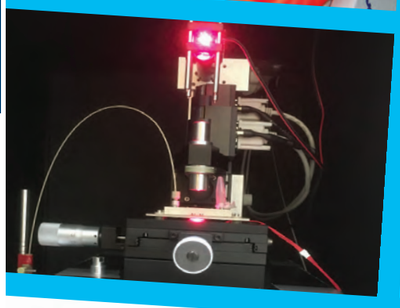
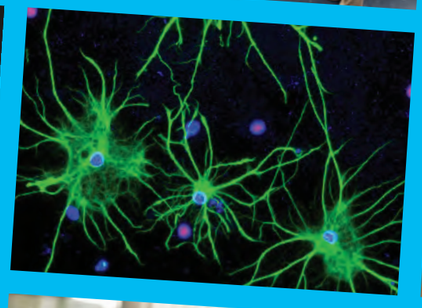
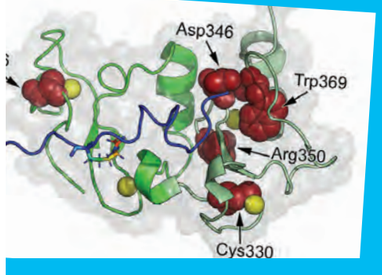
FRIEDRICH-ALEXANDER
UNIVERSITÄT
ERLANGEN-NÜRNBERG
MEDIZINISCHE FAKULTÄT

Universitätsklinikum
Erlangen

IZKF Erlangen
Annual Report 2017






interdisciplinary
Center for
Clinical Research

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IZKF Annual Report 2017

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IZKF Erlangen

Annual Report 2017

Editorial

Developments in the last year were to a large extent marked by activities in the area of promoting young researchers. After lengthy discussions and preparations an important development initiated and intensely promoted by IZKF finally came to fruition: the founding of a joint graduate school between the Department of Biology and the Medical Faculty called "Life@FAU". Under this umbrella all DFG-funded research training groups from both faculties and the IZKF Research Training Group joined forces to offer a structured graduation programme which is also open to independent graduate students, e.g. from DFG-research grants who currently have no access to such structured training. With this framework we aim to improve the training of young graduate students by providing on the one hand a minimum requirement for all and on the other hand harnessing synergistic effects between the activities of the individual training programmes, which are now open for a broader circle of students. This will benefit especially independent students. In addition, the Faculty has now a modern and attractive training structure with high intra- and extramural visibility. IZKF has the biggest training group and therefore it was a natural decision that IZKF Administrative Office also serves as administrative office of Life@FAU. The spokesman of IZKF Junior Scientist Committee and IZKF board member Prof. Christoph Becker was elected as Chairperson of the Steering Committee.

Medical students who usually write their MD-thesis in parallel to their studies will also benefit from this infrastructure. Recipients of IZKF scholarships performing an experimental thesis have already been receiving a comparable structured training programme, although of reduced duration. This programme was now revised to accommodate the new Bavarian regulations agreed by the five Bavarian Medical Faculties in the realm of Bavarian performance-related resource allocation programme (Bayern-LOM). This now includes a compulsory research semester in the lab as well as continued participation in research activities and seminars until their graduation. The structure and diversity of Life@FAU offers students now an even larger spectrum of activities to choose from and also increased flexibility. The aim is to improve the quality and acceptance of experimental MD-thesis and to provide the qualified and motivated trainees with optimal conditions. Thanks to additional funding by the Faculty we were also able to increase the number of scholarships to now 25 per year, allowing us to better support the fortunately high number of interested physicians in training with their thesis.

Establishing a Clinician-Scientist-Programme (CSP) according to the DFG-recommendations was another important issue dealt with by the Board. For many years now, IZKF support for medical trainees has included many major elements of such CSPs, including laboratory rotations for clinicians as well as junior research grants. Other elements usually implemented in CSPs, e.g. tailored scientific training curricula, are available on a voluntary basis but need to be formalized into a compulsory programme. At the end of 2017, the Board decided to implement such a CSP. The details of the programme are currently being worked out and we expect to have a first call for participants already in 2018.

Following the external Faculty evaluation in February 2017 the Board intensely discussed ways to better support the creation of collaborative research programmes. To this end, changes in the funding format for advanced IZKF projects were debated. The idea was that intramural funding should support especially projects that could serve as building blocks for future activities such as DFG collaborative research units or centres (Forschergruppen and SFBs). At first sight this

seemed like an attractive idea, which nevertheless many difficulties surfaced when scrutinised more carefully. In principle this possibility has already been available for many years. Interested scientists could submit coordinated interacting proposals, and indeed several projects funded by IZKF were later integrated into such collaborative programmes. But never a specific initiative was born from within IZKF. The Board discussed restricting future calls to certain topics, but it was realised that selection of these topics would be difficult and conflicting and could jeopardize quality criteria for the sake of an exact fit. Also the experience with such programmes at other centers was mixed at best. After many deliberations the Board finally agreed that the strength of IZKF with its internal scientific evaluation is the selection of projects based on scientific excellence and not so much the setting of scientific priorities and the associated preference of certain research focusses to the detriment of others. IZKF will therefore continue with its proven procedures and programmes of supporting scientists from all areas of the Faculty based on scientific excellence only.

This funding structure has a long track record of success. As always you will find in the back part of this report interesting results and facts of the continued output evaluation by the administrative office documenting the success of the different IZKF funding programmes. Currently funded projects from our last call are still well under way. The scientific description of these projects is in the front part of the annual report. Due to budget limitations we can only have a new call for projects when these ongoing projects are terminated in 2019. In order to ensure the continued access of a broad group of scientists the Board also decided to limit the funding of future advanced projects to one personnel position only.

Starting this year we also report in more detail on the starting programme ELAN following its consolidation in IZKF. We have adapted the terminology and now refer to these projects as pilot projects. Selection criteria and funding volumes remain unaltered. Notably, in 2017 we were able to fund for the first time also projects from the part of the Faculty administered by the University (Kapitel 1519). This became possible thanks to funds from within the Emerging Talents Initiative (ETI). This now fulfils a longstanding aspiration of the Board to ensure equal access to intramural research funding for all institutions of the Faculty.

This annual report appears for the second time in the design of the Faculty of Medicine. This reflects the strong links with the Faculty and our focus on promoting research. Also our webpage has moved to a prominent position of the Faculties' webpage at www.izkf.med.fau.de. The content was revised and the presentation was improved. We discontinued the restricted internal pages, now all content is publicly available. We also expanded the coverage of the Junior Research groups, who now have an ample platform to portray and promote their research activities. I invite you to browse through our webpage and learn about the many activities of IZKF.

I also want to take the opportunity to congratulate our Junior Research Group leader Dr. Paolo Ceppi for obtaining the prestigious „Lung Cancer Young Investigator Award 2017" of the International Association for the study of Lung Cancer (IASLC). Dr. Ceppi heads the Junior Group 1 „Understanding the plasticity of cancer cells" in the focus research area of oncology since 2015. The topics of his research are the mechanisms of plasticity of cancer cells, epithelial-mesenchymal transition (EMT), cancer stemcells and susceptibility of cancer cells to chemotherapy. This prize is an important recognition of his work and that of his team.

Finally, I want to express my heartfelt gratitude to all members of the administrative office for their continued dedication. Without Dr. Faber and her team not only this annual report but also the success of IZKF as a whole would not be possible. And I thank you for your continued interest in IZKF.

Prof. Dr. André Reis
Chairman

Contents

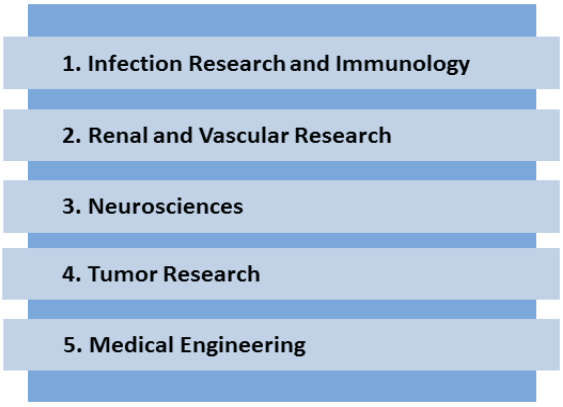
About us

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 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-organised 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 up to 13 members with voting right, up to 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.

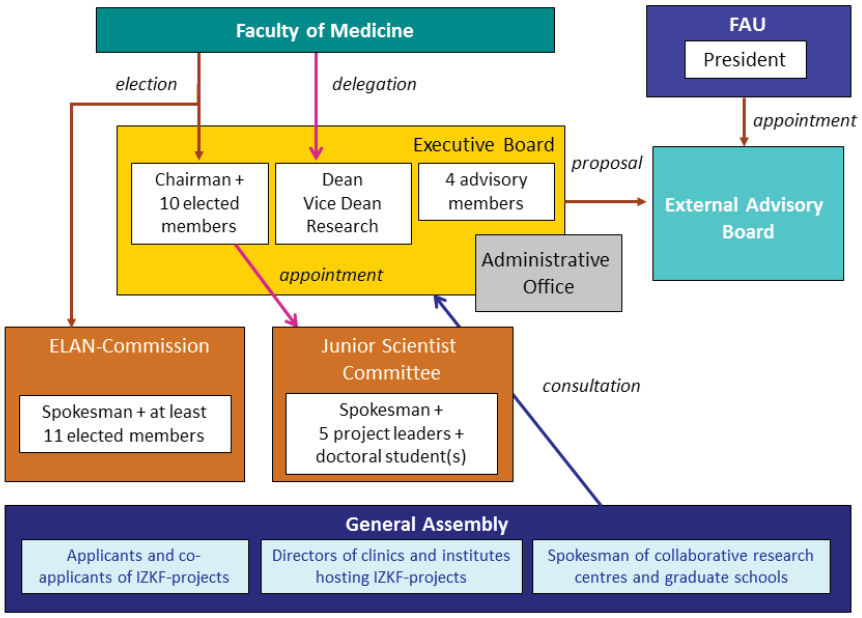
Programmes and the financial framework are reviewed and approved by the External Scientific Advisory Board. This body meets on site every two or three years to oversee the general development of the IZKF and the proposed projects. The board consists of at least 10 internationally recognized scientists (31.12.2017 20) 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 organises 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. Christoph Becker, Department of Medicine 1
Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene
Prof. Dr. Anja Bosserhoff, Institute of Biochemistry
Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I
Prof. Dr. Johann Helmut Brandstätter, Division of Animal Physiology
Prof. Dr. Dr. Raymund Horch, Department of Plastic and Hand Surgery
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

Consultative Members

Prof. Dr. Joachim Hornegger, President of the FAU
Christian Zens, Head of Administration of the FAU
Prof. Dr. 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. Dr. Horch

Prof. Dr. Mackensen

Prof. Dr. Dr. Schüttler

Prof. Dr. Winkler

Prof. Dr. Hornegger

Zens

Prof. Dr. Dr. Iro

Dr. Bender

Current members of the Management Board

About us

ELAN-Commission

Spokesman for pilot projects (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, Department of Stem Cell Biology

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


Prof. Dr. Schulze

Current members of the Junior Scientist Committee

Spokesman for career development programmes

Prof. Dr. Christoph Becker, Department of Medicine 1

Members

Dr. Caroline Bosch-Voskens, Department of Dermatology
Prof. Dr. Aline Bozec, Department of Medicine 3
Dr. Paolo Ceppi, IZKF Junior Research Group 1
Prof. Dr. Felix Engel, Department of Nephropathology
Benjamin Häberle, Institute of Biochemistry
Isabelle Schöpe, Department of Surgery (till 31.10.2017)
Prof. Dr. Schulze, Department of Oto-Rhino-Laryngology - Head and Neck Surgery

Administrative Office


Meyerhöfer-Klee


Dr. Faber


Neufang


Reichel

Current staff of the Administrative Office

Manager

Dr. Katrin Faber

IZKF Administration

Anne Reichel
Kathrin Neufang (since 01.10.2017)
Bianca Meyerhöfer-Klee (part-time)

About us

General Assembly

Surname	Name
Alzheimer	Christian
Amann	Kerstin
Andreev	Katharina
Bäuerle	Tobias
Becker	Christoph
Beckmann	Matthias W.
Behrens	Jürgen
Bender	Albrecht
Bernkopf	Dominic
Bogdan	Christian
Bosch-Voskens	Caroline
Boßerhoff	Anja
Bozec	Aline
Brabletz	Thomas
Brandstätter	Johann Helmut
Buchholz	Björn
Ceppi	Paolo
Cesnjevar	Robert
Dietrich	Peter
Distler	Jörg
Dörfler	Arnd
Dudziak	Diana
Dulin	David
Eberhardt	Esther
Eichler	Anna
Ensser	Armin
Enz	Ralf
Erber	Ramona
Erim	Yesim
Fasching	Peter
Finotto	Susetta
Fromm	Martin

Surname	Name
Full	Florian
Gefeller	Olaf
Georgiadou	Ekaterini
Gerlach	Katharina
Golub	Yulia
Gramberg	Thomas
Grampp	Steffen
Grützmann	Robert
Günther	Claudia
Häberle	Benjamin
Hartmann	Arndt
Hellerbrand	Claus
Hilgers	Karl F.
Horch	Raymund
Iro	Heinrich
Jäck	Hans-Martin
Janko	Christina
Jitschin	Regina
Klingberg	Anika
Klucken	Jochen
Korbmacher	Christoph
Kornhuber	Johannes
Krappmann	Sven
Kremer	Andreas
Krönke	Gerhard
Kruse	Friedrich E.
Lai	Xin
Lang	Roland
Lee	De-Hyung
Lehmann	Christian
Leppkes	Moritz
Lie	Dieter Chichung

Surname	Name
Linker	Ralf
Lutzny-Geier	Gloria
Mackensen	Andreas
Marxreiter	Franz
Mielenz	Dirk
Moll	Gunther
Mougiakakos	Dimitrios
Müller	Christian
Münzner	Julienne
Naschberger	Elisabeth
Neurath	Markus
Nimmerjahn	Falk
Nitschke	Lars
Pachowsky	Milena
Palumbo-Zerr	Katrin
Patankar	Jay
Petter	Michaela
Pilarsky	Christian
Rascher	Wolfgang
Regensburger	Martin
Reis	André
Reuter	Nina
Rother	Ulrich
Schett	Georg
Schlötzer-Schrehardt	Ursula
Schmidt	Manuel
Schöpe	Isabella
Schubert	Ulrich
Schuler	Gerold
Schulze	Holger
Schüttler	Jürgen
Schwab	Stefan

Surname	Name
Schwappacher	Raphaela
Seidel	Thomas
Stamminger	Thomas
Steinkasserer	Alexander
Stürzl	Michael
Tenbusch	Matthias
Traxdorf	Maximilian
Überla	Klaus
Uslu	Ugur
Veelken	Roland
Vöhringer	David
Wahlbuhl-Becker	Mandy
Waldmann	Elisabeth
Waldner	Maximilian
Wegner	Michael
Winkler	Jürgen
Winner	Beate
Wirtz	Stefan
Zhang	Yun
Zimmermann	Katharina
Zweier	Christiane
Zundler	Sebastian

General Assembly of the IZKF (08.11.2017)

About us

External Scientific Advisory Board


Prof. Dr. Häussinger

Chairman

Prof. Dr. Dieter Häussinger (till 31.12.2017)
Düsseldorf University Hospital - Department of Gastroenterology, Hepatology and Infectiology


Prof. Dr. Sendtner

Vice-Chair

Prof. Dr. Michael Sendtner (since 01.01.2018 Chairman)
University Hospital Würzburg - Institute for Clinical Neurobiology

Members

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

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

Prof. Dr. Ulrich Kalinke,
TWINCORE, Centre for Experimental and Clinical Infection Research

Prof. Dr. Thomas Kamradt,
Jena University Hospital, Institute of Immunology

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

Prof. Dr. Tanja Kuhlmann,
University Hospital Münster, Institute of Neuropathology

Prof. Dr. Holger Moch,
University Hospital Zurich, Institute of Pathology and Molecular Pathology

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

Prof. Dr. Jörg Prinz,
LMU München, Department of Dermatology and Allergology

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

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

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

Prof. Dr. Reiner Siebert,
University Hospital Ulm, Institute of Human Genetics

Prof. Dr. Lydia Sorokin,
University of Münster, Institute of Physiological Chemistry and Pathobiochemistry

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

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

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. Klaus Pfeffer (till 31.12.2017)
Düsseldorf University Hospital - Institute of Medical Microbiology

Prof. Dr. Wolff Schmiegel (till 31.12.2017)
Bochum University Hospital - Department of Medicine

Prof. Dr. Thomas Wirth (till 31.12.2017)
University of Ulm - Institute of Physiological Chemistry


Prof. Dr. Busch


Prof. Dr. Hengel


Prof. Dr. Kalinke


Prof. Dr. Kamradt


Prof. Dr. Katschinski


Prof. Dr. Kuhlmann


Prof. Dr. Moch


Prof. Dr. Pavenstädt


Prof. Dr. Pfeffer*


Prof. Dr. Prinz


Prof. Dr. Rieß


Prof. Dr. Schmiegel*


Prof. Dr. Schulz


Prof. Dr. Seufferlein


Prof. Dr. Siebert


Prof. Dr. Sorokin


Prof. Dr. Tiegs


Prof. Dr. Wirth*

External Scientific Advisory Board (31.12.2017)

* till 31.12.2017

Programmes

Programmes

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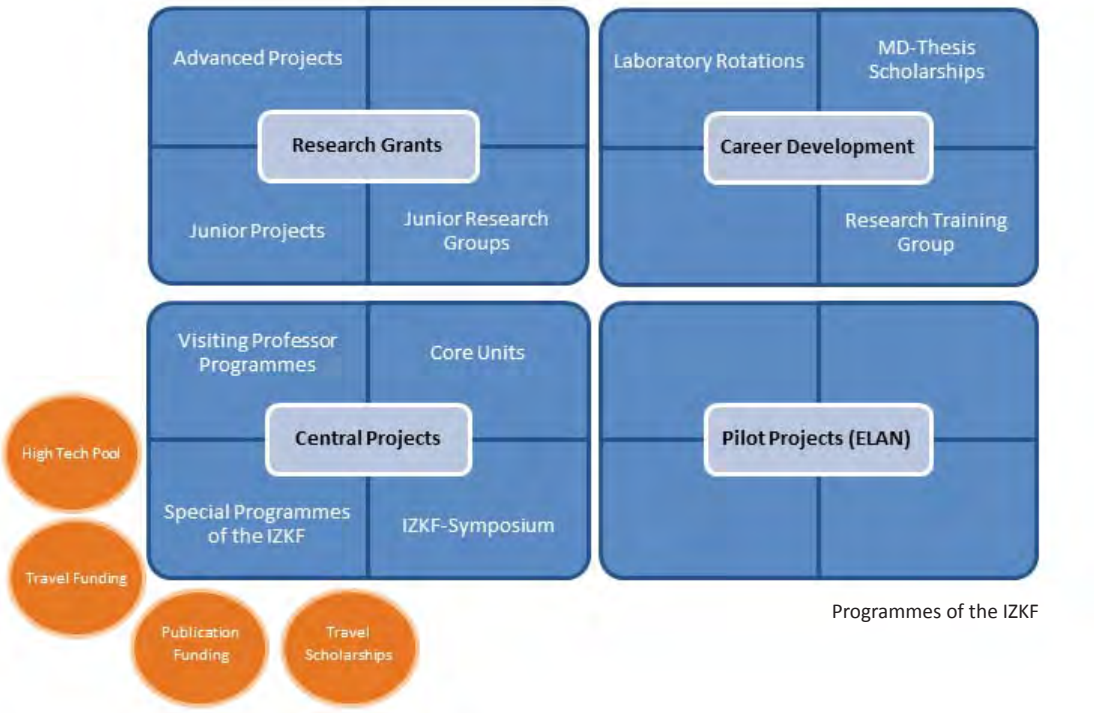
Programmes

Overview

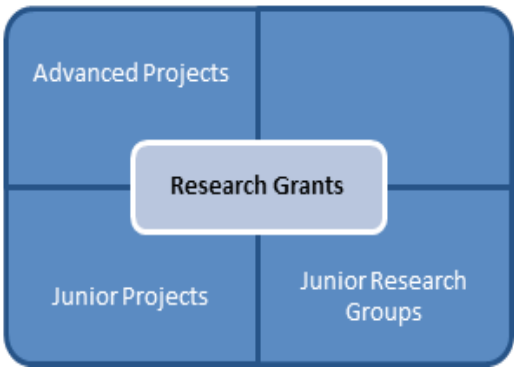
Calls for advanced and junior projects, pilot projects (ELAN), junior research groups, core facilities, MD-thesis scholarships and laboratory rotations are periodically announced within the Faculty of Medicine.



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 include now one personnel position (graduate student or technical assistant). 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	Graduate student or Technical assistant
Consumables	T€ 20 p.a.
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 graduate student 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 Graduate student
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 the ELAN programme 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. Young scientists up to 38 years of age are supported with a maximum of € 50,000 for a period of up to 12 months. Since 2017, also applicants from the part of the Faculty administrated by the University (Kapitel 1519) are approved for the programme, so that young scientists from the entire Faculty of Medicine can apply. In addition, newly appointed Professors can submit their application regardless of age.

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

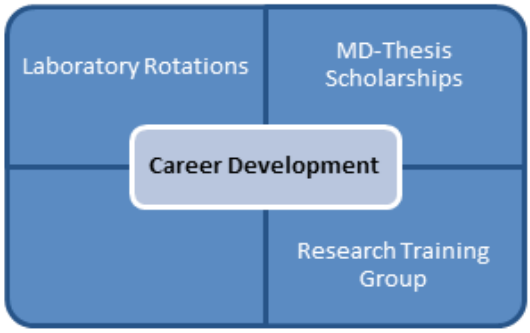
Staff	Group leader Postdoctoral scientist Graduate student Technical assistant
Consumables	T€ 50 p.a.
Others	Participation in the allocation of funds based on performance criteria (LOM) Laboratory space Investment funds
Duration	6 years

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 scientist and one graduate student, 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 (LOM). At the end of 2017 there are two junior research groups. One group (N1) is housed in the Nikolaus Fiebiger Center for Molecular Medicine with its attractive scientific environment and diverse activities; the other (N2) is located at the Kußmaul-Campus at the Optical Imaging Center Erlangen (OICE).

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 up to 6 full-time positions; this equates to 72 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. Up to 25 grants are available for medical students with outstanding performance and commitment in studies. The particiants have to work full-time in the laboratory over a period of 9 months, whereby a scholarship is offered during their research activity of 8 continuous month. Furthermore, the doctoral students have to complete defined training module during their studies. Training modules like guest speaker seminars, soft skills courses and the continuous supervision by a doctoral committee should continue throughout until completion of the doctorate. Every participant of the MD-Thesis Scholarship Programme automatically becomes a member of the IZKF Research Training Group and the Graduate School of Life Sciences at FAU (Life@FAU) inaugurated in October 2017. Thus, the doctoral students can benefit from a structured, interdisciplinary training programme.

Programmes

Research Training Group

The IZKF runs a research training group for all PhD and MD-students of the IZKF. Participation is mandatory for all doctoral candidates in sciences who are not involved in an alternative structured training programme of the Faculty/ University and for doctoral candidates who receive funding as part of an IZKF MD-thesis scholarship. Other students may associate with the research training group.

Aims of the IZKF Research Training Group include fostering networking and scientific self-organisation, 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 are organised by the Junior Scientist Committee. In addition, the participants of the graduate school have to attend guest speaker seminars and to participate in the annual internal retreat. Participation in external congresses and in seminars organised by the doctoral students are mandatory.

The research training group also offers a mentoring programme for all doctoral students. Each doctoral student announces three supervisors. At least one annual meeting of the doctoral student and the supervision committee is expected.

Currently, the IZKF Research Training Group is divided into two areas neuroscience (Neuro) and immunology/infection/oncology/renal and vascular research (T(h)ink). An additional oncology group is in preparation.

In 2017, the IZKF Research Training Group was integrated into the newly created Graduate School of Life Sciences at FAU (Life@FAU). This new umbrella organisation was established by initiative of IZKF. Life@FAU supports interdisciplinary graduate programmes in medicine and science at FAU and offers doctoral candidates in the fields of medicine and the natural sciences a structured, interdisciplinary training programme. The objectives of Life@FAU are to enhance the structured training programmes for doctoral candidates in life sciences at FAU, to create uniform standards in postgraduate education and to ensure the provision of structured training programmes.

Postgraduate Workshop

Every two years, the Junior Scientist Committee organises the IZKF Postgraduate Workshop. The Postgraduate Workshop alternates with the IZKF 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 expected 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 centralised 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.

Next generation sequencing core unit

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:

- Next generation sequencing
- Cell sorting unit with immune monitoring
- Preclinical animal unit
- Small animal imaging – PIPE

Programmes

International IZKF Symposium

IZKF regularly organises 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 administers 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 an amount of up to € 3,000 for travel and accommodation costs for visiting researchers. 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 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 but not all programmes are available to all funding lines of IZKF.

High Tech Pool

IZKF actively encourages the use of modern “omics” technologies in the projects, such as those provided by the Core Unit Next Generation 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. This programme is available for advanced and junior projects.

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.

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.

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
A56	Role of HIG2 in atherosclerosis	01.03.2014-28.02.2017	PD Dr. Warnecke	Department of Medicine 4
A61	Leishmania, iNOS and iron	01.02.2014-31.01.2017	Prof. Bogdan, PD Dr. Schleicher	Institute of Clinical Microbiology, Immunology and Hygiene
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 subpopulations	01.04.2016-30.09.2018	Prof. Dudziak	Department of Dermatology
A66	Genome wide CRISPR/Cas9 knockout for the identification 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 immunity to infection	01.07.2016-31.12.2018	Prof. Lang	Institute of Clinical Microbiology, Immunology and Hygiene
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	01.07.2016-31.03.2019	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	PD Dr. Dr. Günther, PD Dr. Dr. Wirtz	Department of Medicine 1

Oncology

Project No.	Project title	Term	Applicant(s)	Institute
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
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

Advanced Grants

Neurosciences

Project No.	Project title	Term	Applicant(s)	Institute
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, Department of Medicine 4, Department of Psychiatry and Psychotherapy
E14	Role of TRPC5 in trigeminal nociception	01.04.2014-31.03.2017	Prof. Zimmermann	Department of Anaesthesiology
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
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
E21	Modulation of alpha-Synuclein pathology by FoxO-dependent pathways	01.05.2016-31.10.2018	Prof. Lie, Prof. 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	Department of Stem Cell Biology, Department of Anesthesiology
E26	Genetics and pathomechanisms of intellectual disability with microcephaly	01.03.2016-31.08.2018	Prof. 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
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

A56 - Final Report

01.03.2014 - 28.02.2017

Role of HIG2 in atherosclerosis

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

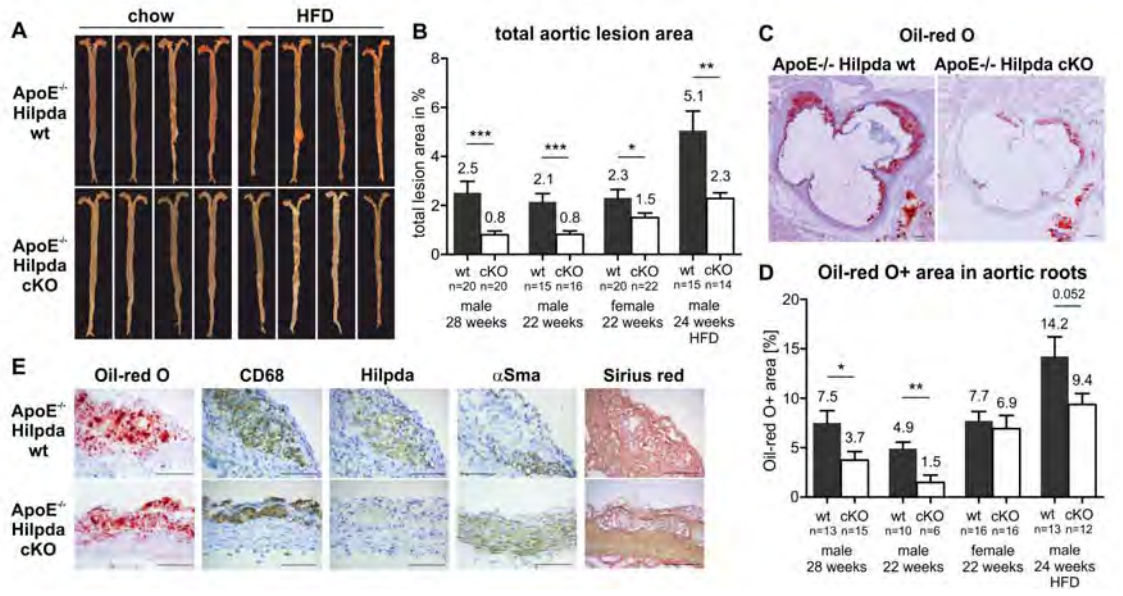
The hypoxia-inducible lipid droplet protein Hig2/Hilpda is highly expressed in atherosclerotic foam cells, but its role in atherogenesis has not been shown. We found that Hilpda is crucial to neutral lipid accumulation, resistance against lipid overload and controlled PGE2 production in macrophages. Tie2-cre- but not lysM-cre-mediated Hilpda depletion in apolipoprotein E-deficient mice reduced total aortic plaque size and macrophage content, and vascular inflammation under normal and 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 did not form LDs after Hif-1 activation or loading with fatty acids (FA) or cholesterol. Hilpda was upregulated by Hif-1 under M1 and M2 conditions, as well as by Ppar ligands and FA. Adrp/Plin2, associated with hypoxic lipid accumulation previously, proved to be a Ppar target, but was hardly induced by Hif-1 in BMDMs. Hif-1α cKO and adrp knockdown reduced, but did not abolish lipid accumulation. Comparison of Hilpda and Adrp/Plin2 indicated that Hilpda mediates LD formation, whereas Adrp stabilizes LDs.

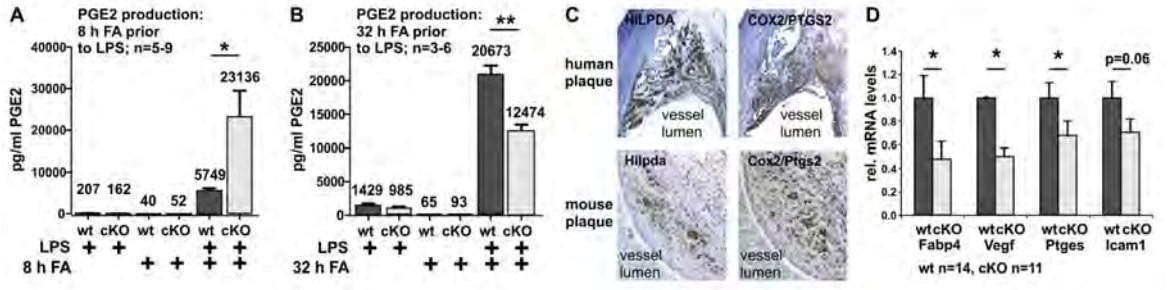
Mechanisms and functional consequences of Hilpda-induced neutral lipid storage.

Hilpda-induced lipid storage did not require increased uptake of lipoproteins or FA. Tracing of “click”-FA confirmed that esterification to di- and triacylglycerol is impaired in hilpda cKO BMDMs, but not uptake and integration of FA into membrane phospholipids. Hilpda-induced LD formation decreased ROS after reoxygenation and protected against membrane damage after FA overload, but did not alter ATP levels. After FA loading for 8 h, which enhanced subsequent LPS-stimulated PGE2 formation, hilpda cKO BMDMs produced more PGE2, whereas after FA loading for 32 h PGE2 production was reduced in cKO BMDMs,



In apoe$^{-/-}$ tie2-cre hilpda cKO mice -/+ HFD total aortic plaque area is reduced (A and B). Aortic root lipid deposition (Oil red O; C and D) and CD68+macrophages are decreased in hilpda cKO mice, whereas α-smooth muscle actin (αSma) is increased (E).

PD Dr. Warnecke



Hilpda-induced LDs restrict PGE2 production after 8 h fatty acid (FA) overload (A), but support PGE2 synthesis 32 h after FA loading (B). Hilpda and Cox2 co-localize in plaques (C). Vegf and inflammatory genes in blood vessels of hilpda wt and cKO mice (D).

indicating that LDs can serve both as buffer and as reservoir for the FA substrates of PGE2 production. Thus, Hilpda-mediated LD formation is crucial to a controlled and inducible production of PGE2, which increases Vegf expression and vascular permeability in acute inflammation and contributes to plaque destabilization in advanced plaques.

Hilpda contributes to atherogenesis in Apolipoprotein E-deficient mice.

In human plaques HILPDA was highly expressed in foam cells surrounding the lipid core and in the vulnerable plaque shoulder. Co-localization with COX2/PTGS2 suggested that HILPDA may contribute to PGE2 production, destabilization and rupture of advanced human plaques.

We analyzed 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 total lesion areas in whole aortas and macrophage content and lipid deposition in aortic roots. We then investigated male apoe-/- tie2-cre hilpda cKO mice after 8 weeks of high fat diet (HFD), expecting to stress differences between cKO and wt mice by Ppar-mediated hilpda induction. However, although HFD doubled total aortic plaque area, it did not increase, but blunted the relative difference in atherogenesis between hilpda cKO and wt mice. In large blood vessels, Fabp4, a marker of atherosclerosis progression, was robustly induced by HFD. Expression of Vegf, Fabp4 and Ptges/mPges1 mRNA were significantly lower in apoe-/- hilpda cKO than in wt mice, and Icam1 showed a tendency towards reduction, indicating attenuated vascular inflammation in hilpda cKO mice.

Thus, Hilpda contributes to early lesion formation and vascular inflammation and probably also to the progression of atherosclerosis.

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Publications during funding period

Maier A, Wu H, Cordasic N, Oefner P, Dietel B, Thiele C, Weidemann A, Eckardt KU, Warnecke C (2017) Hypoxia-inducible protein 2 Hig2/Hilpda mediates neutral lipid accumulation in macrophages and contributes to atherosclerosis in apolipoprotein E-deficient mice. FASEB J. 31(11): 4971-4984

A61 - Final Report

01.02.2014 - 31.01.2017

Leishmania, iNOS and iron

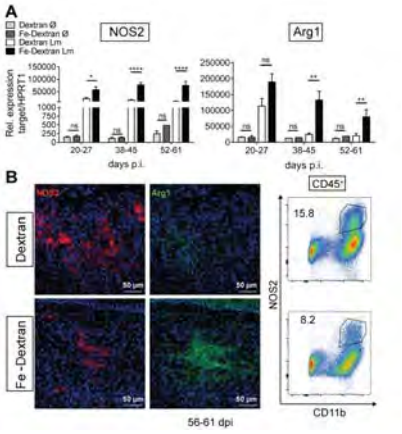
Prof. Dr. Christian Bogdan, PD Dr. Ulrike Schleicher,
Institute of Clinical Microbiology, Immunology and Hygiene

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 and converts L-arginine into citrulline and NO. Based on studies with promastigote *Leishmania,* NO is capable to exert direct leishmanicidal effects. However, whether this is also true for amastigote *Leishmania,* has never been demonstrated. In the light of the known signaling function of NO, possible indirect antimicrobial effects of NO need 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.

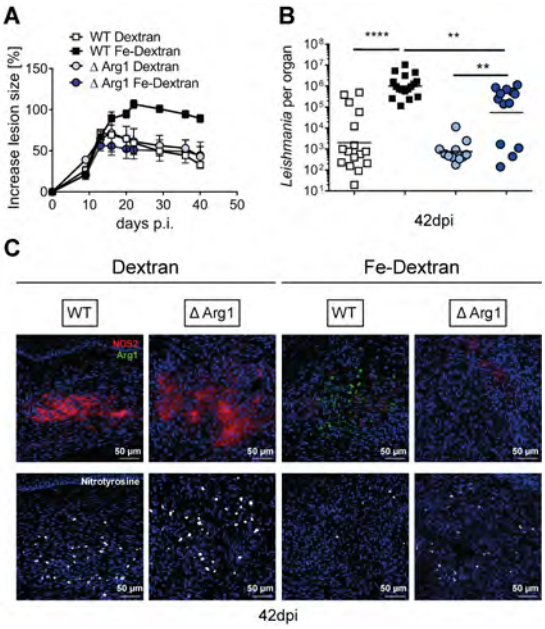
In a reverse approach iron-loaded *L. major*-infected C57BL/6 mice showed disease exacerbation with aggravated skin swelling at the site of infection and higher parasite loads in the lesion. This was paralleled by enhanced cell death in the infected tissue, increased influx of $CD11b^+Ly6G^+Ly6C^+$ neutrophil-like cells lacking suppressor activity and decreased percentages of T-cells, eosinophils, macrophages and dendritic cells. Furthermore, iron overload increased the expression of IFN-gamma, TNF, IL-4, TGF-beta and NOS2 mRNA in skin lesions, whereas NOS2 protein expression and NO production was suppressed compared to control mice. Interestingly, arginase (Arg) 1, which competes with NOS2 for the substrate L-arginine, was upregulated on mRNA and protein level. In Arg1-deficient C57BL/6 mice the aggravated course of disease following iron loading was abolished and NO production in the lesions was partially restored. Similar effects were observed when iron-loaded C57BL/6 mice were treated with anti-IL-4-antibodies indicating that iron-driven Arg1 induction is mainly due to enhanced IL-4 levels.



Expression of NOS2 and Arg1 mRNA (A) or protein (B) in the skin lesions of *L. major*-infected, iron dextran-loaded versus dextran-treated (control) C57BL/6 mice.


Prof. Dr. Bogdan


PD Dr. Schleicher



Course of infection (A), parasite burden (B) and NO production (nitrotyrosine staining, C) in the skin lesions of L. major-infected iron dextran-loaded or dextran-treated (control) C57BL/6 WT versus △Arg1 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 NOS2 and Arg1 expression. Disease exacerbation and impaired parasite control at least partially result from IL-4-mediated Arg1 expression that decreases NO production in iron-loaded mice.

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Invited lectures

6th World Conferences on leishmaniasis, May 16-20, 2017, Toledo, Spain, Arginase 1 during the acute and latent phase of cutaneous leishmaniasis

Publications during funding period

Schleicher U, Liese J, Justies N, Mischke T, Haeberlein S, Sebald H, Kalinke U, Weiss S and Bogdan C (2018) Type I interferon signaling is required for CpG-oligodesoxynucleotide-induced control of Leishmania major, but not for spontaneous cure of subcutaneous cure of primary and secondary L. major infection. Frontiers Immunol. doi: 10.3389/ fimmu.2018.00079

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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

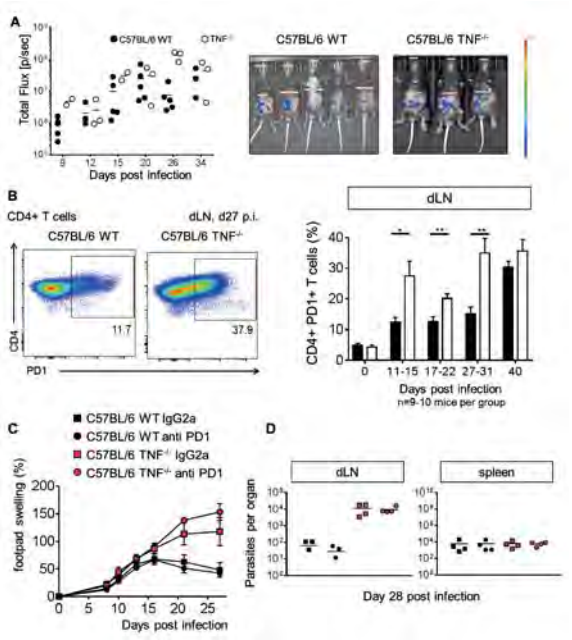
Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene

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.

Tumor necrosis factor (TNF)-deficient C57BL/6 mice fail to control an infection with the intracellular pathogen *Leishmania (L.) major* and develop fatal visceral disease, despite an intact Th1 immune response and expression of type 2 nitric oxide (NO) synthase (NOS2) protein that converts L-arginine into citrulline and leishmanicidal NO. NOS2 activity can be antagonized by arginase (Arg)1 that metabolizes L-arginine into urea and ornithine. In *L. major*-infected $TNF^{-/-}$ mice we discovered a hyperexpression of Arg1, paralleled by suppression of NOS2 activity and NO production at the sites of infection. To test whether in *L. major*-infected $TNF^{-/-}$ mice Arg1 upregulation alone accounts for the detrimental outcome, we generated C57BL/6 mice deficient for both TNF and Arg1. Unexpectedly, $TNF^{-/-}Tie2Cre^{+/-}Arg1^{fl/fl}$ mice developed progressive cutaneous leishmaniasis comparable to $TNF^{-/-}$ mice, despite restored NO release in the infected tissue. These results support the hypothesis that alterations other than the upregulation of Arg1 contribute to the non-healing course of infection in $TNF^{-/-}$ mice.

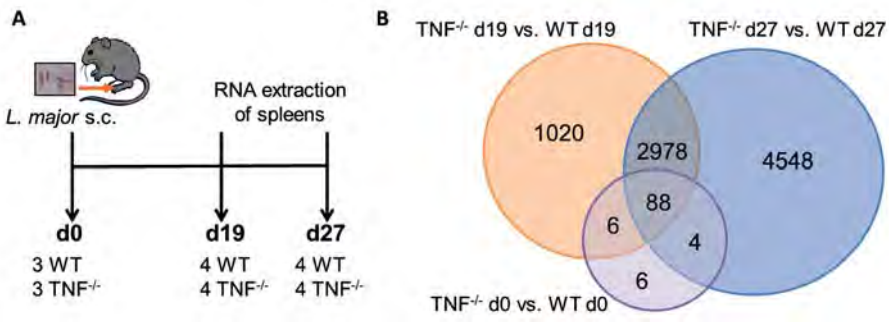
L. major-infected $TNF^{-/-}$ mice exhibited visceral disease with high splenic parasite loads. Previously, we showed that in the spleen parasite control requires the production of reactive oxygen species (ROS) by phagocyte NADPH-oxidase (Phox) rather than NO release by NOS2. As Phox activity is induced by TNF, we investigated the production of ROS in wildtype (WT) versus $TNF^{-/-}$ mice. Surprisingly, an increase of overall ROS production was seen in $TNF^{-/-}$ mice by in vivo imaging (IVIS). Since this method also detects extracellular ROS, we are planning to analyze mRNA and protein expression of Phox components and the generation of ROS specifically in splenocytes.

As TNF-deficiency also affected T cell populations ($CD4^+Tbet^+$, $CD8^+$, $CD4^+FOXP3^+$), we analyzed T cell exhaustion in *L. major*-infected $TNF^{-/-}$ mice. The inhibitory surface molecule programmed cell death protein 1 (PD-1) was upregulated on $CD4^+$ and on $CD8^+$ T cells, suggesting T cell suppression in infected $TNF^{-/-}$ mice. However, blocking of PD-1 by application of anti-PD-1 did not prevent non-healing disease in *L. major.*



(A) ROS production in infected WT and TNF-/- mice; (B) lymph node $PD1^+CD4^+$ T cells in infected WT vs. $TNF^{-/-}$ mice; (C) lesion size and (D) parasite load of infected WT vs. $TNF^{-/-}$ mice after anti-PD-1 or control rat IgG2a treatment

Prof. Dr. Bogdan



(A) RNA extraction from spleens of naive or infected TNF$^{-/-}$ and WT mice for gene expression analysis by RNASeq. (B) Significantly regulated genes in infected WT vs. TNF$^{-/-}$ mice at day 0, 19 and 27 post infection as detected by RNASeq, $p < 0.05$.

In order to discover "novel" TNF-regulated antimicrobial effectors, spleens of *L. major*-infected TNF$^{-/-}$ vs. WT mice at two different time points of infection were analyzed by RNAseq technology (coop. with Dr. Ekici). A high number of differentially expressed genes were identified, some of which were already validated by real-time PCR (e.g. MadCAM-1, Stra6, Erdr1, Fcamr, Fcgr1) and flow cytometry (changes in B cell populations). Pathway analyses (Ingenuity®) and functional studies are performed to link these genes to the susceptibility of TNF$^{-/-}$ mice.

Finally, we are also investigating the regulation of Arg1 by TNF in humans. To this end, Arg1 expression is analyzed in peripheral blood mononuclear cells and neutrophils from rheumatoid arthritis patients prior and during treatment with TNF-antagonists.

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Invited lectures

6th World Conferences on leishmaniasis, May 16-20, 2017, Toledo, Spain, Arginase 1 during the acute and latent phase of cutaneous leishmaniasis

Publications during funding period

Soulat D, Bogdan C (2017) Function of macrophage and parasite phosphatases in leishmaniasis. Frontiers in Immunology 8: 1-21

Leitherer S, Clos J, Liebler-Tenorio EM, Schleicher U, Bogdan C, Soulat D (2017) Characterization of the protein tyrosine phosphatase LmPRL-1 secreted by Leishmania major via the exosome pathway. Infection Immunity 85(8): 1-19

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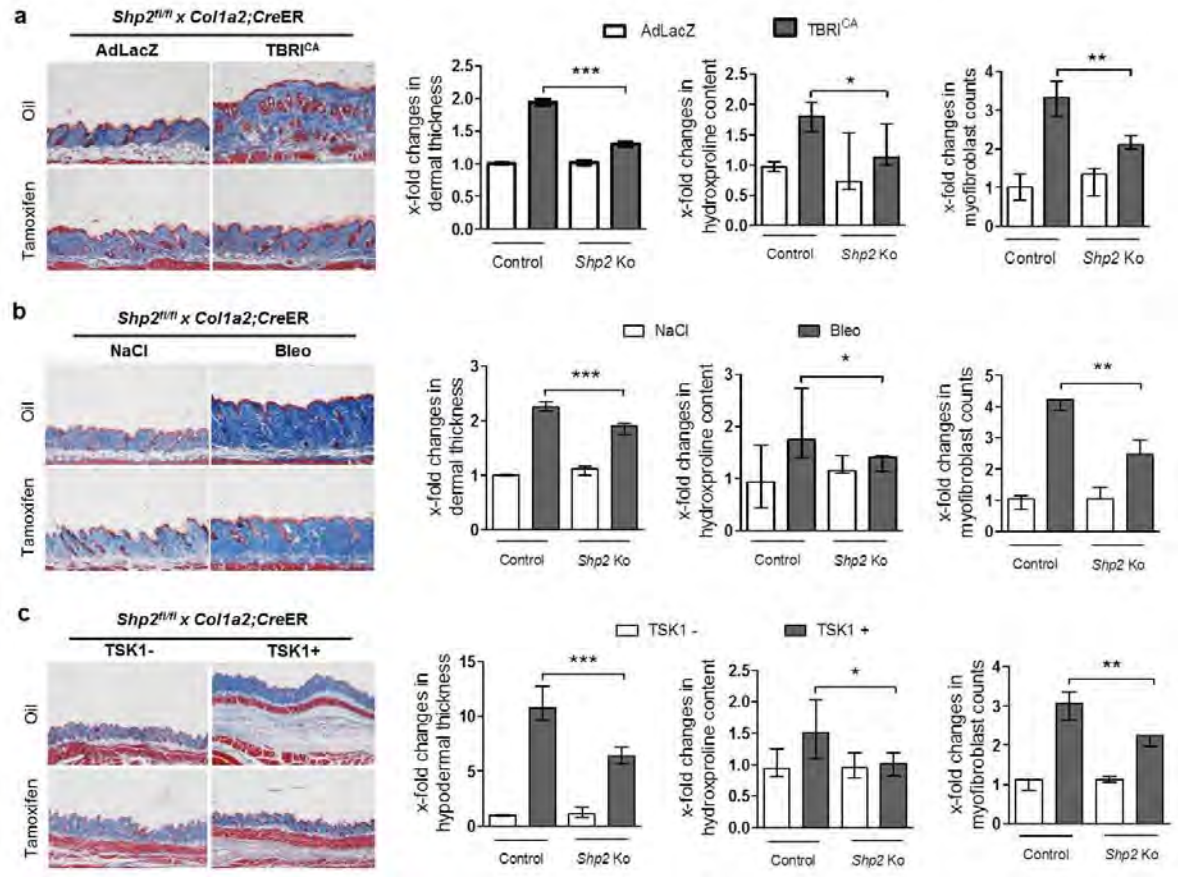
A64 - Progress Report

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.



Fibroblast-specific knockout of Shp2 protects from experimental fibrosis.
a: TBRICA-induced fibrosis. b: Bleomycin-induced skin fibrosis. c: TSK1 model. Representative images of Masson trichrome stained skin shown at 40-fold magnification. Dermal thickness, hydroxyproline content and myofibroblast counts. All groups in all models consisted of ≥ 6 mice each


Prof. Dr. Distler


Prof. Dr. Schett

Uncontrolled activation of TGFβ signaling is a common denominator of fibrotic tissue remodeling. Here we characterize the tyrosine phosphatase SHP2 as a molecular checkpoint for TGFβ-induced JAK2/STAT3 signaling and as a potential target for the treatment of fibrosis. SHP2 is recruited to JAK2 in a TGFβ-dependent manner in fibroblasts and dephosphorylates JAK2 at Y570, which permits subsequent activation of STAT3. Although SHP2 expression is decreased in systemic sclerosis (SSc), the moderate downregulation of SHP2 expression is not sufficient to counterbalance the hyper-activation of TGFβ signaling in SSc. However, augmentation of this endogenous regulation by genetic or pharmacologic inactivation of SHP2 promotes accumulation of JAK2 phosphorylated at Y570, reduces JAK2/STAT3 signaling, inhibits TGFβ-induced fibroblast activation and ameliorates dermal and pulmonary fibrosis. Given the availability of potent SHP2 inhibitors, SHP2 might thus be a potential novel target for the treatment of fibrosis.

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Invited lectures

Annual Meeting of the American College of Rheumatology Meeting, November 2017, San Diego, CA, USA, All roads lead to fibrosis

Keystone Meeting “Injury, Inflammation and Fibrosis“, March 2017, Snowbird, UT, USA, Targeting TGFbeta-Dependent Fibroblast Activation

Publications during funding period

Šumová B, Chakraborty D, Mallano T, Chen CW, Distler A, Distler O, Schett G, Šenolt L, Distler JH (2017) Activation of Signal Transducer and Activator of Transcription 3 (STAT3) integrates common profibrotic pathways to promote fibroblast activation and tissue fibrosis. Nat. Comm 8(1): 1130

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A65 - Progress Report

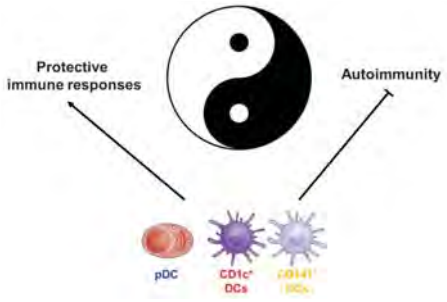
01.04.2016 - 30.09.2018

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 DCs with Toll like receptor (TLR) ligands. After culture, we measured the concentration of secreted cytokines and chemokines by CBA assay from collected supernatants. Strikingly, we found that the cellular surface expression profile did not reflect the secreted 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. We hypothesize 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 understand this tolerizing potential of human thymic DCs as this immunoprivileged site might harbor important aspects also for understanding of tumor development.



Human DC subpopulations and their role in tolerance and immunity.

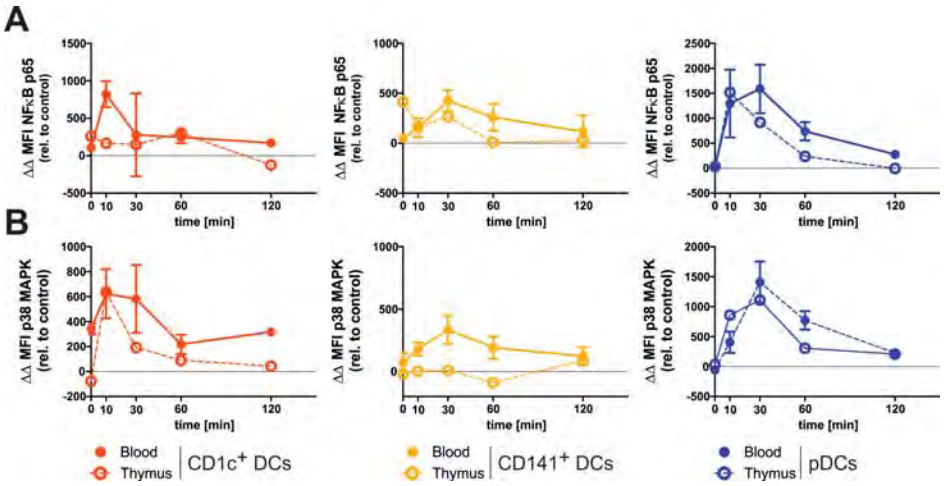
Results in Aim 1: Determination of functional differences between blood and thymic DCs

To strengthen our preliminary findings we performed side-by-side polarization and proliferation assays in a co-culture of isolated and activated thymic and blood DCs with HLA-mismatched naïve $CD4^+$ peripheral blood T cells. Cell-sorted DCs from these tissues were stimulated with TLR ligands. After 24 hrs, the cells were co-cultured with purified allogeneic blood $CD4^+$ T cells. Intracellular FACS analyses revealed that thymic DCs are inhibited in driving TH1 differentiation while the differentiation into TH2 and TH17 was unaltered compared to blood DCs. Next, we will investigate $CD8^+$ driven responses.

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 DCs. Therefore, we measured the phosphorylation of signaling molecules by upon stimulation with TLR ligands of sorted blood and thymic DCs. Notably, we found a differential DC specific phosphorylation profile in that NFκB p65 and p38 MAPK were stronger and longer phosphorylated in R848-stimulated blood DCs compared to thymic DCs. Those data are of specific importance, as we needed more evidence about potential regulatory mechanisms in thymic DCs. Based on our findings, we are currently sorting

Prof. Dr. Dudziak



Differential phosphorylation of signaling molecules in blood and thymic DCs after stimulation with the TLR7/8 ligand R848

non- and TLR activated DCs for epigenetic profiling, including ATAC sequencing combined with RNA-Seq analyses.

