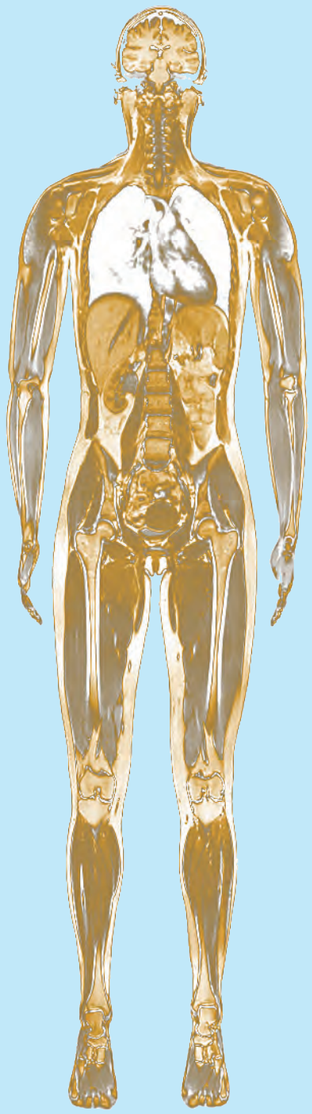
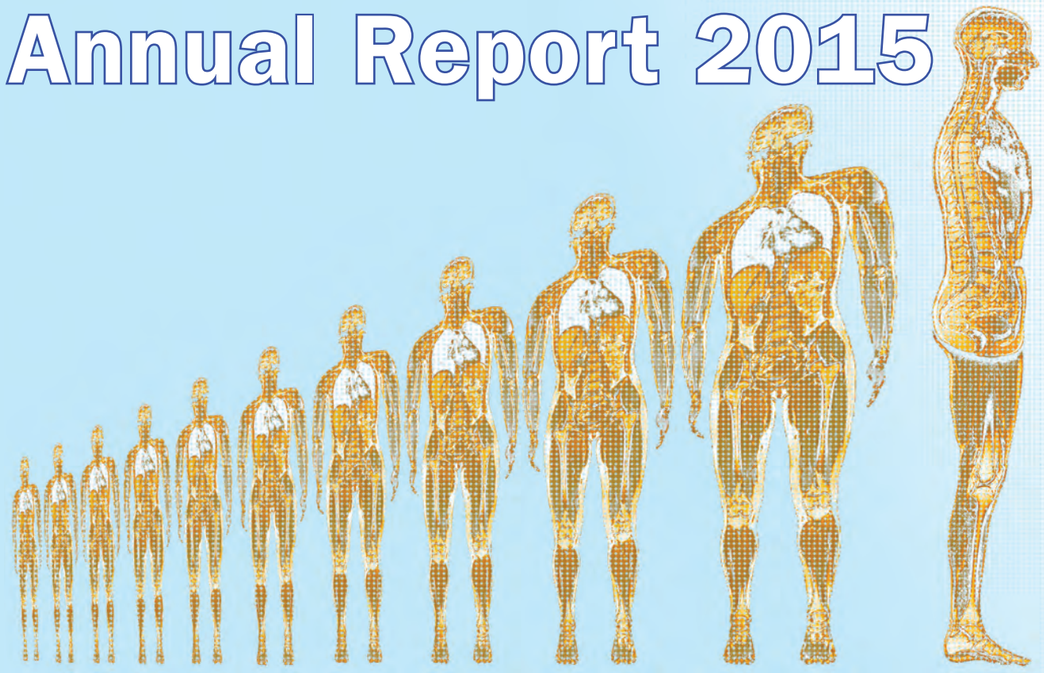


interdisciplinary

Center for Clinical Research Erlangen

IZKF Erlangen Annual Report 2015

Annual Report 2015



Universitätsklinikum Erlangen

FRIEDRICH-ALEXANDER UNIVERSITÄT ERLANGEN-NÜRNBERG
MEDIZINISCHE FAKULTÄT

IZKF Erlangen

IZKF Erlangen

Annual Report 2015

Editorial

The Interdisciplinary Center for Clinical Research Erlangen (IZKF) is the central intramural funding platform of the Faculty of Medicine. This annual report is brought to you as a testimony of our continued effort to achieve transparency on the work done at the IZKF and its performance towards supporting and developing clinically oriented research in Erlangen. After last years' restructuring of the report we are very pleased that it was well received not only for being even more informative in portraying the structure and activities of the IZKF but also for effectively showcasing the research done by the many participating scientists. Likewise the revised web-based presentation was a success, being more informative, transparent and up-to-date than ever.

The main focus of our activities in 2015 was again our biannual call for project proposals. When regularly evaluating large numbers of applications, electronic data handling is a must. Therefore the IZKF introduced in 2015 a new customized online electronic application and reviewing platform. Based on a concept initially established at a smaller scale by the ELAN-Fonds, a system tailored to the special needs of IZKF was developed. After rigorous beta-testing, the software withstood initial scrutiny on occasion of the review of the Junior Projects in the first half of 2015. After fixing some minor glitches, it was fully rolled out for the submission and review of the Advanced Projects later in the year. Overall it worked very well and was positively received, both by applicants and reviewers. This system reduces considerably the administrative work required, improving overall speed and transparency of the evaluation. The system will also have a long-term impact in facilitating monitoring of projects and continuous measurement of performance criteria, both of individual projects and of programmes as a whole.

For this year's call for Advanced Projects some minor changes were introduced, aimed at focusing the applications towards those with the best chances of success. Thus for each applicant the prerequisite of a running third party funded project was introduced. This reduced slightly the number of applications received to 58 (34 projects from a single institution and 24 interdisciplinary projects). Applications were reached in from 26 institutions of the University Hospital and 11 form the FAU. After a first round of written evaluation, 47 projects were selected for oral presentation at a two day symposium on July 6th-7th. Of these, the evaluation board selected a total of 33 projects for full application to the external Scientific Advisory Board (SAB) on occasion of its site visit on November 19th-20th. The SAB praised the selection process and lauded the scientific level of the applications. Due to budget constraints, though, it had to take some difficult decisions and finally reduced the funding level to an equivalent of 28 projects.

As in previous occasions the SAB also evaluated the overall development of the IZKF. In its final report the SAB accredited the IZKF for its continued outstanding scientific, structural and financial development. The programmes are very well structured and funding is clearly oriented towards successful nursing of projects for acquisition of extramural funding. The SAB also praised the continued efforts to monitor the output of projects and the successful collaboration with the Faculty and the University as a whole.

The SAB also made several general recommendations. It strongly emphasized the integration of both intramural funding activities into one unified structure, i.e. the integration of the ELAN-Fonds into the IZKF structure with access of the entire Faculty of Medicine to all funding lines. This way the intramural funding strategy activities of the Faculty would become more recognizable as a coherent strategy tailored to the varying requirements during a scientific career. The Management Board has discussed these recommendations together with the Faculty and I have presented a plan to reform the IZKF structures in preparation for such an integrated intramural funding body. After extensive discussions by the Boards of IZKF and Faculty it is anticipated that, after approval by the Medical Faculty, such a restructured IZKF will become operational by the end of 2016. This will represent an important overhaul of its structures with a full integration into the Faculty in agreement with its mission. On another line we were also advised to

focus our funding activities more towards collaborative projects e.g. through funding of defined innovative topics in dedicated calls. The Management Board will continue the progress review of all funding lines and act where appropriate in time for the next round of applications in 2017.

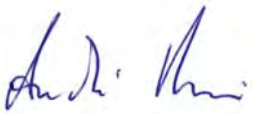
In 2015 the scientific programme committee for our biannual International Meeting in Kloster Banz worked intensively and put together an interesting scientific programme under the general header of „Translational Medicine". We look forward to an exciting meeting on June 16th-17th, 2016 and expect a full house with lots of new ideas and inspiring contacts in the beautiful environment of the baroque monastery of Banz.

The support of young scientists continues to be a main goal of IZKF. As in previous years the call for applications in the Junior Projects programme jointly carried out with the ELAN-Fonds was well received. This programme supports young scientists with first research experience in pursuing interesting ideas and concepts that will hopefully lead to extramural funding after a 2.5 year period. In 2015, proposals for 20 projects were reviewed from which 6 were selected for funding including four applications from physicians. This eased off concerns over the attractiveness of this funding scheme for MDs that arose the previous year, when only PhDs were funded. Also the programme for laboratory rotations was again very successful with a rate of utilisation close to 100%, confirming a continued interest in a physician-scientist career.

On a more experienced level, IZKF supports Junior research groups for up to 6 years. The funding level is comparable to that of ERC starting grants with an international candidate search and competitive allocation. Last year we appointed Dr. Paolo Ceppi from Chicago / USA as the new leader of Junior Group 2 with the title "A novel role for thymidylate synthetase in solid tumors". The group with an oncology focus started August 1st and is housed in the Nikolaus-Fiebiger-Zentrum. We wish him and his team success. In the second half of 2015, the selection process of a new leader for Junior Group 1 in the area of "Physics and Medicine" was initiated and we expect to inaugurate the new group starting September 1st, 2016. The research focus will be in the area of the new "Max-Planck-Center Physics and Medicine", a joint venture between Max-Planck-Institute, FAU, the Faculty of Medicine and the University Hospital. The group will focus on innovative physical (microscopic) methods to study biological processes related to disease. In this way the IZKF wants to support important new research activities of the Faculty.

The graduate school has also matured further with the split into two large groups, one on neuroscience in conjunction with the interdisciplinary Center for Neuroscience (iCN) and one on diverse topics i.e. oncology, immunology, infection, kidney and circulation. This later group is known by its German acronym as T(h)ink-group. In addition to the compulsory members (IZKF graduate students and MD stipend holders), a large number of associated members from other projects and funding sources have joined, indicating a widespread demand for such structured programmes. From these developments it becomes obvious that the graduate school needs to develop even further, to both accommodate the large demand as well as the high heterogeneity. The Board has initiated activities to develop such a comprehensive structure and programme following the initiative of the Junior Scientist Committee chaired by Prof. Becker. The concepts are currently being discussed and will hopefully be implemented later this year. The aim is to join forces with other graduate programmes to create synergies and to better tailor programmes to the individual need of every graduate student.

Finally, at the annual meeting in November, we bid farewell to Prof. Stürzl and Dr. Boos after the end of their respective terms in the Junior Scientist Committee and welcomed Prof. Engel (Nephropathology) and Dr. Bosch-Voskens (Dermatology) as new members. Prof. Becker (Internal Medicine 1) took the chair of this committee from Prof. Stürzl who we especially thank for his dedication to the establishment of the IZKF graduate school.



Prof. Dr. André Reis

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About us

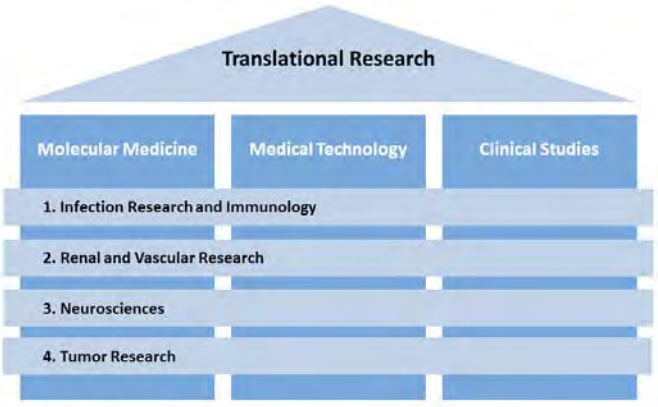
History

The 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

- Immunology and infection research
- Renal and vascular research
- Neurosciences
- Tumor research

In addition, the acquisition of extramural funding has become a central aim of project funding.



Main research areas of the Faculty of Medicine

Mission Statement

The Interdisciplinary Center for Clinical Research (IZKF) is a 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 on 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 assesed in a two-stage review process. Junior projects are subject to a one-stage internal review only.

Governance

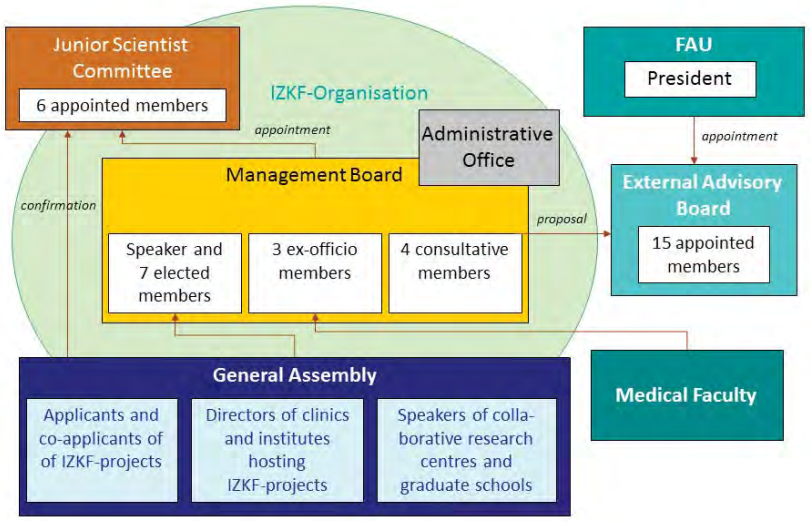
IZKF is a self-organised structure within the Faculty of Medicine. The IZKF has a set of written rules and regulations approved by the IZKF General Assembly and the Faculty of Medicine. Governing bodies include the General Assembly, the Management Board, the Junior Scientist Committee and the External 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 11 members with voting power, eight elected by the general assembly for a three year period and three ex-officio members from the Faculty of Medicine as well as four consultative 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 Speaker 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 years to oversee the general development of the IZKF and the proposed projects. The Board consists of at least 10 internationally recognised scientists (currently 15) 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 Scientists 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 five project leaders, three from research grants, one from junior projects and one of the junior research group leaders. Recently, also a representative from the doctoral students became a member of the Junior Scientist Committee.

The General Assembly convenes once a year to vote on important issues and to approve the annual report of the Speaker. It elects the Speaker, the deputy, representatives of the research areas and the junior research groups for a three-year term. It ratifies the members of the Junior Scientists Committee appointed by the Management Board. 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. All members can stand for office. Every project has one voting delegate and decisions are reached by simple majority. A 2/3 quorum is required.



Governance of the IZKF

About us

Statutary Bodies

Management Board

Speaker

Prof. Dr. André Reis, Institute of Human Genetics

Deputy Speaker

Prof. Dr. Michael Wegner, Institute of Biochemistry

Members

Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene
Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I
Prof. Dr. Kai-Uwe Eckardt, Department of Medicine 4
Prof. Dr. Andreas Mackensen, Department of Medicine 5
Prof. Dr. Markus F. Neurath, Department of Medicine 1
Prof. Dr. Dr. Jürgen Schüttler, Dean of the Faculty of Medicine, Department of Anaesthesiology
Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology
Prof. Dr. Beate Winner, IZKF Junior Research Group 3

Consultative Members

Prof. Dr. Joachim Hornegger, President of the FAU
Dr. Sybille Reichert, Head of Administration of the FAU
Prof. Dr. Heinrich Iro, Medical Director of the University Hospital Erlangen
Dr. Albrecht Bender, Head of Administration of the University Hospital Erlangen

Prof. Dr. Reis

Prof. Dr. Wegner

Prof. Dr. Bogdan

Prof. Dr. Brabletz

Prof. Dr. Eckardt

Prof. Dr. Mackensen

Prof. Dr. Neurath

Prof. Dr. Dr. Schüttler

Prof. Dr. Steinkasserer

Prof. Dr. Winker

Prof. Dr. Winner

Prof. Dr. Hornegger

Dr. Reichert

Prof. Dr. Iro

Dr. Bender

Current Members of the Management Board

About us

Junior Scientist Committee


Prof. Dr. Becker


Prof. Dr. Schulze


Prof. Dr. Winner


Bormann


Prof. Dr. Engel


Dr. Bosch-Voskens

Current Members of the Junior Scientist Committee

Chairman

Prof. Dr. Dr. Michael Stürzl, Department of Surgery (till 09.12.2015)
Prof. Dr. Christoph Becker, Department of Medicine 1 (since 17.12.2015)

Vice Chair

Prof. Dr. Schulze, Department of Oto-Rhino-Laryngology - Head and Neck Surgery

Members

Dr. Anja M. Boos, Department of Plastic and Hand Surgery (till 09.12.2015)
Tobias Bormann, Department of Medicine 1 (since 09.12.2015)
Dr. Caroline Bosch-Voskens, Department of Dermatology (since 09.12.2015)
Prof. Dr. Felix Engel, Department of Nephropathology (since 09.12.2015)
Prof. Dr. Beate Winner, IZKF Junior Research Group 3

Administrative Office


Reichel


Dr. Faber


Meyerhöfer-Klee


Reinwardt

Current staff of the Administrative Office

Manager

Dr. Katrin Faber

IZKF Administration

Anne Reichel

Bianca Meyerhöfer-Klee (part-time)

Miriam Reinwardt (part-time)

About us

General Assembly

Surname	Name
Achenbach	Stephan
Amann	Kerstin
Bach	Christian
Baur	Andreas
Becker	Christoph
Behrens	Jürgen
Bogdan	Christian
Bosch-Voskens	Caroline
Brabletz	Thomas
Ceppi	Paolo
Croner	Roland
Dees	Clara
Dietel	Barbara
Distler	Jörg
Dörfler	Arnd
Eckardt	Kai-Uwe
Engel	Felix
Eulenburg	Volker
Ferrazzi	Fulvia
Finotto	Susetta
Gefeller	Olaf

Surname	Name
Gerlach	Katharina
Grützmann	Robert
Günther	Claudia
Hahn	Alexander
Hartmann	Arndt
Hashemolhosseini	Said
Hildner	Kai
Iro	Heinrich
Klucken	Jochen
Kornhuber	Johannes
Krönke	Gerhard
Küspert	Melanie
Lehmann	Christian
Leppkes	Moritz
Lie	Dieter Chichung
Mackensen	Andreas
Marxreiter	Franz
Mayr	Andreas
Mchedlidze	Tamar
Müller	Christian P.
Naschberger	Elisabeth

Surname	Name
Neufert	Clemens
Neurath	Markus
Nitschke	Lars
Ramming	Andreas
Regensburger	Martin
Reichel	Martin
Reis	André
Reuter	Nina
Schauer, neé Schorn	Christine
Schett	Georg
Schierer	Stephan
Schleicher	Ulrike
Schmidt	Manuel
Schneider-Stock	Regine
Scholtysek	Carina
Schuler	Gerold
Schulze	Holger
Schüttler	Jürgen
Schwab	Stefan
Sonnewald	Uwe
Spriewald	Bernd

Surname	Name
Stamminger	Thomas
Steinkasserer	Alexander
Stürzl	Michael
Thiel	Christian
Überla	Klaus
Völkl	Simon
Waldner	Maximilian
Warnecke	Christina
Wegner	Michael
Winkler	Jürgen
Winner	Beate
Wirtz	Stefan
Xiang	Wei
Zimmermann	Katharina

General Assembly of the IZKF at 09.12.2015

About us

External Scientific Advisory Board

Prof. Dr. Häussinger

Chairman

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

Prof. Dr. Sendtner

Vice-Chair

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

Members

Prof. Dr. Reinhard Büttner,
Cologne University Hospital - Institute of Pathology

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

Prof. Dr. Heinz Höfler (till 31.12.2015),
Technical University of Munich - Institute of Pathology

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

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

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

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

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

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

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

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

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

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

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

Prof. Dr. Frauke Zipp (till 31.12.2015),
Mainz University Medical Center - Department of Neurology


Prof. Dr. Büttner


Prof. Dr. Hengel


Prof. Dr. Katschinski


Prof. Dr. Kelm


Prof. Dr. Kurts


Prof. Dr. Pavenstädt


Prof. Dr. Pfeffer


Prof. Dr. Rieß


Prof. Dr. Schmiegel


Prof. Dr. Schulz


Prof. Dr. Seufferlein


Prof. Dr. Tiegs


Prof. Dr. Wirth

Current External Scientific Advisory Board

Programmes

Programmes

Research Grants
Career Development Programmes
Central Projects

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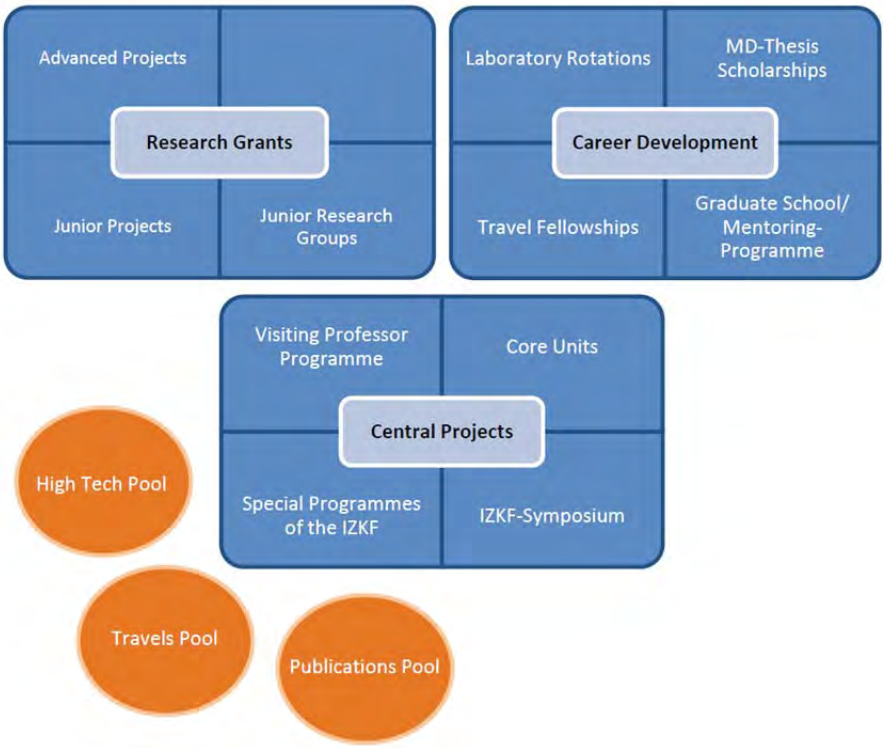
Programmes

Overview

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



Programmes of the IZKF

Research Grants

Advanced Projects

The IZKF offers research grants in all focal research areas of the Faculty of Medicine, i.e. immunology and infection research, renal and vascular research, neurosciences and tumor research. 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 extended for another 6 months.

IZKF projects ordinarily include two personnel positions (postgraduate and technical assistant or two postgraduates, or in exceptional cases a post doctoral scientist). Applicants are expected to have an active publication record and own external funding. Preliminary results should yield the promise of a successful transfer of the project into external funding after the 30-months term. Innovative and original ideas and

concepts are especially valued as well as the clinical relevance and interdisciplinary approaches. Applicants from all clinics, departments and institutes of the Faculty of Medicine and co-applicants from other faculties are entitled with no age limit.

Project funding is allocated after a stringent peer-review process based solely on scientific criteria. Research grants are approved after a two-stage review process. In an initial step, draft proposals are subject to an internal review by the Management Board, the Junior Scientists Committee, members of the ELAN committee and other recognised 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 years.

Staff	Postgraduate scientist Technical assistant	Two postgraduate scientists
Consumables	20 T€ p.a.	20 T€ p.a. / scientist and institution
Others	Special programmes of the IZKF during project duration	
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 in collaboration with the ELAN programme offers starting grants to young postdoctoral physicians and scientists up to 35 years of age without previous significant external funding. Candidates should have a visible publication record and projects should be based on an original idea with first tangible results. Projects include a position for a technician or a postgraduate and consumables for 30 months. After this time it is expected that successful candidates submit an external grant application. If the application is filed within duration of the junior project, the spending period will be extended by another 6 months.

Junior projects are subject to a one-stage internal review only. Full proposals are reviewed by the Management Board, the Junior Scientists Committee and the ELAN committee 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.

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

Junior Research Groups

Junior research groups offer an attractive career development opportunity for outstanding young scientists with a training in medicine or natural sciences and a strong background and reputation in one of the Faculties' main research fields. Over a period of 6 years each junior research group receives funding for the group leader, one postdoctoral and one postgraduate scientist, one technical assistant and consumables. From this position several previous junior research group leaders have been appointed to a professorship or have achieved other attractive positions. The groups operate independently but may be associated to individual clinics or institutes. For physicians a part time involvement in clinical activities is possible. Groups also have access to research funds allocated by the Faculty based on scientific performance criteria. At the end of 2015 two junior research groups exist. They are housed in the Nikolaus Fiebiger Center for Molecular Medicine with its attractive scientific environment and diverse activities.

Staff	Group Leader Postdoctoral scientist Postgraduate scientist Technical assistant
Consumables	50 T€ p.a.
Others	Participation in the allocation of funds based on performance criteria (LOM) Provision of laboratory space Initial provision of investment funds
Duration	6 years

Career Development Programmes

Support for and development of young scientists has been a central goal of the IZKF since its inception. In addition to 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. IZKF supports young scientists in temporary rotating into a laboratory to fully devote themselves to their projects. This rotation can be for 6-12 months in full time or 12-24 months in part time. This programme is open to all young clinicians of the faculty. Junior project leaders can also access this programme in addition to their project funding. The IZKF can allocate 8 full-time positions; this equates to 96 months, which can be used flexibly. The initial grant always consists of 6 months in full time or 12 months in part time. Extensions are conditional on successful evaluation based on oral presentation of work progress and updated work programme.

MD-Thesis Scholarships

This programme was initiated to arouse interest for science in motivated medical students early on in their career. Medical students are supported in performing an experimental thesis in association with the IZKF or externally funded projects. It is expected that they spend a significant time in a laboratory. The IZKF offers 7 months grants and the supervision of a tutorial committee consisting of 2 experts. Up to 18 grants are available for medical students with very good study degrees and a demonstrated scientific interest. The programme provides the participation in specific programmes, events and workshops of the IZKF. In accordance with the recommendations of the Scientific Advisory Board of IZKF, medical doctoral students have been integrated in the IZKF Graduate school. Medical students are required to participate in the structured seminar programme of the Graduate School and to present their projects.

Travel Fellowships

Travel fellowships 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 now possible.

Graduate School

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

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

Aims 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 as well as site visits to other laboratories in academia and industry are organised by the Junior Scientist Committee.

Mentoring-Programme

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

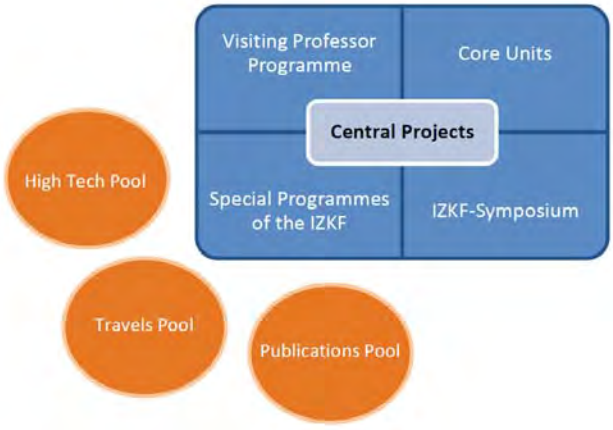
At least one annual meeting between the supervisor, the mentors and the doctoral student is expected. A participation in the IZKF Graduate School and the Postgraduate Workshop is mandatory.

Postgraduate Workshop

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

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

Central Projects



Core Facilities

Modern molecular technologies, such as genomics, proteomics and advanced molecular imaging, require very expensive and complicated instrumentation and are methodologically very demanding. Thus it is often not scientifically worthwhile or cost-effective to establish and maintain these techniques in parallel in different groups. Core facilities or units are 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.

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

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

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

Core units of the Faculty of Medicine currently in operation:

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

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.

Conference hall IZKF Symposium

Visiting Professor Programme

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

IZKF Visiting Professor Programme

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

Visiting Professor at the IZKF Symposium (Prof. P. Lichter)

FAU Visiting Professor Programme

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

Special Programmes

Special programmes provide additional funding for IZKF projects.

High Tech Pool

IZKF actively encourages the use of modern "omics" technologies in the projects, such as those used in the Core Unit Ultra Deep Sequencing. Since these experiments are quite expensive and consumables within IZKF projects are restricted to € 20,000, additional support is available. Costs for consumables are supported with up to € 10,000 per project.

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.

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 financial contribution of the IZKF is € 1,200. This programme is also available for successful applicants for MD-thesis scholarships and laboratory rotations.

Advanced Research Grants

Advanced Research Grants

Progress and Final Reports

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Advanced Grants

Immunology and Infection

Project No.	Project title	Term	Applicant(s)	Institute
A52	cFlip isoforms in the intestinal epithelium	01.11.2013-31.10.2016	Dr. Günther, Prof. Becker	Department of Medicine 1
A53	Th17/piTreg differentiation in vivo	01.10.2013-30.09.2016	Prof. Hildner	Department of Medicine 1
A54	Fam180a in inflammatory diseases	01.11.2013-31.10.2016	PD Dr. Dr. Wirtz, Prof. Waldner	Department of Medicine 1
A55	NR4a1 during immunologic tolerance	01.01.2014-31.12.2016	PD Dr. Krönke	Department of Medicine 3
A56	Role of HIG2 in atherosclerosis	01.03.2014-31.08.2016	PD Dr. Warnecke	Department of Medicine 4
A57	Nr4a1 as a novel target for the treatment of scleroderma-tous chronic graft-versus-host disease	01.01.2014-30.06.2016	Prof. Distler, Prof. Spriewald	Department of Medicine 3, Department of Medicine 5
A58	Characterization of DN T cells from ALPS patients	01.10.2013-30.09.2016	Prof. Mackensen, Dr. Völkl	Department of Medicine 5
A59	IL-10 and lung cancer	01.10.2013-30.09.2016	Prof. Finotto	Department of Molecular Pneumology
A60	Monocyte derived Dendritic cells (Mo-DC) by DC Exosomes	01.10.2013-30.09.2016	Prof. Baur, Dr. Schierer	Department of Dermatology
A61	Leishmania, iNOS and iron	01.02.2014-31.07.2016	Prof. Bogdan, PD Dr. Schleicher	Institute Clinical Microbiology, Immunology and Hygiene
A62	ND10 and interferon-induced gene expression	01.01.2014-30.06.2016	Prof. Stamminger	Institute of Clinical and Molecular Virology
A63	Mechanisms of TNF-Mediated Control of Intracellular Pathogens in Mice and Man	30 months	Prof. Bogdan	Institute of Clinical Microbiology, Immunology and Hygiene
A64	The tyrosine-protein phosphatase SHP2 regulates TGFβ -dependent activation of JAK2/STAT3 in fibrotic diseases	01.02.2016-31.07.2018	Prof. Distler, Prof. Schett	Department of Medicine 3
A65	Tolerizing potential of human dendritic cell subpopu-lations	01.04.2016-30.09.2018	Prof. Dudziak	Department of Dermatology
A66	Genome wide CRISPR/Cas9 knockout for the identifica-tion of antiviral cellular restriction factors	01.07.2016-31.12.2018	Prof. Ensser	Institute of Clinical and Molecular Virology
A67	Analysis of the TRIM5alpha-mediated block to LINE-1 retroelements	01.02.2016-31.07.2018	Prof. Gramberg	Institute of Clinical and Molecular Virology
A68	Analysis of the role of the IL-23/Th17 axis during the control of antibody activity in rheumatoid arthritis	30 months	PD Dr. Krönke, Prof. Nimmerjahn	Department of Medicine 3, Chair of Genetics
A69	Contribution of ATM kinase and the DNA-damage response in the innate response to infection	30 months	Prof. Lang	Institute of Clinical Microbiology, Immunology and Hygiene

Project No.	Project title	Term	Applicant(s)	Institute
A70	Novel targets for antiretroviral therapy - deubiquitinating enzymes regulate HIV-1 replication	01.07.2016-31.12.2018	Prof. Schubert	Institute of Clinical and Molecular Virology
A71	Viral modulation of the protein kinase ULK1	01.07.2016-31.12.2018	Prof. Stamminger	Institute of Clinical and Molecular Virology
A72	Targeted modulation of regulatory T cells and analyses of the underlying mechanisms	01.07.2016-31.12.2018	Prof. Steinkasserer	Department of Immune Modulation
A73	Checkpoint inhibitors as adjuvants for viral vaccines	01.07.2016-30.06.2017	Prof. Überla	Institute of Clinical and Molecular Virology
A74	The Role of Eosinophils in Allergic Bronchopulmonary Aspergillosis	30 months	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	30 months	Dr. Günther, PD Dr. Dr. Wirtz	Department of Medicine 1

Oncology

Project No.	Project title	Term	Applicant(s)	Institute
D19	Role of intestinal epithelial SMAD7 for tumor development	01.11.2013-31.10.2016	Dr. Wittkopf, Prof. Becker	Department of Medicine 1
D20	Collagen 10 and metastasis in CRC	01.11.2013-30.04.2016	Prof. Stürzl, Prof. Croner, PD Dr. Naschberger	Department of Surgery
D21	DAPK and colon cancer	16.10.2013-15.10.2016	Prof. Schneider-Stock, PD Dr. Neufert	Institute of Pathology, Department of Medicine 1
D22	Identification and functional characterisation of novel components of the Wnt/β-catenin signal transduction pathway	01.11.2013-31.10.2016	Prof. Behrens	Chair of Experimental Medicine II
D23	Influence of bone marrow adipocytes on the metastatic niche in experimental bone metastasis	01.01.2016-30.06.2018	Prof. Bozec	Department of Medicine 3
D24	Differentiation-associated Schwann cell transcription factors in melanoma– learning from embryogenesis	30 months	Prof. Bosserhoff, Prof. Wegner	Institute of Biochemistry

Advanced Grants

Project No.	Project title	Term	Applicant(s)	Institute
D25	Interaction of the EGFR- and the ZEB1-pathway in aggressive cancer types	01.05.2016-31.10.2018	Prof. Brabletz	Chair of Experimental Medicine I
D26	Identification of antigen specificity of tumor-infiltrating lymphocytes in triple-negative breast cancer	01.01.2016-30.06.2018	Prof. Mackensen, Prof. Fasching	Department of Medicine 5, Department of Obstetrics and Gynecology
D27	2-Hydroxyglutarate in Acute Myeloid Leukaemia: Novel Molecular Targets and Impact on Immune Escape	30 months	PD Dr. Mougiakakos	Department of Medicine 5
D28	SPARCL1 function in vessel maturation and metastasis of colorectal carcinoma	01.02.2016-31.07.2018	Prof. Stürzl, PD Dr. Naschberger	Department of Surgery
D29	Aging and senescence of the adaptive immune system in colorectal cancer	01.01.2016-30.06.2018	Prof. Waldner	Department of Medicine 1

Neurosciences

Project No.	Project title	Term	Applicant(s)	Institute
E11	H50Q aSyn mutation in PD	01.12.2013-30.11.2016	PD Dr. Klucken, PD Dr. Xiang	Department of Molecular Neurology, Institute of Biochemistry
E12	Adult hippocampal neurogenesis in synucleinopathies	01.04.2014-31.03.2017	Prof. Winkler, Prof. Lie	Department of Molecular Neurology, Institute of Biochemistry
E13	The role of acid sphingomyelinase in depression/ anxiety-induced alcohol addiction	01.04.2014-31.03.2017	Prof. Müller, PD Dr. Reichel, Prof. Kornhuber	Department of Psychiatry and Psychotherapy
E14	Role of TRPC5 in trigeminal nociception	01.04.2014-30.09.2016	Prof. Zimmermann	Department of Anaesthesiology
E15	GlyT1 and neuropathic pain	01.11.2013-31.10.2016	PD Dr. Eulenburg, Prof. Schulze	Institute of Biochemistry, Department of Otorhinolaryngology – Head and Neck Surgery
E16	Regulatory networks in neurogenesis and neurodevelopmental disorders	01.04.2014-31.03.2017	Prof. Lie, Prof. Reis	Institute of Biochemistry, Institute of Human Genetics
E17	The neuromuscular role of Wnt signaling pathways	01.04.2014-30.09.2016	Prof. Hashemolhosseini	Institute of Biochemistry
E18	Assessing developmental potential and differentiation capabilities of NG2-glia in the healthy and diseased central nervous system	01.12.2013-30.11.2016	Prof. Wegner, Prof. Winkler	Institute of Biochemistry, Department of Molecular Neurology
E19	Inhibitory neurotransmission in the cochlea: Glutamate and endocannabinoids	15.02.2016-14.08.2018	Prof. Enz	Institute for Biochemistry
E20	Identification of molecules, receptors and genes involved in chronic pruritus	30 months	Dr. Dr. Kremer, Prof. Zimmermann	Department of Medicine 1, Department of Anesthesiology

Project No.	Project title	Term	Applicant(s)	Institute
E21	Modulation of alpha-Synuclein pathology by FoxO-dependent pathways	30 months	Prof. Lie, PD Dr. Klucken	Institute of Biochemistry Department of Molecular Neurology
E22	The role of Swiprosin-1/EFhd2 in resilience to drug addiction	01.03.2016-31.08.2018	Prof. Alzheimer, PD Dr. Mielenz, Prof. Müller	Institute of Physiology and Pathophysiology, Department of Molecular Immunology, Department of Psychiatry and Psychotherapy
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	30 months	Prof. Winner, Prof. Schüttler	IZKF Junior Research Group 3, Department of Anesthesiology
E26	Genetics and pathomechanisms of intellectual disability with microcephaly	01.03.2016-31.08.2018	PD Dr. Zweier	Institute of Human Genetics
E27	Lysophosphatidic acid-induced pruritus of cholestasis	01.03.2016-31.08.2018	Dr. Dr. Kremer, Prof. Fischer	Department of Medicine 1, Institute of Physiology and Pathophysiology

Renal and Vascular Research

Project No.	Project title	Term	Applicant(s)	Institute
F3	Fam60a in heart and brain development	01.03.2014-31.08.2016	Prof. Engel	Department of Nephropathology
F4	Pathogenesis of the short rib-polydactyly syndrome	01.10.2013-30.09.2016	PD Dr. Thiel	Institute of Human Genetics
F5	The Role of ANO1 in Polycystic Kidney Disease	30 months	Dr. Buchholz	Department of Medicine 4
F6	Renal afferent nerve activity - sympathoinhibitory or sympathoexcitatory?	01.07.2016-31.12.2018	Prof. Veelken, Prof. Amann	Department of Medicine 4, Department of Nephropathology

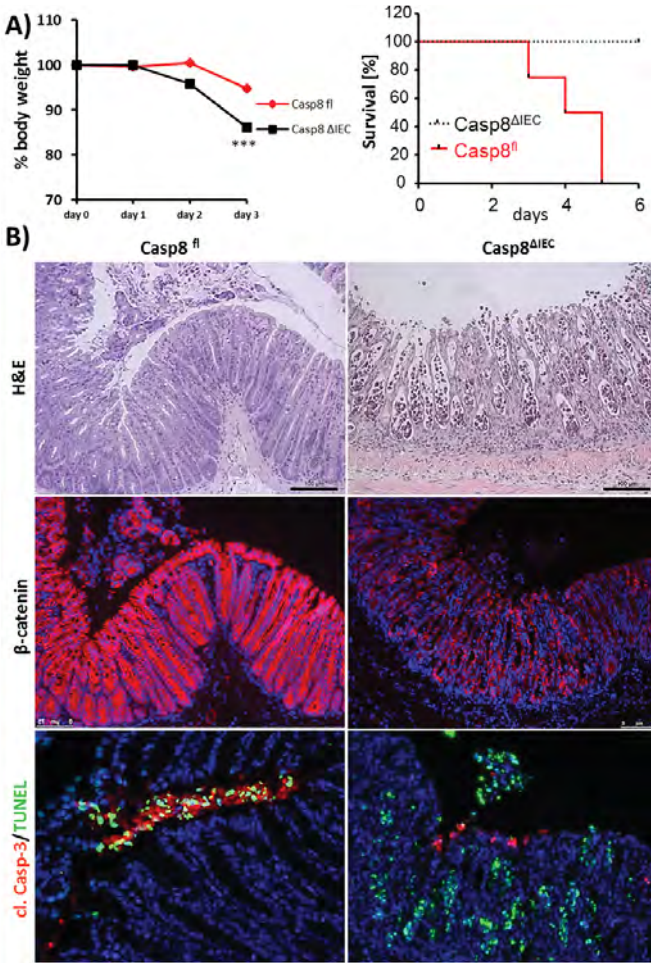
A52 - Progress Report

01.11.2013 - 31.10.2016

cFlip isoforms in the intestinal epithelium

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

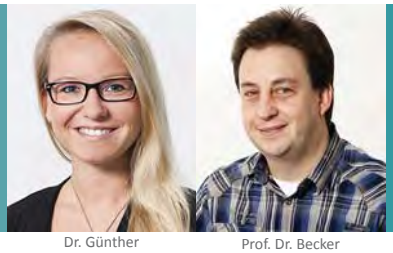
cFLIP is an important regulator of cell death and survival, influencing the activity of caspase-8, finally resulting in the decision whether and which form of cell death is activated. This regulatory network is not only part in the pathogenesis of chronic IBD, but also in infectious colitis. We investigated the role of caspase-8, which is regulated by cFLIP, in the S. Typhimurium model. Our data provide solid evidence for an important and essential function of caspase-8 for infectious colitis.



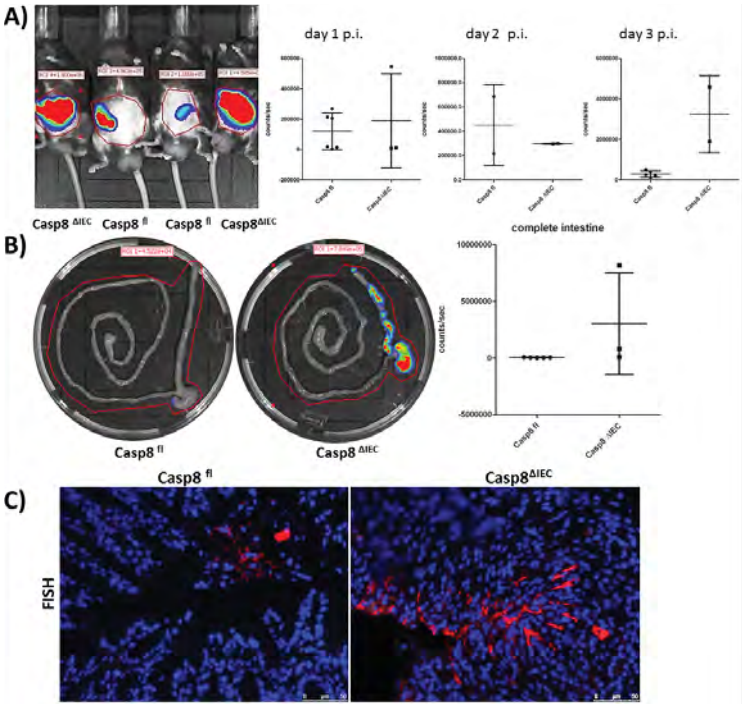
Casp8$^{\Delta IEC}$ mice are highly susceptible towards infection with Salmonella typhimurium. A) Weight curve and Kaplan-Meier plot for survival. B) Colon cross sections stained with H&E, beta-catenin or cleaved caspase-3 and TUNEL of infected Casp8$^{\Delta IEC}$ and control animals.

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gastrointestinal tract. Excessive cell death has been associated with chronic inflammation as seen in patients with Crohn's disease and ulcerative colitis. These patients show increased levels of pro-inflammatory cytokines which activate cell death in the intestinal epithelium. Recent data have demonstrated that the activity status of caspase-8 decides which form of cell death is initiated. After activation of death receptors, caspase-8 is activated and initiates the caspase cascade, leading to classical apoptosis. Thus blocking of caspases appeared to be a potential therapeutic option for patients with IBD. Our recent studies however have shown that inhibition of caspases does not protect from cell death but instead causes a novel form of regulated cell death, denoted as necroptosis. Necroptosis is mediated by effector molecules such as RipK1, RipK3 and MLKL.

Caspase-8 itself is also tightly controlled by the two isoforms of cFLIP, the cellular FLICE inhibitory protein, cFLIP long (cFLIPL) and cFLIP short (cFLIPS). Binding of cFLIPL to the inactive pro-caspase-8 blocks autocatalytic cleavage of caspase-8 and consequently prevents apoptosis. Aditionally, necroptotic cell death is blocked through inactivation of RIPK3, which is an important mediator of necroptosis, by residual catalytic activity of the heterodimer. In contrast to this, cFLIPS also prevents the initial cleavage step of procaspase-8 and therefore



Dr. Günther Prof. Dr. Becker



Casp8$^{\Delta IEC}$ mice show impaired bacterial clearance. A) In vivo imaging and bacterial counts of S. typhimurium in the gut of living mice and B) explanted intestine. C) Invasion of bacteria into subepithelial areas shown by fluorescence in situ hybridization.

cannot contribute to the inactivation of RipK1, which leads to necroptosis.

In addition to the role of caspase-8 in chronic inflammatory diseases, we aimed to elucidate its importance during infection caused by enteric pathogens.

Mice were infected orally with the bioluminescent UK-1 strain of Salmonella. Typhimurium 24 hours after antibiotic treatment. During the infection, Casp8$^{\Delta IEC}$ mice displayed severe weight loss, high lethality and dramatic tissue damage as compared to wild type control animals. An overall higher load of S. typhimurium in the gut of Casp8$^{\Delta IEC}$ mice was detected using the IVIS in vivo imaging system. In contrast to this, control mice were able to clear the bacterial infection after a few days. Barrier break down caused by excessive cell death in the epithelium resulted in septic shock, since commensal bacteria and Salmonella typhimurium were enabled to invade into subepithelial areas, entered the blood stream and reached distant organs.

mRNA data of colonic tissue did not show changes in the expression of antimicrobial peptides in Casp8ΔIEC mice compared to control mice. In contrast to this, immunohistochemical stainings and the expression of pro-inflammatory markers in colon of Casp8$^{\Delta IEC}$ mice showed an increased inflammatory reaction towards the infection. We now seek to investigate the specific roles of the cFLIP isoforms for intestinal homeostasis during Salmonella typhimurium induced infectious colitis.

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Invited lectures

C. Becker: International Congress of Mucosal Immunology, 16.07.2015, Berlin, „Signaling pathways driving the pathogenesis of inflammatory bowel disease"

C. Becker: United International Gastroenterology Week 2015, 27.10.2015, Barcelona, "Role of the Epithelium in Intestinal Inflammation"

C. Günther: 1st Ghent Gut Inflammation Group Conference, Ghent, Belgium, 03/2015. "Cell death regulation in the intestinal epithelium: Still a mystery?"

Publications during funding period

Günther C, Buchen B, He G, Hornef M, Torow N, Neumann H, Wittkopf N, Martini E, Basic M, Bleich A, Watson AJ, Neurath MF, Becker C (2015) Caspase-8 controls the gut response to microbial challenges by TNFalpha -dependent and –independent pathways. Gut 64(4): 601-610

A53 - Progress Report

01.10.2013 - 30.09.2016

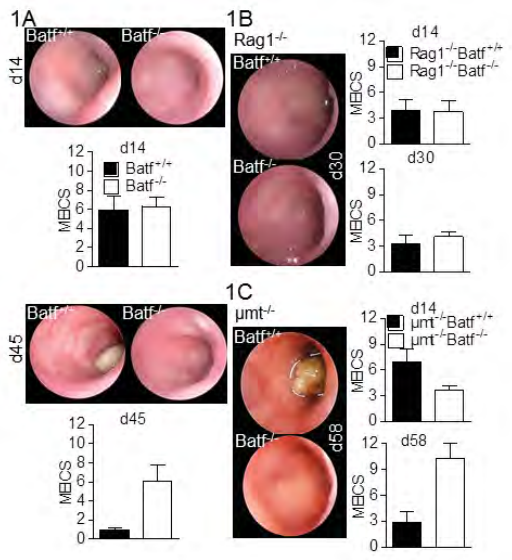
Th17/piTreg differentiation in vivo

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

The AP-1 transcription factor family member Batf is a regulator of Th17 cell differentiation and of at least under lymphopenic conditions de novo developing FoxP3⁺ T cells in vivo. After experimental disruption of the mucosal barrier the absence of Batf-dependent T cells results in abolished intestinal homeostasis and altered colitis-associated colon tumor formation. Hence, Batf plays a so far unappreciated regulatory role during mucosal inflammation und colonic tumorigenesis in vivo.

The AP-1 family member Batf regulates Th17 and de novo Treg formation in vivo

Previously, we identified Batf as a crucial T cell-intrinsic regulator of both colitogenic Th17 cells and de novo forming colitis-mitigating FoxP3⁺ Tregs under lymphopenic conditions. In addition, we found that in the steady state mucosal but not lymphoid tissue-resident Treg populations are diminished in the absence of Batf.



Batf-dependent resolution of 7d-DSS-cycle-induced colitis resides within the T cell compartment. Representative endoscopic images and scores over time derived from Rag1$^{+/+}$(1A), Rag1$^{-/-}$(1B) and µMT-/-(1C) mice with or without Batf expression.

Batf-dependent T cells drive colitis-associated colon cancer (CAC) formation

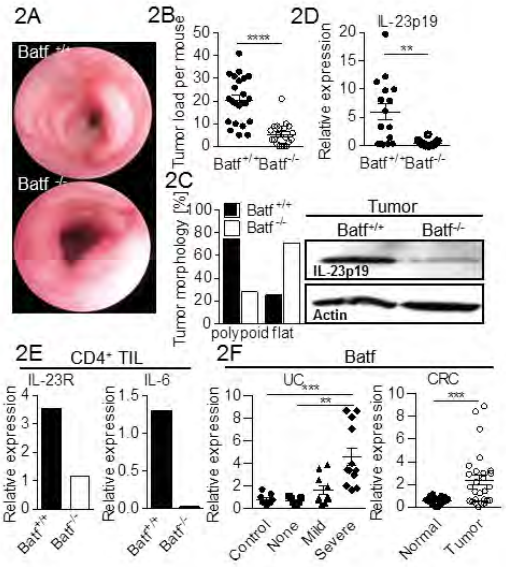
Interestingly, we observed that Batf-expressing T cells critically contribute to the reconstitution of the mucosal homeostasis in response to experimental barrier disruption following dextran sodium sulfate (DSS) treatment p.o. over 7d. In the absence of Batf however, DSS treatment induced a persistent mucosal inflammation and lack of resolution of colitis despite DSS discontinuation. To assess the functional consequences of chronic mucositis in the context of intestinal tumor development, we employed the azoxymethane (AOM) /DSS-driven colitis-associated colon cancer model. Batf$^{+/+}$ and Batf$^{-/-}$ mice were treated once with the carcinogen AOM and two 7d-DSS-cycles and then followed for signs of intestinal tumor development. Unexpectedly, in the absence of Batf-expressing T cells polypoid colitis-associated colon cancer formation was reduced despite overall increased colitis activity. However, flat appearing tumor lesions highly reminiscent of lesions found in ulcerative colitis patients suffering from a highly active, poorly controlled disease course were more abundant in the absence of Batf.

