienhöfer D, Frey B, Lell M, Manger B, Rech J, Naschberger E, Holmdahl R, Krenn V, Harrer T, Jeremic I, Bilyy R, Schett G, Hoffmann M, Herrmann M (2014) Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat Med. 20(5): 511-7

J42 - Final Report

01.04.2014 - 31.03.2016

Molecular Medicine

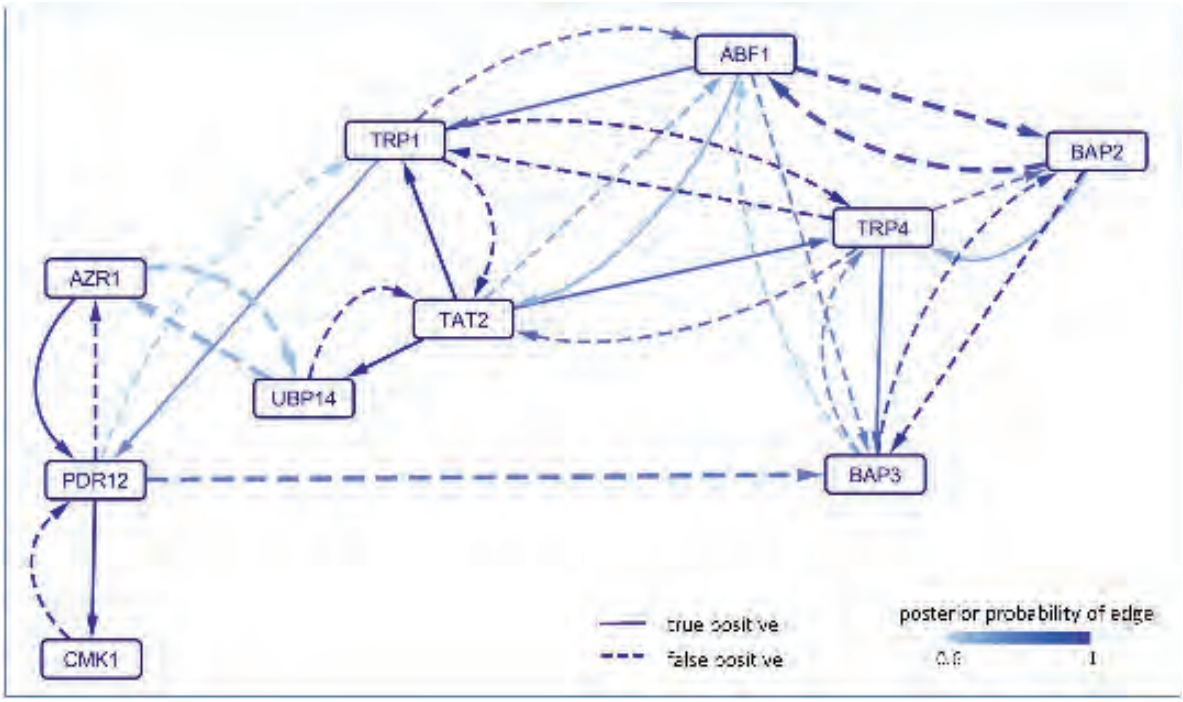
Bayesian reverse engineering of developmental networks

Dr. Fulvia Ferrazzi, Institute of Human Genetics

The project's goal is the development of a Bayesian approach to reverse engineer gene regulatory networks from expression time series and prior knowledge. The methodology was validated on gold-standard networks and afterwards employed to analyze a high resolution temporal expression dataset describing heart development. These data have the potential to shed light on congenital heart disease, cardiac stem cell differentiation, and regeneration.

Dynamic Bayesian networks to integrate expression data and prior knowledge

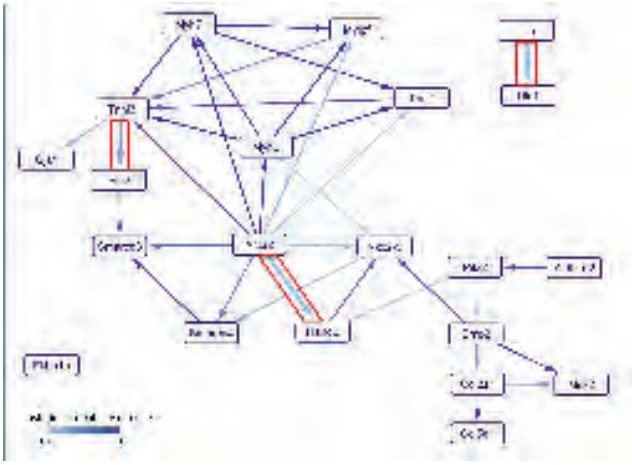
Gene networks offer a flexible framework to represent and analyze interactions between genes. Moreover, they can support the identification of novel hypotheses on regulatory processes. On the basis of measured expression data, reverse engineering methodologies aim at inferring the underlying gene regulatory network. We developed a reverse engineering methodology based on dynamic Bayesian networks (DBNs) to integrate prior knowledge in the learning of gene networks from temporal expression data. DBNs are probabilistic graphical models that can capture intrinsic inference uncertainty and are suitable to describe the dynamics of gene expression. It has been shown in the literature that the introduction of prior knowledge (i.e. curated information available in online repositories) in the network learning process can improve the accuracy of the inferred models. The use of a Bayesian framework is very



Learned network for DREAM 3 Challenge 4. When using prior knowledge sensitivity increases from 0.1 to 1, specificity goes from 0.86 to 0.77 and precision increases from 0.07 to 0.32 with respect to network learning without prior.

Dr. Ferrazzi

interesting as it offers a principled way to integrate prior information in learning. As a source of prior knowledge the STRING database of known and predicted protein interactions was chosen. It combines several resources and currently constitutes one of the most comprehensive interaction repositories. Moreover, information about the strength of the interactions is provided via a confidence score, which we transformed into prior probabilities of network edges to be employed during network learning. In order to search across the space of potential network structures we relied on the MCMC Metropolis-Hasting algorithm.



Learned network for Heart Morphogenesis genes. Red boxes are inferred relationships for which no prior knowledge is available in STRING. These are candidate novel connections.

We validated our methodology on gold-standard networks (i.e. networks for which the real underlying biological regulations are known) taken from past DREAM challenges (http://dreamchallenges.org/). The obtained results show that the inclusion of prior knowledge improves the accuracy of the learned networks.

Application of the methodology to a mammalian heart development dataset

The here developed methodology was subsequently applied to a temporal expression dataset describing rat heart development from embryonic day 11 to postnatal day 10, generated in collaboration with Prof. F.B. Engel. Data analysis identified over 3,000 differentially expressed probe sets, which clustered in groups characterized by markedly different patterns of expression. First, network reconstruction was applied on subsets of genes associated with selected enriched Gene Ontology processes, including “Heart Morphogenesis” (GO:0003007, enrichment p-value < 0.01). Moreover, a “meta-gene” network was learned, in which the network variable is not anymore represented by the expression of a single gene, but by a gene cluster. This approach is promising since it offers a global and concise view of the examined biological system. The inferred network allows the generation of novel hypotheses to be experimentally validated and employed to refine the learned model.

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Invited lectures

Frontiers in Cardiovascular Research International Symposium, 9th March 2016, Singapore, “Systems biology approaches to identify novel regulators of heart development”

Faculty of Informatics Seminar, 10th February 2016, University of Lugano, “Dynamic Bayesian networks to discover novel regulatory mechanisms underlying heart development”

Publications during funding period

Cabrera-Fuentes HA, Aragones J, Bernhagen J, Boening A, Boisvert WA, Bøtker HE, Bulluck H, Cook S, Di Lisa F, Engel FB, Engelmann B, Ferrazzi F, Ferdinandy P, Fong A, Fleming I, Gnaiger E, Hernández-Reséndiz S, Kalkhoran SB, Kim MH, Lecour S, Liehn EA, Marber MS, Mayr M, Miura T, Ong SB, Peter K, Sedding D, Singh MK, Suleiman MS, Schnittler HJ, Schulz R, Shim W, Tello D, Vogel CW, Walker M, Li QO, Yellon DM, Hausenloy DJ, Preissner KT (2016) From basic mechanisms to clinical applications in heart protection, new players in cardiovascular diseases and cardiac theranostics: meeting report from the third international symposium on „New frontiers in cardiovascular research“. Basic Res Cardiol. 111(6): 69

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J43 - Final Report

01.02.2015 - 31.10.2016

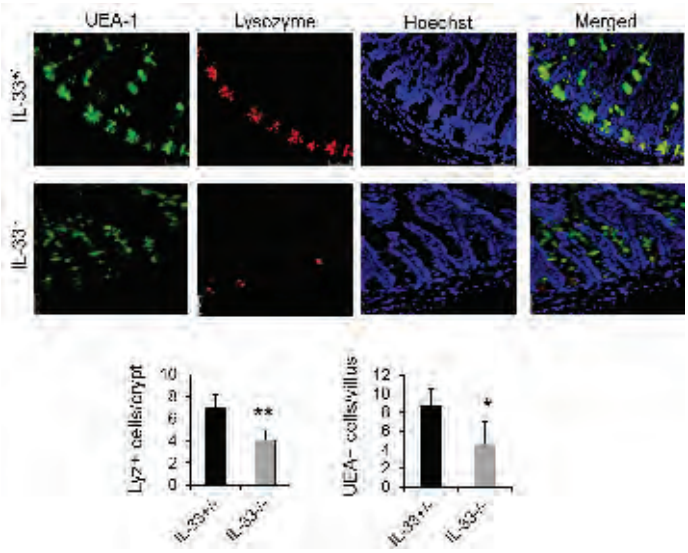
The role of IL-33/ST2 signaling in the development of infectious colitis

Dr. Tamar Mchedlidze, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Gram-negative bacteria of the genus Salmonella induce gastrointestinal infectious disease and thus constitute a significant health problem. Based on comprehensive analysis we found that IL-33 and its signaling pathway plays important role for protection of the host from Salmonella-dependent diseases. Furthermore, we explored the potential mechanism through which IL-33/ST2 axis contributed to disease progression after infection-associated challenge.

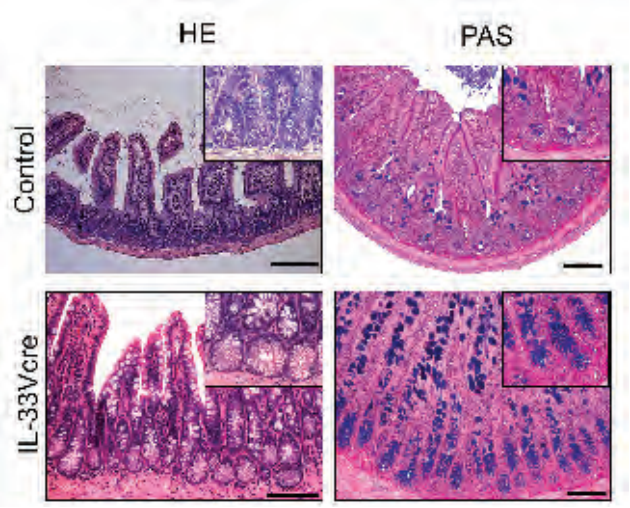
Infections with *Salmonella* strains are considered as a major health problem world-wide with high morbidity and mortality rates. Despite the numerous studies, the precise mechanism how this enteric pathogen interacts with the host immune system during development and resolution of the disease remains incompletely understood. Alarmin-like cytokine IL-33 plays important role in sensing damage during inflammatory conditions and therefore potentially has an important role in immunity against infections. Several studies indicated the role of IL-33 in bacteria-induced sepsis, but the precise role of IL-33 in intestinal diseases remains largely unknown.

We analyzed IL-33 expression in IL-33 LacZ reporter mice and detected strong reporter gene activity after *S. typhimurium* infection. Upregulation of intestinal IL-33 expression was confirmed by quantitative PCR analysis. Upon infection, IL-33$^{-/-}$ mice developed progressive disease symptoms whereas in control animals only mild symptoms were detected. Besides, lack of IL-33 was associated with severe tissue damage and immune cell infiltration, correlating to higher local and systemic bacterial load when compared to wild-type littermates. Similar results were observed in mice lacking the IL-33 receptor chain ST2.



UEA-1 and lysozyme double staining was performed on terminal ileum cross-sections of IL33$^{+/-}$ or IL33$^{-/-}$ mice infected with *S. typhimurium.*

Dr. Mchedlidze



Terminal illeum cross-sections of IL-33Vcre mice were analyzed by Hematoxylin & Eosin (H&E) staining and Periodic acid-Schiff (PAS) staining.

In order to analyze the impact of IL-33 in dissemination of microbial pathogens in more detail, we generated IL-33$^{-/-}$ Nramp1^{wt} (natural resistance-associated macrophage protein 1) mice which control systemic outgrowth of bacterial infections. Similarly, these deficient mice exhibited increased bacterial burden compared to controls. Besides, IL-33$^{-/-}$ mice showed decreased secretory epithelial cells including Paneth cells and goblet cells after *Salmonella* infection which was confirmed by reduced UEA-1 (Ulex europeus agglutinin-1) and lysozyme staining. Furthermore, dramatically reduced antimicrobial peptides in IL-33$^{-/-}$ mice indicated to compromised epithelial antimicrobial defense. In order to analyze if microbial composition could be responsible for increased susceptibility of IL-33$^{-/-}$ mice, we elucidated if IL-33 in the steady-state impacts microbial communities in the gut. Interestingly, 16S-based next generation sequencing analysis of IL-33 deficient and wild-type littermates demonstrated that there are no significant changes in the microbiome.

Additionally, we generated transgenic mice allowing the expression of IL-33 in the intestine in epithelial cell-specific manner after tamoxifen injection (IL-33Vcre mice). Interestingly, histological analysis of these animals confirmed that overexpression of IL-33 in the intestine led to increased numbers of secretory lineage cells (goblet and Paneth cells) whereas absorptive lineage cells were significantly diminished.

Taken together our findings indicated that IL-33 is able to trigger crucial epithelial changes in the context of infection-associated challenge and plays important role during intestinal inflammation.

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Publications during funding period

Mchedlidze T, Kindermann M, Neves ST, Voehringer D, Neurath MF, Wirtz S (2016) IL-27 supresses type 2 immune responses in vivo via direct effects on group 2 innate lymphoid cells. Mucosal Immunology 9:1384-1394

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J45 - Progress Report

01.01.2015 - 30.06.2017

Immunology and Infection

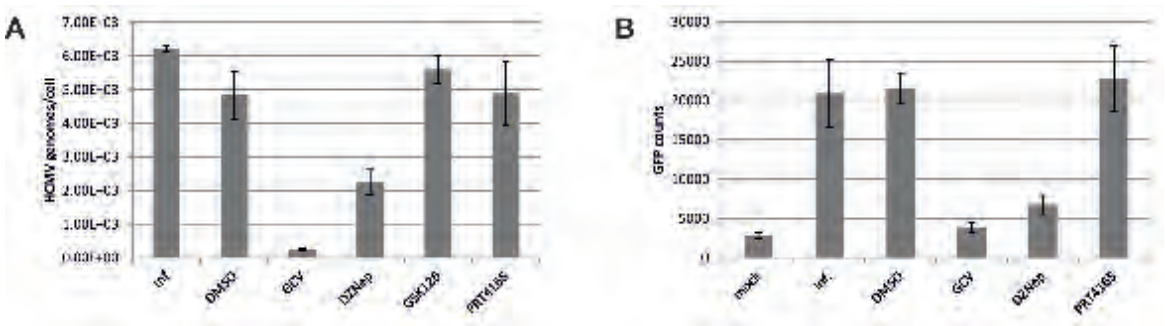
Modulation of PRC2 activity by HCMV IE2

Dr. Nina Reuter, Institute of Clinical and Molecular Virology

Chromatin-based modifications of herpesviral genomes play a crucial role in dictating the outcome of infection. Host cell multiprotein complexes like the nuclear domain 10 (ND10) or the Polycomb repressive complex 2 (PRC2) have been identified as regulators of viral gene expression on the epigenetic level. This proposal aims at investigating the role of PRC2 for HCMV infection as well as elucidating the mechanisms HCMV has evolved to modulate PRC2 function for its own benefit.

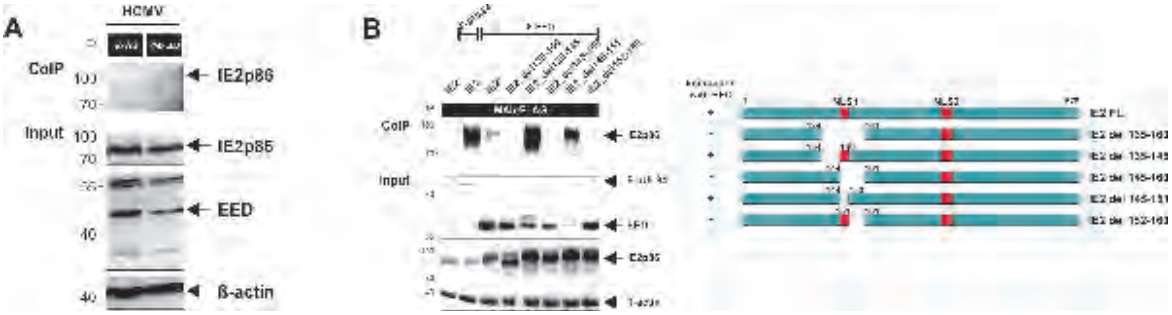
Role of PRC2 during lytic HCMV replication

To address the relevance of PRC2 activity for the productive life cycle of HCMV, we dissected the levels of PRC2 core components following HCMV infection. This revealed an HCMV-induced upregulation of the major PRC2 factors EZH2, SUZ12, and EED on the mRNA as well as protein level. By immunofluorescence staining, we found that all major PRC2 components, which are normally evenly distributed throughout the nucleus, relocalize into viral replication compartments as infection progresses. Interestingly, however, the repressive histone mark H3K27me3 instituted by PRC2 turned out to be specifically excluded from these sites suggesting a differential role of PRC2 independent of its repressor activity. This assumption is further supported by recent findings from the literature showing a novel function of PRC2 in the regulation of DNA replication and the DNA damage response. Intriguingly, both of these cellular processes are known to be required for an efficient HCMV replication. Indeed, addition of the PRC2 inhibitor DZNep following HCMV infection clearly impaired virus replication as it had a negative impact on viral late gene expression which is dependent on an efficient viral genome amplification and resulted in a reduced release of infectious viral particles. In this regard, we tested a series of diverse PRC2 inhibitory substances. Interestingly, only substances which induced a destabilization of the PRC2 complex had the capacity to compromise HCMV replication, while inhibition of PRC2's enzymatic activity alone had no effect. This further supports the idea that it is a repressor-independent activity of PRC2 which is required for an efficient lytic replication of HCMV.



Inhibition of PRC2 negatively affects HCMV replication. A) Quantification of viral genomes by qPCR. B) GFP-based antiviral assay. GCV: Ganciclovir, antiviral nucleoside analogue; DZNep and GSK126: PRC2 inhibitors; PRT4165: PRC1 inhibitor.

