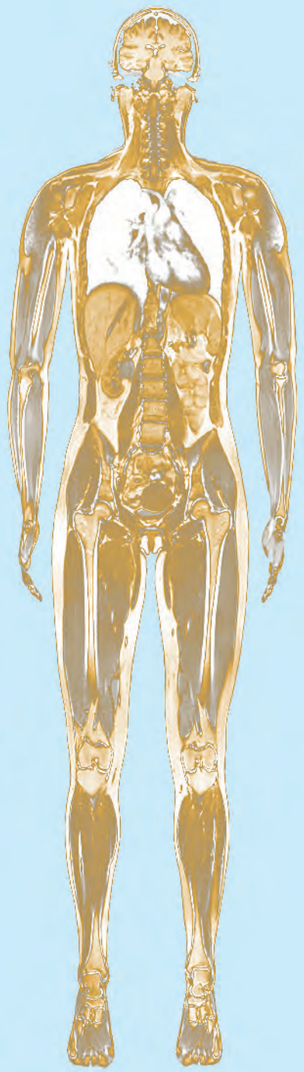
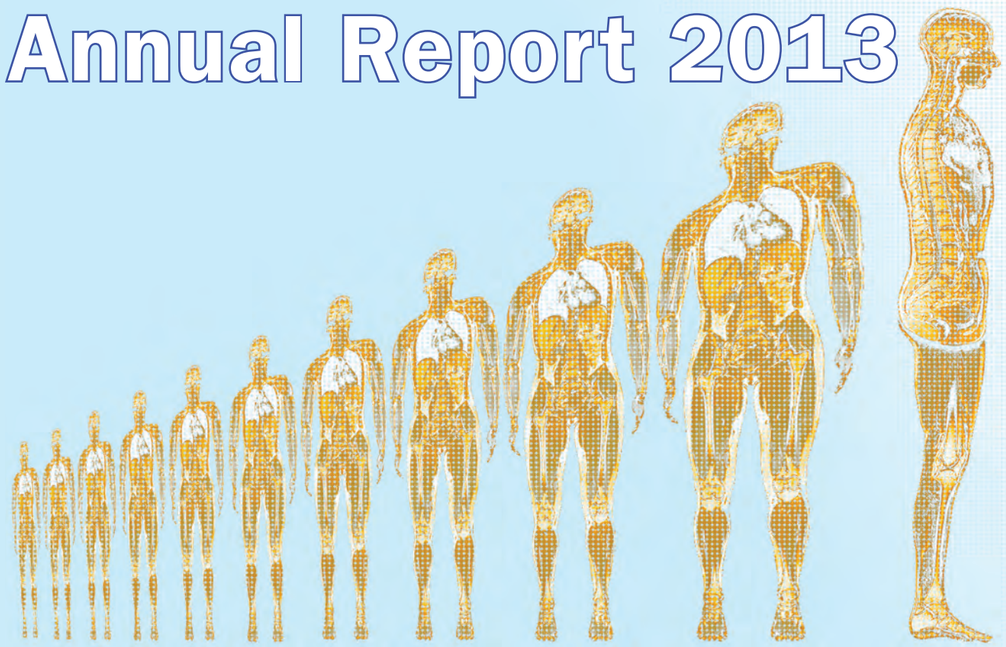


interdisciplinary

Center for Clinical Research Erlangen

IZKF Erlangen Annual Report 2013

Annual Report 2013



Universitätsklinikum Erlangen

FRIEDRICH-ALEXANDER UNIVERSITÄT ERLANGEN-NÜRNBERG
MEDIZINISCHE FAKULTÄT

IZKF Erlangen

Editorial

This annual report is intended to portrait the structure and activities of the Interdisciplinary Center for Clinical Research (IZKF) and to showcase the research of the many participating scientists during last year. The mission of IZKF is to support and develop clinically oriented research independently of organisational structures and based solely on scientific excellence. The key element 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 placed on interdisciplinary research which has become a necessity, as methods and technologies have become even more complex and scientific advances are often achieved at the interfaces between disciplines.

In our efforts to fund excellent research we are supported by the external Scientific Advisory Board (SAB). Last July the SAB visited us again for the evaluation of the overall achievements of IZKF and especially the projects for the next funding period. In total, 25 projects were approved and are scheduled to commence between October 2013 and March 2014. The SAB lauded the developments of IZKF and confirmed the excellence of the work being done. The SAB especially welcomed the positive evaluation of the IZKF goals i.e. publication records, resulting in extramural funding and career development presented by the board and encouraged IZKF to pursue with monitoring of output parameters. The general concept of limiting the project duration to 30 months with an additional period of six months upon presentation of an external grant application has proved to be very successful.

The major restructuring decided by the board last year - to hold biannual calls for projects - was also welcomed as was the organising of an international symposium every other year. The next symposium will be held again in Kloster Banz on May 15 and 16, 2014 under the title of "Translational Medicine" and the programme committee has assembled a very interesting programme. During the evaluation visit, the SAB also confirmed the chairman Prof. Häussinger (Düsseldorf) and vice-chair Prof. Sendtner (Würzburg) and welcomed its new members. The SAB was also very pleased about the continued generous financial support by the Medical Faculty as well as the significant increase of the financial participation of the University. The FAU vice-president Prof. Hornegger gave a welcoming speech at the evaluation session and recognised the work of the IZKF and praised its structure, which served as inspiration for the new FAU funding scheme Emerging Fields Initiative (EFI). Finally, the SAB encouraged IZKF to continue developing its Graduate School and made valuable suggestions which the board started implementing.

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, which was 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 two-year period. Also the programme for laboratory rotations was again very successful with a rate of utilisation of more than 120%, indicating a continued interest in a physician-scientist career by many talented physicians. On a more experienced level, IZKF supports junior research groups for up to 6 years. In an attempt to attract new methodologies and to open new research fields with this funding scheme, the board launched a call for a junior research group in the area of proteomics applied to disease mechanisms in 2012. Candidates for the group leader position were selected during a symposium in March 2013 and the necessary mass-spectrometry instrumentation was applied for at the DFG shortly after. Despite intense efforts it was not possible to fill this vacancy. The board therefore re-evaluated the original concept and decided to refocus the topic of the junior research group.

Following recommendations by the Research Committee on fostering internationalisation of research which were adopted by the Medical Faculty in early 2013, the board decided to use English as the sole language for all scientific communication in the IZKF starting in October 2013.

Finally, the general assembly in November held elections for board members. I myself was reelected for a further term as speaker. Prof. Wegner was elected as new deputy and Prof. Mackensen, Prof. Winkler, Prof. Steinkasserer were confirmed as board members representing the research areas of the Faculty.

Prof. Dr. André Reis

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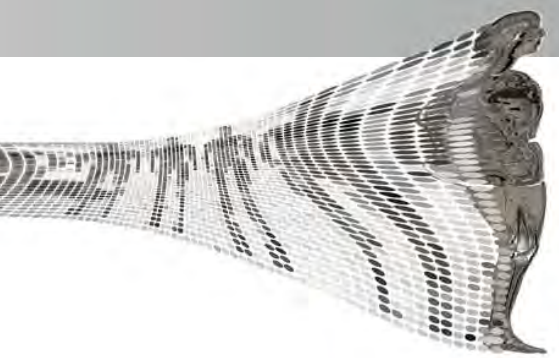
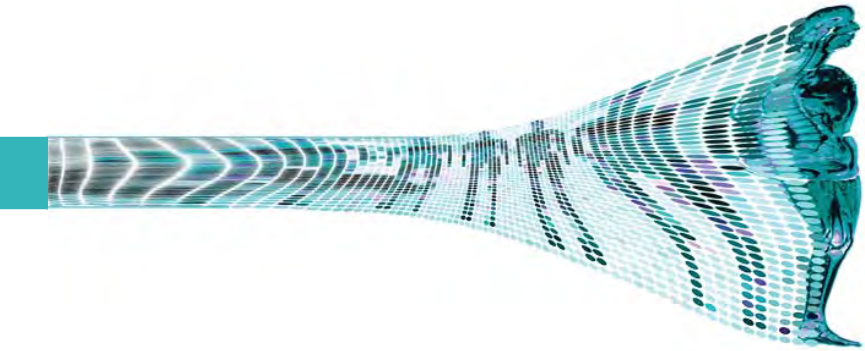
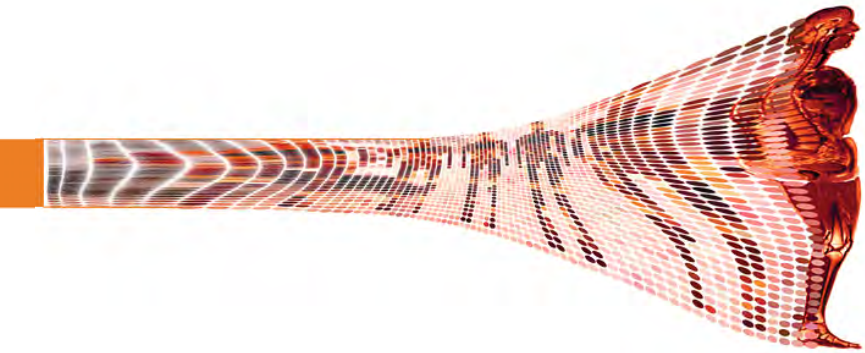
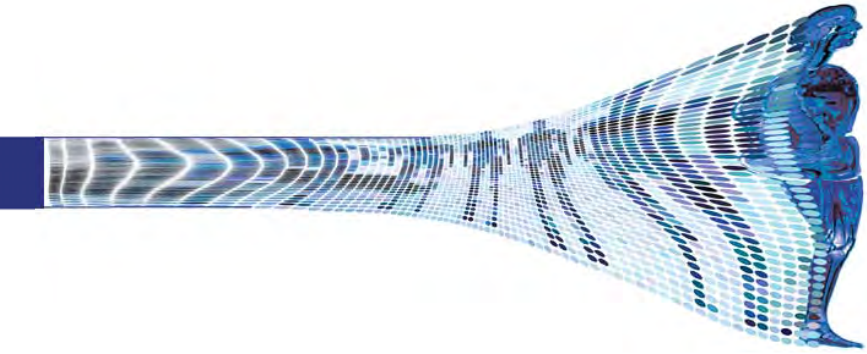




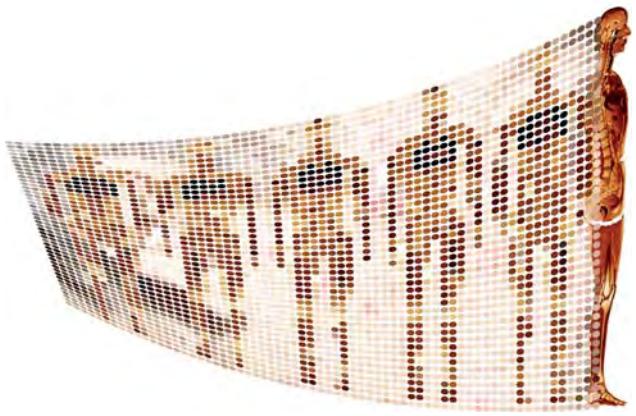




General Information

Mission Statement

The Interdisciplinary Center for Clinical Research (IZKF) is a central structure of research development of the FAU Medical Faculty. Its mission is to improve the overall quality of clinical research at the Medical Faculty, to stimulate interdisciplinary research, to advance the careers of young scientists and to foster the acquisition of extramural funds.



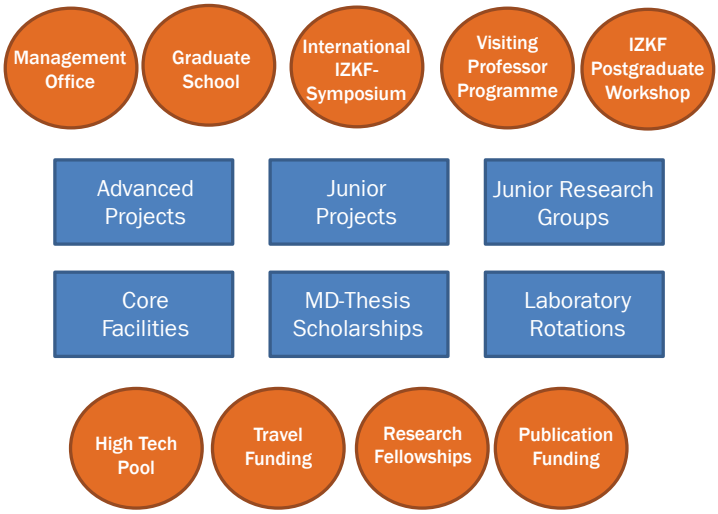
History

The IZKF was founded in 1996 under the leadership of Prof. Joachim Kalden and under the major topic “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 Medical Faculty and the University. The initial scientific focus on inflammation research could be further developed to also accommodate all other focal research areas and interdisciplinary fields of the Faculty without sacrificing this distinctive topic. This allows nearly all institutions of the Medical Faculty to file applications with IZKF.

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

General Information

Programmes



The IZKF activities can be divided into research grants (advanced projects, junior projects, junior research groups), other career development programmes (MD-thesis scholarships, laboratory rotations, Graduate School) as well as core facilities and supporting activities. Advanced and junior projects, junior research groups, core facilities, MD-thesis scholarships and laboratory rotations are periodically requested for proposal within the Medical Faculty.

Research Grants

Advanced Projects

The IZKF offers research grants in all major research areas of the Medical Faculty, i.e. immunology and infection research, renal and vascular research, neurosciences and tumor research. Since the last funding period (2010 – 2013) 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 it is possible to extend the project for another 6 months.

IZKF projects ordinarily include two personnel positions (doctoral student and technical assistant or two doctoral students or in exceptional cases a postdoctoral). 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 Medical Faculty and co-applicants from other faculties are approved for submission in the IZKF. A limit of age does not exist.

Junior Projects

For scientists starting their independent career obtaining their first extramural research funding is an important step. To aid 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 doctoral student and consumables for 24 months. After this time it is expected that successful candidates submit an external grant application. If the application is successfully filed within duration of the junior project, IZKF will extend the project cost-neutral by another 6 months. For if it has been shown that 24 months of grant are predominantly too short to complete the necessary publication for a successful application, they shall be adjusted to the advanced projects and adapt the grant of 30 months.

Currently the following institutions receive project funding within the IZKF:

Institution	Funded Projects 2013
Institutes of FAU	
Department of Experimental Medicine II	D22
Preclinical Institutes	
Institute of Biochemistry	E9, E10, E11, E15, E18, J17, J33
Clinical Theoretical Institutes	
Institute of Pathology	D18, D21, J35
Department of Nephropathology	A45, F1
Institute of Microbiology	A48, A49
Institute of Clinical and Molecular Virology	A50, A51, D17, J26, J30
Institute of Pharmacology and Toxicology	F2
Institute of Human Genetics	F4, Z4
Clinics	
Department of Medicine 1	A37, A38, A39, A52, A53, A54, D16, D19, D21, J12, J27, J28, J36
Department of Medicine 3	A40, A41, J20, J29, J41
Division of Molecular Immunology	E8, J21
Department of Medicine 4	F1, F2, J14, J23, J31
Department of Medicine 5	A42, A58, J18, J24, J34
Department of Molecular Pneumology	A59
Department of Dermatology	J37
Department of Surgery	A43, D20, J19, J22
Department of Plastic and Hand Surgery	J15, J25
Department of Cardiac Surgery	A44
Department of Immune Modulation	A45, A46, A60
Department of Oto-Rhino-Laryngology	E15
Department of Molecular Neurology	E9, E11, E18, J32
Department of Psychiatry and Psychotherapy	E10

Junior projects can be identified by the J in the project number. Advanced projects with the A in the project number belong in the area of immunology and infection research, with F in the area of renal and vascular research, with the E in the area of neurosciences and with D in the area of tumor research. Core facilities starting with the Z in the project number are additionally mentioned here. Projects terminated in 2013 are shown in red.

Junior Research Groups

At the time two positions for 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 up to 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 are independent 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. Both running junior research groups are housed in the Nikolaus Fiebiger Center for Molecular Medicine with its diverse scientific environments and numerous activities. The new Translational Research Center (TRC, move-in scheduled for April 2014) also has premises available to another junior research group. From the end of 2012 to the end of 2013 considerable efforts were made to establish a junior research group in proteomics applied to disease mechanisms at the IZKF. Unfortunately, the concept has not proved to be viable and therefore there will be a re-tendering for the junior research group with a different thematic focus.

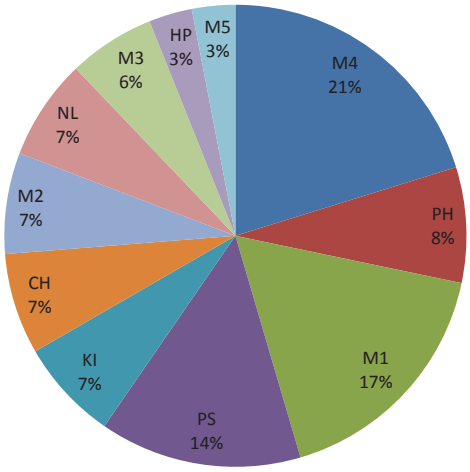
General Information

Other Career Development Programmes

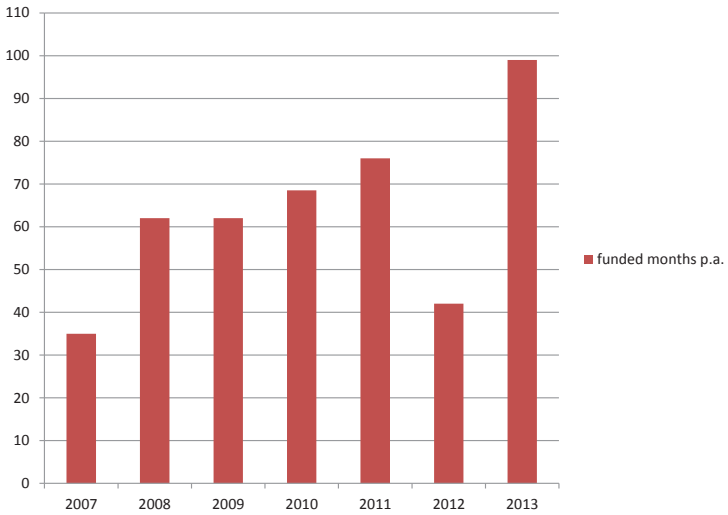
Support and development of young scientists has been a central goal of the IZKF since its inception. In addition to advanced and junior projects and junior research groups, the IZKF also offers other specific programmes for young scientists such as MD-thesis scholarships and laboratory rotations.

MD-Thesis Scholarships

The doctoral programme was initiated to motivate medical students interested in science in a sustainable manner and 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. To compensate for this extra time, the IZKF offers 7 months grants and the supervision of a tutorial committee consisting of 2 experts. Up to 20 grants are available. In 2013 a total of 18 medical doctoral students from 11 institutions were funded and out of which 15 new scholarships where approved. 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. At the end of 2011 an extensive evaluation of the programme took place and was updated in 2013. Between 2007 and 2013 the IZKF supported 58 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. In accordance with the recommendations of the scientific advisory council of IZKF, in the future it shall be compulsory to integrate medical doctoral students in the IZKF graduate school. The rate of scholarship shall be adjusted to the rate of DFG- scholarships.



Distribution of medical students per institutions



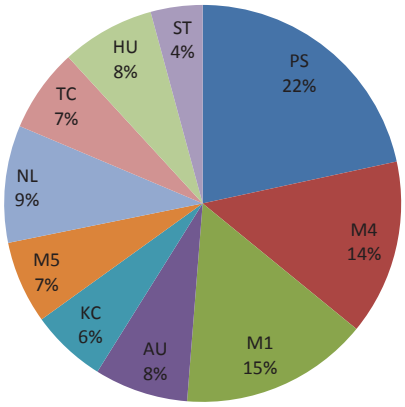
This column graph shows the capacity of the programme from 2007 to 2013 in months of funding. Until September 2011 scholarships were awarded for six months with the possibility to extend to 12 months. Starting September 2011 the duration of funding was changed to 7 months (without the possibility of extension) to assure sufficient laboratory time for the doctoral candidates.

Name	Institution	Funding period
Benjamin Thomas	Department of Plastic and Hand Surgery (HP)	07/2012 - 01/2013
Rafael Heiß	Department of Medicine 4 (M4)	08/2012 - 02/2013
Isabell Schellinger	Department of Medicine 4 (M4)	10/2012 - 04/2013
Julia Friedel	Department of Medicine 1 (M1)	01/2013 - 07/2013
Katrin Brecht	Department of Medicine 1 (M1)	01/2013 - 07/2013
Mateja Condic	Department of Psychiatry and Psychotherapy (PS)	01/2013 - 07/2013
Nadja Michel	Department of Paediatrics and Adolescent Medicine (KI)	01/2013 - 07/2013
Hannes Brandt	Department of Surgery (CH)	01/2013 - 07/2013
Victoria Forschbach	Department of Plastic and Hand Surgery (HP)	01/2013 - 07/2013
Anna Bauereiß	Department of Psychiatry and Psychotherapy (PS)	01/2013 - 08/2013
Rabea Münch	Department of Medicine 2 (M2)	06/2013 - 12/2013
Daniel Heinze	Department of Medicine 4 (M4)	06/2013 - 12/2013
William Laqua	Institute of Pathology (PH)	06/2013 - 12/2013
Patrick Süß	Department of Neurology (NL)	06/2013 - 12/2013
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

MD-Thesis Scholarships 2013

Laboratory Rotations

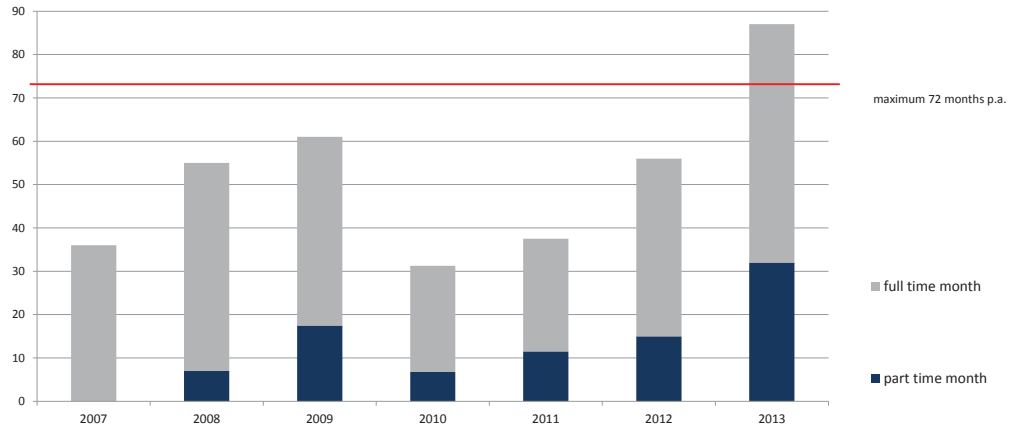
Access to protected research time is essential for young clinicians developing their projects. IZKF supports young scientists in temporary rotating into a laboratory to fully devote themselves to their projects. This rotation can be for 6-12 months in full time or 12 - 24 months in part time. This programme is also open to young clinicians not directly funded by IZKF. Junior projects can also access this programme in addition to their project funding with the contribution of matching funds from their institution. The IZKF can allocate 6 full-time positions; this equates to 72 months, which can be used flexibly and as needed. The initial grant always consists of 6 months in full time or 12 months in part time. In order to receive an extension, the present findings must be presented orally and an updated work programme must be submitted. Due to high demand and high quality of first and follow-up assignments of IZKF, additional funds were provided 2013 for rotation positions. The acceptance of rotation positions is underlined in its use by 10 different institutions within 2013.



Funding distribution of laboratory rotations within the University Hospital Erlangen 2013

Name	Institution	Funding period	Rotating scope
Dr. Christian Knipfer	Department of Oral and Maxillofacial Surgery (KC)	07/2012 - 06/2014	50%
Dr. Teja W. Grömer	Department of Psychiatry and Psychotherapy (PS)	03/2013 - 08/2013	100%
Dr. Martin Regensburger	Department of Neurology (NL)	12/2012 - 05/2013	100%
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. Philipp Spitzer	Department of Psychiatry and Psychotherapy (PS)	01/2013 - 12/2013	100%
Dr. Christiane Zweier	Institute of Human Genetics (HU)	10/2012 - 09/2014	50%
Dr. Vera Schütz	Department of Medicine 1 (M1)	01/2013 - 06/2013	100%
Dr. Steffen Grampp	Department of Medicine 4 (M4)	01/2013 - 12/2013	100%
Dr. Regina Jitschin	Department of Medicine 5 (M5)	04/2013 - 09/2013	100%
Dr. Gheorghe Hundorfean	Department of Medicine 1 (M1)	04/2013 - 03/2014	50%
Dr. Markus Hecht	Department of Radiation Oncology (ST)	09/2013 - 02/2014	100%
Dr. Matthias Türck	Department of Neurology (NL)	09/2013 - 02/2014	100%
Rotations within Junior Projects			
Dr. Christoph Kopp	Department of Medicine 4 (M4)	06/2011 - 03/2013	25%
Dr. Andreas Kremer	Department of Medicine 1 (M1)	07/2013 - 12/2013	50%

Laboratory Rotations 2013



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.

Graduate School

In 2010, a graduate training programme was established for all PhD-students within IZKF. 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.

In 2013 the IZKF Graduate School consisted of 30 members and 22 associate participants as well as 9 medical doctoral students. Andrea Liebl was speaker of the Graduate School and participated in the annual IZKF General Assembly.

According to the recommendations of the external Scientific Advisory Board of IZKF, with beginning of the new grant period a mentor programme shall be established and the binding offers of the IZKF Gra-

duate School will be increased. Furthermore it is planned to give a series of seminars particularly for doctoral students in the field of neuroscience.

Project leaders of the IZKF and other speakers of the Medical Fakulty presented talks to the post graduates in 2013:

- Prof. Dr. R. Schneider-Stock: „Epigenetic regulation in tumors“
- Dr. A. Konrad: „RTCM and Parachip“
- Prof. Dr. F. Haller: „Next Generation Sequencing“
- Dr. V. Eulenburg: „Analysis of Glycine transporter function with biochemical and genetical approaches“
- Dr. R. Palmisano: „Encouraging interdisciplinary coherence in a confined space!“
- Dr. E. Naschberger: „Isolation, Characterization and Use of Tumor Endothelial Cells from Human Colorectal Carcinoma“
- Prof. Dr. K. Schiebel: „Possibilities of project funding within ELAN and IZKF“
- Dr. Ch. Beyer: „Translational Medicine“

Additionaly a scientific writing course was given by Dr. Deborah Bennett in July 2013.