Prof. Dr. Hildner

Batf regulates tumor-promoting IL-23R+ CD4+ T cells displaying a unique expression profile

Interestingly, hampered tumor development occurred despite abundant expression of IL-17a and IL-22 expressed by Non-Th17 cells indicating that bona fide Th17 cell-associated effector molecules are redundant in this context. However, both IL-23R-expressing T cell formation and the establishment of an IL-23^{+} intratumoral milieu -both known to be crucial promotors of colon cancer formation- were highly dependent on T cell-intrinsic Batf expression. Mechanistically, we found that IL-6 derived from Batf-expressing T cells at least in part accounts for intratumoral IL-23 expression overall promoting polypoid tumor formation. Finally, studying human colitis and colon cancer samples confirmed that Batf is highly expressed in the context of mucosal inflammation and tumor formation.

Together, these results reveal a so far unappreciated T cell-intrinsic role for Batf during the maintenance of mucosal homeostasis and colonic tumor formation. Preliminary global gene expression profiling experiments of intestinal T cells reveal a distinct Batf-dependent expression pattern providing molecular hints to explain the diminished ability of Batf-deficient T cells to resolve mucosal inflammation resulting in an altered colitis-associated colon cancer phenotype.



Batf drives colitis-associated colon cancer. 2 A-C Endoscopic and histological scores; 2D IL-23p19 (CAC) and 2E IL-23R and IL-6 expression in tumor-infiltrating lymphocytes (TIL); 2E Batf expression in ulcerative colitis (UC) and colon cancer (CRC).

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Publications during funding period

Punkenburg E, Vogler T, Büttner M, Amann K, Waldner M, Atreya R, Abendroth B, Mudter J, Merkel S, Gallmeier E, Rose-John S, Neurath MF, Hildner K (2015) Batf-dependent Th17 cells critically regulate IL-23 driven colitis-associated colon cancer. Gut. Doi: 10.1136/gutjnl-2014-308227 [Epub ahead of print]

A54 - Progress Report

01.11.2013 - 31.10.2016

Fam180a in inflammatory diseases

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

The molecular mechanisms causing acute as well as chronic inflammatory diseases remain poorly understood. A vastly complex network of pro- and anti-inflammatory factors interplay to create a homeostatic balance. One so far uncharacterized factor promoting inflammation is Fam180a. Our results suggest Fam180a to be an important player in the establishment and maintenance of inflammation.

Fam180a is highly conserved

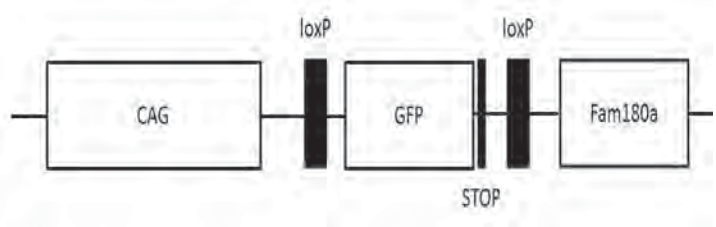
Fam180a is a 19 kDA sized protein with a predicted cleavable signal peptide (SignalP 4.1). It is highly conserved among different species; however our knowledge of the tertiary structure remains vague. There are no highly homologue aa sequences known, leading to a wide range of structural predictions. Fam180a can be found in the supernatant of transfected cells at a significantly larger size than predicted, indicating extensive glycosylation or a dimeric nature.

Fam180a$^{-/-}$ mice are protected in a model of acute intestinal inflammation

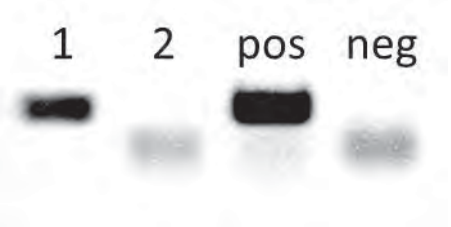
Fam180a$^{-/-}$ mice show significantly diminished inflammation in a model of acute DSS colitis. The mice display reduced weight loss, inflammation score, bowel wall thickening and accumulation of neutrophil granulocytes.

Creation of a Fam180a overexpressing mouse

In order to gain a deeper understanding on the biological functions of Fam180a we aimed to overexpress Fam180a in transgenic mice. Initially, we synthesized the following DNA construct for subsequent integration into the genome: A CAG element (consisting of a cytomegolavirus enhancer element, a chicken beta actin promotor followed by its first exon and intron as well as a rabbit beta-globin splice acceptor) leads to the transcription of GFP, terminated by a stop codon. The GFP containing sequence including the stop codon is flanked by “loxP” sites, allowing for an excision of the flanked sequence in presence of a cre recombinase, subsequently leading to the loss of GFP and transcription of the adjacent codon optimized Fam180a coupled to a flag-tag. This cons-



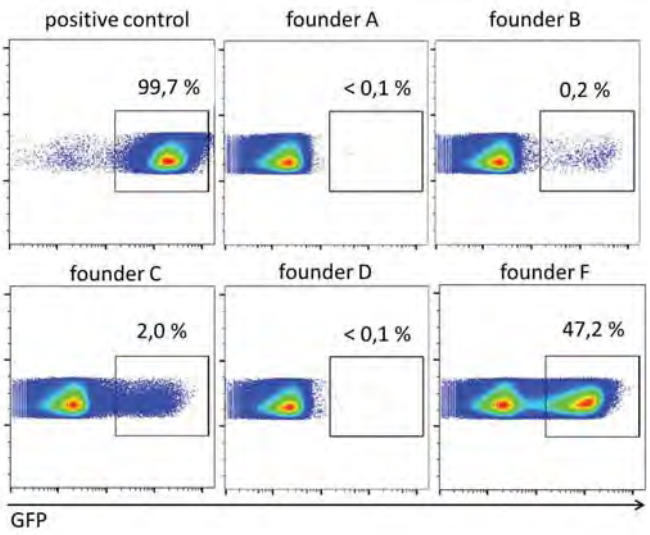
Targeting strategy for the generation of Fam180a transgenic mice.

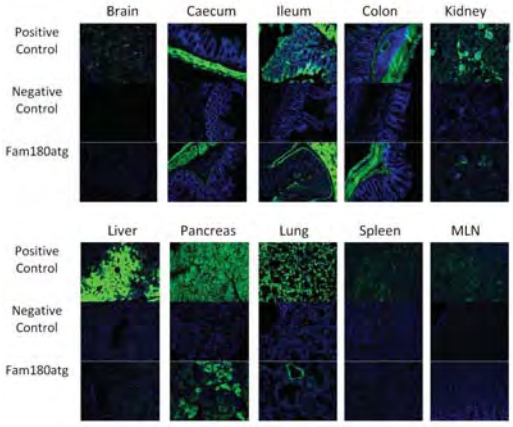


Genotyping of the initial founder generation for the construct. (1)Fam180atg mouse (2) mouse without integration of the construct (pos) construct containing vector (neg) C57Bl/6 mouse

PD Dr. Dr. Wirtz

Prof. Dr. Waldner





left: Investigation of GFP expression in a splenocyte single cell suspensions of different Fam180atg mice by flow cytometry
right: Cryosections of founder F. GFP in green, DAPI in blue

truct was purified and finally introduced into mouse zygotes by microinjection. The oocytes were transplanted into foster-mothers and the offspring was screened for the presence of the construct, leading to the identification of 6 transgenic mice. These mice were further crossed with C57Bl/6 and the resulting offspring showed sufficient GFP expression to proceed with further crossing to mice expressing the cre recombinase. We are now crossing these mice to mice with broad expression of the Cre recombinase in multiple tissues (Actin-Cre) as well as a strain expression Cre recombinase in hematopoietic and endothelial cells (Tie2-cre).

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Invited lectures

M. Waldner: SFB 841 Symposium, 27.03.2015, Hamburg, Titel: „Gut inflammation and colorectal cancer development“

M. Waldner: Workshop „Optische Diagnostik heute und morgen“ des Leibniz Instituts für Photonische Technologien, 4.7.2015, Jena, Titel: „Unmet medical needs: Gastroenterology“

Awards

Thiersch Preis der Medizinischen Fakultät Erlangen, Stefan Wirtz, 04. November 2015, Erlangen

Publications during funding period

none

A55 - Progress Report

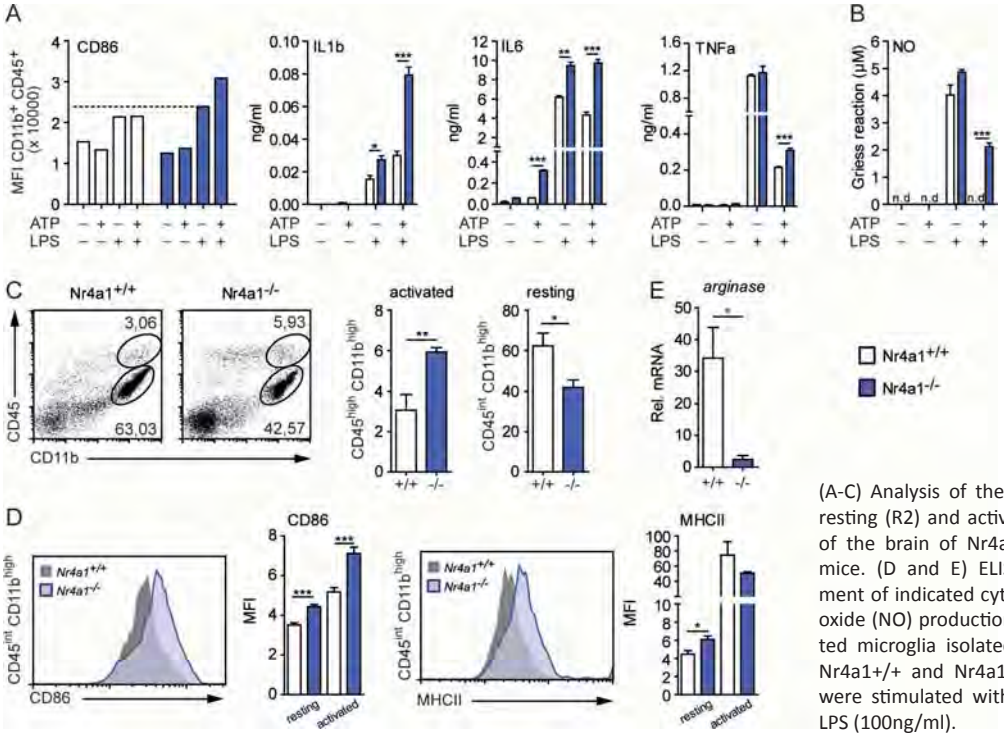
01.01.2014 - 31.12.2016

NR4a1 during immunologic tolerance

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

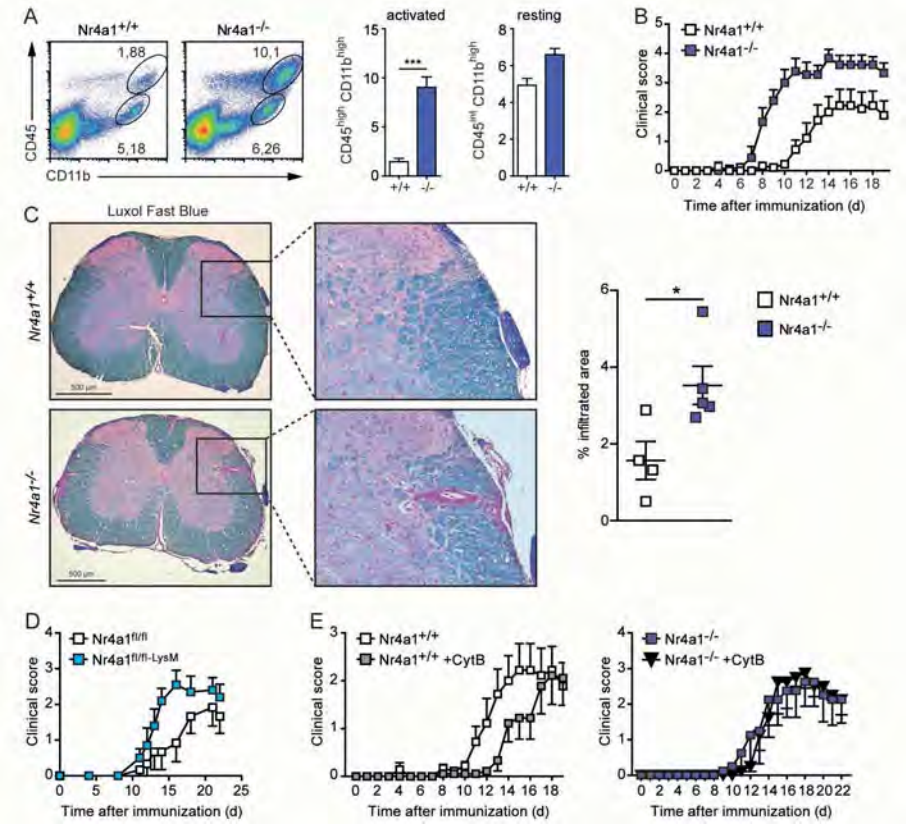
Our peliminary data show an exacerbation of experimental autoimmune encephalomyelitis (a murine model for human multiple sclerosis) after deletion of the nuclear receptor NRa1 suggesting a key role of this transcription factor during the maintenance of self-tolerance and control of inflammation. During the current project we aim addressing the involved molecular events and responsible cell types.

Our data show that the nuclear receptor (NR) Nr4a1 acts as key rheostat controlling the microglia activation threshold and protecting from autoimmune-driven CNS inflammation. In steady state microglia, ubiquitous neuronal-derived stress signals such as ATP induced expression of this NR, which contributed to the maintenance of a resting and non-inflammatory microglia phenotype. Global and myeloid-specific deletion of Nr4a1 triggered the spontaneous and overwhelming activation of microglia and resulted in an accelerated and exacerbated form of experimental autoimmune encephalomyelitis (EAE). Ligand-induced activation of Nr4a1, in turn, strongly ameliorated the course of disease. Our current data thus identify Nr4a1 as regulator of microglia activation and potentially new target for the treatment of inflammatory CNS diseases such as multiple sclerosis.



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

PD Dr. Krönke



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

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Invited lectures

Annual Scientific meeting of the European Society of Clinical Investigation, 27.05-30.05.2015, Cluj Napoca, Romania; "Regulated Sorting of Self and Non-Self"

Annual German Lupus meeting 08.05.-09.05.2015, Heidelberg, Germany; "A tale of two macrophage subsets"

Invited seminar at the ETH Zürich 05.05-06.05.2015, Zürich, Switzerland; "Pros and cons of lipid oxidation during inflammation and immunity"

Publications during funding period

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

Ipseiz N, Uderhardt S, Scholtysek C, Steffen M, Schabbauer G, Bozec A, Schett G, Krönke G (2014) The nuclear receptor Nr4a1 mediates anti-inflammatory effects of apoptotic cells. J Immunol 192(10):4852-8

A56 - Progress Report

01.03.2014 - 31.08.2016

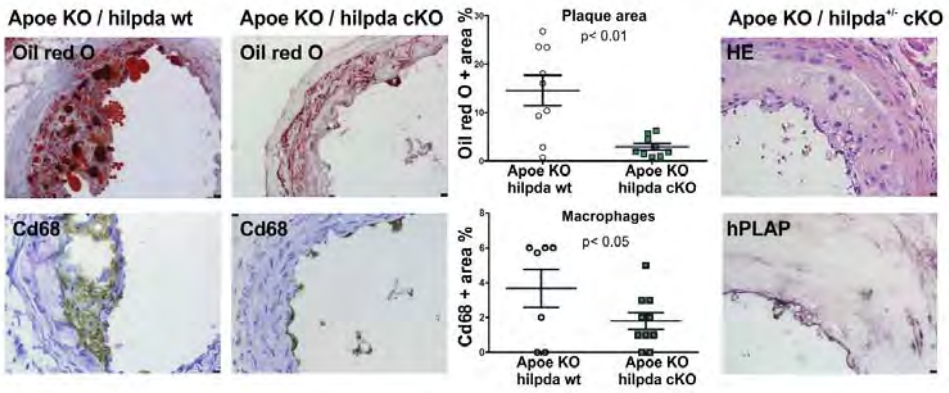
Role of HIG2 in atherosclerosis

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

The lipid droplet protein Hilpda mediates hypoxic and PPAR-induced lipid accumulation. Hilpda is expressed in atherosclerotic plaques, but its role in atherogenesis is not known. Here we investigate the effects of a tie2 cre-mediated hilpda knockout on macrophage function and the development of atherosclerosis in apoe$^{-/-}$ mice. Preliminary results show that lesion size was reduced, plaques appeared more instable and lipid deposition appeared to be shifted towards smooth muscle cells of the media.

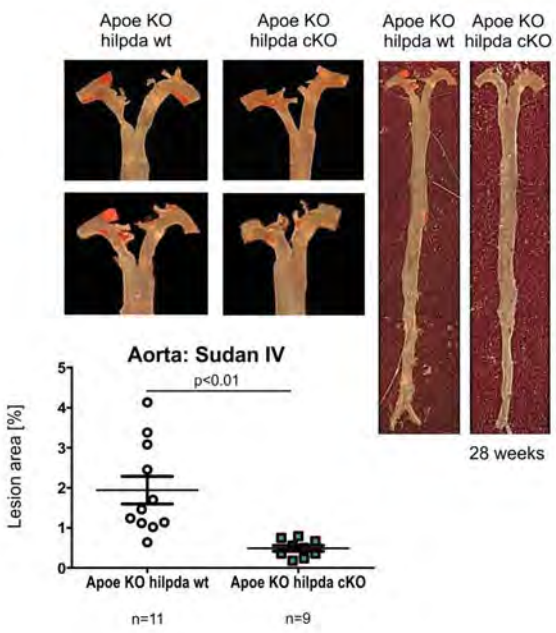
Hilpda is a Hif-1 and a Ppar target in macrophages and is required for lipid droplet formation.

We previously showed that bone marrow-derived macrophages (BMDMs) from tie2 cre hilpda cKO mice cannot store neutral lipids after exposure to hypoxia, pharmacological Hif inducers, LPS, cholesterol or fatty acids. PPARγ and PPARβ/δ ligands transactivate Hilpda as well as Adrp/Plin2, which has also been implicated in hypoxic lipid accumulation. However, compared to Hilpda, Adrp/Plin2 was hardly induced by hypoxia and marginally affected by hif-1a cKO. Comparison of hif-1α and hif-2α cKO BMDMs demonstrated that hilpda is a specific target of Hif-1α. Lipid accumulation was reduced, but not abolished in hif-1α cKO BMDMs, presumably because Hilpda expression was not completely abrogated. Lipid droplets were also reduced after siRNA knockdown of Adrp/Plin2, suggesting that Hilpda leads to lipid accumulation, whereas Adrp may stabilize lipid droplets. Localization, induction kinetics and stability of the two proteins also imply major functional differences.



Plaque lipids (Oil red O) and macrophages (Cd68) in the aortic root were reduced in 22-week-old male apoe KO/ hilpda cKO mice compared to hilpda wt mice, although total aortic plaque area did not differ; hPLAP activity marks hilpda cKO cells.

PD Dr. Warnecke



In 28-week-old male apoe KO/hilpda cKO mice total aortic plaque area was reduced compared to hilpda wt mice in particular in the upper part of the aorta and truncus as shown by quantification of Sudan IV positive areas in En face preparations.

Further characteristics of hilpda cKO BMDMs.

The mechanism of lipid accumulation by Hilpda does not require increased uptake of lipoproteins or fatty acids, but seems to consist in enhanced esterification and sequestration of lipids into lipid droplets at the surface of the ER. Although hilpda cKO BMDMs seemed to be more resistant against severe fatty acid overload as determined by trypan blue exclusion, other methods indicated that fatty acid loading is more harmful to hilpda cKO cells. This discrepancy may be due to altered cell membrane composition of wt and cKO cells after fatty acid loading confounding trypan blue staining. Basic and LPS-induced NO production was not affected in Hilpda cKO BMDMs. However, PGE2 synthesis from arachidonic acid, which may take place on the surface of lipid droplets, appears to be reduced in Hilpda cKO BMDMs.

Hilpda cKO may attenuate atherogenesis in Apolipoprotein E-deficient mice.

In human atherosclerotic plaques HILPDA is expressed in macrophage foam cells surrounding the lipid core and in the plaque shoulder, a region frequently characterized by inflammation and angiogenesis, and prone to rupture.

Analysis of tie2 cre/hilpda$^{fl/fl}$/apoe$^{-/-}$ mice was first performed on 22-week-old mice under normal diet. However, the high variability of plaque formation at this age impeded the interpretation of the results. Nevertheless male hilpda cKO mice exhibited reduced lesion size and macrophages in the aortic root, though not in the whole aorta. We then used 28-week-old male mice and found that total lesion size in the aorta of hilpda cKO mice was decreased compared to hilpda wt mice. Interestingly, lipid deposition frequently seemed to shift from the intima towards the smooth muscle cells of the media.

Since high fat diet (HFD) could accentuate the differences between hilpda cKO and wt mice through Ppar-mediated hilpda induction, we want to extend our studies to mice under HFD.

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Publications during funding period

none

A57 - Progress Report

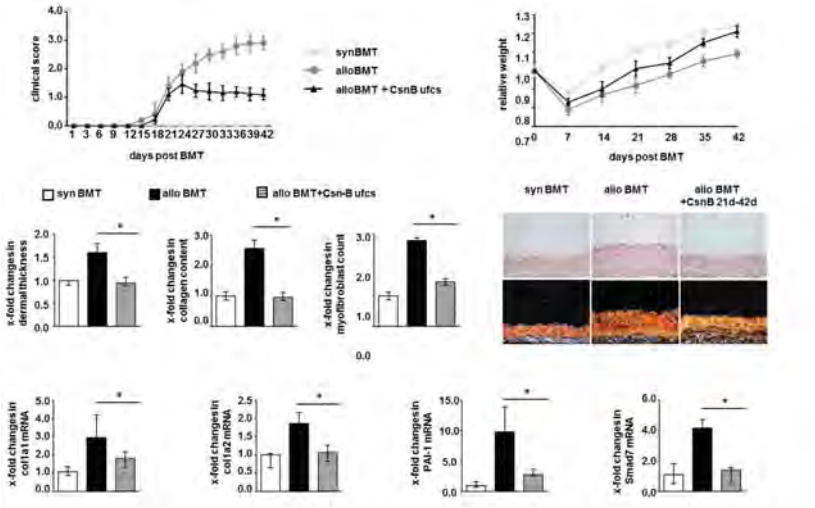
01.01.2014 - 30.06.2016

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

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

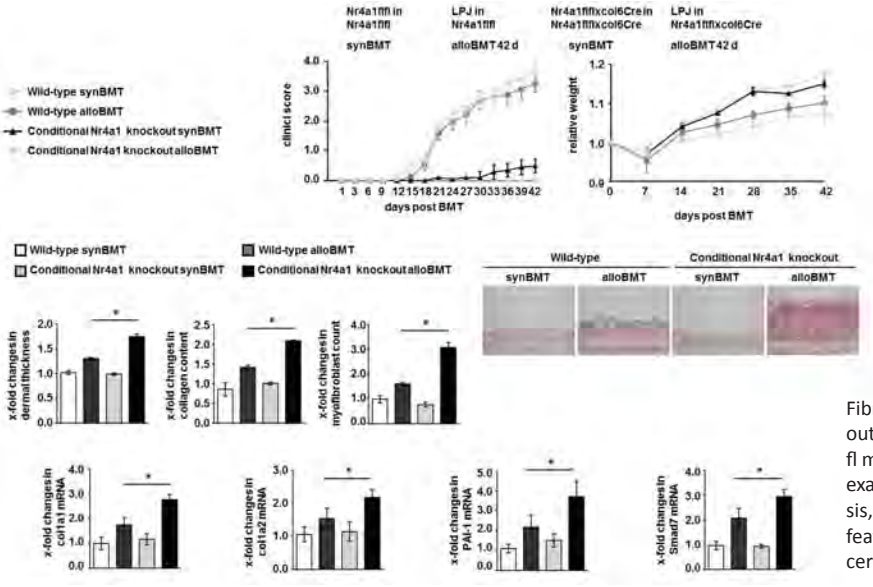
The orphan nuclear receptor Nr4a1 is an endogenous antagonist of TGF-ß, which is inactivated in sclerodermatous cGvHD by phosphorylation. Pharmacologic agonists of Nr4a1 inhibit the phosphorylation-induced inactivation of Nr4a1 in cGvHD, thereby preventing the aberrant activation of fibroblasts and inhibiting tissue fibrosis in experimental cGvHD. Further studies indicate that Nr4a1 may also be a target for therapeutic intervention in other fibrotic diseases.

After demonstrating that Nr4a1 inhibits TGF-β signaling via SP1-dependent transrepression with recruitment of cofactors such as Sin3A, CoREST, LSD1 and HDAC1 and after elucidating GSK3-dependent phosphorylation and HDAC-dependent epigenetic silencing as underlying mechanisms for the inactivation of Nr4a1 upon prolonged TGF-β stimulation, we further evaluated the effects of Nr4a1 in murine sclerodermatous cGvHD and on the graft-versus-leukemia reaction (GvL). We demonstrated that the Nr4a1 agonist Csn-B also ameliorated clinical features of murine cGvHD such as weight loss and the cutaneous cGvHD score, when applied not in preventive, but in therapeutic dosing schedules with dosing initiated only after the onset of first clinical signs of Scl cGvHD on d21 post bone marrow transplantation. Therapeutic dosing of Csn-B also effectively inhibited enhanced TGF-β signaling with decreased mRNA levels of target genes such as Smad7 and PAI-1, reduced myofibroblast differentiation and ameliorated collagen accumulation and skin thickening. In contrast, mice with fibroblast-specific knockout of Nr4a1 (Col1a2 CreER; Nr4a1fl/fl mice with tamoxifen) were more sensitive to cGvHD induced fibrosis than control mice with normal levels of Nr4a1 (Col1a2 CreER; Nr4a1fl/fl mice with corn oil; further referred to as wildtype phenotype). While fibrosis was more severe and the upregulation of TGF-β target genes was more severe in mice with fibroblast-specific knockout of Nr4a1, inflammation-dependent features such as weight loss, skin ulcers and hair loss were comparable between both groups. We further analyzed the effects of Nr4a1 activation on GvL using the P815 mouse model. We demonstrate that allogeneic bone marrow transplantation and subsequent cGvHD enhance survival of mice injected with P815 cells as compared to syngeneic controls. First results suggest that treatment with Csn-B may slightly impair the GvL reaction as first mice treated with Csn-B died slightly earlier than vehicle-treated, allogeneically transplanted control mice.



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

Prof. Dr. Distler Prof. Dr. Spriewald



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

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Invited lectures

Pfizer 2015 Inflammation and Immunology Summit; February 2015, New York, USA; Novel insights into the pathogenesis of fibrotic diseases–Implications for targeted therapies

FASEB congress on TGF-β signaling, July 2015, Snowmass/CO/USA, The nuclear receptor 4A1 (NR4A1) regulates TGF-β signaling during mesenchymal tissue responses

Publications during funding period

Wohlfahrt T, Usherenko S, Englbrecht M, Dees C, Weber S, Beyer C, Gelse K, Distler O, Schett G, Distler JH*, Ramming A* (2015) Type 2 innate lymphoid cell counts are increased in patients with systemic sclerosis and correlate with the extent of fibrosis. Ann Rheum Dis. Doi: 10.1136/annrheumdis-2015-207388 [Epub ahead of print] * contributed equally

Lin NY, Chen CW, Kagwiria R, Liang R, Beyer C, Distler A, Luther J, Engelke K, Schett G, Distler JH (2015) Inactivation of autophagy ameliorates glucocorticoid-induced and ovariectomy-induced bone loss. Ann Rheum Dis. Doi: 10.1136/annrheumdis-2015-207240 [Epub ahead of print]

Huang J, Beyer C, Palumbo-Zerr K, Zhang Y, Ramming A, Distler A, Gelse K, Distler O, Schett G, Wollin L, Distler JH (2015) Nintedanib inhibits fibroblast activation and ameliorates fibrosis in preclinical models of systemic sclerosis. Ann Rheum Dis. 10.1136/annrheumdis-2014-207109 [Epub ahead of print]

Dees C, Beyer C, Distler A, Soare A, Zhang Y, Palumbo-Zerr K, Distler O, Schett G, Sandner P, Distler JH (2015) Stimulators of soluble guanylate cyclase (sGC) inhibit experimental skin fibrosis of different aetiologies. Ann Rheum Dis: 74(8):1621-5

Tomcik M, Palumbo-Zerr K, Zerr P, Sumova B, Avouac J, Dees C, Distler A, Becvar R, Distler O, Schett G, Senolt L, Distler JH (2015) Tribbles homologue 3 stimulates canonical TGF-β signalling to regulate fibroblast activation and tissue fibrosis. Ann Rheum Dis. Doi: 10.1136/annrheumdis-2014-206234 [Epub ahead of print]

Mallano T, Palumbo-Zerr K, Zerr P, Ramming A, Zeller B, Beyer C, Dees C, Huang J, Hai T, Distler O, Schett G, Distler JH (2015) Activating transcription factor 3 regulates canonical TGFβ signalling in systemic sclerosis. Ann Rheum Dis. Doi: 10.1136/annrheumdis-2014-206214 [Epub ahead of print]

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. Nat Med 21: 150–158

A58 - Progress Report

01.10.2013 - 31.03.2016

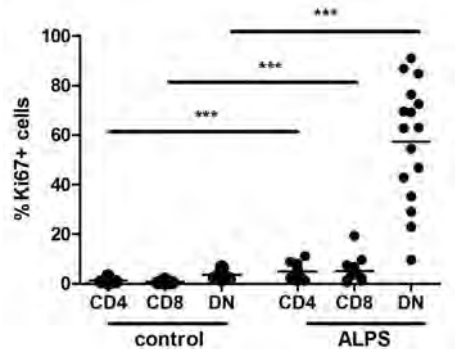
Characterization of DN T cells from ALPS patients

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

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

Abnormally differentiated DN T cells of ALPS patients are highly proliferative in vivo

Most characteristic indication of ALPS is the chronic benign lymphoproliferation with massive, often visible lymphadenopathy and splenomegaly. However, it has been unclear whether proliferative capacity affects all lymphocytes or is restricted to defined cell subsets. To examine in detail the mitotic activity of lymphocyte subsets, we analyzed expression of the nuclear protein Ki67, which is present during active phases of cell cycle (G1 to M) but is absent in resting cells (G0). We observed low but significantly increased frequencies of Ki67+ CD4+ and CD8+ T cells in ALPS patients compared to healthy controls. In contrast, DN T cells from ALPS patients highly expressed Ki67. To confirm that Ki67 expression is associated with cell division, we determined the presence of PCNA (proliferating cell nuclear antigen) and cyclin A, which are expressed during S and G2 phase of the cell cycle. ALPS DN T cells expressed both cell cycle regulators indicating that these cells progress through cell cycle. The majority of ALPS DN T cells are CCR7-, CD45RA+, and CD57+, an expression pattern of terminally differentiated effector memory cells. Further analysis of T cell differentiation markers revealed a linear relationship between the percentage of DN T cells showing a CCR7-/CD45RA+ differentiation state and the fraction of DN T cells with mitotic activity. Thus, in contrast to CCR7-/CD45RA+/CD57+ terminally differentiated T cells of healthy individuals, which are generally associated with poor proliferative potential and low Ki67 expression, ALPS DN T cells expressing the same markers are highly proliferative in vivo.

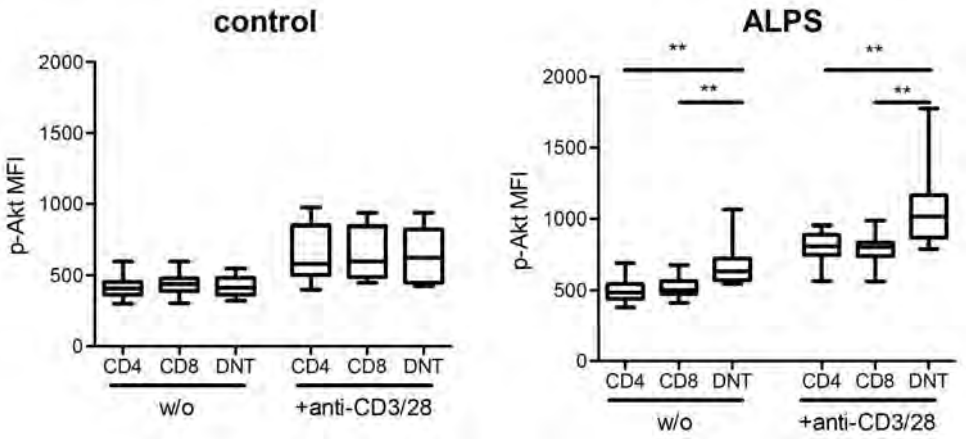


ALPS DN T cells show high proliferative activity in vivo. PBMC from ALPS patients and healthy controls were gated for CD4+, CD8+, and DN T cells and analyzed for Ki67 expression. Graph represents cumulative data of all studied healthy donors and ALPS patients.

ALPS DN T cells show hyperactive mTOR signaling

As ALPS DN T cells vigorously proliferate in vivo but paradoxically express costimulatory receptors CD27 and CD28, we considered that signal transduction downstream of costimulatory receptors might be crucial for accumulation of DN T cells in ALPS. An important molecule, activated downstream of CD28, is the serine/threonine kinase mTOR, which plays a key role in regulating cell proliferation and effector differentiation. We therefore analyzed the phosphorylation status of protein

Prof. Dr. Mackensen Dr. Völkl



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

kinase Akt(S473), mTOR(S2448) and its downstream target ribosomal protein S6 at both phosphorylation sites (S235/6 and S240) in T cells from ALPS patients ex vivo. Intriguingly, ALPS DN T cells showed enhanced basal and activation-induced phosphorylation of Akt, mTOR and S6 as compared to ALPS bulk CD4+ and CD8+ T cells or the respective cell populations from healthy controls. The mTOR inhibitor rapamycin has successfully been used to treat autoimmune cytopenias and lymphoproliferation in ALPS patients. Given that enhanced mTOR signaling is present in ALPS DN T cells but not in CD4+ or CD8+ T cells, we hypothesized that rapamycin predominantly affect this cell subset. We first investigated how rapamycin affects proliferation and survival of ALPS DN T cells in vitro. Notably, even low concentrations of rapamycin induced a substantial inhibition of the mitotic activity of ALPS DN T cells. In contrast, ALPS CD4+ and CD8+ T cells demonstrated a vast insensibility to rapamycin treatment. We next monitored the effect of rapamycin on ALPS DN T cells in vivo. The percentage and mitotic activity of DN T cells was dramatically reduced in ALPS patients under rapamycin therapy. Of interest, a more detailed analysis revealed that the reduction specifically affected ALPS DN T cells displaying the abnormal differentiation phenotype.

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Invited lectures

Annual Meeting of the “Arbeitsgemeinschaft für Pädiatrische Immunologie”, 23.-25.04.2015, Freiburg, “Hyperactive mTOR pathway promotes accumulation of aberrant double-negative T-cells in human autoimmune lymphoproliferative syndrome (ALPS)”

Publications during funding period

Allgäuer A, Schreiner E, Ferrazzi F, Ekici AB, Gerbitz A, Mackensen A*, Völkl S* (2015) IL-7 abrogates the immunosuppressive function of human double-negative T cells by activating Akt/mTOR signaling. The Journal of Immunology 195(7): 3139-48

Rensing-Ehl A*, Völkl S*, Speckmann C, Lorenz MR, Ritter J, Janda A, Abinun M, Pirscher H, Bengsch B, Thimme R, Fuchs I, Ammann S, Allgäuer A, Kentouche K, Cant A, Hambleton S, Bettoni da Cunha C, Huetker S, Kühnle I, Pekrun A, Seidel MG, Hummel M, Mackensen A, Schwarz K, Ehl S (2014) Abnormaly differentiated CD4+ or CD8+ T cells with phenotypic and genetic features of double negative T cells in human Fas deficiency. Blood 124(6): 851-60 [*contributed equally]

A59 - Progress Report

01.10.2013 - 30.09.2016

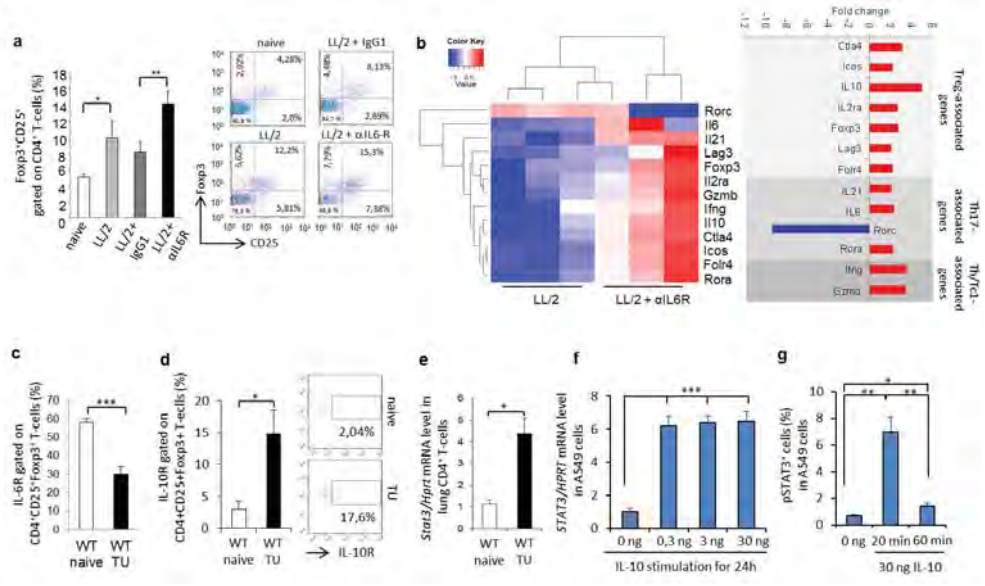
IL-10 and lung cancer

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

Lung carcinoma is the most common cause of cancer deaths worldwide. Although the immune system has the ability to eliminate malignant cells, tumours can develop strategies to escape this rejection. One of these strategies is the production of immune suppressive factors, such as the cytokine IL-10. In this study we aim to achieve a better understanding of the role of IL-10 for the immunopathogenesis of lung carcinoma, using a murine model of lung carcinoma as well as human samples.

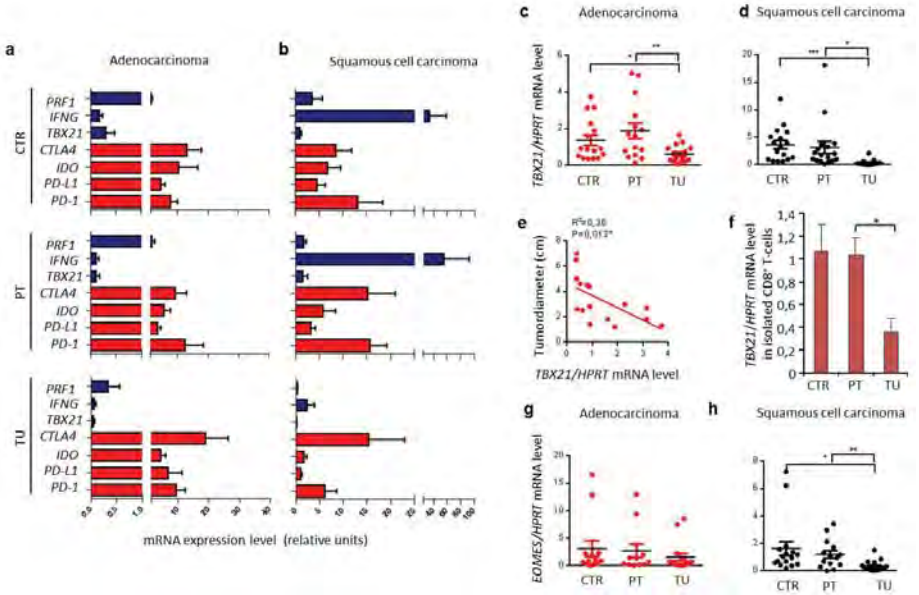
Our analyses in a murine model of lung cancer revealed an increased percentage of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) in tumour bearing as compared to control mice. The intranasal administration of anti-IL-6R antibodies (ab) resulted in a further increase of Treg numbers in the lungs of tumour bearing mice as compared to mice treated with an isotype control. Consistently, mRNA affimetrix array analyses of $CD4^+$ T-cells, isolated from the lungs of tumour bearing mice, revealed that anti-IL-6R ab treatment leads to an up-regulation of Treg-associated genes, such as *Ctla4, Icos* and *Foxp3*. Moreover, these analyses show that *in vivo* blockade of IL-6R results in increased mRNA expression of Th1/Tc1 genes, such as *Ifng* and *Gzmb*. Interestingly, we also found that tumour induction leads to a downregulation of IL-6R protein expression on the surface of Treg cells, suggesting a possible mechanism, which could be used by tumour-infiltrating Treg to evade their IL-6 mediated inhibition.

An important member of the IL-6 signaling pathway is the intracellular mediator Stat3 (signal transducer and activator of transcription 3), which is phosphorylated and activated after the binding of IL-6 to its receptor. However, according to previous studies, Stat3 might be important for Treg expansion. Also, we have previously demonstrated that $CD4^+$ T-cells



Analysis of lymphocyte subpopulations and gene expression in a murine model of lung carcinoma, dependently of tumour induction or anti-IL6R ab treatment (a-e) *Stat3* mRNA and pStat3 protein levels after IL-10-treatment of the lung adenocarcinoma A549 cells (f-g).

Prof. Dr. Dr. Finotto



Human lung samples from ADC and SCC patients. Comparative mRNA expression analysis of Treg and Th1/Tc1-associated genes (a-b). *Tbx21* mRNA expression and correlation with tumour diameter in whole lung and in CD8⁺ cells (c-f). *Eomes* mRNA levels (g-h).

from the lungs of tumour bearing mice show increased mRNA levels of Stat3, despite the reduced IL6R expression on Treg cells in tumour.

Besides IL-6, Stat3 can be activated by other factors, such as IL-10. Indeed, tumour induction is associated with an increased protein expression of IL-10R on CD4⁺ T-cells in general and Treg cells, in particular. These results indicate that IL-10 could be an important activator of Stat3 in the tumour microenvironment, especially regarding Treg cells. In line with this, the stimulation of A549 cells with different doses of IL-10 leads to an up-regulation of *Stat3* mRNA expression as well as to increased phosphorylation of Stat3 protein.

The second part of the project comprises the analysis of human samples from a cohort of patients with non-small cell lung cancer (NSCLC). During the last funding period we analysed the mRNA expression of several T-regulatory genes as well as Th1/Tc1-associated genes, finding a shift of the balance towards the suppressive genes in the tumoural lung region of both adeno- and squamous cell carcinoma patients (ADC and SCC). Besides that, we also observed a significantly reduced mRNA expression of *Tbx21* (T-box expressed in T cells) in whole lung tissue as well as in isolated CD8⁺ T-cells from the TU region of patients with NSCLC. Furthermore, we found an inverse correlation between the tumour diameter and the *Tbx21* expression levels in the lung tissue samples from the patients. Eomes (Eomesodermin), which is another transcription factor involved in CD8⁺ T cell development, was found down-regulated in the tumoural region of patients with SCC but not ADC.

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Publications during funding period

Andreev K, Denis IT, Siegemund R, Rieker R, Hartmann A, Schmidt J, Sirbu H, Finotto S (2015) Impaired T-bet-pSTAT1alpha and perforin-mediated immune responses in the tumoral region of lung adenocarcinoma. British journal of cancer 113: 902-913

Balabko L, Andreev K, Burmann N, Schubert M, Mathews M, Trufa DI, Reppert S, Rau T, Schicht M, Sirbu H, Hartmann A, Finotto S (2014) Increased expression of the Th17-IL-6R/pSTAT3/BATF/RorγT-axis in the tumoural region of adenocarcinoma as compared to squamous cell carcinoma of the lung. Sci Rep.4: 7396.1-10

A60 - Progress Report

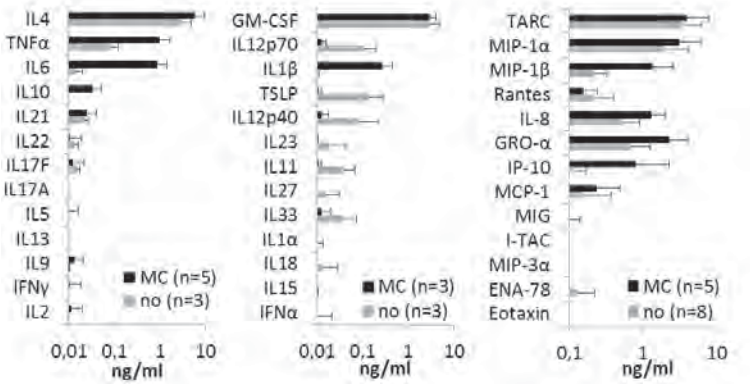
01.10.2013 - 30.09.2016

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

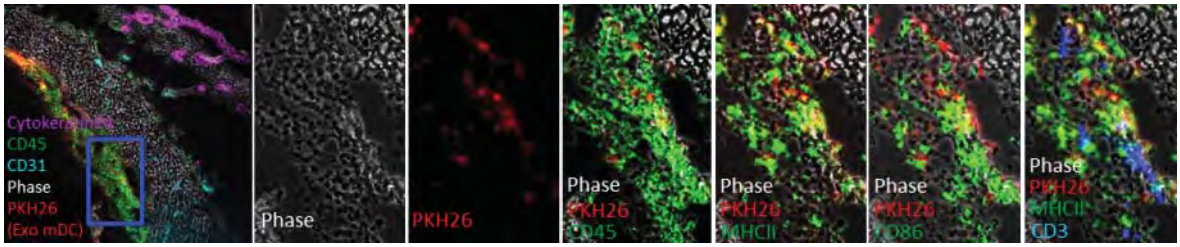
Prof. Dr. Andreas Baur, Dr. Stephan Schierer, Department of Dermatology

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

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



Normalized amounts of vesicles derived by ultracentrifugation of supernatants of unstimulated DC (no) or DC stimulated with cytokine maturation cocktail (MC) were analyzed by antibody based bead array.



One day after intradermally injected labeled Exo analysis of skin cryosections with MELC (Multi-Epitope-Ligand-Cartography) technology revealed that leukocytes (e.g. DC, T cells) infiltrated the area with Exo and DC (MHCII+ CD86+) engulfed the Exo.

Prof. Dr. Baur Dr. Schierer

In order to understand the role of DC Exo we started to analyze effects on resting human PBMC. First experiments of labeled DC Exo incubated with PBMC revealed that mainly monocytes (Mo), and to a minor degree other leukocytes take up DC Exo. Three hours after uptake of Exo DC exclusively Mo showed activation via phosphorylation of Stat5. After 6 days Mo incubated with Exo derived from immature DC (Exo imDC) changed their morphology to a mix of macrophage and immature DC phenotype, whereas Exo derived from mature DC (Exo mDC) lead to a homogenous DC phenotype. In agreement with that only Exo mDC, but not Exo imDC generated DC showed only after maturation with cytokines an allogeneic stimulatory capacity comparable to conventional generated DC. On the other hand, long term incubation (10-13 days) of PBMC with Exo DC lead to prolonged survival and proliferation of DC similar as GM-CSF treated PBMC. Western blot analysis confirmed that both Exo imDC and Exo mDC contain large amounts of GM-CSF. Blocking the essential DC growth factor GM-CSF abrogated the Exo induced survival of Mo. Elucidating their diverse differentiation capability we found that Exo mDC include active TNF-α together with its corresponding active protease Adam17 in contrast to Exo imDC that harbor only the inactive precursors, pro-TNF-α and pro-Adam17. Antibody array analysis of chemokine and cytokine levels supported that especially Exo mDC include known inflammatory cytokines (e.g. Il1β, IL-6, TNF-α) needed for maturation and survival of DC. Furthermore, we found additional chemokines (e.g. IL-8, MIP1-α and -β) and cytokines (e.g. IL3, MIF) in Exo mDC capable to attract and activate a broad range of immune cells. To confirm these predicted effects in-vivo we generated Exo from Bone marrow derived DC (Exo BMDC) that were either not matured (Exo imBMDC) or matured with LPS (Exo BMDC+LPS) or Poly I:C (Exo BMDC+Poly). Array analysis revealed that all Exo BMDC contain the growth factor GM-CSF, but only Exo BDMC+Poly not Exo BMDC+LPS showed elevated levels of cytokines and chemokines. In cooperation with AG Zinser (dermatology) labeled Exo BMDC+Poly were injected intradermally in autologous C57/Bl6 mice. After one-day skin cryosections analyzed with MELC (Multi-Epitope-Ligand-Cartography) technology revealed that leukocytes (DC, T cells) infiltrated the area with Exo and DC (MHCII+ CD86+) ingested the Exo. At the same time Exo appear mainly in medullary macrophages, but also in the DC/T cell area of the draining lymph node.

In summary mDC Exo seem to have a multifunctional capability to recruit, activate and differentiate immune cells especially Mo.

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Publications during funding period

none

A61 - Progress Report

01.02.2014 - 31.07.2016

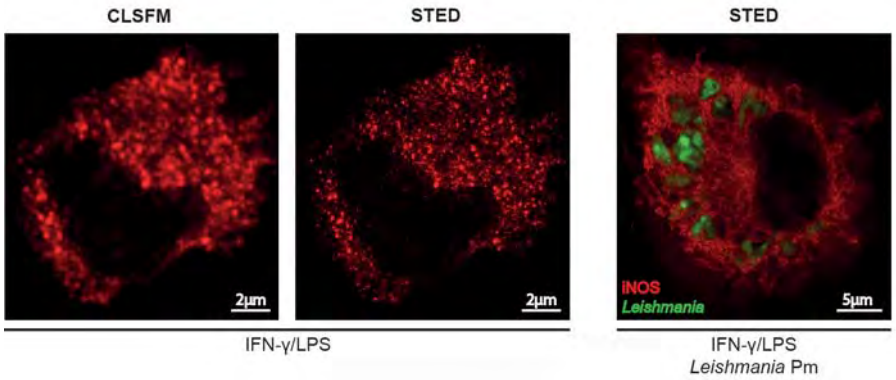
Leishmania, iNOS and iron

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) syntase (iNOS or NOS2). NOS2 is expressed in phagocytes upon stimulation by cytokines (e.g. IFN-γ, TNF) and/or microbial ligands (e.g. lipopolysaccharide [LPS]) and converts L-arginine into citrulline and NO. Based on studies with extracellular (promastigote) Leishmania, NO is certainly capable to exert direct leishmanicidal effects. However, whether this is also the case for intracellular (amastigote) Leishmania, has never been demonstrated. Considering the capacities of NO as a signaling molecule, possible indirect antimicrobial effects of NO have to be taken into account.

As NO is quickly oxidized to less toxic compounds after its release, direct effects on Leishmania requires close vicinity between NOS2 and the pathogen which resides within phago(lyso)somes. Using confocal and high resolution microscopy, we confirmed that NOS2 is expressed in the cytosol and in vesicle-like structures ("nitroxosomes"). Infection with Leishmania did not alter the overall intracellular localization of NOS2. Interestingly, NOS2 staining in IFN-γ/LPS-simulated uninfected or promastigote-infected bone marrow-derived macrophages (BMM) co-localized with EEA1 (early endosomal antigen1) and calnexin (ER marker), but not with LAMP1 (lysosomal-associated membrane protein1). In contrast, in Leishmania amastigote-infected BMM NOS2 colocalized with LAMP1 and calnexin, but not with EEA1 indicating that NOS2 gets recruited to the Leishmania-containing phagolysosomes in infected host cells. Ongoing studies aim to further characterize the NOS2-positive compartment in amastigote-infected BMM.