Dr. Reuter



The HCMV regulatory protein IE2 interacts with the PRC2 core component EED *in vivo* (A) and *in vitro* (B). A) CoIP experiment of HCMV-infected cells. B) Identification of EED binding-deficient mutants of IE2.

Analysis of the regulation of PRC2 activity by the HCMV effector protein IE2p86 (IE2)

By yeast two-hybrid screening and co-immunoprecipitation (CoIP) analysis, we discovered an interaction between the HCMV transactivator protein IE2 and the PRC2 core factor EED. This IE2-EED interaction could also be confirmed in the context of HCMV infection which further underlines the *in vivo* relevance of this finding. By immunofluorescence analysis, we could show that IE2 colocalizes with EED in viral replication centers (VRCs). Furthermore, since we found that the IE2-EED interaction does not result in a destruction of the PRC2 complex, this suggests that the entire PRC2 complex is actively recruited into VRCs by the viral IE2 protein. To elucidate the role of IE2 as a regulator of PRC2 activity, we generated an IE2 mutant which is no longer able to bind to EED. With the help of CoIP experiments we could narrow down the EED interaction interface to amino acids 152-160 of IE2. Next, we generated recombinant viruses expressing this EED interaction-deficient mutant of IE2 in the context of the HCMV laboratory strains AD169 and TB40/E. Characterization of these recombinant viruses will help us to further define the *in vivo* relevance of the IE2-EED interaction.

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Invited lectures

Seminar: Methods in Molecular Virology, 28.11.2016, Institute of Clinical and Molecular Virology, Erlangen, „The human cytomegalovirus immediate-early protein IE2p86 negatively regulates transcription of lentiviral vectors leading to a shut-down of transgene expression“

Publications during funding period

Schilling EM, Scherer M, Reuter N, Schweininger J, Muller YA, Stamminger T (2016) The human cytomegalovirus IE1 protein antagonizes PML nuclear body mediated intrinsic immunity via the inhibition of PML de novo SUMOylation. J Virol [Epub ahead of print]

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Scherer M, Otto V, Stump JD, Klingl S, Müller R, Reuter N, Muller YA, Sticht H, Stamminger T (2015) Characterization of recombinant human cytomegaloviruses encoding IE1 mutants L174P and 1-382 reveals that viral targeting of PML bodies perturbs both intrinsic and innate immune responses. J. Virol. 90(3): 1190-205

Wagenknecht N, Reuter N, Scherer M, Reichel A, Müller R, Stamminger T (2015) Contribution of the Major ND10 Proteins PML, hDaxx and Sp100 to the Regulation of Human Cytomegalovirus Latency and Lytic Replication in the Monocytic Cell Line THP-1. Viruses 7(6): 2884-907

J46 - Progress Report

01.04.2015 - 30.09.2017

Neurosciences

The role of zinc finger protein Zfp276 in glial development of the mouse nervous system

Dr. Melanie Küspert, Institute of Biochemistry

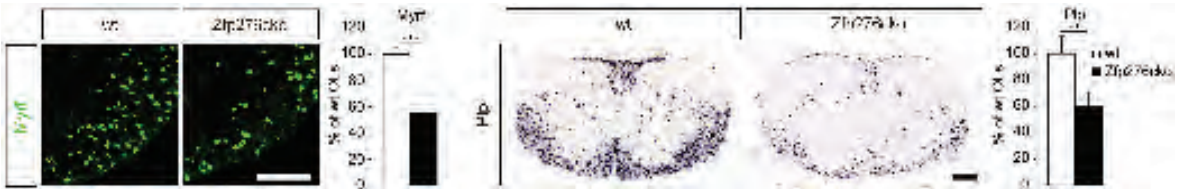
Recently, the transcription factor Zfp276 was identified as a potential new target of Sox10, a key regulator of myelination. Induction of Zfp276 expression in oligodendrocytes and Schwann cells during the onset of myelination argued for a role in the regulation of this process. Direct regulation of Zfp276 expression by Sox10 now could be proven in vitro and in vivo. First studies on Zfp276 function revealed reduced expression of oligodendroglial differentiation markers after depletion of Zfp276.

Background

Myelination is a highly regulated process during development of vertebrate PNS and CNS. The transcription factor Sox10 is a key regulator of Schwann cell and oligodendrocyte differentiation and its deletion leads to complete failure of myelination in the PNS and severe hypomyelination in the CNS in mice. Additionally, several Sox10 mutations are described that lead to hypomyelinating conditions in human patients. Sox10 functions both, via direct activation of myelin gene regulatory elements and induction of other transcriptional activators of myelination. In the PNS several transcription factors were identified, which are both direct targets of Sox10 and transcriptional regulators of myelination and which often act synergistically with Sox10 on shared target genes. One prominent example is the zinc finger transcription factor Egr2, essential for expression of major myelin genes during PNS myelination. In contrast, downstream targets of Sox10 that mediate its function on myelin gene expression are largely unknown for the CNS. Recent transcriptome and ChIP-Seq data identified the poorly characterized zinc finger protein Zfp276 as a potential new target of Sox10 during myelination.

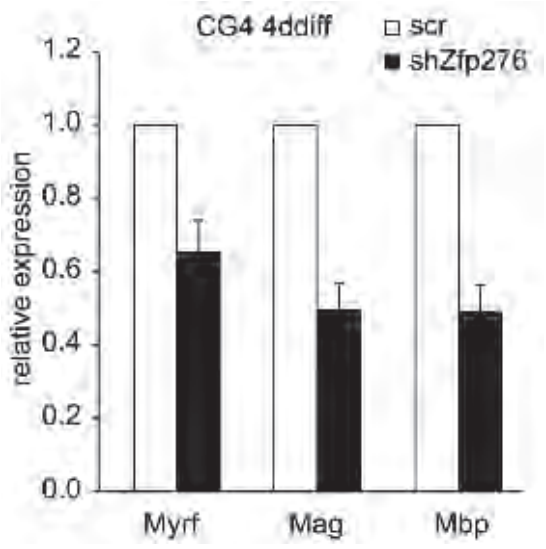
Zfp276 is differentially expressed in myelinating glia and regulated by Sox10 in a dose-dependent manner

Using qRT PCR, *in situ* hybridization and immunohistochemistry induction of Zfp276 mRNA and protein expression in newly differentiated myelinating Schwann cells and oligodendrocytes was detected *in vivo*. The spatio-temporal expression pattern of Zfp276 only in myelinating glial cells but not in non-myelinating glial cells of the dorsal root ganglion argues for a potential role in the regulation of early myelination events. As implicated by Sox10-ChIP-Seq data and by coexpression of both transcription factors a direct effector-target-relationship between Sox10 and Zfp276 could be proven as in early murine postnatal spinal cord devoid of Sox10 *Zfp276* expression was strongly reduced. Based on Sox10-binding regions from ChIP-Seq experiments and on evolutionary conservation an intronic enhancer of *Zfp276* was identified and its dependence on Sox10 was analyzed. Both, overexpression and knockdown experiments for Sox10 demonstrated a dose-dependent change of enhancer activity.



IHC and ISH were performed on spinal cord of P7 mice that either were wildtype (wt) or displayed Zfp276 deletion in Sox10^{+} cells (Zfp276cko). Antibodies for Myrf protein and an antisense probe for *Plp* mRNA were used. IHCs show ventral spinal cord.

Dr. Küspert



Oligodendrocyte-like CG4 cells were transfected with plasmids expressing scrambled shRNA (scr) or shRNA targeting Zfp276 (shZfp276) and differentiated for 4 days. Expression levels of maturation markers were determined via qRT-PCR and normalized to Rpl8.

Zfp276 deletion in oligodendroglial progenitors leads to reduced expression of early and late myelin genes

To decipher the role of Zfp276 during glial differentiation oligodendroglial cells were subjected to Zfp276 knockdown *in vitro* and conditional Zfp276 knockout mice in which Zfp276 was deleted in all Sox10-expressing cells were analyzed in early postnatal stages. Both, the expression of the myelin gene regulatory factor Myrf, as well as the expression of early and late myelin genes were significantly reduced upon Zfp276 depletion. Future experiments will have to elucidate the mechanism by which Zfp276 exerts its function during oligodendrocyte differentiation.

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Publications during funding period

Muth K N, Piefke S, Weider M, Sock E, Hermans-Borgmeyer I, Wegner M, and Küspert M (2016) The Dual-Specificity Phosphatase Dusp15 is Regulated by Sox10 and Myrf in Myelinating Oligodendrocytes. Glia 64(12): 2120-2132

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J47 - Progress Report

01.03.2015 - 31.08.2017

Renal and Vascular Research

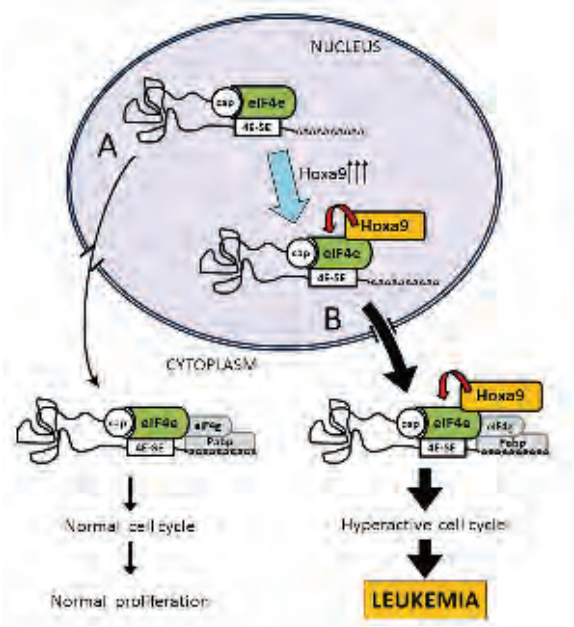
Post-transcriptional regulation by Hoxa9

Dr. Christian Bach, Department of Medicine 5 – Haematology and Oncology

The oncogene Hoxa9 contributes to post-transcriptional regulation by interaction with the RNA export and protein synthesis regulator eIF4e. To date, target genes of this interaction have not been identified. Therefore, this project aims to identify post-transcriptional targets of Hoxa9 and eIF4e by RNA immunoprecipitation. Moreover, analyses of altered RNA-export will be performed as functional validation. In summary, this study will help to clarify the contribution of Hoxa9 to leukemogenesis and provide a solid basis to uncover novel therapeutically relevant targets.

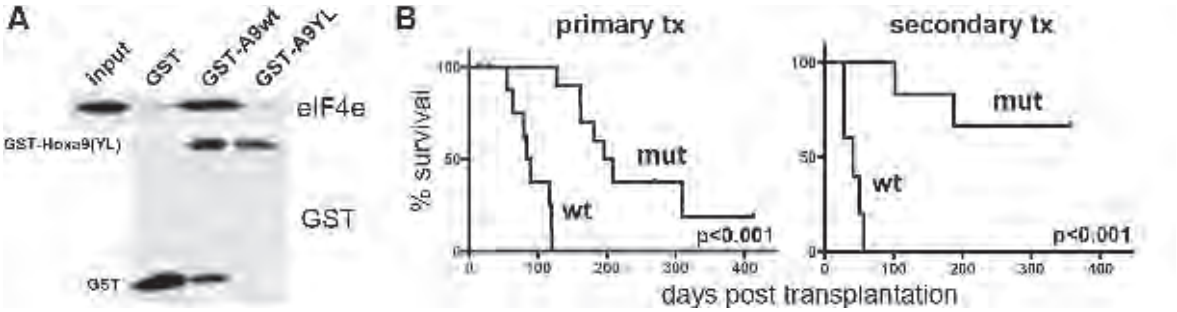
Hoxa9 and the Hoxa9/eIF4e interaction

The Hoxa9 transcription factor is an oncogene frequently upregulated in pediatric and adult acute leukemias. Recently, the interaction of Hoxa9 with the post-transcriptional regulator eIF4e was shown to activate post-transcriptional regulation of several known oncogenes like CyclinD1, c-Myc, and others in leukemia cell lines. Therefore, our aim is to uncover the impact of the Hoxa9-eIF4e interaction on leukemogenesis and regulation of potential target genes both in vitro and in vivo. Previously, we created a variant of Hoxa9 by introducing point mutations into its eIF4e interaction motif. Interestingly, the previously reported Y11A mutation of Hoxa9 did not result in altered colony formation in retroviral transduction and transformation assays despite its' reported disruption of eIF4e interaction. However, we demonstrated that a Hoxa9 variant carrying a double point mutation (Hoxa9YL) is incapable of eIF4e interaction in GST-pulldown experiments and had severely impaired serial replating capacity. This indicated that eIF4e interaction is a key mediator of the oncogenic activity of Hoxa9.



Schematic overview of the hypothesis for leukemia induction by the Hoxa9/eIF4e interaction. Normal (A) eIF4e mediated post-transcriptional cell cycle regulation becomes hyperactivated upon Hoxa9 overexpression (B) resulting in enhanced proliferation and eventually leukemia.

Dr. Bach



Abrogation of Hoxa9/eIF4e interaction leads to severely reduced leukemogenicity. (A) GST-pulldown for wildtype (wt) and mutated (YL) Hoxa9 and eIF4e showing a lack of interaction for the mutant. (B) Survival of mice transplanted with hematopoietic progenitor cells transduced with wildtype (wt) or mutated (mut) Hoxa9. Survival is shown both for primary and secondary transplantations, indicating the crucial role of this interaction for leukemia development.

Hoxa9/eIF4e interaction is critical for the development of full penetrance/low latency AML in vivo

In order to test the leukemogenicity of Hoxa9YL and to generate leukemia cell lines for further analysis we retrovirally transduced murine hematopoietic progenitor cells with both wildtype Hoxa9 (Hoxa9wt) and Hoxa9YL together with the co-factor Meis1. After transplantation into syngenic mice we determined leukemia phenotype and overall survival. Hoxa9YL as well as Hoxa9wt transduced cells gave rise to overt AML. Notably, the resulting leukemias were phenotypically similar in both groups. Disease latency of Hoxa9YL leukemias, however, was significantly prolonged compared to Hoxa9wt leukemias (median disease onset ~190 days vs. ~90 days), concomitant with a reduced disease penetrance for Hoxa9YL (80% vs 100%) despite the persistence of transduced cells in the bone marrow for more than 400 days post transplantation. Importantly, we observed a considerably more pronounced effect on latency (median disease onset ~50 days vs. ~150 days) and penetrance (40% vs 100%) after re-transplantation of leukemic bone marrow from diseased mice into healthy secondary recipients. These findings argue for a persistent cell intrinsic reduction of leukemogenicity conferred by the disruption of the Hoxa9/eIF4e interaction. This implies that the Hoxa9/eIF4e interaction itself as well as targets regulated by this interaction could be attractive targets for therapeutic intervention. Therefore, we generated cytokine-dependent “primary” leukemia cell lines from bone marrow of diseased mice in order to identify potential targets by RNA immunoprecipitation of Hoxa9 and eIF4e as the next step.

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Publications during funding period

Ye M, Zhang H, Yang H, Koche R, Staber PB, Cusan M, Levantini E, Welner RS, Bach CS, Zhang J, Krivtsov AV, Armstrong SA, Tenen DG (2015) Hematopoietic Differentiation Is Required for Initiation of Acute Myeloid Leukemia. Cell Stem Cell 17(5): 611-23

J48 - Progress Report

01.01.2015 - 30.06.2017

Molecular Medicine

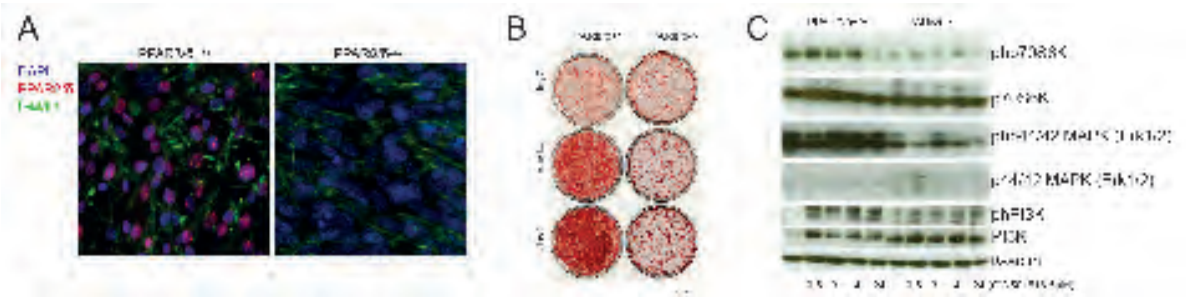
PPARβ/δ in the crosstalk of bone and glucose metabolism

Dr. Carina Scholtysek, Department of Medicine 3 – Rheumatology and Immunology

The nuclear receptor (NR) PPARβ/δ is a central regulator of fatty-acid oxidation thereby reducing dyslipidemia and insulin resistance. So far cellular functions for PPARβ/δ during energy homeostasis are not clear. Since we identified PPARβ/δ as a master regulator of bone turnover and provided evidence for the potential of this NR to serve as a target for the treatment of osteoporosis, we now aim to a potential role for PPARβ/δ during the crosstalk between bone and energy metabolism.

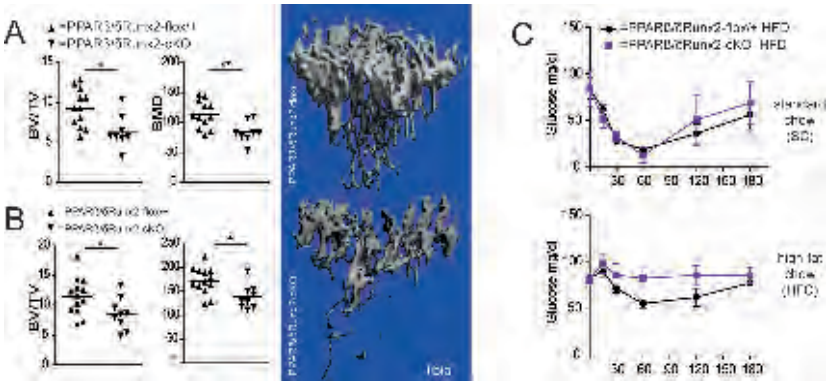
PPARβ/δ controls osteogenic differentiation and metabolism

During the last years, we have focused on common factors involved in the regulation of immune, bone and energy homeostasis. Special attention was given to the role of lipid mediators and nuclear receptors (NRs) in the crosstalk between these systems. Recently published work of our laboratory identified PPARβ/δ as an anabolic regulator of bone turnover. Since its family member PPARγ acts as key regulator during differentiation of mesenchymal stem cells (MSCs) into adipocytes, we subsequently investigated a possible role for PPARβ/δ during differentiation of MSCs into the osteoblast lineage. Immunofluorescence staining of PPARβ/δ during MSCs differentiation implicated a prominent nuclear expression of PPARβ/δ in wild-type MSCs during osteogenic differentiation. Mineralisation assays revealed reduced bone matrix production in PPARβ/δ-deficient MSCs in vitro. Accumulative evidence showed that altered metabolic processes have been implicated to affect MSC differentiation. Therefore we analysed metabolic proteins involved in MSC function in PPARβ/δ wildtype and knockout cells. Notably, we found different expression pattern of metabolic key regulators, such as mTor1 and ERK1/2, between PPARβ/δ wild-type and knockout MSCs. These data highlight a major role for PPARβ/δ in the regulation of MSC homeostasis. A possible function for PPARβ/δ in the transcriptional regulation of MSC and osteoblast metabolism may represent a general mechanism contributing to the wide-ranging functions of energy and bone metabolism.