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Invited lectures

6th Chinese-German Symposium on Immunology, November 2017, Hangzhou China, Exploring dendritic cells in mouse and man for vaccine development in tumor and autoimmune therapy

Annual Meeting of the Autumn School of Immunology, October 9, 2017, Merseburg, How dendritic cells (interact and) activate T cells

47th Annual Meeting of the German Society of Immunology, September 2017, Erlangen, Understanding the transcriptional code of dendritic cells to improve immunotherapeutic approaches

101st Annual Meeting of the German Society of Pathology, June 2017, Erlangen, Understanding the transcriptional code of dendritic cells to improve immunotherapeutic approaches

Guest speaker at the MPI for Infection Research, March 28, 2017, Berlin, Dendritic cells in mouse and man – In-depth analysis for better vaccine design

Guest speaker at the TRR156, March 15, 2017, Heidelberg, Dendritic cells in mouse and man – In-depth analysis for better vaccine design

Guest speaker at the 'Erlanger days of Radiology', March 18, 2017, Erlangen, Vakzinierung mit Dendritischen Zellen

Publications during funding period

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Mokada-Gopal L, Boeser A, Lehmann CHK, Drepper F, Dudziak D, Warscheid B, Voehringer D (2017) Identification of Novel STAT6-Regulated Proteins in Mouse B Cells by Comparative Transcriptome and Proteome Analysis. J Immunol. 198(9): 3737-3745

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A66 - Progress Report

01.07.2016 - 31.12.2018

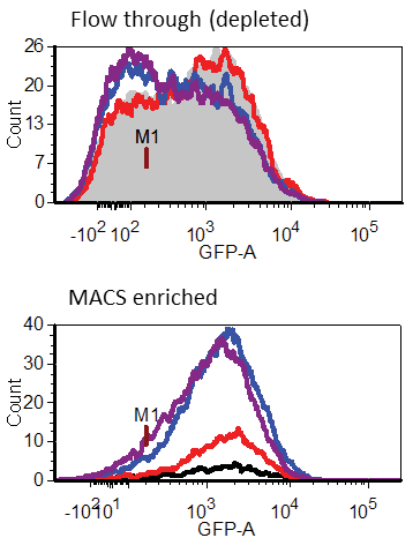
Genome wide CRISPR/Cas9 knockout for the identification of antiviral cellular restriction factors

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.

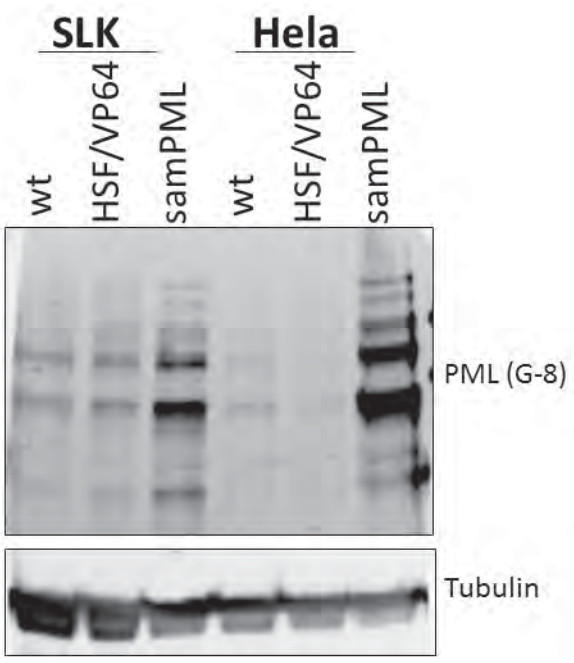
After performing first genome wide screening experiments on KSHV infected SLK target cells, we found the initial screens were limited by the FACS-sorting rate of large epithelial cells, such as SLK(Caki) or HeLa, which allowed to separate ~1500 cells per second, and would have resulted in sort times exceeding 20 hours for a typical sample of 50-100 million cells. Therefore, we 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. The synergistic activation mediator (SAM)



KSHV Bac16 - H2kk, SLK cells infected for 48h, flow cytometric detection of EGFP co-expressed from virus

Magnetic enrichment of cells infected by H-2kk expressing KSHV.

Prof. Dr. Ensser



Synthetic activation mediator (SAM) targeted to PML promoter.

system was functionally verified and established as a complementary approach to validate the sgRNA ko-screen. Its genome-wide sgRNA2.0 library of >70.000 plasmids is now available and the components of the SAM system, NLS-dCas9-VP64 and helper transactivator MS2-p65-HSF1 were tested in model cell lines (e.g. SLK(Caki), HeLa). Furthermore, an improved version of the genome-wide sgRNA library in the pLentiCRISPRv2 vector became recently available. We have obtained and amplified this "Brunello-Library" consisting of 4 sgRNA per cellular gene and 1000 non-targeting control sgRNAs (total 77.441 constructs) and sequence verified it by Illumina NGS on the core units HiSeq 2500 instrument. The Brunello library is more efficient due to its optimized algorithm for sgRNA design, resulting in a lower false discovery rate (FDR), and the lower complexity requires 1/3 less cells to be transduced, and consequently, less sorting time for selection of enriched or depleted cells. In summary, the two complementary screens will 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.

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Publications during funding period

Ensser A (2017) Genome Sequence of the Alcelaphine Gammaherpesvirus 1 Attenuated Laboratory Strain WC11. Genome Announc 5(45)

Full F, Hahn AS, Grosskopf AK, Ensser A (2017) Gammaherpesviral Tegument Proteins, PML-Nuclear Bodies and the Ubiquitin-Proteasome System. Viruses 9(10)

A67 - Progress Report

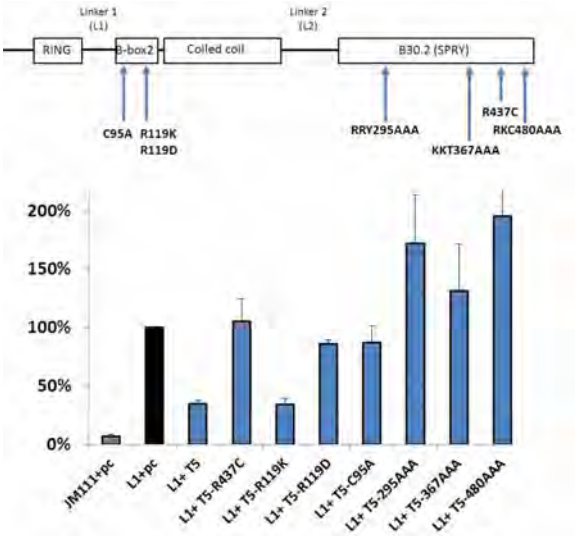
01.02.2016 - 31.07.2018

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

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

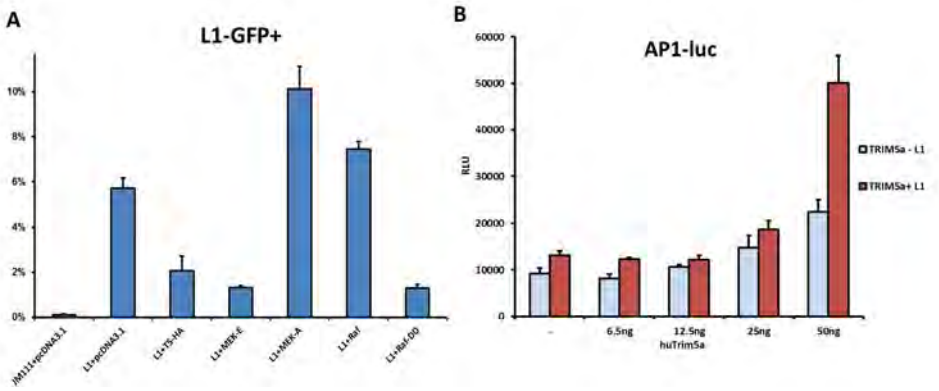
LINE-1 is the only autonomously active retrotransposon in humans. LINE-1 retrotransposition has been shown to cause various genetic disorders and it is key to control LINE-1 to maintain genome integrity. We found that the retroviral restriction factor TRIM5α also inhibits LINE-1 elements. In this study, we will determine the features of TRIM5α important for the block. We will also analyze the mechanism of LINE-1 inhibition and ask if other mobile elements are restricted as well.

Within the first year of funding, we demonstrated that the retroviral restriction factor TRIM5α reduces the retrotransposition frequencies of LINE-1 reporter elements. To identify regions of TRIM5α important for LINE-1 restriction, we analyzed naturally occurring TRIM5α variants, single nucleotide polymorphisms (SNPs), as wells as TRIM5α deletion mutants in LINE-1 GFP reporter assays. We found the N-terminal RING domain of TRIM5α, which harbors an E3 ligase function, to be dispensable for restriction of LINE-1. In contrast, we identified a single SNP located within the SPRY domain of TRIM5α, which directly interacts with retroviral capsids, that causes a complete loss of LINE-1 restriction. To confirm the importance of the SPRY domain and to determine the role of the B-Box domain, which is important for multimerization of TRIM5α, in LINE-1 restriction, we analyzed the retrotransposition efficiency of LINE-1 GFP in the presence of additional SAMHD1 mutants. We found that replacing charged surface amino acids in the SPRY domain abrogated the block to LINE-1 confirming the role of the SPRY domain in LINE-1 restriction. Furthermore, changing critical amino acids within the B-box (C95, R119) also strongly reduced TRIM5α activity against LINE-1, suggesting that multimerization of TRIM5α is important for its activity against LINE-1.



Mutations in B-Box and SPRY-domain of TRIM5α abrogate its anti-L1 activity. 293T cells were transfected with TRIM5α or the indicated mutants and an L1 GFP reporter plasmid. Retrotransposition events were quantified 5 days later and normalized to pcDNA-transfected control cells.

Prof. Dr. Gramberg



AP-1 activation by TRIM5a correlates with L1 inhibition. A) L1-GFP+ 293T cells were transfected with MEK1 and Raf or the active mutants MEK-E and Raf-DD. B) Cells were transfected with an AP1-driven luciferase reporter, L1, and TRIM5α. Luciferase activity was determined after 48h.

Since the restriction of LINE-1 by TRIM5α seems independent of its E3 ligase function, we wondered whether the reported TRIM5α-mediated induction of the MAP-Kinase pathway activating AP-1 and NF-kB transcription factors might play a role in LINE-1 restriction. In 293T cells, we found the replication of LINE-1 GFP to be potently restricted by overexpression of constitutively active mutants of the signaling kinases MEK1 and Raf1. In AP-1 and NfKb-dependent luciferase reporter assay, overexpression of TRIM5a led to NF-kB and AP-1 activation. Interestingly, the TRIM5α-mediated AP-1 signal in the presence of LINE-1 was enhanced, suggesting that TRIM5α induces inhibitory AP-1 signaling in the presence of LINE-1. In summary, the inverse correlation between LINE-1 replication and AP-1 induction by TRIM5α suggests the involvement of the TRIM5a-mediated MAP-Kinase activation in LINE-1 retrotransposition. Our findings suggest that AP-1 restricts the activity of LINE-1 mobile genetic elements and thereby contributes to host genome stability.

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Invited lectures

Guest seminar (SFB) Institut für Virologie, MHH, June 08, 2017, Hannover, Restriction factors block Retroviruses and endogenous Retroelements

Guest seminar Robert Koch Institut, May 08, 2017, Berlin, The role of SAMHD1 in fending off Retroviruses and endogenous Retroelements

Awards

Thiersch Preis, Habilitationspreis der Med. Fakultät, Thomas Gramberg, November 6, 2017, Erlangen

Publications during funding period

none

A68 - Progress Report

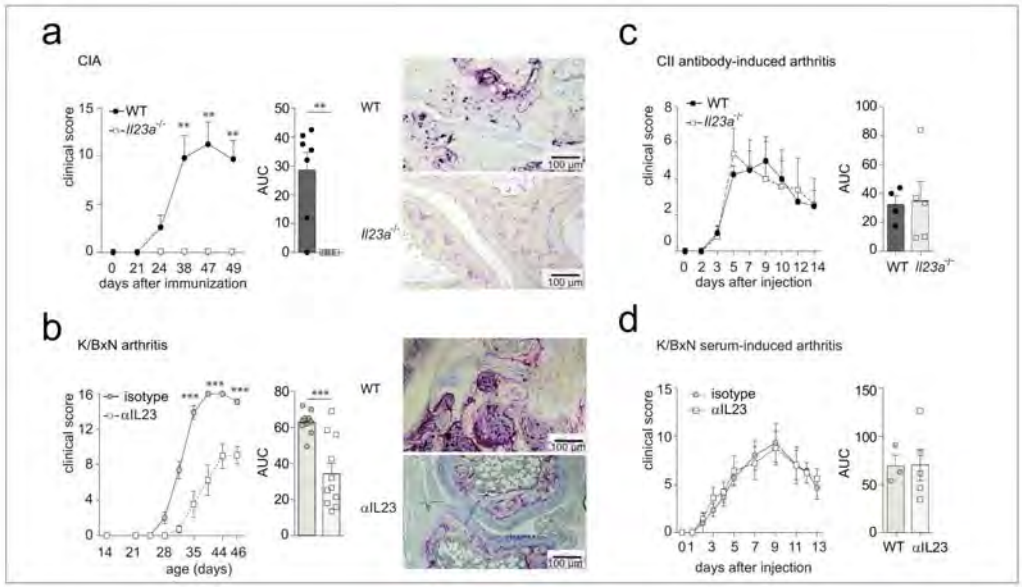
16.06.2016 - 15.12.2018

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

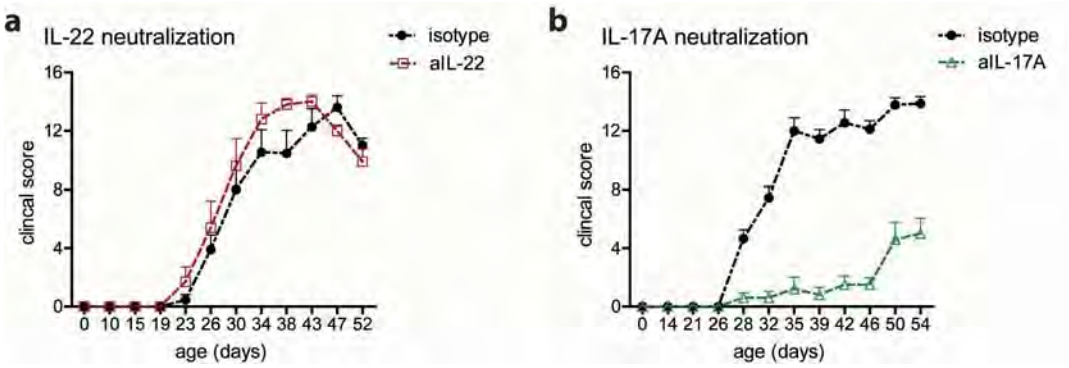
We currently determine the role of the IL-23/TH17 axis during the control of the intrinsic inflammatory activity of autoantibodies during onset of autoimmune arthritis. We have identified a pathway where TH17 cells regulate expression of glycosyltransferases in newly differentiating antibody-producing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

We previously identified the IL-23/TH17 axis as important modulator of the inflammatory activity of autoantibodies during rheumatoid arthritis (RA). 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 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 the RA model of K/BxN arthritis. Currently we are dissecting the role of single TH17-derived cytokines in the control of the B cell response as well as of the glycosylation of antibodies.



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



Impact of an antibody-mediated blockade of (a) IL-17 and (b) IL-22 on the clinical course of K/BxN arthritis.

Here we are focusing on the cytokines IL-17, IL-22, GM-CSF and RANKL. Preliminary data show that In the model of K/BxN arthritis blockade of IL-17A, but not of IL-22 suppresses development of arthritis suggesting a major role of IL-17 in the TH17-dependent regulation of B cell activity in this antibody-mediated model of RA.

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Invited lectures

Antibody Engineering and Therapeutics conference, December 2017, San Diego, Regulation of inflammatory properties of autoantibody glycosylation in autoimmune disesase

Molecular-Medicine Seminar, December 2017, University of Strasbourg, France, Regulatory checkpoints controlling the transition between autoimmunity and inflammation and their impact for human autoimmune disease

Seminar at the Centre for Immunobiology, October 2017, University of Glasgow, UK, Regulatory checkpoints controlling the transition between autoimmunity and inflammation

Awards

Langener Wissenschaftspreis, Gerhard Krönke, November 2017, Langen

Publications during funding period

Rothe T, Ipseiz N, Faas M, Lang S, Perez-Branguli F, Metzger D, Ichinose H, Winner B, Schett G and Krönke G. (2017) The Nuclear Receptor Nr4a1 Acts as a Microglia Rheostat and Serves as a Therapeutic Target in Autoimmune-Driven Central Nervous System Inflammation. J Immunol.198(10): 3878-3885

Pfeifle R, Rothe R, Ipseiz N, Scherer HU, Culemann S, Harre U, Ackermann JA, Seefried M, Kleyer A, n 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 and Krönke G. (2017) Regulation of autoantibody activity by the IL-23/Th17 axis promotes the onset of autoimmune disease. Nat Immunol. 18(1): 104-113

A69 - Progress Report

01.07.2016 - 31.12.2018

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

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

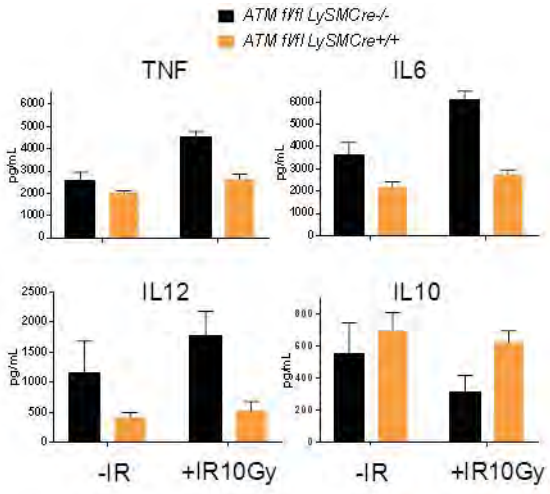
The DNA-damage response (DDR) requires the kinase ATM and is essential for the integrity of the host genome. We observed activation of the ATM kinase pathway in Toll-like receptor (TLR)-stimulated macrophages and a modulation of the inflammatory response by ATM-inhibition. Here, we carry out detailed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

In this project, we employ conditional ATM knockout mice to investigate *in vitro* and in vivo how the DNA damage response regulates the function of macrophages and dendritic cells (DC) upon encounter with microbial stimuli.

In a first set of *in vitro* experiments using bone marrow derived macrophages, we have observed that ionizing irradiation (IR) alters the balance of pro- and anti-inflammatory cytokine production in response to subsequent stimulation with LPS. TNF, IL-6 and IL-12 were significantly increased, whereas IL-10 was down-regulated. This effect of IR was completely dependent on ATM, as shown by Cre-mediated deletion in $ATM^{flox/flox}$ BMDM. Interestingly, non-irradiated ATM-deficient BMDM displayed moderately increased IL-10 and reduced proinflammatory LPS-induced cytokines compared to control BMDM, consistent with autochthonous activation of ATM by TLR4 activation.

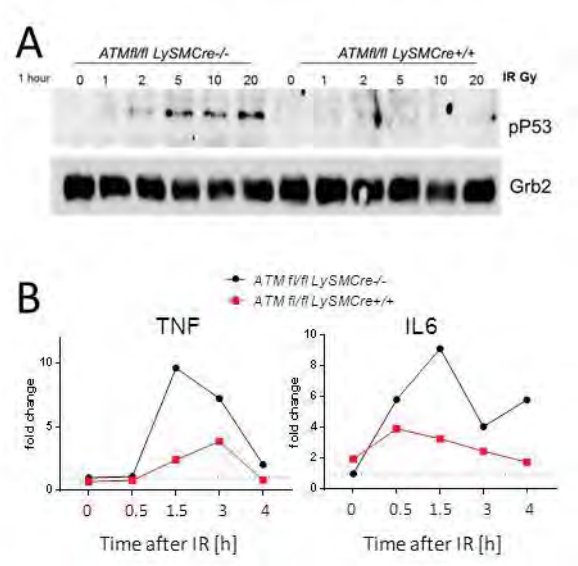
The transcription factor p53 is a canonical downstream target of ATM kinase. In irradiated macrophages, we found strong and dose-dependent phosphorylation of p53 after one hour, which was completely dependent on ATM. We next assessed whether IR *per se* induces cytokine expression and observed a moderate and transient upregulation of mRNA encoding IL-6 and TNF, with a peak between 1.5 – 3 hours. Preliminary data using pharmacological inhibitors suggest that p53 and NFκB activation contribute to the IR-induced cytokine expression. To further dissect the mechanisms of cross-regulation between DNA-damage response and TLR signaling in macrophages, we plan to employ conditional knockout mice for p53 and components of the NFκB pathway.

In vivo challenge with the TLR4-ligand LPS causes rapid systemic cytokine production, which can lead to organ damage and shock. Prior IR of mice with 2 Gy caused a significant reduction of TNF serum levels, whereas IL-6 production remained unaltered, suggesting that the consequences of DNA-damage induction are differentially regulated *in vivo.* The involvement of ATM kinase in immune responses to infection and inflammation is currently investigated using conditional knockout mice with ATM deletion in macrophages or DC. Preliminary data from immunization experiments indicate that ATM-deficiency in CD11c+ DC results in increased induction of Th cells producing IFNγ and IL-17.



Irradiation alters the balance of cytokine production in response to the TLR4 ligand LPS. ATM-deficient and control bone marrow-derived macrophages (BMDM) were irradiated (10 Gy), followed by stimulation with LPS. Cytokines were measured from supernatants.

Prof. Dr. Lang



Irradiation activates p53 and induces transient cytokine transcription dependent on ATM. (A) BMDM were irradiated with the indicated doses. Phosphorylation of p53 was determined after 1 h by Western blot. (B) Kinetics of mRNA expression of Tnf and Il6 in irradiated (10 Gy) ATM-deficient and control BMDM.

Taken together, our results support the notion that the DNA damage response through ATM kinase exerts a significant regulatory effect on the innate response to microbial danger. While macrophage activation status *in vitro* was clearly biased towards a more inflammatory response by DNA damage response, the resulting phenotypes *in vivo* were also compatible with an attenuation of inflammatory cytokine production by DNA damage and ATM. Therefore, in ongoing work we are investigating in more detail and breadth the effect of ATM-deficiency in murine infection and immunization models.

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Invited lectures

Medical Faculty Dresden, April 26, 2017, Dresden, Balancing innate immune responses in infection

Medical Faculty Mainz, September 26, 2017, Mainz, Angeborene Immunität gegen Infektionen: von Detektion zu Resolution

DGfI Autumn School of Immunology Merseburg, 09.10.2017, Merseburg, Ontogeny, differentiation and activation states of macrophages

Publications during funding period

none

A70 - Progress Report

01.07.2016 - 31.12.2018

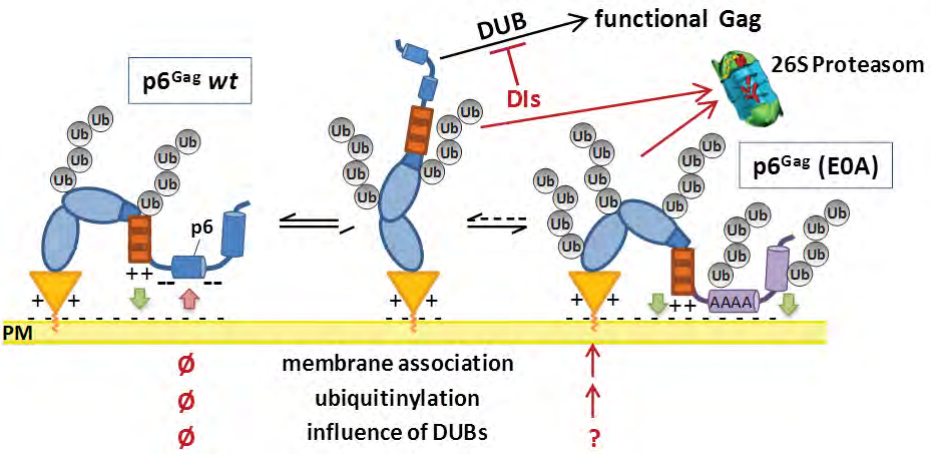
Novel targets for antiretroviral therapy – deubiquitinating enzymes regulate HIV-1 replication

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

We found that certain deubiquitinating enzymes (DUBs) play an essential role in HIV-1 replication. In addition, we have been investigating the role of three HIV-1 proteins, Vpr, Vpu and p6, in the interaction with the ubiquitin proteasome system (UPS). We found that Vpr is involved in HIV-associated fat metabolism diseases and Vpu directs the polyubiquitination of certain host cell-receptors. p6 regulates the polyubiquitination of Gag and is degraded by the insulin-degrading enzyme (IDE)

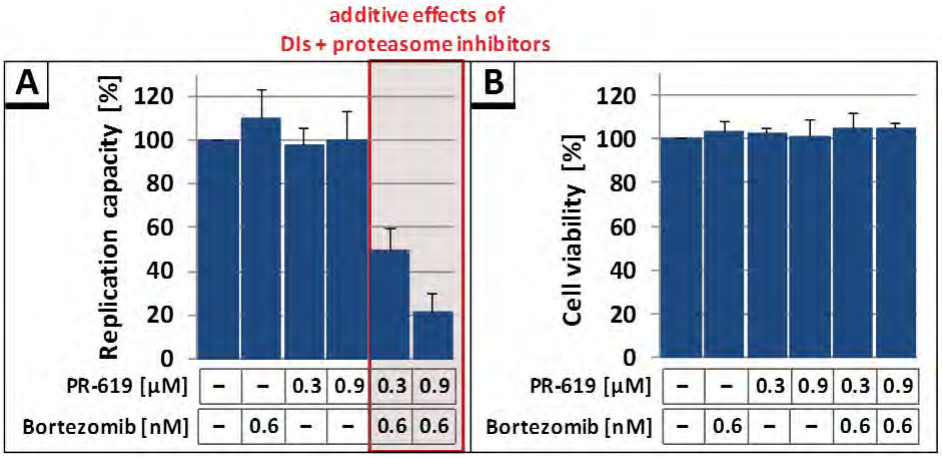
To further unravel the role of the UPS in HIV-1 replication we have been investigating the interaction of small HIV-1 proteins with the UPS.

Currently, it is not clear of whether the ion channel activity of the regulatory HIV-1 Vpu protein is involved in the degradation of host cell receptors, like tetherin or CD4. However, we were able to demonstrate that this activity is conserved throughout the evolution of HIV-1 and its ancestor SIVcpz. In case of the HIV-1 regulatory protein Vpr we could show, together with the group of Dr. Balasubramanyam, Houston, USA, that Vpr, by interacting with the cellular DBB1, DCAF and Cul4A-ligase complex plays an important role in HIV-associated fatty liver diseases. We will now investigate the role of certain DUBs in these processes. The 52 aa HIV-1 p6 Gag protein regulates virus budding, a process that somehow involves the polyubiquitination of Gag. Now we demonstrated 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 found that the p6 represents the first known viral substrate of the ubiquitously expressed cytosolic metalloendopeptidase IDE. Thereby, p6 is approximately 100-fold more efficiently degraded by IDE than its eponymous substrate insulin. This phenomenon could be considered as one explanation for the significantly higher risk for type II diabetes in



Hypothetical model: the role of DUBs in the p6 mediated polyubiquitination of HIV-1 Gag. (E0A=p6 mutant lacking all negative charged amino acids)

Prof. Dr. Schubert



Synergistic inhibition of HIV-1 replication by combinatory treatment of lymphatic tissue with DIs (PR-619) and proteasome inhibitors (Bortezomib).

HIV-1 carriers. Moreover, this proteolysis regulates virus replication in an Env-dependent manner. Until now it is unclear if certain DUBs are involved in the IDE-mediated degradation of p6, a topic which we will further investigate.

For the first time we were able to demonstrate that DUBs are involved in HIV-1 replication by regulating the Gag processing and thus virus infectivity. As only certain DUB-inhibitors (DIs), which specifically inhibit USP47, have anti-retroviral activity, we hypothesized that this DUB candidate plays 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. Furthermore, we could show that the HIV-1 replication during permanent (up to 15 days post infection, p.i.) and even structured (only day 1 and 3 p.i.) treatment of lymphoid tissue with USP47-specific DIs, resulting in a complete block of virus replication. Thereby, virtually no toxicity was detected even at the highest concentrations of the DIs. Most strikingly, combinatory treatment of DIs and proteasome inhibitors in a concentration range, where the inhibitors alone had no influence on virus replication, revealed a synergistic antiretroviral activity of inhibitors that act on both components of the UPS, proteasomes and DUBs.

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Publications during funding period

Agarwal N, Iyer D, Gabbi C, Saha P, Patel SG, Mo Q, Chang B, Goswami B, Schubert U, Kopp JB, Lewis DE, Balasubramanyam A (2017) HIV-1 viral protein R (Vpr) induces fatty liver in mice via LXRα and PPARα dysregulation: implications for HIV-specific pathogenesis of NAFLD. Nature Scientific Reports 17;7(1): 13362

Setz C, Friedrich M, Rauch P, Fraedrich K, Matthaei A, Traxdorf M, Schubert U (2017) Inhibitors of Deubiquitinating Enzymes Block HIV-1 Replication and Augment the Presentation of Gag-Derived MHC-I Epitopes. Viruses 9(8): 222

Hahn F, Schmalen A, Setz C, Friedrich M, Schlößer S, Kölle J, Spranger R, Rauch P, Fraedrich K, Reif T, Karius-Fischer J, Balasubramanyam A, Henklein P, Fossen T, Schubert U (2017) Proteolysis of mature HIV-1 p6 Gag protein by the insulin-degrading enzyme (IDE) regulates virus replication in an Env-dependent manner. PLOS ONE 12(4): e0174254

A71 - Progress Report

01.07.2016 - 31.12.2018

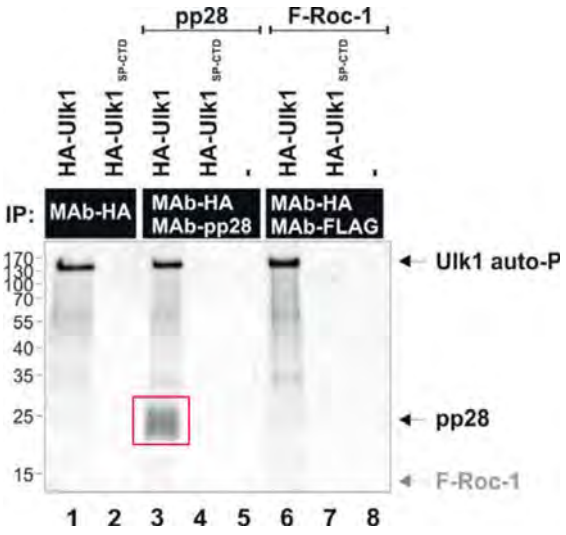
Viral modulation of the protein kinase ULK1

Prof. Dr. Thomas Stamminger, Institute of Clinical and Molecular Virology (till 31.12.2017),
Institute of Virology, Ulm University Medical Center (from 01.01.2018)

The cellular protein kinase Ulk1 is a critical regulatory factor at the intersection of autophagy and innate immunity. We observed that Ulk1 is strongly upregulated after infection with human cytomegalovirus and could show that this upregulation is important for viral replication. Consequently, inhibitors of Ulk1 activity interfered with viral replication revealing a novel antiviral principle that may also dampen hyperinflammation.

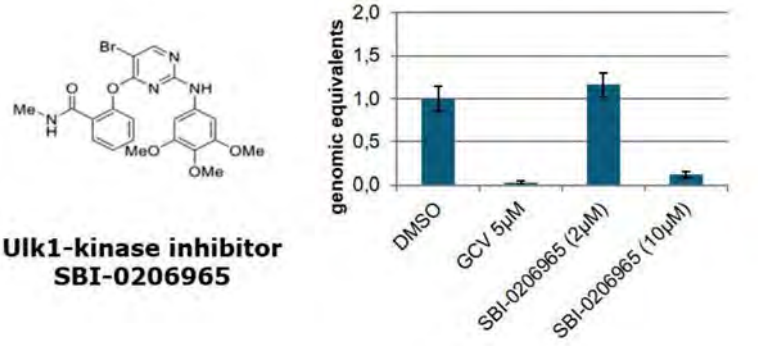
Role of Ulk1 for the replication of human cytomegalovirus

Research of the last years identified the serine/threonine kinase Ulk1 as an important initiator protein of autophagy. Autophagy is a highly conserved catabolic process which results in the lysosomal degradation of cytoplasmic material and may contribute to the elimination of pathogens. We observed that HCMV infection of primary human fibroblasts results in a strong upregulation of Ulk1 at late times of the replicative cycle. This correlates with a hyperphosphorylation of Ulk1 at serine 317, 556, 638 and 758. Since Ulk1 is phosphorylated via AMPK and mTOR we performed infection experiments in the presence of the inhibitors compound C (specific for AMPK) and mTorin1 or rapamycin (specific for mTOR). The substances were added late after infection in order to interfere with the HCMV-induced upregulation of Ulk1. The most important result of these experiments was the observation that compound C was able to abrogate the HCMV-mediated induction of Ulk1. In order to characterize the functional consequences of inhibited Ulk1 upregulation, we analyzed infection kinetics in order to quantify the accumulation of viral proteins and the release of viral particles. Interestingly, while no effect of compound C on the expression of viral proteins was detectable, we observed a significantly reduced release of viral particles. This was a first indication that Ulk1 may affect the steps of viral particle assembly and/or the release. In order to search for viral target molecules we performed in vitro kinase analyses using various viral proteins as substrates. This revealed a strong phosphorylation of the viral tegument protein pp28 by Ulk1. This is important, since pp28 is known to be essential for the secondary envelopment of viral particles. In order to further corroborate this, two-dimensional gel electrophoresis experiments were performed revealing that pp28 is phosphorylated in a Ulk1-dependent manner. Furthermore, we observed an accumulation of Ulk1 in the cytoplasmic virion assembly compartment. Taken together, our results strongly suggest that HCMV utilizes Ulk1 and initial steps of autophagy for an efficient morphogenesis of viral particles.



In vitro kinase assays reveal a strong phosphorylation of the viral tegument protein pp28 by Ulk1

Prof. Dr. Stamminger



Inhibition of viral replication by the Ulk1 kinase inhibitor SBI-0206965

The protein kinase Ulk1 as a novel target for antiviral strategies

So far, we have shown that an interference with Ulk1 phosphorylation via an inhibition of AMPK activity negatively affects the release of viral particles. Thus, we were interested to investigate whether a direct inhibition of Ulk1 kinase activity also acts antiviral. Consequently, several substances with suspected or described Ulk1 inhibitory activity were tested by in vitro kinase assays. This revealed a dose-dependent effect of the substances SU11274, SU6656, MRT68921 and SBI-0206965 on Ulk1 autophosphorylation as well as on the phosphorylation of pp28. In particular, SBI-0206965 also inhibited the release of viral particles and may therefore be suitable for antiviral approaches.

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Invited lectures

Mini-Herpesvirus-Workshop, September 22, 2017, Berlin, The human CMV IE1 protein: an offender of PML nuclear bodies

42nd Annual International Herpesvirus Workshop, July 29 - August 02, 2017, Ghent, Belgium, Inhibitors of the autophagy-initiating protein kinase Ulk1 interfere with HCMV replication

IUMS Conference Singapore, July 17 - 21, 2017, Singapore, The HCMV IE1 protein antagonizes PML nuclear body mediated intrinsic immunity via inhibition of PML de novo SUMOylation

Medical Faculty, Goethe-Universität Frankfurt, April 19, 2017, Frankfurt, Das humane Cytomegalovirus – von molekularen Mechanismen zu neuen Therapien

Annual Meeting of the Society for Virology 2017, March 22 - 25, 2017, Marburg, Innate antiviral defense by PML nuclear bodies

Publications during funding period

none

A72 - Progress Report

01.07.2016 - 31.12.2018

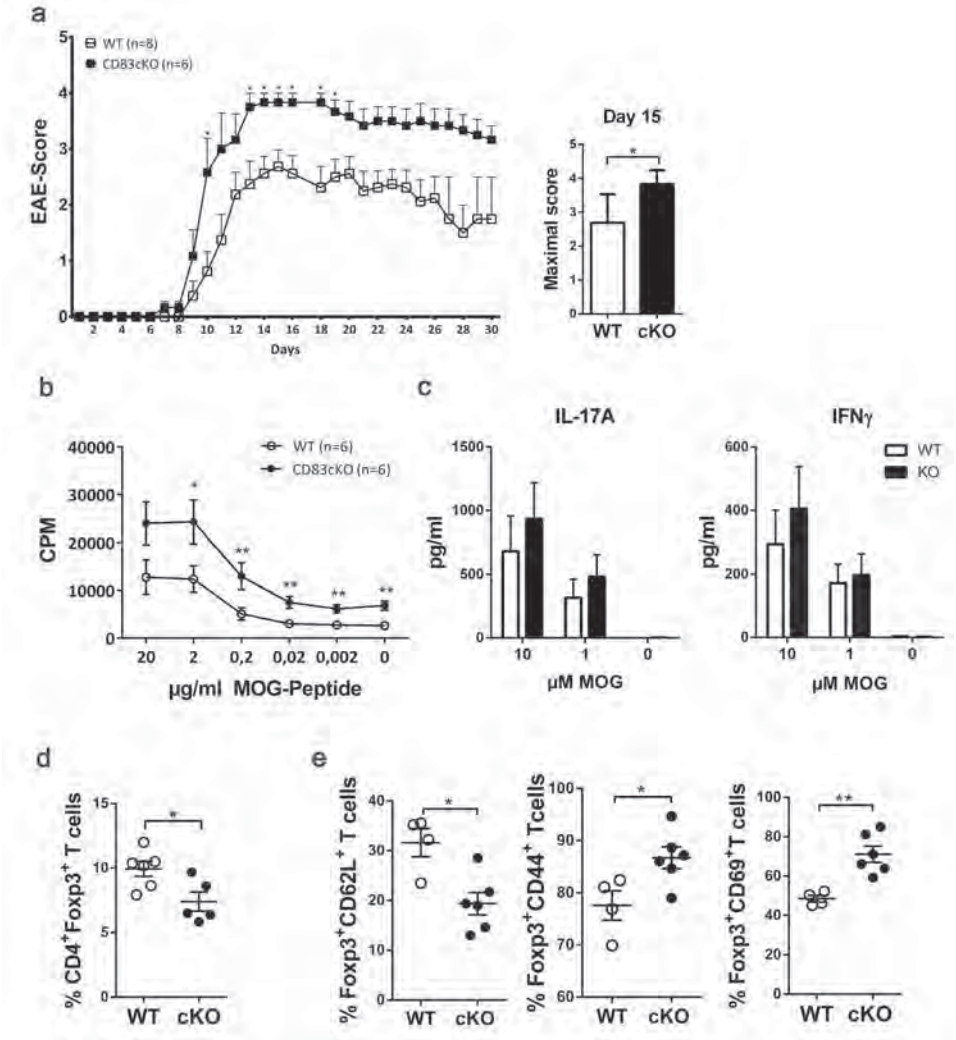
Targeted modulation of regulatory T cells and analyses of the underlying mechanisms

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. Previously we showed that activated murine as well as of human Tregs express the cell surface molecule CD83, indicating that this molecule is functionally important. 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.

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. To further analyze these in vivo findings, we raised the question whether cKO Tregs can be equally expanded as WT Tregs upon activation in vitro. Thus, naïve CD4$^+$CD25$^+$CD62L$^+$ T cells were cultured in the presence of IL-2 and anti-CD3/CD28 expansion beads and after 10 days, cKO Tregs showed equal expansion rates as WT Tregs. However, on mRNA level we detected increased IFNγ levels in cKO Treg cells and a downregulation of GATA3 expression levels. Thus, we conclude that anti-CD3/CD28 and IL-2 stimulated cKO Tregs can be equally activated and expanded, however, cKO derived Tregs showed an altered pro-inflammatory cytokine pattern. To analyze if the suppressive capacity of cKO Tregs is impaired using an additional in vivo model, total CD4$^+$ T cells from WT or cKO mice were isolated and transferred into RAG1$^{-/-}$ mice. The first and very striking effects were a strongly increased mortality rate in RAG1$^{-/-}$ mice, and an increased weight loss. In addition an increased clinical severity score with higher inflammation was observed in cKO cell transferred animals compared to WT controls. Analysis of mesenteric lymph nodes showed a significant lower infiltration of Tregs, whereas pro-inflammatory cytokine production was significantly increased. In contrast, significantly reduced GATA3 expression levels were detected in cKO animals, while TGF-β levels were not affected. Additionally, flow cytometric analyses of mLNs revealed reduced KLRG1+ and CD103+ expression levels among the Foxp3+ T cell population in cKO cell transferred mice. This indicates a reduced number of terminal differentiated Tregs in the gastrointestinal tract of RAG1$^{-/-}$ mice after adoptive CD4$^+$ T cell transfer from CD83cKO mice. Thus, we show that CD83 expression by Tregs is involved in the control of late differentiation and stability of regulatory T cell homeostasis. Since we earlier proved that also in human Treg cells CD83 expression is rapidly and strongly enhanced upon activation, one could envisage that CD83 has similar functions in human Treg cell differentiation.

Prof. Dr. Steinkasserer



CD83cKO mice develop exacerbated EAE pathology (a). Restimulatied splenocytes from EAE mice (b). CBAs of supernatants from restimulated splenocytes (c). Analysis of splenic T cells (d). FACS analyses of naïve T cells, effector memory T cells (e).

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Invited lectures

Research Seminar at the "ForBIMed" Network", January 26, 2017, Regensburg, Characterization of immunological biomarkers in antiviral therapy

Research Seminar at the "Medical University of Innsbruck", April 07, 2017, Innsbruck, Modulation of pathogenic inflammatory responses in autoimmunity and transplantation

Research Seminar at "Genomatix", May 10, 2017, Munich, Transcriptional regulation of CD83 expression in regulatory T cells

Publications during funding period

none

A73 - Progress Report

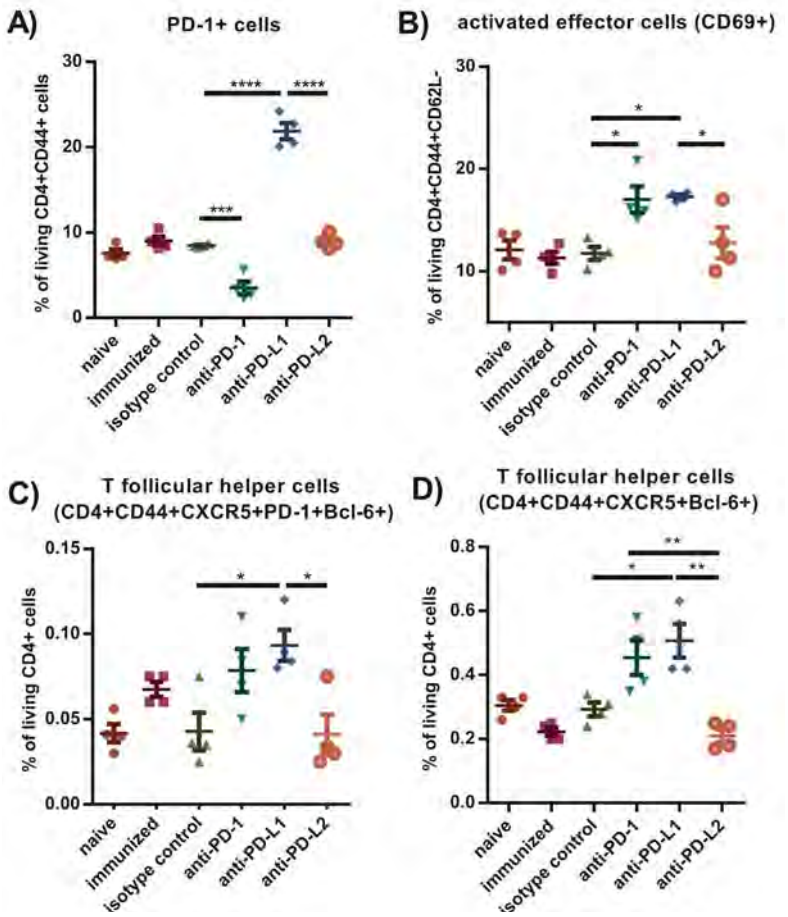
01.07.2016 - 31.03.2019

Checkpoint inhibitors as adjuvants for viral vaccines

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

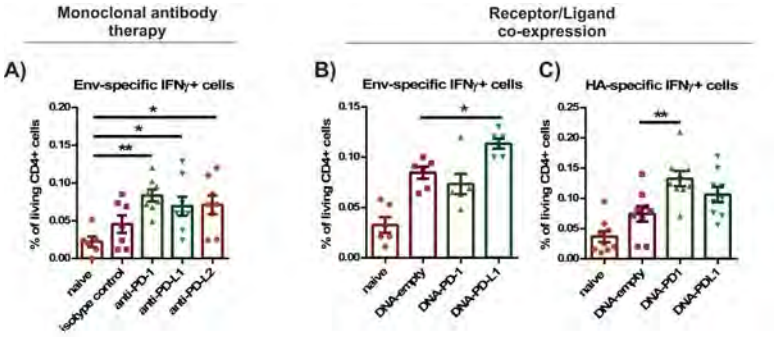
Checkpoint inhibitors (CPI) show great promise in improving immune control of cancer. How these antibodies affect the immunogenicity of vaccines is largely unknown. Using a mouse model, we observed that immune checkpoint inhibition by monoclonal antibody administration during genetic immunization against HIV-1 modulated frequencies of T cell subsets in the spleen. Additionally, the co-application of DNA encoding ectodomains of PD-1 and PD-L1 with DNA vaccines enhanced HIV-1 and Influenza A-specific CD4+ T cell responses.

Monoclonal antibodies targeting co-inhibitory molecules of the immune system show promising results in tumor therapy by counteracting immune-suppressive signals. We applied two different strategies to block immune checkpoints (monoclonal antibody treatment and co-expression of the PD-1 or PD-L1 ectodomains) in order to modulate antigen-specific T cell responses induced by DNA-based vaccines.



Influence of CPI antibody administration on T helper cells populations. Frequency of CD4+CD44+PD-1+ (A), CD4+CD44+CD62L-CD69+ (B), CD4+CD44+CXCR5+PD-1+Bcl-6+ (C) and CD4+CD44+CXCR5+Bcl-6+ (D) cells in the spleen of BALB/c mice two weeks after intramuscular DNA immunization against Env and Gag.

Prof. Dr. Überla



Influence of CPI on vaccine-induced T helper cell responses. Env- and HA-specific IFNγ-producing CD4+ T cells after immune checkpoint blockade by monoclonal antibody administration (left) or co-expression of the ectodomains of PD-1 and PD-L1 (right) two weeks after intramuscular DNA immunization as determined by intracellular cytokine staining.

Influence of systemic CPI treatment on T cell subpopulations and T cell responses

To explore the influence of anti-PD-1, -PD-L1, and -PD-L2 antibodies on T cell subpopulations, mice were immunized once with 30 µg HIV-Env and Gag-Pol expression plasmid by intramuscular electroporation. Starting two days after immunization mice received 200 µg of anti-PD-1, -PD-L1, and -PD-L2 antibodies or an isotype control in three day intervals by the intraperitoneal route. Two weeks after the immunization, changes in T helper cell subsets in the spleen were analyzed. Treatment with the anti-PD-L1 antibody enhanced the percentage of antigen-experienced CD4+ T cells expressing PD-1, while the anti-PD-L2 antibody had no effect. A decrease of antigen-experienced CD4+ T cells expressing PD-1 was observed after anti-PD-1 treatment which could be either due to depletion of PD-1 positive cells or blocking of their staining during the flow cytometric analysis. The fact, that both, anti-PD-1 and anti-PD-L1 treatment enhanced the percentage of activated CD4 effector cells argues for the latter. Interestingly, anti-PD-L1 also enhanced the percentage of T follicular helper (TfH) cells. To avoid ambiguities in PD-1 staining in the anti-PD-1 group, the percentage of T follicular helper cells was also determined without taking PD-1 expression into consideration. Again anti PD-L1 enhanced the percentage of TfH-like cells, while in the anti-PD-1 group a trend to an enhanced percentage of TfH-cells was observed.

Effect of checkpoint receptor/ligand co-administration on vaccine-induced CD4+ T cell responses

Since we observed competitive effects in the antibody responses to the vaccine antigens and the concomittantly administered CPI antibodies, immune checkpoints were also blocked by co-expression of soluble PD-1 or PD-L1 ectodomains. Blocking of PD-1 by co-administration of DNA encoding PD-L1 induced a significant upregulation of Env-specific IFN-producing CD4+ T cells in comparison to immunization with Env-DNA alone. As the HIV Env antigen induces an unusual IgG subtype response, we additionally used DNA vaccines encoding the influenza HA and NP antigens in this exploratory experiment. In contrast to HIV Env immunization, PD-1 DNA applied together with the anti-Influenza DNA vaccine significantly increased the percentage of HA-specific CD4+ T cells expressing IFN.

Together, these data indicate that blocking of immune checkpoints may improve vaccine-induced antigen-specific immune responses and their modulatory effect seems to depend on the nature of the respective antigen.

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Publications during funding period

none

A74 - Progress Report

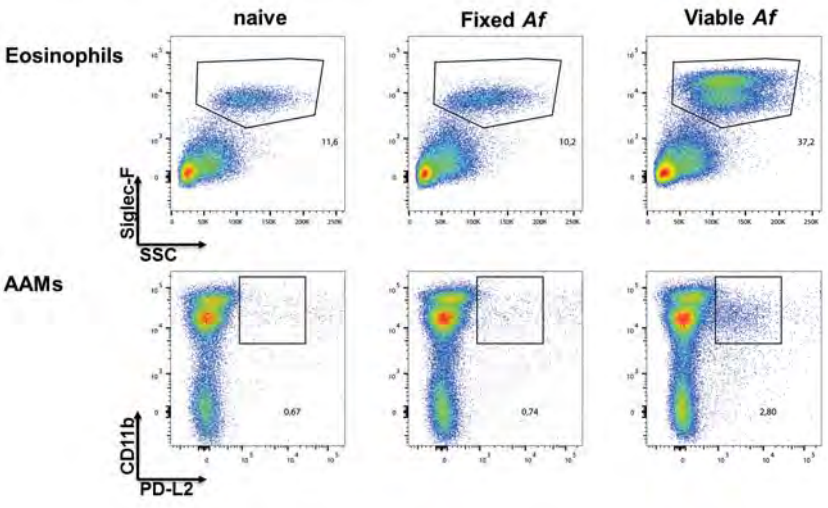
01.06.2016 - 30.11.2018

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

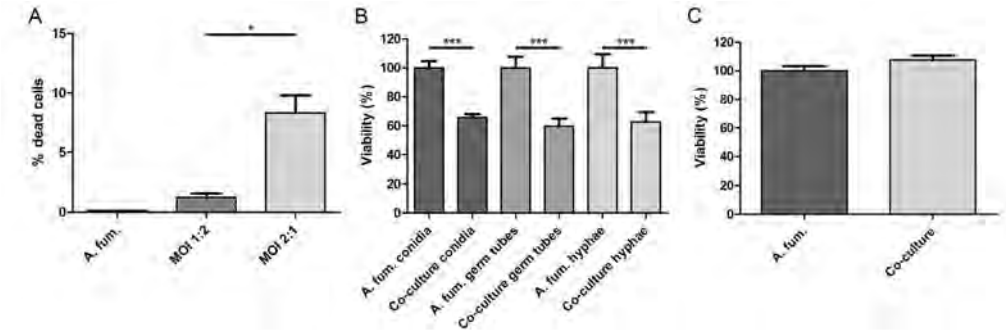
This interdisciplinary project targets the intimate interplay of eosinophilic granulocytes with cells and cellular components of the fungal pathogen Aspergillus fumigatus in the context of allergic pulmonary aspergillosis (ABPA) which mainly affects asthma and cystic fibrosis patients. The main research 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 shed light on the main host and fungal determinants triggering this complex allergic disease.

Using a mouse model with repetitive intranasal application of live or heat-inactivated *A. fumigatus* conidia, we could show that only live conidia elicited eosinophilia, expression of eosinophil-recruiting chemokines including eotaxins (CCL11 and CCL24), Th2 polarization, goblet cell hyperplasia and differentiation of M2 macrophages in the lung. This response was entirely dependent on T cell-derived IL-4/IL-13 and slightly reduced in basophil-deficient mice. In vitro co-culture experiments further revealed that live but not heat-inactivated conidia stimulated eosinophils to release chemokines and cytokines as measured by Multiplex Luminex assays. The most strongly induced factors included IL-4, IL-13, IL-18, IL-23, IL-28 and TNF, MIP 1a, MIP-1b and MCP-1. Since eosinophil activation was dependent on viable conidia, we consider it likely that eosinophils respond either to secreted substances or cell wall components that only become accessible after germination. Interestingly, signaling through Toll-like receptors or C-type Lectin receptors seems not to be required for *A. fumigatus*-induced eosinophil activation since bone marrow-derived eosinophils from Myd88- and Card9-deficient mice showed an unaltered cytokine response. Identification of eosinophil-stimulating substances will be one of the major goals for the next year. To achieve this goal, we will screen a comprehensive *A. fumigatus* transcription factor deletant library and in parallel analyze supernatants of fungal cultures after fractionation by size or charge for the eosinophil-stimulating activity.



Viable *A. fumigatus* induces lung eosinophilia and M2 polarization. Cell populations were assessed from lungs gathered from naïve or infected mice that had received either PFA-fixed or viable Aspergillus fumigatus (Af) conidia.

Prof. Dr. Vöhringer Prof. Dr. Krappmann



Monitoring the *A. fumigatus*/eosinophil interplay in vitro. Live-dead staining (A) and viability (B) of *A. fumigatus* in co-culture with eosinophils. (C) Fungal viability in the absence and presence of eosinophils physically separated.