IZKF Postgraduate Workshop 2013

Postgraduate Workshop

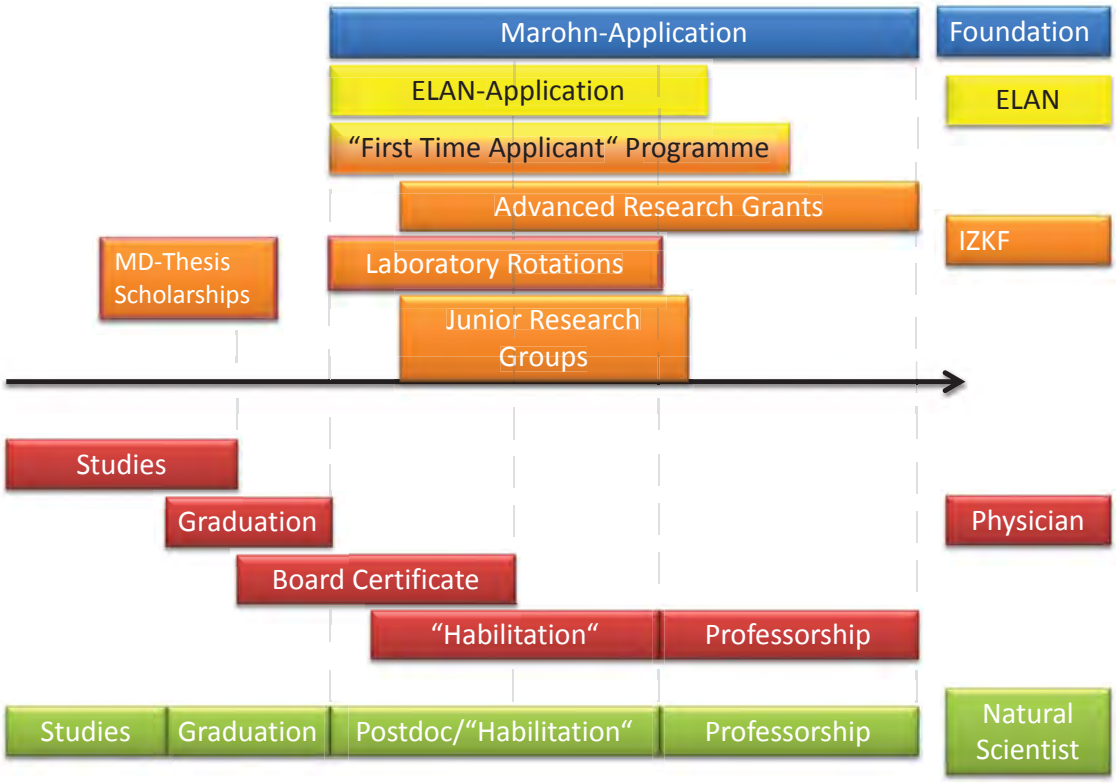
Previously held annually and in future held every two years alternating with the international symposium at Kloster Banz, the Junior Scientist Committee organises the IZKF Postgraduate Workshop. On this occasion lectures are held by internationally recognised speakers on a timely topic. In 2013 Prof. Dr. Tobias Moser, Universitätsmedizin Göttingen, gave a lecture about “Sound encoding in the inner ear: From molecular physiology to disease and restoration”. After the lecture 26 postgraduates presented their work in a poster session. To encourage networking, the poster session was accompanied by beer and pretzels. Subsequently Prof. Dontscho Kerjaschki held a lecture on the topic “The rediscovery of the lymphatic system“.

After the lecture two prizes were awarded to Steven Havlicek for his poster “Gene-dosage dependent neurite defects in a human induced pluripotent stem cell model of SPG4 related heriditary spastic paraplegia“ and to Mousumi Mahapatro for her poster “Gut pericryptal fibroblasts reprogram intestinal stem cell differentiation via Interleukin-33“.

General Information

IZKF and other intramural programmes

The IZKF is a central structure of research development of the Medical Faculty, but not the only one. Besides the IZKF two further programmes for intramural project funding of (young) scientists exist: the ELAN programme and the Johannes und Frieda Marohn-Foundation. IZKF and ELAN work closely together.



Programmes for intramural funding of (young) scientists (MD and PhD) at different stages of scientific career. MD-Thesis Scholarships and Laboratory Rotations are only available for Physicians.

Main purposes of the ELAN programme are the financial support for research projects, the promotion of innovative didactic models and the internationalisation of clinical teaching as well as its evaluation. Funding is provided for projects of highly qualified young investigators and newly established groups. Applicants should not be older than 42 years and must be employees of the University Hospital Erlangen. Application is not restricted to deadlines but the funding committee meets only 5-6 times per year.

According to the founders' will, the purpose of the Johannes and Frieda Marohn-Foundation is the promotion of new innovative projects of the Medical Faculty serving diagnosis, prevention and therapy of diseases in general. Projects dealing with diseases in the field of gastroenterology including all liver and pancreatic diseases with diabetes, cancer and medical data bases shall be supported preferentially. Application is not restricted to deadlines but the funding committee meets only 4 times per year.

The ELAN programme and the Marohn-Foundation provide 6-12 months funding for pilot projects with 30-40 k€. Whereas ELAN is restricted to applicants of the University Hospital Erlangen with an age below 42 years, the Marohn-Foundation is focusing thematically. Simultaneous funding of Marohn and ELAN is excluded. Many „First Time Applicants" received funding previously by either of this two institutions.

IZKF "First Time Applicant" funding is open for young scientists with no previous extramural funding. This funding is co-financed by ELAN and IZKF and is mirrored by cooperation on several levels. The chairman of the ELAN funding committee is full member of the Management Board of the IZKF. ELAN and IZKF are equal partners for selection of applications and financing the programme.

General Information

Core Facilities and Supporting Activities

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 allowing 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 successful, 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. Currently the following core units are in operation:

Core Unit Ultra Deep Sequencing

Next Generation Sequencing (NGS) is a revolutionary technology which is based on massive-parallel sequencing of short DNA-fragments. Millions of DNA fragments are produced in parallel allowing for ultra deep sequencing, which, following sophisticated bioinformatic analyses, can be realigned to a reference sequence. The main application is genome or exome sequencing in humans and animal or plant models. Numerous methodological developments have been made allowing also other applications such as transcriptome and methylome profiling or chromatin-immunoprecipation sequencing, all of which were previously performed using microarray based experimental designs.

This core unit was established within IZKF in 2007 under the denomination of Genomics Unit (Z3) operating an Affymetrix microarray platform. At the end of 2010 a first NGS platform was acquired. Following the rapid technological developments, at the end of 2013 already the third generation of instrumentation was put into operation. Thus the core unit has

Core Unit Ultra Deep Sequencing

Core Unit DNA-extraction platform for biobanking purposes

state-of-the-art instrumentation and methodology as well as a dedicated bioinformatic support providing all major applications to its users. Following the initial IZKF funding, the core unit is funded since late 2012 by the Faculty of Medicine in collaboration with the Biology Department of the Natural Science Faculty.

Core Unit DNA-extraction platform for biobanking purposes

Genetic and genomic studies for complex traits and clinical studies require high quality DNA-samples of probands as well as a rigorous and reliable handling and tracking of large numbers of samples. Starting in 2009, the IZKF core facility Z4 "DNA-Extraktionsplattform (Biobank)" was established offering quality controlled DNA-extraction from blood samples and other body fluids, their handling and aliquoting as well as and their long-term archiving. The core unit has two large scale semi-automatic DNA-extraction extraction platforms, a chemagic magnetic separation modulel (Perkin Elmer) and an Autopure LS (Qiagen) and has a capacity of up to 10,000 samples/year. Both platforms yield high quality DNA samples suitable for all downstream applications such as PCR, SNP genotyping, microarray applications and next-generation sequencing. Part of the instrumentation was co-funded by the Erlangen University Comprehensive Cancer Centre. After conclusion of the IZKF funding in 2013, the core facility continues to offer its services financed through user fees.

Management Office

The management office is responsible for the administrative and financial management of the IZKF. It's main focus is:

- Assistance of the speaker, the Management Board and the Junior Scientist Committee
- Organisation of internal and external evaluations
- Organisation of events for or in association with the IZKF
- Public relations (in particular annual reports and the webpage)
- Administration and counselling of the promoted projects and the special programmes
- Relationship management with the other IZKFs

General Information

International IZKF Symposium

IZKF regularly organises international scientific symposia. Since 2009 the International IZKF-Symposium is being held at the conference center at the baroque monastery of Kloster Banz in the upper Main valley. An attractive programme with many speakers from Germany and abroad is developed by the programme committee. In addition, projects funded by IZKF and ELAN programme present their concepts and results in poster sessions. The unique environment of Kloster Banz is stimulating and interactive. The theme of 2009 was "Molecular Therapies" and in 2012 it was "Individualised Medicine". The next symposium will take place from May 15th to 16th 2014. The theme will be "Translational Medicine". The poster presentation is open to IZKF projects, ELAN-projects as well as other participants who would like to present their own work. In order to assure sufficient time for discussion, there will be 2 poster sessions for the first time this year. A further innovation will be the consideration of two Keynote-Lectures which have been scheduled for Friday. The 2014 symposium will end on Friday.

Symposium 2012

Kloster Banz

Symposium 2012

Visiting Professor Programme

To encourage cooperation and to foster the exchange of ideas, the IZKF promotes visits by external scientists, who are invited by project leaders. Guests are expected to give a lecture for IZKF members. The IZKF supports transportation and hotel accommodation with up to € 3,000.

Since its inception in 2012, it is the responsibility of IZKF to handle the FAU Visiting Professor Programme.

The following lectures were given by external scientists in 2013:

- Prof. Dr. H. Betz, University of Frankfurt, Germany: „Inhibitory postsynapses: assembly and regulation“
- Prof. Dr. E. Gundelfinger, Leibniz Institute for Neurobiology, Germany: „Regulation of synaptic neurotransmitter receptors by the extracellular matrix and cell adhesion molecules“
- Prof. Dr. K.-A. Nave, Max Planck Institute for Experimental Medicine, Germany: „Myelin mutant mice: from genes to therapy“
- Prof. Dr. H. Monyer, University Hospital Heidelberg, Germany: „Synchronus rhythmic activity and spatial coding in the hippocampal-entorhinal formation“
- Dr. E. Leipold, Friedrich Schiller University Jena, Germany: „A de novo gain-of-function mutation in SCN11A causes loss of pain perception“

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 € 15,000, additional support is available. Costs for consumables are supported with up to € 10,000 per project.

Travel Funding

To present results, which were achieved in the IZKF projects to the scientific community, the participation in international meetings and conferences is supported. All applicants are expected to give a lecture or present a poster. 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. These amounts are valid in IZKF for the new funding period (2013-2016) and represent in most cases a doubling of the prior rate. This programme is also available for successful applicants for MD-thesis-scholarships and laboratory rotations.

Research Fellowships

The IZKF also supports young academics, who are working in IZKF projects, in performing important experiments or in learning to use new techniques in external laboratories. Travel grants include transportation and hotel accommodation for up to 3 months.

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.

Animal Breeding Costs

IZKF supports the cost of animal breeding in the core facility Franz-Penzoldt-Zentrum with supplementary funds for the project-specific requirements of approved subprojects up to the upper-limit funded by the German Research Foundation (DFG).

General Information

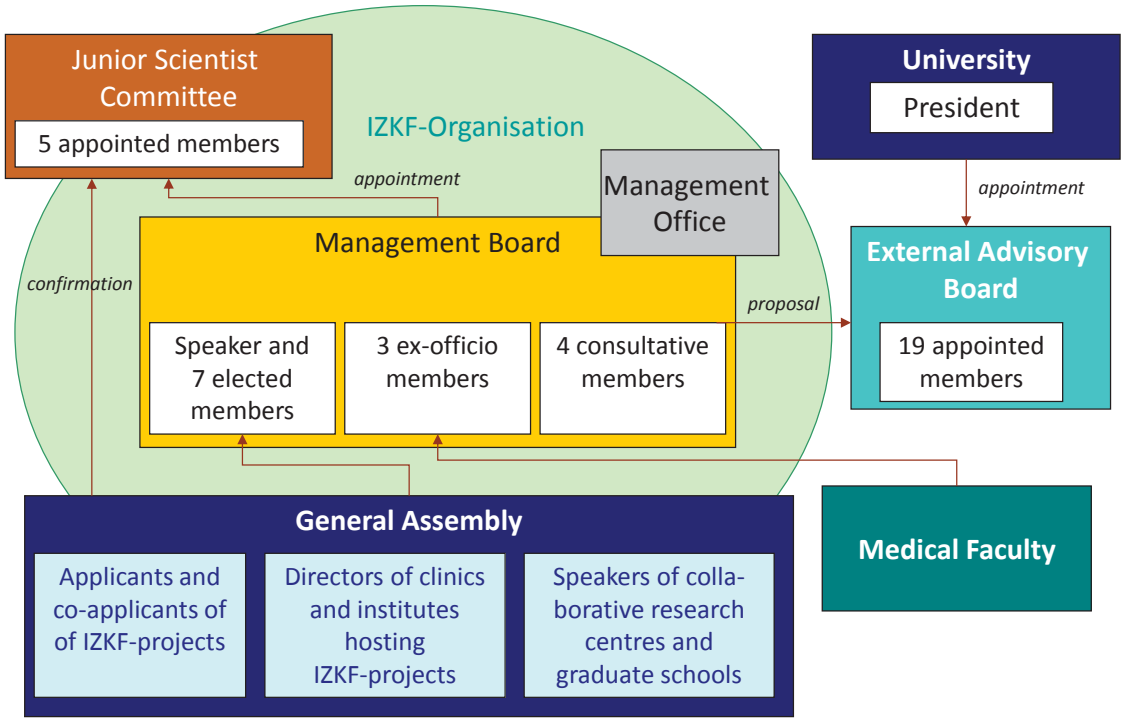
Governance

Structure

IZKF is a self-organised structure within the Medical Faculty. The IZKF has a set of written rules and regulations approved by the IZKF General Assembly and the Medical Faculty. 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 Medical Faculty 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 three years to oversee the general development of the IZKF and the proposed projects. Between the on-site reviews research proposals are sent out for review. In the future on-site reviews will be held in intervals of two years. Thus the next meeting of the SAB is scheduled for November 2015. The Board consists of at least 10 internationally recognised scientists (currently 19) 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.

Procedures

Project funding is allocated after a stringent peer-review process based solely on scientific criteria. Research grants are established 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 a few other recognised scientists of the Medical Faculty 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 were accepted triennially until now, but recently the Management Board suggested changing this to biennial applications.

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 yearly.

General Information

Statutary Bodies

Management Board

Speaker

Prof. Dr. André Reis, Institute of Human Genetics

Deputy Speaker

Prof. Dr. Michael Wegner, Chairman of the ELAN Commission, Institute of Biochemistry

Members

Prof. Dr. Jürgen Behrens, Chair of Experimental Medicine II
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 Medical Faculty, 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
Thomas Schöck, Head of Administration of the University Erlangen-Nuremberg
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. Behrens

Prof. Dr. Eckardt

Prof. Dr. Mackensen

Prof. Dr. Neurath

Prof. Dr. Dr. Schüttler

Prof. Dr. Steinkasserer

Prof. Dr. Winkler

Prof. Dr. Winner

Prof. Dr. Grüske

Schöck

Prof. Dr. Iro

Dr. Bender

Current Members of the Management Board

General Information

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

Administration Office


Reichel


Dr. Faber


Reinwardt


Nothmann

Current staff of the Administration Office

Manager

Dr. Katrin Faber

IZKF Administration

Anne Reichel (from 01.10.2013)
Miriam Reinwardt (part-time)
Sandra Nothmann (from 01.11.2013)
Annika Höft (till 30.06.2013)

General Information

General Assembly

Surname	Name
Achenbach	Stephan
Alzheimer	Christian
Amann	Kerstin
Baur	Andreas
Becker	Christoph
Behrens	Jürgen
Ben Abdallah	Nada
Bender	Albrecht
Beyer	Christian
Bogdan	Christian
Boos	Anja
Bosch-Voskens	Caroline
Croner	Roland
Distler	Jörg
Eckardt	Kai-Uwe
Eulenburg	Volker
Finotto	Susetta
Fleckenstein	Bernhard
Grömer	Teja
Grüske	Karl-Dieter
Günther	Claudia
Hartmann	Arndt

Surname	Name
Herrmann	Martin
Hildner	Kai
Hilgers	Karl F.
Hohenberger	Werner
Horch	Raymund
Iro	Heinrich
Jäck	Hans-Martin
Klucken	Jochen
Kornhuber	Johannes
Kraus	Cornelia
Kremer	Andreas
Kremer	Anita
Kreß	Andrea
Lechmann	Matthias
Leppkes	Moritz
López Posadas	Rocio
Mackensen	Andreas
Mattner	Jochen
Moskalev	Evgeny
Muñoz	Luis
Naschberger	Elisabeth
Neufert	Clemens

Surname	Name
Neurath	Markus
Nitschke	Lars
Reiprich	Simone
Reis	André
Schett	Georg
Schierer	Stephan
Schneider-Stock	Regine
Schöck	Thomas
Schödel	Johannes
Schauer, neé Schorn	Christine
Schuler	Gerold
Schulze	Holger
Schüttler	Jürgen
Sonnewald	Uwe
Stamminger	Thomas
Steinkasserer	Alexander
Stürzl	Michael
Thiel	Christian
Thomas	Marco
Titze	Jens
Völkl	Simon
Waldner	Maximilian

Surname	Name
Wegner	Michael
Winkelmann	Rebecca
Winkler	Jürgen
Winner	Beate
Wirtz	Stefan
Wittkopf	Nadine
Xiang	Wie

General Assembly of the IZKF at 06.11.2013

General Information

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

Prof. Dr. Hartmut Wekerle,
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


Prof. Dr. Büttner


Prof. Dr. Gay


Prof. Dr. Hengel


Prof. Dr. Höfler


Prof. Dr. Katschinski


Prof. Dr. Kelm


Prof. Dr. Kerjaschki


Prof. Dr. Kurts


Prof. Dr. Luger


Prof. Dr. Pavenstädt


Prof. Dr. Pfeffer


Prof. Dr. Rieß


Prof. Dr. Schmiegel


Prof. Dr. Tiegs


Prof. Dr. Wekerle


Prof. Dr. Wirth


Prof. Dr. Zipp

Current External Scientific Advisory Board

General Information

Input and Output

Budget

Since 2004 the IZKF has been fully supported by intramural funds. The main financial contribution comes from the Medical Faculty. Additional contributions are received from the University Erlangen-Nuremberg. The contribution from the Medical Faculty is composed of basic support of € 3,325,000 incremented by a research bonus of € 390,000. In 2013, the Medical Faculty provided € 50,000 supplementary funds for extra MD-thesis scholarships along with an extra amount of € 250,000 for the increase of the remuneration of doctoral students from 50% to 65% in the second project year. The ELAN programme contributes an equal share of funding for junior projects.

About half of the IZKF-budget (€ 1.6 million) goes toward the funding of advanced projects. About 25 advanced projects are funded by IZKF regularly. About € 800,000 are allotted to the funding of junior projects, and € 500,000 to the funding of junior research groups. Further portions of the total budget (about € 600,000) are assigned to other career development programmes (MD-thesis scholarships, laboratory rotations, graduate school), the core facilities, and other supporting activities.

Financial Statements IZKF 2013

Balance forward	2,549 K€
Revenues	
Support by the Medical Faculty	4,014 K€
Support by the University	180 K€
Contribution of ELAN-Fonds for junior projects	350 K€
Contribution of IZKF for junior research groups	- 20 K€
Total revenues 2012	**4,525 K€**
Expenditures	
Research Grants	**2,935 K€**
thereof advanced projects	1,654 K€
thereof junior research groups	437 K€
thereof junior projects	841 K€
Other career development programmes	624 K€
Core facilities and supporting activities	301 K€
Total expenditures 2013	3,857 K€
Balance (2013)	668 K€
Balance (total)	3,217 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 47 scientific projects were actively running: 23 advanced projects, 22 junior projects and 2 junior research groups. In addition, 25 advanced projects and 7 junior projects have just started their work at the end of 2013 or at the beginning of 2014. These 47 funded scientific projects published 60 original articles in 2013 resulting in an average of 1.3 publications per project. The cumulative impact factor (IF) was 406.823, averaging 6.780 per publication. The high quality of many of these publications is reflected in 8 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 13 master and diploma thesis, 58 doctoral theses and four “Habilitationen“ that were in progress or finalised in 2013. In 2013 three patents were awarded to IZKF projects. A total of more than 100 scientists from 22 different institutions are involved in the 47 scientific projects, funded by IZKF.

Some IZKF project leaders were able to achieve outstanding results. Subsequently 14 prizes were awarded to IZKF project leaders and two 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 of 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.

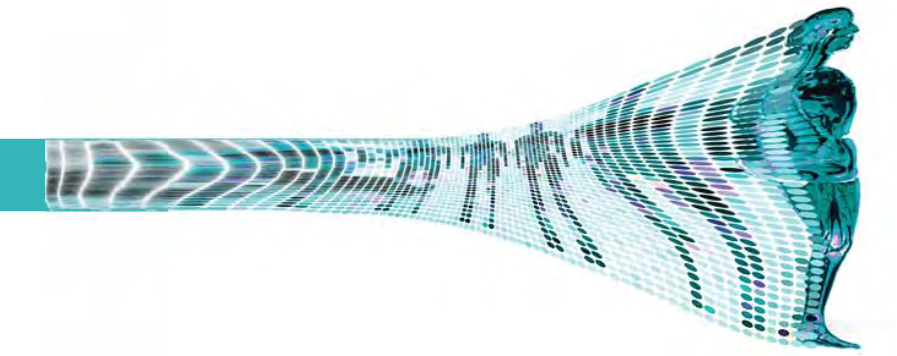
The following table shows the status of third-party funding of projects terminated between 2010 and 2013:

	Number of finished projects	Project terminated (no third-party funding)	application for third-party funding planned	application for third-party funding, review in progress	application for third-party funding, approved for funding
Research projects finalised in 2010	27	14	0	0	13
Research projects finalised in 2011	11	5	0	0	6
Junior Projects finalised in 2011	11	5	0	0	6
Research projects finalised in 2012	1	1	0	0	0
Junior Projects finalised in 2012	2	2	0	0	0
Research projects finalised in 2013	19	6	1	2	10
Junior projects finalised in 2013	12	4	3	2	3
Total	83	37	4	4	38

The table shows the number of projects which could obtain further project funding by external agencies such as BMBF/BMG (4 projects), DFG (21 projects) and others (13 projects).

Advanced Research Grants

Advanced Research Grants



Advanced Grants

Project No.	Applicant(s)	Project Title
A37□	Becker	Caspase-8 in intestinal epithelial cells
A38□	Hildner, Atreya	CD103$^+$ DCs and colitis
A39□	Neufert, Becker	Differentiation and pathogenic role of Th17 cells in inflammatory bowel disease
A40□	Distler, Schett	Canonical Wnt signaling in the pathogenesis of fibrotic diseases
A41□	Herrmann, Munoz	Inflammation in crystal arthropathies
A42□	Ullrich	Phenotypical and functional characterization of human NK cell subsets in different organs
A43□	Stürzl	Paracrine gene functions in Kaposi's sarcoma
A44□	Ensminger	Platelets and transplant arteriosclerosis
A45□	Amann	Podocytes as non hematopoetic professional antigen presenting cells
A46□	Lechmann	Relevance of TSLP in the immune response
A48□	Mattner	Development of new diagnostic tools for primary biliary cirrhosis
A49□	Schleicher, Bogdan	Therapy of cutaneous leishmaniasis with sodium chlorosum
A50□	Schmidt	New antiretroviral restriction factors
A51□	Stamminger	HCMV GPCRs as targets for antiviral therapy
A52*	Günther, Becker	cFlip isoforms in the intestinal epithelium
A53*	Hildner	Th17/piTreg differentiation in vivo
A54*	Wirtz, Waldner	Fam180A in inflammatory diseases
A55*	Krönke	NR4a1 during immunologic tolerance
A56*	Warnecke	Role of HIG2 in atherosclerosis
A57*	Distler, Spriewald	Nr4a1 in cGvHD
A58*	Mackensen, Völkl	Characterization of DN T cells from ALPS patients
A59*	Finotto	IL-10 and lung cancer
A60*	Baur, Schierer	Mo-DC by DC-Exosomes
A61*	Bogdan, Schleicher	Leishmania, iNOS and iron
A62*	Stamminger	ND10 and interferon-induced gene expression
D16□	Wirtz	The role of IL-33/ST2 signalling in the pathogenesis of hepatic fibrosis
D17□	Neipel	The role of Ephrin-A2 receptor-tyrosinkinase in human herpesvirus-8 infection
D18□	Schneider-Stock	miRNAs in normal and malignant intestinal epithelial cells
D19*	Wittkopf, Becker	Role of intestinal epithelial SMAD7 for tumor development
D20*	Stürzl, Croner, Naschberger	Collagen 10 and metastasis in CRC
D21*	Schneider-Stock, Neufert	DAPK and colon cancer
D22*	Behrens	Wnt components

□year of application 2010
*year of application 2013

Project No	Applicant(s)	Project Title
E8□	Mielenz	Swiprosin-1/EFhd2 and tauopathies
E9□	Winkler, Klucken, Wegner	Glia in synucleinopathies
E10□	Eulenburg, Grömer	The role of neuronal glycine transporter 1 (GlyT1) in synaptic transmission
E11*	Klucken, Xiang	H50Q aSyn mutation in PD
E12*	Winkler, Lie	Adult hippocampal neurogenesis in synucleinopathies
E13*	Müller, Reichel, Kornhuber	Sphingomyelinase, depression and alcoholism
E14*	Zimmermann	TRPC5 and tooth pain
E15*	Eulenburg, Schulze	GlyT1 and neuropathic pain
E16*	Lie, Reis	Regulatory networks in intellectual disability
E17*	Hashemolhosseini	Wnt signaling at neuromuscular synapses
E18*	Wegner, Winkler	NG2-positive glia
F1□	Amann, Hilgers	Angiogenesis in chronic renal failure
F2□	Maas, König, Jacobi	AGXT2 and methylarginines
F3*	Engel	Fam60a in heart and brain development
F4*	Thiel	Pathogenesis of the short rib-polydactyly syndrome

□year of application 2010
*year of application 2013

A37 - Final Report

01.11.2010 - 31.10.2013

Caspase-8 in intestinal epithelial cell

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

Increased cell death has been associated with inflammatory bowel disease (IBD). Data from our group and others implicate, that caspase-mediated signalling pathways are important for the pathogenesis of IBD. The aim of this project was to elucidate the role of caspase-8 mediated signalling in the intestinal epithelium by using conditional mouse strains in which caspase-8 or its regulator cFLIP have been inactivated specifically in intestinal epithelial cells.