PPARβ/δ regulates MSC functionalities. (A) Immunofluorescence staining of wildtype (PPARβ/δ +/+) and PPARβ/δ -/- MSCs after 7 days of osteogenic differentiation. (B) Reduced mineralisation in PPARβ/δ-/- MSCs. (C) Decreased expression of metabolic key proteins in PPARβ/δ -/- MSCs.

Dr. Scholtysek



Deletion of PPARβ/δ in osteoblasts results in decreased bone volume (BV/TV) and bone mineral density (BMD) in tibial (A) and vertebral (B) bone. Plasma glucose levels during insulin tolerance tests (C) in mice fed a standard chow and a high-fat chow.

Deletion of PPARβ/δ in osteoblasts results in decreased bone mass and impaired insulin sensitivity

We have recently shown that PPARβ/δ-deficient mice display a decreased bone mass. Since our data showed a key role of this nuclear receptor in osteoblast differentiation we seek to determine whether expression of this nuclear receptor in osteoblasts and osteoblast precursor cells is responsible for this osteopenic phenotype. Therefore we generated mice carrying a conditional deletion of PPARβ/δ in osteoblasts by crossing mice carrying a "floxed" PPARβ/δ allele with mice expression the Cre recombinase under the Runx2 promoter. Micro-CT (µCT) measurement of tibial and vertebral bones revealed an osteopenic phenotype with significantly decreased bone volume and reduced bone mineral density in PPARβ/δ conditional Runx2 Cre knockout mice (PpardRunx2-cKO) compared to their wild-type littermates (Ppardflox/+). These data confirm a crucial role for PPARβ/δ in osteoblasts during bone homeostasis. To determine a potential role of skeletal PPARβ/δ during the regulation of glucose homeostasis, mice received either a standard chow (SC) or a high fat chow (HFC) for 8 weeks. Afterwards, we performed insulin tolerance tests (ITT). After a HFC, but not under a SC diet, deletion of PPARβ/δ in osteoblasts resulted in impaired insulin sensitivity. These data suggest an important role of skeletal PPARβ/δ in the regulation of systemic glucose metabolism.

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Publications during funding period

Palumbo-Zerr K, Zerr P, Distler A, Fliehr J, Mancuso R, Huang J, Mielenz D, Tomcik M, Fürnrohr BG, Scholtysek C, Dees C, Beyer C, Krönke G, Metzger D, Distler O, Schett G, Distler JH (2015) Orphan nuclear receptor NR4A1 regulates transforming growth factor-β signaling and fibrosis. Nature Medicine 21: 62-70

J49 - Progress Report

01.04.2015 - 30.09.2017

Others

Extending statistical boosting algorithms for biomedical research

Dr. Andreas Mayr, Department of Medical Informatics, Biometry and Epidemiology

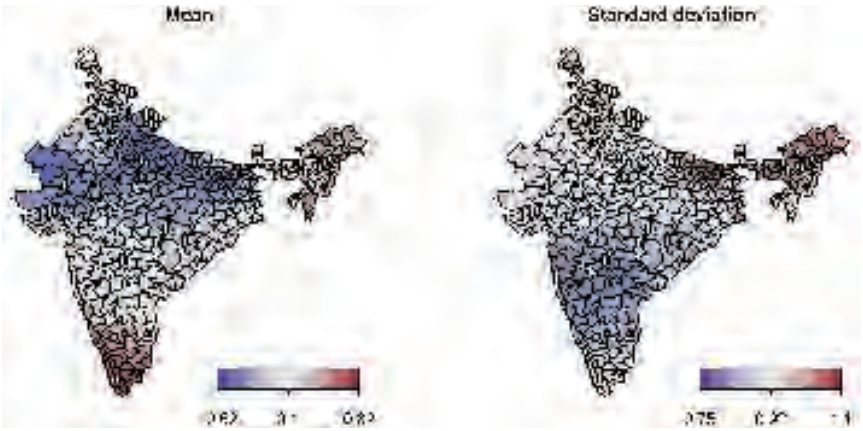
This project focuses on statistical boosting algorithms, particularly for model classes that go beyond the classical regression of the mean. These computational learning algorithms are very flexible and allow to estimate and select predictor effects in statistical models. The aim is to further extend these algorithms and to analyse their properties for specific regression settings that are relevant for medical research.

Boosting the concordance index for survival data

In the construction of biomarkers for time-to-event outcomes, the main focus is often to get a prediction rule that discriminates well between patients with shorter and longer survival times. In most cases, the underlying models are fitted based on standard Cox regression which is not necessarily optimal for this task. To overcome this, we developed a framework to boost the concordance index which results in prediction rules with optimal discriminatory power. This framework was further extended via stability selection in order to enhance the variable selection. We could show that our new approach is able to automatically estimate a biomarker containing only the most relevant variables while outperforming standard approaches with respect to the resulting discriminatory power. The increased sparsity due to stability selection is favourable for interpretational and practical reasons, but does not necessarily lead to increased prediction accuracy.

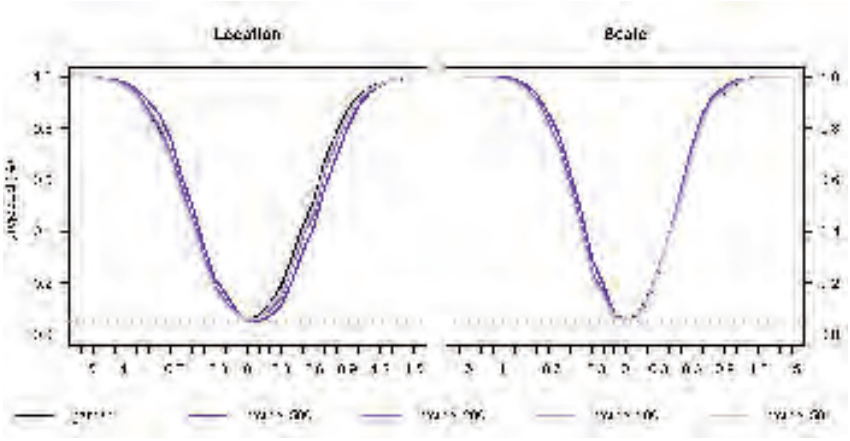
Boosting joint models

In many clinical studies, time-to-event outcomes are measured alongside longitudinal markers. From a methodological perspective, it is favourable to model these two outcomes jointly in order to avoid biased results. In cooperation with the newly started Project J61 we have developed a boosting algorithm to select and estimate joint models for this setting, including two separate sub-predictors. This first boosting algorithm for joint models could be a starting point for a new powerful framework of boosting joint models which offers versatile possibilities for further extensions and enhancements.



Regional partial effects of the boosted distributional regression model on stunted growth of children in India; the left part refers to the mean of the conditional distribution, the right part to the standard deviation.

Dr. Mayr



Rejection rates (power) of bootstrap-based significance tests for boosted location and scale models given different effect sizes (x-axis); the dotted line refers to the significance level.

Boosting generalized additive models for location, scale and shape

The algorithm for boosting distributional regression is implemented in the open source programming environment R via the add-on package gamboostLSS. In order to increase the visibility of the software and to provide an easy entry for other researchers to use our methodology we have published a tutorial paper where we highlight the strengths of our approach in model building and variable selection. We analysed data on childhood malnutrition in India by modelling the conditional distribution of a stunting score based on socio-demographic characteristics of mothers and children.

Boosting in the context of regularized regression

In a structured comparison between statistical boosting and the lasso approach for penalized regression we identified low-dimensional scenarios where both approaches lead to similar results although following a very different methodology. For high-dimensional data, we showed in simulations that the lasso leads to slightly sparser models while boosting tents to yield more accurate predictions. Putting all results into perspective, one could argue that the decision between both methods is probably less important than the optimal tuning of the algorithms (via the penalization parameter for the lasso and the stopping iteration for boosting).

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Invited lectures

International Conference of the ERCIM WG on Computational and Methodological Statistics, Dec. 10, 2016, Sevilla, Boosting Joint Models for longitudinal and time-to-event outcomes.
Insituto de Calculo, UBA, Feb. 23, 2016, Buenos Aires, An introduction to boosting generalized additive models for location, scale and shape.

Publications during funding period

Mayr A, Hofner B and Schmid M (2016) Boosting the discriminatory power of sparse survival models via optimization of the concordance index and stability selection. BMC Bioinformatics 17:288

Hepp T, Schmid M, Gefeller O, Waldmann E and Mayr A (2016) Approaches to regularized regression - A comparison between gradient boosting and the lasso. Methods of Information in Medicine 455(5): 422-430

Hofner B, Mayr A and Schmid M (2016) gamboostLSS: An R package for model building and variable selection in the GAMLSS framework. Journal of Statistical Software 74(1): 1-31

Faschingbauer F, Dammer U, Raabe E, Kehl S, Beckmann M, Schmid M, Schild RL, Mayr A (2016) A new sonographic weight estimation formula for small for gestational age (SGA) fetuses. Journal of Ultrasound in Medicine 35: 1713-1724

Mayr A, Schmid M, Pfahlberg A, Uter W, Gefeller O (2015) A permutation test to analyse systematic bias and random measurement errors of medical devices via boosting location and scale models. Statistical Methods in Medical Research: doi: 10.1177/0962280215581855 [Epub ahead of print]

Analysis of the role of IL-9 in the induction of Colitis-associated cancer (CAC)

Dr. Katharina Gerlach, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Inflammatory bowel disease (IBD) is linked to an increased risk of developing colitis-associated colorectal cancer (CAC). Especially T cells are critical mediators playing important roles in the development of inflammation and cancer. Recently, we have identified Th9 cells as IL-9 producing cells inducing colitis, but the involvement of IL-9 in CAC is not revealed so far. However, high numbers of IL-9-expressing T cells suggest an important role in the development of CAC.

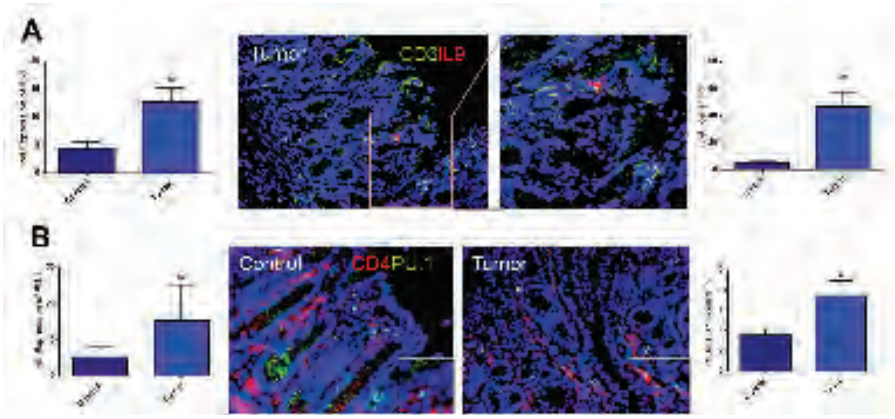
Background and aim

The two chronic types of colonic inflammation are associated with an increased risk for the development of colitis-associated colorectal cancer. About 20% of all forms of cancer arise in association with chronic inflammation and the tumor microenvironment may exhibit extensive inflammatory infiltrates with high levels of pro-inflammatory cytokine production. IL-9 producing T cells were found in a variety of different autoimmune or chronic inflammatory diseases as well as in cancer patients. Nevertheless, the role of Th9 and IL-9-producing cells in cancer immunity is unknown, and their ability to cause inflammation and destruction of tissues which leads to colon cancer might be of strong interest in the therapy of malignancy.

Our initial data showed an up regulation of IL-9-producing T cells in the lamina propria of IBD patients. Functional analysis revealed that IL-9-deficieny led to reduced development of colitis and a significant down regulation of the Th9 cell-related transcription factor PU.1. As these observations indicated that IL-9 controls intestinal inflammation, the aim of our study is to analyse the possible role of this cytokine in colitis-associated tumor growth.

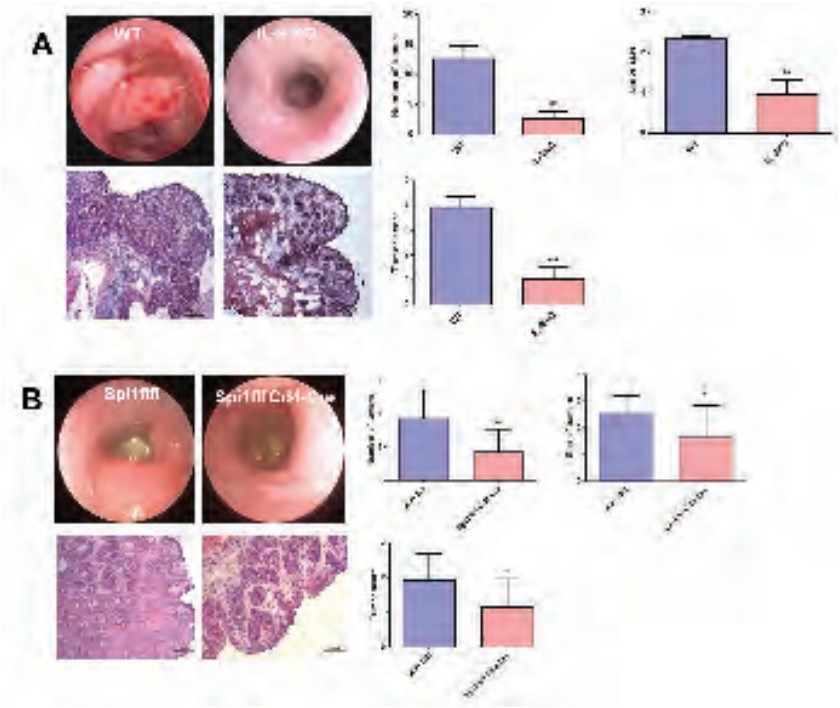
Analysis of Th9 cells in Colitis-associated cancer

To analyse CAC in mice we used the well-established AOM/DSS model and investigated in an initial series of studies the expression of IL-9 and Th9 cells in wildtype mice. Quantitative PCR analysis showed an up regulation of the transcription factor PU.1 and the proinflammatory cytokine IL-9 in AOM/DSS treated mice compared to controls which was additionally confirmed on the protein level by immunofluorescence staining. The detection of IL-9-producing T cells in this model points out the important relevance for the development of CAC. For functional relevance we used IL-9-deficient animals and subjected them to AOM/DSS. IL-9 deficiency led to significant less inflammation consequently followed with a significant reduction of tumors in the experimental model



(A) Higher levels of IL-9 expression in tumor tissue from AOM/DSS treated wildtype mice revealed by qPCR and immunofluorescent staining. (B) qPCR and immunofluorescent staining showed elevated PU.1 levels in AOM/DSS tumors of wildtype mice.

Dr. Gerlach



(A) IL-9 KO mice in the experimental AOM/DSS model had significantly lower numbers of tumors than wildtype mice. (B) Absence of the transcription factor PU.1 in T cells led to significantly reduced tumor growth.

of AOM/DSS. Miniendoscopic analysis as well as histological sections confirmed these findings. To gain a deeper understanding of the regulatory role of Th9 cells in the development of colitis-associated cancer the influence of the transcription factor PU.1 was investigated. Therefore conditional PU.1 KO mice were treated with AOM/DSS. Tumor development was reduced when PU.1 was absent in T cells compared to control mice further underlining the crucial involvement of these cells in CAC.

Our findings uncover a crucial role of IL-9 in the development of colitis-associated neoplasias as IL-9 led to tumor growth in the model of CAC. These data show that IL-9 plays a crucial role in the induction of inflammation and the development of CAC. The increased presence of PU.1-expressing T-cells in tumorigenic tissue illustrates the involvement of Th9 cells in the carcinogenesis.

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Invited lectures

International Congress of Immunology, 24.August 2016, Melbourne Australia, PU.1 expressing Th9 cells promote Colitis-associated cancer (CAC)

Awards

Hans-Hench Award, 27. September 2016 Meeting of the German Society for Immunology, Hamburg Germany

Publications during funding period

Popp V, Gerlach K, Mott S, Turowska A, Garn H, Atreya R, Lehr HA, Ho IC, Renz H, Weigmann B, Neurath MF (2016) Rectal Delivery of a DNAzyme That Specifically Blocks the Transcription Factor GATA3 and Reduces Colitis in Mice.Gastroenterology 152(1): 176-192. e5.

J51 - Progress Report

01.10.2015 - 31.03.2018

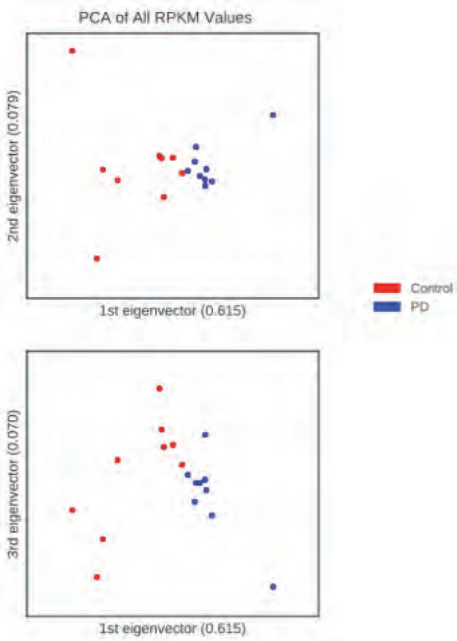
Neurosciences

Inflammatory signature in Parkinson´s disease

Dr. Franz Marxreiter, Department of Neurology (till 30.04.2016),
Department of Molecular Neurology (since 01.05.2016)

Cumulating evidence suggests that activation of the innate immune system is an integral part of the pathophysiology of Parkinson's Disease (PD), even in early stages of the disease. The role of this immunogenic response at onset and during progression of PD is not well understood. Studies aiming at parallel profiling of the prototypical cells of the innate immune system, namely peripheral monocytes or microglia, as well as astrocytes are scarce. Therefore, this project aims at understanding the functional consequences of altered monocytic/microglial/astroglial activity in PD.

During the first episode of the funding period we focused on the innate immune system, more specifically monocytes. We hypothesized that the activation state of the innate immune system is altered in early stage PD patients. We therefore assessed (I) the cytokine profile; (II) the cellular profile; and (III) the monocytic transcriptome signature in early stage PD patients vs. healthy controls. In addition, we assessed whether monocytes show an altered response to a proinflammatory stimulus.