We further analyzed *A. fumigatus*-induced allergic lung inflammation in eosinophil-deficient △dblGata mice. Unexpectedly, we found no obvious differences in various parameters of allergic inflammation (Th2 polarization, M2 differentiation or goblet cell hyperplasia) during the acute response (day 17) of the allergic lung inflammation. However, lower expression levels of IL-4 and the metalloproteases MMP-12 and MMP-13 were observed in eosinophil-deficient as compared to control mice. This correlated with more severe lung damage in eosinophil-deficient mice and suggests that eosinophils may contribute to tissue remodeling and repair. To get a better insight in eosinophil-regulated gene expression profiles of the lung during A. fumigatus-induced allergic lung inflammation and tissue repair we will now perform RNAseq analysis.

To investigate the cellular interaction of eosinophils with morphotypes of *A. fumigatus,* in vitro co-culturing experiments were established. The results of a live-dead-stain revealed that the reduction of fungal viability upon confrontation with murine eosinophils is the result of an actual killing mechanism and not caused by growth inhibition. As phagocytosis could be excluded as predominant killing mechanism, the antifungal effect is most likely induced by oxidative mechanisms. Since no differences in fungal killing rate could be observed after co-culturing eosinophils with *A. fumigatus* conidia, germ tubes, or hyphae, the reduction of viability appears to be independent from the fungal morphotype. Furthermore, the results of transwell experiments imply that direct contact between both cell types is necessary to induce the antifungal effect exerted by eosinophils. Preliminary results deduced from various defined A. fumigatus mutant strains indicate that cell wall carbohydrates play an important role for the extent of the reduction of fungal viability that is induced by eosinophilic granulocytes.

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Publications during funding period

none

A75 - Progress Report

01.07.2016 - 31.12.2018

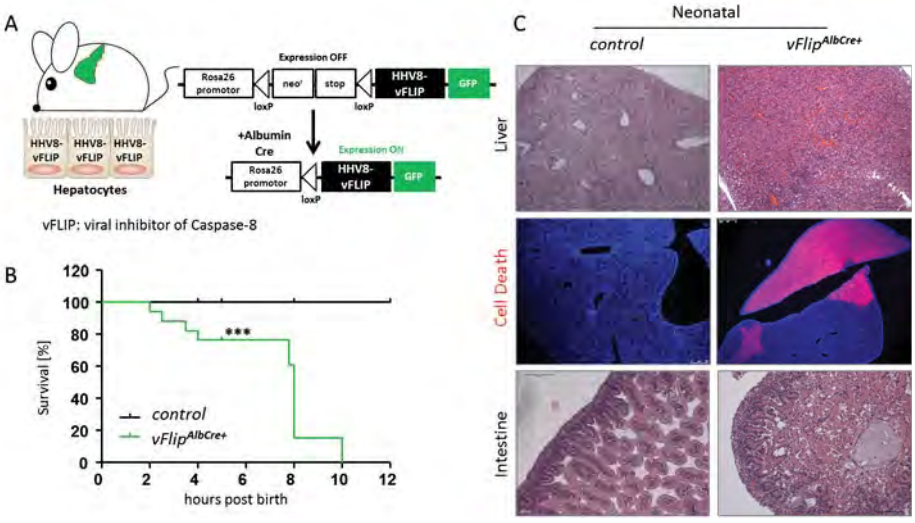
Role of MLKL-dependent programmed necrotic cell death in the pathogenesis of hepatitis

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

In this project, we aim to analyze the role of the pseudokinase MLKL in liver diseases. We now found that MLKL is upregulated in hepatitis C patients. In order to investigate the contribution of MLKL to viral induced hepatitis, we established a new mouse model that is characterized by acute, cell death mediated liver dysfunction. Accordingly, we identified that transgenic expression of vFLIP, a viral Caspase-8 inhibitor causes severe liver injury that finally culminates in multiple organ insufficiency and death of the mice.

Hepatocellular death plays a fundamental role in almost all hepatic diseases and thus, detailed knowledge about molecular mechanisms that mediate cell death responses in the liver is essential to advance therapeutic strategies. Previous concepts on cell death mechanisms have been recently challenged by the description of necroptosis, a novel form of programmed cell death mediated by the activation of RIP-kinases. The contribution of necroptosis to inflammatory liver diseases is controversial and particularly the role of mixed lineage kinase domain-like protein (MLKL), a recently identified key mediator of necroptosis, is largely unknown. The overall goal of this proposal is to identify how cell death is regulated during inflammatory liver injury. Based on our preliminary observations, we particularly want to elucidate the role of MLKL-dependent programmed necrosis in the pathogenesis of hepatitis characterized by hepatocellular necrosis. We anticipate our finding to be a starting point for the identification of novel biomarkers and the development of therapeutic strategies targeting regulated necrosis in hepatic diseases.

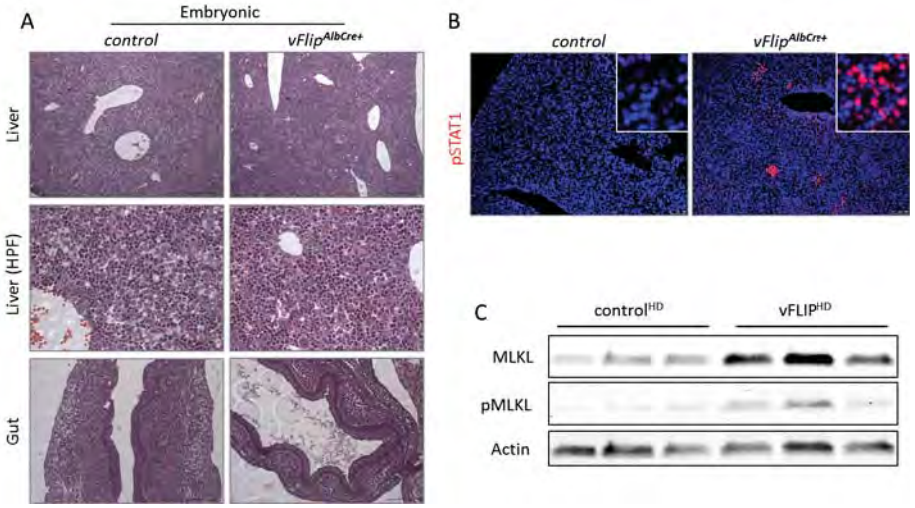
To evaluate the contribution of MLKL to hepatocellular necrosis-induced liver dysfunction we develop a new mouse model that is characterized by acute, cell death mediated liver failure. Interestingly, many pathogens such as bacteria or viruses express molecules that can directly interfere with the host cell death machinery and inhibit host cell death responses. Human Herpesvirus 8 (HHV8), also known as Kaposi sarcoma associated Herpesvirus expresses one of such molecules. This protein shares structural similarities



Transgenic expression of vFLIP induces massive hepatocellular death (A)Model of vFLIP expression in hepatocytes. (B) Kaplan–Meyer survival analysis. (C) Representative images of liver sections stained by H&E or TUNEL assay (red, cell death) and H&E stained small intestine derived from control or vFlip$^{AlbCre+}$ mice 8 hours post birth.

PD Dr. Dr. Günther

PD Dr. Dr. Wirtz



vFLIP triggered cell death is associated with strong upregulation of MLKL (A)Representative pictures of H&E stained embryonic liver and gut cross sections from control and vFlip$^{AlbCre+}$ mice. (B) Representative pSTAT1 (red) staining in neonatal liver tissue. (C) Detection of MLKL and pMLKL protein in tissue lysates. Actin was used as loading control.

with a cellular Caspase-8 inhibitor, the short isoform of cFLIP. Previous studies suggest that vFLIP negatively regulates Caspase-8 activity and thus exerts anti-apoptotic functions, which could further trigger regulated necrosis. Therefore we hypothesized that expression of vFLIP in hepatocytes might induce hepatocellular necrosis and thus represents a novel mouse model to study the molecular regulation of MLKL-dependent programmed necrotic cell death in the pathogenesis of hepatitis. To generate mice expressing vFLIP from HHV8 in hepatocytes, Rosa26. vFLIP mice were crossed to mice which express Cre Recombinase under the control of the hepatocyte-specific Albumin promoter. Transgenic expression of vFLIP causes severe liver tissue injury that finally culminates in multiple organ insufficiency and death of the mice. Accordingly, perinatal lethality of vFlip$^{AlbCre+}$ was observed between 2 and 8 hours post birth. Expression of vFLIP in hepatocytes primarily promotes excessive cell death in the liver but also affects the gut-liver axis and contributes to intestinal damage by yet unknown mechanisms. Notably, we uncovered that embryonic development and tissue homeostasis is unaffected by vFLIP expression, highlighting the importance of controlled host cell death in post-embryonic life, when the body is exposed to a variety of external factors such as microbes or food-derived antigens. Interestingly, vFLIP induced tissue injury was associated with activation of STAT1 and subsequent induction of MLKL gene and protein expression, suggesting that vFLIP induced hepatocellular necrosis might be mediated by MLKL.

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Invited lectures

275th Anniversary Symposium of the Friedrich-Alexander-Universität Erlangen-Nürnberg, January 2018, Erlangen, Induction of Cell death in tumors

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

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 66: 716–723

*These authors share first authorship

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 adipocytes in the melanoma bone metastatic niche using a high-fat diet (HFD) fed mouse model. During the second year of our funding, we have shown that serum from HFD fed mice does not promote growth of the B16F0 cell line in vitro, Furthermore, we showed that HFD is associated with an increase of pro-inflammatory immune cells in the bone marrow metastatic niche which was found to be correlated with a decreased B16F0 tumor load.

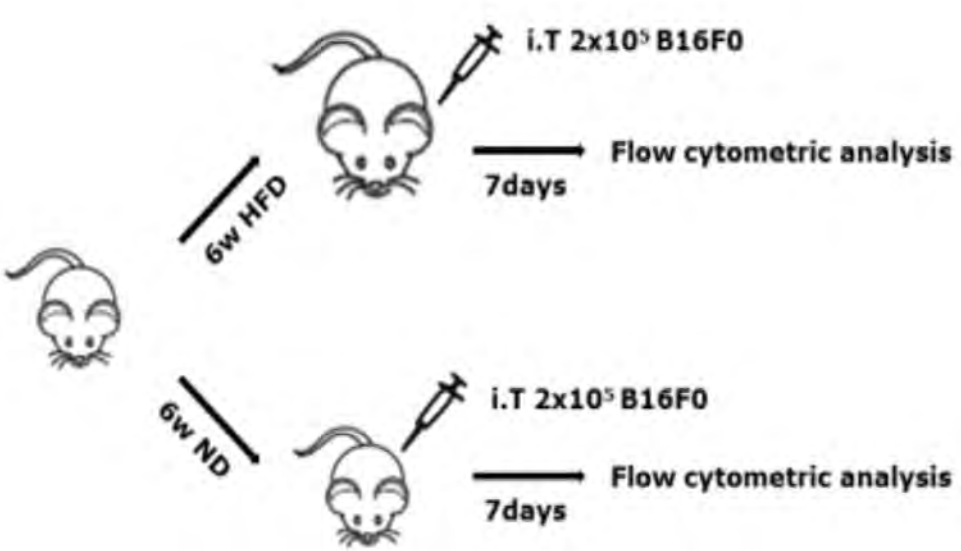
In vitro:

High-fat diet fed derived mouse serum does not affect the proliferation and migration rates of B16F0 in-vitro, as opposed to B16F10 (Chen G et al 2016). We analysed the growth and migration patterns of B16F0 in-vitro when grown in the presence of serum derived from mice fed either a normal or a high-fat diet. Surprisingly we found that addition of either had no impact on the proliferation or migration rate of B16F0 as measured by %confluency and relative wound density respectively.

In vivo:

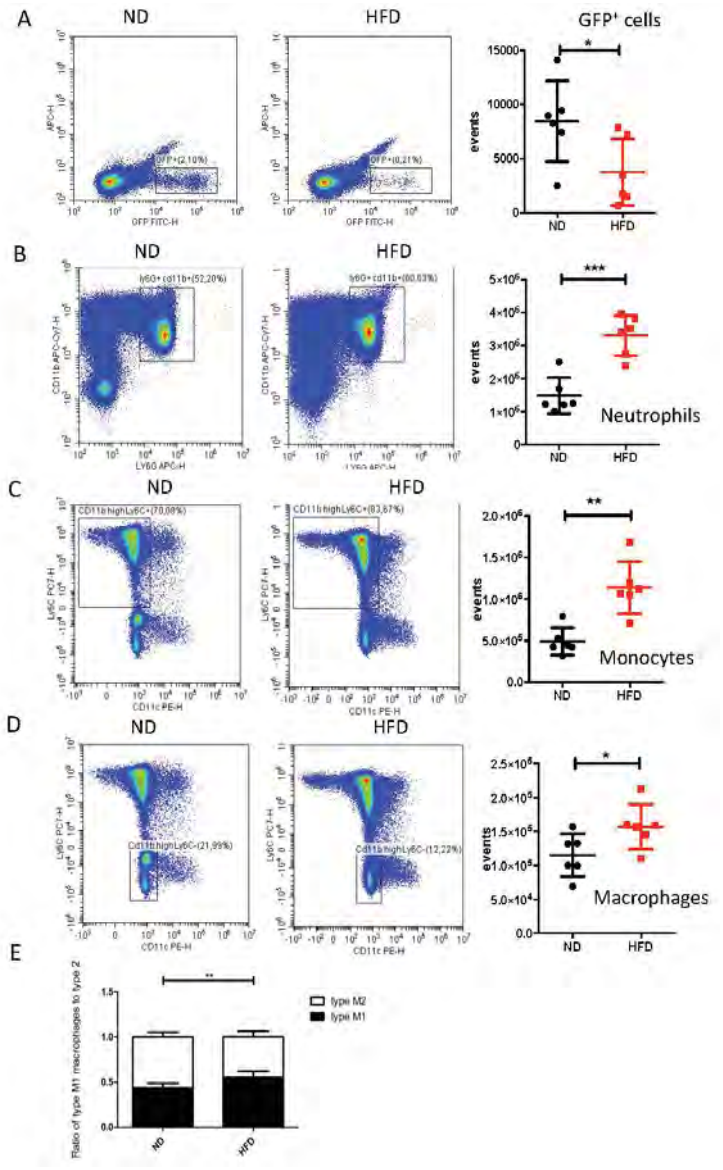
B16F0 cells stably expressing GFP are readily identifiable in the bone marrow: we generated a stable GFP expressing B16F0 cell line, allowing us to perform a quantitative analysis of the number of viable B16F0 cells in vivo in the bone tumor niche.

High-fat diet leads to a decreased tumor load: Flow cytometric analyses show that high-fat diet fed mice have a decreased number of viable B16F0 cells in the bone marrow compared to normal diet fed mice 1 week post tumor cell injection.



A. Schematic of the experimental layout for intra-tibial injection of B16F0GFP cells in mice previously exposed to 6-7 weeks of normal diet (ND) or high fat diet (HFD). B-E. Dot plot and quantification of absolute number of B16F0-GFP+ cells (B), neutrophils (C), monocytes (D) and macrophages (E) in the tibia of tumor cell injected ND and HFD mice.

Prof. Dr. Bozec



A. Dot plot pictures representative of the Flow cytometry gating strategy. B. Dot plot pictures and quantification of the ration of pro inflammatory like (M1) to anti-inflammatory like (M2) cells in the tibia of tumor cell injected ND and HFD mice.

High-fat diet is associated with an increasingly pro-inflammatory bone tumor environment: Flow cytometric analyses of the immune cell fractions in the bone marrow tumor niche show that high-fat died fed mice have a significant increase of neutrophils, monocytes and macrophages. Furthermore, the analysis of the macrophage subpopulations reveals an increased proinflammatory macrophage polarization in the high-fat diet modulated metastatic niche.

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Invited lectures

IZKF summer symposium 2017, Erlangen, Talk: Role of adipocyte within the bone marrow metastatic niche

Kongress der Deutschen Gesellschaft für Innere Medizin 2017, April/May 2017, Mannheim, Talk: Microbiota from obese mice regulate hematopoietic stem cell differentiation by altering the bone niche. Cell Metabolism

Publications during funding period

Hannemann N, Jordan J, Paul S, Reid S, Baenkler HW, Sonnewald S, Bäuerle T, Vera J, Schett G, Bozec A. (2017) The AP-1 Transcription Factor c-Jun Promotes Arthritis by Regulating Cyclooxygenase-2 and Arginase-1 Expression in Macrophages. J Immunol. 15: pii: 1601330

Chen G, Luo Y., Eriksson D, Meng X, Qian C, Bäuerle T., Chen 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

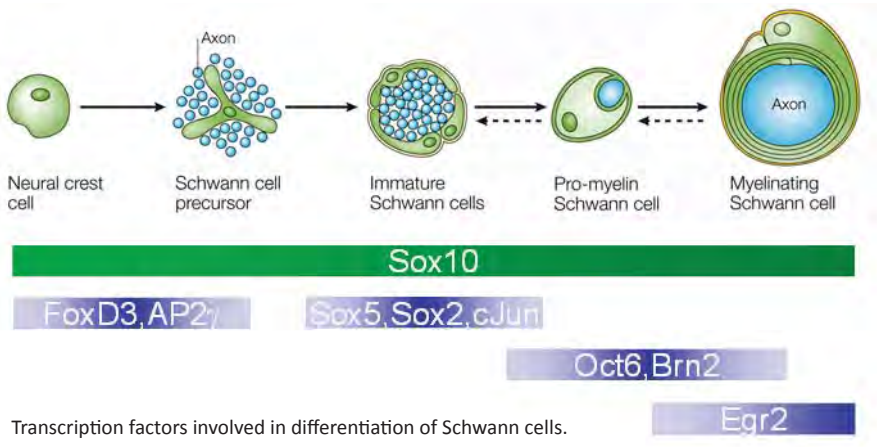
Melanoma is an aggressively disseminating cancer with continuously rising incidence. Melanoma cells derive from melanocytes, which originate from the neural crest and display characteristics of cells of the nervous system. Interestingly, Schwann cells, nervous system cells derived from the neural crest, 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 was successful.

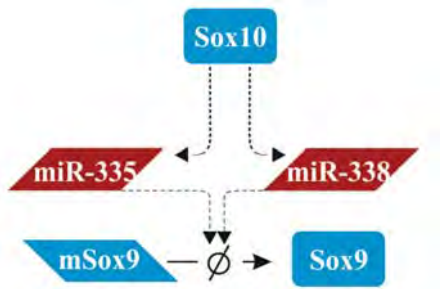
We are now focussing in this collaborative project on the two transcription factors most strongly deregulated and analyse their specific impact on melanoma in detail. We already determined their functional importance in aspects like tumour cell proliferation and metastasis in vitro. An important task will be the definition of target genes of these transcription factors in both melanoma and Schwann cell lines. Here, we are following up on the idea of an impact on myelination of these factors. 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 and the melanoma cell line Mel Im. The resulting transcription factor-deficient cell lines will be compared in their expression



Transcription factors involved in differentiation of Schwann cells.

Prof. Dr. Bosserhoff Prof. Dr. Wegner

profile to the original ones and between melanoma and Schwann cell line. In case of the Sox10-deficient 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. We were, for instance, able to show that CRISPR/Cas9-mediated loss of Sox10 decreases expression of miR335 and mir338. Both microRNAs are direct downstream targets of Sox10 and affect - among others - expression of Sox9. This regulatory loop is likely responsible for coupling and inversely correlating expression levels of Sox9 to those of Sox10. This regulatory circuit may be relevant to melanoma as Sox10 facilitates initiation and progression of melanoma, whereas Sox9 actively inhibits it.



Regulatory circuit by which Sox10 represses Sox9 expression posttranscriptionally.

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 are therefore comparing 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

Hinterzartener Kreis Meeting, March 23, 2017, Cadenabbia, Italy, Aging and Cancer (AB)

Hallmarks of Skin Cancer, November 7, 2017, Heidelberg, New players in melanoma progression (AB)

Current Topics in Myelin Research, February 20-22, 2017, Kassel, Transcriptional Control In Schwann Cells: From Factors To Networks (MW)

ISN-Satelliten-Symposium, August 25-29, 2017, Embiez, France, Transcriptional Control In Schwann Cells & Oligodendrocytes: From Factors To Networks (MW)

Publications during funding period

none

D25 - Progress Report

01.05.2016 - 31.10.2018

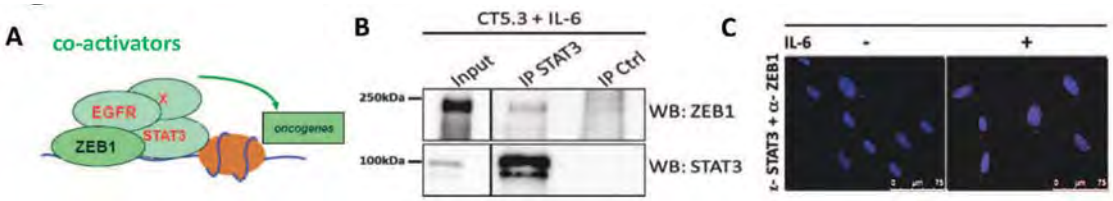
Interaction of the EGFR- and the ZEB1-pathway in aggressive cancer types

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

The EMT-program provides cancer cells with motility, invasiveness and stem cell features. A major EMT inducer is the transcriptional 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 interaction partners identified by MassSpec and ChIP-Seq analyses. We further investigate their relevance for Zeb1 function and cancer progression.

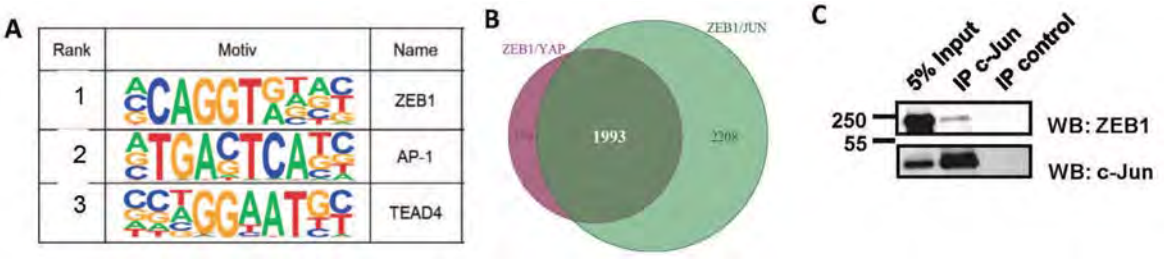
We demonstrated that under certain conditions – e.g. in an oncogenic context of cancer cells – the oncogenic factor ZEB1 can switch from a transcriptional repressor of epithelial genes to a transcriptional activator of tumor promoting genes. We proposed novel nuclear interaction partners and previously exem-plified this by showing interaction with YAP1, a main effector of Hippo signalling, to activate a specific common target gene set. 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.

In the first year of funding, we confirm the nuclear interactions of EGFR and Stat 3 with Zeb1 by applying CoIPs and proximity ligation assays in tumor cells and IL6-activated fibroblasts. In parallel we started analyses assessing a functional cooperation to activate a common target genes set.



(A) Model: EGFR, STAT3 and others (x) act as ZEB1 co-activator of tumor promoting genes. Co-IP (B) and proximity ligation assay (C) of endogenous ZEB1 and STAT3 in nuclear lysates of IL-6 stimulated fibroblasts showing that STAT3 interacts with ZEB1 in the nucleus (red).

Prof. Dr. Brabletz



(A) Zeb1 ChIP-Seq peaks enrich for AP-1 (Jun/Fos) and Tead/Yap motives. (B) Strong overlap of peaks after ChIP-Seqs for Zeb1, Jun and Yap1. (C) CoIP of Zeb1 and Jun, indicating interaction.

Furthermore a second unbiased strategy was applied to identify functional cooperation partners of Zeb1 in promoting tumor progression. To this end we analysed Zeb1 ChIP-Seq-data sets generated in our lab allowing the identification candidate interaction partners on tumor cell enhancers and promoters. Motiv searches on Zeb1 ChIP Seq peaks revealed a strong overlap with binding sites for the transcription factor AP-1 (Jun/Fos) and Tead (confirming the interaction with the Tead partner YAP1). Direct comparison of ChIP Seq peaks for Zeb1, Jun and Yap1 revealed a strong overlap in target enhancers and promoters. The consequent hypothesis is that all three factors functionally interact to activate a common set of tumor promoting genes. First experiments applying CoIPs from cancer cell nuclear extracts support a direct interaction of Zeb1 and AP-1 components Jun and Fra1.

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Invited lectures

International Symposium Control of Cell Motility in Development and Cancer, March 23, 2017, Universitätsklinikum Freiburg, Cellular plasticity in cancer: driving force and therapeutic target

AACR Annual Meeting, April 4, 2017, Washington, USA, EMT, cell plasticity and metastasis

Mildred Scheel Cancer Conference, June 14, 2017, Bonn, Cellular plasticity in cancer: driving force and therapeutic target

Georg Speyer Haus (GSH) Retreat, June 20, 2017, Burg Rothenfels, Cellular plasticity in cancer: driving force and therapeutic target

The Francis Crick Institute, July 25, 2017, London, United Kingdom, Cellular plasticity in cancer: driving force and therapeutic target

GSH-Symposium “Dynamics of adult stem cells and cancer”, October 25, 2017, Frankfurt, Cellular plasticity in cancer: driving force and therapeutic target

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, October 29, 2017, Philadelphia, USA, Cellular plasticity in cancer: driving force and therapeutic target

AEK Autumn School Minimal Residual Disease, Circulating Tumor Cells and Metastasis, November 6, 2017, Berlin, Cellular plasticity in cancer: driving force and therapeutic target

EKFS Cancer Symposium, November 16, 2017, Göttingen, Cellular plasticity in cancer: driving force and therapeutic target

Pathologisches Institut, Universität Bern, November 23, 2017, Switzerland, 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 and HER2 breast cancer.

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 progesterone-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.

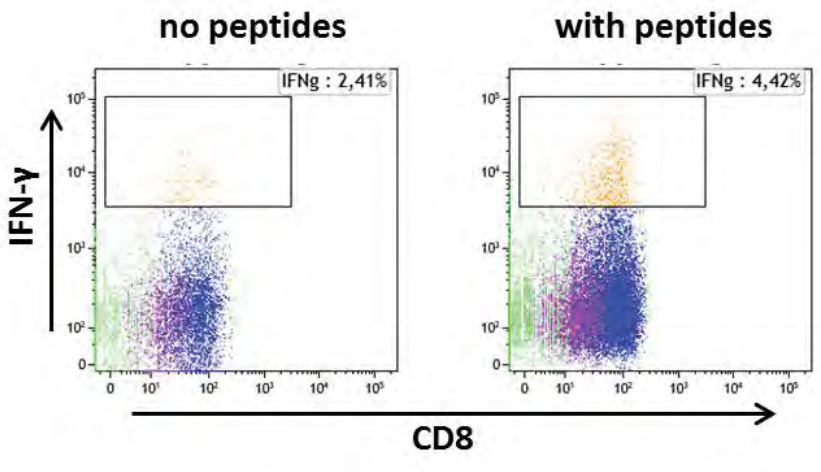
We therefore aimed to identify the targets of tumor-infiltrating T-cells in TNBC with special emphasis being placed on tumor-specific mutations.

Characterization of tumor-infiltrating immune cells

We so far collected and analyzed the infiltrating immune cells of 358 breast biopsies. 50 (14,0%) were derived from patients with initial diagnosis of TNBC, while 52 (14,5%) were Her2+ tumors, 193 (53,9%) hormone-receptor positive and 63 (17,6%) were derived from non-malignant lesions. Patients diagnosed with TNBC had an average age of 57,3 years as compared to 60,1y for Her2+ tumors and 62,1y for patients with hormone receptor positive tumors.

Expansion of tumor-infiltrating T-lymphocytes

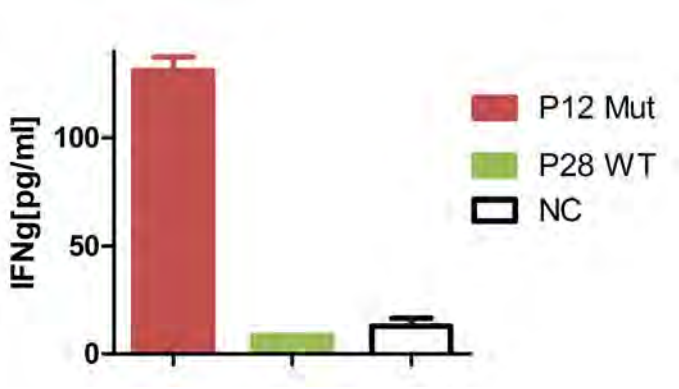
T-lymphocytes in biopsies derived from TNBC and Her2+ were expanded in vitro to average cell number of around 50-60 million T-lymphocytes within 2-3 weeks.



Flow cytometric analysis of T-cell reactivity after stimulation with the peptide mix.

Prof. Dr. Mackensen

Prof. Dr. Fasching



Reactivity of T-cell clone 3E1 against tumor specific mutant peptide (P28) and its wild-type (WT) counterpart loaded on autologous EBV-LCL as tested by INF-γ ELISA. NC: negative control

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.

Identification of neoantigen specific T-cell clones

We so far analyzed T-cell reactivities of two patients by co-culturing expanded TILs with peptide loaded autologous dendritic cells and sorting for activated T-cells. In one patient we could not find any peptide specific T-cells. In the other patient we found 4 peptide reactive T-cell clones. All four clones were CD4 T-cells reactive against the same tumor-specific peptide (P28). By testing reactivity against the wildtype counterpart we could show that these T-cell clones are truly tumor-specific. Currently, we are analyzing HLA-restriction of the T-cell clones and the ability of the identified neoantigen to be endogenously processed. Furthermore, we will go on with the analysis of additional patients.

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Publications during funding period

none

D27 - Progress Report

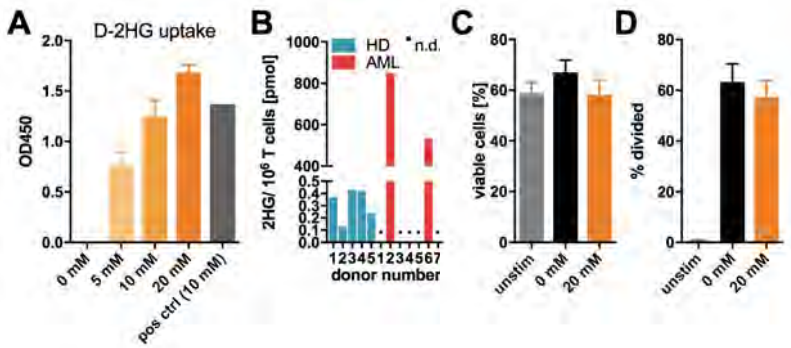
01.07.2016 - 31.12.2018

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

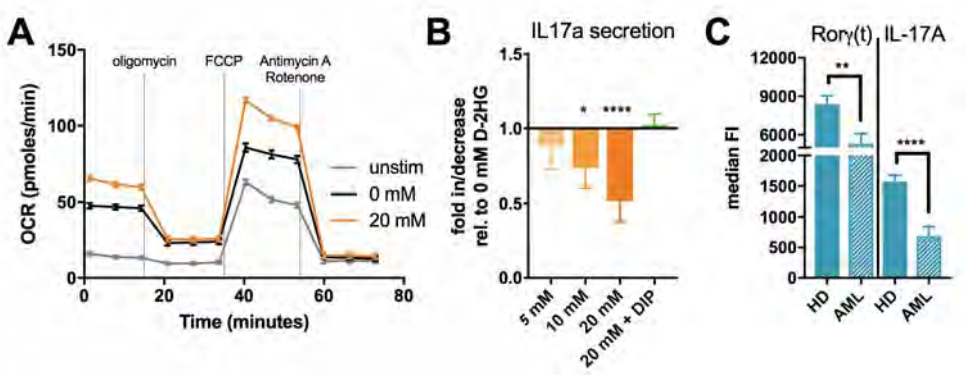
Increased 2-hydroxyglutarate (2-HG) levels are found in 15% of acute myeloid leukaemia (AML) patients. 2-HG overproduction is attributed to mutations in isocitrate dehydrogenase 1/2 (IDH1/2). Our data indicates a link between increased 2-HG levels and c-Myc pathway. AML patients display substantial immune 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 impact malignant potential of cancer cells by promoting amongst others invasiveness and chemo-resistance. 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. 2-HG is an oncometabolite produced and secreted by various tumor entities, including AML, harboring mutations in the isocitrate dehydrogenase genes. These monoallelic mutations result in a loss-of-function in terms of conversion of isocitrate to α-ketoglutarate (αKG) and a gain-of-function leading to the reduction of αKG to 2-hydroxyglutarate (2-HG) instead. Several studies so far have aimed to delineate autocrine effects of 2-HG on the tumor cells. There are reports about the competitive inhibition of αKG-dependent enzymes leading to profound epigenetic alterations about a link between 2-HG content and the cellular redox equilibrium, about the promotion of glutamine metabolism, as well as about interferences with mitochondrial respiration. However, while most studies are focusing on tumor-directed 2-HG effects, its microenvironmental impact and, in particular, its role in AML-mediated immune modulation remains largely unexplored. Therefore, we were interested whether 2-HG holds potential immune regulatory properties by interfering with T-cell function. In this study we found that exogenously applied, non-cell-permeable 2-HG was readily taken up by T-cells, which is underlined by our measurements showing up to 1000-fold enriched levels of 2-HG in T-cells from IDH2-mutated AML patient samples. While via-



2-HG uptake by T-cells. (A) 2-HG uptake by T-cells was measured by a colorimetric assay. (B) 2-HG levels in T-cells from healthy donors (HD) and AML patients (AML) quantified by LS/MS. T-cells were analyzed regarding the 2-HG impact (C) on viability and (D) proliferation.

Prof. Dr. Mougiakakos



Metabolic reprogramming by 2-HG. (A) Respiration (=OCR) was measured in T-cells cultured with/without 2-HG. (B) IL-17 secretion by T-cells was determined in presence of 2-HG with/without DIP stabilizing HIF-1α. (C) Rorα(t) and IL-17 were quantified by FACS in T-cells from healthy donors (HD) and patients (AML).

bility, proliferation and IFNγ production were mainly unaffected by 2-HG, bioenergetics of activated T-cells shifted away from aerobic glycolysis towards respiration. This is at least partly explained by the observed 2-HG-triggered hypoxia inducible factor-1α (HIF-1α) destabilization. In line with previous findings that HIF-1α-dependent (aerobic) glycolysis orchestrates differentiation of Th17 cells, we found a reduced Th17 polarization in presence of 2-HG.

Taken together, our data suggests that abundantly produced 2-HG by malignancies such as AML may act (in addition to its oncometabolic potential) as a novel immunometabolic modulator. The T-cells' bioenergetics are shifted towards oxidative phosphorylation and interference with the stability of HIF-1α diminishes the formation of pro-inflammatory Th17 cells. Self-evidently, more studies on the 2-HG's impact on the various components of the patient's immune system will be necessary in order to put our observation into the context of both anti-tumor immunity as well as protection from infections that are major causes of mortality and morbidity during disease progression.

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Invited lectures

DKZF Seminar, July 18, 2017, Heidelberg, Immune metabolic interplay in chronic lymphocytic leukemia

CCK Seminar, April 21, 2017, Karolinska Institute Stockholm, Sweden, From metabolic reprogramming to metabolic targeting

CCS/CLL conference, April 01, 2017, Salzburg, Austria, Metabolic alterations in CLL: from immune escape to metabolic targeting

DFG Hinterzartener Kreis, March 24, 2017, Cadenabbia, Italy, Metabolic alterations in CLL: from immune escape to metabolic targeting

Seminar University of Freiburg, February 09, 2017, Freiburg, Cancer metabolism: compete and rule

Publications during funding period

Bruns H, Böttcher M, Qorraj M, Fabri M, Jitschin S, Dindorf J, Busch L, Jitschin R, Mackensen A, Mougiakakos D (2017) CLL-cell-mediated MDSC induction by exosomal miR-155 transfer is disrupted by vitamin D. Leukemia 31: 985-988

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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

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 (TECs) in colorectal carcinoma (CRC) and identified SPARCL1 as an important regulatory molecule of this phenotype contributing to vessel homeostasis and vascular-derived inhibition of tumor growth. 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.

The specific aims of the project are

Aim 1: Structure-function analyses of SPARCL1 and isolation of its cellular receptor

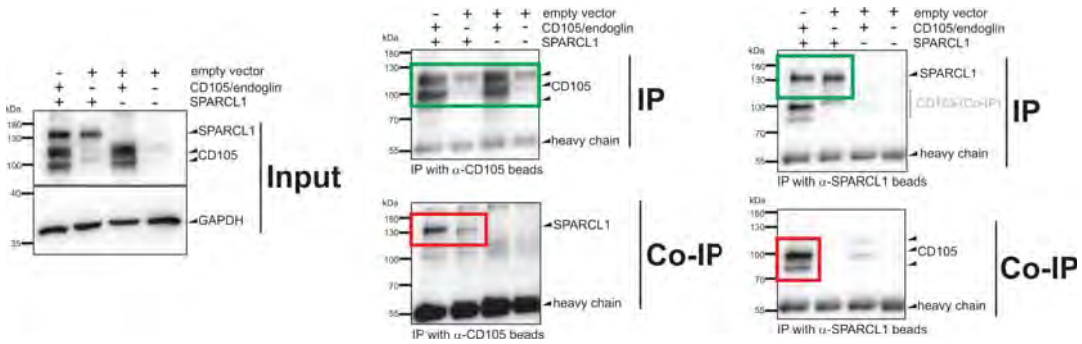
First, wild type recombinant human SPARCL1 has been successfully cloned and purified from supernatants of human eukaryotic cells. The protein showed similar anti-angiogenic activity as compared to commercially available recombinant SPARCL1. At present, subdomains of SPARCL1 are cloned and purified and will be used for a structure-function analysis of SPARCL1 anti-angiogenic activity. Secondly, CD105/endoglin was identified as a cellular receptor of SPARCL1 in Hela. At present, validation experiments in primary endothelial cells are ongoing.

Aim 2: Impact of SPARCL1 on the induction and resolution of angiogenesis

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. An in vivo inhibition of vessel sprouting by addition of rec. murine SPARCL1 to the bones was demonstrated by this assay. At present, metatarsals from wt and SPARCL1-ko mice are comparatively analyzed.

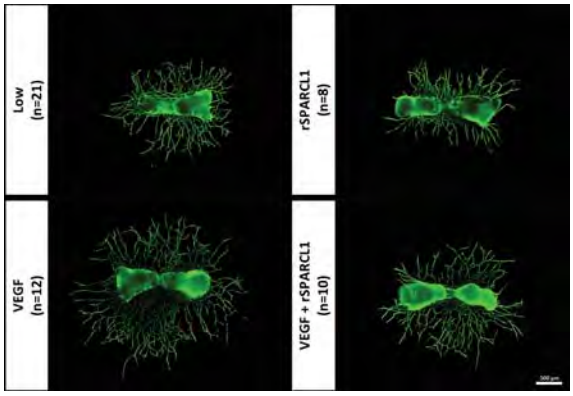
Aim 3: Impact of SPARCL1 on prognosis and therapy response of patients with CRC

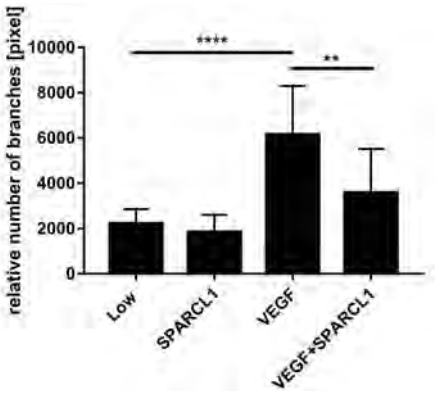
SPARCL1 expression was determined in FFPE-extracted RNA of CRC patient samples (n=614) recruited in the Polyprobe study. Implementation of the corresponding clinical data in adequate software tools (TranSMART, cooperation Christoph/Prokosch, MIK) in order to analyze potential clinical correlations was conducted. A reduced incidence of metastases in the long-term follow up of R0-resected CRC patients with high SPARCL1 expression was identified.



SPARCL1 binds to endoglin/CD105 in HeLa cell lysates after overexpression. Co-immunoprecipitation from Hela cells transfected with SPARCL1, endoglin/CD105 and/or control vector.

Prof. Dr. Stürzl PD Dr. Naschberger





Endothelial sprout outgrowth from metatarsal bones of wt SPARCL1 mice is inhibited by rec. mSPARCL1. Embryonic metatarsals from wt mice were cultivated in the presence or absence of VEGF and/or SPARCL1. Sprouts were visualized using CD31 staining and quantified by ImageJ.

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Invited lectures

5th iTARGET Workshop, March 2017, Erlangen, Tumor-microenvironment dependent imprinting of endothelial cells in human colorectal carcinoma (Elisabeth Naschberger)

Awards

Renate-Wittern-Sterzel-Preis, Gleichstellungspreis der Friedrich-Alexander-Universität, Prof. Dr. Stürzl, November 6, 2017, Erlangen

Publications during funding period

Naschberger E, Geißdörfer W, Bogdan C, Tripal P, Kremmer E, Stürzl M, Britzen-Laurent N (2017) Processing and secretion of guanylate binding protein-1 depend on inflammatory caspase activity. J Cell Mol Med. 21(9): 1954-1966

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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):209

Feiersinger F, Nolte E, Wach S, Rau TT, Vassos N, Geppert C, Konrad A, Merkel S, Taubert H, Stürzl M, Croner RS (2016) MiRNA-21 expression decreases from primary tumors to liver metastases in colorectal carcinoma. PLoS One 11: e0148580

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

The increasing incidence of colorectal cancer (CRC) during lifetime has been largely attributed to an accumulation of genomic damage in tumor cells. Recent data also propose a role for an aged immune system in CRC pathogenesis, as tumor infiltrating T cells with short telomeres have been associated with worse prognosis in CRC. Our project aims at the functional evaluation of aging related pathways such as telomere shortening and cellular senescence in immune cells during CRC development.

Aging is widely described as loss of cellular function that leads to diseases such as diabetes, cardiovascular disorders, neurodegenerative diseases and cancer. On the molecular level, an accumulation of genomic alterations such as DNA damage or telomere shortening results in the activation of protective pathways that induce programmed cell death or cellular senescence. Recent data propose that these mechanisms also affect immune function. For instance, the infiltration of tumor tissue with aged T cells with short telomeres has been associated with a worse prognosis. However, the underlying mechanisms have not been evaluated so far.

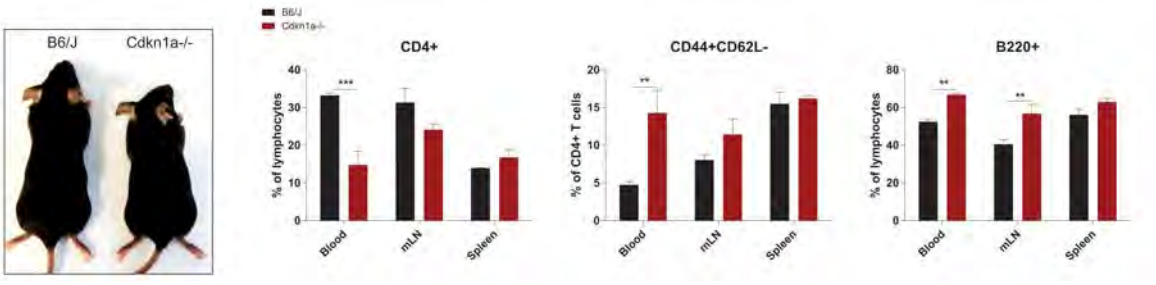
In this project, we evaluate the role of aging and senescence in immune cells in the anti-tumor immune response against CRC. Previously, we described our initial results on the role of aging in immune cells using mTERC-knockout mice. Here we present recent data on the role of cellular senescence by using mice with deletion of p21 (*Cdkn1a -/-* mice) in CRC models.

Effect of p21 deficiency on immune cells under steady state conditions

According to data from the literature, Cdkn1a -/- mice undergo normal development. However, due to the inability to arrest in G1 phase, mice develop spontaneous tumors at an old age. When we investigated the effect of a *Cdkn1a*-loss on the immune system, we observed that untreated Cdkn1a -/- mice have higher frequencies of B cells in blood and mesenteric lymph nodes compared to wildtype mice. Moreover, we noticed that *Cdkn1a*-loss affects CD4+ T cell abundance with an accumulation of effector memory T cells. How these results can be explained by the loss of Cdkn1a on the molecular level is currently under investigation.

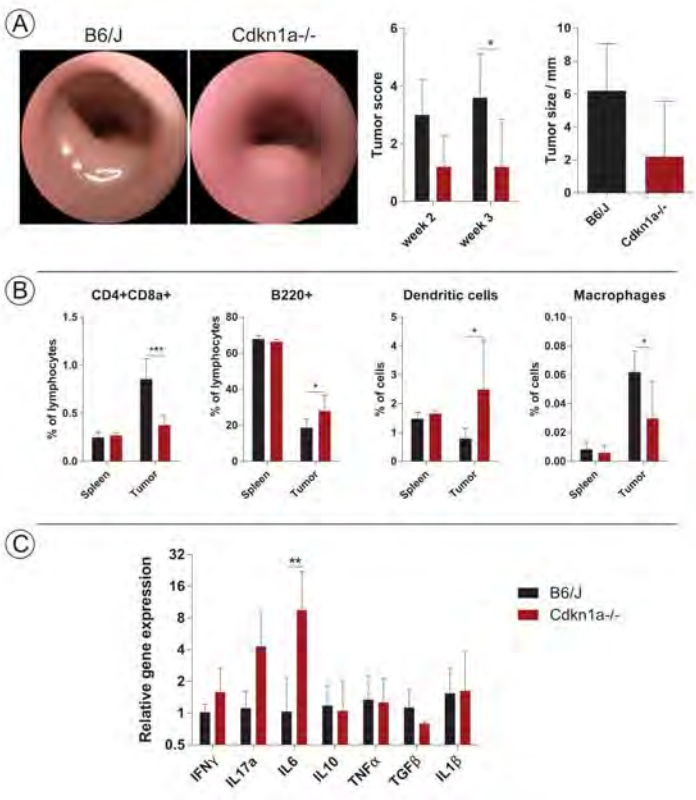
p21 deficiency in the tumor microenvironment protects against CRC growth

To further investigate the role of p21 in immune cells during CRC development, we exposed *Cdkn1a* -/- mice to an orthotopic CRC model using wildtype murine CRC cells (MC38). Upon endoscopic scoring,



Characterization of the immune system of Cdkn1a -/- mice under steady-state conditions: no obvious phenotype was observed during development. Flow cytometry data showing relevant immunological differences.

Prof. Dr. Waldner



Cdkn1a -/- mice in CRC model. (A) Endoscopic tumor assessments after orthotopic tumor injection. (B) Flow cytometry data of immune cells from spleen and tumor tissue. (C) Relative cytokine expression of immune cells infiltrated in tumors.

knockout mice developed significantly smaller tumors than wildtype mice. Analyzing the composition of infiltrating immune cells in the tumor microenvironment, we observed higher frequencies of B cells and dendritic cells in tumor tissue of *Cdkn1a* -/- mice. Furthermore, decreased CD4+ CD8+ T cells and macrophage frequencies were detected. mRNA analysis of *Cdkn1a* -/- tumors revealed a higher expression of pro-inflammatory cytokines such as IL-6 and IL-17a, but not IFNγ or TGFβ.

In summary, our data show that p21 deficiency leads to reduced tumor growth and this phenotype might be influenced by a role of p21 in tumor infiltrating immune cells. However, as Cdkn1a is systemically deleted in Cdkn1a -/- mice, we cannot exclude a functional relevance for p21 in other cells such as fibroblasts, endothelial cells etc. Therefore, we will utilize more specific approaches in subsequent experiments including the use of Cdkn1a bone marrow chimeras and conditional knockout mice for p16 and p53 in various T cell subsets.

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Invited lectures

Workshop von Leibniz-IPHT und Jenoptik „Ex-vivo und in-vivo optisch molekulare Pathologie: Potenziale und Trends“, March 3, 2017, Jena, Gastroenterologie - Anforderung an eine verbesserte endoskopische Diagnostik

Netzwerktreffen BioPhotonik des Kompetenznetzwerks BayernPhotonics, April 25, 2017, Erlangen, Innovative optische Verfahren zur Diagnostik gastrointestinaler Erkrankungen

101. Jahrestagung der Deutschen Gesellschaft für Pathologie, June 23, 2017, Erlangen, Neue optische Verfahren zur Diagnostik gastrointestinaler Erkrankungen

Laser World of PHOTONICS 2017, June 27, 2017, München, Unmet needs for the endoscopic diagnosis of gastrointestinal diseases

SPECTARIS-Forum Photonik 4.0 - Optische Gesundheitstechnologien, November 6, 2017, Berlin, Biophotonik in der Gastroenterologie: Diagnostik der Zukunft

Publications during funding period

Knieling F, Neufert C, Hartmann A, Claussen J, Urich A, Egger C, Vetter M, Fischer S, Pfeifer L, Hagel A, Kielisch C, Gortz RS, Wildner D, Engel M, Rother J, Uter W, Siebler J, Atreya R, Rascher W, Strobel D, Neurath MF, Waldner MJ (2017) Multispectral Optoacoustic Tomography for Assessment of Crohn's Disease Activity. N Engl J Med 376: 1292-1294

E12 - Final Report

01.04.2014 - 31.03.2017

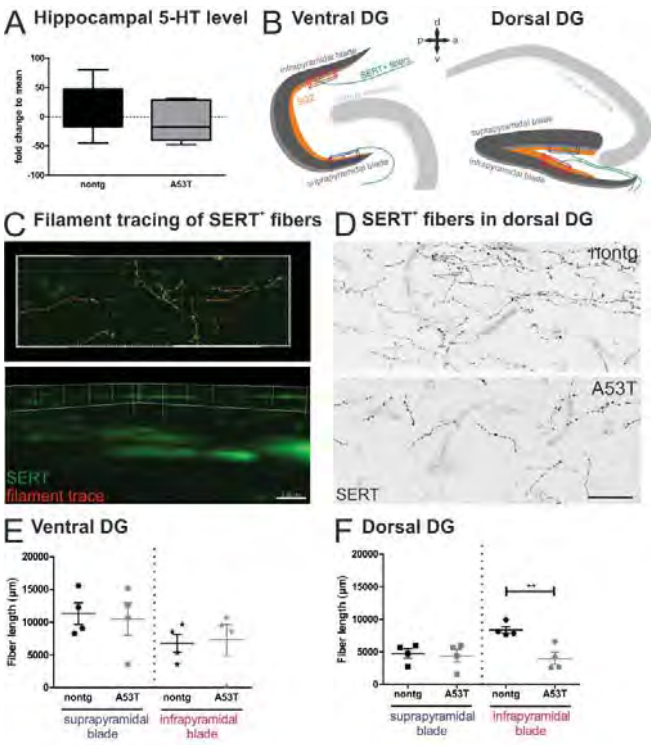
Adult hippocampal neurogenesis in synucleinopathies

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) and related synucleinopathies. Using different animal models, we identified the impact of α-synuclein (α-syn) on the hippocampal serotonergic system affecting hippocampal neurogenesis, which might contribute to NMS in PD. Moreover, we aimed to decipher new molecular targets for the treatment of these highly debilitating NMS.

Analysis of the hippocampal serotonergic system in BAC transgenic α-syn rats and A53T α-syn mice 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, 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 newborn neurons, together with an early anxiety-like phenotype. Furthermore, transgenic A53T α-syn mice present with a less dense serotonergic innervation of the dorsal DG of the hippocampus at 12 months of age. Interestingly, we observed a compromised increase in doublecortin expressing neuroblasts after chronic treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine at the site of reduced serotonergic innervation, the infrapyramidal blade of the dorsal DG. These data further stress the findings of a compromised intra-hippocampal circuitry in different models for synucleinopathies as underlying cause for NMS in these diseases.



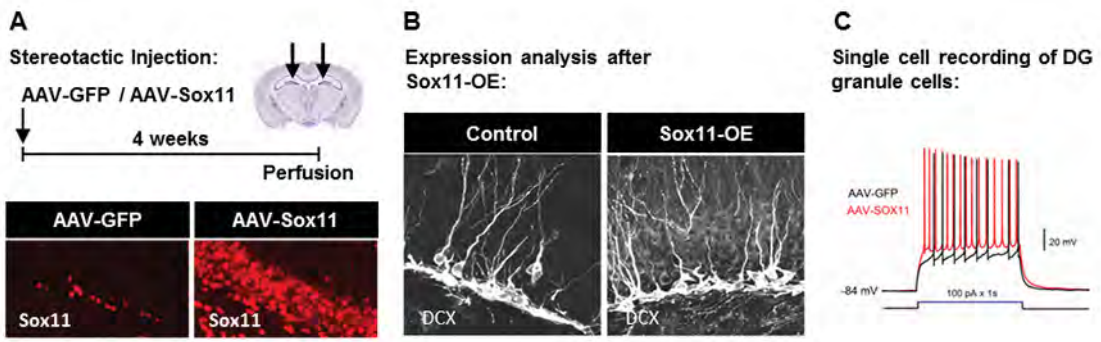
(A) Unchanged hippocampal 5-HT levels in non-tg vs. A53T mice. (B-F) Imaris® software based filament tracing (red) of SERT+ fibers (green) revealed a reduction in the dorsal infrapyramidal blade of the DG in A53T mice, while innervation of the ventral DG was similar in A53T and non-tg mice.

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 SSRI class increase the expression of the transcription factor Sox11 in DG neurons. We have now established that the induction of Sox11 in DG neurons is regu-

Prof. Dr. Winkler

Prof. Dr. Lie



(A) AAV-mediated overexpression of Sox11 (red). Sox11-overexpression enhances expression of the microtubule associated protein DCX (grey, B) and increases excitability of dentate granule neurons (C).

lated by neuronal activity and that the activity-dependent expression of Sox11 is specific for the DG. Our electrophysiological and behavioral analyses revealed that Sox11 expression substantially alters DG neuron plasticity and hippocampus-dependent memory consolidation. In addition, RNA-Sequencing analysis showed Sox11 activity altered expression of neuronal cytoskeleton-associated genes, ion channels, and regulators of pre- and postsynaptic development was changed in DG neurons. Collectively, these data identify Sox11 as a novel DG-specific regulator of neuronal plasticity, which may be involved in the actions of antidepressants.