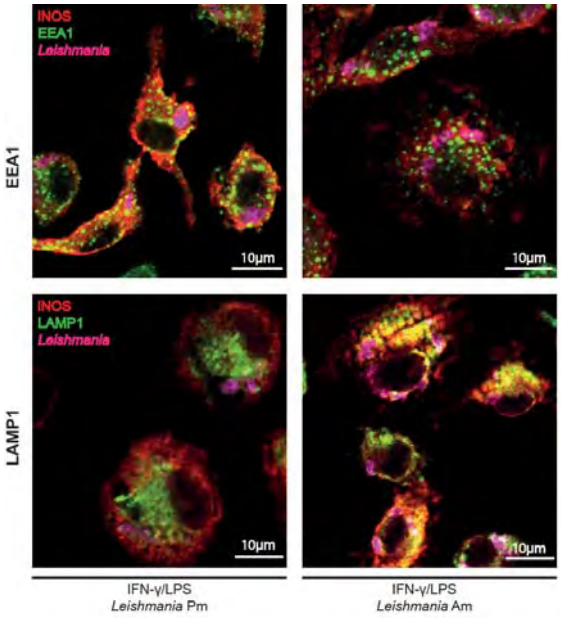


Confocal (CLSFM) and high resolution (STED) microscopy of NOS2 expression in IFN-γ/LPS-stimulated BMM, which were uninfected or infected with Leishmania promastigotes.

In order to test the hypothesis that the antileishmanial effect of NO not only relies on direct destruction of the parasites by damaging their DNA and proteins, but is also a result of the withdrawal of iron from the microenvironment of amastigotes, we performed killing assays with infected BMM that were stimulated for endogenous NO production

Prof. Dr. Bogdan

PD Dr. Schleicher



Confocal microscopy (CLSFM) of NOS2, EEA1, Lamp1 and Leishmania in IFN-γ/LPS-stimulated BMM, which were infected with Leishmania promastigotes (Pm) or amastogotes (Am).

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 analyzed whether the expression of ferroportin-1 (Fpn1), the only known cellular export system for Fe^{2+}/Fe^{3+}, is increased in a NO-dependent manner. So far, we could not detect any NO-dependent regulation of Fpn1 and of the iron storage protein ferritin. To definitively rule out a role of Fpn1, experiments with BMM of Tie2cre $Fpn1^{fl/fl}$ mice will be performed.

In a reverse approach we could demonstrate that iron-overloading of Leishmania-infected mice caused an exacerbation of the infection. Whether iron-dependent regulation of NOS2 and/or modulation of other effector pathways are responsible for this effect, is currently under investigation.

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Invited lectures

49th Meeting of the European Society of Clinical Investigation, May 27-30, 2015, Cluji-Naboca. C. Bogdan: Myeloid Cells as Inducers, Effectors or Suppressors of the Anti-Leishmania Immune Response (invited plenary lecture)

29th Annual Meeting of the European Macrophage and Dendritic Cell Society, September 11-13, 2015, Krakow. C.Bogdan: Macrophages and dendritic cells in antileishmanial defense and relation to reactive chlorine intermediates (invited plenary lecture)

Publications during funding period

Bogdan C (2015) Nitric oxide in innate and adaptive immunity: an update. Trends in Immunology 36(3): 161-178

Jantsch J, Schatz V, Friedrich D, Schröder A, Kopp C, Siegert I, Maronna A, Wendelborn D, Linz P, Binger KJ, Gebhardt M, Heinig M, Neubert P, Fischer F, Teufel S, David JP, Neufert C, Cavallaro A, Rakova N, Küper C, Beck FX, Neuhofer W, Muller DN, Schuler G, Uder M, Bogdan C, Luft FC, Titze J (2015) Cutaneous Na+ storage strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense. Cell Metabolism 21(3): 493-501

Siegert I, Schödel J, Nairz M, Schatz V, Dettmer K, Dick C, Kalucka J, Franke K, Ehrenschwender M, Schley G, Beneke A, Sutter J, Moll M, Hellerbrand C, Wielockx B, Katschinski DM, Lang R, Galy B, Hentze MW, Koivunen P, Oefner PJ, Bogdan C, Weiss G, Willam C, Jantsch J (2015) Ferritin-Mediated Iron Sequestration Stabilizes Hypoxia-Inducible Factor-1α upon LPS Activation in the Presence of Ample Oxygen. Cell Reports 13(10): 2048-55

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Mahnke A, Meier, RJ, Schatz V, Hofmann J, Castiglione K, Schleicher U, Wolfbeis OS, Bogdan C, Jantsch J (2014) Hypoxia in Leishmania major Skin Lesions Impairs the NO-Dependent Leishmanicidal Activity of Macrophages. The Journal of Investigative Dermatology 134: 2339-2346

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A62 - Progress Report

01.01.2014 - 30.06.2016

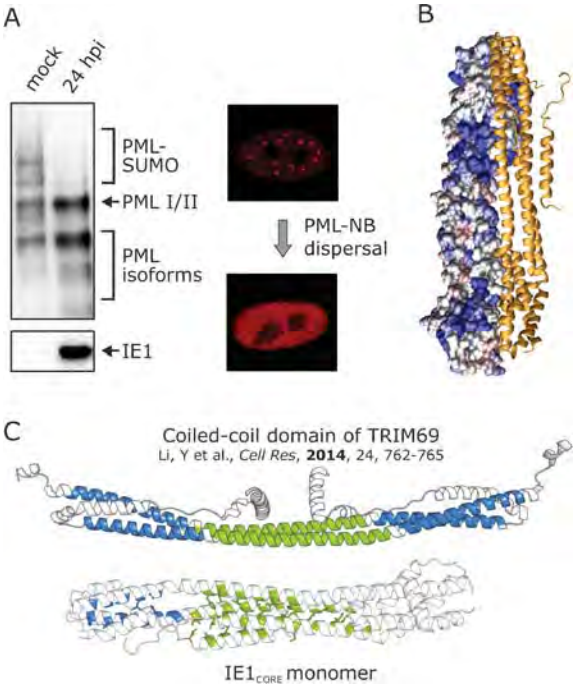
ND10 and interferon-induced gene expression

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

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

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

PML is the organizer of cellular structures termed PML nuclear bodies or nuclear domain 10 (ND10). We have shown that PML, and other ND10 components like hDaxx and Sp100 act as cellular restriction factors against human cytomegalovirus (HCMV) and a variety of other viruses. Interestingly, PML is an interferon-stimulated gene (ISG) and its expression is strongly increased by interferons (IFNs). Intriguingly, we observed that PML-depleted cells exhibit a reduced expression of the IFN-β-stimulated MHC-II gene HLA-DR/DP/DQ, compared to control cells. Furthermore, it is known that the antiviral function of ND10 is antagonized by viral regulatory proteins such as the immediate-early protein IE1 of HCMV. IE1 binds to the coiled-coil domain of the TRIM protein PML through its globular core domain (IE1CORE) and induces ND10 disruption in order to initiate lytic HCMV infection. During the last year, we investigated the consequences of a point mutation (L174P) in IE1CORE, which abrogates the interaction with PML, for lytic HCMV infection. This revealed that a recombinant HCMV encoding IE1-L174P displays a severe growth defect comparable to that of an IE1 deleted virus. Our data provide evidence that the IE1-L174P mutation affects the structural integrity of IE1 and this correlated with abrogation of the IFN-antagonistic function of this viral protein. Moreover, we could show that IE1CORE as expressed by a recombinant HCMV encoding IE1 1-382 is not only required to antagonize PML-mediated intrinsic immunity, but affects a recently described function of PML in innate immune signaling. We demonstrate a co-regulatory role of PML in type-I and type-II interferon-induced gene expression and provide evidence that upregulation of interferon-induced genes is inhibited by IE1CORE. In conclusion, our data suggest that targeting PML by viral regulatory proteins represents a strategy to antagonize both intrinsic and innate immune mechanisms.

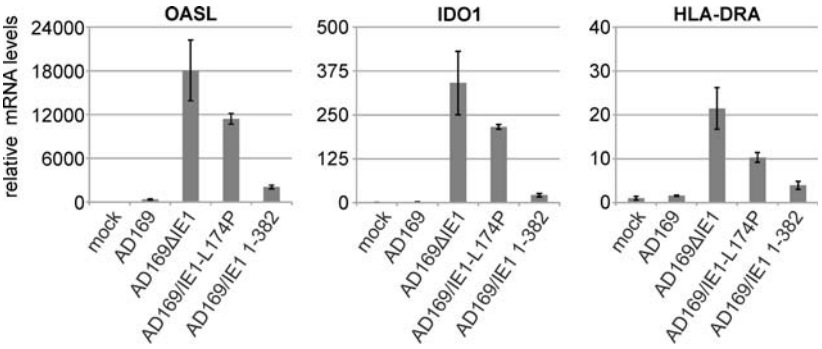


Function and structure of the PML-NB antagonistic protein IE1 of human cytomegalovirus. A) De-SUMOylation and PML-NB dispersal by IE1. B) Structure of IE1CORE. C) Structural homology of IE1CORE to the coiled-coil domain of TRIM proteins.

Prof. Dr. Stamminger

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

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



Suppression of IFN signaling by HCMV encoding IE1 1-382 which contains an intact PML binding domain but lacks the STAT2 interaction region.

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Invited lectures

Seminar of the SFB900, March 26, 2015, Hannover, Innate antiviral defense by PML nuclear bodies.

5th International Congenital CMV Conference and 15th International CMV/BetaHerpesvirus Workshop, April 20-24, 2015, Brisbane, Australia, Human cytomegalovirus IE1: an enigmatic regulatory protein antagonizing innate immune defense mechanisms.

Gordon Research Conference Viruses&Cells, June 21-26, 2015, Girona, Spain, Crystal structure of cytomegalovirus IE1 protein reveals targeting of TRIM family member PML via coiled-coil interactions

Second International Conference of the SFB796 "Mechanisms of Microbial Host Cell Manipulation", October 4-6, 2015, Erlangen, A viral G protein-coupled receptor interacts directly with TRAF6 to induce a highly regulated canonical NF-kB activation

Lecture at the Institute of Virology, MedUni Wien, September 21, 2015, Vienna, Austria, Das humane Cytomegalovirus – von molekularen Mechanismen zu neuen Therapien

MRC Human Immunology Unit Seminar, November 25, 2015, Weatherall Institute of Molecular Medicine, University of Oxford, UK, Human cytomegalovirus IE1: an enigmatic regulatory protein antagonizing innate immune defense mechanisms mediated by PML nuclear bodies

Seminar at Dep. of Medicine of SKKU (Sung Kyun Kwan University Seoul), December 2, 2015, Seoul, Korea, Innate antiviral defense by PML nuclear bodies

Publications during funding period

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): 1190-1205

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), 2884-2907

Scherer M, Stamminger T (2014) The human CMV IE1 protein: past and present developments. Future Virology 9: 415-430

Scherer M, Klingl S, Sewana M, Otto V, Schilling E-M, Stump JD, Müller R, Reuter N, Sticht H, Muller YA, Stamminger T (2014) Crystal structure of cytomegalovirus IE1 protein reveals targeting of TRIM family member PML via coiled-coil interactions. PLOS Pathogens 10 (11): e1004512

D19 - Progress Report

01.11.2013 - 31.10.2016

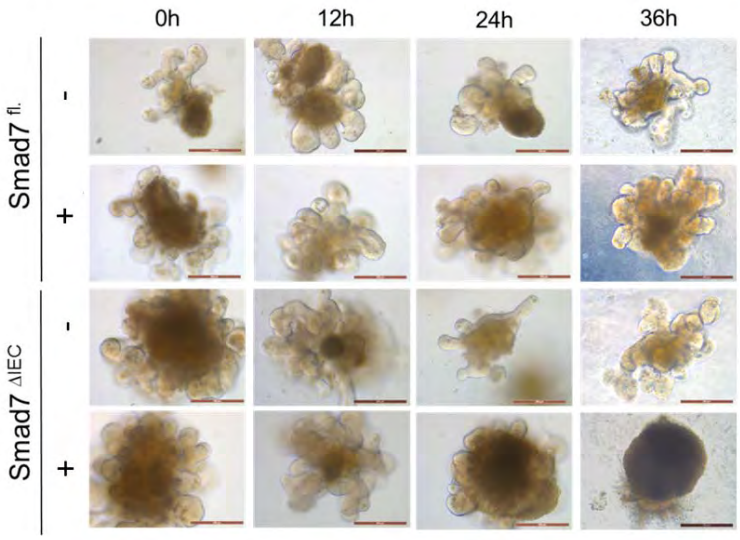
Role of intestinal epithelial Smad7 for tumor development

Dr. Nadine Wittkopf (till 28.02.2015), Prof. Dr. Christoph Becker,
Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Since polymorphisms in the Smad7 gene have been associated with an increased risk for developing colorectal cancer, we want to investigate the role of Smad7 in the intestinal epithelium for gut homeostasis and development of intestinal cancer. We will study the molecular mechanisms influenced by Smad7 using mice with an intestinal epithelial cell specific deletion of Smad7 and aim to create a basis for the development of innovative therapeutic approaches.

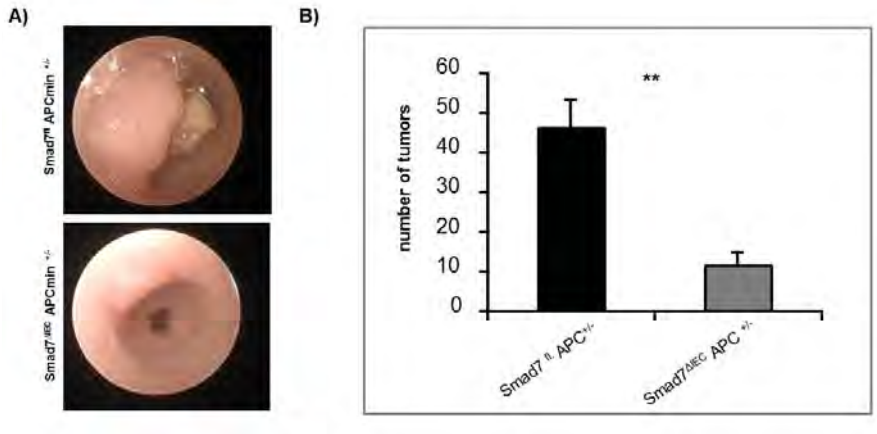
Mice with a conditional knockout for Smad7 in the intestinal epithelium (Smad7$^{\Delta IEC}$) were generated to evaluate its function in the intestinal epithelium. First analyses indicated no postnatal developmental gut defects in Smad7$^{\Delta IEC}$ mice and furthermore, no macroscopic intestinal lesions could be detected in control (Smad7^{fl}) or knockout animals. Histological and morphometric evaluations moreover revealed no differences in epithelial structure of small and large intestine. An additional analysis of differentiated intestinal epithelial cell types like Paneth or goblet cells as well as enterocytes and enteroendocrine cells showed no differences between control and Smad7$^{\Delta IEC}$ animals. The presence of immune cells in small intestine and colon did not differ between the two groups of mice.

In previous experiments we could show, that in a colitis associated tumor model (AOM/DSS) mice with Smad7 deficiency developed less tumors in comparison to the control group. To further investigate the role of Smad7 during tumor development independent of colitis, we used a sporadic tumor mouse model (APCmin). Therefore, we analyzed the tumor formation in the small intestine and colon at different ages. Mice with APCmin alleles and an additional deletion of Smad7 in intestinal epithelial cells (APC$^{+/-}$ Smad7$^{\Delta IEC}$) showed a reduced tumor number as well as tumor size in small intestine and colon when compared to the control group (APC$^{+/-}$ Smad7^{fl}). Additionally, it could be shown, that APC$^{+/-}$ Smad7$^{\Delta IEC}$ mice



Representative pictures of small intestinal organoid cultures of control and Smad7$^{\Delta IEC}$ mice stimulated with (+) or without (-) TGF-β for an overall of 36 hours (h). Smad7$^{\Delta IEC}$ mice show an increased susceptibility for the stimulation with TGF-ß after 24 hours resulting in cell death.

Dr. Wittkopf Prof. Dr. Becker



A) Representative colonoscopic picture of control (Smad7^{fl} APC-min$^{+/-}$) and Smad7$^{\Delta IEC}$ mice with a mutated APC gene (Smad7$^{\Delta IEC}$ APCmin$^{+/-}$). B) Statistical analysis of the tumor number of control and Smad7$^{\Delta IEC}$ Apc$^{+/-}$ mice showed a reduced tumor size in mice deficient for Smad7 in IECs.

had a higher life span than APC$^{+/-}$ Smad7^{fl} mice. These data support the hypothesis that Smad7 influences the process of tumor development.

We further performed *in vitro* analyses of small intestinal organoids from Smad7fl and Smad7$^{\Delta IEC}$ mice. Surprisingly, organoids from Smad7$^{\Delta IEC}$ mice reacted more sensitive to TGF-β stimulation and increased cell death could be detected after 36 hours.

Taken together our data show that a deletion of Smad7 in intestinal epithelial cells leads to reduced tumor burden in an colitis associated tumor model and in a sporadic tumor model in mice. The detailed mechanism on how Smad7 influences the TGF-ß signaling pathway or if also other pathways like NF-κB or Wnt are involved has still to be elucidated. Further experiments will investigate the role of Smad7 on these side pathways to understand its role during colon tumorigenesis.

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Invited lectures

C. Becker: International Congress of Mucosal Immunology, 16.07.2015, Berlin, „Signaling pathways driving the pathogenesis of inflammatory bowel disease"

C. Becker: United European Gastroenterology Week 2015, 27.10.2015, Barcelona, "Role of the Epithelium in Intestinal Inflammation"

Publications during funding period

Wittkopf N, Neurath MF, Becker C (2014) Immune-epithelial crosstalk at the intestinal surface. J Gastroenterol 49: 375-87

D20 - Progress Report

01.11.2013 - 30.04.2016

Collagen 10 and metastasis in CRC

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

We previously showed that the expression of the collagen 10 mRNA is increased in primary lesions of metastasizing colorectal carcinomas. In this project tumor stage-related expression of collagen 10 will be validated at the protein level and the collagen 10 producing cells will be determined. Moreover, the role of collagen 10 in the regulation of metastasis will be analyzed in mouse model systems. The project will deliver new insights into the function of matrix components in the metastasis of colorectal carcinoma.

Positive correlation of collagen 10 RNA and protein in colorectal carcinoma

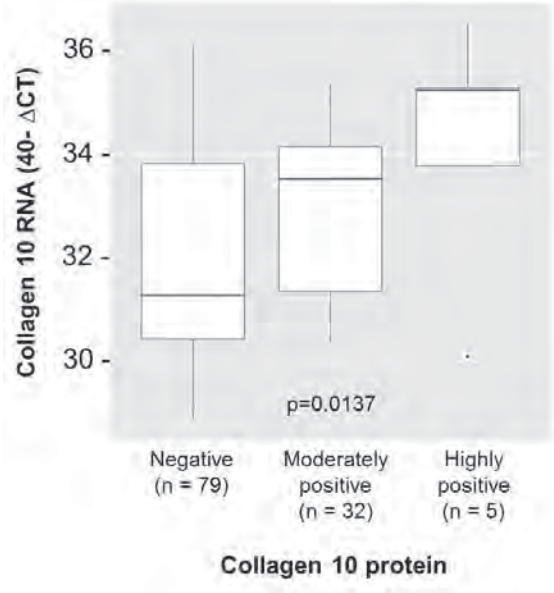
Immunohistochemical staining with a specific monoclonal antibody (provided by Prof. von der Mark, Inst. of Experimental Medicine 1) revealed that the collagen 10 protein is present in colorectal carcinoma (CRC) tissues (n=116) and significantly increases with progression of the disease. Protein expression was categorized and correlated significantly with collagen 10 mRNA (TaqMan-qPCR) expression in consecutive tissue sections of these patients.

Collagen 10 protein is detected in vimentin- and PDGF-β-receptor positive but not α-SMA-positive stromal cells in CRC

Immunofluorescent co-localization studies revealed that collagen 10 is expressed in vimentin- and PDGF-β-receptor positive but not α-SMA-positive stromal cells in CRC tissues. These analyses suggest that the major source of collagen 10 in CRC might be tumor-associated fibroblasts or mural cells.

Establishing of collagen 10 knockout mice

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

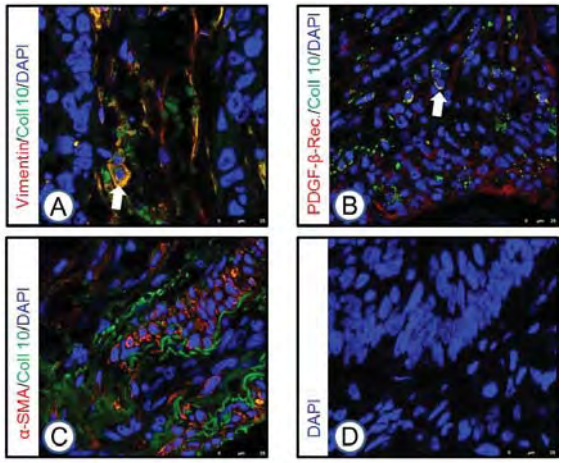


Expression of collagen 10 at the mRNA and protein level is significantly correlated in colorectal carcinoma tissues.


Prof. Dr. Dr. Stürzl


Prof. Dr. Croner


PD Dr. Naschberger



Collagen 10 is expressed in vimentin (A) and PDGF-β-receptor (B) positive but not in α-smooth muscle actin (C) positive cells in colorectal carcinoma tissues. Negative control staining (D).

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Awards

Karl-Heinrich Bauer Award 2015 for surgical tumor research, German Society of Surgery, Prof. Roland Croner.

Publications during funding period

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Schaal U, Grenz S, Merkel S, Rau TT, Hadjihannas MV, Kremmer E, Chudasama P, Croner RS, Behrens J, Stürzl M, Naschberger E (2013) Expression and localization of axin 2 in colorectal carcinoma and its clinical implication. Int J Colorectal Dis 11:1469-78

D21 - Progress Report

16.10.2013 - 15.10.2016

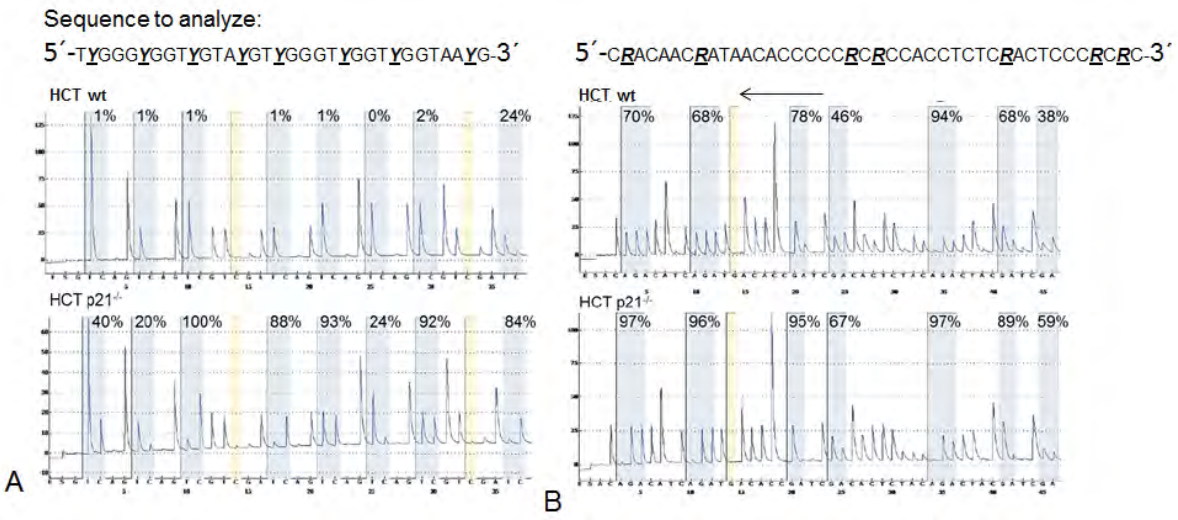
DAPK and colon cancer

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

DAPK levels were decreased or even lost in disseminating tumor cells at the invasion front of colorectal cancer which was associated in vitro with its inhibitory role in migration. The regulation of DAPK at the invasion front is only poorly understood but might be connected to EMT. Since EMT-positive HCT116 p21ko colorectal cancer cells do not express DAPK we examined the molecular regulation of DAPK in these cells. Furthermore, 3D crypt organoids of normal and DAPK-ko mice were generated.

DAPK promoter hypermethylation analyses

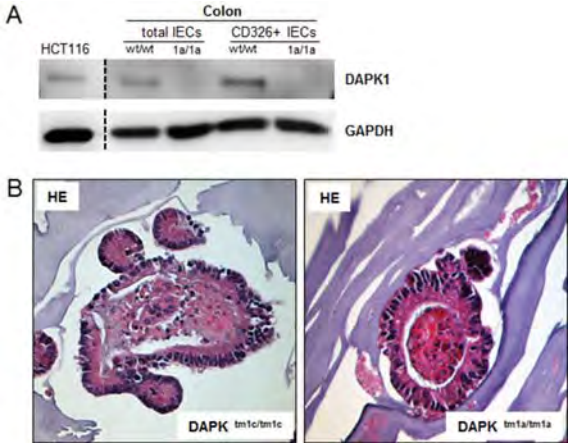
Hypermethylation of gene promoters is the major mechanism for transcriptional silencing. We studied DAPK promoter methylation using pyrosequencing technique. Five different regions in the very large CpG island of the DAPK promoter were analyzed. Altogether, these assays covered 35 different CG dinucleotides. The DAPK promoter was heavily methylated in the mesenchymal HCT116 p21ko cells compared to the parental epithelial HCT116 cells. Interestingly, in HCT116 parental cells there was a heterogeneous methylation pattern with a gradual increase in promoter hypermethylation close to the transcription start. In contrast, HCTp21$^{-/-}$ cells showed homogeneously a high degree of methylation. In a next step, we plan to analyze promoter hypermethylation in the tumor center and at the invasion front of colon cancer to evaluate the potential translational relevance of our recent findings.



Pyrosequencing of two promoter regions to detect DAPK hypermethylation in parental HCT and HCTp21-/- cells. (A) distant (B) close to transcription site; (A) Y: C or T (B) R: G or A (antisense). Methylation % is shown as peak for each CG dinucleotide.

Prof. Dr. Schneider-Stock

PD Dr. Neufert



(A) Validation of the loss of DAPK protein in Dapk1tm1a/tm1a mice by Western blot.
(B) HE-staining of organoids from different mice with modified Dapk1 alleles (genotypes as indicated). 10x40 magnification.

DAPK knockout mice studies

We could validate that our transgenic founder strain C57BL/6NTac carrying the homozygous insertion allele (Dapk1$^{tm1a(EUCOMM)Hmgu}$) results in a knockout (KO) of DAPK1 on protein level in lysates of total colon and CD326-enriched intestinal epithelial cells (IECs). Mice with conditional deletion of DAPK in IECs (Dapk1$^{\Delta IEC}$) were generated by crossbreeding of Dapk1tm1a/tm1a mice with Flippase deleter mice resulting in mice with Dapk1^{tm1c} alleles and subsequent crossbreeding with Villin-Cre mice resulted in mice with Dapk1^{tm1d} alleles.

Ex vivo crypt organoid cultures were developed for DAPK deficient IECs and analyzed for morphology and growth characteristics of crypts. The typical villus domain in the organoid center and peripheral crypt structures was similar in organoids of DAPK deficient and control IECs. However, there is evidence for a more irregular morphology of DAPK deficient IECs compared to controls. To enrich the number of preserved crypts per paraffin block, we established a novel technique of paraffin embedding. Further growth statistics, kinetic studies, determination of organoid specific cell populations (stem cells, Paneth cells, goblet cells, enterocytes and enteroendocrine cells) and quantification of proliferation/cell death ratios are under investigation.

Thus, we could validate that mice carrying Dapk1$^{tm1a(EUCOMM)Hmgu}$ alleles homozygously do not express DAPK protein including IECs of the colon. DAPK deficiency in IECs seems to influence the growth morphology of crypt organoid cultures. Meanwhile, colon tissue and primary IECs of different mice with modified DAPK1 alleles (Dapk1$^{tm1a/tm1a}$, Dapk1$^{tm1c/1m1c}$ and Dapk1$^{tm1d/tm1d}$) are available for further analysis.

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Publications during funding period

Ivanovska J, Zlobec I, Forster S, Karamitopoulou E, Dawson H, Hendrik Koelzer V, Agaimy A, Garreis F, Söder S, Laqua W, Lugli A, Hartmann A, Rau TT, Schneider-Stock R (2015) DAPK loss in colon cancer tumor buds: implications for migration capacity of disseminating tumor cells. Oncotarget Nov 3;6(34):36774-88

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* Both authors share first authorship. # Both authors share senior authorship.

D22 - Progress Report

01.11.2013 - 31.10.2016

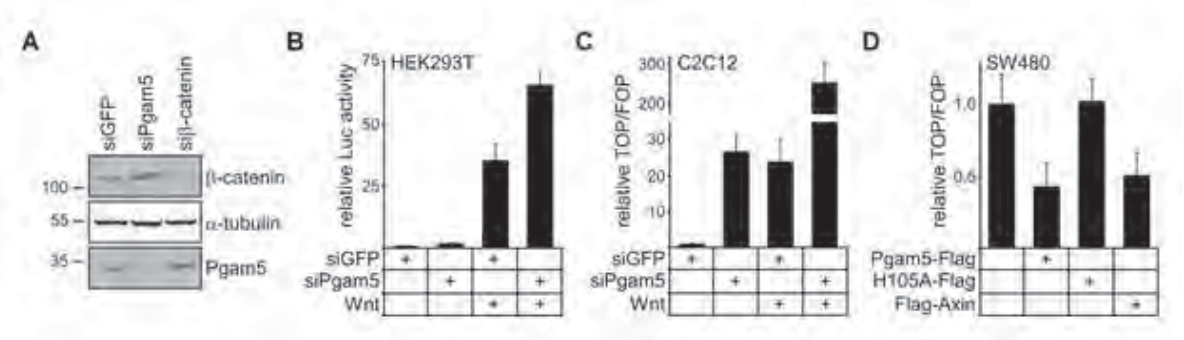
Identification and functional characterisation of novel components of the Wnt/β-catenin signal transduction pathway

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

We will search for novel components of the Wnt signal transduction pathway at the level of Wnt receptors and the β-catenin destruction complex using yeast two hybrid screens and proteomic analyses, and determine their function by cell biological and developmental studies. Through this work we wish to achieve a better understanding of the signaling pathway in order to identify possible targets for interference in disease processes.

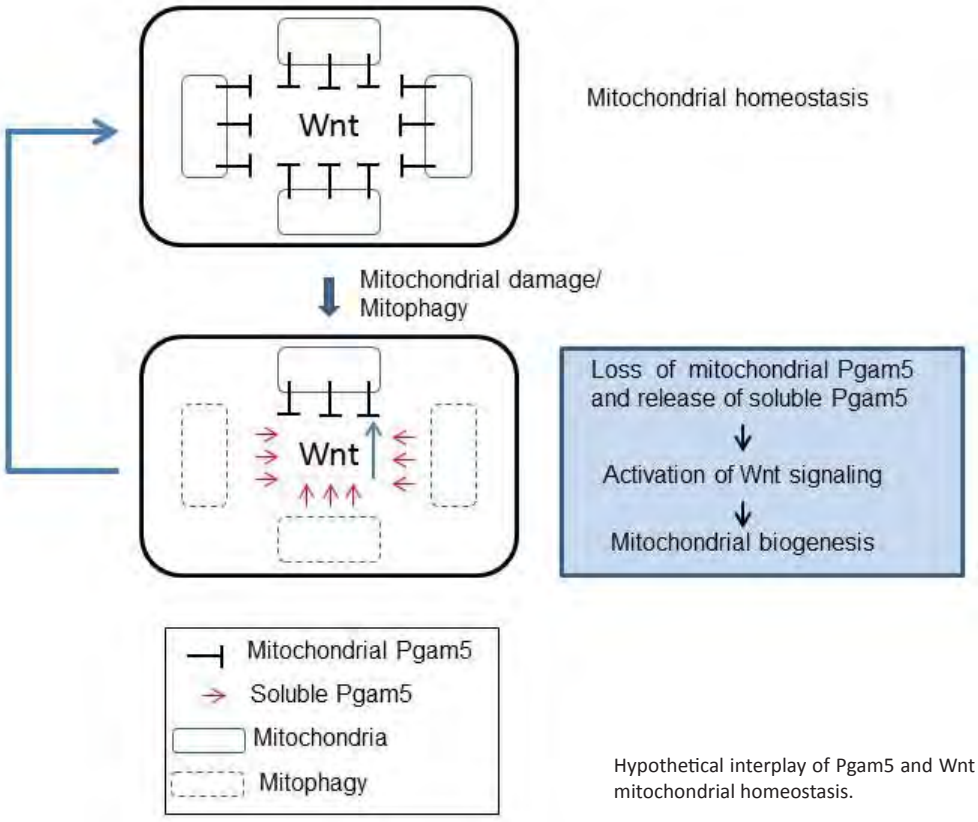
We are interested in widening the spectrum of possible regulators of the Wnt signaling pathway by identifying interactors of the core components of the pathway. For instance, by yeast two hybrid screens novel putative binding proteins of the E3 ligases regulating the Wnt receptors frizzled and LRP6, RNF43 and ZNRF4 were isolated and functionally characterized. Here we will focus on results obtained on the previously identified interaction of the negative regulator of Wnt signaling, Axin with the protein phosphatase Pgam5 which is associated with mitochondria. We found that knockdown of Pgam5 increased β-catenin stability and Wnt dependent transcription in reporter assays, suggesting that Pgam5 is a negative regulator of Wnt signaling. In line, overexpression of Pgam5 in colorectal tumor cells reduced constitutive Wnt signaling activity. Data from our collaboration partner Dr. Alexandra Schambony (Dept. of Biology, Erlangen) showed that morpholino-dependent downregulation of Xenopus laevis Pgam5 led to phenotypes similar to depletion of the negative Wnt pathway regulator Dickkopf 1, i.e. posterization of the body axis, in line with a negative regulatory role of Pgam5 in Wnt signaling during development. Moreover, co-expression of Pgam5 was sufficient to antagonize ectopic axis duplication induced by ventral overexpression of Wnt-8 or β-Catenin which further confirmed that Pgam5 antagonizes Wnt/β-Catenin signaling. Thus, Pgam5 has characteristics of a negative regulator of the Wnt pathway suppressing β-Catenin stability, TCF/β-Catenin dependent transcription, and Wnt dependent morphogenesis.

Surprisingly, Pgam5 lacking the first 24 amino acids, which is no longer associated with mitochondria and located in the cytoplasm has an activating role in Wnt signaling, as revealed by increased β-Catenin levels and reporter activity after its overexpression. This form is generated by mitochondrial damage induced by exposure of mitochondria to membrane depolarization agents such as the substance CCCP.



Effect of Pgam5 knockdown (A-C) and over expression (D) on TCF/β-catenin signaling by analyzing cytoplasmic β-catenin in Western blots (A) and TCF/βcatenin dependent reproter assays (B-D).

Prof. Dr. Behrens



Hypothetical interplay of Pgam5 and Wnt signaling in controlling mitochondrial homeostasis.

Because Wnt signaling was previously shown to stimulate mitochondrial biogenesis, i.e. increase the numbers of mitochondria per cell we hypothesize that conditions leading to reduced mitochondrial numbers such as mitochondrial damage translate into decreased Pgam5 levels leading to activation of Wnt signaling and compensatory mitochondrial biogenesis. Conversely, high numbers of mitochondria might curb Wnt signaling via increased Pgam5 levels. In addition, soluble Pgam5 generated as a consequence of the loss of mitochondrial membrane potential might activate Wnt signaling thereby inducing mitochondrial biogenesis. In that way, the Pgam5-Wnt interplay might act as a sensor for maintaining mitochondrial homeostasis.

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Invited lectures

Wnt Symposium 2015, Heidelberg, May27, 2015, “Regulation of Axin by phosphorylation”

Biocenter Würzburg, July 15, 2015, „Two sides of the same coin? Axin and Conductin/Axin2 in Wnt pathway regulation, cell cycle and feedback“

Publications during funding period

none

E11 - Progress Report

01.12.2013 - 30.11.2016

H50Q aSyn mutation in PD

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

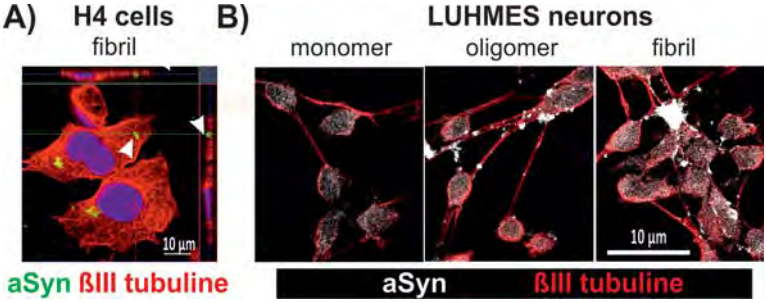
Alpha-Synuclein (aSyn) is a key protein in the neurodegeneration of Parkinson's disease (PD). We aim to elucidate the role of oxidative stress (OS) dependent Histidine50 (H50) modification in aSyn aggregation, toxicity and propagation. We revealed substantial effects of posttranslational modification of H50 and the novel PD-linked mutation (H50Q) on aSyn aggregation and toxicity. Currently, we intensify our studies on the relevance of OS and aSyn modification for both extra- and intracellular pathways linked to the pathogenesis of PD.

H50 alterations trigger aSyn aggregation and toxicity

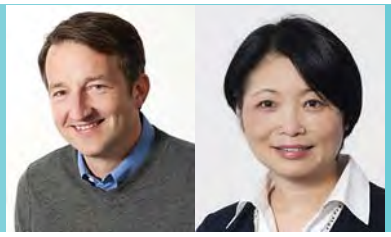
HNE is a reactive lipid peroxidation product found in increased levels in brains of PD patients. We revealed that HNE-induced posttranslational modification (PTM) of aSyn histidine 50 (H50) significantly increases aSyn oligomerization and its toxicity to cells. Furthermore, we studied the effects of the novel PD-causing aSyn mutation (H50Q) and found that this mutation increases the aggregation propensity of aSyn both in vitro and in cells. Additionally, overexpression of H50Q-aSyn promotes apoptosis-associated cell death, which is even more pronounced under oxidative stress. Thus, either mutation or PTM of aSyn H50 increases aSyn pathology, suggesting a crucial role of H50 in aSyn-mediated neurodegeneration in PD.

The role of extracellular aSyn modified at H50 in the propagation of pathology

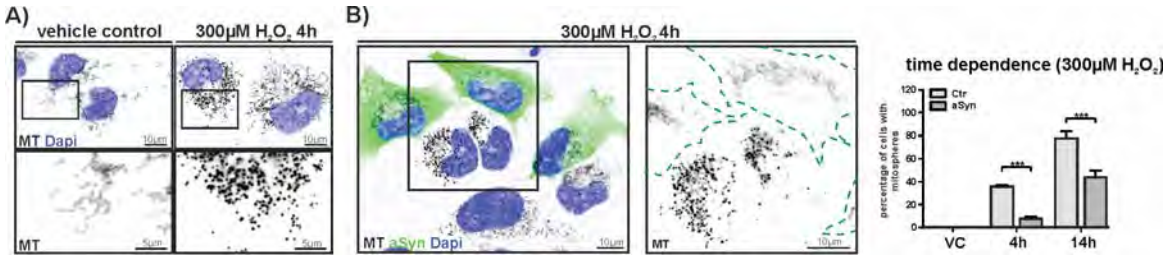
Current knowledge suggests an important role of extracellular aSyn in the progression of PD. Here, we investigate the mechanisms by which aSyn pathology propagates. We observed that extracellular aSyn, including aSyn modified by HNE at H50 (HNE-aSyn), can be taken up by different neural cell types, probably by clathrin- and lipid raft-dependent endocytic pathways. The uptake of aggregated aSyn, such as HNE-aSyn, appears to be more efficient than monomeric aSyn. We detected a co-localization of internalized aSyn with endosomal and lysosomal markers, suggesting that internalized aSyn is processed through endosomal/lysosomal pathways in recipient cells. We also observed a strong accumulation of aggregated aSyn in the cytosol, indicating that aggregated aSyn bypasses protein degradation pathways. Importantly, internalized aggregated aSyn induces the aggregation of intracellular aSyn in recipient cells.



A) Confocal microscopy shows the uptake of aggregated aSyn exposed to H4 cells. B) Extracellular aggregated aSyn (HNE-aSyn oligomers and fibrils) applied to LUHMES neurons induces strong accumulation and aggregation of intracellular aSyn in neurons.



PD Dr. Klucken PD Dr. Xiang



A) Oxidative stress-induced hyperpolarized mitospheres are stained by mitochondrial membrane potential-dependent mitotracker in H4 neuroglioma cells. B) WT aSyn overexpression substantially prevents the formation of oxidative stress-related mitospheres.

The role of aSyn in oxidative stress-induced mitochondrial dysfunction

Due to the association of mitochondrial dysfunction, oxidative stress and aSyn with the progression of PD, we recently aim to decipher the effect of wild type (WT) aSyn on oxidative stress-induced mitochondrial alterations. Our results show that H2O2-induced oxidative stress provokes the formation of spherically shaped and hyperpolarized mitochondria in different cell types including neurons. The formation of these mitospheres depends on mitochondrial fission processes, is not linked to Parkin-associated mitophagy, and precedes Caspase3 activation. Intriguingly, we found that WT aSyn prevents mitosphere formation, accompanied by a reduction of apoptosis, supporting a protective function of WT aSyn in the interplay between mitochondrial dysfunction and oxidative stress. Future experiments will be performed to answer the question whether aSyn mutations (e.g. H50Q) and oxidative stress-related alterations impact this protective role of WT aSyn.

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Invited lectures

Salzburg, Österreich – Invited lecture "Autophagy modulates extracellular aSyn pathology – a novel role for exosomes in Parkinson's Disease", 08./09. Juli 2015 - Prof. Aigner

Aarhus, Dänemark - Neurodin Meeting – "Extra-/Intracellular pathology of alpha Synuclein" 24-26.Mrz 2015 - Prof. Ramero-Ramos

Publications during funding period

Schreglmann SR, Regensburger M, Rockenstein E, Masliah E, Xiang W, Winkler J, Winner B (2015) The temporal expression pattern of alpha-synuclein modulates olfactory neurogenesis in transgenic mice. PLoS One 10(5): e0126261

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

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E12 - Progress Report

01.04.2014 - 31.03.2017

Adult hippocampal neurogenesis in synucleinopathies

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

Non-motor symptoms like anxiety and depression play an important role in Parkinson's disease (PD), frequently occurring prior to the onset of motor symptoms. First data from alpha-synuclein (a-syn) transgenic animal models indicate that a-syn severely impairs the hippocampal serotonergic system prior to the onset of motor symptoms resulting in a compromised neuritic hippocampal circuitry and reduced neurogenesis.

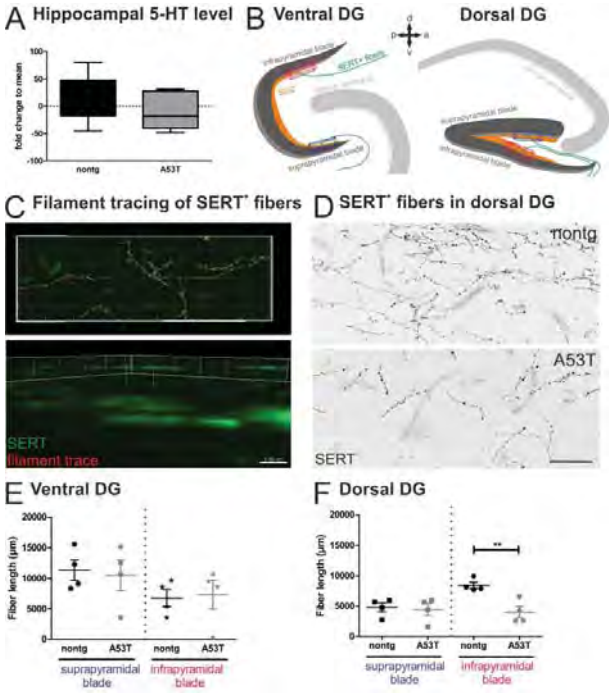
The aim of this study is to analyze non-motor neuropsychiatric symptoms (NMS) related to hippocampal neurogenesis in transgenic PD animal models. Moreover, we aim to decipher the underlying pathogenesis of these symptoms to identify new molecular targets for the treatment of NMS.

Decreased fluoxetine responsiveness in A53T a-syn mice

The hippocampal serotonergic (5-HT) system of A53T a-syn mice was analyzed since a-syn was expressed in 5-HT neurons associated with reduced hippocampal neurogenesis. Although we did not observe changes in hippocampal 5-HT levels we detected differentially reduced serotonergic innervation of hippocampal subregions. Intriguingly, 5-HT neurites were less present in the dorsal infrapyramidal blade of the dentate gyrus (DG), the region predominately involved in cognition. Importantly, neuroblast maturation was less efficiently stimulated by selective serotonin reuptake inhibitor (SSRI) fluoxetine, indicating a pivotal role of the spatial 5-HT innervation for hippocampal plasticity.

Early hippocampal changes associated with NMS in BAC transgenic a-syn rats

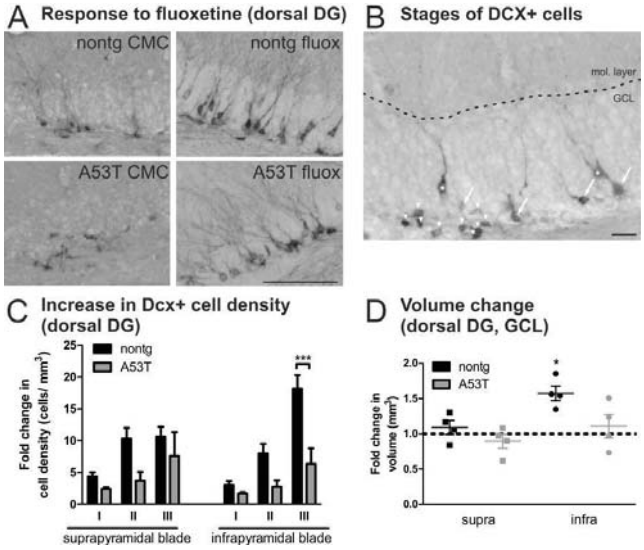
To analyze early hippocampal changes in PD, we used BAC transgenic a-syn rats. Prior to the onset of motor symptoms we observed a severe 5-HT dysfunction in the hippocampus of 4 month old rats, as detected by reduced serotonergic innervation, low 5-HT levels, and altered 5-HT receptor expression in the DG/CA3 subfield of the hippocampus. These structural changes were accompanied by a severely impaired hippocampal neurogenesis, namely a profound reduction of doublecortin (lower case) neuroblasts and dendrites. Importantly, BAC alpha-synuclein rats showed an early anxiety-like phenotype consisting of reduced exploratory behavior and feeding.



(A) Comparable hippocampal 5-HT levels in nontransgenic and 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 nontransgenic mice.

Prof. Dr. Winkler

Prof. Dr. Lie



(A-D) Fluoxetine-mediated stimulation of hippocampal neuroblasts. (C) Fluoxetine-mediated increase in late Doublecortin+ cell density within the infrapyramidal blade was reduced in A53T mice. (D) Fluoxetine treatment resulted in a significant increase of GCL volume in the dorsal infrapyramidal blade of nontg mice only.

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

We previously identified the Sox C transcription factor Sox11 as a critical regulator of neuronal differentiation of stem cells in the adult hippocampal DG. Our recent data strongly suggested that Sox11 may also be involved in hippocampal plasticity and hippocampus-dependent regulation of mood. Firstly, we found that Sox11 expression is transiently induced in hippocampal DG granule neurons in response to novel environments and exploratory behavior. Secondly, data from our laboratory and others revealed that antidepressant treatment such as electroconvulsive shocks (ECS) and application of SSRIs strongly increases the expression of Sox11 in DG neurons.

We have now established that Sox11 alters the plasticity of DG granule neurons. In addition, we have found that Sox11 expression in mature DG granule neurons changes the molecular composition of the neuronal cytoskeleton. We are presently conducting experiments to establish how these Sox11-induced changes alter hippocampus-dependent behavior.

Using mass-spectrometry we found evidence that Sox11 can be post-translationally modified by phosphorylation. We have now identified a candidate Serin-residue whose phosphorylation appears to be critical for Sox11 transcriptional activity, stability and subcellular localization.

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Invited lectures

DCL: Colloquium Translational Medicine, 27 January 2016, Department of Surgery, University Clinic Würzburg.

JW: International Symposium 'Induced Pluripotent Stem Cells' (ForIPS), 3 July 2015, Siemens Foundation, Schloss Nymphenburg, München

Publications during funding period

Deusser J, Schmidt S, Ettle B, Plötz S, Huber S, Müller CP, Masliah E, Winkler J, Kohl Z (2015) Serotonergic dysfunction in the A53T alpha-synuclein mouse model of Parkinson's disease. J Neurochem. 135(3):589-97

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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

E13 - Progress Report

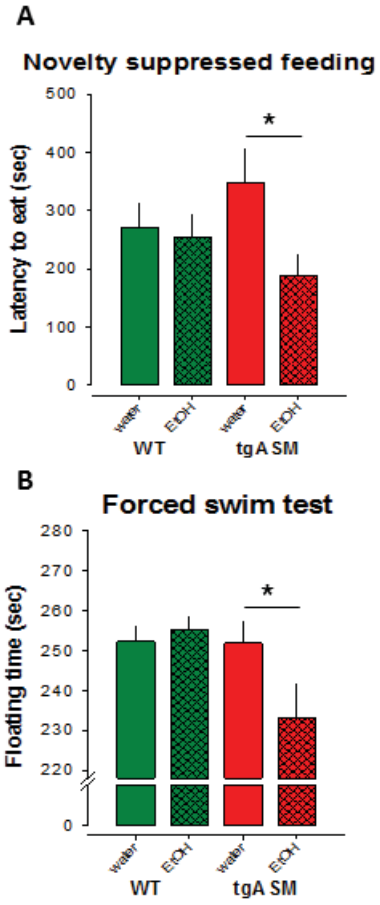
01.04.2014 - 31.03.2017

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

Prof. Dr. Christian P. Müller, PD Dr. Martin Reichel, 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 could replicate enhanced alcohol consumption of the tgASM mice compared to WT controls. When animals were subsequently tested in a series of anxiety- and depression tests we found that free-choice alcohol drinking reduced depression-like behaviour in tgASM animals in the novelty-suppressed feeding (NSF), Forced-swim-test (FST) and open field test compared to water drinking tgASM mice, without affecting locomotor activity. However, while alcohol reduced depression in tgASM mice, it enhanced anxiety-related behaviour in the elevated-plus-maze (EPM).



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).