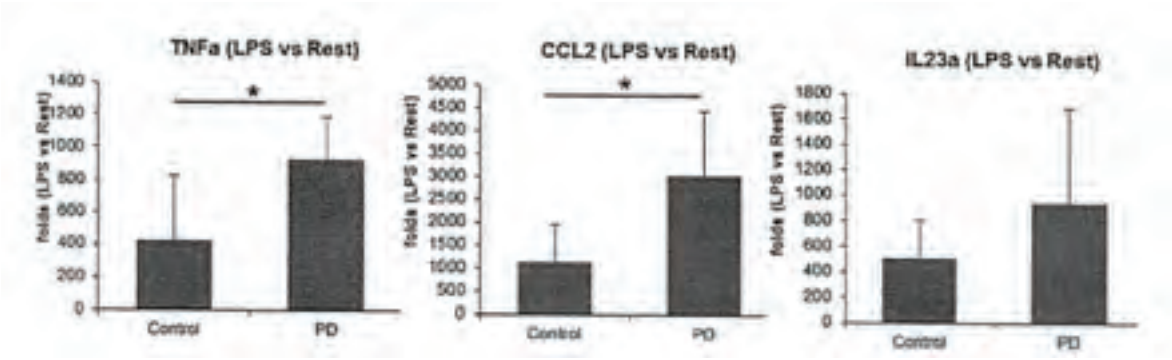
Principal component analysis of the differentially expressed genes indicates a subtle, but distinct monocytic transcriptional signature that separates PD from controls.

Fasting blood collection was performed according to a standardized procedure in patients with early stage PD and controls. White blood cell count and serum cytokine levels were determined by the diagnostic laboratory of the Department of Internal Medicine 3, FAU Erlangen-Nuernberg. We could not detect significant differences between both groups (data not shown).

To detect differences in the composition of monocyte subpopulations, we performed a FACS based analysis of monocyte subpopulations in the patient cohort. There was no significant difference with regard to classical ($CD14^{+}CD16^{-}$) and non-classical monocyte subpopulations (data not shown). Taken together, the cytokine and monocytic profile in PD is not altered.

Next, we analyzed the monocytic gene expression signature of early PD patients using RNA-sequencing (RNA-seq) of whole monocyte populations of 12 PD patients and 9 controls. To identify genes that showed modest but consistent differences between controls and PD patients, we filtered genes according to the Irreproducible Discovery Rate (IDR) for each gene. Kruskal Wallis test identified 41 differentially expressed genes. The majority of genes were down-regulated in PD patients (32 down-regulated and 9 up-regulated genes), 40 of the 41 genes were validated by 5-fold stratified cross validation ($p<0.05$). Principal component analysis on these genes suggests that these genes form a signature that separates PD from controls.

Dr. Marxreiter



Monocytes from PD patients show an altered response to the TLR4 agonist LPS compared to controls ex vivo. Expression of TNF-alpha, CCL2 mRNA and IL23a in monocytes 3 hours after stimulation with 10 µg/ml LPS as determined by qPCR and normalized to GAPDH expression (n = 5 per group; t-test; *p<0.05; mean ± SEM).

Next we analyzed, whether the monocytes of PD patients show an altered response to lipopolysaccharide (LPS). We determined TNF-α and IL-1β mRNA levels 3 hours after stimulation with LPS in monocytes isolated from controls and PD. Stimulation with LPS showed an increased pro-inflammatory response indicated by a significant up-regulation of TNFα in the PD group compared to controls. Despite lower baseline levels, IL-1β mRNA was similar in both groups.

In conclusion our findings suggest that, while the cytokine profile and the monocytic composition are not altered in PD, there is a distinct gene expression profile in monocytes from PD patients that separates them from controls. In addition, monocytes derived from early-stage PD patients are more susceptible to exogenous inflammatory stimuli and show a state of enhanced immunogenic reactivity.

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Publications during funding period

none

J52 - Progress Report

01.11.2015 - 30.04.2018

Modeling cortical dysfunction of SPG11 spastic paraplegia using patient-derived pluripotent stem cells

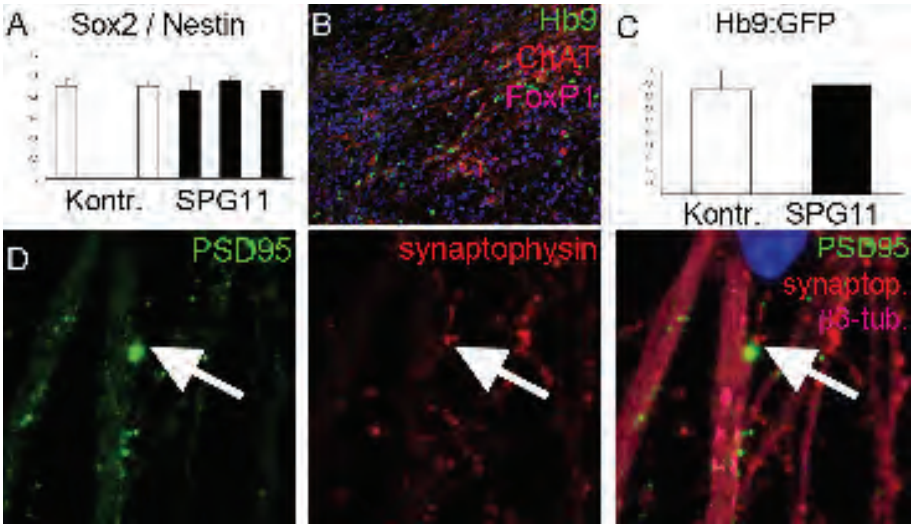
Dr. Martin Regensburger, Department of Neurology

Mutations in SPG11 are the most frequent cause of complicated autosomal-recessive hereditary spastic paraplegia. A subset of patients develop severe axonal motor neuropathy. As a disease model, we have set up the differentiation of SPG11-patient induced pluripotent stem cells (iPSC) into motor neuron progenitors (MNP) and alpha motor neurons (aMN). Survival and proliferation of MNP and aMN were not impaired in SPG11-derived cells. Future studies will focus on the axonal compartment in SPG11 aMN.

Previous studies indicated that patients with homozygous or compound heterozygous mutations in SPG11 patients exhibit a phenotypic spectrum with substantial interindividual variability. Peripheral axonal motor neuropathy causes disabling amyotrophy in SPG11 and there is no experimental model of neuropathy in SPG11 so far. Therefore, we have set up a model of neuropathy in the relevant cell type of aMN derived from SPG11 patient-specific iPSC.

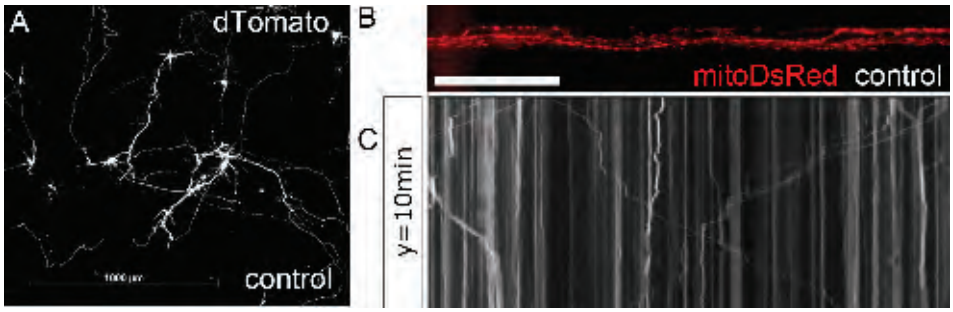
Generation of SPG11 iPSC

Patients and healthy controls were recruited within the movement disorders outpatient clinic of the Division of Molecular Neurology and underwent detailed phenotypic analyses of complicating symptoms including motor neuropathy. iPSC from SPG11 cases with different levels of peripheral neuropathy were reprogrammed from patient-derived fibroblasts. Lines showing a normal caryotype and a low number of copy number variations were selected for subsequent analyses.



Characterization of MNP and mature aMN. A) The ratio of Sox2-/Nestin-positive cells was at least 80% in SPG11 and controls. B) Mature aMN (day 30) express Hb9, choline acetyltransferase and FoxP1. C) The ratio of Hb9-positive cells was at least 85%. D) Mature aMN express pre- and postsynaptic markers (arrow).

Dr. Regensburger



Morphological and functional neurite analysis of aMN A) Neurite morphology is determined by quantification of length and branching of single dTomato-transfected aMN. B) aMN are grown in microfluidic chambers which allow unidirectional growth of the longest axon-like neurites. *Scale bar* 50µm. C) Sample kymograph of mitochondrial movement within the axon shown in (B) over 10 minutes.

Differentiation into MNP and aMN

The modified dual SMAD inhibition protocol yields a high ratio of Sox2-/Nestin-positive MNP and a high ratio of mature aMN. MNP are generated by treatment of iPSC with small molecules and caudalizing/ventralizing morphogens. Maturation from MNP into aMN is achieved by treatment with neurotrophic growth factors over a period of at least 2 weeks. Differentiation was verified by gene and protein expression of Hb9, choline acetyltransferase and Islet-1. Formation of synapses was shown by synaptophysin-/PSD95-colocalization analysis. aMN showed electrophysiological properties of mature neurons. Proliferation of MNP, as determined by PCNA staining and BrdU incorporation assays, was unchanged in SPG11. We also found that levels of cell death were not altered in SPG11 when compared to aMN derived from controls.

Analysis of the SPG11 aMN axon

In summary, we have established an aMN differentiation paradigm to model motor neuropathy in SPG11-HSP. As previous *in vitro* studies of corticospinal motor neurons and neuropathological *post-mortem* evidence suggest a dying-back axonopathy in SPG11, future analyses will focus on neuritic and specifically axonal morphology and function in SPG11 aMN.

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Invited lectures

Regionaltreffen des HSP-Fördervereins „Gehn mit HSP“, 01.10.16, Ingolstadt, Genetische Grundlagen der HSP

24. Jahreskongress der Deutschen Interdisziplinären Gesellschaft für Außerklinische Beatmung e.V., 04.06.16, Bamberg, Amyotrophe Lateralsklerose – Neues zu Pathophysiologie und zu klinischen Studien

Publications during funding period

none

J53 - Progress Report

03.08.2015 - 02.02.2018

Neurosciences

Diffusion tensor imaging of the visual pathway in pseudoexfoliation glaucoma

Dr. Manuel Schmidt, Department of Neuroradiology

Pseudoexfoliation syndrome (PEX) is an aging-related systemic disorder of the extracellular matrix. Some patients with PEX develop glaucoma (PEXG). There is a strong genetic component (e.g. common SNPs of the LOXL1 gene are associated with PEX(G)). However, genetic testing is not suitable to identify those with PEX at increased risk for developing secondary glaucoma. Aim of this project is to explore the role of injury of the visual pathway in affected patients with structural MR-imaging (DTI).

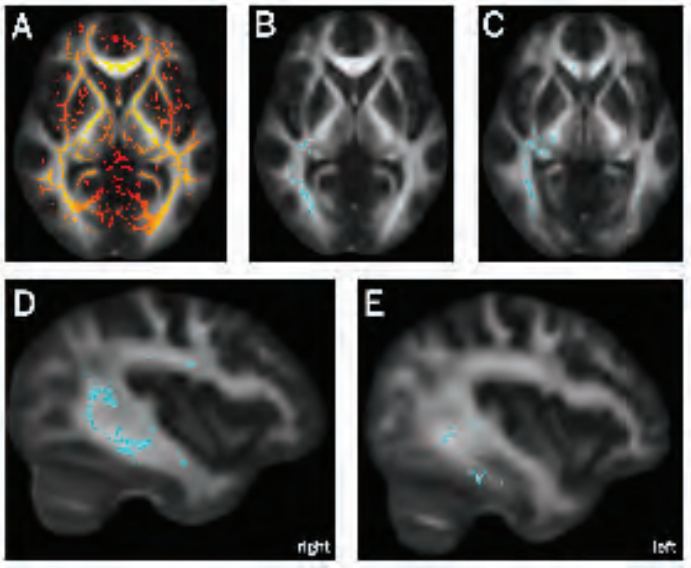
In PEX-associated glaucoma, protein deposits block normal drainage of aqueous humor and lead to an elevated intraocular pressure (IOP) and subsequently to secondary glaucoma. However, this theory is not satisfying as crucial questions regarding the pathogenesis of PEXG are still not answered and factors other than elevated IOP have to be considered due to the known systemic disorder.

We visualize and quantify the extent of neuronal degradation along the visual pathway in PEXG to identify subgroups based upon structural DTI patterns with regard to genetic prognostic factors for populations at risk for developing PEXG.

We use a sophisticated, customized DTI sequence with 2 mm spatial resolution at a clinical high-field scanner. This is a multiplexed echo planar imaging diffusion tensor sequence (M-EPI). For best correction of susceptibility artifacts, we use two acquisitions in diametrically different phase encoding direction (0/180 degrees) which are combined through post processing.

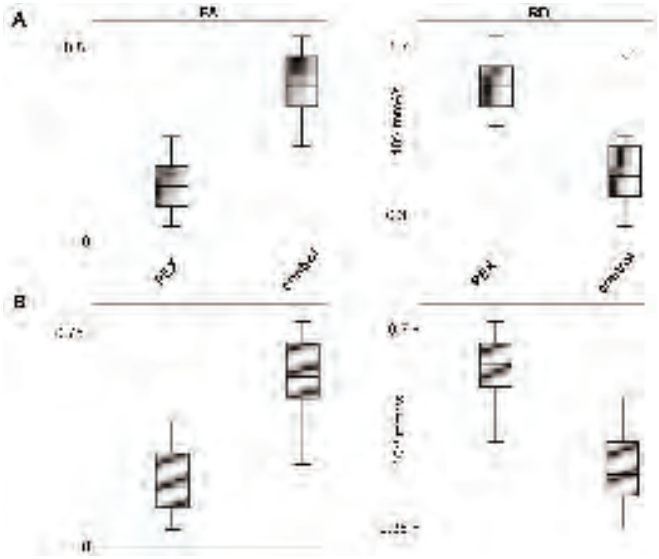
Data analysis is performed in a two-step approach: Voxel-wise statistical analysis of whole brain diffusion maps (TBSS) is followed by a ROI based evaluation of conspicuous structures to obtain the tensor-derived diffusion values.

One important clinical feature of PEXG is an impaired stereoacuity resulting in depth perception deficits. With distinct disparity cells sensitive to binocular disparity, located in the primary visual cortex and extrastriate areas representing the neuronal basis of stereovision, injury of the central visual pathway is suspected in PEXG. Indeed, our cohort of PEXG patients shows rarefaction of the optic radiation on diffusivity maps. We discovered markedly reduced fractional anisotropy (FA) in the optic radiation of PEXG patients suggesting axonal damage. In order to specify the underlying cause of rarefied optic radiation fibers we also evaluated radial diffusivity (RD).



(A) Mean FA skeleton of all tracts common to the group. (B-E) Clusters of voxels with reduced FA in PEXG vs controls (p = 0.15). Brain mask for anatomical orientation. (B) and (C) show axial reformations, (D) and (E) sagittal reformations of the optic radiation.

Dr. Schmidt



(A) Decreased fractional anisotropy (FA) and corresponding increased radial diffusivity (RD) of the optic tract shows neuronal degradation. (B) Similarly altered diffusivity can be found in the optic radiation (4th neuron of the visual pathway).

Radial diffusivity is known to be modulated by myelin in white matter structures. Our findings suggest that dysmyelination and demyelination contribute to rarefaction of the optic radiation in PEXG and may result in impaired stereoacuity. However, neuronal damage is not limited to the optic radiation in these glaucoma patients. We discovered pathological diffusivity in the prechiasmal visual pathway implying widespread neuronal degradation in PEXG. Further exploration of genetic risk factors (TLR4, LOXL1) will help us define the relevance of myelin and axonal dysfunction in PEXG.

The structural imaging methods developed within the scope of this project have already found their way into other scientific projects regarding neurodegenerative diseases (MSA, SPG4, Parkinson's disease). For the second year of the project, we plan further measurements of PEXG patients with a newly installed ultra high-field 7T scanner. Structural imaging at 7T will yield improved evaluation of crossing fiber tracts (HARDI) combined with higher spatial resolution for detailed anatomical visualization.

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Invited lectures

Deutscher Röntgenkongress, Mai 2016, Leipzig, Ultra-Hochfeld MRT der Sehbahn bei 7T - erste Ergebnisse bei Glaukom

RSNA 2016, Annual Meeting of the Radiological Society of North America, December 2016, Chicago, USA, Ultra-high-field DTI of the visual pathway at 7T

Awards

RSNA Travel Award for young investigators, Dr. Manuel Schmidt, December 2016 Chicago

Publications during funding period

none

J54 - Progress Report

01.11.2015 - 30.04.2018

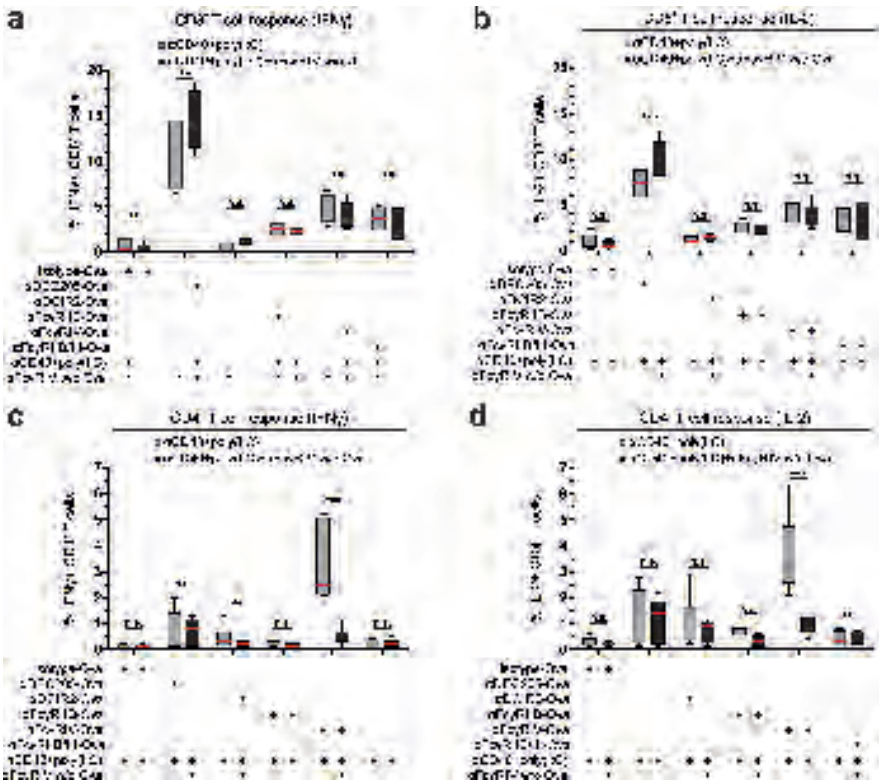
Oncology

Analysis of alternative mechanisms of tumor rejection

Dr. Christian Lehmann, Department of Dermatology

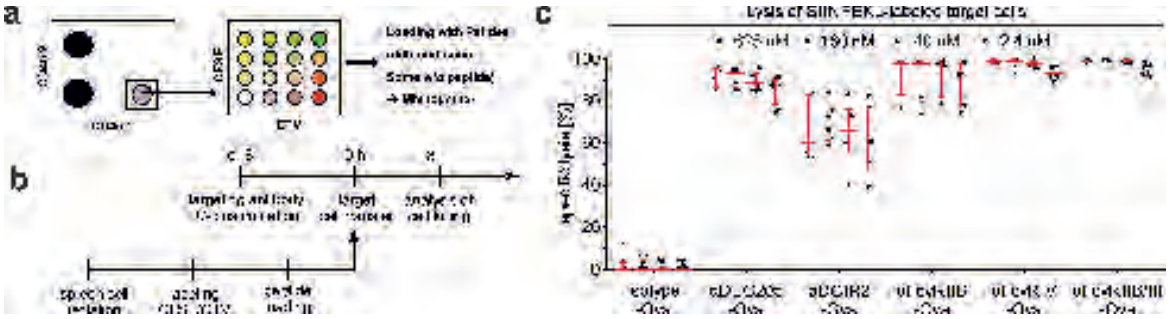
Immunologic tumor therapies aim to prolong patient survival mainly by inducing cytotoxic CD8⁺ T cell responses to a limited number of tumor epitopes. We have demonstrated in a murine melanoma model the independency of prolonged survival from the strength of induced CD8⁺ T cell responses. We speculate that a major part of this effect is due to the protection from tumor metastases formation. We would now like to investigate the underlying mechanisms to provide hints for tumor therapy improvement.