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Invited lectures

Keystone Symposium „Neurogenesis during Development and in the Adult Brain“, January 8-12, 2017, Olympic Valley, USA, Autophagy-dependent regulation of neurogenesis

Publications during funding period

Beckervordersandforth R, Ebert B, Schäffner I, Moss J, Fiebig C, Shin J, Moore DL, Ghosh L, Trinchero MF, Stockburger C, Friedland K, Steib K, von Wittgenstein J, Keiner S, Redecker C, Hölter SM, Wurst W, Jagasia R, Schinder AF, Ming G, Toni N, Jessberger S, Song H, Lie DC (2017) Role of mitochondrial metabolism in the control of early lineage progression and ageing phenotypes in adult hippocampal neurogenesis. Neuron 93(3): 560-573

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E13 - Final Report

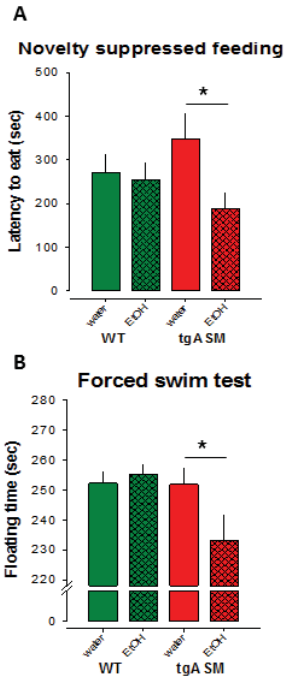
01.04.2014 - 31.03.2017

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

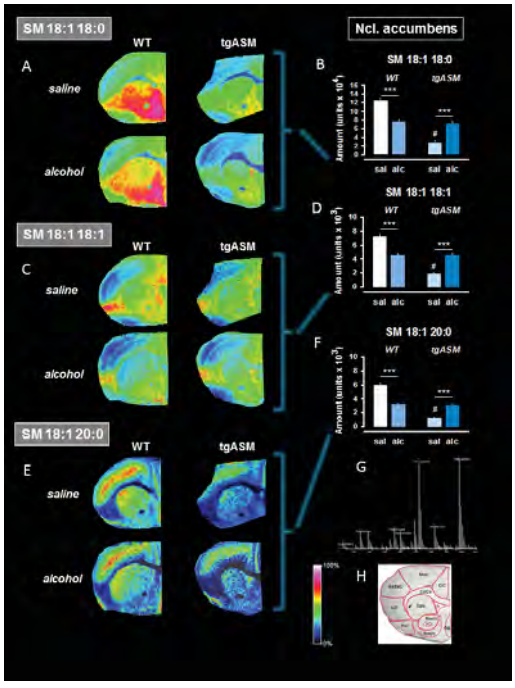
Prof. Dr. Christian P. Müller, Department of Psychiatry and Psychotherapy
PD Dr. Martin Reichel, Department of Medicine 4 – Nephrology and Hypertension
Prof. Dr. Johannes Kornhuber, Department of Psychiatry and Psychotherapy

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,



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

we found rather opposite effects compared to a free-choice administration. Alcohol enhanced depression-like behaviour. 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

3. Hamburger Fachgespräch Alkoholabhängigkeit, March 11 2017, Hamburg, Germany, Lipide als Risikofaktoren und Biomarker für chronischen Alkoholkonsum

Universiti Sains Malaysia, Center for Drug Research, April 4 2017, Penang, Malaysia, Sphingolipids and the transition from depression to alcoholism

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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 - Final Report

01.04.2014 - 31.03.2017

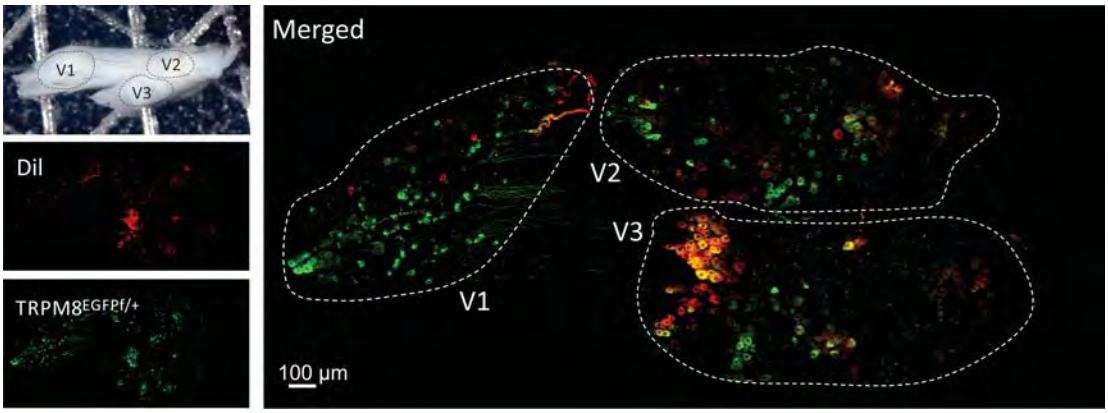
Role of TRPC5 in trigeminal nociception

Prof. Dr. Katharina Zimmermann, Department of Anaesthesiology

In dental hypersensitivity one of the strongest triggers to induce pain is cold. The trigeminal nociceptive system exerts unique features in its organization and protein expression profile. To provide insight into the molecular organization of mouse tooth pulp nociceptors we studied the contribution of identified cold transduction channels including TRPC5 and found functional and morphological evidence for its involvement in cold transduction in mouse and human tooth nociceptors.

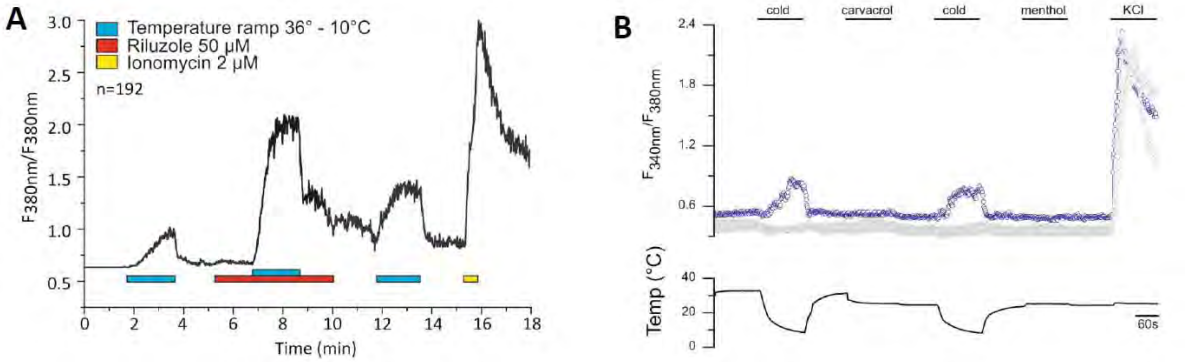
Identification of mouse dental primary afferent neurons in the trigeminal ganglion

Unambiguous recognition of dental primary afferent neurons (DPAN) within the trigeminal ganglion requires the use of retrograde axoplasmic transport of fluorescent dye. Retrograde DiI-based labeling is routinely used in molars of rats, but in the mouse, the neurotoxic Fluoro-Gold (FG) is standard because of its superior membrane penetration. This is essential, because anatomic proportions in the oro-facial region differ substantially between rats and mice and murine teeth can only borrow a fraction of the dye harbored in a rat molar (less than ¼). Yet for live cell imaging studies in neurons DiI is standard as it leaves physiological properties unaltered. We successfully established retrograde labeling in mouse molars with DiI by using a formulation called NeuroTrace® which is optimized for superior axonal membrane penetration, but not with conventional crystalline DiI.



Two-Photon Laser Scanning Fluorescence Microscopy reconstruction of an entire mouse trigeminal ganglion showing TRPM8 in green and DPAN in red (DiI); cells of the ophthalmic (V1), maxillary (V2) and mandibular (V3) nerves are marked with dashed lines.

Prof. Dr. Zimmermann



Cold responses registered with calcium microfluorimetry and presumably mediated by TRPC5 in (A) HEK293T cells transiently transfected with murine TRPC5 and (B) retrograde labeled dental primary afferent neurons of a TRPM8/A1-deficient mouse.

TRPC5 and TRPM8 are cold transducers in mouse tooth nociceptors

We have obtained cultured DiI-labeled DPAN from C57BL/6J mice and subjected to cold stimulation and treatment with pharmacological agonists of the TRPM8 and TRPA1 cold transducers. We found that DPAN's cold sensitivity relies to 45% on TRPM8 and to a negligible fraction on TRPA1 which leaves room for a cold transduction mechanism independent of TRPM8 and TRPA1. Evidence for TRPC5 as cold transducer was found in DPAN of mice deficient of TRPM8 and TRPA1, where cold responses are reduced by half and share a similar shape with the cold responses obtained in HEK cells heterologously expressed with TRPC5. In addition, the cold responses recorded with calcium microfluorimetry were decreased by the specific TRPC5 antagonist ML204. To investigate the contribution of TRPC5 to cold sensitivity in real tooth nociceptors we developed a specific jaw-nerve preparation which allows to record from axons of the inferior alveolar nerve innervating molars and incisor in the mouse with suction electrodes. We found that the number of cold sensitive fibers is much reduced in TRPC5-deficient mice. ML204 reliably blocked cold responses and lost its blocking effect in nociceptors from TRPC5-deficient mice. Nevertheless, we found cold sensitive fibers to be highly reduced in TRPM8/A1- and TRPC5/A1-deficient mice. Since cold responses from TRPC5/A1-deficient mice do display a reduced number of action potentials and reduced thresholds of activation, TRPM8 remains as second important cold transducer. Likely the tooth cold transduction mechanism is of complex nature and involves heteromultimers of at least two channels, TRPC5 and TRPM8.

Together with our results of an upregulation of TRPC5 in human pulpitic teeth, TRPC5 presents a highly interesting target for the treatment of inflammatory tooth pain and dentine hypersensitivity to cold – frequent dental problems often leading to the extraction of healthy teeth.

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Invited lectures

Wolfson Centre for Age-Related Diseases at King's College London, January 25, 2017, London, Heat-resistant action potentials in nociceptors require TTX-resistant sodium channels subtypes

Publications during funding period

Kadala A, Sotelo-Hitschfeld P, Ahmad Z, Tripal P, Schmid B, Mueller A, Bernal L, Winter Z, Brauchi S, Lohbauer U, Messlinger K, Lennerz JK, Zimmermann K (2017) Fluorescent Labeling and 2-Photon Imaging of Mouse Tooth Pulp Nociceptors. Journal of Dental Research: doi 10.1177/0022034517740577

E16 - Final 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

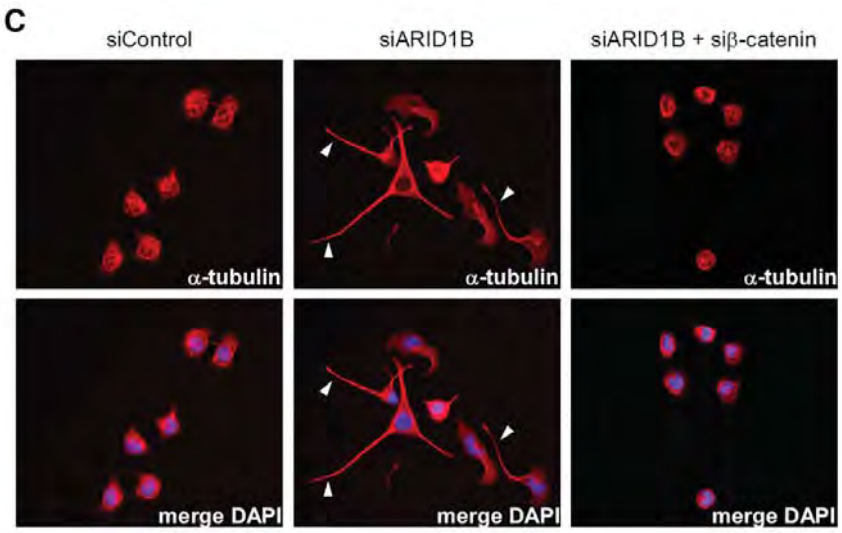
Genetic defects are responsible for the vast majority of intellectual disability (ID) cases in countries with high standard of living. There is evidence that ID-gene encoded proteins are connected in pathways that regulate neurodevelopment and –plasticity. This project aims to identify common pathophysiological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

Functional Studies of Chromatin-Remodeling-Factor ARID1B

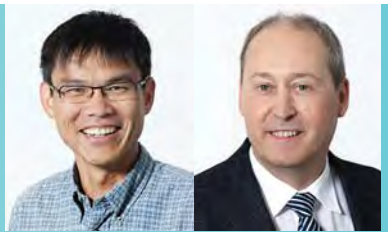
ARID1B encodes the DNA-binding component of the BAF-complex and is the gene mutated in the majority of patients with Coffin-Siris Sydrome (CSS). ARID1B mutations are also observed in ID patients with a more unspecific, broader clinical presentation. Our studies of patients' material and of cellular knock-down 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. We have now established 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

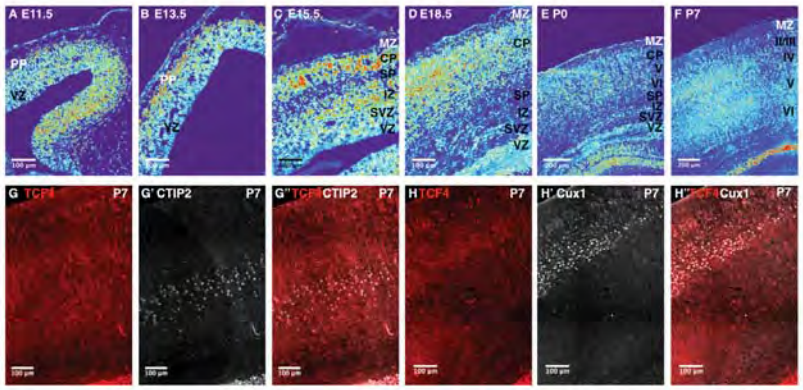
In exome sequencing studies of ID patients with symptoms overlapping with those seen in CSS but lacking a mutation in BAF-complex members we identified mutations in candidate genes. In a series of female ID-patients from laboratories worldwide we identified missense variants of the X-linked gene NAA10, 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.



Knockdown of ARID1B in Neuro2A Cells Leads to Neurite Outgrowth through ß-Catenin. Immunofluorescence of Neuro2A cells transfected with siRNAs against control or ARID1B and ß-catenin as indicated, stained for a-tubulin. Arrowheads point to neurites (image from Vasileiou et al., Am J Hum Genet, 2015).



Prof. Dr. Lie Prof. Dr. Reis



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.

Functional analysis of the ID-linked transcription factor Sox11

The BAF complex regulates the expression of the transcription factor Sox11. Mutations in Sox11 were identified as a cause for a subset of CSS cases. We found that Sox11 regulates differentiation and synaptic integration during neurogenesis. To investigate the pathophysiological mechanisms underlying Sox11-mutation associated CSS we have now generated iPSCs with mutations in the Sox11 gene.

In proteomic analyses, we identified the transcription factor TCF4 as interactor of SOX11. TCF4 mutations cause Pitt-Hopkins syndrome, a disorder characterized by developmental delay and ID. We found that TCF4 is highly expressed in the developing and adult cortex and hippocampus. In ongoing analyses of TCF4-heterozygote knockout mice, we observed hypoplasia of cortical and hippocampal structures. This phenotype is aggravated by Sox11 heterozygosity. These data suggest a role for TCF4 in the regulation of cortical and hippocampal neurogenesis and support the hypothesis that TCF4 and Sox11 cooperatively regulate developmental pathways.

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Invited lectures

Keystone Symposium „Neurogenesis during Development and in the Adult Brain", January 8-12 2017, Olympic Valley, USA, Autophagy-dependent regulation of neurogenesis

Publications during funding period

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Cernilogar FM, Di Giaimo R, Rehfeld F, Cappello S, Lie DC (2015) RNA interference machinery-mediated gene regulation in mouse adult neural stem cells. BMC Neurosci. 16:60 doi 10.1186/s12868-015-0198-7

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E17 - Final Report

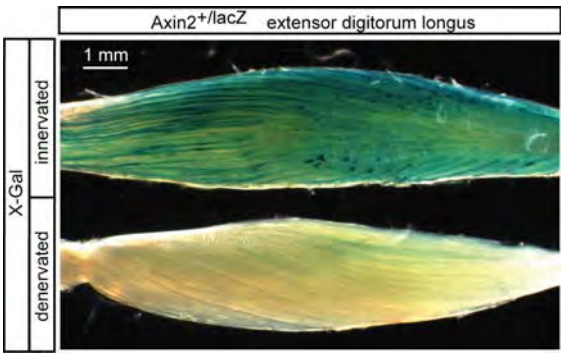
01.04.2014 - 31.03.2017

The neuromuscular role of Wnt signaling pathways

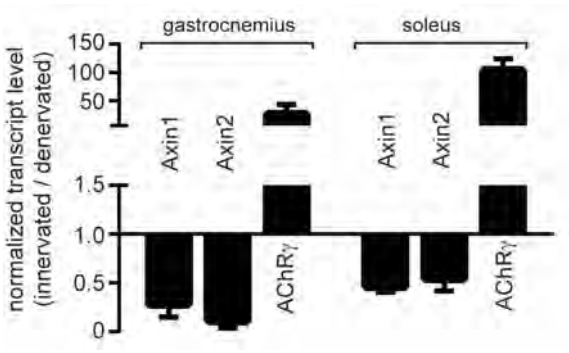
Prof. Dr. Said Hashemolhosseini, Institute of Biochemistry

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.



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

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.

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Publications during funding period

Giacomazzi G, Holvoet B, Trenson S, Caluwé E, Kravic B, Deroose C, Huylebroeck D, Hashemolhosseini S, Janssens S, McNally E, Quattrocelli M, Sampaolesi M (2017) MicroRNAs regulate myogenic propensity of mesodermal iPSC-derived progenitors. Nat Commun. 8(1): 1249-1262

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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

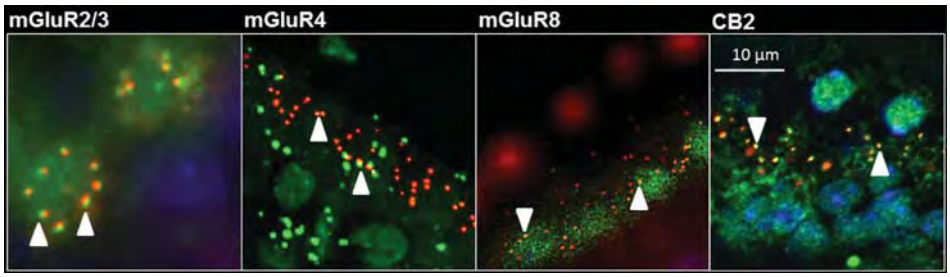
Neuronal signal transduction is largely guided by a synaptic expression of neurotransmitter receptors. These receptors binding regulatory proteins, such as enzymes and scaffolds that regulate their trafficking, localization, ligand affinity, desensitization behaviour and surface concentration. In this 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.

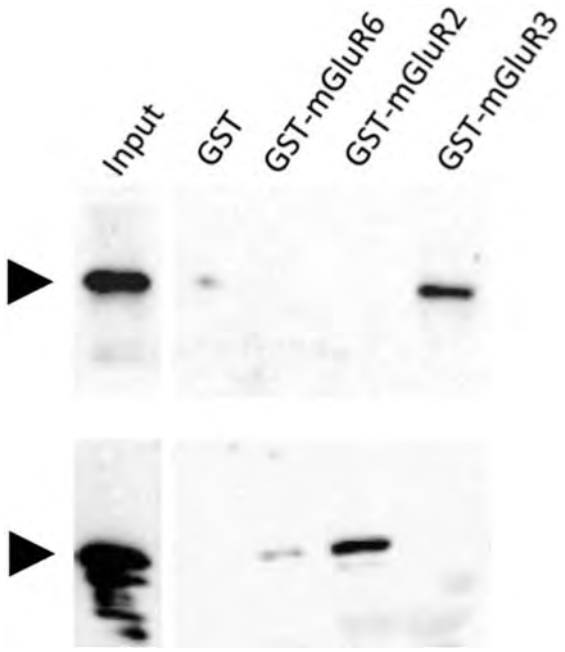
Which pre-synaptic mGluR and CB receptors are expressed in the cochlea?

Expression and localization of the mostly pre-synaptically localized mGluR2, mGluR3, mGluR4, mGluR7a, mGluR7b, mGluR8a, mGluR8b and CB1 in the inner ear is largely unknown. We detected transcripts and proteins for all receptor types analysed in the mouse cochlea. Using CTBP2 as a marker for pre-synaptic ribbons, we could co-localize mGluR2/3, mGluR4, mGluR8 and CB2 with ribbon synapses in cochlear wholemounts.



CTBP2 labels pre-synaptic ribbons of hair-cells in the cochlea (red). These synapses also express metabotropic glutamate receptors (mGluR) and cannabinoid receptors (CB), shown in green. Co-localization is indicated by arrowheads (yellow). Nuclei were counterstained with DAPI (blue).

Prof. Dr. Enz



Western-blots showing examples of 2 proteins (arrowheads) identified in our yeast 2-hybrid screens that interact with different mGluR types expressed at ribbon synapses of hair-cells in the cochlea in GST pull-down assays. GST serves as negative control.

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 function. Protein interactions were identified in individual yeast 2-hybrid screens using intracellular C-termini of mGluR2, mGluR3, mGluR4, mGluR7a, mGluR7b, mGluR8a, mGluR8b, CB1 and CB2 as baits for a cochlear cDNA-library. These screens yielded several hundred potential interaction partners that were clustered in functional groups, representing proteins involved in post-translational modifications, trafficking, cell adhesion or of the cytoskeleton. Of these, 10 proteins were selected for further characterization. Pull-down assays showed robust and reproducible interaction of 3 proteins with the receptors´ C-termini. Currently, we test binding of these 3 proteins against all mGluR and CB receptor types known, and compare the localization of interacting proteins at hair-cell synapses in the cochlea by immunohistochemistry and electron microscopy.

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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

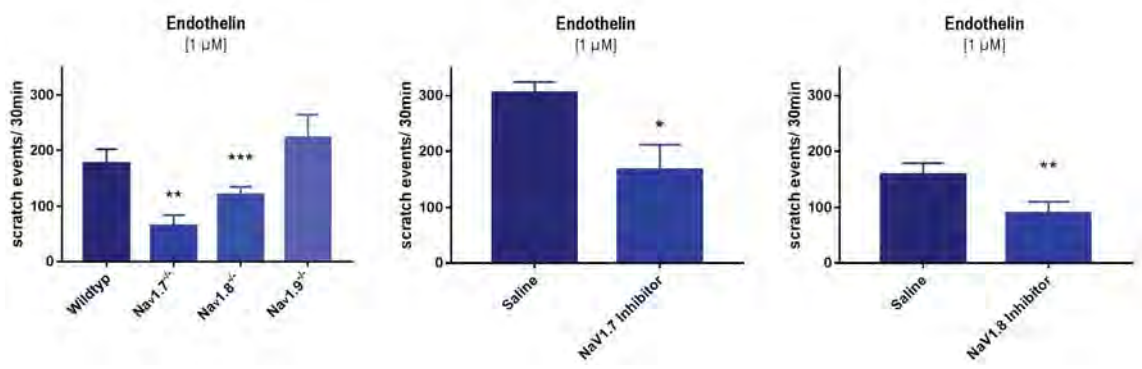
Chronic pruritus is an agonizing symptom accompanying many dermatological and systemic disorders. Aim of this project is to identify pruritogens in plasma of patients suffering from chronic pruritus, to characterize the specific NaV channel subtypes that generate and propagate the action potentials in 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.

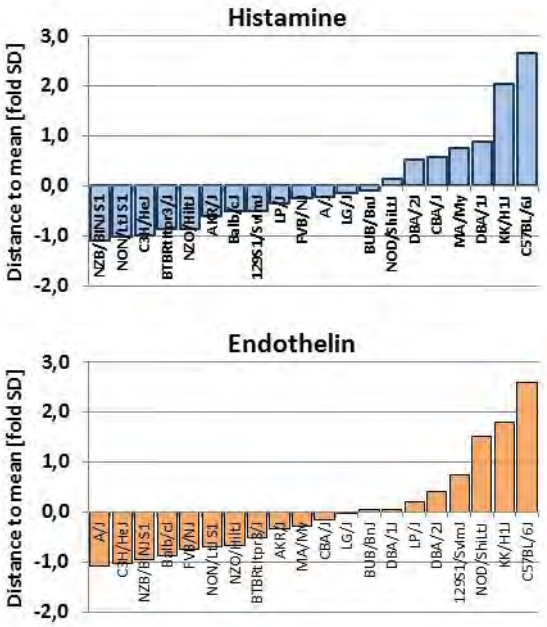
Identification of specific NaV channel subtypes required for itch signaling

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. DRG neurons of all knockout mice showed an equal depolarization upon stimulation with pruritogens measured by calcium imaging and exhibited only minor neurophysiological differences. However, scratching behavior upon intradermal injection of different histaminergic and non-histaminergic pruritogens was significantly impaired in knockout-animals compared to wildtype mice. These observations were reproduced using pharmacological inhibition of the respective NaV subtypes in wildtype mice.



Genetic deletion (A) or pharmacological inhibition of Nav1.7 (C) and Nav1.8 (B) attenuates endothelin-induced scratching compared to wildtype mice.

Dr. Dr. Kremer Prof. Dr. Zimmermann



Z-transformed data of scratch events following intradermal injection of histamine (top) and endothelin (bottom) in male mice of 20 different inbred strains (n=9-13 per strain).

Quantification of acute itch after pruritogen injection in 20 different inbred mouse strains

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 physical parameters. 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 quantified scratching behaviour in a body of inbred strains. So far, we phenotyped twenty inbred mouse strains and observed strong differences between number and time of scratch bouts following the intradermal injection of 10 different pruritogens (histamine, chloroquine, lysophosphatidic acid, serotonin, endothelin, BAM8-22, compound48/80, SLIGRL, β-alanine and trypsin). As control parameters we measured several parameters in the phenomaster cage system including in-cage activity and indirect calorimetry. Once we have analyzed all pruritogens in all 20 inbred mouse strains we will perform haplotype-based computational genetic mapping (HBCGM) of the data and further investigate genes with high correlation to the phenotypic trait differences.

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Invited lectures

4th Porto Liver Meeting, June 23, 2017, Porto, Portugal, Pruritus management in liver diseases

Hepatologie-Update, Hamburger Lebertage, May 12-13, 2017 Hamburg, Juckreiz bei Lebererkrankungen – was kann man therapeutisch tun

PBC in Motion, February 27, 2017, London, UK, Managing symptoms of disease

Expert summit on viral hepatitis, February 12-13, 2017, Berlin, Primär biliäre Cholangitis – nicht nur der Name ist neu

Awards

Young Investigator Award, A.E. Kremer, 2017, International Liver Meeting, EASL, Amsterdam

Publications during funding period

He GW, Günther C, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S, Becker C (2017) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut. 66(4): 716-723

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

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

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

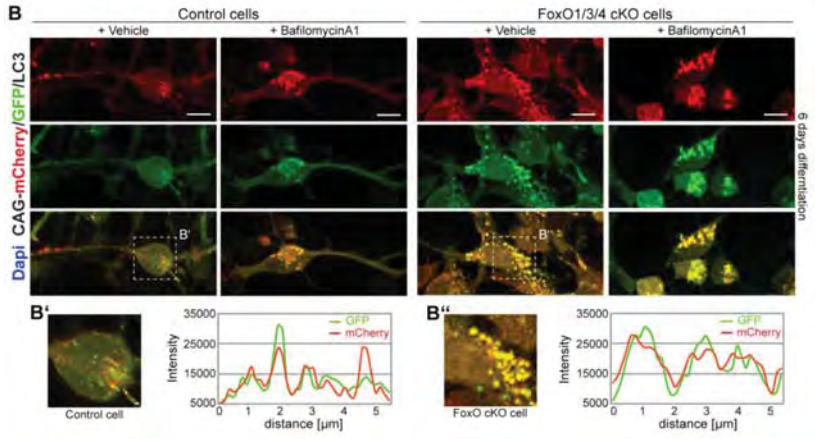
Dysregulation of autophagy, the central cellular self-clearance mechanism, is impaired in synucleinopathies including Parkinson´s disease and has been implicated in the cell-to-cell transfer of aSyn potentially leading to disease progression. This project addresses the currently unresolved question of how ageing accelerates aSyn-related toxicity and cerebral spreading. In the second project phase, we have investigated the dependency of autophagy on the ageing-associated FoxO-pathway.

FoxO transcription factors potently modulate autophagy in neural cells

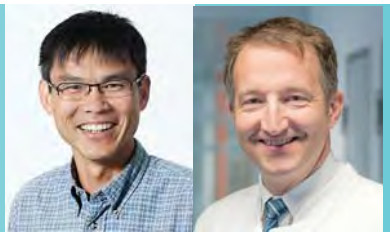
We found that FoxO-deficiency impaired the function and homeostasis of neural precursor cells and neurons. Thus, FoxO-deficient neural precursor cells showed excessive proliferation and premature differentiation, while FoxO-deficient neurons displayed aberrant dendritogenesis and synaptogenesis, enlarged mitochondria, and enhanced sensitivity to degeneration. Using molecular, biochemical, and imaging analyses we found that FoxO-deficiency resulted in a strong impairment of autophagic flux in neural precursor cells and neurons. Most importantly, we could stimulate autophagic flux in FoxO-deficient neurons in vivo, and ii) to almost completely rescue the in vitro and in vivo progenitor and neuronal phenotype caused by FoxO-deficiency. Collectively these findings reveal a novel link between FoxO transcription factors, autophagic flux, and the development and homeostasis of neurons.

Impairment of autophagic flux modulates aSyn containing exosome profile

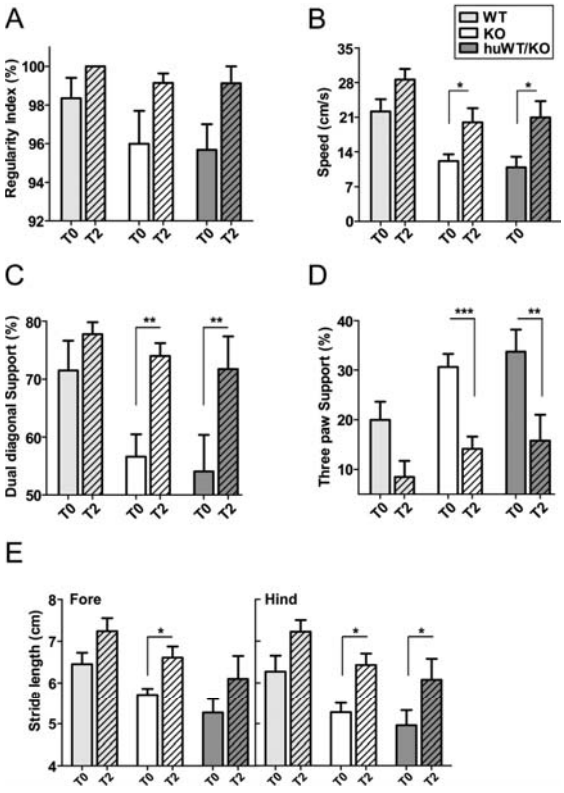
The second goal of the project was to understand the interplay of cellular autophagy with systemic changes related to aging. In this phase of the project we analyzed the characteristics of the exosomes that are released by neural cells after autophagy inhibition and identified a specific protein pattern representing a distinct exosome species which we termed autophagoexosomes. This species could be identified in the human CSF of affected patients only and supported the spreading of synucleinopathy in rodent brains. In a follow up project, we have initiated to study the effect of exercise on brain and system autophagy level, since exercise – similar to starvation – are important trigger for autophagy and might link the interplay between cellular autophagy, systemic (protective) effects and age-related FoxO dependent signal transduction pathways. Initial data supports that exercise in deed induces systematic (but not brain specific) autophagy flux, but, however, substantially improves the phenotype (e.g. gait pattern) of rodent synucleinopathy models.



Stem cells expressing the autophagy reporter LC3-GFP-mCherry were differentiated into neurons. While control cells harbored autophagosomes (yellow) and autophagolysosomes (red), FoxO-deficient cells only contained autophagosomes, revealing impaired autophagosome processing.



Prof. Dr. Lie Prof. Dr. Klucken



Treadmill exercise in mouse models of synucleinopathy (WT: wild type, KO: aSyn knock out; huWT: human aSyn) recorded by Catwalk XT improved gait parameters: Velocity (A,B), dynamic postural control (C,D), step cycle (E). T0 (baseline) vs. T2 (after 1 month exercise).

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Invited lectures

20 years of alpha-synuclein in Parkinson's Disease and related synucleinopathies, September 7-10, 2017, Vravrona Athens, Greece, Presentation for the poster "Autophagy inhibition promotes alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophago/exosome-like phenotype" (G. Minakaki)

International Symposium „Human induced pluripotent stem cells" (ForIPS), December 6, 2017, Autophago-exosomes; linking lysosomal inhibition to disease progression in synucleinopathies (G. Minakaki)

Keystone Conference „Neurogenesis during Development and in the Adult Brain", January 9 -12, 2017, Olympic Valley, USA. Autophagy-Dependent Control of Neurogenesis (DC. Lie)

Instituto de Biomedicina de Valencia, September 27, 2017, Valencia, Spain, New players in adult hippocampal neurogenesis (DC. Lie)

Spanish Society of Neuroscience, September 29, 2017, Alicante, Spain, Role of mitochondria and autophagy in adult hippocampal neurogenesis (DC. Lie)

Awards

Poster Award in the frame of the international IZKF Graduate Workshop, G. Minakaki, October 19, 2017, Erlangen

Poster Award DGN (Kongress der Deutschen Gesellschaft für Neurologie), S. Menges, September 20-23, 2017, Leipzig

Poster Award in the frame of the international meeting «20 years of alpha-synuclein in Parkinson's Disease and related synucleinopathies: from the bedside to the bench and back to the patient», G. Minakaki, September 7-10, 2017, Vravrona, Athens, Greece

Publications during funding period

Menges S, Minakaki G, Schaefer I, Meixner H, Prots I, Schlotzer-Schrehardt, Friedland K, Winner B, Outeiro T, Winklhofer J, von Arnim C, Xiang W, Winkler J and Klucken J (2017) Alpha-synuclein prevents the formation of spherical mitochondria and apoptosis under oxidative stress. Sci Rep. 7: 42942

Beckervordersandforth R, Ebert B, Schaffner I, Moss J, Fiebig C, Shin J, Moore DL, Ghosh L, Trinchero MF, Stockburger C, Friedland K, Steib K, von Wittgenstein J, Keiner S, Redecker C, Holter SM, Xiang W, Wurst W, Jagasia R, Schinder AF, Ming GL, Toni N, Jessberger S, Song H and Lie DC (2017) Role of Mitochondrial Metabolism in the Control of Early Lineage Progression and Aging Phenotypes in Adult Hippocampal Neurogenesis. Neuron 93(6): 1518

E22 - Progress Report

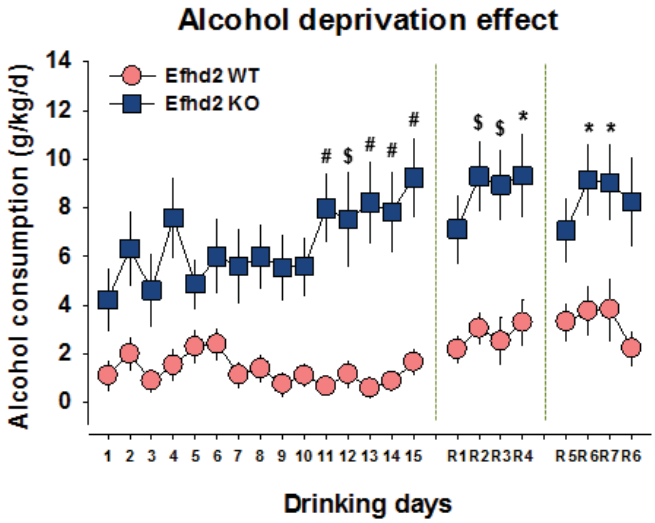
01.03.2016 - 31.08.2018

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-, cocaine- and methamphetamine addiction.

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 □-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 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. These findings showed that EFhd2 is a conserved re-

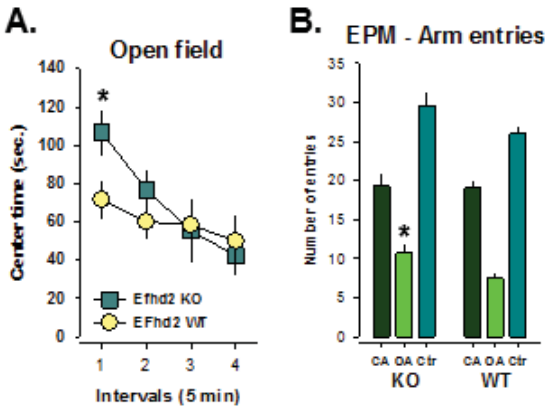


The lack of Swirprosin-1/EFhd2 in mice leads to enhanced consumption of alcohol in a free-choice drinking paradigm and spontaneous escalation of consumption. Withdrawal from alcohol for three weeks (dotted green lines) increases alcohol consumption in wild type mice (alcohol deprivation effect), but 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).

silience 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. In a parallel study we found that EFhd2 KO mice show a faster and more efficient establishment of the conditioned rewarding effects of cocaine and methamphetamine, two psychostimulant type drugs. These findings support the view that EFhd2 may not only provide resilience for alcohol addiction, but for drug addiction in general.

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Invited lectures

Autumn school of the German society for immunology (DGFI), October 10, 2017, How B cells control immunity

Publications during funding period

Lang SC, Harre U, Purohit P, Dietel K, Kienhöfer D, Hahn J, Baum W, Herrmann M, Schett G, Mielenz D (2017) Neurodegeneration Enhances the Development of Arthritis. J Immunol 198: 2394–2402

Mielenz D, Reichel M, Jia T, Quinlan EB, Stöckl T, Mettang M, Zilske D, Kirmizi-Alsan E, Schönberger P, Praetner M, Huber SE, Amato D, Schwarz M, Purohit P, Brachs S, Spranger J, Hess A, Büttner C, Ekici AB, Perez-Branguli F, Winner B, Rauschenberger V, Banaschewski T, Bokde AL, Büchel C, Conrod PJ, Desrivières S, Flor H, Frouin V, Gallinat J, Garavan H, Gowland P, Heinz A, Martinot JL, Lemaitre H, Nees F, Paus T, Smolka MN, IMAGEN Consortium, Schambony A, Bäuerle T, Eulenburg V, Alzheimer C, Lourdusamy A, Schumann G, Müller CP (2017) EFhd2/Swiprosin-1 is a common genetic determinator for sensation-seeking/low anxiety and alcohol addiction. Mol Psychiatry: doi 10.1038/mp.2017.63

E23 - Progress Report

01.01.2016 - 30.06.2018

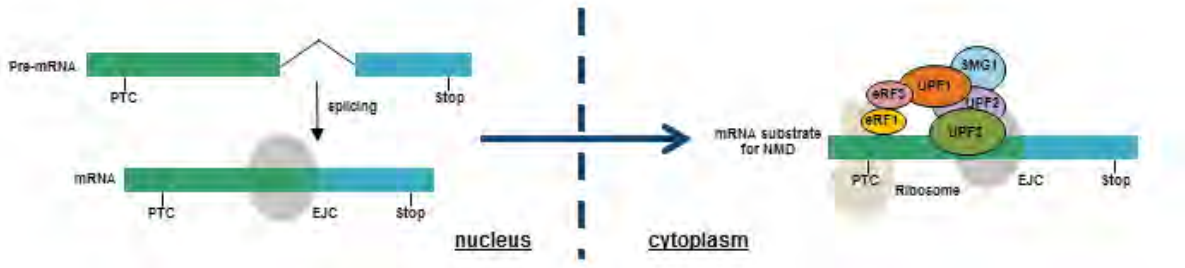
Identification and characterization of LOXL1 risk variants for pseudoexfoliation syndrome and glaucoma

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

Pseudoexfoliation (PEX) syndrome represents a 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, 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 disease.

To search for sequence variants influencing transcriptional output of LOXL1, we conducted a genome-wide association scan on 771 German PEX patients and 1350 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. We found that the rs11638944:C>G transversion exerts 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 associated with nonsense-mediated decay. These mechanisms eventually lead to reduced levels of LOXL1 mRNA in cells and tissues of risk allele carriers. These findings uncover a functional mechanism by which common noncoding variants influence LOXL1 expression and thereby predispose to connective tissue alterations and susceptibility to glaucoma (Pasutto et al. 2017).

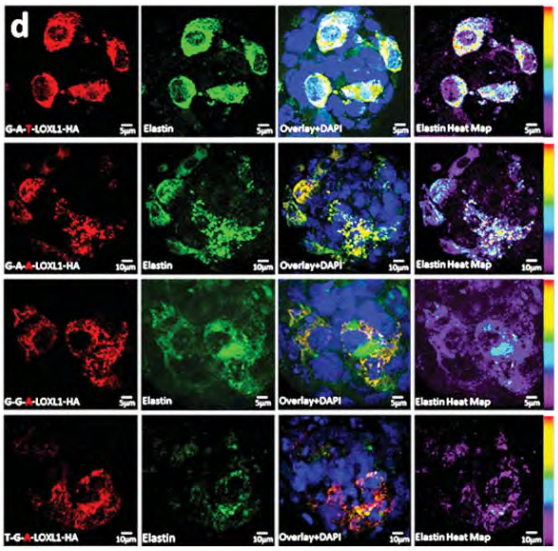
Alternative splicing and nonsense-mediated mRNA
We further investigated the involvement of alternative mRNA splicing coupled to nonsense-mediated decay (NMD) in the regulation of LOXL1 expression in response to PEX-associated pathophysiologic factors. Alternatively spliced LOXL1-a transcripts, characterized by inclusion of an additional exon introducing a premature termination codon in exon 2, were upregulated by NMD inhibitors puromycin and caffeine or after knockdown of NMD core factors. Exposure of cells to various PEX-associated (stress) factors, including TGF-β1, UV-B light and oxidative stress, enhanced LOXL1-a transcript levels while levels of wildtype LOXL1 were reduced. These findings provide evidence for a functional role of alternative splicing coupled to NMD in the posttranscriptional regulation of LOXL1 gene expression and suggest this mechanism to represent a dynamic mode of adapting LOXL1 expression to PEX-associated environmental and nutritional cues (Berner et al. 2017).



Schematic illustration of the nonsense-mediated decay (NMD) pathway as quality control mechanism that degrades unproductive premature termination codon (PTC)-containing mRNA transcripts during translation

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

As members of the International Glaucoma Genetics Consortium (IGGC), we participated in a genome-wide association study (GWAS) of >10,000 PEX cases and >100,000 controls from 24 countries followed by replication in 18 countries. We identified five new loci, underlined by POMP, TMEM136, AGPAT1, RBMS3 and SEMA6A, associated with PEX. 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. We further identified a rare protective allele at LOXL1 (p.Phe407) predicted to affect protein function through deep resequencing. Functional assays showed an effect of the protective p.407F allele on increased elastin and fibrillin synthesis compared to the wild-type p.407Y allele. These findings provide new biological insights into the pathology of PEX and highlight a potential role for naturally occurring rare LOXL1 variants in disease biology (Aung et al. 2017).



Immunofluorescent staining of HA-tagged LOXL1 variants overexpressed in HLEC cells labelled with anti-HA for detection of LOXL1 (red), elastin (green) and DAPI (blue) (from: Aung T et al., Nat Genet. 2017)

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Invited lectures

7th World Glaucoma Congress, June 28 – July 1, 2017, Helsinki, Finland, Etiology and pathophysiology of exfoliation syndrome

7th World Glaucoma Congress, June 28 – July 1, 2017, Helsinki, Finland, The dual role of LOXL1 in exfoliation syndrome/glaucoma

Publications during funding period

Pasutto F*, Zenkel M*, Hoja U, Berner D, Uebe S, Ferrazzi F, Schödel J, Liravi P, Ozaki M, Paoli D, Frezzotti P, Mizoguchi T, Nakano S, Kubota T, Manabe S, Salvi E, Manunta P, Cusi D, Gieger C, Wichmann HE, Aung T, Khor CC, Kruse FE, Reis A, Schlötzer-Schrehardt U (2017) Pseudoexfoliation syndrome-associated genetic variants affect transcription factor binding and alternative splicing of LOXL1. Nat Commun. 8: 15466

Aung T*, Ozaki M*, Lee MC*, Schlötzer-Schrehardt U*, Thorleifsson G*, et al. (2017) Genetic association study of exfoliation syndrome identifies a protective rare variant at LOXL1 and five new susceptibility loci. Nat Genet. 49: 993

Berner D, Zenkel M, Pasutto F, Hoja U, Liravi P, Gusek-Schneider GC, Kruse FE, Schödel J, Reis A, Schlötzer-Schrehardt U (2017) Post-transcriptional regulation of LOXL1 expression via alternative splicing and nonsense-mediated mRNA decay as an adaptive stress response. Invest Ophthalmol Vis Sci. 58: 5930-5940

[*authors contributed equally]

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

Aggregation of alpha-synuclein (aSyn) associated with demyelination and inflammation is a hallmark of neurodegenerative disorders like multiple system atrophy. Recently, increased aSyn levels were also observed in multiple sclerosis patients. Previous results showing impaired maturation and myelination of aSyn overexpressing primary oligodendrocytes suggest a detrimental role of aSyn aggregates in myelin homeostasis. The aim of this project is to study the role of aSyn under inflammatory demyelination conditions.

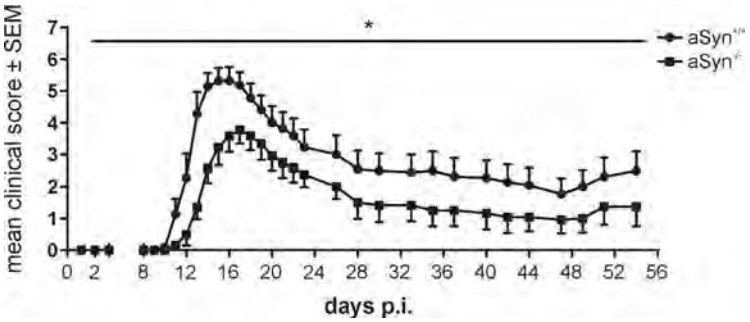
Neurodegenerative and -inflammatory diseases, like multiple system atrophy and multiple sclerosis (MS), are characterized by myelin loss and activation of immune responses in central and peripheral tissues which are accompanied by aggregation of aSyn. The specific contribution of aSyn to degenerative and regenerative processes as well as inflammation remains to be elucidated. Therefore, our project aims at investigating the role of aSyn in inflammatory demyelination and regulation in the context of MS. For this purpose, next to cell culture experiments, we employ two different animal models: the experimental autoimmune encephalomyelitis (EAE) model, a chronic model of MS reflecting inflammation and demyelination in the central nervous system (CNS) and the Cuprizone model, a model of acute demyelination.

Effect of aSyn on inflammatory processes

In the first year, our project focused on the interaction of aSyn and inflammatory processes. In vitro experiments revealed that aSyn is able to modulate microglial immune responses by uptake of aSyn fibrils (Hoffmann et al., 2016). Furthermore, we observed physiological aSyn expression by different immune cell subsets such as CD4- and CD11b/c-positive cells. To analyze the functional role of endogenous aSyn in neuroinflammation, we induced EAE in wildtype and aSyn-knockout mice. Our results suggest that in the acute phase of EAE endogenous aSyn acts as a new regulator of Th1 responses in neuroinflammation (Ettle, Kuhbandner et al., 2016).

Role of aSyn in chronic EAE

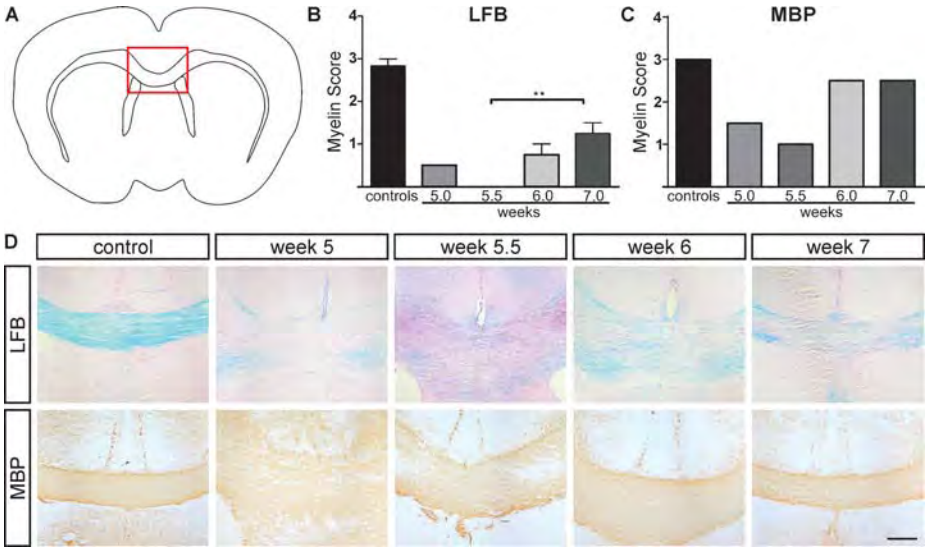
To further elucidate the role of aSyn in inflammatory demyelination in the chronic phase of EAE, we monitored wildtype and aSyn-deficient mice for 8 weeks after EAE induction. Interestingly, aSyn-knockout mice showed an ameliorated disease course compared to wildtype mice. At the peak of motor dysfunction, these mice exhibited mild gait ataxia, while the control group suffered from moderate paralysis of the hind limbs. Furthermore, aSyn-knockout mice showed fast recovery and displayed a difference of 1 score point to wildtype mice at the end of the observation period.



Clinical course of MOG-EAE. EAE was induced in aSyn+/+ or aSyn-/- mice (n=11/12 per group) by active immunization with MOG35-55 peptide and clinical signs were assessed using a 10-point scale (*p<0.05).


Prof. Dr. Winkler


Prof. Dr. Linker



C57BL/6 mice were fed 0.2% CPZ for 5 weeks. (A) Analyzed area of the corpus callosum. Myelin quantification by Luxol Fast Blue (LFB) (B) and MBP (C) staining (n=3, **p<0.01). (D) Representative pictures of brain slices stained with LFB or by anti-MBP antibody (scale bar = 200 µm).

Impact of aSyn deficiency on myelination processes

Next to the EAE model, we aim to study the impact of aSyn deficiency in the Cuprizone model, an acute de- and remyelination model lacking peripheral immunological processes. Here, toxic demyelination is induced by feeding mice a diet containing 0.2% Cuprizone (CPZ), a chopper chelator, which leads to oligodendroglial cell death followed by demyelination. After termination of the CPZ diet, new oligodendrocyte progenitor cells begin to form new myelin sheaths and rapid remyelination occurs.

First, we performed preliminary experiments with wildtype mice to standardize the temporary structure analysis. For the examination of toxin-induced de- and remyelination, mice brains were removed 5, 5.5, 6 and 7 weeks after starting the CPZ diet. Luxol fast blue (LFB) staining revealed complete demyelination after 5.5 weeks and immunohistochemical staining with anti-MBP antibody indicated advanced remyelination one week after withdrawal of CPZ.

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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

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

E25 - Progress Report

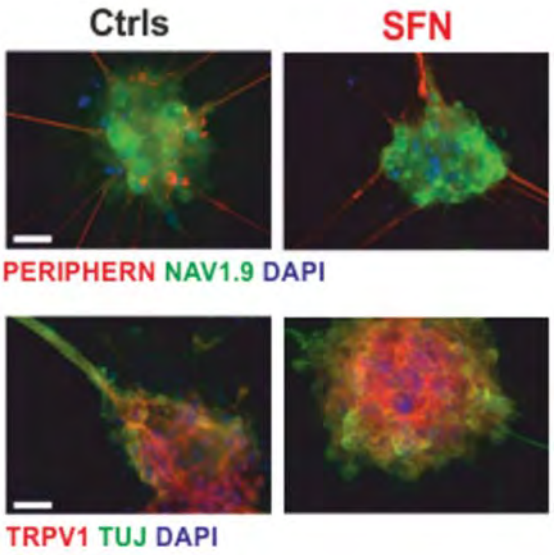
01.07.2016 - 31.12.2018

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

Prof. Dr. Beate Winner, Department of Stem Cell Biology
Prof. Dr. Jürgen Schüttler, Department of Anesthesiology

Our aim is to understand the role of the sodium channel subtype Nav1.9 in chronic pain. 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 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 these patients with painful peripheral neuropathy with small fibre neuropathy (SFN).

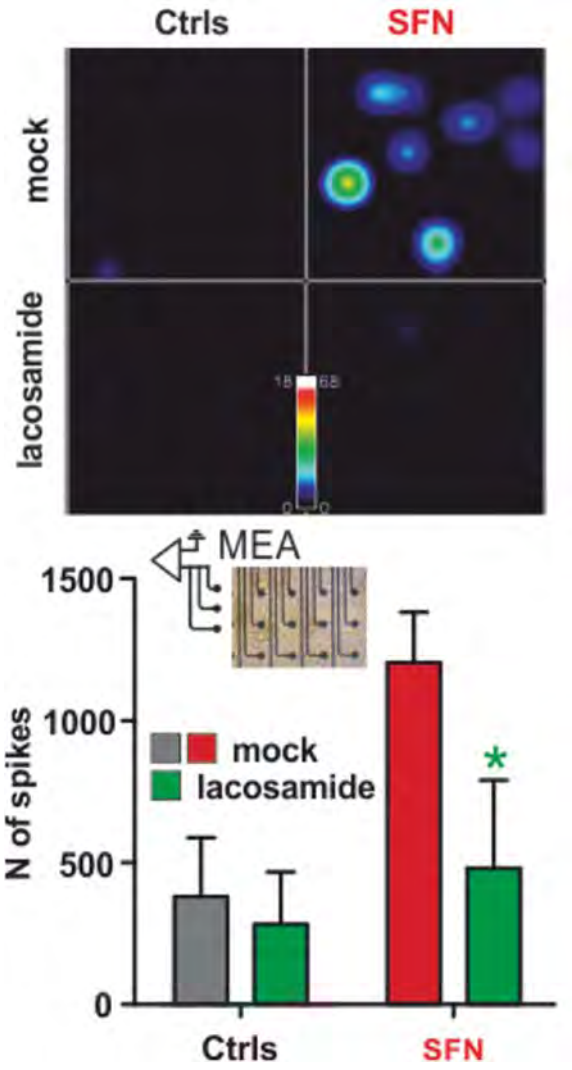


Immunostaining of SFN- and Ctrl-iPSC-derived nociceptors for PERIPHERIN (red), Nav1.9 (green), TRPV1 (red), TUJ1 (green) and DAPI (blue).