Alcohol consumption had no effect on depression-related behaviour in WT mice. tgASM mice show enhanced ASM activity in the hippocampus. Alcohol drinking partially reversed this genotype effect. In a second study we asked whether the depressive phenotype can be reversed by the pharmacological effects of the alcohol alone, or whether the free-choice and, thus, self-titration, was a crucial element in this action. In this study, animals had no free-choice, but received repeated alcohol-injections (i.p.). When depression/anxiety behaviour was tested afterwards, we found rather opposite effects compared to a free-choice administration. Alcohol enhanced depression-like behaviour in the FST, but reduced anxiety in the EPM. This alcohol effect was observed in both, tgASM and WT mice. This is in line with alcohol effects on ASM activity in the hippocampus. When given i.p., alcohol 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. In collaboration with Dr. Witt (Bruker, Bremen) we used

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

mass spectrometric analysis of brain slices performed with a FTMS SolariX XR 12 T. In tgASM mice, several sphingomyelin species were 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. So far, our findings suggest the ASM-sphingomyelin/ceramide pathway as a potential mediator of depression-induced alcohol preference, and possibly, addiction, by controlling sphingolipid homeostasis in specific parts of the brain reward system.

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Invited lectures

Christian P. Müller:

International Behavioral Neuroscience Society - Annual Meeting, 03.06.2015, Victoria, Canada, Sphingolipids: From depression and anxiety to alcoholism

European Behavioral Pharmacology Society/ EBBS Society - Joint meeting, 13.09.15, Verona, Italy, A new sphingolipid pathway in depression-induced alcoholism.

Arbeitsgemeinschaft für Neuropsychopharmakologie - Symposium, 24.09.15, München, Sphingolipids in alcohol abuse and addiction

Johannes Kornhuber:

Arbeitsgemeinschaft für Neuropsychopharmakologie - Symposium, 25.09.15, München, Das Ceramid-System als neues Target antidepressiver Therapie

Martin Reichel:

Arbeitsgemeinschaft für Neuropsychopharmakologie - Symposium, 24.09.15, München, Sphingolipids in alcohol abuse and addiction

Publications during funding period

Müller CP, Quednow BB, Lourdusamy A, Kornhuber J, Schumann G, Giese KP (2015) CaM kinases – From memories to addiction. Trends in Pharmacological Sciences, doi: 10.1016/j.tips.2015.11.001. [Epub ahead of print]

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

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E14 - Progress Report

01.04.2014 - 30.09.2016

Role of TRPC5 in trigeminal nociception

Prof. Dr. Katharina Zimmermann, Department of Anaesthesiology

Cold hyperalgesia and cold hypersensitivity are common dental problems. TRPC5 is present in normal teeth and undergoes strong upregulation in sensory neurons innervating root, pulp and dentine of pulpitic human teeth. To investigate its role in tooth pain and characterize it as treatment target we developed a mouse labeling model of primary afferent dental neurons, and employed a previously developed high throughput screening assay to search Griffith University's Nature Bank sample catalogue for TRPC5 modulators. We identified more than 150 natural compounds with TRPC5 modulating properties.

Model of retrograde labeling of mouse dental primary afferents

In the previous progress report, we described a model to label dental primary afferent neurons (DPAN) in the rat via retrograde axoplasmic transport of fluorescent DiI. Briefly holes are drilled in all maxillary molars, dye is placed in the tooth cavities, and cavities are closed with light-sensitive dental cement. The dye is transported axonally to the trigeminal ganglia within 5 days. In the last months we have honed many technical details in order to achieve a transfer of the model to the mouse and obtain a reproducible amount and consistent practical yield of labeled mouse DPAs. Technical details included to validate characteristics of different carbocyanine dye formulations (paste vs crystal), develop techniques to drill holes in mouse molars (less than a quarter of the size of the rat molars), minimize contamination of adjacent oral cavity tissue and reduce off-target and background staining in the ganglia, determine the optimal traveling time of the dye in the mouse to reliably and reproducibly label all DPAN. We determined the optimal travel time to 120h. We also found that NeuroTrace® DiI tissue-labeling paste is superior to crystalline application because it stains cells more intensive and allows a better distinction of the cells from background; it thereby leads to more constant labeling results. This method is ready to study properties of DPAN innervating normal and inflamed teeth using calcium imaging, Patch-Clamp and full transcriptome.

Screening for TRPC5 modulators in Griffith university's Nature Bank

Previously, a calcium-based High-Throughput-Screening assay for testing modulatory effects compounds on heterologous expressed TRPC5 on a FLIPRTETRA® Platform was developed based on a HEK 293t cell line stably expressing inducible hTRPC5. A high extracellular calcium concentration (5.8mM) or Riluzole, a TRPC5 agonist, are used to produce reproducible TRPC5-mediated calcium signals in cells loaded with Fluo-4. Using this FLIPR assay we already had tested several cone snail venoms but were not successful in identifying TRPC5 modulators. Now we tested 16,000 samples of natural compounds from the Nature Bank project at Griffith University's Eskitis Institute for Drug Discovery which included natural product fractions from a large array of Australian plants and marine invertebrates (currently 200,000 available mixtures). We identified 52 blocking and 104 activating fractions which need to be further characterized. While TRPC5 blocking agents were exclusively isolated from animal kingdom, agonist included also a few samples from plants. We are primarily attracted by the blockers, because some of them may be suitable as tooth pain analgesic precursor compounds; we list the species where blockers where found in the table. Nevertheless, agonists may be very suitable scientific tools, because today there are only very few activators of TRPC5 and the physiological function of this ion channel is still largely unknown. Blockers were included in fractions from Australian marine animals: 38 are from sponges, 5 from chordates, 4 from cnidaria, two from tunicates, two from echinoderms, and the last

Prof. Dr. Zimmermann

# hits	Phylum	Class	Order	Family	Genus	Species
1	Bryozoa	Stenolaemata	Horneridae	Horneridae	*Hornera*	*foliacea*
4	Chordata	Ascidiacea	Didemnidae	Didemnidae	*Tridemnum*	*cerebriforme*
1	Chordata	Ascidiacea	Pyuridae	Pyuridae	*Pyura*	*australis*
3	Cnidaria	Anthozoa	Ellisellidae	Ellisellidae	*Junceella*	*fragilis*
1	Cnidaria	Anthazoa	Alcyonacea	Alcyoniidae	*Sarcophyton*	N.n.
1	Echinoderm.	Echinoidea	Diadematidae	Diadematidae	*Echinotrix*	N.n.
1	Echinoderm.	Asteroidea	Valvatida	Oreasteridae	*Culcita*	N.n.
1	Porifera	Anthozoa	Alcyoniidae	Alcyoniidae	*Sarcophyton*	*trocheliophorum*
11	Porifera	Demospong.	Petrosiidae	Petrosiidae	*Neopetrosia*	*pacifica*
1	Porifera	Demospong.	Petrosiidae	Petrosiidae	*Neopetrosia*	*exigua*
2	Porifera	Demospong.	Niphatidae	Niphatidae	*Niphates*	*1122 and 3126*
2	Porifera	Demospong.	Chalinidae	Chalinidae	*Haliclona*	*chrysa*
2	Porifera	Demospong.	Ianthellidae	Ianthellidae	*Ianthella*	*basta and flabelliformis*
2	Porifera	Demospong.	Callyspong.	Callyspong.	*Callyspongia*	*2520 and 2392*
2	Porifera	Demospong.	Phloeodict.	Phloeodict.	*Oceanapia*	*renieroides*
2	Porifera	Demospong.	Phloeodict.	Phloeodict.	*Oceanapia*	*3100 and 2531*
1	Porifera	Demospong.	Raspailiidae	Raspailiidae	*Thrinacophora*	*cervicornis*
1	Porifera	Demospong.	Chalinidae	Chalinidae	*Haliclona*	*1954*
1	Porifera	Demospong.	Thorectidae	Thorectidae	*Strepsichordaia*	*lendenfeldi*
1	Porifera	Demospong.	Thorectidae	Thorectidae	*Aplysinopsis*	*sp*
1	Porifera	Demospong.	Ancorinidae	Ancorinidae	*Stelletta*	*splendens*
2	Porifera	Demospong.	Irciniidae	Irciniidae	*Ircinia*	*1242 and 3968*
1	Porifera	Demospong.	Spongiidae	Spongiidae	*Spongia*	*1386*
2	Porifera	Demospong.	Axinellidae	Axinellidae	*Reniochalina*	*stalagmitis*
1	Porifera	Demospong.	Desmox.	Desmox.	*Myrmekioderma*	*granulata*
1	Tunicata	Ascidiacea	Enterogona	Polyclinidae	*Polyclinum*	*vasculosum*
1	Tunicata	Ascidiacea	Enterogona	Didemnidae	*Tridemnum*	*sibogae*

Taxonomy of hits from Griffith University's Nature Bank screening on TRPC5. TRPC5 blocking compounds were found in fractions from Animalia, but not Plantae kingdom. Abbreviated names: Echinodermata, Demospongiae, Callyspongiidae, Phloeodictyidae, Desmoxyidae.

one from a moss animal. The next step in the identification process will be to confirm activity of these compounds and to determine the concentration-response relation. The fractions with highest activity will then be further separated to pure compounds using activity guided HPLC and retested in the FLIPR assay to identify the active compounds including its concentration-response profile and its selectivity for action on TRPC5 (using heterologous expression systems of various other TRP channels).

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Publications during funding period

none

E15 - Progress Report

01.11.2013 - 31.10.2016

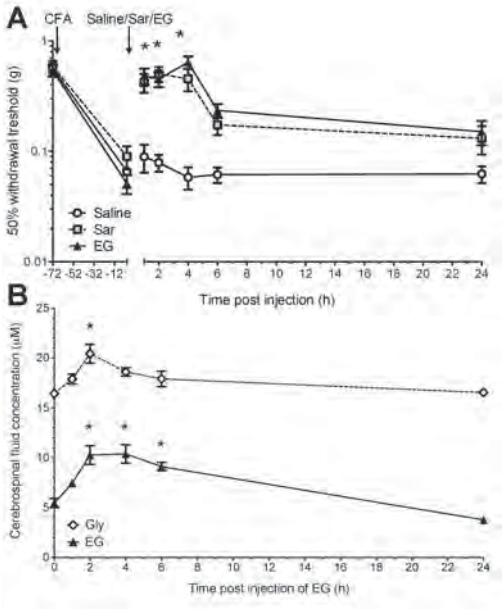
GlyT1 and neuropathic pain

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

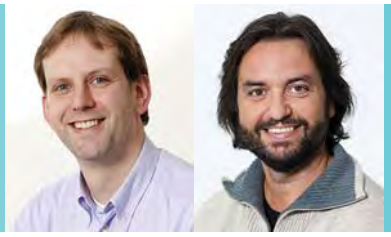
The treatment of chronic pain is - despite intense research - still challenging. We have shown that the glycine transporter GlyT1 is a drug target for the treatment of chronic pain. Here we show that the anti-hyperalgesic effect of systemic lidocaine involves inhibition of GlyT1 activity. Furthermore we established a new technique to analyse plastic changes in cortical somatosensory information processing that will be crucial to analyse the mechanism how GlyT1 influences pain perception.

For patients, chronic pain causes a devastating cut back in life quality. Despite of intense research, the treatment options for chronic pain are still limited and the therapeutic outcome in many cases is not satisfactory. It has been shown previously that the long term systemic treatment of chronic pain patients with the local anaesthetic lidocaine results in an amelioration of the pain symptoms via a mechanism that is unlikely to involve voltage gated sodium channels, i.e. the principal drug target of lidocaine. We have shown that application of the lidocaine metabolite N-Ethylglycine (EG) in animal models for neuropathic and inflammatory pain mimics the beneficial effect of the parental substance, suggesting that not lidocaine itself but its metabolite is causal for the anti-hyperalgesic effect of lidocaine. Using electrophysiological recordings from X. laevis oocytes, we demonstrated that EG acts specifically as a substrate on the glycine transporter GlyT1 whereas other glycine responsive proteins like GlyT2, glycine receptors, or NMDA receptors were unaffected. Using HPLC based analysis we showed that after systemic application EG reaches the cerebrospinal fluid and causes an increase of the glycine concentration. The time courses of the EG and glycine concentrations corresponded with the anti-nociceptive effect observed after EG application. Together these findings suggest that modulation of the GlyT1 may be a useful therapeutic agent for the treatment of pathological pain states.

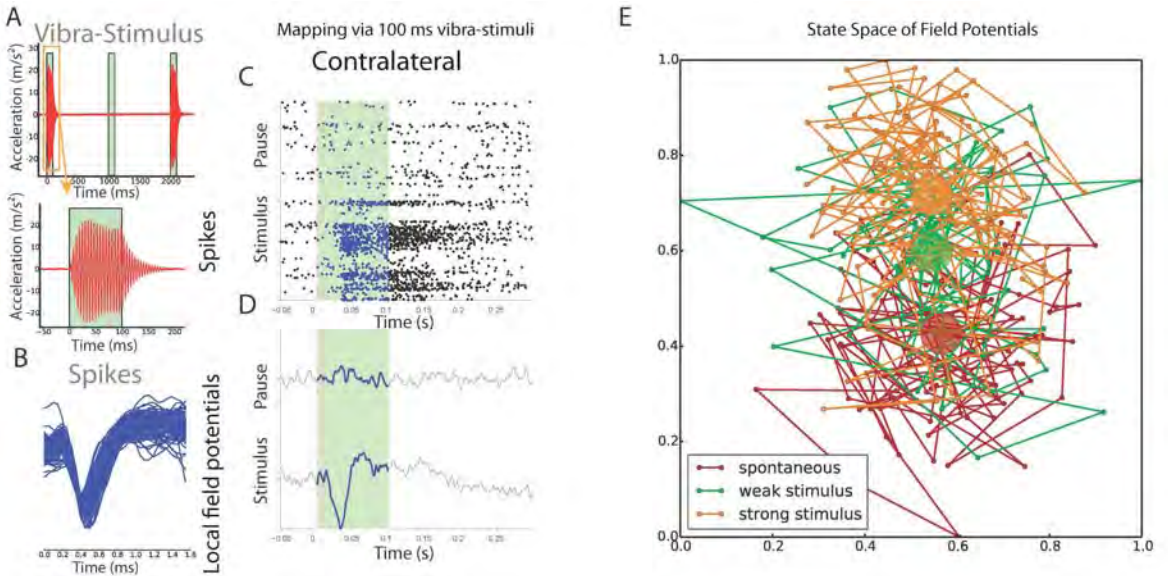
To elucidate the mechanism how GlyT1 active substances influence the processing of nociception on a systemic level, we have established a novel method for the analysis of neuronal processing of somatosensory stimuli in the neocortex of mice using multidimensional scaling. The primary somatosensory cortex (SI) is the central area for the perception of mechanosensory information like touch or vibration, but also for pain. In contrast to imaging techniques like fMRI or PET that allow for the analysis of the large-scale networks within the brain but show only low temporal and spatial resolutions, multichannel recordings of electrical activity in SI allow us to determine small changes in activation patterns of neurons with a high temporal and spatial resolution.



A: A single application of EG reduces nociceptive behaviour in inflammatory pain. B: Cerebrospinal fluid samples before and at the indicated timepoints after subcutaneous injection of EG (200 mg/kg) or Sarcosine (200 mg/kg).



PD Dr. Eulenburg Prof. Dr. Schulze



A: Tactile vibration stimuli. B: Action potentials of SI single unit. C: Timing of B. D: Local field potential recorded simultaneously at the same recording site as C. E: Analysis of spatio-temporal response patterns via multidemensional scaling.

To determine small differences in the network activity after stimulation, the time course of the field potentials of 16 electrodes are regarded as trajectories in a 16 dimensional state space. For visualization and further analysis, the trajectories are projected onto a 2D state space where all pairwise distances are preserved. This method will allow us to determine differences in neuronal processing of noxious and non-noxious stimuli which cannot be seen with imaging techniques. Furthermore, this method will be used to analyse changes in the functional network structure during the development of neuropathic pain.

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Publications during funding period

Werdehausen, R., Mittnacht, S. , Bee, L.A., Minett, M.S. Armbruster, A., Bauer, I., Wood, J.N. Hermanns, H. Eulenburg, V. (2015). The lidocaine metabolite N-ethylglycine has antinociceptive effects in experimental inflammatory and neuropathic pain. PAIN 156 (9); 1647-1659

E16 - Progress Report

01.04.2014 - 31.03.2017

Regulatory networks in neurogenesis and neurodevelopmental disorders

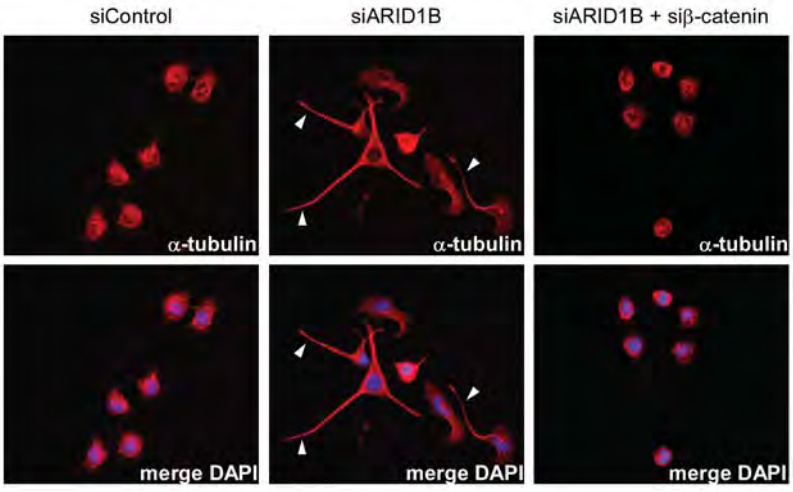
Prof. Dr. Dieter Chichung Lie, Institute of Biochemistry
Prof. Dr. André Reis, Institute of Human Genetics

Genetic defects are responsible for the vast majority of intellectual disability (ID) cases in countries with highly developed health systems. Our data suggest that a number of ID causing genes is connected via a SOX11-dependent network and that perturbation of this network contributes to the pathophysiology of ID. This project aims to determine the developmental function of such hypothesized network and to probe network components as novel etiological genes in ID.

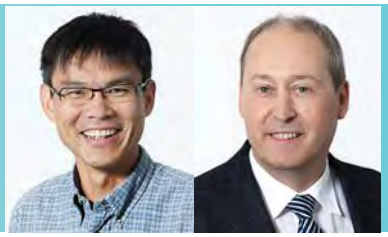
To date, causal mutations in more than 750 genes have been identified in intellectual disability (ID), and it is expected that numerous other ID genes will be discovered in the next years. As each gene accounts only for a small fraction of ID cases, it is highly unlikely that targeting each single gene defect will develop into a therapeutic mainstay. There is increasing evidence that ID-gene encoded proteins are connected in molecular pathways that regulate neurodevelopment and –plasticity. This observation provides a new prospect for development of therapies in ID as they suggest that targeting specific deregulated pathways may benefit a larger population of ID individuals. A major challenge for ID therapy development is to further specify deregulated pathways, to identify the ID-genes connected to these pathways, and to define the specific impact of these pathways on neurodevelopment and -plasticity.

Functional analysis of the ID-linked transcription factor Sox11

De novo mutations in the SoxC group transcription factor Sox11 were identified as genetic causes for a subset of individuals with Coffin-Siris syndrome– a developmental disorder associated with ID. Our previous data revealed that Sox11 and the closely related SoxC transcription factor Sox4 are highly expressed in immature neurons but down-regulated upon maturation. To begin to explore the impact of SoxC transcription factors on the late maturation/functional integration phase, we determined the impact of extended expression of Sox11 on neuronal development using neurogenesis in the hippocampal dentate gyrus of adult mice after viral transduction as a model system. In contrast to controls, a large fraction of Sox11 over-expressing neurons maintained the expression of immature neuronal markers. Moreover, Sox11 over-expressing neurons displayed decreased spine density and exhibited electrophysiological properties that were highly similar to the electrophysiological properties of immature neurons.



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

Collectively, these data indicate that Sox11 is critically involved in the timing of neuronal maturation and synaptic integration.

Chromatin-Remodeling-Factor ARID1B Represses Wnt/β-Catenin Signaling

De novo mutations in components of the BAF chromatin remodeling complex (a.k.a. SWI/SNF-A complex) such as ARID1B have also been found in some individuals with unspecific ID and with Coffin-Siris syndrome. To explore the underlying molecular mechanism(s) we performed whole-transcriptome analysis in such individuals with ID and ARID1B loss-of-function mutations and found Wnt/ß-catenin target genes to be upregulated. Using cellular models of low and high Wnt/ß-catenin activity, we demonstrated that knockdown of ARID1B activates Wnt/ß-catenin target genes and Wnt/ß-catenin-dependent transcriptional reporters in a ß-catenin-dependent manner. Reciprocally, forced expression of ARID1B inhibited Wnt/ß-catenin signaling downstream of the ß-catenin destruction complex.

Both endogenous and exogenous ARID1B associated with ß-catenin and repress Wnt/ß-catenin-mediated transcription through the BAF core subunit BRG1. Accordingly, mutations in ARID1B leading to partial or complete deletion of its BRG1-binding domain, as is often observed in ID, compromise association with ß-catenin, and the resultant ARID1B mutant proteins fail to suppress Wnt/ß-catenin signaling. Finally, knockdown of ARID1B in mouse neuroblastoma cells lead to neurite outgrowth through ß-catenin. Thus ARID1B is a repressor of Wnt/ß-catenin signaling. The data also suggest that aberrations in chromatin-remodeling factors, such as ARID1B, might contribute to neurodevelopmental abnormalities through deregulation of developmental pathways, such as the Wnt/ß-catenin signaling pathway.

Sox11 biochemically interacts with the ID factor TCF4

To obtain further insight into the Sox11 transcriptional network, proteomic analysis for SOX11 interactors was performed in collaboration with Dr. Johannes Glöckner (University of Tübingen). Interestingly, we identified several ID-related proteins including the transcription factor TCF4 as biochemical interactors of SOX11.

TCF4 mutations cause Pitt-Hopkins syndrome, a disorder characterized by developmental delay and intellectual disability. To begin to understand the function of TCF4 in CNS development and plasticity, detailed analysis of TCF4 expression in the developing and adult central nervous system (CNS) was performed. During development, TCF4 expression is present in neural stem cells and is maintained in developing neurons. In the adult CNS, high TCF4 expression was observed in areas, where stem cells continue to generate neurons throughout adulthood. In contrast to embryonic neurogenesis, TCF4 was absent from stem cells and was instead initiated at the time of neuronal fate commitment of precursor cells. Moreover, TCF4 expression was observed in almost all neurons with a particularly high expression in hippocampal neurons and neurons of the piriform cortex. These data suggest that TCF4 serves distinct functions in embryonic and adult neurogenesis. Moreover, the widespread neuronal expression of TCF4 raises the possibility that TCF4 plays a role in neuronal function and maintenance in the adult CNS.

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Publications during funding period

Beckervordersandforth R, Zhang CL, Lie DC (2015). Transcription-Factor-Dependent Control of Adult Hippocampal Neurogenesis. Cold Spring Harb Perspect Biol. 7(10):2015 Oct 1;7(10):a018879. doi: 10.1101/cshperspect.a018879.

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.

Vasileiou G, Ekici AB, Uebe S, Zweier C, Hoyer J, Engels H, Behrens J, Reis A, Hadjihannas MV (2015). Chromatin-Remodeling-Factor ARID1B Represses Wnt/beta-Catenin Signaling. Am J Hum Genet 97: 445-56

E17 - Progress Report

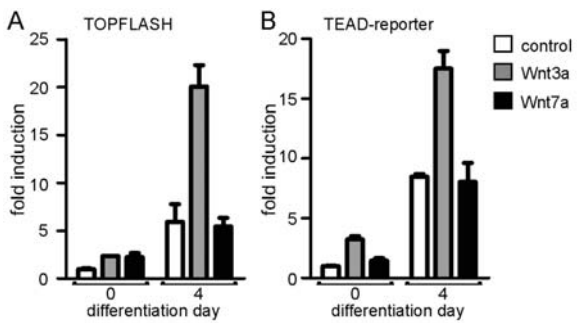
01.04.2014 - 30.09.2016

The neuromuscular role of Wnt signaling pathways

Prof. Dr. Said Hashemolhosseini, Institute of Biochemistry

Canonical Wnt/β-catenin signaling plays an important role in myogenic differentiation, but its role in muscle fibers, muscle stem cells and at neuromuscular synapses is unknown. We detected Wnt/β-catenin signaling by Axin2, a negative regulator and itself target of Wnt/β-catenin signaling, together with Hippo pathway members in a subset of fast muscle fibers and during differentiation of cultured myoblasts. Absence of Axin1/2 interfered with proliferation and myotube formation. Upon injury, Wnt/β-catenin signaling was induced and fiber formation affected in Axin2-deficient fibers.

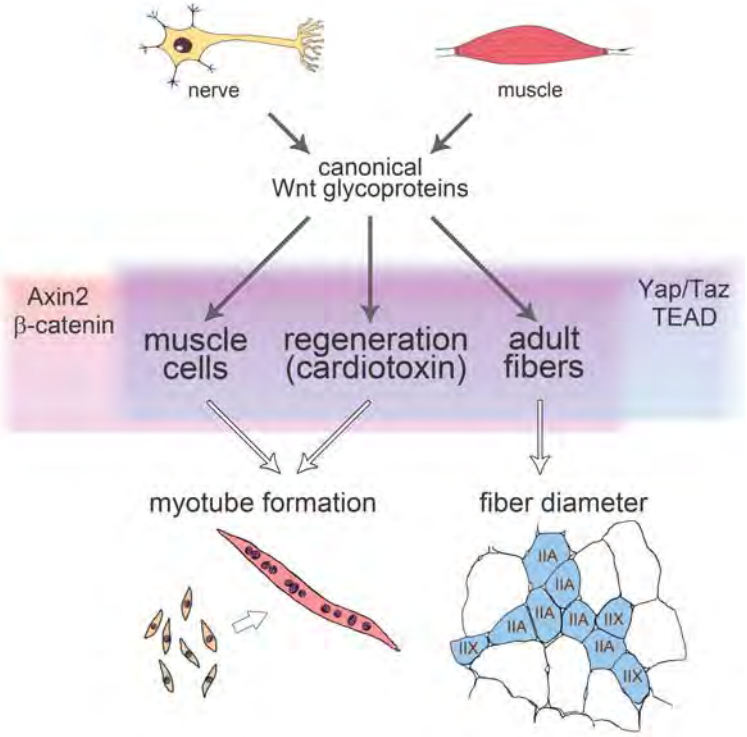
Wnt signaling plays a critical and complex role in myogenesis. So far, the importance of canonical Wnt/β-catenin signaling in resting adult myofibers is completely unknown. Therefore we investigated the expression profile of Axin2 (using Axin2-lacZ mice) as a marker of canonical Wnt signaling in various adult skeletal muscles. Muscles of Axin2-lacZ reporter mice were dissected and stained with X-Gal. While myofibers of wildtype littermates did not show any positive staining, many myofibers of heterozygous muscles turned blue and were accompanied by positive immunofluorescence staining for β-catenin. Muscle fiber types expressing Axin2-lacZ are type IIa and, most likely, IIx, and are smaller in diameter. We did not observe signs of myopathy in homozygous Axin2 deficient mice. Previously, a role for Hippo pathway member TAZ in Wnt signaling was identified and, later, confirmed, that YAP/TAZ is incorporated into the β-catenin destruction complex, which is orchestrating the Wnt response. Moreover, the Hippo pathway effector YAP was evidenced as a critical regulator of skeletal muscle fiber size. Here, Hippo pathway members YAP/TAZ and TEAD1 co-localized with canonical Wnt signaling in skeletal muscle fibers. All β-galactosidase positive nuclei belong to muscle fiber nuclei; Axin2 was not expressed in satellite cells. Next, the expression of Axin2 was analyzed during regeneration of skeletal muscle using cardiotoxin injection. Expression of β-galactosidase was identified in newly formed myofibers (marked by centrally located nuclei) and single cells in regenerating muscle. Presumably, those single cells are myoblasts since satellite cells (Pax7 positive) were negative for β-galactosidase expression. Our results also suggest that injured Axin2 deficient muscle regenerates differently from wild type and heterozygous Axin2-lacZ mice. In toto, Axin2 is not expressed in resting satellite cells, but in regenerating muscle after injury. In cultured muscle cells, Axin2 expression increased in C2C12 cells cultures together with TEAD1 target gene expression. To test whether Axin2 upregulation observed upon myotube formation is mediated by secreted Wnt proteins, differentiating C2C12 cells were treated with soluble Dickkopf protein (Dkk-1), which specifically inhibits the canonical Wnt signaling pathway by disruption of the Wnt-Frizzled-LRP complex. After 3 days of differentiation, incubation of myotubes with Dkk-1 resulted in a nearly complete loss of Axin2 expression. This observation demonstrates that the canonical Wnt/β-catenin pathway is activated in differentiating myocytes by



Primary myoblast cultures were transfected with canonical Wnt/β-catenin (TOPFLASH) or Hippo pathway (TEAD) luciferase reporter and Wnt expression plasmids. Both (A) TOPFLASH and (B) TEAD reporter activity were induced upon differentiation and enhanced in response to canonical Wnt3a stimulation.

Prof. Dr. Hashemolhosseini

canonical Wnt ligands, and that this activation is responsible for the expression of Axin2. To identify the possible sources of extracellular Wnt glycoproteins in adult muscle, we asked whether Wnt protein release might be nerve- or muscle-dependent, or even be released by both tissues. As a first step, we denervated the hindlimb of heterozygous Axin2-lacZ reporter mice and observed complete loss of β-galactosidase activity.



Our data demonstrate for the first time that nerve- and/or muscle-derived Wnt glycoproteins act on skeletal muscle cells through both canonical Wnt/β-catenin-dependent and Hippo members YAP/TAZ/TEAD1-mediated signaling, which play an important role in muscle cell differentiation, regeneration and fiber type diameter.

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Invited lectures

Protein kinase CK2 interacts at the neuromuscular synapse with Rapsyn, Rac1, 14-3-3γ and Dok-7, and phosphorylates the latter two.44th European Muscle Conference, Muscle Research in Health and Disease, September 21 - 25, 2015, Warsaw, Poland.

The role of protein kinase CK2 at NMJs and within muscle fibers. EMC Satellite Meeting on Muscle Synapse, Nencki Institute of Experimental Biology. September 25 - 26, 2015, Warsaw, Poland.

Mitochondrial protein import is regulated by CK2-dependent phosphorylation of outer mitochondrial membrane protein Tom22 in mouse skeletal muscles. Interuniversity Institute of Myology, XII IIM-Myology Meeting, 1 – 4 October 2015, Reggio Emilia, Italy.

Canonical Wnt/β-catenin signaling through Axin2, YAP/TAZ and TEAD1, is essential for myotube formation and small diameter adult fiber types. Interuniversity Institute of Myology, XII IIM-Myology Meeting, 1 – 4 October 2015, Reggio Emilia, Italy. Talk presented by Danyil Huraskin.

Awards

Danyil Huraskin, M.Sc.: Luise Prell Award 2015

Dustin Herrmann, B.Sc.: Sofie Wallner Award 2015

Publications during funding period

Herrmann D, Straubinger M, Hashemolhosseini, S (2015) Protein kinase CK2 interacts at the neuromuscular synapse with Rapsyn, Rac1, 14-3-3β and Dok-7, and phosphorylates the latter two. J Biol Chem, J Biol Chem 290: 22370-22384.

E18 - Progress Report

01.12.2013 - 30.11.2016

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

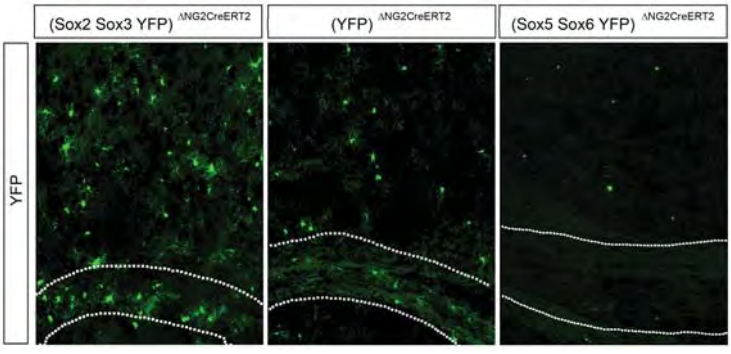
Prof. Dr. Michael Wegner, Institute of Biochemistry
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

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

The aim of this study is to analyze the consequences of altered Sox gene expression in adult NG2-glia and to investigate and exploit resulting changes in developmental potential and differentiation capacity of these cells in disease models. The disease that we primarily focus on is multiple system atrophy (MSA), a fast progressing neurodegenerative disease characterized by alpha-synuclein (aSyn)-positive glial cytoplasmic inclusions (GCIs) within mature oligodendrocytes, and widespread myelin loss as a neuropathological hallmark. During the second year of the project, we started to analyze the consequences of altered Sox gene expression in NG2-glia of healthy adult mice. Additionally, we obtained a better understanding for the pathomechanisms in MSA.

Analysis of mouse mutants

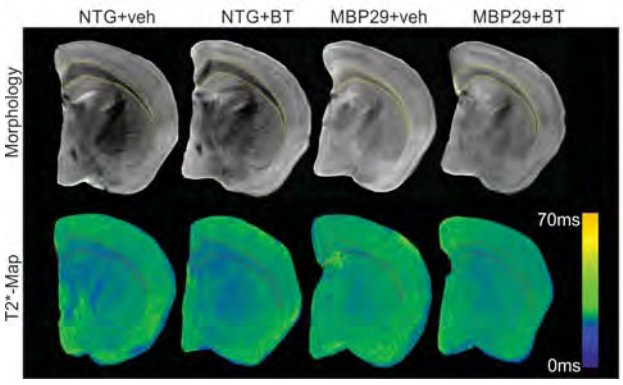
To study the consequences of altered Sox gene expression in adult NG2-glia, we generated compound mouse mutants that allow adult-onset, tamoxifen-induced, and NG2-CreERT2-mediated Sox gene deletion or overexpression. While adult NG2-glia exhibited increased proliferation and impaired differentiation capacities preferentially in the cortical gray matter of mice with combined Sox2 and Sox3 gene deletions, joint loss of the closely related Sox5 and Sox6 led to a dramatic loss of affected NG2-glia throughout gray and white matter. Most of the NG2-glia disappeared shortly after the Cre-dependent recombination event, most likely by apoptosis. The few remaining cells failed to keep their identity as NG2-glia in the absence of Sox5 and Sox6 and overwhelmingly differentiated into oligodendrocytes. Overexpression of Sox10 in NG2-glia also promoted differentiation into oligodendrocytes, but did not affect viability. It can be concluded that Sox5 and Sox6 are required for survival of NG2 glia and maintenance of the undifferentiated state, whereas increased Sox10 levels promote differentiation, with more multifaceted, region-specific effects of Sox2 and Sox3. We currently analyze how these altered properties of NG2-glia will affect their response to injury and their behavior in disease conditions.

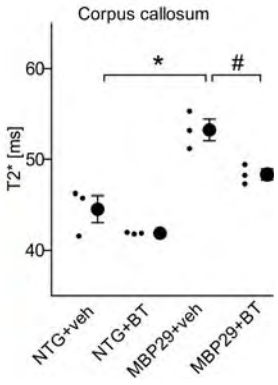


Detection of NG2:CreERT2-recombined NG2-glia in mouse forebrain by YFP. In YFP-positive glia, Sox2 and Sox3 (left panel) or Sox5 and Sox6 (right panel) were deleted. The middle panel shows the control. Cortical gray matter is in the upper part; corpus callosum is marked by stippled lines.

Prof. Dr. Wegner

Prof. Dr. Winkler





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

Defining the pathophysiological events during MSA

In MSA, comprehensive studies investigating the behavior of NG2-glia and their remyelination capacities are lacking. Thus, we generated a cellular system that gave us important insights into these processes by studying the effect of human aSyn accumulation on primary rat NG2-glia and their maturation in culture. Both upon lentiviral overexpression of aSyn and uptake of recombinant aSyn from the culture medium, the differentiation potential of NG2-glia was severely impaired (Ettle et al., 2014). In the second year of the project, we extended these findings and showed that an intervention using the differentiation-promoting small molecule benztropine is able to restore the aSyn-induced differentiation defect in vitro and in vivo.

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Invited lectures

M. Wegner:

Joint Meeting of the German and French Societies of Developmental Biologists, 11.03.-14.03.2015 in Nürnberg: “Epigenetic and transcriptional control of glia in the vertebrate nervous system”

XII European Meeting on Glial Cells in Health and Disease in Bilbao, Spain, 15.07.-18.07.2015: “The role of chromatin remodeling in myelinating glia”

J. Winkler:

Department of Neurosciences, University of California San Diego, 15.10.2015: “Targeting myelin deficit: a novel avenue for restoration in MSA?”

Publications during funding period

Ettle, B., Schlachetzki, J.C., Winkler, J. (2015) Oligodendroglia and Myelin in Neurodegenerative Diseases: More Than Just Bystanders? Molecular neurobiology. doi:10.1007/s12035-015-9205-3. [Epub ahead of print]

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

Stolt CC, Wegner M. (2015) Schwann cells and their transcriptional network: Evolution of key regulators of peripheral myelination. Brain Res. doi: 10.1016/j.brainres.2015.09.025. [Epub ahead of print]

Küspert M, Wegner M (2015) SomethiNG 2 talk about-Transcriptional regulation in embryonic and adult oligodendrocyte precursors. Brain Res. doi: 10.1016/j.brainres.2015.07.024. [Epub ahead of print]

Ettle, B., Reiprich, S., Deusser, J., Schlachetzki, J.C., Xiang, W., Prots, I., Masliah, E., Winner, B., Wegner, M., Winkler, J. (2014) Intracellular alpha-synuclein affects early maturation of primary oligodendrocyte progenitor cells. Molecular and cellular neuroscience. 2014 Sep;62:68-78.

F3 - Progress Report

01.03.2014 - 31.08.2016

Fam60a in heart and brain development

Prof. Dr. Felix Engel, Department of Nephropathology

fam60a is a member of the SIN3-HDAC complex. The aim of this project is to determine its in vivo function during zebrafish development. Our data suggest Fam60a as an important regulator of thalamus regionalization by controlling the spatial expression of neuronal differentiation genes.

fam60a is expressed in brain during early zebrafish development

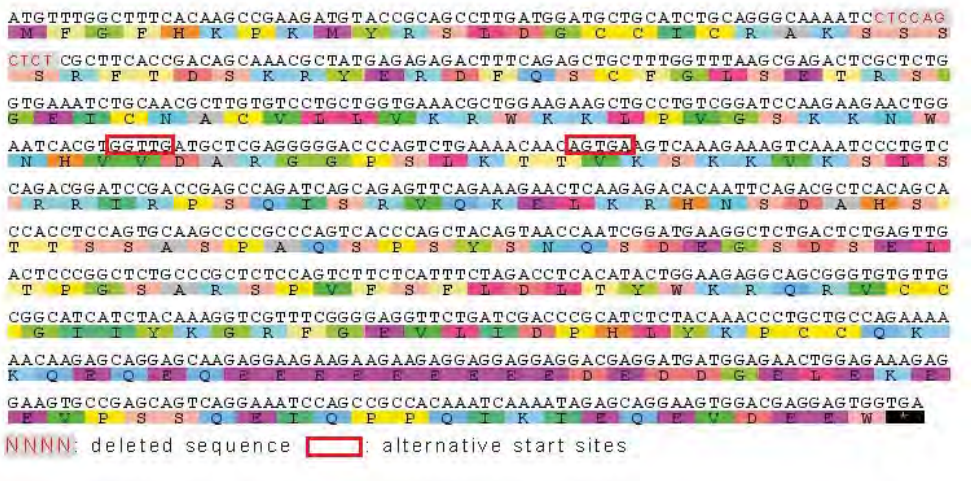
Immunofluorescence analyses confirmed that FAM60A is a nuclear protein expressed in the neural tube and in dorsal root ganglia in mice. Whole mount in situ hybridization (WISH) in zebrafish detected fam60a mRNA expression in the brain primordium and in the otic vesicle.

The small nuclear protein FAM60A possesses a NLS

In silico analyses identified in fam60a two overlapping NLS sequences. Six C-terminal GFP constructs were generated with partial NLSs. Densitometric analyses of western blots and quantitative confocal microscopy of transfected HEK 293T cells suggest, that Fam60a contains a bipartite NLS composed of two conserved stretches of amino acids connected by a not conserved linker sequence.

fam60a knockdown disrupts brain development leading to increased her6 and reduced 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. ngn1 expression was reduced in the caudal thalamus and slightly increased in the telencephalon. Importantly, injec-



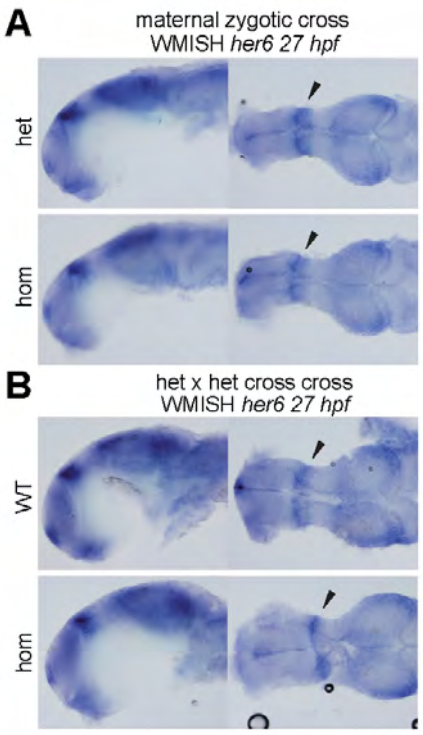
Fam60a sequence indicating the TALEN-mediated deletion in the fam60a mutants as well as alternative start sites.

Prof. Dr. Engel

tion 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 ngn1 and ascl1b in the mid-diencephalic organizer to drive formation of the rostral thalamus, the prethalamus and the caudal thalamus.

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. Thus, in future experiments we will generate additional fam60a mutants. Furthermore, we will perform deep sequencing experiments to determine compensatory mechanisms. Finally, we will inject MOs in fam60a mutant embryos. In case that the fam60a mutation induces a compensatory mechanism the MO injection should not cause a phenotype.



fam60 a mutant fails to exhibit expanded her6 expression in the thalamus (arrowhead). Whole mount in situ hybridization for her6 (blue).

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Publications during funding period

Ferrazzi F, Bellazzi R, Engel FB (2014) Gene network analysis: from heart development to cardiac therapy. Thromb Haemost. 113(3):522-31.

F4 - Progress Report

01.10.2013 - 30.09.2016

Pathogenesis of the short rib-polydactyly syndrome

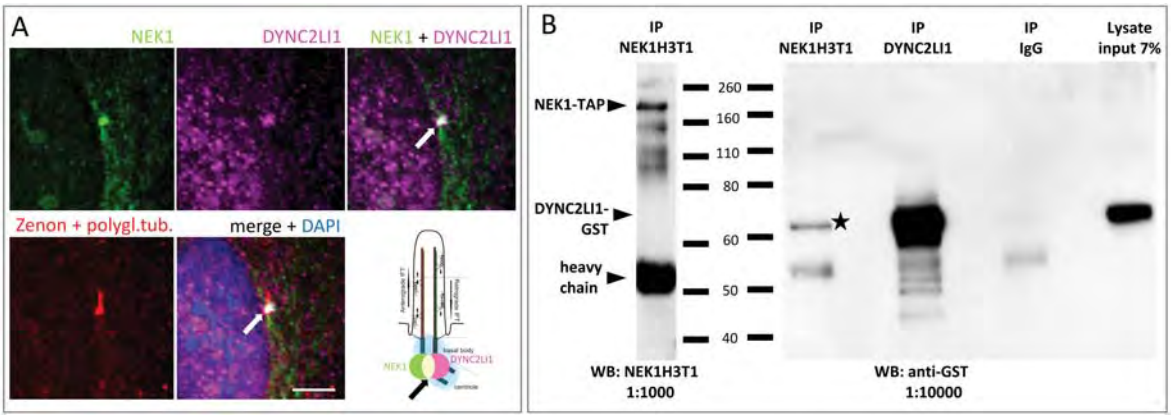
PD Dr. Christian T. Thiel, Institute of Human Genetics

The regulation of the primary cilium is a key factor in the maintenance of individual and cellular growth. Defects of ciliogenesis have been implicated in a wide range of human phenotypes and play a crucial role in signal transduction and cell cycle coordination. We successfully performed a Y2H screen of NEK1 and identified NEK1 interaction partners. This broadens the clinical spectrum of ciliary defects by identification of mutations in MAP4 and DYNC2LI1 in patients with growth defects.

The primary cilium in the pathogenesis of growth defects

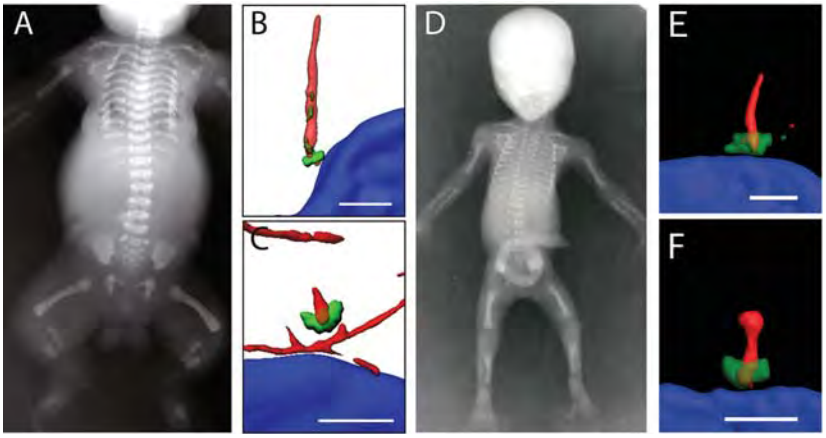
The primary cilium is a nearly ubiquitous organelle of non-proliferating vertebrate cells. It consists of the basal body complex on the cytoplasmic side of the cell membrane, and the ciliary axoneme. Herewith, it detects extracellular stimuli to initiate intracellular transduction cascades (Hedgehog, Wnt, planar cell polarity, FGF, Notch, mTor, PDGF and the Hippo signaling pathways). Thus, cilia play important roles in differentiation, migration, proliferation, determination of left-right asymmetry during development.

Based on the diverse function, defects of cilia associated genes lead to a pleiotropic spectrum of phenotypic effects, including brain malformations, polydactyly, kidney cysts, retinal degeneration, and skeletal abnormalities. Features present in short-rib polydactyly type Majewski (SRPS II), a lethal osteochondrodysplasia, where we identified mutations in NEK1. Absence of NEK1 leads to severely reduced cilia number and alters cilia morphology in vivo. To establish a genotype-phenotype correlation we screened 28 further patients and identified 4 patients with NEK1 and 6 patients with mutations in DYNC2H1, a dynein-2 complex component. Using exome sequencing in one mutation negative patient we identified and functionally characterized MAP4 as a novel growth related gene.



NEK1 and DYNC2LI1 co-localization and co-immunoprecipitation. (A) Immunofluorescence presentation of the co-localization of NEK1 and DYNC2LI1 at the basal body region of the primary cilium. (B) Co-IP showed an interaction between both proteins.

PD Dr. Thiel



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

Identification and characterization of the NEK1 interaction partner DYNC2LI1

To fully understand the role of NEK1 in the context of the primary cilium we performed Y2H with a cilia cDNA library. We identified 81 NEK1 interacting proteins of which 66 have yet not been associated with the primary cilium before. In immunofluorescence analysis and co-immunoprecipitation experiments with our custom made antibody we identified and confirmed DYNC2LI1, a further dynein-2 complex component, as a novel candidate gene for SRPS. DYNC2LI1 depleted cells showed similar altered cilia morphology as observed in NEK1 and other defects of ciliary components. In addition, we observed an accumulation of proteins at the ciliary tip reported in other osteochondrodysplasias. Thus, we expect to identify mutations in our novel ciliary genes in further patients.

NEK1 effect on ciliary signaling pathways

We performed expression experiments of genes encoding key members of the hedgehog, Wnt, and PDGF pathways under normal and starvation condition in fibroblasts. This proposed a compensatory up-regulation with higher expression ratios after starvation induced ciliogenesis. To further understand how NEK1 defects can negatively affect signal transduction in the various pathways involved, we established NEK1 deficient cell lines with the CRISPR-Cas9 system. Here, genome wide RNA sequencing (RNASeq) might provide a global characterization of the functional roles of ciliary proteins in the manifestation of phenotypic features.

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Publications during funding period

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

Kessler K, Wunderlich I, Uebe S, Falk NS, Gießl A, Brandstätter JH, Popp B, Klinger P, Ekici AB, Sticht H, Dörr H-G, Reis A, Roepman R, Seemanová E, Thiel CT. (2015) DYNC2LI1 mutations broaden the clinical spectrum of dynein-2 defects. Scientific Research 5:11649

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

Newly started Projects

Immunology and Infection

A63 30 months

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


Prof. Dr. Bogdan

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

Tumor necrosis factor (TNF)-deficient mice fail to control the intracellular protozoan pathogen *Leishmania major,* despite an intact IFN-γ (Th1) immune response and a sustained expression of type 2 nitric oxide synthase (NOS2) mRNA and protein, which is the key antileishmanial effector pathway. Here, we will test the hypothesis that TNF conveys protection by alternative mechanisms including the induction of NOS2 cofactors and the downregulation of arginine-metabolizing enzymes such as arginase 1.

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A64 01.02.2016 - 31.07.2018

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


Prof. Dr. Distler


Prof. Dr. Schett

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

Fibrotic diseases impose a major burden on modern societies and there is huge medical need for anti-fibrotic therapies. Our preliminary results demonstrate that SHP2 positively regulates TGFbeta signaling by increasing the activation JAK2 and STAT3. Inactivation of SHP2 prevents fibroblast activation and ameliorates experimental fibrosis. We aim to further characterize the molecular mechanisms of SHP2 signaling in fibroblasts and to validate SHP2 as a therapeutic target in fibrotic diseases.

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A65 01.04.2016 - 30.09.2018

Tolerizing potential of human dendritic cell subpopulations


Prof. Dr. Dudziak

Prof. Dr. Diana Dudziak, Department of Dermatology

DCs are important regulators of the innate and adaptive immune system, but also control central and peripheral tolerance. We found in contrast to blood DCs that human DC subsets of the thymus were unable to secrete cytokines and chemokines upon stimulation with various TLR ligands, although they upregulated typical DC activation markers. The analysis of the tolerogenic potential of human thymus DCs will form the basis for future clinical treatment of various autoimmune or cancer diseases.

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A66 01.07.2016 - 31.12.2018

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


Prof. Dr. Ensser

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

We propose a systematic, unbiased approach at identifying cellular restriction factors of DNA viruses using the powerful CRISPR/Cas9 knockout technology, through a lentiviral sgRNA library targeting each human gene with several independent constructs. Our focus is on cellular factors that restrict or promote the replication of Herpesviruses and/or limit the growth of tumor cells transformed by KSHV and EBV. These factors represent primary therapeutic targets.

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Newly started Projects

A67 01.02.2016 - 31.07.2018

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


Prof. Dr. Gramberg

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

LINE-1 retroelements have been shown to cause mutations resulting in various genetic disorders. It is therefore essential to control their replication to maintain genome integrity. A strong block to retroviral infection is mediated by TRIM5a. In first experiments, we found that TRIM5a also inhibits the retrotransposition of LINE-1. Thus, this study will analyze this novel function of TRIM5a and its contribution to genome stability by blocking the replication of endogenous retroelements.