One of the major goals in immunological tumor therapies is the induction of strong CD8⁺ T cell responses, as these cells are able to kill cancer cells directly and specifically by different mechanisms. The major measurement techniques for the strength of such responses in human beings are based on the cytokine production of such effector CD8⁺ T cells. These include ELISpot, intracellular cytokine FACS and capture assays. It is generally believed that a strong production of Th1 cytokines, such as IFNγ is a prerequisite and an adequate measurand to determine the efficacy of an immunological anti-tumor response. However, by the specific induction of immune responses via in vivo antigen targeting, we have been able to demonstrate that the functional capacity of CD8⁺ T cells (by cytokine production) does not directly correlate with their potential to lyse target cells.



Induction of T cell responses in naïve mice. (a-d) C57BL/6 mice were immunized +αCD40+poly(I:C) +/-αFcγRIV w/o Ova. 14 d later, cells were re-stimulated with an ovalbumin peptide pool. Intracellular (a, c) IFNγ and (b, d) IL-2 by flow cytometry.

Dr. Lehmann



Lysis of target cells. (a) Labeling (b) Experimental design (c) Lysis of SIINFEKL labeled (2.4 to 625 nmol) target cells 8 days after immunization with targeting antibodies +αCD40+poly(I:C) by in vivo killing assay.

Investigation of newly established antibodies for antigen targeting

In the past we mainly worked with targeting antibodies specific for the major classical DC subsets ($CD8^+$ and $CD8^-$ DCs), which preferentially induce $CD8^+$ or $CD4^+$ T cell responses, respectively. Lately, we have been able to also clone antibodies specific for FcγRIIB, FcγRIV, and FcγRIIB/III. With these antibodies, in particular αFcγRIV-Ova, we have been able to induce strong simultaneous $CD4^+$ and $CD8^+$ T cell responses in a transgenic T cell system. We now could show that these targeting antibodies are also capable to induce Ova specific responses in naïve mice. This is clearly shown by the induction of IFNγ and IL2 in $CD8^+$ and $CD4^+$ T cells.

Killing of target cells after antigen targeting

To gain more insights into the functionality of immune responses, we used our previously established in vivo killing assay and determined the strength of the immune reaction by using different targeting antibodies. The assay itself is based on the transfer of so-called target cells, which have been isolated from a naïve congenic mouse (e.g. $CD45.1^+$). After isolation, the cells are divided into 25 equal proportions, which are labeled with different concentrations of CFSE and cell trace violet (CTV). Afterwards, these populations can be loaded with various peptides or diverse concentrations of these peptides. Shortly before transfer into the previously immunized mice, the 25 populations are mixed together. Its specific labeling can identify each single population. Thereby it is possible to analyze the lysis of many different peptide loaded cells in one single mouse. We found that all used targeting antibodies can induce an immune response capable of killing peptide loaded target cells.

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Publications during funding period

Heidkamp, GF*, Sander J*, Lehmann CHK, Heger L, Eissing N, Baranska A, Lühr JJ, Hoffmann A, Reimer KC, Lux A, Söder S, Hartmann A, Zenk J, Ulas T, McGovern N, Alexiou C, Spriewald B, Mackensen A, Schuler G, Schauf B, Forster A, Repp R, Fasching PA, Purbojo A, Cesnjevar R, Ullrich E, Ginhoux F, Schlitzer A, Nimmerjahn F, Schultze JL*, Dudziak D* (2016) Human lymphoid organ dendritic cell identity is predominantly dictated by ontogeny, not tissue microenvironment. Science Immunology 1, eaai7677; 1-17

Lehmann CHK, Baranska A, Heidkamp GF, Kiessling M, Spoulat D, Krug A, Ravetch JV, Leussen JHW, Nimmerjahn F, Dudziak D (2016) 364 Antigen targeting of Fc-receptors induces strong T cell responses in vivo independent of ITAM signaling. Journal of Investigative Dermatology 136(9): 223

Lehmann CHK, Heger L, Heidkamp GF, Baranska A, Lühr, JJ, Hoffmann, A, and Dudziak D (2016) Direct delivery of antigens to Dendritic cells via antibodies specific for endocytic receptors as a promising strategy for future therapies. Vaccines 4, 8

The role of microRNA-188-5p dysregulation in hepatocellular carcinoma development and progression

Dr. Peter Dietrich, Institute of Biochemistry

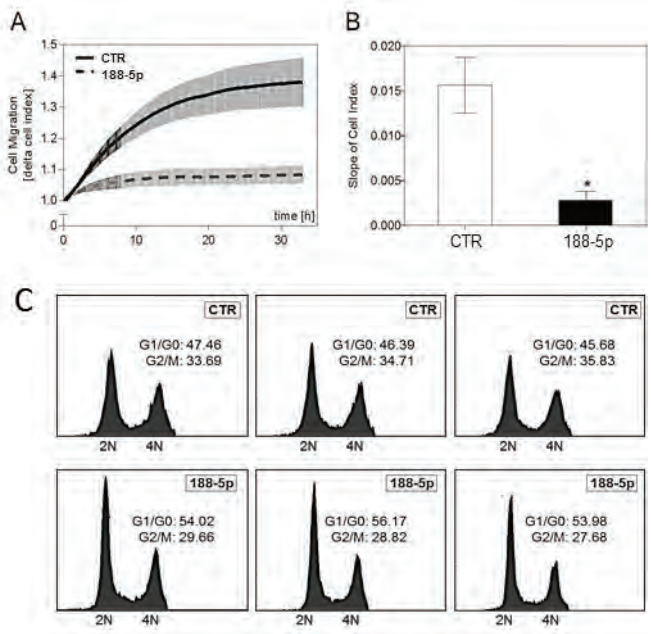
Aberrant microRNA-expression correlates with severity and prognosis of hepatocellular carcinoma (HCC). Our results demonstrate that microRNA-188-5p (miR-188-5p) is strongly downregulated in HCC cell lines and tissues. Re-expression of miR-188-5p strongly inhibited proliferation, clonogenicity, and migration of HCC cell lines. Using in silico and cDNA-expression array analysis, we identified potential novel target genes for the miR-188-5p in HCC that will be further evaluated.

Background

Hepatocellular carcinoma (HCC) has poor prognosis. Dysregulated microRNAs (miRs) are more and more recognized to play crucial roles in HCC development and progression. Recently, we showed that miR-188-5p is downregulated in activated synovial fibroblasts in rheumatoid arthritis (RASF) that display tumor-like functions such as tissue invasion, enhanced proliferation and migration. Re-expression of miR-188-5p strongly reduced migration in RASF. Therefore, we asked if miR-188-5p could also play a functional role in tumor development and progression. We found that miR-188-5p was markedly downregulated in HCC cell lines and tissues. Therefore, the aim of this study is to investigate the regulation and function of miR-188-5p in HCC and to reveal potential novel target genes.

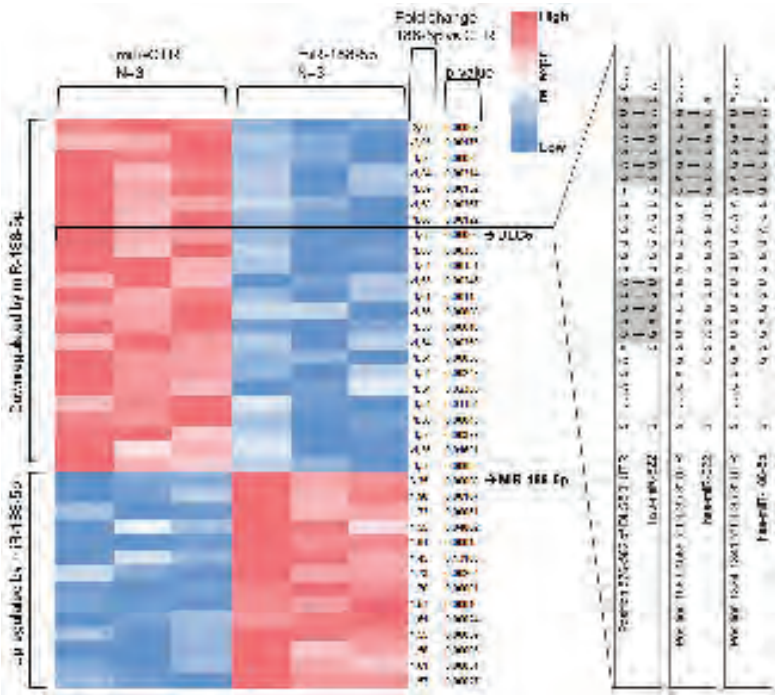
Results

Preliminary qRT-PCR analysis revealed that microRNA-188-5p is downregulated in different HCC cell lines as well as primary and metastatic tissue samples from HCC patients. To get insights of miR-188-5p function in HCC, we re-expressed miR-188-5p. We found a significant inhibition of HCC cell proliferation after re-expression of miR-188-5p. In contrast, downregulation of endogenous miR-188-5p by transfection of an anti-miR-188-5p construct enhanced the



(A) Migration after transfection with miR-188-5p (188-5p) as compared to control-transfected (CTR) HCC cells (e.g. cell line PLC). (B) Summarized analysis of migration for PLC as depicted in (A) (*: $p<0.05$ vs. CTR). (C) Flow cytometric propidium iodide staining depicts percentage of cells (PLC) in cell cycle fractions (G1/G0, G2/M) after 188-5p-transfection as compared to controls (CTR).

Dr. Dietrich



cDNA array analysis comparing control-transfected (miR-CTR) and miR-188-5p-transfected HCC cells (Hep3B). Significantly downregulated genes with 3'UTR binding sites for the miR-188-5p (e.g. DLG5) will be further analyzed.

proliferation of HCC cells. Moreover, we performed flow cytometric cell cycle analysis, and detected enhanced G1 and reduced G2 cell cycle fractions after microRNA-188-5p transfection in HCC cells. Clonogenicity assays revealed marked inhibition of stem-cell-like behavior of miR-188-5p-transfected HCC cells. Cell migration experiments revealed markedly reduced migration of HCC cells after miR-188-5p transfection. In the following, we performed cDNA-array expression analysis of miR-188-5p-transfected HCC cells and control-transfected cells. About 60 genes revealed significant downregulation after miR-188-5p transfection, pointing to possible direct or indirect regulation by miR-188-5p. In the following, these genes were pre-selected by *in silico* analysis of conserved 3'UTR binding sites for the miR-188-5p. Afterwards, these genes were further selected by their potential to reveal novel oncogenic functions in HCC. The remaining genes (19 genes) will be evaluated regarding their regulation by miR-188-5p and their functional roles in HCC.

Conclusions/Outlook

MiR-188-5p is a strong tumorsuppressive microRNA which is markedly downregulated in HCC. Investigation of miR-188-5p induced regulation of gene expression using cDNA array- and *in silico*-based analysis revealed multiple potential novel target genes for HCC.

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Publications during funding period

none

Newly started Projects

J56 01.01.2017 - 30.06.2019 Immunology and Infection

Epigenetic reprogramming of macrophages


Dr. Palumbo-Zerr

Dr. Katrin Palumbo-Zerr, Department of Medicine 3 – Rheumatology and Immunology

Our preliminary data show that recognition of apoptotic cells (ACs) by macrophages dramatically affects chromatin remodelling processes within these phagocytes, resulting in an anti-inflammatory macrophage phenotype. We hypothesize that these events essentially contribute to the non-immunogenic clearance of dying cells. In the proposed project, we plan to dissect the underlying molecular mechanisms and determine the role of these pathways during the resolution of injury-induced inflammation.

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J57 01.01.2017 - 30.06.2019 Immunology and Infection

Herpesviruses and DUX4


Dr. Full

Dr. Florian Full, Institute of Clinical and Molecular Virology

DUX4 is a developmental transcription factor exclusively expressed in early embryogenesis. Aberrant expression of DUX4 in muscle cells is the cause of Facioscapulohumeral muscular dystrophy. Preliminary data showed, that DUX4 expression is also induced upon herpesviral infection, leading to the upregulation of hundreds of DUX4 target genes. This proposal aims at identifying the mechanism of herpesviral DUX4 induction and the role of DUX4 and its target genes on viral replication.

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J58 01.09.2016 - 28.02.2019 Oncology

Counteracting Wnt signaling


Dr. Bernkopf

Dr. Dominic Bernkopf, Chair of Experimental Medicine II

Axin and conductin are paralogous negative regulators of Wnt/β-catenin signaling. Polymerisation, which is essential for axin activity, is inhibited in conductin by its RGS domain making it less active than axin. Conductin polymerisation could be enforced by a RGS-binding protein or by introducing the axin RGS domain in conductin thereby increasing degradation of β-catenin. We will study how conductin polymerisation is regulated with the goal to inhibit β-catenin driven colorectal cancer growth.

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J59 30 months Oncology

Immunotoxin induced anti-tumor immunity


Dr. Müller

Dr. Fabian Müller, Department of Medicine 5 – Haematology and Oncology

Preclinical and first clinical data suggest that treatment with recombinant immunotoxin (rITs) targeting mesothelin induced anti-tumor immunity in patients. The goal of this study is to test CD22-targeting rITs for their capacity of inducing anti-lymphoma immunity in a newly established syngeneic mouse model. The model will be used to understand molecular mechanisms central to the lymphoma-host-interaction and to optimize immune-modulating combination treatment based on lymphoma-targeting rITs.

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Newly started Projects

J60 01.10.2016 - 31.03.2019 Molecular Medicine

The role of Hck/Lyn in Vesicles secretion

Dr. Lee

Dr. Jung-Hyun Lee, Department of Dermatology

In recent work I detected that plasma extracellular vesicles (pEV) from HIV-infected individuals and tumor patients contain either significant amounts of Hck or Lyn. In the here presented grant proposal we suggest to investigate the molecular mechanism behind this finding. We will (1) analyze the molecular interactions of Hck and Lyn with identified factors of the pEV secretion complex, and (2) clarify at which subcellular compartment these molecular events take place.

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J61 01.01.2017 - 30.06.2019 Others

Extending joint models in biomedical outcomes

Dr. Elisabeth Waldmann, Department of Medical Informatics, Biometry and Epidemiology

Dr. Waldmann

Research on the association between longitudinal and time of event measurements is crucial in biomedical studies. This association however can only be modelled reliably if the two quantities are modelled jointly. The aim of this project is to extend those joint models in both: on one side to include the ability to model longitudinal processes more appropriately for different data structures and on the other side in the important question of variable selection.

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Pilot Projects

Pilot Projects

Overview

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Pilot Projects

Ongoing Projects

Project No.	Project title	Applicant	Term	Institute
AU-14-06-04-1	Gene therapy to protect corneal endothelial cells during cultivation in eye banks and after transplantation	Prof. Dr. Dr. Fuchsluger	01.05.2015-15.03.2017	Department of Ophtalmology
AU-14-10-06-1	Qualification to „Master of Medical Education“	Dr. Menzel-Severing	15.09.2015-14.02.2017	Department of Ophtalmology
CH-15-04-27-1	Identification of actin binding domains within GBP1 and their role in actin remodeling	Dr. Unterer	01.02.2016-31.01.2017	Department of Surgery
DE-15-12-15-1	Generation of better designer dentritic cells for therapeutic cancer vaccination by electroporation of MRNA encoding constitutively active TRAF6	Dr. Hoyer	01.04.2016-31.03.2017	Department of Dermatology
DE-16-08-11-1	The simultaneous siRNA-mediated knock-down of inhibitory receptors to improve engineered CAR/TCR-T-cell functionality	Dr. Uslu	01.01.2017-31.12.2017	Department of Dermatology
FK-15-11-18-1	Quantification of the surface and transmembrane domain of Syncytin-1 for diagnosis and prognosis of placental disorders and breast cancer	Dr. Rübner	01.06.2016-30.04.2017	Department of Obstetrics and Gynecology
HP-15-10-07-1	Mesenchymal stem cells and myoblast differentiation under GDF-11 and IGFBP stimulation on PCL-collagen-nanofiber scaffolds	Dr. Cai	01.07.2016-30.06.2017	Department of Plastic and Hand Surgery
ID-15-12-22-1	Herpes simplex virus type 1 mediated modulation of dendritic cell adhesion and migration	Dr. Heilingloh	01.07.2016-30.06.2017	Department of Immune Modulation
M1-15-12-16-1	Epithelial cell shedding in the absence of geranylgeranylation	Dr. López Posadas	01.11.2016-31.10.2017	Department of Medicine 1
M1-16-02-17-1	Analysis of α4β7 and β7 integrin blockade in inflammatory bowel diseases	Dr. Zundler	01.10.2016-30.09.2017	Department of Medicine 1
M3-15-08-06-1	The mechanisms of glucocorticosteroids- and estrogens- regulated autophagy in osteoclasts	Dr. Lin	01.04.2016-31.03.2017	Department of Medicine 3
M3-15-08-16-1	Female sex steroids impact on antibody glycosylation	Dr. Engdahl	02.02.2016-31.01.2017	Department of Medicine 3
M3-15-08-17-1	The impact of Siglec-9 and Siglec-E on osteoclastogenesis	Dr. Harre	01.03.2016-28.02.2017	Department of Medicine 3
M3-15-08-25-1	Crosstalk between FcgR and IFNgR signaling pathways during OC differentiation	Dr. Herbort (Groetsch)	01.07.2016-30.06.2017	Department of Medicine 3
M3-16-03-30-1	Evaluation of the impact of medium to long chain fatty acids on bone resorbing cells.	Dr. Zaiss	16.10.2016-15.10.2017	Department of Medicine 3
M4-15-11-10-1	Identification of interleukin-1alpha as a key cytokine in crystal-induced renal failure.	Dr. Knauf	01.05.2016-30.04.2017	Department of Medicine 4
M5-15-12-16-1	GMP-compliant manufacturing of bispecific T cells through retroviral TCR transduction	Dr. Gary	01.10.2016-30.09.2017	Department of Medicine 5
MP-16-05-24-1	The role of Tbet expressing Foxp3+ regulatory T cells in lung carcinoma	Dr. Andreev	01.12.2016-30.11.2017	Department of Molecular Pneumology
NT-15-10-28-1	The β-Catenin/Wnt-Pathway as Cross-Talk-Mechanism between Podocytes and Parietal Epithelial Cells in the Development of Focal-Segmental Glomerulosclerosis	Dr. Pfister	01.09.2016-31.08.2017	Department of Nephropathology
NT-16-01-04-1	Mechanism and functional role of MTOC translocation during heart development	Dr. Vergarajauregui	01.06.2016-31.05.2017	Department of Nephropathology