To investigate the functional relevance of SCN11A variants in a human system, we received skin biopsies. 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 patients and controls all 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. 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.

To assess the overall cellular excitability in larger cell populations, we seeded SFN patient- and control-derived nociceptors onto multielectrode array (MEA) chips and assessed their spontaneous activity. Patient-derived nociceptors displayed a significantly increased excitability: more spikes, more active electrodes, and a higher burst frequency were detected. Lacosamide is an FDA approved sodium channel modifier used to treat epilepsy. In MEA recordings of one patient-derived nociceptors, lacosamide strongly reduced the number of spikes, but did not have an effect on controls, indicating that pathological hyperactivity was impaired, but general action potential generation was not inhibited. Based on this preclinical prediction derived from MEA testing, one

Prof. Dr. Winner Prof. Dr. Schüttler



upper panel: MEA spike firing rate heat map of spontaneously active SFN- and Ctrl-nociceptors either mock treated or with 500 µM lacosamide.. lower panel: number of spikes per well. inset: nociceptors on MEA chip.

patient started off-label treatment with lacosamide. Within five days the patient's pain was tremendously reduced, and peak pain ratings in the evenings dropped from VAS 7.5 to 1.5. To assess if the clinical response resulted from treating the hyperexcitability of the patient's C-fibers, we clinically used microneurography while the patient was under lacosamide treatment with low pain levels. The proportion of spontaneously active C-fibers was dramatically reduced. This shows that lacosamide, which was individually identified as a treatment option on iPSC-derived nociceptors in-vitro, specifically modified the patient's peripheral neurons to revert pathology.

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Invited lectures

ForIPS Symposium, December 6, 2017, München, iPSC disease modeling – current aspects

BMBF research group meeting, November 16, 2017, Köln, Human stem cell based disease modeling – an update

Publications during funding period

none

E26 - Progress Report

01.03.2016 - 31.08.2018

Genetics and pathomechanisms of intellectual disability with microcephaly

Prof. Dr. 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.

De novo missense variants in *RHOBTB2* cause a developmental and epileptic encephalopathy

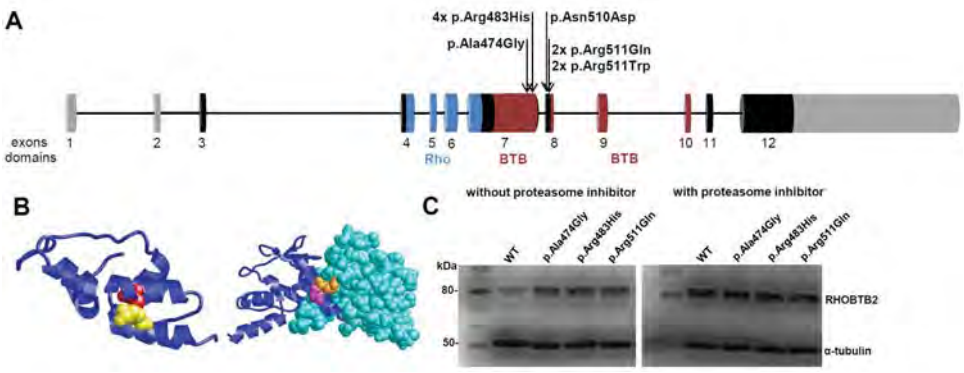
By trio exome sequencing and collaboration with colleagues worldwide, we identified *de novo* missense variants in *RHOBTB2* in ten individuals with a developmental and epileptic encephalopathy. The highly similar phenotype includes early onset seizures, severe intellectual disability, postnatal microcephaly, movement disorders and developmental regression.

RHOBTB2 encodes an atypical RhoGTPase, which is activated by interaction with other proteins that relieves its auto-inhibited conformation and is inactivated by auto-ubiquitination and degradation in the proteasome. The latter is mediated by binding of the BTB domains of RHOBTB2 to the ubiquitin ligase scaffold cullin 3 and thus to a cullin dependent ubiquitin ligase complex.

Three of the four different variants we identified are recurrent, and all cluster within the BTB domain encoding region, thus pointing to a domain specific pathomechanism.

RHOBTB2 variants result in increased protein levels

Co-immunoprecipitation did not show impaired binding of mutant RHOBTB2 to CUL3. However, 24 h after transfection of HEK293 cells we found increased levels of mutant RHOBTB2 compared to wildtype. When adding proteasome inhibitor, protein levels were equal between mutant and wildtype. These observations indicate that the identified variants in RHOBTB2 might impair its proper ubiquitination and degradation in the proteasome. This is probably not mediated by a direct interaction with CUL3 but by impaired BTB domain stability or dimer formation. Increased levels of RHOBTB2 and other RHOBTB2-dependent substrates might therefore be relevant for the disease phenotype.



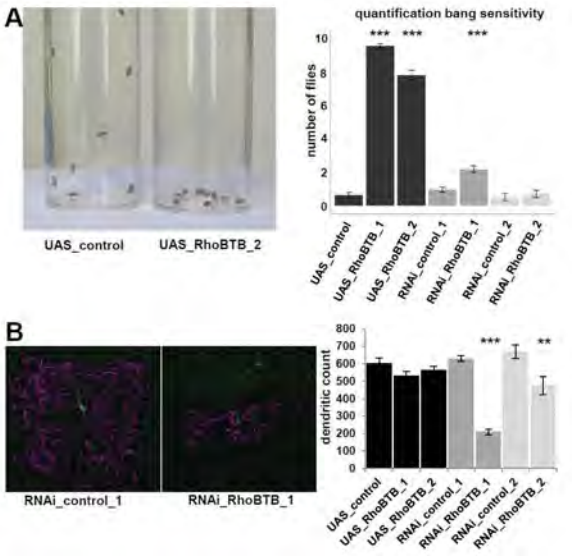
A: Identified mutations in RHOBTB2 cluster and are B: located within the first BTB or at the interface of the second BTB domain. C: Increased protein levels of mutant RHOBTB2 compared to wildtype and equal levels when adding proteasome inhibitor.

Prof. Dr. Dr. Zweier

Altered dosage of RhoBTB in *Drosophila* results in seizure susceptibility and other neurological phenotypes

As the identified variants in RHOBTB2 seem to result in impaired degradation and thus increased amount of protein, manipulating RhoBTB dosage in *Drosophila melanogaster* by tissue specific knockdown and particularly overexpression appeared to be a very suitable modelling approach. In accordance with the human phenotype we found seizure susceptibility in the fly that was more severe upon pan-neuronal overexpression than upon knockdown. Furthermore, severe, gross neurological, locomotor defects were observed upon overexpression of RhoBTB in all neurons and particularly in motoneurons, while complex learning and memory processes were unaffected. This might possibly support a contributory effect of epileptic activity to the severity of intellectual disability in the affected human individuals. The observation of more severe neurological phenotypes upon overexpression of RhoBTB than upon knockdown is in line with the assumption from humans that increased RHOBTB2 levels contribute to the disease causing mechanism.

Additionally, we found knockdown of RhoBTB in *Drosophila* dendritic arborization neurons resulting in a decreased number of dendrites, thus suggesting a role of RhoBTB in dendritic development.



A: Bang sensitivity: after vortexing, flies overexpressing RhoBTB (UAS) remain trembling and shaking on the bottom. Mild or no bang sensitivity upon knockdown (RNAi). B: Dendritic arborization neurons with fewer dendrites upon knockdown of RhoBTB.

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Invited lectures

TMF Workshop Omics in Medial Research, December 5, 2017, Berlin, Undiagnosed Pediatric Diseases

7th european course in clinical dysmorphology „what I know best“, November 6-7, 2017, Rome, Italy, 3p25 deletion syndrome

Publications during funding period

Popp B, Ekici AB, Thiel CT, Hoyer J, Wiesener A, Kraus C, Reis A, Zweier C (2017) Exome Pool-Seq in neurodevelopmental disorders. Eur J Hum Genet 25: 1364-1376

Reuter MS, Krumbiegel M, Schlüter G, Ekici AB, Reis A, Zweier C (2017) Haploinsufficiency of NR4A2 is associated with a neurodevelopmental phenotype with prominent language impairment. Am J Med Genet A 173: 2231-2234

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

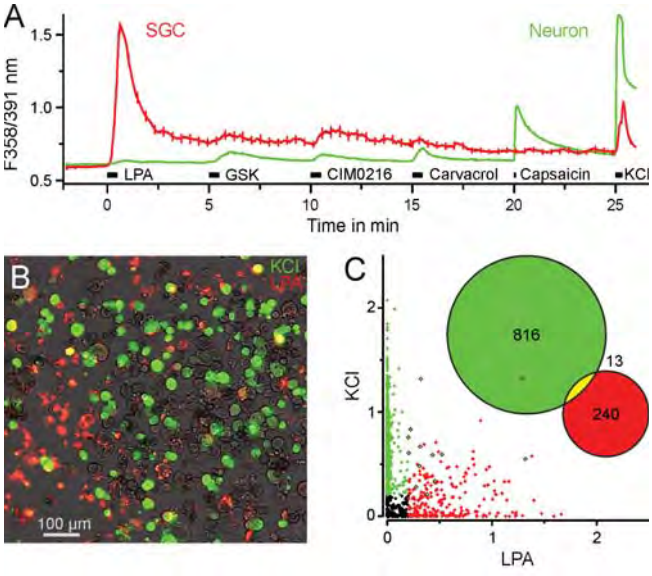
Lysophosphatidic acid-induced pruritus of cholestasis

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. 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. Only 1.6% (13 of 829) 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 correlated inversely ($r = -0.37$, $p<0.001$, $n=1237$, 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 18:1 caused only a marginal activation of heterologously expressed TRPV1, and responses in dorsal root ganglion cultures from TRPV1-deficient mice were similar to controls. The LPA 18:1-induced increase in cytoplasmatic calcium stems from the endoplasmatic reticulum. In combination with LPA receptor expression results from DRGs and Schwann cells, pharmacological results indicate a signaling pathway through LPA receptor 1.

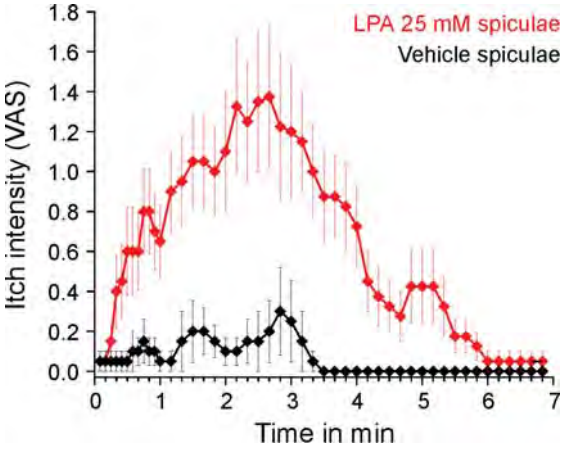


LPA activates satellite glia cells but only 1.6% of neurons. Left panel: Overlay of the transmission image (greyscaled) and the response intensity to LPA (red) and to KCl (green). Right panel: Scatterplots of the ratio increase for every cell to LPA and Capsaicin.

Dr. Dr. Kremer Prof. Dr. Fischer

LPA-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. In preliminary, using microneurography LPA activated both human mechosensitive (CM) and mechanoinsensitive (CMI) nerve fibres.



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.

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Invited lectures

Expert summit on viral hepatitis, 2017, Berlin, Primär biliäre Cholangitis – nicht nur der Name ist neu

PBC in Motion, 2017, London, UK, Managing symptoms of disease

Hepatologie-Update, Hamburger Lebertage, 2017, Hamburg, Juckreiz bei Lebererkrankungen – was kann man therapeutisch tun

4th Porto Liver Meeting, 2017 Porto, Portugal, Pruritus management in liver diseases

Federation of the European Neuroscience Societies, 2017, Pecs, Hungary, Tissue acidosis-induced pain

FEPS 2017, Wien, Austria, TRPA1 and TRPV1 photosensitization by 7-Dehydrocholesterol

FEPS 2017, Wien, Austria, Human sensors for tissue acidosis

FEPS 2017, Wien, Austria, Lysophosphatidic acid activates peripheral glial cells

Awards

Young Investigator Award, A.E. Kremer, 2017, International Liver Meeting, EASL, Amsterdam

Publications during funding period

Babes A, Ciotu CI, Hoffmann T, Kichko TI, Selescu T, Neacsu C, Sauer SK, Reeh PW, Fischer MJM (2017) Photosensitization of TRPA1 and TRPV1 by 7-dehydrocholesterol: implications for the Smith-Lemli-Opitz syndrome. Pain 158(12): 2475-2486

Mack K, Fischer MJM (2017) Disrupting sensitization of TRPV4. Neuroscience 352: 1-8

Schwarz MG, Namer B, Reeh PW, Fischer MJ (2017) TRPA1 and TRPV1 antagonists do not inhibit human acidosis-induced pain. J Pain 18(5): 526-534

He GW, Günther C, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S, Becker C (2017) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut 66(4): 716-723

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

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

F3 - Final Report

01.03.2014 - 28.02.2017

Fam60a in heart and brain development

Prof. Dr. Felix Engel, Department of Nephropathology

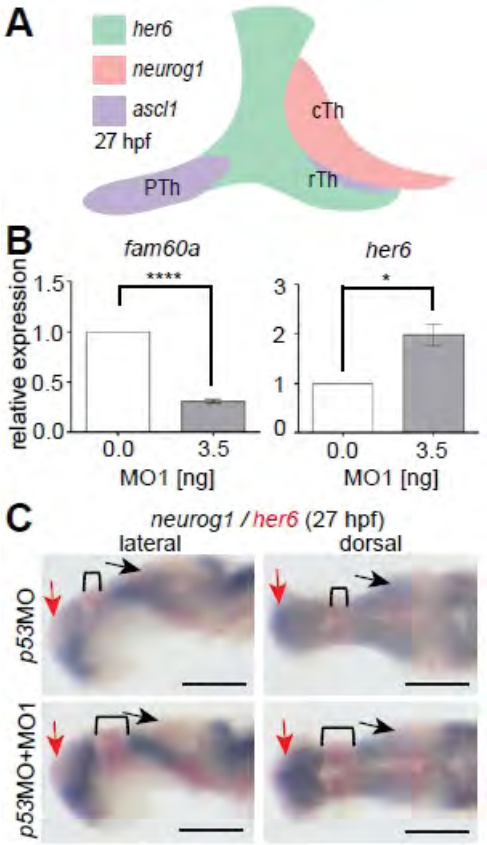
Neurodevelopmental disorders are the most common and disabling long-term complication of congenital heart diseases and thus the NHLBI stated "one of the most important challenges in the 21st century for CHD patients is to improve neurological deficits." The goal of this project is to better understand 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.

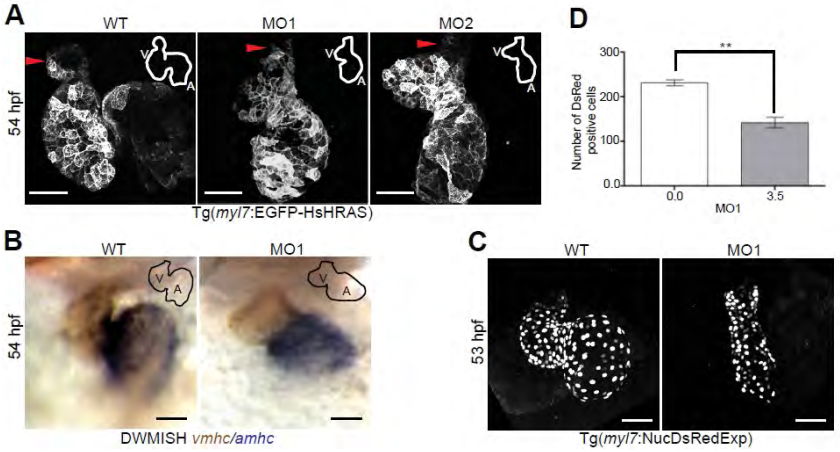
fam60a knockdown disrupts brain development altering her6, neurog1, and ascl1b expression

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 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.

Prof. Dr. Engel



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.

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.

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.

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

Max-Planck-Institut für molekulare Biomedizin, April 24, 2017, Münster, Cardiac regeneration: from zebrafish to mammals

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

F5 - Progress Report

01.07.2016 - 31.12.2018

The Role of ANO1 in Polycystic Kidney Disease

Dr. Björn Buchholz, Department of Medicine 4

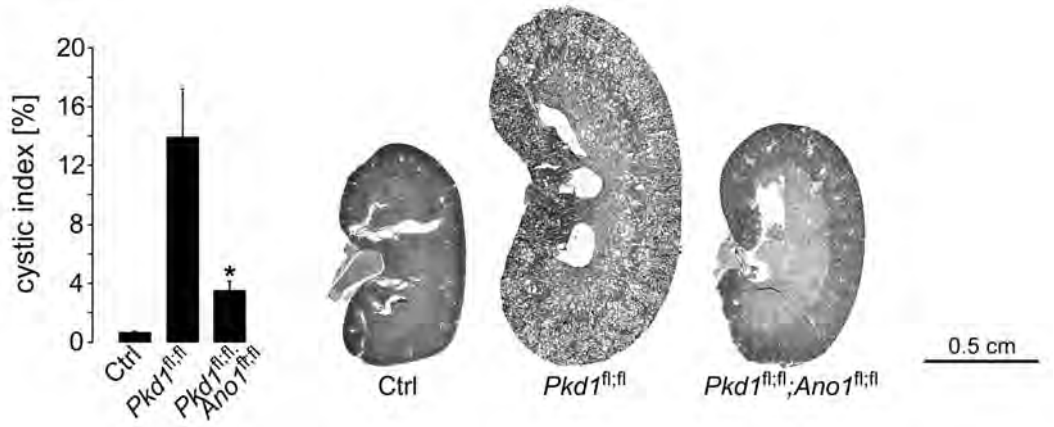
Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a frequent renal disorder which is characterized by continuous secretion-dependent growth of multiple cysts in both kidneys often resulting in end stage renal disease. We have shown that the calcium-activated chloride channel anoctamin 1 (ANO1) significantly promotes cyst growth in vitro. Now we want to characterize the role of ANO1 in vivo in an ADPKD mouse model and want to analyse the mechanisms leading to ANO1 activation.

ANO1 promotes cyst growth in an ADPKD mouse model

We have established an ADPKD mouse model in collaboration with Prof. Peters (Dept. of Human Genetics, Leiden). This mouse model is characterized by an inducible tubule-specific deletion of the PKD1-gene ($KSPCreER^{T2}$;$PKD1^{lox;lox}$), the main affected gene in human ADPKD. Deletion of PKD1 by application of tamoxifen at postnatal day 20 resulted in significant polycystic kidney disease within 9 weeks. In contrast, double knockout of PKD1 and ANO1 significantly ameliorated the renal cystic phenotype. At the moment, we are testing several ANO1 inhibitors in our established *in vitro* cyst model for their efficacy and toxicity in order to find a potential candidate to be additionally tested *in vivo.*

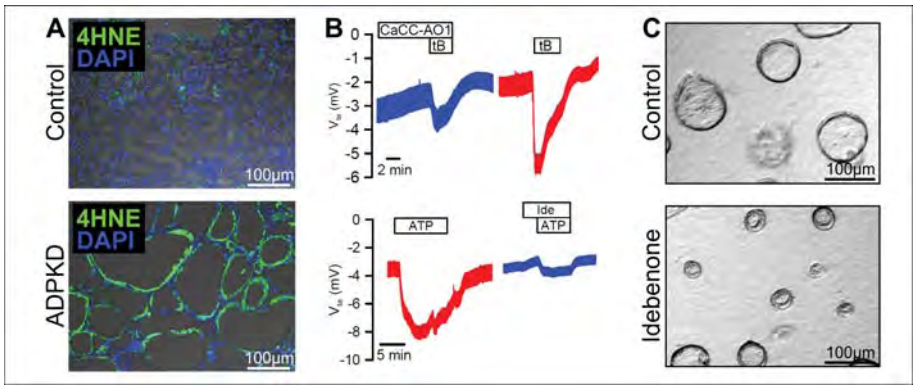
P2Y2R mediates ANO1-dependent chloride secretion and cyst expansion

Cyst growth is driven by chloride secretion. Since ANO1-dependent chloride conductance is activated by cytosolic increase of calcium, we wanted to understand the underlying mechanism. We found that ATP, which has been shown to accumulate in the cyst fluid, leads to strong activation of ANO1. In line with these findings, we found that the Gq-coupled purinergic receptor P2Y2R mediates ANO1-dependent chloride secretion and *in vitro* cyst expansion. Furthermore, we could show that P2Y2R is a target gene of the hypoxia inducible transcription factor HIF-1α which is in line with our previous findings that showed that HIF-1α promotes secretion-dependent cyst enlargement. Additionally, we have generated *in vivo* data further supporting the role of HIF-1α for cyst growth which has recently been submitted.



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



A 4HNE = indicator for ROS. B Effect of lipid peroxidizing tert-butyl hydroperoxide (tBHP) on transepithelial voltage ± ANO1 inhibitor CaCC-A01 and the impact of idebenone on ATP-dependent chloride secretion. C In vitro cyst growth ± idebenone.

Reactive oxygen species (ROS) lead to activation of ANO1 via lipid peroxidation

Recently, we found that mouse and human ADPKD kidneys are stained positive for 4-Hydroxynonenal (4-HNE), an indicator for oxidative stress. Interestingly, idebenone, a ROS scavenger, has been reported as a direct inhibitor of ANO1. We now have significant data that show that idebenone is not a direct inhibitor of ANO1 but prevents lipid peroxidation like other antioxidants and ROS scavengers which consecutively prevents activation of ANO1. In line with these findings, ROS activation promotes cyst growth in vitro, which can be inhibited by antioxidants like idebenone but also by direct inhibitors of ANO1 like CaCC-inhA01. These findings are of importance since idebenone is a dietary supplement that is currently tested in clinical trials for a number of diseases with pro-oxidant/pro-inflammatory alterations, particularly neurodegenerative diseases and therefore could qualify as a therapeutic option to retard renal cyst growth. A manuscript has been prepared and is currently under review.

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Invited lectures

Annual congress of the Deutsche Gesellschaft für Nephrologie (DGfN), September 15, 2017, Mannheim, The impact of hypoxia on cyst growth in polycystic kidney disease

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, 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.

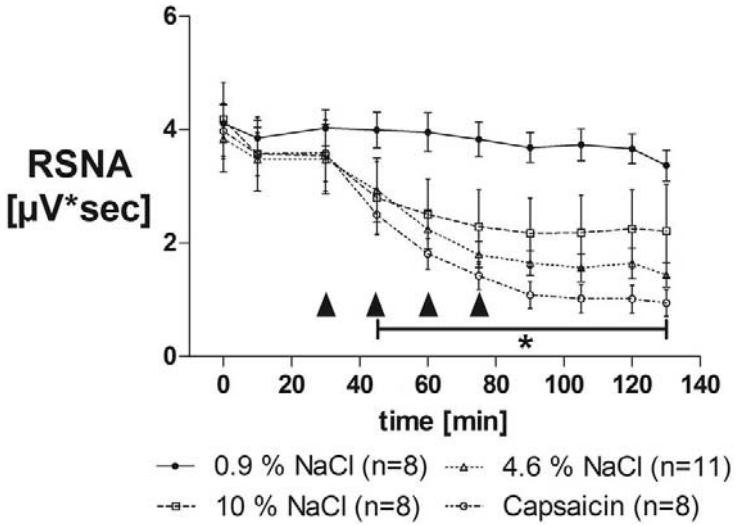
Stimulation of Renal Afferent Nerves?

Since only neurons with renal projections from stenotic kidneys in a rat model of renal hypertension produced action potentials upon respective stimulation - a finding that could not explained by intrarenal pressure alterations or inflammation in vivo - it was assumed at first that rather altered perfusion occurring in a clipped kidney with renal stenosis could be of pivotal importance. However, there is no doubt that renal afferent nerve units are bimodal in that they respond to mechanical and chemical stimuli.

In clipped kidneys of the renovascular rat model the activity of the renin-angiotensin system is intrarenally increased. Increased intrarenal angiotensin II level will alter renal sodium handling. Hence, it could be possible that renal afferent nerve fibers could respond to altered or increased sodium concentrations in the vicinity of interstitially located intrarenal afferent nerve endings.

Na^+ – a strong stimulator of neurons with afferent axons from the kidneys

Hence, in a next step before the above mentioned hypothesis could be tested in experimental renovascular hypertension we investigated in how far increasing sodium concentrations could alter currents and the activity of neurons with renal afferents in vitro. Furthermore, it had to be tested in how far intraarterial bolus applications of increasing concentrations of sodium into the kidney in vivo would induce sympathetic nerve activity decreases. It turned out that in vitro administering of NaCl to cultivated DRG neurons with projections from the kidney elicited higher inward currents and also increased the production



Decreases of renal sympathetic nerve activity (RSNA) in vivo due to intrarenal boli of high salt (4.6% and 10 %) and capsaicin (3.3*10-7M–3.3*10-6M) compared to control (applications: arrow heads). (* $p < 0.05$). Intrarenal capsaicin is reference drug for eliciting sympathoinhibition.

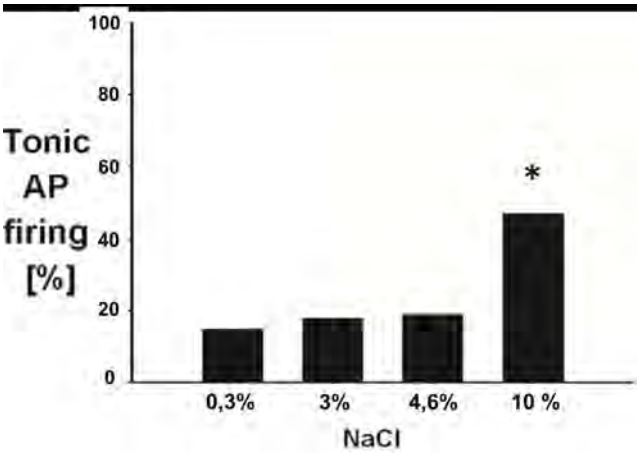

Prof. Dr. Veelken


Prof. Dr. Amann

of action potentials. Furthermore, efferent renal sympathetic nerve activity was decreased due to intrarenal salt application for several hours. These findings support our hypothesis that sodium too is able to elicit a prominent sympathoinhibition via stimulation of renal afferent nerve fibers. In how far this sympathoinhibitory sodium dependent mechanism is impaired in renovascular hypertension is to be tested.

Bradykinin – Sympathoexcitation via renal afferent nerves?

Reports on the effects of bradykinin were most challenging concerning our hypothesis in that bradykinin might have elicited a sympathoexcitatory response via afferent renal nerves in this respect. However, we could demonstrate that intrarenally administered bradykinin only elicited short-term increases of renal sympathetic nerve activity (pain fibers from renal capsule?) whereas the same dose of bradykinin induced a strong, longlasting decrease in renal sympathetic activity. This observation supports the general hypothesis of the project even further.



The percentage of renal neurons in vitro with higher firing activity (> 4 action potentials (AP) in 10 seconds so called tonic neurons) increased due to stimulation with higher saline concentrations (* $p < 0.05$).

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Invited lectures

Experimental Biology 2017, April 22-26, 2017, Chicago, Illinois, USA, Bimodal action of intrarenal afferent stimulation by Bradykinin on RSNA: Tonic inhibition after short excitation (Martin Hindermann)

Publications during funding period

none

Junior Groups / Projects

Progress and Final Reports

112

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 research appointments:

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 Internship 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 theme 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 in part to 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 therapeutical 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 novel drugs even in initially 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 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 inter-conversion 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.

Aim of the research: The Junior Group aims at discovering fundamental druggable 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. By high-throughput approaches we have already identified a number of potential EMT/CSC-regulating metabolic mechanisms, which we aim to validate by the analysis of human samples and functionally investigate by the use of cell and molecular biology techniques. This approach may ultimately lead to the identifications of novel targets for therapeutic intervention.

Third-party funding

Paolo Ceppi, German Cancer Aid Research Grant, Determination of the role of aldose reductase AKR1B1 and associated pathways in epithelial-to-mesenchymal transition and cancer stem cells, 2017-2020

Paolo Ceppi, International Association for the Study of Lung Cancer, The role of thymidylate synthase in epithelial-to-mesenchymal transition in NSCLC, 2017-2018

Paolo Ceppi, DFG Research Grant, Whole-genome CRISPR/Cas9 mediated identification of miR-200 repressors, 2018-2021

N1 - Progress Report

01.08.2015 - 31.07.2021

Understanding the plasticity of cancer cells

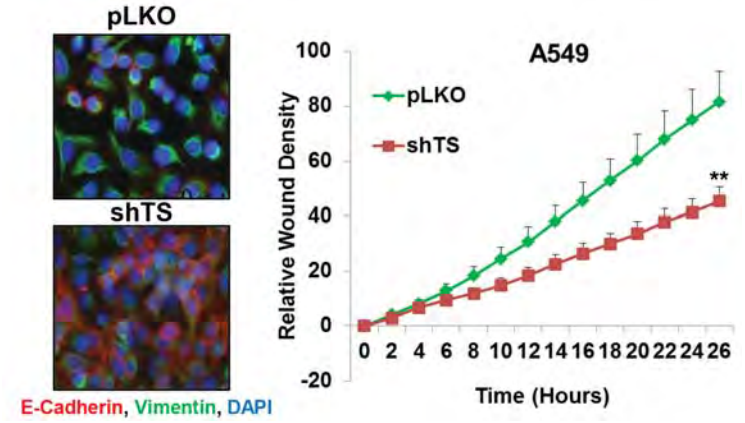
Dr. Paolo Ceppi, IZKF - Junior Research Group 1

The group focuses on 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. The final goal is the development of more effective drugs and therapeutic strategies.

The activity of the lab during the reported period has been mainly focused in two projects exploring the role of two metabolic enzymes in the plasticity of cancer cells:

1) The role of thymidylate synthase in EMT and cancer stemness (Siddiqui et al. The Journal of Pathology, 2017).

Thymidylate synthase (TS) is a fundamental enzyme in the nucleotide metabolism and one of the oldest anti-cancer targets. We discovered a significant correlation between TS and the markers of EMT, a developmental process that allows the cancer cells to acquire features of aggressiveness, like motility and chemoresistance. TS levels were found significantly augmented in mesenchymal-like compared to epithelial-like cancer cells, and up-regulated following EMT induction by TGF-Beta. Moreover, mesenchymal-like cells were found more resistant to TS-inhibiting drugs. Importantly, a particularly relevant association was found between TS and the powerful EMT driver ZEB1, which was confirmed in clinical specimens from lung tumors and in a genetic mouse model of pancreatic cancer with ZEB1 deletion. A bioinformatic analysis revealed that TS expression was negatively correlated with that of several EMT-suppressing microRNAs (miRNA)s. By luciferase assays we could confirm the role of EMT-suppressing miRNAs in regulating TS, and we identified a novel specific role for miR-375 in targeting TS 3'UTR. Functionally, we found that ZEB1 could indirectly increase TS levels through the regulation of miR-375. Interestingly, TS itself showed to have a regulatory role on EMT in cancer cells. Conversely, TS overexpression could promote EMT and stem-like markers. All together, these data indicate the existence of an unprecedented functional loop involving the TS enzyme, ZEB1 and EMT-related miRNAs that govern cancer differentiation.

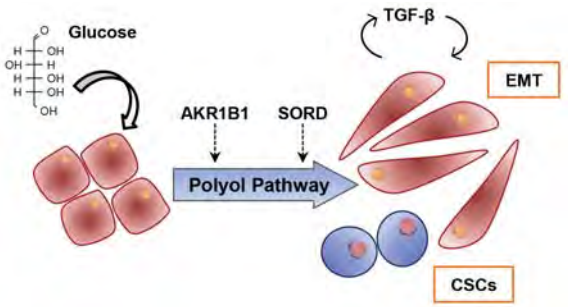


Left, Thymidylate synthase (shTS) knockdown suppresses EMT in lung adenocarcinoma cells increasing E-Cadherin and reducing Vimentin levels (compared to controls, pLKO). Right, a real-time migration assay reveals a significantly reduced motility in cancer cells upon TS knockdown.

Dr. Ceppi

2) The role of polyol pathway in linking glucose metabolism to the aggressiveness of cancer cells (Schwab et al. Submitted).

By performing a transcriptomic analysis we identified the glucose-transforming polyol pathway (PP) gene aldo-keto-reductase-1-member-B1 (AKR1B1) as strongly correlated with epithelial-to-mesenchymal transition (EMT). This association was confirmed staining samples from lung cancer patients and from an EMT-driven colon cancer mouse model with p53 deletion. In vitro, mesenchymal-like cancer cells showed increased AKR1B1 levels and AKR1B1 knockdown was sufficient to revert EMT. An equivalent level of EMT suppression was measured by targeting the downstream enzyme sorbitol-dehydrogenase (SORD), further pointing at the involvement of the PP. Comparative RNA sequencing profiling confirmed a profound alteration of EMT in PP-deficient cells, revealing a strong repression of TGF-β signature genes. Mechanistically, excess glucose was found to promote EMT through autocrine TGF-β stimulation, while PP-deficient cells were refractory to glucose-induced EMT. PP represents a molecular link between glucose metabolism and cancer differentiation and aggressiveness, and a novel potential therapeutic target.



Scheme of the identified role of polyol pathway (PP) genes in EMT and cancer stem cells (CSCs). PP genes drive/support cancer EMT and the generation of cells with the phenotype of CSCs by an autocrine TGF-Beta stimulation.

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Invited lectures

I Conference of Cancer Pharmacology Research, December 13, 2017, St. John's University, New York City, USA, A novel role for thymidylate synthase in regulating EMT and determining tumor aggressiveness

IX Chinese-German Lung Cancer Cooperative Group Meeting 2017, September 1, 2017, Shanghai, China. Targeting stemness and the mechanisms of treatment resistance in Lung Cancer

Seminars of the Institute for Medical Microbiology and Hygiene, May 11, 2017, University of Regensburg, Metabolic pathways as regulators of epithelial-to-mesenchymal transition

5th iTarget Workshop on Tumor Microenvironment, March 15, 2017, Uniklinikum Erlangen, The role of CD95 and CD95 ligand in cancer

Awards

Young Investigator Award of the International Association for the Study of Lung Cancer, Paolo Ceppi, May 4, 2017

Publications during funding period

Rasheed SAK, Leong HS, Lakshmanan M, Raju A, Dadlani D, Chong FT, Rajarethinam R, Skanthakumar T, Tan EY, Hwang JSK, Lim KH, Tan DS, Ceppi P, Wang M, Tergaonkar V, Casey PJ, Iyer NG (2017) GNA13 expression promotes drug resistance and tumor-initiating phenotypes in solid tumors. Oncogene doi: 10.1038/s41388-017-0038-6

Siddiqui A, Vazakidou ME, Schwab A, Napoli F, Fernandez-Molina C, Rapa I, Stemmler MP, Volante M, Brabletz T, Ceppi P (2017) Thymidylate synthase is functionally associated with ZEB1 and contributes to the epithelial-to-mesenchymal transition of cancer cells. The Journal of Pathology 242: 221-233

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 viral and cellular transcription and replication at the single-molecule level using biophysical techniques.

Before starting his lab, Dr. Dulin graduated his Bachelor in physics and mathematics at the University of Bordeaux (France) in 2004 and his Master "Laser, Matter and Nanoscience" in 2006.

Between 2006 and 2009, he was a PhD candidate in the Laboratory Charles Fabry of the Institut d'Optique (Paris) 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 then moved to a first postdoctoral position in the lab of Prof. N. Dekker at TU Delft (The Netherlands), where he stayed until August 2014. There, he developed new magnetic tweezers approaches for high throughput and high-resolution study of polymerases and helicases kinetics. In particular, he studied the mechanism of misincorporation and antiviral nucleotide analogue incorporation by viral polymerase.
He then moved to the University of Oxford (UK) for a second postdoctoral position, where he studied bacterial transcription initiation dynamics using single-molecule FRET in the lab of Prof. A. Kapanidis, until being appointed in Erlangen.

From the left: E. Ostrofet, F. Stal Papini, M. Seifert

Research Focus

The Dulin lab aims at understanding the fundamental processes involved in the central dogma of molecular biology, i.e. replication, transcription and translation, using high-end microscopy. Each step in gene expression involves complex molecular motors, e.g. DNA polymerase, RNA polymerase (RNAP), ribosome. Much has been learned related to these motors using standard ensemble biochemical assays, but their detailed kinetic characterization remains elusive. Indeed, these enzymes do not progress linearly along their template, but rather through burst of successive catalytic reactions interrupted by pauses of various origins, e.g. co-factors binding, misincorporation, which makes gene expression highly stochastic, and impacts the organisms phenotype. By accessing enzymatic processes one molecule at a time, and not the average behavior of many enzyme, single-molecule biophysics has changed our view on biology, offering an understanding of the rare, transient and stochastic — but important — events that interrupt enzymatic activity. Our lab develops high-end microscopy techniques, such as magnetic tweezers and single-molecule Fluorescence Resonant Energy Transfer (FRET), to describe in great details (1) how RNA viruses replicate their genome and (2) how the human mitochondrial genome is transcribed.

1- Flavivirus replication mechanism

RNA viruses represent an important class of human and animal pathogens. They are responsible of numerous pandemics worldwide, with an important economical and societal cost. Amongst RNA viruses, the Flavivirus genus is the most resurgent and emergent family of virus. Members of this family, e.g. Dengue, West Nile and Zika, are responsible for hundreds of million of viral infections every years, with little or none therapeutic options. One key target for drug development is the replication machinery of these viruses. However, little is known concerning the mechanisms of genome replication in flavivirus, limiting the potential development of drugs. Using magnetic tweezers and single-molecule FRET, we aim at understanding how the viral enzymes constituting the flavivirus replicase are recruited and how they work together during viral genome replication.

2- Transcription in human mitochondria

Mitochondria are dynamic, double-membrane-bound organelles that are essential components of the eukaryotic cell. They are involved in many cellular processes, but they are mainly known as the powerhouse of the eukaryotic cell by providing the major source of cellular energy, i.e. ATP. Due to the importance of the mitochondria in many cellular processes, abnormal mitochondria activity is linked to several disorders, including diabetes, obesity, cardiovascular disease, Parkinson's, Alzheimer's and cancer. The mitochondria genome is a gene-dense ~16 kb circular genome that is transcribed by the mitochondrial RNA polymerase (mtRNAP), which initiates transcription out of three promoters. Though the mitochondria transcription complex is relatively simple, e.g. only two other factors in addition to mtRNAP necessary for transcription initiation, little is known on the regulation and kinetics of mitochondria transcription initiation, elongation and termination. Using magnetic tweezers and single-molecule FRET assays, we investigate the mechanism of mitochondrial transcription.

N2 - Progress Report

01.09.2016 - 31.08.2022

Physics and Medicine

Dr. David Dulin, IZKF - Junior Research Group 2

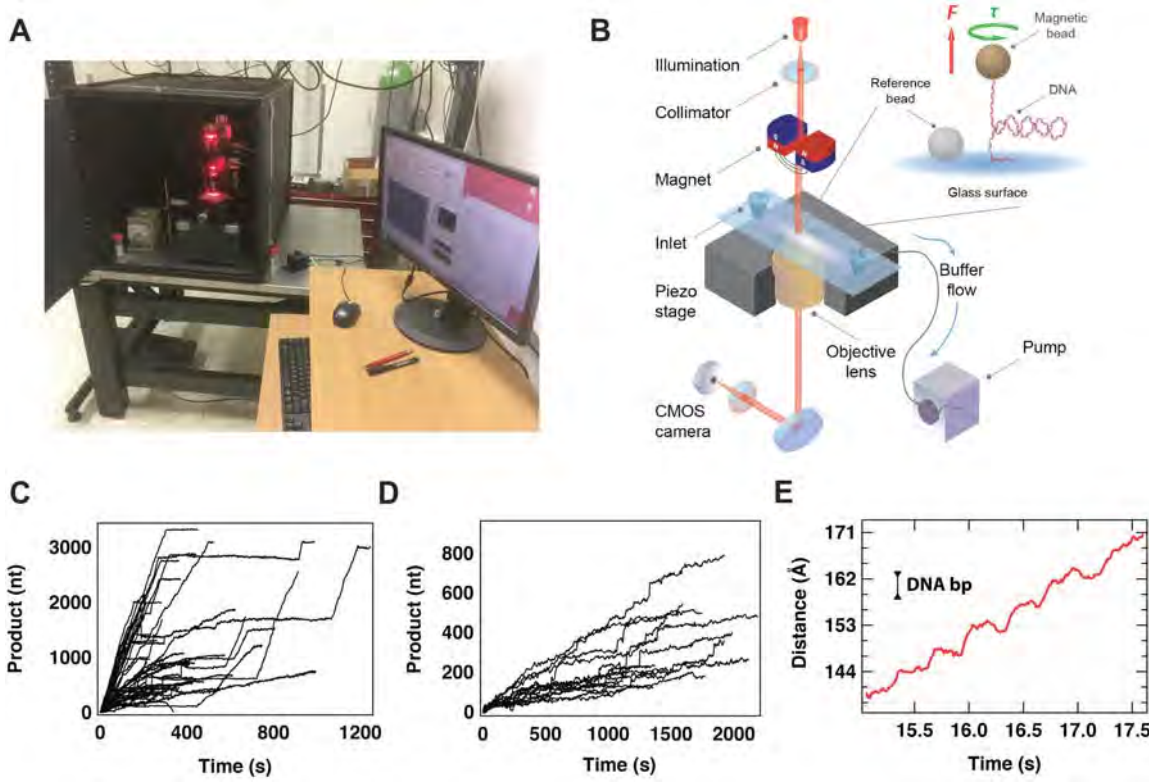
The Junior Group aims at understanding the molecular processes that regulate gene expression using high-end microscopy. We therefore develop single-molecule biophysics apparatuses to access enzymatic processes at the single-molecule level with high spatial (~nm) and temporal (~ms) resolution, to understand how nucleic acids are replicated and transcribed. In particular, we aim at characterizing flaviviruses (Dengue, Zika) genome replication and human mitochondrial genome transcription.

In September 2016, the Junior Research Group N2 has started a new research activity in Erlangen, focused on the study of molecular processes with high-end microscopy. During the first six months, we have established a fully functional molecular biology lab to synthesize the nucleic acids scaffolds used in magnetic tweezers experiments. We have also established two magnetic tweezers assays in the microscopy lab. The molecular biology lab space was operational according to biosafety requirement from the end of October 2016, and was subsequently equipped with all the major equipment for nucleic acids synthesis, purification and evaluation. We also developed a microfluidic workshop to assemble the microfluidic flow chambers used in microscopy experiments. In addition, the lab has grown with new members: Dr. Flavia Stal-Papini (research assistant, October 2016), Eugen Ostrofet (PhD candidates, May 2017) and Mona Seifert (PhD candidates, January 2018). A new PhD candidate, Monika Spermann, will join us in February 2018.

To perform single-molecule magnetic tweezers experiments, it is necessary to design and synthesize specific DNA scaffold. Though using standard molecular biology techniques, the making of a nucleic acid scafold with a high yield and high purity remains challenging. We have now established protocols to synthesize DNA and RNA hairpins, linear DNA and RNA for the different experiments we performs in our lab. We are preparing an article that describes the new protocols we have developed. Magnetic tweezers are a force and torque spectroscopy technique, i.e. one uses them to apply force and torque on nucleic acids. However, a complete force calibration needs to be performed first, which necessitates DNA scaffolds of a defined length. Following the fabrication of the appropriate DNA scaffold, we have calibrated in force our magnetic tweezers apparatus with a novel approach, which will result in a new publication.

Following these preparatory steps, we have performed magnetic tweezers experiments on viral polymerase replication activity, and we have successfully observed poliovirus and dengue polymerases replication. We have further tested the resolution of our assay, and show that we can measure steps down to ~0.3 nm. We are now collecting preliminary results for grant applications.

Dr. Dulin



(A) Magnetic tweezers set up. (B) Schematic description of the magnetic tweezers in (A). (C) Traces of individual poliovirus polymerase from a single experiment. (D) Same as in (C) with Dengue polymerase. (E) 0.3 nm steps detected using high-resolution magnetic tweezers.

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Invited lectures

FASEB science research conference, June 2017, Saxtons River, Vermont, USA, Mechanism and Regulation of Prokaryotic Transcription

SFB960-Symposium, October 2017, Regensburg, The Biology of RNA-Protein Complexes

Publications during funding period

Kriegel F, Ermann N, Forbes R, Dulin D, Dekker NH, Lipfert J (2017) Probing the salt dependence of the torsional stiffness of DNA by multiplexed magnetic torque tweezers. Nucl. Acids Res. 45 (10): 5920-5929

Dulin D, Arnold JJ, van Laar T, Oh H-S, Lee C, Harki DA, Depken M, Cameron CE, and Dekker NH (2017) Signatures of Nucleotide Analogue Incorporation by an RNA-Dependent RNA Polymerase Revealed Using High-Throughput Magnetic Tweezers. Cell Reports 21 (4): 1063-1076

Junior Projects

Immunology and Infection

Project No.	Project title	Term	Applicant	Institute
J45	Modulation of PRC2 activity by HCMV IE2	01.01.2015-30.06.2018	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
J62	Mechanisms of neutrophil infiltration in rheumatoid arthritis	01.08.2017-31.01.2020	Dr. Klingberg	Department of Medicine 3
J63	IL-3 in inflammatory bowel disease	01.12.2017-31.05.2020	Dr. Zundler	Department of Medicine 1

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. 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	01.07.2018-31.12.2019	Dr. Müller	Department of Medicine 5
J67	Metabolic reprogramming of AML MDSCs	01.01.2018-30.06.2020	Dr. Dr. Jitschin	Department of Medicine 5
J68	Role of GATA4 in Intestinal Inflammation & Cancer	01.10.2017-31.03.2020	Dr. Patankar	Department of Medicine 1

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	01.10.2015-31.03.2019	Dr. Marxreiter	Department of Molecular Neurology
J52	Modeling cortical dysfunction of SPG11 spastic paraplegia using patient-derived pluripotent stem cells	01.11.2015-31.10.2018	Dr. Regensburger	Department of Neurology

Project No.	Project title	Term	Applicant	Institute
J53	Diffusion tensor imaging of the visual pathway in pseudoexfoliation glaucoma	03.08.2015-02.02.2018	Dr. Schmidt	Department of Neuroradiology
J66	ß subunits: adding pieces to the puzzle of pain	01.01.2018-30.06.2020	Dr. Eberhardt	Department of Anaesthesiology

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
J64	Nephroprotection by HIF-hydroxylase inhibitors	01.10.2017-31.03.2020	Dr. Grampp	Department of Medicine 4
J65	T-System Regulation by Glucocorticoids	01.11.2017-30.04.2020	Dr. Seidel	Institute of Cellular and Molecular Physiology

Molecular Medicine

Project No.	Project title	Term	Applicant	Institute
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	01.10.2016-30.04.2018	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

Modulation of PRC2 activity by HCMV IE2

Dr. Nina Reuter, Institute of Clinical and Molecular Virology

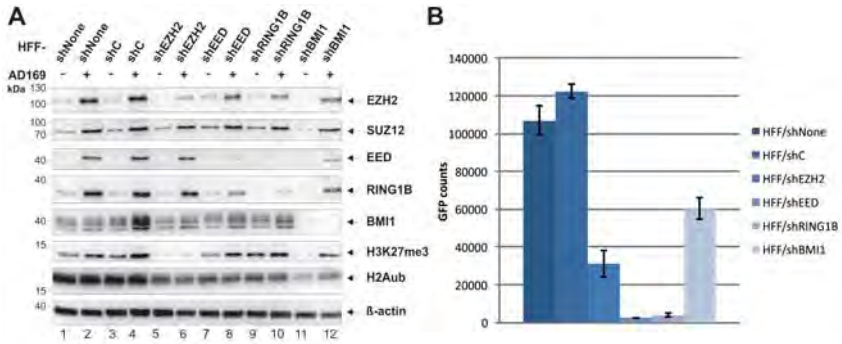
Chromatin-based modifications of herpesviral genomes play a crucial role in dictating the outcome of infection. Host cell multiprotein complexes like PML bodies or the Polycomb repressive complex 2 (PRC2) have been identified as regulators of viral gene expression on the epigenetic level. This proposal aims at investigating the role of PRC1/2 for HCMV infection as well as elucidating the mechanisms HCMV has evolved to modulate PRC1/2 function for its own benefit.

Role of PRC1/2 during lytic HCMV replication

To address the relevance of PRC1/2 activity for the productive life cycle of HCMV, we dissected the levels of PRC1/2 core components following HCMV infection. This revealed an HCMV-induced upregulation of the major PRC1/2 factors on the mRNA as well as protein level. By immunofluorescence staining, we found that all major PRC1/2 components, which are normally evenly distributed throughout the nucleus, relocalize into viral replication compartments as infection progresses. Interestingly, however, the repressive histone marks instituted by PRC1/2 turned out to be specifically excluded from these sites suggesting a differential role of both complexes independent of their repressor activity. This assumption is further supported by recent findings from literature showing a novel function of PRC1/2 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. In accordance with this, we observed a clearly impaired virus replication upon depletion of individual PRC1/2 core factors by shRNAs. Especially viral late gene expression, which is dependent on an efficient viral genome amplification, turned out to be negatively affected by the loss of PRC1/2 components. Indeed, by qPCR we could finally confirm a diminished viral DNA synthesis in the absence of PRC1/2 core factors indicating a requirement of polycomb group (PcG) proteins for an efficient HCMV DNA replication.

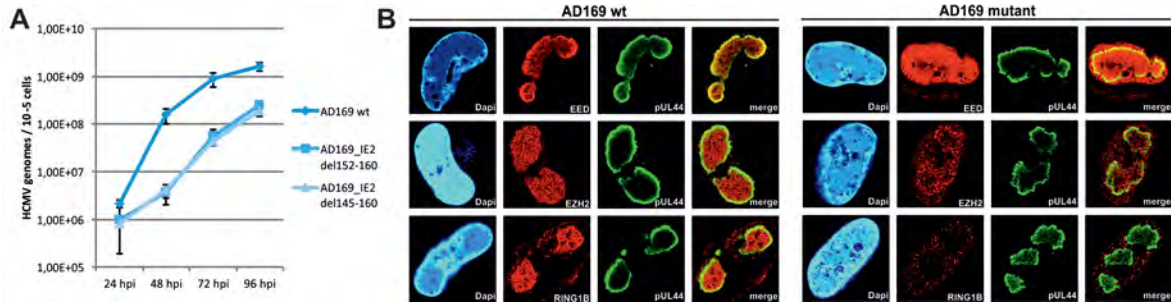
Analysis of the regulation of PRC1/2 activity by the HCMV effector protein IE2p86 (IE2)

By yeast two-hybrid screening and co-immunoprecipitation analysis, we discovered an interaction between the HCMV transactivator protein IE2 and the PcG protein EED, which is a shared component of both complexes, PRC1 and 2. 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 and all other PcG factors in viral replication centers



PRC1/2 core factors are required for an efficient HCMV replication. A) Verification of an efficient shRNA-mediated knockdown of individual PcG proteins. B) GFP-based assay of PcG protein-knockdown and control HFF cells infected with HCMV-GFP.

Dr. Reuter



IE2 redirects PRC1/2 into VRCs for an efficient viral genome amplification. A) Impaired viral DNA synthesis and B) inefficient PcG protein recruitment into VRCs of EED binding-deficient IE2 mutant viruses in comparison to wt HCMV.

(VRCs). This suggests that PRC1/2 are actively recruited into VRCs by the viral IE2 protein. To further elucidate the role of IE2 as a regulator of PRC1/2 activity, we generated recombinant viruses lacking the EED interaction interface within IE2. Multi-step growth curve analysis revealed a severe growth defect of the EED interaction-deficient IE2 mutants in comparison to wildtype (wt) HCMV. In accordance with our hypothesis, we observed an impaired intracellular accumulation of newly synthesized viral DNA in case of the mutant viruses which resulted from an incomplete relocalization of PcG proteins into VRCs when compared to wt HCMV. In summary, we identified a novel interaction between IE2 and EED, which contributes to the recruitment of PcG proteins into VRCs for efficient viral DNA replication.

