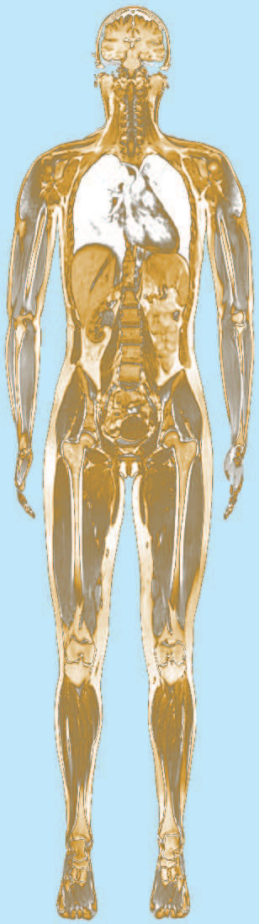
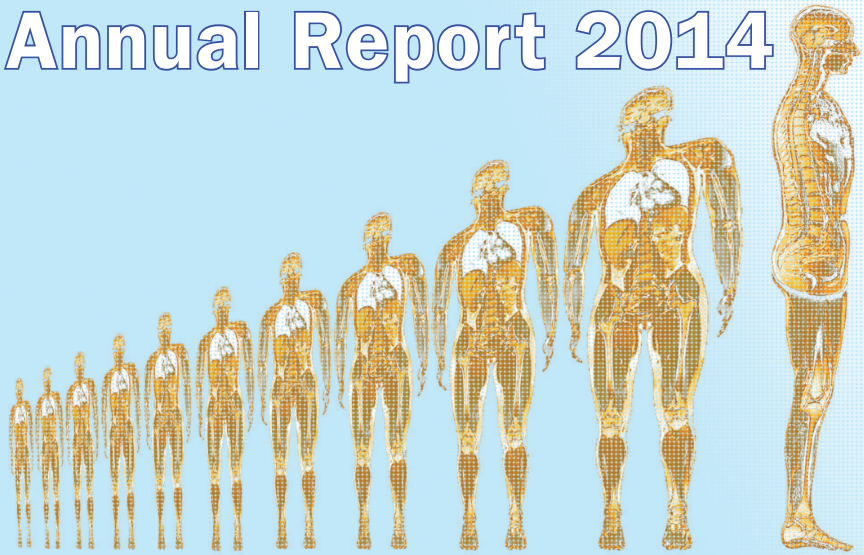


interdisciplinary

Center for Clinical Research Erlangen

IZKF Erlangen Annual Report 2014

Annual Report 2014



Universitätsklinikum Erlangen

FAU FRIEDRICH-ALEXANDER UNIVERSITÄT ERLANGEN-NÜRNBERG
MEDIZINISCHE FAKULTÄT

IZKF Erlangen

IZKF Erlangen

Annual Report 2014

Editorial

This years' annual report has been restructured with the aim of being even more informative, not only portraying the structure and activities of the IZKF but also monitoring performance towards achieving our goals. In addition we also intend to showcase the research done by the many participating scientists during the last year. The Interdisciplinary Center for Clinical Research Erlangen (IZKF) is the central intramural funding platform of the Faculty of Medicine and the IZKF spends a noticeable part of the Faculties budget. Therefore it is of great importance to achieve transparency on how money is spent and how the institution performs in its aim to support and develop clinically oriented research based solely on scientific excellence.

Despite the overall trend towards electronic dissemination of information, this classical print product is still very popular and widely used. Nevertheless, we recognise the importance of web-based information and have invested considerable efforts in upgrading our internet presentation. Under the leadership of Dr. Katrin Faber the team at the Administrative Office has prepared a completely refurbished webpage (www.izkf.uk-erlangen.de) which after extensive testing went online in July. It is now entirely bi-lingual German/English and more informative, transparent and up-to-date than ever.

A key element of IZKF is the competitive nature of its funding with transparent access aiming to support innovative ideas and excellent scientists and to increase competitiveness of external funding applications. A special emphasis is on interdisciplinary research as scientific advances are often achieved at the interfaces of disciplines. To promote this aim, the Management Board decided to favour these projects from two cooperating institutions by increasing the allowed budget for consumables starting with the 2015 call for advanced projects. The Management Board also decided to moderately increase the generally allowed funding for consumables to compensate for general inflation. The upper limit had not been modified in more than a decade. At the same time the Board also decided to reduce the administrative burden by allocating funds for animal housing as a lump sum together with consumables. This should also achieve equal funding levels for all projects independently of the experimental approach used.

Achieving equal opportunities for women in science and reconciling work and family life are a continuous challenge for any faculty. More than half of scientists supported by IZKF especially in the junior project programme are female. Pregnancy or parental leave periods can seriously disturb these projects which aim at preparing these young scientists for starting an independent career and obtaining extramural support for their research. Thus the Management Board took a bold step in generously supporting young mothers in science. Female scientists can now interrupt their projects with full funding for up to one year of maternity leave. During their absence the personnel paid by the project can continue their work with full funding, provided that another scientist from the institution co-supervises the work. Thus the groups are maintained unaltered and ongoing work is only minimally affected. After their return, PIs are expected to work at least half-

time and their projects continue as initially scheduled. These measures result de facto in up to one year prolongation of the projects which is intended to counterweight possible disadvantages of maternal leave. We hope that this will allow motivated young female scientists to better reconcile a career in science with family life.

The support of young scientists continues to be a main goal. As in previous years the call for applications for Junior Projects in the "First time applicant" 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 2014, proposals for 20 projects were reviewed from which 7 were selected for funding. Also the programme for laboratory rotations was again very successful with a rate of utilisation of more than 100%, indicating a continued interest in a physician-scientist career. The Management Board, though, is concerned that for the first time this year no physicians were among the PIs of the successful junior grant projects. The Board will closely monitor this development and search for appropriate measures to more effectively support young physicians in science.

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. Following the appointment by the Faculty of two new full professors in the research area of oncology, the Board decided to contribute to strengthening of this area. Thus in fall 2014, a call for a junior research group in the area of molecular oncology was launched, with a focus in signal transduction, chromatin remodelling or tumour microenvironment. Candidates for the group leader position were selected during a symposium in January 2015 and we hope the group will start their operation in the summer of 2015.

During the last years, we have initiated an extensive evaluation of our funding schemes. In 2014 we have continued to monitor outcome of advanced and junior projects, the results of which are detailed in novel News and Figures section of this report. Overall, the programmes are very successful, with about two thirds of the projects applying for extramural funding. The funding sums obtained exceed those invested by intramural funding. Thus many projects initiated in the IZKF become competitive in an ever more difficult external funding environment. We thus can proudly state that the money is well spent.

Since 2013 the IZKF moved to biannual calls for projects alternating with the organisation of an international research symposium. Thus 2014 a symposium was held under the topic "Translational Medicine" in the beautiful venue of Kloster Banz on May 15 and 16. The programme committee assembled a very interesting programme with 10 renowned external speakers, 7 international and 3 national, supplemented by talks from 9 scientists from Erlangen. In addition, one hundred posters of high quality were on display highlighting the research done in Erlangen and fostering scientific exchange. The excellent participation of 220 scientists almost exceeded the maximum capacity of Kloster Banz. The feedback of participants and speakers was unanimously very positive and the programme was judged balanced and very interesting. It was also noted that the participation from Erlangen came from all research areas of the Faculty not just from projects funded by the IZKF or ELAN-Fonds.

Finally, in the general assembly in November we bid farewell to Prof. Jürgen Behrens who after 9 years reached the statutory time limit as a member of the Management Board. The Assembly thanked him for his longstanding valuable support and elected Prof. Christian Bogdan as his successor.

Prof. Dr. André Reis

Contents

About us

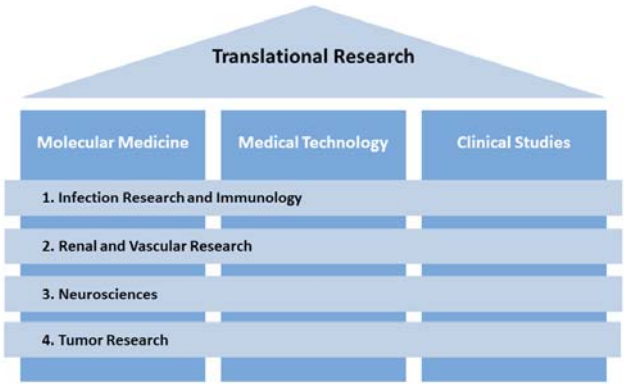
History

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 Medical Faculty 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

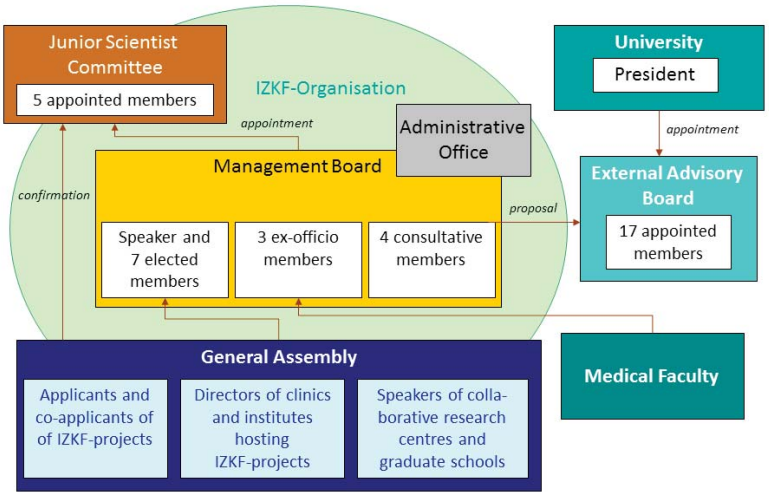
IZKF is a self-organised structure within the Faculty of Medicine. The IZKF has a set of written rules and regulations approved by the IZKF General Assembly and the Faculty of Medicine. Governing bodies include the General Assembly, the Management Board, the Junior Scientist Committee and the External 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, seven elected by the general assembly for a three year period and four 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 17) 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 and one from junior projects as well as one of the junior research group leaders.

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. Jürgen Behrens, Chair of Experimental Medicine II (till 05.11.2014)
Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene (since 05.11.2014)
Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I (since 01.10.2014)
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. Karl-Dieter Grüske, President of the University Erlangen-Nuremberg (till 31.03.2015)
Prof. Dr. Joachim Hornegger, President of the University Erlangen-Nuremberg (since 01.04.2015)
Thomas Schöck, Head of Administration of the University Erlangen-Nuremberg (till 15.06.2014)
Dr. Sybille Reichert, Head of Administration of the University Erlangen-Nuremberg (since 16.06.2014)
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. Winkler

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. Winner


Prof. Dr. Becker


Prof. Dr. Dr. Stürzl


Prof. Dr. Schulze


Dr. Boos

Current Members of the Junior Scientist Committee

Chairman

Prof. Dr. Dr. Michael Stürzl, Department of Surgery

Members

Prof. Dr. Beate Winner, IZKF Junior Research Group 3
Prof. Dr. Christoph Becker, Department of Medicine 1
Prof. Dr. Schulze, Department of Oto-Rhino-Laryngology - Head and Neck Surgery
Dr. Anja M. Boos, Department of Plastic and Hand Surgery

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)

General Assembly

Surname	Name
Achenbach	Stephan
Amann	Kerstin
Baur	Andreas
Becker	Christoph
Behrens	Jürgen
Bogdan	Christian
Boos	Anja
Bosch-Voskens	Caroline
Croner	Roland
Dees	Clara
Dietel	Barbara
Distler	Jörg
Eckardt	Kai-Uwe
Engel	Felix
Eulenburg	Volker
Ferrazzi	Fulvia
Finotto	Susetta
Fleckenstein	Bernhard
Günther	Claudia
Hartmann	Arndt
Hashemolhosseini	Said
Hildner	Kai
Hohenberger	Werner
Iro	Heinrich
Klucken	Jochen
Kornhuber	Johannes
Kremer	Andreas
Kreß	Andrea
Krönke	Gerhard
Leppkes	Moritz
Lie	Dieter Chichung
Mackensen	Andreas
Moskalev	Evgeny
Müller	Christian P.
Naschberger	Elisabeth
Neufert	Clemens

Surname	Name
Neurath	Markus
Nitschke	Lars
Ramming	Andreas
Reichel	Martin
Reiprich	Simone
Reis	André
Schauer, neé Schorn	Christine
Schett	Georg
Schierer	Stephan
Schleicher	Ulrike
Schneider-Stock	Regine
Schödel	Johannes
Schuler	Gerold
Schulze	Holger
Schüttler	Jürgen
Sonnewald	Uwe
Spriewald	Bernd
Stamminger	Thomas
Steinkasserer	Alexander
Stürzl	Michael
Thiel	Christian
Thomas	Marco
Titze	Jens
Völkl	Simon
Waldner	Maximilian
Warnecke	Christina
Wegner	Michael
Winkler	Jürgen
Winner	Beate
Wirtz	Stefan
Wittkopf	Nadine
Xiang	Wie
Zimmermann	Katharina

General Assembly of the IZKF at 06.11.2013

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. Steffen Gay (till 31.03.2014),
Zürich University Hospital - Department of Rheumatology and Institute of Physical Medicine

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

Prof. Dr. Heinz Höfler,
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. Dontscho Kerjaschki (till 31.03.2014),
University of Vienna - Clinical Institute of Pathology

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

Prof. Dr. Thomas A. Luger (till 31.03.2014),
Münster University Hospital - Department of Dermatology

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 (since 01.04.2014),
University Hospital Aachen - Department of Neurology

Prof. Dr. Thomas Seufferlein (since 01.04.2014),
University Hospital Ulm - Internal Medicine I

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

Prof. Dr. Hartmut Wekerle (till 31.03.2014),
Max-Planck-Institute of Neurobiology, Martinsried - Department of Neuroimmunology

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

Prof. Dr. Frauke Zipp,
Mainz University Medical Center - Department of Neurology

Current External Scientific Advisory Board

Programmes

Programmes

Research Grants
Career Development Programmes
Central Projects

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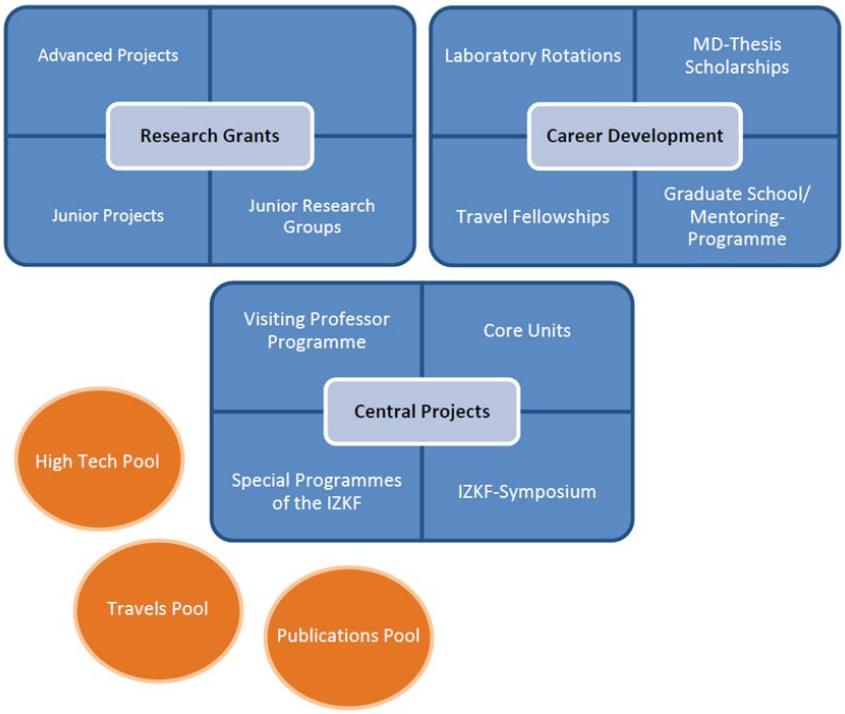
Programmes

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.

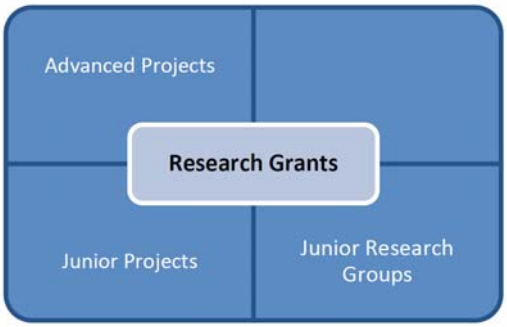


Programmes of the IZKF

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

Programmes

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. Currently two junior research groups exist. They are housed in the Nikolaus Fiebiger Center for Molecular Medicine with its diverse scientific environments and numerous 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 by IZKF. Junior project leaders can also access this programme in addition to their project funding. The IZKF can allocate 6 full-time positions; this equates to 72 months, which can be used flexibly. The initial grant always consists of 6 months in full time or 12 months in part time. Extensions are conditional on successful evaluation based on oral presentation of work progress and updated work programme.

MD-Thesis Scholarships

This programme was initiated to arouse interest for science in motivated medical students early on in their career. Medical students are supported in performing an experimental thesis in association with the IZKF or externally funded projects. It is expected that they spend a significant time in a laboratory. 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 council 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 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. Recently 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.

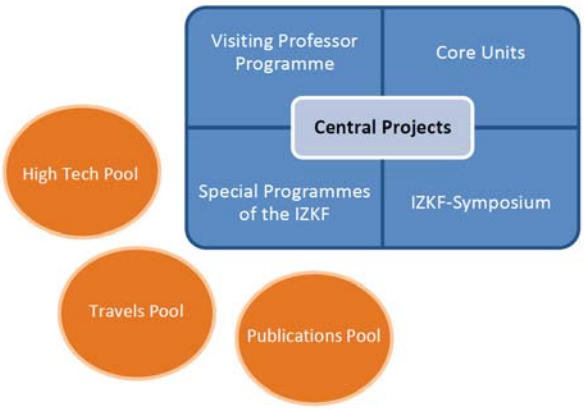
Postgraduate Workshop

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.

Programmes

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, projects funded by IZKF and ELAN programme 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, the 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

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 subprojects, 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 IZKF 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.

Programmes

Impressions of the IZKF-Symposium 2014









loster Ba

Advanced Research Grants

Advanced Research Grants

Progress and Final Reports

26

Advanced Grants

Immunology and Infection

Project No.	Project title	Term	Applicant(s)	Institute
A43	Systematic analysis of paracrine mechnisms in HHV-8-regulated tumorigenesis of Kaposi's sarcoma	15.02.2011-14.02.2014	Prof. Stürzl	Department of Surgery
A46	Role of the Cytokine TSLP in the pathology of Th1/Th17- and Th2- mediated immune reactions	20.01.2011-19.01.2014	PD Dr. Dr. Lechmann	Department of Immune Modulation
A52	Analysis about the functional role of cFLIP isoforms in intestinal epithelial cells	01.11.2013-30.04.2016	Dr. Günther, Prof. Becker	Department of Medicine 1
A53	Molecular mechanisms of Th17 and piTreg differentiation in vivo	01.10.2013-30.09.2016	Prof. Hildner	Department of Medicine 1
A54	Fam180A: A new important mediator of inflammatory diseases?	01.11.2013-30.04.2016	PD Dr. Dr. Wirtz, Prof. Waldner	Department of Medicine 1
A55	The nuclear receptor NR4a1 as modulator of immunologic self tolerance	01.01.2014-30.06.2016	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	Nr4a1as a novel target for the treatment of sclerodermatous 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	01.10.2013-31.03.2016	Prof. Mackensen, Dr. Völkl	Department of Medicine 5
A59	Immunosuppressive role of IL-10 in lung cancer	01.10.2013-30.09.2016	Prof. Finotto	Division of Molecular Pneumology
A60	Monocyte-derived Dendritic Cells (Mo-DC) by DC-Exosomes	01.10.2013-31.03.2016	Dr. Baur, Dr. Schierer	Department of Dermatology
A61	Cross-regulation of inducible NO synthase and iron in cutaneous and visceral leishmaniasis	01.02.2014-31.07.2016	Prof. Bogdan, PD Dr. Schleicher	Institute of Clinical Microbiology, Immunology and Hygiene
A62	ND10 and interferon-induced gene expression	01.01.2014-30.06.2016	Prof. Stammiger	Institute of Clinical and Molecular Virology

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	Significance of Death-associated protein kinase (DAPK) in colorectal carcinogenesis	16.10.2013-15.04.2016	Prof. Schneider-Stock, 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	01.11.2013-30.04.2016	Prof. Behrens	Chair of Experimental Medicine II

Neurosciences

Project No.	Project title	Term	Applicant(s)	Institute
E10	The role of neuronal glycine transporter 1 (GlyT1) in synaptic transmission	01.04.2011-31.03.2014	Dr. Eulenburg, PD Dr. Grömer	Institute of Biochemistry, Department of Psychiatry and Psychotherapy
E11	Oxidative stress-mediated posttranslational modification of alpha-synuclein: The role of His50 of alpha-synuclein in Parkinson disease	01.12.2013-31.05.2016	PD Dr. Klucken, PD Dr. Xiang	Division of Molecular Neurology, Institute of Biochemistry
E12	Adult hippocampal neurogenesis in synucleinopathies	01.04.2014-31.03.2017	Prof. Winkler, Prof. Lie	Division of Molecular Neurology, Institute of Biochemistry
E13	Sphingomyelinase, depression and alcoholism	01.04.2014-31.03.2017	Prof. Müller, 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	Glycine dependent neurotransmission and neuropathic pain	01.11.2013-30.04.2016	Dr. Eulenburg, Prof. Schulze	Institute of Biochemistry, Department of Otorhinolaryngology – Head and Neck Surgery
E16	Regulatory Networks in Intellectual Disability	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-positive cells in the healthy and diseased central nervous system	01.12.2013-31.05.2016	Prof. Wegner, Prof. Winkler	Institute of Biochemistry, Division of Molecular Neurology

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	Division of Nephropathology
F4	Pathogenesis of the Short rib-Polydactyly syndrome	01.10.2013-31.03.2016	PD Dr. Thiel	Institute of Human Genetics

A43 - Final Report

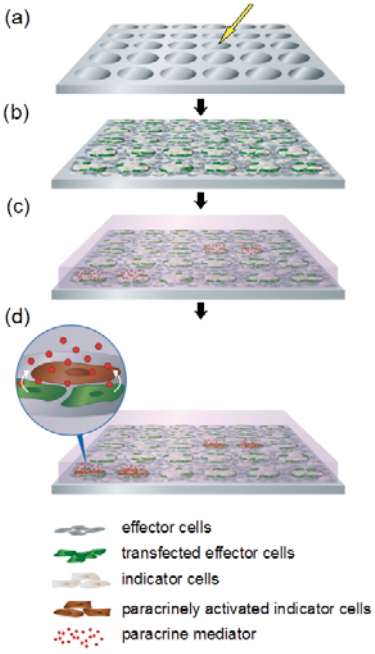
15.02.2011 - 14.02.2014

Paracrine gene functions in Kaposi‘s sarcoma

Prof. Dr. Dr. Michael Stürzl, Department of Surgery

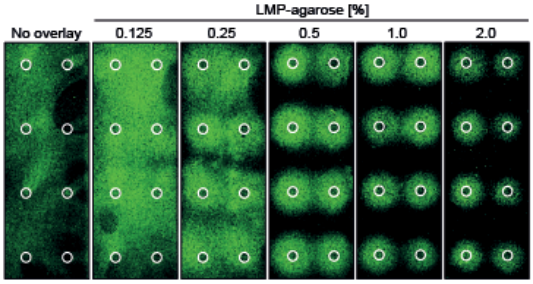
Kaposi’s sarcoma-associated herpesvirus (KSHV) is the etiologic agent of Kaposi’s sarcoma (KS), an endothelial cell-derived tumour. Previous in vivo observations indicated that paracrine effects from infected cells on none-infected cells may constitute a prominent driving force of tumor growth. The aim of this proposal was to identify (i) viral genes inducing paracrine effects and (ii) respective paracrinely acting factors which are released form infected cells and may contribute to KS tumorigenesis.

In the first part of the project a novel chip-based transfection assay allowing systematic high throughput analysis of paracrine gene functions has been successfully developed (Kuhn et al., 2012). In parallel, we searched for paracrinely-acting proteins released from KSHV-infected cells. To this goal we compared cell culture supernatants harvested from latently KSHV-infected and uninfected cells using 2-dimensional differential in gel electrophoresis (2D-DIGE). In this framework the most appropriate cell culture model was a cell line which was originally isolated from KS and regarded to be an immortal KS tumor cell line (SLK). A derivative of this line (iSLK cell line) has been engineered recently expressing the major activator of KSHV lytic replication in a doxycycline (DOX)-inducible manner. It has been reported that this cell line can be efficiently infected by KSHV, with a very low background of lytic infection in the absence and a very efficient lytic replication in the presence of DOX. Following routine initial characterization protocols in our laboratory SLK and iSLK cells (retrieved from different sources) were subjected to short tandem repeat (STR) profiling using the recommended 9 STR loci. Comparison with an international reference database of cell line STRs revealed that the SLK cell line as well as its derivative exhibited a STR profile similar to the clear cell renal carcinoma cell line Caki-1. Subsequent analysis of all available lots of SLK cells including the AIDS reagents repository confirmed that SLK cells were cross-contaminated (Stürzl et al., 2012). For further experiments we substituted SLK cell by telomerase-immortalized human umbilical vein endothelial cells (HUVEC-TI) and lymphatic endothelial cells (LEC-TI) as more appropriate model systems for KS. HUVEC-TI/LEC-TI were infected with a recombinant KSHV strain (KSHV.219) expressing constitutively GFP to allow monitoring of infection efficiency. Supernatants of these cells and uninfected cells were harvested and subjected to 2D-DIGE. This approach identified several proteins, which were present in different amounts in the supernatants from infected and uninfected cells. Differentially expressed proteins were extracted from the gel and identified by mass spectrometry. Among these EGF-containing fibulin-like extracellular matrix protein 1 precursor (EFEMP-1) and glucose-regulated protein 94 (GRP94) were found to be present in increased concentrations in the cell culture supernatants.



Scheme of the Parachip. (a) Slide with transfection spots. (b) Overlay with effector and indicator cells. Indicator cells are selectively transfected. (c) Overlay with a diffusion restricting matrix. (d) Activation of indicator cells by the paracrine mediator.

Prof. Dr. Dr. Stürzl



Paracrine induction of the large GTPase GBP-1 (green) in human fibroblasts by IFN-γ released from HEK293T cells transfected with an IFN-γ expression plasmid (IFN γ). Chips were overlaid with low melting point (LMP) agarose in increasing concentrations to restrict the diffusion of IFN-γ.

tants of KSHV-infected HUVEC-TI/LEC-TI. Strikingly, EFEMP-1 has been shown to have pro-tumorigenic effects in pancreatic cancer. GRP94 has been shown to exert anti-apoptotic effects and has been detected in cell-released exosomes. Differential secretion of GRP94 from KSHV infected cells could be confirmed using immunoprecipitation experiments and an increased expression of the grp94 gene in KS lesions was detected. The contribution of both proteins to the growth of KS is currently investigated.

Contact:
Prof. Dr. Dr. Stürzl
phone: +49 9131 85 33109
e-mail: michael.stuerzl@uk-erlangen.de

Publications during funding period

Publications directly related to the project:

Chudasama P, Konrad A, Jochmann R, Lausen B, Holz P, Naschberger E, Neipel F, Britzen-Laurent N, Stürzl M (2014) Structural proteins of Kaposi's sarcoma-associated herpesvirus antagonize p53-mediated apoptosis. Oncogene (in press)

Jochmann R, Holz P, Sticht H, Stürzl M (2013) Validation of the reliability of computational O-GlcNAc prediction. Biochim Biophys Acta Dec 9. pii: S1570-9639(13)00424-X. doi: 10.1016/j.bbapap.2013.12.002. [Epub ahead of print]

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Hahn AS, Kaufmann JK, Wies E, Naschberger E, Panteleev-Ivlev J, Schmidt K, Holzer A, Schmidt M, Chen J, König S, Ensser A, Myoung J, Brockmeyer NH, Stürzl M, Fleckenstein B, Neipel F (2012) The ephrin receptor tyrosine kinase A2 is a cellular receptor for Kaposi's sarcoma–associated herpesvirus. Nat Med. 18(6):961-6

Kuhn E, Naschberger E, Konrad A, Croner RS, Britzen-Laurent N, Jochmann R, Münstedt H, Stürzl M (2012) A novel chip-based parallel transfection assay to evaluate paracrine cell interactions. Lab Chip 12(7):1363-72

Konrad A, Jochmann R, Kuhn E, Naschberger E, Chudasama P, Stürzl M (2011) Reverse transfected cell microarrays in infectious disease research. Methods Mol Biol 706: 107-18

Alkharsah KR, Singh VV, Bosco R, Santag S, Grundhoff A, Konrad A, Stürzl M, Wirth D, Dittrich-Breiholz O, Kracht M, Schulz TF (2011) Deletion of Kaposi's sarcoma herpesvirus FLICE inhibitory protein (vFLIP) from the viral genome compromises the activation of STAT-1 responsive cellular genes and spindle cell formation in endothelial cells. J Virol 85: 10375-88

Jochmann R, Lorenz P, Chudasama P, Zietz C, Stürzl M, Konrad A (2011) KSHV Paracrine Effects on Tumorigenesis. Intech – Herpes Viruses ISBN 978-953-307-750-5

Publications acknowledging the IZKF funding, because based on the co-operative working structure in our laboratory interaction occurred.

Stein MF, Lang S, Winkler TH, Deinzer A, Erber S, Nettelbeck DM, Naschberger E, Jochmann R, Stürzl M, Slany RK, Werner T, Steinkasserer A, Knippertz I (2013) Multiple IRF- and NFkB-sites cooperate in mediating cell type- and maturation-specific activation of the human CD83 promoter in dendritic cells. Mol Cell Biol 33: 1331-44

Britzen-Laurent N, Lipnik K, Ocker M, Naschberger E, Schellerer VS, Croner RS, Vieth M, Waldner M, Steinberg P, Hohenadl C, Stürzl M (2013) GBP-1 acts as a tumor suppressor in colorectal cancer cells. Carcinogenesis 34: 153-62

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 28: 1469-78

Ostalecki C, Konrad A, Thurau E, Schuler G, Croner RS, Pommer AJ, Stürzl M (2013) Combined multi gene analysis at the RNA and protein levels in single FFPE tissue sections. Exp Mol Pathol 95: 1-6

Serafin V, Persano L, Moserle L, Esposito G, Ghisi M, Curtarello M, Bonanno L, Masiero M, Ribatti D, Stürzl M, Naschberger E, Croner RS, Jubb AM, Harris AL, Koeppen H, Amadori A, Indraccolo S (2011) Notch3 signaling promotes tumor growth in colorectal cancer. J Pathology 224: 448-60

Naschberger E, Schellerer V, Rau TT,Croner RS, Stürzl M (2011) Isolation of endothelial cells from human tumors. Methods Mol Biol. 731: 209-18.

A46 - Final Report

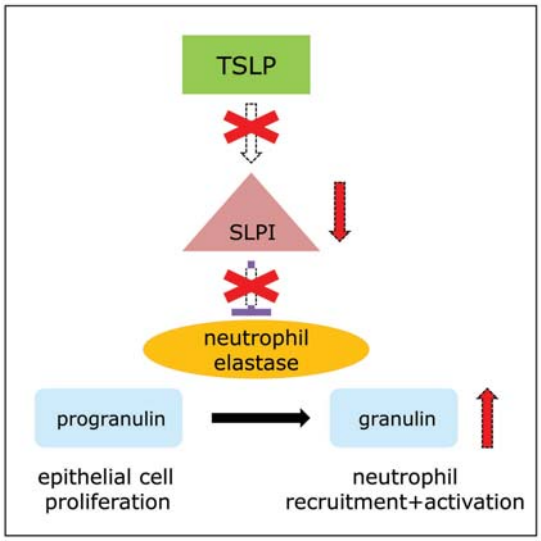
20.01.2011 - 19.01.2014

Relevance of TSLP in the immune response

PD Dr. Dr. Matthias Lechmann, Department of Immune Modulation

Tslp is a key regulator of Th2-driven inflammation and allergic diseases. But its exact role in the balance of immune responses is obscure. Using our Tslp ko mouse, we study the immunoregulatory mechanisms of Tslp in the pathogenesis of different diseases in vivo. So far, we could show for the first time that Tslp is essential for recovery following colitis. Further, we showed that deletion of TSLP resulted in an amelioration of EAE symptoms with reduced inflammatory infiltrates in the brain.

The aim of this project is to evaluate the functions of the cytokine Tslp in autoimmune diseases and infections. Tslp is proposed to be a master regulator of Th2-driven inflammation. There is strong evidence that Tslp plays a crucial role in allergic diseases. In contrast, it has been reported to have an essential protective function in inflammatory responses of the gut. Several studies also suggested a possible role of Tslp in regulatory T cell differentiation and it might shift T cells towards a Th17 phenotype. Thus, the exact role of Tslp in B and T cell development has recently become unclear.

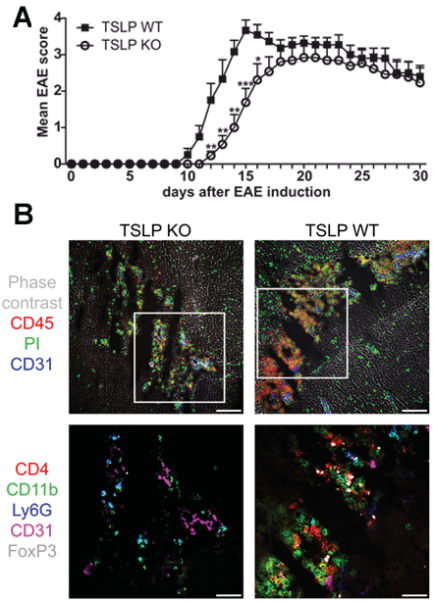


Our data showed that loss of the cytokine Tslp reduced SLPI expression increasing neutrophil elastase activity and the pro-inflammatory granulin in murine colon.

To clarify the role of Tslp, we generated a Tslp knockout (ko) mouse. First, we analyzed this mouse in different murine disease models of colitis. Strikingly, we could show that Tslp is essential for recovery following colitis. Our data showed that Tslp functions as a critical mediator controlling the balance between host defence and wound repair, but does not restrict the production of Th1 type cytokines or affect the translocation of gut bacteria. Tslp-deficiency was associated with an increase in neutrophil elastase activity and a decrease of the endogenous secretory leukocyte peptidase inhibitor. Our data demonstrated for the first time that Tslp can act directly on intestinal epithelial cells in an autocrine manner and is a key facilitator of wound repair following intestinal injury (Reardon et al, 2011).

Next, we evaluated the role of Tslp for the balance of Th1, Th17 and Th2 cells and for the functionality of Tregs in our Tslp ko mice using the Experimental-Autoimmune-Encephalomyelitis (EAE) model. Tslp ko mice were immunized s.c. with myelin oligodendrocyte glycoprotein (MOG) in CFA at day 0 to induce the EAE. In addition, pertussis toxin was administered i.p. at day 0 and 2. The clinical symptoms of the mice were scored from 0 to 5. We found that Tslp-deficient mice displayed a delayed onset of disease and an ameliorated form of EAE compared with Tslp wt mice. This delayed onset was accompanied by reduced inflammatory infiltrates in brain and spinal cord visualized by the Multi-Epitope-Ligand-Carto-

PD Dr. Dr. Lechmann



Tslp ko mice show a reduced EAE severity. A: Mean clinical EAE score of Tslp ko and Tslp wt mice. B: MELC images of brain at day 12 after EAE induction. Inflammatory foci of Tslp ko mice contain fewer leukocytes than inflammatory foci of Tslp wt.

graphy (MELC) technique. The MELC-technique was adapted to identify a large panel of murine leukocyte subpopulations in a whole frozen section to compare non-inflamed versus inflamed tissues (Eckhardt et al., 2012). Interestingly, T cells from Tslp ko mice show reduced encephalitogenic capacities and a diminished expression of proinflammatory cytokines, due to impaired activation. $CD3^+$ T cells isolated in the preclinical EAE-phase from MOG-immunized Tslp ko mice showed a reduced response after secondary exposure to MOG in comparison to $CD3^+$ T cells isolated from Tslp wt mice. The addition of recombinant (rec.) Tslp further increased T cell proliferation during MOG restimulation in vitro. In addition, the Tslp deficiency in the ko mice was compensated by the injection of rec. Tslp. In summary, these data demonstrate that expression of, and immune activation by Tslp significantly contributes to the immunopathology of EAE.

Contact:
PD Dr. Dr. Lechmann
phone: +49 9131 85 34746
e-mail: matthias.lechmann@uk-erlangen.de

Publications during funding period

Eckhardt J*, Ostalecki C*, Kuczera K, Schuler G,. Pommer AJ*, Lechmann M* (2012) Murine Whole Organ Immune Cell Populations Revealed by Multi-epitope Ligand Cartography. J Histochem Cytochem published online 16 November 2012. DOI:10.1369/0022155412470140

Reardon C*, Lechmann M*, Brüstle A, Gareau MG, Shuman N, Philpott D, Ziegler SF, Mak TW (2011) Thymic Stromal Lymphopoetin-Induced Expression of the Endogenous Inhibitory Enzyme SLPI Mediates Recovery from Colonic Inflammation. Immunity 35(2):223-35. Epub 2011 Aug 4

*These authors contributed equally to this work.

A52 - Progress Report

01.11.2013 - 30.04.2016

cFlip isoforms in the intestinal epithelium

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

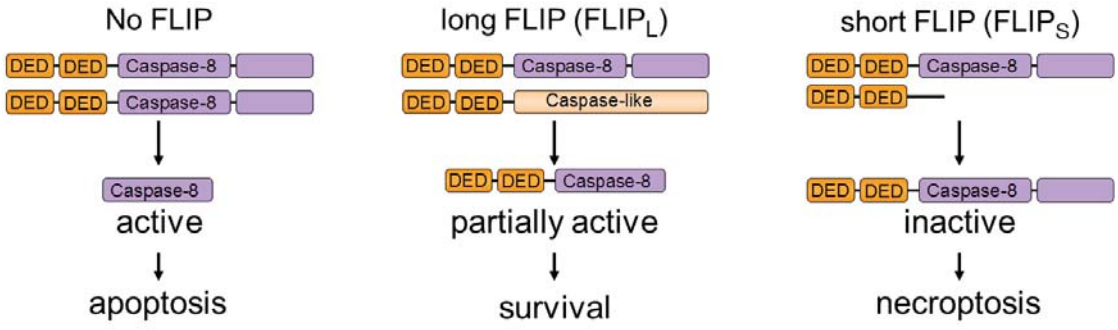
cFLIP is a central regulator of cell death and survival. Recent data provide evidence, that cFLIP proteins can also decide which form of cell death is activated in a cell. Previous data additionally demonstrate a potential role of cFLIP isoforms for the regulation of intestinal epithelial necroptosis in inflammatory bowel disease patients. The aim of this project is a differential analysis of the role of cFLIP variants for the pathogenesis of infectious and inflammatory bowel diseases.

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 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. 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. This RipK3-dependent cell death pathway is negatively regulated by caspase-8.

The activation status of caspase-8 therefore decides not only on cell survival and death, it also regulates the mode of cell death, demonstrating that caspase-8 needs to be tightly controlled.

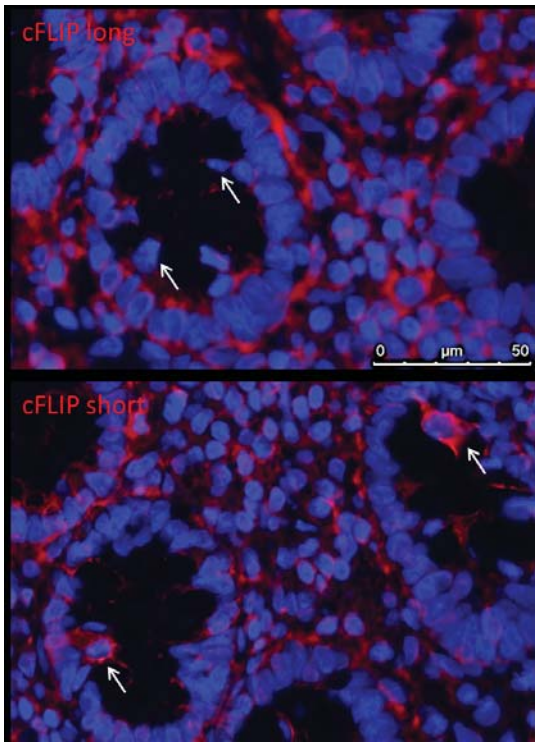
A crucial regulator of caspase-8 is cFLIP (cellular FLICE-inhibitory protein), which exists in two different isoforms, cFLIP long ($cFLIP_L$) and short ($cFLIP_S$). $cFLIP_L$ binds to procaspase-8 via its two death effector domains (DEDs). An additional pseudocaspase-like domain is able to block the autocatalytic cleavage of caspase-8 and therefore prevents apoptosis. Furthermore, a residing catalytic activity of the heterodimer has been shown to be sufficient to inactivate the Rip-kinases and therefore necroptosis. Combining these effects, the association of caspase-8 with $cFLIP_L$ protects cells from apoptotic and necroptotic cell death and ultimately enables them to survive.



Regulation of caspase-8 activity by cFLIP isoforms.


Dr. Günther


Prof. Dr. Becker



The short isoform of cFLIP is expressed in dying intestinal epithelial cells in Crohn's disease patients. The protective long isoform is seems not to be expressed in these cells.

In addition to $cFLIP_L$, the shorter isoform $cFLIP_S$ does not contain a pseudocaspase domain. $cFLIP_S$ unlike $cFLIP_L$ prevents the initial cleavage step of procaspase-8 and therefore cannot contribute to the inactivation of RipK1. RipK1 and RipK3 associate, get phosphorylated and can promote necroptosis.

The aim of this project is the evaluation of the importance of the individual isoforms of cFLIP in chronic and infectious IBD.

We could already demonstrate an increased level of $cFLIP_S$ mRNA in whole gut specimens collected from Crohn´s disease patients. We performed immunohistochemical analyses on biopsies of inflamed tissue from patients. Using antibodies specific for the individual isoforms, we could detect $cFLIP_S$ in dying epithelial cells. In contrast to this, dying cells did not show $cFLIP_L$ presence, supporting the data, which show that cFLIPL protects cells from death via apoptosis or necroptosis.

In further studies we want to investigate the effect of $cFLIP_{L/S}$ expression in human and murine cells. The influence on intestinal epithelial cells in in vivo models using isoform specific knockout mice will also be important.

Contact:
Prof. Dr. Becker
phone: +49 9131 85 35886
e-mail: christoph.becker@uk-erlangen.de

Invited lectures

Jahrestagung der Deutschen Gesellschaft für Mukosale Immunologie und Mikrobiom, Essen, 14.- 15.11.2014 „Regulation of intestinal epithelial cells by inflammatory cytokines"

3rd Conference "Translational Medicine on Pathogenesis and Therapy of Immune-Mediated Diseases", Mailand, 29. – 30.10.2014, "Is Crohn's disease an immune defect or an immune excess?"

Summer School "Inflammation at Interfaces", Schleswig, 08. - 10.10.2014 Keynote lecture "Regulation of intestinal epithelial barrier function by cytokines"

Forum Intestinale Immunologie, Berlin 05. - 06.10.2014 "Regulation der Darmbarriere durch Zytokine"

Rehbrücker Kolloquium, Potsdam, 06.08.2014 „Regulation of intestinal epithelial cell functions during inflammation and infection"

„Hot Topics in Gastroenterology", Rom, Italien, 11.04.2014 "Inflammatory pathways in the control of colon carcinogenesis"

IRC Workshop „Innate Immunity, Signaling and Cell Death", Ghent, Belgium, 16.12.2013, "From inflammation to cancer: cell death regulation in the intestinal epithelium"

Publications during funding period

Takahashi, N., L. Vereecke, M.J.M. Bertrand, L. Duprez, S.B. Berger, T. Divert, A. Goncalves, M. Sze, B. Gilbert, S. Kourula, V. Goossens, S. Lefevre, C. Günther, C. Becker, J. Bertin, P.J. Gough, W. Declercq, G. van Loo, P. Vandenabeele. (2014). RIPK1 is a guardian of intestinal homeostasis protecting the epithelium against apoptosis. Nature. 513(7516):95-9

Günther C., B. Buchen, G. He, M. Hornef, N. Torow, H. Neumann, N. Wittkopf, E. Martini, M. Basic, A. Bleich, A.J. Watson, M.F. Neurath, C. Becker. (2014) Caspase-8 controls the gut response to microbial challenges by TNFalpha -dependent and –independent pathways. Gut. doi: 10.1136/gutjnl-2013-305947. [Epub ahead of print]

Günther, C., B. Buchen, M.F. Neurath, C. Becker. (2014) Regulation and pathophysiological role of epithelial turnover in the gut. Semin Cell Dev Biol. S1084-9521(14):177-3

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A53 - Progress Report

01.10.2013 - 30.09.2016

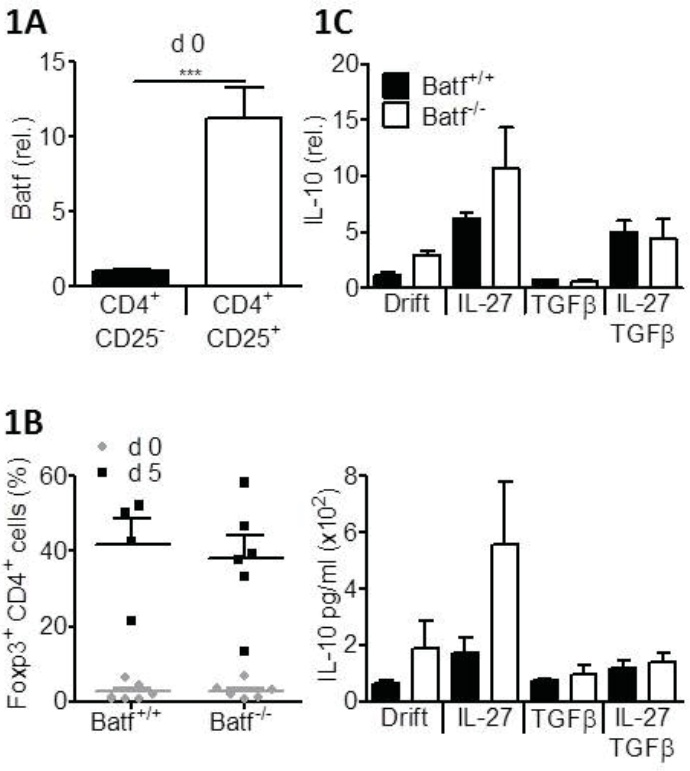
Th17/piTreg differentiation in vivo

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

T cell differentiation into Interleukin 17a$^+$ Th17 cells critically depends on the AP-1 transcription factor family member Batf. Furthermore, Batf is a crucial T cell intrinsic regulator of the de novo differentiation of peripherally induced regulatory FoxP3$^+$ T cells (iTregs) under lymphopenic conditions in a clinically relevant setting in vivo. In contrast to Th17 cells, iTreg generation is not affected in vitro. Hence Batf plays a dichotomous role during Th17/iTreg development in vivo.