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A68 30 months

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


PD Dr. Krönke


Prof. Dr. Nimmerjahn

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

Preliminary data show a central role of the IL-23/Th17 axis during the control of autoantibody activity during rheumatoid arthritis (RA). During the proposed project, we aim to elucidate underlying molecular mechanisms and evaluate their relevance in patients suffering from RA.

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A69 30 months

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


Prof. Dr. Lang

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 modulatioon of the inflammatory response by ATM-inhibition. We propose now detailed studies to elucidate the molecular mechanisms and the consequences of ATM/DDR activation for the host response, protection and immunopathology during infection.

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A70 01.07.2016 - 31.12.2018

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


Prof. Dr. Schubert

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

We could show that certain deubiquitinating enzymes (DUBs) play an essential role in HIV-1 replication, at least by regulating Gag processing, virus infectivity, and entry of Gag into the MHC-I pathway. DUB-inhibitors offer a new way for antiretroviral therapy as they (1) target genetically stable cellular factors, (2) interfere with virus spread, and (3) have the potential to improve immune recognition of HIV-1+-cells.

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Newly started Projects

A71 01.07.2016 - 31.12.2018

Viral modulation of the protein kinase ULK1


Prof. Dr. Stamminger

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

Research of the last years revealed that the cellular protein kinase ULK1 exerts critical regulatory functions at the intersection of autophagy, innate immunity and inflammatory disorders. We observed that ULK1 is strongly upregulated during human cytomegalovirus (HCMV) infection and a knockdown of ULK1 severely inhibited viral replication. This project aims to characterize how HCMV manipulates ULK1 since this may reveal a novel viral strategy to avoid hyperinflammation.

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A72 01.07.2016 - 31.12.2018

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


Prof. Dr. Steinkasserer

Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation

The aim of this project is to elucidate the function of CD83 expressed on Tregs. Recently we reported that after activation Tregs rapidly upregulate CD83 expression, providing a new phenotypic marker for activated Tregs. However, the functional importance of this expression is completely unknown. Using our recently generated conditional KO animals, whereby CD83 expression is exclusively deleted on Tregs we aim to elucidated the role of CD83 for activation and suppressive capacity of Tregs.

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A73 01.07.2016 - 30.06.2017

Checkpoint inhibitors as adjuvants for viral vaccines


Prof. Dr. Überla

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

Checkpoint inhibitors (CPI) show great promise in improving immune control of tumors. However, the coinhibitory receptors targeted by CPIs also play an important role during the induction of immune responses by viral vaccines. Therefore the aims of the project are to better understand the consequences of CPI therapy on vaccine-induced adaptive immune responses and to explore the potential of CPIs as a systemic and local immunomodulatory vaccine adjuvant.

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A74 30 months

The Role of Eosinophils in Allergic Bronchopulmonary Aspergillosis


Prof. Dr. Vöhringer


Prof. Dr. Krappmann

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

Allergic bronchopulmonary aspergillosis (ABPA) is an immunologic hypersensitivity pneumonitis caused by an inflammatory response towards antigens of the mould *Aspergillus fumigatus.* Based on the applicants' complementary expertise, this project monitors eosinophil recruitment and identifies sources of crucial cytokines. The project further aims at characterization of cellular host-pathogen interactions and identification of fungal factors contributing to the development of ABPA.

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Newly started Projects

A75 30 months

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


Dr. Günther


PD Dr. Wirtz

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

Although cell death is of fundamental importance in almost all hepatic diseases such as autoimmune hepatitis, there is still no precise knowledge about the underlying mechanisms. The aim of this proposal is to elucidate the role of MLKL-dependent programmed cell death in the pathogenesis of liver diseases.

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Oncology

D23 01.01.2016 - 30.06.2018

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


Prof. Dr. Bozec

Prof. Dr. Aline Bozec, Department of Medicine 3

Aim of the project is to elucidate the pathophysiologic role of adipocytes in the bone marrow microenvironment of osteolytic metastasis using mouse models. For this purpose, non-invasive imaging in vivo and molecular assays in vitro will be applied to investigate processes like tumor cell proliferation, bone resorption and angiogenesis as well as adipocyte expressed factors in osteolytic bone metastasis.

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D24 30 months

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


Prof. Dr. Bosserhoff


Prof. Dr. Wegner

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

Melanoma is an aggressive cancer derived from melanocytes. Interestingly, melanocytes and Schwann cells, both of neural crest origin, can transdifferentiate into each other. As two transcriptions factors with roles in Schwann cell differentiation were recently shown to be involved in melanoma development, this project aims to combine the expertise of both PIs and characterize further central Schwann cell transcription factors in melanoma to enhance the molecular understanding of this tumour.

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D25 01.05.2016 - 31.10.2018

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


Prof. Dr. Brabletz

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

We have demonstrated that the EMT-activator and transcriptional repressor ZEB1 is crucial for tumor progression, including metastasis and drug resistance. By Mass-Spec analysis we identified the prominent oncogenic factor EGFR as binding partner of ZEB1, which could explain the strong tumor promoting function of both factors. In this project we want to characterize the molecular background, as well as the functional and clinical relevance of the ZEB1/EGFR interaction in aggressive human cancers.

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Newly started Projects

D26 01.01.2016 - 30.06.2018

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


Prof. Dr. Mackensen


Prof. Dr. Fasching

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

Triple negative breast cancer (TNBC) is characterized by aggressive growth, mainly affects younger women and is difficult to treat. Interestingly, the density of immune cell infiltrates in the primary tumor positively correlates with outcome. However, it is so far unknown which antigens are targeted by the tumor infiltrating T-cells. The aim of this project is to identify the targets of tumor infiltrating T-cells in TNBC with main focus on tumor-specific mutations.

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D27 30 months

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


PD Dr. Mougiakakos

PD Dr. Dimitrios Mougiakakos, Department of Medicine 5

Metabolic alterations represent a hallmark of cancer and promote malignant features. Increasing evidence suggests that metabolic communication between tumor cells and the immune system contributes to immune escape. Elevated levels of the metabolite 2-hydroxyglutarate (2-HG) were observed in AML patients and represent a negative prognostic marker. We want to (A) identify novel (targetable) pathways driving 2-HG accumulation and to (B) investigate whether 2-HG exerts immune modulating effects.

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D28 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


Prof. Dr. Stürzl


PD Dr. Naschberger

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

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D29 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


Prof. Dr. Waldner

Recent studies provide evidence for a dramatic dysfunction of the immune system due to aging. In this regard, an attenuated anti-tumor immune response could explain the increased cancer incidence in elderly people. As functional data supporting this hypothesis are scarce, this project will analyze the role of an aged adaptive immune system in a murine model of colorectal cancer.

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Newly started Projects

Neurosciences

E19 15.02.2016 - 14.08.2018

Inhibitory neurotransmission in the cochlea: Glutamate and endocannabinoids

Prof. Dr. Enz

Prof. Dr. Ralf Enz, Institute for Biochemistry

Sensory organs need tailor-made signal transduction pathways. Pre-synaptic glutamate- and endocannabinoidreceptors 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 molecular mechanisms in hair-cells of the cochlea are largely unknown. Here, we will investigate pre-synaptic receptor expression in hair-cells and elucidate their regulation by interacting proteins.

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E20 30 months

Identification of molecules, receptors and genes involved in chronic pruritus

Dr. Dr. Kremer

Prof. Dr. Zimmermann

Dr. Dr. Andreas Kremer, Department of Medicine 1
Prof. Dr. Katharina Zimmermann, Department of Anesthesiology

Chronic pruritus is an agonizing symptom accompanying many dermatological and systemic disorders. The aim of this project is to identify pruritogens in plasma of patient suffering from chronic pruritus, to unravel specific NaV channel subtypes that define itch pathways, and to identify and characterize novel gene products that predispose to or protect from itch by in silico genetic mapping in mice. These results may pave the way towards novel treatment targets for this dreadful symptom.

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E21 30 months

Modulation of alpha-Synuclein pathology by FoxO-dependent pathways


Prof. Dr. Lie


PD Dr. Klucken

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

Synucleinopathies are age-associated neurodegenerative disorders characterized by the intracellular accumulation of alpha-synuclein (aSyn) aggregates. This project will address the currently unresolved question of how ageing accelerates aSyn toxicity and cerebral spreading. Specifically, it will investigate whether modulation of autophagy by the ageing-associated FoxO-pathway affects exosomal release of aSyn, transcellular propagation, and acceleration of aSyn-toxicity.

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E22 01.03.2016 - 31.08.2018

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


Prof. Dr. Alzheimer


PD Dr. Mielenz


Prof. Dr. Müller

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

Drug addiction is a major psychiatric disorder. In the first Swiprosin-1/EFhd2 knock out model we found that EFhd2 works as resilience factor against addiction. We will now investigate how and where in the brain EFhd2 regulates the reinforcing effects of alcohol and methamphetamine, by controlling neurochemical, electrophysiological, and gene expression effects during addiction establishment. These insights will allow for a better understanding of resilience mechanisms against drug addiction.

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Newly started Projects

E23 01.01.2016 - 30.06.2018

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


Prof. Dr. Schlötzer-Schrehardt


Prof. Dr. Reis

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

Pseudoexfoliation (PEX) syndrome represents an age-related systemic connective tissue disorder, which is frequently associated with severe complications including glaucoma and aortic aneurysms. Genetic association studies have linked it to common variants in the LOXL1 (lysyl oxidase-like 1) gene, but their impact on disease development remains unexplored. The aim of this project, therefore, is to analyze how functional regulatory LOXL1 variants confer susceptibility to PEX syndrome/glaucoma.

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E24 01.01.2016 - 30.06.2018

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


Prof. Dr. Winkler


Prof. Dr. Linker

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

Increasing evidence indicates that neuroinflammatory and -degenerative disorders are far more interconnected than previously assumed. Since intracellular alpha-synuclein (a-syn) aggregates in synucleinopathies affects the maturation of primary oligodendrocyte progenitor cells, the project will investigate the hypothesis whether a-syn has a detrimental role for myelin homeostasis in a prototypical model of multiple sclerosis with inflammation and demyelination in the central nervous system.

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e-mail: ralf.linker@uk-erlangen.de

E25 30 months

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


Prof. Dr. Winner


Prof. Dr. Schüttler

Prof. Dr. Beate Winner, IZKF Junior Research Group 3
Prof. Dr. Jürgen Schüttler, Department of Anesthesiology

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

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E26 01.03.2016 - 31.08.2018

Genetics and pathomechanisms of intellectual disability with microcephaly


PD Dr. Zweier

PD Dr. Christiane Zweier, Institute of Human Genetics

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

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Newly started Projects

E27 01.03.2016 - 31.08.2018

Lysophosphatidic acid-induced pruritus of cholestasis


Dr. Dr. Kremer


Prof. Dr. Fischer

Dr. Dr. Andreas Kremer, Department of Medicine 1,
Prof. Dr. Michael Fischer,
Institute of Physiology and Pathophysiology

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.

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Renal and Vascular Research

F5 30 months

The Role of ANO1 in Polycystic Kidney Disease


Dr. Buchholz

Dr. Björn Buchholz, Department of Medicine 4

In Polycystic Kidney Diseases (PKD) continuous growth of renal cysts due to proliferation and fluid secretion causes renal failure. Although the underlying mechanisms remain incompletely understood, they appear to be related to the primary cilium. We aim to analyze the functional role of the calcium-activated chloride channel ANO1 for progression of PKD in a PKD mouse model and in PKD cells and to study its impact on ciliary calcium homeostasis and cell proliferation.

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F6 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


Prof. Dr. Veelken


Prof. Dr. Amann

In spite of clear evidence of its importance a basic feature of renal innervation - the regulation of sympathetic activity by afferent renal nerves - is not yet understood. It is particularly unclear whether renal afferents, i.e. the dorsal root ganglion neurons with renal projections, stimulate or inhibit sympathetic activity. We want to demonstrate in a model of experimental hypertension that afferent renal nerve activity acts rather sympathoinhibitory but not sympathoexcitatory.

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Junior Groups / Projects

Junior Groups / Projects

Progress and Final Reports

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Junior Research Group 1

Dr. Paolo Ceppi

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Biographical Sketch

The Junior Group Leader started his appointment at the Interdisciplinary Center for Clinical Research (IZKF), Friedrich-Alexander University Erlangen-Nürnberg in Erlangen on August 1st, 2015. Below is a list of the previous reseach employements:

Mar 2011-Jun 2015 Postdoctoral fellow at the Division of Hematology/Oncology, Feinberg School of Medicine, Robert H. Lurie Comprehensive Cancer Center Northwestern University, Chicago, USA (Prof. M. Peter).

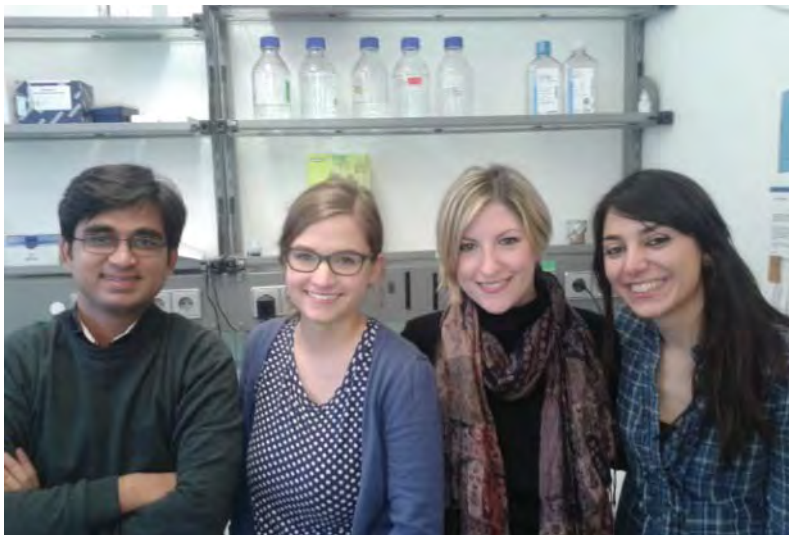
Feb 2009-Dec 2009 Visiting PhD student at the Department of Experimental Surgery and Molecular Oncology of Solid Tumors, Medical Faculty Mannheim, University of Heidelberg and DKFZ Heidelberg, Germany (Prof. H. Allgayer).

Jan 2007-Dec 2010 PhD student in the Pathology Division of the Department of Clinical and Biological Sciences, University of Turin, Italy (Prof. M. Papotti).

Jul 2004-Dec 2006 Research assistant at Thoracic Oncology Unit and the Pathology Division of the Department of Clinical and Biological Sciences, University of Turin, Italy (Prof. G. Scagliotti and Prof. M. Papotti).

Dec 2004-Jun 2005 Visiting Research scholar at Department of Biochemistry and Molecular Biology, Norris Cancer Center, University of Southern California, Los Angeles, USA (Prof. P. Danenberg). Training at ResponseGenetics Inc. Los Angeles, USA (Dr. K. Danenberg).

Mar 2002-Jul 2004 Research student at the Department of Genetics, Biology and Biochemistry, University of Turin, Italy (Prof. F. Malavasi).



From the left: Aarif Siddiqui, Annemarie Schwab, Maria Eleni Vazakidou, Francesca Napoli

Research Focus

The focus of the Junior Group 1 is „Understanding the plasticity of cancer cells".

Background and Rationale:

Despite the progresses made in the last years with the development of novel molecularly targeted agents, cancer is still a very deadly disease. This could be attributable to several aspects, including the fact that only a minority of selected patients benefit from the novel compounds (such as those targeting oncogenic drivers like EGFR, BRAF, HER2 and many others), while poor therepeutcal options are available for the vast majority of the patients in which a targetable driving oncogenic mutation is undetermined. Moreover, the pathway redundancy and the very frequent occurrence of mutations limit the efficacy of these drugs even in potentially responding patients. There is therefore an urgent need for the identification of novel fundamental mechanisms of cancer biology and of relevant determinants of chemoresistance in order to develop more effective drugs and therapeutic strategies. The recent discovery of epithelial-to-mesenchymal transition (EMT), cancer stem cells (CSCs) and of their functional association and interdependence represent some of the most promising advances in the last two decades of cancer research. CSCs are defined as a subpopulation of undifferentiated cancer cells with stem-like features responsible for tumors' heterogeneity and for some of the most lethal features of cancers: tumorigenicity, metastatic spread, relapse and chemoresistance. The 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. Understanding the metabolic pathways associated with EMT and CSCs could provide new important insights in the biology of cancer, leading to the identification of novel targets for therapeutic intervention.

Aim of the research:

The Junior Group aims at identifying novel fundamental mechanisms and molecular determinants that regulate the plasticity and the aggressiveness of cancer cells and at studying the association between cancer differentiation and sensitivity to chemotherapy. We are particularly interested in studying the metabolic changes associated with EMT and CSCs and in understanding how to possibly interefere with these pathways to reduce the aggressiveness and the lethality of cancer cells (in terms of growth, metastasis development and intrinsic and acquired chemoresistance). The Group uses several cell and molecular biology techniques, mouse models, and the analysis of human samples as well as omics and high-throughput approaches.

Third-party funding

Third-party funding are not yet available for this Group.

N1 - Progress Report

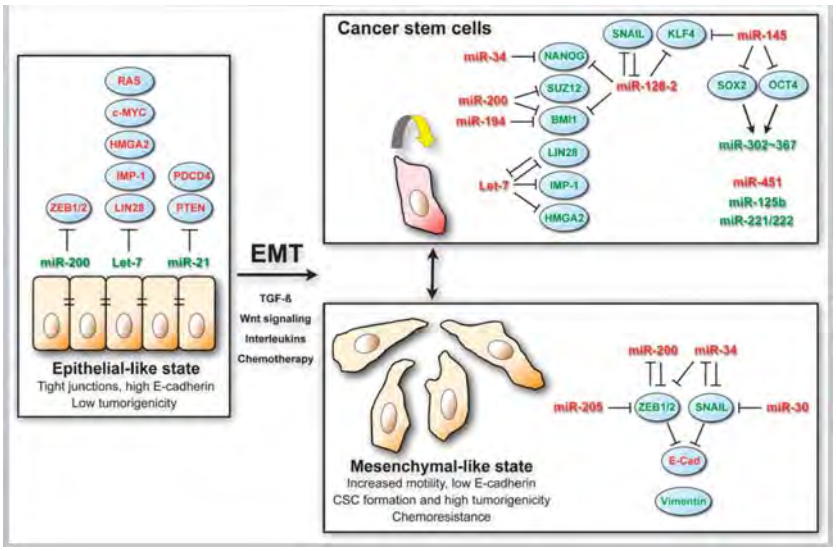
01.08.2015 - 31.07.2021

Understanding the plasticity of cancer cells

Dr. Paolo Ceppi, IZKF - Junior Research Group 1

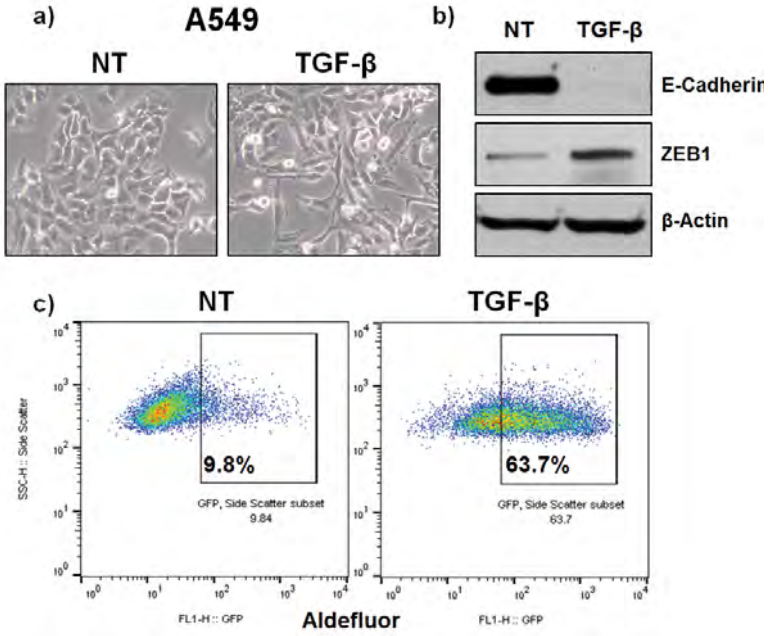
The Junior Group aims at identifying novel fundamental mechanisms that regulate the plasticity of cancer cells and at studying the association between cancer differentiation and sensitivity to chemotherapy. We are particularly interested in studying the metabolic changes associated with EMT and CSCs and in understanding how to possibly interfere with these pathways to reduce the aggressiveness and the lethality of cancer cells.

For the first months of activity, the Junior Group N1 has dedicated most of the time and efforts in settling and establishing the labspace in the location of the first floor of the Nikolaus-Fiebiger-Zentrum in Erlangen. The spaces were made available around the end of the month of September. From the end of November/ beginning December all the major equipments have been acquired and since then the lab is actively working to produce preliminary data for grant applications (see decription below). Two PhD students have been hired since the beginning of the Junior Group: Mr. Mohammed Aaif Siddiqui and Ms. Annemarie Schwab. Moreover, the recruitment of a postdoctoral fellow has been very recently completed, with the selected candidate (Dr. Maria Eleni Vazakidou) expected to start on February 1st 2016. During the course of 2016, an additional Research Assistant (Ms. Francesca Napoli from University of Torino, Italy) will be joining the lab and working on the projects of the Group for a period of six months.



Signals from the microenvironment or chemotherapy can trigger EMT in cancer cells. miRNAs can contribute to these alterations by targeting key-components of differentiation pathways (Ceppi P. Oncogene, 2013).

Dr. Ceppi



In vitro model of EMT/CSCs. Lung carcinoma A549 cells exposed to transforming growth factor Beta 1 (TGFB) for 5 days, showing a) morphological changes, b) reduction of E-cadherin and increase of ZEB1, and c) increase of the CSC marker ALDH1.

Current work of the group: As outlined above, the main area of research of the Junior Group is the metabolism of cancer cells and we are currently working on the identification of metabolic genes with a role in cancer plasticity, EMT and CSCs. By the use of bioinformatic approaches on large data sets, we have identified a list of candidate metabolic genes higly correlating (positively and negatively) with the expression of CSC and EMT markers in cancer cells. These candidate molecules have also been screened for their correlation with CSCs and EMT markers in tissues from patients with cancer of different origin (all solid malignancies) using molecular signatures available in the literature and very recently published by others. The group is currently working on the validation and assessing the functional relevance of some of the most interesting candidate genes identified (for instance those related to the metabolic energy pathways) in a panel of cancer cell lines in vitro. The plan for the next months will also include the test of the expression and the role of these candidate metabolic genes in tissues of cancer patients and in mouse cancer models, as well as the establishment of internal and external scientific collaborations.

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Own publications on the subject (from the previous research experiences)

Ceppi P, Peter ME. microRNAs Regulate Both Epithelial-to-Mesenchymal Transition and Cancer Stem Cells. Oncogene. 2013 Mar 4.

Ceppi P, Hadji A, Kohlhapp FJ, Pattanayak A, Hau A, Liu X, Liu H, Murmann AE, Peter ME. CD95 and CD95L promote and protect cancer stem cells. Nature Communications, Nov 2014,5:5238.

Ceppi P, Mudduluru G, Kumarswamy R, Rapa I, Scagliotti GV, Papotti M, Allgayer H: Loss of miR-200c expression induces an aggressive, invasive, and chemoresistant phenotype in non-small cell lung cancer. Molecular Cancer Research, 2010, 8:1207-1216.

Junior Research Group 2

Prof. Dr. Jens Titze

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Biographical Sketch

Dr. Titze studied Medicine at Humboldt University and Free University of Berlin from 1989 to 1996. From 1997 to 2000 he followed specialty training in internal medicine (residency) at the Stauferklinik Schwäbisch Gmünd, University of Ulm. He joined the FAU Erlangen-Nuremberg in 2000 first as a postdoctoral and clinical fellow in internal medicine (2000-2004), then as a consultant in internal medicine and renal fellow (2004-2009). In 2009 he became head of the IZKF Junior Research Group 2. The main goal of his preclinical research was discovery of extrarenal regulation of salt and water balance. His main contributions to the field are the demonstration that Na+ is stored in the skin and in muscle, and that immune cells regulate interstitial electrolyte homeostasis and blood pressure through cutaneous lymph capillaries. The main goal of his patient-oriented research activity was a strong clinical focus on Na+ storage as a cardiovascular risk factor and the transfer of the findings from basic research into clinical practice in 23NaMRI imaging studies. Through these studies, he has developed a strong expertise in interstitial physiology, immune cell and vascular biology, and quantitative approaches for innovative phenotyping of electrolyte storage in preclinical and clinical research. With grants of NIH, the American Heart Association, the German Research Foundation (DFG), and Federal Ministry of Economics and Technology (BMWi), he has laid ground for a research program on extrarenal regulation of electrolyte homeostasis in cardiovascular biology and medicine. He now expands this approach to the role of interstitial Na+ storage in the tumor microenvironment for tumor growth and spreading.

From the left: P. Dietsch, St. Perisic, D. Amslinger, P. Neubert, U. Goller, P. Linz, J. Goß, J. Titze, A. Birukov, Mrs. Dietsch, A. Dahlmann and N. Rakova.

Research Focus

The main goal of our preclinical research is discovery of extrarenal regulation of salt and water balance. Our main contributions to the field are the demonstration that Na+ is stored in the skin and in muscle, that immune cells regulate interstitial electrolyte homeostasis and blood pressure through cutaneous lymph capillaries, and that salt accumulation promotes the immunological host defense response and auto-immune responses. The main goal of our patient-oriented research activity is transfer of these findings into clinical practice in 23NaMRI imaging studies. Through these studies, we have developed a strong expertise in interstitial physiology, immune cell and vascular biology, and quantitative approaches for innovative phenotyping of electrolyte storage in preclinical and clinical research. We have a strong clinical focus on Na+ storage as a cardiovascular risk factor, and recently expand this approach to the role of Na+ storage in immunity and tumor growth and spreading.

Third-party funding

Ongoing Research Support

Titze, J (PI), American Heart Association, Lowering tissue Na+ stores to reduce blood pressure in aging humans, AHA 14SFRN20770008, 2014-2018

Titze, J (PI), National Institutes of Health, Lymphatic regulation of skin electrolyte metabolism and blood pressure, NIH R01 HL118579-03, 2013-2018

Titze, J; Jantsch, J (PIs): German Research Foundation SFB 643/4, Immune system regulation of electrolyte metabolism under homeostatic and inflammatory conditions, 2013-2016

Titze, J (PI), Federal Ministry for Economics and Technology, Long-term control of body Na+ and body fluid homeostasis during long-term simulation of a space flight, 2013-2016

Completed Research Support (last 3 years)

Titze, J (PI), German Research Foundation, Lymphangiogenesis in response to Na+ storage in the skin, 2009-2012

Titze, J (PI), Federal Ministry for Economics and Technology, Long-term control of body Na+ and body fluid homeostasis during long-term simulation of a space flight, 2009-2012

N2 - Final Report

01.11.2009 - 31.10.2015

Immune system as regulator of volume and blood pressure

Prof. Dr. Jens Titze, IZKF - Junior Research Group 2

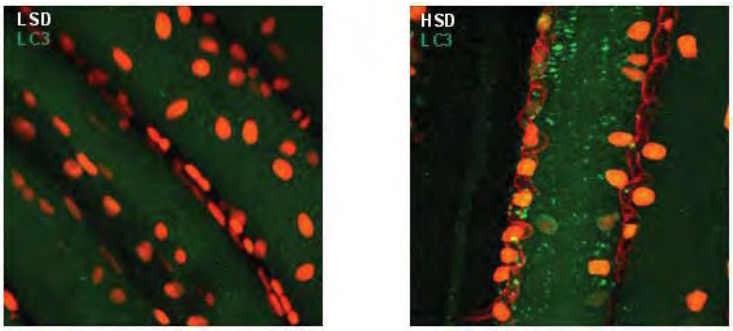
We have found that the immune system regulates salt and water balance, and that tissue Na+ storage significantly boosts innate and adaptive immune responses. The finding has opened an entirely new perspective on immune function that extends ancient protection from invaders to physiological adaptation to environmental conditions and blood pressure control. We have developed 23Na magnetic resonance imaging methods for rapid transfer of our basic research findings into the clinical arena.

Extrarenal regulation of sodium balance by homeostatic immune cells. We have shown that immune cells regulate lymphatic electrolyte and water clearance in the skin. Blocking this physiological response leads to salt accumulation and arterial hypertension (Nature Medicine 2009, Hypertension 2010, J Clin Invest 2013).

Salt leads to proinflammatory immune cell polarization. Our and we collaborators have shown that local salt accumulation leads to immune cell polarization to promote host defense, but also aggravates immune disease (Nature 2013, Cell Metabolism 2015, J Clin Invest 2015).

Humans store sodium in the skin and in muscle. We have implemented 23Na-MRI technology to non-invasively visualize Na+ reservoirs in humans. We find that our basic research program is rapidly transferable into the clinical arena and finds broad application in clinical research studies (Hypertension 2012, Kidney Int 2012, Hypertension 2013, Kindey Int 2015, Cell Metabolism 2015).

Long-term sodium balance studies question existing concepts on how fluid and electrolyte homeostasis is regulated. We have performed the first ultra-long term sodium and water balance studies in man. Studying daily sodium balance for months in men during a simulated space flight to Mars, we have found that steady state sodium balance is characterized by endogenous weekly rhythmical storage and release of body sodium rather than by rigidly constancy of the sodium content (Cell Metabolism 2013). This finding questions the validity of 24 hrs urine samples as a good measure of salt intake (Hypertension 2015). We recently study how sodium storage induces urea production and catabolic muscle wasting.



Expression of the autophagy marker LCIII (green) in mice with a low-salt (LSD) or a high-salt diet (HSD). Salt leads to muscle wasting and autophagy to provide with protein for urea generation.

Prof. Dr. Titze

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Invited lectures

More than 20 invited lectures, including 3 keynote lectures, at international conferences.

Awards

No new awards in 2015.

Selected publications during funding period

Binger KJ, Gebhardt M, Heinig M, Rintisch C, Schroeder A, Neuhofer W, Hilgers K, Manzel A, Schwartz C, Kleinewietfeld M, Voelkl J, Schatz V, Linker RA, Lang F, Voehringer D, Wright MD, Hubner N, Dechend R, Jantsch J, Titze J, Müller DN. High salt reduces the activation of IL-4- and IL-13-stimulated macrophages. J Clin Invest. 2015 Nov 2;125(11):4223-38.

Lerchl K, Rakova N, Dahlmann A, Rauh M, Goller U, Basner M, Dinges DF, Beck L, Agureev A, Larina I, Baranov V, Morukov B, Eckardt KU, Vassilieva G, Wabel P, Vienken J, Kirsch K, Johannes B, Krannich A, Luft FC, Titze J. Agreement between twenty-four hour salt ingestion and sodium excretion in a controlled environment. Hypertension 2015;66:850-7.

Jantsch J, Schatz V, Friedrich D, Schroder A, Kopp C, Siegert I, Maronna A, Wendelborn D, Linz P, Binger KJ, Gebhardt M, Heinig M, Neubert P, Fischer F, Teufel S, David JP, Neufert C, Cavallaro A, Rakova N, Kuper C, Beck FX, Neuhofer W, Muller DN, Schuler G, Uder M, Bogdan C, Luft FC, Titze J. Cutaneous Na+ storage strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense. Cell Metabolism. 2015;21:493-501

Linz P, Santoro D, Renz W, Rieger J, Ruehle A, Ruff J, Deimling M, Rakova N, Muller DN, Luft FC, Titze J, Niendorf T. Skin sodium measured with 23na mri at 7.0 T. NMR in Biomedicine. 2015;28:54-62

Dahlmann A, Dorfelt K, Eicher F, Linz P, Kopp C, Mossinger I, Horn S, Buschges-Seraphin B, Wabel P, Hammon M, Cavallaro A, Eckardt KU, Kotanko P, Levin NW, Johannes B, Uder M, Luft FC, Muller DN, Titze JM. Magnetic resonance-determined sodium removal from tissue stores in hemodialysis patients. Kidney International. 2015;87:434-441

Schlote J, Schröder A, Dahlmann A, Karpe B, Cordasic N, Daniel C, Hilgers KF, Titze J, Amann K, Benz K. Cardiovascular and renal effects of high salt diet in GDNF+/- mice with low nephron number. Kidney Blood Press Res. 2013; 37(4-5):379-91.

Wiig H, Schröder A, Neuhofer W, Jantsch J, Kopp C, Karlsen TV, Boschmann M, Goss J, Bry M, Rakova N, Dahlmann A, Brenner S, Tenstad O, Nurmi H, Mervaala E, Wagner H, Beck FX, Müller DN, Kerjaschki D, Luft FC, Harrison DG, Alitalo K, Titze J. Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. J Clin Invest. 2013; 123: 2803-2815.

Kopp C, Linz P, Dahlmann A, Hammon M, Jantsch J, Müller DN, Schmieder RE, Cavallaro A, Eckardt KU, Uder M, Luft FC, Titze J. 23Na magnetic resonance imaging-determined tissue sodium in healthy subjects and hypertensive patients. Hypertension. 2013;61:635-640

Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Yosef N, Linker RA, Müller DN, Hafler DA. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. Nature. 2013;496:518-522

Rakova N, Jüttner K, Dahlmann A, Schröder A, Linz P, Kopp C, Rauh M, Goller U, Beck L, Agureev A, Vassilieva G, Lenkova L, Johannes B, Wabel P, Moissl U, Vienken J, Gerzer R, Eckardt KU, Müller DN, Kirsch KA, Morukov B, Luft FC, Titze J. Long-term space flight simulation reveals infradian rhythmicity in human Na+ balance. Cell Metabolism. 2013;17:125-131

Kopp C, Linz P, Hammon M, Schofl C, Grauer M, Eckardt KU, Cavallaro A, Uder M, Luft FC, Titze J. Seeing the sodium in a patient with hypernatremia. Kidney International. 2012;82:1343-1344

Kopp C, Linz P, Wachsmuth L, Dahlmann A, Horbach T, Schöfl C, Renz W, Santoro D, Niendorf T, Müller DN, Neininger M, Cavallaro A, Eckardt KU, Schmieder RE, Luft FC, Uder M, Titze J. 23Na magnetic resonance imaging of tissue sodium. Hypertension. 2012;59:167-172.

Machnik A, Dahlmann A, Kopp C, Goss J, Wagner H, van Rooijen N, Eckardt KU, Muller DN, Park JK, Luft FC, Kerjaschki D, Titze J. Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhance binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. Hypertension. 2010;55:755-761

Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, Park JK, Beck FX, Muller DN, Derer W, Goss J, Ziomber A, Dietsch P, Wagner H, van Rooijen N, Kurtz A, Hilgers KF, Alitalo K, Eckardt KU, Luft FC, Kerjaschki D, Titze J. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. Nat Med. 2009;15:545-552

Junior Research Group 3

Prof. Dr. Beate Winner

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Biographical Sketch

Dr. Winner studied Medicine in Regensburg, Würzburg and Toronto from 1992 to 1999. Her MD thesis was carried out in the laboratory of Prof. J. Galle at the Department of Medicine, University of Würzburg. From 1999 to 2007 she worked at the Department of Neurology, University of Regensburg (board examined neurologist 2005). The clinical focus was neurodegenerative diseases. Her postdoctoral training was performed in the Neuroregeneration Laboratory of the Department of Neurology with Profs. G. Kuhn, J. Winkler, and L. Aigner. After completing the postdoctoral lecturer qualification in neurology in 2007, she joined the Laboratory of Genetics (Prof. FH Gage) at the Salk Institute, La Jolla as a Feodor-Lynen fellow. Dr. Winner joined the FAU Erlangen-Nürnberg in 2010 to start her own laboratory as head of the IZKF junior research group III. In addition she was awarded a BMBF research group neuroscience in 2011 and an associated junior group within the BioSysNet consortium in 2012. She acquired the permits for the use of human embryonic stem cells according to the German Stem Cell Act and as the first group at the FAU Erlangen-Nürnberg set up the tools to perform disease modeling using induced pluripotent stem cell technology. The focus of her lab is to model rare neurologic diseases using induced pluripotent stem cell derived neuronal models.

From the left: Dr. Francesc Perez-Branguli, Daniela Gräf, Annika Sommer, Marius Brazdis (at the back), Tania Rizo (in the middle), Sandra Loskarn (in the front), Katrin Simmnacher, Himanshu Mishra, Dr. Iryna Prots, Prof. Beate Winner, Holger Wend

Research Focus

NEURODEGENERATION IN STEM CELL-BASED MODELS

Neurons in the central nervous system (CNS) are only taken for biopsy under rare conditions and previously our understanding about disease-related neuronal phenotypes in humans was mainly derived from analyzing postmortem brain tissues. This inability to sample live brain cells limited our knowledge of human neuropathological abnormalities during the course of neurodegenerative diseases. Therefore stem cell derived human neurons represent a means of exploring patient-specific pathological mechanisms and test individualized therapeutic interventions. The aim is to use these individualized induced pluripotent stem cell derived models as read-out systems for testing of small compounds and the reversibility of cellular phenotypes and eventually go back to the patients.

Within the Universitätsklinikum Erlangen the human induced pluripotent stem cell technology is able to bridge basic and translational research. We receive somatic cells (e.g. blood, fibroblasts) from patients from clinicians and then turn these into induced pluripotent stem cells and from there into the cell type of interest (mostly neural cells). The focus of my research is to define disease phenotypes of neurodegenerative diseases using stem cell based in vitro neuronal models. We started by comparing controls and patients with monogenic motor neuron diseases called hereditary spastic paraplegias. More recently we started to target monogenic forms of cognitive dysfunction disorders, and pain. We are specifically interested in understanding connectivity of neurons, both at the level of synaptic function and axonal transport.

Third-party funding

Steven Havlicek, Bayerische Forschungsstiftung, Modeling familial motor-neuron disease by the use of human induced pluripotent stem cells (hiPSCs), 2010-2011.

Beate Winner, Francesc Perez-Branguli, BioSysNet, Transcriptome analysis to delineate genes involved in synaptic dysfunction in synucleinopathies. 2012-2017.

Beate Winner, BMBF, Disease modeling and target identification of motor neuron disease using induced pluripotent stem cells. 2011-2018.

Iryna Prots, ELAN, Distinct alpha-synuclein species interfere with neuronal transport mechanisms. 2011-2012.

Martin Regensburger, ELAN, Neuroprotective role of EFhd2 (swiprosin-1) in neuronal development and neurodegeneration. 2014.

Beate Winner, Zacharias Kohl, Jürgen Winkler, ForIPS, Forschungsverbund Induzierte Pluripotente Stammzellen. TP1: Zentralprojekt ForIPS: humane Induzierte pluripotente Stammzellen. 2013-2017.

Iryna Prots, Beate Winner, TP11: Humanes in vitro Modell für Neuroinflammation. 2013-2017.

Angelika Lampert, Beate Winner, Johannes und Frieda Marohn-Stiftung, Neuronale Differenzierung von peripheren Neuronen aus humanen induzierten pluripotenten Stammzellen (hiPSC). 2013-2015.

Beate Winner, Zacharias Kohl, Jürgen Winkler, Tom-Wahlig Stiftung, Individualized human in vitro model for hereditary spastic paraplegia. 2009-2016.

Beate Winner, Jürgen Schüttler, IZKF, Modeling pain syndromes using human induced pluripotent stem cell-derived nociceptors. 2016-2019.

Beate Winner, DFG, Subproject GRK2162, Examining the impact of the hereditary spastic paraplegia gene SPG11 on neuronal development and maintenance. 2016-2020.

Beate Winner, Florian Krach, Stefan Aigner, BaCaTec, TDP-43 pathology and cellular phenotypes in genome-engineered and iPSC-derived neurons of patients with ALS. 2016-2017.

Beate Winner, im Rahmen des BMBF Forschungsverbunds: Netzwerk für kognitive Störungen durch veränderte Chromoatindynamik (Sprecher, Prof. Reis). TP7: Generierung von humanen krankheitsspezifischen kortikalen Neuronen. 2016-2018.

N3 - Progress Report

01.10.2010 - 30.09.2016

Modeling neurodegenerative diseases using stem cells

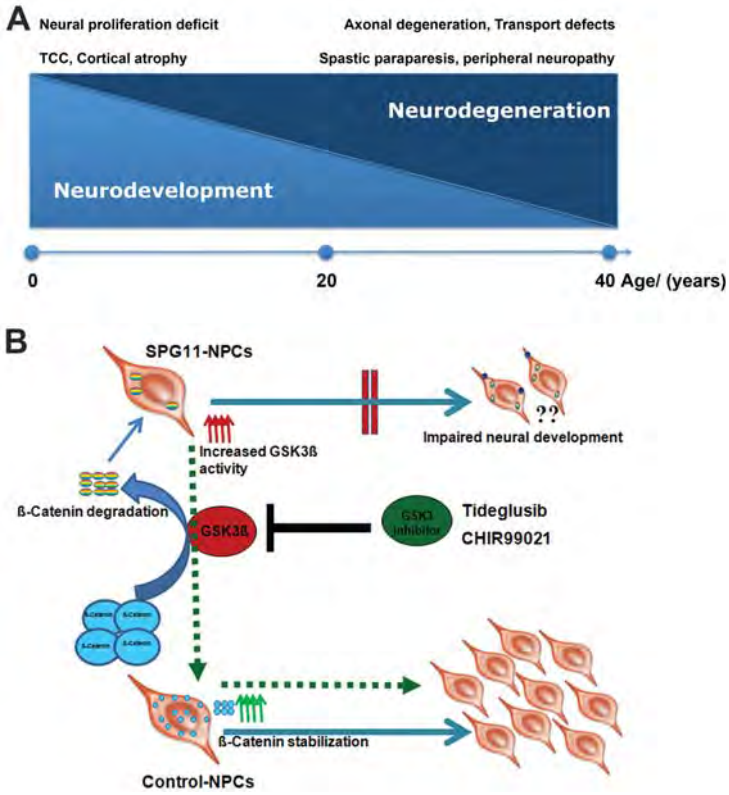
Prof. Dr. Beate Winner, IZKF - Junior Research Group 3

The overall goal in our laboratory is to investigate neurodegeneration using human stem cell derived models. In this respect, we investigated neural phenotypes in the most frequent complicated autosomal recessive (SPG11) form of hereditary spastic paraplegia (HSP). A major finding was a dual role for spatacsin in neurodevelopment and neurodegeneration due to GSK3ß-dependent dysregulation of neurodevelopment in SPG11-patient induced pluripotent stem cell derived neurons.

Dual role for spatacsin in neurodevelopment and neurodegeneration

1. Dysfunction of spatacsin leads to axonal pathology in SPG11 linked hereditary spastic paraplegia

One of our main research interests is to understand the role of spatacsin (mutated in SPG11 linked hereditary spastic paraplegia) in induced pluripotent stem cell derived neurons from patients. In these neurons we recently reported an accumulation of vesicle-like structures and inclusions within the neurites from SPG11 patients indicating axonal pathology. These data provided the first evidence that human SPG11 mutations and loss of function of spatacsin share axonal pathologies and show that SPG11 is implicated in axonal maintenance and cargo trafficking (Perez-Branguli, Mishra et al., HMG 2014).



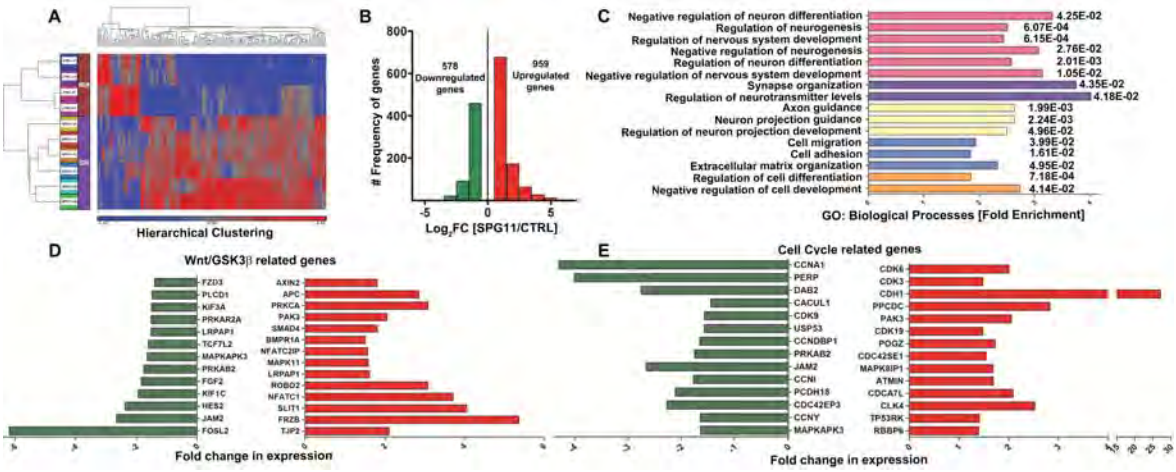
A) Two distinct stages of SPG11 disease pathology: Neurodevelopmental and neurode- genera!ve phenotype. B) Potential mechanism.

Prof. Dr. Winner

2. GSK3ß-dependent dysregulation of neurodevelopment in SPG11-patient iPSC derived neurons

Clinical symptoms of SPG11-linked hereditary spastic paraplegia are a thin corpus callosum and severe cortical atrophy. The specific transcriptome signature showed that roughly half of the differentially expressed genes in SPG11-neural progenitor cells (NPCs) were related to neural development and included transcriptional changes in the regulation of neurogenesis, neuronal differentiation, nervous system development and axonal projection. Cortical NPCs derived from SPG11 patients had a reduced proliferative potential. This proliferation defect could be linked to a loss of function of spatacsin and cell cycle abnormalities. Fewer NPCs were present at the S phase and G2/M phase, paralleled by a decreased expression of these checkpoint genes in SPG11-NPCs. Furthermore, our study revealed an impaired GSK3ß/ß-Catenin signaling in SPG11-NPCs that could be rescued by antagonists of GSK3 (Mishra et al., resubmitted to Annals of Neurology).

In addition to the previously known adult-onset motor neuron disease phenotype, our recent data provide a novel link between a GSK3 dependent pathway and a cortical developmental phenotype in SPG11 patients. Our data point towards distinct temporal functions of spatacsin, causing axonal transport deficits in corticospinal projections in mature neurons in vitro and most likely during adulthood in patients. Our current model of SPG11 disease pathology starts with an early onset neurodevelopmental phenotype (first two decades), consisting of a proliferation deficit and cortical neurogenesis abberations. This results in impairment of axonal outgrowth and guidance, consistent with a TCC and cortical atrophy. After transition around the 2nd and 3rd decade of life, an additional neurodegenerative phenotype is observed. The major adult-onset phenotype is reflected by axonal degeneration, resulting in impaired axonal transport, with the clinical correlates of spastic paraparesis and peripheral neuropathy.



A) Heat map of gene expression analysis of NPCs generated from CTRL and SPG11-iPSCs. ((B) Histographs (C) Gene Ontology term analysis of important biological processes enriched within differentially expressed genes. (D, E) Differentially regulated pathways in SPG11-NPCs.

N3 - Progress Report

01.10.2010 - 30.09.2016

Invited lectures

Beate Winner:

Symposium: Sensory Neuropathies – Peripheral Neurodegeneration, January 8th, 9th 2015, Vaals, NL, Examining the impact of the hereditary spastic paraplegia gene SPG11 on neuronal development and maintenance in the peripheral nervous system.

11th Göttingen meeting of the German Neuroscience Society, Symposium 34: Modeling evolution, neuronal development and neurodegenerative disorders using mammalian induced pluripotent stem cells, March 21st 2015, Göttingen, Organizer and speaker: A tale of traffic jams and bumpy roads and more?

Tom Wahlig Symposium, HSP, April 17th 2015, Graz, Human in vitro modeling of HSP

Bavarian Research network induced pluripotent stem cells, ForIPS Symposium, Munich, July 3rd 2015, Munich, Human in vitro modeling of Hereditary Spastic Paraplegia

DGPPN Kongress, Berlin, November 26th 2015, Modellierung der Neuroentwicklung und -degeneration anhand von Patienten-abgeleiteten induzierten pluripotenten Stammzellen

Iryna Prots:

International Summer School „Pluripotent Stem Cells“, September, 28th - October, 2nd 2015 at Studienhaus Gut Schönwag, Using of human Pluripotent Stem Cells for investigation of Neurodegeneration

„Science meets School“ of the BioSysNet Concortium, October 12th 2015 at the FAU Erlangen-Nürnberg, Nutzung der induzierten pluripotenten Stammzellen für die Untersuchung der Neurodegeneration bei Menschen

Awards

Paper of the Month der Deutschen Physiologischen Gesellschaft (Eberhardt, Havlicek, and Schmidt et al. 2015, Stem Cell Reports)

Selected publications during funding period

Eberhardt E*, Havlicek S*, Schmidt D*, Link AS, Neacsu C, Kohl Z, Hampl M, Kist AM, Klinger A, Nau C, Schüttler J, Alzheimer C, Winkler J, Namer B, Winner B#, Lampert A#. Pattern of functional TTX-resistant sodium channels reveals developmental stage of human iPS- and ES cell-derived nociceptors. Stem Cell Reports, 2015 5(3):305-13. *# contributed equally

Schreglmann S, Regensburger M, Rockenstein E, Masliah E, Xiang W, Winkler J, Winner B. The temporal expression pattern of alpha-Synuclein modulates olfactory neurogenesis in transgenic mice. PlosOne, 2015 11;10(5):e0126261.

Link AS, Kurinna S, Havlicek S, Lehnert S, Reichel M, Kornhuber J, Winner B, Huth T, Zheng F, Werner S, Alzheimer C. Kdm6b and Pmepa1 as targets of bioelectrically and behaviorally induced Activin A signaling. Mol Neurobiol. 2015 Jul 28. [Epub ahead of print].

Pérez-Brangulí F, Mishra HK, Prots I, Havlicek S, Kohl Z, Saul D, Rummel C, Dorca-Arevalo J, Regensburger M, Graef D, Sock E, Blasi J, Groemer TW, Schlötzer- Schrehardt U, Winkler J, Winner B. Dysfunction of spatacsin leads to axonal pathology in SPG11 linked hereditary spastic paraplegia. HMG, 2014 23(18):4859-74.

Havlicek S, Kohl Z, Mishra HK, Prots I, Eberhardt E, Denguir N, Wend H, Plötz S, Boyer S, Marchetto MCN, Aigner S, Sticht H, Groemer TW, Hehr U, Lampert A, Schlötzer-Schrehardt U, Winkler J, Gage FH, Winner B. Gene dosage dependent rescue of HSP neurite defects in SPG4 patients' neurons. HMG, 2014; 23(10):2527-41.

Purohit P*, Perez-Branguli F*, Prots I*, Borger E, Gunn-Moore F, Welzel O, Loy K, Wenzel EM, Grömer TW, Brachs S, Holzer M, Buslei R, Fritsch K, Regensburger M, Böhm KJ, Winner B, Mielenz D. The Ca2+ sensor protein Swiprosin-1/EFhd2 is present in neurites and involved in kinesin-mediated transport in neurons. Plos One, 2014; 9(8):e103976. *contributed equally

Ettle B, Reiprich S, Deusser J, Schlachetzki JC, Xiang W, Prots I, Masliah E; Winner B, Wegner M, Winkler J. Intracellular alpha-synuclein affects early maturation of primary oligodendrocyte progenitor cells. Molecular and Cellular Neuroscience. 2014;62:68-78.