Role of caspase-8 in the intestinal epithelium for intestinal homeostasis.

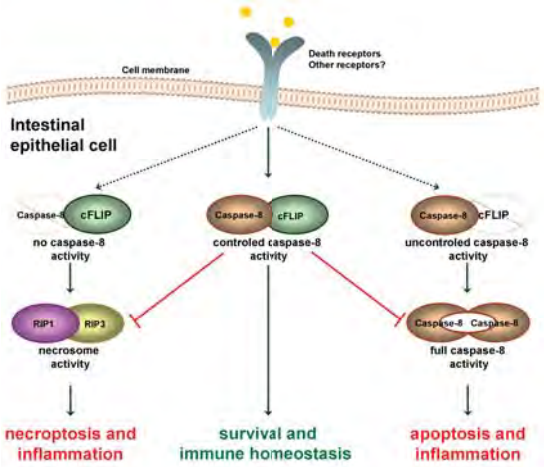
Surprisingly, intestinal epithelial cell specific caspase-8 deficient mice developed spontaneous ileitis and were hypersensitive to DSS-induced colitis. Caspase-8 deficiency resulted in the specific absence of Paneth cells and reduced numbers of goblet cells, implicating defects in the innate defence against luminal bacteria. Epithelial cell loss was associated with increased necrotic cell death in crypts of these mice. Detailed analysis of cell death in Casp8$^{\Delta IEC}$ mice indicated that Paneth cells died from necroptosis a recently identified novel form of cell death that shares microscopical features of necrosis, but occurs in a programmed (regulated) fashion. In marked contrast to the absence of Paneth cells in Casp8$_{DIEC}$ mice in vivo, intestinal derived organoids grown from Casp8ΔIEC mice showed Paneth cells indistinguishable in localization and number from organoids cultured from control littermate mice, indicating that necroptosis requires an external trigger. Indeed we figured out that Casp8$^{\Delta IEC}$ mice were hypersensitive to TNF-α challenge, a cytokine that is known to induce cell death. On a molecular level necroptosis is mediated by a protein complex recently denoted ripoptosome due to the presence of the proteins RIPK1 and RIPK3 which are necessary for necroptosis. We could demonstrate that necroptosis in Casp8$^{\Delta IEC}$ mice could be blocked by additional deletion of RIPK3 in vivo. Finally, we identified high levels of RIPK3 in human Paneth cells and increased necrosis in the terminal ileum of patients with Crohn's disease, suggesting a potential role of necroptosis in the pathogenesis of this disease.

Thus, our data uncover an unexpected function of caspase-8 in regulating necroptosis of intestinal epithelial cells and in maintaining immune homeostasis in the gut.

Role of the caspase-8 regulator cFLIP in the intestinal epithelium for intestinal homeostasis.

In the second part of our project we investigated the control of caspase-8 activation, by analysing mice with a specific deletion of the caspase-8 regulator cFlip in the intestinal epithelium. Surprisingly deletion of cFLIP specifically in IECs results in embryonic lethality, suggesting an important role for this caspase-8 regulator in the intestinal epithelium during embryonic development. To further investigate the role of cFlip for the intestinal homeostasis, we generated mice (cFlip$^{i\Delta IEC}$) that allowed IEC-specific cFlip deletion in adult mice. Deletion of cFlip by tamoxifen gavage results in the death of these animals within only a few days. Histological analysis revealed severe villous atrophy, destruction of the mucosal structure and a high amount of dying epithelial cell in cFlipi$^{\Delta IEC}$ mice. Detailed analysis identified this form of cell death as caspase-mediated apoptosis. In contrast to the lethal effect of cFlip deficiency in vivo, cFlip deficient intestinal organoids did not die in vitro, indicating that apoptosis is not mediated via epithelial cell intrinsic apoptosis pathways. Moreover these results suggested that cFlip regulated cell death is dependent on external factors that are present in vivo. Indeed we could show that targeting death receptors by addition of a low dose of TNF-α or CD95L to the culture medium resulted in death of Flip$^{i\Delta IEC}$ but not control derived organoids. To investigate the potential contribution of necroptosis in the cFlip

Prof. Dr. Becker



Model of cFlip and caspase-8 as central regulators of intestinal epithelial cell death and intestinal immune homeostasis.

deficient background, cFlip$^{i\Delta IEC}$ mice were crossed to mice lacking the necroptosis mediator RIPK3. Treatment of these double deficient mice with tamoxifen resulted in the same phenotype as cFlip$^{i\Delta IEC}$ mice. Moreover, we also investigated the influence of autophagosomal cell death by crossing cFlipi$^{\Delta IEC}$ mice to mice with an epithelial specific deletion of ATG7. However we could not observe any protection after tamoxifen induced deletion of cFlip in these double deficient mice. Overall this demonstrates that neither necroptosis nor autophagy, but rather the extrinsic apoptosis pathway is responsible for epithelial cell death induced by cFlip deficiency.

In summary our data demonstrate that the activation state of caspase-8 has to be tightly controlled as excessive caspase-8 activation in intestinal epithelial cells induces apoptosis, whereas a lack of caspase-8 activation induces necroptosis. In any case, dysregulation of caspase-8 leads to epithelial cell death, barrier dysfunction and inflammation. Thus both caspase-8 as well as its regulator cFlip have essential functions in maintaining intestinal tissue homeostasis by controlling cell death in a way that is compatible with intestinal epithelial cell survival.

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

Invited lectures

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

Annual Meeting of the German Society of Immunology (DGfI), Mainz, Germany, 11. – 14.09.2013, Plenary lecture “Cytokine regulation of intestinal barrier function”

Awards

Claudia Günther: 1st Research prize; 6th Seeon Conference-Microbiota, Probiota and Host

Claudia Günther: 1st Poster prize; Falk Gastro-Conference “Inflammatory Bowel Diseases: Microbiota versus the Barrier”

Publications during funding period

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A38 - Final Report

01.10.2010 - 31.09.2013

CD103^{+} DCs and colitis

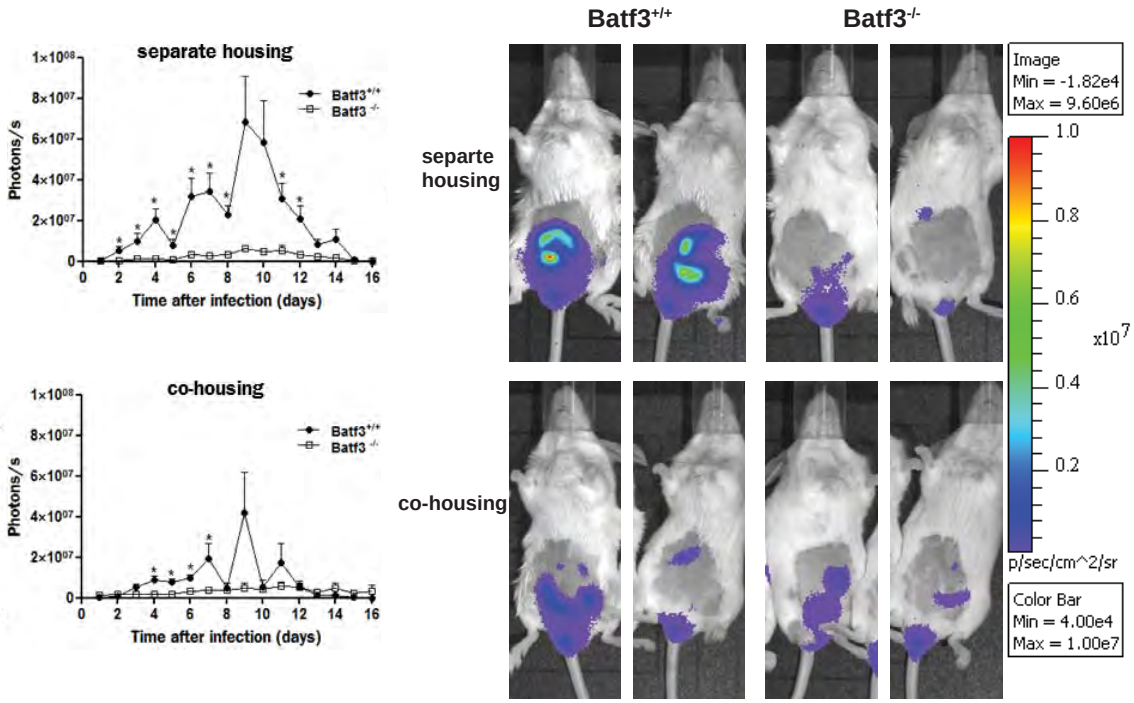
Prof. Dr. Kai Hildner, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology
Prof. Dr. Raja Atreya, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

We investigate the role of mucosal CD103+ dendritic cells (cDCs) in an infectious Citrobacter rodentium (C.r.) colitis model. Batf3$^{-/-}$ mice lack the CD103+CD11b- DC subset and show diminished bacterial burden and prolonged survival even in the absence of B and T cells. The relative resistance against infection and colonization by C.r. depends on the presence and integrity of the intestinal flora of Batf3$^{-/-}$ mice and is communicable by co-housing and stool transplantation to Batf3$^{+/+}$ mice.

Background and summary of previous results

Citrobacter rodentium (C.r.) is related to EHEC and EPEC and provides an excellent in vivo model to investigate host-pathogen interactions. Batf3$^{-/-}$ mice lack CD103^{+}CD11b^{-} cDCs within nonlymphoid tissues. Using Batf3$^{-/-}$ mice, we are analyzing the specific role of CD103^{+} DCs during infectious colitis.

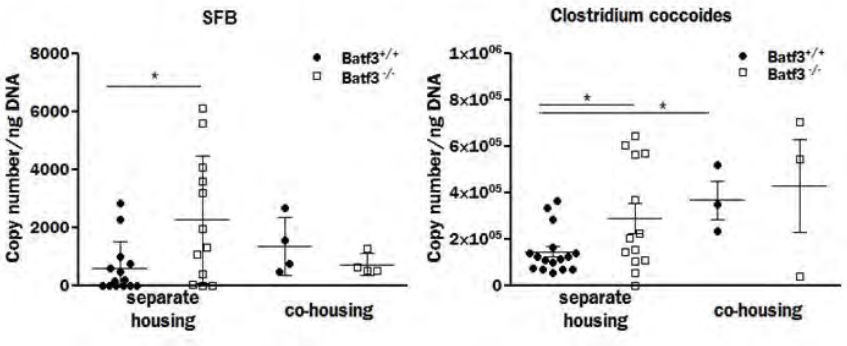
As reported previously (2011-2012), Batf3$^{-/-}$ mice are virtually protected against the expansion of C.r. in vivo. The resistance against infection is independent of the presence of T and B cells and is not directly due to the absence of CD103^{+} cDCs as primary targets of C.r. since even Batf3-independent CD11b^{+} DCs are less infected in the absence of Batf3.



Relative resistance of Batf3$^{-/-}$ mice against C. rodentium infection is communicable to Batf3$^{+/+}$ mice by co-housing of both mouse strains. All values are mean +/- SEM, and represent data from 3 pooled experiments. p< 0,05.

Prof. Dr. Hildner

Prof. Dr. Atreya



Batf3$^{-/-}$ mice have an altered microbial composition in the gut compared to Batf3$^{+/+}$ mice. Co-housing induces significant changes in the gut microbiota of Batf3$^{+/+}$ mice. All values are mean +/- SEM representing data from 2 pooled experiments. p< 0,05.

Infection resistance can be transferred from Batf3$^{-/-}$ to Batf3$^{+/+}$ mice

To address the question whether the resistance against C.r. infection can be communicated between mice, Batf3$^{+/+}$ and Batf3$^{-/-}$ mice were housed together in the same cage followed by C.r. inoculation. Interestingly, in this setting Batf3$^{+/+}$ mice showed diminished bacterial loads comparable to the course of infection of Batf3$^{-/-}$ mice. In addition, transplantation of stool from Batf3$^{-/-}$ mice into Batf3$^{+/+}$ mice followed by C.r. infection resulted in a similarly reduced C.r. content as determined by whole body bioluminescence imaging.

Targeted disruption of the intestinal flora of Batf3$^{-/-}$ mice abrogates infection resistance

The co-housing and stool transplantation data imply that the resident intestinal flora plays a key role during the resistance of Batf3$^{-/-}$ mice after C.r. infection. To functionally prove the role of the microbiota of Batf3$^{-/-}$ mice in conferring protection Batf3$^{+/+}$ and Batf3$^{-/-}$ mice were intensely treated with antibiotics prior p.o. infection with C.r. By this conditioning a significant reduction of the cultivable flora could be detected. Interestingly, both Batf3$^{+/+}$ and Batf3$^{-/-}$ mice showed an altered, however, comparable course of infection after C.r. inoculation and importantly Batf3$^{-/-}$ mice lacked the relative resistance against infection after abrogation of the resident flora.

Intestinal flora in the steady state differs significantly between Batf3$^{+/+}$ and Batf3$^{-/-}$ mice

Preliminary analysis of the composition of the intestinal microbiota in the absence of Batf3 supports the hypothesis that Batf3 deficiency results in a modification of the gut flora. However, in depth analysis of the quality and quantity of alterations are currently planned.

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Publications during funding period

Atreya R, Neumann H, Neufert C, Waldner MJ, Billmeier U, Zopf Y, Willma M, App C, Münster T, Kessler H, Maas S, Gebhardt B, Heimke-Brinck R, Reuter E, Dörje F, Rau TT, Uter W, Wang TD, Kiesslich R, Vieth M, Hannappel E, Neurath MF. In vivo imaging using fluorescent antibodies to TNF predicts therapeutic response in Crohn's disease. Nature Medicine. In press.

A39 - Final Report

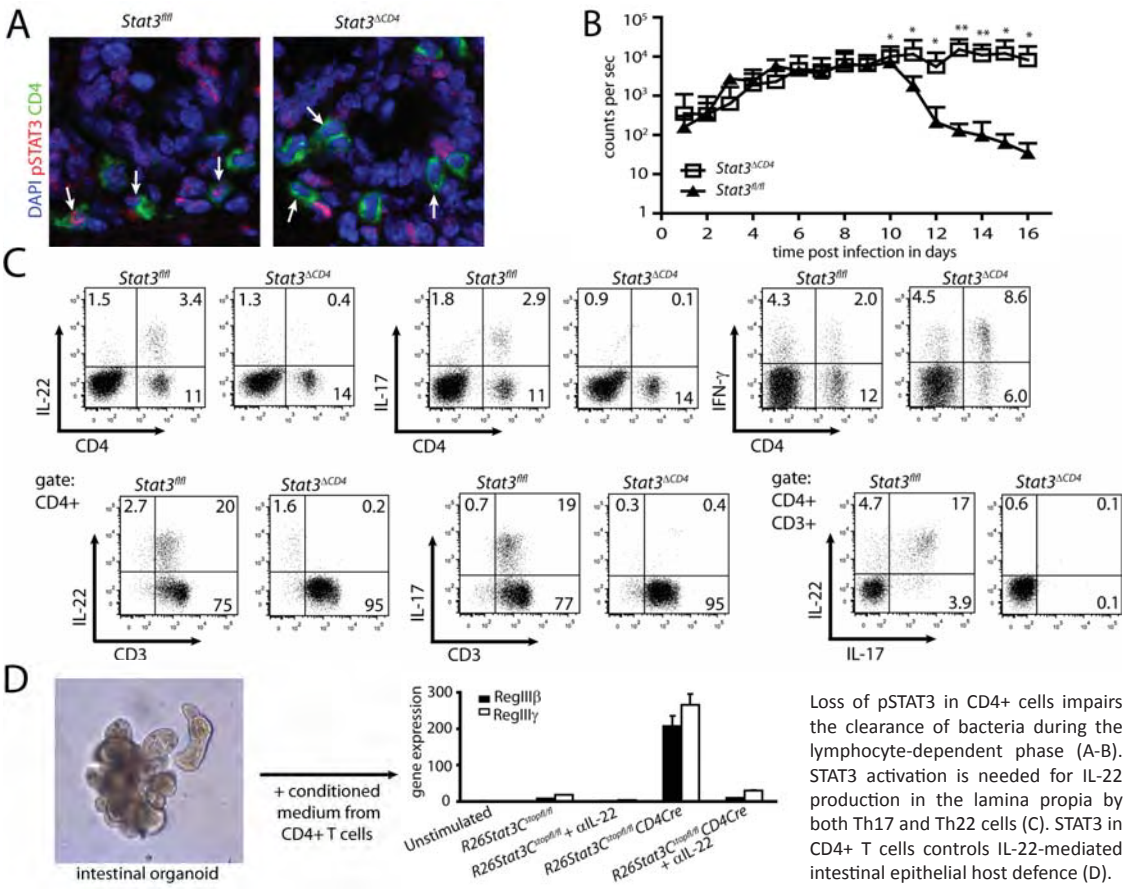
01.10.2010 - 30.09.2013

Differentiation and pathogenic role of Th17 cells in inflammatory bowel disease

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

Our work indicates a critical role of STAT3 activation in both Th17 and Th22 cells for control of the IL-22-mediated host defence in the gut. Strategies expanding STAT3 activated CD4 lymphocytes may be considered as future therapeutic options for improving intestinal barrier function in intestinal inflammation triggered by enteropathogenic bacteria.

Genome wide association studies have connected STAT3 to intestinal inflammation, and defective epithelial barrier function increases susceptibility to enteropathogenic bacteria. The C. rodentium model mimics such infection by enteropathogenic bacteria, and requires sequential contributions from various immune cell populations including CD4⁺ innate lymphoid cells and CD4⁺ lymphocytes. However, the role of STAT3 activation in CD4⁺ cells during host defense against enteropathogenic bacteria has not been clarified yet.



Loss of pSTAT3 in CD4+ cells impairs the clearance of bacteria during the lymphocyte-dependent phase (A-B). STAT3 activation is needed for IL-22 production in the lamina propia by both Th17 and Th22 cells (C). STAT3 in CD4+ T cells controls IL-22-mediated intestinal epithelial host defence (D).

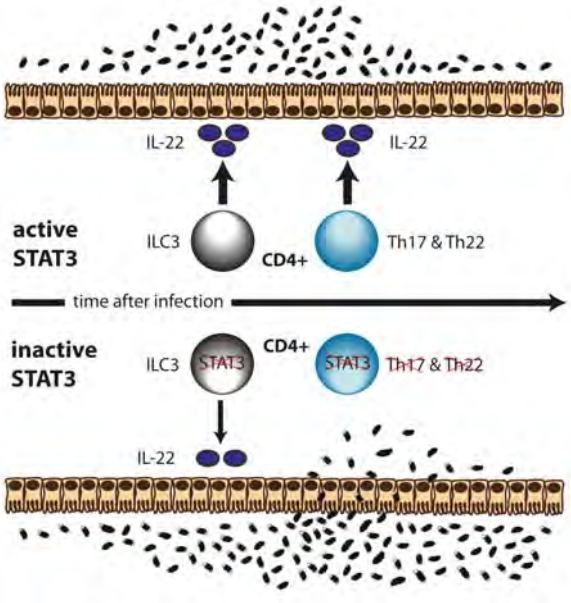

Dr. Neufert


Prof. Dr. Becker

We have analyzed the course C. rodentium infection in mice with a CD4-specific inactivation of STAT3 or with a CD4 specific constitutive activation of STAT3. Serial studies were performed by bioluminescence imaging in vivo and with immunomonitoring by flow cytometry. In addition, a recently established minicircle-vector based expression system was used for investigations into STAT3 dependent effector functions mediated by $CD4^+$ cells.

In mice with defective STAT3 in $CD4^+$ cells, the course of infection was significantly altered during the lymphocyte-dependent phase; mice exhibited marked epithelial barrier dysfunction facilitating systemic bacterial spread and impeding clearance of infection. Immunomonitoring of lamina propria cells revealed complete loss of IL-22 producing $CD4^+$ lymphocytes suggesting that STAT3 activation was necessary for IL-22 expression not only by Th17 cells, but also by Th22 cells. In contrast, IL-22 production was only partly reduced in CD3-$CD4^+$ cells. Constitutive activation of STAT3 in $CD4^+$ cells induced potent epithelial barrier function in vitro and in vivo, and promoted protection from C. rodentium.

In conclusion, our studies highlight a key role of STAT3 activation in $CD4^+$ lymphocytes for shaping epithelial host defence mechanisms, and approaches increasing the number and/or promoting the function of STAT3 activated $CD4^+$ lymphocytes may be considered as future therapeutic options in infectious.



Model for the role of STAT3-activation in $CD4^+$ cells during infectious colitis.

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Invited lectures

Annual Meeting of the German Society of Immunology (DGfI), Mainz, Germany, 11. – 14.09.2013, Plenary lecture "Cytokine regulation of intestinal barrier function" (C. Becker)

Awards

Abstract Prize 2013 of United European Gastroenterology, C. Neufert, 15.10.2013, Berlin

Publications during funding period

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A40 - Final Report

01.12.2010 - 30.11.2013

Canonical Wnt signaling in the pathogenesis of fibrotic diseases

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

Canonical Wnt signaling is activated in fibrotic diseases and stimulates fibroblast activation and tissue fibrosis. Inhibition of Wnt signaling by different genetic and pharmacologic approaches exerts potent anti-fibrotic effects in preclinical models of various fibrotic diseases. Transforming growth factor-β (TGF-β) stimulates the activation of canonical Wnt signaling by inhibiting the expression of the endogenous Wnt inhibitor Dkk-1. Activation of canonical Wnt signaling is required for TGF-β mediated fibrosis and inhibition of Wnt signaling ameliorates TGF-β induced fibrosis.

Our studies characterize canonical Wnt signaling as a key-pathway in the pathogenesis of fibrotic diseases and promising target for anti-fibrotic therapies. Canonical Wnt signaling stimulates resting fibroblasts to differentiate into activated myofibroblasts and induces fibrosis in different mouse models such as transgenic overexpression of Wnt10b, inhibition of GSK3 or fibroblast-specific overexpression of degradation-resistant β-catenin. The aberrant activation of canonical Wnt signaling in systemic sclerosis (SSc), in idiopathic pulmonary fibrosis (IPF) and in liver cirrhosis is in part mediated by overexpression of the Wnt proteins Wnt1 and Wnt10b, and in part by decreased expression of endogenous Wnt antagonists such as Dkk1. TGF-β contributes to the activation of canonical Wnt signaling by inhibiting the expression of Dkk1 in a Smad-independent, p38 dependent manner. Inhibition of TGF-β signaling prevents the decrease in Dkk1 and reduces the aberrant activation of canonical Wnt signaling in fibrotic conditions. In addition to the direct, p38 dependent inhibition of Dkk-1 transcription, TGF-β also downregulates the expression of Dkk-1 and of other endogenous Wnt inhibitors by epigenetic mechanisms. TGF-β stimulates the expression of DNA-methyltransferases and induces hypermethylation of the Dkk-1 promoter to silence Dkk-1 transcription and foster canonical Wnt signaling. TGF-β mediated activation of Wnt signaling is required for the pro-fibrotic of TGF-β as inhibition of canonical Wnt signaling significantly ameliorates TGF-β induced fibrosis.

Besides studying the molecular mechanisms underlying the increased activation of Wnt signaling in fibrosing disorders and the effects of canonical Wnt signaling on fibroblasts, we also evaluated the therapeutic potential of targeting canonical Wnt signaling in fibrotic diseases. After canonical Wnt signaling has long been considered as “undruggable”, several interesting approaches for targeted therapies have been identified during the last years. We evaluated the efficacy and tolerability of targeting tankyrases, porcupine and the interactions of β-catenin with CBP or TCF with small molecule inhibitors in different preclinical models. All inhibitors exerted potent anti-fibrotic effects in bleomycin- and TGF-β-induced fibrosis as well as in a tight-skin-1 mouse model with inhibitors of tankyrases and of porcupine being particularly effective. Wnt inhibitors demonstrated anti-fibrotic effects in preventive as well as in therapeutic settings in experimental skin fibrosis. Pharmacologic inhibition of canonical Wnt signaling is also effective in preclinical models of other fibrotic diseases such as pulmonary fibrosis and chronic sclerodermatous graft-versus-host-disease. Moreover, first studies in preclinical models demonstrate that the combined inhibition of other stem cell pathways such as hedgehog and notch signaling in addition to Wnt signaling exert additive anti-fibrotic effects and may further reduce adverse effects, e.g. on stem cell regeneration. Together, these data suggest that inhibition of canonical Wnt signaling maybe an interesting approach for targeted therapies in fibrotic diseases.

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Publications during funding period

Dees C, Schlottmann I, Funke R, Distler A, Palumbo-Zerr K, Zerr P, Lin N-Y, Beyer C, O Distler O, Schett G, Distler JH. The Wnt antagonists DKK1 and SFRP1 are downregulated by Promoter Hypermethylation in Systemic Sclerosis (2013). Ann Rheum Dis. [Epub ahead of print].