The AP-1 family member Batf regulates Th17 differentiation and Th17-related tissue pathology

Previous data of our group showed that Batf controls the ability of a naïve T helper cell to become an IL-17a$^+$ Th17 cell in vitro and in vivo. Hence, Batf$^{-/-}$ T cells fail to induce tissue inflammation as e.g. experimental autoimmune encephalomyelitis (EAE) in a T cell intrinsic manner. In accordance with this finding, Batf$^{-/-}$ T cells are not colitogenic in two colitis models under lymphopenic conditions.



In vitro characterization of Batf$^{-/-}$ regulatory T cells (Tregs). 1A Batf mRNA expression in naïve vs. Treg T cells. 1B Differentiation of TGF-ß-induced Foxp3$^+$ iTreg. 1C Batf-dependent IL-10 mRNA and protein expression in IL27/TGF-ß induced Tr1 cells.

Prof. Dr. Hildner

Batf is dispensable for the generation of FoxP3$^+$ iTregs in vitro

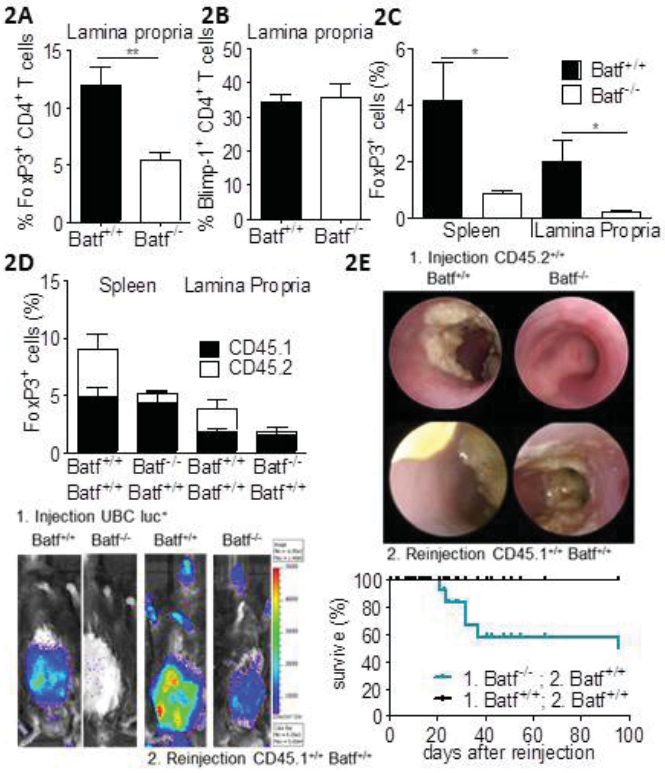
Batf expression in developing T cells is induced by T cell receptor- and IL-6/Stat3-dependent signaling. Interestingly, steady state splenic CD4$^+$CD25$^+$ T cells enriched for lymphoid tissue-resident Tregs constitutively express elevated Batf expression levels ex vivo compared to naïve T cells. However, various in vitro culture systems testing de novo Treg formation failed to reveal Batf-dependent differences in respect to either the number or frequency of FoxP3$^+$ iTreg or IL-10$^+$ Tr1 cells.

Batf deficient mice display diminished colonic but not splenic FoxP3$^+$ T cells in vivo

Furthermore, steady state analyses failed to identify reduced numbers of FoxP3$^+$ Tregs in lymphoid tissues of Batf$^{-/-}$ mice. In contrast, the frequency of FoxP3$^+$ colonic lamina propria (cLP) T cells was reduced while Blimp1 expressing cLP T cells were unaltered in Batf$^{-/-}$ compared to Batf$^{+/+}$ mice.

Batf is crucial for the de novo formation of FoxP3$^+$ T cells under lymphopenic conditions in vivo

To test whether Batf$^{-/-}$ T cells are hampered to differentiate de novo into FoxP3$^+$ T cells in vivo, we transferred CD45.1$^+$ Batf$^{+/+}$ and CD45.2$^+$ Batf$^{-/-}$ T cells either into the same or separate lymphopenic hosts and assessed the formation of FoxP3$^+$ T cells 14d later. Strikingly, we detected a reduced frequency of FoxP3$^+$ Tregs in all assessed compartments (spleen and cLP). In contrast to mice previously given Batf$^{+/+}$ T cells, mice previously receiving Batf$^{-/-}$ T cells developed a fatal systemic inflammation upon secondary challenge with Batf$^{+/+}$ T cells. Molecular studies are under way to reveal molecular cues contributing to this phenomenon in the absence of Batf.



Batf-dependent Tregs in vivo. 2A, 2B FoxP3$^+$ and Blimp1$^+$ T cells (%) ex vivo. 2C, 2D top Tregs (%) upon individual or combined naïve T cell transfer into Rag1$^{-/-}$ mice. 2D bottom, 2E Colitis induction and survival upon indicated transfer of T cells.

Contact:
Prof. Dr. Hildner
phone: +49 9131 85 45173
e-mail: kai.hildner@uk-erlangen.de

Publications during funding period

none

A54 - Progress Report

01.11.2013 - 30.04.2016

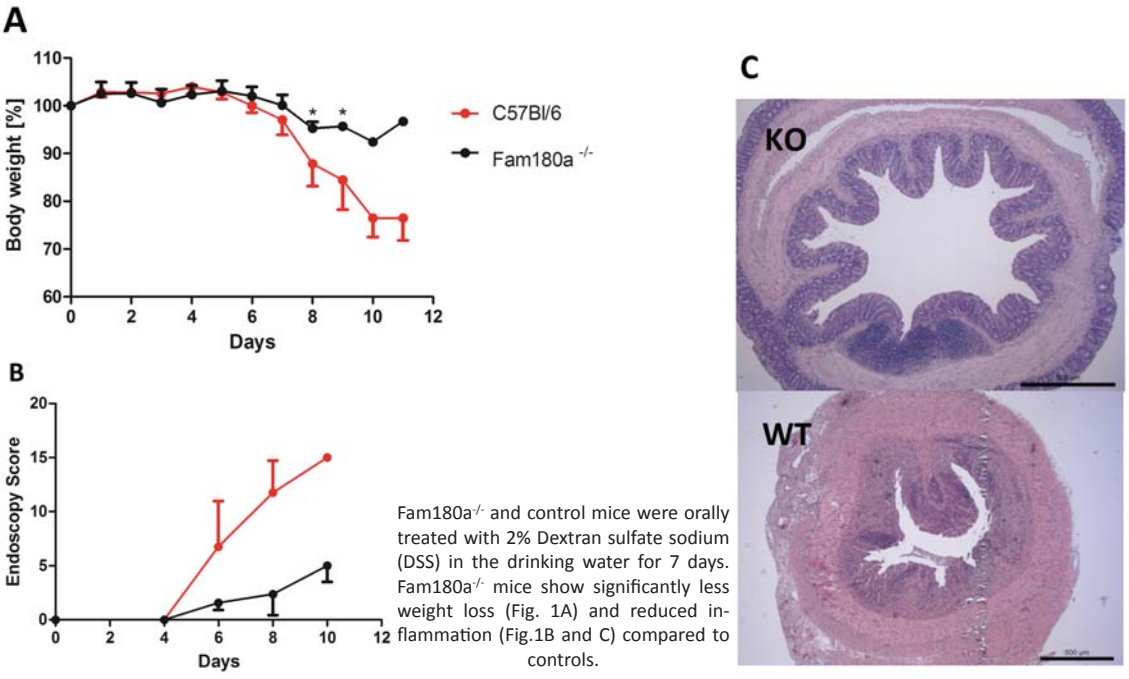
Fam180a in inflammatory diseases

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

Acute as well as chronic inflammatory diseases are caused by a complex immunological network of cell types and their mediators, a system that is still only poorly understood. One so far uncharacterized player in this network, Fam180a, resembles a cytokine-like factor promoting inflammation. In this project we investigated its expression as well as the phenotype of Fam180a deficient mice in different animal models. Our results suggest Fam180a to be a secreted protein responsible for the maintenance of an inflammatory phenotype by interacting with neutrophil granulocytes in a so far unknown manner.

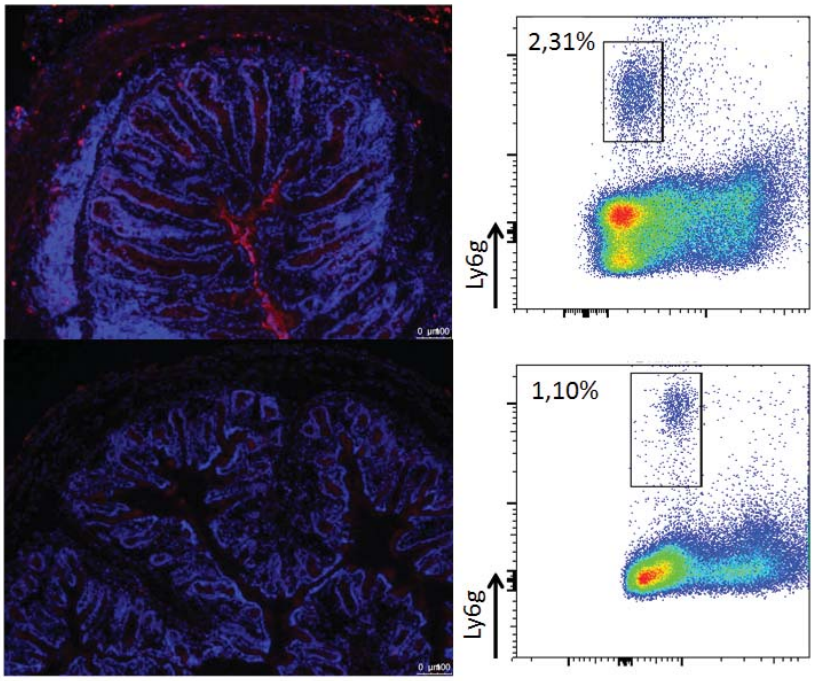
Fam180a is highly expressed in cells of the innate immune system and shows similarities to other cytokines

Fam180a is a 19-kDa large protein highly expressed in CD11b^{+} and Ly6g^{+} cells. The protein contains a N-terminal aminoacid sequence closely resembling a signal peptide, suggesting an extracellular function. We reinforced this notion by locating Fam180a in secretory vesicles of transfected macrophages (J774). Fam180a is highly conserved across different species and composes an α-helix bundle, similar to numerous cytokines like IL-2, IL-4 or GM-CSF. In addition, our results from transfected fibroblasts indicate that Fam180a is active as a dimer based on labelling of the secreted protein at a significantly larger size (35kDa) than expected. Furthermore, a recombinant Fam180a monomer produced in E. coli is not soluble



Fam180a$^{-/-}$ and control mice were orally treated with 2% Dextran sulfate sodium (DSS) in the drinking water for 7 days. Fam180a$^{-/-}$ mice show significantly less weight loss (Fig. 1A) and reduced inflammation (Fig.1B and C) compared to controls.

PD Dr. Dr. Wirtz Prof. Dr. Waldner



Staining of colonic sections 13 days after the first DSS administration (left) as well as FACS data from isolated lamina propria cells (right). Fam180a$^{-/-}$ mice show overall less tissue damage as well as reduced numbers of neutrophils (DAPI, MPO/Ly6G) compared to control mice.

without the addition of chaotrophic reagents, indicating the need for a binding partner, chaperones or specific conditions for a native folding.

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

Fam180a deficient mice show no phenotype in the steady state. In an acute model of DSS induced colitis however, these mice display significantly reduced weight loss and decreased mucosal inflammation compared to wildtype mice, which was also the case on a RAG deficient background. We found a major reduction in the amount of Ly6g^{+} cells in the inflamed colon at different time points during inflammation, indicating a link between Fam180a and neutrophil granulocytes. A similar connection could be established in a model of pancreatitis, where Fam180a deficient mice showed less organ damage and reduced immune cell infiltrations.

Neutrophils from Fam180a$^{-/-}$ mice are more prone to cell death

Analysis of BM-neutrophils isolated from Fam180a$^{-/-}$ mice revealed an increased mortality rate compared to controls. This phenotype could not be reversed by the addition of Fam180a derived from transfected culture supernatants. Possible explanations are the lack of a functional binding partner or species incompatibility, however it may also be the case that the presence of Fam180a is required for a normal neutrophil development.

Contact:
Prof. Dr. Waldner
phone: +49 9131 85 35894
e-mail: maximilian.waldner@uk-erlangen.de

Awards

Endoskopie-Forschungspreis der Olympus Europa Stiftung und der DGVS an Maximilian Waldner, 17-20.09.2014, Leipzig

Publications during funding period

none

A55 - Progress Report

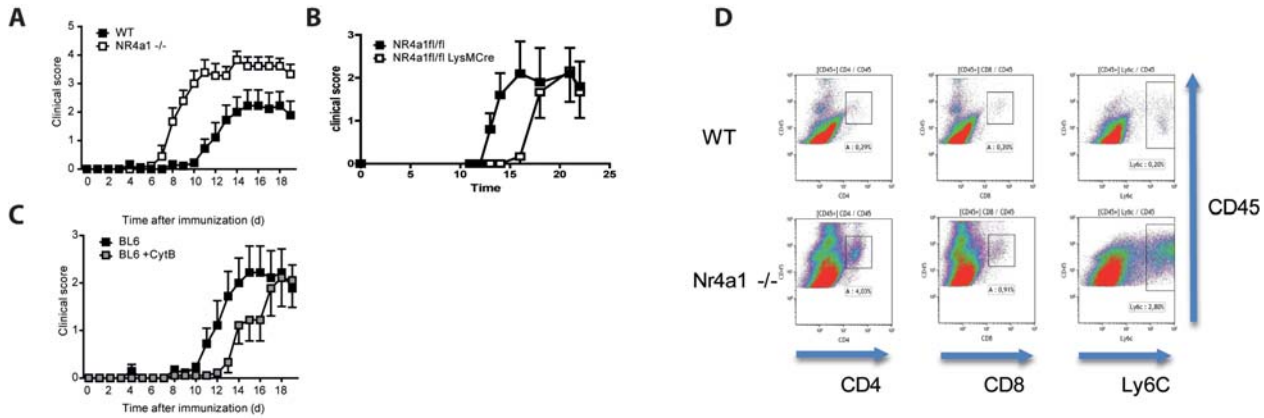
01.01.2014 - 30.06.2016

NR4a1 during immunologic tolerance

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

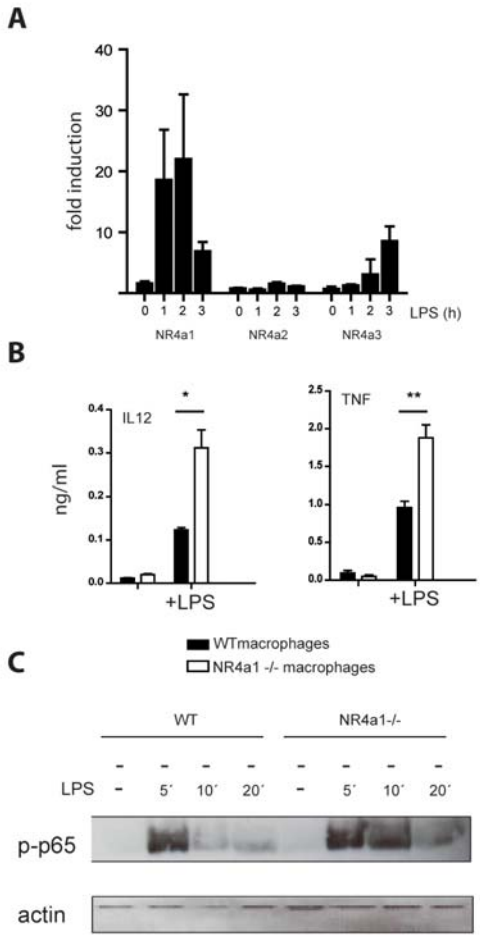
Our peliminary data shows an exacerbation of different autoimmune diseases after deletion of the nuclear receptor NRa1 suggesting a key role of this transcription factor during the maintenance of self-tolerance. During the current project we aim to adress the involved molecular events and responsible cell types.

To determine the role of NR4a1 during autoimmunity and self-tolerance, we performed the model of experimental autoimmune encephalomyelitis in WT and NR4a1$^{-/-}$ mice. Notably, NR4a1-deficient animals developed an accelerated and augmented disease that was characterized by an increased influx of monocytes, macrophages and T-cells into the inflamed CNS.Ligand-induced activation of NR4a1 by injection of the specific R4a1 agonist Cytosporone B, in turn, resulted in an attenuated course of disease confirming a central role of this receptor in the regulation of autoinflammatory disorders. To determine the underlying molecular and cellular mechanisms, we subsequently analyzed mice carrying a conditional deletion of NR4a1 in distinct cell types including macrophages, T-cells and dendritic cells. Here, the macrophage-specific (LysM-Cre-mediated) deletion of this nuclear receptor triggered an exacerbated form of EAE, thus displaying the same phenotype as the full KO mouse and suggesting a key role of NR4a1 in this myeloid cell subset. In accordance with a major role of NR4a1 in the control of macrophage activation, our in vitro data reveal an exacerbated inflammatory response of NR4a1-deficient macrophages, which is characterized by an increased production of pro-inflammatory cytokines and prolonged activation of NfkB.



(A) Exacerbation of EAE in Nr4a1$^{-/-}$ mice and (B) mice carrying a macrophage-specific deletion of NR4a1. (C) Ameliorated EAE after Cytosporone B-induced activation of NR4a1. (D) Increased infiltration of inflammatory leukocytes into the CNS of NR4a1$^{-/-}$ mice after induction of EAE.

PD Dr. Krönke



(A) Measurement of the LPS-induced expression of NR4a family members in bone marrow-derived macrophages. (B) Measurement of the LPS-induced cytokine secretion by WT and NR4a1-/- macrophages. (C) Analysis of NfκB activation (p65 phosphorylation) in macrophages after incubation with LPS.

Additional data show that uptake of apoptotic cells by macrophages resulted in a rapid induction of NR4a1, which subsequently enabled a non-immunogenic clearance of apoptotic-cell-derived autoantigens. Deletion of NR4a1 resulted in elevated levels of IL12 in response to the uptake of dying cells and provoked a break in self-tolerance in the murine model of pristane-induced lupus.

Contact:
PD Dr. Krönke
phone: +49 9131 85 43012
e-mail: gerhard.kroenke@uk-erlangen.de

Invited lectures

Eurolupus Meeting, 23.4.2014, Athens, "12/15-Lipoxygenase orchestrates the clearance of apoptotic cells"

Seminar at the University of Lübeck (Kolloquium Molekulare Zellbiologie), 10.4.2014, "Pros and cons of lipid oxidation during inflammation and immunity"

Publications during funding period

Ipseiz N, Scholtysek C, Culemann S, Krönke G. (2014) Adopted orphans as regulators of inflammation, immunity and skeletal homeostasis. Swiss Med Wkly. 2014 Dec 4;144:w14055

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. 2014 May 15;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

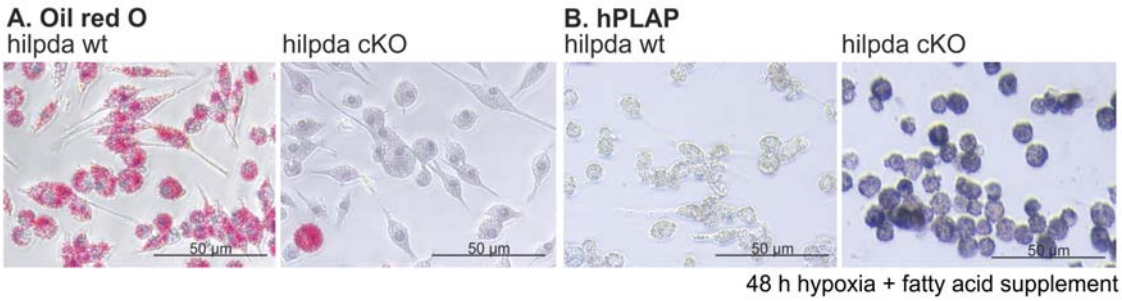
The hypoxia-inducible lipid droplet associated protein Hig2/Hilpda mediates lipid accumulation under hypoxic conditions, but also stimulates lipid droplet formation after lipid loading in normoxia. Although Hilpda is highly expressed in foam cells, its contribution to atherogenesis is not known. In the present project we are delineating the effects of a conditional hilpda knockout on the development of atherosclerosis in apolipoprotein $E^{-/-}$ mice in vivo and on macrophage function in vitro.

Hilpda knockout (KO) macrophages cannot store neutral lipids under hypoxia or after lipid loading.

Like most cells, bone marrow-derived macrophages (BMDMs) accumulate neutral lipids under hypoxic conditions. Tie2 cre-driven hilpda KO completely abolished lipid accumulation in BMDMs after exposure to hypoxia (1% O_2), chemical activators of hypoxia-inducible factors (HIF) such as DMOG and DP, or TLR4 activation with LPS, even under serum-free conditions. LPS activated HIF-1 and downregulated HIF-2, suggesting that hilpda is a HIF-1 target in BMDMs. The hilpda-mediated lipid accumulation was independent of the HIF-mediated glycolytic switch, as it was not reduced by dichloroacetate, an inhibitor of PDK. In addition, the effect of the hilpda KO was not reversed by siRNA-knockdown of the hPLAP reporter, which is expressed instead of Hilpda in KO cells.

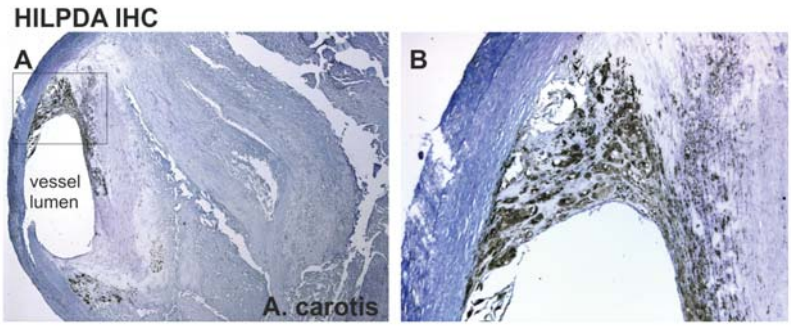
Hilpda is also inducible by PPAR activation.

Loading BMDMs with cholesterol-cyclodextrin complex revealed that hilpda KO BMDMs are not able to store cholesteryl esters. Loading BMDMs with fatty acid supplement or oleic acid/BSA markedly increased lipid droplet size and number. Cholesterol and fatty acids robustly induced hilpda mRNA expression even in normoxia. Fatty acids increased hilpda mRNA independent of HIF-1. In agreement with our results, hilpda was recently identified as a novel PPAR target gene (Mattijssen et al., J. Biol. Chem. 2014). This suggests that hilpda could be induced in atherosclerosis not only by HIF-1, but also by hyperlipidemia. The fatty acid-induced, hilpda-mediated lipid droplet formation was very robust, delayed but not abolished in normoxia, and not affected by statins, rapamycin, PKC, PI3 kinase or MAP kinase inhibitors.



Hilpda is required for neutral lipid storage.
Hilpda cKO (tie2 cre) and wildtype macrophages were exposed to hypoxia and fatty acid supplement and stained for neutral lipids (A) or hPLAP (B), which is expressed instead of Hilpda in cKO cells.

PD Dr. Warnecke



HILPDA in a human atherosclerotic plaque.
Immunohistochemistry (IHC) shows the characteristic HILPDA signals in macrophages in the plaque shoulder. A, 2.5x; B, magnif. of detail marked in (A). Section was provided by B. Dietel, Med. 2., UKE.

Further characteristics of hilpda KO BMDMs.

Hilpda KO cells proved to be more resistant than wildtype cells against cell stress induced by fatty acid overload combined with DP, whereas ATP levels under hypoxia were not reduced. Lipid accumulation by hilpda does not involve increased fatty acid uptake, but appears to be mediated by increased intracellular sequestration of esterified fatty acids in lipid droplets.

Hilpda KO did not lead to marked alterations in mRNA expression of genes related to lipid metabolism or inflammation, although cox2/ptgs2 expression was frequently reduced.

HILPDA in atherosclerosis.

In human plaques HILPDA was highly expressed in macrophage foam cells surrounding the lipid core and in the plaque shoulder, a region frequently characterized by active inflammation and prone to destabilization and rupture.

Analysis of apoE$^{-/-}$ mice was established including Enface preparations of aortae, histology and immunohistochemistry. Based on a timeline experiment, a mouse age of 22 weeks was determined for further analyses, because all apoE$^{-/-}$ mice had visible plaques at that age.

Since November, apoE$^{-/-}$/hilpda cKO mice are being analyzed in parallel with their cre-negative siblings. The histological examination and statistics will reveal the details.

Contact:
PD Dr. Warnecke
phone: +49 9131 85 39562
e-mail: christina.warnecke@uk-erlangen.de

Awards

IZKF poster award, Anja Maier, 16th May 2014, IZKF Symposium 2014, 15th-17th May 2014, Kloster Banz

Publications during funding period

none

A57 - Progress Report

01.01.2014 - 30.06.2016

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

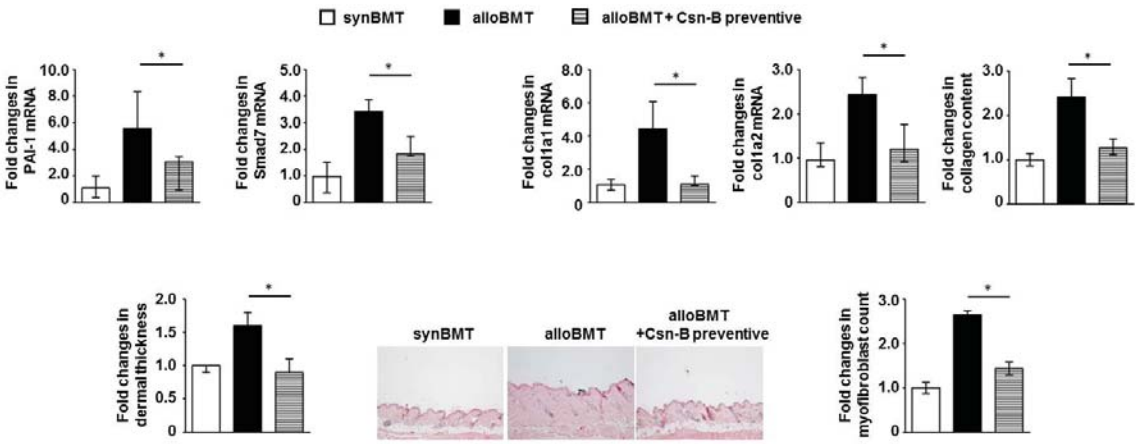
Prof. Dr. Jörg Distler, Department of Medicine 3 – Rheumatology and Immunology
Prof. Dr. Bernd Spriewald, Department of Medicine 5 – Haematology and Oncology

The orphan nuclear receptor Nr4a1 is an endogenous antagonist of TGF-ß, which is inactivated in scleroder-matous 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 tis-sue fibrosis in experimental cGvHD. Further studies indicate that Nr4a1 may also be a target for therapeutic intervention in other fibrotic diseases.

Nr4a1 inhibits TGF-β signaling by trans-repression

Analysis of the col 1a1 promoter revealed neither NBREs nor NurRE nor DR5 elements within 10 kbp upstream of both promoters. Apart from gene-re-pression by sequence-specific DNA binding, nuclear receptors commonly interact with other transcrip-tional regulators to trans-repress gene expression. To address the hypothesis that Nr4a1 binds to the promoters of type I collagens as Nr4a1/Sp1 comple-xes, we first showed that TGF-β stimulates binding of Nr4a1 to Sp1 in fibroblasts with a shift from Smad3/Smad4/Sp1 complexes at early time points to Nr4a1/Sp1 complexes at later time points. ChIP assays further demonstrated that TGF-β induces binding of Nr4a1-containing complexes to the Sp1 binding site -242 bp in the col 1a1 promoter, which was inhibited by siRNA mediated knockdown of Sp1.

Silencing of NCOR, SMRT, CtBP1 or CtBP2 did not re-duce the inhibitory effects of Nr4a1 on the expressi-on of TGF-β responsive genes. Knockdown of Sin3A and CoREST, however, released the block of Nr4a1 on TGF-β signaling. siRNA-mediated knockdown of HDAC1 or LSD1 restored the responsiveness of Nr4a1 overexpressing fibroblasts to TGF-β, demonstrating that both components are functionally required for Nr4a1 dependent trans-repression. Nr4a1 therefore recruits Sp1/Sin3A/CoREST/LSD1/HDAC1 complexes to inhibit the transcription of type I collagens and other TGF-β targets genes.



CsnB inhibits TGF-β signaling and ameliorates clinical and histological features of murine cGvHD.

Prof. Dr. Distler Prof. Dr. Spriewald

Inactivation of Nr4a1 signaling in fibrotic diseases

We next investigated whether persistently elevated TGF-β levels may lead to desensitization of the Nr4a1 response. The levels of Nr4a1 mRNA and pan-Nr4a1 protein rapidly declined after an initial peak. In contrast to Nr4a1, induction of other TGF-β-responsive genes did not decrease upon chronic exposure to TGF-β. Chronically activated TGF-β signaling also failed to continuously induce Nr4a1 in vivo. We hypothesized that the desensitization of Nr4a1 transcription might be mediated by epigenetic mechanisms. Indeed, incubation with pan-HDAC inhibitor TSA, but not with 5-aza, prevented the decline of Nr4a1 upon long-term stimulation with TGF-β.

Moreover, chronically elevated levels of TGF-β induced S351 phosphorylation of Nr4a1 in cultured fibroblasts. Progressive accumulation of P-Nr4a1 was also observed in mice overexpressing TBRI and in experimental models of fibrosis. P-Nr4a1 accumulated in human fibrotic tissues. Inactivation of Akt, but not of GSK3-β or JNK abrogated phosphorylation of Nr4a1 upon long-term stimulation with TGF-β. Overexpression of non-mutated Nr4a1 prevented the induction of TGF-β target genes at early time-points, but its inhibitory effects faded upon prolonged TGF-β stimulation. In fibroblasts overexpressing mutated Nr4a1, however, the TGF-β-responsive genes remained suppressed throughout the observation period. Mechanistically, P-Nr4a1 can no longer bind to Sp1, thereby preventing Sp1 dependent trans-repression of TGF- β target genes by Nr4a1.

Contact:
Prof. Dr. Distler
phone: +49 9131 85 43008
e-mail: joerg.distler@uk-erlangen.de

Invited lectures

Annual Meeting of the European League against Rheumatism, June 2014, Paris, What is new - Systemic sclerosis

Annual Meeting of the „Deutschen Gesellschaft für Rheumatologie“, September 2014, Düsseldorf, SSc im Jahr 2014 - ein Update

Awards

Rudolf-Schoen-Award of the „Deutschen Gesellschaft für Rheumatologie“ 2013

Heisenberg Professorship from the German Research Association for Jörg Distler

Publications during funding period

Zerr P, Palumbo-Zerr K, Huang J, Tomcik M, Sumova B, Distler O, Schett G, Distler JH (2014). Sirt1 regulates canonical TGF-β signalling to control fibroblast activation and tissue fibrosis. Ann Rheum Dis. 2014 Sep 1. [Epub ahead of print]

Tomcik M, Palumbo-Zerr K, Zerr P, Avouac J, Dees C, Sumova B, Distler A, Beyer C, Cerezo LA, Becvar R, Distler O, Grigorian M, Schett G, Senolt L, Distler JH (2014). S100A4 amplifies canonical TGF-ß induced fibroblast activation in systemic sclerosis. Ann Rheum Dis. Apr 7 [Epub ahead of print]

Beyer C, Huang J, Beer J, Zhang Y, Palumbo-Zerr K, Zerr P, Distler A, Dees C, Maier C, Munoz L, Krönke G, Uderhardt S, Distler O, Jones S, Rose-John S, Oravecz T, Schett G, Distler JH (2014). Activation of liver X receptors inhibits experimental fibrosis by interfering with interleukin-6 release from macrophages. Ann Rheum Dis. Mar 11 [Epub ahead of print]

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Zerr P, Vollath S, Palumbo-Zerr K, Tomcik M, Huang J, Distler A, Beyer C, Dees C, Gela K, Distler O, Schett G, Distler JH (2014). Vitamin D receptor regulates TGF-β signalling in systemic sclerosis. Ann Rheum Dis. Jan 21 [Epub ahead of print]

Distler A, Lang V, Del Vecchio T, Huang J, Zhang Y, Beyer C, Lin NY, Palumbo-Zerr K, Distler O, Schett G, Distler JH (2014). Combined inhibition of morphogen pathways demonstrates additive antifibrotic effects and improved tolerability. Ann Rheum Dis. Jan 20 [Epub ahead of print]

Zhang Y, Dees C, Beyer C, Lin NY, Distler A, Zerr P, Palumbo K, Susok L, Kreuter A, Distler O, Schett G, Distler JH (2014). Inhibition of casein kinase II reduces TGFβ induced fibroblast activation and ameliorates experimental fibrosis. Ann Rheum Dis. 2014 Jan 15 [Epub ahead of print]

A58 - Progress Report

01.10.2013 - 31.03.2016

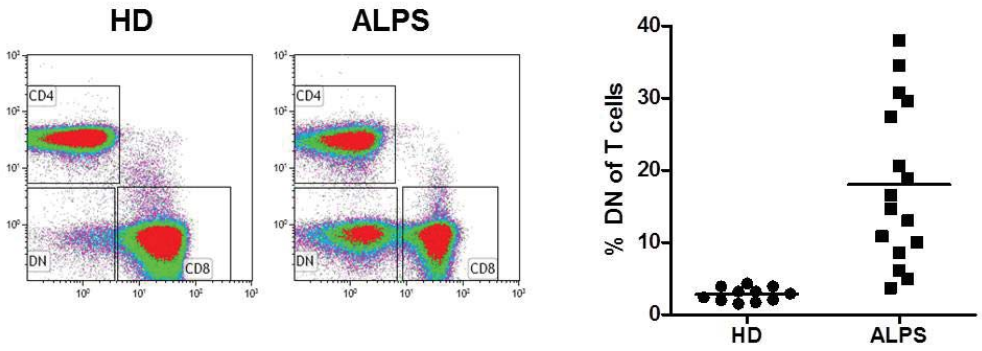
Characterization of DN T cells from ALPS patients

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. The most prominent feature of ALPS is the accumulation of CD3$^+$ TCR$\alpha\beta^+$ 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. In this project we aim to characterize the mechanisms leading to an accumulation of DN T cells and the functional properties of these cells.

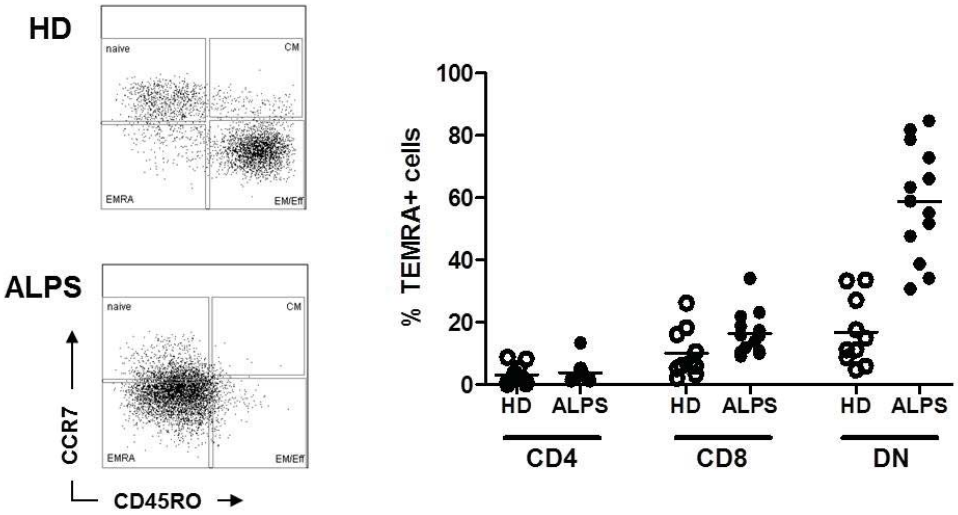
DN T cells from ALPS patients exhibit a distinct differentiation pattern

In close collaboration with Prof. Dr. Stephan Ehl (CCI, University of Freiburg) we compared the cellular phenotype of ALPS DN T cells to that of CD4$^+$ or CD8$^+$ T cells. The majority of ALPS DN T cells expressed markers associated with terminal differentiation (TEMRA), whereas in healthy donors most DN T cells showed an effector-memory phenotype. However, the surface receptor KLRG1, which is normally coexpressed with CD57 and PD-1, was almost absent on ALPS DN T cells. Furthermore, ALPS DN T cells highly expressed CD27 and CD28, which are typically downregulated on TEMRA cells. In contrast, DN T cells from healthy donors were CD27$^+$ CD28$^+$ KLRG1$^+$ CD57low, consistent with an effector-memory phenotype. Thus, ALPS DN T cells clearly differed from healthy donor DN T cells and also from typical CD4$^+$ or CD8$^+$ TEMRA cells; thereby, they could not be grouped into a defined differentiation subset. We then analyzed the expression of the T-box transcription factor T-bet and its paralog eomesodermin, which represent a pair of regulatory factors crucially involved in determination of T cell fate, phenotype, and function. ALPS DN T cells showed high expression of eomesodermin, which was also observed in CD4$^+$ or CD8$^+$ TEMRA cells. However, DN T cells from ALPS patients completely lacked expression of T-bet, suggesting an abnormal programming of these cells.



Representative plots showing percentages of CD4$^+$, CD8$^+$, and DN T cells among all T cells. Graph represents cumulative data of all studied healthy donors and ALPS patients.

Prof. Dr. Mackensen Dr. Völkl



PBMC from healthy donors and ALPS patients were analyzed for CCR7 and CD45RO expression. Dot plots are gated for DN T cells, graph represents cumulative data of all ALPS patients and healthy donors.

Abnormal expression profile can be detected in subsets of CD4⁺ or CD8⁺ T cells

We next asked whether we could identify cells with this abnormal phenotype among ALPS CD4⁺ or CD8⁺ T cells. ALPS patients showed slightly increased percentages of CD4⁺ and CD8⁺ TEMRA cells, which predominantly expressed CD57 and KLRG1. However, we detected a small subset of KLRG1⁻ CD27⁺ CD28⁺ TEMRA cells within the ALPS CD4⁺ and CD8⁺ T cell population, whereas these cells were completely absent in healthy donor T cells. Notably, these uncommon CD4⁺ and CD8⁺ TEMRA cells also exhibited the high eomesodermin to T-bet expression ratio. To further examine the relationship of these cell subsets to ALPS DN T cells, we analyzed their coreceptor expression. Although TEMRA CD4⁺ and CD8⁺ T cells from healthy donors had normal coreceptor expression, TEMRA cells of ALPS patients consisted of 2 populations, a CD27⁻ CD28⁻ population with normal CD4 or CD8 expression ("conventional"), and an abnormal CD27⁺ CD28⁺ population with reduced CD4 or CD8 levels ("DN-like"). We then performed TCRβ deep sequencing in sorted T-cell populations of an ALPS patient. Of note, a significant fraction of identical TCRβ CDR3 sequences shared by CD4⁺ TEMRA and DN T cells, as well as by CD8⁺ TEMRA and DN T cells could be detected, indicating that both CD4⁺ and CD8⁺ T cells represent precursors of DN T cells.

Contact:
Dr. Völkl
phone: +49 9131 85 43168
e-mail: simon.voelkl@uk-erlangen.de

Publications during funding period

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

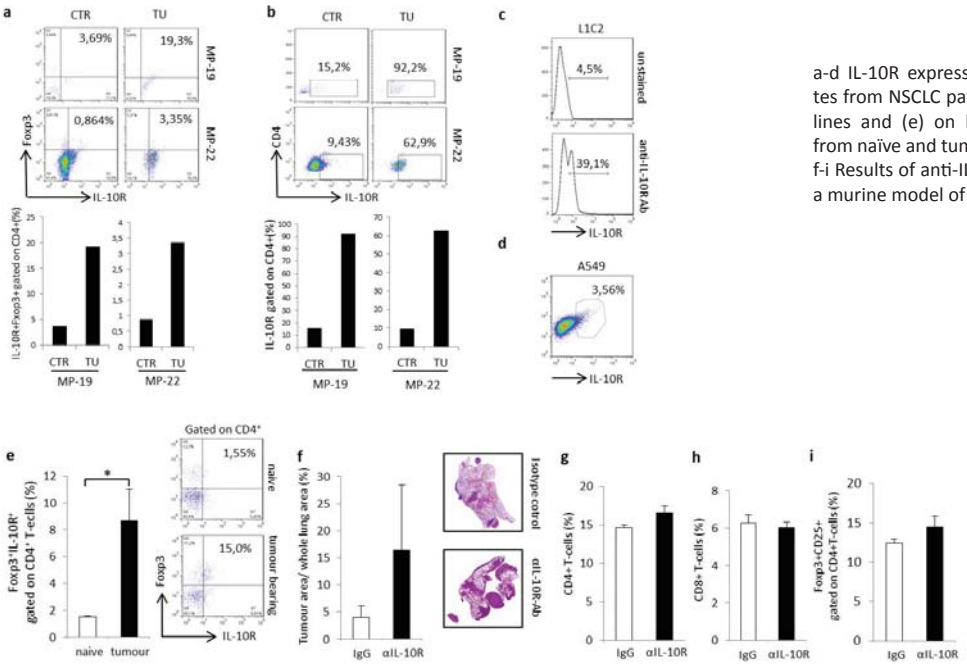
01.10.2013 - 30.09.2016

IL-10 and lung cancer

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

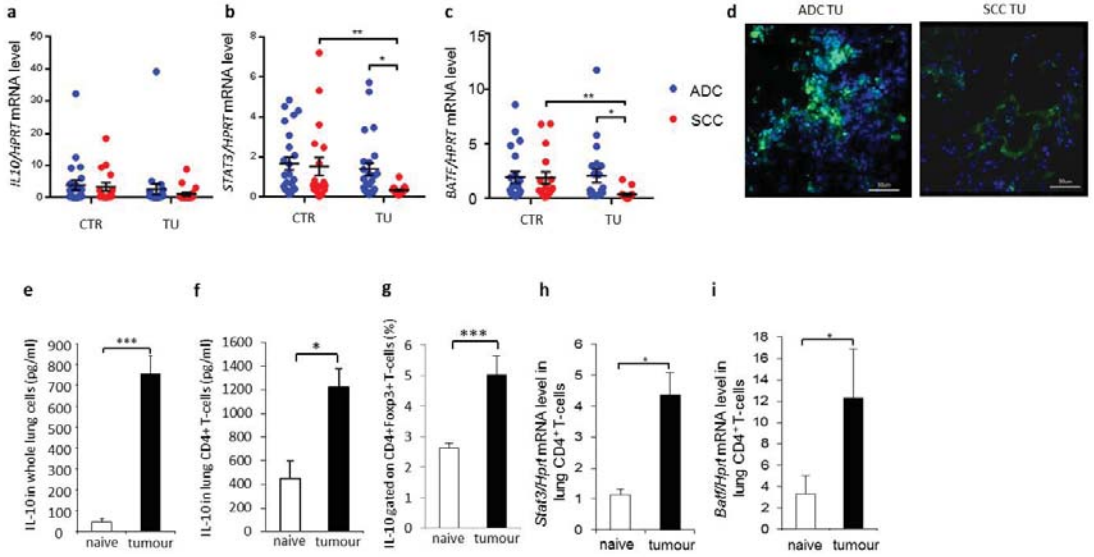
IL-10 is a strong immunosuppressive cytokine. In this project we first addressed the question of how IL-10 gene expression is regulated in lung cancer. IL-10 gene expression is controlled by different transcription factors, such as T-bet, STAT3, BATF and IRF4. In this part of the study, we investigated differential expression of IL-10 and IL-10R as well as the transcription factors regulating IL-10 in lung tissue and cells, obtained after lung tumour surgery and in a murine model of disease.

In the first period of the project we analyzed IL-10 and IL-10R in the lungs of patients with Non-Small-Cell-Lung Cancer (NSCLC). To this aim, we analysed samples from two different regions of the lungs, namely the so called tumoural area (TU), taken directly from the solid tumour and the control region (CTR), being a tumour free lung tissue that is at least 5 cm away from the solid tumour. Hereby, we also compared the gene expression in patients with adenocarcinoma and squamous cell carcinoma, the two major subtypes of NSCLC. Although we could not detect a difference in the mRNA levels of IL-10 between the control and the tumoural area of adeno– as well as squamous cell carcinoma, we found increased numbers of IL-10R expressing $CD4^+$ T-cells and $CD4^+Foxp3^+$ T-cells in the tumoural area of NSCLC patients. Moreover, we analysed the mRNA expression of STAT3 and BATF (Basic leucine zipper transcription factor, ATF-like), two transcription factors that are involved in the regulation of IL-10 production, which were both up-regulated in the tumoural region of patients with adenocarcinoma as compared to those with squamous cell carcinoma. In the control region, both STAT3 and BATF were increased in the squamous carcinoma, whereas it remained steadily up-regulated in adenocarcinoma patients.



a-d IL-10R expression on lymphocytes from NSCLC patients, tumour cell lines and (e) on lung cells derived from naïve and tumour bearing mice. f-i Results of anti-IL-10R treatment in a murine model of lung cancer.

Prof. Dr. Dr. Finotto



RNA and protein expression of IL10, STAT3 and BATF in samples from NSCLC patients and in lung cells, derived from naïve and tumour bearing mice.

To further analyse the role of IL-10 and the IL-10R in an experimental model of lung cancer, we injected mice with a lung tumour cell line intravenously (L1C2 or LL2-luc-M38 cells). A previously performed flow cytometry analysis revealed that about 30-40% of the murine L1C2 lung tumour cells express the receptor for IL-10, whereas only 3-4% of the human lung tumour cells line A549 were found to express the IL-10R. In our murine model for lung cancer we found that total lung cells from mice bearing tumour release significantly more IL-10 as compared to lung cells, isolated from tumour-free mice. We then sorted out $CD4^+$ T-cells from the total lung cell pool, finding higher levels of IL-10 protein as well as of Batf and Stat3 mRNA in $CD4^+$ T-cells from tumour bearing mice as compared to those from naïve mice. In addition, we found increased numbers of IL-10 producing $CD4^+Foxp3^+$ T regulatory cells in mice bearing tumour as compared to naïve mice. In accordance with our human data also $CD4^+Foxp3^+$T regulatory cells from mice bearing tumour were found to express increased IL-10R on their surface indicating an IL-10 autocrine loop on the surface of T regulatory cells in mice bearing tumour that needs to be further investigated. We next treated lung tumour bearing mice with a neutralizing anti-IL-10R antibody or an Isotype control (on day 4 and day 7 respectively) and anaylsed the tumour area in the lungs of the mice. We found no significant difference of the tumour load between mice treated with anti-IL-10R antibody and the isotype control. We also did not observe any significant difference concerning the numbers of $CD4^+$ helper T-cells, $CD8^+$ cytotoxic T-cells or $CD4^+CD25^+Foxp3^+$ regulatory T-cells. Taken together we discovered a not yet identified role of STAT3/BATF on IL-10 production in CD4+ T cells as well as in T regulatory cells infiltrating the lungs bearing tumour that we are further investigating. These data will set up the basis for new immunotherapy for lung cancer.