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Invited lectures

Summer Symposium, Research Highlights at IZKF, July 21, 2017, Erlangen, The role of polycomb group (PcG) proteins in HCMV infection

42nd Annual International Herpesvirus Workshop, August 1, 2017, Ghent, Belgium, SUMOylation of IE2p86 is required for efficient autorepression of the human cytomegalovirus major immediate-early promoter

42nd Annual International Herpesvirus Workshop, August 1, 2017, Ghent, Belgium, Immediate-early protein 2 (IE2) of HCMV recruits polycomb repressive complex 2 into viral replication compartments for efficient viral DNA synthesis

Awards

Travel Award, 42nd Annual International Herpesvirus Workshop, Nina Reuter, July 2017, Ghent, Belgium

Priscilla Schaffer Memorial Award, 42nd Annual International Herpesvirus Workshop, Adriana Svrlanska, August 2017, Ghent, Belgium

Publications during funding period

Reuter N, Schilling EM, Scherer M, Müller R, Stamminger T (2017) The ND10 component promyelocytic leukemia protein acts as an E3 ligase for SUMOylation of the major immediate early protein IE1 of human cytomegalovirus. J. Virol. 91(10): 2335-16

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Kahle T, Volkmann B, Eissmann K, Herrmann A, Schmitt S, Wittmann S, Merkel L, Reuter N, Stamminger T, Gramberg T (2015) TRIM19/PML Restricts HIV Infection in a Cell Type-Dependent Manner. Viruses 8(1)

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 - Final Report

01.04.2015 - 30.09.2017

Neurosciences

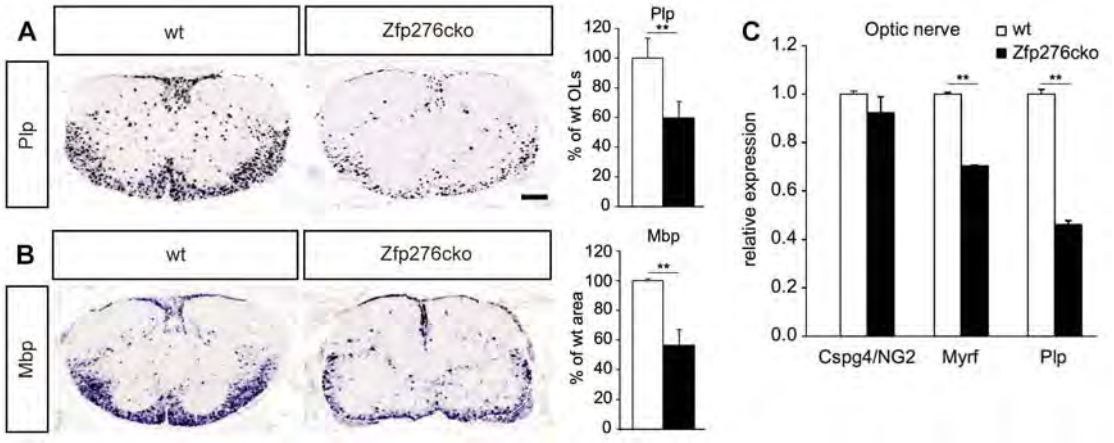
The role of zinc finger protein Zfp276 in glial development of the mouse nervous system

Dr. Melanie Küspert, Institute of Biochemistry

The transcription factor Zfp276 was recently identified as a candidate target of Sox10 during myelination. Induced Zfp276 expression in oligodendrocytes and Schwann cells during the onset of myelination argued for a regulatory role during this process. Direct regulation of Zfp276 by Sox10 now could be proven in vitro and in vivo. Analyses on Zfp276 function revealed reduced oligodendroglial differentiation after depletion of Zfp276 and increased differentiation upon precocious Zfp276 expression in oligodendrocyte precursors.

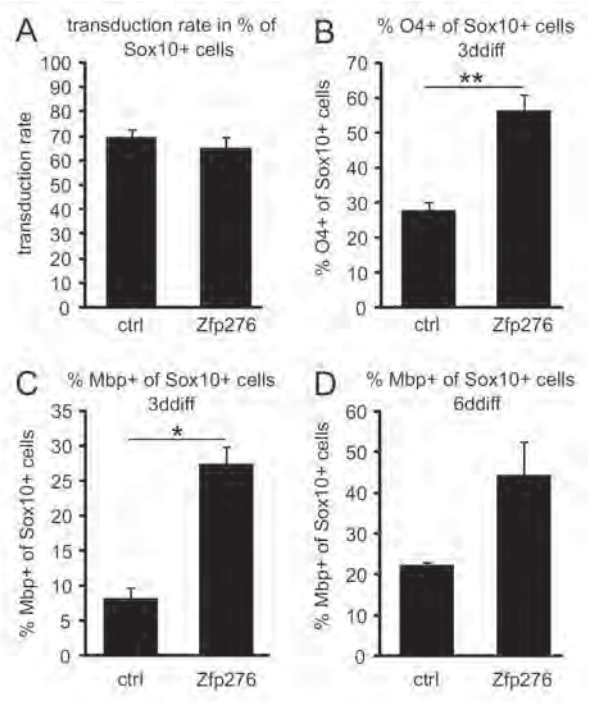
Background

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. 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.



(A, B) ISH was performed on spinal cord sections of control (wt) and conditional Zfp276 knockout (Zfp276cko) mice at P7. Antisense probes for Plp and Mbp mRNA were used. (C) qRT PCR was performed on total mRNA from optic nerve tissue of the same mice.

Dr. Küspert



Rat primary oligodendroglia were transduced with GFP-expressing (ctrl) and Zfp276/GFP co-expressing (Zfp276) retroviruses and differentiated in vitro for 3 (3ddiff) or 6 days (6ddiff) prior to IHC for Sox10 and O4 or Sox10 and Mbp.

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 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 argued 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.

Expression of early and late myelin genes is regulated by Zfp276 in oligodendroglial cells

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 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. Consistently, precocious retroviral expression of Zfp276 already in oligodendrocyte precursors led to increased numbers of immature and mature oligodendrocytes compared to control-transduced cells. Future experiments will have to elucidate the mechanism by which Zfp276 exerts its function during oligodendrocyte differentiation.

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Invited lectures

12th Göttingen Meeting of the German Neuroscience Society, March 22-25, 2017, Göttingen, Dusp15 in myelinating oligodendrocytes

Publications during funding period

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

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

Post-transcriptional regulation by Hoxa9

Dr. Christian Bach, Department of Medicine 5 – Haematology and Oncology

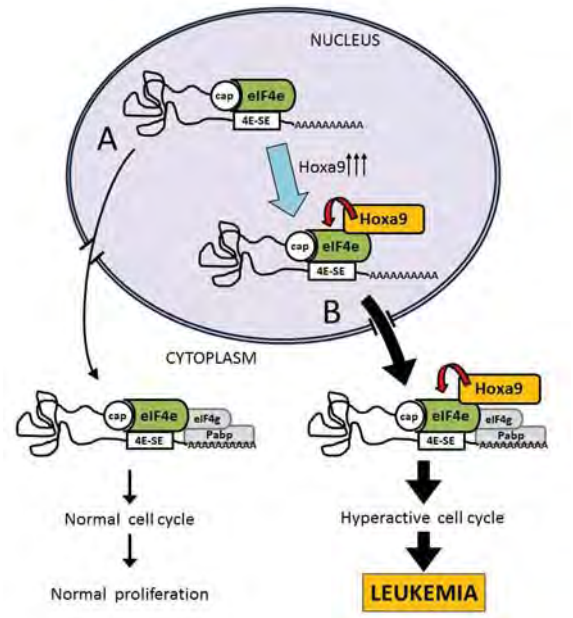
The oncogene Hoxa9 contributes to post-transcriptional regulation by interaction with the RNA export and protein synthesis regulator eIF4e. To date, target genes of this interaction have not been identified. Therefore, this project aims to identify post-transcriptional targets of Hoxa9 and eIF4e by RNA 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/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. We demonstrated that a Hoxa9 variant carrying two point mutations (Hoxa9YL) within its eIF4e interaction motif is incapable of eIF4e interaction and had severely impaired serial replating capacity in vitro. This indicated that eIF4e interaction is a key mediator of the oncogenic activity of Hoxa9.

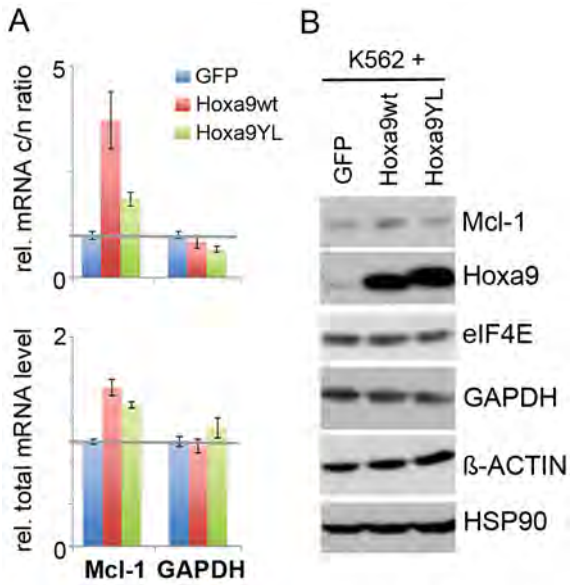
Hoxa9/eIF4e interaction is critical for the development of full penetrance/low latency AML in vivo

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 compared overall survival between both groups. Hoxa9YL as well as Hoxa9wt transduced cells gave rise to overt AML with a similar phenotype. 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 non-malignant Hoxa9YL 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 indicate a persistent cell intrinsic reduction of leukemogenicity conferred by the disruption of the Hoxa9/eIF4e interaction.



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



Hoxa9/eIF4e interaction promotes nuclear export of Mcl-1. (A) mRNA distribution (top) and total mRNA level (bottom) of Mcl-1 and GAPDH (neg. control) in K562 cells transduced with GFP (neg. control), Hoxa9wt and Hoxa9YL. (B) Western Blot for Mcl-1 and control proteins in transduced K562 cells.

Hoxa9/eIF4e interaction promotes nuclear export of eIF4e targets

We performed DNA binding, microarray and RNA export analyses in 'primary' leukemia cell lines isolated from diseased mice to elucidate the mechanisms by which Hoxa9/eIF4e interaction exacerbates leukemogenesis. Importantly, neither DNA binding nor steady-state mRNA levels were altered by introduction of the YL mutation. However, transduction of K562 cells (without endogenous Hoxa9 expression) with Hoxa9wt and Hoxa9YL lead to an increase in the cytoplasmatic to nuclear ratio of Mcl-1 mRNA (a known eIF4e target). This is consistent with the Hoxa9/eIF4e interaction positively affecting nuclear mRNA export and resulted in increased Mcl1 protein expression in Hoxa9wt transduced cells. Currently, we are performing the transcriptome-wide analysis of Hoxa9/eIF4e mRNA targets by nuclear RNA immunoprecipitation in order to identify novel potential therapeutic candidates.

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Awards

Next Generation Award der Deutschen Gesellschaft für Immungenetik, Christian Bach, June 2, 2017, Mannheim

Publications during funding period

Bach C, Steffen M, Roesler W, Winkler J, Mackensen A, Stachel KD, Metzler M, Spriewald BM (2017) Systematic comparison of donor chimerism in peripheral blood and bone marrow after hematopoietic stem cell transplantation. Blood Cancer J. 7(6): e566

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

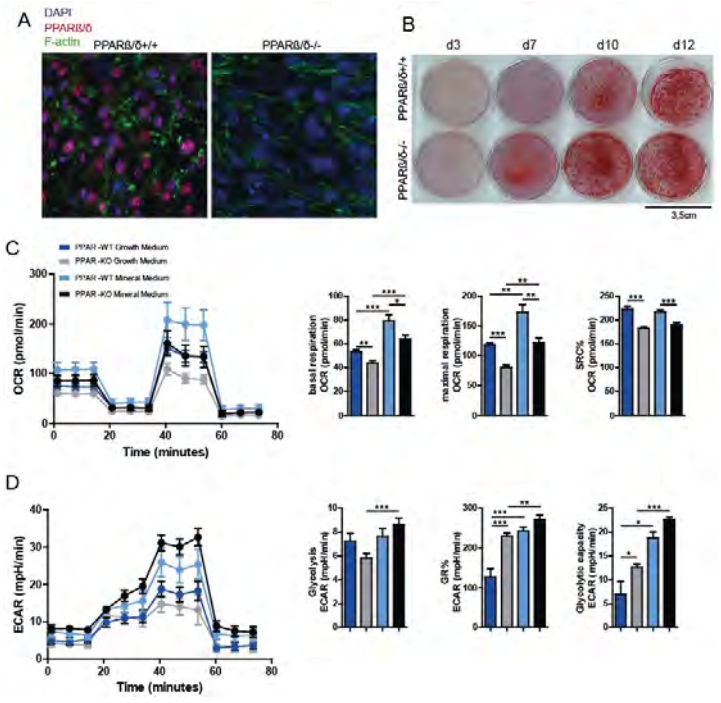
PPARβ/δ in the crosstalk of bone and glucose metabolism

Dr. Carina Scholtysek, Department of Medicine 3 – Rheumatology and Immunology

Peroxisome proliferator-activated receptors (PPARs) are metabolic key-checkpoints regulating cellular development and metabolism. Since PPARß/δ expression in osteoblasts could potentially contribute to beneficial effects on energy and bone metabolism we seek to evaluate the function of this nuclear receptor as a regulator of osteoblast metabolism and uncover the specific role of PPARß/δ in osteoblasts during regular bone turnover and systemic energy homeostasis.

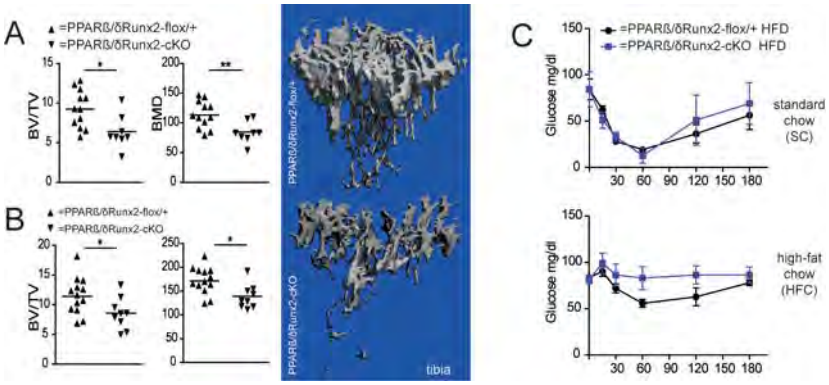
PPARβ/δ controls osteogenic differentiation and metabolism

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 PPARɣ 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 exacerbated differentiation and bone mineralisation in PPARβ/δ-deficient MSCs in vitro. Accumulative evidence showed that altered metabolic processes have been implicated to affect cellular differentiation. To study the potential role of PPARβ/δ in the control of osteoblast metabolism, we applied OCR and ECAR measurements with Seahorse Cellular Flux Assays to compare metabolic profiles between PPARβ/δ wild-type and PPARβ/δ-deficient osteoblasts. The results indicate that PPARβ/δ-deficient osteoblasts use glycolysis as the preferential metabolic pathway to meet the energy demands upon differentiation instead of oxidative phosphorylation as present in wildtype osteoblasts. These data highlight PPARβ/δ as a metabolic checkpoint during osteoblastogenesis and could represent a general mechanism for PPARβ/δ contributing to the wide-ranging functions of energy and bone metabolism.



(A) Immunofluorescence staining of PPARβ/δ +/+ and PPARβ/δ -/- MSCs after 7 days of osteogenic differentiation. (B) Increased mineralisation in PPARβ/δ-/- MSCs. (C) Decreased oxidative phopsphorylation and increased glycolysis (D) 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 in vivo

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 expressing 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 - Final Report

01.04.2015 - 30.09.2017

Others

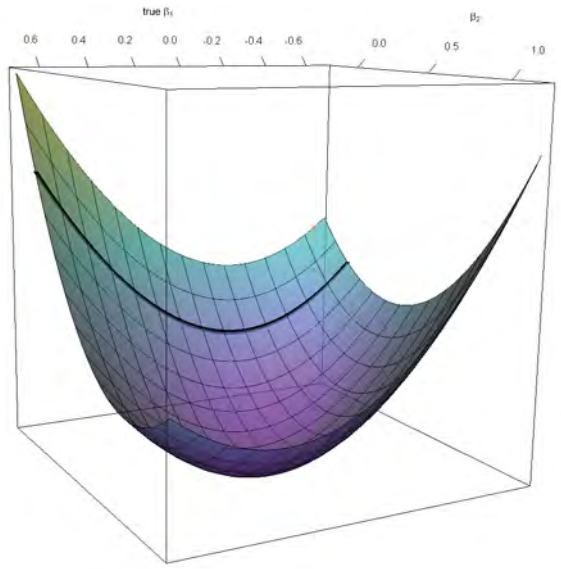
Extending statistical boosting algorithms for biomedical research

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.

Promoting the usage of statistical boosting

Although providing multiple advantages like automated variable selection and robustness in high-dimensional data, the application of statistical boosting algorithms is still somehow limited to groups with biostatistical background or competence in machine learning. To foster the usage of these type of algorithms we published two tutorial articles and one review article providing insights in the usage of the available software as well as illustrative step-by-step analyses of biomedical research questions. Additionally, we developed a specific software focusing on beta regression models for health-related quality of life outcomes providing a more user-friendly interface. In an additional research article, we further analysed the similarities of prediction models resulting from boosting and models from more popular regularized regression methods.



3D plot representing the log-likelihood as global loss function for different potential combinations of coefficients for two different predictors. The dark line represents the initialization of the algorithm and affects the steepness of early updates.

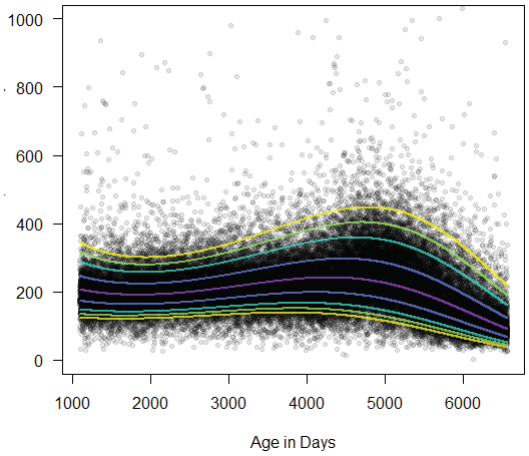
Extending the algorithm

The variable selection properties of boosting are controlled by the number of iterations the algorithm carries out, which is typically determined via resampling procedures. We proposed a new method based on permutated shadow variables that allows stopping the algorithm directly without fitting various models. Furthermore, we adapted the boosting algorithm for distributional regression so it can be applied in combination with stability selection, leading to sparser models. Therefore, we changed the update procedure in order to let the algorithm decide for which dimension of the model the update serves best to optimize the overall likelihood. In an approach to assess measurement errors, we developed a permutation procedure to construct significance tests for systematic bias and random errors of clinical devices.

Boosting new model classes

In many clinical studies, survival times are measured alongside longitudinal markers. From a methodological perspective, it is favourable to model these two outcomes jointly in order to avoid biased results. We proposed a boosting algorithm for this type of joint models, allowing for the first time to estimate them in the presence of high-dimensional data while simultaneously incorporating variable selection. The methodological development was accompanied by an illustrative application on a cystic fibrosis registry. In a second project, we focused on the construc-

Dr. Mayr



Reference intervals for blood levels of children, estimated on a sample with unlabelled pathological and healthy observation based on mixture distributions.

tion of biomarkers for survival data. We proposed a boosting algorithm based on the concordance index, therefore effectively avoiding the restrictive assumptions of the popular Cox procedure and combined it with stability selection. We illustrated the advantages of our new approach in an application to estimate biomarkers for the metastases-free survival of breast cancer patients. Currently, we are working on extending the framework of distributional regression to multivariate distributions, providing an algorithm to estimate the joint conditional distribution of multiple clinical endpoints as well as on mixtures of distributions from pathological and healthy patients.

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Invited lectures

Institut für Medizinische Informationsverarbeitung Biometrie und Epidemiologie, May 4, 2017, Munich, A non-technical introduction to boosting distributional regression models with an application to health-related quality of life outcomes

Institut für Statistik, May 3, 2017, Munich, An introduction to boosting distributional regression models

Publications during funding period

Mayr A, Hofner B (2018) Boosting for statistical modelling – a non-technical introduction. Statistical Modelling. doi:10.1177/1471082X17748086

Mayr A, Schmid M, Pfahlberg A, Uter W, Gefeller O (2017) 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 26(3): 1443-1460

Waldmann E, Taylor-Robinson D, Klein N, Kneib T, Schmid M and Mayr A (2017) Boosting joint models for longitudinal and time-to-event data. Biometrical Journal 59(6): 1104-1121

Mayr A, Hofner B, Waldmann E, Hepp T, Meyer S and Gefeller, O (2017) An Update on Statistical Boosting in Biomedicine. Computational and Mathematical Methods in Medicine. doi: 10.1155/2017/6083072

Gefeller, O, Hofner, B, Mayr, A, Waldmann, E (2017) Predictive Modelling Based on Statistical Learning in Biomedicine. Computational and Mathematical Methods in Medicine. doi: 10.1155/2017/4041736

Thomas J, Hepp T, Mayr A and Bischl, B (2017) Probing for sparse and fast variable selection with model-based boosting. Computational and Mathematical Methods in Medicine. Doi: 10.1155/2017/1421409

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

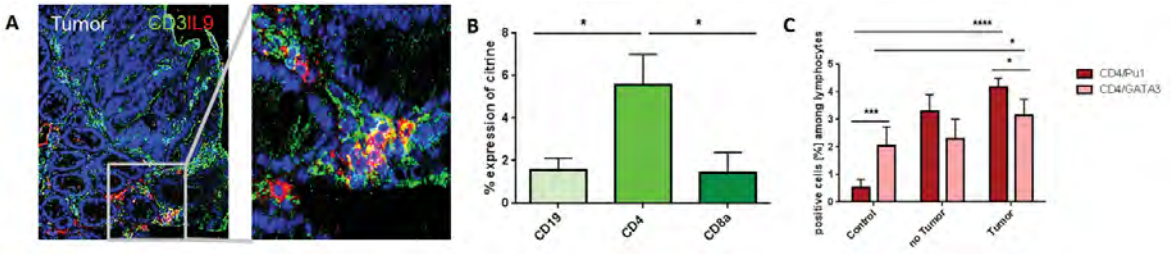
Analysis of the role of IL-9 in the induction of Colitis-associated cancer (CAC)

Dr. Katharina Gerlach, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Inflammatory bowel disease (IBD) is linked to an increased risk for developing colitis-associated cancer (CAC). Here, we uncovered a significant expansion of IL-9-expressing PU.1+ T cells in CAC. Deficiency of IL-9 led to less tumor growth in the AOM/DSS model. Functionally, high IL-9 levels maintained IL-9 receptor upregulation on epithelial cells increasing SOCS-3 levels and reducing proliferation of these cells. Thus our findings highlight a pivotal role for IL-9 in the establishment of CAC.

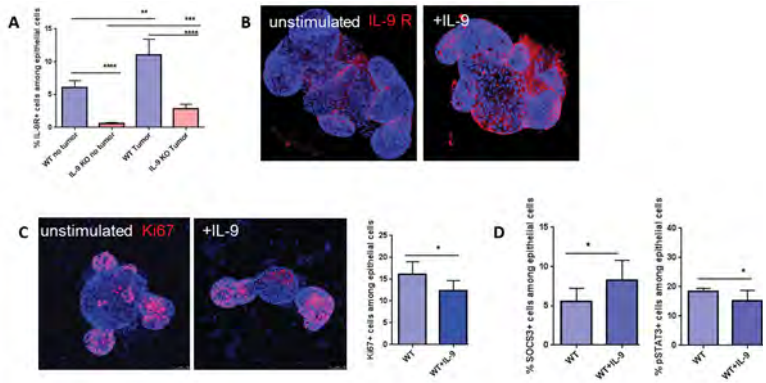
Th9 cells producing IL-9 are induced in colitis-associated cancer

A potential relevance for Th9 cells in CAC was highlighted by our finding that IL-9-expressing T cells are present in colitis-associated neoplasia. We proposed that IL-9 is important for CAC development and analysed which cell type produces the highest levels of IL-9 in CAC tumors. For that we took advantage of IL-9citrine reporter mice out of which we isolated LPMCs from AOM/DSS tumors and measured the expression of IL-9 in different cell populations using flow cytometry. We noticed that mainly CD4+ cells were induced to produce high amounts of IL-9 in CAC suggesting that these cells were Th9 cells. As Th9 cells can also convert from Th2 cells we next analysed LPMCs from AOM/DSS treated wildtype mice for the expression of the Th2 cell transcription factor GATA3 and the Th9 cell transcription factor PU.1. We found more GATA3+ T cells compared to PU.1+ cells in the control tissue of wildtype mice. However, in tumor tissue Th9 cells were significantly upregulated in contrast to control tissue and levels even exceeded Th2 cells in the tumor. These data illustrate that Th9 cells are potentially the crucial T helper cell subset for the maintenance of colitis-associated tumors.



(A) Immunofluorescent analysis demonstrated an induction of mucosal IL-9 expressing T cells in CAC (B) FACS analysis of tumor tissue from CitrineIL-9 reporter showed highest IL-9 expression in CD4+ T cells. (C) In contrast to GATA3+ T cells PU.1+ T cells dominate in the tumor tissue of AOM/DSS treated WT mice.

Dr. Gerlach



(A) IL-9 receptor is mainly expressed on epithelial cells of tumor tissue. (B) IL-9 led to the induction of IL-9 receptor expression on epithelial cells. (C) *In vitro* IL-9 was able to down-regulate proliferation. (D) FACS analysis of organoids incubated with IL-9 led to increased SOCS-3 and decreased pSTAT3 expression.

IL-9 regulates IL-9 receptor expression and controls proliferation in colitis-associated cancer

To investigate specific target cells for the proinflammatory cytokine IL-9 in CAC we checked which cells express the IL-9 receptor. When we performed FACS analysis we found that the IL-9 receptor is mainly expressed on epithelial cells of tumor tissue. For *in vitro* studies we created organoids as a model for epithelial cell structures and treated them with recombinant IL-9. Staining by immunofluorescence for the IL-9 receptor and subsequent confocal analysis of the organoids revealed that the proinflammatory cytokine was able to upregulate IL-9 receptor expression on epithelial cells suggesting that these cells were target cells for IL-9. In a next set of experiments, we analysed the influence of IL-9 on epithelial cell proliferation during CAC development. Therefore we investigated organoids stimulated with recombinant IL-9 by flow cytometry for the proliferation marker Ki67. Here we found a decreased proliferation rate of epithelial cells when IL-9 was present suggesting an anti-proliferative effect of IL-9 on epithelial cells. In a next step we looked at signaling components in epithelial cells during CAC development. We found that the STAT3 inhibitor SOCS3 was significantly upregulated in the epithelial cells of tumor tissue when high levels of IL-9 were present. In accordance with the higher SOCS3 expression we found a significantly lower number of pSTAT3+ cells in epithelial cells. These findings propose that IL-9 controls proliferation in CAC through SOCS3 and pSTAT3 regulation.

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Invited lectures

Deutsche Gesellschaft für Immunologie, September 24, 2017, Erlangen, The transcription factor NFATc3 promotes intestinal inflammation by suppression of regulatory T cells

T-Cell Meeting, June 29, 2017, Marburg, The contribution of Th9 cells to colitis and colitis-associated cancer

Publications during funding period

Reissig S, Tang Y, Nikolaev A, Gerlach K, Wolf C, Davari K, Gallus C, Masri J, Mufazalov I A, Neurath M F, Wunderlich F T, Schattenberg J M, Galle P R, Weigmann B, Waisman A, Glasmacher E, Hovelmeyer N (2017) Elevated levels of Bcl-3 inhibits Treg development and function resulting in spontaneous colitis. Nature communications 8: 15069

Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath M F (2017) Chemically induced mouse models of acute and chronic intestinal inflammation. Nature protocols 12: 1295-1309

J51 - Progress Report

01.10.2015 - 31.03.2019

Inflammatory signature in Parkinson´s disease

Dr. Franz Marxreiter, Department of Molecular Neurology

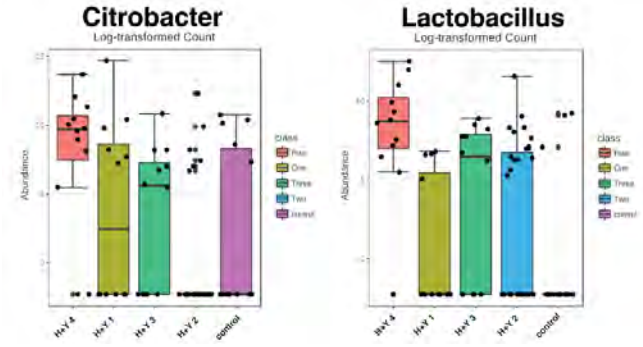
Altered gut microbiota may contribute to, or trigger the pathologic processes in the enteric nervous system in Parkinson's disease (PD), causing a proinflammatory environment in the enteric nervous system. In recent years, several neurological diseases have been linked to altered gut microbiota (i.e. multiple sclerosis), however with different bacterial genus and/or families being altered in different study cohorts. In PD, one study reported altered abundance of a number of bacterial families in a finnish cohort. To further evaluate the role of gut microbiota in PD we focused the second part of this project on alterations of the gastrointestinal microbiome in different disease stages.

We aimed to evaluate, whether altered gut microbiota may play a role in early pathological processes in PD. Thus, we hypothesized that, the gastrointestinal microbiome is altered already at early disease stages in the course of PD, and remains altered during the course of the disease.

Between Nov 2016 and Nov 2017 we enrolled 150 participants in this observational study. In PD patients, motor performance was measured using the Unified Parkinson's Disease Rating Scale (UPDRS) and disease stage was measured using the modified Hoehn & Yahr scale (H&Y). Severity of non-motor symptoms was assessed using the Non-Motor Symptoms Scale (NMS). The degree of constipation was additionally assessed using the Wexner constipation score.

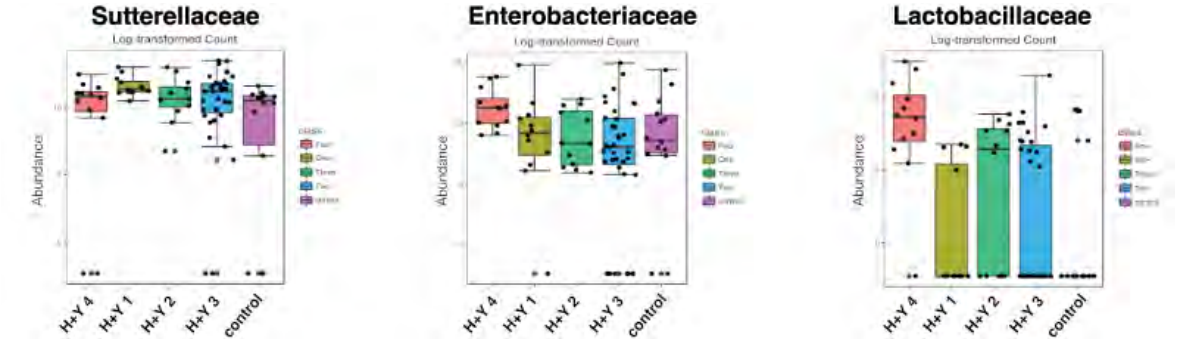
The participants collected the fecal samples at home in a DNA stabilizing solution (Stool Collection Tubes with Stool DNA Stabilizer; Stratec®, Stratec Molecular, Berlin, Germany). Participants had to return the collection tubes within 3 days after collection. Total DNA was extracted from the samples. Afterwards polymerase chain reaction amplification and pyrosequencing the V3 and V4 regions of the bacterial 16S ribosomal RNA gene was performed and used for taxonomic assignment.

So far, we have analyzed the microbiome of 71 PD patients (mean age: 66+10.6; 44% females) and 14 controls (mean age: 64.9+7.0; 36% females) in close collaboration with the group of Prof. Dr. Markus Neurath and PD Dr Stefan Wirtz (Medicine 1, University Hospital Erlangen). Our preliminary results



Abundances of Citrobacter and Lactobacillus are significantly altered between PD disease stages and controls (Citrobacter p= 0.00041294, Lactobacillus p= 0.0010838); H+Y= Hoehn and Yahr stage

Dr. Marxreiter



Abundances of Sutterellaceae (p= 0.015532), Enterobacteriaceae (p= 0.047262), and Lactobacillaceae (p= 0.0010838) differ between PD disease stages and controls; H+Y= Hoehn and Yahr stage

indicate, that there is no change in alpha- or beta-diversity between controls and different PD disease stages. Abundances of individual bacterial families, especially Lactobacillaceae, Sutterellaceae, and Enterobacteraiceace, however differ between disease stages and controls, and may be particularly altered at higher disease stages, only. On the genus level, abundances of Citrobacter and Lactobacillus were significantly altered, also particularly at higher disease stages. This indicates that some bacterial families may be altered at early disease stages already, whereas others may be altered in particular at higher disease stages. This suggests, that increased abundances of specific bacterial families may contribute to the pathologc processes early in the course of PD while alterations in other bacterial families may be a result of PD and PD related non-motor symptoms like constipation since they are only altered at later disease stages.

During the final part of the funding period, we will enroll more control subjects. Thereafter, a more detailed statistical analysis will be performed, correcting for relevant confounders in order to strengthen the preliminary results presented here.

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Invited lectures

Fränkischer Parkinsontag 2017, July 29, 2017, Erlangen, Beginnt Parkinson im Darm?

Publications during funding period

Marxreiter F, Winkler J, Uhl M, Madžar D (2017) A Case Report of Severe Delirium after Amantadine Withdrawal. Case Reports in Neurology 9(1), 44–48

Schmidt MA, Engelhorn T, Marxreiter F, Winkler J, Lang S, Kloska S, Goelitz P, Doerfler A (2017) Ultra high-field SWI of the substantia nigra at 7T: reliability and consistency of the swallow-tail sign. BMC Neurology 17:194

Modeling cortical dysfunction of SPG11 spastic paraplegia using patient-derived pluripotent stem cells

Dr. Martin Regensburger, Department of Neurology

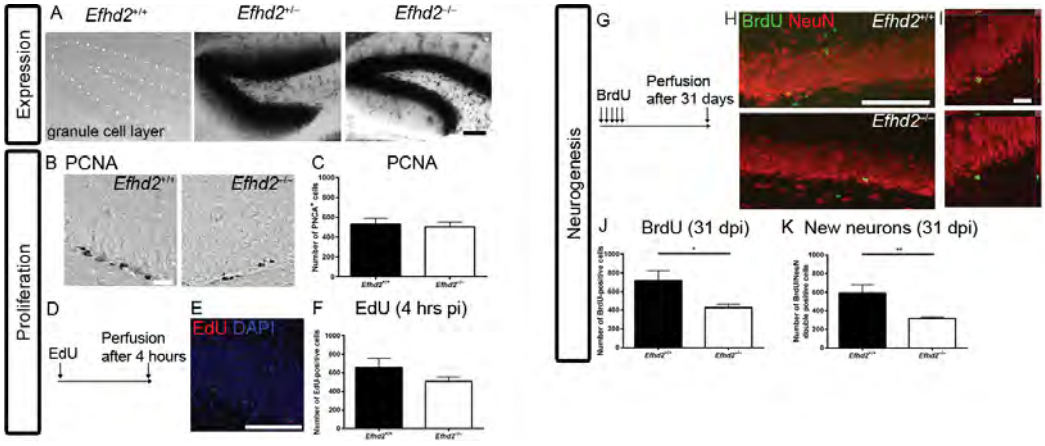
Efhd2 is highly expressed in the adult hippocampal neurogenic niche and has been implicated in neurodegenerative diseases. We observed a severe reduction of the survival of adult newborn neurons in Efhd2 knockouts. Dendrite morphology of newborn neurons was compromised in full Efhd2 knockouts, but not upon cell autonomous Efhd2 deletion. These data connect Efhd2 to impaired synaptic plasticity and identify a role of Efhd2 in neuronal survival and synaptic integration in the adult hippocampus.

Background

The aim of my project is to characterize neurodegeneration using neural stem cell based models. Swiprosin-1/Efhd2 (Efhd2) is highly expressed in the CNS during development and in the adult. EFHD2 is regulated by Ca^{2+} binding, stabilizes F-actin and promotes neurite extension. Previous studies indicated a dysregulation of EFHD2 in human Alzheimer's disease brains on the protein level.

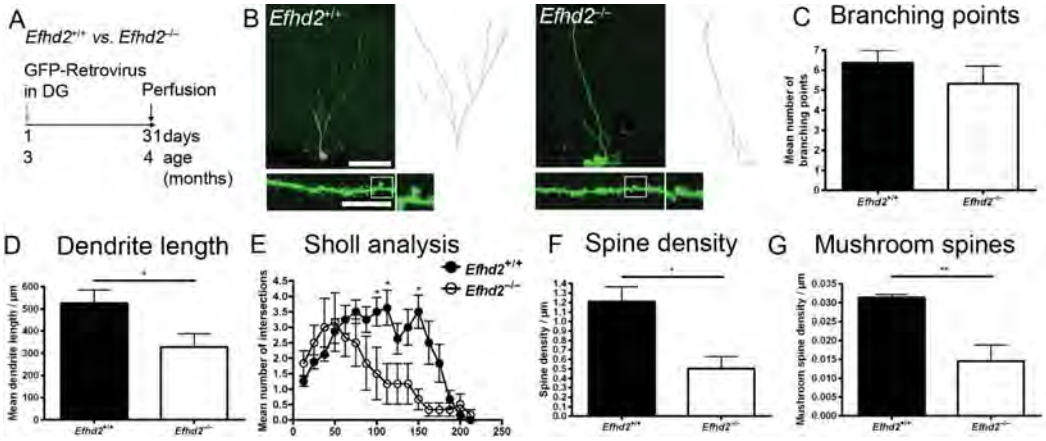
Impaired neurogenesis in Efhd2$^{-/-}$

We hypothesized a detrimental effect of genetic ablation of Efhd2 on hippocampal integrity and specifically investigated adult hippocampal neurogenesis. To this end, the hippocampus of adult Efhd2$^{-/-}$ / LacZ knock-in mice was analyzed. This mouse model was previously generated in the group of PD Dr. Dirk Mielenz. Efhd2 was expressed throughout adult neuronal development, i.e. in Sox2-positive neural stem cells of the dentate gyrus, in doublecortin-positive neuroblasts, and in mature NeuN-positive neurons. Analysis of proliferating cells labeled with the thymidine analogon EdU showed no changes in Efhd2$^{-/-}$. However, there was a marked reduction of doublecortin-positive cells involving all developmental subpopulations (early, intermediate and mature morphology). Additionally, the number of newborn neurons, as determined by the number of BrdU-/NeuN double-positive cells 1 month after labeling, was significantly reduced in the Efhd2$^{-/-}$ hippocampus.



Efhd2 knockout impairs survival of newborn neurons. A) Hippocampal Efhd2 expression in Efhd2$^{-/-}$/lacZ knock-in mice. B-F) Unchanged hippocampal proliferation in Efhd2$^{-/-}$. G-K) Reduced number of BrdU-positive cells and newborn neurons in Efhd2$^{-/-}$.

Dr. Regensburger



Compromised dendrite and spine morphology in Efhd2$^{-/-}$. A-B) Paradigm of newborn neuron labeling. C) Unchanged branching points in Efhd2$^{-/-}$. D-G) Reduced dendrite length, dendrite complexity, spines and mushroom spines of newborn neurons in Efhd2$^{-/-}$.

Cell-extrinsic role of Efhd2 on dendritic spine formation

Next, we analyzed dendrite morphology of adult newborn neurons in Efhd2$^{-/-}$ by use of a GFP-expressing retrovirus. Dendrite length, dendrite complexity (as determined by Sholl analysis), and spine density of newborn neurons were reduced in Efhd2$^{-/-}$. To investigate the cell-autonomous role of Efhd2 in dendrite morphology, we made use of an Efhd2$^{fl/fl}$ mouse model where exon 2 of Efhd2 is deleted upon expression of Cre recombinase. Specific expression of Cre within single newborn neurons upon retroviral delivery did not change dendrite morphology, indicating a cell-extrinsic role of Efhd2. Prompted by previous reports of altered levels of EFHD2 in Alzheimer's disease brains, we analyzed tau pathology in Efhd2$^{-/-}$ and found markedly increased levels throughout the adult hippocampus.

Conclusion

These data connect Efhd2 to impaired synaptic plasticity as present in neurodegenerative disease and identify a role of Efhd2 in neuronal survival and synaptic integration in the adult hippocampus.

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Invited lectures

90. Kongress der Deutschen Gesellschaft für Neurologie, September 20, 2017, Leipzig, Metabolische Dysregulation bei SPG11-assoziierter hereditärer spastischer Paraplegie

Regionaltreffen des HSP-Fördervereins „Gehn mit HSP", October 21, 2017, Ingolstadt, Metabolische Dysregulation bei HSP

18. Rummelsberger Parkinson Symposion mit Patientenseminar, December 2, 2017, Parkinson – Neues aus der Forschung

Publications during funding period

Regensburger M, Schreglmann SR, Stoll S, Rockenstein E, Loskarn S, Wei Xiang, Masliah E, Winner B (2017) Oligomer-prone E57K-mutant alpha-synuclein exacerbates integration deficit of adult hippocampal newborn neurons in transgenic mice. Brain Struct Funct. 2017 Nov 9; 25:239

Schneider-Gold C, Dekomien G, Regensburger M, Schneider R, Trampe N, Krogias C, Lukas C, Bellenberg B (2017). Monozygotic twins with a new compound heterozygous SPG11 mutation and different disease expression. J Neurol Sci. 381: 265-268

Regensburger M, Türk M, Pagenstecher A, Schröder R, Winkler J (2017) VCP-related multisystem-proteinopathy presenting as early-onset Parkinson's disease. Neurology 89(7): 746–8

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. 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 central visual pathway in affected patients with functional and structural MR-imaging.

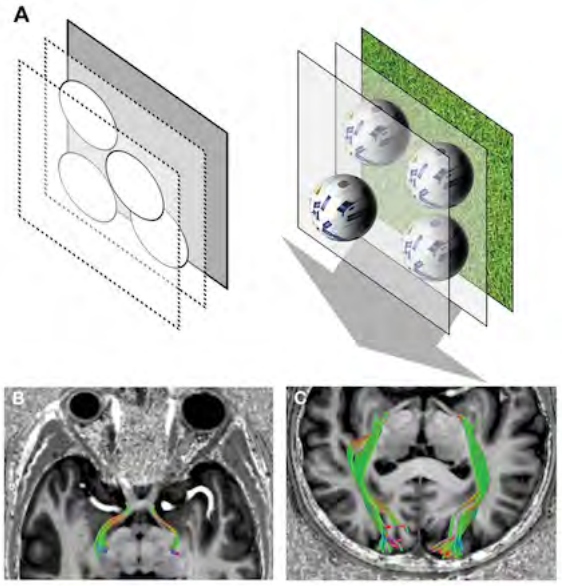
In PEX-associated glaucoma, protein deposits block normal drainage of aqueous humor and lead to an elevated intraocular pressure (IOP) and subsequently to secondary glaucoma. However, this theory is not satisfying as crucial questions regarding the pathogenesis of PEXG are still not answered and factors other than elevated IOP have to be considered.

Genome-wide association studies with PEX/PEXG patients identified common SNPs in the lysyl oxidase-like 1 (LOXL1) gene on chromosome 15q24.1 as the main genetic risk factors of the disease. Our preliminary data confirms that the high-risk haplotype is frequently more common in PEXG compared to POAG. Since PEX is a systemic disease with microfibrillar deposits accumulating not only in ocular tissues, an affection of the intracranial part of the visual pathway (most of the 3rd neuron and the 4th neuron) is possible in PEX patients who develop secondary glaucoma.

One important clinical feature of PEXG is an impaired stereoacuity resulting in depth perception deficits. We examined the stereopsis performance in PEXG patients using a dynamic stereo test that provides a moving stereoscopic stimulus on a background with grass texture. The visual targets consisted of four spheres with the same soccer ball texture. Three of those virtual soccer balls were located on the screen plane; one had an enlarged disparity and appeared in front of the screen plane. In this configuration, the balls move out of the screen towards the observer by continuously enlarging only their disparities. The subject's task was to detect the leading ball as fast as possible. Stereoacuity - measured in arcsec as a discrimination of two points - is impaired in PEXG compared to controls (322.2 vs 29.3, p=0.01). 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 suggestive in PEXG. Indeed, PEXG patients show rarefaction of the optic radiation on diffusivity maps. We discovered markedly reduced fractional anisotropy in the optic radiation (right: 0.648 vs. 0.547, p = 0.002; left: 0.638 vs. 0.554, p= 0.003) of PEXG patients suggesting axonal damage.

The structural imaging methods developed within the scope of this project have already found their way into other scientific projects regarding neurodegenerative diseases (Encephalomyelitis disseminata, Parkinson's disease). We plan to continue our work within the scope of a project funded by the Deutsche Forschungsgemeinschaft. In this project, we aim to develop and validate (ex vivo and in vivo) a knew MR-imaging contrast called chemical exchange saturation transfer (CEST-imaging) that allows the presence of low-concentration proteins (in the range of µM to mM) to be imaged indirectly. CEST imaging could serve as an elegant method to explore protein deposits in neuronal tissues and their role regarding axonal degradation.

Dr. Schmidt



(A) Illustration of dynamic stereopsis evaluation. The targets are constantly moving towards the observer. The subject's task is to detect the leading ball as fast as possible. (B and C) Selective tractography of the optic tract and the optic radiation.

	Haplotype	Cases	Controls	χ2	P value
PEXG	GG	0.85	0.475	12.579	4.0×10^{-4}
	TG	0.125	0.35	5.591	0.0181
	GA	0.025	0.175	5.0	0.0253
POAG	GG	0.375	0.475	0.462	0.4965
	TG	0.312	0.35	0.072	0.7889
	GA	0.312	0.175	1.283	0.2573

Preliminary data Analysis of two common SNPs (rs1048661G>T and rs3825942G>A) of the LOXL1 gene in our PEXG population (n=20 PEXG, n=20 controls, n=8 POAG). The high-risk haplotype GG is significantly more frequent in PEXG compared to controls.

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Publications during funding period

Schmidt MA, Engelhorn T, Marxreiter F, Winkler J, Lang S, Kloska S, Goelitz P, Doerfler A (2017) Ultra high-field SWI of the substantia nigra at 7T: reliability and consistency of the swallow-tail sign. BMC Neurol 17(1): 194

Engelhorn T, Schmidt MA, Dörfler A, Michelson G (2017) Diffusion tensor imaging of the visual pathway in glaucomatous optic nerve atrophy. Ophthalmologe 114(10): 906–921

Schmidt MA, Linker RA, Lang S, Lücking H, Engelhorn T, Kloska S, Uder M, Cavallaro A, Dörfler A, Dankerl P (2017) FLAIRfusion Processing with Contrast Inversion: Improving Detection and Reading Time of New Cerebral MS Lesions. Clin Neuroradiol. doi: 10.1007/s00062-017-0567-y [Epub ahead of print]

Analysis of alternative mechanisms of tumor rejection

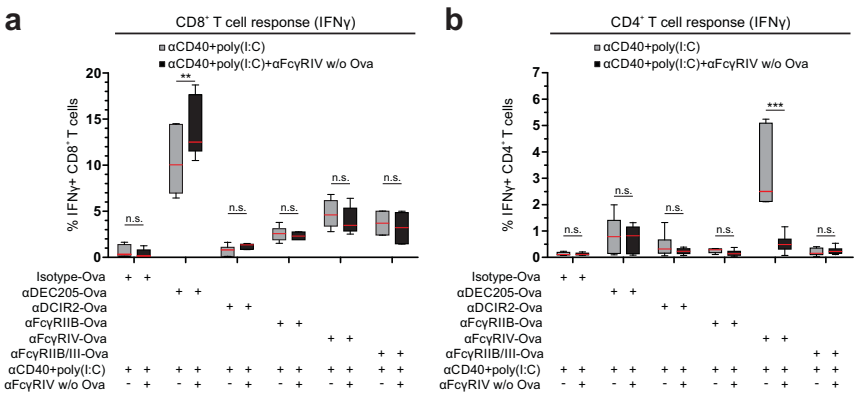
Dr. Christian Lehmann, Department of Dermatology

The current immunological tumor therapies aim to induce or reactivate cytotoxic CD8+ T cell responses to a limited number of epitopes. However, we demonstrated that the survival benefit is independent from the strength of CD8+ T cell responses in a melanoma model. We speculate that a major part of this phenomenon is due to the protection from lethal metastases. In this project, we are focusing on the underlying mechanisms to provide insights to improve future tumor therapies.

CD8+ T cells are a focus of immunological therapies as they can directly lyse cancer cells, thereby fostering the treatment or providing even a cure of the disease. In patients, the effectiveness of a given therapy is mainly measured by cytokine capture assays, ELISAs, intracellular FACS staining and/or ELISpot assays. It is accepted that a strong production of Th1 cytokines, such as IFNγ, is an adequate measurand and prerequisite for the efficacy of an immunological anti-tumor response. However, by taking advantage of an antigen targeting approach to select dendritic cell subsets in vivo, we could demonstrate that the cytokine production does not directly correlate with the capacity to lyse target cells. In some diseases, such as melanoma, the primary tumor is not a direct cause of tumor-associated deaths, but rather its metastases in other organs. Therefore, prevention or the destruction of these secondary tumor formations is another important feature of an effective therapy. Our previous results revealed that even in the absence of strong CD8+ T cell responses, mice can be protected from tumor outgrowth, which has been recapitulated in a therapeutic model.

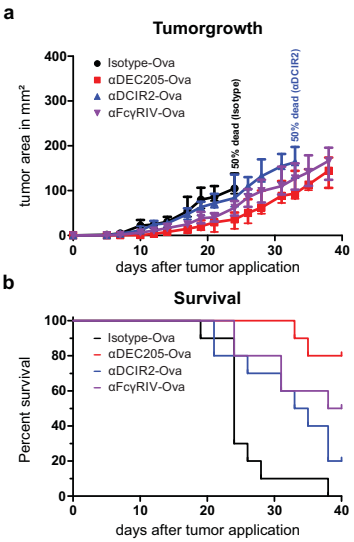
Investigation of new targeting-antibodies in a murine melanoma model

Previously, we mainly focused on targeting the two major classical DC subsets (CD8+ and CD8- DCs), which mainly induce CD8+ or CD4+ T cell responses, respectively. As we hypothesized that a concomitant induction of both kinds of T cells could be beneficial, we widened our spectra to our recently cloned targeting antibodies specific for FcγRIIB, FcγRIV, and FcγRIIB/III. In particular, αFcγRIV-Ova was able to induce strong CD4+ and CD8+ T cell responses simultaneously. In the naïve system this was demonstrated



Induction of naïve T cell responses. (a, b) C57BL/6 mice were immunized with named targeting antibodies +αCD40+poly(I:C) +/-αFcγRIV w/o Ova. 14 d later, cells were re-stimulated with a peptide pool. Intracellular (a, b) IFNγ by flow cytometry.

Dr. Lehmann



MCA101-Ova as tumor model. Naïve C57Bl/6J mice were immunized with named targeting antibodies +αCD40+poly(I:C) 14 days before tumor application. (a) Tumor growth and (b) Survival after s. c. application of MCA-Ova cells.

by the induction of IFNγ and IL2 in CD8+ and CD4+ T cells as well as the killing of SIINFEKL-loaded target cells in vivo.

Introduction of the MCA101-Ova tumor model and antigen targeting to αFcγRIV

The observed protective capacity of the antigen targeting antibodies, which only induced weak T cell responses in naïve mice (such as αDCIR2-Ova) could be due to the epitope spreading: The small number of CD8+ T cells might lyse a certain number of tumor cells. By this, other tumor antigens become more easily accessible for antigen-presenting cells, which might in turn foster the induction of new or the re-activation of already present T cell responses. To get more insights into this mechanism, we are investigating different closely related tumor models, such as B16F10-Ova (MO4) and B16F10 or MCA101-Ova and MCA101.

The first experiments with B16F10 revealed that there is only a very short time for intervention, as the cells grow very aggressive. Therefore, we started to use MCA101-Ova cells, a well-established fibrosarcoma model. By preventive treatment of naïve mice with αDEC205-Ova, αDCIR2-Ova, αFcγRIV-Ova (+αCD40/pIC), we could demonstrate their efficacy to protect challenged mice from tumor outgrowth and prolong survival.

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Invited lectures

„International Young Immunologists“ at the 2017 Annual Meeting of the Chinese Society for Immunology, October 29, 2017, Tianjin, China, Antigen targeting of C-type lectin and Fc receptors for the induction of anti-tumor responses in vivo

Publications during funding period

Schneppenheim J, Loock AC, Hüttl S, Schweizer M, Lüllmann-Rauch R, Oberg HH, Arnold P, Lehmann CHK, Dudziak D, Kabelitz D, Lucius R, Lennon-Duménil AM, Saftig P, Schröder B (2017) The Influence of MHC Class II on B Cell Defects Induced by Invariant Chain/CD74 N-Terminal Fragments. J Immunol. 199(1): 172-185

Lehmann CHK, Baranska A, Heidkamp GF, Heger L, Neubert K, Lühr JJ, Hoffmann A, Reimer KC, Brückner C, Beck S, Seeling M, Kießling M, Soulat D, Krug AB, Ravetch JV, Leusen JHW, Nimmerjahn F*, Dudziak D* (2017) DC subset-specific induction of T cell responses upon antigen uptake via Fcγ receptors in vivo. J Exp Med. 214(5): 1509-1528

Mokada-Gopal L, Boeser A, Lehmann CHK, Drepper F, Dudziak D, Warscheid B, Voehringer D (2017) Identification of Novel STAT6-Regulated Proteins in Mouse B Cells by Comparative Transcriptome and Proteome Analysis. J Immunol. 198(9): 3737-3745

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. Sci Immunol. 1(6)

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(2): 8

*contributed equally

The role of microRNA-188-5p dysregulation in hepatocellular carcinoma development and progression

Dr. Dr. Peter Dietrich, Institute of Biochemistry

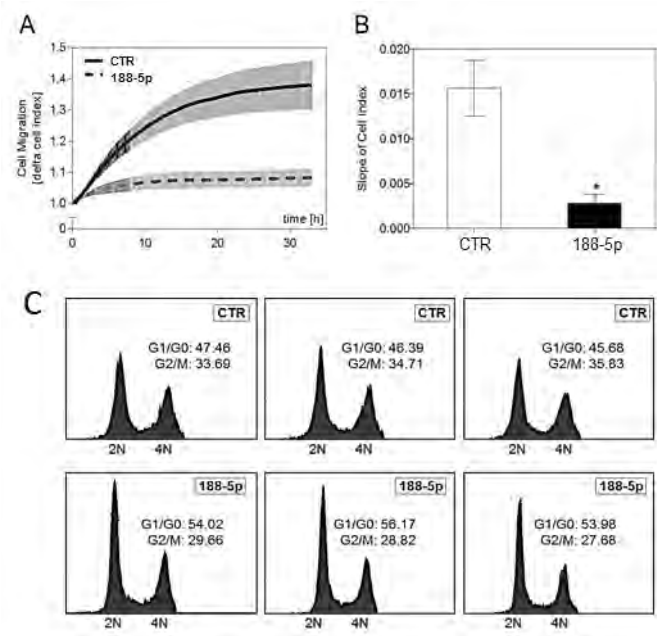
Aberrant microRNA-expression correlates with severity and prognosis of hepatocellular carcinoma (HCC). Our results demonstrate that microRNA-188-5p (miR-188-5p) is strongly downregulated in HCC cell lines and tissues. Re-expression of miR-188-5p markedly inhibits proliferation, clonogenicity, and migration of HCC cell lines. Using in silico and cDNA-expression array analysis, we identified novel target genes (e.g. KLF12, IL6ST, DLG5) for the miR-188-5p in HCC that will be further evaluated.