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A41 - Final Report

01.01.2011 - 31.12.2013

Inflammation in crystal arthropathies

Prof. Dr. Dr. Martin Herrmann, Department of Medicine 3 – Rheumatology and Immunology
Dr. Dr. Luis Muñoz, Department of Medicine 3 – Rheumatology and Immunology

Deposition of monosodium urate (MSU) crystals in tissues and joints causes gouty arthritis. The uptake of MSU is accompanied by release of pro-inflammatory cytokines/chemokines from granulocytes and leads to a strong inflammation in vitro and in vivo. Furthermore, MSU induce neutrophil extracellular traps (NETs) after incubation with PMN in a ROS-dependent manner: antioxidants decrease NETosis in human blood cells and in whole blood from ROS-deficient Ncf1** mice NETosis was almost abrogated.

MSU induce in vitro and in vivo a pro-inflammatory response

5x106 granulocytes per milliliter, comparable with the cell density during the early phase of a gouty attack, were incubated in autologous plasma with or without MSU crystals. 18 hours later pro-inflammatory cytokines/chemokines were quantified in the supernatants. Under these conditions a huge amount of proinflammatory cytokines (IL-1β, IL-6, TNF) and chemokines (IL-8, IP-10, MCP-1, MIP-1 β) were released.

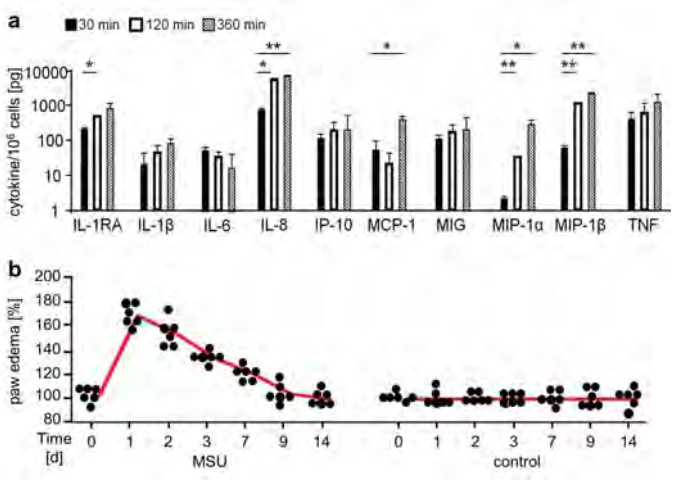
To analyze the pro-inflammatory potential of MSU in vivo we injected the crystals s.c. into the foot pads of wild type (WT) mice. This led to a rapid and strong inflammation in the paws with erythema and swelling. As comparable with an acute untreated gouty attack the inflammation induced in WT mice resolved self-dependently after approximately one week.

MSU induce the formation of neutrophil extracellular traps (NETs)

To analyze the interaction of human neutrophils with MSU we performed fluorescence microscopic video analyses. Neutrophils were incubated with MSU and the DNA of the cells was stained with Hoechst 33342. We observed the rearrangement of the neutrophils´ nuclear structures followed by a sudden and rapid cellular disintegration. NETosis upon stimulation with MSU was also confirmed by specific staining of NETs with neutrophil elastase (NE), MPO and LL37. To ensure that the changes we observed in MSU-treated neutrophils are really due to NETosis and not by necrosis, we compared and quantified morphological changes in the nuclei of neutrophils treated with MSU and a panel of known inducers of necrosis. After induction of necrosis we did not detect cloudy/filamentous structures co-stained for DNA and NE, comparable to those that could readily be observed in cells exposed to MSU.

MSU induced NETosis depends on ROS

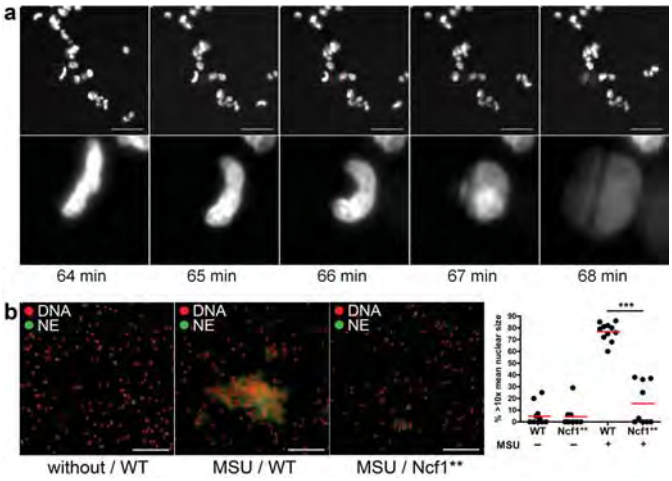
It was previously shown that NET formation induced by chemicals, various pathogens, or cytokines is strongly augmented by the presence of ROS. Since



MSU induce inflammation in vitro and in vivo
(a) 5x106 PMN/ml were incubated with MSU or PBS. Values of PBS-treated PMN are subtracted. *P < 0,05;**P < 0,01. (b) MSU or PBS were injected s.c. into the foot pad of WT mice. One dot represents one mouse.

Prof. Dr. Dr. Herrmann

Dr. Dr. Muñoz



(a) PMN + MSU stained with DAPI. Pictures show remodeling of PMNs´ nuclear structures and release of NETs. (b) Analysis of NETting cells in blood from oxidative burst-deficient Ncf1** and WT mice. Scale bars, 200 µm.

we observed that MSU is a potent inducer of NETs, we analyzed if MSU-initiated NETosis is accompanied by and dependent on the formation of ROS in human whole blood ex vivo cultures. Already 30 min after the addition of the crystals ROS was to be detected and reached its maximum after 4.5h. The incubation of human whole blood cultures with MSU resulted in the formation of NETs containing extranuclear DNA as detected by DAPI staining. The ex vivo culture of human whole blood with the antioxidants butylated hydroxytolune, butylated hydroxytolune and ascorbic acid clearly reduced the sizes of the NETs formed by blood granulocytes in the presence of >90% plasma.

Ncf1** mice carry a single mutation in Neutrophil cytosolic factor (Ncf) 1, a regulatory subunit of the NOX2 complex, that completely abrogates production of ROS by NOX2. WE analyzed the MSU induced NETs formation ex vivo in whole blood cells from Ncf1** mice compared to WT mice. The formation of NETs was drastically reduced in blood cells from ROS-deficient Ncf-1** mice.

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Invited lectures

3rd Forum of oriental gout, 6.9.2013, Nangchong, China, Mononuclear Phagocytes and Granulocytes contribute to MSU- crystal-induced inflammation

Annual Meeting of Taiwan Rheumatology Association, 7.12.2013, Hsinchu, Taiwan, Mononuclear Phagocytes and Granulocytes contribute to MSU- crystal-induced inflammation

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Frischholz B, Wunderlich R, Rühle PF, Schorn C, Rödel F, Keilholz L, Fietkau R, Gaipl US, Frey B (2013) Reduced secretion of the inflammatory cytokine IL-1β by stimulated peritoneal macrophages of radiosensitive Balb/c mice after exposure to 0.5 or 0.7Gy of ionizing radiation. Autoimmunity. 46(5):323-8

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A42 - Final Report

15.10.2010 - 14.04.2013

Phenotypical and functional characterization of human NK cell subsets in different organs

Prof. Dr. Evelyn Ullrich, Department of Medicine 5 – Haematology and Oncology

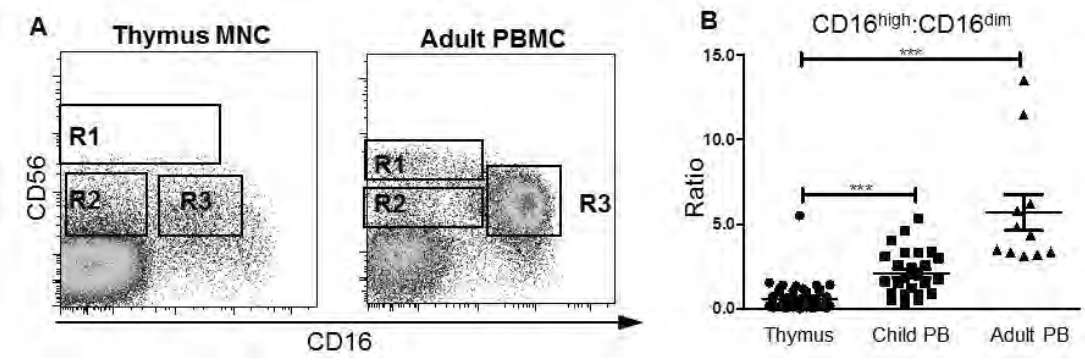
Natural Killer (NK) cells are a heterogeneous population of immune cells with cytotoxic capacity and multiple immunoregulatory properties. In this study, we performed an extensive phenotypical, genomic and functional characterization of human NK cell subsets from different organs. The specific aim of this was project the functional analysis of children thymic NK cells in comparison to peripheral blood NK cells from children and adults.

NK cell subset isolation from human thymus

During the whole funding period of this IZKF project, more than 200 preparations of human thymic tissues removed during pediatric heart surgery from children with different age have been performed. Our study aimed to characterize the detailed phenotype, genotype and functional aspects of thymic NK cell subsets in direct comparison to peripheral blood from children and adults and revealed important differences concerning the NK cells subsets and their distribution. Peripheral blood NK cells are classified according to the expression of CD56 and CD16 mostly into two different subpopulations, immunoregulatory, cytokine-producing $CD56^{high}CD16^{dim}$ and cytotoxic $CD56^{dim}CD16^{high}$ NK cells. It is widely accepted that NK cell development takes place in secondary lymphoid tissues leading to the maturation from $CD56^{high}CD16^{dim}$ into $CD56^{dim}CD16^{high}$ NK cells. In thymic tissue, NK cells did not only express lower levels of CD56, there was also an absence of the $CD56^{high}CD16^{dim}$ NK cell subset and an accumulation of a so far not well described third NK cell subpopulation ($CD56^{dim}CD16^{dim}$).

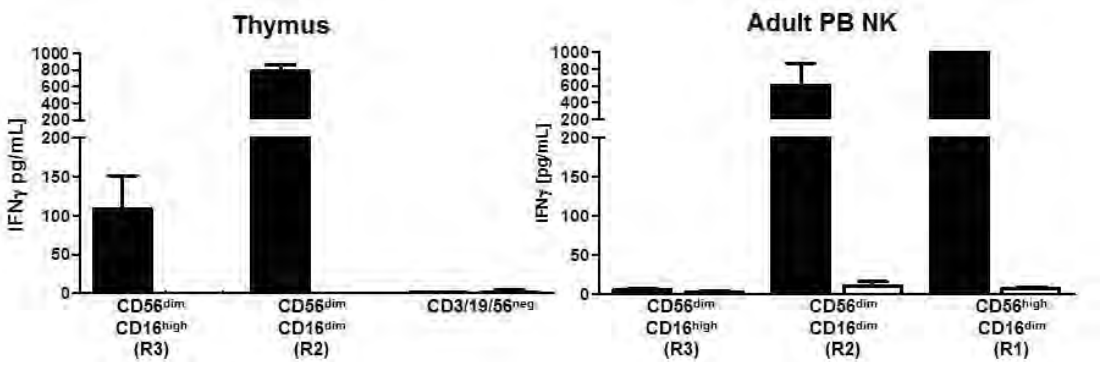
Specific phenotype of thymic NK cell subsets

The phenotypical analyses of thymic and peripheral blood NK cells displayed a significant downregulation of Killer-cell Immunoglobulin-like receptors (KIRs; CD158a/h, CD158b and CD158e/k) and some Natural Cell Cytotoxicity Receptors (NCRs; NKp30 and NKp46) as well as other activating receptors (NKG2D) on either one or both thymic NK cell subpopulations compared to peripheral blood NK cells. On the other hand, another NCR (NKp44) and the inhibitory co-receptor NKG2A were up-regulated on thymic NK cells. Of note, some of those markers further displayed an age-dependency on thymic NK cells.



(A) Representative flow cytometry of thymus MNC (left) and adult PBMC (right) used to identify three NK cell subsets (R1-3) inbetween all CD3/CD19-negative cells.. (B) Scatter blot showing ratios of $CD16^{high}$ (R3) to $CD16^{dim}$ (R1+R2) NK cells of child thymus, child PB and adult PB.

Prof. Dr. Ullrich



IFN-γ production of thymic NK cell subsets (left panels) and adult PB NK subsets (right panels) cultivated for 72 hours without (white) or with addition of 1000 U/ml IL-2 (black).

Functional comparison of NK cell subsets isolated from thymus and peripheral blood

Functional analyses showed remarkable differences between the thymic NK cell subsets concerning IFN-γ production and killing capability. Both IFN-γ production and cytotoxicity were significantly reduced in freshly isolated thymic NK cells compared to peripheral blood NK cells. Interestingly, IFN-γ secretion as well as cytotoxicity of thymic NK cell subsets could be triggered by a prolonged stimulation period with 1000 U/ml IL-2.

In summary, this comparative study of NK cell subsets provides important information on the NK cell tissue distribution in human organs and may be of great value for the understanding of NK cell development and for the further improvement of NK cell therapeutic concepts.

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Invited lectures

15th International Congress of Immunology, 08/2013, Milano, Italy, "NK cell subsets isolated from human thymus differ from peripheral blood NK cells in their phenotype and their cytotoxic and cytokine secreting capabilities"

Awards

Award for the best oral presentations at the 18th World Congress on Advances in Oncology, dedicated to Evelyn Ullrich, 10/2013, Crete, Greece.

Publications during funding period

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A43 - Progress Report

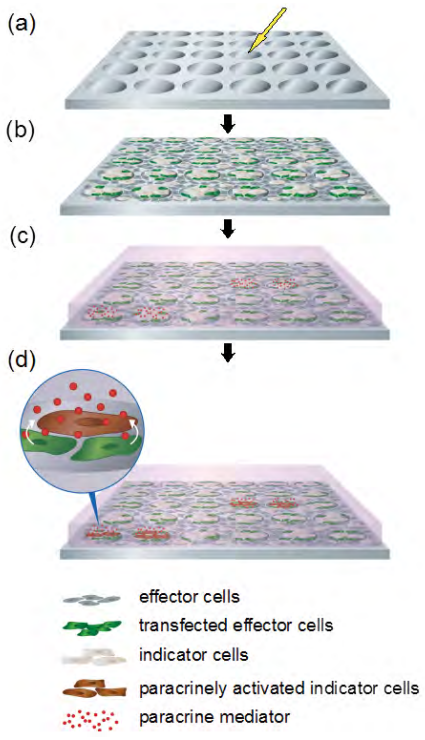
15.02.2011 - 14.02.2014

Paracrine gene functions in Kaposi‘s sarcoma

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

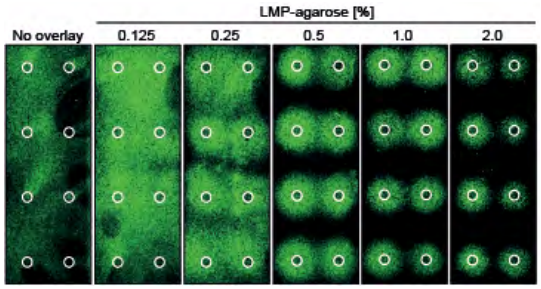
Kaposi’s sarcoma-associated herpesvirus (KSHV) is the etiologic agent of Kaposi’s sarcoma (KS), an endothelial cell-derived tumour. Previous in vivo observations indicated that paracrine effects from infected cells on none-infected cells may constitute a prominent driving force of tumor growth. The aim 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 analyses 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 analyses of all available lots of SLK cells including those from 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



Scheme of the Parachip. (a) Slide with transfection spots (arrow). (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.

be present in increased concentrations in the cell culture supernatants 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.

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A44 - Final Report

01.10.2010 - 31.03.2013

Platelets and transplant arteriosclerosis

Prof. Dr. Stephan Ensminger, Department of Cardiac Surgery

This project investigates interactions of human platelets with human endothelium during the development of transplant-arteriosclerosis in a humanised mouse model. Human mammary arteries are implanted in Rag2-/-, yc-/- mice which are reconstituted with human platelets and different subsets of leucocytes. Therefore, we are able to analyse platelet effector-mechanisms and interactions with allogeneic endothelial cells in the absence of T, B and NK cells and their impact on chronic rejection.

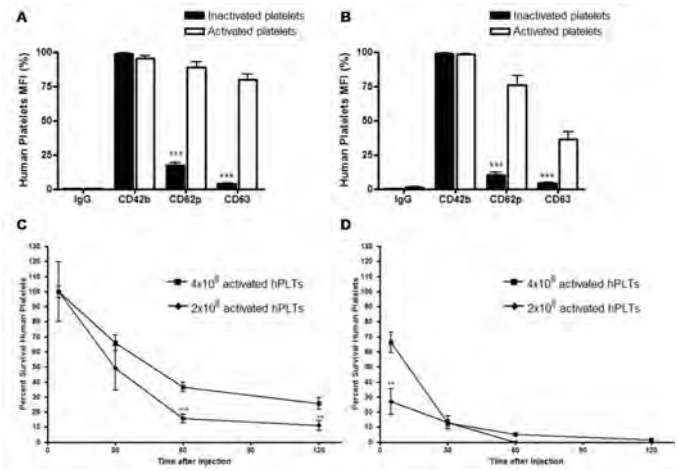
During the last year a reliable platelet transfusion protocol was established. Thereby we are now able to ensure that phenotyped human platelets (hPlts) are circulating within the peripheral blood of the Rag2-/-, γc-/- mice during the entire experimental protocol.

Activation status of human platelets

In a first step hPlts were analysed and phenotyped for a variety of surface markers including CD62p and CD63 by flow cytometry to get detailed information about their activation status. The platelets had low levels of CD62p (+/- 20%) as well as low levels of CD63 (+/- 5%). In order to differentiate activated platelets from quiescent platelets, TRAP6 was used for stimulation of hPlts. After TRAP6 stimulation, expression of CD62p and CD63 on hPlts was markedly increased (CD62p +/- 89%; CD63 +/- 80%) and therefore revealed the phenotype of activated platelets.

In-vivo recovery of hPlts in Rag2-/-, γc-/- mice

Activated or inactivated hPlts (2x108 in 100µl and 4x108 in 200µl) were injected into the lateral tail vein of Rag2-/-, γc-/- mice. At predetermined time points (5, 30, 60 and 120 minutes) after transfusion, whole blood of mice was collected and the human platelet recovery within the circulation was determined by flow cytometry. Human and mouse platelets in mouse whole blood were labeled with monoclonal anti-human CD41a-APC- and anti-mouse CD42b-FITC-conjugated antibodies. The recovery of inactivated hPlts at 5 minutes after injection was set as 100% recovery. At 5, 30, 60 and 120 minutes the inactivated hPlts (4x108) had recoveries of +/- 100%; +/- 65,62; +/- 36,71% and +/- 25,73%. In contrast the activated hPlts (4x108) at 5, 30, 60 and 120 minutes after injection had recoveries of +/- 66,39%; +/- 12,66; +/- 5,06% and +/- 1,48%. The inactivated and activated hPlts with a concentration of 2x108 had fewer recoveries. According to this we decided to use a daily injection of 4x108 inactivated or activated hPlts in implanted Rag2-/-, γc-/- mice.



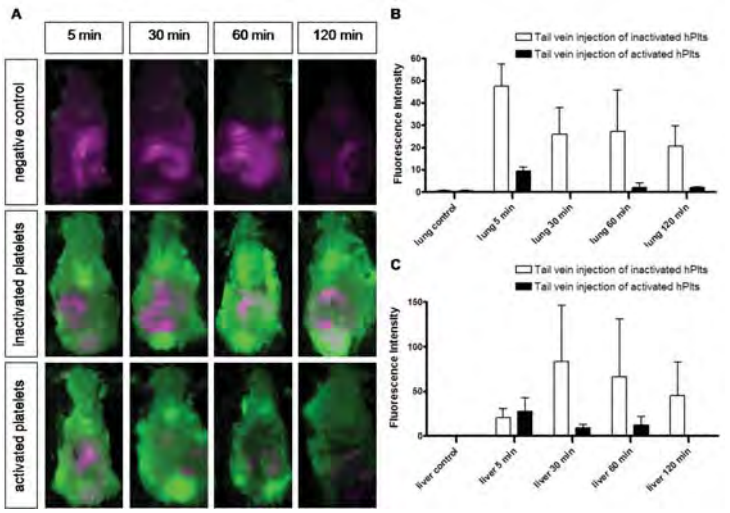
Activation status of human platelets (hPlts) (A) Without DCF (B) With DCF. Injection of hPlts into the lateral tail vein of Rag2-/-, γc-/- mice (C) Survival rate of inactivated hPlts (D) Survival rate of activated hPlts.

In-vivo fluorescence imaging of hPlts

In addition it was necessary to label the hPlts, in order to trace them by in-vivo fluorescence imaging. Therefore we used the fluorescent dye called 2'-7' dichlorofluorescein (DCF). This protocol resulted

Prof. Dr. Ensminger

in significant improved in vivo tracking of the injected hPlts as well as only minor interference with the platelet activation status. According to this we were able to detect the localization of intravenously injected platelets. The DCF-labeled hPlts (4x108) were injected into the lateral tail vein of mice and detected with an in-vivo fluorescence imager. At 5, 30, 60 and 120 minutes after injection of inactivated hPlts the fluorescence intensity of liver and lung decreased less than after injection of activated hPlts. Especially after 30 minutes we detected a major difference between the fluorescence signals of activated and inactivated hPlts.



In-vivo Imaging of DCF-labeled activated or inactivated hPlts (green fluorescence) at predetermined time points after lateral tail vein injection (A) Whole body image of injected mice (B) Fluorescence intensity lung (C) Fluorescence intensity liver.

Outlook

Finally, the first group of implanted Rag2-/-, γc-/- mice which has been reconstituted daily with human inactivated platelets is currently analysed and the still remaining in vivo experiments are performed. We are confident to have them finished until end of August 2013.

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Publications during funding period

Goldmann K, Hoffmann J, Eckl S, Spriewald BM, Ensminger SM (2012) Attenuation of transplant arteriosclerosis by oral feeding of major histocompatibility complex encoding chitosan-DNA nanoparticles. Transpl Immunol. 2012 Dec 5. pii: S0966-3274(12)00130-X.

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A45 - Final Report

01.11.2010 - 30.10.2013

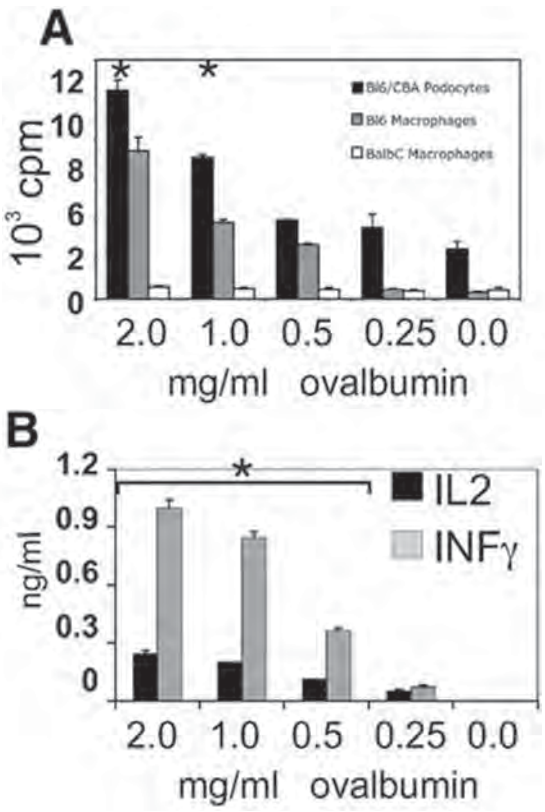
Podocytes as non hematopoetic professional antigen presenting cells

Prof. Dr. Kerstin Amann, Department of Nephropathology

Podocytes are highly differentiated epithelial cells of the kidney, which can present antigen and can initiate a specific T cell response in vitro and in vivo. As previously shown only for hemopoietic cells, podocytes have the capability to activate naive T cells by MHC class I, MHC class II and crosspresentation. Thus, podocytes may represent novel targets for immunotherapy of inflammatory kidney diseases and potentially also for prevention of kidney rejection.

Podocytes Activate Naive OT-II Cells

We addressed the question of whether proteins taken up by podocytes were processed as peptide–MHC complexes for presentation to T cells. Podocytes loaded with ovalbumin induced proliferation of ovalbumin-specific CD4+ T cells in a dose-dependent manner. OT-II T cells also upregulated the activation marker CD25. In addition to undergoing activation and proliferation, the CD4+ T cells secreted the Th1 cytokines IL-2 and IFN-γ. We next asked whether podocytes could also activate CD8+ T cells. In the mixed lymphocyte reactions performed, podocytes were also able to activate allogeneic CD8+ T cells. Interestingly, podocytes mainly activated allogeneic CD8+ T cells, whereas their capacity to activate CD4+ T cells was markedly lower. Because the podocytes were generated from CBA ($H2^k$) × C57BL/10 ($H2^b$) mice, we were able to analyze the activation of alloreactive cells and ovalbumin-reactive T cells in a mixture of unsorted spleen cells from OT-II transgenic C57BL/6 ($H2^b$) mice simultaneously in one experimental setting. In the presence of ovalbumin, a very strong allotypic reaction of the Vα2-negative nontransgenic cells, together with the ovalbumin-specific activation of the transgenic Vα2-positive OT-II T cells, was detected as CD62L down- and CD69 upregulation on the T cells. In contrast, in the absence of ovalbumin, we detected only an allospecific reaction. Podocytes activate CD8+ T cells by MHC I presentation. LPS-activated bone marrow DCs, BMMs, or podocytes were used. In all experiments, podocytes were more potent activating naïve CD8+ T cells than macrophages, and bone marrow DCs were superior to podocytes and additionally able to activate allogeneic CD4+ T cells.



Podocytes activate CD4+ T cells by MHC II presentation. (A) podocytes induced proliferation of ovalbumin-specific MHC class II-restricted CD4+ T cells from OT-II mice.(B) Supernatants were collected after 48 hours and analyzed for IL-2 and IFN-γ expression by ELISA.