Contact:
Prof. Dr. Dr. Finotto
phone: +49 9131 85 35883
e-mail: susetta.finotto@uk-erlangen.de

Publications during funding period

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 - 31.03.2016

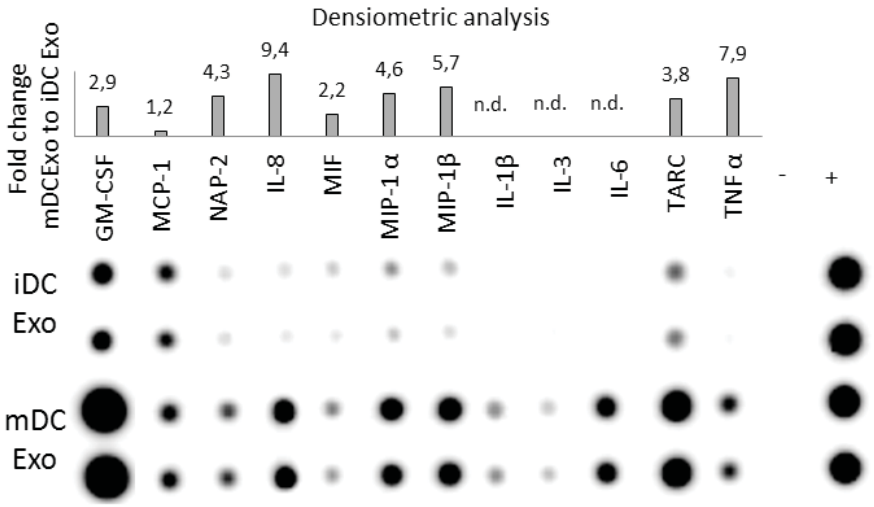
Monocyte derived Dendritic cells (Mo-DC) by DC Exosomes

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 like cells. Furthermore intradermally injection of mDC Exo led to infiltration of DC and T cells in draining lymph node reflecting their composition of several immuno-stimulatory and -attracting factors.

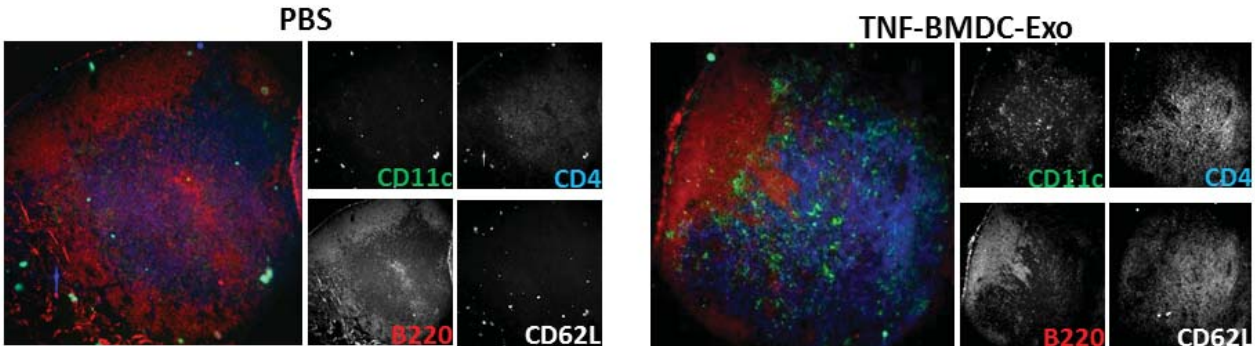
Exosomes (Exo) are secreted membrane vesicles from endosomal origin, with the size of 40-100nm, which contain active biologic cytosolic- (e.g. miRNA and cytokines) and surface-derived molecules. Regarding exosomes derived from Dendritic cells (DC Exo) were mainly applied for therapeutic cell free vaccination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

In order to understand the role of DC Exo we started to analyze effects on resting 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 DC Exo exclusively Mo showed activation via phosphorylation of Stat5. After 6 days Mo incubated with Exo derived from immature DC (iDC Exo) changed their morphology to a rather immature (stretched, short microvilli), whereas Exo derived from mature DC (mDC Exo) lead to a more mature DC phenotype (round, veiled). Flow cytometric analysis confirmed that Mo treated with mDC Exo showed increased expression of maturation marker compared to Mo incubated with iDC Exo. In agreement with that mDC Exo, but not iDC Exo generated DC showed an allogeneic stimulatory capacity comparable to conventional generated DC. On the other hand long term incubation (10-13 days) of PBMC with DC Exo lead to prolonged survival and proliferation of DC



Antibody (chemokine and cytokine) array of iDC and mDC Exo. (duplicate factors and neg. (-) and pos. (+) controls are shown). Graph: Comparative densiometric analysis; Fold change of mDCExo to iDC Exo is depicted.

Dr. Baur Dr. Schierer



Draining lymph nodes of intradermally-injected TNF-BMDC Exo show infiltration of $CD11c^+$ and $CD4^+CD62L^+$ cells. MELC analysis of lymph node cryosections derived from one mouse 48h after simultaneous injection of Exo in left and PBS into right back flank.

similar as GM-CSF treated PBMC. Western blot analysis confirmed that both iDC and mDC Exo contain large amounts of GM-CSF. Blocking the essential DC growth factor GM-CSF abrogated the DC Exo induced differentiation of Mo. Elucidating their different maturation capability we found that mDC Exo include active TNF-α together with its corresponding active protease Adam17 in contrast to iDC Exo that harbor only the inactive precursors, pro-TNF-α and pro-Adam17. Antibody array analysis of chemokine and cytokine levels supported that especially mDC Exo include known inflammatory cytokines (e.g. Il1β, IL-6, TNF-α) needed for maturation of DC in vitro. Furthermore we found additional chemokines (e.g. IL-8, MIP1-α and -β, Nap-2) and cytokines (e.g. IL3, MIF) in mDC Exo capable to attract and activate a broad range of immune cells. To confirm these predicted effects in-vivo we generated TNF matured Bone marrow derived DC-Exo (TNF-BMDC Exo). Array analysis revealed that TNF BMDC Exo also contain growth factors, inflammatory cytokines and chemokines similar to human mDC Exo. In cooperation with AG Zinser (dermatology) TNF-BMDC Exo were injected intradermally in autologous C57/Bl6 mice. After two days draining lymph node appeared swollen and analysis of sections with MELC (Multi-Epitope-Ligand-Cartography) technology revealed that this was mainly the result of infiltrated DC ($CD11c^+$) and naïve T-cells ($CD4^+CD62L^+$). In cooperation with AG Schleicher/Bogdan (A61) the effect of BMDC Exo will further analyzed in infection Leishmania model.

In summary mDC Exo seem to have a multifunctional capability to recruit, activate and differentiate immune cells especially Mo, probably to prepare immune system for foreign/danger situation

Contact:
Dr. Schierer
phone: +49 9131 85 31127
e-mail: stephan.schierer@uk-erlangen.de

Publications during funding period

none

A61 - Progress Report

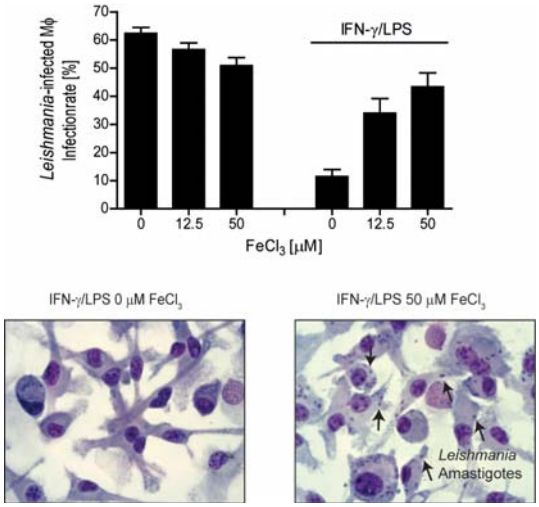
01.02.2014 - 31.07.2016

Leishmania, iNOS and iron

Prof. Dr. Christian Bogdan, PD Dr. Ulrike Schleicher,
Institute of Microbiology - 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 syntase (iNOS or NOS2). NOS2 is expressed in phagocytes upon stimulation by cytokines (e.g. interferon [IFN]-γ, tumor necoris factor [TNF]) and/or microbial ligands (e.g. lipopolysaccharide [LPS]) and converts the amino acid L-arginine into citrulline and nitric oxide (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 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.



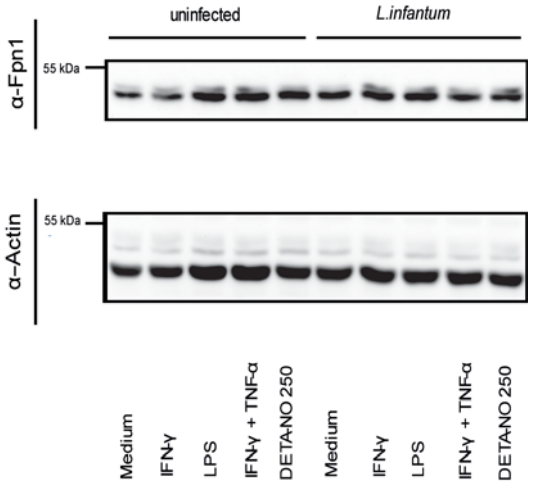
Killing of Leishmania in IFN-γ/LPS-stimulated bone marrow-derived macrophages in the absence or presence of exogenous Fe^{3+}.

In order to test the hypothesis that the antileishmanial effect of NO not only relies on direct destruction of the parasites by damaging Leishmania DNA, structural proteins and/or metabolic enzymes, but is also a result of the withdrawal of iron from the microenvironment of intracellular Leishmania, we performed killing assays with L. major- and L. infantum-infected macrophages that were stimulated by IFN-γ plus LPS in the presence or absence of exogenous Fe^{2+} or Fe^{3+}, added in the form of $FeCl_3$ or $FeSO_4$. Both compounds proved to be able to at least partly reverse the killing of intracellular Leishmania in IFN-γ/LPS-stimulated mac-

Prof. Dr. Bogdan PD Dr. Schleicher

rophages. The rescuing effect of Fe^{2+}/Fe^{3+} was also observed when macrophages were exposed to an exogenous NO donor instead of being activated for the generation of endogenous NO. These data indicate that deprivation of iron might be one of the mechanisms of NOS2-dependent control of Leishmania. To further elucidate the underlying mechanisms, we started to analyze whether the expression of ferroportin-1 (Fpn-1), the only known cellular export system for Fe^{2+}/Fe^{3+}, is increased in a NO-dependent manner both in vitro in infected macrophages and in vivo during L. major and L. infantum infections. At least in vitro with bone marrow-derived macrophages, neither Fpn-1 nor the iron storage protein ferritin seemed to be regulated by NO on a mRNA and protein level.

In a reverse approach we could demonstrate that iron-overloading of Leishmania-infected mice caused an exacerbation of the infection. Correspondingly, iron loading of macrophages reduced the production of NO indicating that iron-dependent regulation of NOS2 might influence the outcome of Leishmania infection.



Expression of ferroportin-1 protein in Leishmania-infected and uninfected macrophages incubated in medium or stimulated with cytokines (IFN-γ, TNF), LPS or the NO-donor DETA-NO (250 µM).

Ongoing analyses further address the antimicrobial function and intracellular localisation of NOS2 and its crossregulation with iron.

Contact:
Prof. Dr. Bogdan
phone: +49 9131 85 22551
e-mail: christian.bogdan@uk-erlangen.de

Invited lectures

13th International Congress of Parasitology, August 10-15,2014, Mexico City, C. Bogdan Immunoregulatory and wound-healing effects of reactive oxygen species in cutaneous leishmaniasis (plenary lecture)

Graduate School of Immunology, University of Kopenhagen, International Summer School Meeting, September 3-5, 2014, Sandbjerg, C. Bogdan Cutaneous and visceral leishmaniasis – Clinical manifestations, immune response and novel radical-based mechanisms of control and therapy (plenary lecture)

Publications during funding period

Bode, S.F., Bogdan, C., Beutel, K., Behnisch, W., Greiner, J., Henning, S., Jorch, N., Jankofsky, M., Jakob, M., Schmid, I., et al. (2014). Hemophagocytic lymphohistiocytosis in imported pediatric visceral leishmaniasis in a nonendemic area. The Journal of Pediatrics 165, 147-153 e141

Mahnke, A., Meier, R.J., Schatz, V., Hofmann, J., Castiglione, K., Schleicher, U., Wolfbeis, O.S., Bogdan, C., and 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

Stahl, H.C., Ahmadi, F., Schleicher, U., Sauerborn, R., Bermejo, J., Amirih, M., Sakhayee, I., Bogdan, C., and Stahl, K.W. (2014). A randomized controlled phase IIb wound healing trial of cutaneous leishmaniasis ulcers with 0.045% pharmaceutical chlorite (DAC N-055) with and without bipolar high frequency electro-cauterization versus intralesional antimony in Afghanistan. BMC Infectious Diseases 14, 619

A62 - Progress Report

01.01.2014 - 30.06.2016

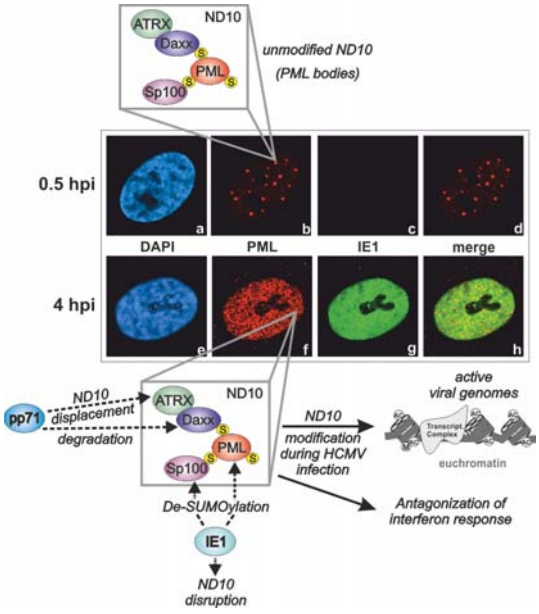
ND10 and interferon-induced gene expression

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, is frequently modified during infection with various viruses. This proposal investigates whether ND10 structures have a co-regulatory function for interferon-induced gene expression. We propose that viruses may antagonize specific aspects of the interferon reponse by manipulating ND10.

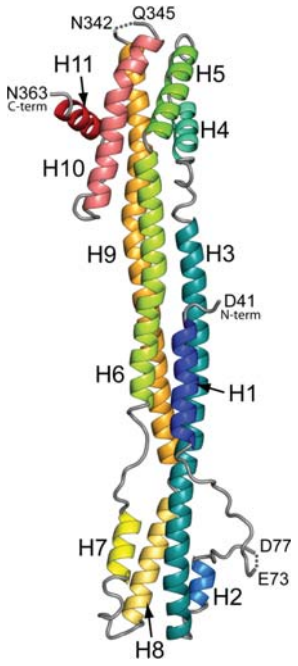
Role of the ND10 protein PML for IFN-type I and II induced gene expression

In our previous studies we have shown that the nuclear domain 10 (ND10) components PML, hDaxx, and Sp100 mediate an intrinsic immune defense against human cytomegalovirus (HCMV) and other herpesviruses. PML, in addition to other ND10 components, is encoded by an interferon-stimulated gene (ISG) and its expression is strongly increased by interferons (IFNs). Intriguingly, we observed that PML-depleted cells show a reduced expression of an IFN-γ–stimulated gene, namely the MHC-II gene HLA-DR/DP/DQ, compared to control cells. In order to explore whether PML itself is involved in the induction of further ISGs, we treated PML-depleted primary human fibroblasts (HFFs) as well as control HFFs with IFN-γ and analyzed the cellular gene expression profiles by a cDNA array experiment. We identified numerous genes that displayed a reduced expression in PML-depleted cells. Next, we performed quantitative RT-PCR to analyze the expression of single genes in more detail. These experiments revealed that PML depletion impairs gene activation induced by both type I- and type II- IFNs. These results strongly suggest a co-regulatory role of PML in the IFN-induced upregulation of a distinct set of ISGs. Moreover, IFN stimulation of HFFs with inducible expression of the globular PML interaction domain of IE1 revealed an inhibitory effect of this viral effector protein on ISG induction. In conclusion, IE1 may not only antagonize the ND10-mediated intrinsic defense, but may also counteract specific aspects of the interferon response by manipulating ND10 proteins.



Architecture of the subnuclear structure ND10 and its modification during human cytomegalovirus infection. During HCMV infection the viral proteins pp71 and IE1 modify ND10 to ensure viral gene expression and to antagonize the interferon response.

Prof. Dr. Stamminger



Crystal structure of the globular core domain of the ND10-antagonistic cytomegalovirus protein IE1. The structure displays an all α-helical, femur-shaped fold which shares secondary structure features with the coiled-coil domain of TRIM proteins.

3D structure of the ND10 antagonistic viral effector protein IE1 of human cytomegalovirus

Since IE1 of HCMV acts as an important ND10-antagonistic protein we wished to delineate the molecular basis for this. By solving the crystal structure for the evolutionary conserved primate cytomegalovirus IE1 proteins we could show that IE1 consists of a globular core ($IE1_{CORE}$) flanked by intrinsically disordered regions. The 2.3 Å crystal structure of $IE1_{CORE}$ displays an all α-helical, femur-shaped fold, which lacks overall fold similarity with known protein structures but shares secondary structure features recently observed in the coiled-coil domain of TRIM proteins. Yeast two-hybrid and coimmunoprecipitation experiments demonstrated that $IE1_{CORE}$ binds efficiently to the TRIM family member PML and is able to induce PML deSUMOylation. Importantly, we show that PML deSUMOylation by $IE1_{CORE}$ is sufficient to antagonize PML-NB-instituted intrinsic immunity. Moreover, co-immunoprecipitation experiments demonstrate that $IE1_{CORE}$ binds via the coiled-coil domain to PML and also interacts with TRIM5α.We propose that $IE1_{CORE}$ sequesters PML and possibly other TRIM family members via structural mimicry using an extended binding surface formed by the coiled-coil region.

Contact:
Prof. Dr. Stamminger
phone: +49 9131 85 26783
e-mail: thomas.stamminger@viro.med.uni-erlangen.de

Invited lectures

Colloquium at the Institute for Virology, Charité Campus Mitte, May 22, 2014, Berlin, Crosstalk between human cytomegalovirus and PML nuclear bodies

The Fifth Weissenburg Symposium Biriciana: Epigenetics – a different way of looking at genetics, Sept. 15-17, 2014, Weissenburg, Viral silencing mediated by components of PML nuclear bodies

Publications during funding period

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, J.D., Müller, R., Reuter, N., Sticht, H., Muller, Y.A., 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

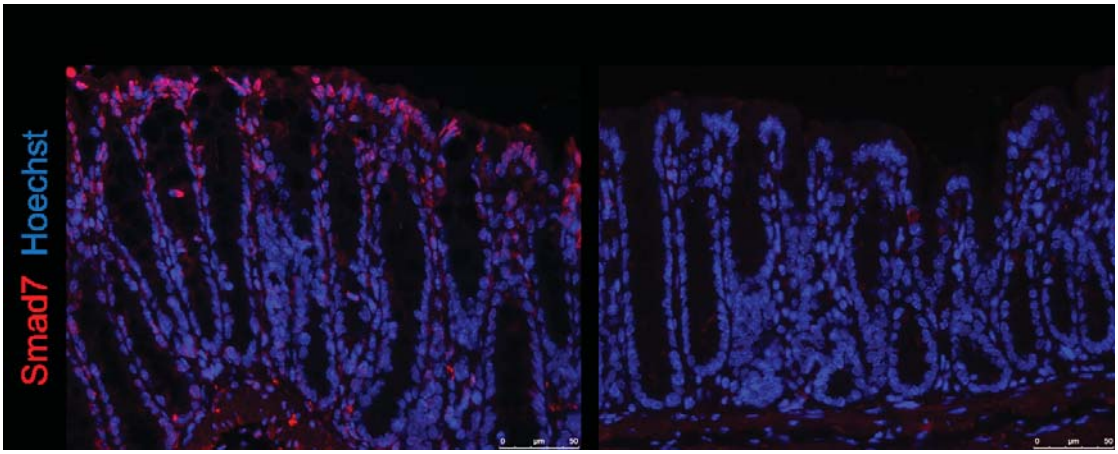
Role of intestinal epithelial SMAD7 for tumor development

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

Polymorphisms in Smad7 – an important inhibitor of the TGF-β signaling pathway – were recently associated with an increased risk for the development of colon cancer. Besides the inhibitory function of Smad7 in the TGF-β signaling pathway, Smad7 has recently been shown to be an important cross regulator of other pathways such as NF-κB and β-catenin signaling. Using mice with conditional deletion of Smad7 in IEC we now investigate the functional role of Smad7 for development of intestinal cancer.

In order to analyze the functional role of Smad7 in intestinal epithelial cells (IEC) for colon cancer development we generated mice with a conditional knockout for Smad7 in the intestinal epithelium ($Smad7^{\Delta IEC}$) and investigated tumor development in these mice using a colitis-associated tumor model (AOM/DSS). Strikingly, Smad7 deficient mice developed a lower number of tumors than control mice ($Smad7^{fl.}$). To investigate whether the decreased number of colon tumors in $Smad7^{\Delta IEC}$ mice was dependent on colitis in the AOM/DSS model, we used a sporadic tumor mouse model (APCmin). Comparison of $APCmin^{+/-}$ $Smad7^{\Delta IEC}$ mice and control mice ($APCmin^{+/-}$) revealed that $APCmin^{+/-}$ $Smad7^{\Delta IEC}$ mice not only showed a higher survival rate but also a reduced number of tumors strongly supporting our hypothesis that Smad7 plays a crucial role in the development of colon tumors in-vivo – independent from inflammation. In fact, histochemical stainings revealed that Smad7 deficiency in the intestinal epithelium itself did not affect the immunological status of the gut. In addition, Smad7 deficiency did not cause any detectable changes in intestinal pathology under unchallenged conditions.

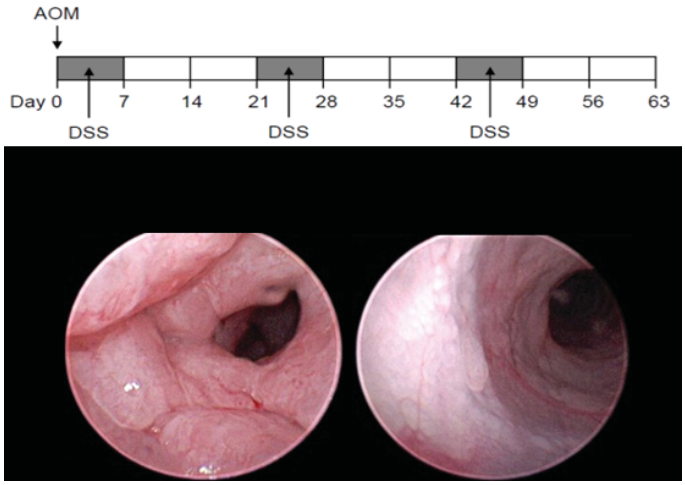
Further analysis now aimed to clarify the reason for reduced tumor development in $Smad7^{\Delta IEC}$ mice in the AOM/DSS and APCmin mouse models. In the reporting period, we focused on possible implications of Smad7 deficiency on proliferation and apoptosis. First results did not reveal increased apoptosis rate in $Smad7^{\Delta IEC}$ mice. However, analysis of isolated Smad7-deficient intestinal epithelial cells and in-vitro analysis of IEC-organoids derived from $Smad7^{\Delta IEC}$



Immunhistochemical stainings confirm deletion of Smad7 in the intestinal epithelium of $Smad7^{\Delta IEC}$ mice. The pictures show representative staining of colonic tissue. Smad7 is shown in red, Hoechst stained nuclei are shown in blue.


Dr. Wittkopf


Prof. Dr. Becker



Upper panel: Time course of colitis associated tumor model (AOM/DSS). Lower panel: Analysis by endoscopy revealed reduced tumor numbers in the colon of Smad7$^{\Delta IEC}$ mice compared to controls (Smad7$^{fl.}$) in AOM-DSS colitis.

mice point towards a decreased rate of intestinal epithelial cell proliferation. In addition, quantitative analysis of gene transcription of Smad7$^{\Delta IEC}$ organoids also point towards reduction of the differentiation marker c-Myc. However, no differences in the amount of terminally differentiated cells such as Paneth cells, goblet cells or enterocytes were detected. On which level Smad7 influences tumor development and whether the decreased proliferation observed in Smad7 deficient IEC is sufficient to explain the strong phenotype will now be investigated using inducible conditional knockout mice. Deletion of Smad7 selectively in the tumor will not be induced at the beginning but at later stages of the AOM/DSS experiment revealing whether Smad7 deficiency can actively reduce tumor size, stop tumor growth or do not influence tumors on later states. Future experiments will further investigate the role of the newly reported cross links of Smad7 to side pathways such as NF-κB, β-catenin and gene transcription during colon tumorigenesis.

Contact:
Prof. Dr. Becker
phone: +49 9131 85 35886
e-mail: christoph.becker@uk-erlangen.de

Invited lectures

Hot Topics in Gastroenterology, Rome, Italy, 11.04.2014
Forum Intestinale Immunologie, Berlin, 05.-06.10.2014
Summer School “Inflammation at interfaces“, Schleswig, 08.-09.10.2014

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

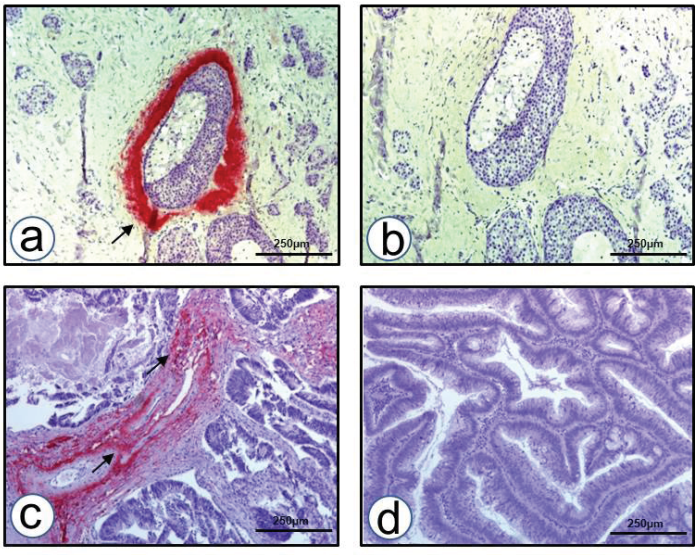
We previously showed that the expression of collagen 10 mRNA is increased in primary lesions of metastasizing colorectal carcinomas. Therefore, the function of collagen 10 in the regulation of metastasis will be analyzed in the present project. The focus will be on the role of collagen 10 in the formation of stem cell-like tumor-initiating cells and epithelial mesenchymal transition. The project will deliver new insights into the function of matrix components in the metastasis of colorectal carcinoma.

Detection of collagen 10 protein in colorectal carcinoma and breast carcinoma

Immunohistochemical staining with a specific monoclonal antibody (provided by Prof. von der Mark, Inst. of Experimental Medicine 1) showed that the collagen 10 protein is present in colorectal carcinoma (CRC) tissues. The protein was found in extracellular deposits preferentially around blood vessels, most likely arteries. Stromal fibroblasts showed also cytoplasmic collagen 10 staining indicating that these cells may be the major source of the protein. Besides CRC high expression of collagen 10 mRNA was detected in breast carcinoma. These results could also be confirmed at the protein level using immunohistochemistry. Collagen 10 in breast carcinoma was preferentially localized around the tumor ducts, blood vessels and tumor cells.

Role of collagen 10 in epithelial mesenchymal transition

The p52 cell line (provided by Prof. von der Mark) releases recombinant human collagen 10 in high amounts. This recombinant collagen 10 was isolated with more than 98% purity from supernatants of p52 cells using ion exchange chromatography. Increasing concentrations of purified collagen 10 were applied onto different colorectal carcinoma cell lines (HT29, SW480) and the breast carcinoma cell line MCF7. The impact on epithelial mesenchymal transition (EMT) of the cells was analyzed *via* the expression of E-cadherin and vimentin in these cells. TGF-β1 treatment was used as a positive control according to published reports in the literature. Interestingly, collagen 10 decreased E-cadherin expression in HT29 and increased vimentin expression in the SW480 cells. These results suggest that collagen 10 may contribute to EMT in colorectal carcinoma.

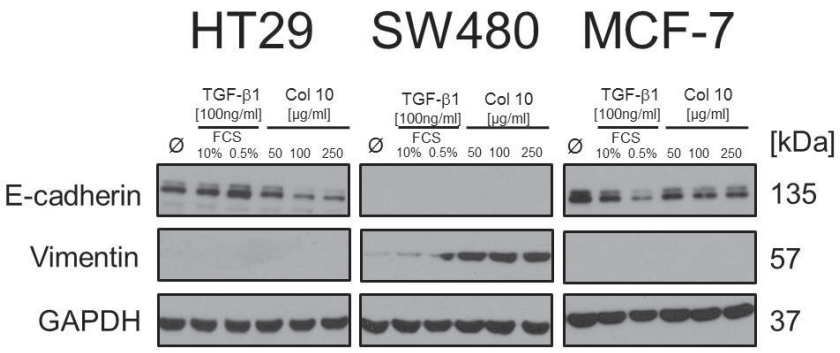


Collagen 10 expression (arrows) was increased in colorectal carcinoma (a) and breast carcinoma (c). Respective isotype control antibody stainings were negative (b, d).


Prof. Dr. Dr. Stürzl


Prof. Dr. Croner


PD Dr. Naschberger



Collagen 10 up-regulates the expression of vimentin in SW480 cells (colorectal carc.) and down-regulates the expression of E-Cadherin in HT29 (colorectal carc.) and MCF7 cells (breast carc.).

Production of a collagen 10 knockout mouse

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 are expected in the following generation. 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).

Contact:
Prof. Dr. Dr. Stürzl
phone: +49 9131 85 39522
e-mail: michael.stuerzl@uk-erlangen.de

Publications during funding period

Hävemeier A, Gramolelli S, Pietrek M, Jochmann R, Stürzl M, Schulz TF (2014) Activation of NF-κB by the Kaposi's sarcoma herpesvirus K15 protein involves recruitment of the NF-κB-inducing kinase, IκB kinases and phosphorylation of p65. J Virol 22:13161-72

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Schellerer VS, Langheinrich M, Hohenberger W, Croner RS, Merkel S, Rau TT, Stürzl M, Naschberger E (2014) Tumor-associated fibroblasts isolated from colorectal cancer tissues exhibit increased ICAM-1 expression and affinity for monocytes. Oncol Rep 1:255-61

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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

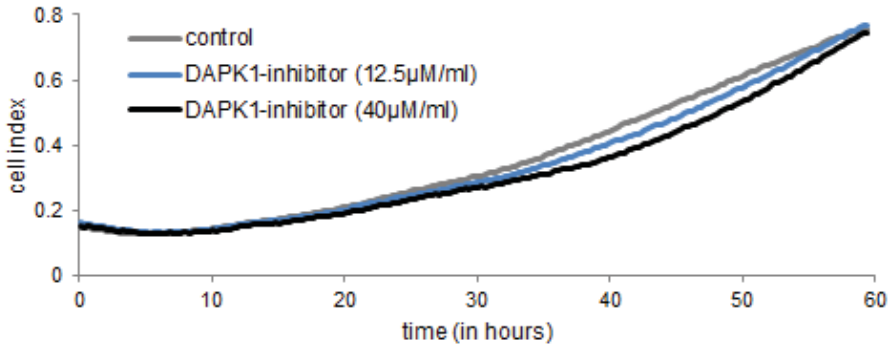
16.10.2013 - 15.04.2016

DAPK and colon cancer

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

Colorectal cancer (CRC) is one of the most common tumor diseases worldwide. We have shown previously that the Death-associated protein kinase 1 (DAPK), a cytoskeletal associated serine/threonine kinase, has an impact on the carcinogenesis of colorectal cancer. Loss of expression reported in numerous tumor cells, cell lines, and cancer biopsies leading to selective advantages for tumor tissue suggests a role as a tumor suppressor. Interestingly, it shows antagonistic duality in different experimental settings. In the first phase of the project we established different in vitro tools for studying the impact of a deficient kinase activity or DAPK scaffold in normal and malignant intestinal epithelial cells.

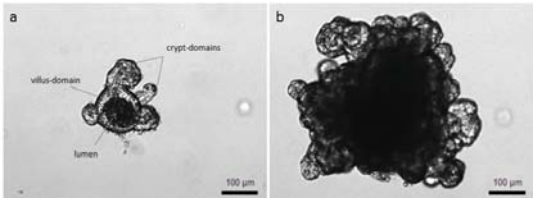
For investigations into the functional role of DAPK1 in colorectal tumors, we are generated a CRISPR DAPK ko cell line. A murine dimethyl-hydrazine-induced C57BL/6 colon adenocarcinoma MC38 DAPK-knockout cell line will serve as a tool for transcriptome, miRNome and proteome analysis and *in vitro* model for further DAPK-dependent functional analyses. MC38 cells are highly aggressive grade III adenocarconima mouse tumor cells with an immense metastatic and invasive potential to the liver. We started to implement a stable DAPK1-knockout (KO) in MC38 cells by using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 nuclease) technology. The stable MC38-DAPK-KO will be finally verified by fluorescence-activated cell sorting and generating colonies from single cell clones. This cell line will be further analyzed using different in vitro functional assays (migration, proliferation, anoikis, colony formation). Preliminary data of clonogenic assay, migration assays, xCelligence proliferation assay and soft agar colony formation testing a specific DAPK1-inhibitor on MC38 cells suggest no major influence of DAPK kinase activity on migration, proliferation and colony formation. Obviously, substrate phosphorylation by DAPK is not necessary for these cellular functions. Thus, MC38-DAPK-KO cells will assist to corroborate the DAPK-dependent functional scaffolds. Using a stable DAPK sh knockdown in HCT116 colon cells we showed that DAPK is a migration inhibitor. This corresponds to its loss in highly migrating cells seen at the tumor invasion front in human colon tumors.



DAPK kinase activity and proliferation: MC38 cells were treated with a DAPK1-inhibitor (12.5µM; 40µM) and proliferation was examined using an xCelligence cell analyzer. Runs were done in duplicates (2.500 cells per well) after an adaptation time of 10h. Single data points were measured each 30s.


Prof. Dr. Schneider-Stock


Dr. Neufert



Growth time course of a crypt-derived organoid from the small intestine of a wild type C57BL/6J mouse: A) day 6: typical structure with the lumen in the center surrounded by the epithelium of the villus- and crypt domains.B) day 15: multiple crypt-villi-like domains and increased size.

In addition, ex vivo organoid cultures for studying the role of DAPK in the intestine were established in close collaboration with the group of Dr. C. Becker (Medical Clinics 1). We showed that the number and quality of the crypts evaluated by light microscopy was suitable for studying functional effects of gene knockouts or drugs. We used intestinal epithelial tissues of DAPK wildtype and KO mice to study the influence of DAPK on crypt architecture. After isolating crypts we let them grow in matrigel. They showed the first signs of crypt outgrowth after 48h-72h. Organoids with multicellular three-dimensional crypt-villus like structures were visible after 3-6 days of culture. They differed in structure, morphology, size, and proliferation capacity dependent on their genotype. Using different cell viability assays and apoptosis staining we will evaluate the effect of two different DAPK inhibitors. Providing essential features of their respective organ, organoids may serve as a new surrogate system with high physiological relevance. Therefore, they constitute an adequate tool for determination of specific gene defects in the respective cells and compartments.

In summary, our most recent studies provide additional evidence for a differential role of epithelial DAPK in the regulation of the intestinal homeostasis and the development of colorectal cancer. The new established *in vitro* tools will help to further understand the function of DAPK.

Contact:
Prof. Dr. Regine Schneider-Stock
phone: + 9131 85 26069
e-mail: regine.schneider-stock@uk-erlangen.de

Dr. Clemens Neufert, PhD
phone: + 9131 85 45062
e-mail: clemens.neufert@uk-erlangen.de

Invited lectures

Regine Schneider-Stock: German Congress of Pathology, 12.6.-14.6.2014, plenary lecture, Topic: Role of DAPK in inflammatory carcinogenesis

Awards

Clemens Neufert: Kußmaul award (Franconian gastroenterologists), 27.06.2014

Publications during funding period

Benderska N, Ivanovska J, Rau TT, Schulze-Luehrmann J, Mohan S, Chakilam S, Gandesiri M, Ziesche E, Fischer T, Soeder S, Agaimy A, Distel L, Sticht H, Vijayalakshmi M, Schneider-Stock R. DAPK–HSF1 interaction as a positive-feedback mechanism stimulating TNF-induced apoptosis in colorectal cancer cells. J Cell Science 2014;127:5273–87

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D22 - Progress Report

01.11.2013 - 30.04.2016

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

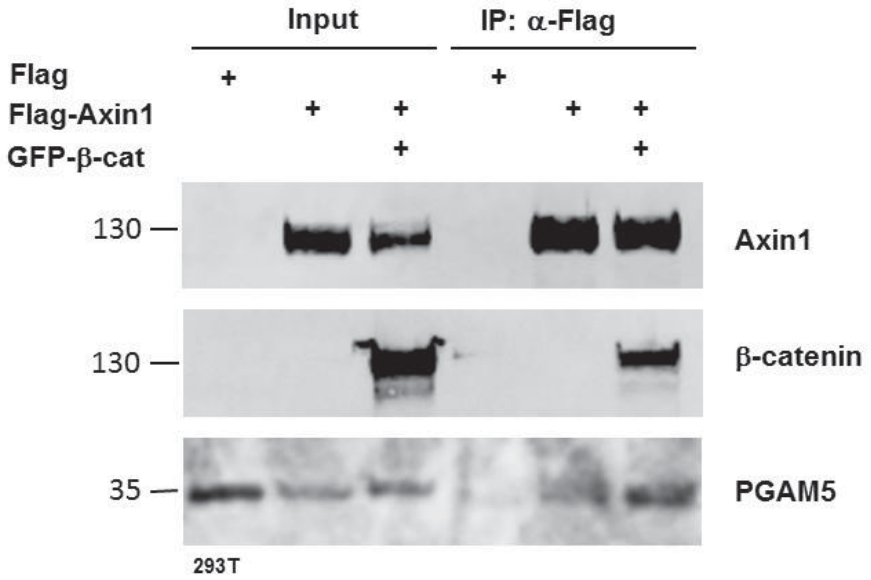
We analyse novel components of the Wnt signal transduction pathway at the level of Wnt receptors and the β-catenin destruction complex that have been found by yeast two hybrid screens and proteomic analyses, and determine their function by cell biological and developmental studies. Through this work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

APC Interaction partners

ATAD3A, GIRDIN, WDR26 have been described previously by Hilger and Mann (J. Proteome Res. 2012, 11, 982-994) to be present in complexes with APC and Axin, however the functional relevance of these interactions was not reported. We performed knockdown of these proteins followed by β-catenin-dependent reporter assays to evaluate a possible role in Wnt signaling. Knockdown had no effects on reporter assays suggesting that these proteins are not centrally involved in the Wnt pathway.

LRP6/ZNRF3 interaction partners

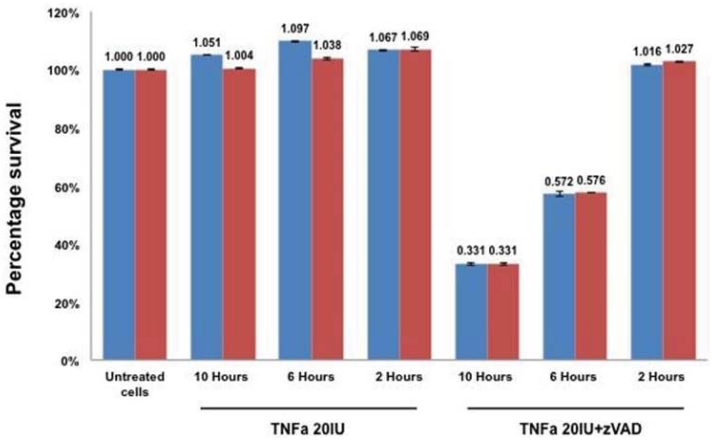
LRP6 acts as a coreceptor of the Frizzled receptors for binding of Wnt ligands. Its cytoplasmic domain is a docking site for the β-catenin destruction complex and blocks β-catenin degradation. To find novel interaction partners of this domain we performed yeast two hybrid screens. Only few candidates were identified so far which did not show solid interactions. We also performed screens with ZNRF3 as bait. ZNRF3 is an E3 ligase involved in downregulating Wnt receptors including LRP6 and thereby blocks



Co-immunoprecipitation of endogenous PGAM5 with transiently expressed axin is enhanced in the presence of β-catenin.

Prof. Dr. Behrens

Wnt signaling. We identified LIM domain containing proteins which showed robust interaction with the cytoplasmic domain of ZNRF3. These proteins are currently further analyzed for a role in Wnt signaling.



Effect of increased Wnt signaling on necroptosis. Blue bars represent control media treated cells and red bars represent Wnt ligand containing media treated cells. All cells were preincubated for 10 hours followed by the chemical cocktail treatment.

Axin/Axin2 interaction partners

Mass spectrometry data performed in collaboration with Drs. Alexandra Schambony, (University Erlangen) and Marc Gentzel (MPI Dresden) suggested that PGAM5 could be an interacting partner of axin. This interaction was confirmed by co-immunoprecipitation experiments which also showed that β-catenin enhances the interaction. PGAM5 is a member of the PGAM protein family of phoshoglyceromutases but it is known to have an alternate ser/thr protein phosphatase activity. PGAM5 is involved in cellular stress responses and necroptosis. In order to test whether Wnt signaling pathway influences necroptosis, we developed a cell viability assay. We used mouse L929 cells which when subjected to a chemical cocktail treatment consisting of TNFα and zVAD-FMK (pan caspase inhibitor), undergo cell death via the necroptotic pathway. We incubated the cells with or without Wnt3a containing media for 10 hours followed by the chemical cocktail treatment. There was no effect of Wnt3a treatment on cell death. We also studied whether the downstream proteins involved in canonical Wnt signaling have an effect on necroptosis. Transfection of L929 with Axin1, β-catenin or stabilized β-catenin (S33Y) expression vectors did not significantly change necroptosis induced by the chemical cocktail treatment. These experiments indicate that canonical Wnt signaling in L929 has no effect on necroptosis. We therefore focused on the question of whether PGAM5 might have a role in canonical Wnt signaling. Initially, we found that in the SW480 cell line, PGAM5 overexpression leads to a repression in reporter assays. This result is currently being further investigated.

Contact:
Prof. Dr. Behrens
phone: +49 9131 85 29109
e-mail: juergen.behrens@fau.de

Invited lectures

Physics of Cancer, 26.9.2013 Leipzig, Macromolecular protein complexes in Wnt signaling
Sarkomkonferenz 2014, 20.3.2014 Berlin, β-Catenin and Wnt signaling

Publications during funding period

none

E10 - Final Report

01.04.2011 - 31.03.2014

The role of neuronal glycine transporter 1 (GlyT1) in synaptic transmission

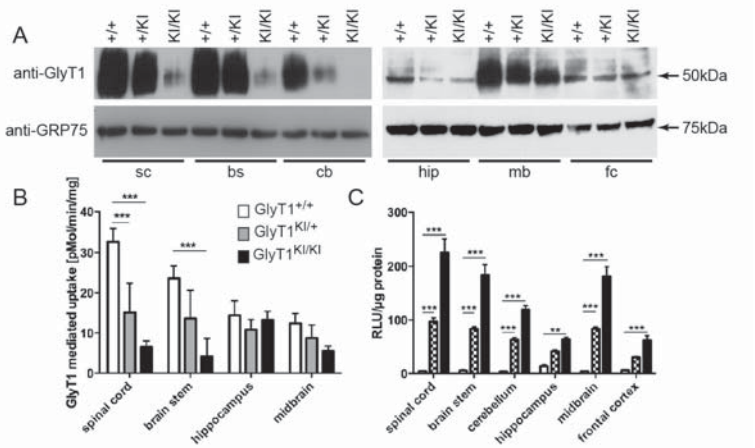
Dr. Volker Eulenburg, Institute of Biochemistry
PD Dr. Teja W. Grömer, Department of Psychiatry and Psychotherapy

The glycine transporter GlyT1 is involved in the modulation of glycine dependent neurotransmission and inhibition of GlyT1 has beneficial effects as add on therapy for psychotic diseases. GlyT1 is expressed in multiple isoforms that are generated from a single gene by alternative promoter usage and/or splicing. Here, we show by a loss of function approach that GlyT1b has important function in adult animals and that GlyT1b deficiency causes episodes of pronounced hypoactivity.

Glycine acts as an inhibitory neurotransmitter but also constitutes an essential coagonist for glutamate receptors of the NMDAR subtype. It was suggested that GlyT1, a high affinity transporter for glycine that is important for the regulation of the extracellular glycine concentration, is expressed in different isoforms (GlyT1a –c) with GlyT1b being expressed by neurons. By a loss of function approach we show that GlyT1b is expressed predominantly in caudal brain regions and contributes to the regulation of inhibitory synapses.

Initial characterization of GlyT1 b/c knock-in mice

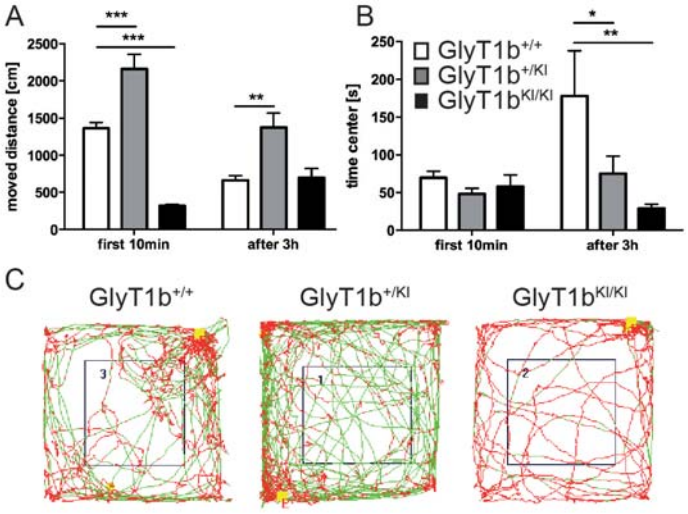
In this project GlyT1b/c knock-in mice carrying a Luciferase-RFP fusion protein under control of the endogenous GlyT1b promoter have been generated by an ES cell based approach. GlyT1b$^{KI/KI}$ were born at the expected Mendelian frequency and survived to adulthood. Initial analysis did not reveal any severe phenotype. Analysis of reporter expression was performed on basis of luciferase activity in brain homogenates of GlyT1b$^{KI/KI}$ mice. Here, highest Luciferase activity was determined in caudal brain regions like brain stem and spinal cord. In line with GlyT1b deficiency in this brain region, GlyT1 activity and protein expression was reduced in mice carrying the LucR reporter knock in homozygously. Interestingly, GlyT1$^{KI/KI}$ mice developed pronounced episodes of generalized hypotonia including a total suppression of motor activity upon exposure to mild stress. These episodes, however, were transient and fully reversible, without affecting the viability of GlyT1b$^{KI/KI}$ mice. These data are consistent with GlyT1b contributing to the control of the glycine concentration at inhibitory synapses. Behavioral analysis revealed that GlyT1b$^{KI/KI}$ are hypoactive whereas heterozygous GlyT1b^{KI} mice displayed hyperactivity consistent with GlyT1b being additionally involved in the regulation of forebrain associated functions most likely associated with excitatory synapses. Immunhistochemical analysis suggested that GlyT1b is not exclusively expressed by neurons but in addition also by astrocytes and/or oligodendrocytes.