Background

The molecular landscape underlying hepatocellular carcinoma (HCC) is known to be decisively shaped by the regulatory functions of microRNAs (miRs). Based on our previous findings that downregulation of microRNA-188-5p contributes to the aggressive phenotype of activated synovial fibroblasts in rheumatoid arthritis (Ruedel, Dietrich et al., 2015), we hypothesized that miR-188-5p could also affect HCC. Therefore, the aim of this study was to analyze the function of miR-188-5p and to reveal novel potential diagnostic and therapeutic target genes for this microRNA in liver cancer.

Results

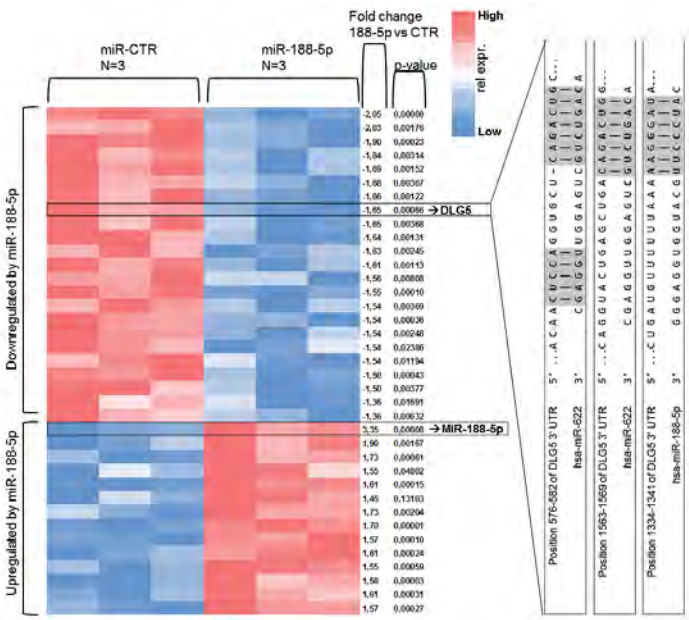
MiR-188-5p was strongly downregulated in HCC and re-expression of miR-188-5p exerted strong tumorsuppressive effects (reduced migration, proliferation, clonogenicity and induction of a G0/G1 cell cycle arrest) in HCC cell lines. RNA-expression arrays revealed a list of potential novel target genes for miR-188-5p that are unknown HCC including Krueppel-like factor 12 (KLF12), Interleukin-6 signal transducer (IL6ST) and Discs large MAGUK scaffold protein 5 (DLG5). MiR-188-5p-mediated regulation of these target genes was confirmed in vitro. Using tissue patient samples, KLF12, IL6ST and DLG5 were found to be elevated in HCC as compared to non-tumorous liver tissues. IL6ST knockdown significantly altered HCC cell migratory behavior. Moreover, functional analysis using si-RNA-Pool-mediated gene knockdown identified KLF12 as a novel therapeutic target for HCC.



(A) Migration after transfection with miR-188-5p (188-5p) as compared to control-transfected (CTR) HCC cells (e.g. HCC cell line PLC). (B) Summarized analysis of migration for PLC as depicted in (A) (*: $p<0.05$). (C) Flow cytometric propidium iodide staining depicts percentage of cells (PLC) in cell cycle fractions (G1/G0, G2) after 188-5p-transfection as compared to controls (CTR).

Dr. 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.



Conclusions/Outlook

By combining global gene expression analysis with a systematic in silico and experimental screening process, we identified several novel target genes of the tumor-suppressive miR-188-5p in HCC. KLF12 and IL6ST were identified as important modulators of oncogenic functions in HCC cells and could serve as molecular targets for HCC therapy in the future.

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Invited lectures

BioSysNet/si-Tools Symposium, January 24, 2017, Munich, MicroRNA target identification and subsequent functional validation using RNAi-mediated specific gene silencing reveals novel therapeutic target genes in cancer

Awards

Congress Travel Award of the FALK Foundation e.V., FALK Workshops, Peter Dietrich, January 1, 2017, Essen

Poster Award (Poster Highlights 2017) of the German Association of the Study of the Liver (GASL), January 1, 2017, Essen

Patents/ Licenses during funding period

European patent No. 16179431.8 (14.07.2016) Bosserhoff, A., Dietrich, P.: "KRAS inhibitor for use in treating cancer"

Publications during funding period

Dietrich P, Freese K, Mahli A, Thasler WE, Hellerbrand C, Bosserhoff AK (2017) Combined effects of PLK1 and RAS in hepatocellular carcinoma reveal rigosertib as promising novel therapeutic „dual-hit" option. Oncotarget, Advance Online Publications [Epub ahead of print]

Dietrich P, Fritz V, Koch A, Hartmann A, Bosserhoff A, Hellerbrand C (2017). Wildtype Kirsten rat sarcoma is a novel microRNA-622-regulated therapeutic target for hepatocellular carcinoma and contributes to sorafenib-resistance. Gut. doi: 10.1136/gutjnl-2017-315402 [Epub ahead of print]

Dietrich P, Kuphal S, Spruss T, Hellerbrand C, Bosserhoff A (2017) Wildtype KRAS is a Novel Therapeutic Target for Melanoma Contributing to Primary and Acquired Resistance to BRAF Inhibition. Oncogene 2017 Oct 23. doi: 10.1038/onc.2017.391. [Epub ahead of print]

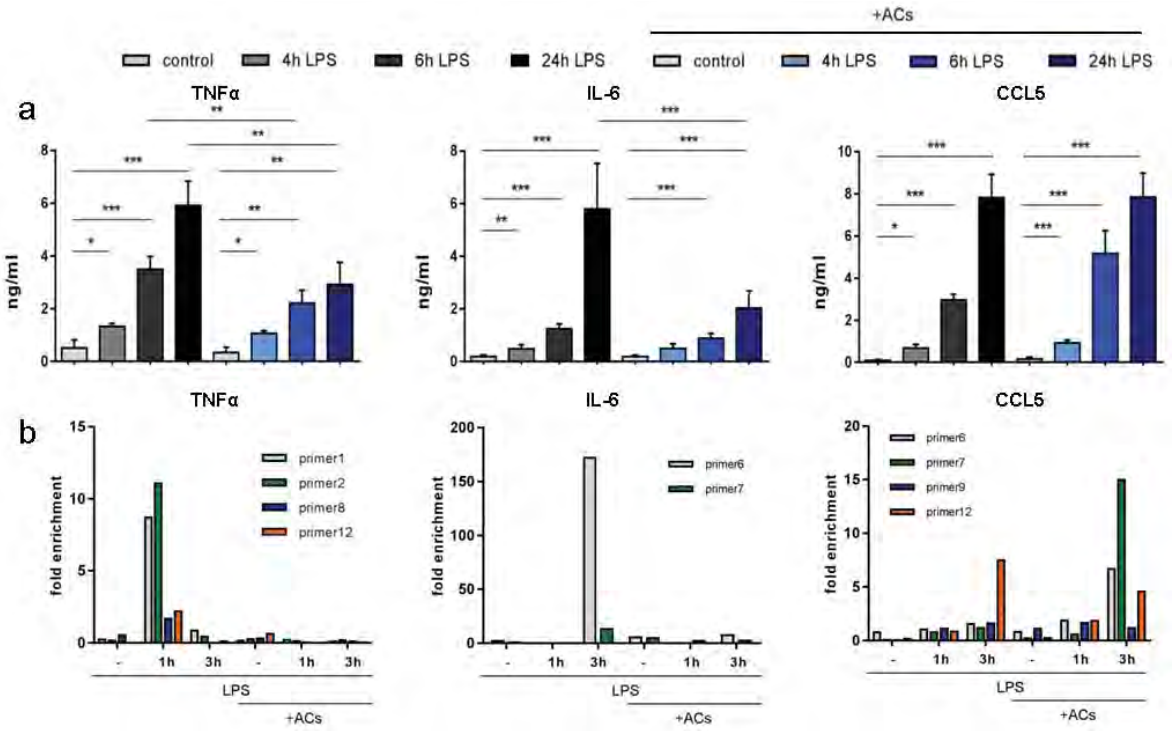
Mahli A, Saugspier M, Koch A, Sommer J, Dietrich P, Lee S, Thasler R, Schulze-Luehrmann J, Luehrmann A, Thasler WE, Müller M, Bosserhoff A, Hellerbrand C. ERK activation and autophagy impairment are central mediators of irinotecan-induced steatohepatitis. Gut. doi: 10.1136/gutjnl-2016-312485. [Epub ahead of print]

Epigenetic reprogramming of macrophages

Dr. Katrin Palumbo-Zerr, Department of Medicine 3 – Rheumatology and Immunology

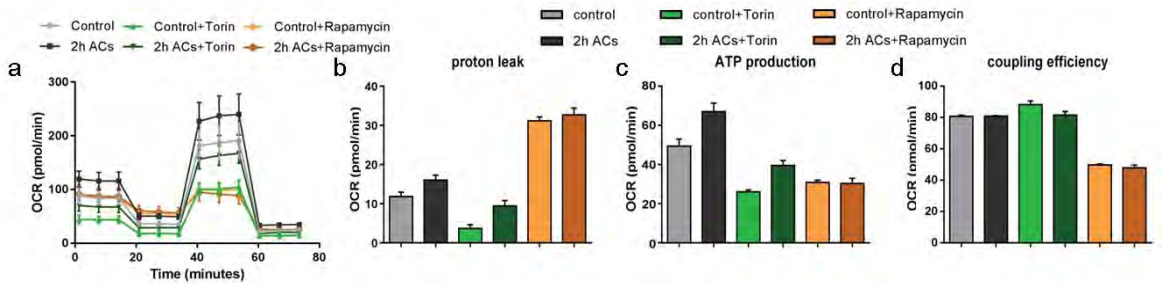
Clearance of apoptotic cells (ACs) is a key step during the resolution of inflammation and the maintenance of self-tolerance. In the current project we aim to dissect the immunometabolic consequences of the clearance of AC by macrophages. Uptake of ACs results in an anti-inflammatory reprogramming of macrophages and our data suggest that these events are linked to a metabolic reprogramming and fundamental epigenetic changes in these cells.

Our results show that uptake of apoptotic cells (ACs) results in the differential regulation of the expression of LPS-induced cytokines in macrophages. Whereas we observe an inhibition of pro-inflammatory cytokines such as TNFα and IL-6, several other cytokines such as CCL5 are not blocked. These differential changes in cytokine expression correlate with changes in histone (H3K27) acetylation at the respective promoters. Moreover, we determined metabolic changes within the macrophage and detected a drastic increase in oxidative phosphorylation and ATP production in macrophages that ingested ACs. Interestingly, these metabolic changes were linked to activation of the mTOR pathway as inhibition of mTOR by Torin and Rapamycin blocked the increase in oxygen consumption rate (OCR) in macrophages



Phagocytosis of ACs results in distinct expression pattern, time kinetics and differential blockade of cytokines. (a) ELISA-based analysis of TNFα, IL-6 and CCL5 cytokine release. (b) ChIP analysis of histone acetylation on H3K27 at the respective promoters.

Dr. Palumbo-Zerr



Inhibition of mTOR by Torin and Rapamycin blocks the increase in oxygen consumption in macrophages after uptake of ACs. Seahorse analysis of oxygen consumption rate (OCR).: (a) mitochondrial respiration, (b) proton leak, (c) ATP production and (d) coupling efficiency.

after uptake of ACs. Together these data suggest a fundamental immunometabolic reprogramming of macrophages during the clearance of ACs. Future research will focus on the interconnection between the observed changes and the responsible molecular pathways to identify novel targets for the treatment of chronic inflammatory diseases.

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Publications during funding period

none

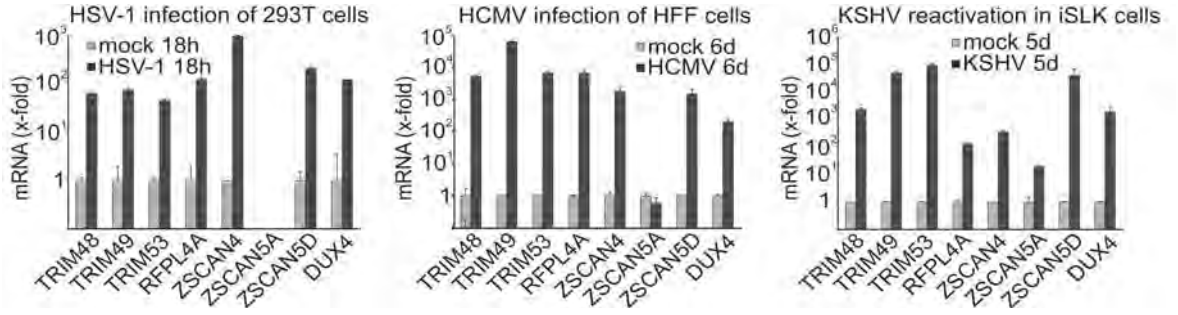
Herpesviruses and DUX4

Dr. Florian Full, Institute of Clinical and Molecular Virology

Expression of the embryonic transcription factor DUX4 in adults causes Facioscapulohumeral Muscular Dystrophy (FSHD) and is associated with various forms of cancer. We show that DUX4 and hundreds of its target genes are activated upon reactivation/replication of herpesviruses. We are currently elucidating the molecular mechanism of how herpesviruses upregulate DUX4 expression, how DUX4-induced genes affect herpesviral replication and whether DUX4 expression has an impact on herpesviral disease.

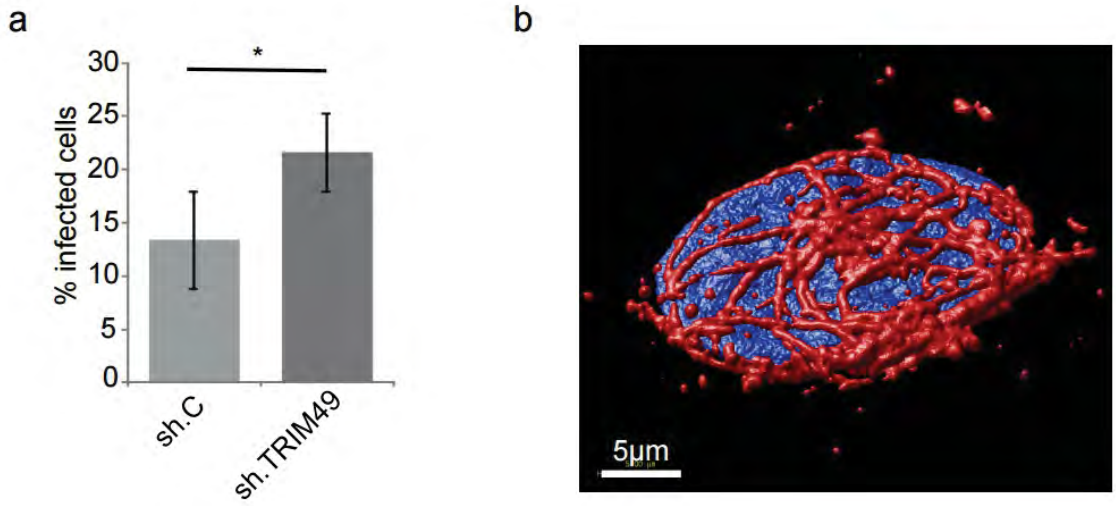
Herpesviral infection is the cause of significant morbidity and mortality in humans worldwide, especially in immunocompromised individuals. Furthermore, two of the eight human herpesviruses, Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV), are classified as human carcinogens. Despite differences in pathogenesis and carcinogenicity, herpesviruses share common features in their replication strategies, most notably in the switch from latency to lytic replication. As such, molecular insight into the factors that modulate the latent-to-lytic switch of herpesviral replication is of fundamental importance for the development of novel anti-herpesviral therapies.

Using RNA-Seq experiments we identified DUX4 as a transcription factor that is activated upon lytic replication of Herpes simplex virus (HSV). Further experiments also confirmed expression of DUX4 upon lytic replication of Human Cytomegalovirus (HCMV) and lytic reactivation of Kaposi's sarcoma-associated herpesvirus (KSHV). DUX4 is a germline transcrip tion factor that is exclusively expressed during early embryonic development in healthy individuals. However, aberrant expression of DUX4 in muscle cells is the cause of Facioscapulohumeral Muscular Dystrophy (FSHD). In addition, DUX4 overexpression is also associated with various forms of cancer. Strikingly DUX4 upregulation could be observed



Upregulation of mRNA of DUX4 and DUX4-target genes upon replication of HSV-1, HCMV and KSHV at indicated time points assessed by quantitative real time PCR.

Dr. Full



a) Intracellular FACS staining of HSV-1 ICP-0 protein after infection of 293T cells with HSV-1 upon shRNA mediated kd of TRIM49. n=(4)
b) 3D reconstruction of TRIM49 protein localization in HeLa cells red: TRIM49, blue: DAPI

upon infection with herpesviruses of all subgroups pointing at a conserved herpesviral mechanism of DUX4 induction. We could further demonstrate that DUX4 expression upon herpesviral replication leads to the upregulation of hundreds of DUX4 target genes, among them several members of the TRIM, PRAMEF and ZSCAN protein families. Notably, most of the DUX4 target genes are minimally or not at all expressed in normal (uninfected) differentiated cells and their biological functions have yet to be characterized. We started with the characterization of DUX4 target genes by i) analyzing the influence of knockdown/overexpression on viral replication, ii) analyzing the subcellular localization of uncharacterized proteins and iii) identification of interaction partners by mass spectrometry. In addition we generated various CRISPR/CAS9 knockout cell lines for DUX4 and its most important target genes to further analyze their effect on herpesviral infection.

Taken together, our results indicate that α–, β– and γ-herpesviruses induce a DUX4-dependent germline-specific transcriptional program. We are currently elucidating the molecular mechanism of how herpesviral infection upregulates DUX4 expression, and how DUX4-induced genes affect herpesviral replication.

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Publications during funding period

Full F, Hahn AS, Großkopf AK, Ensser A (2017) Gammaherpesviral Tegument Proteins, PML-Nuclear Bodies and the Ubiquitin-Proteasome System. Viruses 2017 Oct 21;9(10)

J58 - Progress Report

01.09.2016 - 28.02.2019

Oncology

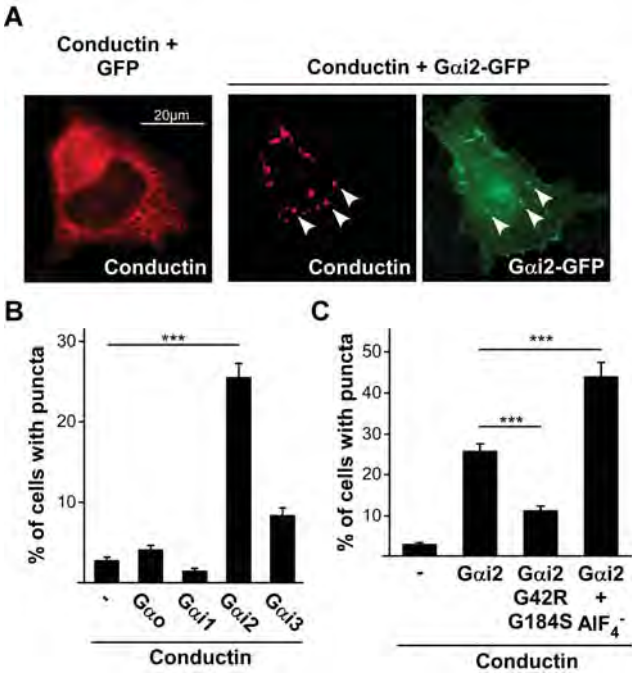
Counteracting Wnt signaling

Dr. Dominic Bernkopf, Chair of Experimental Medicine II

Wnt/β-catenin signaling is the major driving force of colorectal cancer making it an interesting therapeutic target. Our project focuses on conductin/axin2 a negative regulator of Wnt/β-catenin signaling sharing high homology with axin. Since axin-mediated β-catenin degradation correlates with axin polymerization and conductin does not polymerize, we hypothesize that induction of polymerization will enhance conductin-mediated β-catenin degradation, which could by exploited for cancer therapy.

In the course of this project we want to understand why conductin does not polymerize in contrast to its paralog axin, investigate whether conductin polymerization can be enforced and analyze whether this suffices to inhibit Wnt/β-catenin signaling in colorectal cancer.

We could previously show that deletion of the conductin RGS (regulator of G-protein signaling) domain or replacement by the axin RGS domain results in efficient polymerization of conductin indicating that the RGS domain of conductin prevents polymerization. Since the conductin RGS domain shows high homology to RGS domains in GAPs (GTPase-activating proteins) which bind to Gα subunits of trimeric G-proteins and catalyze GTP hydrolysis, we wondered whether binding of Gα proteins to the conductin RGS domain affects conductin polymerization. Indeed, co-expression of Gαi2 induced polymerization of transiently expressed conductin, as seen by the formation of microscopically-visible spherical structures called “puncta”. Induction of conductin polymerization was not observed with Gαo, Gαi1 nor Gαi3 suggesting a rather specific mechanism. Interestingly, out of the four tested Gα proteins Gαi2 also showed the strongest interaction with the conductin RGS domain in GST-pulldown assays suggesting that Gα-RGS interaction is important for induction of polymerization. In line, weakening of the Gαi2-RGS interaction by introducing two published mutations in Gαi2 (G42R G184S) significantly reduced induction of

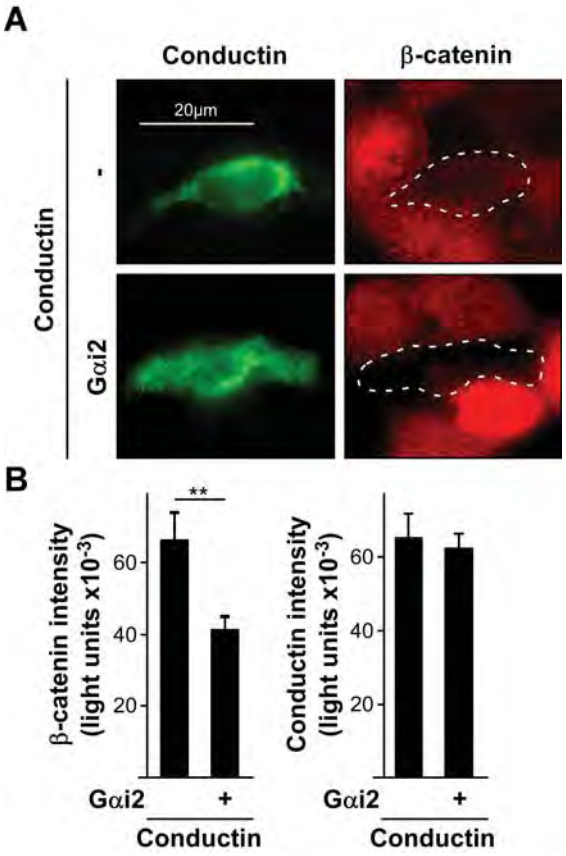


Conductin polymerization is induced by Gαi2 binding A) Immunofluorescence staining. Arrowheads: co-localization. B+C) Quantification of cells with conductin puncta induced by different Gα proteins or AlF4- treatment, as indicated.***p<0.001 (n=1500).

Dr. Bernkopf

conductin polymerization. Moreover, treatment of cells co-expressing Gαi2 and conductin with AlF4-, which stabilizes the conformation of Gα proteins with the highest affinity to RGS domains and thereby strengthens the Gαi2-RGS interaction, increased conductin polymerization. Together these data suggest that Gαi2 induces conductin polymerization via direct binding to the conductin RGS domain. Next, we analyzed whether induction of conductin polymerization by Gαi2 increases β-catenin degradation by conductin in colorectal cancer cells (SW480). Transient expression of conductin reduced β-catenin levels, as previously described. Importantly, β-catenin levels were even lower in cells co-expressing conductin together with Gαi2. Gαi2 alone did not significantly reduce β-catenin levels. These data suggest that Gαi2 enhances conductin-mediated β-catenin degradation potentially by inducing conductin polymerization.

We will continue to study (i) how the conductin RGS domain prevents polymerization, (ii) how this is counteracted by Gαi2 binding and (iii) whether enhancing conductin-mediated β-catenin degradation by Gαi2 can be exploited for colorectal cancer treatment.



Conductin-mediated degradation of β-catenin in colorectal cancer cells (SW480) is enhanced by Gαi2 A) Immunofluorescence staining. B) Quantification of β-catenin and conductin intensities of three independent experiments as in A. **p<0.01 (n=30).

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Publications during funding period

Rauschenberger V, Bernkopf DB, Krenn S, Jalal K, Heller J, Behrens J, Gentzel M, Schambony A (2017) The phosphatase Pgam5 antagonizes Wnt/beta-Catenin signaling in embryonic anterior-posterior axis patterning. Development 144: 2234-2247

J59 - Progress Report

01.07.2017 - 31.12.2019

Oncology

Immunotoxin induced anti-tumor immunity

Dr. Fabian Müller, Department of Medicine 5 – Haematology and Oncology

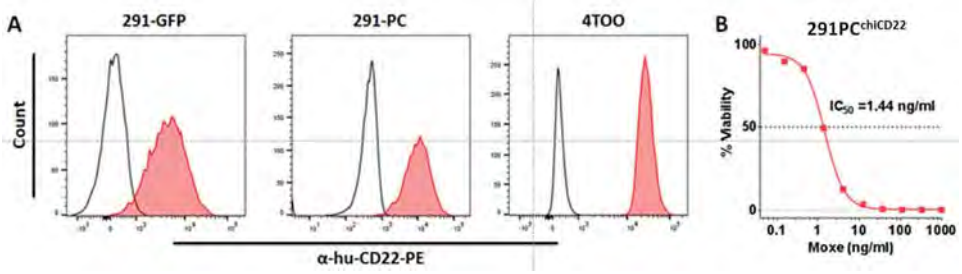
The immunotoxin Moxetumomab pasudotox consists of a CD22 targeting antibody and Pseudomonas exotoxin. It specifically lyses human B-cell lymphomas. Immunotoxins can induce an anti-tumor immune response in patients with solid tumors. Hypothesizing that Moxe similarly induces an anti-lymphoma immune response, we aim to establish and characterize an immune competent murine lymphoma model expressing human CD22 to then determine the lymphoma infiltrating immune cells and the changes induced by Moxe.

The goal of this project is to establish an immune competent lymphoma mouse model that facilitates the testing of Moxetumomab pasudotox (Moxe) and its effects on the murine immune system. Because Moxetumomab binds only to human CD22, we generated a chimeric protein (chiCD22) consisting of the intracellular domains of the murine and the extracellular domains of the human CD22. We hyposezised that Moxe binds the human parts on the cell surface and that the intracellular murine parts ensure the correct transport of Moxe through the various intracellular compartments.

Murine lymphoma cell lines including the multiple myeloma 4TOO and the Burkitt's lymphoma 291PC were transduced with chiCD22 using newly constructed murine lentiviruses. The successfully transduced cell lines were FACS-sorted for highest expression of chiCD22 and single cloned by limited dilution. Then, the chiCD22-positive cell lines were specifically killed by Moxe in a dose dependent manner supporting that the chimeric protein efficiently transports Moxe and thus, sensitizes the mouse cells to a drug which exclusively targets human CD22.

Next, we tested whether the C57BL/6-derived 291PC^{chiCD22} cells would grow in mice and found that wild-type C57BL/6 mice rejected 291PC^{chiCD22}. Prof. Nischke, Dept. of Biology, FAU Erlangen developed a C57BL/6$^{tm(chiCD22)}$ where the murine CD22 was replaced by the chiCD22 by homologue recombination. In a collaborative effort, we subcutaneously injected the 291PC^{chiCD22} in the flank of C57BL/6$^{cm(chiCD22)}$ mice where they engrafted and formed tumors. The 291PC^{chiCD22} which grew in mice maintained the high expression of chiCD22. Preliminary results demonstrated a major macrophage and NK-cell fraction among the tumor-infiltrating immune cells. The phenotypic and functional assessment of these tumor infiltrating immune cells is ongoing.

In parallel, we aimed to develop a second mouse model independent of lentiviral transduction. C57BL/6$^{\lambda\text{-myc}}$ mice overexpress the myc-oncogene in B-cells and thus, spontaneously develop lymphomas. Together with Prof. Nitschke's group, we cross-bred the C57BL/6$^{tm(chiCD22)}$ mice and the C57BL/6$^{\lambda\text{-myc}}$ mice. The resulting C57BL/6$^{\lambda\text{-myc/tm(chiCD22)}}$ mouse strain spontaneously

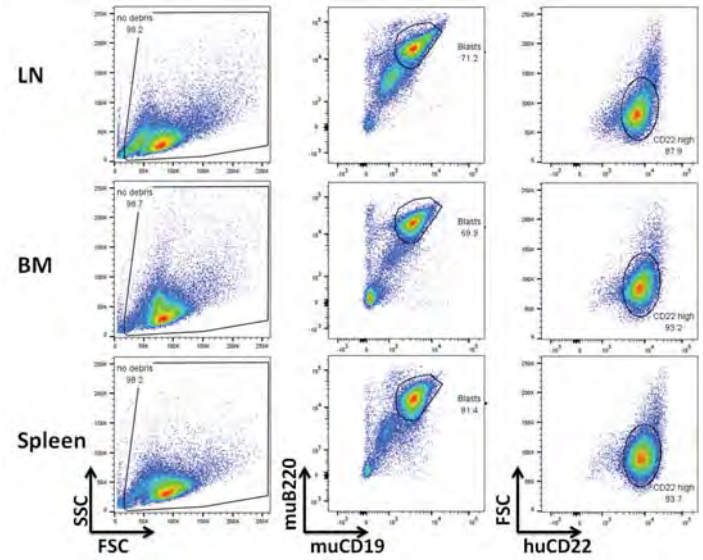


Murine B cell lymphomas transduced with chimeric CD22 become sensitive to Moxe. A Indicated murine B-cell lymphomas were transduced with chimeric CD22 and single cloned. B 291PCchiCD22 cells are sensitive to Moxe in a dose-dependent manner.

Dr. Müller

developed a B-cell lymphoma which highly expressed the chiCD22. These chiCD22 positive lymphoma cells were extracted and serially transplanted into recipient C57BL/6$^{tm(chiCD22)}$ mice. As for the 291PC^{chiCD22} model, the characterization of the lymphoma infiltrating immune cells is ongoing.

During the remaining funding period we will use the two model systems to closer characterize the tumor infiltrating cells. In addition to a detailed phenotypic assessment, the functional state of the tumor-associated macrophages and NK cells will be determined. Both model systems will then be used to measure the efficacy of Moxe against the chiCD22 positive murine tumor cells in vivo and ultimately, to determine the effects of Moxe on the tumor-infiltrating host immune cells.



Novel chiCD22 positive, myc-driven murine B-cell lymphoma. A BL6tm(chi22)/λ-myc mouse developed lymphadenopathy and was sacrificed. Lymph nodes (LN), spleen, and bone marrow (BM) show a predominant B-cell clone suggesting chiCD22 positive lymphoma.

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Invited lectures

Meeting of the German society of hematology and oncology (DGHO), September 2017, Stuttgart, Paclitaxel synergizes with exposure time adjusted CD22-targeted immunotoxins against B-cell malignancies

Patents/ Licenses during funding period

EP 17 209 379.1 (pending)

Publications during funding period

none

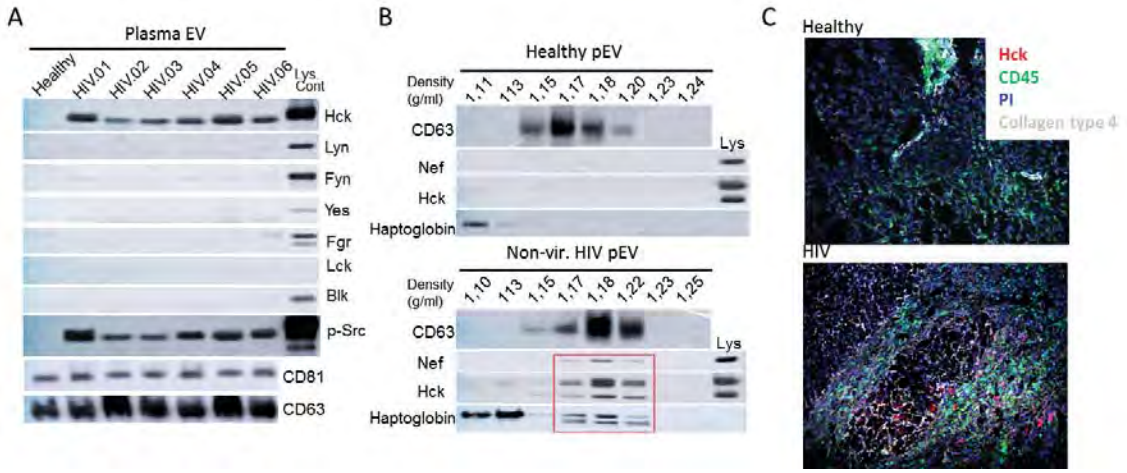
The role of Hck/Lyn in Vesicles secretion

Dr. Jung-Hyun Lee, Department of Dermatology

Research of the last years revealed that ADAM proteases-containing extracellular vesicles (EV) contributed to the HIV pathogenesis. In the present project we aim to understand and elucidate the underlying molecular and cellular mechanism of the secretion of EV. We discovered that EV secretion was governed by the tyrosine kinases Hck. HIV infection induced the activation of Hck and triggered the Hck-mediated cytoskeleton reorganization of infected cells through induction of HAS3.

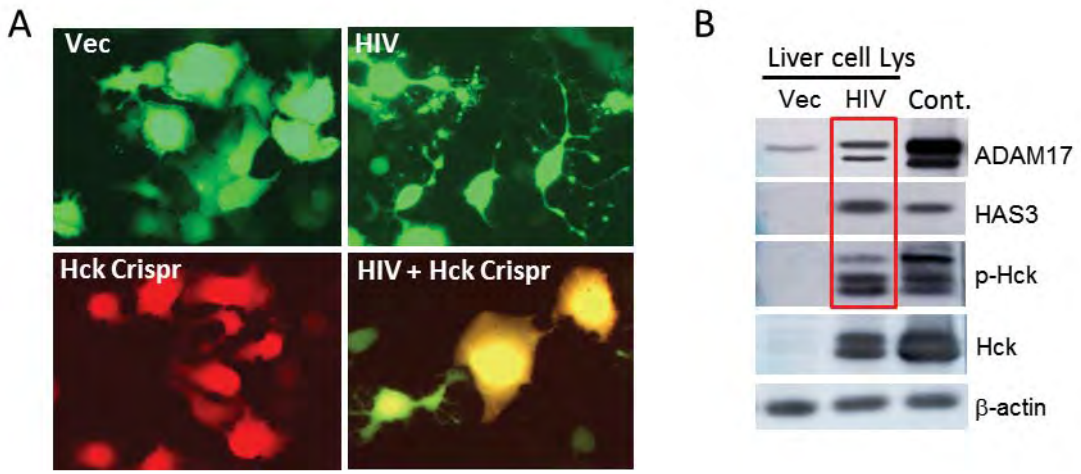
The activated Hck is detected in pEV and liver tissue of HIV patients

In vitro, the tyrosine kinase was essential for the uploading of ADAM17 into EV and was also present in EV in large amounts. We therefore speculated that plasma EV (pEV) from patients may harbor a tyrosine kinase. Purified pEV from 6 non-viremic patients and 1 healthy control were blotted for 7 tyrosine kinases. Hck was identified in all HIV samples but not in controls. In addition, a phopho-Src (p-Src) was detected, suggesting that the pEV-associated tyrosine kinase was activated. Interestingly, we detected haptoglobin, an acute phase protein mainly secreted by liver cells in HIV pEV. This liver-specific factor was found in sucrose gradient fractions of HIV pEV along with activated Hck and Nef, but not in respective fractions of healthy control pEV. These data suggested that liver could be one of cellular source of EV secretion in HIV infection. To confirm this idea, we analyzed liver tissue from a HIV-infected non-viremic individual who died of liver cirrhosis-associated complications. Staining of liver samples showed that strong increased Hck expression was detected in HIV patient liver, but not in healthy donor liver. These results suggested that Hck activation was potentially connected to the generation and/or function of HIV pEV and liver as the likely cellular origin of HIV pEV.



(A) Blotting of indicated tyrosine kinases in pEV purified by differential centrifugation from HIV patients and healthy donor. (B) Western blot analysis of pEV purified by sucrose gradient from a HIV patient and a healthy donor. (C) Multi-epitope ligand cartography (MELC) analysis of liver.

Dr. Lee



(A) Florescent microscopy analysis of indicated constructs expressed liver cells. (B)Western blot analysis of indicated constructs transfected liver cells.

Hck mediates the cytoskeleton reorganization for vesicular secretion

We noticed that HIV infection induced membrane protrusions, similar as described Hck function for F-actin reorganization to form protrusions of the plasma membrane in a Cdc42- and Rac-dependent manner. To analyze this effect we co-transfected GFP. Transfection of HIV proviral construct induced the membrane protrusions, while Hck knockdown (Hck Crispr/cas9-RFP) blocked this function. We hypothesized that one of regulatory protein might be related to this induction of membrane protrusions by Hck activation. Recent studies showed that Hyaluronan acid synthase 3(HAS3) was related to the regulation of cytoskeleton reorganization through induction of cell protrusions. In line with these findings, translation of HAS3 was induced by HIV infection along with activated ADAM17 and Hck. We concluded that HIV infection increased the secretion of ADAM proteases-containing EV through Hck activation, which induced a plasma membrane reorganization that possibly allowed vesicular secretion.

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Invited lectures

Extracellular Vesicle Research Mini-Symposium, July 19, 2017, Erlangen, HIV activates the tyrosine kinase Hck in hepatocytes to secrete ADAM protease-containing extracellular vesicles

Publications during funding period

none

J61 - Progress Report

01.01.2017 - 30.06.2019

Others

Extending joint models in biomedical outcomes

Dr. Elisabeth Waldmann, Department of Medical Informatics, Biometry and Epidemiology

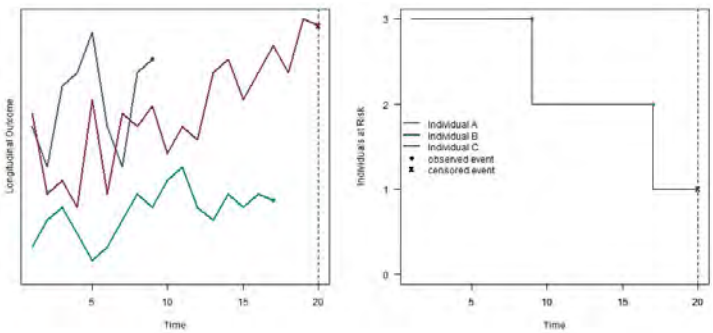
Research on the association between longitudinal and time of event measurements is crucial in biomedical studies. This association, however, can only be modelled reliably if the two quantities are modelled jointly. The aim of this project is to extend those joint models in both: on one side to include the ability to model longitudinal processes more appropriately for different data structures and on the other side in the important question of variable selection.

Joint Modelling

There are several kinds of models, referred to as joint model. We are dealing with a joint model for longitudinal and time-to-event outcomes. This model has to estimate the impact of covariates on those two outcomes: a longitudinal measurement, such as any kind of biomarker, and an event time, such as death (hence the term survival analysis). Assuming those two outcomes are depending on each other, it is preferable to use a joint model, which accounts for the influence of covariates as well as the relationship between the outcomes. The covariates can be divided in three groups: variables having an impact on the longitudinal outcome (from now on referred to as the longitudinal sub-predictor), variables having an impact on the event-time-outcome (from now on referred to as the survival sub-predictor) and variables having an impact on both (from now on referred to as the shared sub-predictor).

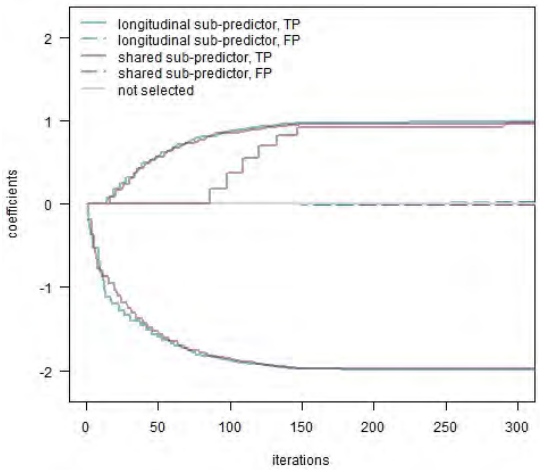
Variable Selection via Boosting

The question of variable selection was until recently completely ignored in the field of joint modelling. We hence concentrated on the question of how to select variables in joint modelling frameworks. Gradient boosting is a special inference method originating in the machine learning context, which has lately been used more and more in the statistical community. Boosting methods are famous for their ability to estimate and select effects in regression models simultaneously. The method furthermore prevents from overfitting by an inherent shrinkage mechanism. We therefor developed a special boosting algorithm for joint models, partly based on the findings from the previous IZKF Junior Project J49. This first basic algorithm, which includes longitudinal and shared sub-predictor, is already implemented and published. We are currently working on an extension which includes a survival sub-predictor.



Example of a data structure joint modelling is suitable for. The left side displays longitudinal outcomes, the right side event times, respectively censoring. While events for Individuals A & B are observed, censoring occurs for Individual C.

Dr. Waldmann



Coefficient paths for a simulation study on the selection and allocation algorithm. The not-selected grey line consists of 2500 non-informative variables. TP: true positive, i.e. correctly selected, FP: false positive, i.e. wrongly selected.

Variable Allocation via Boosting

Since researchers do not necessarily know which covariate has an influence on which part of the outcome, we are working on an algorithm which is able to do both - select and allocate variables to the correct sub-predictor. We are currently working on an extension of our boosting algorithm which is able to do so and first simulation studies are already showing that our concept is working.

Comparison of Joint Modelling Approaches

Joint modelling has lately been implemented in various different ways, most of them based on likelihood approaches. Those implementations vary in performance, but also in model class. One of our goals is to compare those implementations and give insights into which approach is appropriate for which kind of data set.

Beyond the Mean Regression

First steps have also been done in the direction of extending the joint model towards beyond the mean regression. In this context, we added a further set of sub-predictors to model the variance of the longitudinal outcome based on covariates. Those sub-predictors can then again be linked to the survival model.

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Invited lectures

Research Seminar Department of Applied Statistics, JKU, April 27, 2017, Linz, Austria, Joint Modelling of Longitudinal and Time-to-Event Data -from classical approaches to machine learning

Research Seminar Department of Statistics LMU, May 31, 2017, Munich, Joint Modelling of Longitudinal and Time-to-Event Data -from classical approaches to machine learning

International Conference of the ERCIM WG on Computational and Methodological Statistics, December 16, 2017, London, UK, Variable selection and allocation in joint models for longitudinal and time-to-event data via boosting

Publications during funding period

Waldmann E, Taylor-Robinson D, Klein N, Kneib T, Pressler T, Schmid M, Mayr A (2017) Boosting Joint Models for Longitudinal and Time-to-Event Data. Biometrical Journal, 59(6): 1104-1121

Mayr A, Hofner B, Waldmann E, Hepp T, Meyer S, Gefeller O (2017) An update on statistical boosting in biomedicine. Computational and Mathematical Methods in Medicine Vol. 2017, Article ID 6083072, doi:10.1155/2017/6083072

Gefeller O, Hofner B, Mayr A, Waldmann E (2017) Predictive Modelling Based on Statistical Learning in Biomedicine, Computational and Mathematical Methods in Medicine Vol. 2017, Article ID 4041736, doi:10.1155/2017/4041736

Newly started Projects

J62 01.08.2017 - 31.01.2020 Immunology and Infection

Mechanisms of neutrophil infiltration in rheumatoid arthritis


Dr. Klingberg

Dr. Anika Klingberg, Department of Medicine 3 – Rheumatology and Immunology

Neutrophil granulocytes play a central role in innate immunity, but their function regarding pathogenesis of autoimmune diseases like rheumatoid arthritis is poorly understood. Previous studies were restricted to cell-unspecific models or cell culture experiments. Thus, we aim to study this cell type via novel cutting-edge imaging techniques and newly available high specific mouse models in vivo.

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J63 01.12.2017 - 31.05.2020 Immunology and Infection

IL-3 in inflammatory bowel disease


Dr. Zundler

Dr. Sebastian Zundler, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

The role of IL-3 in the pathogenesis of inflammatory bowel diseases (IBD) has not been explored so far. Preliminary data show that the course of oxazolone colitis is aggravated in IL-3 deficient mice vs. controls and that the expression of IL-3 is significantly increased in active ulcerative colitis. Therefore, this project is designed to address the role of IL-3 in experimental colitis and human IBD. This could lead to the identification of novel therapeutic targets for the treatment of IBD.

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J64 01.10.2017 - 31.03.2020 Renal and Vascular Research

Nephroprotection by HIF-hydroxylase inhibitors


Dr. Grampp

Dr. Steffen Grampp, Department of Internal Medicine 4, Nephrology and Hypertension

Acute kidney injury has an increasing incidence and no specific pharmacological treatment options. A great body of evidence from rodent models suggests that the stabilization of hypoxia-inducible factors leads to an improved kidney function. A new generation of specific PHD-inhibitors has been introduced for the treatment of anemia in chronic kidney disease. Aim of this study is to investigate the nephroprotective potential of novel HIF-hydroxylase inhibitors in human renal tubular cells.

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J65 01.11.2017 - 30.04.2020 Renal and Vascular Research

T-System Regulation by Glucocorticoids


Dr. Seidel

Dr. Thomas Seidel, Institute of Cellular and Molecular Physiology

The transverse tubular system (t-system) of ventricular cardiomyocytes is crucial for cardiac contractility. Stabilizing t-system structure is a target of therapy and prevention because the t-system depletes or structurally declines in heart failure. However, mechanisms of t-system regulation remain largely unknown. Our preliminary data suggest t-system regulation by corticoid receptor signaling. The proposed project shall identify underlying signaling pathways and associated proteins.

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Newly started Projects

J66 01.01.2018 - 30.06.2020 Neurosciences

β subunits: adding pieces to the puzzle of pain

Dr. Esther Eberhardt, Department of Anaesthesiology

Dr. Eberhardt

The objective of this study is to identify the role of regulatory proteins of voltage-gated sodium channels (β subunits) as potential contributors and new therapeutic targets of pain. Main focus of this work will be on mutations of β1 and β3 that have recently been found in patients suffering from the pain syndrome erythromelalgia. Using human stem cell-derived nociceptors these mutations will be functionally characterised with electrophysiological and molecular biology methods.

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J67 01.01.2018 - 30.06.2020 Oncology

Metabolic reprogramming of AML MDSCs

Dr. Dr. Regina Jitschin, Department of Medicine 5 - Hematology and Medical Oncology

Dr. Dr. Jitschin

AML is the most common acute leukemia in adults. Emerging evidence suggests that immune alterations favor leukemogenesis and relapse. Myeloid derived suppressor cells (MDSCs) are mediators of tumor immune escape. Here, we aim to decipher the interconnection between metabolic reprogramming and MDSC abundance in AML and to unravel the role of AML-derived exosomes in this context. A better understanding is key for improving immune-based therapeutic approaches in AML.

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J68 01.10.2017 - 31.03.2020 Oncology

Role of GATA4 in Intestinal Inflammation & Cancer

Dr. Patankar

Dr. Jay V. Patankar, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

GATA4 is expressed in the intestinal epithelia and regulates metabolic functions in physiological states. We have shown that a microbiota-derived signal regulates epithelial GATA4. Epigenetic silencing of GATA4 frequently occurs in colon cancer patients. However, its role in bridging gut barrier, inflammation and carcinogenesis remains poorly defined. We seek to identify pathways and strategies to restore GATA4 in cancer to provide new therapeutic alternatives in the treatment of colon cancer.

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Pilot Projects

Pilot Projects

Overview

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Pilot Projects

Ongoing 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	16.01.2017-15.01.2018	Department of Surgery
HN-16-08-05-1	Targeted induction of immunogenic cell death by drug-loaded nanoparticles for tumor therapy	Dr. Janko	13.02.2017-12.02.2018	Department of Otorhinolaryngology – Head and Neck Surgery
HN-16-08-22-1	Automated sleep stage analysis based on global EEG pattern analysis	Dr. Traxdorf	01.02.2017-31.01.2018	Department of Otorhinolaryngology – Head and Neck Surgery
M3-16-10-05-1	Interleukin 9 is an essential factor for resolution of arthritis	Dr. Ramming	01.04.2017-31.03.2018	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	01.02.2017-31.01.2018	Institute of Clinical Microbiology, Immunology and Hygiene
P001	A systems biology approach to identify microRNAs associated with dendritic cell-mediated immunogenicity	Dr. Lai	01.04.2017-31.03.2018	Department of Dermatology
P002	CD22-targeting Pseudomonas exotoxin – Mertansine duo-toxin for the antibody-based therapy of B-cell malignanciess	Dr. Müller	01.05.2017-30.04.2018	Department of Medicine 5
P003	Characterization of transformed intestinal epithelial cells with a rhabdoid sarcomatoid phenotype	Dr. Münzner	01.05.2017-30.04.2018	Institute of Pathology
P004	Trauma-related disorders and psycho-social resources in refugees from Syria with residence permit	Dr. Georgiadou	01.04.2017-31.03.2018	Division of Psychosomatics and Psychotherapy
P005	Evaluation of collagen gel in cartilage repair and biochemical high-field MR imaging using T2 mapping	Dr. Pachowsky	01.12.2017-30.11.2018	Department of Surgery
P006	JAK1-dependent transphosphorylation of JAK2 limits the anti-fibrotic effects of selective JAK2 inhibitor	Dr. Zhang	01.10.2017-30.09.2018	Department of Medicine 3
P007	CRISPR/Cas9 based gene knock out to identify new susceptibility genes for pancreatic cancer chemotherapy	Prof. Pilarsky	01.08.2017-31.07.2018	Department of Surgery
P009	Influence of CD40 receptor blockade on development of CD19+ B cells to acquire immunoregulatory function	Dr. Wahlbuhl-Becker	13.12.2017-12.12.2018	Department of Paediatrics and Adolescent Medicine
P010	Assessment of the FGFR1 amplicon as potential prognostic and predictive marker in breast cancer	Dr. Erber	01.09.2017-31.08.2018	Institute of Pathology
P011	Effects of physical exercise and influence of motor proteins on muscle atrophy in cancer patients	Dr. Schwappacher	01.08.2017-31.07.2018	Department of Medicine 1

Project No.	Project title	Applicant	Term	Institute
P013	Effects of prenatal trauma on maternal hormone balance, placenta function and expression of HPA-axis related genes in fetal brain and placenta	Dr. Golub/ Dr. Frey	15.08.2017-14.08.2018	Division of Child and Adolescent Mental Health
P014	Identification of factors making steatotic liver tissue to an attractive metastatic niche for melanoma	Prof. Hellerbrand	01.11.2017-31.10.2018	Institute of Biochemistry

Terminated Projects

Project No.	Project title	Applicant	Term	Institute
AU-14-10-06-1	Qualification to „Master of Medical Education“	Dr. Menzel-Severing	15.09.2015-25.09.2017	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

Pilot Projects

Project No.	Project title	Applicant	Term	Institute
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
JP-16-05-11-1	Prenatal depressive symptoms and distress: Associations with child epigenome, cortisol release and mental health	Dr. Eichler	01.01.2017-31.12.2017	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	01.02.2017-30.11.2017	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	01.02.2016-31.01.2017	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)	01.04.2016-30.10.2017	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

Newly Started Projects

Project No.	Project title	Applicant	Term	Institute
P008	Induction of mucosal immune responses by gene-based vaccines against viral respiratory tract infections	Prof. Tenbusch	01.01.2018-31.12.2018	Institute of Clinical and Molecular Virology
P012	The role of RIPK4 during intestinal inflammation and colorectal cancer development	Dr. Martini	01.01.2018-31.12.2018	Department of Medicine 1
P015	Modulation of human purinergic receptor P2X4 by bile acids	Dr. Ilyaskin	01.05.2018-31.10.2018	Institute of Physiology
P016	Establishment of a laboratory method for the detection of novel contrast media for ultrasound imaging	Dr. Knieling	01.02.2018-31.01.2019	Department of Pediatric and Adolescent Medicinie
P017	Electronic support for perioperative risk evaluation - a pilot study and proof of concept	Dr. Wagner	12 mo.	Department of Anaesthesiology
P019	The involvement of the enteric nervous system in the immunopathogenesis of multiple sclerosis	Prof. Kürten	11 mo.	Institute of Anatomy
P021	Estimation of bone strength of the distal radius and meta-carpal head using micro finite element analysis from high-resolution peripheral quantitative computed tomography (HR-pQCT) in rheumatoid arthritis	Dr. Kleyer	01.03.2018-31.08.2018	Department of Medicine 3
P022	Vascularization and bone formation of cell-loaded hydrogel matrices in the rat AV loop model	Dr. Steiner	12 mo.	Department of Plastic and Hand Surgery
P023	Dipeptidyl-peptidase-4 (DPP4) characterizes a subpopulation of fibrosis-promoting fibroblasts and is a molecular target for the treatment of fibrosis	Dr. Soare	12 mo.	Department of Medicine 3
P024	The contribution of butyrophilins in the pathogenesis of Rheumatoid Arthritis	Dr. Sarter-Zaiss	12 mo.	Department of Medicine 3

News and Figures

News and Figures

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News and Figures

Overview

The following figures 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. In total 92 master thesis, doctoral thesis and habilitations were ongoing in 2017 and 75 publications were completed.