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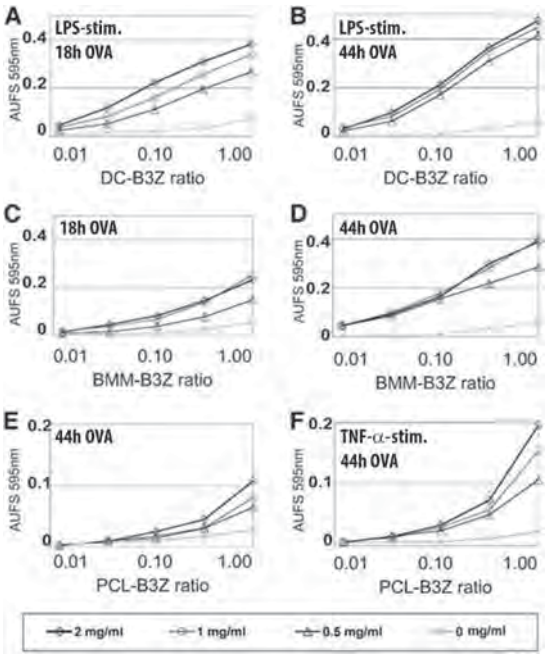
Crosspresentation by Podocytes

We also wanted to know if podocytes are able to take up exogenous antigen and crosspresent it to CD8+ T cells. For comparison, DCs and BMMs were equally loaded with different concentrations of ovalbumin and cocultivated with a mouse T cell hybridoma line, which sensitively recognizes H2Kb loaded with the ovalbumin peptide. LPS-stimulated DCs were very potent activators of antigen-specific CD8+ T cells. Antigen crosspresentation by BMMs was less potent than for DCs. We also observed antigen crosspresentation by podocytes showing a lower capacity than observed for DCs and BMMs, but it was significantly enhanced by the addition of TNF-α. Toll-like receptors (TLRs) are essential in DCs for sensing microbial molecules and consequently, inducing maturation. We found that podocytes expressed high levels of mRNA for almost all TLRs. Furthermore, we found that six mostly T cell-attracting chemokines were expressed by PCL cells. Interestingly, the subunits of the immunoproteasome (Lmp-2 and Lmp-7) and two proteins essential for efficient crosspresentation (nox-2 and pg91) are also expressed by podocytes.



Podocytes activate T cells by crosspresentation. Crosspresentation and activation of the ovalbumin-specific H2-K^b–restricted T cell hybridoma B3Z. DCs, BMMs, or podocytes (PCLs) were incubated with different concentrations of ovalbumin.

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Publications during funding period

Goldwich A, Burkard M, Olke M, Daniel C, Amann K, Hugo C, Kurts C, Steinkasserer A, Gessner A (2013) Podocytes are nonhematopoietic professional antigen-presenting cells. J Am Soc Nephrol. May; 24(6): 906-16

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A46 - Progress Report

20.01.2011 - 19.01.2014

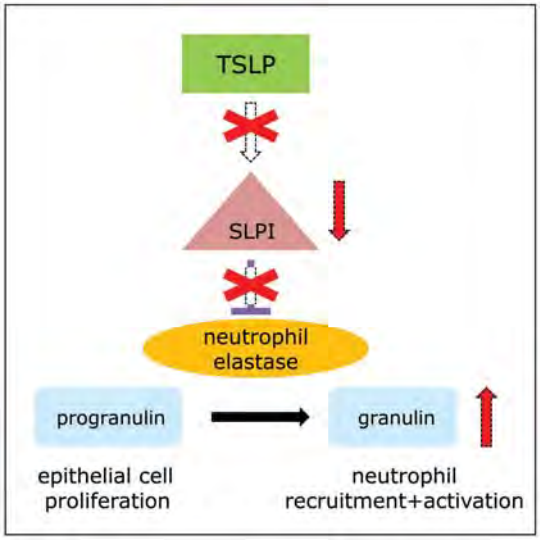
Relevance of TSLP in the immune response

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.

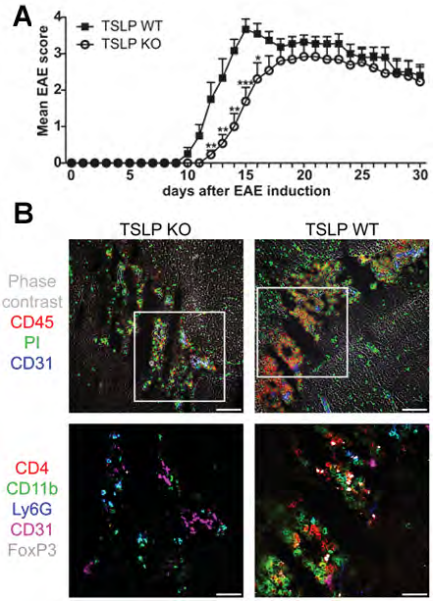
To clarify the role of Tslp, we generated a Tslp knockout (ko) mouse. First, we analyzed this mouse in different murine disease models of colitis. Strikingly, we could show that Tslp is essential for recovery following colitis. Our data showed that Tslp functions as a critical mediator controlling the 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).



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

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-Cartography (MELC) technique. The MELC-technique was adapted to identify a large panel of murine leukocyte subpopulations in a whole frozen section to compa-

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.

re 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.

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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.

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*: These authors contributed equally to this work

A48 - Final Report

01.01.2011 - 31.12.2013

Development of new diagnostic tools for primary biliary cirrhosis

Prof. Dr. Jochen Mattner, Institute of Microbiology - Clinical Microbiology, Immunology and Hygiene

Autoimmunity is driven by complex interactions of environmental factors with genetic traits. Primary biliary cirrhosis (PBC) has been associated with the ubiquitous alphaproteobacterium Novosphingobium (Novo) and allelic variations within Cd101. Thus, we will evaluate whether PBC patients suffer from chronic Novo-infections by analyzing immune responses against different Novo antigens. We will also define the regulation of CD101 expression in PBC patients compared to healthy individuals.

Identification of Novo-antigens, which are specifically recognized by the sera of PBC patients

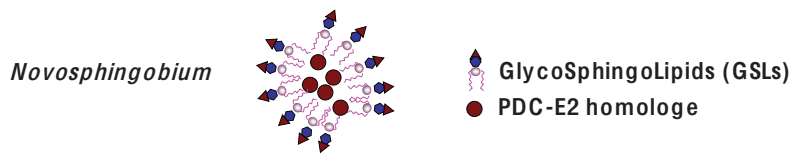
We utilized western blot analysis for testing the reactivity of serum antibodies from PBC patients and healthy individuals against protein and lipid extracts of Novo. Antigen-specific immune responses were detected by using monoclonal antibodies recognizing human IgG bound to antigens on the western blot membrane.

Sera of PBC patients reacted to various protein antigens of Novo including the Novo PDC-E2 homologue, the signature antigen in PBC and flagellin. As most of the protein antigens elicited unspecific immune reactions, we chose to test the serum-reactivity of PBC patients to the GlykoSphingoLipid (GSL) antigens of Novo that substitute for lipopolysaccharide (LPS) in the Novo cell wall. Although PBC patients, but not healthy individuals reacted against these unique Novo cell wall compounds, patients with other inflammatory disorders of the gastrointestinal tract exhibited immune reactions against these GSL and other polysaccharide antigens as well. In order to define the immune reactions of different patient populations against intestinal bacterial GSL and polysaccharide antigens, we have been utilizing a novel glycan array in collaboration with Prof Seeberger and colleagues at the Max Planck Institute in Potsdam.

Evaluation of CD101 expression levels in PBC patients and its correlation with functional phenotypes.

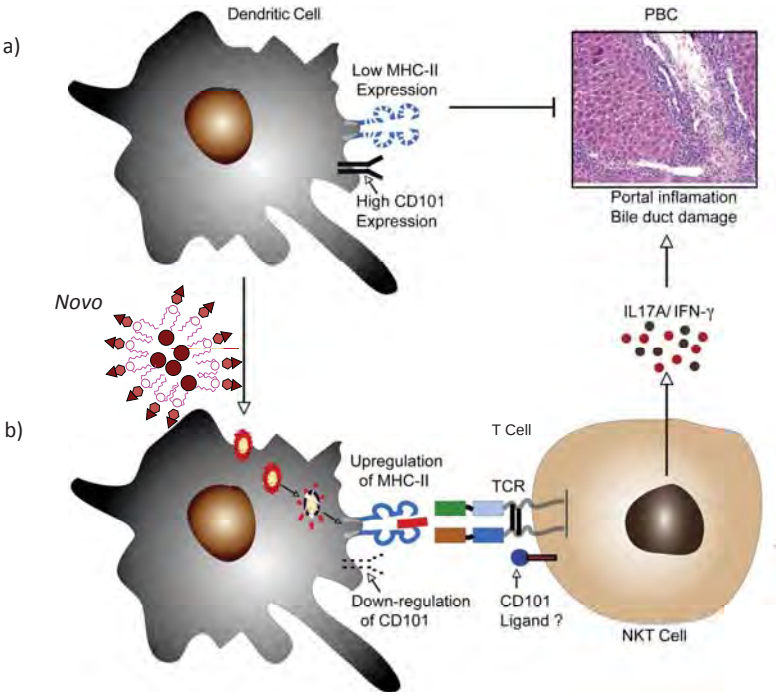
CD101 is a negative costimulatory molecule that is expressed on different subsets of T cells, macrophages, monocytes and dendritic cells (DCs) as well as on all mature granulocytes.

Similarly as in our infection-induced mouse model of PBC, we observed reduced expression levels of CD101 on monocytes, DCs and granulocytes in the peripheral blood of PBC patients compared to healthy individuals by flow cytometry. In contrast to mice, where CD101 expression on T cells is preferentially restricted to regulatory T cells and memory T cells, CD101 expression was more widely distributed among human T cell populations. PBC patients revealed also a reduced expression of CD101 on T cells in contrast to the mouse model system in which Novo-infection did not modulate the number of CD101-positive T cells. However, in mice and in humans, T cells from infected and diseased individuals showed enhanced T cell responses to different Novo antigens, correlating with the reduced expression levels of CD101.



Novosphingobium express highly conserved PDC-E2 molecules and unique GSL antigens. The intracellular PDC-E2 homologues and the cell wall GSL compounds are displayed in a schematically represented Novo bacterium.

Prof. Dr. Mattner



Cd101 regulates the susceptibility to Novo-induced liver disease.
a) The resistance Cd101 allele stabilizes CD101 expression and promotes tolerogenic T cells.
b) The susceptibility Cd101 allele triggers biliary disease due to the suppression of CD101.

We detected not only decreased CD101 expression levels in the peripheral blood, but also at the site of inflammation in internal organs. These exciting observations suggesting CD101 as a biomarker for visceral inflammatory diseases form also the basis for the continued funding of the project by the DFG (DFG MA 2621/3-1).

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Publications during funding period

Mattner J (2013) Natural killer T (NKT) cells in autoimmune hepatitis. Curr Opin Immunol. 25:697-703.

Danzer D, Mattner J (2013) Impact of microbes on autoimmune diseases (2013) Archivum Immunologiae et Therapiae Experimentalis. 61:175-186.

Mohammed JP, Fusakio ME, Rainbow DB, Moule C, Fraser HI, Clark J, Todd JA, Peterson LB, Savage PB, Wills-Karp M, Ridgway WM, Wicker LS , Mattner J (2011) Identification of Cd101 as a susceptibility gene for Novosphingobium aromaticivorans-induced liver autoimmunity. J. Immunol. 187:337-349

Rainbow DB, Moule C, Fraser HI, Clark J, Howlett SK, Burren O, Christensen M, Moody V, Steward CA, Mohammed JP, Fusakio ME, Masteller EL, Finger EB, Houchins JP, Naf D, Koentgen F, Ridgway WM, Todd JA, Bluestone JA, Peterson LB, Mattner J, Wicker LS (2011) Evidence that Cd101 is an autoimmune diabetes gene in nonobese diabetic mice. J. Immunol.187:325-336.

A49 - Final Report

01.03.2011 - 31.08.2013

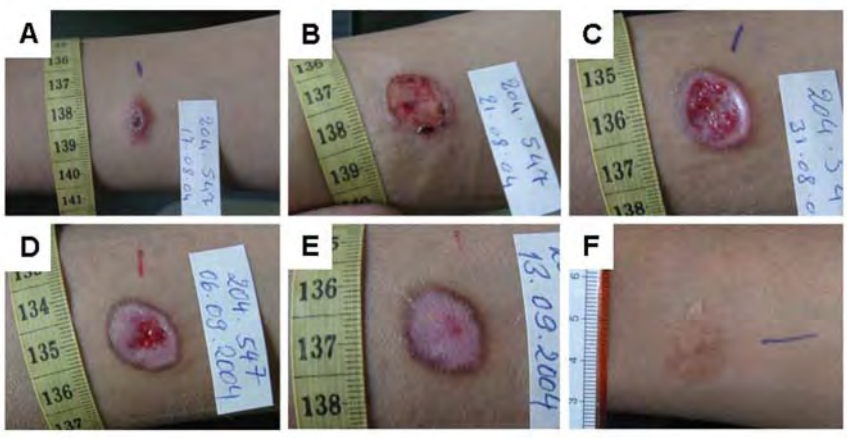
Therapy of cutaneous leishmaniasis with sodium chlorosum

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

Therapy of chronic and slowly healing skin lesions of Old World cutaneous leishmaniasis by local application of pharmaceutical sodium chlorite (sodium chlorosum, DAC N-055) after removal of necrotic tissue led to a strongly accelerated wound healing. Based on these clinical observations possible anti-parasitic, immunomodulating and/or wound healing effects of DAC N-055 have been investigated in vitro and in vivo in order to establish its mechanism of action.

In two clinical trials we performed in Afghanistan, the healing of skin lesions (ulcers) of patients with cutaneous leishmaniasis (CL) was more rapid after moist wound treatment (MWT) with DAC N-055 following removal of necrotic tissue by electrocauterization (EC) compared to the standard intralesional sodium stibogluconate (SSG) injections. Especially patients with high parasite numbers showed a clear benefit from the treatment with DAC N-055. Under acidic conditions (pH<6) as they exist in the skin, in the presence of heme (Fe^{3+}), or after exposure to UV radiation the chlorite and/or the chlorine peroxide compound contained in DAC N-055 may release ClO_2 and/or singlet oxygen, both of which are strong microbicidal oxidants. Besides its direct leishmanicidal effect on extracellular promastigotes of several *Leishmania* species, DAC N-055 caused a concentration-dependent arrest in the proliferation of intracellular amastigotes in infected human peripheral blood mononuclear cells (PBMC) enriched for monocytes, but not in mouse bone marrow-derived macrophages (BMMφ).

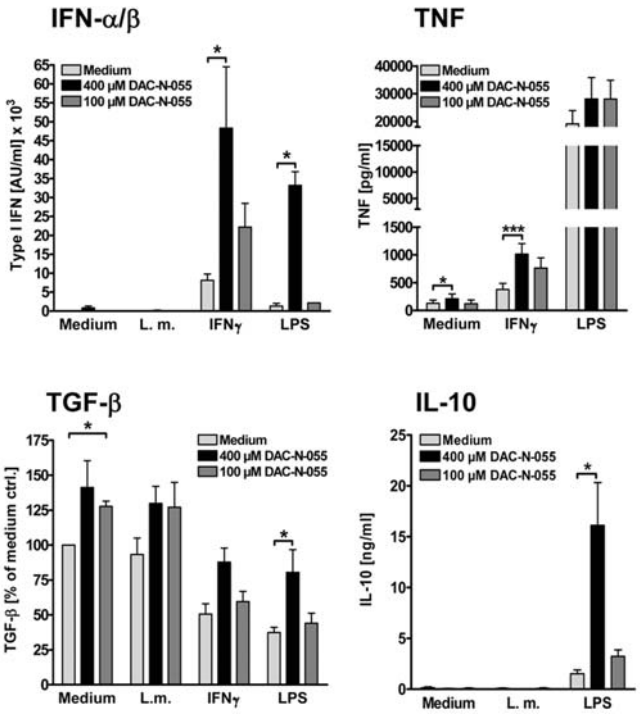
To elucidate the immunoregulatory activities of DAC N-055 which might contribute to the acceleration of the wound healing, effects on myeloid cells were investigated. *In vitro*, DAC N-055 was able to augment the production of inflammatory cytokines (e.g. tumor necrosis factor [TNF], interferon [IFN]-αβ, interleukin [IL]-6) by different immune cells including mouse BMMφ, mouse bone marrow-derived dendritic cells (BMDC) or human PBMC either on its own or in synergism with a second stimulus such as IFN-γ. In contrast, production of IL-12 by BMDC was not affected by DAC N-055. In addition, DAC N-055 promoted the transcription of inducible nitric oxide synthase (iNOS) in IFN-γ-stimulated mouse BMMφ which was independent of IFN-α/β-signaling. The enhanced production of IFN-α/β following exposure to DAC



Wound healing in a patient with cutaneous leishmaniasis treated with moist wound care including DAC N-055 after removal of necrotic tissue by electrocauterization

PD Dr. Schleicher

Prof. Dr. Bogdan



Up-regulation of pro- and anti-inflammatory cytokines by DAC N-055 in mouse bone marrow-derived macrophages cultured in medium or stimulated by *L. major* promastigotes (*L.m.*), IFN-γ or LPS

N-055 was not related to an increased activation of IRF transcription factors (IRF1, 3, 7). We neither observed a boost in the NF-κB activation nor accelerated phosphorylation of STAT transcription factors in DAC N-055-treated myeloid cells, but the immunomodulatory effects of DAC N-055 in BMMφ described above were partially dependent on MAP kinases (ERK, p38, JNK). To clarify, which chemical compound of DAC N-055 might be involved in its immunomodulatory capacities, chemicals such as NaClO, $NaClO_2$ and H_2O_2 were tested. Since $NaClO_2$ showed similar effects as DAC N-055 we concluded that the DAC N-055-induced immunoregulatory phenotype is at least partially due to sodium chlorite. Interestingly, DAC N-055 not only increased the production of inflammatory cytokines, but simultaneously also promoted the release of anti-inflammatory cytokines such as IL-10 or tumor growth factor (TGF)-β. To get a broader idea of the activities of DAC N-055 gene array analyses have been performed, which are currently under evaluation.

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Invited lectures

Bogdan C. Chemical Aspects of the Immunological Control of Leishmania Parasites. Ringberg Conference on the Chemistry and Biology of Carbohydrate Vaccines, Max Planck-Society, Rottach-Egern, October 14-18, 2013.

Publications during funding period

Jebran A*, Schleicher U*, Steiner R, Wentker P, Mahfuz F, Stahl H-C, Amin F, Bogdan C**, Stahl K.-W** (2014) Rapid Healing of Cutaneous Leishmaniasis by High-Frequency Electrocauterization and Hydrogel Wound Care with or without DAC N-055: A Randomized Controlled Phase IIa Trial in Kabul, PLoS Neglected Tropical Diseases, accepted for publication (Dec 27, 2013).

**shared first authorship, **shared senior authorship

Anish C, Martin CE, Wahlbrink A, Bogdan C, Ntais P, Antoniou M, Seeberger PH (2013) Immunogenicity and diagnostic potential of synthetic antigenic cell surface glycans of Leishmania. ACS Chem Biol 8(11): 2412-2422.

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Wiese M, Gerlach RG, Popp I, Matuszak J, Mahapatro M, Castiglione K, Chakravortty D, Willam C, Hensel M, Bogdan C, Jantsch J (2012) Hypoxia-mediated impairment of the mitochondrial respiratory chain inhibits the bactericidal activity of macrophages. Infect. Immun. 80(4): 1455-1466

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A50 - Final Report

01.10.2010 - 31.03.2013

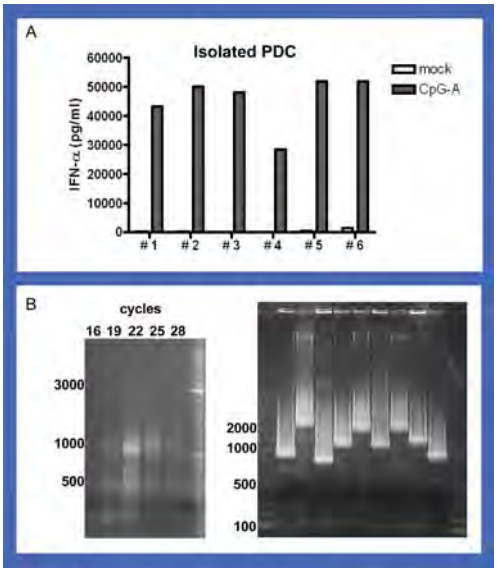
New antiretroviral restriction factors

Prof. Dr. Barbara Schmidt, Institute of Clinical and Molecular Virology (till 30.09.2012),
University of Regensburg, Institute of Medical Microbiology and Hygiene (from 01.10.2012)

Plasmacytoid dendritic cells (PDC), the major producers of type I interferons (IFN), can be infected by HIV-1, but do not support virus replication unless exposed to CD40 ligand (CD40L). The enhanced CD40:CD40L interaction silences IFN-alpha production in HIV-1 infection in vitro and in vivo. To identify potential restriction factors in these cells, we aimed to construct a PDC library. Using a chip array of CD40L-exposed PDC, we investigated the mechanism of IFN-alpha silencing in more detail.

HIV-1 restriction factors

Until now, five retroviral restriction factors have been identified, three of them counteracted by viral accessory proteins: APOBEC-3G (vif) (2002), TRIM5-alpha (2004), Tetherin (vpu) (2008), SAMHD1 (vpx) (2011), and Schlafen 11 (2012). These factors act at different stages of the HIV-1 replication cycle, starting from uncoating and reverse transcription and ending with budding of newly synthesized viral particles. We showed that PDC, the major producers of type I IFN in the blood, can be infected by HIV-1, but viral replication is severely impaired. Notably, virus replication can be enhanced by exposure of PDC to CD40 ligand (CD40L), a costimulatory member of the tumor necrosis factor family. CD40L is upregulated upon acute and chronic immune activation. Therefore, we hypothesized that PDC harbor (new) HIV-1 restriction factors, which are downregulated upon exposure to CD40L.



Construction of the PDC library. (A) PDC were cultivated with(out) CpG-A for 6h. (B) Using the In-Fusion SMARTer cDNA library construction kit, the cDNA was cloned into pSMART2IF. Sizes between 500-2000 bp confirmed the integrity of the library.

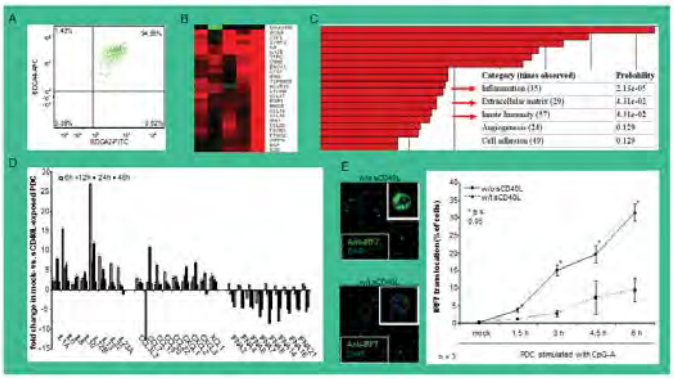
Construction of a PDC library

To clone the PDC transcriptome, PDC were isolated from six healthy donors. The cells were mock stimulated or exposed to an agonist of Toll-like receptor 9 (CpG-A) for six hours. The cDNA was amplified using the In-Fusion SMARTer cDNA library construction kit and subsequently cloned into the pSMART2IF vector. Sequencing of individual clones revealed expected fragment sizes of 500-2000 bp, confirming the integrity of the library. We planned to clone this library into the pRetroLib vector, which would have resulted in recombinant viruses, each expressing a single PDC transcript. Unfortunately, several approaches failed, including direct cloning of the PDC transcriptome into pRetroLib, amplification of the pSMART2IF library by PCR as well as restriction digestion of the pSMART2IF library with subsequent cloning into pRetroLib. Current efforts focus on cloning single promising candidate genes into pRetroLib, which will then be tested for susceptibility or resistance to HIV-1 infection.

CD40L silences IFN-alpha production in HIV-1 infection

To investigate the effect of CD40L on the PDC transcriptome, we performed an Agilent Whole Human Genome Oligo 4x44 v2 Microarray (Miltenyi Biotec) of PDC cultivated with or without CD40L for 6-12-24-48h. Genes involved in inflammation and extracellular matrix were upregulated upon expo-

Prof. Dr. Schmidt



Effects of CD40L. CD40L-exposed PDC of controls (A) were subjected to a chip array (B). Functional grouping (C) revealed a dichotomous regulation of the NKkB and IFN pathway (D). CD40L significantly reduced translocation of IRF7 into the nucleus (E).

sure to CD40L, whereas type I IFNs were selectively downregulated. The upregulation of proinflammatory cytokines, in particular IL-6, was also shown on the protein level. These data suggest a dichotomous regulation upon exposure to CD40L, namely upregulation of the NFkB pathway and downregulation of the IFN pathway. Realtime analyses showed downregulation of IRF7, a key regulator of the IFN pathway, but only to a minor extent. However, the translocation of IRF7 into the nucleus was significantly reduced upon exposure to CD40L. Thus, our data support a model in which the chronic immune stimulation in HIV-1 infection silences IFN-alpha production via CD40L, and thus contributes to the immunopathogenesis of AIDS.

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Invited lectures

23rd Annual Meeting of the Society of Virology, 7.03.2013, Kiel, "CD40 ligand silences alpha interferon production via reduced translocation of IRF7 into the nucleus"

Deutsch-Österreichischer AIDS-Kongress (DÖAK), 15.6.2013, Innsbruck, „Eine neue Hypothese zur Entstehung des Immunrekonstitutionssyndroms"

Invited Talk, Retreat of SFB796, 30.7.2013, Kloster Banz, "Chronic immune activation in HIV-1 infection: a role for mitochondrial DNA ?"

Lecture for the prize "Klinische Virologie 2013", 26.9.2013, Hannover, „Forschung trifft Klinik"

Autumn School of Immunology, 7.10.2013, Merseburg, „How innate immunity senses (retro)viruses"

Invited talk, Congress of the Deutsche STI-Gesellschaft (DSTIG), 9.11.2013, Cologne, „HIV-Superinfektionen"

Awards

Wissenschaftspreis „Klinische Virologie 2013"; Prof. Dr. Barbara Schmidt, 26.9.2013, von der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten e.V. (DVV) und Gesellschaft für Virologie e.V. (GfV)

Publications during funding period

Ries M, Schuster P, Thomann S, Donhauser N, Vollmer J, Schmidt B. (2013) Identification of novel oligonuleotides from mitochondrial DNA that spontaneously induce plasmacytoid dendritic cell activation. J. Leukocyte Biology 94: 123-135.