Western-blots of brain samples from GlyT1b^{KI} mice showed a strong reduction in GlyT1 expression in caudal brain regions (A). GlyT1 activity was reduced in these brain regions (B). Brain regions with large reductions in GlyT1 expression showed high Luciferase activity (C).

Dr. Eulenburg

PD Dr. Grömer



GlyT1b$^{KI/KI}$ mice were hypoactive in the initial phase of open field test whereas GlyT1b$^{KI/+}$ mice showed pronounced hyperactivity (A). Moreover, GlyT1^{KI} mices showed increased anxiety (B). Overall, GlyT1$^{KI/KI}$ mice moved slower as compared to wildtype mice.

Taken together, the characterization of GlyT1b^{KI} mice has significantly contributed to our understanding of GlyT1 function in the mature nervous system.

Influence of GlyT1 on synaptic transmission

To test the influence of GlyT1 on the presynaptic vesicular release induced by electrical stimulation we used synapto-pHluorin transfected rat hippocampal neurons. Here, glycine application produced a small increase in synapto-pHluorin fluorescence intensity, representing synaptic vesicle exocytosis and incubation with the GlyT1 specific inhibitor ALX5407 resulted in a decrease in the number of vesicles released after glycine application, indicating a modulatory effect of glycine on synaptic transmission in this system. The evoked response under glycine treatment, however, did not change, indicating a minor effect of glycine on synaptic transmission evoked by electrical stimulation.

Contact:
Dr. Eulenburg
phone: +49 9131 85 26206
e-mail: volker.eulenburg@fau.de

Invited lectures

7th symposium of the SFB35 „Transmembrane transporters in health and disease, , Vienna, 9-11.10.2014, „glycine transporters; different tissues different functions?“

Publications during funding period

Jung J, Loy K, Schilling EM, Röther M, Brauner JM, Huth T, Schlötzer-Schrehardt U, Alzheimer C, Kornhuber J, Welzel O, Groemer TW (2014) The Antidepressant Fluoxetine Mobilizes Vesicles to the Recycling Pool of Rat Hippocampal Synapses During High Activity. Mol Neurobiol 49:916-930

Jung J, Weisenburger S, Albert S, Gilbert DF, Friedrich O, Eulenburg V, Kornhuber J, Groemer TW. (2013) Performance of scientific cameras with different sensor types in measuring dynamic processes in fluorescence microscopy. Microsc Res Tech. 76(8):835-43

Lall D, Armbruster A, Ruffert K, Betz H, Eulenburg V (2012) Transport activities and expression patterns of glycine transporters 1 and 2 in the developing murine brain stem and spinal cord. Biochem Biophys Res Commun. 423 .661-6

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Wenzel EM, Morton A, Ebert K, Welzel O, Kornhuber J, Cousin MA, Groemer TW (2012) Key physiological parameters dictate triggering of activity-dependent bulk endocytosis in hippocampal synapses. PLoS One 7 e38188

E11 - Progress Report

01.12.2013 - 31.05.2016

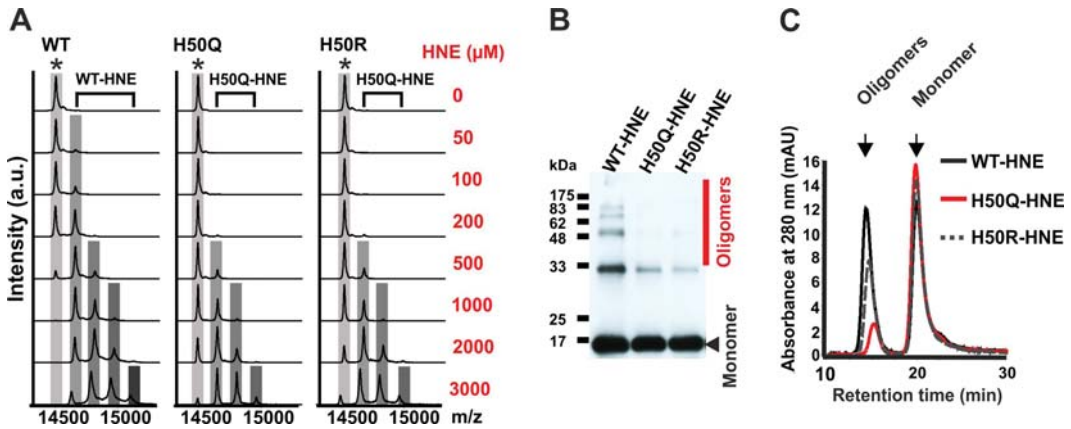
H50Q aSyn mutation in PD

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

Aggregation and neurotoxicity of alpha-synuclein (aSyn) are key factors in the pathogenesis of Parkinson's disease (PD). We aim to elucidate the role of histidine 50 (H50) of aSyn, which is not only a target residue for a specific posttranslational modification (PTM), but also involved in a novel PD associated aSyn mutation (H50Q). Our results show that both the PTM and mutations of H50 increase aSyn aggregation and toxicity, suggesting an essential role of H50 in aSyn pathology.

H50 is crucial for HNE-mediated aSyn modification, oligomerization, and cytotoxicity

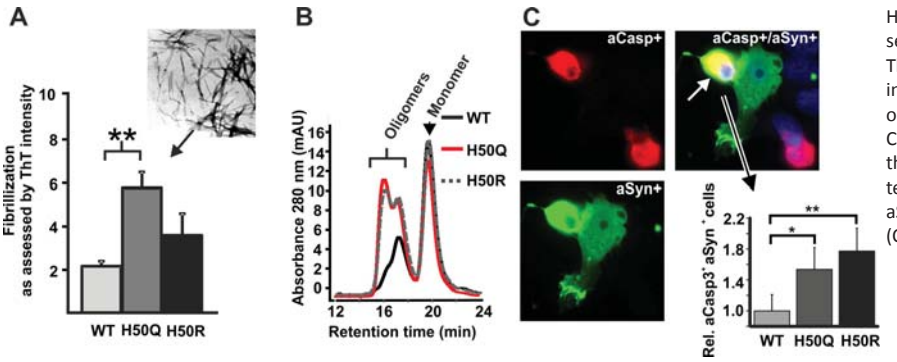
HNE is a toxic lipid peroxidation product found in brain tissue of patients with neurodegenerative diseases. It reacts with histidine, lysine, and cysteine residues of proteins. We were able to show that posttranslational modification of aSyn by HNE directly increases aSyn oligomerization and cytotoxicity. Since aSyn contains only one histidine residue (H50), a candidate for HNE modification, we exchanged H50 by a glutamine or an arginine residue (H50Q/R) via side-directed mutagenesis to study the molecular role of H50 in aSyn. Mass spectrometric analysis revealed that H50Q/R substantially reduced HNE modification of aSyn compared to WT aSyn. This result indicates that H50 is the most reactive target residue for HNE modification. Western blot analysis and size exclusion chromatography (SEC) showed that the mutation of H50 reduced HEN-induced aSyn oligomerization. Furthermore, analysis of cell viability revealed that overexpression of aSyn H50Q/R mutants reduced the susceptibility of cells to HNE as compared to WT aSyn. These findings underline a crucial role of HNE modification at the H50 residue in aSyn-mediated oligomerization and neurotoxicity.



Mass spectrometric analysis of HNE aSyn adducts (A), Western blot (B) and SEC analysis (B) of aSyn oligomers show that H50 is the most reactive target residue for HNE modification and is also crucial for HNE-induced oligomerization of aSyn.

PD Dr. Klucken

PD Dr. Xiang



H50Q/R mutations increase the fibrillization (EM and ThT, A) and oxidative stress-induced aSyn oligomerization (SEC, B). ICC analysis of Caspase^{+}/aSyn^{+} cells shows that H50Q/R mutations potentiate the apoptosis of aSyn overexpressing cells (C).

H50 mutations increase the aggregation propensity of aSyn and trigger cell damage

In order to clarify whether the mutation of H50 also impacts aSyn aggregation and toxicity, we analyzed oligomerization and fibrillization of H50 aSyn mutants both in vitro by using recombinant aSyn and in cells overexpressing the aSyn variants. In vitro, H50Q mutation increased fibrillization of recombinant aSyn as assessed by Thioflavin T (ThT) assay. In presence of an oxidative/nitrative agent, SEC analysis showed that H50Q mutant aSyn was more prone to oligomerization than WT aSyn. This in vitro finding suggests an increased oligomerization propensity of H50 mutant aSyn under oxidative stress. Consistently to the in vitro observations, we also detected an increased level of aSyn oligomers in cells overexpressing H50Q mutant aSyn. Immunocytochemical analysis revealed that transient transfection of aSyn H50Q/R mutants resulted in an increased apoptosis in mutant aSyn overexpressing cells as compared to WT aSyn overexpressing cells. Moreover, under oxidative stress cell damage was even more pronounced for cells overexpressing aSyn H50 mutants.

In summary, both posttranslational and genetic modifications of aSyn at H50 increase aggregation (oligomerization or fibrillization) and cytotoxicity of aSyn, supporting an essential role of aSyn H50 in PD. Thus, our study provides novel insights into the mechanisms underlying oxidative stress-associated pathogenesis of sporadic and H50Q-associated monogenic PD.

Contact:
PD Dr. Klucken
phone: +49 9131 85 39324
e-mail: jochen.klucken@uk-erlangen.de

PD Dr. Xiang
phone: +49 9131 85 26206
wei.xiang@biochem.uni-erlangen.de

Invited lectures

Workshop, Lecture, German Society of Neurology, 17.09.14, Munich, Klucken et al.; "Autophagie als Modulator der alpha-Synuclein Degradation und Freisetzung"

CNMPB-Lecture, 03.12.14, University Hospital Göttingen, Klucken et al.; „Autophagy modulates spreading and extracellular toxicity of alpha-synuclein"

Awards

Luise-Prell Price of the Medical Faculty, Friedrich-Alexander University Erlangen-Nürnberg (FAU) for outstanding master thesis – Molecular Medicine „The role of Histidine 50 in alpha-synuclein pathology", Stefanie Menges; 01.11.2014, Erlangen

Publications during funding period

Poehler, AM, Xiang W, Spitzer P, May V, Meixner H, Rockenstein E, Chutna O, Outeiro T, Winkler J, Masliah E, Klucken J (2014) Autophagy modulates SNCA/alpha-synuclein release, thereby generating a hostile microenvironment. Autophagy: e36436

Casadei N, Pöhler AM, Tomás-Zapico C, Torres-Peraza J, Schwedhelm I, Witz A, Zamolo I, De Heer R, Spruijt B, Noldus LP, Klucken J, Lucas JJ, Kahle PJ, Krüger R, Riess O, Nuber S (2014) Overexpression of synphilin-1 promotes clearance of soluble and misfolded alpha-synuclein without restoring the motor phenotype in aged A30P transgenic mice. Hum Mol Genet: 23(3) 767-781

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 a transgenic alpha-synuclein (α-syn) rat model indicate that α-syn severely impairs the hippocampal serotonergic system prior to the onset of motor symptoms resulting in a compromised axonal and synaptic hippocampal circuitry and reduction of hippocampal neurogenesis.

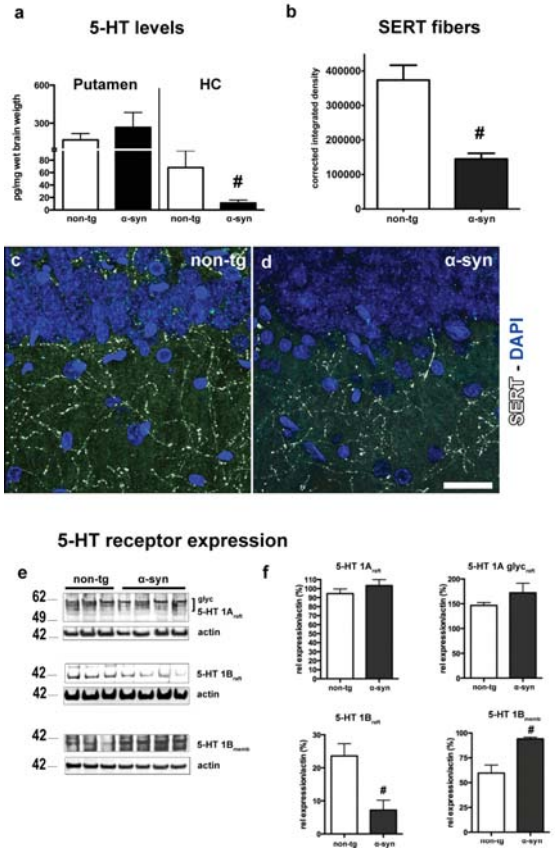
The aim of this study is to analyze non-motor neuropsychiatric symptoms related to the hippocampus and its capacity to generate new neurons in models of synucleinpathies, in particular Parkinson's disease (PD). Moreover, we try to decipher the molecular pathophysiology of these symptoms and to identify potential therapeutic targets.

Impaired hippocampal serotonergic system in BAC transgenic alpha-synuclein rats

To characterize the interplay between the serotonin (5-HT) system and the hippocampus, we used a recently generated α-syn transgenic rat model. At 12 months of age, these animals develop key features of PD such as pathological α-syn accumulation and dopamine decline accompanied with motor deficits. Prior to the onset of this phenotype, we observed severe 5-HT dysfunction in the hippocampus of 4-month-old animals, as detected by reduced input of 5-HT transporter (SERT) expressing neurites, low 5-HT levels, and altered 5-HT receptor expression in the hippocampal dentate gyrus (DG)/CA3 region.

Hippocampal 5-HT dysfunction result in reduced hippocampal neurogenesis

Impaired hippocampal neurogenesis contributes to neuropsychiatric symptoms, presumably by altered serotonergic signaling. Consecutively, the hippocampal serotonergic deficit was accompanied by a severely impaired hippocampal neurogenesis in transgenic animals, namely by a profound reduction of neuroblasts and new-born neurons. We further detected an impaired dendritogenesis of DCX+ neuroblasts in α-syn rats possibly affecting the integration of new neurons into neural networks. In addition, we analysed axons from DG neurons (mossy fibres)

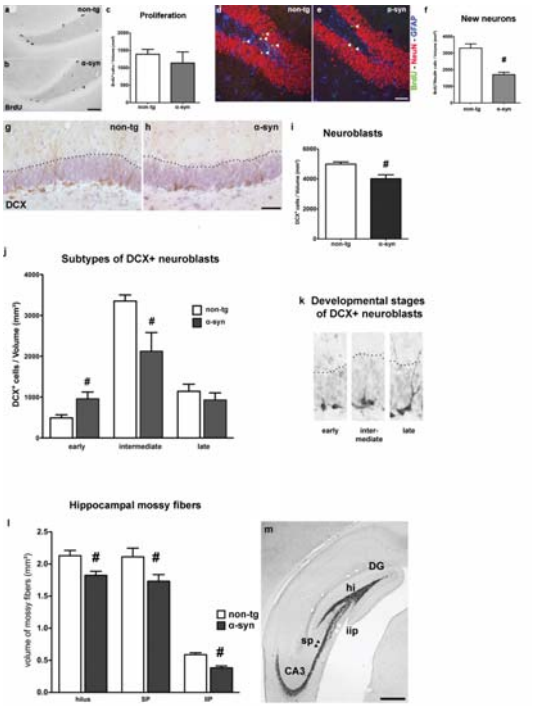


Reduced serotonin (5-HT) levels and fibers expressing the 5-HT transporter SERT in the hippocampus of α-syn rats (a-d). Unchanged expression of 5-HT receptor 1A, while 5-HT 1B, localized to synaptic vesicles, shows down-regulation (raft fraction) in α-syn animals (e, f).

expressing ZnT3, projecting to the hippocampal CA3 subregion, and observed a reduced volume of all three subfields of the mossy fibre tract in α-syn rats,

Prof. Dr. Winkler

Prof. Dr. Lie



Decreased adult neurogenesis in α-syn rats (a-i). Here, post-mitotic DCX+ neuroblasts showing an intermediate phenotype are mainly affected (j, k). Decreased volumes of all three regions of the mossy fibre tract representing the axonal projections of DG neurons (l, m).

indicative for an impaired axonal output from DG neurons, altogether representing a compromised intra-hippocampal circuitry in this PD model.

Transcriptional and posttranslational regulation of the putative antidepressant target Sox11

Antidepressant treatments such as electroconvulsive shock (ESC) and selective serotonergic reuptake inhibitors strongly increase the expression of the SoxC transcription factor Sox11 in hippocampal DG neurons, rendering this transcription a candidate target for the actions of antidepressants. Our recent electrophysiological analysis showed that increased expression of Sox11 alters the plasticity of DG granule neurons. To understand the regulation of Sox11 in response to antidepressant treatment we studied the epigenetic modification of the Sox11 locus. This analysis revealed that ECS treatment altered the methylation of the Sox11 gene. We also obtained preliminary evidence that the increased expression of Sox11 may be controlled by the differential methylation of the 3' untranslated region of the Sox11 gene. In addition, we found evidence that Sox11 function may be controlled by differential phosphorylation, which in turn may affect Sox11's subcellular localization.

Contact:
Prof. Dr. Winkler
phone: +49 9131 85 39323
e-mail: juergen.winkler@uk-erlangen.de

Prof. Dr. Lie
phone: +49 9131 85 24622
e-mail: chi.lie@fau.de

Invited lectures

Prof. Dr. Chichung Lie:

Keystone meeting "Adult Neurogenesis", 12.-17. Mai 2014, Stockholm, Sweden, Impact of mitochondrial function on adult neurogenesis

5. Symposium des IZKF Erlangen, 16.-17. Mai 2014, Kloster Banz, Germany, Molecular regulation of adult hippocampal neurogenesis

Route 28 Workshop "Adult Neurogenesis", 6.-10. September 2014, Frauenchiemsee, Regulatory pathways in adult neurogenesis

BMBF Symposium "Neural Stem Cells", 5.-6. November 2014, Heidelberg, Germany, Impact of mitochondrial function on adult neurogenesis

Awards

Benjamin Häberle: Fritz und Maria Hofmann-Preis für hervorragende wissenschaftliche Abschlussarbeit im Masterstudiengang Molekulare Medizin

Publications during funding period

Rockenstein E, Nuber S, Overk CR, Ubhi K, Mante M, Patrick C, Adame A, Trejo-Morales M, Gerez J, Picotti P, Jensen PH, Campioni S, Riek R, Winkler J, Gage FH, Winner B, Masliah E. Accumulation of oligomer-prone α-synuclein exacerbates synaptic and neuronal degeneration in vivo. Brain. 2014 May;137(Pt 5):1496-513

Steib K, Schäffner I, Jagasia R, Ebert B, Lie DC (2014) Mitochondria modify exercise-induced development of stem cell-derived neurons in the adult brain. J Neurosci. 34(19):6624-33

E13 - Progress Report

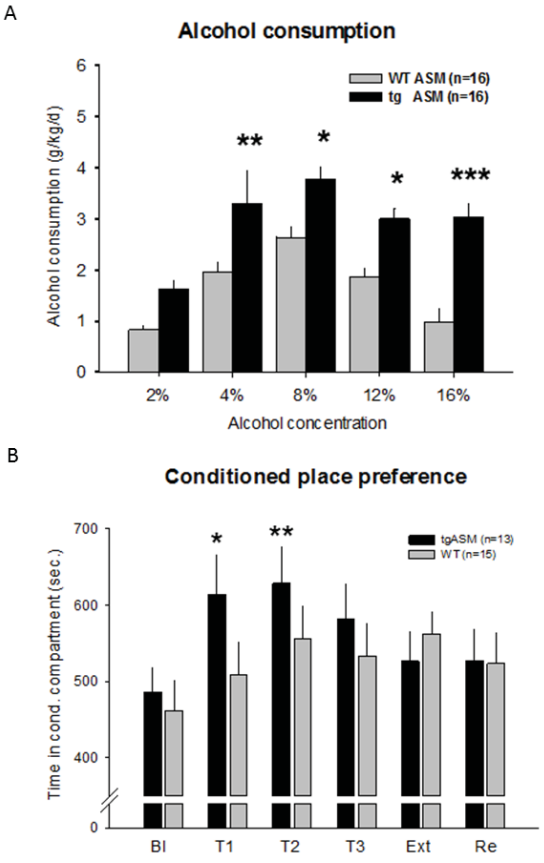
01.04.2014 - 31.03.2017

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

Prof. Dr. Christian P. Müller, 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.

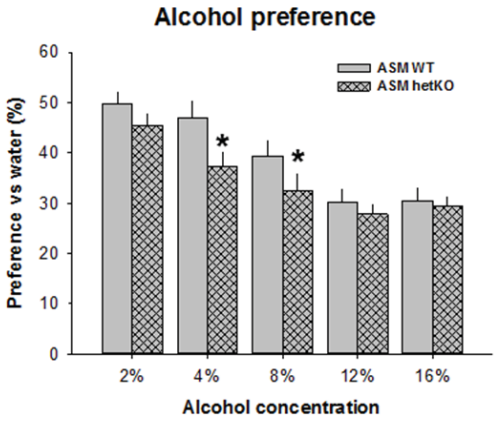
Mice with a transgenic over-expression of acid sphingomyelinase (tgASM) and enhanced ceramide levels in the brain show anxiety and depression-related behaviour and drink more alcohol than wild type (WT) controls. We have tested these mice and found that they learn significantly faster to associate a neutral place with the rewarding effects of alcohol in a conditioned place preference (CPP) paradigm. While alcohol induced comparable acute locomotor effects in tgASM and WT animals, repeated alcohol administration led to a sensitization and establishment of hyperlocomotion only in tgASM animals. Conditioned locomotor effects were also established faster in tgASM mice. This suggests that the enhanced preference and drinking of alcohol is due to a faster establishment of alcohol's rewarding effects. We then tested whether the role of ASM in reinforcement learning is specific for alcohol or would generalize also for natural reinforcer. We found that the reinforcing action of preferred food was not different between tgASM and WT mice in a CPP paradigm, which suggests an alcohol specific role of ASM. Heterozygous ASM knock out mice prefer alcohol less than WT mice at a medium dose range. We investigated these animals for the reinforcing effects of alcohol and found that sub-chronic alcohol administration was unable to induce reinforcing effects in the CPP test. While acute locomotor effects of alcohol were preserved in het ASM KO mice, sensitization after repeated application was significantly attenuated. These findings suggest that full ASM activity is required for the rewarding action of alcohol. We furthermore investigated the role of sphingolipids and ceramide in normal behavioural plasticity. It is known that the extinction of an operant behaviour,



Transgenic mice over-expressing ASM (tgASM) show A. enhanced alcohol drinking and B. faster establishment of alcohol-induced conditioned place preference (Bl-baseline; T-test, Ext-extinction, Re-reinstatement).

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

A



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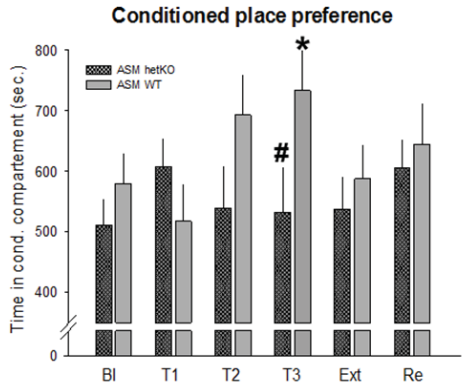


Heterozygous ASM knock out (het ASM KO) mice show A. attenuated alcohol preference and B. a complete lack of alcohol-induced conditioned place preference (Bl-baseline; T-test, Ext-extinction, Re-reinstatement).

e.g. a behavioural response for a food reinforcer requires neuronal plasticity. We hypothesized that this is in part mediated by alterations in ASM activity and ceramide levels in distinct brains structures. In collaboration with J.P. Huston (University of Düsseldorf) and B. Kleuser (University of Potsdam) we investigated animals which were trained for operant responding. Thereafter, an expected reward was withheld and the operant behaviour extinguished. Analysis of sphingolipids in the hippocampus showed a specific decline in ceramide 22:0 and ceramide 24:0, while there was an increase in sphingomyelin species 24:1 compared to a non-extinguished control group. These very specific alterations were accompanied by an increase in neutral sphingomyelinase (NSM) activity and a decrease in neutral ceramidase (NC) activity, but no alteration in ASM or acid ceramidase (AC) activity in this brain region. These findings suggest that sphingolipid composition in the brain changes in a regionally selective manner already in response to normal behavioral adaptations, and may, by altering membrane properties, provide an additional mechanism for synaptic plasticity.

Contact:
Prof. Dr. Müller
phone: +49 9131 85 36896
e-mail: christian.mueller@uk-erlangen.de

Invited lectures

Christian P. Müller: 2nd International Workshop on Molecular Medicine of Sphingolipids, 16.10.2014, Kloster Banz, From Depression to Alcoholism: The role of acid sphingomyelinase

Awards

Annika Liese Preis 2014, an: AG Kornhuber und AG Gulbins, 15.11.2014, Medizinische Hochschule Hannover, Congress Center

Publications during funding period

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

E14 - Progress Report

01.04.2014 - 30.09.2016

TRPC5 and tooth pain

Prof. Dr. Katharina Zimmermann, Department of Anaesthesiology

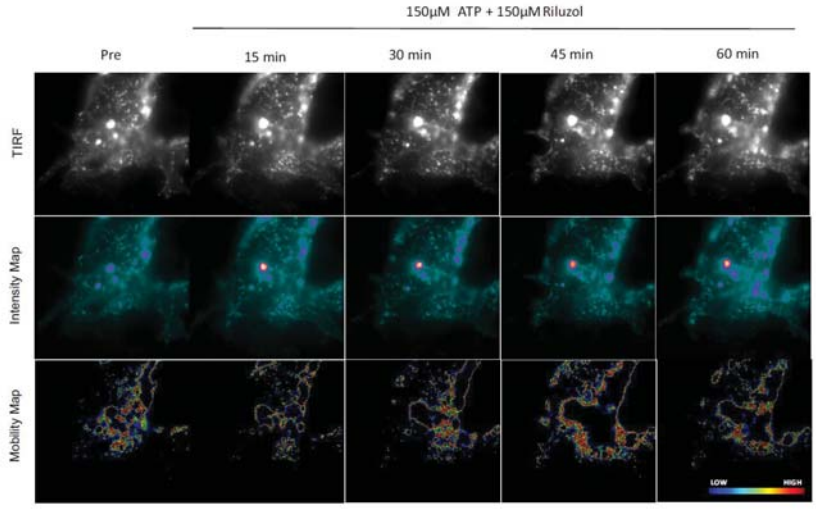
Cold hyperalgesia and cold hypersensitivity are common dental problems. TRPC5 is present in normal teeth and undergoes strong upregulation in sensory neurons innervating root, pulp and dentine of pulpitic human teeth. To investigate its role in tooth pain and characterize it as treatment target we studied TRPC5 channel turnover at the plasma membrane, established a rat labeling model of primary afferent dental neurons, and developed a high throughput screening assay for compound screening.

TRPC5 channel turnover at the plasma membrane

We used TIRF Microscopy recently to study the turnover of the cold transducer TRPM8 at the plasma membrane (PM) and found functional channels to be recruited within seconds after agonist stimulation (Toro et al., J Neurosci 2015, 35(2):571-582). Now we used TIRF microscopy to study the possibility of TRPC5 recruitment to the PM, e.g. of relevance in inflammatory conditions. TRPC5 remains stable in the majority of cells in presence of agonist. Nevertheless a small fraction of transfected cells shows particle movement and increased recruitment to the PM after long agonist exposure (60 min). Future experiments will investigate the effect of GPCR activation, including PLC activation on TRPC5 particles in the PM.

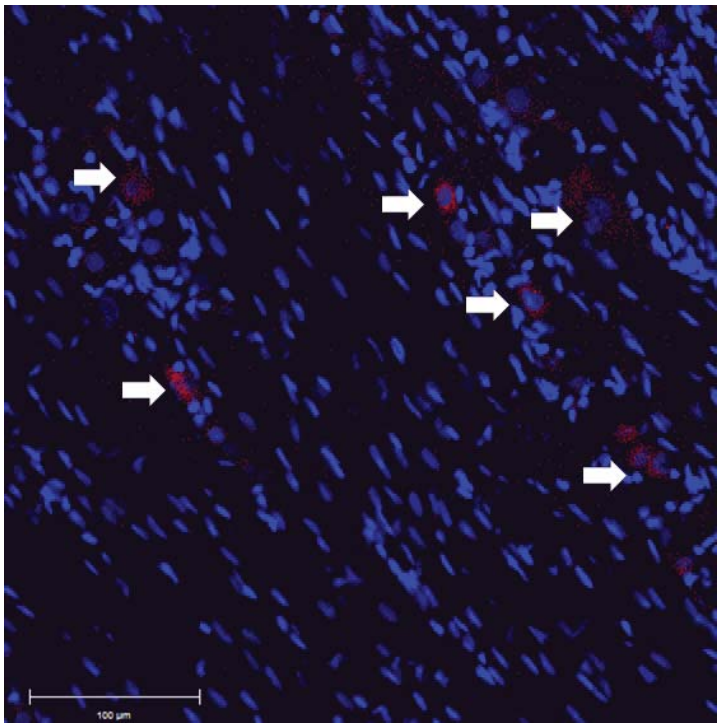
Model of retrograde axoplasmic transport of DiI

Dental primary afferent neurons (DPAN) can be labelled through retrograde axoplasmic transport of fluorescent DiI from their peripheral terminals to their cell bodies. We drill holes in maxillary molars place retrograde labeling dye in the tooth cavities, close them with light-sensitive dental cement and keep the rat under analgesic and antibiotic treatment while the dye is transported axonally. After formalin-perfusion of the animal the trigeminal ganglia are removed, cryopreserved and the labeled cells counted in cryosections using confocal microscopy. Optimal traveling time of the dye is determined to obtain the minimum timeframe necessary to reliably and reproducibly label all DPAN. This method is currently established and the precondition to start



Effect of agonist stimulation on TRPC5-EGFP particles in the plasma membrane in transfected HEK293t cells; top line: TIRF (total internal reflection microscopy) images representing the overall presence of the channel; middle line: EGFP fluorescence intensity; lower line: mobility of TRPC5 within the plasma membrane. Color-coded mobility images reflect variation of a position after stimulus. Red shades represent most mobile regions. Blue shades represent less mobile zones.

Prof. Dr. Zimmermann



Anterograde DiI tracing of rat trigeminal ganglia innervating dental primary afferent neurons of maxillary molars. Dental primary afferent neurons are marked with white arrows. They are labelled by axoplasmic transport of fluorescent DiI from the peripheral terminals to the cell bodies. Ganglia innervating contralateral molars not subjected to dye application contain zero labelled cells (not shown).

studying properties of DPAN innervating normal and inflamed teeth in various models (calcium imaging, Patch-Clamp, full transcriptome).

Screening for TRPC5 modulators in Conus venom peptides (Conus geographus and Conus aulicus)

First, a calcium-based High-Throughput-Screening assay for testing modulatory effects of venom fractions on heterologous expressed TRPC5 on a FLIPR® Platform was developed based on a HEK 293t cell line stably expressing hTRPC5. Riluzole, a TRPC5 agonist, is used to produce reproducible TRPC5-mediated calcium signals in cells loaded with Fluo-4. Cone snail venom was fractionated using HPLC. The fractions were tested using the FLIPR assay. Initially antagonist activity was found in C. aulicus venom, but after further fractionation the antagonist activity could not be confirmed. Furthermore C. geogaphus and C. aulicus both contained early eluting fractions (possibly salts/small molecules/polyamines) that elicit an agonist-like response; the next step would be to test the specificity of these activator responses for TRPC5; for this purpose currently more material is acquired from cone snails. In addition to these fractions we will start screening samples from the Nature Bank project at Griffith University's Eskitis Institute which include extracts from a large array of plants and seeds (currently 200.000 available mixtures) to hopefully identify and pharmacologically characterize a compound that blocks TRPC5.

Contact:
Prof. Dr. Zimmermann
phone: +49 9131 85 33102
e-mail: katharina.zimmermann@fau.de

Publications during funding period

none

E15 - Progress Report

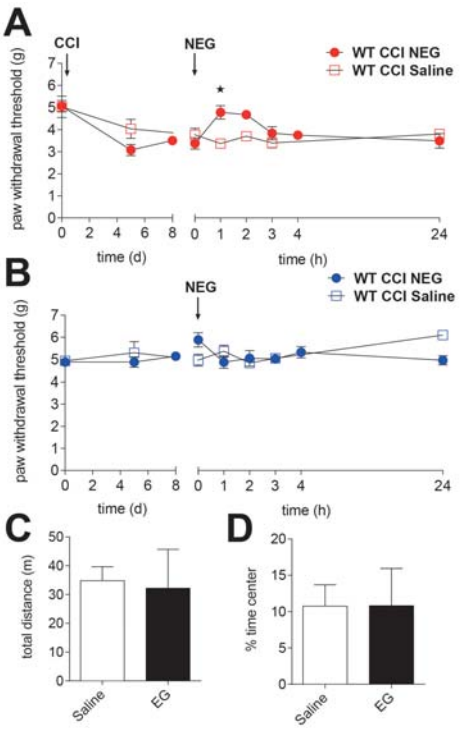
01.11.2013 - 30.04.2016

GlyT1 and neuropathic pain

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

The treatment of neuropathic pain is difficult and the therapeutic results in many cases are not satisfactory. We have shown that substances acting on glycine transporters are beneficial for the treatment of neuropathic pain. Here we plan to investigate the influence of glycine dependent neurotransmission on neuropathic pain syndromes by means of biochemical, behavioural, and electrophysiological approaches. Thereby we hope to determine the therapeutic potential of glycine transporters for this disease.

The perception of pain allows the body an appropriate reaction to potential noxious stimuli and thereby prevents the body from potential damage. Chronic inflammation, systemic diseases like viral infections or cancer as well as injuries to the pain processing somatosensory system can result in long lasting maladaptations and thereby facilitated and/or exaggerated pain perception, called neuropathic pain. For patients neuropathic pain is extremely burdensome and the success of treatments is not satisfactory in many cases. We have shown previously that inhibition of the glycine transporter GlyT1 efficiently ameliorated both mechanical allodynia and thermal hyperalgesiea induced by a chronic constriction of the nervus ischiadicus. Interestingly, inflammatory pain was also ameliorated, suggesting that high GlyT1 driven glycine clearance is required for the facilitated pain response in both scenarios. In follow up experiments we could show that systemic application of GlyT1 substrates, like the Lidocaine metabolite N-Ethyl-glycine (EG), produce a transient amelioration of the facilitated pain response. Using oocytes expressing GlyTs, glycine receptors, or NMDA receptors it was demonstrated that EG acts specifically on GlyT1 and has no effect on the functions of GlyT2, glycine receptors, or NMDA receptors. Using HPLC based analysis we showed that after systemic application in mice, EG was detectable in the cerebrospinal fluid (CSF), demonstrating that EG passes the blood brain barrier. Here, EG was detected in concentrations sufficient for binding to GlyT1. Interestingly, systemic application of even a high dose of EG did not result in hyperactivity or respiratory distress, i.e. side



After constriction (CCI) of the N. ischiadicus, the mechanosensory sensitivty of the paw was determined. A single EG application caused an ameioration of the CCI effect (A), without affecting acute pain (B), or the open field behaviour (C,D).

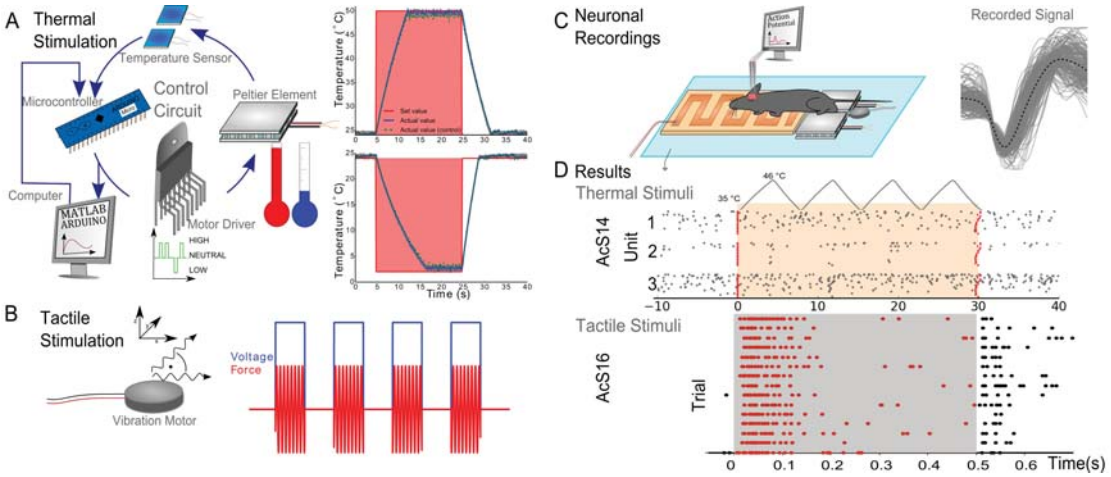

Dr. Eulenburg


Prof. Dr. Schulze

effects that have been reported after application of a high dose of GlyT1 inhibitors. Taken together our findings suggest that reduction of the GlyT1 uptake capacity is a new promising strategy for the treatment of pathological pain conditions.

To determine a possible mechanism how plastic changes in the somatosensory circuitry affects the information processing within central brain regions, a method to record electrophysiological responses within the somatosensory cortex (S1) elicited by thermal or mechanical stimuli was established. Sensory stimulation was performed via peltier elements (for thermal stimulation) or via a coin vibration motor (for mechanical stimulation). First results of S1 recordings demonstrated the correct positioning the electrodes and operational reliability of the stimulation setup. In both stimulation paradigms we found a clear dependency of spike rates and stimulus intensity, with typical onset-responses.

In the next year we will concentrate our efforts to determine the mechanism how a reduction of the GlyT1 transport capacity results in the antihyperalgesic effects observed in the context of inflammatory and neuropathic pain and will compare S1 neuronal responses before and after its induction.



A: Thermal stimulation setup (peltier element), B: Tactile stimulation setup (vibration motor), C: Setup for neuronal recordings (amplifier electronics not shown), D: S1 neuronal responses

Contact:
Dr. Eulenburg
phone: +49 9131 85 26206
e-mail: volker.eulenburg@fau.de

Prof. Dr. Schulze
phone: +49 9131 85 43845
e-mail: holger.schulze@uk-erlangen.de

Invited lectures

V. Eulenburg: 7th symposium of the SFB35 „Transmembrane transporters in health and disease, Vienna, 9-11.10.2014, „glycine transporters; different tissues different functions?“

Publications during funding period

none

E16 - Progress Report

01.04.2014 - 31.03.2017

Regulatory networks in intellectual disability

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

Genetic defects are responsible for the vast majority of intellectual disability (ID) cases in countries with high standard of living. 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 450 genes have been identified in 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 patients. 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.

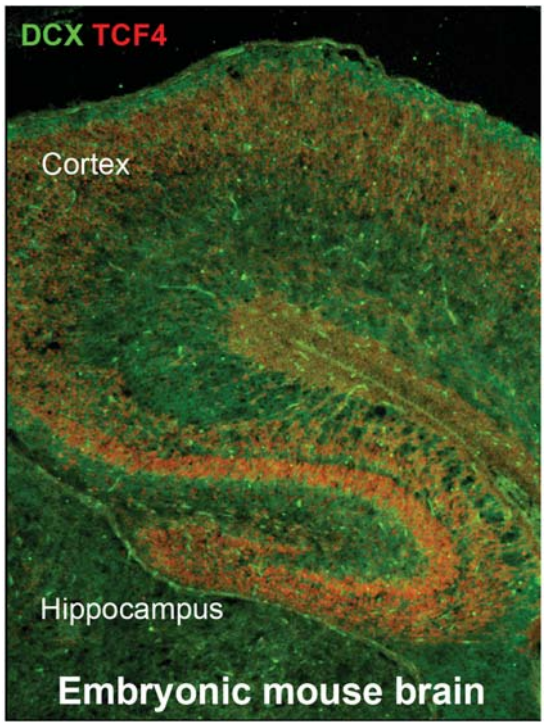
Sox11 biochemically interacts with the ID factor TCF4

We recently identified the transcription factor Sox11 as a key regulator of neuronal fate determination of stem cells in neurogenesis. Intriguingly, de novo mutations in the Sox11 gene were identified as genetic causes for a subset of patients with Coffin-Siris syndrome – a developmental disorder associated with ID.

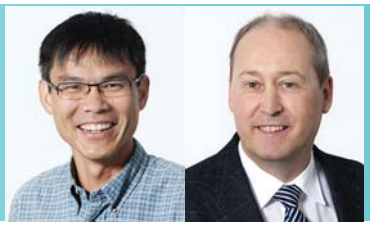
To obtain further insight into the Sox11 transcriptional network, unbiased proteomic analysis for Sox11 interactors was performed in collaboration with Dr. Johannes Glöckner (University of Tübingen). Interestingly, we identified several ID-related proteins including the transcription factor TCF4 as biochemical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

Immunohistochemical analysis reveals widespread expression of TCF4 in developing and mature neurons

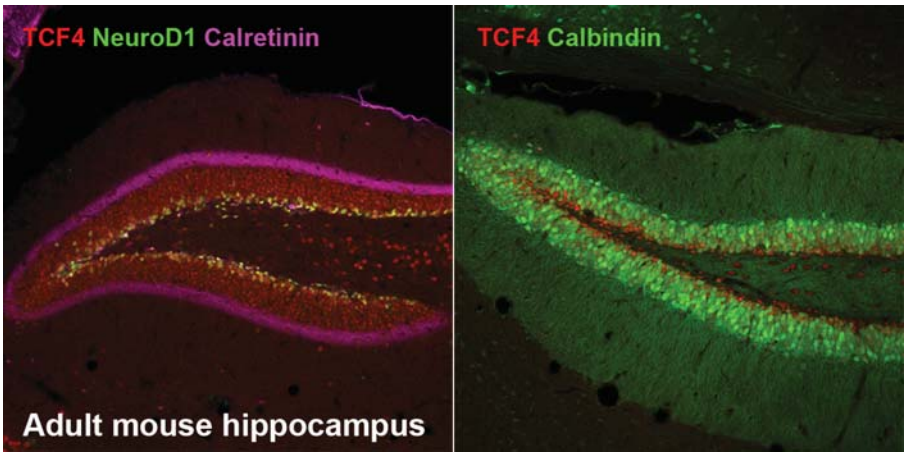
TCF4 mutations cause Pitt Hopkins syndrome, a disorder characterized by developmental delay and intellectual disability. To begin to understand the function of TCF4 in CNS development and plasticity, detailed immunohistochemical analysis of TCF4 ex-



TCF4 (red) is expressed in developing neurons identified via the marker doublecortin (DCX, in green) during embryonic corticogenesis and hippocampal development.



Prof. Dr. Lie Prof. Dr. Reis



TCF4 expression in the adult hippocampus

pression in the developing and adult central nervous system (CNS) was performed. During development, TCF4 expression is readily 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 neural 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. Collectively, 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.

We are presently developing conditional knockout mice to study the impact of TCF4 deletion on neurogenesis and neuronal plasticity.

Contact:
Prof. Dr. Chichung Lie
phone: + 9131 85 24622
e-mail: chi.lie@fau.de

Prof. Dr. Reis
phone: + 9131 85 22318
e-mail: andre.reis@uk-erlangen.de

Invited lectures

Keystone meeting “Adult Neurogenesis”, 12.-17. Mai 2014, Stockholm, Sweden, Impact of mitochondrial function on adult neurogenesis

5. Symposium des IZKF Erlangen, 16.-17. Mai 2014, Kloster Banz, Germany, Molecular regulation of adult hippocampal neurogenesis

Route 28 Workshop “Adult Neurogenesis”, 6.-10. September 2014, Frauenchiemsee, Regulatory pathways in adult neurogenesis

BMBF Symposium “Neural Stem Cells”, 5.-6. November 2014, Heidelberg, Germany, Impact of mitochondrial function on adult neurogenesis

Publications during funding period

Steib K, Schäffner I, Jagasia R, Ebert B, Lie DC (2014) Mitochondria modify exercise-induced development of stem cell-derived neurons in the adult brain. J Neurosci. 34(19):6624-33

E17 - Progress Report

01.04.2017 - 30.09.2016

Wnt signaling at neuromuscular synapses

Prof. Dr. Said Hashemolhosseini, Institute of Biochemistry

In disease, Wnt signaling pathways are associated with carcinomas, but are also involved in synaptic neurodegenerative disorders. Moreover, members of Wnt signaling pathways have been identified at neuromuscular synapses (Wnt3a, Wnt11r, APC, Dishevelled, β-Catenin). However, it is completely unknown which of the Wnt signaling pathways are active at neuromuscular synapses. The aim of this approach is to enlighten the neuromuscular role of Wnt signaling pathways in health and disease.

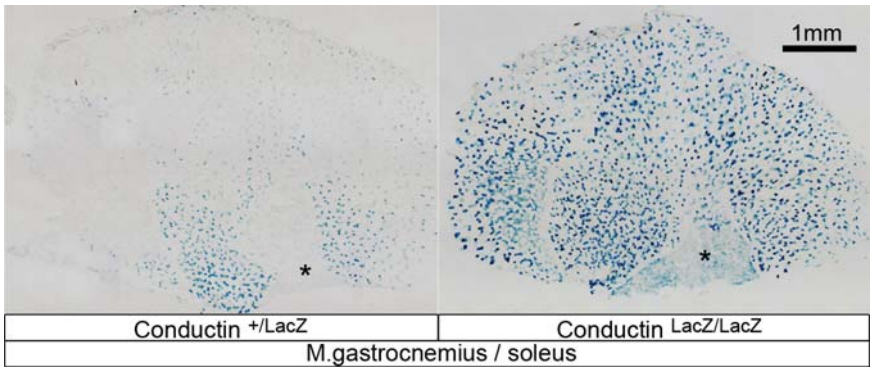
Conductin (also called Axin2), and Axin1 are part of the canonical Wnt signaling pathway. Using Conductin-lacZ reporter mice, X-Gal positive stained tissues are known to reflect active canonical Wnt signaling. X-Gal staining of skeletal muscles of these mice localized a potential neuromuscular role of canonical Wnt signaling (1) in muscle fibers, (2) at neuromuscular synapses, (3) in muscle stem cells / satellite cells, and (4) in terminal / perisynaptic schwann cells.

Axin1 and Conductin are expressed by skeletal muscle cells

In stable cell lines, Conductin is not expressed in myoblasts, but is expressed in myotubes. In muscles diaphragm, extensor digitorum longus, tibialis anterior, and gastrocnemius, Conductin expression is more prominent at the central part of the muscle fibers. In soleus muscle Conductin expression is barely detectable. Interestingly, adult muscle fibers of all other muscle types than soleus, express Conductin almost exclusively by glycolytic muscle fiber types IIa and, most likely, IIx.