May VE, Ettle B, Poehler AM, Nuber S, Ubhi K, Rockenstein E, Winner B, Wegner M, Masliah E, Winkler J. Alpha-synuclein impairs oligodendrocyte progenitor maturation in multiple system atrophy. Neurobiology of Aging, 2014;35(10):2357-68.

Rockenstein E, Nuber S, Overk CR, Ubhi K, Mante M, Patrick C, Adame A, Trejo-Morales M, Riek R, Winklder J, Gage FH, Winner B, Masliah E. Synaptic accumulation of oligomer prone a-synuclein exacerbates synatpci degeneration and neuronal loss in a transgenic mouse model. Brain, 2014;137(5):1496-513.

Winner B, Marchetto MC, Winkler J, Gage FH. Human-induced pluripotent stem cells pave the road for a better understanding of motor neuron disease. HMG, 2014; 23(R1):R27-34.

Marxreiter F, Ettle B, May VE, Esmer H, Patrick C, Kragh CL, Klucken J, Winner B, Riess O, Winkler J, Masliah E, Nuber S. Glial A30P alpha-synuclein pathology segregates neurogenesis from anxiety-related behavior in conditional transgenic mice. Neurobiol Dis. 2013;59:38-51.

Prots I, Veber V, Brey S, Campioni S, Buder K, Riek R, Böhm KJ, and Winner B (2013) Alpha-synuclein oligomers impair neuronal microtubule-kinesin interplay. J. Biol. Chem. 288: 21742-21754

Winner B, Regensburger M, Schreglmann S. Boyer L, Prots I, Rockenstein E, ManteM, Zhao C, Winkler J, Masliah E, Gage FH (2012). Role of α-synuclein in adult neurogenesis and neuronal maturation in the dentate gyrus. J Neurosci, 32(47):16906-16916.

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Kohl Z, Winner B, Ubhi K, Rockenstein E, Mante M, Münch M, Barlow C, Carter T, Masliah E, Winkler J (2012). Fluoxetine rescues impaired hippocampal neurogenesis in a transgenic A53T synuclein mouse model. Eur J Neurosci, 35(1):10-9.

Winner B, Kohl Z, Gage FH. (2011) Neurodegenerative disease and adult neurogenesis. Eur J Neurosci. 33(6):1139-51.

Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, Hetzer C, Loher T, Vilar M, Campioni S, Tzitzilonis C, Soragni A, Jessberger S, Mira H, Consiglio A, Pham E, Masliah E, Gage FH, Riek R. (2011) In vivo demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A. 108(10):4194-9.

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Junior Projects

Immunology and Infection

Project No.	Project title	Term	Applicant	Institute
J37	Adoptive cell therapy with ex-vivo expanded NK and γδT cells in metastatic melanoma	01.07.2013-30.04.2016	Dr. Bosch-Voskens	Department of Dermatology
J38	MCS-18 for the treatment of atherosclerosis	01.02.2014-31.01.2016	Dr. Dietel	Department of Medicine 2
J39	Hypermethylation of SOCS3 in fibrotic diseases	01.01.2014-31.12.2015	Dr. Dees	Department of Medicine 3
J40	PU.1 signalling in fibrotic diseases	01.01.2014-31.12.2015	Dr. Ramming	Department of Medicine 3
J41	Resolution of inflammation in gout	01.12.2013-30.11.2016	Dr. Schauer	Department of Medicine 3
J43	The role of IL-33/ST2 signaling in the development of infectious colitis	01.02.2015-31.07.2017	Dr. Mchedlidze	Department of Medicine 1
J44	Rhadinovirus Entry Receptors	01.04.2015 - 31.12.2015	Dr. Hahn	Institute of Clinical and Molecular Virology
J45	Modulation of PRC2 activity by HCMV IE2	01.01.2015-30.06.2017	Dr. Reuter	Institute of Clinical and Molecular Virology
J50	Analysis of the role of IL-9 in the induction of Colitis-associated cancer (CAC)	16.10.2015 - 15.04.2018	Dr. Gerlach	Department of Medicine 1

Oncology

Project No.	Project title	Term	Applicant	Institute
J54	Analysis of alternative mechanisms of tumor rejection	01.11.2015 - 30.04.2018	Dr. Lehmann	Department of Dermatology
J55	The role of microRNA-188-5p dysregulation in hepato-cellular carcinoma development and progression	01.01.2016 - 30.06.2018	Dr. Dietrich	Institute of Biochemistry

Neurosciences

Project No.	Project title	Term	Applicant	Institute
J33	Sox2 in the CNS: regulating myelination by microRNAs	01.02.2013-31.01.2015	Dr. Reiprich	Institute of Biochemistry
J46	The role of zinc finger protein Zfp276 in glial development of the mouse nervous system	01.04.2015-30.09.2017	Dr. Küspert	Institute of Biochemistry
J51	Inflammatory signature in Parkinson's disease	01.10.2015-31.03.2018	Dr. Marxreiter	Department of Neurology
J52	Modeling cortical dysfunction of SPG11 spastic paraplegia using patient-derived pluripotent stem cells	01.11.2015-30.04.2018	Dr. Regensburger	Department of Neurology
J53	Diffusion tensor imaging of the visual pathway in pseudoexfoliation glaucoma	03.08.2015 - 02.02.2018	Dr. Schmidt	Department of Neuroradiology

Renal and Vascular Research

Project No.	Project title	Term	Applicant	Institute
J31	Function of a novel, HIF-regulated transcript	01.02.2013-31.01.2015	Dr. Dr. Schödel	Department of Medicine 4
J47	Post-transcriptional regulation by Hoxa9	01.03.2015-31.08.2017	Dr. Bach	Department of Medicine 5

Molecular Medicine

Project No.	Project title	Term	Applicant	Institute
J36	Identification of molecular signalling pathways in cholestatic pruritus	01.09.2013-31.08.2015	Dr. Dr. Kremer	Department of Medicine 1
J42	Bayesian reverse engineering of developmental networks	01.04.2014-31.03.2016	Dr. Ferrazzi	Institute of Human Genetics
J48	PPARβ/δ in the crosstalk of bone and glucose metabolism	01.01.2015-30.06.2017	Dr. Scholtysek	Department of Medicine 3

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

Function of a novel, HIF-regulated transcript

Dr. Dr. Johannes Schödel, Department of Medicine 4 – Nephrology and Hypertension

Adaptation of cells and whole organisms to reduced oxygen conditions (hypoxia) is essential for survival. Hypoxia-inducible transcription factors (HIF) are crucially involved in hypoxic gene regulation. HIFs induce a variety of RNA species including non-coding RNAs. We have identified a novel hypoxia inducible transcript (Nici) on chromosome 12 using mRNA-sequencing of MCF-7 breast cancer cells. The aim of this junior project is to characterise expression, regulation and function of this transcript in the context of hypoxic gene regulation.

Background

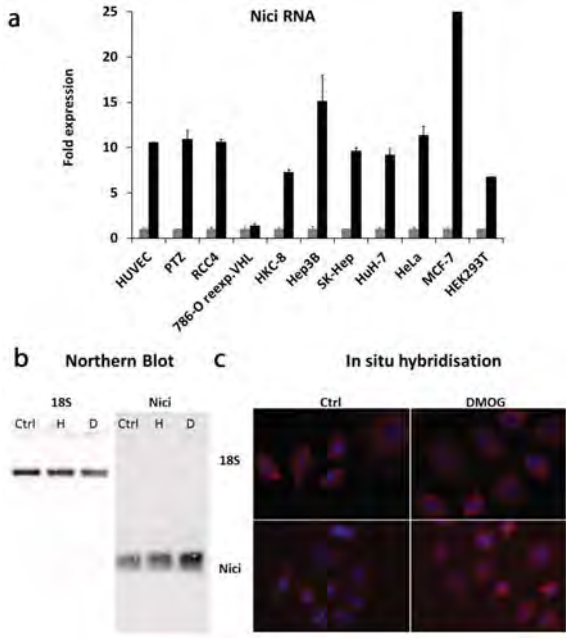
Long non-coding RNAs have been recently discovered and are involved in many intracellular processes including regulation of DNA accessibility and transcription by directly interacting with regulatory DNA elements or protein complexes of transcriptional repressors or activators. Under hypoxic conditions HIF transcription factors are stabilised in cells and mainly act as activators of a transcriptional program that aims to increase oxygen supply and optimize cell metabolism. The role of long non-coding RNAs in hypoxic gene regulation and potentials crosstalk with the HIF response are poorly understood.

Nici - a novel long non-coding RNA regulated via the HIF-pathway

In previous work, we described a set of high stringency HIF DNA-binding sites identified by ChIP-seq in MCF-7 breast cancer cells. Using RNA-seq to examine the transcriptional response on a genome-wide level in combination with the HIF-binding sites we discovered a novel hypoxia inducible intergenic transcript (Nici) on chromosome 12 in MCF-7 breast cancer cells. Nici is strongly upregulated via the HIF-pathway, i.e. it is associated with a highly significant HIF-binding site (18th out of 400) in the promoter region and fold induction of the transcript is comparable to other highly inducible HIF-targets. The genomic locus and the transcript have genetic features of a long non-coding RNA such as an active promoter, a two exon configuration and the absence of an open reading frame.

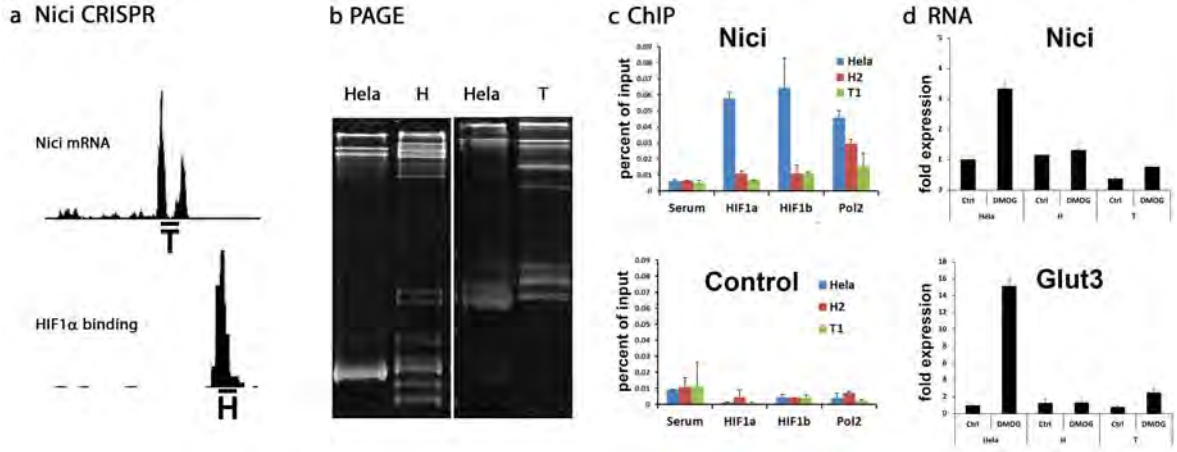
Nici is ubiquitously induced via the HIF-pathway

We first focused on testing whether expression and regulation of the transcript is present in other cell types. Using a variety of human malignant and non-malignant cell lines derived from different tissue origins we found that Nici is ubiquitously induced by hypoxia or pharmacological stabilisation of HIF. Importantly, we observed a correlation of hypoxic induction of Nici expression levels and mRNA le-



a) Relative expression levels of the Nici transcript in different cell lines exposed to the hypoxia mimetic DMOG (dimethyloxallylglycine). b) Northern Blot of total RNA from Hela cells subjected to normoxia (Ctrl), hypoxia (H) and DMOG (D). c) In situ hybridization of control and DMOG stimulated Hela cells.

Dr. Dr. Schödel



a) CRISPR constructs against the Nici transcript (T) and the HIF-binding site (H). b) PAGE analysis of DNA from CRISPR/Cas transfected cell clones identifies mutant clones. c) Binding of HIF-1α, HIF-1β and Polymerase II (Pol2) to the Nici locus is disrupted in the mutant clones. d) Induction of Nici and Glut3 by HIF is reduced in the clones.

vels of the neighboring gene, glucose transporter member 3 (Glut3), which is located approx. 25kb upstream of Nici. In addition, HIF-DNA-binding to the hypoxia responsive elements is conserved across cell lines. Comparing expression levels in normal renal tissue versus renal cancer tissue, in which HIFs are frequently stabilized by the loss of von Hippel-Lindau tumor suppressor protein, we detected a strong upregulation of Nici in tumors. These findings strengthen the hypothesis that Nici commonly contributes to the hypoxic response in human cells and HIF-associated tumors.

Functional role of Nici in the context of hypoxic gene regulation

We used CRISPR/Cas induced knock-out of the HIF-binding site or the Nici transcript to gain further insights into the functional role of Nici. Knock-out of the HIF-binding site abolished HIF-binding to the promoter induction of Nici RNA. We also determined a reduced induction of the neighboring gene GLUT3 in hypoxic conditions suggesting a functional link between the two transcripts. Since GLUT3 is an important regulator of energy supply and is dysregulated in several cancer types future work will focus on the specific impact of Nici expression on hypoxic cell metabolism.

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Publications during funding period

Jantsch J, Schödel J (2015) Hypoxia and hypoxia-inducible transcription factors in myeloid cell-driven host defense and tissue homeostasis. Immunobiology 220, 2: 305-314

Choudhry H, Albukhari A, Morotti M, Hider S, Moralli D, Smythies J, Schödel J, Green CM, Camps C, Buffa F, Ratcliffe P, Ragoussis J, Harris AL, Mole DR (2015) Tumor hypoxia induces nuclear paraspeckle formation through HIF-2α dependent transcriptional activation of NEAT1 leading to cancer cell survival. Oncogene 34: 4482-4490

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*contributed equally

J33 - Final Report

01.02.2013 - 31.01.2015

Neurosciences

Sox2 in the CNS: regulating myelination by microRNAs

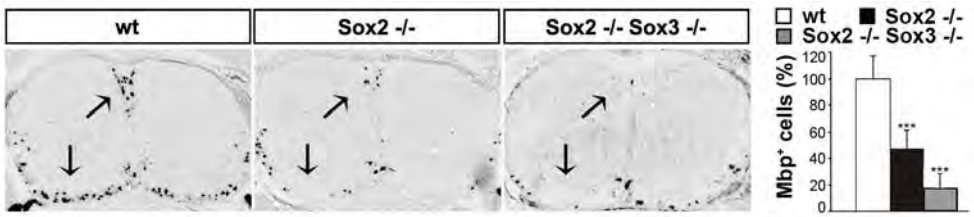
Dr. Simone Reiprich, Institute of Biochemistry

In the central nervous system, differentiation of myelinating oligodendrocytes requires the presence of the transcription factor Sox2. Sox2 supports differentiation directly by activating myelin gene expression and indirectly by repressing microRNA miR145. Thereby, it prevents miR145 from inhibiting pro-differentiation factors. This represents one of the few cases where the stem cell factor Sox2 is associated with differentiation rather than precursor functions.

Oligodendrocytes as the myelinating glia of the central nervous system provide electrical isolation and nutritional support to neurons. In the embryonic and early postnatal mouse spinal cord, the transcription factor Sox2 and its close relative Sox3 are expressed from neural precursor cells through oligodendrocyte progenitors to early differentiating oligodendrocytes. After the onset of myelination, expression of Sox2 and Sox3 slowly fades. The major function ascribed to Sox2 is maintenance of progenitor characteristics and pluripotency. Therefore, we aimed to analyze the role of Sox2 expression in differentiating glial cells in the mouse model.

Sox2 and Sox3 influence terminal differentiation of oligodendrocytes

When Sox2 was deleted in the oligodendrocyte progenitor stage, expansion or distribution of oligodendroglia at pre-differentiation stages was normal. Instead, Sox2 had an influence on terminal differentiation of oligodendrocytes, so that in its absence, myelination was reduced. Sox2 shared this function with its close relative Sox3 resulting in a stronger myelination defect in the combined absence of both transcription factors. We found Sox2 bound to myelin gene regulatory elements, but with minor transactivating potential in reporter gene assays when compared to other known activators of myelin gene expression. More strikingly, deletion of Sox2 and Sox3 came along with a strong reduction in the expression of Sox9 as another important regulator of oligodendrocyte development. This reduction was only observed at the protein, but not at the mRNA level.



In situ hybridization for Mbp (myelin basic protein) on perinatal mouse spinal cord sections shows the myelination defect in the absence of Sox2 which is aggravated by additional deletion of Sox3.

Dr. Reiprich

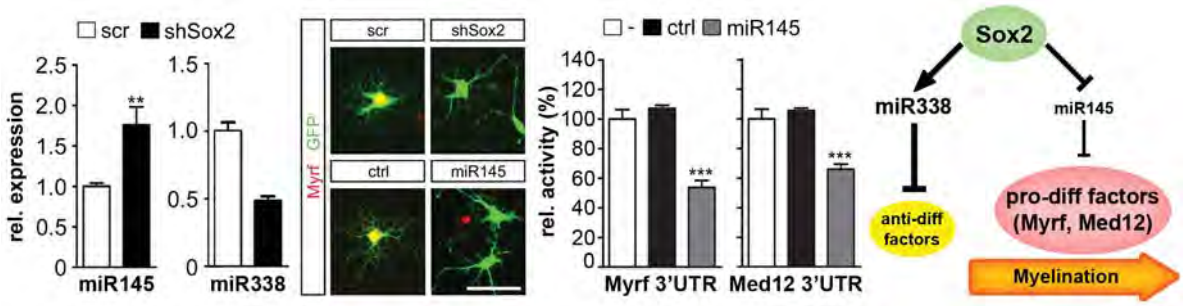
Sox2 controls expression of microRNAs in oligodendroglia

Post-translational repression is typically achieved by microRNAs. In oligodendroglial cell lines, knockdown of Sox2 resulted in an upregulation of miR145 and a downregulation of miR338. Hence, Sox2 represses miR145 and induces miR338. miR338 is known as an activator of myelination by repressing anti-differentiation factors. With miR145 we newly identified a microRNA that is negatively regulated by Sox2 and in turn represses Sox9. However, loss of Sox9 alone would not cause a myelination defect.

miR145 negatively regulates expression of factors required for oligodendrocyte differentiation

Search for further targets of miR145 among the factors required for oligodendrocyte differentiation elucidated Myrf (myelin gene regulatory factor) and Med12 as being targeted by miR145. Both these factors are essentially implicated in terminal differentiation of oligodendroglia. Our results point to a mechanism where miR145 is de-repressed in the absence of Sox2 and can therefore negatively control expression of Sox9, Myrf and Med12, which results in a myelination defect.

Both, repression of miR145 and induction of miR338 by Sox2 are supportive for differentiation and hence myelination. These results add another aspect to the functions of Sox2 beyond its well-characterized role as a stem cell factor.



Knockdown of Sox2 (shSox2) induces miR145, represses miR338 and inhibits Myrf expression as signs of impaired differentiation. Overexpression of miR145 inhibits Myrf or Med12 by targeting the 3'UTRs as shown in cell culture or in reporter gene assays.

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Publications during funding period

Reiprich S, Wegner M (2015) From CNS stem cells to neurons and glia: Sox for everyone. Cell and Tissue Research 359(1): 111-124

Hoffmann S, Hos D, Küspert M, Lang RA, Lovell-Badge R, Wegner M, Reiprich S (2014) Stem cell factor Sox2 and its close relative Sox3 have differentiation functions in oligodendrocytes. Development 141(1) : 39-50

J36 - Final Report

01.09.2013 - 31.08.2015

Molecular Medicine

Identification of molecular signalling pathways in cholestatic pruritus

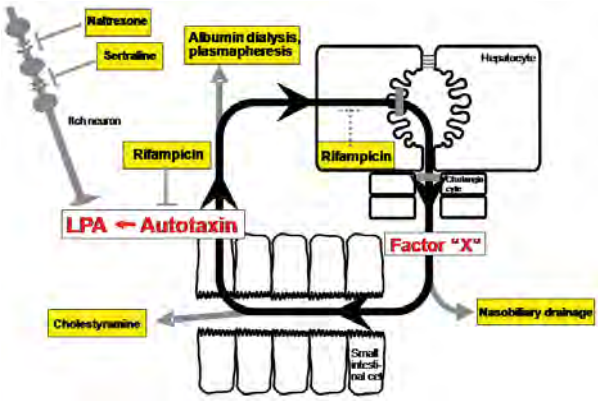
Dr. Dr. Andreas E. Kremer, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Pruritus is a common and often agonizing symptom of various hepatobiliary disorders. The mediators of cholestatic pruritus remain largely elusive. Recently, we could identify the enzyme autotaxin and its product, lysophosphatidic acid (LPA), as potential mediators of cholestatic pruritus. Aim of this project is to unravel the cellular origin of increased autotaxin levels during cholestasis and the expression of LPA receptors and autotaxin in skin of cholestatic patients.

In sera of patients suffering from cholestatic pruritus we could recently identify lysophosphatidic acid (LPA) as potential neuronal mediator. Intradermally injected LPA induced scratching activity in mice. Autotaxin, the enzyme forming LPA, was strongly increased in patients suffering from pruritus compared to non-pruritic controls and autotaxin activity strongly correlated with itch intensity and response to various treatments. It is our hypothesis that an unknown factor "X" drives autotaxin expression in tissues of the enterohepatic circulation. So far, we have generated the following results:

High autotaxin expression in human small intestine

In various human tissues involved in the enterohepatic circulation autotaxin expression was quantified using rt-PCR. We observed a high expression in the small intestine compared to large intestine, liver, bile duct and bile bladder. These results stand in contrast to murine autotaxin expression which was very low in small and large intestine and significantly higher in liver and other tissues. Thus, we aimed to have a closer look in human small intestine to elucidate the cell type responsible for autotaxin expression.



Model of development of cholestatic pruritus and influence by therapeutic interventions. A circulating factor X drives autotaxin expression in small intestine. This factor can be removed by albumin dialysis, nasobiliary drainage or bile acid resins.

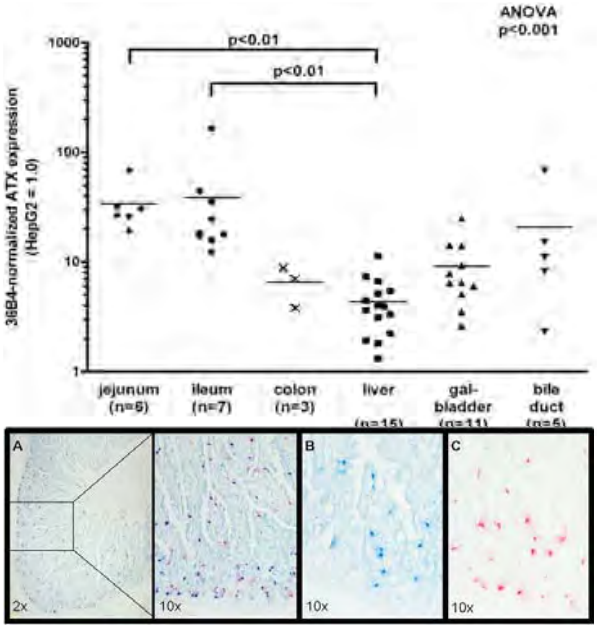
Enteroendcrine cells as source for circulating autotaxin

Performing autotaxin staining in human small intestine we observed a positive staining in a small subset of flat cells mainly located in the epithelial layer. Using various co-stainings (e. g. tryptase for mast cells, CD3 for T cells, CD68 for macrophages, etc.) we observed a large overlap in cells staining positive for chromogranin A. Thus, we could identify autotaxin expressing cells as enteroendocrine cells. In human large intestine we could not find cells staining positive for autotaxin. As expected, we could not observe a positive staining in murine small and large intestine, which was in line with the observed mRNA expression.

HDAC inhibitors rise autotaxin expression in human small intestine

Next we were interested in the regulation of autotaxin expression in human enteroendocrine cells. We therefore obtained a human enterendocrine cell line KRJ-1 (kindly provided by M. Kidd and I. Modlin, Yale, US). While neither cholephilic substances such as bile salts nor serum or bile of cholestatic patients did not increase autotaxin expression in KRJ-1, the HDAC inhibitor trichostatin A increased autotaxin expression 3-4 fold. Intriguingly, after pre-treatment with trichostatin A we observed an induction of ATX by bile salts and serum of patients

Dr. Dr. Kremer



High autotaxin expression in human small intestine on mRNA level. Protein expression of ATX (blue) was detected in chromogranin A positive cells (red) which correspond to enteroendocrine cells.

suffering from pruritus. We further aim to unravel this ATX inducting factor in serum of patients.

Increased autotaxin levels in atopic dermatitis

Interestingly, we and others could show that autotaxin was also increased in patients suffering from atopic dermatitis and that these levels correlated with itch intensity. Thus, autotaxin inhibitors and LPA receptors blockers could represent novel antipruritc treatment strategies in a broad number of patients suffering from chronic pruritus.

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Invited lectures

4. Allergiesymposium des interdisziplinären Allergiezentrums der MHH, November 7, 2015, Hannover, „Darm mit Charme für Allergologen“

5. Hepatologie-Update, Hamburger Lebertage, May 29-30, 2015, Hamburg, “Haut, Juckreiz und die Leber“

197. Falk Symposium, May 8-9, 2015, Lissabon, “Pathogenesis and management of pruritus in PBC and PSC”

121. Jahrestagung der Deutschen Gesellschaft für Innere Medizin (DGIM), April 18-21, 2015, Mannheim, „Juckreiz – ein interdisziplinäres Problem; Juckreiz bei Cholestase“

73rd Annual Meeting of the American Association for Dermatology (AAD), March 20-24, 2015, San Francisco, “Chronic Pruritus: Bedside to Bench Perspectives”

Forscherseminar Charité Berlin, January 19, 2015, Berlin, “Cholestatic pruritus – facts and fiction”

Awards

German Liver Foundation Awardee, Dr. Andreas Kremer, January 30, 2015, München

Publications during funding period

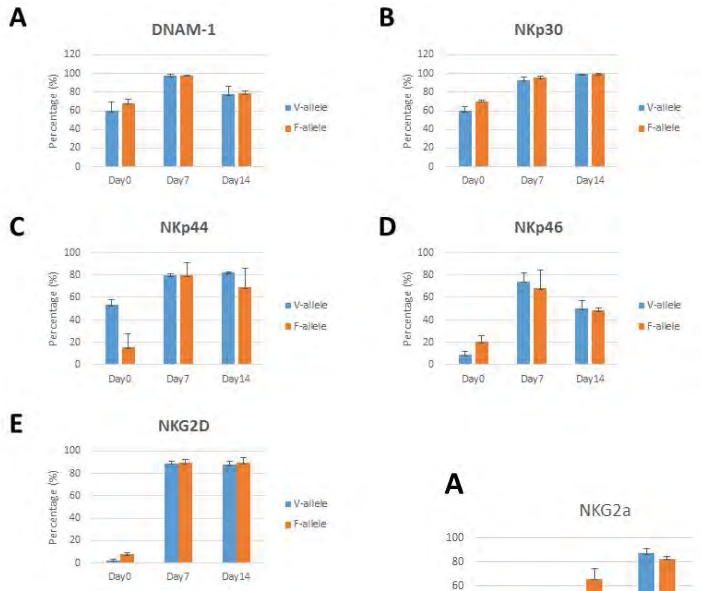
Kremer AE, Gonzales E, Schaap FG, Oude Elferink RP, Jacquemin E, Beuers U (2015). Serum autotaxin activity correlates with pruritus in pediatric cholestatic disorders. J Ped Gastro Nutr. Doi: 10.1097/MPG.0000000000001044 [Epub ahead of print]

Kremer AE, Namer B, Bolier R, Fischer MJ, Oude Elferink RP, Beuers U (2015). Pathogenesis and management of pruritus in PBC and PSC. Dig Dis 33: 164-175

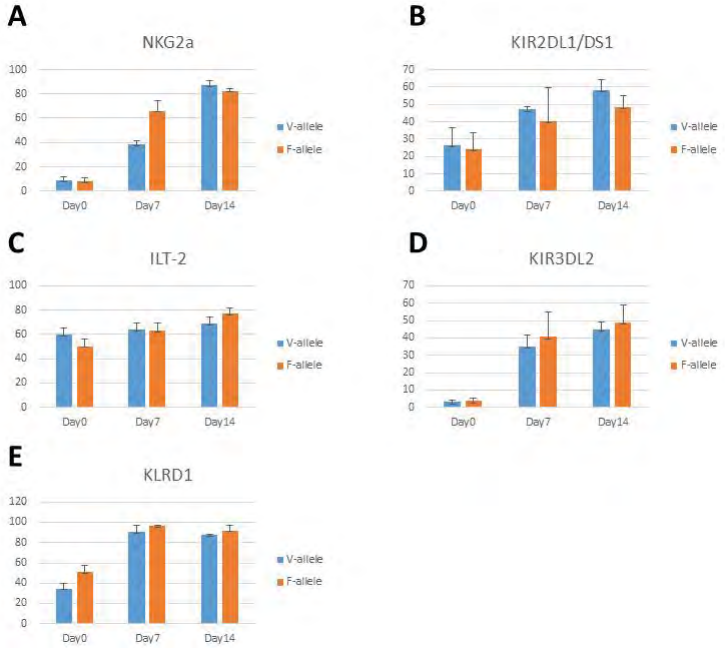
Adoptive cell therapy with ex-vivo expanded NK and γδT cells in metastatic melanoma

Dr. Caroline Bosch-Voskens, Department of Dermatology

Tumor cells can escape a T cell attack in many ways, including by down-regulation of HLA class I molecules. Innate immune cells kill tumor cells in a HLA-unrestricted fashion and as such, the adoptive transfer of natural killer (NK) cells and γδT cells is an attractive strategy to boost T cell immunity. This project aims to develop a GMP-compliant protocol to expand NK and γδT cells from melanoma patients and simultaneously tests the significance of FcγRIIIa polymorphisms on NK and γδT cell activation.



Activating receptors on NK cells from healthy donors expressing one V-allele or two F-alleles. Expression levels of DNAM-1 (A), NKp30 (B), NKp44 (C), NKp46 (D) and NKG2D (E) were determined before (day 0), during (day 7) and after (day 14) NK cell expansion.



Inhibitory receptors on NK cells from healthy donors expressing one V-allele or two F-alleles. Expression levels of NKG2a (A), KIR2DL1/DS1 (B), ILT-2 (C), KIR2DL2 (D) and KLRD1 (E) were determined before (day 0), during (day 7) and after (day 14) NK cell expansion.

Dr. Bosch-Voskens

Successful cancer immunotherapy does not solely depend on the effective activation or transfer of cytotoxic T cells. It requires the design of therapeutic combinations which augment anti-tumor responses and simultaneously overcome tumor-specific escape mechanisms. Tumor cells can escape an T cell attack in many ways, including by down-regulation of HLA class I molecules. Innate immune cells kill tumor cells in a HLA-unrestricted fashion and as such, the adoptive transfer of natural killer (NK) and γδT cells is an attractive strategy to boost T cell immunity.

One means to augment the therapeutic benefit of adoptively transferred NK and γδT cells is to define the patients most likely to respond. Growing experience with antibody therapy shows that select patients experience superior clinical outcomes based upon FcγRIIIa polymorphisms. The most relevant polymorphism depends on the presence of a phenylalanine (F) or valine (V) at amino acid position 158 within the FcγRIIIa receptor. In general, NK cells derived from individuals expressing the FcγRIIIa polymorphism with higher affinity for IgG1 (V/V genotype) show superior natural cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC). In the current funding period a close collaboration was established with the research group of Prof. B. Sriewald (Department of internal medicine 5, Hematology and Oncology, Friedrich-Alexander Universität Erlangen). Within this collaboration, by conventional PCR predefined F- and V-allele frequencies were re-evaluated by a highly sensitive TaqMan® SNP Genotyping assay. Using this assay, 8,7% of patients were homozygous for the V-allele, 42,0% of patients were homozygous for the F-allele and 49,3% of patients were heterozygous and expressed both an V- and an F-allele. These frequencies are similar to those published in prior studies with predominantly Caucasian populations. Unfortunately, due to the limited number of patients solely expressing an V-allele, no analysis could be performed with this subgroup of patients. In the next funding period, a second set of melanoma patients will be genotyped for FcγRIIIa polymorphisms in order to perform comparison studies between patients who are homozygous for the V- and F-allele, respectively.

Alternatively, as a first step, we analyzed the expression of receptors associated with NK cell activation and inhibition in healthy donors bearing an V/V-, V/F- or F/F polymorphism before and after ex vivo NK cell expansion. While initial differences in expression levels of the receptors NKp44, NKp46 and KLRD1 were observed, these differences were overcome after NK cell expansion. These data may suggest that resting NK cells derived from V/V individuals are better natural killers compared to NK cells from F/F individuals. Additional studies are ongoing to define the impact of FcγRIIIa genotype on NK cell degranulation and cytolytic activity against NK cell sensitive tumor cells.

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Publications during funding period

none

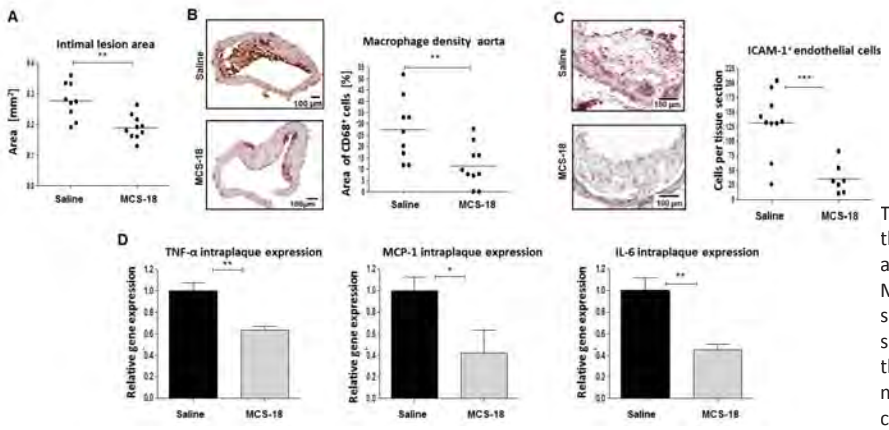
MCS-18 for the treatment of atherosclerosis

Dr. Barbara Dietel, Department of Medicine 2 – Cardiology and Angiology

Progression of atherosclerosis is associated with pronounced inflammatory processes, such as the recruitment of leukocytes and their adhesion to the endothelium. The aim of this project was, to investigate the impact of the herbal substance MCS-18, an anti-inflammatory root compound of helleborus purpurascens which has been shown to exhibit protective effects in murine atherosclerosis onset, on plaque progression in a mouse model of advanced atherosclerosis and on proatherogenic processes in vitro.

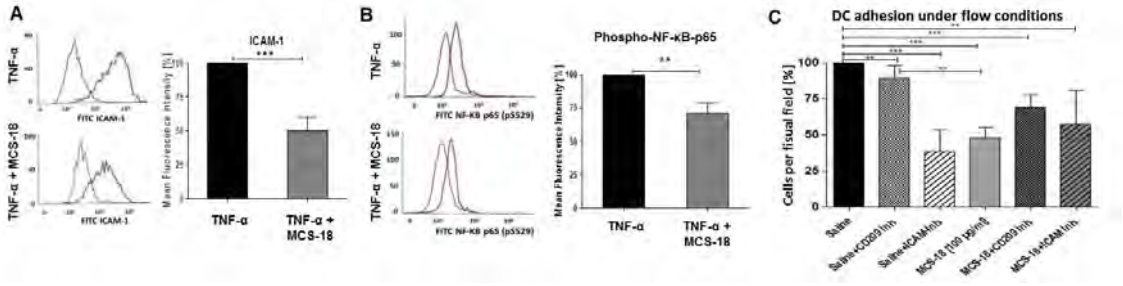
Therapeutic impact of MCS-18 on advanced atherosclerosis

Atherosclerosis is associated with chronic inflammatory responses of the arterial blood vessels. Here, we investigated the impact of the anti-inflammatory compound MCS-18 on murine plaque progression in advanced atherosclerosis and on proatherogenic processes. ApoE-deficient mice were fed a high-fat diet for 12 weeks to induce atherosclerosis, followed by normal chow and intraperitoneal injections of either MCS-18 or saline for another 12 weeks. Plaque size was reduced in MCS-18 treated mice compared to controls, which was associated with a reduced size of the lipid core, indicating an increased plaque stability. In addition, MCS-18 led to significantly lower counts of apoptotic cells and an increased collagen content in atherosclerotic lesions, which also suggests a more stable plaque phenotype. While no changes were detectable with regard to DC maturation in lymphoid organs and numbers of plaque infiltrated mature DCs, immunohistochemical analyses showed that macrophage density and number of ICAM-1 expressing endothelial cells was considerably decreased in plaques of MCS-18-treated mice. Apart from that, quantitative real-time PCR was performed from atherosclerotic lesions of the carotid arteries, which demonstrated reduced transcription levels of intraplaque proinflammatory cytokines (TNF-α, MCP-1 and IL-6) following MCS-18 treatment, confirming a suppressed inflammation in plaques of those mice compared to control treatment.



Therapeutic impact of MCS-18 on the size and inflammatory state of advanced atherosclerotic lesions. MCS-18 reduces intimal lesion size (A), lesional macrophage density (B), ICAM-1 expressing endothelial cells (C) and intraplaque mRNA levels of proatherogenic cytokines and chemokines (D).

Dr. Dietel



Antiatherogenic impact of MCS-18 on HUVECs under flow conditions. MCS-18 decreases levels of ICAM-1 (A) and of intracellular phosphorylated NF-κB-p65 (B) in perfused HUVECs. DC adhesion to a HUVEC monolayer is reduced to the same extent as observed following blocking of ICAM-1 (C).

Impact of MCS-18 on proatherogenic processes in vitro

In addition, human and murine dendritic cells (DCs) and human umbilical vein endothelial cells (HUVECs) were treated with MCS-18 to analyze cell migration and adhesion under flow conditions. In human DCs, flow cytometric analyses showed that MCS-18 reduces the expression of CD209, which is involved in cell rolling along the endothelium. However, blocking of CD209 did not lead to a pronounced reduction of DC migration and adhesion. In addition, MCS-18 also showed a pronounced impact on endothelial cells in vitro. Accordingly, it reduced levels of ICAM-1 and of phospho-NF-κB-p65 in HUVECs under flow conditions, which might depict an essential mechanism for a hampered transmigration of leukocytes into the intima. In the performed in vitro dynamic flow experiments, MCS-18 reduced DC adhesion to the endothelial cell layer in regions of laminar and non-uniform shear stress. While blocking of CD209 in DCs only slightly reduced their adhesion rate, blocking of ICAM-1 in HUVECs led to a significant reduction of DC adhesion. As the co-incubation with MCS-18 and the ICAM-1 inhibitor did not cause any additional reduction of DC adhesion, we speculate that the MCS-18-induced suppression of ICAM-1 in endothelial cells is an important mechanism underlying its antiatherogenic impact.

In summary, our data show that MCS-18 exhibits interesting therapeutic effects in advanced murine atherosclerosis, in which a suppressed adhesion to the endothelium due to downregulation of endothelial ICAM-1 expression might be involved.

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Invited lectures

European Atherosclerosis Society Congress (EAS), March 2015, Glasgow, Anti-inflammatory Effects of MCS-18 on Dendritic Cells and Endothelial cells - Impact on Advanced Atherosclerosis in ApoE-deficient Mice

Annual Meeting of the German Cardiac Society, April 2015, Mannheim, Modulation proatherogener Leukozyteninteraktionen durch die anti-inflammatorische Substanz MCS-18 – Auswirkungen im vorangeschrittenen Atherosklerosemodell der ApoE-/- Maus

Awards

Young Investigator Fellowship Award of the 83th European Atherosclerosis Society Congress, Constanze Kühn, 23th March 2015 in Glasgow

Publications during funding period

Kuehn C, Tauchi M, Stumpf C, Daniel C, Bäuerle T, Schwarz M, Kerek F, Steinkasserer A, Zinser E, Achenbach S, Dietel B (2015) Suppression of Proatherogenic Leukocyte Interactions by MCS-18 - Impact on Advanced Atherosclerosis in ApoE-Deficient Mice. Atherosclerosis. 245:101-110

Dietel B, Muench R, Kuehn C, Kerek F, Steinkasserer A, Achenbach S, Garlichs CD, Zinser E (2014) MCS-18, a natural product isolated from helleborus purpurascens, inhibits maturation on of dendritic cells in apoe-deficient mice and prevents early atherosclerosis progression. Atherosclerosis. 235:263-272

Hypermethylation of SOCS3 in fibrotic diseases

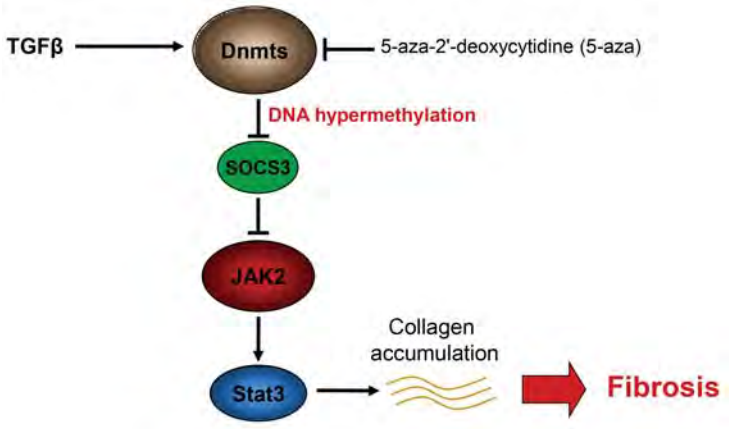
Dr. Clara Dees, Department of Medicine 3 – Rheumatology and Immunology

The project evaluates the role of promoter hypermethylation of SOCS3 in the pathogenesis of fibrotic diseases. Using both pharmacological and genetic approaches like conditional knockout mice, the project examines the mechanisms of TGFβ-induced DNA methylation in fibrogenesis. Given that inhibitors of DNA-methyltransferases are in clinical use for other indications, our study may have direct translational implications.

DNA methylation of CpG islands within promoter regions of genes is a major mechanism of epigenetic gene regulation. In contrast to mutations in the nucleic sequence, DNA methylation is reversible and can be targeted by several pharmacological inhibitors. Our project aimed to analyze the effects of the repression of Suppressor of Cytokine Signaling 3 (SOCS3) by promoter methylation and to evaluate the mechanism of TGFβ-induced DNA methylation in the context of fibrosis.

Our previous findings showed that inhibition of DNA methyltransferases (Dnmts) by 5-aza-2'-deoxycytidine (5-aza) prevented fibrosis in different mouse models and induced regression of pre-established fibrosis. We also could demonstrate a TGFβ-induced silencing of SOCS3 by promoter hypermethylation. The knockdown of SOCS3 in fibroblasts resulted in increased myofibroblast differentiation and collagen release in vitro and in exaggerated dermal fibrosis in different mouse models in vivo.

During further analyses of the mechanism of TGFβ-induced DNA methylation, we showed that the promoter methylation by TGFβ is mediated by a time-dependent induction of Dnmt3a and Dnmt1. In contrast to Dnmt1, whose expression is indirectly dependent on TGFβ and requires protein neosynthesis, the expression of Dnmt3a is directly regulated by Smad3/4 as several Smad binding elements (SBE) have been found in the promoter of Dnmt3a by in silico analyses, and knockdown of Smad3 or Smad4 inhibited the TGFβ-induced expression of Dnmt3a.

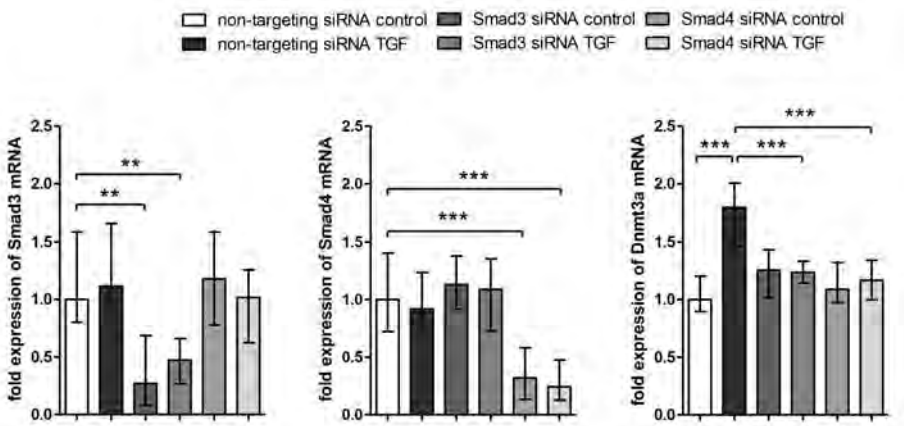


Proposed mechanism of action. Based on our findings, we postulate the following mechanism: The expression of Dnmts is upregulated by TGFb leading to the silencing of SOCS3 by DNA hypermethylation, which then results in increased JAK2-STAT3 signaling.

Dr. Dees

In order to evaluate the influence of Dnmt3a on TGFβ-induced collagen accumulation and silencing of SOCS3, we performed knockdown experiments by transfection of siRNA directed against Dnmt3a. SiRNA-mediated knockdown of Dnmt3a not only blocked the increased collagen expression and release induced by TGFβ, but also prevented the TGFβ-mediated downregulation of SOCS3.

We further confirmed the results obtained in vitro by inducing fibrosis in mice with fibroblast-specific knockout of Dnmt3a. Fibroblast-specific deficiency for Dnmt3a prevented bleomycin-induced fibrosis as well as fibrosis induced by intracutaneous injections of type 5 AAVs expressing constitutively active TGFβ receptor type I (TGFβRIact-AAV5). In both models, mice deficient for Dnmt3a in fibroblasts showed significantly reduced dermal thickness and hydroxyproline content as compared to mice with normal levels of Dnmt3a. Additionally, we also observed a decrease in the differentiation of resting fibroblasts into metabolically active α-smooth muscle actin (αSMA) positive myofibroblasts.



The TGFβ-induced expression of Dnmt3a is dependent on canonical Smad3/4 signaling. Transfection of siRNA targeted against Smad3 or Smad4 efficiently reduced the increase in Dnmt3a expression upon stimulation with TGFβ.

To evaluate the inhibition of Dnmts as treatment for other fibrotic diseases than dermal fibrosis, the treatment with 5-aza in bleomycin-induced lung fibrosis is currently ongoing.

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Publications during funding period

none

PU.1 signalling in fibrotic diseases

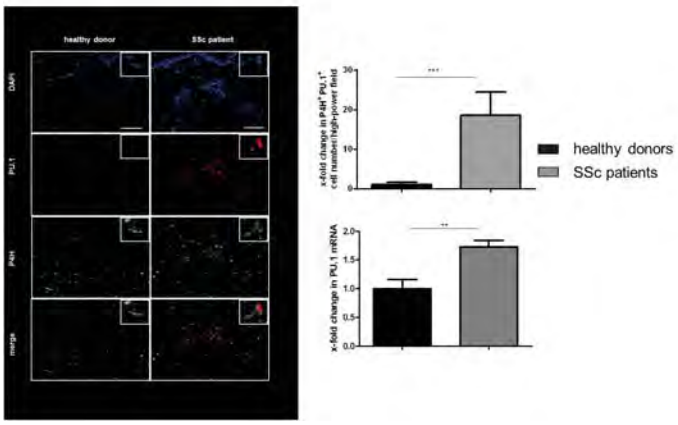
Dr. Andreas Ramming, Department of Medicine 3 – Rheumatology and Immunology

PU.1 belongs to the E26-transcription-specific (Ets) family of proteins and plays an important role in maturation, differentiation and proliferation of hematopoietic cells. In mesenchymal cells like fibroblasts PU.1 is normally quiescent. However, we detected high levels of PU.1 in fibroblasts from patients with systemic sclerosis (SSc). Moreover, PU.1 is highly responsive to TGF-β stimuli in SSc fibroblasts whereas it remains silent in fibroblasts from healthy individuals.

The transcription factor PU box binding-1 (PU.1) belongs to the E26 transformation-specific (ETS) family of proteins and plays an important role in the functional competence of several haemopoietic lineages such as B cells and macrophages. In PU.1 deficient mice, wound healing appears without granulation tissue formation and fibrosing. However, the impact of PU.1 on the development of fibrotic diseases is unknown.

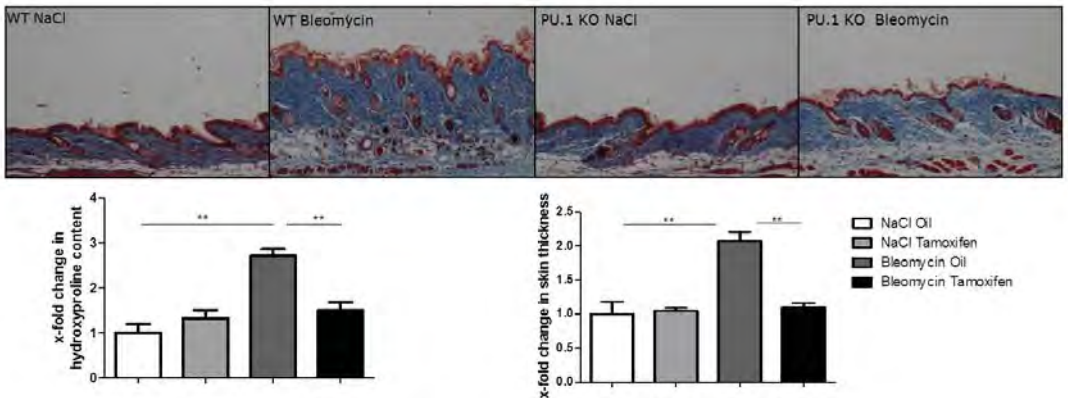
Fibrotic diseases can affect virtually every organ system. They can be restricted to single organs as in idiopathic pulmonary fibrosis (IPF), or may affect multiple organs as in systemic sclerosis (SSc). The histopathological feature of SSc is an excessive accumulation of extracellular matrix that often disrupts the physiological architecture of the affected tissue. Fibroblasts are the principle source of extracellular matrix and have been identified as key players of fibrotic disorders. Although the exact pathomechanisms in SSc remain unclear, there is considerable evidence that transforming growth factor-beta (TGF-β) is a key regulator of fibroblast activation. The activated fibroblasts can differentiate into myofibroblast-like cells, leading to an excessive release of extracellular matrix proteins, especially collagens, which accumulate in the fibrotic tissue.

Here, we aimed to characterize the role of PU.1 in the pathogenesis of fibrotic diseases such as SSc. We identified PU.1 as a TGF-β target gene that is outstandingly up-regulated in SSc fibroblasts and in the fibrotic tissue. After in vitro stimulation with TGF-β, PU.1 expression increased specifically in fibroblasts of SSc patients. In contrast, PU.1 is not expressed in fibroblasts of healthy individuals suggesting the hypothesis of PU.1 as a pathophysiologically important protein in fibrotic disorders. Moreover, PU.1 is up-regulated in the mouse model of bleomycin-induced fibrosis. Whereas bleomycin-induced fibrosis serves as a model of early inflammatory stages of SSc, in which fibroblasts are mainly activated by pro-fibrotic mediators released from infiltrating leukocytes, the Tsk-1 model resembles less inflammatory stages of SSc with endogenous activation of resident fibroblasts. Notably, increased levels of PU.1 were also detected in the skin of Tsk-1 mice suggesting a potential role of PU.1 in early as well as in



Skin sections of healthy donors and SSc patients stained for DAPI, prolyl-4-hydroxylase (P4H), and PU.1, 200x magnification and 600x magnification.