Terminated Projects

Project No.	Project title	Applicant	Term	Institute
AN-14-08-08-1	Identification of psychophysical parameters for prediction of migraine attacks	Dr. Fraunberger	01.03.2015-31.05.2016	Department of Anesthesiology
DE-14-05-14-1	Receptor-transfected gamma/delta T cells; the new magic bullets against cancer?	PD Dr. Schaft	01.03.2015-29.02.2016	Department of Dermatology
DE-14-07-22-1	Exosomic miRNAs as mediators of the interaction between the tumor and the immune system. A Systems Biology perspective	Prof. Dr. Vera-González	16.02.2015-15.02.2016	Department of Dermatology
DE-14-10-17-1	Analysis of the development of Dendritic Cells in human lymphoid organs	Dr. Heidkamp	01.04.2015-30.04.2016	Department of Dermatology
DR-14-09-10-1	Ultra high field MR-microscopy of human hippocampi: Morphologic and multiparametric characterization of hippocampal sclerosis	Dr. Gillmann	01.05.2015-30.04.2016	Department of Neuroradiology
HC-14-12-19-1	Effects of Serotonin Receptor Antagonists on the Development of Transplant Vasculopathy	Dr. Heim	01.01.2016-31.12.2016	Department of Cardiac Surgery
HP-13-12-11-1	Paracrine and cell-cell interactions of adipose-derived stem cells and mammary epithelial cells in breast cancer	Dr. Weigand	01.12.2014-31.05.2016	Department of Plastic and Hand Surgery
JP-14-10-15-1	Influence of different light expositions on sleep and attention/arousal in teenagers	Dr. Studer	01.04.2015-31.03.2016	Department of Child and Adolescent Mental Health
KI-14-05-21-1	Altered nephrogenesis and renal regeneration after acute nephron loss in a model of prematurity.	PD Dr. Menendez-Castro	15.02.2015-14.08.2016	Department of Paediatrics and Adolescent Medicine
KI-15-02-18-1	Establishment of new screening methods for cancer predisposition in young patients with rare tumors	Dr. Brecht	01.12.2015-30.11.2016	Department of Paediatrics and Adolescent Medicine
M1-14-05-21-1	Role of Eomes in the immunological control of colitis-associated tumorigenesis.	Dr. Atreya	01.01.2016-31.12.2016	Department of Medicine 1
M2-14-10-22-1	Prediction of fluoroscopic angulations for transcatheter aortic valve implantation by CT angiography: Influence on procedural parameters	Dr. Hell	01.06.2015-29.02.2016	Department of Medicine 2
M2-15-01-12-1	Learning curve for the detection of coronary artery stenoses with coronary computed tomography	Dr. Schuhbäck	01.11.2015-31.10.2016	Department of Medicine 2
M2-15-06-02-1	Suppression of Th1 response by soluble antigens of Litomosoides sigmodontis and its impact on murine atherosclerosis	Dr. Dietel	01.12.2015-30.11.2016	Department of Medicine 2
M3-14-05-26-1	Physical activitiy of patients with rheumatoid arthritis and the effect of interventional training on etiopathology	Dr. Liphardt	16.01.2015-15.07.2016	Department of Medicine 3
M3-14-12-17-1	Non canonical Wnt signaling in Systemic Sclerosis	Dr. Bergmann	01.10.2015-30.09.2016	Department of Medicine 3
M4-14-10-10-1	Effects of HIF-induced and mitochondrially targeted PHD3 in primary renal tubular cells	Dr. Bihlmaier	16.05.2015-15.05.2016	Department of Medicine 4
M5-14-10-26-1	The role of HLA-DO in the pathogenesis of type 1 diabetes	PD Dr. Kremer	01.02.2015-31.01.2016	Department of Medicine 5
NC-14-03-04-1	Dynamics of flow and cerebral proteins in the cerebrospinal fluid in hydrocephalus patients	Dr. Brandner	01.10.2014-31.03.2016	Department of Neurosurgery
NT-14-07-04-1	Identification of novel regulators of cell division	Prof. Dr. Engel	01.02.2015-31.07.2016	Department of Nephropathology

Pilot Projects

Project No.	Project title	Applicant	Term	Institute
NT-15-06-14-1	ADAMTS13 and miR-532-3p: Glomerular in vivo analysis and regulatory mechanisms in in vitro and in vivo models of humoral rejection	Dr. Bockmeyer	01.01.2016-31.12.2016	Department of Nephropathology
PH-14-12-16-1	TERT Core Promotor Mutations and HRAS Mutation Mosaicism in Early-Onset Bladder Cancer	Dr. Giedl	01.10.2015-31.03.2016	Institute of Pathology
SO-14-03-14-1	Coping styles and unmet needs of patients with cancer with special consideration of the migration background	Dr. Morawa	02.09.2014-30.04.2016	Department of Psychosomatic Medicine and Psychotherapy
SO-15-03-18-1	Prospective, randomised, double-blind, placebo-controlled clinical trial on the effects of an estrogen-progestin combination in adult female patients suffering from anorexia nervos	PD Dr. Paslakis	01.01.2016-31.12.2016	Department of Psychosomatic Medicine and Psychotherapy
ST-15-02-16-1	Mechanism of the synergistic anti-cancer effect of Efavirenz and ionizing radiation	Dr. Hecht	01.11.2015-31.10.2016	Department of Radiation Oncology
UR-14-07-11-1	The microRNA profile of circulating tumor cells (CTCs) in prostate carcinoma	Dr. Lieb	01.04.2015-31.03.2016	Department of Urology
VI-15-04-07-1	Role of the host cyclin-dependent kinase 7 (CDK7) for human cytomegalovirus replication	Dr. Hutterer	01.09.2015-31.08.2016	Institute of Clinical and Molecular Virology

Newly Started Projects

Project No.	Project title	Applicant	Term	Institute
CH-16-06-26-1	Measurements of kidney perfusion after transplantation by intraoperative fluorescence angiography	Dr. Rother	12 months	Department of Surgery
HN-16-08-05-1	Targeted induction of immunogenic cell death by drug-loaded nanoparticles for tumor therapy	Dr. Janko	12 months	Department of Otorhinolaryngology – Head and Neck Surgery
HN-16-08-22-1	Automated sleep stage analysis based on global EEG pattern analysis	Dr. Traxdorf	12 months	Department of Otorhinolaryngology – Head and Neck Surgery
JP-16-05-11-1	Prenatal depressive symptoms and distress: Associations with child epigenome, cortisol release and mental health	Dr. Eichler	12 months	Department of Child and Adolescent Mental Health
M1-16-10.04-1	Molecular mechanisms of chromatin decondensation in the process of neutrophil extracellular trap formation	Dr. Leppkes	12 months	Department of Medicine 1
M3-16-10-05-1	Interleukin 9 is an essential factor for resolution of arthritis	Dr. Ramming	12 months	Department of Medicine 3
MH-16-10-12-1	Functional investigation of the putative ATP-dependent chromatin remodeler PfSwr1 and its role in the deposition of histone variants in the malaria parasite Plasmodium falciparum	Dr. Petter	12 months	Institute Clinical Microbiology, Immunology and Hygiene

News and Figures

News and Figures

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News and Figures

Overview

The following figures impressively show the broad acceptance and the great interest of the Faculty of Medicine members in the programmes of the IZKF. The IZKF gives financial support to projects in all focal areas of the Faculty of Medicine and into a large number of different institutions. Exactly 120 scientific theses were ongoing in 2016 and nearly 70 publications have appeared.

Advanced Projects	**56**
Immunology and Infection	24
Oncology	11
Neurosciences	17
Renal and Vascular Research	4
Tandem projects between different departments and institutes	19
Junior Research Groups	**3**
Junior Projects	**16**
Immunology and Infection	6
Oncology	3
Neurosciences	4
Molecular Medicine	2
Others	1
Thereof projects completed in 2016	23
Institutions with funded projects	**30**
Employees of the IZKF	**149**
Number of scientists (including laboratory rotations)	105
Number of non-scientists	44
Pilot Projects	**54**
Newly granted in 2016	7
Projects completed in 2016	27
Appointments of IZKF project leaders to W2/ W3 - positions	5
Ongoing scientific theses in 2016	**120**
Master theses	12
Doctoral theses	104
Habilitations	4
Laboratory rotations	15
MD-thesis scholarship holders	30
Participants Graduate School	**118**

T(h)INK - Oncology, Immunology and Infection, Renal and Vascular Research	**62**
PhD students from IZKF projects	18
Associated participants	27
MD-thesis scholarships holders	17
Neurosciences	**56**
PhD students from IZKF projects	20
Associated participants	31
MD-thesis scholarships holders	5
Number of awards (2016)	15
Publications (2016)	**69**
Cumulative impact factor	495.939
Average impact factor per publication	7.188
Average publications per project	0.92
Publications in journals with IF ≥ 10	19
Total expenditures IZKF in 2016	**5,944 K€**

Summary of important figures 2016

News and Figures

20 years of the IZKF

01.10.1996	The IZKF starts its work Prof. Kalden becomes 1st Spokesman of the IZKF
01.10.1998	Integration of the research area ostheo-arthritis
01.01.1999	Start of Junior Research Group 1 (Dr. Körner)
01.10.1999	Start of Junior Research Group 2 (Dr. Sorokin)
01.04.2000	The Strategic Commission (later Junior Scientist Committee) starts its work
01.07.2001	Integration of the IZKF Administrative Office in the administration of the University Hospital and recruitment of Dr. Katrin Faber
01.04.2002	Appointment of the External Scientific Advisory Board by the principal of the University
13.05.2002	Prof. Sorg becomes Chairman of the External Scientific Advisory Board, Prof. Müller-Hermelink becomes Vice Chairman
01.10.2002	Establishment of the Core Unit „non-invasive small animal imaging“
01.10.2003	Start of Junior Research Group 2 (Dr. Voll) Establishment of the Chip-Pool (central funds for specific support of funded projects)
01.01.2004	Start of Junior Research Group 3 (Dr. Wiesener)
01.10.2004	Fusion of the former research areas A and B to the new research area Inflammation and Autoimmunity The MD-thesis scholarship programme starts (6 scholarships p.a. + accompanying activities)
01.04.2005	Prof. Müller-Hermelink becomes Chair of the External Scientific Advisory Board, Prof. Bruckner becomes Vice Chairman
01.10.2005	Integration of the new research area D Inflammation and cellular plasticity in tumors
01.02.2007	Establishment of a BayGene-Junior Group (Dr. Nimmerjahn) as an associated Junior Research Group 1
01.10.2007	Integration of the new research area E Pathomechanisms of neuronal signal transduction Prof. Reis becomes new Spokesman of the IZKF Establishment of the Core Unit Genomics The travel scholarship programme is approved (4 scholarships p.a.)
01.01.2009	Establishment of the Core Unit DNA-extraction platform (for biobanking purposes)
14.05.2009	For the first time, the IZKF symposium takes place in Kloster Banz (Topic Molecular Therapies)
13.07.2009	First call for proposals of the Junior Projects
01.11.2009	Start of Junior Research Group 2 (Dr. Titze)
01.10.2010	Assumption of the research areas of the Faculty of Medicine, Integration of the research area Renal and Vascular Research Extension requests are only possible in exceptional cases Start of Junior Research Group 3 (Dr. Winner) The IZKF Graduate School is founded Prof. Häussinger becomes Chairman of the External Scientific Advisory Board, Prof. Sendtner becomes Vice Chairman
01.08.2015	Start of Junior Research Group 1 (Dr. Ceppi)
01.09.2016	Start of Junior Research Group 2 (Dr. Dulin)
20.09.2016	100th and last meeting of the former Management Board
01.10.2016	Integration of the ELAN-Fonds into the IZKF Newly elected Management Board Prof. Reis becomes Chairman (Institute of Human Genetics), Prof. Wegner becomes Vice Chairman (Institute of Biochemistry)

Milestones of the IZKF

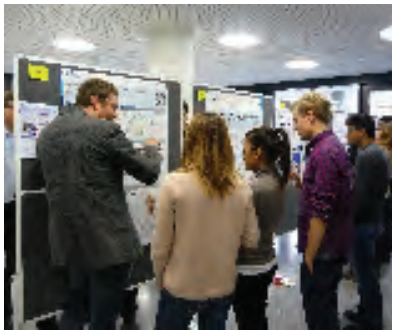

Universitätsklinikum
Erlangen
Portraits of the IZKF (2002-2016)
Zentrum für
Klinische Forschung

News and Figures

Facts and numbers

For 20 years the IZKF promotes clinically oriented research. Over the years it has implemented various innovative and quality-driven research funding programmes. The support of young researchers also holds a prominent position within the IZKF. Today the IZKF is an integral element of the Faculty of Medicine.

Endowed with its own research budget and management, it is further able to initiate specific site-related innovative funding programmes. Some impressive figures show the attractiveness of the IZKF as the central structure of research development with a special focus on the support of youngs researchers at the Faculty of Medicine over many years:



8
funding periods

253
funded projects

9
Junior Research Groups

61
funded Junior Projects

4
funded Core Units

261
project leaders in advanced and junior projects

80
laboratory rotations *2005-2016

78,000 K€
expenditures thereof 49,000 K€ between 2005-2016

268
participants in the IZKF Graduate School *2010-2016

423
publications of the funded projects *2005-2016


Universitätsklinikum Erlangen
Junior Research Groups (1999-2016)
Heiner Körner, Lydia Sorokin
Reinhard Voll, Michael Wiesener
Heiner Körner, N1 – 1999-2004
Cosinokine und Cosinokinrezeptoren bei Infektionserkrankungen
New Star Professor at the Menzies Research Institute Tasmania, focused on Cellular Immunology and Infection Immunology
Lydia Sorokin, N2 – 1999-2002
Zell-Matrix Interaktionen in der Entzündung
University of Münster, Institute of Physiological Chemistry and Pathobiochemistry
Reinhard Voll, N2 – 2003-2008
Rolle des Transkriptionsfaktors NF-κB bei der Pathogenese entzündlicher Erkrankungen
Freiburg University Hospital, Department of Rheumatology and Clinical Immunology, Medical Director
Michael Wiesener, N3 – 2007-2008
Bedeutung von Hypoxie-induzierbaren Transkriptionsfaktoren in Pathogenese, Entstehung und Progression von Nierenzellkarzinomen
University Hospital Erlangen, Department of Medicine 4, W2-Professorship
Falk Nimmerjahn, N1 – 2007-2011
BayGene-Nachwuchsgruppe
FAU Head of Division of Genetics
Jens Titze, N2 – 2009-2015
Immune system as regulator of volume and blood pressure
Vanderbilt University Nashville Tennessee, USA, Associate Professor
Beate Winner, N3 – 2010-2016
Modeling neurodegenerative diseases using stem cells
University Hospital Erlangen, Institute of Human Genetics, W2-Professorship
Paolo Ceppi, N1 – 2013-2021
Understanding the plasticity of cancer cells
ZKF
David Dolin, N2 – 2016-2022
Physics and Medicine
ZKF
Falk Nimmerjahn
Jens Titze
Beate Winner
Paolo Ceppi
David Dolin
Interdisziplinäres Zentrum für Klinische Forschung

News and Figures












Impressions from the IZKF-Symposia 2012-2016

News and Figures

News

Start of Junior Research Group 2 „Physics and Medicine“ of Dr. David Dulin

Dr. David Dulin joined us in September 2016 and started his Junior Research Group in the field of “Physics and Medicine” in the OICE (Optical Imaging Center Erlangen) on the Kußmaul campus in Erlangen. The Junior Research Group will develop and use physics-based approaches to study biological processes. In particular, Dr. Dulin investigates how enzymes process nucleic acids, e.g. DNA or RNA, and proteins. These processes are at the heart of the replication, expression and maintenance of the genome and are therefore key aspects of the life cycle of every organism.

Dr. David Dulin, N2

Prof. Dr. Beate Winner, N3

Project end of Junior Research Group 3

In September 2016, the term of Junior Research Group “Modeling neurodegenerative diseases using stem cells” of Prof. Dr. Winner was concluded. Specifically, Prof. Winner investigates neurodegeneration and regeneration in synucleinopathies including Parkinson’s disease (PD). Numerous doctoral thesis, publications and grant applications resulted from the Junior Research Group. Prof. Winner was appointed a W2-professorship in stem cells models of rare neuronal diseases. Since 01.01.2017 Prof. Winner is Head of the Department of Stem Cell Biology at the University Hospital Erlangen. We wish Prof. Winner and her team continued success in their research.

New structures

By decision of the Faculty of Medicine, the structure of IZKF was modified on October 1st, 2016, resulting in the integration of the ELAN-Fonds and the creation of a consolidated single intramural funding body. Funding of pilot projects was integrated as a new funding line. The revised bylaws stipulate that the entire Management Board is elected by the Faculty of Medicine.

IZKF-Symposium 2016 „Translational Medicine“

From 16th-17th June 2016 the International IZKF-Symposium „Translational Medicine“ was held at the conference centre at the baroque monastery of Kloster Banz. An accompanying poster session was organised presenting concepts and results of projects within the major research areas of the Faculty of Medicine.

IZKF Symposium 2016

From the left: Prof. Dr. Christoph Becker, Thomas Wohlfahrt, Danyil Huraskin, Benjamin Ettle, Benjamin Abendroth, Prof. Dr. André Reis

The four poster prizes were awarded to:

• Benjamin Abendroth (Department of Medicine 1): „The role of Batf-dependent T-cells in the immunopathogenesis of intestinal GvHD”

• Benjamin Ettle (Department of Molecular Neurology): “α-Synuclein impairs myelin formation: a novel pathomechanism and interventional target in Multiple System Atrophy”

• Danyil Huraskin (Institute of Biochemistry): „Wnt/b-catenin signaling via Axin2 is required for myogenesis and active in IIa/IIx fibers together with Hippo pathway members”

• Thomas Wohlfahrt (Department of Medicine 3): „Characterization and evaluation of a novel targets of fibrotic disease treatment”

Publication prize

Every two years the IZKF awards the Publication Award for Young Scientists which is endowed with 1,000 €. The Junior Scientist Committee selected a winner who was announced and honored as a part of the Translational Medicine Symposium on June 17th, 2016 at Kloster Banz. The awardee was also included in the programme with a short lecture.