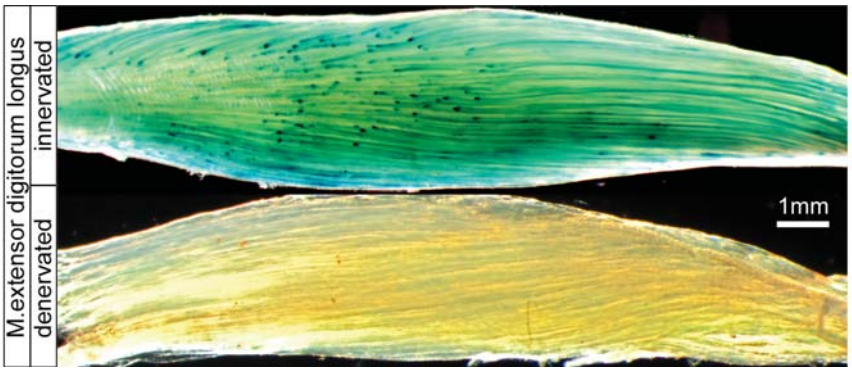
Conductin might be expressed by muscle stem cells/ satellite cells

Additional accumulation of X-Gal stained spots were detected next to the periphery of fiber tubes. These spots might reflect cell nuclei belonging either (1) to the muscle fibers themselves, (2) to synaptic terminal / perisynaptic schwann cells, or (3) to muscle stem cells / satellite cells. Our preliminary data indicate that, at least several of these extra-synaptic spots might co-localize with muscle stem cell / satellite cell marker Pax7.



Typical X-Gal stained cross-section of muscles gastrocnemius and soleus of heterozygote and homozygote Conductin-lacZ mice are shown. Note, not all fibers are stained in the gastrocnemius, and soleus completely lacks staining (asterisk).

Prof. Dr. Hashemolhosseini



Whole-mount X-Gal stained muscles extensor digitorum longus are shown without and with sciatic denervation. Note, conductin expression additionally appears as blue spots at neuromuscular synapses, and is completely absent after denervation.

Conductin expression at neuromuscular synapses

Those blue spots, which localize at the synapse, partially co-localize with synaptic muscle nuclei. This finding correlates with results obtained by in situ hybridization of diaphragms of Conductin-lacZ mice showing accumulation of in situ hybridization signal at the central, synapse containing, endplate zone of muscle fibers. To identify whether Conductin is expressed as a synaptic gene, sciatic nerve lesion was applied to heterozygote Conductin-lacZ mice. It is known that lack of motor activity induces all muscle nuclei to re-transcribe synaptic genes. Surprisingly, all the blue staining was lost in muscle extensor digitorum longus. Loss of Conductin transcription was confirmed by PCR. However, even Axin1 transcription is absent in denervated muscles. This is surprising as Axin1 expression is believed to be not regulated.

Complete loss of X-Gal staining after denervation argues for (1) Conductin expression of type IIa and IIx muscle fibers along the whole fiber depending on motor activity, (2) nuclear X-Gal staining of either origin, synaptic, terminal schwann cell, or satellite cell, to respond to denervation. Up to now, our data do not exclude Conductin expression in terminal schwann cells. It is known that at least in terminal schwann cells, marker expression might not solely depend on evoked motor activity, but might also depend on other morphological changes occurring after denervation.

Contact:
Prof. Dr. Hashemolhosseini
phone: +49 9131 85 24634
e-mail: said.hashemolhosseini@fau.de

Publications during funding period

none

E18 - Progress Report

01.12.2013 - 31.05.2016

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

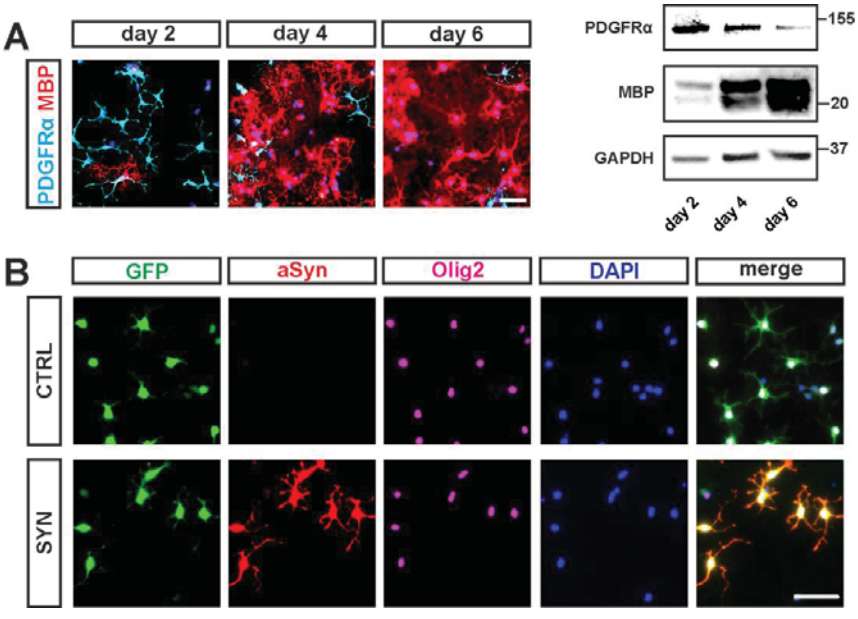
Prof. Dr. Michael Wegner, Institute of Biochemistry
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

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, 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 first year of the project, we generated mice that allow an altered Sox gene expression in adult NG2-glia. Additionally we obtained a better understanding for the pathophysiological mechanisms of MSA.

Generation of mouse mutants

To be able to study the consequences of Sox gene loss in adult NG2-glia, we generated compound mouse mutants that allow specific gene deletion. These mice combine floxed alleles for particular Sox genes with an NG2-CreERT BAC transgene. An additional Rosa26-stopflox-EYFP allele is included to identify the cells in which Cre activity has led to gene deletion. The following floxed Sox alleles were combined with the NG2-CreERT: (i) Sox2 and Sox3; (ii) Sox5 and Sox6; (iii) Sox10. In two mouse lines several floxed Sox alleles were combined because NG2-glia express both closely related Sox genes simultaneously. During the first year mouse colonies were successfully established for all three lines and expanded sufficiently to allow analysis. We have also started to treat adult mice with tamoxifen, and thereby induce



A) Differentiation of primary rat NG2-glia within 6 days from PDGFRα-positive precursors to myelin basic protein (MBP) expressing mature oligodendrocytes. B) Using lentiviral vectors coding for GFP solely (CTRL) or human aSyn and GFP (SYN), we obtained human aSyn expressing NG2-glia and respective control cells.

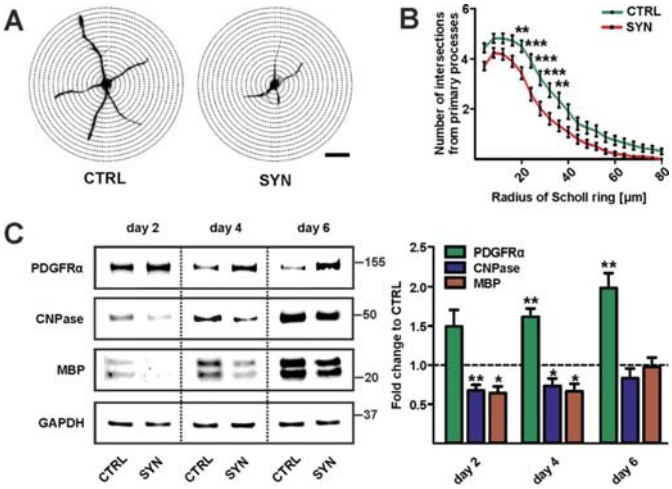

Prof. Dr. Wegner


Prof. Dr. Winkler

Cre-dependent recombination events. First experiments show that 20-30% of all NG2-glia undergo gene deletion. This correlates well with the published deletion rate for the NG2-CreERT transgene, and is sufficient to follow the developmental fate and differentiation capacity of NG2-glia in the absence of the various Sox proteins under study.

Defining the pathophysiological events during MSA

In demyelinating diseases, NG2-glia are normally recruited to sites of demyelination, and attempt to differentiate in order to replace dysfunctional or destroyed mature oligodendrocytes and thereby ameliorate disease symptoms. However, the differentiation capacity of NG2-glia is often limited. In the case of MSA, comprehensive studies investigating the behavior of NG2-glia and their remyelination capacities are completely lacking. We therefore generated a cellular system that gave us some insights into these processes by studying the effect of human aSyn (h-aSyn) on primary rat NG2-glia and their maturation in culture. Upon lentiviral transduction or uptake of extracellular recombinant h-aSyn, h-aSyn containing NG2-glia exhibit fewer and shorter primary processes at the initiation of differentiation. In the following days, h-aSyn expressing NG2-glia further show a severely delayed maturation evidenced by reduced myelin gene expression and increased levels of the progenitor marker platelet derived growth factor receptor-alpha (PDGFRα). However, after 6 days in culture, differentiation resumes and myelin gene expression catches up. This is paralleled by decreased intracellular h-aSyn levels indicating a reverse correlation of h-aSyn and the differentiation potential of OPCs. Taken together, these findings suggest a tight link between the intracellular level of h-aSyn and maturation capacity of NG2-glia.



A) Morphology of NG2-glia upon CTRL and SYN transduction (day 2). B) Scholl analysis reveals fewer and shorter primary processes upon aSyn expression (50 cells analyzed each). C) Altered expression profile of PDGFRa and myelin genes (CNPase, MBP) in aSyn expressing NG2-glia.

Contact:
Prof. Dr. Wegner
phone: +49 9131 85 24620
e-mail: michael.wegner@fau.de

Prof. Dr. Winkler
phone: +49 9131 85 39323
e-mail: juergen.winkler@uk-erlangen.de

Invited lectures

M. Wegner: 4th International Sox Meeting, 8.9.-12.9.2014 in Cleveland, USA: "Sox genes in myelinating glia"

EMBO-Workshop "Development and regeneration of the spinal cord", 1.10.-4.10.2014 in Sitges, Spanien: "Sox genes as critical regulators of glial fate and myelination"

Awards

Benjamin Ettle, MSc, Luise-Prell-Award 2013

Publications during funding period

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

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. Morpholino-mediated loss of fam60a caused expanded her6 expression, reduced ascl1b expression in the midbrain, the prethalamus and the rostral thalamus as well as reduced ngn1 expression in the caudal thalamus. Our data suggest Fam60a as an important regulator of thalamus regionalization by controlling the spatial expression of neuronal differentiation genes.

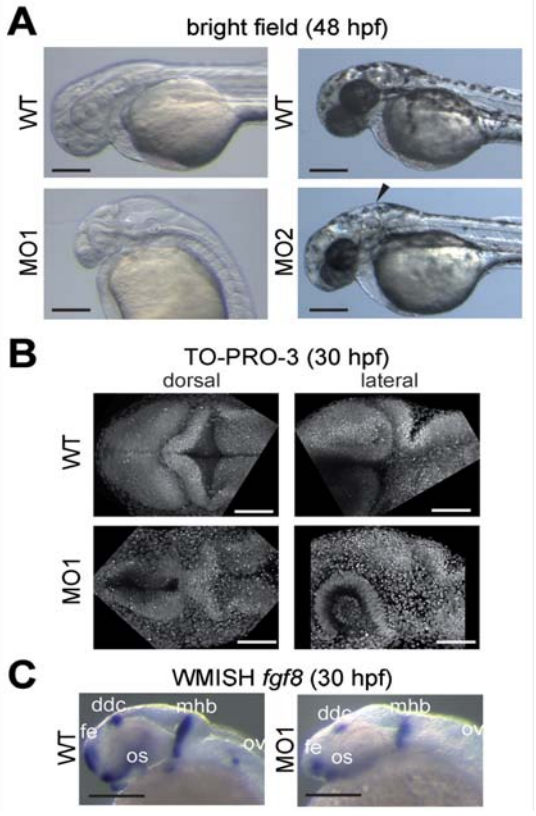
fam60a knockdown disrupts brain development

To assess the in vivo function of fam60a in zebrafish, we used a morpholino (MO)-based approach to knockdown fam60a expression. Injection of two different MOs caused a severe brain phenotype with the formation of a hydrocephalus. Injection of fam60a mRNA rescued the hydrocephalus phenotype in a subset of MO-injected embryos and had only a minor effect on brain development when injected alone indicating that the MO-mediated brain phenotype is due to Fam60A depletion.

To assess whether the morphological abnormalities in morphant brains are caused by primary patterning abnormalities within the neuroepithelium, we analyzed fgf8 expression. Whole mount in situ hybridization showed no obvious changes in fam60a morphants. In contrast, our analyses revealed that fam60a is required for proper inter-segmental patterning of the hindbrain rhombomeres.

fam60a depletion leads to increased her6 and reduced ascl1b expression in the thalamus

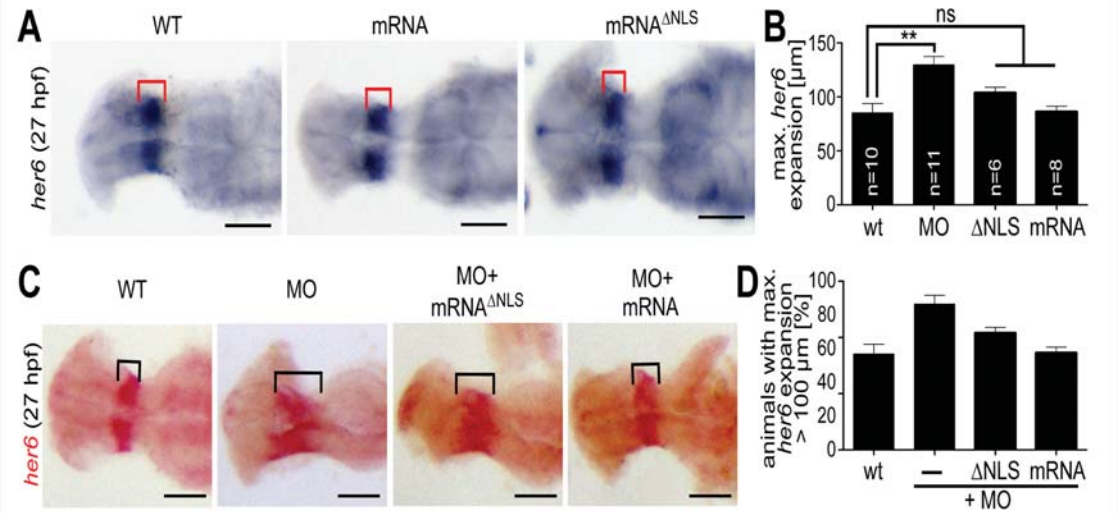
In fam60a morphants qPCR experiments indicated that expression of her6 is increased. Whole mount in situ hybridization showed an expansion of her6-positive cells in the thalamus. In addition, following MO injection ascl1b was significantly reduced in the midbrain and the prethalamus and was not detectable in the rostral thalamus while ngn1 expression was reduced in the caudal thalamus and slightly increased in the telencephalon. The mid-diencephalic organizer was unaffected based on shh expression patterns. Together these results suggest that fam60a



Brain phenotype. (A) wt and MO-injected embryos. Arrowhead: hydrocephalus. Scale bar: 200 µm. (B) TO-PRO-3-stained wt and MO-injected embryos. Scale bar: 50 µm. (C) fgf8 whole mount in situ hybridization. Scale bar 100 µm.

Prof. Dr. Engel

negatively regulates her6 expression and that loss of fam60a is sufficient to increase her6 levels, which in turn leads to reduced pro-neural gene expression in the developing zebrafish brain.



Rescue experiments. (A) In situ hybridization and (B) quantification showing her6 expression in the thalamus. (C) Functional fam60a mRNA but not ΔNLS mRNA rescues MO-mediated her6 expansion. (D) Quantification of C.

Co-injection of fam60a mRNA rescues her6 expansion phenotype

To prove that the observed her6 expansion brain phenotype is specific to Fam60A depletion we performed rescue experiments by co-injecting capped fam60a mRNA or mRNA encoding Fam60a lacking its NLS (mRNAΔNLS) along with MO. Injection of fam60a mRNA and mRNAΔNLS did not significantly alter the her6 expression pattern. In contrast, MO injection resulted in a markedly increased expansion. fam60a mRNA injection along with MO was able to restore her6 expression in the thalamus in the majority of the animals (80 ± 7%, $p < 0.01$). In contrast, co-injection with fam60a mRNAΔNLS did not rescue the her6 phenotype.

Taken together, our data indicate that nuclear Fam60A is required for the correct spatial expression of her6 to control the expression pattern of the proneural genes ngn1 and ascl1b in the mid-diencephalic organizer to drive formation of the rostral thalamus, the prethalamus and the caudal thalamus.

Contact:
Prof. Dr. Engel
phone: +49 9131 85 43635
e-mail: felix.engel@uk-erlangen.de

Publications during funding period

Ferrazzi F, Bellazzi R, Engel FB (2014) Gene network analysis: from heart development to cardiac therapy. Thromb Haemost. 113(1). [Epub ahead of print]

F4 - Progress Report

01.10.2013 - 31.03.2016

Pathogenesis of the short rib-polydactyly syndrome

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

Individual and cellular growth is maintained by many factors. One mechanism involves the regulation by the primary cilium observed on nearly all mammal cell lines. 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 will functional characterize the NEK1 associated ciliary defects to gain insight into the developmental regulation by the primary cilium.

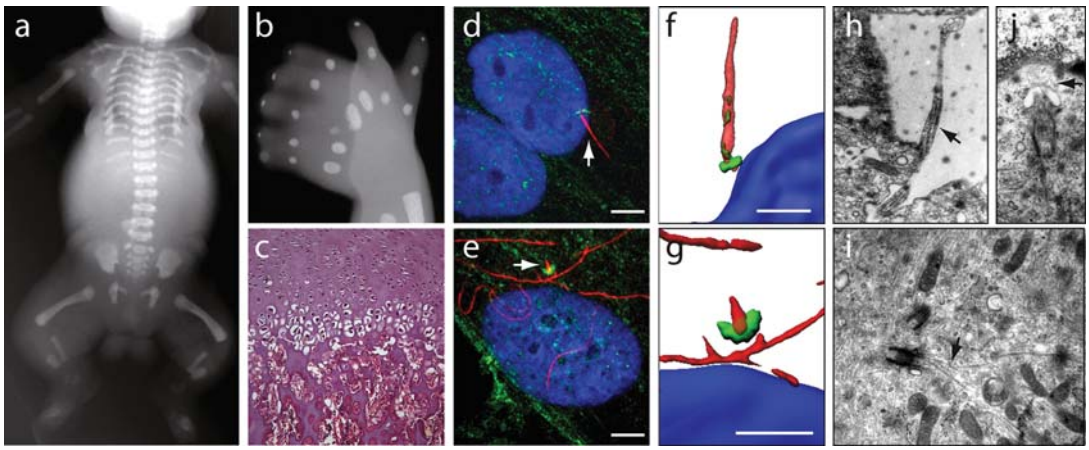
Function of the primary cilium

The primary cilium is a nearly ubiquitous organelle of non-proliferating vertebrate cells. It consists of two main components, the basal body complex on the cytoplasmic side of the cell membrane, and the ciliary axoneme. Herewith, it detects extracellular stimuli via various ciliary membrane receptors and transmits these signals into the cell to initiate intracellular transduction cascades. Those include the 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 and are important for the embryonic and postnatal development and proper organ function in adulthood.

Base on the diverse function, defects of cilia associated genes lead to a pleiotropic spectrum of associated phenotypic effects. These include brain malformations, polydactyly, kidney cysts, retinal degeneration, and skeletal abnormalities.

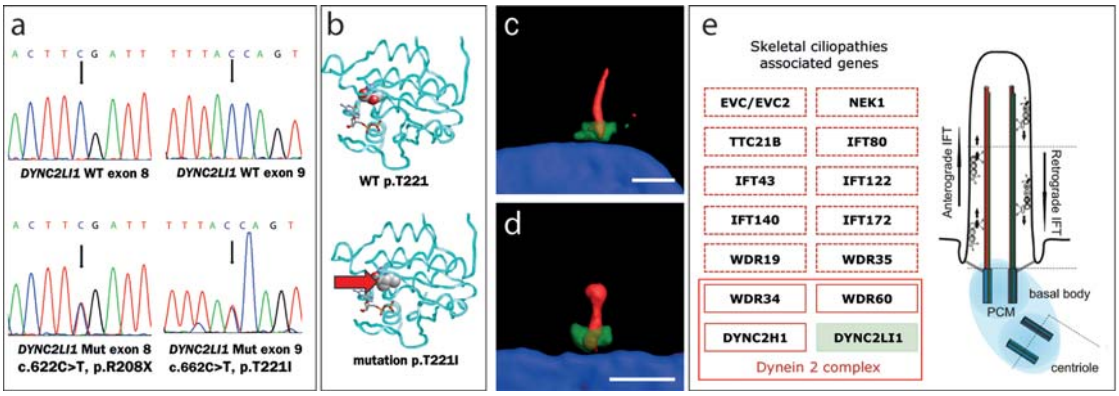
NEK1 associated defects of ciliogenesis

We previously identified mutations in NEK1 as the underlying cause of the short-rib polydactyl type Majewski (SRPS II), a lethal osteochondrodysplasia. NEK1 encodes a serine/threonine kinase with proposed function in DNA double-strand repair, neuronal development, and coordination of cell cycle-associated ciliogenesis. We found that absence of functional full-length NEK1 severely reduces cilia number and alters ciliar morphology in vivo. This assigns this entity to the spectrum of ciliary osteochondrodysplasias.



(a, b) Radiographic features of a patient with short-rib polydactyly type Majewski. (c) Histology of the growth plate. (d, f) Immunofluorescence presentation of a normal cilium compared to a shortened cilium in patient cells (e, g). (h-j) Electron microscopy confirms an arrest of cilia in the stage 1 of ciliogenesis.

PD Dr. Thiel



(a) Sanger confirmation of the 2 DYNC2LI1 mutations. (b) Protein model of the missense mutation at position 221 proposed a conformational effect. (c, d) DYNC2LI1 defects lead to shortened and structural abnormal cilia. (e) Structure of the primary cilium and known genes with a skeletal phenotype (modified from A. Gießl).

To establish a genotype-phenotype correlation we screened 23 patients with suspected SRPS for NEK1 mutations and mutations in DYNC2H1, associated with the clinical overlapping Jeune syndrome. This resulted in the identification of 4 further patients with NEK1 mutations and 3 patients with DYNC2H1 mutations. The proposed hypothesis of a digenic diallelic inheritance could not further substantiated for this patients. Though, these results confirmed the overlapping spectrum of ciliary osteochondrodysplasia.

To further understand how NEK1 defects can negatively affect signal transduction in the various pathways involved, 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.

Identification and characterization of the NEK1 interaction partner DYNC2LI1

To fully understand the role of NEK1 in the context of the primary cilium we established a yeast 2-hybrid assay with a cilia cDNA library. We identified 21 NEK1 interacting proteins. Interestingly, one potential interacting protein, DYNC2Li1, was also been identified as a novel candidate gene for Jeune syndrome in our group. DYNC2LI1 interacts with DYNC2H1 to form the dynein-2 complex important for retrograde intraflagellar transport. In DYNC2LI1 depleted cells we identified a significantly reduced cilia length and altered cilia morphology with broadened ciliary tip as observed in DYNC2H1 defects. In summary, these results already contribute to the clinical spectrum of ciliopathies.

Contact:
PD Dr. Thiel
phone: +49 9131 85 44637
e-mail: christian.thiel@uk-erlangen.de

Publications during funding period

none

Junior Groups / Projects

Junior Groups / Projects

Progress and Final Reports

86

Junior Research Group 2

Prof. Dr. Jens Titze

Contact:
phone: +49 9131 85 39300
e-mail: jens.titze@uk-erlangen.de

Biographical Sketch

The main goal of my preclinical research activity is to discover how immune cells regulate internal environment composition and thereby control blood pressure. My main contributions to the filed are the demonstration that Na^+ is stored in the skin, and that macrophages regulate interstitial electrolyte homeostasis and thereby systemic blood pressure by modulating interstitial electrolyte clearance through cutaneous lymph capillaries (homeostatic immune function). The main goal of my patient-oriented research activity is a transfer of these findings into clinical practice. Through these studies, I 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. As a PI on several grants of the German Research Foundation (DFG), Federal Ministry for Education and Research (BMBF), and Federal Ministry of Economics and Technology (BMWi) of the Federal Republic of Germany, and with the help of from my friends, I laid the ground work for an independent research program on extrarenal regulation of electrolyte homeostasis and blood pressure, and have worked on making our basic research findings enter the clinical arena. The exceptional interdisciplinary research resources and the cooperative research approach at Vanderbilt University have prompted me to transfer this research program from Europe to the United States.

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

Our research focuses on internal salt (NaCl) homeostasis, blood pressure and its regulation by the immune system. We detected hypertonic Na+ accumulation in tissue. This previously unrecognized Na^+ storage escaped the control by the kidney. Immune cells infiltrating Na^+ stores in skin exerted homeostatic immune function by lymphatic electrolyte clearance via a VEGF-C dependent pathway. Blockage of the immune regulation resulted in hypertension, indicating a pivotal role of tissue Na^+ handling for blood pressure control. Furthermore, we found that increased Na^+ concentration in skin boosts pro-inflammatory immune-cell response by macrophages (Mϕ; via NOS2 generation), resulting in bactericidal activity, while on the other hand hypertonic Na^+ accumulation was also able to promote an autoimmune phenotype by IL-17 polarization of T-cells. We aim to further elucidate the complex interplay between electrolyte accumulation and the immune system.

To transfer our basic research findings into the clinical arena, we developed ^{23}Na magnetic resonance imaging methods and implemented them into clinical trials dealing with hypertension, dialysis, scleroderma, and hypernatremia. The purpose of these methods is a non-invasive detection and evaluation of Na+ concentrations in human tissues, particularly in the muscle and in the skin. Our previous studies showed an increase of muscle and skin sodium concentration in hypertensive patients and patients suffering from hyperaldosteronism. Na^+ also increases with age and is higher in males than in females. To clarify whether salt could be an independent cardiovascular risk factor, we are following up on hypertensive patients and patients dependent on dialysis treatment.

Third-party funding

AHA 14SFRN20770008 2014-2018
"Lowering tissue Na^+ stores to reduce blood pressure in aging humans"
Role: PI; Vanderbilt University Strategically Focused Prevention Research Center, Director: Dr. David Harrison

NIH R01 HL118579-01 2013-2018
"Lymphatic regulation of skin electrolyte metabolism and blood pressure"
Role: PI

NIDDK 2R01DK062794-11A1 (PI: Harris, R), 2014-2018
"The role of cyclooxygenase-2 in salt-sensitive hypertension"
Role: Co-Investigator

German Research Foundation Collaborative Research Project Program SFB 643/4, 2013-2016
"Immune system regulation of electrolyte metabolism under homeostatic and inflammatory conditions"
Role: PI

Federal Ministry for Economics and Technology (BMWi), 2013-2016
"Long-term control of body Na^+ and body fluid homeostasis during long-term simulation of a space flight", collaborative project of the German Space Agency, the European Space Agency, and the Russian Academy of Science.
Role: PI

German Research Foundation (DFG), 2009-2012
"Lymphangiogenesis in response to Na^+ storage in the skin", Identification and characterization of the TonEBP / VEGF-C regulatory axis by which MPS cells control electrolyte homeostasis and blood pressure.
Role: PI

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

N2 - Progress 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 ^{23}Na magnetic resonance imaging methods for rapid transfer of our basic research findings into the clinical arena.

Understanding Na^+ storage in humans

We have implemented ^{23}Na-MRI technology to non-invasively visualize Na^+ reservoirs in humans. Now skin tissue can be imaged up to a resolution of 0.9 mm. The improved images directly show that human skin sodium is predominantly found in the upper layers of the skin (dermis + epidermis) but only little in the subcutaneous fat layer. Nephrological, metabolical and dermatological questions can be studied with this technique. In 2014, we have published a clinical study on tissue Na^+ removal in dialysis patients. Age and inflammation augmented Na^+ storage in the patients, and prevented Na^+ mobilisation with therapy.

Immune cells are physiologic regulators of salt water balance and blood pressure control

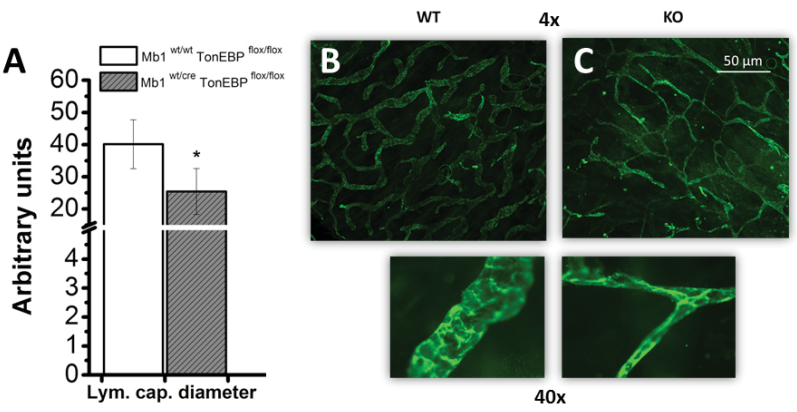
We showed that Na+ storage in the skin and the resulting disequilibrium in interstitial Na^+ concentration attracts macrophages, which then exert a homeostatic-regulatory or autoimmune phenotype.

We could show that a hypertonic microenvironment increases the ability of macrophages to eliminate microbes and serves as barrier against infections. We have extended this work to the role of B-cells (collaboration: Prof. Jäck) and the finding that B cells are critical homeostatic regulators of tissue Na^+ and systemic blood pressure.

Mars500 salt balance studies reveal that high-salt intake induces catabolism in humans

We have performed the first ultra-long term Na+ balance study in humans where we identified weekly (circaseptan) rhythms in human Na^+ balance, and showed that dietary salt intake activates the immune system in humans. We recently study the effect of salt intake on protein catabolism in the Mars500 series.

Contact:
Prof. Dr. Titze
phone: +49 9131 85 39300
e-mail: jens.titze@uk-erlangen.de



Left to right: Panel A-C. Lymph capillaries in control (mb1wtTonEBPflox/flox) and TonEBP-deficient mice (mb1creTonEBPflox/flox). TonEBP-deficiency in B cells reduces the size of the lymph capillary network. Clearance, and leads to a 15-20 mmHg increase in mean arterial blood pressure (not shown). The findings identify B cells as regulators of blood pressure homeostasis.

Prof. Dr. Titze

Invited lectures

06/01/2014 “The interstitium as a sodium sensor and the immune system as a regulator of volume and BP homeostasis” 51st ERA-EDTA Congress, Amsterdam, The Netherlands

05/29/2014 “Skin and Sodium Rhythms in Human Body” Salt in Human Health and Sickness: Building on the Current Scientific Evidence Working Groups” National Heart, Lung, and Blood Institute of the National Institute of Health, Bethesda, USA

04/28/2014 “Hypertension is only skin deep”, Renal Grand Rounds, Division of Nephrology/Hypertension, Feinberg School of Medicine, Northwestern University

10/03/2014 „Lymph vessels and immune cells control skin electrolyte composition and blood pressure“ Gordon Research Conference on Molecular Mechanisms in Lymphatic Function & Disease, Il Giocco Resort Lucca (Barga), Italy

07/03/2014 “Sodium, blood pressure, and homeostatic immune function” Cardiovascular Science Institute Seminars, University of Edinburgh, Edinburgh, USA

06/02/2014 “Sodium balance is not just a renal affair” Center for Cardiovascular Research Seminars, Washington University, St. Louis, USA

01/26/14 Keynote Address “Non-osmotic sodium storage as a pathogenetic factor in hypertension in CDK & ESRD” 16th International Conference on Dialysis, Las Vegas, Nevada, USA

Awards

2014 Election to the American Society of Clinical Investigation, Jens Titze, April 25th, Chicago, USA

2014-2018: AHA 14SFRN20770008 “Lowering tissue Na^+ stores to reduce blood pressure in aging humans”. Role: Principal Investigator

2014-2018: NIDDK 2R01DK062794-11A1 (PI: Harris, R) “The role of cyclooxygenase-2 in salt-sensitive hypertension”. Role: Co-Investigator

Patents/ Licenses during funding period

US Patent Application 20130096415 Method to determine sodium values describing the content of $23Na^+$, and local coil for use in such a method

Selected publications during funding period

Dahlmann A, Dörfelt K, Eicher F, Linz P, Kopp C, Mössinger I, Horn S, Büschges-Seraphin B, Wabel P, Hammon M, Cavallaro A, Eckardt KU, Kotanko P, Levin N, Johannes B, Uder M, Luft FC, Müller DN, and Titze J (2014) Magnetic resonance-determined sodium removal from tissue stores in hemodialysis patients. Kidney International: Aug 6. doi: 10.1038/ki.2014.269. [Epub ahead of print]

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 (2013) Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. J Clin Invest 123: 2803-2815

Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Yosef N, Linker RA, Müller DN, Hafler DA (2013). Sodium chloride drives autoimmune disease by the induction of pathogenic Th17 cells. Nature 496: 518-22

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 (2013) Long-term space flight simulation reveals infradian rhythmicity in human Na^+ balance. Cell Metabolism 17: 125-31

Kopp C, Linz P, Dahlmann A, Hammon M, Jantsch J, Müller DN, Schmieder RE, Cavallaro A, Eckardt KU, Uder M, Luft FC, Titze J (2013) 23Na magnetic resonance imaging-determined tissue sodium in healthy subjects and hypertensive patients. Hypertension 61: 635-40

Helle F, Karlsen TV, Tenstad O, Titze J, Wiig H (2013) High-salt diet increases hormonal sensitivity in skin pre-capillary resistance vessels. Acta Physiol (Oxf) 207(3): 577-81

Kopp C, Linz P, Hammon M, Schofl C, Grauer M, Eckardt KU, Cavallaro A, Uder M, Luft FC, Titze J (2012) Seeing the sodium in a patient with hypernatremia. Kidney Int 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 (2012) 23Na magnetic resonance imaging of tissue sodium. Hypertension. 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 (2010) 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 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 (2009) Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. Nat Med 15: 545-552

Junior Research Group 3

Prof. Dr. Beate Winner

Contact:
Phone: + 9131 85 39301
e-mail: beate.winner@med.uni-erlangen.de

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 basic research 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 III. In addition she was rewarded 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.

From the left: Vanesa Veber, Dr. Iryna Prots, Dr. Haixin Zhang, Dr. Martin Regensburger, Daniela Gräf, Holger Wend, Naime Denguir, Dr. Steven Havlicek, Dr. Francesc Perez-Branguli, Prof. Beate Winner, Himanshu Mishra

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 from analyzing postmortem brain tissues. This mainly derived 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 the reversibility of cellular phenotypes and eventually go back to the patients.

Within the University hospital 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 (HSP). More recently we started to target sporadic neurodegenerative diseases and try to decipher the cascades of aggregation in synucleinopathaies and TDP-43opathies. 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).

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

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

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

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

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

Iryna Prots, Beate Winner, ForIPS, Forschungsverbund Induzierte Pluripotente Stammzellen. TP11: Humanes in vitro Modell für Neuroinflammation.

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

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

N3 - Progress Report

01.10.2010 - 30.09.2016

Modeling neurodegenerative diseases using stem cells

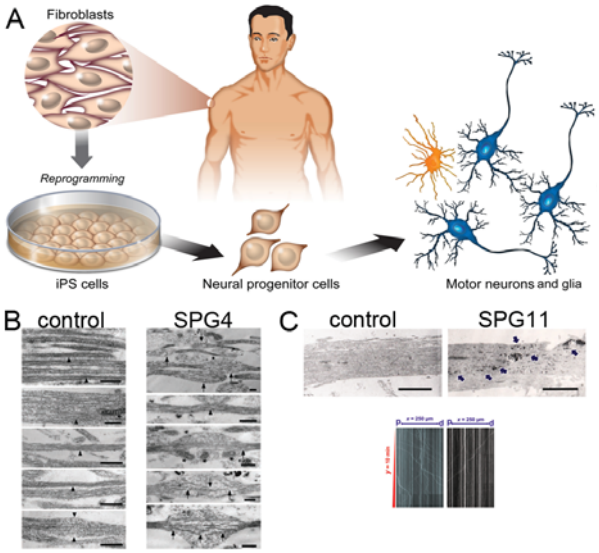
Prof. Dr. Beate Winner, IZKF - Junior Research Group 3

The overall goal in our laboratory is to investigate neurodegeneration using human stem cell derived models. During the last year, we investigated neuronal phenotypes in the most frequent autosomal dominant (SPG4) and recessive (SPG11) forms of hereditary spastic paraplegia (HSP). We were able to show a gene-dosage dependent rescue of impairments of the microtubule structure in patients' neurons with SPG4 mutations and axonal pathology in patients' neurons with SPG11.

The hereditary spastic paraplegias (HSP) are a heterogeneous group of motoneuron diseases characterized by progressive spasticity and paresis of the lower limbs. We generated neuronal cultures from induced pluripotent stem cells (iPSC) from patients' fibroblasts to model the two most frequent genetic forms (SPG4 and SPG11) that cause degeneration in the corticospinal tract.

Gene dosage dependent rescue of HSP neurite defects in SPG4 patients' neurons

Spastin, the protein encoding SPG4, is a member of the ATPase-associated (AAA) family of proteins with the main function to sever microtubuli. We investigated patients with an identical heterozygous nonsense mutation (p.R562X). The levels of Spastin expression and its isoforms were significantly decreased in SPG4 neurons. The neurite complexity of SPG4 glutamatergic projection neurons was severely impaired. Moreover these neurites displayed abundant neurite swellings, with loosely arranged, interrupted microtubules, and an imbalance of axonal transport, with an increase in retrograde transport for mitochondria. An important finding of this study is that elevation of Spastin levels by lentiviral expression of Spastin at low levels led to restoration of neurite complexity and reduction of neurite swellings in SPG4 neurons. Interestingly, there was also a decrease in Spastin expression in the fibroblasts of these patients, indicating a potential role for patient-derived fibroblasts as a pharmacological screening tool in the future.



A) Work flow for investigating disease phenotypes in human stem cell derived cells. B) Neurite swellings in SPG4 neurons. C) Axonal pathology and impaired axonal transport in SPG11 neurons.

In summary, we could show that the gene dosage of spastin (mutated in SPG4 linked HSP) determines the neuritic complexity. Moreover we provided the proof of principle that cellular phenotypes caused by the haploinsufficiency of spastin can be reverted by gene dosage dependent repair (Havlicek et al., HMG 2014).

Prof. Dr. Winner

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

Another study investigated the impact of spatacsin (mutated in SPG11) on neurons. An accumulation of vesicle-like structures and inclusions in human neurites from SPG11 patients points towards neurite pathologies in human neurons with SPG11 mutations. In mouse cortical neurons, spatacsin was located in a punctuated fashion in axons and dendrites. It colocalized with actin, tubulin and synaptic vesicle markers, and was present in synaptosomes. Knockdown of spatacsin evidenced that the loss of function of spatacsin leads to axonal instability by down-regulation of acetylated tubulin. Furthermore, time–lapse assays in spatacsin-silenced neurons highlighted an overall reduction in synaptic vesicles and anterograde vesicle trafficking indicative of impaired axonal transport.

The present study provides the first evidence that human SPG11 mutations and loss of function of spatacsin share neurite pathologies and show that SPG11 is implicated in axonal maintenance and cargo trafficking. Understanding the cellular functions of spatacsin will allow deciphering mechanisms of motor cortex dysfunction in autosomal recessive hereditary spastic paraplegia. (Perez-Branguli, Mishra et al., HMG 2014).

Contact:
Prof. Dr. Beate Winner
Phone: +49 9131 85 39301
e-mail: beate.winner@med.uni-erlangen.de

Invited lectures

Seminar Series, Institute of Physiology, 21.01.2014, Uniklinik RHTW Aachen, Modeling motor neuron disease using human iPSC derived neurons (Beate Winner)

Keystone symposia, Adult Neurogenesis, 15.05.2014, Stockholm, Adult Neurogenesis in Parkinson's Disease (Beate Winner)

Awards

Neurowind e.V. Travelgrant for German Stem Cell Network Conference, Nov. 4th 2014, Bonn, Annika Sommer, PhD student

Travel grant for Early Career Researchers in Overseas for 37th Annual Meeting of the Molecular Biology Society of Japan, November 25 - 27, 2014, RIKEN Center for Integrative Medical Sciences, Yokohama City (Japan) to Himanshu Mishra, PhD student

Award "Best project proposal: The nature of stem cells in adult neurogenesis" at the 8th Route 28 Summit in Neurobiology, September 5-11, 2014 Frauenchiemsee, Germany, to Martin Regensburger, MD

Selected publications during funding period

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

Junior Projects

Immunology and Infection

Project No.	Project title	Term	Applicant(s)	Institute
J27	Impact of posttranslational protein geranyl-geranylation in intestinal epithelial cells in gut homeostasis	01.11.2012-31.10.2014	Dr. López Posadas	Department of Medicine 1
J28	Pathomechanisms of inflammation dependent fibrogenesis	16.11.2012-15.11.2014	Dr. Leppkes	Department of Medicine 1
J29	Interaction of morphogene pathways in the development of fibrotic diseases	01.10.2012-30.09.2014	Dr. Beyer	Department of Medicine 3
J30	Characterization of cytoplasmic activities of human cytomegalovirus pUL69 and its putative role as an antagonist of intrinsic immunity	01.01.2013-31.12.2014	Dr. Thomas	Institute of Clinical and Molecular Virology
J37	Adoptive cell therapy with ex-vivo expanded NK and γδT cells in metastatic melanoma	01.07.2013-30.06.2015	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	Neutrophil-induced resolution of inflammation in gouty arthritis	01.12.2013-30.11.2015	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 - 30.09.2017	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

Oncology

Project No.	Project title	Term	Applicant(s)	Institute
J25	Establishing of an autonomous lymphatic vessel network in the AV-loop model using lymnphatic endothelial cells and mesenchymal stem cells	01.02.2012-31.01.2014	Dr. Boos	Department of Plastic and Hand Surgery
J34	Indirect presentation of HLA class II restricted tumor antigens	15.08.2012-14.08.2014	PD Dr. Anita Kremer	Department of Medicine 5
J35	The role of long non-coding RNAs from human HOX loci in aberrant epi-genetic programming of gastrointestinal stromal tumours (GIST)	01.12.2012-30.11.2014	Dr. Moskalev	Institute of Pathology

Neurosciences

Project No.	Project title	Term	Applicant(s)	Institute
J32	Molecular and structural analysis of neuro-psychiatric symptoms in transgenic models of Parkinson's disease	01.09.2012-31.08.2014	Dr. Ben Abdallah	Department of Molecular Neurology
J33	Sox2 in the CNS: regulating myelination by microRNAs	01.02.2013-31.01.2015	Dr. Reiprich	Institute of Biochemistry
J46	The role of Zfp276 in glial development	01.04.2015-30.09.2017	Dr. Küspert	Institute of Biochemistry

Renal and Vascular Research

Project No.	Project title	Term	Applicant(s)	Institute
J31	Function of a novel, HIF-regulated transcript	01.02.2013-31.01.2015	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(s)	Institute
J36	Identification of molecular signalling pathways in cholestatic pruritus	01.09.2013-31.08.2015	Dr. Andreas Kremer	Department of Medicine 1
J42	Bayesian reverse engineering of gene regulatory networks in heart development	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(s)	Institute
J49	Extending statistical boosting algorithms for biomedical research	01.02.2015-31.07.2017	Dr. Mayr	Department of Medical Informatics, Biometry and Epidemiology

J25 - Final Report

01.02.2012 - 31.01.2014

Establishing of an autonomous lymphatic vessel network

Dr. Anja M. Boos, Department of Plastic and Hand Surgery

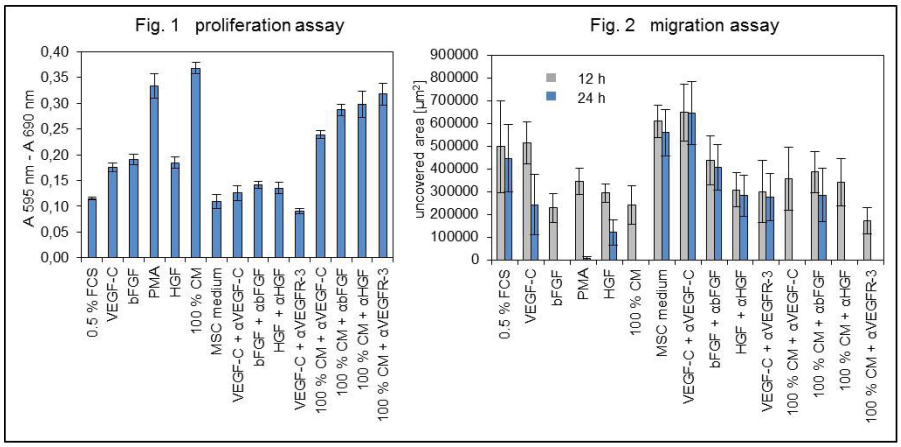
Molecular mechanisms of lymphangiogenesis are still rarely explored. This project addresses the interaction between mesenchymal stem cells (MSC) and lymphatic endothelial cells (LEC) by an in vitro part and aims at identifying novel paracrine factors. In the following in vivo part the arteriovenous (AV) loop model provides a perfectly isolated environment to investigate the lymphangiogenic cascade and could subsequently be used for lymphangiogenesis, anti-lymphangiogenesis and metastasis research.

Background

Lymphatic metastasis is one of the main prognostic factors concerning long term survival of cancer patients. Reliable experimental models are critical to further decipher the lymphangiogenic cascade and to validate novel lymphangiomanipulatory drugs. Many manipulatory in vivo models are complicated by the contribution of the surrounding tissue. The AV-loop model therefore provides a perfectly isolated environment only communicating via the vascular axis with the rest of the organism.