Tennert K, Schneider L, Bischof G, Korn K, Harrer E, Harrer T, Schmidt B for the German Competence Network HIV/AIDS (2013) Elevated CD40 ligand silences alpha interferon production in an HIV-related immune reconstitution inflammatory syndrome. AIDS 27: 297-301.

Ries M, Pritschet K, Schmidt B (2012) Blocking type I interferon production - a new therapeutic option to reduce the HIV-1 induced immune activation. Clin. Dev. Immunol., 534929.

Donhauser N, Pritschet K, Helm M, Harrer T, Schuster P, Ries M, Bischof G, Vollmer J, Smola S, Schmidt B. for the German Competence Network HIV/AIDS (2012) Chronic immune activation in HIV-1 infection contributes to reduced interferon alpha production via enhanced CD40:CD40 ligand Interaction. PLoS ONE 7(3):e33925.

Pritschet K, Donhauser N, Schuster P, Ries M, Haupt S, Kittan NA, Korn K, Pöhlmann S, Holland G, Bannert N, Bogner E, Schmidt B. (2012) CD4- and dynamin-dependent endocytosis of HIV-1 into plasmacytoid dendritic cells. Virology 423: 152-164.

Patents/ Licenses during funding period

License for AbD13070, a phage display antibody to label human and rhesus macaque PDC (together with AbDSerotec/Morphosys)

A51 - Final Report

01.11.2010 - 31.12.2013

HCMV GPCRs as targets for antiviral therapy

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

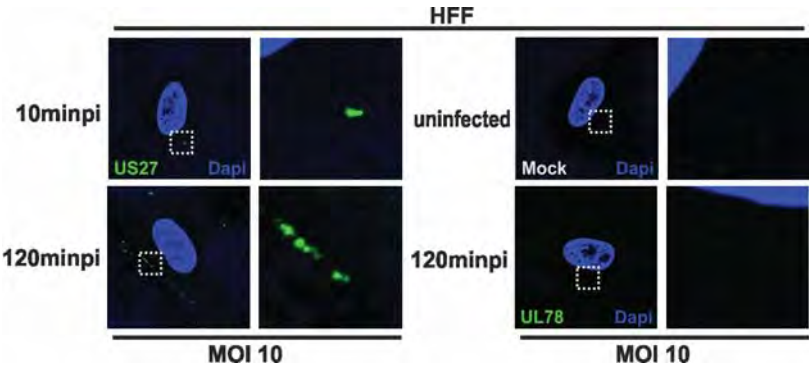
Human cytomegalovirus encodes four proteins (US27, US28, UL33, UL78) with homology to G-protein coupled receptors (GPCRs) that could serve as promising target molecules for antiviral approaches. This project aimed at a functional characterization of the vGPCRs US27 and UL78 during viral infection. Our studies revealed a novel signaling capacity of US27 that involves TRAF factors and leads to a strong activation of NF-kB dependent gene expression.

Intracellular trafficking of the human cytomegalovirus-encoded 7-trans-membrane protein homologues pUS27 and pUL78 during viral infection.

In contrast to the extensively characterized vGPCRs pUS28 and pUL33, knowledge concerning pUS27 and pUL78 is limited. To explore the subcellular localization of both receptors during viral infection, we constructed recombinant HCMVs expressing tagged vGPCRs. Colocalization analyses revealed a predominant association of pUS27 or pUL78 with the trans-Golgi network or the endoplasmic reticulum, respectively. Intriguingly, our data emphasize that protein sorting is highly regulated by viral functions since we detected dramatic changes in the colocalization of pUS27 and pUL78 with endosomal markers during progression of HCMV replication. Furthermore, we observed cell type-dependent differences in trafficking of both vGPCRs between fibroblasts and epithelial cells. Most importantly, infection experiments with a recombinant HCMV carrying tagged versions of pUS27 and pUL78 simultaneously, revealed that these two proteins do not colocalize during viral infection. This contrasts to results of transient expression experiments. In conclusion, our results highlight the importance to investigate vGPCR trafficking in a viral context.

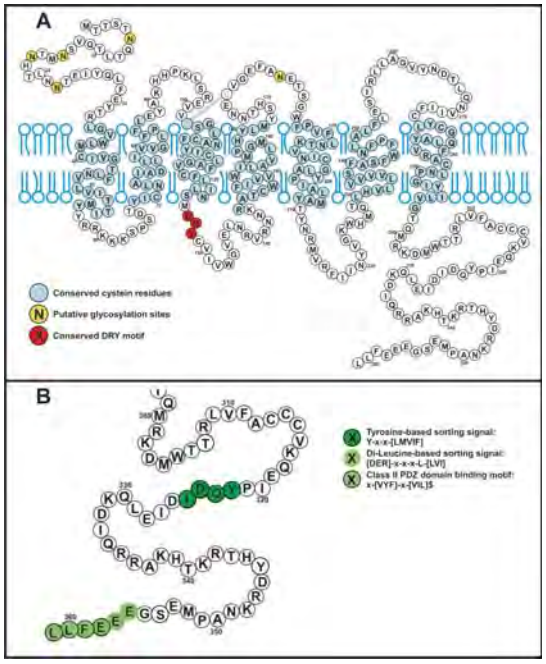
The cytoplasmic domain of the human cytomegalovirus-encoded GPCR pUS27 acts as a strong regulator of NF-kB signaling

G protein-coupled receptors (GPCRs) are key regulators of numerous cellular processes. To determine whether pUS27 or pUL78 display any signaling properties, we performed luciferase reporter assays with CREB- and NF-κB-specific reporter constructs. Our experiments demonstrate for the first time that pUS27 activates NF-κB dependent gene expression. Intriguingly, it turned out that NF-κB activation differed significantly depending on whether N- or C-terminally tagged versions of pUS27 were applied: while transfection of N-terminally tagged pUS27 did not activate NF-κB, the expression of C-terminally tagged versions strongly induced NF-κB signaling. Disruption of a putative PDZ domain binding motif induced high NF-κB signaling suggesting that pUS27



HFF cells were infected with recombinant HCMVs (MOI 10) expressing EYFP-tagged US27 or UL78 as indicated. In contrast to UL78, US27 could clearly be detected at the plasma membrane of infected cells.

Prof. Dr. Stamminger



Bioinformatic analysis of the vGPCR US27. In panel B putative sorting motifs within the C-terminus of US27 are shown.

may be negatively regulated via a PDZ domain protein. Bioinformatic analysis revealed the existence of three putative TRAF binding motifs within the C-terminus of pUS27. Interestingly, mutation of a predicted TRAF6 binding motif led to a complete loss of NF-κB signaling. To answer the question whether the C-terminal region of pUS27 alone is essential and sufficient for NF-κB activation, we generated chimeric proteins with either CD8 or GFP. These chimeras strongly activated NF-κB signaling independent of their localization. Moreover, we could show that pUS27 specifically induced the canonical NF-κB pathway through TRAF6 by using either ACHP, an inhibitor of IKKβ phosphorylation, or a dominant negative IκBα. Taken together, our data reveal a novel and highly complex signaling capability of the HCMV GPCR pUS27.

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Invited lectures

Medizinische Fakultät der LMU München, 12.03.2013, Max von Pettenkofer-Institut, München, Regulatorische Proteine des humanen Cytomegalovirus – von molekularen Mechanismen bis hin zu neuen Therapien

Medizinische Fakultät der Universität Düsseldorf, 30.04.2013, Düsseldorf, Regulatorische Proteine des humanen Cytomegalovirus – von molekularen Mechanismen bis hin zu neuen Therapien

ESV-Workshop „Seminars in Virology“, 28.07.-30.07.2013, Bertinoro, Italy, The IE1 protein of HCMV as an antagonist of intrinsic cellular defense mechanisms

16. Klinisch-Mikrobiologisch-Infektiologisches Symposium, 05.12.-07.12.2013, Berlin, Konnatale CMV-Infektion

Publications during funding period

Niemann I, Reichel A, Stamminger T (2014) Intracellular trafficking of the human cytomegalovirus-encoded 7-trans-membrane protein homologues pUS27 and pUL78 during viral infection: a comparative analysis. Viruses, accepted for publication

Scherer M, Reuter N, Wagenknecht N, Otto V, Sticht H, Stamminger T (2013) Small ubiquitin-related modifier (SUMO) pathway-mediated enhancement of human cytomegalovirus replication correlates with a recruitment of SUMO-1/3 proteins to viral replication compartments. J Gen Virol 94: 1373-1384

Zielke B, Wagenknecht N, Pfeifer C, Zielke K, Thomas M, Stamminger T (2012) Transfer of the UAP56 interaction motif of human cytomegalovirus to its murine cytomegalovirus homolog converts the protein into a functional mRNA export factor that can substitute for pUL69 during viral infection. J Virol 86(13): 7448-7453

Abele-Ohl S, Heim C, Eckl S, Weyand M, Stamminger T, Ensminger SM (2012) Procurement regimens to reduce ischemia reperfusion injury of vascular grafts. Europ Surg Res 49: 80-87

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Newly started Projects

A52 01.11.2013 - 30.04.2016

cFlip isoforms in the intestinal epithelium


Dr. Günther


Prof. Dr. Becker

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 from of cell death is activated in a cell. Our own unpublished data additionally demonstrate a potential role of cFLIP isoforms for the regulation of intestinal epithelial necroptosis in IDB patients. The aim of this application is a differential analysis on the role of cFLIP variants for the pathogenesis of chronic and infectious inflammatory bowel disease.

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A53 01.10.2013 - 30.06.2016

Th17/piTreg differentiation in vivo


Prof. Dr. Hildner

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

The AP-1 family transcription factor Batf is a crucial regulator of the differentiation of naive CD4+ T cells in Interleukin 17a+ Th17 cells. We show that under lymphopenic conditions Batf additionally controls the de novo differentiation into peripherally induced regulatory FoxP3+ T cells. Our data suggest a bidirectional role of Batf during Th17/ Treg differentiation. The goal of our study is the identification of the underlying molecular mechanism.

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A54 01.11.2013 - 30.04.2016

Fam180A in inflammatory diseases


Dr. Wirtz


Dr. Waldner

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

The immunological processes involved in acute and chronic inflammatory diseases are complex and still only poorly understood. Preliminary work of our group led to the identification of the so far uncharacterized protein Fam180A as a cytokine-like factor promoting inflammation. This project will aim at analyzing the biological function of Fam180A within the immune re-sponse and inflammatory conditions at the molecular level.

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A55 01.01.2014 - 30.06.2016

NR4a1 during immunologic tolerance


Dr. Krönke

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

Our preliminary data indicate a crucial Role of the nuclear receptor NR4a1 during the maintenance of immunologic tolerance. In the proposed project, we plan to identify the involved cell types and underlying molecular mechanisms. Furthermore, we aim to evaluate the therapeutic potential of a pharmacologic activation of NR4a1 during the treatment of autoimmune disease.

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Newly started Projects

A56 01.03.2014 - 31.08.2016

Role of HIG2 in atherosclerosis

PD Dr. Warnecke

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

The hypoxia-inducible lipid droplet associated protein HIG2 is required for cellular lipid accumulation under hypoxic conditions. To what extent HIG2 contributes to foam cell formation in atherosclerosis is not yet known. Hypoxia and HIG2 are detectable in human and murine plaques. Therefore we want to investigate the effects of a conditional HIG2 knockout in macrophages and endothelial cells on the development of atherosclerosis in the apolipoprotein E-deficient mouse model.

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A57 01.01.2014 - 30.06.2016

Nr4a1 in cGvHD

PD Dr. Distler

Prof. Dr. Spriewald

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

We characterized the orphan nuclear receptor Nr4a1 as an endogenous antagonist of TGF-ß, which is inactivated in sclerodermatous cGvHD by phosphorylation. First results demonstrated that Nr4a1 agonists can overcome the lack of active Nr4a1, prevent the aberrant activation of fibroblasts and inhibit the tissue fibrosis in experimental cGvHD. We now aim to further evaluate Nr4a1 as a target for therapeutic intervention in cGvHD and other fibrotic diseases.

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A58 01.10.2013 - 31.03.2016

Characterization of DN T cells from ALPS patients


Prof. Dr. Mackensen


Dr. Völkl

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

The autoimmune lymphoproliferative syndrome is characterized by a highly elevated frequency of circulating $CD3^+$ $TCR\alpha\beta^+$ $CD4^-/CD8^-$ (double-negative, DN) T cells. By now, the origin and functionality of this T-cell subpopulation still remain elusive. In this project we aim to characterize the mechansims leading to an accumulation of DN T cells and the functional properties of these cells. Moreover, we want to determine whether DN T cells facilitate the pathogenesis of ALPS.

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A59 01.10.2013 - 31.03.2016

IL-10 and lung cancer


Prof. Dr. Dr. Finotto

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

Lung cancer usually develops from epithelial cells and produce immunosuppressive cytokines like TGF-beta which induces Foxp-3, a marker of T regulatory cells. In preliminary studies we have found that Interleukin 10 which can be also released by lung tumour, is induced along with Foxp-3, in the tumour region in lung adenocarcinoma as compared to squamous carcinoma. Understanding the role of IL-10 in lung tumour cells and in tumour infiltrating lymphocytes will help to set up tailored therapies.

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Newly started Projects

A60 01.10.2013 - 31.03.2016

Mo-DC by DC-Exosomes

Dr. Baur Dr. Schierer

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

Monocyte-derived Dendritic cells (Mo-DC) can be generated in-vitro using cytokines. By which mechanism Mo-DC are naturally induced is unclear yet. Based on preliminary data we hypothesize that Mo-Dc develop when monocytes ingest exosomes from mature dendritic cells. We will determine, how Exosomes induce the maturation of Mo-DC, how these Mo-DC compare to other DC and whether Mo-DC play a role during DC-vaccination of tumor patients.

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A61 01.02.2014 - 31.07.2016

Leishmania, iNOS and iron

Prof. Dr. Bogdan PD Dr. Schleicher

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 is prevented by the activity of inducible nitric oxide synthase (iNOS). The main aim of the project is to test the hypothesis that iron promotes the replication of the parasites by inhibition of iNOS and, conversely, that the antileishmanial effect of iNOS is due to an increased expression of ferroportin-1 which results in an enhanced cellular iron-export and subsequently in an iron-depletion of the parasites.

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A62 01.01.2014 - 30.06.2016

ND10 and interferon-induced gene expression

Prof. Dr. Stamminger

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 role for interferon-induced gene expression. Thus, viruses may antagonize specific aspects of the interferon response by manipulating ND10.

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D16 - Final Report

01.11.2010 - 30.10.2013

The role of IL-33/ST2 signalling in the pathogenesis of hepatic fibrosis

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

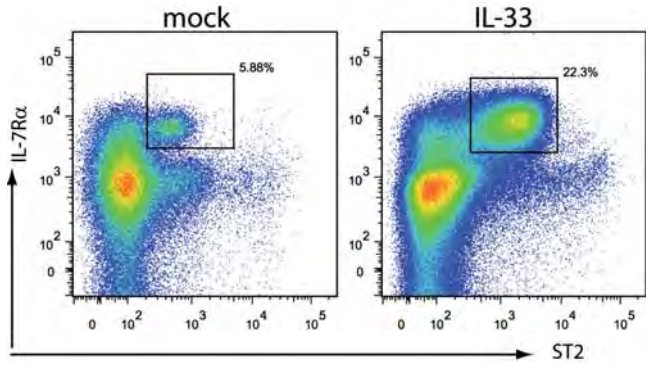
Liver fibrosis is a consequence of chronic liver diseases and thus a major cause of mortality and morbidity. In this study we found that hepatic expression of the IL-1-related cytokine IL-33 was both required and sufficient for severe hepatic fibrosis in vivo. We identified for the first time innate lymphoid cell derived IL-13, acting through STAT6 signaling and hepatic stellate cell activation, as the critical downstream cytokine of IL-33-dependent tissue remodeling and fibrosis in the liver.

IL-33 is elevated in liver fibrosis and drives hepatic ECM depositions

To address its potential contribution to human fibrotic liver disease, we evaluated IL-33 levels in patients with liver cirrhosis. Significantly higher serum levels of IL-33 were observed in fibrotic patients compared to healthy controls. Such elevated IL-33 levels were also observed across different mouse models of hepatic fibrosis. Further studies revealed that IL-33 overexpression in hepatocytes was sufficient to induce excessive hepatic collagen depositions as determined by Sirius red staining and hydroxyproline assay. To address if IL-33 was not only sufficient but also required for hepatic fibrosis, IL-33$^{-/-}$ mice were challenged in models of hepatic fibrosis. In both models employed, IL-33$^{-/-}$ animals developed significantly reduced fibrosis compared to controls

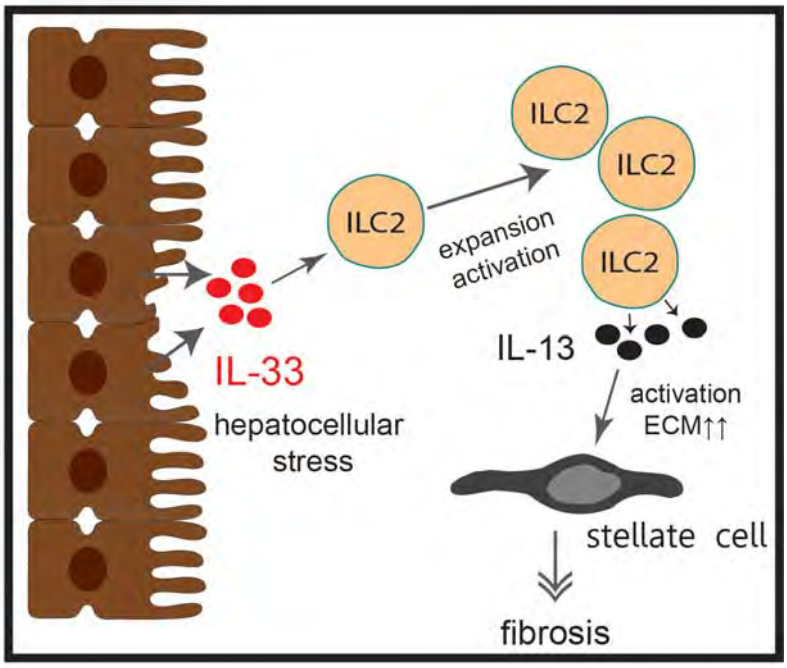
IL-13 dependent type II IL-4R signaling in HSC mediates IL-33 dependent fibrosis

Transcriptional profiling identified the cytokine IL-13 as one of the most prominently upregulated genes in IL-33 dependent liver fibrosis. Indeed, IL-13$^{-/-}$ and IL-4R$\alpha^{-/-}$ mice were markedly protected from disease suggesting that IL-33 and IL-13 constitute a profibrotic axis in the course of the disease. This concept was further supported by our studies demonstrating IL-33- or IL-13-dependent hepatic activation of STAT6 in vivo and direct activation of signaling and gene expression of hepatic stellate cells (HSC) in vitro. Consistently, we show elevated hepatic mRNA and protein expression of the components of the functional IL-13 receptor in human liver cirrhosis. This indicates increased sensitization to IL-13 dependent signals in human fibrotic liver disease and suggests at least partial functional congruence of IL-33 dependent pro-fibrotic hepatic networks in mice and humans.



IL-33 expands liver resident type-2 innate lymphoid cells (ILC2) Hepatic immune cell populations of mock or IL-33 treated Rag1-/- mice were isolated. The expression of IL-7Rα (CD127) and IL-33R (ST2) on lineage negative cells was analyzed by FACS.

Dr. Wirtz



Proposed model for the profibrotic role of IL-33 in chronic liver diseases

Type 2 innate lymphoid cells (ILC2) are essential mediators of IL-33 dependent liver fibrosis

A number of different cell types have been identified as candidates for IL-13 production after IL-33 stimulation. We identified in a series of experiments a non-T/-B cell lymphoid cell population as strong IL-13 producer and pathogenic cell type in our system. These cells express a panel of receptors and transcription factors including ST2, IL-7R, ICOS, KLRG1, Sca-1, GATA3 and RORa characteristic of ILC2, a recently described cell type playing a key role in host defense to viral or parasitic infections. Adoptive transfer of sort purified liver ILC2 into IL-33 unresponsive ST2$^{-/-}$ mice restored this strains' susceptibility to disease indicating that intra-hepatic ILC2 promote IL-33-mediated liver fibrosis in vivo. Moreover, anti Thy1.2 antibody mediated ILC2 depletion significantly ameliorated fibrotic liver disease in mice supporting the concept of modulation of IL-33/ILC2 bioactivity for therapeutic purposes.

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Publications during funding period

Mchedlidze T, Waldner M, Zopf S, Walker J, Rankin AL, Schuchmann M, Voehringer D, McKenzie AN, Neurath MF, Pflanz S, Wirtz S. (2013) Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. Immunity 39(2):357-71.

D17 - Final Report

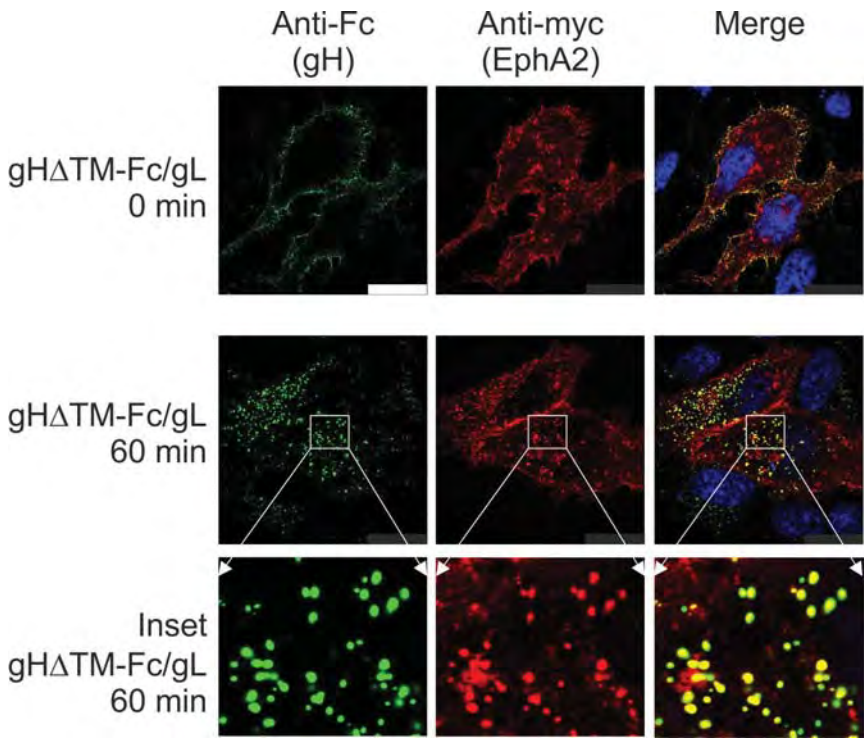
01.10.2010 - 30.09.2013

The role of Ephrin-A2 receptor-tyrosinkinase in human herpesvirus-8 infection

PD Dr. Frank Neipel, Institute of Clinical and Molecular Virology

Human herpesvirus 8 (HHV-8), also termed Kaposi sarcoma-associated herpesvirus (KSHV), is the causative agent of Kaposi's sarcoma (KS) and at least two lymphoproliferative malignancies. Glycoproteins H and L (gH-gL) are required for entry of all herpesviruses into host cells. We identified the first cellular receptor for HHV-8 glycoproteins H and L (gH/gL): the EphrinA2 receptor-tyrosinkinase (EphA2). This project is focused on the role of EphA2 in KSHV infection and pathogenesis.

EphA2 is an essential receptor for KSHV on endothelial cells. Infection of epithelial cells with KSHV as was increased upon overexpression of EphA2. Antibodies against EphA2 and siRNAs directed against EphA2 inhibited infection of endothelial cells. Incubation of the viral inoculum with soluble EphA2-Fc but not Fc reduced the infection by about 90%. Homozygous knock-out of EphA2 resulted in resistance to KSHV infection.



Immunofluorescence analysis of EphA2 and gH/gL localization. HeLa cells transfected with an EphA2-Myc expression plasmid were incubated with the crosslinked gH/gL at 4 °C, washed, shifted to 37 °C and fixed with 3% paraformaldehyde at the timepoints indicated.

PD Dr. Neipel

EphA2-expression correlates with KSHV infection. Plotting the EphA2/actin mRNA ratio against the percentage of infected cells for ten endothelial cells and cell-lines revealed a linear correlation. We examined the expression of EphA2 and KSHV infection in tissue sections from KS by immunohistochemistry. In all KS tissues LANA-1 and EphA2 were expressed in the KS spindle cells. KS tissues showed increased numbers of KSHV positive cells and increased EphA2 staining. Uninvolved skin tissues of the KS patients were negative for LANA-1 and stained only slightly positive for EphA2 in the epithelial cells of the epidermis.

Binding of gH/gL to EphA2 triggers EphA2 phosphorylation and endocytosis. Binding of Ephrin receptors to their ligands like EphrinA1 induces autophosphorylation. Increased autophosphorylation of EphA2 was also detectable within 10 minutes of incubation with KSHV. Interestingly, stimulation with soluble EphrinA1 was barely more effective. To determine whether this effect is of biological significance for KSHV entry, we transiently overexpressed either full-length EphA2 or EphA2 without the intracellular kinase domain in 293T cells. Only EphA2 with intact kinase domain enhanced KSHV infection. To ascertain whether EphA2 is able to mediate uptake of gH /gL, EphA2-expressing cells were incubated with cross-linked gH/gL at 4 °C. Directly after incubation at 4 °C, EphA2 and gH/gL colocalized at the membrane, whereas after a shift to 37 °C for 60 min both proteins colocalized at vesicular structures within the cells.