2016 Prize Winner

Dr. Christine Schauer (Department of Medicine 3): Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines, Nature Medicine 2014 April, 20(5): 511-517.

News and Figures

20 years IZKF

In 2016, the IZKF celebrated its 20th anniversary. On that account, an anniversary symposium took place in the lecture halls of the Faculty of Medicine on October 12th, 2016. Numerous current and former members of the External Scientific Advisory Board, Management Board, project leaders and PhD students of the IZKF as well as representatives of the University, Faculty and the University Hospital followed their invitation.
On occasion of the anniversary, a duz special was published in June 2016 to outline the successes and achievements of the IZKF. The duz special of the IZKFs Aachen, Erlangen, Münster and Würzburg can be reached via the link www.duz.de/duz-special/.

Prof. Dr. Dieter Häussinger, Prof. Dr. Esther von Stebut-Borschitz, Prof. Dr. André Reis

duz special of the IZKFs Aachen, Erlangen, Münster and Würzburg

Prof. Dr. Aline Bozec

Heinz Maier-Leibnitz price was awarded to IZKF project leader Aline Bozec

Junior professor Dr. Aline Bozec was awarded by the German Research Foundation (DFG) with the most important price for junior researchers in Germany. On May 18th, biochemist Prof. Bozec, who is at the Department of Medicine 3 since 2011, received the Heinz Maier-Leibnitz price endowed with € 20,000. We congratulate Prof. Bozec for this great success.

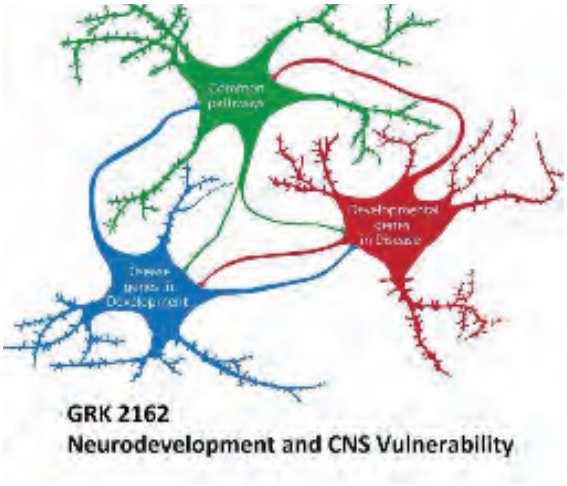
Raja Atreya receives Heisenberg professorship

In April 2016, the former IZKF project leader Prof. Raja Atreya from the Department of Medicine 1 was awarded a Heisenberg professorship. The Heisenberg programme honors acknowledged scientists. The IZKF congratulates Prof. Atreya for this promotion.

Prof. Dr. Raja Atreya

Research Training Group 2162 approved

On July 1st, the Research Training Group 2162 "Neurodevelopment and CNS Vulnerability" (Speaker: Prof. Dr. D.C. Lie, Institute of Biochemistry) was started. Research Training Group 2162 is one of 16 new research training groups funded by the German Research Foundation (DFG) with the aim to provide a structured, high-quality PhD training programme.



Farewell to Prof. Winner, Prof. Steinkasserer and Prof. Neurath

Within the General Assembly on November 9th, Prof. Dr. Beate Winner (2011-2016), Prof. Dr. Alexander Steinkasserer (2007-2016) and Prof. Dr. Markus Neurath (2011-2016) were officially honored for their long-standing and dedicated engagement in the Management Board. We would like to thank them for their service and advice for the IZKF.

Prof. Dr. Beate Winner

Prof. Dr. Alexander Steinkasserer

Prof. Dr. Markus Neurath

News and Figures

Figures

Research Grants

The IZKF research grants can be divided into Advanced Projects, Junior Projects and Junior Research Groups. In 2016, 56 advanced and 16 junior projects received funding of the IZKF. These projects cover the major research areas of the Faculty of Medicine, i.e. immunology and infection research, renal and vascular research, neurosciences and tumor research.

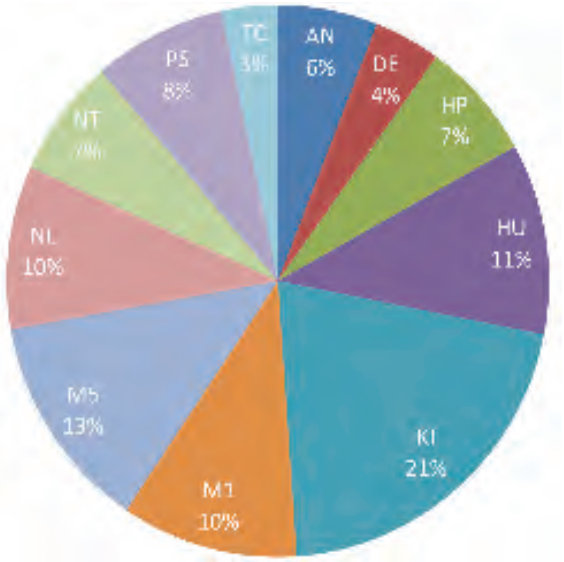
Institute	Immunology and Infection	Oncology	Neurosciences	Renal and Vascular Research	Others
Chair of Experimental Medicine I		x			
Chair of Experimental Medicine II		x			
Department of Anesthesiology			x		
Department of Dermatology	x	x			
Department of Immune Modulation	x				
Department of Infection Biology	x				
Department of Medical Informatics, Biometry and Epidemiology					x
Department of Medicine 1	x	x	x		
Department of Medicine 2	x				
Department of Medicine 3	x	x			x
Department of Medicine 4	x			x	
Department of Medicine 5	x	x			
Department of Molecular Immunology			x		
Department of Neurology			x		
Department of Neuroradiology			x		
Department of Obstetrics and Gynaecology		x			
Department of Ophthalmology			x		
Department of Otorhinolaryngology - Head and Neck Surgery			x		
Department of Psychiatry and Psychotherapy			x		
Department of Surgery		x			
Division of Genetics	x				
Department of Molecular Neurology			x		
Department of Molecular Pneumology	x				
Department of Nephropathology				x	
Institute of Biochemistry		x	x		
Institute of Clinical and Molecular Virology	x				
Institute of Clinical Microbiology, Immunology, and Hygiene	x				
Institute of Human Genetics			x	x	x
Institute of Pathology		x			
Institute of Physiology and Pathophysiology			x		

This table shows the institutes and departments which received project funding within the IZKF in the year 2016 and the respective association to the focal research areas of the Faculty.

Laboratory Rotations

The rotation programme is aimed at young physicians who are interested in research. In the context of the rotation programme they receive protected time either part-time or full-time within clearly defined research projects for up to twelve months full-time or 24 months in part-time.

In 2016 candidates from 11 different institutions were supported.

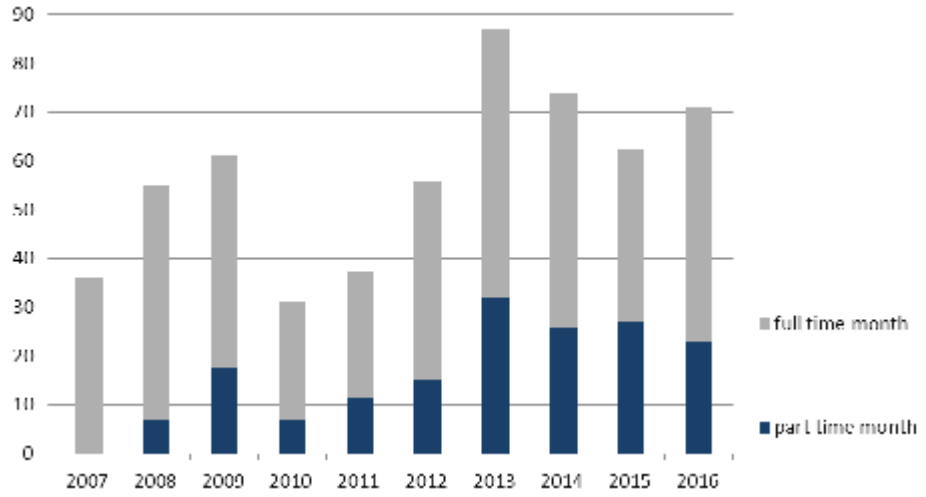


Funding distribution of laboratory rotations 2016

Name	Institution	Funding period	Full-time/ part-time
Dr. Esther Eberhard	Department of Anaesthesiology (AN)	09/2016 - 02/2017	100%
Dr. Ugur Uslu	Department of Dermatology (DE)	04/2015 - 03/2016	100%
Dr. Ingo Ganzleben	Department of Medicine 1 (M1)	07/2015 - 06/2016	100%
Dr. Sebastian Zundler	Department of Medicine 1 (M1)	02/2015 - 02/2016	100%
Dr. Fabian Müller	Department of Medicine 5 (M5)	11/2016 - 04/2017	100%
Prof. Dr. Dimitios Mougiakakos	Department of Medicine 5 (M5)	01/2015 - 12/2016	50%
Dr. Clemens Bockmeyer	Department of Nephropathology (NT)	04/2016 - 03/2017	50%
Dr. Fabian Fahlbusch	Department of Paediatrics and Adolescent Medicine (KI)	10/2016 - 03/2017	100%
Dr. Ferdinand Knieling	Department of Paediatrics and Adolescent Medicine (KI)	02/2016 - 01/2017	100%
Dr. Rebekka Götzl	Department of Plastic and Hand Surgery (HP)	04/2016 - 03/2017	50%
Dr. Timo Oberstein	Department of Psychiatry and Psychotherapy (PS)	08/2016 - 07/2017	100%
Dr. Dumirita Gafencu	Department of Thoracic Surgery (TC)	05/2015 - 04/2016	50%
PD Dr. Ulrike Hüffmeier	Institute of Human Genetics (HU)	09/2015 - 08/2017	50%
Rotations of Junior Project leaders			
Dr. Franz Marxreiter	Department of Molecular Neurology (MN)	05/2016 - 10/2016	100%
Dr. Martin Regensburger	Department of Neurology (NL)	07/2016 - 06/2017	100%

Laboratory Rotations

News and Figures



Man months funded in the rotation programme.

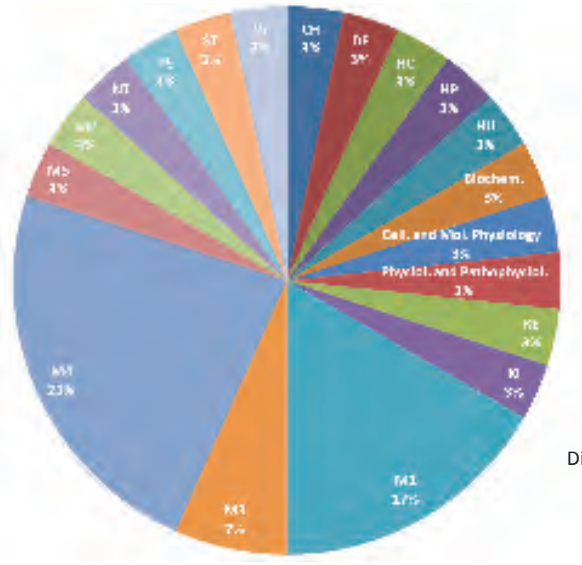
MD-thesis Scholarships

Within the doctoral programme 18 scholarships for medical doctoral students are awarded each year, each granted for a period of 7 months with a monthly allowance of € 773. This support is given with the expectation of a full-time dedication to the thesis.

In 2016, a total of 30 medical doctoral students from 19 institutions were funded. Selection is done by the Junior Scientist Committee and is based on academic performance and first experience in laboratory work.

Since its inception in 2007, IZKF supported 108 medical students with a scholarship. Medical students often initiate experimental work on their doctoral thesis during their studies. They will finish the thesis, though, only several years later when they graduate.

By the end of 2015, 47 students had completed their studies, 29 within the standard study duration. The remainder extended their studies only by one semester, still faster than the average. 30 of the 47 students who completed their studies also completed their doctoral work. Interestingly, 17 students (57%) even obtained the highest degree possible, summa cum laude. This compares very favourably to the average 3-5% of all MD thesis presented and is testimony to the excellent quality of MD thesis performed within this programme.



Distribution of MD-thesis scholarships per institution.

Name	Institution	Funding period
Fritz, Niklas	Department of Cardiac Surgery (HC)	10/2016 - 04/2017
Lamprecht, Ricarda	Department of Dermatology (DE)	07/2015 - 01/2016
Düll, Miriam	Department of Medicine 1 (M1)	08/2015 - 02/2016
Fastancz, Petra	Department of Medicine 1 (M1)	11/2016 - 05/2017
Kappes, Leonie	Department of Medicine 1 (M1)	03/2016 - 09/2016
Offensperger, Laura	Department of Medicine 1 (M1)	10/2016 - 04/2017
Wurm, Lina	Department of Medicine 1 (M1)	04/2016 - 10/2016
Fischer, Kim	Department of Medicine 3 (M3)	11/2016 - 05/2017
Schulz, Oscar	Department of Medicine 3 (M3)	10/2016 - 04/2017
Dambietz, Thomas	Department of Medicine 4 (M4)	01/2016 - 07/2016
Göth, Daniel	Department of Medicine 4 (M4)	01/2016 - 07/2016
Luz, Hannah	Department of Medicine 4 (M4)	11/2016 - 05/2017
Neumeier, Laura	Department of Medicine 4 (M4)	04/2016 - 10/2016
Rentschler, Lukas	Department of Medicine 4 (M4)	11/2016 - 05/2017
Tonner, Louise	Department of Medicine 4 (M4)	04/2016 - 10/2016
Westergerling, Parisa	Department of Medicine 4 (M4)	07/2016 - 01/2017
Stueven, Anna Kathrin	Department of Medicine 5 (M5)	10/2015 - 04/2016
Bailer, Max	Department of Molecular Pneumology (MP)	01/2016 - 07/2016
Mayer, Anna-Lena	Department of Nephropathology (NT)	11/2016 - 05/2017
Stegmann, Hedwig	Department of Paediatric Cardiology (KE)	11/2016 - 05/2017
Kossel, Clara	Department of Paediatrics and Adolescent Medicine (KI)	11/2016 - 05/2017
Hardt, Moritz	Department of Plastic and Hand Surgery (HP)	09/2016 - 03/2017
König, Loretta	Department of Psychiatry and Psychotherapy (PS)	09/2016 - 03/2017
Seeberg, Jacob	Department of Radiation Oncology (ST)	07/2016 - 01/2017
Bieniek, Pawel	Department of Surgery (CH)	01/2016 - 07/2016
Jung, Matthias	Institute of Biochemistry	07/2015 - 01/2016
Steffen, Jan	Institute of Cellular and Molecular Physiology	07/2016 - 01/2017
Böhm, Magdalena	Institute of Clinical and Molecular Virology (VI)	07/2016 - 01/2017
Oberste-Lehn, Lea	Institute of Human Genetics (HU)	07/2015 - 01/2016
Schwarz, Matthias	Institute of Physiology and Pathophysiology	07/2016 - 10/2016

MD-thesis scholarships

News and Figures

Graduate School

The IZKF Graduate School provides doctoral candidates with a structured training programme and promotes networking. Membership is compulsory for all candidates funded by IZKF, both those with medical or natural sciences background. Associated members from other programmes or funding sources are also welcome. The Graduate School is organized in two areas: neuroscience and immunology/ infection/ oncology/ renal and vascular research. The neuroscience section was merged with similar activities at the Interdisciplinary Center for Neurosciences (ICN).

In 2016 the Graduate School included 118 members.

Participants Graduate School	**118**
Neuro	**56**
Participants from IZKF projects	20
Associated participants	31
MD-thesis scholarships	5
T(h)INK - Oncology, immunology and infection, renal and vascular research	**62**
Participants from IZKF projects	18
Associated participants	27
MD-thesis scholarships	17

Participants of the Graduate School 2016 (09.11.2016)

Until November 2016, Tobias Bormann was speaker of the Graduate School for the area oncology, immunology, renal and vascular infection. Isabella Schöpe was elected his successor. Benjamin Häberle was re-elected as speaker of the Graduate School for the neuroscientists.

T(h)INK-Group

Speaker	Deputy Speaker
Tobias Bormann till 11/2016 *Department of Medicine 1*	**Kristina Scheibe** till 11/2016 *Department of Medicine 1*
Isabella Schöpe since 11/2016 *Department of Surgery*	**Victoria Langer** since 11/2016 *Department of Surgery*

NEURO-Group

Speaker	Deputy Speaker
Benjamin Häberle *Inst. of Biochem.* *since 12/2015*	**Diana Schmidt** *N3, IZKF* *till 09/2016* **Stephanie Hartmann** *Physiology* *since 09/2016*

The following soft skills courses were given:

- Presentation Skills, Dr. Deborah Bennett, 21.-23.11.2016
- Scientific Writing, Dr. Deborah Bennett, 18.-20.05.2016 and 24.-26.10.2016
- Kommunikation und Rhetorik, Gerhard Kranz, 21.-22.09.2016
- Microscopy course on sample preparation and two channel confocal imaging, Dr. Ralf Palmisano, 07.-10.06.2016
- Biostatistics, Dr. Matthias Englbrecht, 23.-24.09.2016

Visiting Professor Programme

The visiting professor programme promotes visits by external researchers, thereby encouraging collaborations and supporting the exchange of ideas. Two related programmes are administrated by IZKF with partly different target groups. One is funded by FAU, the other by IZKF directly. Following lectures were given by external scientists in 2016.