Dr. Ramming



(A) Model of bleomycin induced skin fibrosis. Masson trichrome staining of skin tissue from control mice (Wild-type (WT) treated with sodium chloride), bleomycin treated mice and mice with fibroblast specific knockout of PU.1 treated with either sodium chloride or bleomycin. (B) Dermal thickness. (C) Hydroxyproline content. *p<0.05; **p<0.01; ***p<0.001.

established fibrosis. The functional impact of PU.1 in the pathogenesis of fibrosis could be demonstrated in two genetic approaches. The selective PU.1 deficiency in fibroblasts led to an almost complete protection from fibrosis in the mouse models of bleomycin induced skin/lung fibrosis and the sclerodermatous chronic Graft-versus-Host disease (scGvHD) model.

Together, our studies give rise for direct translational implications of targeting PU.1 signaling as a new anti-fibrotic strategy of treatment. The acquired data will be tested in further experimental models of fibrosis to evaluate the relevance in fibrotic settings and to further validate the translational implications of PU.1 signaling in fibrotic diseases.

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Publications during funding period

Wohlfahrt T, Usherenko S, Englbrecht M, Dees C, Weber S, Beyer C, Gelse K, Distler O, Schett G, Distler JH*, Ramming A* (205) Type 2 innate lymphoid cell counts are increased in patients with Systemic Sclerosis and correlate with the extent of fibrosis. Ann Rheum Dis. 2015 doi: 10.1136/annrheumdis-2015-207388. [Epub ahead of print] * contributed equally

Ramming A, Dees C, Distler JH (2015) From pathogenesis to therapy - Perspective on treatment strategies in fibrotic diseases. Pharmacological research: the official journal of the Italian Pharmacological Society. 100: 93-100

Huang J, Beyer C, Palumbo-Zerr K, Zhang Y, Ramming A, Distler A, et al. (2015) Nintedanib inhibits fibroblast activation and ameliorates fibrosis in preclinical models of systemic sclerosis. Ann Rheum Dis. doi: 10.1136/annrheumdis-2014-207109. [Epub ahead of print]

Mallano T, Palumbo-Zerr K, Zerr P, Ramming A, Zeller B, Beyer C, et al. (2015) Activating transcription factor 3 regulates canonical TGFbeta signalling in systemic sclerosis. Ann Rheum Dis. doi: 10.1136/annrheumdis-2014-206214. [Epub ahead of print]

Resolution of inflammation in gout

Dr. Christine Schauer (née Schorn), Department of Medicine 3 – Rheumatology and Immunology

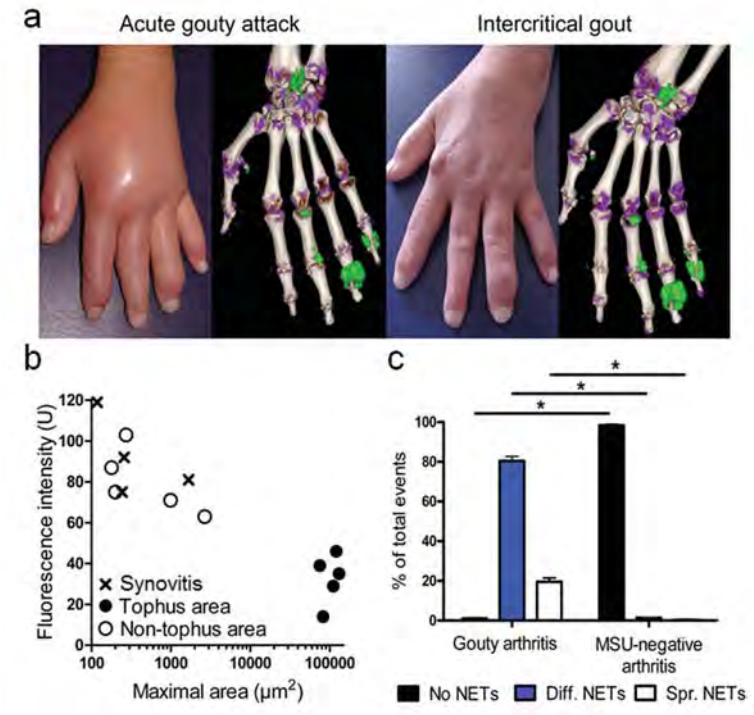
Acute gouty arthritis is a self-limiting process despite persistent monosodium urate (MSU) crystals. For this big enigma of gouty arthritis we propose the following model: In the early phase, MSU crystals induce the formation of solitaire neutrophil extracellular traps (NETs) and huge amounts of pro-inflammatory cytokines. In the late phase in the presence of a high neutrophil density, NETs aggregate and form dense gouty tophi. The latter immobilize MSU and degrade pro-inflammatory mediators.

Characterization of NETs in tophi and synovial fluid from human patients with gout

Clinically overt gouty tophi and subclinical urate deposits in humans can be detected by dual energy computed tomography (DECT). In DECT images of individuals with gout, we noticed that deposits of urate detected during a highly inflammatory phase persisted during a clinically silent (intercritical gout) phase.

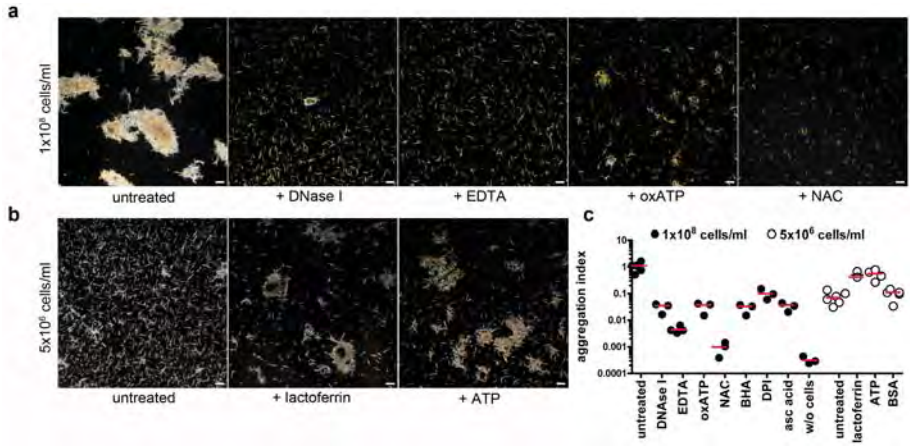
Next we analyzed whether NETs, extranuclear DNA structures colocalizing with material from neutrophil granules, can be found in human tissue sections of subjects with gout and control individuals. Extranuclear DNA colocalizing with NE was abundant in the tophus, whereas in the non-tophus area and in MSU-negative synovitis, neutrophils were sparse, and the DNA exhibited a predominantly nuclear appearance. Quantification of the area covered by propidium iodide (PI)-positive material and of the fluorescence intensity of the PI signal indicated large stretches of diluted extracellular DNA spread over the tophus as compared to a more regular nuclear morphology in the adjacent tissue and in non-gouty synovitis.

We analyzed cytospins from synovial fluid obtained from individuals with gout and MSU-negative arthritis and detected extracellular thready chromatin colocalizing with NE only when the synovial fluid contained MSU crystals. After classifying NETs



(a) DECT scans during acute inflammation and intercritical phase. (b) Fluorescence intensity and PI-positive area in patients with tophaceous gout and MSU-free arthritis. (c) Nuclear morphology in synovial fluids from patients with gout and MSU-negative arthritis.

Dr. Schauer



(a) Polarization microscopy of PMN cultured in high (a) and low (b) densities with MSU and inhibitors. (c) Quantitative analysis of the polarization pictures of (a) and (b).

into diffused and spread NETs as described previously, we found a significantly higher number of cells that had undergone NETosis in the samples from gout than in those from MSU-negative arthritis.

Aggregation of NETs depends on lactoferrin and ATP

Inflamed tissues are characterized by dense infiltration of neutrophils. To mimic the situation during acute inflammation in vivo, we increased the density of neutrophils in our in vitro NETosis assays to values typically encountered in densely infiltrated tissue (10^8 neutrophils ml−1). Under these conditions, MSU crystals induced aggregation of NETs within 10 min. In cryosections of these aggregates, we found extracellular DNA colocalized with granule proteins that resembled gouty tophi. High doses of DNase I, the bivalent cation-chelating agent EDTA, the ATP antagonist oxidized ATP (oxATP) and the ROS inhibitor N-acetylcysteine (NAC) inhibited the formation of these aggNETs.

MSU crystals promoted aggregation in a dose-dependent manner and at a concentration as low as 50 µg ml−1, suggesting that the aggregation we observed in vitro could also occur under physiological conditions in inflamed tissue. In contrast, in low-density cultures (5 × 10^6 neutrophils ml−1), we observed NETosis but no aggregation of NETs after incubating with MSU. The size of the aggNETs depended on the density of neutrophils in the culture only and was not influenced by the addition of peripheral blood mononuclear cells. However, addition of the autocrine neutrophil stimulants ATP or lactoferrin to low-density cell cultures promoted the formation of aggNETs, whereas treatment with BSA as a control had no such effect.

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Publications during funding period

Schauer C, Janko C, Munoz LE, Zhao Y, Kienhöfer D, Frey B, Lell M, Manger B, Rech J, Naschberger E, Holmdahl R, Krenn V, Harrer T, Jeremic I, Bilyy R, Schett G, Hoffmann M, Herrmann M (2014) Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat Med 20(5): 511-7

Bayesian reverse engineering of developmental networks

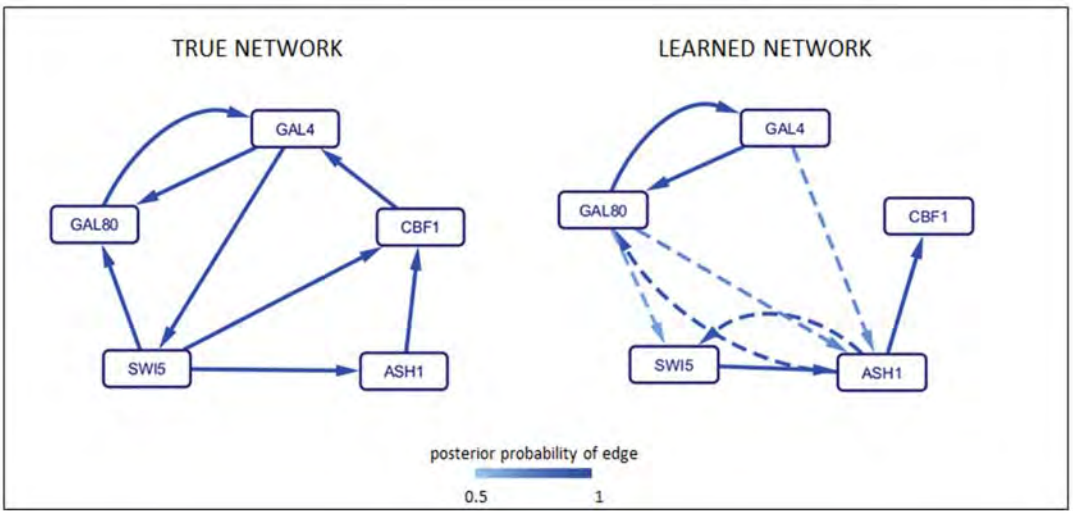
Dr. Fulvia Ferrazzi, Institute of Human Genetics

The project aims at developing a Bayesian approach to reverse engineer gene regulatory networks from expression time series and prior knowledge. The methodology was validated on gold-standard datasets and afterwards employed to analyze a high resolution temporal expression dataset describing heart development. These data have the potential to shed light on congenital heart disease, cardiac stem cell differentiation and regeneration.

Gene networks offer a flexible framework to represent and analyze interactions between genes. Moreover, they can support the identification of novel hypotheses on regulatory processes. On the basis of measured expression data, reverse engineering methodologies aim at inferring the underlying gene regulatory network. It has been shown that the introduction of prior knowledge (i.e. curated information available in online repositories) in the network learning process can improve the accuracy of the inferred models.

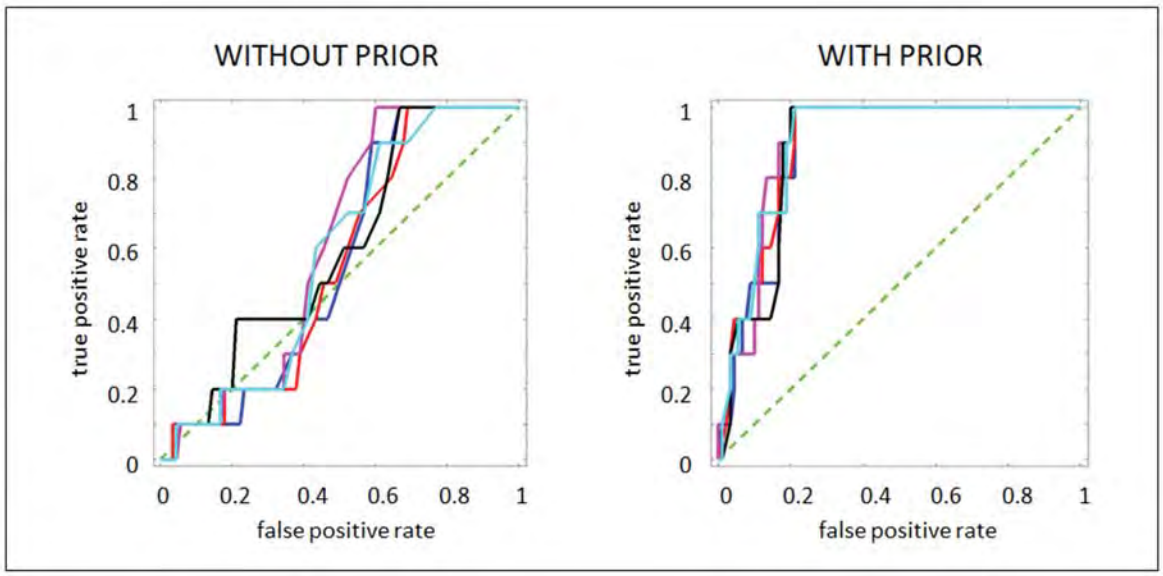
Dynamic Bayesian networks to integrate expression data and prior knowledge

We developed a reverse engineering methodology based on dynamic Bayesian networks (DBNs) to integrate prior knowledge in the learning of gene networks from temporal expression data. DBNs are probabilistic graphical models that are able to describe the dynamics of gene expression. The use of a Bayesian framework can capture intrinsic inference uncertainty and in addition offers a principled way to integrate prior information in learning. As a source of prior knowledge the STRING database of known and predicted protein interactions was chosen. It combines several resources and currently constitutes one of the most comprehensive interaction repo-



True yeast five-gene network from DREAM2 (left) and learned network (right; dashed edges are false positive connections). Introduction of prior knowledge increases sensitivity from 0.25 to 0.5 and precision from 0.29 to 0.44.

Dr. Ferrazzi



Receiver operating characteristic (ROC) curves for the yeast ten-gene network of DREAM3 obtained in five different MCMC runs. With prior integration the area under the curve (AUC; calculated taking into account all the runs) increases from 0.58 to 0.89.

sitories. Moreover, information about the strength of the interactions is provided via a confidence score, which we transformed into prior probabilities of gene network edges to be employed during network learning. In order to search across the space of potential network structures we relied on the MCMC Metropolis-Hasting algorithm.

We validated our methodology on gold-standard networks, taken from past DREAM challenges (http://dreamchallenges.org/) and for which the real underlying biological regulations are known. The obtained results show that the inclusion of prior knowledge improves the accuracy of the learned networks.

Application of the methodology to a mammalian heart development dataset

The here developed methodology was subsequently applied to a temporal expression dataset describing rat heart development from embryonic day 11 to postnatal day 10, generated in collaboration with Prof. F.B. Engel. In this case, a “meta-gene” network was learned: the network variable is not anymore represented by the expression of a single gene, but by a gene cluster. This approach is promising since it offers a global and concise view of the examined biological system. Computational analyses of the network allow the generation of novel hypotheses to be experimentally validated and employed to refine the learned model.

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Invited lectures

Clinical Bioinformatics Course - Systems Biology and Network Medicine, University of Pavia, Italy, 29th May 2015, “Network-based approaches for the study of cardiac regeneration”

Publications during funding period

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

The role of IL-33/ST2 signaling in the development of infectious colitis

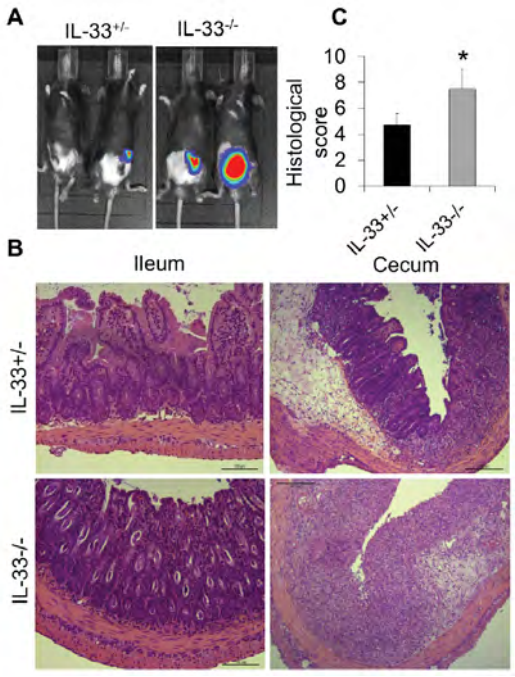
Dr. Tamar Mchedlidze, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Infections caused by gram-negative bacteria of the genus Salmonella are considered as a world-wide health problem. Our results indicate that IL-33/ST2 axis is important for protection from severe Salmonella-dependent gastrointestinal disease. We plan to elucidate the mechanisms through which the IL-33 pathway contributes to various stages of disease manifestation and progression after Salmonella infection.

The aim of the project is to investigate the role of the alarmin-like cytokine IL-33 in the context of gastrointestinal infection induced by the proteobacterial model organism S. *typhimurium*. IL-33 is crucial in sensing damage during inflammatory conditions and therefore potentially plays an important role in immunity against infections. Previous studies implicated this cytokine in bacterial sepsis, but still the precise role of IL-33 in intestinal diseases remains poorly understood.

Our initial data showed upregulation of IL-33 after S. *typhimurium* infection both on protein and mRNA level.

In order to analyze the impact of IL-33 in dissemination of microbial pathogens in more detail, we generated IL-33$^{-/-}$ mice that possess an Nramp1 (natural resistance-associated macrophage protein 1) wild-type allele, allowing them to control systemic outgrowth of bacterial infections. Infection of IL33$^{-/-}$ Nramp1^{wt} mice with S. *typhimurium* resulted in increased bacterial burden in IL33-deficient ani-

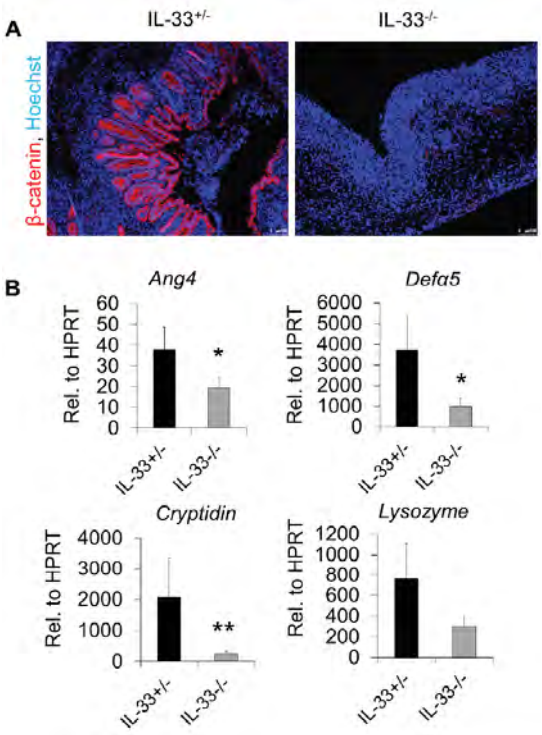


A. IL-33$^{-/-}$ or IL-33$^{+/-}$ mice were infected orally with bioluminescent S. *typhimurium* and measured by in-vivo imaging. B. Ileal and cecal paraffin sections were stained with H&E. C. Cecum of infected mice were histologically scored for tissue damage.

Dr. Mchedlidze

mals compared with littermate controls. Besides, lack of IL-33 was associated with increased immune cell infiltration and tissue damage in cecum and ileum. Moreover, extensive epithelial cell loss was detected by immunofluorescent staining of β-catenin. In addition, secretory epithelial cells (including Paneth and goblet cells), which are known to be crucial for the host defense against intestinal pathogens, were dramatically reduced in knock-out mice. Quantitative PCR analysis of Paneth cell-derived antimicrobial peptides such as Angiogensin 4, Defensin α5, Cryptidin and Lysozyme demonstrated a substantial decrease in IL-33$^{-/-}$ mice compared to controls. This defect in antimicrobial defense in the absence of IL-33 might explain the more severe phenotype of the knock-out mice.

Commensal intestinal microflora is known to strongly affect bacterial colonization. To analyze if microbial composition could be responsible for increased susceptibility of IL-33$^{-/-}$ mice, we elucidated if IL-33 in the steady-state impacts microbial communities in the gut. Interestingly, 16S-based next generation sequencing analysis of IL-33 deficient and wild-type littermates demonstrated that there are no significant changes in the microbiome.



A. Cecal cross-sections of S. *typhimurium* infected IL-33$^{-/-}$ or IL-33$^{+/-}$ mice were stained for β-catenin. B. Quantitative PCR analysis of anti-microbial peptides: Angiogenin-4, Defensis α5, cryptidin and Lysozyme.

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Awards

Bright sparks award (Efis-Biolegend), Mchedlidze T., 6-9 September 2015, 4th European Congress of Immunology"- Austria.

Publications during funding period

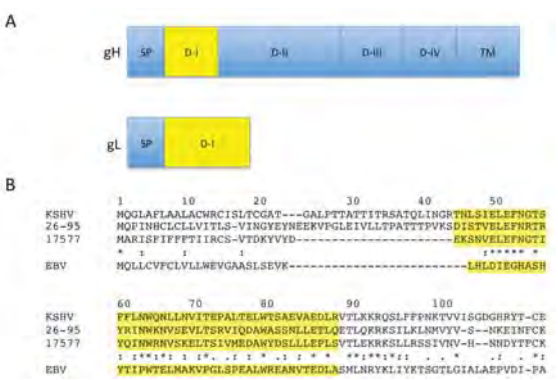
none

Rhadinovirus Entry Receptors

Dr. Alexander Hahn, Institute of Clinical and Molecular Virology

Through comparative studies of Kaposi's sarcoma-associated herpesvirus and the related rhesus monkey rhadinovirus, we were able to identify a conserved binding site for cellular Eph receptors in the gH/gL glycoprotein complex of both viruses. Mutation of this site demonstrates that at least RRV can also make use of alternative receptors for infection of target cells.

Kaposi's sarcoma-associated herpesvirus (KSHV) is a human oncogenic virus associated with Kaposi's sarcoma (KS) and two B-cell proliferative disorders. Together with the closely related rhesus monkey rhadinovirus (RRV) - an animal model virus for KSHV - it belongs to the family of rhadinoviruses (gamma2-herpesviruses). KSHV and RRV share many biological features, such as a highly similar genome structure, use of B cells as a site of latency, and association with a similar range of lymphomas.

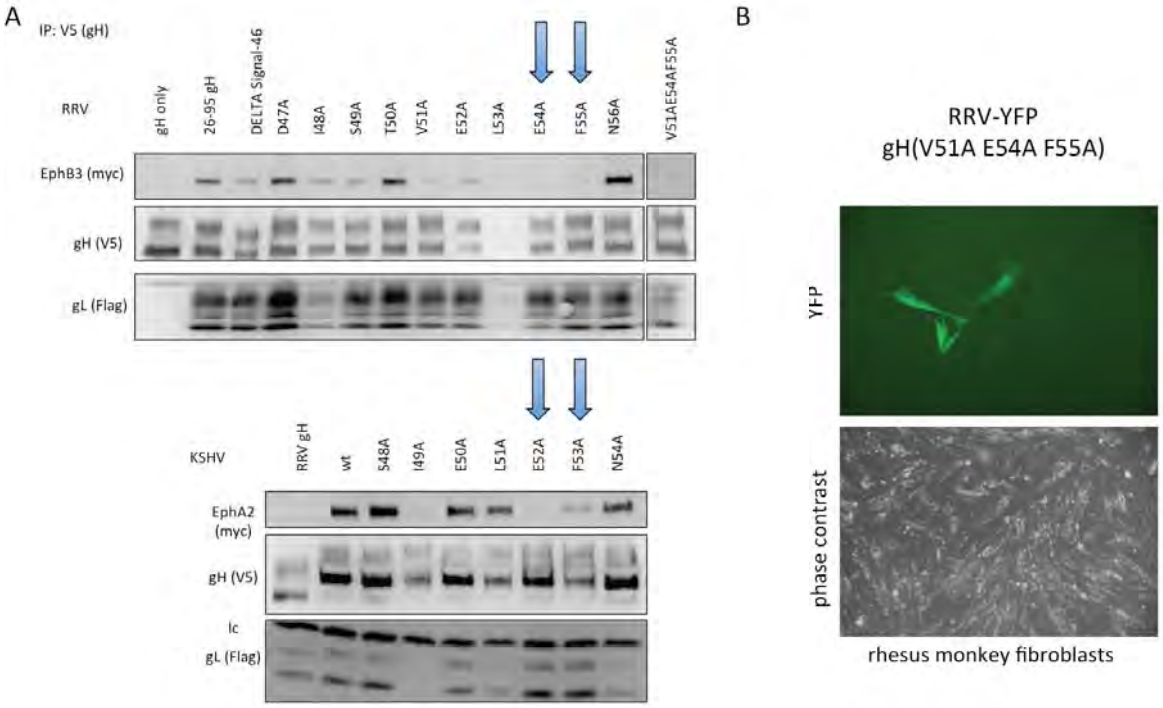


A conserved amino acid motif in the N-terminal domain of the RRV and KSHV gH/gL complex. (A) Putative domain structure of RRV/KSHV gH/gL. (B) Sequence comparison of domain I of two RRV subtypes, KSHV, and EBV.

Recently, we identified Eph family receptor tyrosine kinases (Ephs) as cellular receptors for the gH/gL glycoprotein complex of KSHV (Hahn et al, 2012) and RRV (Hahn et al, 2013). Interaction with Ephs was critical for entry of RRV into endothelial and B cells but not fibroblasts (Hahn et al, 2013). To further characterize the interaction between Ephs and gH/gL of both KSHV and RRV we performed comparative sequence analysis and mutational screens. We identified conserved residues in the N-terminal domain of both RRV and KSHV gH that are critical for Eph binding. Mutation of the putative binding sites resulted in a loss of interaction with soluble versions of Eph receptors in in vitro assays. Based on these results we generated mutant RRV and KSHV negative for Eph binding. The mutant viruses are currently being compared to the corresponding wildtype virus in regard to binding, infectivity and virus yield on different cell types. In agreement with our previous results that postulated Eph-independent infection of fibroblasts by RRV, both an Eph binding-negative gL deletion mutant of RRV and RRV mutated in the Eph binding site on gH are replication-competent on fibroblasts. These results clearly indicate that RRV can make use of alternative receptors for the infection of certain cell types.

Taken together, our results establish the N-terminal domain of the rhadinoviral gH/gL complex as an evolutionarily conserved receptor-binding domain and support a model of cell-specific receptor use, at least for RRV, which would in that respect be similar to e.g. the related Epstein-Barr virus or the cytomegalovirus. Our Eph binding-negative virus mutants will now allow us to identify additional cellular factors important for entry and to characterize the exact function of the Eph proteins during rhadinovirus infection.

Dr. Hahn



(A) Alanine scanning identifies conserved residues that are critical for Eph receptor binding. (B) An Eph binding-deficient RRV is replication-competent on fibroblasts.

Dr. Alexander Hahn has taken up an appointment as group leader at the German Primate Center and will continue his studies there.

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Publications during funding period

none

Modulation of PRC2 activity by HCMV IE2

Dr. Nina Reuter, Institute of Clinical and Molecular Virology

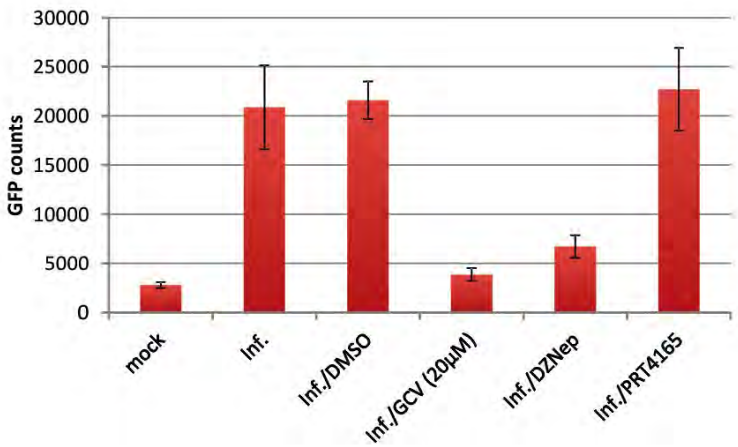
Chromatin-based modifications of herpesviral genomes play a crucial role in dictating the outcome of infection. Host cell multiprotein complexes like the nuclear domain 10 (ND10) or the Polycomb repressive complex 2 (PRC2) have been identified as regulators of viral gene expression on the epigenetic level. This proposal aims at investigating the role of PRC2 for HCMV infection as well as elucidating the mechanisms HCMV has evolved to modulate PRC2 function for its own benefit.

Chromatin-based regulation of HCMV replication

Epigenetic control of HCMV gene expression plays a key role in determining the outcome of infection. Thus, HCMV has to cope with, modulate or utilize the host cell's chromatin machinery to promote an efficient lytic replication or establish a persistent-latent state. Recently, host cell repressor complexes like the nuclear domain 10 (ND10) or the Polycomb repressive complex 2 (PRC2) have been identified that substantially contribute to chromatin-based regulation of HCMV infection. In our preliminary work we could demonstrate that HCMV has to manipulate ND10 functions in order to be able to initiate the productive life cycle. Thus, it is tempting to speculate that also PRC2 activity is modulated by HCMV for its own benefit.

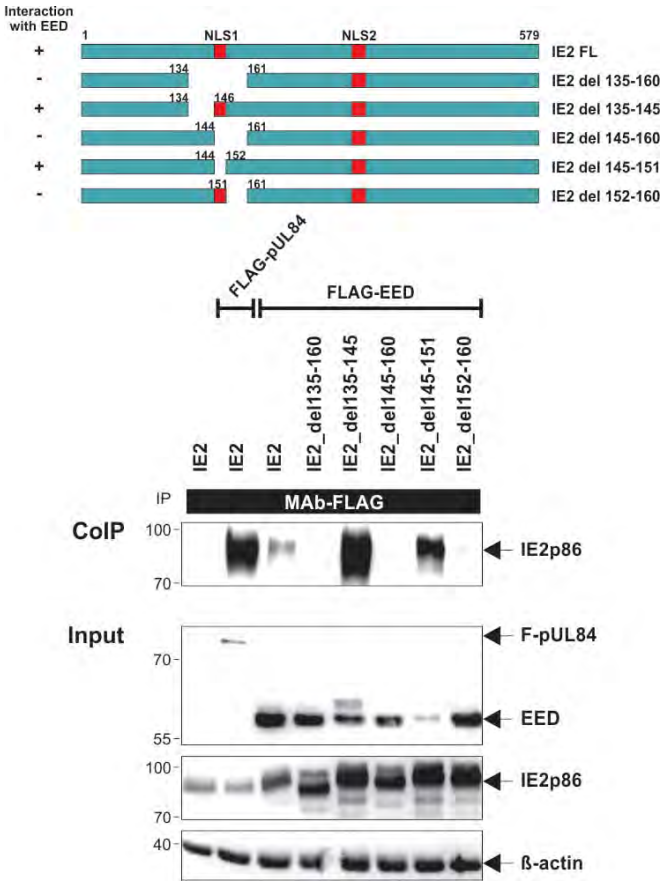
Role of PRC2 during lytic HCMV replication

In order to address the relevance of PRC2 activity for the productive life cycle of HCMV, we dissected the protein levels of PRC2 core components following HCMV infection. Western blot experiments revealed an HCMV-induced upregulation of the major PRC2 factors EZH2, SUZ12, and EED as well as of the PRC2 catalyzed histone modification H3K27me3. By immunofluorescence staining, we found that all major PRC2 components, which are normally evenly distributed throughout the nucleus, relocalize into viral replication compartments as infection progresses. Interestingly, however, the repressive histone mark H3K27me3 instituted by PRC2 turned out to be specifically excluded from these sites. Finally, we compromised PRC2 function following HCMV infection by applying a specific inhibitor of PRC2 methyltransferase activity known as DZNep. Inhibition of PRC2 function clearly impaired HCMV replication as it had a negative impact on viral early and late gene expression which resulted in a reduced release of infectious viral particles. Thus, our findings indicate that PRC2 activity is required for an efficient lytic HCMV replication.



Inhibition of PRC2 activity negatively affects HCMV replication. GFP-based antiviral assay of HFF cells infected with recombinant HCMV-GFP in the presence or absence of inhibitory substances. GCV: Ganciclovir, antiviral nucleoside analogue; DZNep: PRC2 inhibitor; PRT4165: PRC1 inhibitor.

Dr. Reuter



The HCMV regulatory protein IE2 interacts with the PRC2 core component EED. Verification of EED binding-deficient mutants of IE2 by co-immunoprecipitation experiments.

Analysis of the regulation of PRC2 activity by the HCMV effector protein IE2p86 (IE2)

By yeast two-hybrid screening and co-immunoprecipitation (CoIP) analysis, we discovered an interaction between the HCMV transactivator protein IE2 and the PRC2 core factor EED. Furthermore, we could show that IE2 colocalizes with EED in viral replication centers. Hence, this suggests a potential regulation of the repressive multiprotein complex by IE2. In order to elucidate the role of IE2 as a regulator of PRC2 activity, we generated an IE2 mutant that is no longer able to bind to EED. With the help of CoIP experiments we could narrow down the EED interaction domain to amino acids 152-160 of IE2. The generation of a recombinant HCMV expressing this EED interaction-deficient mutant of IE2 in the context of the laboratory strain AD169 will further help us to define the in vivo relevance of the IE2-EED interaction.

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Publications during funding period

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 doi: 10.3390/v8010002 [Epub ahead of print]

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. doi: 10.1128/JVI.01973-15 [Epub ahead of print]

Wagenknecht N, Reuter N, Scherer M, Reichel A, Müller R, Stamminger T (2015) Contribution of the Major ND10 Proteins PML, hDaxx and Sp100 to the Regulation of Human Cytomegalovirus Latency and Lytic Replication in the Monocytic Cell Line THP-1. Viruses 7(6):2884-907

J46 - Progress Report

01.04.2015 - 30.09.2017

Neurosciences

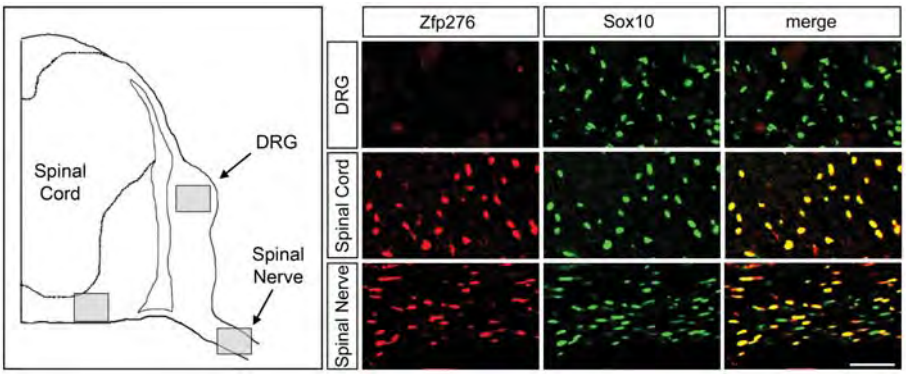
The role of zinc finger protein Zfp276 in glial development of the mouse nervous system

Dr. Melanie Küspert, Institute of Biochemistry

Recently, the poorly characterized transcription factor Zfp276 was identified as a potential new target of the key regulator of PNS and CNS myelination, Sox10. Induced expression of Zfp276 in differentiating oligodendrocytes and Schwann cells during the onset of myelination argued for a role in the regulation of this process. In reporter gene assays dose-dependent activation of the newly identified Zfp276 intronic enhancer by Sox10 was proven. Furthermore, Zfp276 expression was strongly downregulated in vivo in Sox10-deleted mouse oligodendrocytes.

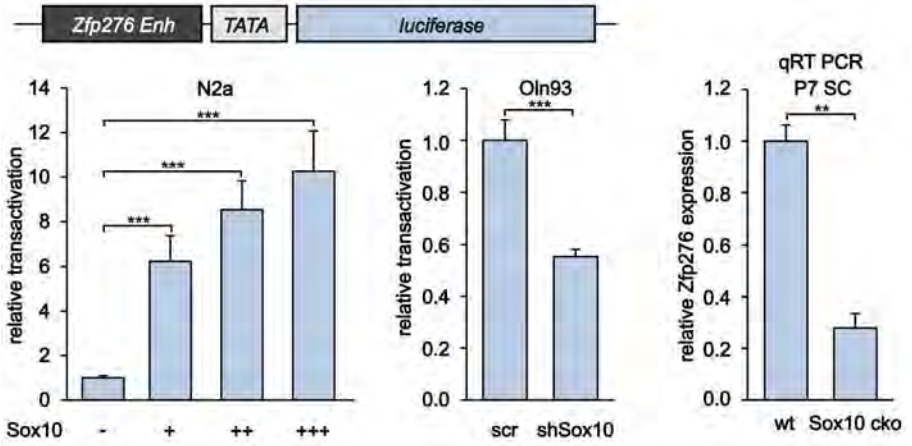
Background

Myelination is a highly regulated process during development of vertebrate PNS and CNS and the transcription factor Sox10 is a known key regulator of Schwann cell and oligodendrocyte differentiation. Deletion of Sox10 leads to complete failure of myelination in the PNS and severe hypomyelination in the CNS of 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 that synergistically act with Sox10 on shared target genes. One prominent example is the zinc finger transcription factor Egr2, known to be essential for expression of Mbp and other major myelin genes during PNS myelination. In contrast, downstream targets of Sox10 that mediate its function on myelin gene expression are largely unknown for the CNS and no zinc finger transcription factor was identified until now that acts equivalently to Egr2 in 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.



Immunohistochemical staining against Zfp276 (red) and the glial marker Sox10 (green) was performed on transverse sections from the forelimb level of P16 wildtype mice. Zfp276 expression is restricted to myelinating glial cells in the PNS and CNS.

Dr. Küspert



Sox10-dependence of the Zfp276 enhancer was shown using a luciferase reporter under control of the Zfp276 enhancer and overexpression of Sox10 or scrambled and Sox10-specific shRNAs. In Sox10-deleted spinal cord Zfp276 transcripts were strongly decreased compared to wildtype.

Zfp276 is differentially expressed in myelinating glia of the PNS and CNS

Using qRT PCR, in situ hybridization and immunohistochemical staining an upregulation of Zfp276 mRNA and induced expression of Zfp276 protein in newly differentiated myelinating Schwann cells and oligodendrocytes was detected in vitro and in vivo. The spatio-temporal expression pattern of Zfp276 within the peri- and early postnatal spinal cord and sciatic nerve as well as co-labeling of Zfp276-positive cells with antibodies directed against the glial factor Sox10 and against the major myelin protein Mbp showed a restricted Zfp276 expression only in myelinating glial cells but not in non-myelinating glial cells of the dorsal root ganglion, arguing for a potential role in the regulation of early myelination events.

Zfp276 expression is regulated by Sox10 in a dose-dependent manner

As indicated by Sox10-ChIP-Seq data and by coexpression of both transcription factors a direct effector-target-relationship between Sox10 and Zfp276 was assumed. Affirming this assumption, Zfp276 expression was strongly reduced in early postnatal Sox10-deficient mouse spinal cord. Based on the Sox10-binding regions in ChIP-Seq experiments and the evolutionary conservation between mammalian species an intronic enhancer of Zfp276 was identified and tested in reporter gene assays for its dependence on Sox10. Both, overexpression and knockdown experiments for Sox10 demonstrated a dose-dependent change of activity of the Zfp276 enhancer. Further experiments will focus on the elucidation of Zfp276 function during myelination in vitro and in vivo.

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Publications during funding period

Küspert M, Wegner M (2015) SomethiNG 2 talk about - Transcriptional regulation in embryonic and adult oligodendrocyte precursors. Brain Research. doi:10.1016/j.brainres.2015.07.024 [Epub print ahead]

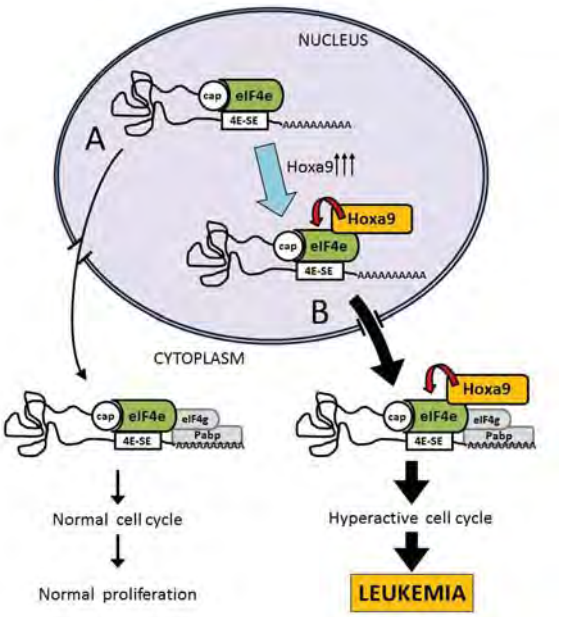
Post-transcriptional regulation by Hoxa9

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.

Background

Hoxa9 is a leukemogenic transcription factor upregulated in aggressive pediatric acute leukemias as well as 50% of adult acute myeloid leukemias (AML). Despite experimental evidence of Hoxa9 playing a major role during leukemia initiation the exact mechanism leukemic transformation by Hoxa9 is poorly understood. Recently, a hitherto unexpected association of Hoxa9 with the post-transcriptional regulator eIF4e was demonstrated, implying that the transcription factor Hoxa9 also acts as a post-transcriptional regulator. One function of eIF4e is to enhance nuclear export of a subset of mRNAs, characterized by the presence of an eIF4e sensitivity element. These mRNAs are predominantly coding for proliferation associated proteins, such as cell cycle regulators. Consequently, eIF4e itself acts as a potent oncogene and is highly expressed in approximately 50% of all human malignancies. Since Hoxa9 was demonstrated to increase eIF4e acitivity in vitro, our aim is to uncover the impact of the Hoxa9-eIF4e interaction on leukemogenesis in general and on the regulation of potential target genes in particular.

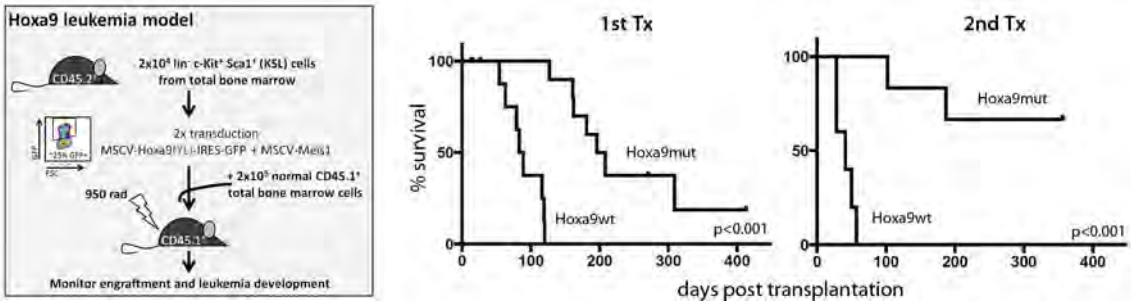


Schematic overview of the hypothesis for leukemia induction by the Hoxa9/eIF4e interaction. Normal (A) eIF4e mediated post-transcriptional cell cycle regulation becomes hyperactivated upon Hoxa9 overexpression (B) resulting in enhanced proliferation and eventually leukemia.

Dr. Bach

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

Previously, we created a variant of Hoxa9 by introducing point mutations into its eIF4e interaction motif. Accordingly, this mutant (Hoxa9YL) was incapable of eIF4e interaction. In order to test the leukemogenicity of Hoxa9YL and to generate leukemia cell lines for further analysis we retrovirally transduced murine hematopoietic progenitor cells with both wildtype Hoxa9 (Hoxa9wt) and Hoxa9YL together with the co-factor Meis1. After transplantation into syngenic mice we determined leukemia phenotype and overall survival. Hoxa9YL as well as Hoxa9wt transduced cells gave rise to overt AML. Notably, the resulting leukemias were phenotypically similar in both groups. Disease latency of Hoxa9YL leukemias, however, was significantly prolonged compared to Hoxa9wt leukemias (median disease onset ~190 days vs. ~90 days), concomitant with a reduced disease penetrance for Hoxa9YL (80% vs 100%) despite the persistence of transduced cells in the bone marrow for more than 400 days post transplantation. Importantly, we observed a considerably more pronounced effect on latency (median disease onset ~50 days vs. ~150 days) and penetrance (40% vs 100%) after re-transplantation of leukemic bone marrow from diseased mice into healthy secondary recipients. These findings argue for a persistent cell intrinsic reduction of leukemogenicity conferred by the disruption of the Hoxa9/eIF4e interaction. This implies that the Hoxa9/eIF4e interaction itself as well as targets regulated by this interaction could be attractive targets for therapeutic intervention. Therefore, we generated cytokine-dependent "primary" leukemia cell lines from bone marrow of diseased mice in order to identify potential targets by RNA immunoprecipitation of Hoxa9 and eIF4e as the next step.



Abrogation of Hoxa9/eIF4e interaction leads to severely reduced leukemogenicity in the Hoxa9 in vivo leukemia model in both primary and secondary bone marrow transplantations, indicating the crucial role of this interaction for leukemia development.

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Publications during funding period

Ye M, Zhang H, Yang H, Koche R, Staber PB, Cusan M, Levantini E, Welner RS, Bach CS, Zhang J, Krivtsov AV, Armstrong SA, Tenen DG (2015). Hematopoietic Differentiation Is Required for Initiation of Acute Myeloid Leukemia. Cell Stem Cell. 17(5):611-23

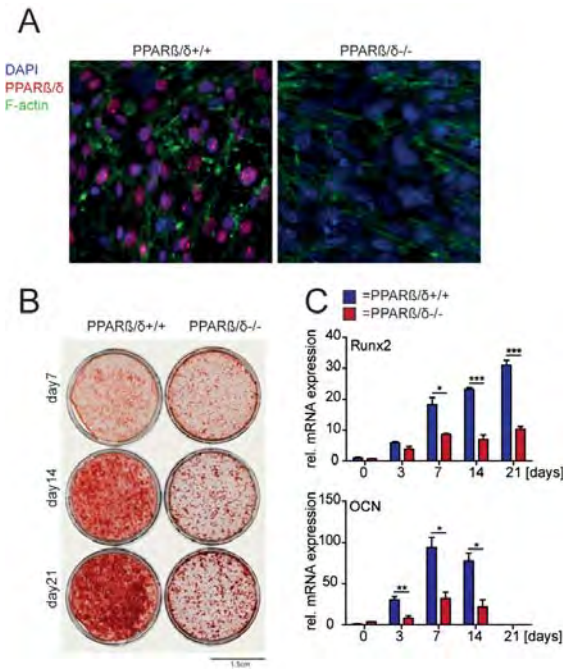
PPARβ/δ in the crosstalk of bone and glucose metabolism

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

The nuclear receptor (NR) PPARβ/δ is a central regulator of fatty-acid oxidation thereby reducing dyslipidemia and insulin resistance. So far cellular functions for PPARβ/δ during energy homeostasis are not clear. Since we identified PPARβ/δ as a master regulator of bone turnover and provided evidence for the potential of this NR to serve as a target for the treatment of osteoporosis, we now aim to a potential role for PPARβ/δ during the crosstalk between bone and energy metabolism.

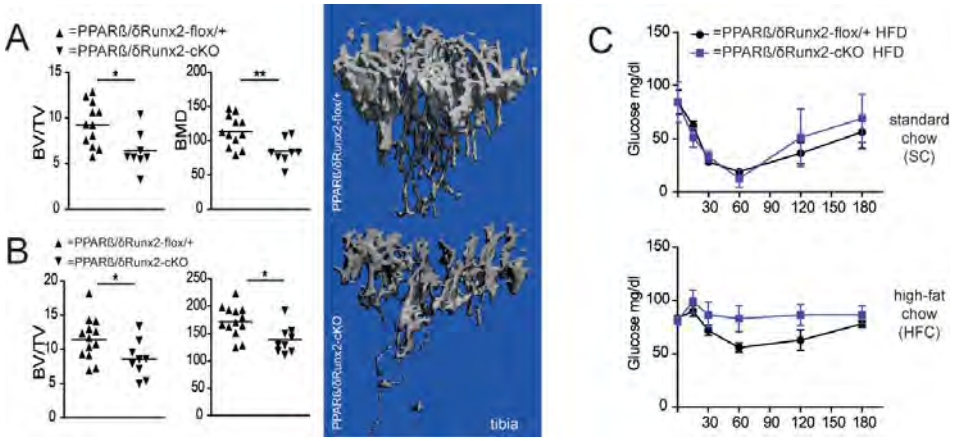
PPARβ/δ controls the differentiation of MSCs into osteoblasts.

During the last years, we have focused on common factors involved in the regulation of immune, bone and energy homeostasis. Special attention was given to the role of lipid mediators and nuclear receptors (NRs) in the crosstalk between these systems. Recently published work of our laboratory identified PPARβ/δ as an anabolic regulator of bone turnover. Since its family member PPARγ acts as key regulator during differentiation of mesenchymal stem cells (MSCs) into adipocytes, we subsequently investigated a possible role for PPARβ/δ during differentiation of MSCs into the osteoblast lineage. Immunofluorescence staining of PPARβ/δ during MSCs differentiation implicated a prominent nuclear expression of PPARβ/δ in wild-type MSCs. Mineralisation assays revealed that PPARβ/δ-deficient MSCs failed to differentiate into bone matrix secreting osteoblasts in vitro. Notably, we found the two master genes of osteoblast differentiation, Runx2 and osteocalcin (OCN), significantly decreased in PPARβ/δ-deficient MSCs. These data indicate a major role for PPARβ/δ in MSC differentiation and osteoblast function in vitro. Since OCN is a protein linking bone to energy homeostasis we suggest a functional crosstalk between PPARβ/δ, OCN and energy metabolism.