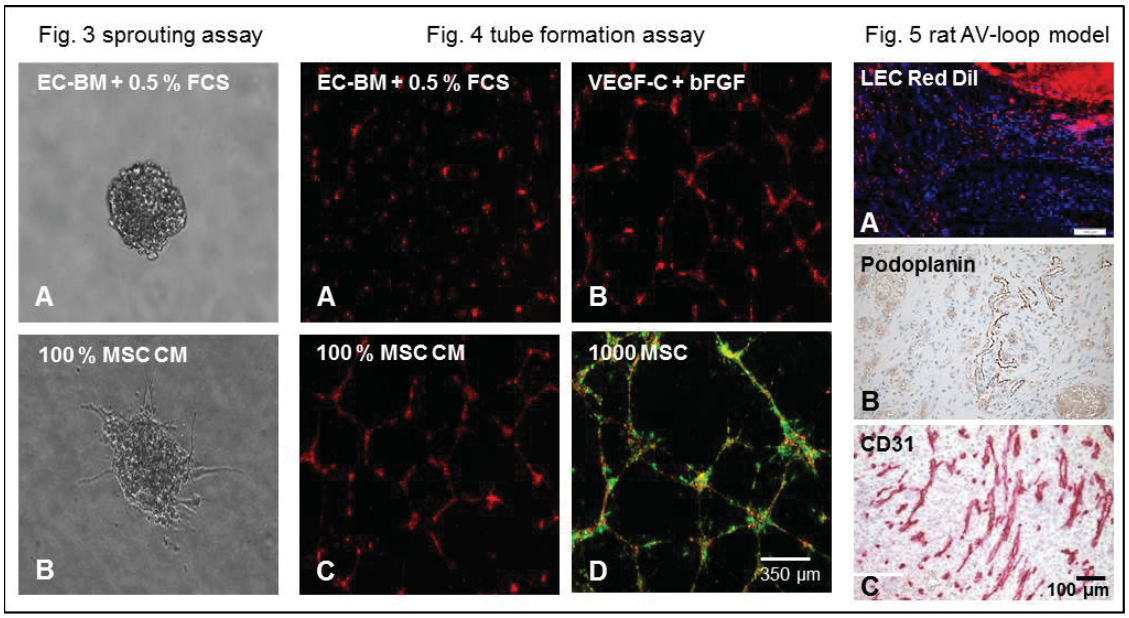
In vitro evaluation of interaction of LEC and MSC

First, primary LEC were tested in different in vitro angiogenesis assays. LEC proliferation was assessed using MTT assay. Cells were stimulated with control medium (endothelial cell basal medium, EC-BM + 0.5 % FCS), VEGF-C: 100 ng/µl, bFGF: 50 ng/µl, HGF: 50 ng/µl, PMA: 50 ng/µl or 100 % MSC conditioned medium (CM) or MSC growth medium. LEC proliferation could be potently enhanced by MSC secreted factors. Soluble antibodies against the growth factors blocked the stimulative effect in the control medium. Addition of soluble antibodies to the MSC CM resulted in a lower LEC proliferation compared to the group with CM alone indicating that the above mentioned growth factors play a role in the MSC – LEC interplay. At the moment an unknown factor is still not deciphered because the effect of the CM on LEC proliferation could only be blocked partly by the known factors. LEC migration was tested by a horizontal and a transmigration assay. LEC were stimulated as mentioned above and 250 000, 500 000 or 750 000 MSC were added. In both assays LEC were stimulated to migrate by MSC secreted factors in a higher extent than by VEGF-C + bFGF. Addition of soluble antibodies against known growth factors blocked LEC migration in the control medium but only to a minor



Cells were stimulated with media as mentioned above. LEC were stimulated to proliferate or migrate by MSC secreted factors. Soluble antibodies against growth factors resulted in lower LEC proliferation and migration.

Dr. Boos



LEC were stimulated with different media. Sprouting and tube formation were stimulated by MSC secreted factors. LEC labeled red / MSC green. LEC spheroids and MSC (each 500 000) were implanted in immunodeficient rats for 2 weeks. Lymphatic positive structures could be found.

extend in the MSC CM. To investigate these effects more in detail, an analysis of the composition of the MSC CM by ELISA and Western Blot is ongoing. LEC spheroids were embedded in collagen and fibrin gels and stimulated analogous to the other assays. Sprouting was stimulated with MSC secreted factors. To evaluate tube formation capacity LEC were plated on matrigel and stimulated as mentioned above. In addition 1 000 or 2 000 MSC were added to the assay. MSC secreted factors enhanced LEC tube formation directly.

Ongoing experiments are focusing on loss-of-function and gain-of-function experiments and aiming at identifying the unknown MSC-derived lymphangiogenic activity.

Establishing of a lymphatic network in the immunodeficient rat AV-loop model

The establishment of the LEC network in the AV-loop model in immunodeficient rats is in progress. LEC spheroids were implanted alone or with MSC. Podoplanin and Lyve1 positive lymphatic endothelial like structures could be found in the immunohistological evaluation.

Further steps would be the use of growth factors, soluble antibodies and transduced LEC – depending on the in vitro results. The explants will be analyzed using immunohistochemical and molecular biological methods. Quality of vessel network formation and connection to the blood vessel system will be evaluated. In the future the focus will lie on loss-of-function and gain-of-function experiments to get deeper insights into the lymphangiogenic processes.

Contact:
Dr. Boos
phone: +49 9131 85 33277
e-mail: anja.boos@uk-erlangen.de

Invited lectures

"Mesenchymal stem cells promote angiogenic properties of lymphatic endothelial cell" - GRC Conference: Molecular Mechanisms in Lymphatic Function & Disease, 09.03.2014 – 14.03.2014, Barga, Italien

Publications during funding period

none

J27 - Final Report

01.11.2012 - 31.10.2014

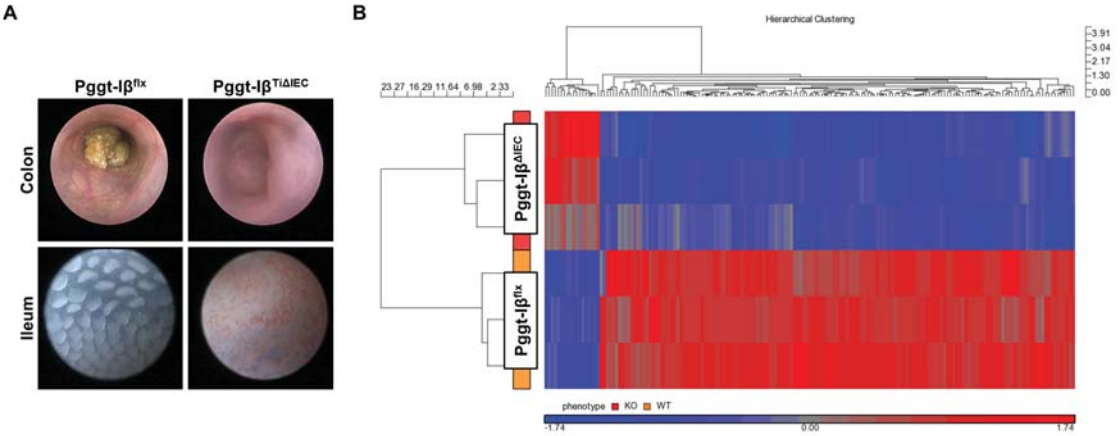
GGTase-I in intestinal epithelial cells

Dr. Rocío López Posadas, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Although it is known that statins mediate their anti-inflammatory effects via inhibition of posttranslational prenylation, the exact role of prenylation in chronic inflammation remains undefined. Motivated by an impressive phenotype of mice lacking the prenylation-catalyzing enzyme geranylgeranyltransferase-1 (GGTase-I) in intestinal epithelial cells (IECs), we will now concentrate on the functional relevance of GGTase-I for gut homeostasis and the mechanism underlying this phenotype.

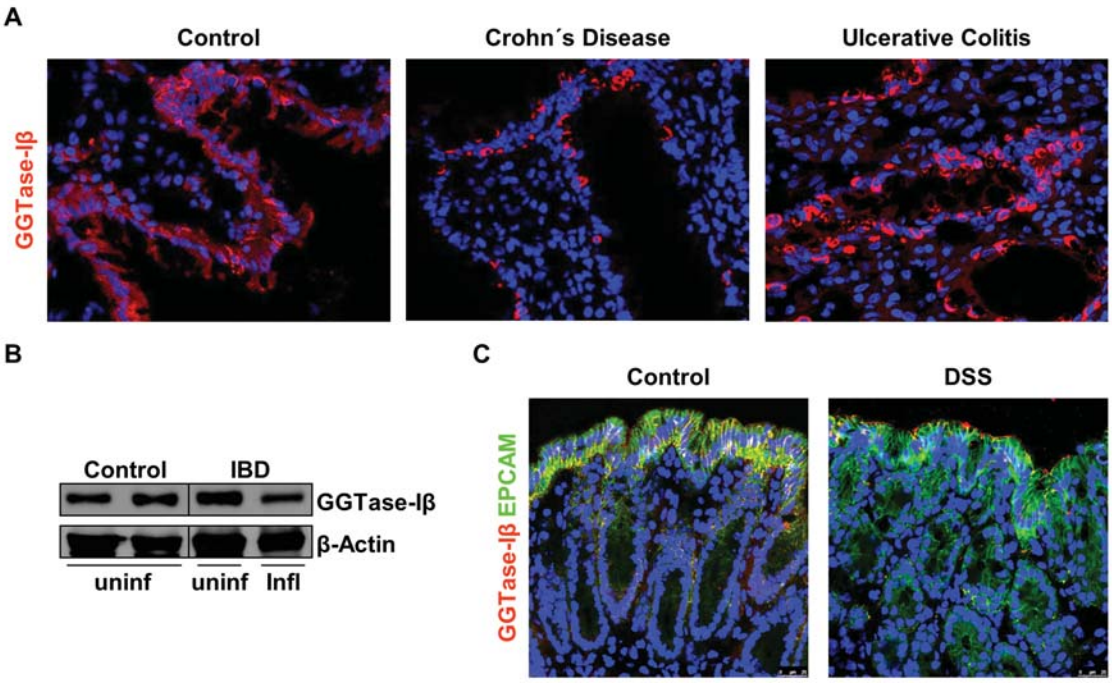
Our data demonstrated a crucial role of GGTase-Iβ expression within IECs for the maintenance of intestinal homeostasis. Deletion of GGTase-Iβ in IECs leads to embryonic lethality. Tamoxifen induced deletion of the Pggt-Iβ gene in IECs (Pggt-Iβ^{TiΔIEC} mice) lead to a lethal intestinal disease. Intestinal mucosa appeared extremely damaged, epithelial architecture destroyed and intestinal permeability dramatically increased. Electron microscopy analysis showed a clear modification of epithelial morphology, disposition and integrity. Our in vitro studies in intestinal epithelial organoids demonstrated that the breakdown of intestinal homeostasis is intrinsic of IEC.

Gene expression as well as proteomic assay clearly demonstrated the dramatic impact of geranylgeranylation in IECs biology (725 genes and 322 proteins showed an altered expression; fold change ≥ 2, and P ≤0,05). Gene ontology analysis shows the involvement of IEC integrity, morphology, and architecture in this effect. Regarding the mechanism underlying this striking phenotype, our data can rule out intestinal microbiota, cell death and proliferation as key mediators. However, our study strongly supports cytoskeleton rearrangement as main consequence of abrogation of geranylgeranylation in IECs. Accordingly, actomyosin complex showed an abnormal disposition in IECs from Pggt-Iβ^{TiΔIEC} mice; and in vivo analysis of epithelial cell shedding showed the dramatic loss of epithelial barrier function. This mechanism was associated with a dysfunction of Rho-A pathway after GGTase-I deletion. Together, we propose the following mechanism: GGTase-Iβ expression in IECs is important for the physiological function of Rho



Intestinal inflammation and epithelial cell biology alterations due to induced deficiency of GGTase-Iβ. A. Mini endoscopic pictures from colon and ileum. B. Gene expression assay array. Genes which expression is up or down regulated in IECs isolated from Pggt-Iβ^{TiΔIEC} in comparison with control mice (174 genes; fold change ≥ 2, and P ≤0,05; n=3).

Dr. López Posadas



GGTase-Iβ profile within IECs in human and murine intestinal inflammation. A. GGTase-Iβ immunostaining in gut tissues from control, and IBD patients (CD and UC). B. GGTase-Iβ western blot in IECs isolated from control and IBD patients. C. GGTase-Iβ immunostaining in gut tissues from control and DSS-exposed mice.

A. Lack of geranylgeranylation in IECs resulted in an altered cytoskeleton arrangement and aberrant cell shedding, finally leading to destruction of intestinal architecture, increased intestinal permeability and loss of intestinal homeostasis.

Lethality due to abrogation of GGTase-Iβ expression in IECs meant a clear limitation for the performance of colitis and colorectal cancer experimental models. Therefore, we analyzed Heterozygous mice in this context. In vivo data showed no modification of DSS colitis or AOM-DSS colorectal cancer in heterozygous Pggt1b$^{fl/wt}$Villin-Cre mice.

Regarding the expression profile of GGTase-Iβ, the enzyme could be detected in murine intestinal epithelium. Moreover, epithelial GGTase-Iβ expression is reduced in T-cell dependent (adoptive transfer) and T cell-independent (DSS) experimental colitis. Performing similar expression analysis in human intestinal samples, our data implicated a decreased GGTase-Iβ expression in the gut of Ulcerative colitis as well as of Crohn´s disease patients. Focusing on prenylation targets, we have found that Rho-A in IECs from IBD patients is not modified in terms of expression, but it is accumulated into the cytosol, which reflects an impaired function of this small GTPase.

Contact:
Dr. López Posadas
phone: +49 9131 85 39602
e-mail: rocio.lopez-posadas@uk-erlangen.de

Awards

Poster of distinction, Digestive Disease Week (DDW) 2014. , Spontaneous intestinal inflammation in mice lacking geranylgeranylation in epithelial cells due to RhoA-mediated cytoskeleton alterations and breakdown of gut homeostasis. Rocío López-Posadas, Martin 0. Bergo, Christoph Becker, Kerstin Amann, Stefan Tenzer, Raja Atreya, Markus Neurath and Imke Atreya. May 2014, Chicago, USA

Publications during funding period

none

J28 - Final Report

16.11.2012 - 15.11.2014

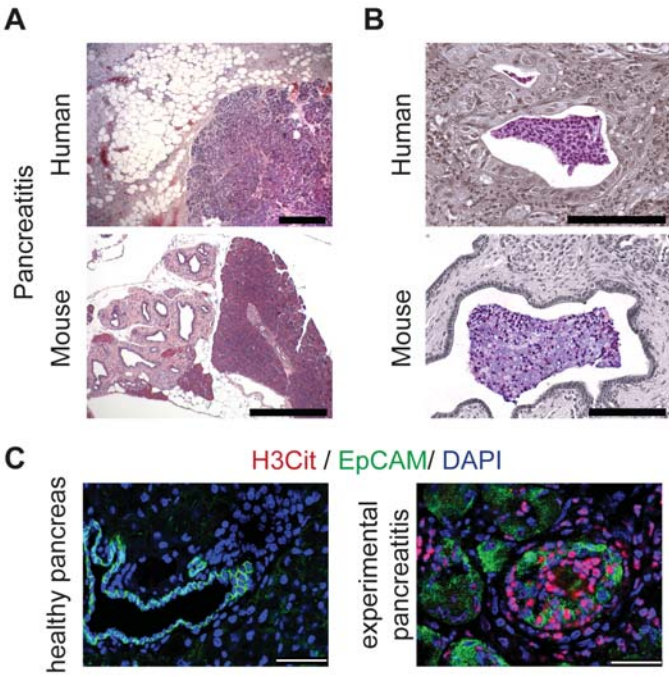
Pathomechanisms of inflammation dependent fibrogenesis

Dr. Moritz Leppkes, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

IL-17A is an important regulator of the granulocyte pool. I have shown that the forced expression of IL-17A leads to the development of chronic pancreatitis. Neutrophils play an important role in this disease. Together with Prof. M. Herrmann (Med 3), I have shown, that neutrophils enter pancreatic ducts and form aggregated neutrophil extracellular traps (aggNETs). We propose that intraductal aggNET formation precipitates focal pancreatic inflammation by interference with pancreatic secretion.

Neutrophils enter pancreatic ducts and form aggregated NETs

The development of an IL-17A expression vector, which leads to a stable systemic expression of IL-17A (Dr. Wirtz) opened the possibility to analyse the functional role of specific cell types and molecular mediators in the pathogenesis of IL-17A-induced pancreatitis. In two to four weeks after vector injection into wild-type B6/J mice, a massive infiltration of neutrophil granulocytes into the murine pancreas is noted, which leads to the segmental destruction of the exocrine pancreas and consecutive tissue fibrosis. The depletion of neutrophil granulocytes using anti-Ly6G antibodies prevented the development of IL-17A-induced pancreatitis, thereby implementing neutrophil granulocytes as the main pathogenic cell type in this model. Neutrophils may extrude decondensed chromatin leading to neutrophil extracellular traps (NETs). In high cellular density, these cluster and form aggregated NETs (aggNETs). In pancreatic inflammation, neutrophils enter pancreatic ducts and form aggNETs. In a novel pathogenic model, I propose, that the increased presence of extracellular chromatin in the pancreatic juice decreases fluidity and leads to ductal occlusion and stasis of the juice. This may facilitate premature zymogen activation in dependent acini and offers a compelling explanation for the focal distribution of the disease.

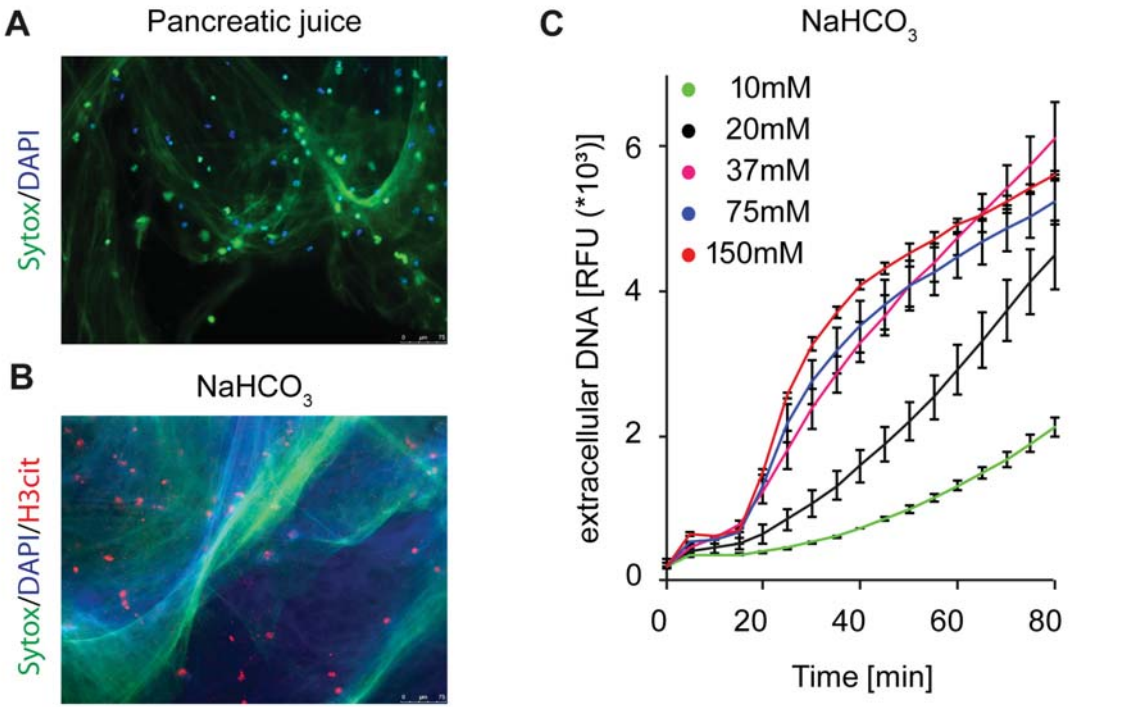


(A, B) H&E stains of human and murine chronic pancreatitis displaying A the segmental inflammation and B intraductal neutrophil clusters. C EpCAM and citrullinated Histone H3 (H3cit) co-labeling reveals H3cit in inflamed murine pancreatic ducts only.

Sodium Bicarbonate induces aggNET formation in human neutrophils

Given the fact, that IL-17A was induced systemically, I wanted to identify tissue-specific factors explaining, why the pancreas is prone to chronic neutrophilic inflammation in this model. Pancreatic juice contains inactive digestive zymogens and elevated

Dr. Leppkes



A Cytospins revealed the presence of web-like chromatin in pancreatic juice. B Human neutrophils develop similar 3-D webs, immunopositive for H3cit, in response to NaHCO3. C Neutrophils dose-dependently extrude chromatin in response to $NaHCO_3$.

levels of sodium bicarbonate in order to neutralize the duodenal content after meals. Interestingly, we found that sodium bicarbonate dose-dependently induces chromatin extrusion in human neutrophils leading to the formation of aggNETs similar to those found in pancreatic juice. Bicarbonate-induced neutrophil chromatin showed citrullinated histone implying chromatin decondensation in this process. Taken together, IL-17A induces chronic pancreatitis mediated by neutrophil granulocytes. Granulocytes enter the pancreatic tissue, infiltrate pancreatic ducts and form aggregated NETs in response to sodium bicarbonate. This model of pathogenesis will influence future therapeutic approaches to pancreatic inflammation.

Contact:
Dr. Leppkes
phone: +49 9131 85 35909
e-mail: moritz.leppkes@uk-erlangen.de

Publications during funding period

Leppkes M, Roulis M, Neurath MF, Kollias G, Becker C. Pleiotropic functions of TNF-alpha in the regulation of the intestinal epithelial response to inflammation. Int Immunol. 2014 May 12. PubMed PMID: 24821262

J29 - Final Report

01.10.2012 - 30.09.2014

Interaction of morphogen pathways in the development of fibrotic diseases

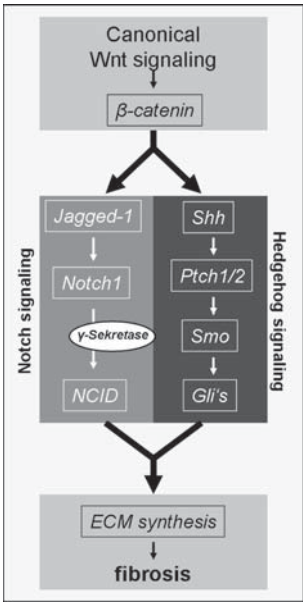
Dr. Christian Beyer, Department of Medicine 3 – Rheumatology and Immunology

Re-activation of the developmental morphogen pathways Wnt, Hedgehog and Notch is driving many fibrotic diseases. Each single pathway has potent pro-fibrotic effects, but their crosstalk in the context of fibrosis remains to be defined. Our project investigates the interaction of morphogen pathways in experimental models of fibrosis. Using both pharmacological and genetic approaches, our project may have important translational implications in the treatment of fibrotic diseases.

The morphogen pathways Wnt, Hedgehog and Notch are emerging as key drivers of fibrotic processes. The current project aims to understand (a) how these pathways interact in the fibrotic process and (b) if specific (pharmacological) targeting of the morphogen network can inhibit fibrosis. Preliminary findings postulate a hierarchy of the morphogen pathways in fibrosis in which Wnt signaling induces Hedgehog and Notch signaling to transmit pro-fibrotic effects. So far, we have generated the following important results:

Pharmacological blockade of morphogen pathways inhibits experimental fibrosis.

Using different models of inflammatory and inflammation-independent fibrotic diseases, we showed that pharmacological and genetic blockade of different members of the Wnt signaling pathway inhibited experimental fibroblast activation and collagen release. We observed that targeting tankyrases that mediate the stability of the β-catenin destruction complex (Distler A et al., ARD, 2013) or blocking complex formation of β-catenin with its co-factors and the TCF transcription factor family (Beyer C et al, ARD, 2013) prevented and treated dermal fibrosis. In previous projects, we demonstrated that inhibition of either Hedgehog signaling (Horn A et al, ARD, 2012; Horn A et al, Arthritis Rheum, 2012; Zerr et al, Blood, 2013) or Notch signaling (Dees C et al, ARD, 2011; Dees C et al, Arthritis Rheum, 2011) also had potent anti-fibrotic effects in various experimental models of fibrosis. In the context of clinical application, we observed that combined blockade of different morphogen pathways in clinically well-tolerated doses had increased therapeutic efficacy in the treatment of experimental fibrosis compared to interference with a single pathway (Distler A et al, ARD, 2014).

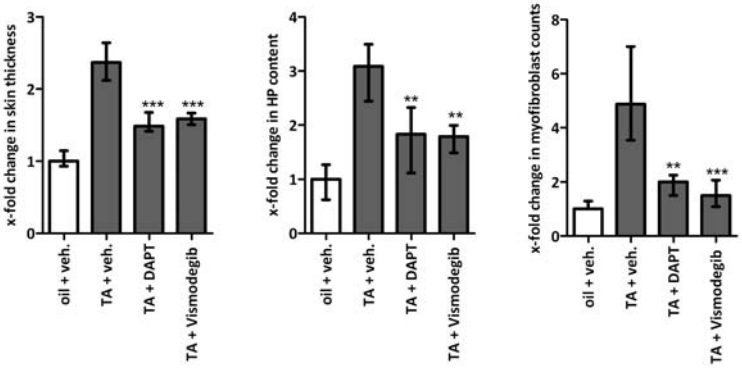


Hypothesis – Hierarchy of Morphogen Pathways in Fibrosis
Based on our preliminary findings, we postulate that Wnt signaling induces the Hedgehog and Notch signaling cascades in fibrosis.

Wnt signaling activates Hedgehog and Notch signaling in fibrosis.

We used two different mouse models to study the effects of Wnt on Hedgehog and Notch in fibrosis. In mice overexpressing a constitutively active form of β-catenin in fibroblasts (β-catenin ΔExon 3 $^{fl/fl}$ x Col1a2; CreER mice) and in mice overexpressing Wnt10b (under the control of a FABP4 promoter), Wnt activation stimulated the expression of Sonic hedgehog, Ptch-1 and Ptch-2 (ligand and target genes of Hedgehog signaling) as well as Jag-1 and Hes-1 mRNA (ligand and target genes of Notch signaling). On protein levels, Shh, Gli-proteins and Jag-1 were up-regulated. Surprisingly, mRNA of the Notch-1 receptor and protein levels of the Notch intracellular domain were downregulated, which is under further investigation.

Dr. Beyer



Pharmacological Blockade of Hedgehog or Notch Signaling Inhibits Wnt-driven Fibrosis
Pharmacological inhibition of Hedgehog (with Vismodegib) und Notch (with DAPT) inhibits Wnt-driven fibrosis in a model with a constitutively active β-catenin in fibroblasts as assessed by skin thickness, hyodroxyproline content, and myofibroblast numbers.

Pharmacological blockade of Hedgehog and Notch signaling inhibits Wnt-driven, experimental fibrosis.

β-catenin ΔExon 3 $^{fl/fl}$ x Col1a2; Cre-ER mice and Wnt-10b mice develop spontaneous dermal fibrosis. In both models, treatment with the Hedgehog inhibitor vismodegib (FDA-approved for basal cell carcinoma) significantly reduced fibroblast activation, collagen release and tissue fibrosis. Similarly, treatment of mice overexpressing a constitutively active form of β-catenin with the Notch inhibitor DAPT reduced the numbers of activated fibroblasts, collagen content and skin thickness. These data suggest that blocking the stimulatory effects of Wnt on Notch and Hedgehog can inhibit fibrosis, further supporting our hypothesis of an hierarchical interaction of morphogen pathways in fibrotic diseases.

Contact:
Dr. Beyer
phone: +49 9131 85 43023
e-mail: christian.beyer@uk-erlangen.de

Awards

Abstract-Award EULAR meeting 2014: “Signature of Circulating MicroRNAs in Osteoarthritis” (06/2014)

Publications during funding period

Distler A, Lang V, Del Vecchio T, Huang J, Zhang Y, Beyer C, Lin NY, Palumbo-Zerr K, Distler O, Schett G, Distler JH (2014) Combined inhibition of morphogen pathways demonstrates additive antifibrotic effects and improved tolerability. Ann Rheum Dis. 73(6): 1264-8

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Zerr P, Palumbo-Zerr K, Distler A, Tomcik M, Vollath S, Munoz LE, Beyer C, Dees C, Egberts F, Tinazzi I, Del Galdo F, Distler O, Schett G, Spriewald BM, Distler JH (2012) Inhibition of hedgehog signaling for the treatment of murine sclerodermatous chronic graft-versus-host disease. Blood 120(14): 2909-17

J30 - Final Report

01.01.2013 - 31.12.2014

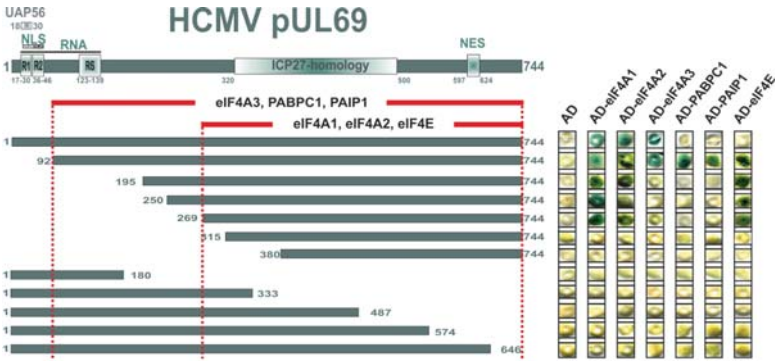
Cytoplasmic functions of human cytomegalovirus pUL69

Dr. Marco Thomas, Institute of Clinical and Molecular Virology

Human cytomegalovirus encodes for the multifunctional regulatory protein pUL69 which has an important role for viral mRNA export and a so far uncharacterized cytoplasmic function. Here we reconfirm the interaction of pUL69 with cytoplasmic PABPC1 or eIF4A1 and identified additional translation factors as novel pUL69-interactors. Furthermore we demonstrate that pUL69 is posttranslationally modified by protein arginine methyltransferase 6, which critically affects efficient virus multiplication.

Background

HCMV pUL69 is an RNA-binding, nucleocytoplasmic shuttling protein that facilitates the cytoplasmic accumulation of unspliced mRNAs via recruitment of the cellular mRNA export factors UAP56/URH49 (Zielke & Thomas et al., 2011). Besides its important function for viral mRNA export, HCMV pUL69 seems to have additional, so far uncharacterized functions as it interacts with the cytoplasmic translation factors PABPC1 or eIF4A1 (Aoyagi et al., 2010). We therefore set out to unravel its impact on protein translation and analyzed whether, and if so how, pUL69 is regulated by posttranslational protein modification.



Yeast two-hybrid analyses of pUL69/-mutants fused to GAL4-BD and translation factors fused to GAL4-AD. Positive interactions are indicated by blue staining and regions that are sufficient for interaction are highlighted by red bars.

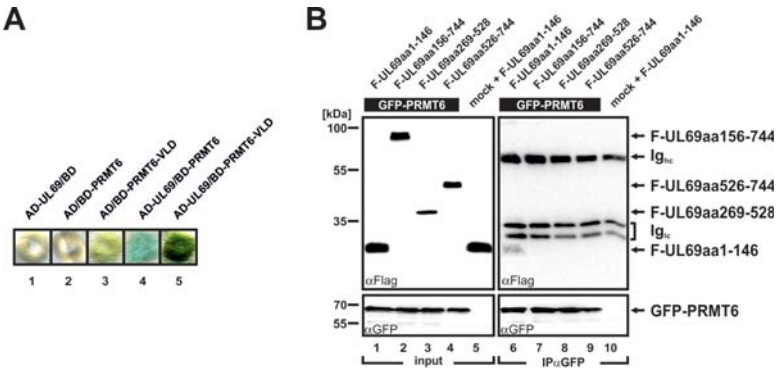
HCMV pUL69 interacts with translation initiation factors

In our current study protein-protein interactions were determined by yeast two-hybrid analyses. For this, yeast were transformed with plasmids encoding truncated versions of pUL69 fused to GAL4-BD together with vectors coding for components of the translation initiation complex fused to GAL4-AD. Hereby we could reconfirm the interaction of pUL69 with eIF4A1 or PABPC1. In addition, an interaction of pUL69 and eIF4A2, eIF4A3, eIF4E or PAIP1 was observed. Mapping studies narrowed down the region encompassing amino acids 92 to 744 of pUL69 as its interaction domain with eIF4A3, PABPC1 and PAIP1. Moreover, for interaction with eIF4A1, eIF4A2 and eIF4E a shorter region of pUL69 encoding the amino acids 269 to 744 was sufficient. Taken the results together, one can assume an important role of pUL69 during translation.

HCMV pUL69 is posttranslationally modified by protein arginine methyltransferase 6 (PRMT6)

Moreover, colocalization and interaction studies were performed to demonstrate a direct interaction of viral pUL69 with cellular PRMT6. Western blot experiments revealed a significant gel shift upon overexpression of catalytically active PRMT6. We identified crucial arginine residues within the functional important N-terminus of pUL69 that were required for interaction with UAP56 as well PRMT6 but did not affect the alpha-helical structure of the respective interaction region. Moreover, by performing a well-established mRNA-export assay we unambiguously confirm that the UAP56/PRMT6-binding de-

Dr. Thomas



Interaction of pUL69 and PRMT6 as determined by yeast two-hybrid (A) or coimmunoprecipitation analyses from transfected HEK293T cells (B).

ficient mutants lost their mRNA export activity. Furthermore, we demonstrated that arginines 28 and 29 were crucial for pUL69-mediated mRNA export while UAP56/PRMT6-interaction were not affected. We therefore identified a novel posttranslational modification within the multifunctional regulatory protein pUL69 of HCMV and strongly suggest that arginine methylation has a significant impact on pUL69´s function in vivo.

Conclusion

Based on our previous work and data of this study we propose that pUL69 binds to a specific subset of cellular and viral transcripts, facilitates their export into the cytoplasm, where it likely regulates their translation. The diverse functions of pUL69 are regulated via posttranslational protein modification by phosphorylation and arginine-methylation.

Contact:
Dr. Thomas
phone: +49 9131 85 22100
e-mail: marco.thomas@viro.med.uni-erlangen.de

Invited lectures

Workshop: 39th International Herpesvirus Workshop (IHW 2014), Kobe, Japan, July 2014; Dr. M. Thomas: „PRMT6-mediated arginine-methylation of the viral mRNA export factor pUL69 and its impact on human cytomegalovirus replication"

Workshop: 24th Annual Meeting of the Society of Virology, Alpbach, Austria, March 2014; Dr. M. Thomas: „Characterization of PRMT6-mediated arginine-methylation of the HCMV-encoded mRNA export factor pUL69"

Seminar: Methods in Molecular Virology; 16.12.2013; Institute for Clinical and Molecular Virology, University Hospital Erlangen, Germany; Dr. M. Thomas: „Posttranslational modifications of HCMV pUL69 and identification of mRNAs recruited by pUL69 during infection"

Publications during funding period

Thomas, M., Zielke, B., Reuter, N. and Stamminger, T. (2014); Methods to study the nucleocytoplasmic transport of macromolecules with respect to their impact on the regulation of human cytomegalovirus gene expression.; Methods in Molecular Biology, Vol. 1119: 197-216, edited by Yurochko, A. D.; Miller, W. E.; doi: 10.1007/978-1-62703-788-4_12

J31 - Progress Report

01.02.2013 - 31.01.2015

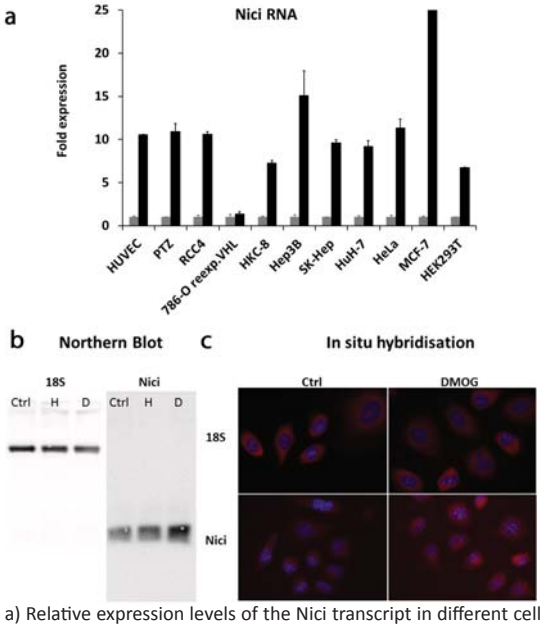
Function of a novel, HIF-regulated transcript

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.



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.

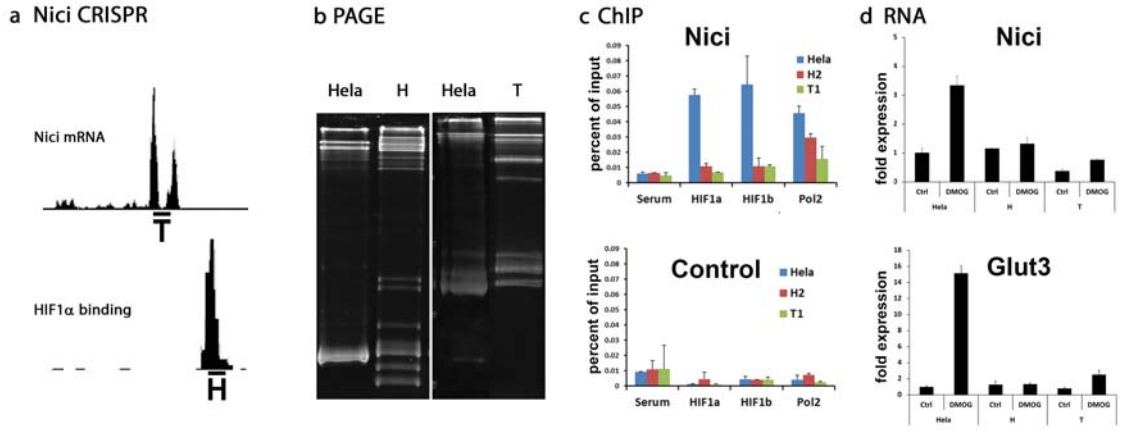
Nici – a novel long non-coding RNA regulated via the HIF-pathway:

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 levels of the neighboring gene, glucose transporter member 3 (Glut3), which is located approx. 25kb

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.

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.

Contact:
Dr. Dr. Schödel
phone: +49 9131 85 39560
e-mail: johannes.schoedel@uk-erlangen.de

Invited lectures

SFB 699 Seminar, 29.01.2014, Regensburg, “Genetic, epigenetic and transcriptional mapping of the cellular response to hypoxia”

Awards

Else-Kröner Exzellenzstipendium, Dr. Dr. Johannes Schödel, 10.12.2014, Bad Homburg v.d.H.

Publications during funding period

Choudhry H*, Schödel J*,Oikonomopoulos S, Camps C, Grampp S, Harris AL, Ratcliffe PJ, Ragoussis J, Mole DR (2014) Extensive regulation of the non-coding transcriptome by hypoxia: role of HIF in releasing paused RNApol2. EMBO Rep, 15, 70-76

*contributed equally

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 (2014) Tumor hypoxia induces nuclear paraspeckle formation through HIF-2α dependent transcriptional activation of NEAT1 leading to cancer cell survival. Oncogene, [Epub ahead of print] doi: 10.1038/onc.2014.378

J32 - Final Report

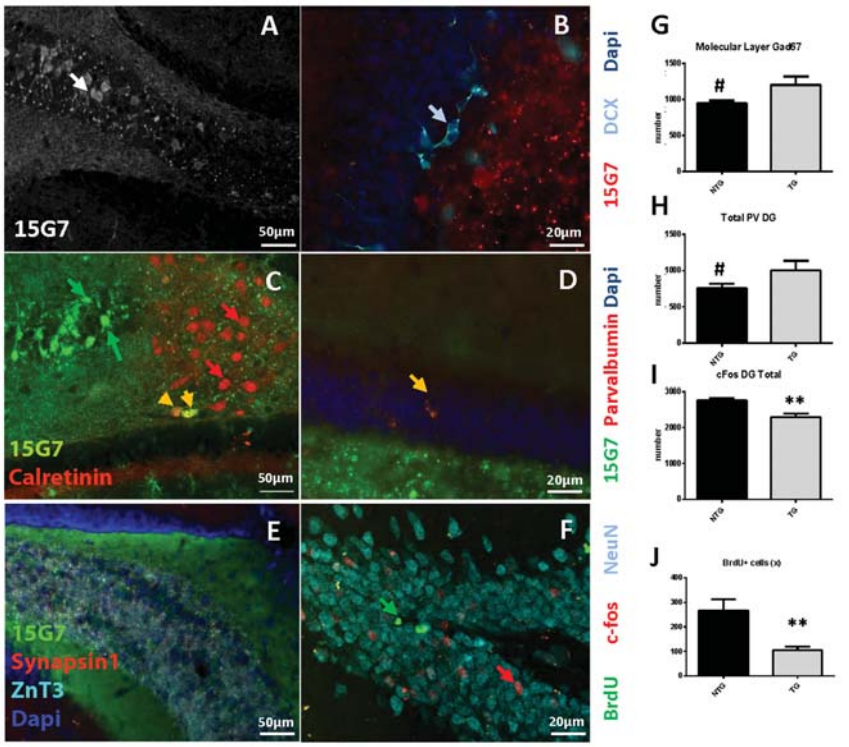
01.09.2012 - 30.08.2014

Neuropsychiatric symptoms in Parkinson's disease

Dr. Nada Ben Abdallah, Department of Molecular Neurology

Cognitive and affective changes in Parkinson's disease may arise from hippocampal dysfunctioning. In a mouse model with forebrain-overexpression of human wildtype alpha-synuclein we observed novelty-induced reactivity and altered habituation, changes reminiscent of hippocampal impairment. This was paralleled by decreased hippocampal neurogenesis, altered hippocampal cfos expression and enrichment of the transgene within the hippocampal GABAergic interneurons and the presynaptic compartment.

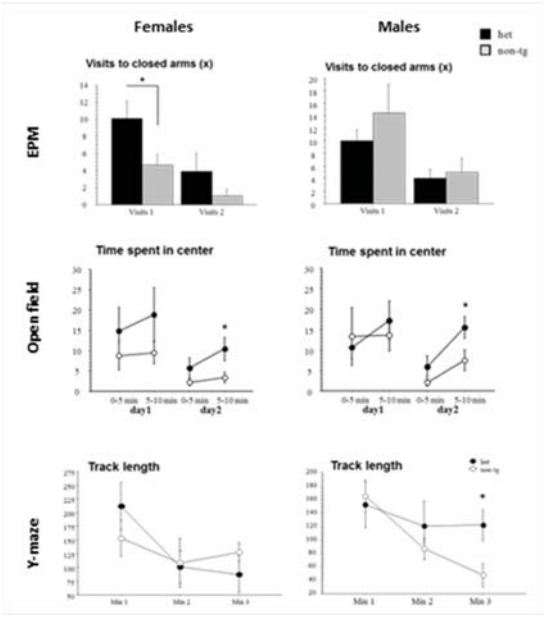
Affective and cognitive changes often occur early before the onset of motor symptoms in Parkinson's disease (PD), and may be associated with accumulation of alpha-synuclein (α-syn) within the hippocampus circuitry. In the present project we investigated a plausible link between hippocampal α-syn distribution, adult neurogenesis, and related functional changes in a transgenic PD mouse model. We used transgenic (TG) mice overexpressing human wildtype α-syn under the PDGF-ß promoter leading to a pronounced neuronal expression within the forebrain. We found significant reduction in newborn cell survival and neuronal differentiation in TG mice compared to non-transgenic littermates (NTG; Figure 1). The transgene was strongly present in all hippocampal subregions, and was detected in GABAergic interneurons (GABA standing for Gamma-aminobutyric acid), hilar mossy cells and dentate newborn neurons. This expression pattern is predictive of modified circuitry within the hippocampus. Indeed the number of interneurons in the dentate gyrus and the subiculum were altered in TG mice. We also detected strong colocalization of α-syn in hippocampal presynaptic compartment, i.e. axonal projections of dentate granule neurons onto CA3 pyramidal neurons known as mossy fibers, without altering the mossy fibers volume. Contextual enrichment entailed



α-synuclein in different neuronal populations of the hippocampus (A-D). More interneurons in TG mice (G,H). α-synuclein in zinc-enriched and synapsin-1+ mossy fibers (E). Less cFos activation and cell survival in enriched TG mice (F-J).

Dr. Ben Abdallah

activation of the immediate early gene cfos within all hippocampal subregions. Absolute numbers and density of cfos-expressing cells were however significantly lower in TG mice, suggesting a possible functional alteration. To investigate this notion, 18 females (10 TG and 8 NTG) and 14 males (8 TG and 6 NTG) of 7 months of age were tested in a battery of behavioral tests including the rotarod to evaluate motor performances, and the open field and elevated plus maze to evaluate exploration, habituation and anxiety-like behaviors, as well as the novel object test and the Y-maze to evaluate spatial short-term memory. No significant motor deficits were detected in either experimental group (data not shown). In the open field however TG mice spent more time in the center area of the arena compared to their NTG littermates (Figure 2-A1 and A2). Moreover, TG females made more visits between closed arms of the plus maze compared to NTG mice (Figure 2-B1). Both observations suggest a lack of habituation to new environments indicative of probable hyperarousal. This is confirmed in the novel object test where male TG mice spent more time in the center area containing the novel object during the first two minutes of the test, compared to NTG males (data not shown). Altered habituation in TG males was also observed in the Y-maze test, where they moved longer distances throughout the test session (Figure 2-C2). Our results suggest altered functionality of the hippocampal formation in relation to α-syn distribution. Future perspectives should aim at understanding the relevance of early non-motor symptoms as a predictive indicator for PD.



Behavioural investigation in a battery of tests including the open field (A1, A2; time in center zone), the elevated plus-maze (EPM; B1, B2; visits to closed arms), and the spatial Y-maze test (C1, C2; distance moved).

Contact:
Dr. Ben Abdallah
phone: +49 9131 85 35881
e-Mail: nada.benabdallah@uk-erlangen.de

Publications during funding period

Ben Abdallah NM, Filipkowski RK, Pruschy M, Jaholkowski P, Winkler J, Kaczmarek L, Lipp HP (2013) Impaired long-term memory retention: common denominator for acutely or genetically reduced hippocampal neurogenesis in adult mice. Behav. Brain Res. 1: 275-286

J33 - Progress Report

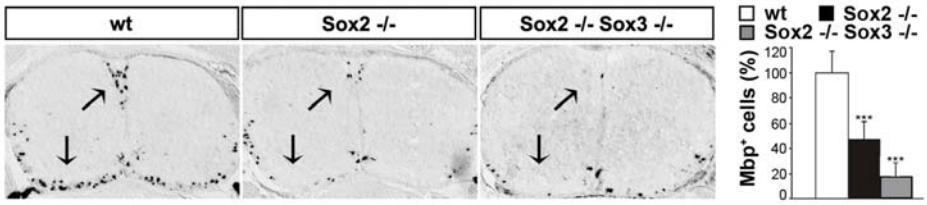
01.02.2013 - 31.01.2015

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.



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.

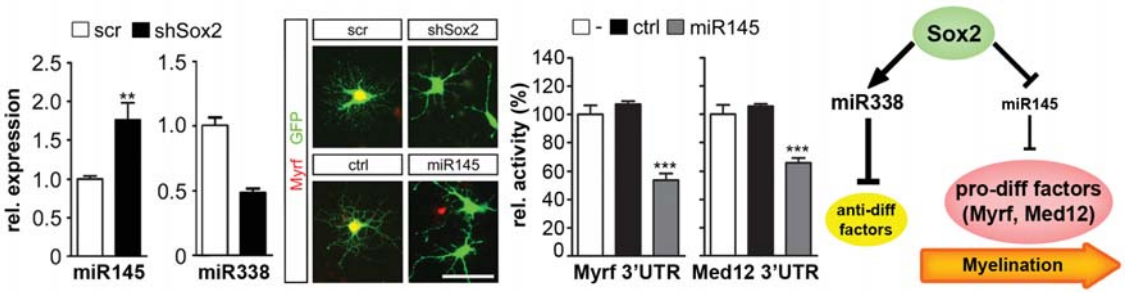
Sox2 and Sox3 influence terminal differentiation of oligodendrocytes

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.

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.

Dr. Reiprich



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.

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.