Scientist	Institute	Lecture title
Prof. Kazuhiro Ikenak	Div. of Neurobiology and Bioinformatics, National Institut for Physiological Science, Okazaki, Japan	Regulation of oligodendrocyte generation by sonic hedgehog signaling in mouse spinal cord
Prof. Carol Carter	Department of Molecular Genetics and Microbiology, Adjunct Professor, Department of Physiology & Biophysics, New York, USA	HIV-1 & Ist ESCRTs: Lessons From Patients
Prof. Ken R. Chien	Karolinska Institutet, Department of Medicine, Sweden	Modified RNAs as therapeutic approaches
Prof. Dr. Eliezer Masliah	UC San Diego Health Sciences San Diego, USA	Immunotherapy: a novel treatment for neurodegenerative disorders at the horizon?
Prof. Sebastian Jessberger	University of Zurich, Brain Research Instiute, Switzerland	Stem cells aging and cell division
Prof. Christian Jobin	Division of Gastroenterology, Hepatology & Nutrition, Department of Medicine in the College of Medicine, Florida, USA	Microbial genomics in colorectal cancer
Prof. Raghu Kalluri	The University of Texas, MD Anderson Center, Houston, USA	Fibroblasts as nexus of inflammation and cancer
Prof. Carl F. Nathan	Weill Cornell Medicine, New York, USA	Killing Persistent Forms of Mycobacterium tuberculosis
Prof. Michel Nussenzweig	The Rockefeller University, New York, USA	The HIV vaccine problem
Prof. Sidonia Fagarasan	Center for Integrative Medical Sciences, Laboratory for Mucosal Immunity, Yokohama City, Japan	Mucosal immunity

FAU-Visiting Professor Programme

Scientist	Institute	Lecture title
Dr. Anbarasu Lourdusamy	Division of Child Health, Obstetrics and Gynaecology, School of Medicine, University of Nottingham, UK	Network biology: application to brain disorders
Prof. Richard Wyatt, PhD	Department of Immunology and Microbial Sciences, The Scripps Research Institute, La Jolla, CA, USA	A Darwinian dilemma dictates B cell responses to the HIV trimmer

IZKF-Visiting Professor Programme

News and Figures

IZKF Funding and Output

Budget

Since 2004, the IZKF has been fully supported by intramural funds. The main financial contribution is given by the Faculty of Medicine. Additional contributions are received from the FAU. The junior project programme is jointly funded by IZKF and the ELAN-Fonds. For the fiscal year 2016, IZKF and ELAN-Fonds were still administered seperately. In 2017, a single consolidated budget will be reported.

About € 3.7 millions go towards the funding of 56 advanced projects, while € 807,000 are allocated to the funding of junior projects, and € 488,000 to the funding of junior research groups. Further portions of the total budget are assigned to other career development programmes (MD-thesis scholarships, laboratory rotations, graduate school; total sum € 596,000). Expenditures for other supporting activities sum up to € 319,000. Part of the expenditures of 2016 were financed from residual funds of the previous years.

Financial Statement IZKF 2016

Revenues	
Support of the Medical Faculty	3,482 K€
Support of the University	268 K€
Contribution of ELAN-Fonds for junior projects	310 K€
Contribution of IZKF for junior research groups (basic funding)	- 30 K€
Total revenues 2016	**4,378 K€**
Expenditures	
Research Grants	5,029 K€
thereof advanced projects	3,734 K€
thereof junior research groups	488 K€
thereof junior projects	807 K€
Other career development programmes	596 K€
Supporting activities	319 K€
Total expenditures 2016	**5,944 K€**

Output and Evaluation

Various parameters are used to assess compliance with the mission of the IZKF in advancing clinically oriented research at the Faculty. Scientific publications and academic success of young scientists are the most obvious and straightforward ones. Additionally, the acquisition of extramural funding is an explicit objective of IZKF. Furthermore, patents, scientific prizes and offers of professorships are relevant parameters. Other important parameters for the IZKF are the number of different institutions and scientists, who are involved in the IZKF, the number of interdisciplinary projects as well as the number of joint publications.

In the reporting period altogether 75 scientific projects were actively running: 56 advanced projects, 16 junior projects and 3 junior research groups. In addition, 6 junior projects started their work in 2016 or in the beginning of 2017. These 75 funded scientific projects published 69 original articles in 2016 resulting in an average of 0.92 publications per project. The cumulative impact factor (IF) was 495.939, averaging 7.188 per publication. The high quality of many of these publications is reflected in 19 publications with an IF of more than 10. Being part of IZKF allows intensive networking and direct access to collaborations, which can be seen in 20 publications that were generated in a cooperation of multiple projects. Additional articles of finalised projects are in preparation, submitted or accepted. Publications that have already been accepted are listed in the corresponding final reports.

Intense academic activity within IZKF projects is reflected in 12 master theses, 104 doctoral theses and four habilitations that were in progress or finalised in 2016. A total of 105 scientists from 30 institutions are involved in 75 scientific projects funded by IZKF.

Some IZKF project leaders were able to achieve outstanding results. 15 prizes were awarded to IZKF project leaders. Five professorships were offered, three of them were accepted.

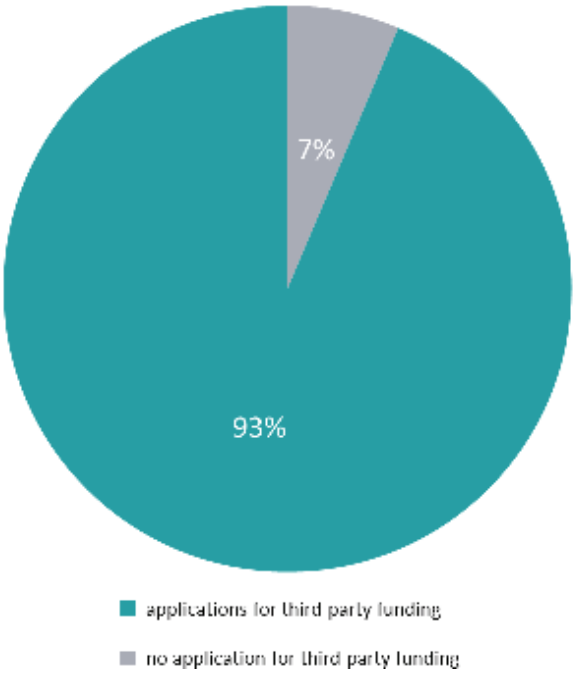
In many instances funding by the IZKF starts at an early phase of the project, thus it must be considered as a high risk funding programme. It is nevertheless reassuring that most of the projects are successful and many of them are continued after termination of intramural funding. To support this with figures, results of a detailed survey of acquired third-party funding by IZKF-projects are given on the next pages.

Beginning with the funding period 2010-2013, grants were awarded for a period of 30 months with an extension by 6 months, if these projects are submitted for external funding. Within the last funding period (2013 – 2016) all projects submitted third party funds applications and therefore received the 6 month funding extension. When considering the last two funding periods (2010 – 2016) 47 projects were funded by the IZKF of which 44 (94%) submitted third party funds applications. Of these, 34 projects (77%) were granted extramural funding, 3 (7%) are still under review and only 7 (16%) were not funded. This impressive success is also reflected by the fact that IZKF funding resulted in the acquisition of more extramural funds than were originally spent.

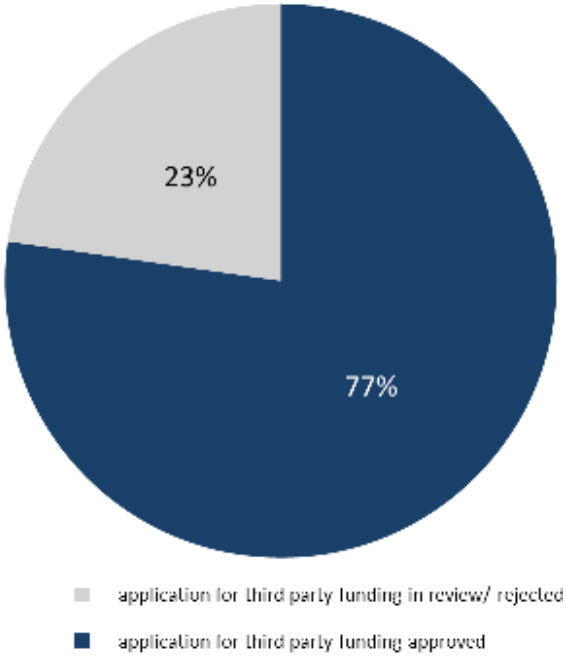
Similarly, the junior projects lead to a high number of extramural funding applications with a very high success rate. This developement has been stable over the entire duration of the programme.

News and Figures

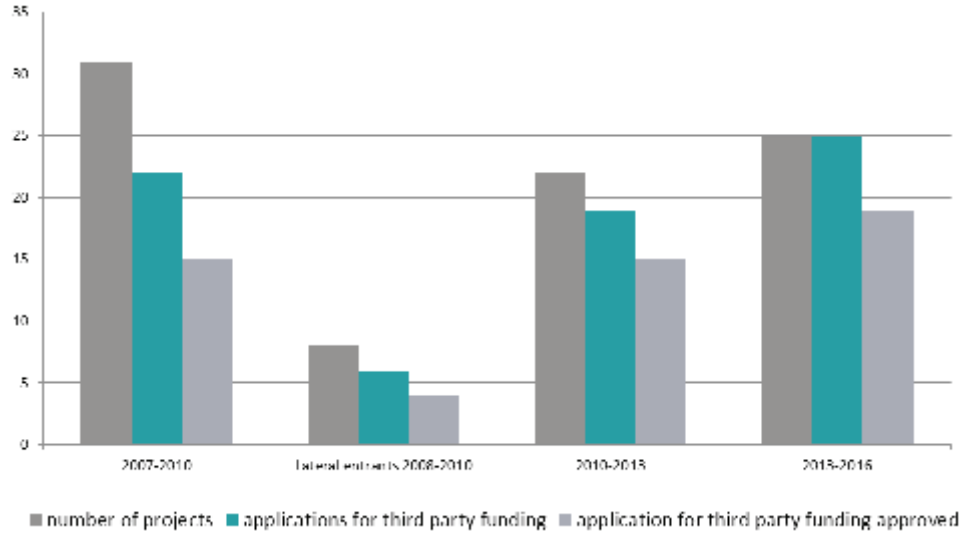
Acquisition of third-party funding advanced projects



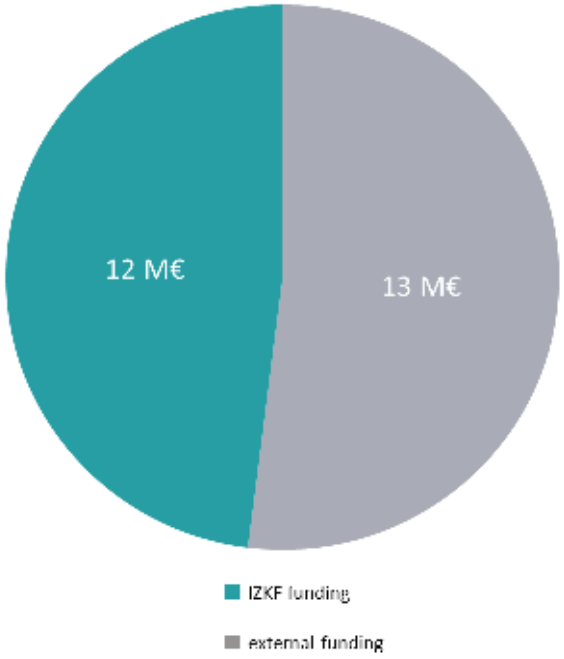
Applications for third-party funding submitted by advanced projects between 2010 and 2016



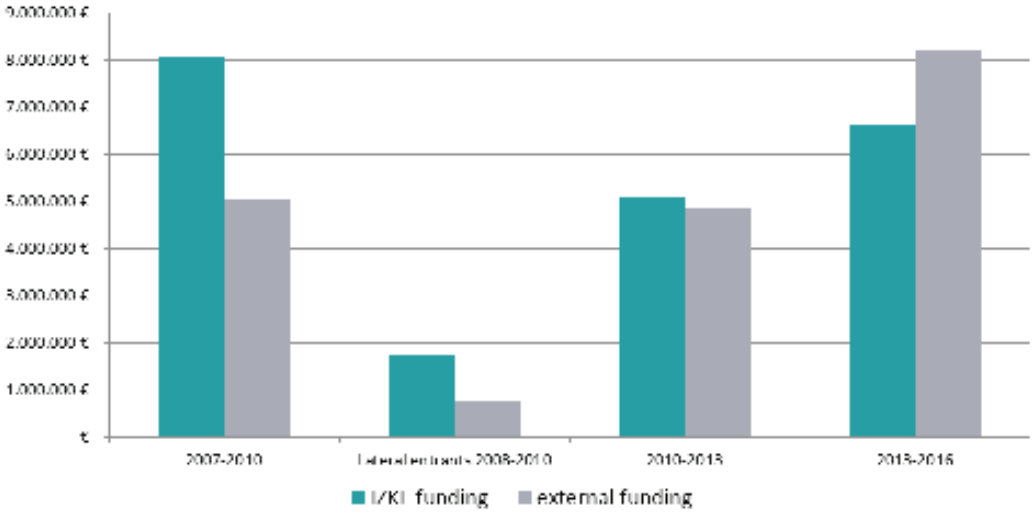
Approved applications for third-party funding of advanced projects between 2010 and 2016



This column graph compares the number of advanced projects with that for the submitted and approved applications for external funding in each funding period.



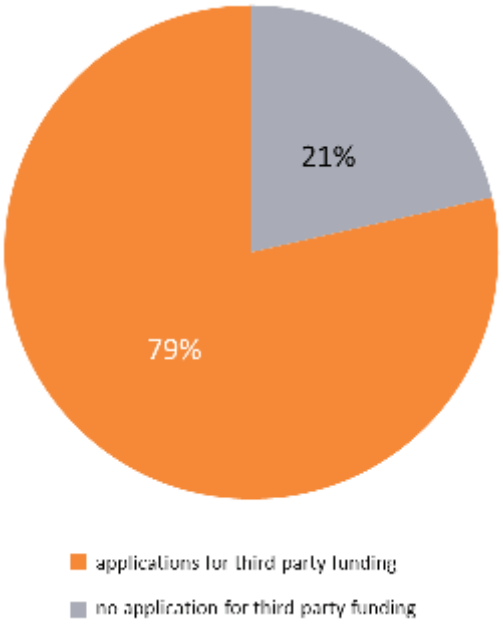
External funding received from advanced projects between 2010 and 2016.



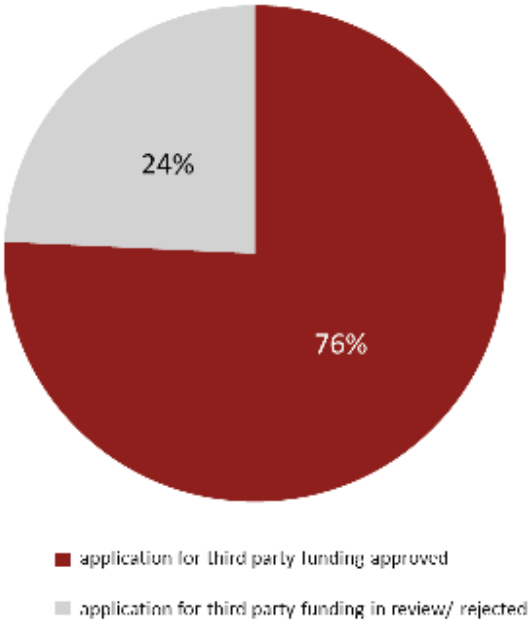
External funding received from advanced projects between 2007 and 2016.

News and Figures

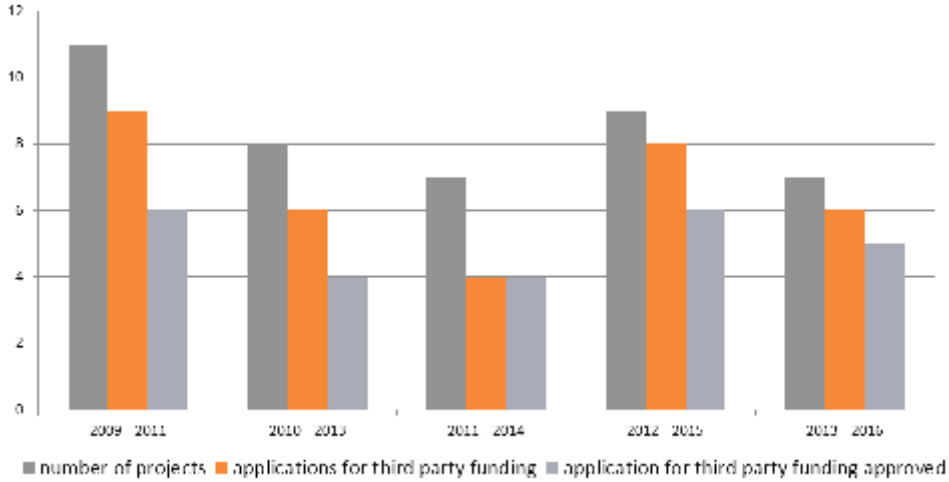
Acquisition of third-party funding junior projects



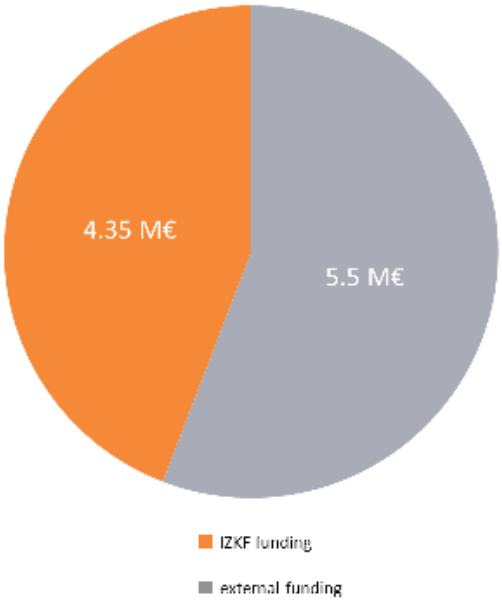
Applications for third-party funding submitted by junior projects (projects initiated between 2009 and 2013).



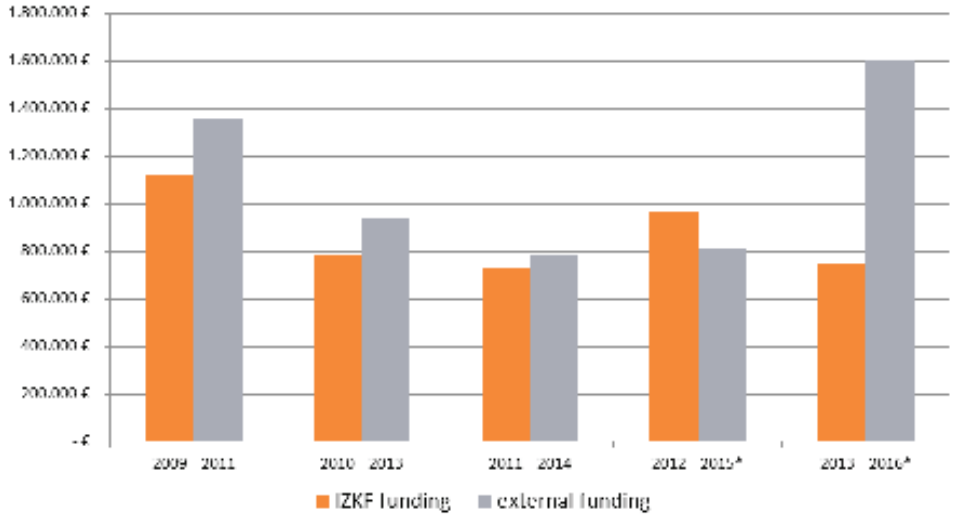
Approved applications for third-party funding of junior projects (projects initiated between 2009 and 2013).



Success-rate of junior projects. Further applications of projects initiated in 2013 are planned.



External funding received from all junior projects
initiated between 2009 and 2013.



External funding received from junior projects initiated between 2009 and 2013.
* Several grant applications are still under evaluation.

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