PPARβ/δ controls the differentiation of MSCs into osteoblasts. (A) Immunofluorescence staining of WT and PPARβ/δ -/- MSCs after 7 days of osteogenic differentiation. (B) Reduced mineralization in PPARβ/δ-/- MSCs and decreased mRNA of Runx2 and OCN.

Dr. Scholtysek



Deletion of PPARβ/δ in osteoblasts results in decreased bone volume (BV/TV) and bone mineral density (BMD) in tibial (A) and vertebral (B) bone. Plasma glucose levels during insulin tolerance tests (C) in mice fed a standard chow and a high-fat chow.

Deletion of PPARβ/δ in osteoblasts results in decreased bone mass and impaired insulin sensitivity

We have recently shown that PPARβ/δ-deficient mice display a decreased bone mass. Since our data shows a key role of this nuclear receptor in osteoblast differentiation we seek to determine whether expression of this nuclear receptor in osteoblasts and osteoblast precursor cells is responsible for this osteopenic phenotype. Therefore we generated mice carrying a conditional deletion of PPARβ/δ in osteoblasts by crossing mice carrying a "floxed" PPARβ/δ allele with mice expression the Cre recombinase under the Runx2 promoter. Micro-CT (µCT) measurement of tibial and vertebral bones revealed an osteopenic phenotype with significantly decreased bone volume and reduced bone mineral density in PPARβ/δ conditional Runx2 Cre knockout mice (PpardRunx2-cKO) compared to their wild-type littermates (Ppardflox/+). These data confirm a crucial role for PPARβ/δ in osteoblasts during bone homeostasis. To determine a potential role of skeletal PPARβ/δ during the regulation of glucose homeostasis, mice received either a standard chow (SC) or a high fat chow (HFC) for 8 weeks. Afterwards, we performed insulin tolerance tests (ITT). After a HFD, but not under a regular chow 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 2015 Feb; 21(2):150-8

J49 - Progress Report

01.04.2015 - 30.09.2017

Others

Extending statistical boosting algorithms for biomedical research

Dr. Andreas Mayr, Department of Medical Informatics, Biometry and Epidemiology

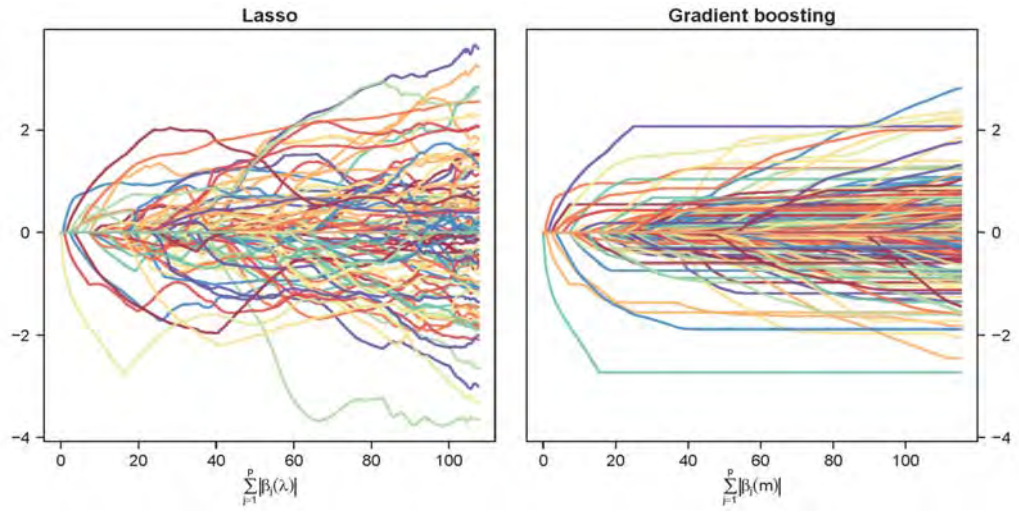
This project focuses on statistical boosting algorithms, particularly for model classes that go beyond the classical regression of the mean. These computational learning algorithms are very flexible and allow to estimate and select predictor effects in statistical models. The aim is to further extend these algorithms and to analyse their properties for specific regression settings that are relevant for medical research.

Extending the inference of statistical boosting

A current drawback of statistical boosting is that due to the iterative fitting design it is not possible to provide standard errors for the effect estimates. This makes standard methods for the construction of confidence intervals and hypothesis tests infeasible. To overcome this issue, we developed a permutation test to simultaneously assess the significance of predictor effects in generalized additive models for location, scale and shape. We incorporated this test in a newly developed framework to analyse measurement errors based on this specific model class for distributional regression. We applied the new approach to simultaneously assess systematic bias and random measurement errors of two devices to measure skin pigmentation in an epidemiological study carried out at our department.

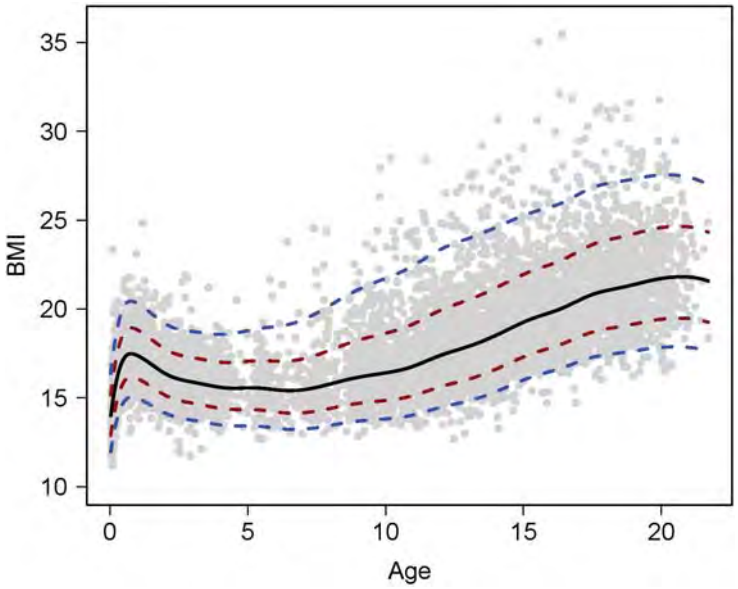
Boosting sonographic weight estimation

In cooperation with the Department of Obstetrics and Gynaecology we applied statistical boosting approaches to develop a new sonographic weight estimation formula for small for gestational age (SGA) fetuses. SGA fetuses have higher rates of neonatal mortality and morbidity than normal birth weight infants, which make the accurate prediction of the birth weight clinically more relevant for these cases. The new formula drastically outperformed all currently used formulas from the literature even when we re-fitted those for our specific sample.



Coefficient paths resulting from penalized regression via the lasso and gradient boosting in case of high block-wise correlations. Boosting penalizes the travelled path of the coefficients, the lasso only their size.

Dr. Mayr



The development of the Body Mass Index for boys depending on age. Regression curves represent quantiles based on a model for the Box-Cox Power Exponential distribution.

Analysing the properties of the algorithm

We are currently working on a structured comparison between statistical boosting and the popular lasso approach for penalized regression. We are identifying settings where the solutions of both algorithms coincide (positive cone condition) and provide guidance how this can be checked in practice. For high-dimensional data, we are conducting extensive simulation studies for various scenarios in order to compare the performance of both approaches regarding variable selection and prediction accuracy. Our results suggest that the lasso leads to slightly sparser models while boosting tends to yield more accurate predictions. A general advantage of boosting is its modular nature which makes the development of new extensions more attractive.

Extending boosting towards multiple outcomes and multivariate distributions

In cooperation with the University of Liverpool and the Chair of Statistics in Göttingen we are currently working on extending statistical boosting to the joint modelling of longitudinal and survival data in clinical studies. The advantages of the boosting algorithm makes it possible for the first time to estimate joint models in high-dimensional data settings including more explanatory variables than observations.

Additionally, we are working on extending the more general framework of distributional regression toward multiple correlated outcomes in order to model their combined distribution.

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Invited lectures

International Conference of the ERCIM WG on Computational and Methodological Statistics, Dec. 13, 2015, London, Boosting distributional regression models for multivariate responses.

Publications during funding period

Mayr A, Schmid M, Pfahlberg A, Uter W, Gefeller O (2015) A permutation test to analyse systematic bias and random measurement errors of medical devices via boosting location and scale models. Statistical Methods in Medical Research: doi: 10.1177/0962280215581855 [Epub ahead of print]

Faschingbauer F, Dammer U, Raabe E, Kehl S, Beckmann M, Schmid M, Schild RL, Mayr A (2015) A new sonographic weight estimation formula for small for gestational age (SGA) fetuses. Journal of Ultrasound in Medicine: accepted.

Newly started Projects

J50 16.10.2015 - 15.04.2018 Immunology and Infection

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


Dr. Gerlach

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

IBD in humans is combined with a risk for developing CAC. Previously, we could identify Th9 cells as drivers of IBD and therefore we will analyse the role of IL-9 in CAC. The functional role will be investigated with specific KO mice for IL-9, IL-9R and PU.1 in an experimental CAC model as well as in tumor patients. The aim is to understand the IL-9 driven induction of CAC and to develop a therapeutical concept for CAC.

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J51 01.10.2015 - 31.03.2018 Neurosciences

Inflammatory signature in Parkinson´s disease


Dr. Marxreiter

Dr. Franz Marxreiter, Department of Neurology

In Parkinson’s disease (PD), peripheral monocytes and the CN immune system, namely microglia and astroglia play an important inflammatory role in mediating disease progression. The hypothesis of this proposal is tha the inflammatory profile of these cells in human PD and its models is altered. I aim to characterize the distinct monocytic, microglial and astroglial phenotypes and their interactions. Moreover, the anti-inflammatory effects of the flavone apigenin will be analyzed.

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J52 01.11.2015 - 30.04.2018 Neurosciences

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

Dr. Regensburger

Dr. Martin Regensburger, Department of Neurology

Mutations in SPG11 are the most frequent cause of complicated hereditary spastic paraplegia (HSP). Neurons differentiated from SPG11-patient-iPSC show reduced neurite growth. Our preliminary experiments additionally indicate a reduced proliferation of neural progenitor cells in SPG11-HSP. In the proposed project, this proliferation deficit will be comprehensively characterized in order to identify underlying molecular pathways and potential targets for interventions.

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J53 03.08.2015 - 02.02.2018 Neurosciences

Diffusion tensor imaging of the visual pathway in pseudoexfoliation glaucoma

Dr. Schmidt

Dr. Manuel Schmidt, Department of Neuroradiology

A proportion of patients with pseudoexfoliation syndrome (PEX) will develop glaucoma (PEXG). There is a strong genetic component in PEX as common SNPs of the LOXL1 gene are supposed to be associated with PEX and PEXG. However, genetic testing is not suitable to identify those with PEX at increased risk for developing secondary glaucoma. Aim of this project is to elucidate the role of primary injury of the 4th neuron of the visual pathway in patients with PEXG with structural MR-imaging (DTI).

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Newly started Projects

J54 01.11.2015 - 30.04.2018 Oncology

Analysis of alternative mechanisms of tumor rejection


Dr. Lehmann

Dr. Christian Lehmann, Department of Dermatology

Immunologic tumor therapies aim to prolong patient survival mainly by inducing cytotoxic CD8+ T cell responses to a limited number of tumor epitopes. We have demonstrated in a murine melanoma model the independency of prolonged survival from the strength of induced CD8+ T cell responses. We speculate that a major part of this effect is due to the protection from tumor metastases formation. We would now like to investigate the underlying mechanisms to provide hints for tumor therapy improvement.

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J55 01.01.2016 - 30.06.2018 Oncology

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

Dr. Peter Dietrich, Institute of Biochemistry

Dr. Dietrich

Hepatocellular carcinoma (HCC) is a deadly cancer with poorly understood pathological features. It is known that several microRNAs are dysregulated in HCC. We found that a mostly unknown microRNA in cancer - miR-188-5p - is down-regulated in HCC cells and first experiments reveal potent tumorsuppressive functions of miR-188-5p. Therefore, in this project, we want to explore the role of miR-188-5p dysregulation and function in HCC development and progression and its potential therapeutic benefit.

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News and Figures

News and Figures

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News and Figures

The following figures impressively show the broad acceptance and the great interest of the Faculty of Medicine members in the programmes of the IZKF. The IZKF gives financial support to projects in all focal areas of the Faculty of Medicine and into a great number of different institutions. Nearly 100 scientific theses were running in 2015 and nearly 60 publications appeared.

Advanced Projects	**25**
Immunology and Infection	11
Oncology	4
Neurosciences	8
Renal and Vascular Research	2
Tandem projects between different departments and institutes	7
Junior Research Groups	**3**
Junior Projects	**16**
Immunology and Infection	8
Neurosciences	2
Renal and Vascular Research	2
Molecular Medicine	3
Others	1
Thereof projects completed in 2015	6
Institutions with funded projects	**20**
Employees of the IZKF	**114**
Number of scientists (including laboratory rotations)	78
Number of non-scientists	36
Appointments of IZKF project leaders to W2/ W3 - positions	1
Ongoing scientific theses in 2015	**68**
Bachelor theses	2
Master theses	7
Doctoral theses	55
Habilitations	4
Laboratory rotations	14
MD-thesis scholarship holders	23
Participants Graduate School	**110**

T(h)INK - Oncology, Immunology and Infection, Renal and Vascular Research	**55**
PhD students from IZKF projects	20
Associated participants	19
MD-thesis scholarships holders	16
Neurosciences	**55**
PhD students from IZKF projects	15
Associated participants	34
MD-thesis scholarships holders	6
Number of awards (2015)	8
Publications (2015)	**59**
Cumulative impact factor	416,638
Average impact factor per publication	7,062
Average publications per project	1,4
IF ≥ 10	16
Total expenditures IZKF in 2015	**4,624 K€**

Summary of important figures 2015

News and Figures

News

Start of the Junior Research Group 1 of Dr. Paolo Ceppi

In August 2015, Dr. Paolo Ceppi started his work on “Understanding the plasticity of cancer cells” in the Nikolaus-Fiebiger-Centre. After establishing his group Dr. Ceppi is now focusing on the mechanisms that regulate cancer plasticity and at studying the epithelial-to-mesenchymal transition, cancer stem cells and the association between cancer differentiation and sensitivity to chemotherapy.

Dr. Paolo Ceppi, N1

Prof. Dr. Jens Titze, N2

Project end of Junior Research Group 2

Prof. Jens Titze‘s group „Immune system as regulator of volume and blood pressure“ concluded in October 2015. Numerous publications and grant applications grew out of the successful research group. Prof. Titze now holds a shared professorship between the FAU and Vanderbilt University (USA). We wish Prof. Titze and his team continued success in their research.

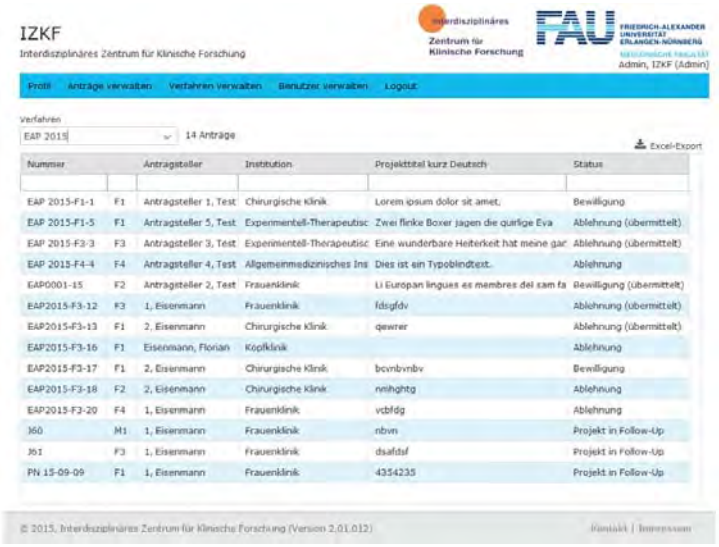
New Junior Research Group „Physics and Medicine”

In August 2015, the IZKF initiated recruitment of a new junior research group leader in the field of „Physics and Medicine”. The candidate´s research activities will exploit physical methods to investigate fundamental problems related to one or several of the main research topics of the Faculty. On 18th of December, 2015, a symposium to select candidates was held at the Translational Research Center. We are pleased to announce the appointment of Dr. David Dulin (University of Oxford, Department of Physics), who will join us starting September 2016. The research group will be located at the OICE on the Kußmaul campus in Erlangen.

IZKF Review 2015

On November 19th and 20th, 2015, the biannual on site review by the External Scientific Advisory Board (SAB) took place. The programmes and the financial framework of the IZKF were reviewed and approved. We are pleased that the SAB confirmed the outstanding research and the exceptional structural and financial development of IZKF. A funding recommendation for 31 advanced projects was pronounced.

From the left: Prof. Dr. Gisa Tiegs, Prof. Dr. Diana Dudziak, Dr. Christian Lehmann



Establishment of the IZKF application tool

In 2015, IZKF introduced a new customized online electronic application and reviewing platform to handle the large amounts of applications. The system reduces considerably the administrative work required, improving overall speed and transparency of the evaluation. In addition, the system will have a long-term impact in facilitating monitoring of projects and continuous measurement of performance criteria, both of individual projects and of programmes as a whole. Our application tool can be reached via the link: https://med- fak.rrze.uni-erlangen.de/izkf

Meeting of the IZKF managers

On November 30th to December 01st, 2015, the meeting of the IZKF Manager took place in Erlangen. The managers of the IZKFs Würzburg, Aachen, Erlangen and Münster came together at the premises of the Faculty of Medicine to discuss administrative and managerial issues including the planned publication „duz special“ and a brochure on the occasion of the IZKF 20th anniversary in 2016.

From the left: Dr. Andrea Thelen-Fröhlich (IZKF Würzburg), Karen DeBruyne (IZKF Aachen), Dr. Katrin Faber (IZKF Erlangen) and Dr. Sabine Blass-Kampmann (IZKF Münster)

News and Figures

Insight into biotechnology companies: Site visit of the IZKF Graduate School

A 2-day excursion of the IZKF Graduate School took place in March. Two biotechnology companies were visited. Led by Prof. Stürzl, the site visit with about 30 doctoral students gave young researchers the opportunity to gain exciting insights into the research of the companies.

Members of the IZKF Graduate School and Prof. Dr. Michael Stürzl

In Penzberg, the doctoral students of the IZKF Erlangen gained an insight into the research-intensive processes of Roche. The production of a biotechnology company was illustrated at Sandoz (a Novartis company) in Kundl. All participants considered that the site visit had a positive effect in their training.

From the left: Prof. Dr. Cornelius Schwarz, Prof. Dr. Alan Clarke, Miriam Düll, Andrea Liebl, Prof. Dr. Michael Stürzl

IZKF Postgraduate Workshop

On October 8th, 2015, the 14th IZKF Postgraduate Workshop took place in the Translational Research Center (TRC). Two interesting lectures were given:

Prof. Dr. Alan Clarke (European Cancer Stem Cell Research Institute, Cardiff School of Biosciences), "Murine models of colorectal cancer: Investigating the roles of Wnt pathway modulators" and

Prof. Dr. Cornelius Schwarz (Werner Reichardt Centre for Integrative Neuroscience, Hertie Institute for Clinical Brain Research, Tübingen), "Function of neocortex and tacile perception. Studies in the rodent whisker system."

Within the poster session 42 doctoral candidates from the IZKF Graduate School presented their projects. The selection committee awarded the two poster prizes to:

Andrea Liebl (Department of Surgery): "Tumor-microenvironment-dependent imprinting of endothelial cells in human colorectal carcinoma" and

Miriam Düll (Department of Medicine 1): "Effects of Methylglyoxal on human nociceptors"

Farewell to Prof. Stürzl

Prof. Dr. Michael Stürzl ended his term as member and chairman of the Junior Scientist Committee in December 2015. During his term the committee successfully established the IZKF Graduate School. The IZKF thanks him for many years of dedication to supporting and promoting young researchers.

From the left: Prof. Reis, Prof. Stürzl

News and Figures

Figures

Research Grants

The IZKF research grants can be divided into Advanced Projects, Junior Projects and Junior Research Groups. In 2015, 25 advanced and 16 junior projects received funding of the IZKF. These projects cover all major research areas of the Faculty of Medicine, i.e. immunology and infection research, renal and vascular research, neurosciences and tumor research.

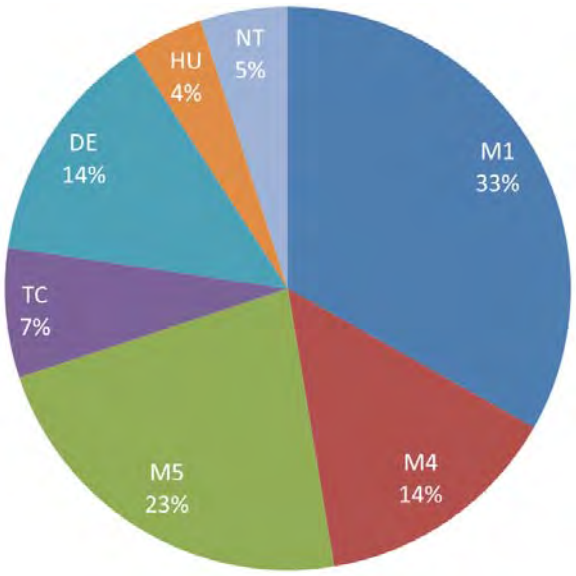
Institute	Immunology and Infection	Oncology	Neurosciences	Renal and Vascular Research	Others
Chair of Experimental Medicine II		x			
Department of Anesthesiology			x		
Department of Dermatology	x				
Department of Medical Informatics, Biometry and Epidemiology					x
Department of Medicine 1	x	x			x
Department of Medicine 2	x				
Department of Medicine 3	x				x
Department of Medicine 4	x			x	
Department of Medicine 5	x			x	
Department of Otorhinolaryngology - Head and Neck Surgery			x		
Department of Psychiatry and Psychotherapy			x		
Department of Surgery		x			
Division of Molecular Neurology			x		
Division of Molecular Pneumology	x				
Division of Nephropathology				x	
Institute of Biochemistry			x		
Institute of Clinical and Molecular Virology	x				
Institute of Clinical Microbiology, Immunology, and Hygiene	x				
Institute of Human Genetics			x	x	x
Institute of Pathology		x			

This table shows the institutes and departments which received project funding within the IZKF in the year 2015 and in which main research areas they are located.

Laboratory Rotations

The rotation programme is aimed at young physicians who are interested in research. In the context of the rotation programme they receive protected time either part-time or full-time within clearly defined research projects for up to twelve months full-time or 24 months in part-time.

In 2015 candidates from 7 different institutions were supported.

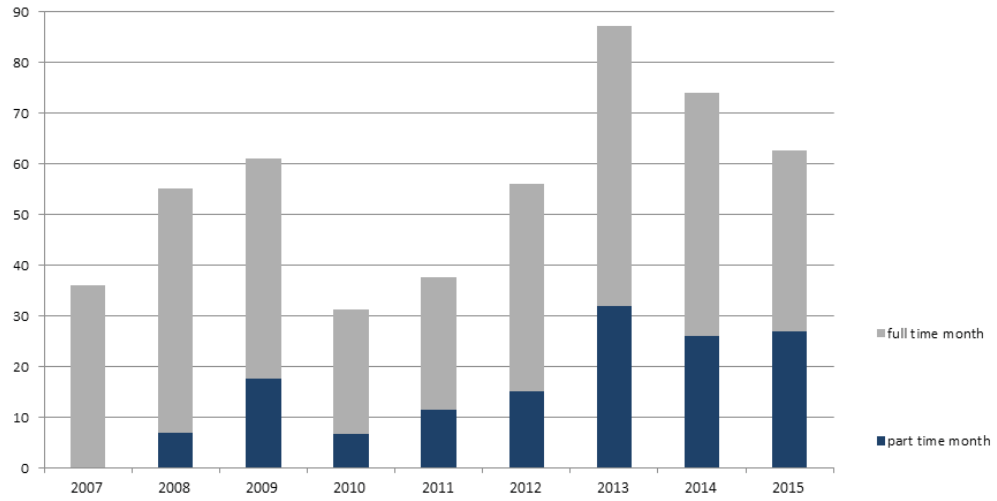


Funding distribution of laboratory rotations 2015

Name	Institution	Funding period	Full-time/ part-time
Dr. Gheorghe Hundorfean	Department of Medicine 1 (M1)	04/2013 - 03/2015	50%
Dr. Timo Rath	Department of Medicine 1 (M1)	04/2014 - 03/2015	100%
Dr. Ingo Ganzleben	Department of Medicine 1 (M1)	07/2015 - 06/2016	100%
Dr. Sebastian Zundler	Department of Medicine 1 (M1)	02/2015 - 02/2016	100%
Dr. Karl Bihlmaier	Department of Medicine 4 (M4)	10/2014 - 09/2015	50%
Dr. Tim Schröder	Department of Medicine 4 (M4)	10/2014 - 09/2015	50%
PD Dr. Dimitrios Mougiakakos	Department of Medicine 5 (M5)	01/2015 - 12/2016	50%
Dr. Merjeta Qorraj	Department of Medicine 5 (M5)	02/2015 - 07/2015	100%
Dr. Merjeta Qorraj	Department of Medicine 5 (M5)	10/2015 - 12/2015	50%
Dr. Dumitrita Gafencu	Department of Thoracic Surgery (TC)	05/2015 - 04/2016	50%
Dr. Uslu Ugur	Department of Dermatology (DE)	04/2015 - 03/2016	100%
PD Dr. Ulrike Hüffmeier	Institute of Human Genetics (HU)	09/2015 - 08/2016	50%
Dr. Frederick Pfister	Department of Nephropathology (NT)	07/2014 - 06/2015	50%
Rotations of Junior Project leaders			
Dr. Johannes Schödel	Department of Medicine 4 (M4)	02/2014 - 01/2015	100%

Laboratory Rotations

News and Figures



Man months funded in the rotation programme.

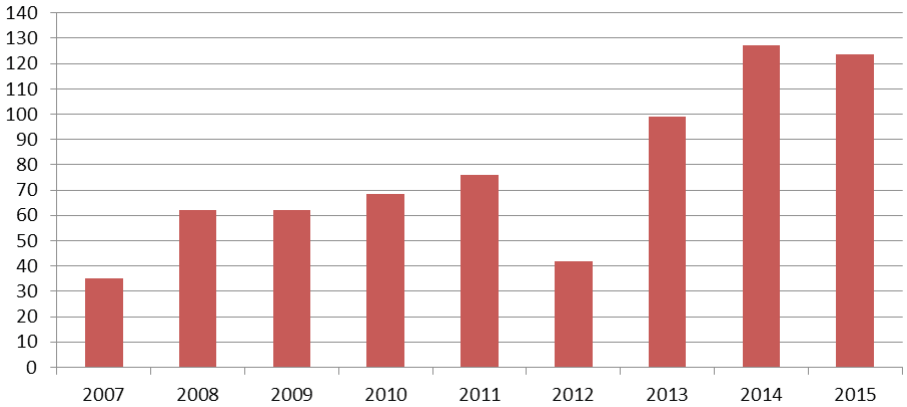
MD-Thesis Scholarships

Within the doctoral programme 18 scholarships for medical doctoral students are awarded each year, each granted for a period of 7 months with a monthly allowance of € 773. This support is given with the expectation of a full-time dedication to the thesis.

In 2015, a total of 23 medical doctoral students from 14 institutions were funded. Selection is done by the Junior Scientist Committee of the IZKF and is based on academic performance and the presence of first experience in laboratory work.

Since its inception in 2007 IZKF supported 92 medical students with a scholarship. Medical students can already start their doctoral thesis during their studies. It can take several years until students finish their studies and afterwards their doctoral thesis.

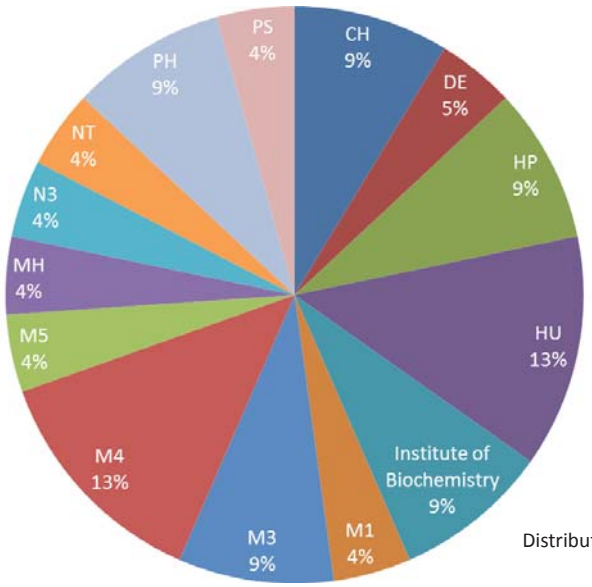
At the end of 2015, 47 students had completed their studies, 29 within the standard duration of study. The extension of the other 18 students was mainly by 1 semester. 30 of the 47 students which have completed their studies also completed their doctoral work (18 of those 30 students have finished their studies within the standard period of study). 17 students even received a MD with summa cum laude (57 % of the completed). This is an outstanding result since the average of summa cum laude theses at the Faculty is around 3 - 5 %.



Man months funded in the MD-thesis scholarship programme.

Name	Institution	Funding period
Schray Annika	Junior Research Group 3 (N3)	10/2014 - 04/2015
Düll Miriam	Department of Medicine 1 (M1)	08/2015 - 02/2016
Haberland Konrad	Department of Medicine 3 (M3)	04/2015 - 10/2015
Rauner Jan	Department of Medicine 3 (M3)	04/2015 - 10/2015
Bickenbach Lena	Department of Medicine 4 (M4)	10/2014 - 04/2015
Pfann Victoria	Department of Medicine 4 (M4)	04/2015 - 10/2015
Erdmann Laura	Department of Medicine 4 (M4)	10/2015 - 11/2015
Stueven Anna-Kathrin	Department of Medicine 5 (M5)	10/2015 - 04/2016
Bardenbacher Marco	Department of Surgery (CH)	12/2014 - 06/2015
Schütz Manuela	Department of Surgery (CH)	04/2015 - 10/2015
Tasbihi Kereshmeh	Department of Plastic and Hand Surgery (HP)	10/2014 - 04/2015
Weber Maximilian	Department of Plastic and Hand Surgery (HP)	10/2014 - 04/2015
Hellwig Konstantin	Department of Plastic and Hand Surgery (HP)	01/2015 - 07/2015
Lamprecht Ricarda	Department of Dermatology (DE)	07/2015 - 01/2016
Fischer Fabian	Institute of Microbiology (MH)	04/2015 - 10/2015
Sighart Regina	Institute of Human Genetics (HU)	10/2014 - 04/2015
Grüner Johanna	Institute of Human Genetics (HU)	04/2015 - 10/2015
Oberste-Lehn Lea	Institute of Human Genetics (HU)	07/2015 - 01/2016
Dorsch Melissa	Institute of Pathology (PH)	10/2014 - 04/2015
Lieb Konstantin	Institute of Pathology (PH)	04/2015 - 10/2015
Röder Sebastian	Department of Nephropathology (NT)	10/2014 - 04/2015
Seidel Sabrina	Institute of Biochemistry	12/2014 - 06/2015
Jung Matthias	Institute of Biochemistry	07/2015 - 01/2016

MD-thesis scholarships



Distribution of medical students per institution

News and Figures

Graduate School

The IZKF Graduate School provides doctoral candidates with a structured training programme and promotes networking. Membership is compulsory for all candidates funded by IZKF, both those with medical or natural sciences background. Associated members from other programmes or funding sources are also wellcome. The Graduate School is organized in two areas: neuroscience and immunology/ infection/ oncology/ renal and vascular research. The neuroscience section was merged with similar activities at the Interdisciplinary Center for Neurosciences (ICN).

In 2015 the Graduate School included 110 members.

Participants Graduate School	**110**
Neuro	**55**
Participants from IZKF projects	20
Associated participants	19
MD-Thesis scholarships	16
T(h)INK - Oncology, immunology and infection, renal and vascular research	**55**
Participants from IZKF projects	15
Associated participants	34
MD-Thesis scholarships	6

Participants of the Graduate School 2015 (09.12.2015)

In 2015, Tobias Borman was re-elected as speaker of the Graduate School for the area oncology, immunology, renal and vascular infection. Lian Ye was speaker of the Graduate School for the neuroscientists until December 2015 when Benjamin Häberle became his successor.

T(h)INK-Group

Speaker	Deputy Speaker
Tobias Bormann since 10/2014 *A54, M1*	**Kristina Scheibe** since 10/2014 *D21, M1*

NEURO-Group

Speaker	Deputy Speaker
Lian Ye *Mol. Clin. Phar. till 12/2015* **Benjamin Häberle** *Inst. of Biochem. since 12/2015*	**Andrea Link** *Physiology* **Benjamin Ettle** *E18, MN* **Diana Schmidt** *N3, IZKF*

The following soft skills courses were given:

- Presentation Skills, Dr. Deborah Bennett, 30./31.01.2015 and 06./07.02.2015
- Microscopy course on sample preparation and two channel confocal imaging, Dr. Ralf Palmisano, 21.-24.04.2015
- Biostatistics, Daniela Keller, 29./30.05.2015 and 17./18.05.2015

Visiting Professor Programme

The visiting professor programme promotes visits by external researchers, thereby encouraging collaborations and supporting the exchange of ideas. Two related programmes are administrated by IZKF with partly different target groups. One is funded by FAU, the other by IZKF directly. Following lectures were given by external scientists in 2015.

Scientist	Institute	Lecture title
Prof. David Ian Cook	UNSW Sydney, School of Medical Sciences, Gastroenterology and Hepatology, Sydney, Australia	Epithelial responses to pathogens
Prof. Martin Flajnik	University of Maryland, School of Medicine, Department of Microbiology and Immunology, Baltimore, USA	Discovery of cellular immunology and acquired tolerance
Prof. David Nemazee	The Scripps Research Institute, Department of Immunology and Microbial Science California Campus, La Jolla, USA	T and B cell tolerance
Prof. Subbaya Subramanian	University of Minnesota, Department of Surgery, Division of Basic and Translational Research, Minneapolis, USA	MicroRNAs in the Pathobiology of Osteosarcoma
Prof. Kai W. Wucherpfennig	Harvard University, Dana-Farber Cancer Institute, Harvard Medical School, Department of Neurology, Boston, USA	The Role of E-Proteins in Th17 and Regulatory T cell induction
Prof. Bernard Zalc	Institut du Verveau et de la Moelle épinière, GH Pitié-Salpêtrièr, Paris, France	The acquisition of myelin: an evolutionary seccess story am
Prof. Warren Strober	National Institute of Allergy and Infectious Diseases, Mucosal Immunity Section, Bethesda, USA	The Role of E-Proteins in Th17 and Regulatory T cell induction
Prof. Michael Caplan	Department of Cellular and Molecular Physiology, Yale University, New Haven, USA	Novel Protein Trafficking and Signaling Pathways in Renal Epithelial Cells

FAU-Visiting Professor Programme

Scientist	Institute	Lecture title
Dr. Maria C. Marchetto	Salk Institute for Biological Sciences, La Jolla, USA	News and views: human iPSC derived neuronal models
Prof. Juan Manuel Encinas	Achucarro Basque Center for Neuroscience, Zamudio, Spain	Reactive neural stem cells in the adult brain
Prof. Maurilio Sampaolesi	Translational Cardiomyology Laboratory, Stem Cell Biology and Embryology, Dept. of Development and Regeneration, KU Leuven, Belgium	Cell reprogramming and miRNA-based treatments for skeletal and cardiac muscle repair
Prof. Luca Gattinoni	National Cancer Institute (NCI), Bethesda, USA	T-cell metabolism and antitumor immunity
Prof. Dr. Tiago Fleming Outeiro	Department of Neuro Degeneration and Restorative Research, University Medical Center Göttingen	Zooming in into the role of posttranslational modifications in synucleinopathies
Prof. Dr. Kostas Vekrellis	Division of Basic Neurosciences, Foundation for Biomedical Research of the Academy of Athens	Proteolytic clearance of alpha-synuclein in vivo: novel targets in Parkinson's disease transmission
Prof. Dr. Riccardo Bellazzi	Biomedical Informatics Labs "Mario Stefanelli", Dipartimento di Ingegneria Industriale e dell'Informazione, University of Pavia, Italy	Data and knowledge integration: methods and technologies to support the fusion of clinical and molecular data
Prof. Miriam Jasiulionis	Universidade Federal de São Paulo (UNIFESP), Escola Paulista de Medicina (EPM), São Paulo, Brazil	Loss of contact: Cellular stress, epigenetic alterations and malignant transformation
Prof. Maria Gazouli	Department of Basic Medical Science, School of Medicine, University of Athens, Greece	Stem cells in Inflammatory bowel disease
Prof. Marcus Peter	Division of Hematology/Oncology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, USA	Killing cancer cells by targeting tumor suppressors
Dr. Agnes Kittel	Dep. Of Pharmacology, Institute of Experimental Medicine, Hunagrian Academy of Sciences, Budapest, Hungary	Emergence of the extracellular vesicles from the point of view of an electron microscopist

IZKF-Visiting Professor Programme

IZKF Funding and Output

Budget

Since 2004, the IZKF has been fully supported by intramural funds. The main financial contribution is given by the Medical Faculty. Additional contributions are received from the FAU. The junior project programme is jointly funded by IZKF and the ELAN-Fonds.

About half of the IZKF-budget (€ 2.4 millions) goes toward the funding of 25 advanced projects, while € 756,000 are allocated to the funding of junior projects, and € 570,000 to the funding of junior research groups. Further portions of the total budget are assigned to other career development programmes (MD-thesis scholarships, laboratory rotations, graduate school; total sum € 511,000). Expenditures for other supporting activities sum up to € 342,000.

Financial Statements IZKF 2015

Revenues	
Support by the Medical Faculty	3,876 K€
Support by the University	268 K€
Contribution of ELAN-Fonds for junior projects	300 K€
Contribution of IZKF for junior research groups (basic funding)	- 24 K€
Total revenues 2015	**4,420 K€**
Expenditures	
Research Grants	3,771 K€
thereof advanced projects	2,443 K€
thereof junior research groups	572 K€
thereof junior projects	756 K€
Other career development programmes	511 K€
Supporting activities	342 K€
Total expenditures 2015	**4,624 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 44 scientific projects were actively running: 25 advanced projects, 16 junior projects and 3 junior research groups. In addition, 6 junior projects started their work in 2015 or in the beginning 2016. These 44 funded scientific projects published 59 original articles in 2015 resulting in an average of 1.3 publications per project. The cumulative impact factor (IF) was 416.638, averaging 7.062 per publication. The high quality of many of these publications is reflected in 16 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 7 master and diploma theses, 55 doctoral theses and four habilitations that were in progress or finalised in 2015. Around 80 scientists from 20 different institutions are involved in 44 scientific projects funded by IZKF.

Some IZKF project leaders were able to achieve outstanding results. 8 prizes were awarded to IZKF project leaders and two professorships were offered and accepted.

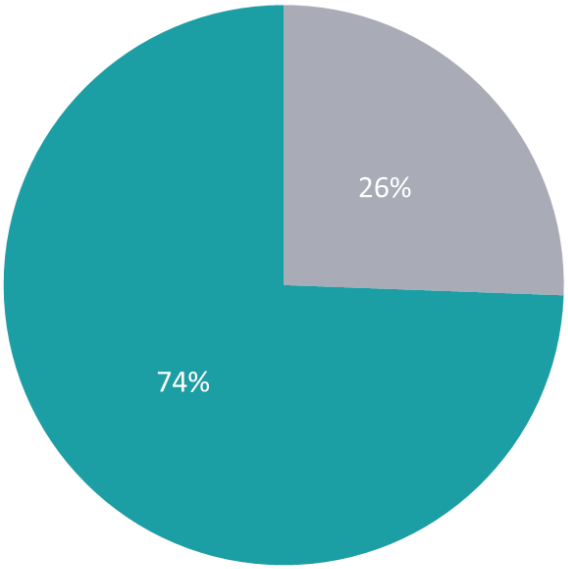
In many instances funding by the IZKF starts at an early phase of the project, thus it must be considered as a high risk funding programme. It is nevertheless reassuring that most of the projects are successful and many of them are continued after termination of intramural funding. To support this with figures, a detailed survey of acquired third-party funding by IZKF-projects, which were completed since 2010 is 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. When comparing the funding period 2010- 2013 with earlier funding periods, it becomes obvious that the number of applications for external funding increased significantly. The data also show that the extension period leads to an earlier acquisition of third-party funding.

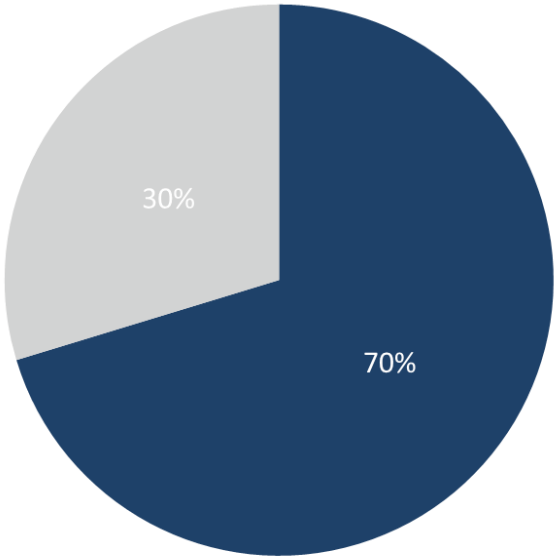
Similarly, the junior projects lead to an high number of extramural funding applications with a very high success rate. This developement has been stable over the entire duration of the programme.

News and Figures

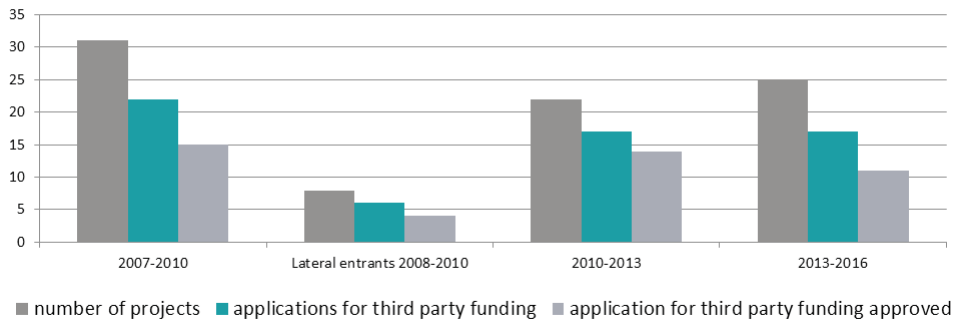
Acquisition of third-party funding advanced projects



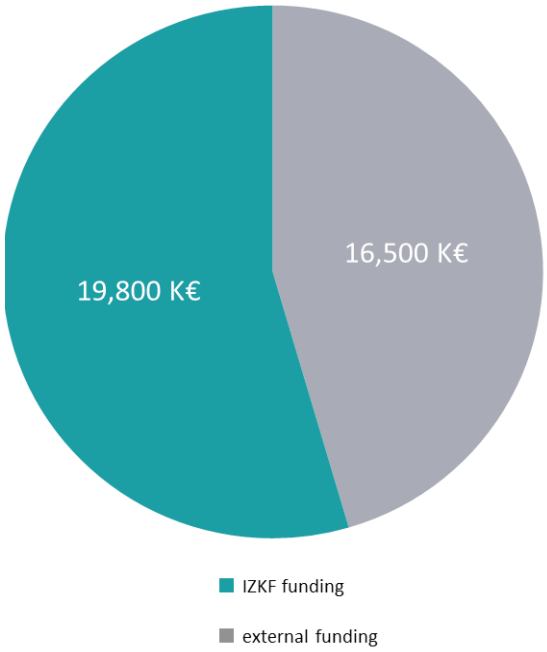
Applications for third-party funding submitted by advanced projects between 2007 and 2015



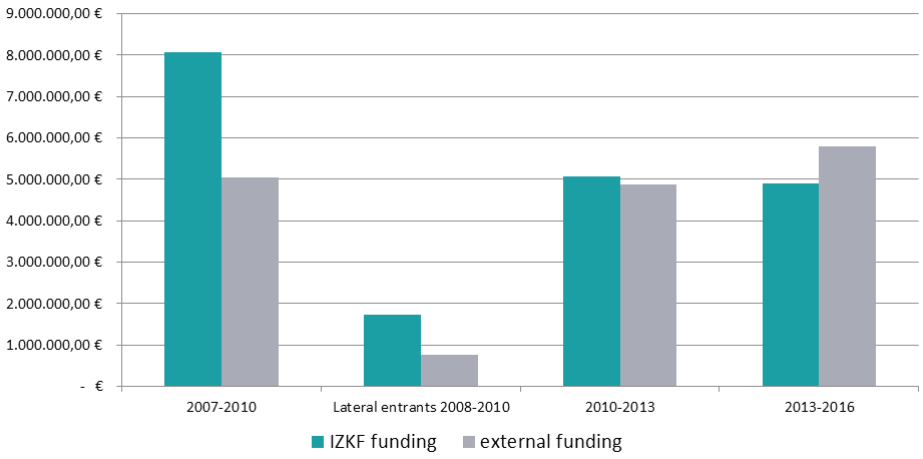
Approved applications for third-party funding of advanced projects between 2007 and 2015



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



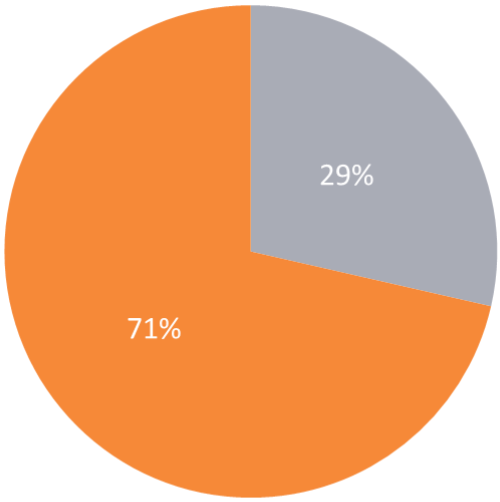
External funding received from advanced projects between 2007 and 2015.



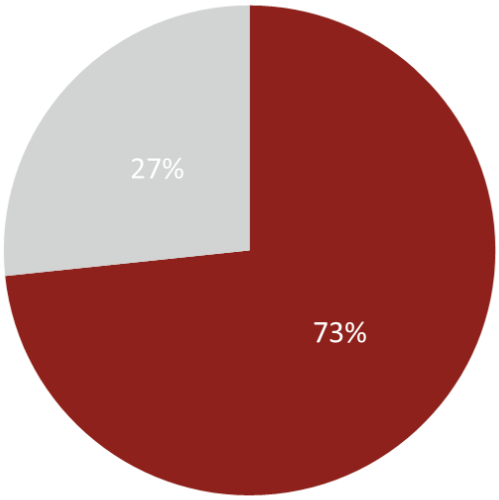
External funding received from advanced projects between 2007 and 2015.

News and Figures

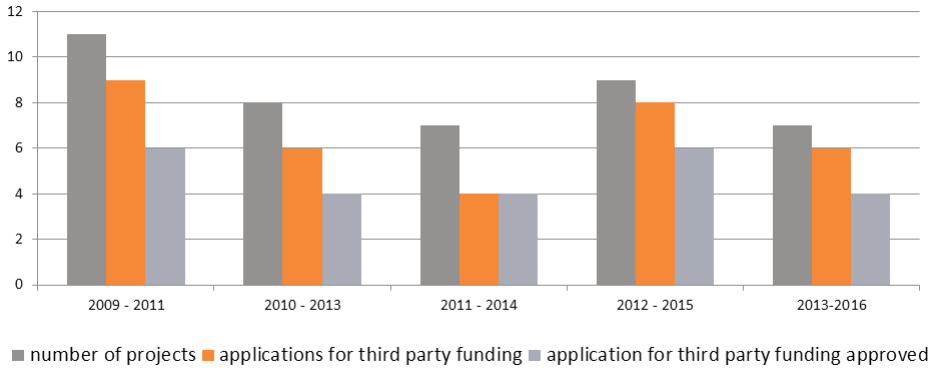
Acquisition of third-party funding junior projects



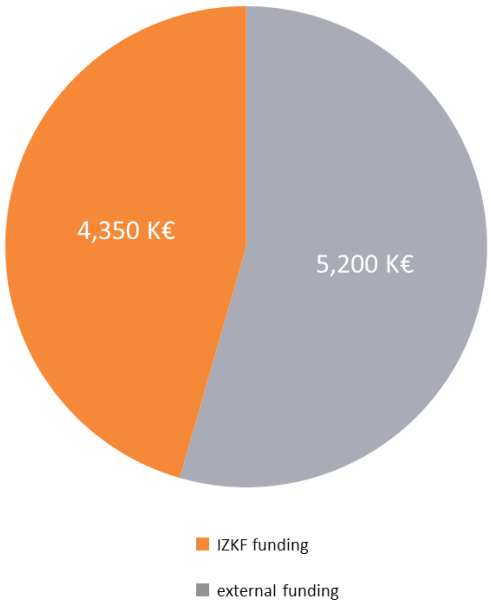
Applications for third-party funding submitted by junior projects (projects initiated between 2009 and 2013).



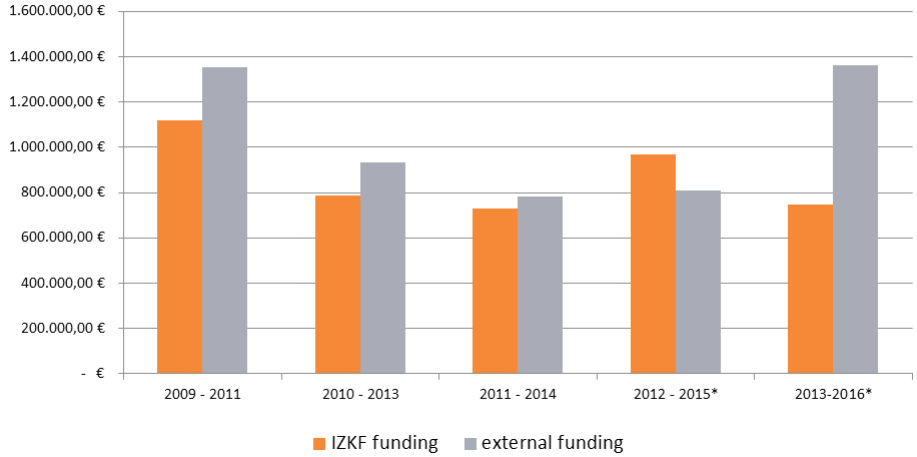
Approved applications for third-party funding of junior projects (projects initiated between 2009 and 2013).



Success-rate of junior projects. Further applications of projects initiated in 2013 are planned.



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



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

Index of Names

IMPRINT

Annual Report 2015

Publisher

Interdisziplinäres Zentrum für Klinische Forschung (IZKF)
Speaker: Prof. Dr. André Reis
www.izkf.uk-erlangen.de

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Cover and colour design

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Höhenweg 12
91094 Langensendelbach

Layout and setting

Bianca A. Meyerhöfer-Klee
IZKF Administrative Office

Photo credits

p. 155 Uni-Klinikum Erlangen

Printing

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

Print run

350 copies

April 2016