Contact:
Dr. Simone Reiprich
phone: + 9131 85 24638
e-mail: simone.reiprich@fau.de

Publications during funding period

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

J34 - Final Report

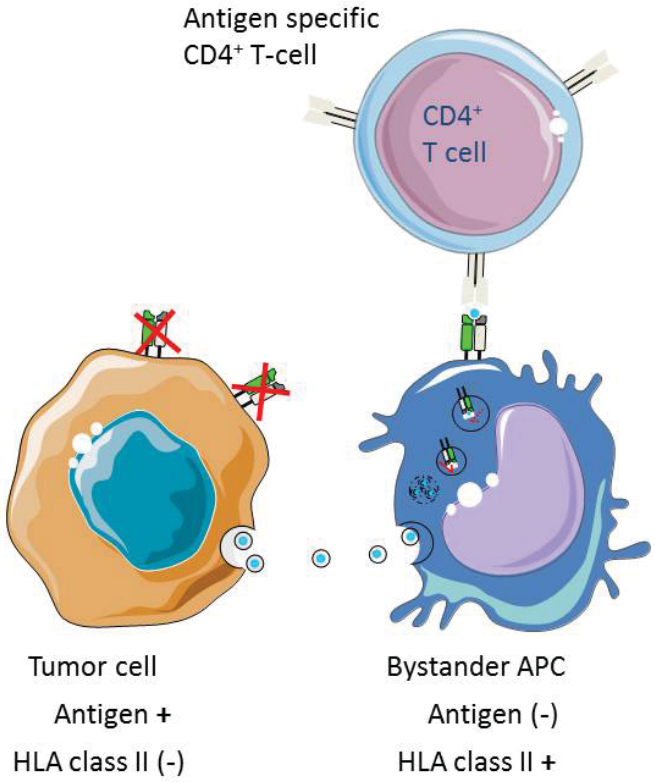
15.08.2012 - 14.08.2014

Indirect presentation of HLA class II restricted tumor antigens

PD Dr. Anita Kremer, Department of Medicine 5 – Hematology and Oncology

CD4⁺ T-cells can induce eradication of HLA class II negative tumors via recognition of indirectly presented tumor associated antigens on surrounding cells. The mechanisms involved in the secretion of tumor associated antigens are analysed in vitro and in vivo in this project. A better understanding of these processes on the long run might enable advanced targeted tumor immunotherapy with reduced risk of escape variants.

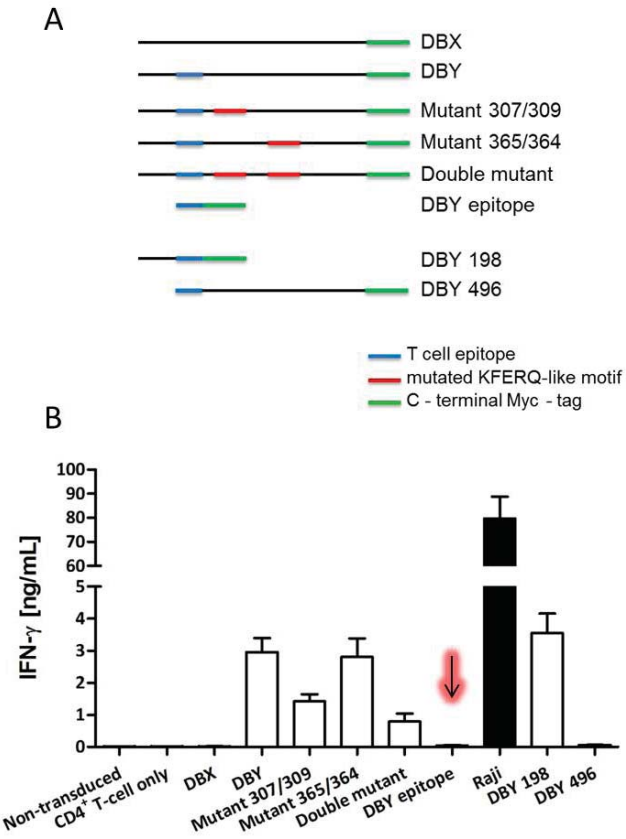
The presentation of endogenous antigens on surrounding HLA class II positive cells leading to activation of CD4⁺ T-cells is an established mechanism in solid organ transplantation and it seems progressively important in anti-tumor immunity as well. However, it is unclear whether intercellular transfer of antigens is due to an active transport or mere release of intracellular content by cell death. In our previous results we have observed that intercellular transfer is restricted to certain antigens including Y-chromosome antigen DBY. We also had indications that this selectivity is due to binding of cytosolic antigens to chaperone hsc70 and subsequent invagination into intraluminal vesicles of the late endosome. To further analyze the mechanism of intercellular antigen transfer, we generated tumor cell lines retrovirally transduced with the full length human wildtype antigen (DBY), its X-chromosome homologue (DBX), the T-cell epitope of DBY (epitope) and full length antigen with mutations in either one or both putative hsc70 bindings sites.



Schematic overview of indirect antigen presentation.

We verified expression of our transgenes by flow cytometric quantification of marker gene expression on the cell surface, western blot analyses, immunofluorescence and qPCR. To confirm the capability of our antigens to be processed, presented and recognized on the cell surface we additionally transduced them into HLA class II positive EBV-transformed B-cells. Thereby, we could show that all transgenes were highly expressed in our cell lines and that all of them were able to activate DBY-specific CD4⁺ T-cells upon direct presentation.

PD Dr. Kremer



(A) DBY constructs are schematically depicted. (B) T-cell recognition of DBY constructs after intercellular antigen transfer. Mean release of IFN-γ by the DBY specific T-cell clone is depicted. The male B-cell line Raji was used as positive control.

By co-incubation experiments we could show that wildtype DBY as well as DBY with a mutated hsc70 binding site in position 364/365 led to strong activation and IFN-γ secretion of the T-cell clone. The DBY constructs including a mutation in position 307/309 either in combination with the second mutation or alone led to a weaker activation of the T-cell clone, possibly indicating a role of this putative binding site in the intercellular transfer of the antigen. Most strikingly, we did not observe any intercellular transfer for the epitope construct. This points to an additional regulatory element outside the T-cell epitope influencing the intercellular transfer of DBY. Therefore we cloned additional constructs spanning either the N-terminus up to the T-cell epitope (198) or from the T-cell epitope to the C-terminus of the protein (496). While construct 496 was unstable, variant 198 showed high expression and was in contrast to the epitope construct indirectly presented. These data indicate a regulatory structure in the N-terminus of the protein allowing intercellular transfer of the antigen. Subsequent experiments will involve identification of this structure by screening a library of randomly mutated DBY constructs. To test in vivo tumor rejection as a function of antigen composition we generated the murine homologues of our DBY constructs and are currently establishing a mouse model.

Contact:
PD Dr. Kremer
phone: +49 9131 85 43183
e-mail: anita.kremer@uk-erlangen.de

Invited lectures

Medical Immunology Campus Erlangen, 4/2013: HLA class II restricted minor histocompatibility antigens in Graft-versus-Leukemia and Graft-versus-Host disease

Awards

Ernst-Jung Karriere-Förderpreis (Anita Kremer, 2013)

Jon van Rood Award of the EBMT Immunobiology Working Party (Anita Kremer, 2013)

Publications during funding period

none

J35 - Final Report

01.12.2012 - 30.11.2014

lncRNA-directed epigenetic programming of *HOX* loci in GIST

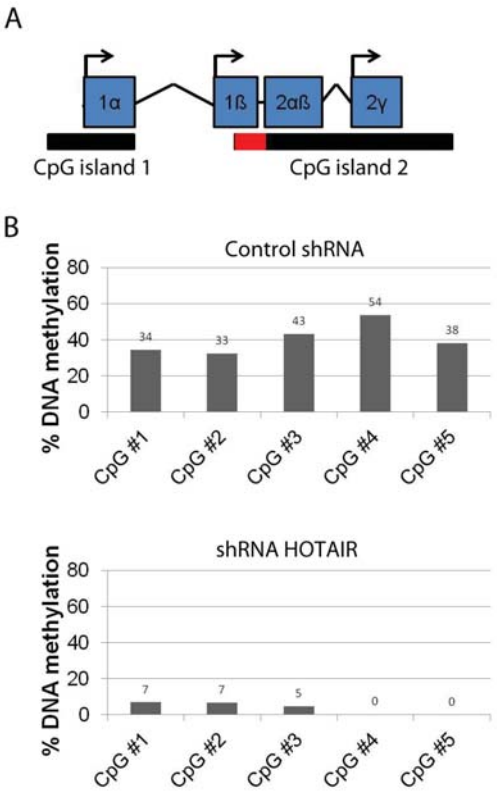
Dr. Evgeny Moskalev, Institute of Pathology

LncRNAs are functionally distinct transcripts of high regulatory potential. To ascertain their contribution to oncogenesis, we performed expression profiling in GIST and identified transcripts specific for distinct clinico-pathological groups. The lncRNA HOTAIR was upregulated in high risk GIST. Global alterations of DNA methylation patterns were induced upon HOTAIR knockdown, including a tumour suppressor RASSF1. The results suggest that HOTAIR determines specificity of DNA methylation in GIST.

An abundant class of lncRNAs is increasingly recognised as key regulators of diverse cellular processes. Few characterised lncRNA species orchestrate epigenetic programmes, nuclear organisation and other core processes. Clear indications exist for the role of certain lincRNAs in oncogenesis. This field remains, however, incompletely explored to date. Here, we aim at understanding the functional role of lncRNAs with a particular focus on epigenetic deregulation by using gastrointestinal stromal tumours (GISTs) as a model.

Novel lncRNA species of nuclear localization are specific for GISTs of different risk groups

Differentially expressed transcripts were revealed in GISTs of low vs. high risk groups by using GeneChip Human Gene 2.0 ST Arrays of Affymetrix. The expression of most prominent transcripts was further validated by qPCR suggesting biomarker potential for the disease. Analysis of subcellular localization was performed by fractionation of nucleus and cytoplasm followed by quantification of expression by qPCR. The nuclear localization of the transcripts of interest further suggests the functional contribution as transcriptional regulators. Functional analysis is in progress, which will provide mechanistic insights into their function.

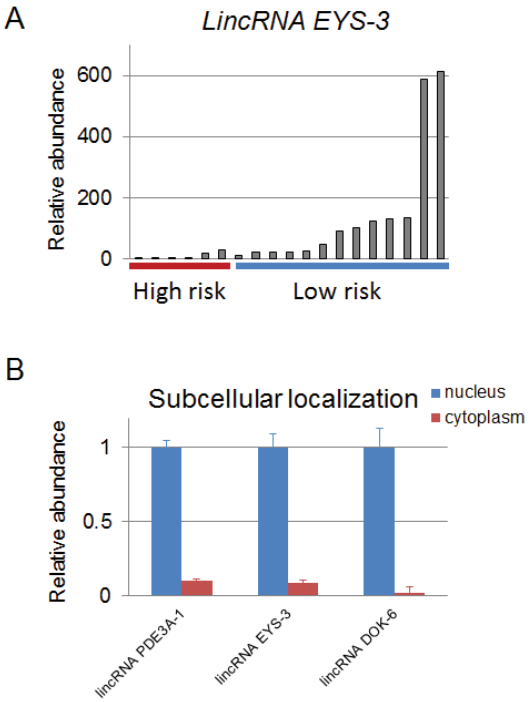


DNA methylation analysis of the *RASSF1* gene (A) by pyrosequencing. (B) DNA methylation percentages (vertical axis) of five CpG sites (horizontal axis) in control GIST T1 cells (upper graph) and upon *HOTAIR* knockdown (lower graph).

Long non-coding RNA HOTAIR affects DNA methylation patterns in GIST

To study if cancer specific deregulation of DNA methylation both in *HOX* genes and beyond can be determined by abnormal expression of lncRNA species within *HOX* clusters, we addressed the epigenetic related *HOX antisense intergenic RNA (HOTAIR).* This transcript was significantly upregulated in patient samples (n=73) of high risk compared to low and intermediate risk groups. Besides, very high levels of endogenous expression were detected in GIST cell lines GIST T1 and GIST48b. Stable knockdown

Dr. Moskalev



(A) A representative example of lincRNA *EYS-3* that is differentially expressed (vertical axis) in GISTs of high risk (red bar) vs. low risk (blue bar, horizontal axis) and – along with other transcripts of interest – is localized in the nucleus (B).

of *HOTAIR* in both cell lines was achieved by RNAi using lentiviral transduction, and genome-wide DNA methylation profiling was performed by using the Infinium HumanMethylation450 BeadChip platform. A total of 218 CpG sites got hypomethylated upon the *HOTAIR* knockdown in GIST T1 and GIST48b cells (β>0.3, FDR<0.05) including potential tumour suppressors, transcription factors, tumour-specific antigens, genes related to angiogenesis or involved in metabolism. As confirmed by bisulfite pyrosequencing, CpG methylation of 64% degree at *RASSF1* in GIST T1 cells could be entirely erased upon *HOTAIR* knockdown.

Taken together, the results suggest that *HOTAIR* is one of the factors that enable target specificity of DNA methylation in GIST. While the molecular mechanism remains to be elucidated, it is plausible to assume a recruitment of DNA methyltransferases by the Polycomb repressive complex 2, which target specificity is determined by *HOTAIR*. The results suggest the feasibility of manipulating DNA methylation patterns in a targeted manner and are of potential interest in context of epigenetic cancer therapy.

Contact:
Dr. Moskalev
phone: +49 9131 85 43631
e-mail: evgeny.moskalev@uk-erlangen.de

Invited lectures

Keystone Symposia Conference Long Noncoding RNAs: Marching toward Mechanism, 27.02-4.03.2014, Santa Fe, Genome-wide expression profiling reveals lincRNA and snoRNA species associated with aggressive behaviour of gastrointestinal stromal tumours (GISTs)

Cold Spring Harbor Laboratory Meeting Regulatory and Non-Coding RNAs, 26-30.08.2014, Cold Spring Harbor, Expression of lincRNA and snoRNA species is associated with aggressive behaviour of gastrointestinal stromal tumours (GISTs)

Publications during funding period

none

J36 - Progress Report

01.09.2013 - 31.08.2015

Identification of molecular signalling pathways in cholestatic pruritus

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.

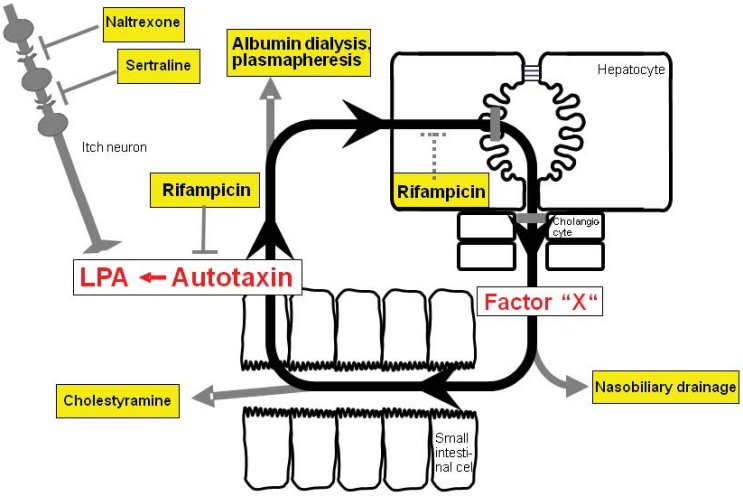
In sera of patients suffering from cholestatic pruritus we could recently identify lysophosphatidic acid (LPA) as potential neuronal mediator. Intradermally injected LPA induced scratching activity in mice. Autotaxin, the enzyme forming LPA, was strongly increased in patients suffering from pruritus 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.

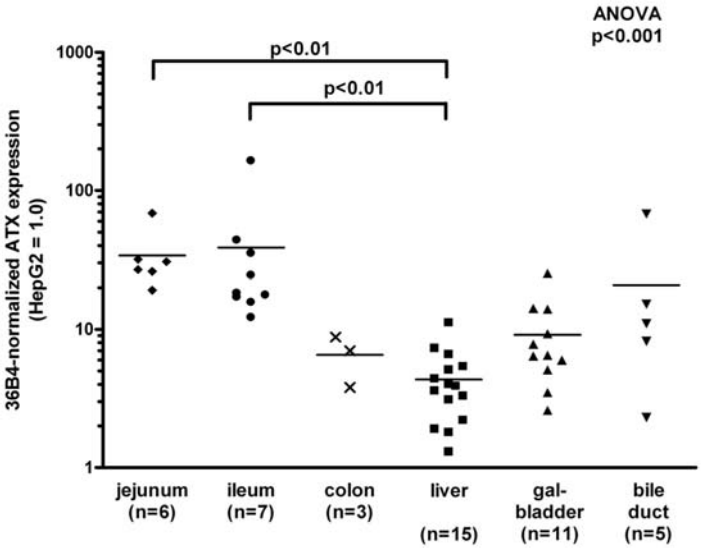
Enteroendcrine cells as source for circulating autotaxin

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 po-



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.

Dr. Kremer



High autotaxin expression in human small intestine.

sitive 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. We are currently investigating the combined effects and other factors of epigenetic modulation.

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.

Contact:
Dr. Kremer
phone: +49 9131 85 45015
e-mail: andreas.kremer@uk-erlangen.de

Invited lectures

23rd Congress of the European Academy of Dermatology and Venerology (EADV), October 8–12, 2014, Amsterdam, “Treatment algorithm for cholestatic pruritus”

4. Münsteraner Pruritussymposium, September 19–20, 2014, Münster, „Pathogenese des cholestatischen Pruritus“

Gastro-Kolloq, MHH, June 6, 2014, „Cholestatischer Pruritus: Fakten und Fiktion“

20. Falk Symposium Aktuelle Hepatologie im Rahmen der DGIM, April 25–27, 2014, Wiesbaden, „Primär biliäre Zirrhose – Standard und Zukunft“

European Association for the Study of the Liver (EASL), April 9–13, 2014, London, “Liver disease in pregnancy”

58. Jahreskongress der Saarländisch-Pfälzischen Internistengesellschaft (SPIG), March 06-08, 2014, Neustadt, “Juckreiz – auch ein internistisches Problem”

191. Falk Gastro-Conference, October 4–5, 2013, London, “Pathogenesis and treatment of pruritus in cholestasis”

Awards

UK-PBC Bursary Award, Dr. Andreas Kremer, April 08, 2014, London

Publications during funding period

none

J37 - Progress Report

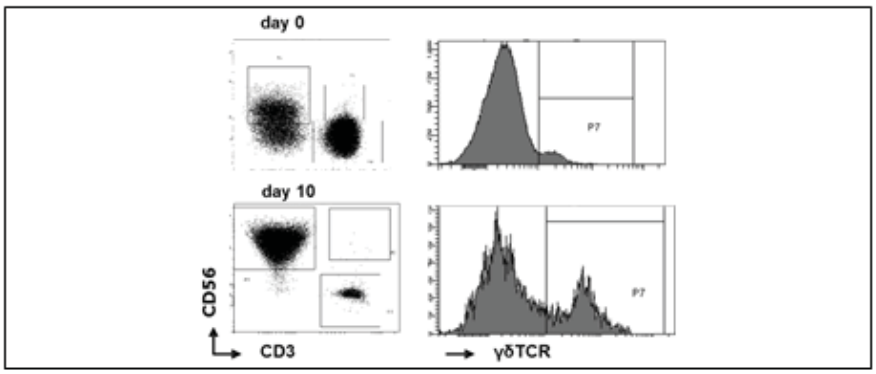
01.07.2013 - 30.06.2015

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 NK and γδT cells is an attractive strategy to boost T cell immunity. This project aims to develop a GMP-compliant protocol to expand large numbers of NK and γδT cells from melanoma patients and simultaneously tests the significance of FcγRIIIa polymorphisms on NK and γδT cell activation.

To date, only a few studies have explored the use of autologous NK and γδT cells as a form of immunotherapy for treatment of melanoma. In general, implementation of adoptive cell transfer of autologous NK and γδT cells is hampered by (i) the small number of NK and γδT cells in peripheral blood that could be isolated relative to the number of cells that would be required to be effective, (ii) the difficulties associated with large-scale production of cytolytic NK and γδT cells in compliance with GMP and (iii) the constraints imposed by autologous inhibitory receptor-ligand interactions. Importantly, successful cancer immunotherapy does not solely depend on the effective transfer of cytotoxic NK and γδT cells. It requires the design of therapeutic combinations which augment NK and γδT cell-mediated antitumor responses and concurrently overcome tumor-specific escape mechanisms. One means to augment the therapeutic benefit of adoptively transferred NK and γδT cells is to define the population most likely to respond, through improved characterization of mode of action. Growing experience with antibody therapy demonstrates that select patients experience superior clinical outcomes based upon FcγRIIIa polymorphisms. The most relevant polymorphisms depend on the presence of phenylalanine (F) or valine (V) at amino acid position 158 within the Fc-receptor and NK cells derived from individuals harboring an FcγRIIIa polymorphism with higher affinity for IgG1 (eg. V/V phenotype) show superior natural cytotoxicity and ADCC. To date, no studies have determined the significance of FcγRIIIa polymorphisms on NK and γδT cell expansion and natural cytotoxicity in melanoma patients.



NK cells and γδT cells are efficiently expanded from PBMC derived from Stage IV melanoma patients. In the presence of K562-IL15-4-1BBL expanded cells become significantly enriched in NK cells (defined by CD3-CD56+ by flow cytometry) and γδT cells (defined by γδTCR+ by flow cytometry).

Dr. Bosch-Voskens

Fcγ-Receptor Phenotype	Patient (n=87)	Healthy Donor (n=14)
FF	39	10
FV	22	2
VV	26	2

Current PCR-typing results of FcγRIIIa polymorphisms in melanoma patients and healthy donors

In order to evaluate the significance of FcγRIIIa polymorphisms in melanoma, we first determined the frequencies of a F-allele and V-allele at amino acid position 158 of the FcγRIIIa in a large set of cell fractions from melanoma patients derived from the freezer inventory of the department of dermatology. PCR analysis showed a distribution of polymorphic classes of 44.8% for F/F, 25,3% for F/V and 29.9% for V/V.

To achieve large-scale expansion of NK and γδT cells, peripheral blood mononuclear cells (PBMC) derived from melanoma patients were co-cultured in a 1 to 1.5 ratio with lethally irradiated K562 cells expressing membrane-bound IL-15 and 4-1BBLigand in culture media containing IL-2 and zometa. After 14 days of culture, PBMC became enriched in NK cells (defined as CD3-CD56+ by flow cytometry) and γδT cells (defined as γδTCR+ by flow cytometry).

Additional studies are ongoing to define the impact of FcγRIIIa genotype on NK and γδT cell expansion efficiency, activation-receptor repertoire and cytolytic activity against autologous melanoma cells.

Contact:
Dr. Bosch-Voskens
phone: +49 9131 85 45816
e-mail: caroline.bosch-voskens@uk-erlangen.de

Publications during funding period

none

J38 - Progress Report

01.02.2014 - 31.01.2016

MCS-18 for the treatment of atherosclerosis

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

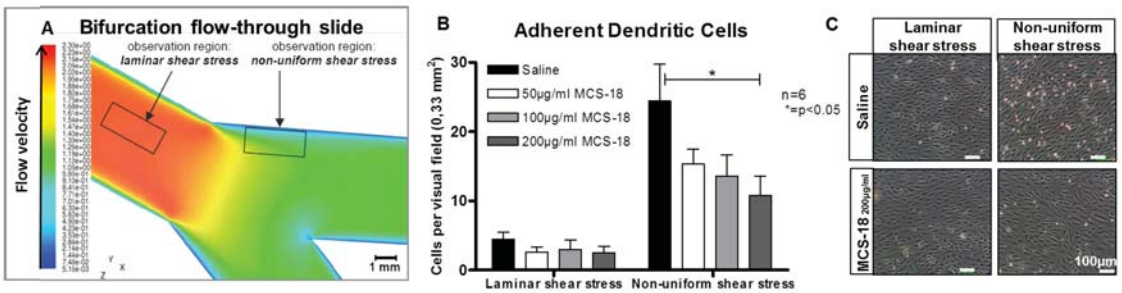
Progression of atherosclerosis is associated with pronounced inflammatory processes, such as the recruitment of leukocytes and their adhesion to the endothelium. The aim of this project is, to investigate the impact of the herbal substance MCS-18, an antiinflammatory root compound of helleborus purpurascens 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.

Impact of MCS-18 on proatherogenic processes in vitro

Atherosclerosis is a vascular disease, characterized by an excessive inflammatory response. Dendritic cells (DC), which link both adaptive and innate immunity, are recruited to atherosclerotic lesions and play a crucial role in plaque progression. To investigate the effect of the antiinflammatory substance MCS-18 on the chemotactic behaviour of DCs, the cells were treated with different concentrations of MCS-18 (50-200µg/ml) or saline for three days during incubation with a maturation cocktail, whereupon DC migration towards MIP-3β was determined. Compared to saline treatment, a significant decrease in DC migration was detected following pretreatment with the highest concentration of MCS-18 (200µg/ml).

Apart from that, we analyzed the adhesion of MCS-18 treated DCs to a monolayer of human umbilical vein endothelial cells (HUVEC) under different patterns of shear stress using dynamic flow experiments.

While no differences were observed in regions of laminar shear stress, a significant reduction of DC adhesion was detected following treatment with 200µg/ml MCS-18 compared to saline treatment in regions of non-uniform shear stress. This was associated with a decreased expression of the C-type lectin CD209 in DCs and of ICAM-1 and NFkB-p65 in MCS-18 pretreated HUVECs.



Dynamic flow experiments to analyze DC-adhesion to an endothelial cell monolayer under flow conditions. (A) Illustration of different patterns of shear stress in a bifurcation slide. Quantitative analysis (B) and exemplary images (C) of adherent DCs.

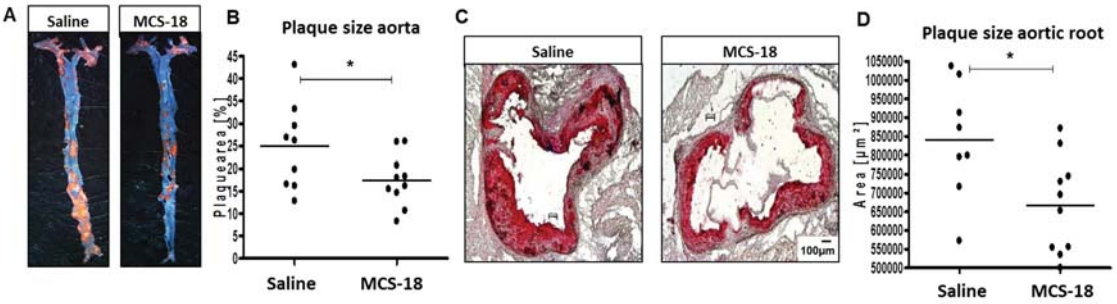
Dr. Dietel

Impact of MCS-18 on advanced atherosclerosis in ApoE-deficient mice

To investigate the impact of MCS-18 on advanced atherosclerotic lesions *in vivo,* ApoE-knockout mice were fed a high-fat Western-type diet (1.25% cholesterol, 21% fat) for three months. Thereupon, mice received normal chow for the following three months and were divided into a saline treated and a MCS-18 treated group (500µg i.p. twice a week). After six months, mice were sacrificed. En-face preparation of the isolated aortas showed a reduced plaque size in the aortic arch and the thoracoabdominal aorta of MCS-18 treated mice.

While *ex vivo* DIXON-MRI measurements, which were performed of the isolated hearts, only showed a slight decrease in the lipid content, the plaque size detected by Oil-red O staining of cross-sections from the aortic root was significantly reduced following MCS-18 treatment.

TUNEL-staining of the aortic root revealed a decreased density of apoptotic cells in plaques of MCS-18 compared to saline treated mice. While serum concentration of cholesterol and triglycerides showed no differences in both groups, concentration of the proatherogenic cytokines IL-6, IL-18 and IL-22 was decreased in serum of MCS-18 treated mice.



Impact of MCS-18 on plaque size. (A) Exemplary images of plaques (red) in enface prepared aortas. (B) Quantitative analysis: plaques size aorta.(C) Exemplary images of Oil-red O stained aortic root. D) Quantitative analysis: plaque size aortic root. * p<0.05.

In conclusion, our results show that MCS-18 has a pronounced impact on migration and adhesion of human DCs to the endothelium. As potential mechanisms, downregulation of CD209 in DCs and of ICAM-1 in HUVECs should be considered. While less pronounced than in atherosclerosis onset, our experiments do show that the therapeutic application of MCS-18 has a beneficial impact on plaque progression in a mouse model of advanced atherosclerosis.

Contact:
Dr. Dietel
phone: +49 9131 85 45375
e-mail: barbara.dietel@uk-erlangen.de

Invited lectures

80th Annual Meeting of the German Cardiac Society, April 2014, Mannheim, „Modulation proatherogener Leukozyteninteraktionen durch die antiinflammatorische Substanz MCS-18 – Auswirkungen im vorangeschrittenen Atherosklerosemodell der ApoE$^{-/-}$ Maus“

82nd European Atherosclerosis Society Congress, May 2014, Madrid, “Dixon Imaging allows quantification of murine plaque lipids by ex vivo MRI”

Annual Meeting of the European Macrophage & Dendritic Cell Society, October 2014, Vienna, “Anti-inflammatory Effects of MCS-18 on Dendritic Cells and Endothelial cells - Impact on Advanced Atherosclerosis in ApoE-deficient Mice”

Publications during funding period

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 of dendritic cells in apoe-deficient mice and prevents early atherosclerosis progression. Atherosclerosis. 235:263-272

J39 - Progress Report

01.01.2014 - 31.12.2015

Hypermethylation of SOCS3 in fibrotic diseases

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

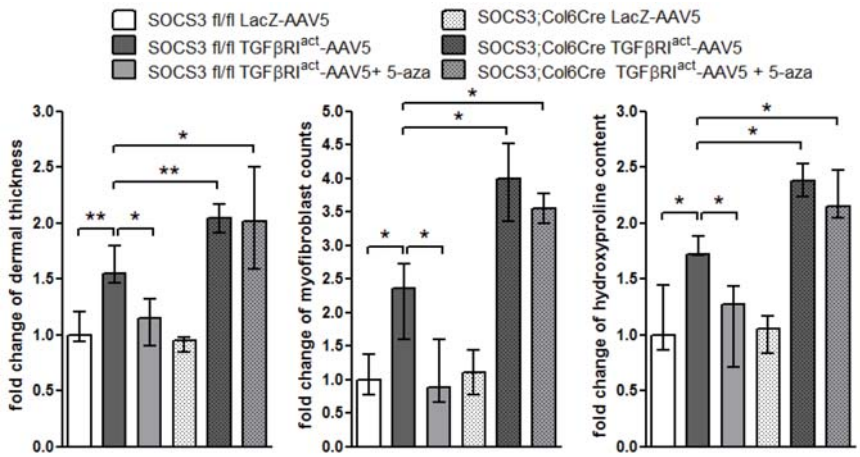
This project evaluates the role of promoter hypermethylation of Suppressor of Cytokine Signaling (SOCS) 3 in the pathogenesis of fibrotic diseases. Using both pharmacological and genetic approaches like conditional knockout mice, the project examines the mechanisms of TGFβ-induced DNA methylation in fibrosis, particularly in systemic sclerosis (SSc). Given that inhibitors of DNA methyltransferases are in clinical use for other indications, our study may have direct translational implications.

Epigenetic gene regulation is defined as heritable changes in gene expression which are not encoded in the nucleic sequence, but are mainly mediated by secondary modifications of DNA and chromatin and by non-coding RNAs. The current project aims to (1) analyze the role of gene repression by increased DNA methylation of the SOCS3 promoter and (2) evaluate the underlying mechanisms of TGFβ-induced DNA methylation in the context of fibrosis.

Our preliminary findings showed potent inhibitory effects of the DNA methyltransferase (Dnmt) inhibitor 5aza on TGFβ-induced collagen expression in vitro and on bleomycin-induced dermal fibrosis in vivo. Furthermore, we found SOCS3 downregulated by promoter hypermethylation in skin and fibroblasts of SSc patients as well as upon TGFβ stimulation. Based on these findings, we have generated the following results:

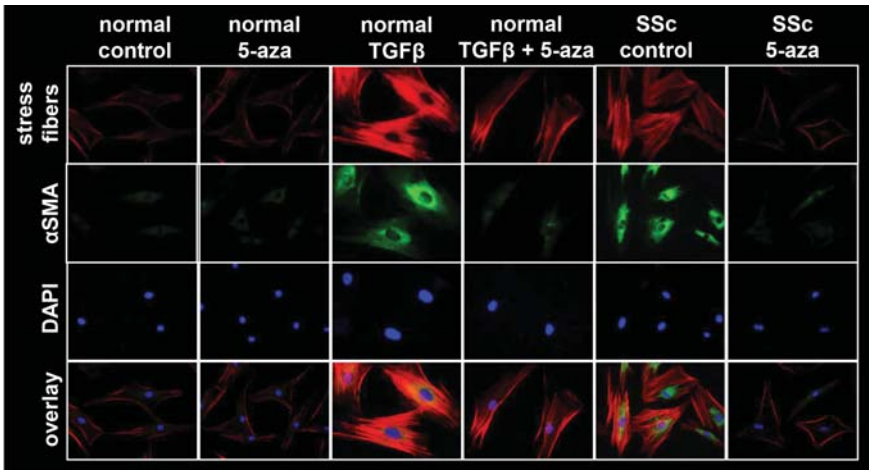
In order to evaluate the anti-fibrotic potential of 5-aza on TGFβ-induced fibrosis in vivo, we induced fibrosis in mice by intracutaneous injections of type 5 AAVs expressing constitutively active TGFβ receptor type I (TGFβRI^{act}-AAV5). Treatment with 5aza efficiently reduced TGFβRI^{act}-induced fibrosis with decreased dermal thickness and hydroxyproline content. In addition, the counts of metabolically active α-smooth muscle actin (αSMA) positive myofibroblasts were significantly reduced.

The inhibitory effect of 5aza on myofibroblast differentiation was also observed in vitro, as incubation of fibroblasts with 5aza reduced TGFβ-induced αSMA expression and stress fiber formation in normal fibroblasts and also decreased basal αSMA levels and stress fibers in SSc fibroblasts.



Fibroblast-specific knockdown of Socs3 aggravates dermal fibrosis induced by injection of type 5 AAV expressing constitutively active TGFβ receptor type I (TGFβRI^{act}-AAV5). Control mice received injections with LacZ expressing AAV5 (LacZ-AAV5).

Dr. Dees



Inhibition of Dnmts prevents myofibroblast differentiation. Incubation of normal fibroblasts with 5aza reduced TGFβ-induced SMA expression and stress fiber formation. 5aza also decreased the basal levels of αSMA and stress fibers in SSc fibroblasts.

In line with the TGFβ-induced hypermethylation of SOCS3, chronically increased TGFβ levels, which are also found in fibrotic tissue, potently reduced SOCS3 expression on the mRNA as well as on the protein levels in normal fibroblasts in vitro. In contrast, overexpression of SOCS3 efficiently blocked the stimulatory effects of TGFβ on collagen synthesis. These inhibitory effects of SOCS3 may be mediated by blocking JAK2 signaling as additional knockdown of JAK2 prevented the increase of collagen synthesis induced by knockdown of SOCS3.

Further on, we induced fibrosis in mice with fibroblast-specific knockout of SOCS3 by bleomycin as well as by TGFβRI^{act}-AAV5. In both models, mice deficient for SOCS3 in fibroblasts showed exaggerated fibrosis as compared to mice with normal SOCS3 levels.

In our preliminary results we have also shown that TGFβ upregulates the expression of Dnmt3a. To evaluate whether targeting Dnmt3a exhibits antifibrotic effects, we induced fibrosis by injections of bleomycin in mice lacking Dnmt3a selectively in fibroblasts. Indeed, mice with fibroblast-specific deficiency of Dnmt3a were protected from bleomycin-induced fibrosis with decreased dermal thickening and reduced hydroxyproline contents and myofibroblast counts as compared to mice with normal expression of Dnmt3a.

Contact:
Dr. Dees
phone: +49 9131 85 36331
e-mail: clara.dees@uk-erlangen.de

Publications during funding period

none

J40 - Progress Report

01.01.2014 - 31.12.2015

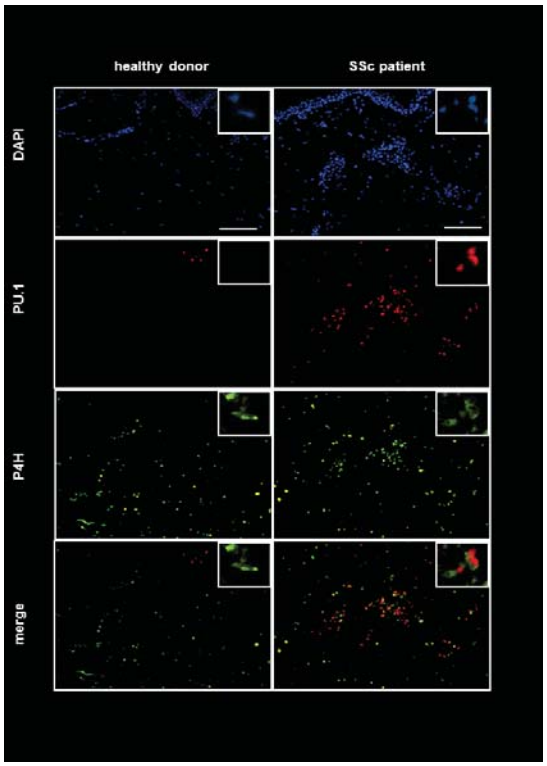
PU.1 signalling in fibrotic diseases

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.

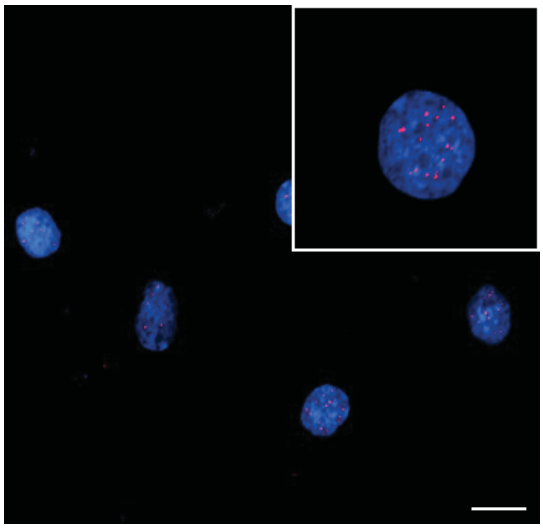
The transcription factor PU box binding-1 (PU.1) belongs to the E26 transformation-specific (ETS) family of proteins and plays an important role in the functional competence of several haemopoietic lineages such as B cells and macrophages. In PU.1 deficient mice, wound healing appears without granulation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



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



Human SSc fibroblast cells stained for DAPI and PU.1. 600 x magnifications.

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 even not after stimulation with TGF-β 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 established fibrosis.

We aim to further characterize the molecular mechanisms of PU.1 signaling in fibrosis and to further validate the inhibition of PU.1 and PU.1 target genes as therapeutic approach in fibrotic diseases.

Contact:
Dr. Ramming
phone: +49 9131 85 43048
e-mail: andreas.ramming@uk-erlangen.de

Publications during funding period

none

J41 - Progress Report

01.12.2013 - 30.11.2015

Resolution of inflammation in gout

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

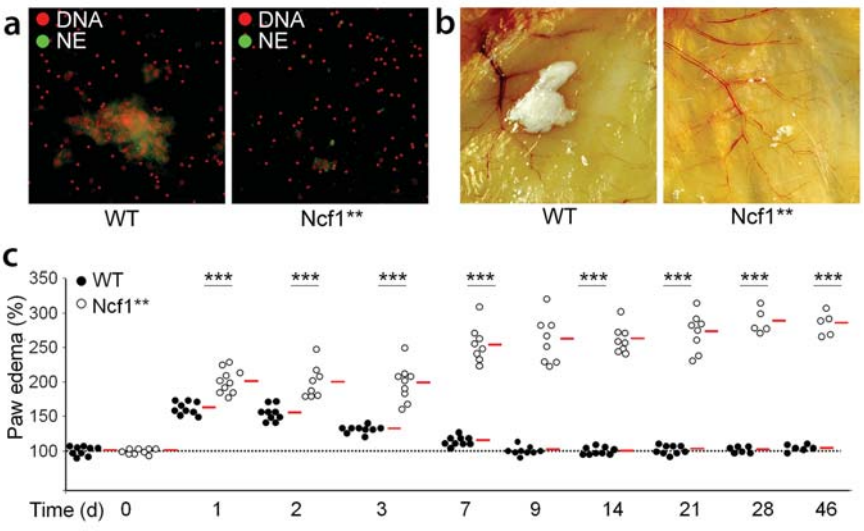
Acute gouty arthritis is a self-limiting process despite persistent monosodium urate (MSU) crystals. For this big enigma of gouty arthritis we propose the following model: In the early phase, MSU crystals induce the formation of solitaire neutrophil extracellular traps (NETs) and huge amounts of pro-inflammatory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

MSU crystals trigger NETosis and aggregation of NETs in cultured human neutrophils.

In low-density cultures (5 × 10^6 neutrophils ml^{-1}), typically for the early phase of gouty arthritis, we observed NETosis but no aggregation of NETs after incubating with MSU crystals in vitro. To mimic the situation during acute inflammation in vivo, we increased the density of neutrophils in our in vitro NETosis assays to values typically encountered in densely infiltrated tissue (10^8 neutrophils ml^{-1}). Under these conditions, MSU crystals induced aggregation of NETs (aggNETs). In cryosections of these aggregates, we found extracellular DNA colocalized with granule proteins that resembled gouty tophi.

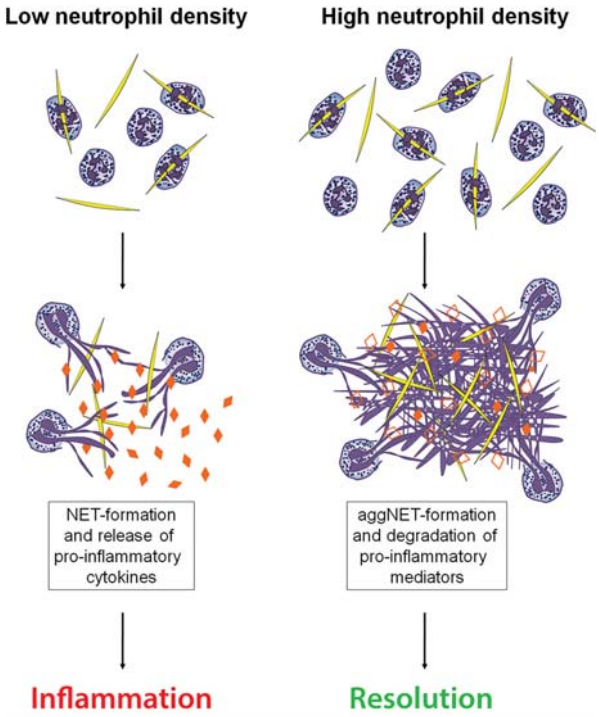
MSU-induced NETosis and aggNET formation depend on ROS.

Reactive oxygen species (ROS) inhibitors (N-acetylcysteine, butylated hydroxyanisole, diphenylene iodonium) strongly decreased the formation of NETs and aggNETs in in vitro analyses. Furthermore, NETosis was also reduced after incubation with MSU in blood ex vivo from human and murine individuals with mutations in the Ncf1 subunit encoding the NADPH oxidase. To analyze NET formation in vivo, we injected MSU crystals into air pouches of wild-type (WT) and Ncf1** mice. The NET aggregation was strongly reduced in air pouches of Ncf1** mice.



Impaired aggNET formation results in exacerbated and chronic inflammation.
NET aggregation is decreased in peripheral blood neutrophils (a) and air pouches (b) of Ncf1** compared to WT mice. Reduced aggNET formation leads to a chronic course of inflammation (c).

Dr. Schauer



Induction and resolution of inflammation in gout. In low neutrophil densities, neutrophils induce single NETs concomitant with release of inflammatory mediators. In high neutrophil densities cells form aggNETs that trap and degrade inflammatory mediators.

AggNETs degrade neutrophil-derived inflammatory mediators via proteases.

Next we analyzed the inflammatory mediators released during MSU-induced NETosis and aggregation of NETs. Whereas cytokines and chemokines were detected at high concentrations in supernatants from low-density cultures (5×10^6 neutrophils ml^{-1}), their concentrations were substantially reduced in supernatants from high-density neutrophil cultures (10^8 cells ml^{-1}). Then we incubated aggNETs with exogenous cytokines and chemokines and monitored their concentrations in the supernatants. We observed a time-dependent decrease in the concentration of proinflammatory cytokines and chemokines in the media containing aggNETs. Blockade of proteinase 3 and elastase inhibited the inflammatory mediator degradation by aggNETs.

Inability to form NETs results in chronic MSU-induced neutrophilic inflammation.

After MSU injection, we detected higher amounts of proinflammatory cytokines and chemokines in lavages of air pouches of Ncf1** as compared to WT mice. To assess the pathogenic implications of these findings in vivo, we induced gout by subcutaneous injection of MSU crystals into the foot pads of WT and Ncf1** mice. In WT mice, injection of MSU crystals led to rapid but self-limited paw erythema and swelling. In contrast, Ncf1** mice exhibited a chronic course, with paw swelling that was apparent for several weeks and higher concentrations of inflammatory mediators in the chronically inflamed paws. These results indicate that NETosis and the aggregation of NETs (tophus formation) promote resolution of MSU-induced inflammation in gouty arthritis.

Contact:
Dr. Schauer
phone: +49 9131 85 24787
e-mail: christine.schauer@uk-erlangen.de

Awards

Avrion Mitchison Preis, Preisträgerin: Dr. Christine Schauer, Verleihung: 02.12.2014, Berlin

Publications during funding period

Schauer C., Janko C., Munoz L.E., 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. May;20(5):511-7

J42 - Progress Report

01.04.2014 - 31.03.2016

Bayesian reverse engineering of developmental networks

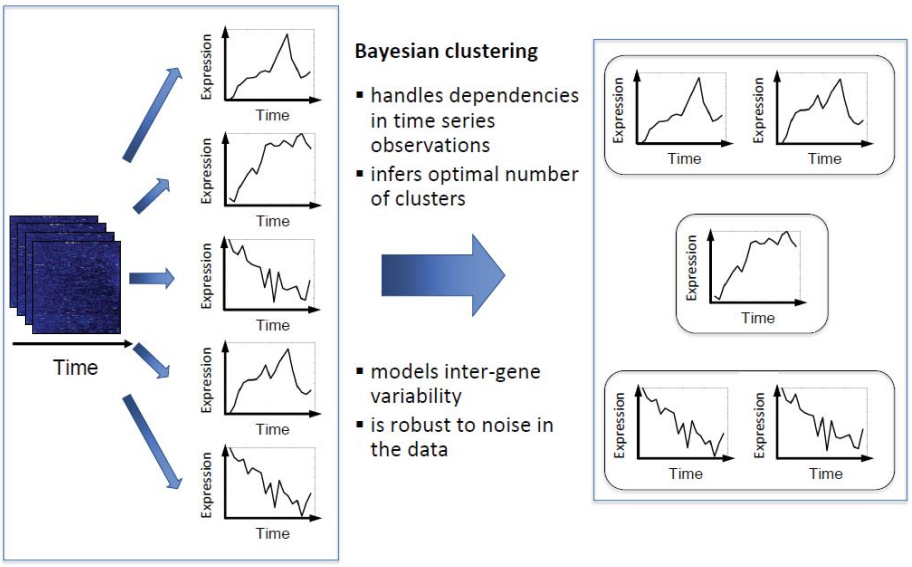
Dr. Fulvia Ferrazzi, Institute of Human Genetics

The project aims at developing a Bayesian approach to reverse engineer gene regulatory networks from expression time series and prior knowledge. In silico analyses of the inferred networks will allow the prioritization of experimentally testable hypotheses. The approach will be applied to a high resolution temporal expression dataset describing rat heart development. These data have the potential to shed light on congenital heart disease, cardiac stem cell differentiation, and regeneration.