EphA2 is required for both virion endocytosis and membrane fusion. We used several techniques including cell fractionation, quantitative DNA PCR and dual wavelength fluorescence microscopy of KSHV virions labelled with lipophilic dyes to determine the steps of the infection process that depend on EphA2. This revealed unequivocally that EphA2 is not required for KSHV attachment to endothelial cells. However, endocytosis of KSHV was not detectable in the absence of EphA2. When endocytosis of attached virions was blocked e.g. by incubation at 0°C, fusion of the cytoplasma membrane with the envelope of attached extracellular KSHV virions could be triggered by acidification. This pH-dependent fusion was possible with EphA2-positive but not EphA2-negative cells. The use of EphA2 deletion mutants revealed that in addition to the extracellular domain an intracellular region of about 50 amino acids containing several tyrosine residues was also required for virion endocytosis. In summary, EphA2 is required for both endocytosis of KSHV virions into endothelial cells and membrane fusion.

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Invited lectures

Gastseminar am Institut für Mikrobiologie, Virologie und Hygiene, Universitätsklinikum Regensburg, 24.1.2013, Regensburg, zelluläre Rezeptoren des Kaposi-Sarkom assoziierten Herpesvirus

Publications during funding period

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

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.

Schmidt K, Wies E, Neipel F. (2011) Kaposi's sarcoma-associated herpesvirus viral interferon regulatory factor 3 inhibits gamma interferon and major histocompatibility complex class II expression. J. Virol. 85(9):4530-7.

D18 - Final Report

15.10.2010 - 14.04.2013

miRNAs in normal and malignant intestinal epithelial cells

Prof. Dr. Regine Schneider-Stock, Institute of Pathology

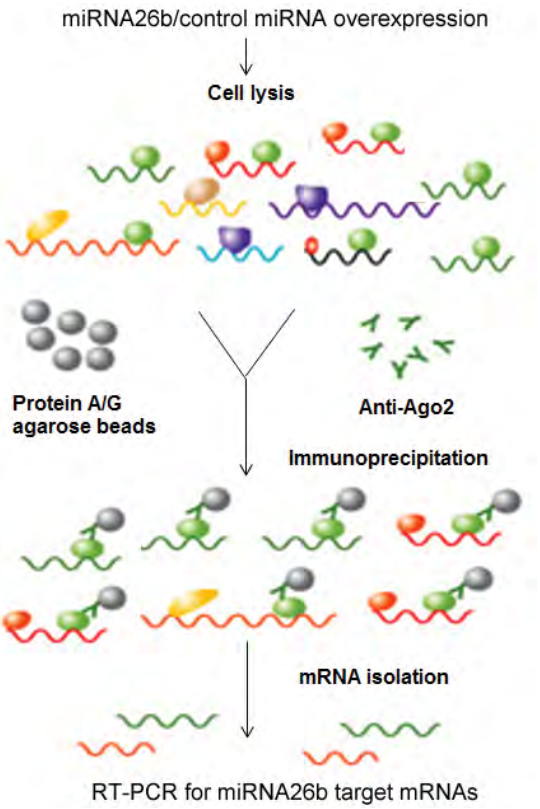
miRNAs are small non-coding RNA molecules regulating posttranscriptional gene expression. They are involved in the regulatory network of many pathological processes such as cell death and inflammation. The role of miRNAs in chronic inflammatory bowel disease such as Ulcerative colitis (UC) is poorly understood. Since UC is strongly correlated with the action of the pro-inflammatory cytokine TNF, we aimed to elucidate the role of miRNAs in TNF-induced apoptosis in epithelial cells of the gut.

Identification of an inflammation-associated miRNA

miRNAs may serve as non-invasive biomarkers for prognosis of cancer patients. We established a new method for detection of miRNAs from sera. Comparing sera from patients suffering from celiac disease and patients from a normal control group (collaboration with Dr. Fahlbusch, Pediatric Clinic, Erlangen) we observed a remarkable increase in miRNA26b expression in the diseased group showing that RNA has the potential to be a general marker for inflammation.

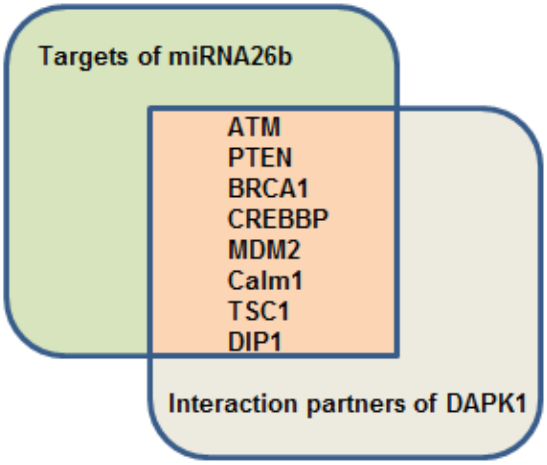
A new experimental approach: RNA-Binding Protein Immunoprecipitation (RIP) assay

miRNA26b seems to be an excellent candidate miRNA in the regulation of inflammatory processes. In addition to the *in vivo* data, we showed *in vitro* that normal intestinal epithelial cells chronically treated with TNF significantly up-regulated miRNA26b expression. The detection of the responsible target genes is a big challenge and is so far performed via luciferase reporter assays. To detect direct interactions between miRNA26b and its target mRNAs in a larger scale we established the RIP assay. First a miRNA26b mimic and a negative control was transfected into HCT116 colorectal tumor cells. Then, Ago2 bound miRNAs and their target mRNAs were precipitated from a cell lysate by magnetic beads coated with an Anti-Ago2 antibody. The RNA was isolated from the precipitated protein-RNA-complex and was further analyzed by real-time RT-PCR. Potential candidates were identified by an alignment of *in silico* analysis of potential miRNA26b targets and DAPK1 interaction partners. The experiment yielded five direct targets of miRNA26b: MDM2, DIP1,



Strategic workflow for RIP-assay to identify new miRNA26b targets

Prof. Dr. Schneider-Stock



miRNA26b targets were aligned with known DAPK1 interaction partners showing an overlap of 8 proteins. miRNA26b targets were predicted by 5 prediction programs (DIANA-mT, miRANDA, miRDB, miRWalk, TargetScan). DAPK interaction partners were found using BioGRID and String 9.05 databases.

BRCA1, CREBBP, and PTEN. How these proteins are involved in CU or in inflammation-associated tumor transformation has to be further examined.

miRNAs und alternative splicing of the anti-inflammatory and pro-apoptotic DAPK

The two DAPK isoforms produced by alternative splicing mediate different cellular functions and are suggested to play also a diverse role in inflammation (Benderska and Schneider-Stock, 2013). We investigated if TNF treatment of normal and malignant intestinal epithelial cells induced a differential up-regulation of the two DAPK isoforms. Interestingly, we observed a remarkable increase of the DAPKß mRNA under TNF only in normal cells which could explain the observed apoptosis resistance under TNF (Chakilam et al. 2013). Furthermore, two miRNAs (miRNA191, miRNA504) localized at the 3´UTR of DAPK at the known splice site were differentially regulated in normal and malignant cells. HCT116 cells with overexpressed miRNA504 did not show any DAPKß expression suggesting a possible role of this miRNA for the specific degradation of the DAPKß mRNA.

This project identified a number of promising inflammation-associated miRNAs that have to be further characterized functionally in human subjects suffering from UC.

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Publications during funding period

Benderska N, Schneider-Stock R (2013) Transcription control of DAPK. Apoptosis; 2014 Feb;19(2):298-305

Chakilam S, Gandesiri M, Rau TT, Agaimy A, Vijayalakshmi M, Ivanovska J, Wirtz RM, Schulze-Luehrmann J, Benderska N, Wittkopf N, Chellappan A, Ruemmele P, Vieth M, Rave-Fränk M, Christiansen H, Hartmann A, Neufert C, Atreya R, Becker C, Steinberg P, Schneider-Stock R (2013) Death-associated protein kinase controls STAT3 activity in intestinal epithelial cells. Am J Pathol 182(3): 1005-1020

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Patents/ Licenses during funding period

European patent Application Nr. EP 13 155 510.4 “Death-associated protein kinased, inhibitors and activators thereof for use in pharmaceutical compositions and in predictive medicine”

Newly started Projects

D19 01.11.2013 - 30.04.2016

Role of intestinal epithelial SMAD7 for tumor development


Dr. Wittkopf


Prof. Dr. Becker

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

Since polymorphisms in the SMAD7 gene have been associated with an increased risk for developing colorectal cancer, we want to investigate the role of SMAD7 in the intestinal epithelium for gut homeostasis and development of intestinal cancer using mice with an intestinal epithelial cell specific deletion of SMAD7. In particular, we will study the molecular mechanisms influenced by SMAD7 and whether modulation of SMAD7 in the intestinal epithelium shows a therapeutic effect on tumorigenesis.

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D20 01.11.2013 - 30.04.2016

Collagen 10 and metastasis in CRC


Prof. Dr. Dr. Stürzl


Prof. Dr. Croner


PD Dr. Naschberger

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

Own clinical studies showed that the expression of the collagen 10 gene is increased in primary lesions of metastasizing colorectal carcinomas. The function of collagen 10 in the regulation of metastasis will be analysed cell biologically and using animal models. A focus will be on the formation of stem cell like tumor initiating cells and on epithelial mesenchymal transition. The project will deliver new insights into the function of matrix components in the metastases of colorectal carcinoma.

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D21 16.10.2013 - 15.04.2016

DAPK and colon cancer


Prof. Dr. Schneider-Stock


Dr. Neufert

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

Colorectal cancer (CRC) is a frequent malignant disease with limited therapeutic options. Scientific data on the role of DAPK1 in CRC including recent own studies appear controversial, because DAPK1 can act both as a tumor suppressor or an oncogene dependent on the specific molecular context. Using established model systems and novel experimental tools including unpublished genetically modified mice, our project aims to clarify the functional role of DAPK1 for intestinal tumorigenesis.

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D22 01.11.2013 - 30.04.2016

Wnt components


Prof. Dr. Behrens

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

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

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E8 - Final Report

01.10.2010 - 30.09.2013

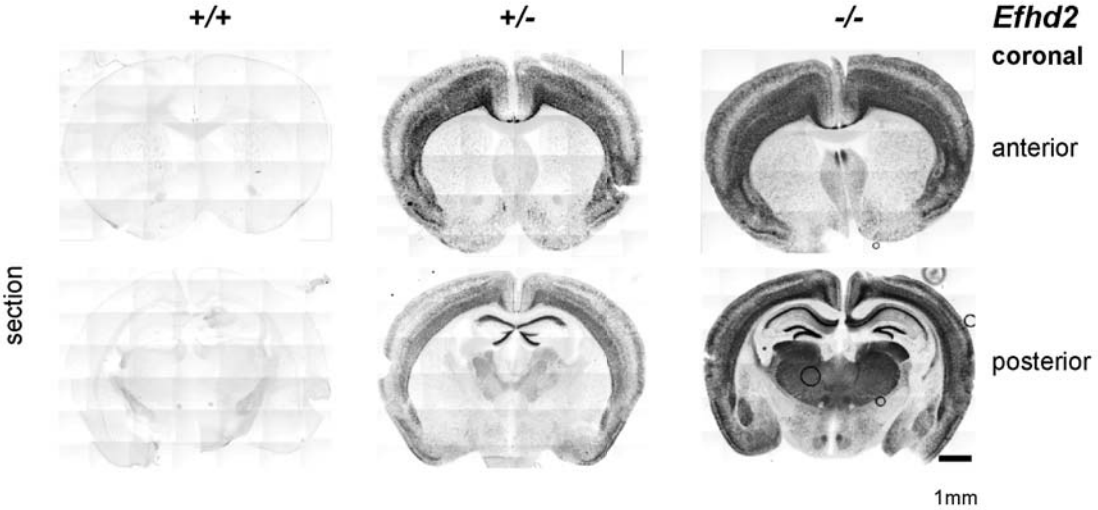
Swiprosin-1/EFhd2 and tauopathies

Dr. Dirk Mielenz, Division of Molecular Immunology

Here we assessed the neurological function of the adaptor protein Swiprosin-1/EFhd2 (EFhd2). We identified EFhd2 as a neuronal, pre- and postsynaptic protein, which controls tau phosphorylation. Both endocytosis and exocytosis in primary hippocampal EFhd2$^{-/-}$ neurons were unaltered, but axonal transport was enhanced in EFhd2$^{-/-}$ primary hippocampal neurons. Finally, adult neurogenesis, as well as expression of genes involved in adult neurogenesis, were significantly reduced in EFhd2$^{-/-}$ mice.

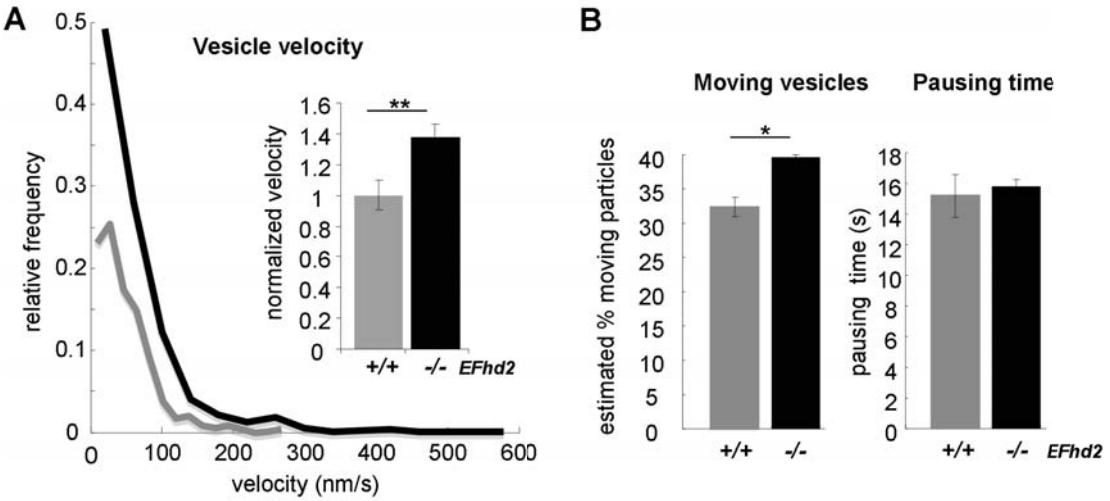
Basic characterization of the function of Swiprosin-1/EFhd2

Swiprosin-1/EFhd2 (EFhd2) is a cytoskeletal Ca^{2+} sensor protein strongly expressed in the brain. It has been shown to interact with mutant tau, which can promote neurodegeneration, but nothing is known about the normal physiological function of EFhd2 in the nervous system. To elucidate this question, we analyzed EFhd2$^{-/-}$/LacZ reporter mice and showed that LacZ was strongly expressed in the cortex, the dentate gyrus, the CA1 and CA2 regions of the hippocampus, the thalamus and the olfactory bulb. Immunohistochemistry and western blotting confirmed this pattern and revealed expression of EFhd2 during neuronal maturation. In cortical neurons, EFhd2 was mostly found in dendrites and co-localized with pre- and post-synaptic markers with approximately one third of EFhd2 protein associated with synaptosomes, where EFhd2 was mostly confined to the cytosolic and plasma membrane fractions. Both endocytosis and exocytosis in primary hippocampal EFhd2$^{-/-}$ neurons were unaltered but axonal transport was enhanced in EFhd2$^{-/-}$ primary hippocampal neurons, and notably EFhd2 inhibited kinesin mediated microtubule gliding. Therefore, we have found that EFhd2 is a neuronal synaptic protein that interferes with kinesin motor activity and axonal transport.



Coronal sections of anterior and posterior parts of whole mount lacZ reporter gene stained brains from adult EFhd2$^{+/+}$, EFhd2$^{+/-}$ and EFhd2$^{-/-}$ mice. This experiment was performed by Dr. Martin Regensburger (IZKF N3, Prof. Dr. Beate Winner).

Dr. Mielenz



(A) Velocity and (B) number and pausing times of marked moving vesicles in primary hippocampal neurons of EFhd2$^{+/+}$ and EFhd2$^{-/-}$ mice. This experiment was performed by Dr. Teja Grömer et al., Dept. of Psychiatry.

Swiprosin-1/EFhd2 is involved in phosphorylation of tau

Tau is a microtubule stabilizing protein whose aberrant phosphorylation through mutations, such as tau P301S, P301L or △K280, can contribute to neurodegenerative diseases, possibly through tau tangles. Tau P301S and tau P301L tg mice develop a neurodegenerative phenotype. EFhd2 has been shown to interact with mutant tau P301L by others. Here we investigated whether EFhd2 could also interact with endogenous, unmutated tau. In fact, EFhd2$^{-/-}$ mice showed increased abundance of phosphorylated and unmutated endogenous tau. This result suggested that EFhd2 controls microtubule stability. Interestingly, a GST-EFhd2 fusion protein but not GST alone inhibited tau mediated microtubule stabilization in a cell-free system and in transfected 293 cells. We were furthermore able to generate EFhd2$^{-/-}$ x tau P301S tg mice. We expect that tau P301S is hyperphosphorylated in the complete absence of EFhd2, and that loss of EFhd2 mediates tau induced neurodegeneration. We conclude that EFhd2 is a synaptic protein that interferes with tau function and phosphorylation.

Involvement of Swiprosin-1/EFhd2 in adult neurogenesis

Microenviromental changes are altered during neurodegeneration. These changes modulate the multipotent state of neuronal precursor cells, as well as incorporation of newly formed neurons into the brain circuitry. Gene expression analysis of adult EFhd2$^{+/+}$ and EFhd2$^{-/-}$ brains revealed a down-regulation of genes involved in adult neurogenesis. In accordance, stereotactic injection of a GFP-expressing retrovirus showed that adult neurogenesis was significantly reduced in brains of EFhd2$^{-/-}$ mice.

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Publications during funding period

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Hagen S, Brachs S, Kroczek C, Fürnrohr B, Lang C, Mielenz D (2012) The B cell receptor-induced calcium flux involves a calcium mediated positive feedback loop. Cell Calcium, 51, 411-417

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E9 - Final Report

01.10.2010 - 30.09.2013

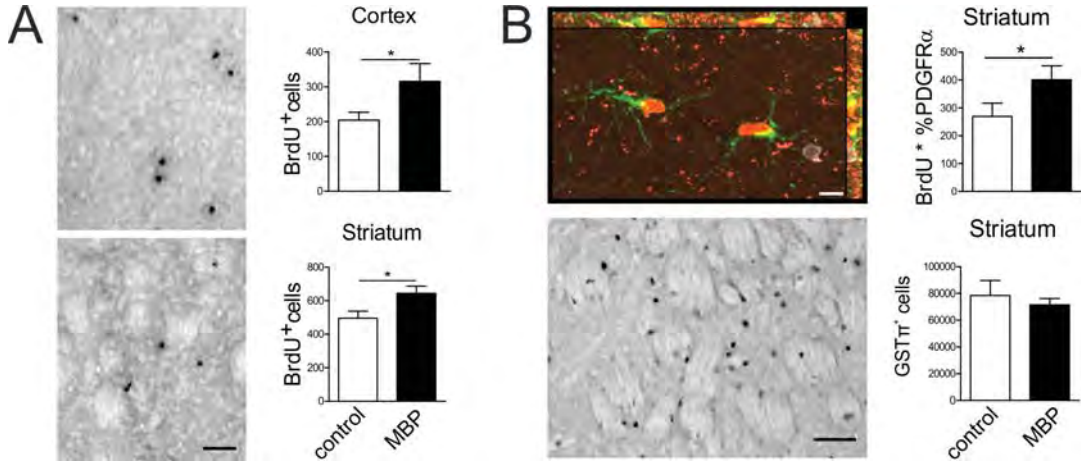
Glia in synucleinopathies

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

The project aims to characterize alpha-synuclein (aSyn) aggregation in glial cell culture and mouse models of the atypical Parkinson Syndrome „Multiple System Atrophy – MSA". In particular, we studied the effect of aSyn overexpression in a transgenic MSA mouse model and in the oligodendroglial cell line CG4. Here, intracellular aSyn leads to impaired oligodendrocyte progenitor maturation. Additionally, we generated a transgenic mouse model that allows human aSyn overexpression in the enteric nervous system.

The atypical Parkinson syndrome multiple system atrophy (MSA) is a rare, neurodegenerative synucleinopathy characterized by fast progression and severe disability. MSA is histopathologically defined by aSyn+ glial cytoplasmatic inclusions (GCIs) observed within oligodendrocytes. Several mouse models expressing human aSyn under different oligodendroglial promoters (e.g. myelin basic protein (MBP)) have been generated that recapitulate the aSyn induced pathology observed in MSA. However, detailed studies analyzing the underlying aSyn mediated cellular and molecular mechanisms are lacking.

In MBP::aSyn transgenic mice, we detected increased numbers of newborn bromo-deoxy-uridine (BrdU)+ cells both in cortex and striatum. Determining the identity of these cells, we observed significantly increased numbers of platelet-derived growth factor receptor-alpha (PDGFRα)+ oligodendrocyte progenitors in the striatum only. In contrast to precursor cells, the number of glutathione-s-transferase pi (GSTπ)+ mature oligodendrocytes is not affected in MBP::aSyn mice. In order to investigate underlying molecular mechanisms, we established an *in vitro* system. Using the oligodendroglial CG4 cell line and transposon mediated transfection, we successfully generated a human wild type aSyn expressing as well as a YFP-venus expressing oligodendroglial cell line (CG4_wt-a-syn and CG4_venus, respectively). Upon aSyn expression, significantly less MBP was expressed in fewer differentiated CG4 cells as determined by counting MBP-positive cells as well as by

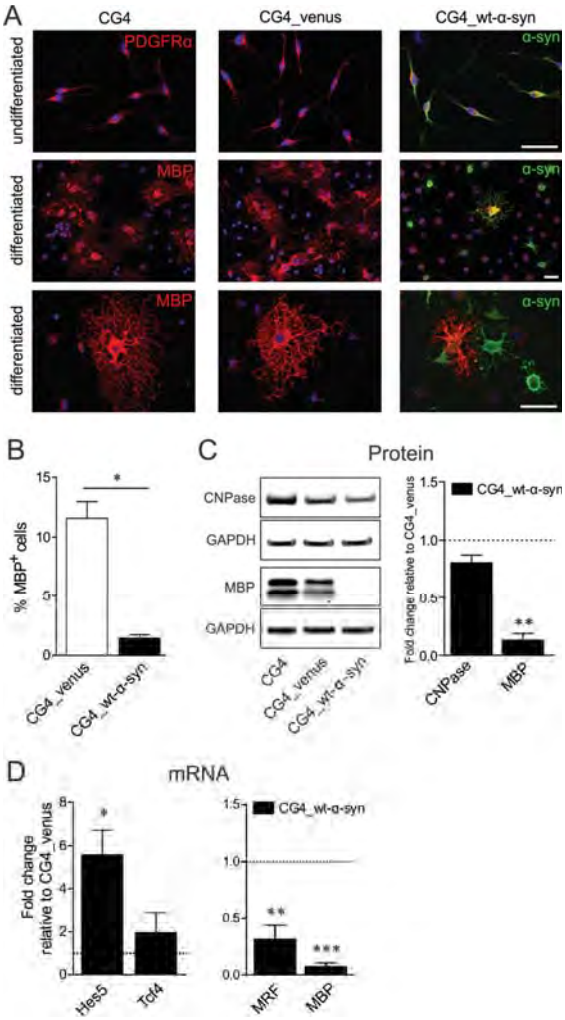


A) Increased number of newborn BrdU+ cells in the cortex and the striatum of MBP::aSyn transgenic mice (9 months of age). B) A representative pair of new born (BrdU+, red) striatal oligodendrocyte progenitors (PDGFRα+, green) is shown. Numbers of newborn PDGFRα+ oligodendrocyte progenitors are increased in MBP::aSyn transgenic mice compared to control mice in the striatum, whereas numbers of mature oligodendrocytes are unaffected. Mature oligodendrocytes are stained with glutathione-S-transferase-pi (GSTπ).


Prof. Dr. Winkler


PD Dr. Klucken


Prof. Dr. Wegner



A) Immunocytochemistry of CG4 cells (parent line and CG4_wt-α-syn and CG4_venus stable transfectants) for PDGFRα (undifferentiated) and MBP (differentiated). Bar: 20 µm. B) Quantification of MBP+ cells. C) Western Blot analysis including densitometry. D) qPCR analysis comparing CG4_wt-α-syn and CG4_venus cells.

Western blot. Furthermore, we analyzed expression profiles of stage specific transcription factors using qPCRs. We confirmed the interference of aSyn with oligodendroglial maturation by showing markedly reduced expression levels of the maturation promoting myelin gene regulatory factor mRNA (MYRF) and reciprocally upregulated levels of the maturation inhibiting hairy enhancer of split-5 (Hes5). Our data revealed a maturation inhibiting effect of aSyn in oligodendrocytic cells. This finding may be helpful in defining novel strategies for compensating the severe myelin loss observed in MSA.

To generate an *in vivo* model of aSyn propagation, we established a transgenic mouse model with aSyn overexpression in the enteric nervous system. In these mice, human aSyn is expressed in wild type or mutant (A53T) version under control of the U3 Sox10 enhancer and the minimal hsp68 promoter. The U3 enhancer drives expression of aSyn (and EGFP) throughout the developing enteric nervous system during embryogenesis and specifically in enteric glia after birth. The obtained transgenic mouse lines are currently analyzed after appropriate aging for aSyn expression, aggregation, and toxicity in the enteric nervous system. Additionally, the propagation of aSyn from the enteric nervous system to dorsal vagal nuclei will be tested.

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Publications during funding period

Klucken J, Poehler AM, Ebrahimi-Fakhari D, Schneider J, Nuber S, Rockenstein E, Schlötzer-Schrehardt U, Hyman BT, McLean PJ, Masliah E, Winkler J (2012) Alpha-synuclein aggregation involves a bafilomycin A1-sensitive autophagy pathway. Autophagy 8:5: 754-66

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E10 - Progress Report

01.04.2011 - 31.03.2014

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

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

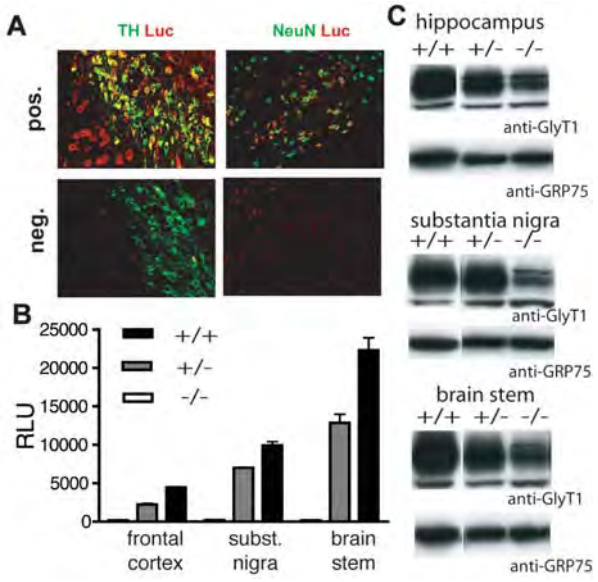
The glycine transporter GlyT1 is involved in the modulation of glycine dependent neurotransmission and inhibition of GlyT1 has beneficial effects as add on therapy for psychotic diseases. GlyT1 is expressed in multiple isoforms that are generated from a single gene by alternative promoter usage and/or splicing. Here we analyse the function of the isoform GlyT1b by a reporter gene approach. This will allow us the identification of GlyT1 expressing cells and the analysis of GlyT1b function in vivo.