Advanced Projects	**39**
Immunology and Infection	15
Oncology	7
Neurosciences	14
Renal and Vascular Research	3
Tandem projects between different departments and institutes	14
Junior Research Groups	**2**
Junior Projects running in 2017	**22**
Immunology and Infection	6
Oncology	5
Neurosciences	4
Renal and Vascular Research	3
Molecular Medicine	2
Others	2
Thereof projects completed in 2017	4
Projects approved in 2017, starting in 2018	2
Institutions with funded projects	**27**
Employees of the IZKF	**111**
Number of scientists (including laboratory rotations)	72
Number of non-scientists	39
Pilot Projects running in 2017	**38**
Newly granted in 2017	17
Projects completed in 2017	21
Projects approved in 2017, starting in 2018	10
Appointments of IZKF project leaders to W2/ W3 - positions	5

Ongoing scientific theses	**92**
Master theses	11
Doctoral theses	78
Habilitations	3
Laboratory rotations	18
MD-thesis scholarship holders	39
Participants research training group	**116**
T(h)INK - Oncology, Immunology and Infection, Renal and Vascular Research	**60**
PhD students from IZKF projects	19
Associated participants	22
MD-thesis scholarships holders	19
Neurosciences	**56**
PhD students from IZKF projects	16
Associated participants	37
MD-thesis scholarships holders	3
Number of patents	2
Number of awards	16
Publications	**75**
Cumulative impact factor	620.577
Average impact factor per publication	8.274
Average publications per project	1.29
Publications in journals with IF ≥ 10	26
Total expenditures IZKF	**6,250 K€**

Summary of important figures 2017

News and Figures

News

Junior Research Group Leader Dr. Paolo Ceppi is awarded with the Young Investigator Research Award

Dr. Paolo Ceppi, leader of the Junior Research Group 1 „Understanding the plasticity of cancer cells“ was awarded with the Young Investigator Award - Lung Cancer“ by the International Association for the study of Lung Cancer (IASLC). This prestigeous price is awarded once a year.

Dr. Ceppi

The award was given to Dr. Paolo Ceppi for his contribution to the molecular characterisation of lung tumors, which improved the therapeutic stratification of the patients and promoted the introduction of the histological determination in the clinical decision-making in non-small cell lung cancer. This funding will be used to support the current lab projects on determining the role of thymidylate synthase in the epithelial-to-mesenchymal transition in non-small cell lung cancer.

Summer Symposium 2017

In July 2017, the IZKF organised a one-day summer symposium “Research Highlights at IZKF”. Selected current and former IZKF project leaders and IZKF junior research group leaders presented research results achieved in connection with IZKF funding. The summer symposium was held on July 21st in the newly inaugurated Harald zur Hausen-lecture hall of the Faculty of Medicine. After an attractive programme, a networking party took place in the Nikolaus-Fiebiger Center where advanced and junior project leaders exchanged ideas about their research.

Life@FAU

In October 2017, the Graduate School for Life Sciences (Life@FAU) was launched following an initiative from IZKF. It offers an interdisciplinary structured training programme for doctoral students at the Faculty of Medicine and the Department of Biology. The Faculty of Medicine and the Department of Biology at the Faculty of Sciences are involved on equal footing. All research training groups of both faculties are members of Life@FAU including the IZKF Research Training Group. The objectives of Life@FAU are to enhance structured training programmes for doctoral candidates at FAU, to create uniform standards in postgraduate education in the field of life sciences and to ensure the provision of structured training programmes. A steering committee with Prof. Christoph Becker (Department of Medicine 1) as chairman was elected.

IZKF Postgraduate Workshop 2017

On October 15th, 2017, the 15th IZKF Postgraduate Workshop took place in the "Neues Hörsaalzentrum". Two interesting talks were given. Prof. Dr. Loren Field (Indiana University School of Medicine, Wells Center for Pediatric Research) gave a lecture on the topic "Monitoring S-phase activity in the adult heart" and Prof. Dr. Martin Kerschensteiner (Institute of Clinical Neuroimmunology, University Hospital and Biomedical Center (BMC), Ludwig-Maximilians University Munich) talked about "A dynamic view of neuroinflammation."

Within the poster session 33 doctoral candidates presented their projects. The selection committee awarded the two poster prizes to

• Georgia Minakaki (Department of Molecular Neurology): „Autophagy inhibition promotes alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophago/exosome-like phenotype "

• Julia von Wittgenstein (Institute of Biochemistry): „Sox11 - a novel activity-regulated gene with dentate gyrus-specific expression "

From left to right: Prof. Dr. Becker, G. Minakaki, Prof. Lie

Figures

Research Grants

The IZKF offers research grants in the funding lines advanced projects, junior projects, pilot projects and junior research groups. In 2017, 39 advanced and 17 junior projects received funding of the IZKF. These projects cover the main research areas of the Faculty of Medicine, i.e. immunology and infection research, renal and vascular research, neurosciences and tumor research.

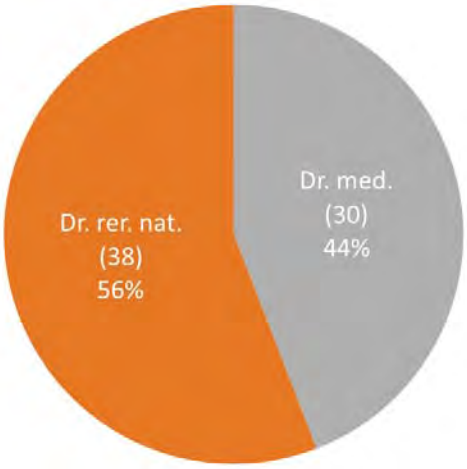
Institute	Immunology and Infection	Oncology	Neurosciences	Renal and Vascular Research	Others
Chair of Experimental Medicine I		x			
Chair of Experimental Medicine II		x			
Department of Anesthesiology			x		
Department of Dermatology	x	x			
Department of Immune Modulation	x				
Department of Infection Biology	x				
Department of Medical Informatics, Biometry and Epidemiology					x
Department of Medicine 1	x	x	x		
Department of Medicine 3	x	x			x
Department of Medicine 4	x			x	
Department of Medicine 5		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 Psychiatry and Psychotherapy			x		
Department of Stem Cell Biology			x		
Department of Surgery		x			
Division of Genetics	x				
Department of Molecular Neurology			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		
Institute of Physiology and Pathophysiology			x		

Institutes and departments running advanced or junior projects in 2017 and the respective association to the focal research areas of the Faculty.

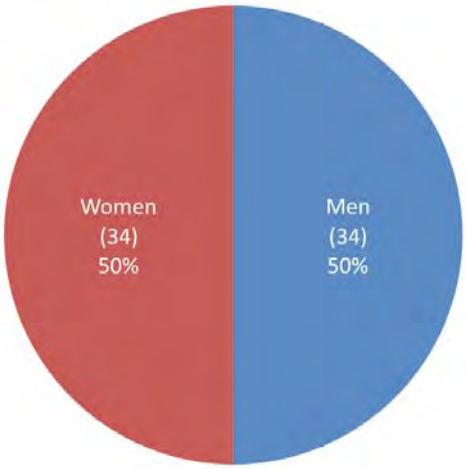
Junior Projects

The junior projects are established to support young postdoctoral physicians and scientists to obtain their first third-party funding. In 2017, 19 proposals were reviewed and 7 (37%) of them were selected for funding. The approved projects cover all of the focal research areas of the Faculty of Medicine. The successful applicants work in 6 different institutions within the Faculty of Medicine. In total, 5 (71%) are physicians and 2 (29%) scientists; 4 (57%) of the successful applicants are men and 3 (43%) are women. The median of age of them was 33 years.

The first call for junior projects was in 2009. Since then the programme is announced annually. Overall 68 junior projects were selected for funding between 2009 and 2017. Between 2009 and 2017, 30 (44%) physicians received funding and 38 (56%) scientists. 20 (67%) of the physicians requested a laboratory rotation, thereof 7 (35%) were women and 13 (65%) men. Over the entire period we equally supported men and women: 34 successful applicants were women and 34 men. The median of age was 32 at time of application, for both women and men.

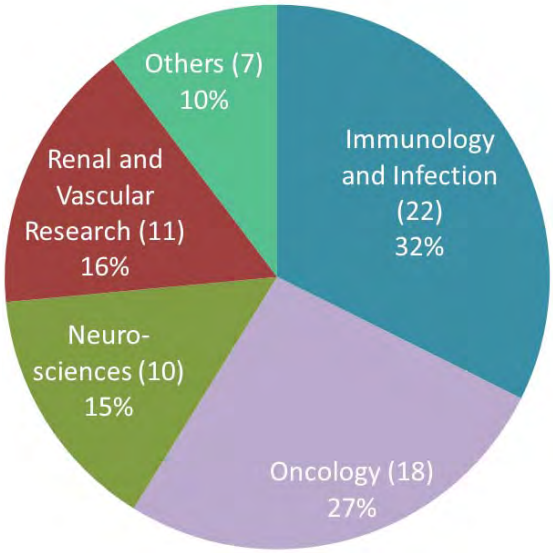


Distribution of physicians and scientists between 2009 and 2017.



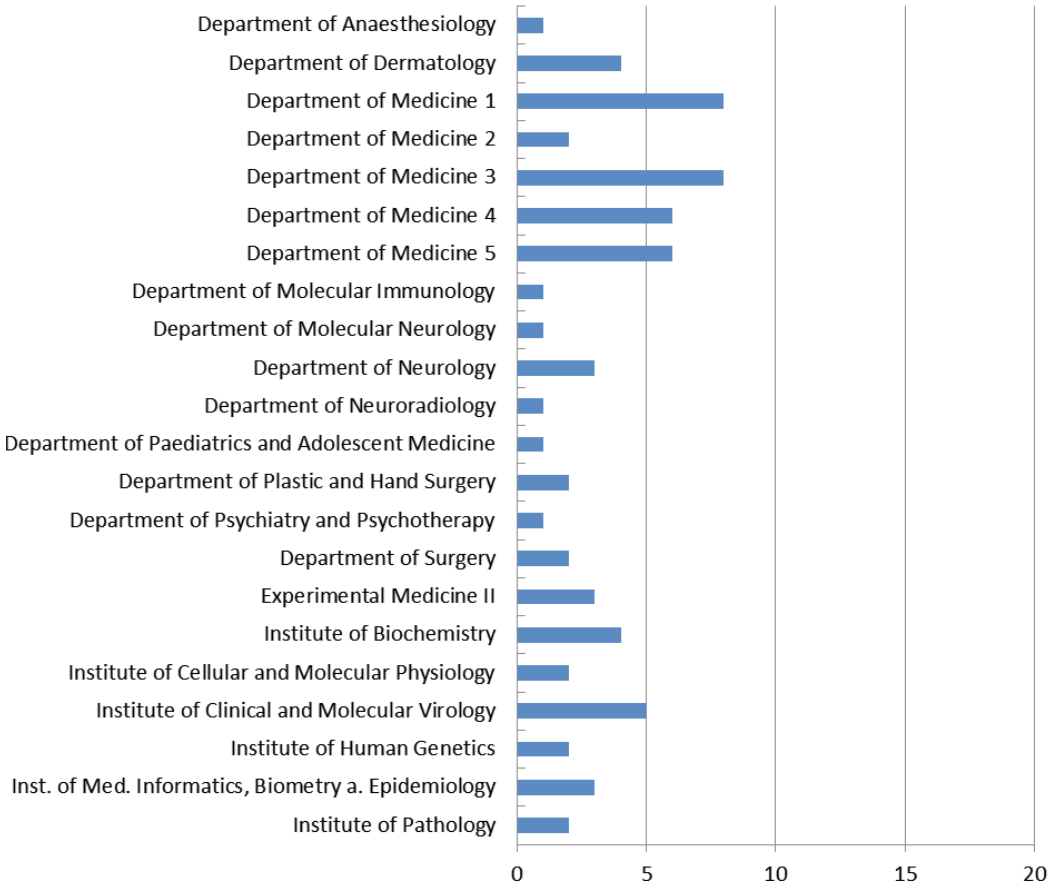
Gender distribution of junior projects between 2009 and 2017.

All focal research areas of the Faculty are represented with Immunology and Infection (32%) and Oncology (27%) being the most successful over the years.



Distribution of junior projects among main research areas of the Faculty of Medicine between 2009 and 2017.

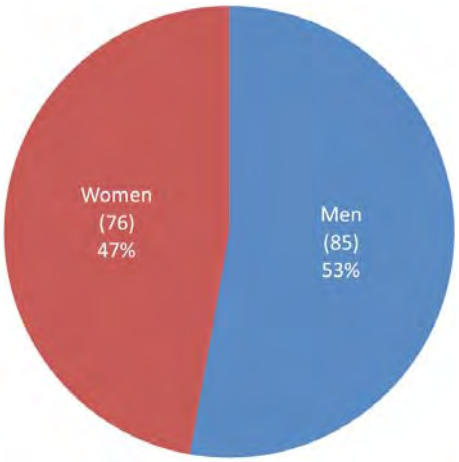
Overall candidates from 22 different institutions within the Faculty of Medicine were successful.



Distribution of junior projects among institutes between 2009 and 2017.

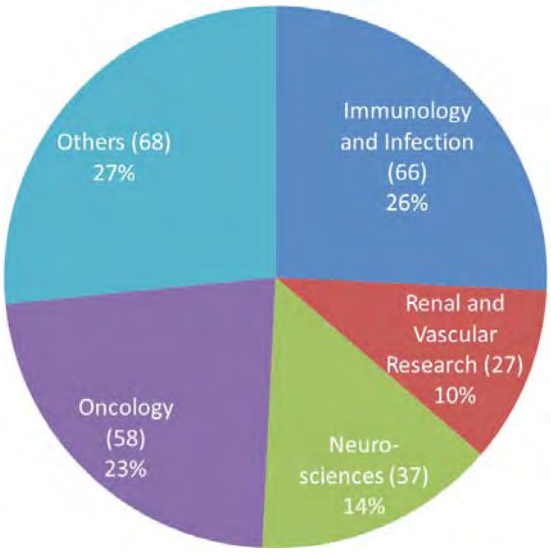
Pilot Projects

In 2017 the pilot project programme was newly integrated into IZKF. Pilot projects are intended to support scientists at an early stage. In the reporting period 2017 27 proposals were discussed during the meetings of the ELAN commission. Thereof, 21 (78%) received funding. The approved projects cover nearly all the focal research areas of the Faculty of Medicine: oncology 8, immunology and infection 7, neurosciences 1 and others 5 projects. In 2017, pilot project leaders from 15 different institutions were reviewed. In total, 11 (52%) of the successful applicants are men and 10 (48%) are women. The median of age was 32 for all project leaders. Women had a median of age of 32 and men of 33 years.



Gender distribution of pilot project leaders between 2012 and 2017.

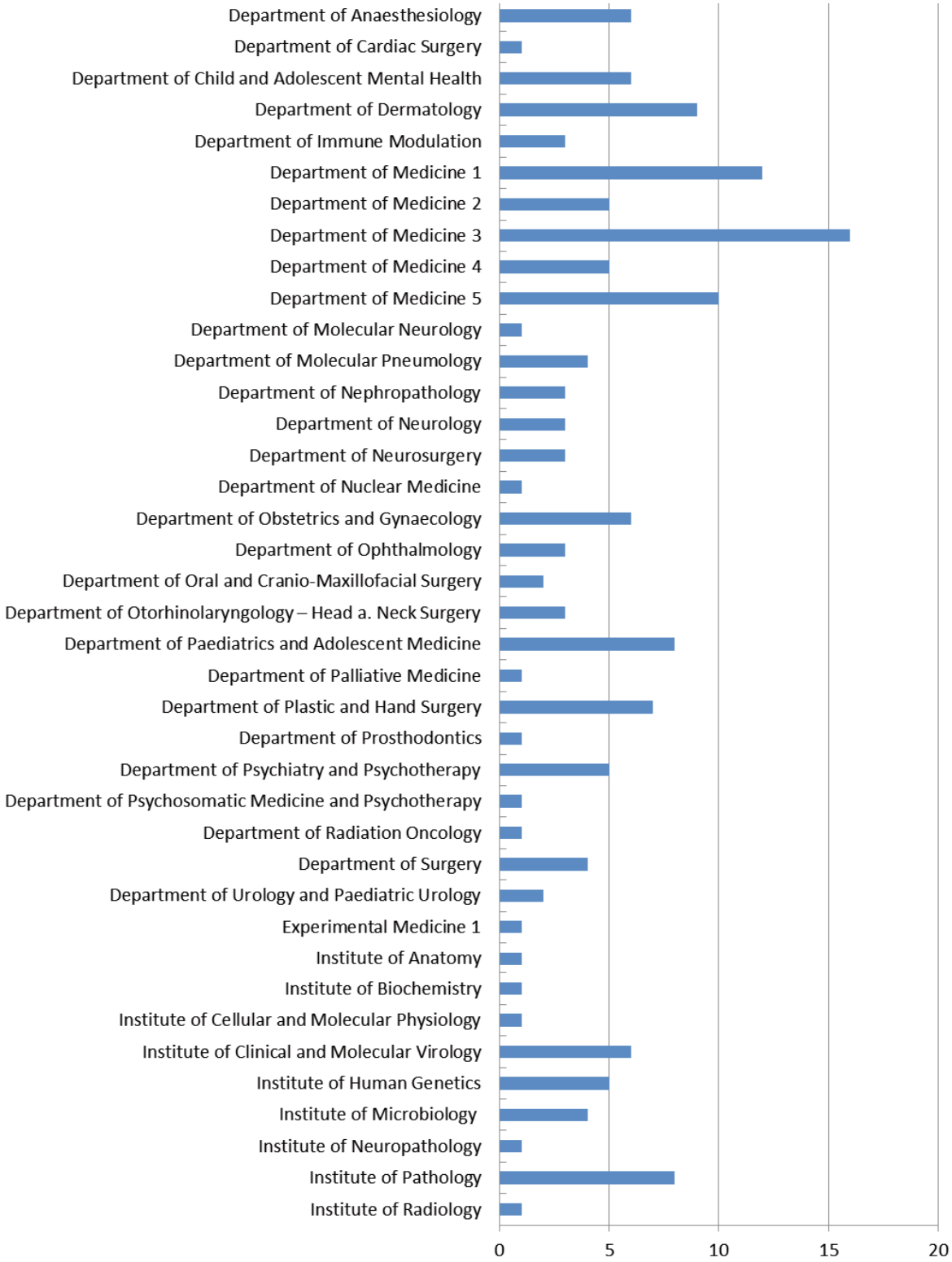
Applications for pilot projects can be submitted at any time. Since 2012 an electronic application using the ELAN-Tool is expected. The ELAN commission meets 5-6 times a year and selects projects for funding. Between 2012 and 2017 a total of 229 proposals for pilot projects were reviewed by the ELAN commission. Overall, 161 (70%) projects were granted for funding. Between 2012 and 2017 in total 76 women (47%) and 85 men (53%) applied successfully for pilot projects. The median of age was 34 years for all, 32 years for women and 34 years for men.



Distribution of pilot projects per main research areas between 2012 and 2017.

All focal research areas of the Faculty are represented with Immunology and Infection (26%) and Oncology (23%) being the most successful over the years. 59 (37%) of 161 projects were completed with a publication.

Candidates from 39 different institutions within the Faculty of Medicine were successful.

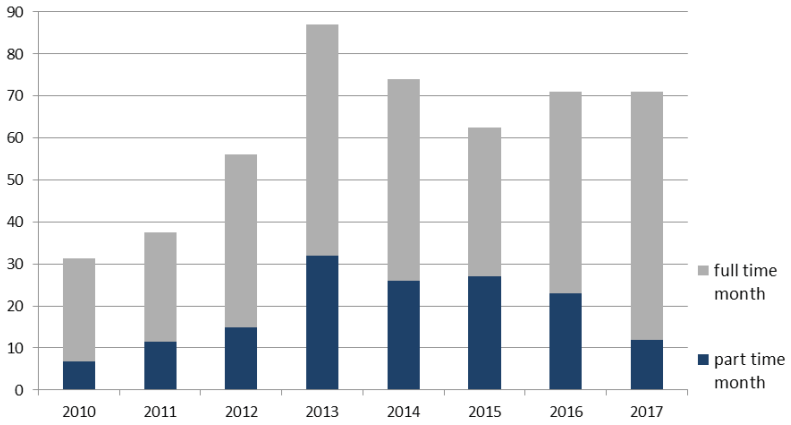


Distribution of pilot projects among institutes between 2012 and 2017.

Laboratory Rotations

In 2017 candidates from 11 different institutions were supported in the Laboratory Rotation Programme. Six new proposals were reviewed by the Management Board in 2017. Five (83%) of them were granted and just one proposal was rejected. Two (40%) of the granted candidates are women and 3 (60%) of them men. The median of age was 33 years at the time when the proposal was submitted.

Between 2012 and 2016 37 laboratory rotations have been funded. 26 (70%) of the funded candidates were men and 11 (30%) were women. The successful candidates were at the age of 29 to 40 years (average of women 34.5 years, average of men 35.5 years).

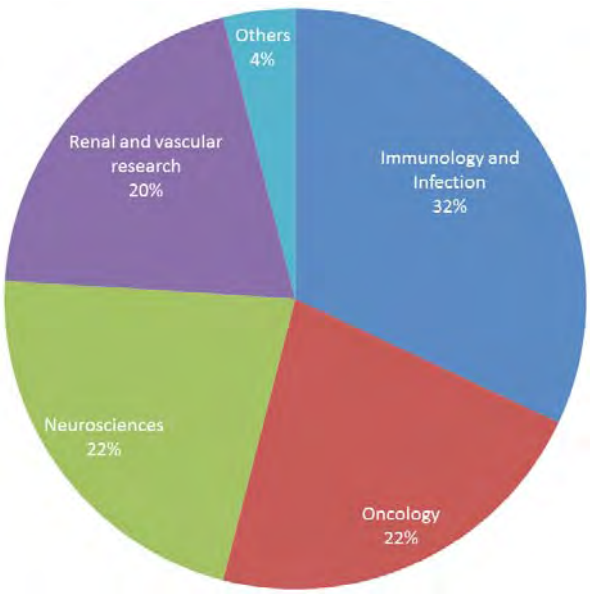


Man months funded in the rotation programme.

The IZKF monitors the career developement of all participants of the rotation programme. Six (16%) of them already finished their habilitation procedure. Another 10 (27%) habilitations are ongoing. Seven (19%) physicians obtained a position as group leader.

Many participants successfully applied for external funding. 23 (62%) of 37 participants received funding including both intramural and external funded ones.

Participants of the laboratory rotation programme work on research projects from all main research areas of the Faculty of Medicine.



Distribution of laboratory rotations among the main research areas from 2009 until 2017.

Name	Institution	Funding period	Full-time/ part-time
Dr. Esther Eberhard	Department of Anaesthesiology (AN)	09/2016 - 02/2017	100%
Dr. Marcel Vetter	Department of Medicine 1 (M1)	04/2017 - 09/2018	100%/50%
Dr. Wajima Safi	Department of Medicine 4 (M4)	07/2017 - 12/2017	100%
Dr. Benedikt Jacobs	Department of Medicine 5 (M5)	07/2017 - 12/2017	100%
Dr. Lisa Meintker	Department of Medicine 5 (M5)	07/2017 - 12/2017	100%
Dr. Clemens Bockmeyer	Department of Nephropathology (NT)	04/2016 - 03/2017	50%
Dr. Manuel Weber	Department of Oral and Cranio-Maxillofacial Surgery	06/2017 - 05/2018	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. Jakob Zierk	Department of Paediatrics and Adolescent Medicine (KI)	02/2017 - 07/2017	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%
PD Dr. Ulrike Hüffmeier	Institute of Humane Genetics (HU)	09/2015 - 08/2017	50%
Rotations of Junior Project leaders			
Dr. Fabian Müller	Department of Medicine 5 (M5)	11/2016 - 10/2017	100%
Dr. Martin Regensburger	Department of Neurology (NL)	07/2016 - 06/2017	100%

Laboratory Rotations running in 2017

MD-thesis Scholarships

Within the doctoral programme 18 scholarships for medical doctoral students were awarded each year until 2017, each granted for a period of 7 months with a monthly allowance of € 773. In 2017, the guidelines of the programme changed. Since April 2017 up to 25 grants are available for medical students. The scholarships with a monthly allowance of € 735 are offered during the research activity of 8 continuous months. A full-time dedication to the thesis is expected.

In 2017, a total of 39 medical doctoral students from 17 institutions were funded. Due to the fact that some scholarships granted in 2016 ended in 2017, the number of funded doctoral students is higher than the number of scholarships available. Overall, 27 applications for the MD-thesis scholarship programme have been received in 2017. The Junior Scientist Committee approved 23 applications (85%), 9 (39%) of the successful applicants were females and 14 (61%) males. The median was 23 years.

Since its inception in 2007, IZKF supported a total of 133 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 after they graduate. By the end of 2017, 46 (35%) students had already completed their doctoral thesis. Interestingly, 20 students (44%) obtained the highest degree possible, summa cum laude. This compares very favourably to the average 5% of all MD thesis presented and is testimony to the excellent quality of MD thesis performed within this programme.

Name	Institution	Funding period
Brandt, Amelie	Department of Medicine 3 (M3)	04/2017 - 10/2017
Böhm, Magdalena	Institute of Clinical and Molecular Virology (VI)	07/2016 - 01/2017
Eitler, David	Department of Otorhinolaryngology – Head and Neck Surgery (HN)	10/2017 - 05/2018
Fastancz, Petra	Department of Medicine 1 (M1)	11/2016 - 05/2017
Fischer, Kim	Department of Medicine 3 (M3)	11/2016 - 05/2017
Fritz, Niklas	Department of Cardiac Surgery (HC)	10/2016 - 04/2017
Greif, Vicky	Department of Medicine 1 (M1)	12/2017 - 07/2018
Hackenbracht, Julia	Institute of Neuropathology (NP)	01/2017 - 07/2017
Hanka, Isabella	Department of Cardiac Surgery (HC)	04/2017 - 10/2017
Hardt, Moritz	Department of Plastic and Hand Surgery (HP)	09/2016 - 03/2017
Hekler, Daniel	Department of Psychiatry and Psychotherapy (PS)	01/2017 - 07/2017
Heß, Merlin	Department of Paediatrics and Adolescent Medicine (KI)	04/2017 - 10/2017
Hoffmann, Lucas	Institute of Neuropathology (NP)	12/2017 - 07/2018
Hug, Karsten	Department of Medicine 2 (M2)	10/2017 - 05/2018
Kaul, Frederik	Institute of Biochemistry	04/2017 - 10/2017
Klingler, Anika	Department of Surgery (CH)	04/2017 - 10/2017
König, Loretta	Department of Psychiatry and Psychotherapy (PS)	09/2016 - 03/2017
Kossel, Clara	Department of Paediatrics and Adolescent Medicine (KI)	11/2016 - 05/2017
Luz, Hannah	Department of Medicine 4 (M4)	11/2016 - 05/2017
Mayer, Anna-Lena	Department of Nephropathology (NT)	11/2016 - 05/2017
Miedl, Markus	Department of Nephropathology (NT)	10/2017 - 05/2018
Mittag, Nora	Institute of Biochemistry	08/2017 - 03/2018
Offensperger, Laura	Department of Medicine 1 (M1)	10/2016 - 04/2017
Osterloh, Justus	Department of Medicine 1 (M1)	10/2017 - 03/2018
Prochnicki, Ania	Department of Nephropathology (NT)	04/2017 - 10/2017
Rentschler, Lukas	Department of Medicine 4 (M4)	11/2016 - 05/2017
Ritter, Maximilian	Department of Surgery (CH)	10/2017 - 05/2018
Rösel, Nadine	Department of Psychiatry and Psychotherapy (PS)	10/2017 - 05/2018
Rümmele, David	Department of Nephropathology (NT)	10/2017 - 03/2018
Schleier, Lena	Department of Medicine 1 (M1)	01/2017 - 07/2017
Schmid, Jonas	Department of Medicine 1 (M1)	10/2017 - 05/2018
Schnell, Alexander	Department of Paediatrics and Adolescent Medicine (KI)	01/2017 - 07/2017
Scholz, Julia	Department of Medicine 4 (M4)	10/2017 - 05/2018
Schulz, Oscar	Department of Medicine 3 (M3)	10/2016 - 04/2017
Seeberg, Jacob	Department of Radiation Oncology (ST)	07/2016 - 01/2017
Stegmann, Hedwig	Department of Paediatric Cardiology (KE)	11/2016 - 05/2017
Steinfeldt, Jakob	Department of Nephropathology (NT)	04/2017 - 10/2017
Steffen, Jan	Insitute of Cellular and Molecular Physiology	07/2016 - 01/2017
Westergerling, Parisa	Department of Medicine 4 (M4)	07/2016 - 01/2017

MD-thesis scholarships

News and Figures

Research Training Group

In 2017 the IZKF Research Training Group included 116 members.

Until November 2017, Isabella Schöpe was speaker of the IZKF Research Training Group for the area oncology, immunology, renal and vascular infection. Iris Stolzer was elected her successor. Benjamin Häberle was reelected as speaker of the IZKF Research Training Group for the area of neurosciences.

Participants IZKF Research Training Groupl	**116**
Neurosciences (ICN)	**56**
Participants from IZKF projects	16
Associated participants	37
MD-thesis scholarships	3
T(h)INK - Oncology, immunology and infection, renal and vascular research	**60**
Participants from IZKF projects	19
Associated participants	22
MD-thesis scholarships	19

Participants of the IZKF Research Training Group 2017

Soft skill and statistic courses given in 2017

- Scientific Writing, Dr. Deborah Bennett, 19.-21.07.2017, 15.-17.11.2017
- Presentation Skills, Dr. Deborah Bennett, 26.-28.04.2017, 22.-24.11.2017
- Kommunikation und Rhetorik, Gerhard Kranz, 11.-12.07.2017
- Microscopy course on sample preparation for two channel confocal fixed sample and spinning disc live cell imaging, Dr. Ralf Palmisano, 20.-23.06.2017
- Biostatistics, Dr. Matthias Englbrecht, 12.-13.05.2017, 06.-07.10.2017

Visiting Professor Programme

Lectures given by external scientists in 2017 - FAU-Visiting Professor Programme

Scientist (Proposed by)	Institute	Lecture title
Prof. Katharina Brandl (Prof. Beate Winner)	Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, USA	How to engage the classroom?
Dr. Mária Dux (Prof. Karl Meßlinger)	Department of Physiology, University of Szeged, Hungary	Veränderungen im trigeminovaskulären System nach Hochfett- und Hochzucker-Diät bei Nagern - Relevanz für die Pathophysiologie der Migräne
Prof. Carl June (Prof. Andreas Mackensen)	Department of Pathology and Laboratory Medicine, Univeristy of Pennsylvania, Philadelphia, USA	CAR T cells in leukemia and myeloma
Prof. Byung In Lee (Prof. Michael Buchfelder)	Department of Neurology, Yonsei University, Seoul, South Korea	Diagnosis and treatment of symptomatic epilepsies (with special regards to neurosurgery)

Scientist (Proposed by)	Institute	Lecture title
Prof. Sylvie Le Gall (Prof. Klaus Überla)	Ragon Institute of MGH, MIT and Harvard, Cambridge, USA	Antigen processing and presentation in HIV infection
Prof. Dingenus Meijer (Prof. Michael Wegner)	Centre for Neuroregeneration, University of Edinburgh, UK	Functional and structural differentiation of the axon
Prof. Hans Schreiber (Prof. Andreas Mackensen)	Division of Pathology, University of Chicago, USA	argeting cancer-specific mutations for T-cell Therapy
Prof. Stuart Shankland (Prof. Kerstin Amann)	Division of Nephrology, University of Washington, USA	Glomerular regeneration from Resident stem/ progenitor cells
Prof. Olaf Stüve (Prof. Ralf Linker)	Department of Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, USA	The Pathogenic Role of Antigen-Specific CD4+ T Cells in Neuromyelitis Optica
Dr. Scott L. Thomson (Prof. Michael Döllinger)	Department of Mechanical Engineering, Brigham Young University, Provo, USA	Computational and experimental studies of the biomechanics of human voice production

Lectures given by external scientists in 2017 - IZKF-Visiting Professor Programme

Scientist (Proposed by)	Institute	Lecture title
Prof. Francesco Ferraguti (Prof. Ralf Enz)	Institute of Pharmacology, Medical University of Innsbruck, Austria	The intercalated cell masses as a relay station for hippocampal and somatosensory inputs to the amygdala: Implications for fear lerning
Dr. Felix Hol (Dr. Dulin)	Department of Bioengineering, Stanford University, USA	Interrogating Mosquito-pathogen Communities using High-throughput Microfluidics
Dr. Sebastian Illes (Prof. Beate Winner)	Department of Physiology at Institute of Neuroscience and Physiology, University of Gothenburg, Sweden	Human induced pluripotent stem cell-based functional cortical circuits for personalised neuropsychiatric disease modelling
Dr. Bilal Kerman (Prof. Jürgen Winkler)	Histology and Embryology Department, Istanbul Medipol University School of Medicine, Turkey	In vitro modelling of myelination and demyelination
Prof. Jonel Trebicka (Dr. Peter Dittrich)	Department of Internal Medicine, University of Bonn, Germany	Renin-Angiotensin-System and development of liver disease and complications
Prof. Barbara Treutlein (Prof. Beate Winner)	Department for Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany	Reconstructing human neurogenesis using single-cell transcriptomics

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. In 2017, a single consolidated budget for the IZKF and the former ELAN Fonds is reported for the first time.

Part of the expenditures of 2017 were financed from residual funds of the previous years.

Financial Statement IZKF 2017

Revenues	
Support of the Medical Faculty	5,483 K€
Support of the University	364 K€
Contribution of IZKF for junior research groups	- 24 K€
Total revenues 2017	**5,823 K€**
Expenditures	
Advanced projects	3,081 K€
Pilot projects	975 K€
Career development	1,939 K€
thereof junior research groups	437 K€
thereof junior projects	911 K€
thereof laboratory rotations	446 K€
thereof MD-thesis scholarships	129 K€
thereof research training group	16 K€
Central projects	96 K€
Administration	159 K€
Total expenditures 2017	**6,250 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 58 scientific projects were actively running: 39 advanced projects, 17 junior projects and 2 junior research groups. In addition, 7 junior projects started their work in 2017 (5) or in the beginning of 2018 (2). These 58 funded scientific projects published 75 original articles in 2017 resulting in an average of 1.29 publications per project. The cumulative impact factor (IF) was 620.577, averaging 8.274 per publication. The high quality of many of these publications is reflected in 26 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 13 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 11 master theses, 78 doctoral theses and three habilitations that were in progress or finalised in 2017. A total of 72 scientists from 27 institutions are involved in 58 scientific projects funded by IZKF.

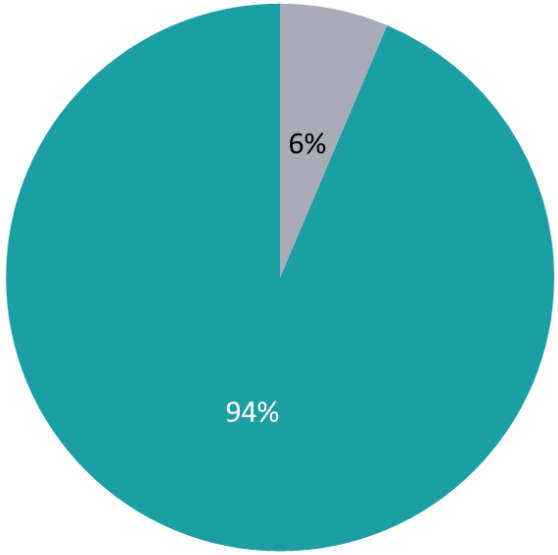
Some IZKF project leaders were able to achieve outstanding results. 16 prizes were awarded to IZKF project leaders. Five professorships were offered, two of them were accepted.

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.

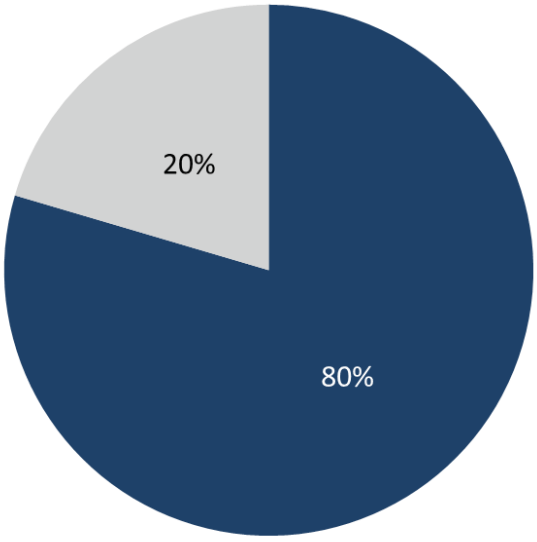
Beginning with the funding period 2010-2013, grants were awarded for a period of 30 months with an extension by 6 months, if these projects are submitted for external funding. Within the funding period 2013-2016 all projects submitted third party funds applications and therefore received the 6 months 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, 35 projects (80%) were granted extramural funding and only 9 (20%) 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 development has been stable over the entire duration of the programme.

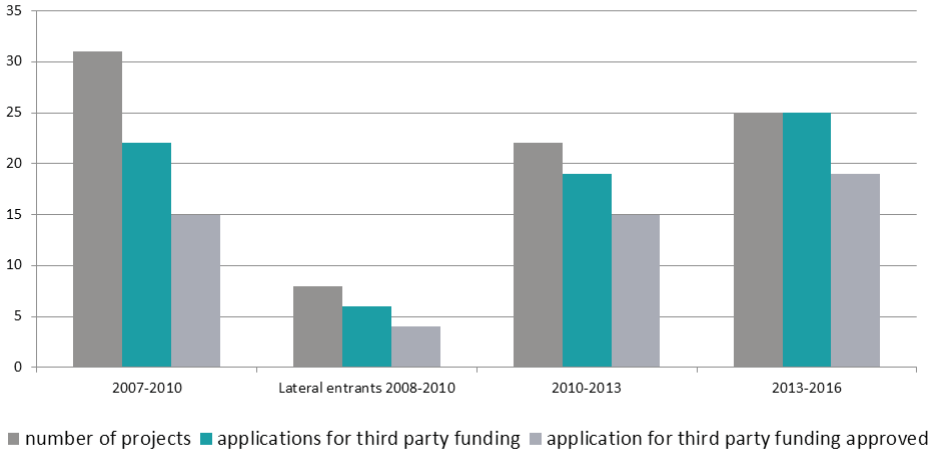
Acquisition of third-party funding by advanced projects



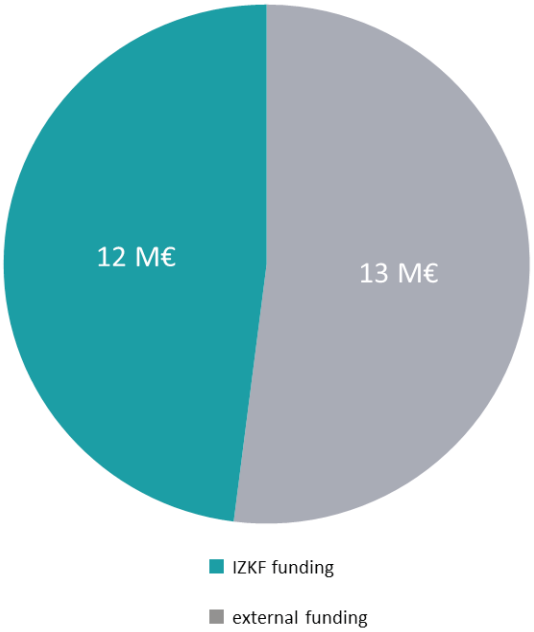
Applications for third-party funding submitted by advanced projects between 2010 and 2017



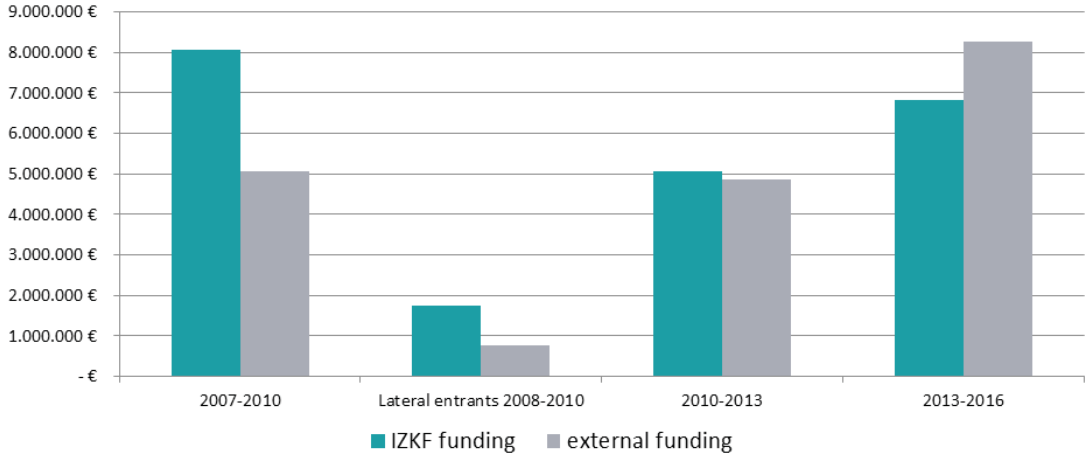
Approved applications for third-party funding of advanced projects between 2010 and 2017



This column graph compares the number of advanced projects with that for the submitted and approved applications for external funding in each funding period.



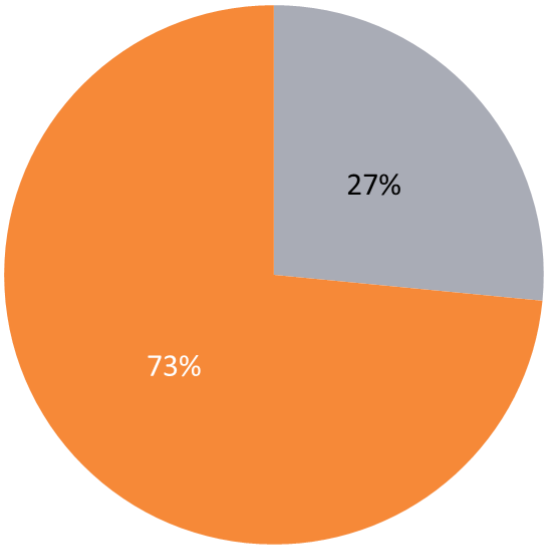
External funding received from advanced projects between 2010 and 2017



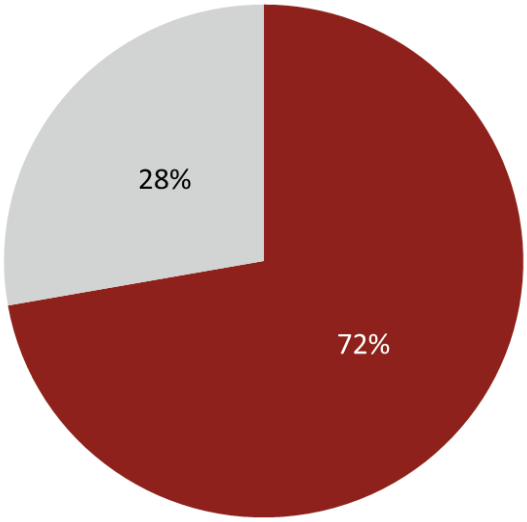
External funding received from advanced projects between 2007 and 2017.

News and Figures

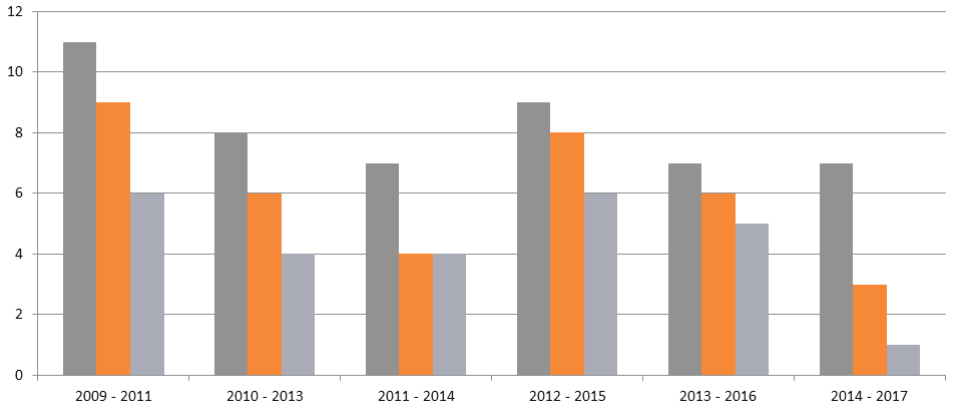
Acquisition of third-party funding by junior projects



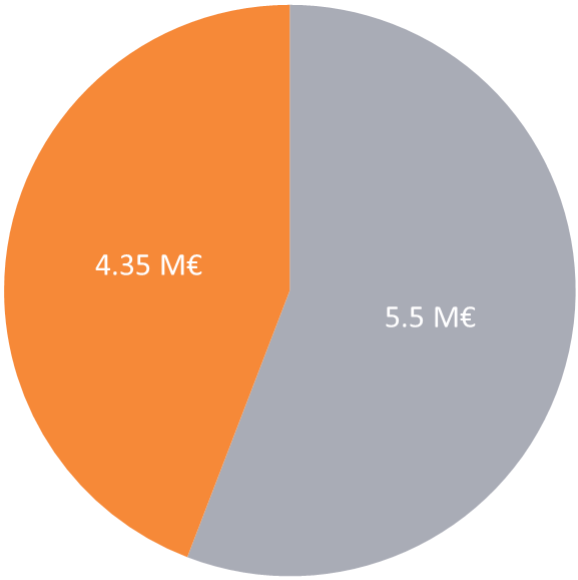
Applications for third-party funding submitted by junior projects (projects initiated between 2009 and 2014).



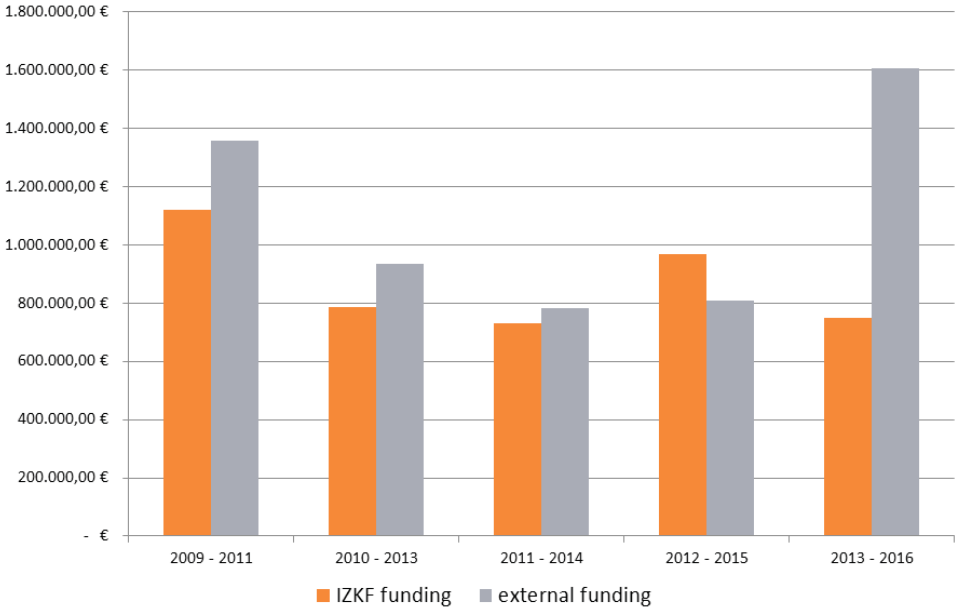
Approved applications for third-party funding of junior projects (projects initiated between 2009 and 2014).



Success-rate of junior projects. Initiated 2009-2013.



External funding received from all junior projects initiated between 2009 and 2013.

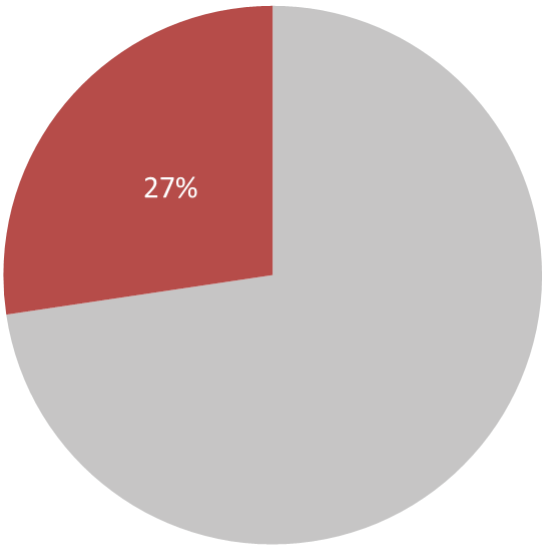


External funding received from junior projects initiated between 2009 and 2013.
* Several grant applications are still under evaluation.

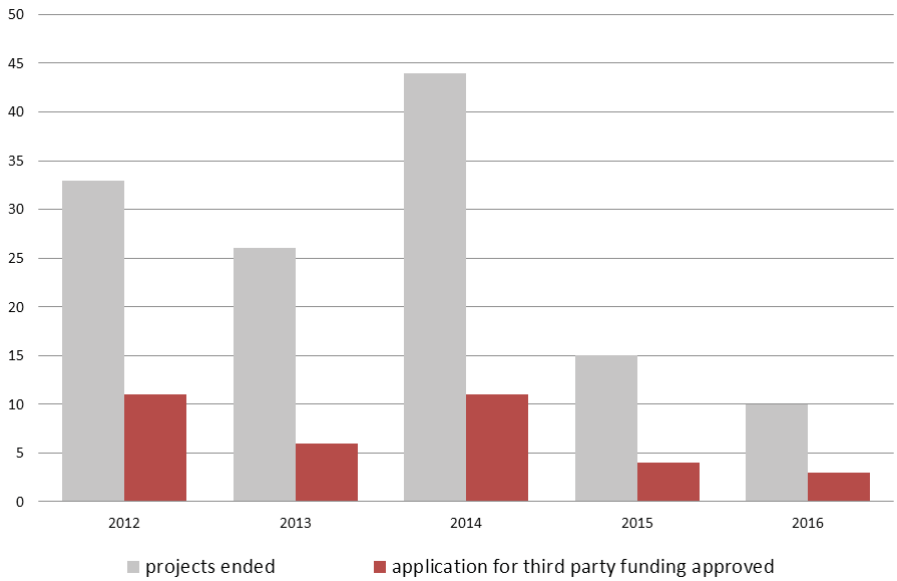
News and Figures

Acquisition of third-party funding by pilot projects

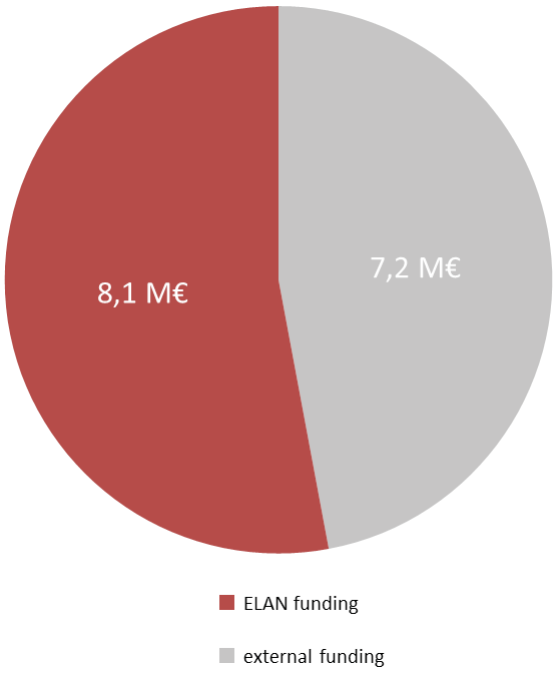
For this output analysis 128 pilot projects concluded between 2012 and 2016 were evaluated. In total 35 of them (27%) obtained external funding for the project.



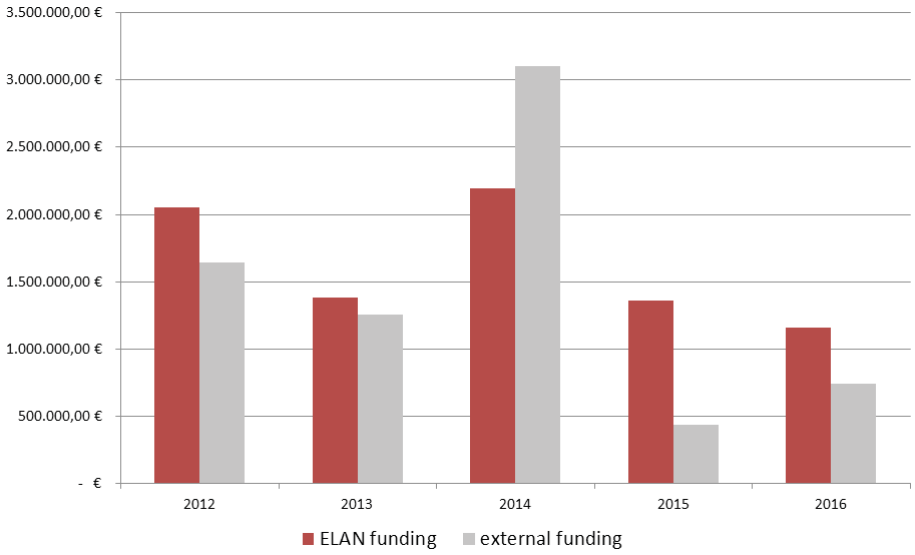
Approved applications for third-party funding of pilot projects (projects completed between 2012 and 2016).



Success-rate of pilot projects. Further applications of projects initiated in 2016 are planned.



External funding received from all pilot projects completed between 2012 and 2016



External funding from pilot projects completed between 2012 and 2016.

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Annual Report 2017

Publisher

Interdisziplinäres Zentrum für Klinische Forschung (IZKF)
Chairman: Prof. Dr. André Reis
www.izkf.med.fau.de

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Cover

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Layout and setting

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Photo credits

Cover: lecture hall, Uni-Klinikum Erlangen
p. 160 Eberhardt/p. 161 Patankar, Uni-Klinikum Erlangen

Printing

Druckhaus Haspel Erlangen e. K.
Willi-Grasser-Straße 13a
91056 Erlangen

Print run

350 copies

April 2018