Systems biology aims at analyzing biological systems through the study of the complex interplay of cellular components, including genes, proteins, and metabolites. In particular, gene networks inferred from expression data can support the identification of novel hypotheses on regulatory processes. We will concentrate on Bayesian methodologies, which are particularly interesting because they can accommodate the intrinsic variability of cellular systems and the presence of noise in the data as well as offer a flexible and powerful framework to integrate prior knowledge in network learning. The application of the approach to a high resolution temporal expression data describing rat heart development, generated in collaboration with the group of Prof. Engel, will support the elucidation of the regulatory mechanisms underlying heart development.

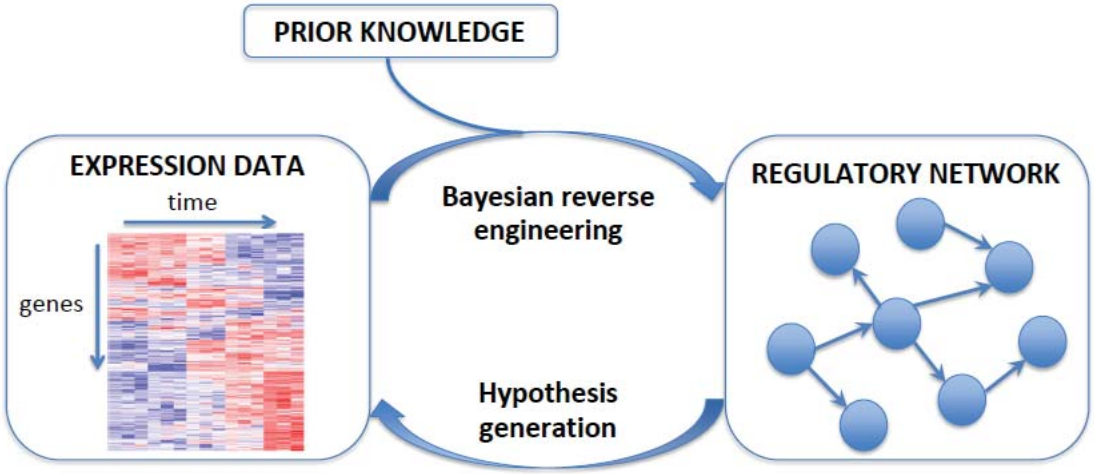
Temporal expression analysis of rat heart development

We have isolated RNA from rat hearts at different developmental stages, from embryonic day 11 to postnatal day 10 at intervals of 12 hours, and measured expression with Affymetrix arrays. Data analysis allowed the identification of over 3,000 differentially expressed probe sets. We clustered the time series using a Bayesian clustering method for temporal expression data, which we had previously developed.



Key features of the Bayesian method used to cluster the heart development time series.

Dr. Ferrazzi



The project's goal is to reverse engineer gene regulatory networks from temporal expression data and prior knowledge and use the inferred networks to generate novel biological hypotheses to be experimentally tested.

Clustering revealed groups characterized by markedly different patterns of expression, such as down-/up-regulated, transiently or bi-phasically expressed. Functional enrichment analysis of the clusters together with the current knowledge of functional and morphological changes during development allowed us to predict the function of uncharacterized genes. Network-level analysis of the data will provide insights into gene regulatory networks underlying heart development.

Network-level analysis of expression time series

As the complexity of both network learning and in silico analyses increases with larger amount of genes, we initially focused on small datasets. On the one hand we explored different methodologies for network learning (Bayesian approaches but also methods based on differential equations) and compared their performance on a widely used benchmark dataset (Cantone et al., Cell 2009). On the other hand we integrated different sources of prior knowledge to build a 'prior network' relative to a subset of the heart development genes and we explored different graph-based centrality measures as a tool to rank candidate genes. We are currently working on the integration of expression data and prior knowledge in reverse engineering and on the extension of the analysis to larger groups of genes.

Contact:
Dr. Ferrazzi
phone: +49 9131 85 26101
e-mail: fulvia.ferrazzi@uk-erlangen.de

Invited lectures

Medical Immunology Campus Erlangen Seminar Series, 8th April 2014, Erlangen, „Systems biology approaches to elucidate gene regulatory networks"

EMBO Conference Series "From Functional Genomics to Systems Biology", 8th-11th November 2014, EMBL Heidelberg, "Nucleosome Organization in Drosophila"

Publications during funding period

Ferrazzi F, Bellazzi R, Engel FB (2014) Gene network analysis: from heart development to cardiac therapy. Thromb Haemost. 113(1) [Epub ahead of print]

Newly started Projects

J43 01.02.2015 - 31.07.2017

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


Dr. Mchedlidze

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

Infections with proteobacteria of the genus *Salmonella* are a significant health problem. We observed that intestinal levels of the cytokine IL-33 are increased in infected intestines and that IL-33$^{-/-}$ mice are highly susceptible to *Salmonella*-dependent infectious enteritis. Therefore, we aim in this proposal to comprehensively analyze the molecular and cellular mechanisms through which the IL-33 pathway contributes to physiological immune reactions in the context of gastrointestinal infections.

e-mail: tamar.mchedlidze@uk-erlangen.de

J44 01.04.2014 - 30.09.2017

Rhadinovirus Entry Receptors


Dr. Hahn

Dr. Alexander Hahn, Institute of Clinical and Molecular Virology

Kaposi's sarcoma-associated herpesvirus (KSHV) and the closely related rhesus monkey rhadinovirus (RRV) engage Eph family receptors through the gH/gL glycoprotein complex for entry into target cells. We will investigate how Ephs contribute to viral entry. In addition, our data shows that RRV can use alternative receptors to enter certain cell types and binds additional membrane proteins through gH. Using RRV as a model, we will investigate the existence of alternative receptors for both viruses.

e-mail: arhahn@viro.med.uni-erlangen.de

J45 01.01.2015 - 30.06.2017

Modulation of PRC2 activity by HCMV IE2


Dr. Reuter

Dr. Nina Reuter, Institute for 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 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 the HCMV regulatory protein IE2p86 in modulating the function of PRC2 during lytic as well as latent HCMV infection by interacting with the core component EED.

e-mail: nina.reuter@viro.med.uni-erlangen.de

J46 01.04.2015 - 30.09.2017

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


Dr. Küspert

Dr. Melanie Küspert, Institute of Biochemistry

Transcription factor Sox10 plays essential roles in myelinating glia and its loss causes severe demyelinating conditions. Downstream targets and interaction partners in the nervous system are only partly known. Recent transcriptome and ChIP-Seq data identified transcription factor Zfp276 as a potential Sox10 target. Its expression overlapped strongly with Sox10. I plan to verify the effector-target relationship between Sox10 and Zfp276 and to characterize the functions of Zfp276 as a mediator of Sox10-dependent regulation of glial differentiation and myelin maintenance.

e-mail: melanie.kuespert@fau.de

Newly started Projects

J47 01.03.2015 - 31.08.2017

Post-transcriptional targets of Hoxa9 in myeloid leukemogenesis


Dr. Bach

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, we aim 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.

e-mail: christian.bach@uk-erlangen.de

J48 01.01.2015 - 30.06.2017

PPARβ/δ in the crosstalk of bone and glucose metabolism


Dr. Scholtysek

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

Preliminary data revealed a key role of PPARβ/δ during the differentiation of osteoblasts and the osteoblast-coordinated regulation of systemic glucose homeostasis. We therefore aim to elucidate the molecular role of PPARβ/δ during the differentiation of mesenchymal stem cells into osteoblasts and plan to generate mice carrying an osteoblast-specific deletion or overexpression of PPARβ/δ to study these animals in terms of their skeletal and metabolic phenotypes.

e-mail: carina.scholtysek@uk-erlangen.de

J49 01.04.2015 - 31.09.2017

Extending statistical boosting algorithms for biomedical research

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

Dr. Mayr

This project aims to extend statistical 'beyond the mean' boosting algorithms for particular biomedical research questions: The main topics are (1) the development of methods to compute confidence intervals and to assess significance, (2) the development of specific loss functions to optimize point predictions and prediction intervals for biomedical forecasting and (3) the extension of multi-dimensional boosting algorithms for joint modelling of multiple endpoints in clinical studies.

e-mail: andreas.mayr@fau.de

News and Figures

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News and Figures

Overview

The following figures impressively show the broad acceptance and the great interest of the Medical Faculty members in the programmes of the IZKF. The IZKF gives financial support to projects in all focal areas of the Medical Faculty and into a great number of different institutions. Nearly 100 scientific theses were running in 2014 and more than 60 publications were published.

Advanced Projects	**26**
Immunology and Infection	11
Oncology	5
Neurosciences	8
Renal and Vascular Research	2
Tandem projects between departments and institutes	5
Projects completed in 2014	3
Junior Research Groups	**2**
Junior Projects	**18**
Immunology and Infection	9
Oncology	3
Neurosciences	3
Renal and Vascular Research	1
Molecular Medicine	2
Projects completed in 2014	8
Institutions with funded projects	**21**
Employees of the IZKF	**82**
Number of scientists (including laboratory rotations)	61
Number of non-scientists	21
Appointments of IZKF project leaders on W2/ W3 - positions	3
Ongoing scientific theses in 2014	**95**
Bachelor theses	1
Master theses	11
Doctoral theses	61
Habilitations	7
Laboratory rotations	15
MD-thesis scholarship holders	26

Participants Graduate School	**88**
T(h)INK - Oncology, Immunology and Infection, Renal and Vascular Research	**46**
PhD students from IZKF projects	24
Associated participants	12
MD-thesis scholarships holders	11
Neurosciences	**42**
PhD students from IZKF projects	12
Associated participants	29
MD-thesis scholarships holders	1
Number of patents (2014)	1
Number of awards (2014)	21
Publications (2014)	**66**
Cumulative impact factor	435.780
Average impact factor per publication	6.467
Average publications per project	1,4
IF ≥ 10	5
Total expenditures 2014	**3,964 K€**

Summary of important figures 2014

News

Junior Research Group in the research field of molecular oncogenesis

The IZKF is going to establish a new Junior Research Group in the research field of molecular oncogenesis as of August 2015. An outstanding scientist with training in medicine or natural sciences and a strong background and reputation in research areas such as signal transduction including chromatin remodeling or tumor microenvironment on any of the tumors of the CCC focus was sought. The Junior Research Group will be housed in the Nikolaus Fiebiger Center and will have access to the scientific facilities and research activities of this center. On 27th of January, 2015, the colloquium for the Junior Research Group leader position took place at the Nikolaus Fiebiger Center. The IZKF is currently in negotiations with the listed candidates.

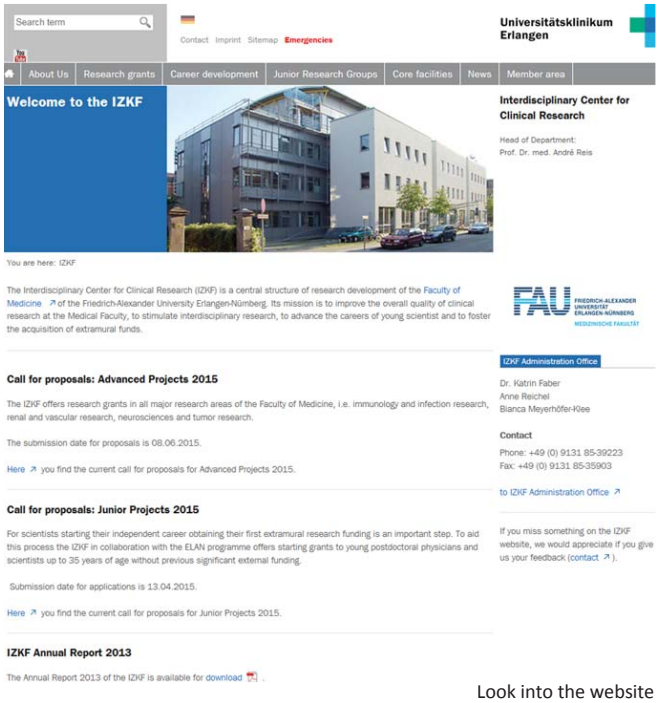
Nikolaus Fiebiger Center

News and Figures

The new IZKF homepage went online

We are pleased to announce that the new homepage of the IZKF went online in July 2014. The new website can be reached via the known links: http://www.izkf.uk-erlangen.de/ or http://izkf-erlangen.de/.

Similar to the annual report, all projects are presented on our website with the most important data and images. The English version of the IZKF homepage has been expanded.



Look into the website

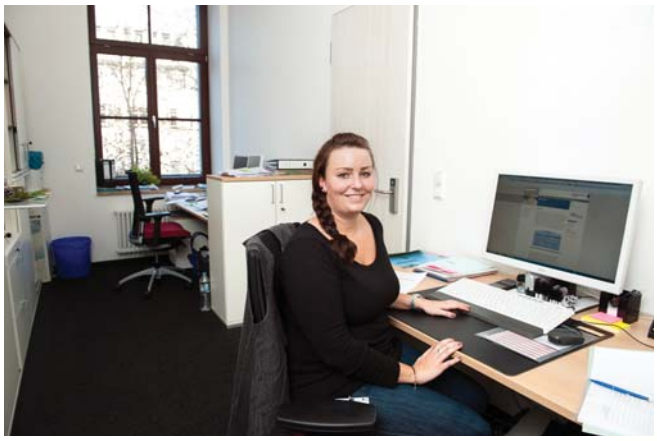
Thanks to a long-standing member

As of November 2014, the third term of Prof. Behrens ended as a representative of the main research areas within the Management Board of the IZKF. Professor Behrens completed his maximum terms as a member of the Management Board. He is succeeded by Prof. Bogdan, who was elected within the annual General Assembly. We would like to thank Prof. Behrens for many years of service and advice in the IZKF Management Board.

Certificate for a long-standing member

Relocation of the IZKF Administrative Office

Since May 2014, the IZKF Administrative Office is located in their new premises in the Krankenhausstraße 12. After many years in the offices of Maximiliansplatz 2, the IZKF Administrative Office moved to the renewed offices in the Krankenhausstraße 12. The IZKF Administrative Office is now located in close proximity to the administration of the Faculty of Medicine leading to an enhanced integration. The new premises are now also offering the possibility to use the conference room of the Medical Faculty for meetings of the boards.



Administrative Office in the Krankenhausstraße 12

Conference Room

News and Figures

Figures

Research Grants

The IZKF research grants can be divided into Advanced Projects, Junior Projects and Junior Research Groups. In 2014, 26 advanced and 18 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.

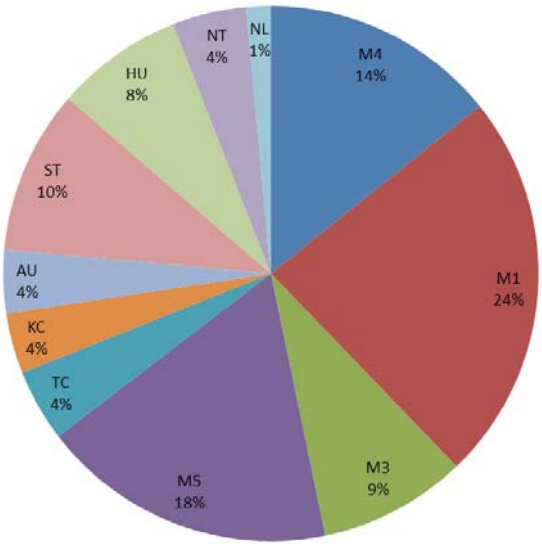
Institute	Immunology and Infection	Oncology	Neurosciences	Renal and Vascular Research	Others
Chair of Experimental Medicine II		x			
Department of Anesthesiology			x		
Department of Dermatology	x				
Department of Medicine 1	x	x			x
Department of Medicine 2	x				
Department of Medicine 3	x				
Department of Medicine 4	x			x	
Department of Medicine 5	x	x			
Department of Otorhinolaryngology - Head and Neck Surgery			x		
Department of Plastic and Hand Surgery		x			
Department of Psychiatry and Psychotherapy			x		
Department of Surgery	x	x			
Division of Immune Modulation	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 2014 and in which main research areas they are located.

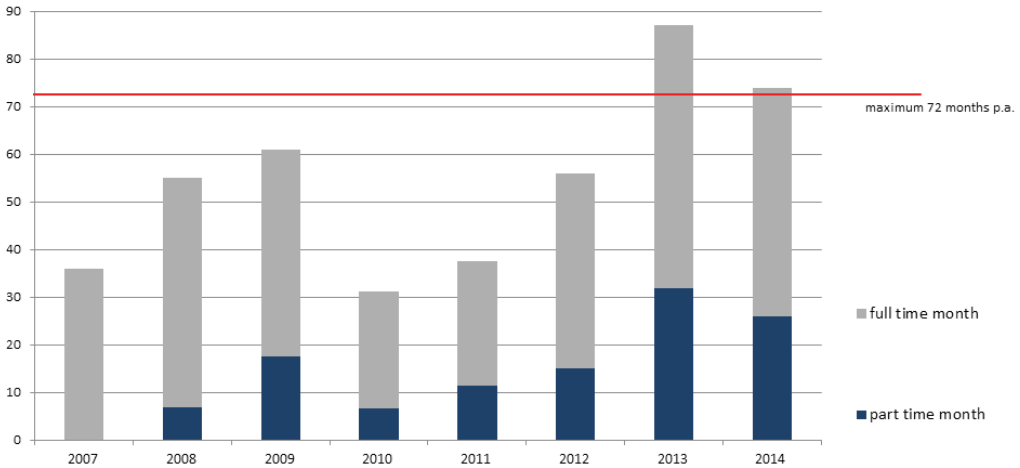
Laboratory Rotations

The rotation programme is aimed at young physicians involved in clinical practice who are interested in research and who completed their doctorate. In the context of the rotation programme, they can be released from their clinical work duties either part-time or full-time in order to devote themselves to their own clearly defined research projects for a set period of time.

Due to high demand and high quality of first and follow-up assignments of IZKF, additional funds were provided for rotation positions in 2014. In 2014, the acceptance of rotation positions is underlined in its use by 11 different institutions.



Funding distribution of laboratory rotations within the University Hospital Erlangen 2014



The table shows the claimed months related to full time in each year. Due to the lifespan of 12-24 months, the rotations usually last over a period of 2-3 calendar years.

Name	Institution	Funding period	Rotating scope
Dr. Christian Knipfer	Department of Oral and Maxillofacial Surgery (KC)	07/2012 - 06/2014	50%
Dr. Cord Huchzermeyer	Department of Ophthalmology (AU)	07/2012 - 07/2014	50%
Dr. Denis Trufa	Division of Thoracic Surgery (TC)	07/2012 - 07/2014	50%
Dr. Christiane Zweier	Institute of Human Genetics (HU)	10/2012 - 09/2014	50%
Dr. Gheorghe Hundorfean	Department of Medicine 1 (M1)	04/2013 - 03/2015	50%
Dr. Markus Hecht	Department of Radiation Oncology (ST)	09/2013 - 08/2014	100%
Dr. Matthias Türck	Department of Neurology (NL)	09/2013 - 02/2014	100%
Dr. Carla Kellermann	Department of Medicine 5 (M5)	07/2014 - 12/2014	100%
Dr. Yazid Resheq	Department of Medicine 5 (M5)	03/2014 - 08/2014	100%
Dr. Timo Rath	Department of Medicine 1 (M1)	04/2014 - 03/2015	100%
Dr. Stefan Uderhardt	Department of Medicine 3 (M3)	04/2014 - 09/2014	100%
Dr. Frederick Pfister	Department of Nephropathology (NT)	07/2014 - 06/2015	50%
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%
Rotations within Junior Projects			
Dr. Johannes Schödel	Department of Medicine 4 (M4)	02/2014 - 01/2015	100%

Laboratory Rotations

MD-Thesis Scholarships

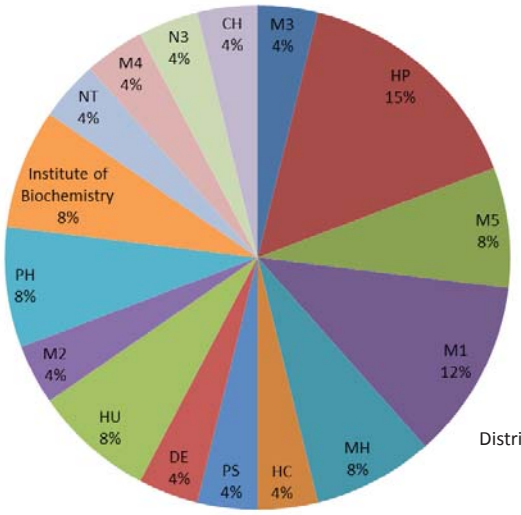
Within the doctoral programme, there are now 18 scholarships for medical doctoral students available every year, granted for a period of 7 months each. Until September 2011, scholarships were awarded for six months with the possibility of extension to 12 months. Starting from September 2011, the duration of funding was changed to 7 months (without the possibility of extension) to assure sufficient laboratory time for the doctoral candidates. Since 2014, the number of scholarships was reduced from 20 to 18 because of an increased support (now € 773/month).

In 2014, a total of 26 medical doctoral students from 16 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. Between 2007 and 2014 the IZKF supported 75 medical students with a scholarship.

By the end of 2013, 37 students completed the studies of medicine, 20 students within the standard period of study. The extension of the other 17 students was mainly approximately by 1 semester. 21 students have completed their doctoral work (12 of those students have finished their studies within the standard period of study). 10 students even received a MD with summa cum laude (48 % of the completed). Since the average of summa cum laude promotions at the Faculty in total ranges between 3 - 5 %, this represents an outstanding result. The next data collection is planned for 2015.

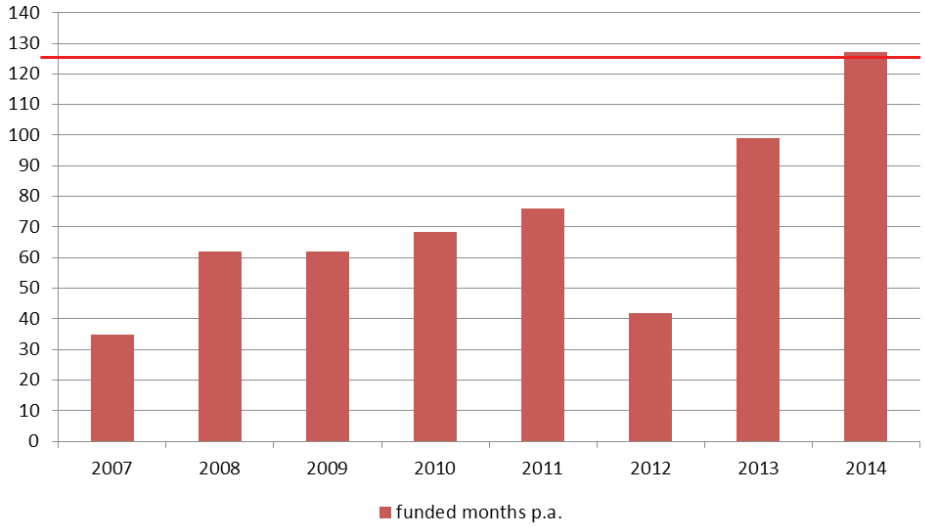
Name	Institution	Funding period
Andrej Stoll	Department of Medicine 3 (M3)	07/2013 - 01/2014
Inge Horn	Department of Plastic and Hand Surgery (HP)	10/2013 - 04/2014
Tobias Middendorf	Department of Medicine 5 (M5)	10/2013 - 04/2014
Jonas Schiemer	Department of Medicine 1 (M1)	10/2013 - 04/2014
Hupfer Thomas	Institute of Microbiology (MH)	01/2014 - 07/2014
Bourjau Yvonne	Department of Medicine 1 (M1)	01/2014 - 07/2014
Diesendorf Helene	Department of Cardiac Surgery (HC)	03/2014 - 09/2014
Tiesel Jens	Department of Psychiatry and Psychotherapy (PS)	03/2014 - 09/2014
Mihatsch Patrick	Department of Dermatology (DE)	03/2014 - 09/2014
Schuhmann Sarah	Institute of Human Genetics (HU)	03/2014 - 09/2014
Witt Ramona	Department of Plastic and Hand Surgery (HP)	03/2014 - 09/2014
Purtak Martin	Institute of Microbiology (MH)	03/2014 - 09/2014
Schacher Nora	Department of Medicine 2 (M2)	06/2014 - 12/2014
Forster Stefan	Institute of Pathology (PH)	06/2014 - 12/2014
Karna Manoj	Department of Medicine 1 (M1)	06/2014 - 12/2014
Sautier Charlotte	Institute of Biochemistry	06/2014 - 12/2014
Gloger Robert	Department of Medicine 5 (M5)	06/2014 - 12/2014
Röder Sebastian	Department of Nephropathology (NT)	10/2014 - 04/2015
Dorsch Melissa	Institute of Pathology (PH)	10/2014 - 04/2015
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
Bickenbach Lena	Department of Medicine 4 (M4)	10/2014 - 04/2015
Schray Annika	Junior Research Group 3 (N3)	10/2014 - 04/2015
Sighart Regina	Institute of Human Genetics (HU)	10/2014 - 04/2015
Seidel Sabrina	Institute of Biochemistry	12/2014 - 06/2015
Bardenbacher Marco	Department of Surgery (CH)	12/2014 - 06/2015

MD-thesis scholarships



Distribution of medical students per institution

News and Figures



This column graph shows the capacity of the programme from 2007 to 2014 in months of funding.

Graduate School

The IZKF Graduate School provides doctoral candidates with a structured training programme and promotes networking among them in various ways. Recently, the Graduate School was divided into two areas: neuroscience and immunology/ infection/ oncology/ renal and vascular research. Members of the ICN (Interdisciplinary Center for Neurosciences) were integrated as associate members into the neuroscience part of the IZKF Graduate School.

In 2014 the Graduate School consisted of 88 members.

Participants Graduate School	**88**
Neuro	***42***
Participants from IZKF projects	12
Associated participants	29
MD-Thesis scholarships	1
T(h)INK - Oncology, immunology and infection, renal and vascular research	**46**
Participants from IZKF projects	24
Associated participants	12
MD-Thesis scholarships	11

Participants of the Graduate School 2014 (05.11.2014)

Until September 2014, Andrea Liebl was speaker of the Graduate School. In the re-election 2014, Tobias Borman was elected as speaker of the Graduate School for the area oncology, immunology, renal and vascular infection. Lian Ye became speaker of the Graduate School for the neuroscientists. Within the scope of the seminars of the Graduate School, Prof. Heinrich Körner, former IZKF Junior Project Group leader, presented his talk „30 years of Tumor Necrosis Factor (TNF) research: It still does not cease to surprise“ to the postgraduates in 2014.

T(h)INK-Group

Speaker	Deputy Speaker
Andrea Liebl from 10/2012 *D20, AMEC*	**Patrick Holz** from 10/2013 *A43, AMEC*
Tobias Bormann since 10/2014 *A54, M1*	**Kristina Scheibe** since 10/2014 *D21, M1*

Additionally, the following soft skill courses were given:

- Presentation Skills, Dr. Deborah Bennett, 07.02.-08.02.2014 + 14.02.-15.02.2014, 24.-28.11.2014
- Kommunikation und Rhetorik, Gerhard Kranz, 11.-12.07.2014
- Microscopy course on sample preparation and two channel confocal imaging, Dr. Ralf Palmisano, 22.09., 07./08./15.10.2014
- Scientific Writing, Dr. Deborah Bennett, 27.-29.10.2014, 01.-03.12.2014

NEURO-Group

Speaker	Deputy Speaker
Lian Ye *Mol. Clin. Phar.*	**Andrea Link** *Physiology* **Benjamin Ettle** *E18, MN* **Diana Schmidt** *N3, IZKF*

ICN/ IZKF Graduate Retreat

16 ICN/IZKF graduate students participated in the 1st ICN/IZKF Graduate Retreat which took place in the Kolping House, Würzburg, from 5th to 7th December 2014. The scientific program of the meeting started on Friday 5th with two invited speakers; Dr. Wörsdörfer, Institute of Anatomy and Cellular Biology, University of Würzburg ("Transdifferentiation from fibroblasts to neurons") and Priv. Doz. Dr. Blum, Institute for Clinical Neurobiology, University Hospital Würzburg ("The role of BDNF – from molecules to behavior"). On Saturday 6th and Sunday 7th, four distinct sessions - in the form of challenging scientific competitions and general discussions - took place, namely "Data Blitz", "Elevator Pitch Talk", "SPOT the BEE", and "Ethics in Science". We would like to emphasize that all sessions were organized by different graduate student-volunteers and all participants of the ICN/IZKF were very actively engaged in all sessions.

Participants of the ICN/IZKF retreat 2014

News and Figures

International IZKF Symposium

From 15th – 16th May 2014 the International IZKF-Symposium "Translational Medicine" was held at the conference centre at the baroque monastery of Kloster Banz. The interesting lectures, lively discussions but also the accompanying poster session received a positive response. The poster session presented concepts and results of projects within the major research areas of the Faculty of Medicine. Four poster prizes were awarded to:

• Dr. Sebastian Försch (Department of Medicine 1): „Bypass of cellular senescence by VEGFR-2 signaling promotes colorectal tumorigenesis directly in intestinal epithelial cells"

• Lukas Heger (Department of Dermatology): "Specialization of human mycloid Dendritic Cells Type 1 for extracellular pathogens"

• Anja Maier (Department of Medicine 4): „The hypoxia-inducible lipid droplet-associated protein HILPDA/HIG2 mediates hypoxic lipid accumulation and may contribute to macrophage foam cell formation in atherosclerosis"

• Socher Eileen (Bioinformatics): „The stability of a nonfibrillar Amyloid-β tetramer structure depends critically on C-terminal peptide length"

Publication prize

Every two years the IZKF confers the Publication Award for Young Scientists which is endowed with 1.000 €. The Junior Scientist Committee selected a winner who was announced and honored as a part of the Translational Medicine Symposium on May 16, 2014 at Kloster Banz. The awardee was also included in the programme with a short lecture.

Prize Winner

Dr. Carina Scherbel (Scholtysek), Department of Medicine 3, „PPARβ/δ governs Wnt signaling and bone turnover"; Nature Medicine 2013 May; 19(5): 608-13

Visiting Professor Programme

The visiting professor programme promotes visits by external researchers, thereby encouraging collaborations to be developed and supporting the exchange of ideas. Next to the IZKF visiting professor programme, there is the FAU visiting professor programme. Following lectures were given by external scientists in 2014.

Scientist	Institute	Lecture title
Prof. Agnes Fogo	Department of Pathology, Microbiology and Immunology, Vanderbuilt University School of Medicine, Nashville, USA	The CDK Epidemic- Can we regress glomerulosclerosis?
Prof. Heinrich Körner	Menzies Research Institute Tasmania, Australia	The TNF and TNF-receptor cytokine superfamily: role in anti-infectious immune responses
Prof. Hans van Bokhoven	Radboud University Medical Center, Molecular Neurogenetics, Nijmegen, Netherlands	Identification of commonly disrupted molecular networks in intellectual disability creates novel diagnostics and therapeutic opportunities
Dr. Jerome Galon	Centre de Recherche des Cordeliers, Laboratory of Integrative Cancer Immunology, Paris, France	Use of systems biology allows to define the immune contexture in human cancer
Dr. Sébastien Jacquemont	Lausanne University Hospital, Faculté de biologie et médecine, Lausanne, Switzerland	Fragile X syndrome: from gene discovery to therapy

Scientist	Institute	Lecture title
Prof. Luiz Carlos de Lima Silveira	Universidade Fedral do Pará, Belém, Brasil	Visual dysfunction following mercury exposure - Necessity of norms for Amazonian populations
Prof. Asghar Rastegar	Yale International Health Program, Yale School of Medicine	Euvolemic hyponatremia - moving beyond SIADH. A case based discussion
Prof. Rainer Storb	Fred Hutchinson Cancer Research Center, Transplantation Biology Program, Seattle, USA	Allogeneic and Hematopoietic Cell Therapy for Blood Disorders: the Road to Success
Prof. Carlo Maria Croce	Department of Molecular Virology, Immunology and Medical Genetics, Ohio State University, USA	Therapeutical role of miRNAs
Prof. Ton Rabelink	Leiden University Medical Center, Department of Nephrology, Leiden, Netherlands	The endothelial glycocalyx: a critical determinant of tissue homeostasis
Prof. Ronald Roepmann	Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands	Understanding cilium (dys-) function by studying the ciliary proteine-protein interaction landscape
Dr. Andrew Feinberg	John Hopkins University, Baltimore, USA	The Epigenetic Basis of Common Human Disease
Prof. Ashok Balasubramanyam	Baylor College of Medicine, Department of Medicine, Diabetes, Endocrinology and Metabolism, Houston, USA	Pathogenesis of HIV-associated adipose dysfunction - role of Vpr
Prof. Lucas Pelkmans	University of Zurich, Institute of Molecular Life Sciences, Zurich, Switzerland	A Hierarchical Map of Regulatory Genetic Interactions in Membrane Trafficking

FAU-Visiting Professor Programme

Scientist	Institute	Lecture title
Prof. Ronald Roepmann	Radbound University Medical Center, Netherlands	Understanding cilium (dys-) function by studying the ciliary protein-protein interaction landscape
Dr. Alejandro Schinder	Leloir Institute Buenos Aires, Argentina	Continuous remodeling of hippocampal circuits by adult neurogenesis
Dr. Evan Reid	Department of Medical Genetics, Addenbrook's Hospital Cambridge, United Kingdom	Spastin, a story of tubules, microtubules and axonal degeneration
Prof. Paul Lucassen	SILS – Center for Neuroscience, University of Amsterdam, Netherlands	Plasticity changes in relation to (early life) stress, nutrition and depression
Dr. Leah Boyer	Salk Institute of Biological Studies, La Jolla, USA	Modeling neurological disease using human pluripotent stem cells
Prof. Moritz Rossner	Ludwig-Maximilian University, Munich, Germany	Gene dosage and environmental factors modulate cognitive deficits in TCF4 mouse models
Dr. Cedric Bardy	Salk Institute of Biological Studies, La Jolla, USA	Modeling human eurological disorders with iPSC: From single neurons to clinical phenotypes of Parkinson patients

IZKF-Visiting Professor Programme

News and Figures

IZKF Funding and Output

Budget

Since 2004, the IZKF has been fully supported by intramural funds. The main financial contribution is given by the Medical Faculty. Additional contributions are received from the University Erlangen- Nuremberg. The contribution from the Medical Faculty is composed of basic support of € 3,396,250 incremented by a research bonus of € 400,441. In 2014, the Medical Faculty provided additionally € 47,500 supplementary funds for extra MD-thesis scholarships. The ELAN programme contributes an equal share of funding for junior projects.

About half of the IZKF-budget (€ 1.9 million) goes toward the funding of advanced projects. About 25 advanced projects are funded by IZKF regularly. About € 760,000 are allotted to the funding of junior projects, and € 500,000 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 € 538,000). Expenditures for core facilities and other supporting activities sum up to € 300,000.

Financial Statements IZKF 2014

Balance forward	3,217 K€
Revenues	
Support by the Medical Faculty	3,844 K€
Support by the University	268 K€
Contribution of ELAN-Fonds for junior projects	310 K€
Contribution of IZKF for junior research groups	- 20 K€
Total revenues 2014	**4,402 K€**
Expenditures	
Research Grants	3,126 K€
thereof advanced projects	1,861 K€
thereof junior research groups	499 K€
thereof junior projects	766 K€
Other career development programmes	538 K€
Core facilities and supporting activities	300 K€
Total expenditures 2014	**3,964 K€**
Balance (2014)	438 K€
Balance (total)	3,655 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 46 scientific projects were actively running: 26 advanced projects, 18 junior projects and 2 junior research groups. In addition, 6 junior projects started their work at the beginning of 2014. These 46 funded scientific projects published 66 original articles in 2014 resulting in an average of 1.4 publications per project. The cumulative impact factor (IF) was 435.780, averaging 6.467 per publication. The high quality of many of these publications is reflected in 5 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 12 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 subprojects is reflected in 11 master and diploma theses, 61 doctoral theses and seven habilitations that were in progress or finalised in 2014. In 2014, one patent was awarded to IZKF projects. Around 90 scientists from 21 different institutions are involved in 46 scientific projects funded by IZKF.

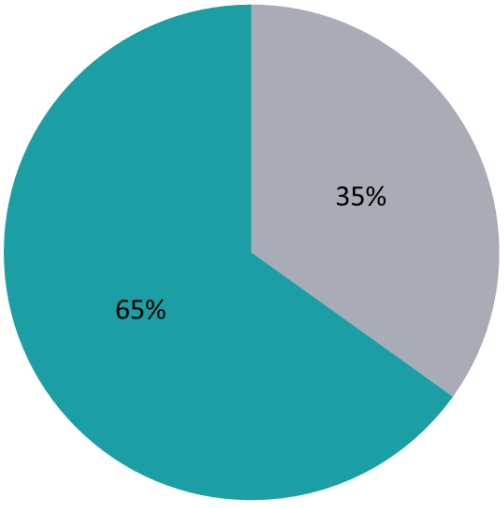
Some IZKF project leaders were able to achieve outstanding results. Subsequently, 21 prizes were awarded to IZKF project leaders and three professorships were offered and accepted.

The IZKF-projects will be completed as part of an outgoing financing. 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, was carried out.

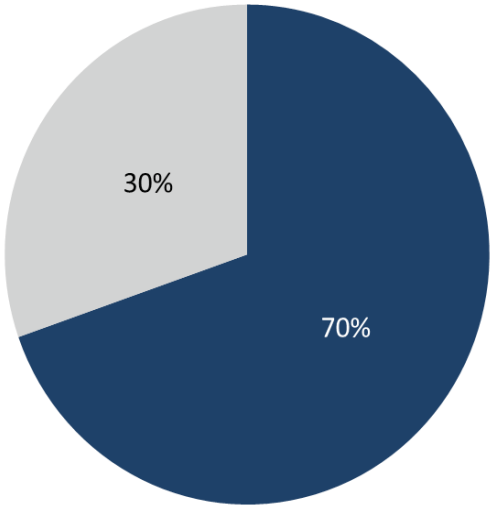
Beginning with the funding period 2010-2013, grants were awarded for a period of 30 months with an extension by 6 months, if these projects are submitted for external funding. When comparing the funding period 2010-2013 with earlier funding periods, it becomes obvious that, owing to the change in regulations, 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.

News and Figures

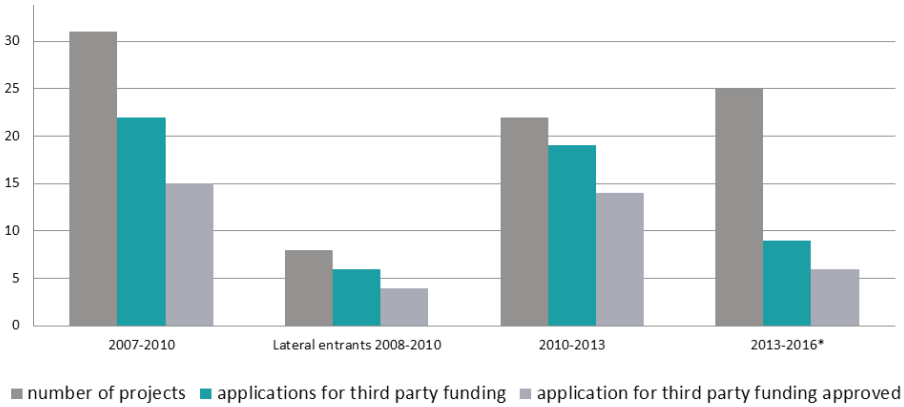
Acquisition of third-party funding advanced projects



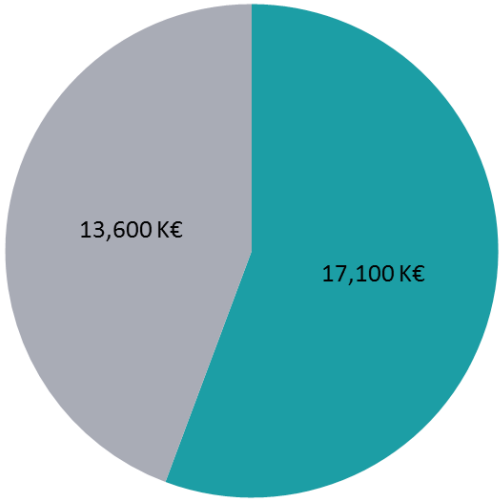
Applications for third-party funding submitted by advanced projects between 2007 and 2014.



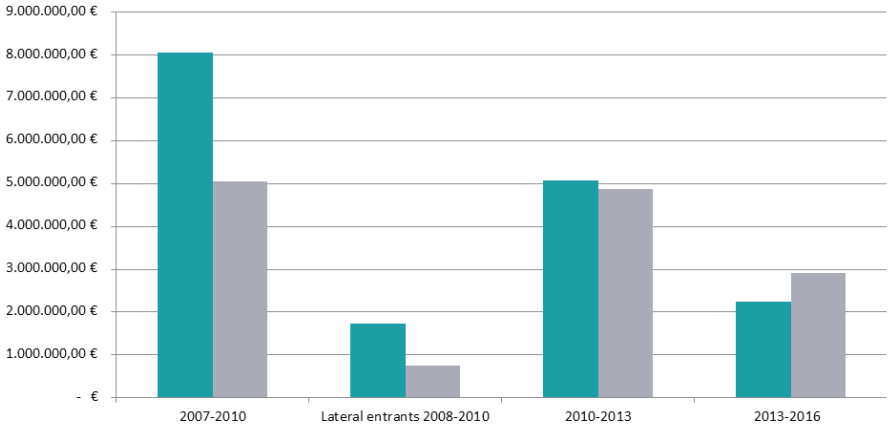
Approved applications for third-party funding of advanced projects between 2007 and 2014.



This column graph compares the number of projects, submitted and approved applications for external funding between the different funding periods of advanced projects.
* Despite the fact that most projects are still ongoing, several have already applied for and obtained extramural funding.



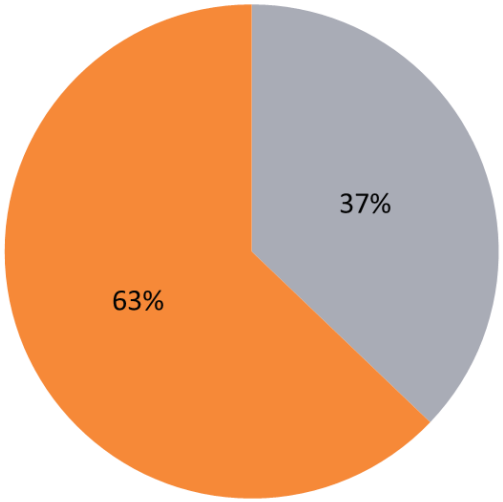
External funding received from advanced projects between 2007 and 2014.



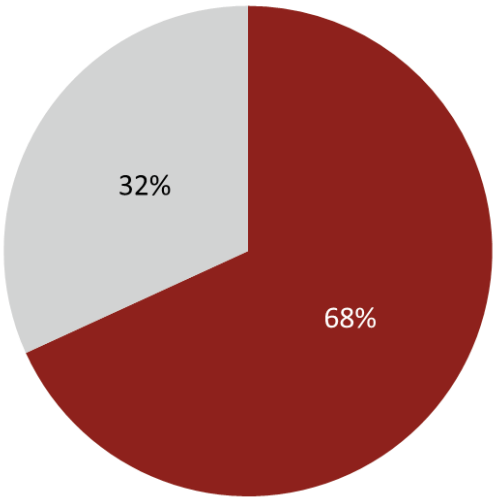
External funding received from advanced projects between 2007 and 2014.

News and Figures

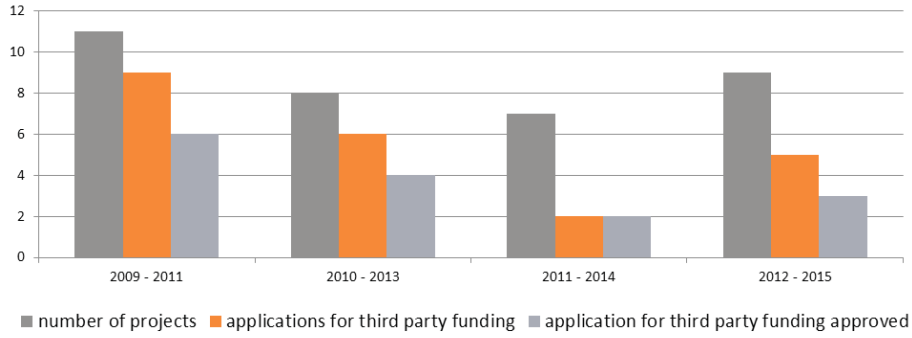
Acquisition of third-party funding junior projects



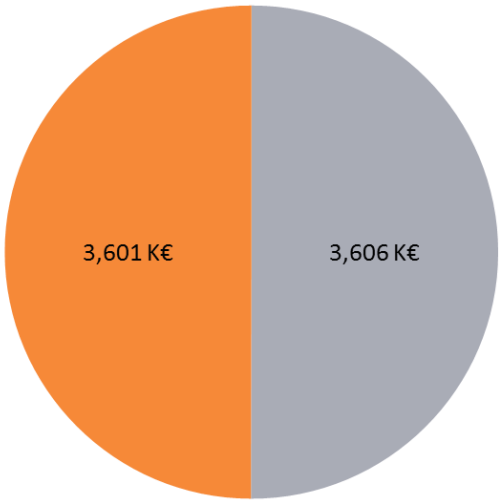
Applications for third-party funding submitted by junior projects started between 2009 and 2012.



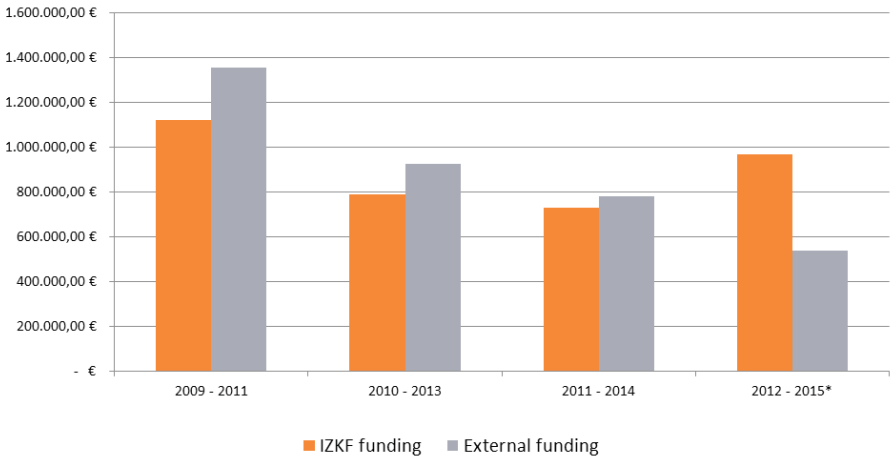
Approved applications for third-party funding of junior projects started from 2009-2012.



This column graph compares the number of projects, submitted and approved applications for external funding between the funding starts. Further applications of projects started in 2012 are planned.



External funding received from all junior projects started between 2009 and 2012.



External funding received from junior projects started between 2009 and 2012.
* Several grant applications are still under evaluation.