Glycine acts as an inhibitory neurotransmitter in caudal regions of the central nervous system but also constitutes a coagonist for glutamate receptor of the NMDAR subtype. The extracellular glycine concentration is controlled by high affinity glycine transporters, the GlyTs. Whereas in caudal region the majority of the GlyT1 activity is present in astrocytes, a significant proportion of the forebrain GlyT1 activity is in neurons. It was speculated that here the GlyT1b isoform is most prevailing. To analyse the function of GlyT1b directly and identify the cells expressing GlyT1b in vivo we set out to generate a mouse line carrying a Luciferase RFP (LucR) reporter knock in downstream of the endogenous start-codon of this transporter isoform.

Initial characterization of GlyT1 b/c knock in mice

Mouse embryonic stem cell clones that were shown to carry the modified GlyT1 allel were used for blastocyte injections. Mating of chimeras obtained from these injections to female mice revealed that chimeras from at least 3 clones transmitted the modified allele to their progeny. Brain sections from heterozygous reporter mice were analyzed for the LucR expression pattern. Luciferase antibody stainings revealed highest expression of LucR reporter within a relative small cell population of the substantia nigra. Co-staining with antibodies against several marker proteins revealed that LucR is exclusively expressed in neurons and that a major fraction (but not all) of LucR expressing cells in this brain region are also expressing tyrosine hydroxylase, a marker protein for dopaminergic neurons.

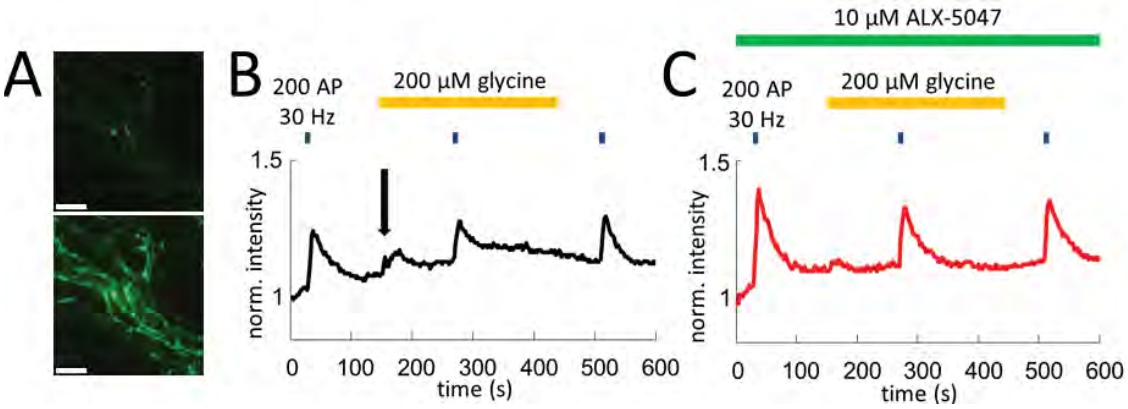
Measurement of the luciferase activity in various brain regions from adult mice carryring the GlyT1 KI allele homozygously revealed that the LucR reporter is expressed throughout all major brain regions. Membrane preparations of individual brain regions of these mice were subjected to Western-blot analysis. Here we could show that GlyT1 expression was reduced in mice carrying the LucR reporter knock in homozygously. These data suggest that the expression pattern of the neuronal GlyT1b isoform is broader than anticipated initailly.



A. GlyT1b reporter expression in substantia nigra: GlyT1b is expressed in NeuN positive neurons including Tyrosinehydroxylase positive neurons. B. Luciferase activity is detected in all brain regions of GlyT1b KI mice. C. Western blots revealed reduction of GlyT1 expression homozygous GlyT1b KI mice.


Dr. Eulenburg


Dr. Grömer



Influence of glycine on synaptic transmission; A) Image of synapto-pHluorin-transfected neurons before (top) and during (bottom) stimulation (Scale 10 µm). Fluorescence time courses of B) control and C) ALX-5407 (Glyt1 specific inhibitor) treated neurons. Arrow: response on glycine application.

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. Thus we could demonstrate that glycine is able to induce release of vesicles from presynaptic pools. Incubation with a GlyT1 specific inhibitor 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.

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Invited lectures

V. Eulenburg: Satellite Symposium of the ISN Meeting 2013 „Brain in Flux“, 25.4-29.4.2013, Cancun, Mexico, „in vivo Glycine transporter 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 (2013) The Antidepressant Fluoxetine Mobilizes Vesicles to the Recycling Pool of Rat Hippocampal Synapses During High Activity. Mol Neurobiol 49(2):916-30

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Newly started Projects

E11 01.12.2013 - 31.05.2016

H50Q aSyn mutation in PD


PD Dr. Klucken


Dr. Xiang

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

Aggregation and neurotoxicity of alpha-synuclein (αSyn) are crucial in the pathogenesis of Parkinson's disease (PD). Our previous work shows that a posttranslational modification (PTM) of aSyn on histidine 50 (His50) modulates aSyn aggregation and toxicity specifically to dopaminergic neurons. Intriguingly, a novel aSyn mutation, H50Q, was recently reported in PD. Thus, the present application aims to characterize the structural and functional role of the novel PD-associated H50Q mutation.

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E12 30 months

Adult hippocampal neurogenesis in synucleinopathies


Prof. Dr. Winkler


Prof. Dr. Lie

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

Synucleinopathies are frequently accompanied by neuropsychiatric symptoms. There is increasing evidence that impairment in adult hippocampal neurogenesis is linked to the development of these symptoms. We will use structural and behavioral analysis to test the ability of two compounds to revert neurogenesis and behavior deficits in synucleinopathy models. In addition, transcriptomic and bioinformatic analyses will be used to identify pathways underlying the beneficial response to the compounds.

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E13 01.04.2014 - 30.09.2016

Sphingomyelinase, depression and alcoholism


Prof. Dr. Müller


Dr. Reichel


Prof. Dr. Kornhuber

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.

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E14 30 months

TRPC5 and tooth pain


PD Dr. Zimmermann

PD Dr. Katharina Zimmermann, Institute of Physiology

Cold hyperalgesia and cold hypersensitivity are common dental problems. We discovered that the novel cold transducer TRPC5 undergoes strong upregulation in sensory neurons innervating root, pulp and dentin of pulpitic human teeth. Objective is to delineate the function of cold sensing TRPC5 in normal vs. inflamed teeth to provide new insight into mechanisms of tooth pain. Potentially our study will reveal a promising novel target for the treatment of tooth pain and dentin hypersensitivity to cold.

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Newly started Projects

E15 01.11.2013 - 30.04.2016

GlyT1 and neuropathic pain


Dr. Eulenburg


Prof. Dr. Schulze

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 are often 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 will determine the therapeutic potential of glycine transporters for this disease.

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E16 30 months

Regulatory networks in intellectual disability


Prof. Dr. Lie


Prof. Dr. Reis

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

Intellectual disability (ID) is a debilitating neurodevelopmental disorder. Our data suggest that a number of ID causing genes is connected via a SOX11-dependent transcriptional network and that perturbation of this network contributes to the pathophysiology of ID. We will combine human genetics, mouse genetics, transcriptomics and bioinformatics to determine the developmental function and targets of such hypothesized network and to probe network components as novel etiological genes in ID.

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E17 30 months

Wnt signaling at neuromuscular synapses


Prof. Dr. Hashemolhosseini

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). But it is completely unknown which of the Wnt signaling pathways are active at neuromuscular synapses. The aim of this scientific approach is to fully enlighten the neuromuscular role of Wnt signaling pathways in health and disease.

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E18 01.12.2013 - 31.05.2016

NG2-positive glia


Prof. Dr. Wegner


Prof. Dr. Winkler

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

In the healthy central nervous system, NG2-positive glia differentiate mostly to oligodendrocytes. In this project Sox gene expression will be altered 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-positive glia will be analyzed for their impact on disease in a mouse model of multiple system atrophy, a fast progressing atypical form of Parkinson disease.

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F1 - Final Report

01.01.2011 - 31.12.2013

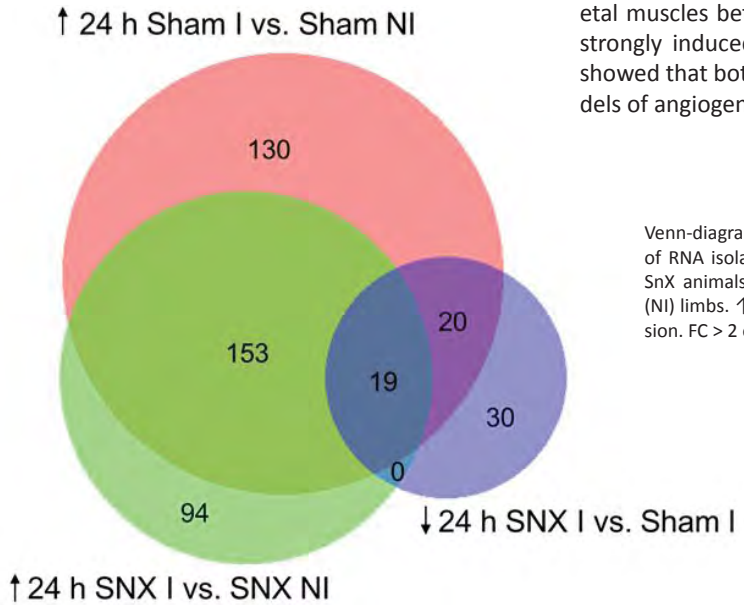
Angiogenesis in chronic renal failure

Prof. Dr. Kerstin Amann, Department of Nephropathology
Prof. Dr. Karl F. Hilgers, Department of Medicine 4 – Nephrology and Hypertension

Mortality rate is still very high in patients with chronic kidney disease (CKD) and mainly based on cardiovascular complications. Present data suggest that impaired angiogenesis in response to hypertrophy or ischemia plays an important pathophysiological role. Using an animal model of CKD and bone marrow transplantation we investigated if angiogenesis in CKD is associated with changes in the expression pattern of angiogenic factors or reduced recruitment of hematopoietic stem cells in the heart as well as in the hind limb ischemia model.

Identification of CKD dependent changes in gene expression during angiogenesis

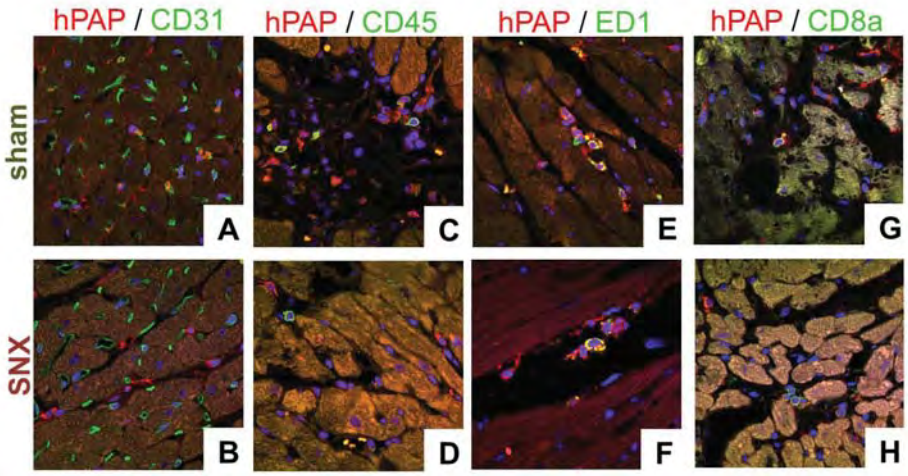
Microarray analysis was used to identify changes in gene expression in ischemic and non-ischemic hind limbs from animals with normal renal function (sham) as well as subtotal nephrectomized (SNX) animals. Therefore, 8wks after induction of CKD by SNX, a rat model of skeletal muscle ischemia by ligating and resecting a part of the superficial femoral artery was used. 24hs later, samples from skeletal muscle were collected. In cooperation with the IZKF Core Unit (Dr. Ekici) Affymetrix cDNA array was performed. Different strategies were then subsequently combined with each other to limit the number of genes of interest rationally. Comparison between ischemic and non-ischemic limbs of SNX vs sham rats showed a broad overlap of upregulated genes (Fold-Change ≥ 2) although 130 genes were upregulated in sham rats only. Combination with genes downregulated by SNX vs sham in ischemic limbs, i.e. both upregulation in ischemia and decreased gene expression in SNX, results in 39 genes of interest. From these, two potential targets (CCl7 and MT1A) were selected for further studies. Both are among the 10 most differentially regulated genes in ischemic skeletal muscles between SNX and sham, and both are strongly induced in ischemia. Work of others also showed that both play a functional role in other models of angiogenesis.



Venn-diagram based on genes of Affymetrix cDNA array of RNA isolated of M. gastrocnemius of both sham and SnX animals respectively ischemic (I) and non-ischemic (NI) limbs. ↑ increased transcription, ↓ reduced expression. FC > 2 or FC < -2, $p < 0.05$

Prof. Dr. Amann

Prof. Dr. Hilgers



Representative staining of hPAP (red), CD31, CD45, ED1, CD8a (green) or double positive (yellow) cells for detection of BMDC-infiltration into the heart in sham (upper panel) and SNX (lower panel) rats.

Tracking and characterization of bone marrow derived cells (BMDC) in chronically diseased kidneys and in the heart

Recruitment of BMDC to the injured kidney and to the heart was investigated by lethally irradiation of wildtype F344 rats followed by reconstitution with bone marrow from human placental alkaline phosphatase (hPAP) transgenic rats. Chimeric recipients were assigned in 2 groups (SNX or sham). After successful bone marrow transplantation (about 47% hPAP positive CD45 cells) high amounts of hPAP positive cells could be detected in chimeric recipients whereas the number of BMDC was nearly equal in kidneys and hearts from SNX compared to sham. Interestingly, in both groups BMDC infiltration into the kidney was nearly tenfold higher compared to infiltration into the heart. Migrated BMDC were then characterized by using immunofluorescence double staining for hPAP and different cell markers. Firstly, in both groups there were neither in the kidney nor the heart CD31/hPAP double positive cells. Thus, BMDC are obviously not a substantial source for renal or cardiac endothelial cells. In contrast, several immune cells were positive for hPAP. In detail, bone marrow derived leukocytes, macrophages, T-cells as well as cytotoxic T-cells could be identified. Quantification of these immune cells revealed only an absolute increase of both T-cells and cytotoxic T-cells in the kidney of sham and SnX animals whereas the other cell types were similarly distributed. Furthermore, there were no significant changes in immune cell recruitment (quantification of double positive cells) in SNX in comparison to sham.

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Publications during funding period

Arend N, Hilgers KF, Campean V, Karpe B, Cordasic N, Klanke B, Amann K (2014) Darbepoetin alpha reduces oxidative stress and chronic inflammation in atherosclerotic lesions of apo E deficient mice in experimental renal failure. PLoS One (in press)

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F2 - Final Report

01.10.2010 - 30.09.2013

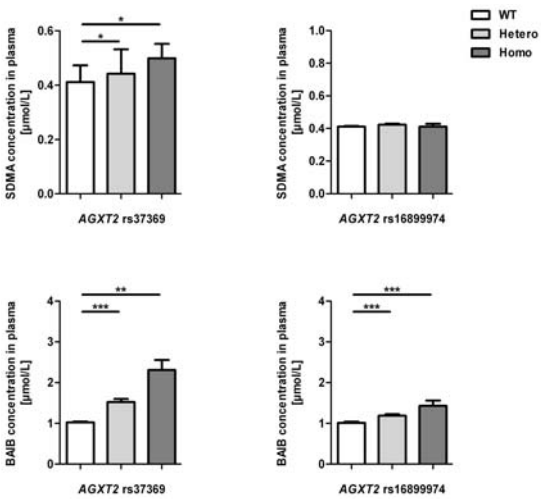
AGXT2 and methylarginines

Prof. Dr. Renke Maas, Institute of Experimental and Clinical Pharmacology and Toxicology
Prof. Dr. Jörg König, Institute of Experimental and Clinical Pharmacology and Toxicology
PD Dr. Johannes Jacobi, Department of Medicine 4 – Nephrology and Hypertension

In this project we characterized the enzyme alanine-glyoxylate-aminotransferase 2 (AGXT2) as a new target in methylarginine metabolism. We established assays to investigate AGXT2 activity and kinetics and put them into context with related metabolic pathways such as dimetyhlarginine dimethylaminohydrolases (DDAH). We investigated the pathophysiological consequences of activation or inhibition of AGXT2/DDAH activity in mice and assessed AGXT2 and DDAH polymorphisms in humans.

Gene polymorphisms affecting AGXT2-mediated metabolism.

In order to investigate the functional relevance and relative contributions of common single nucleotide polymorphisms in the AGXT2 gene to plasma and urinary concentrations of methylarginines as well as beta-aminoisobutyric acid (BAIBA, the prototypical endogenous substrate of AGXT2) we genotyped 400 healthy volunteers for the coding AGXT2 SNPs rs37369 and rs16899974. Principal findings are that in volunteers heterozygous or homozygous for the minor allele of the AGXT2 SNP rs37369 plasma SDMA and BAIB levels were elevated, while in the urine only the BAIB concentration was elevated in carriers of minor alleles. A haplotype analysis revealed that the second investigated AGXT2 SNP (rs16899974) further aggravates the effect of rs37369 with respect to BAIBA. These are the first data linking SDMA and BAIBA metabolism on an intraindividual basis. In vitro studies in cells stably overexpressing wild-type or mutant (AGXT2 p.Val140Ile; rs37369) AGXT2 protein showed decreased metabolism of 2[H]-labeled SDMA as well as reduced formation of a AGXT2-specific SDMA metabolite.



AGXT2 SNPs and biochemical measures in 400 healthy volunteers.

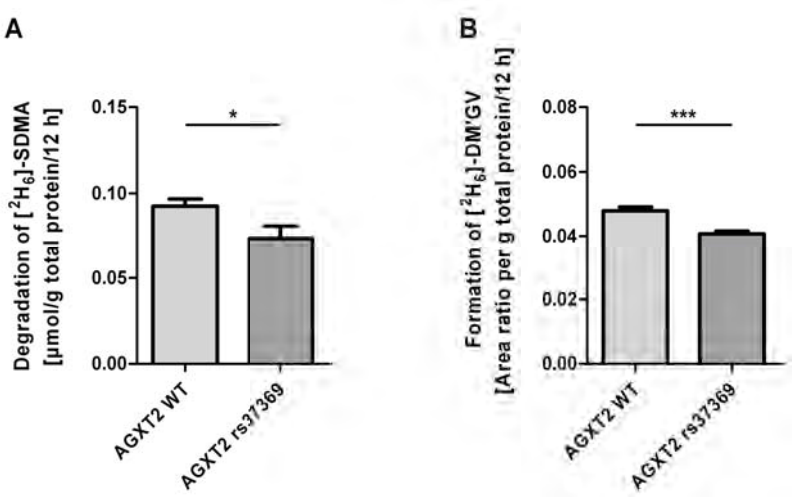
Alteration of methylarginine metabolism in mice.

In a cooperation with colleagues from the universities of Dresden and Magdeburg we investigated the effect of BAIB excess, as it can be seen in humans with hyper-β-aminoisobutyric aciduria, on methylarginine metabolism in mice. Infusion of BAIB increased plasma ADMA and SDMA concentrations by 27% and 31%, respectively, (both $p<0.05$) while plasma DMGV levels decreased 24% ($p<0.05$). Expression of Agxt2 was not altered by the infusion of BAIB. Taken together these data show for the first time that BAIB can inhibit Agxt2-mediated metabolism of dimethylarginines and that endogenous Agxt2 is involved in the regulation of systemic ADMA, SDMA and DMGV levels. The effect of lowering asymmetric dimethylarginine (ADMA) on vascular pathology was assessed in an ApoE-deficiency model in mice. Overexpression of DDAH1 was associated with significantly lower ADMA levels in all treatment groups. In contrast (and unexpected) subtotal nephrectomy had no effect on ADMA levels and only a minor effect on SDMA levels. Furthermore, overexpression of DDAH1 could not protect ApoE-deficient mice with renal impairment from atherosclerosis.


Prof. Dr. Maas


Prof. Dr. König


PD Dr. Jacobi



Characterization of HEK cell lines overexpressing human wild-type and mutant (rs37369, p.Val140Ile) AGXT2 protein; Symmetrical dimethylarginine - SDMA, (N,N'-dimethyl-guanidino) valeric acid - DM'GV

Alternative pathways of methylarginine metabolism involving DDAHs.

In the cohort of healthy volunteers mentioned above polymorphisms of DDAH1 were associated with higher methylarginine concentrations in plasma. In a complementing medical thesis work supported by an IZKF student grant to Fabian Geldmacher we found that alterations of methylarginine levels in plasma of DDAH2-deficient mice were of borderline significance and DDAH activity in tissues mainly expressing DDAH2 was not significantly altered. Moreover, DDAH2-deficient mice show no specific cardiovascular phenotype and no evidence for impaired endothelial function. However, likely new splice variants of the DDAH2 gene were identified.

Contact:
Prof. Dr. Renke Maas
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e-mail: renke.maas@pharmakologie.uni-erlangen.de

Awards

DGPT Posterpreis Klinische Pharmakologie, Anja Kittel, 07.03.2013, Halle/Saale, Germany

Publications during funding period

Kittel A, Maas R (2013) Pharmacology and Clinical Pharmacology of Methylarginines used as Inhibitors of Nitric Oxide Synthases. Curr Pharm Des. [Epub ahead of print] PMID: 24180385

Kittel A, Maas R, König J, Mieth M, Weiss N, Jarzebska N, Hohenstein B, Martens-Lobenhoffer J, Bode-Böger SM, Rodionov RN (2012) In vivo evidence that Agxt2 can regulate plasma levels of dimethylarginines in mice. Biochem Biophys Res Commun 2013 Jan 4;430(1): 84-9.

Newly started Projects

F3 01.03.2014 - 31.08.2016

Fam60a in heart and brain development

Prof. Dr. Felix Engel, Department of Nephropathology

Prof. Dr. Engel

Understanding heart development helps to identify mediators of congenital heart disease, the leading cause of birth defect-related deaths. In addition, it promotes the establishment of therapies for adult heart diseases. Our preliminary work suggests Fam60a as a novel regulator of the fate of progenitor cells in heart and brain development. Moreover, Fam60a has been associated with Alzheimer's disease. Thus, our aim is to elucidate the molecular role of Fam60a during zebrafish development.

e-mail: felix.engel@uk-erlangen.de

F4 01.10.2013 - 31.03.2016

Pathogenesis of the short rib-polydactyly syndrome

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

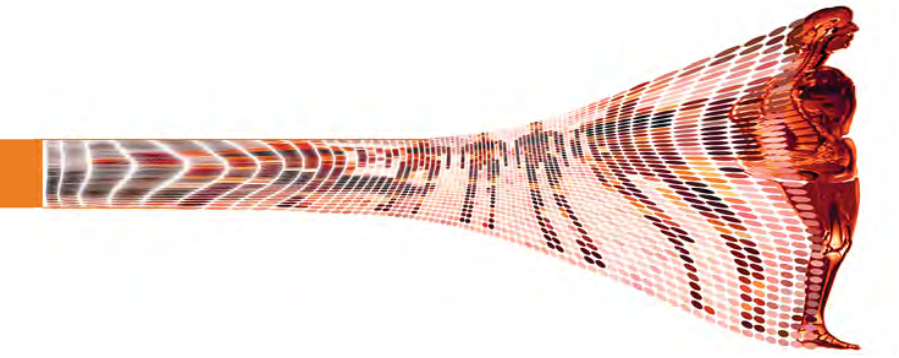
PD Dr. Thiel

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. Ciliary dysfunction (ciliopathy) has been associated with a wide spectrum of human phenotypes. The functional characterization of the NEK1 associated ciliary defects involved in Short rib-Polydactyly syndromes will gave insight into the complex mechanisms of the pathways involved in developmental regulation by the primary cilium.

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

Career Development

Career Development



Junior Research Groups

Junior Research Group N2

Title: Immune system as regulator of volume and blood pressure

Prof. Dr. Jens Titze

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

Junior Research Group N3

Title: Modeling neurodegenerative diseases using stem cells

Prof. Dr. Beate Winner

From the left: N. Zagha, S. Havlicek, V. Veber, H. Wend, T. Halder, Dr. I. Prots, PD Dr. B. Winner, H. Mishra, D. Gräf

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 a rapid transfer of our basic research findings into the clinical arena.

Understanding Na^+ storage in humans

We have implemented ^{23}NaMRI technology to non-invasively visualize Na^+ reservoirs in humans. We are now pursuing clinical studies to better understand Na+ balance in health and disease.

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

We showed that Na^+ storage in the skin and the resulting disequilibrium in interstitial Na^+ concentration attracts immune cells which then exert a homeostatic-regulatory or autoimmune phenotype. The cells either regulate interstitial electrolyte homeostasis, or deteriorate auto-immune disease.

Mars500 salt balance studies reveal body's Na^+ rhythms

At constant salt intake, daily Na^+ excretion in humans exhibits aldosterone-dependent, weekly (circaseptan) rhythms. The findings go beyond the currently accepted steady-state concept of Na^+ balance. The paper has been included into the "Best of Volumes 16 and 17" supplement by Cell Press (http://www.cell.com/bestof).

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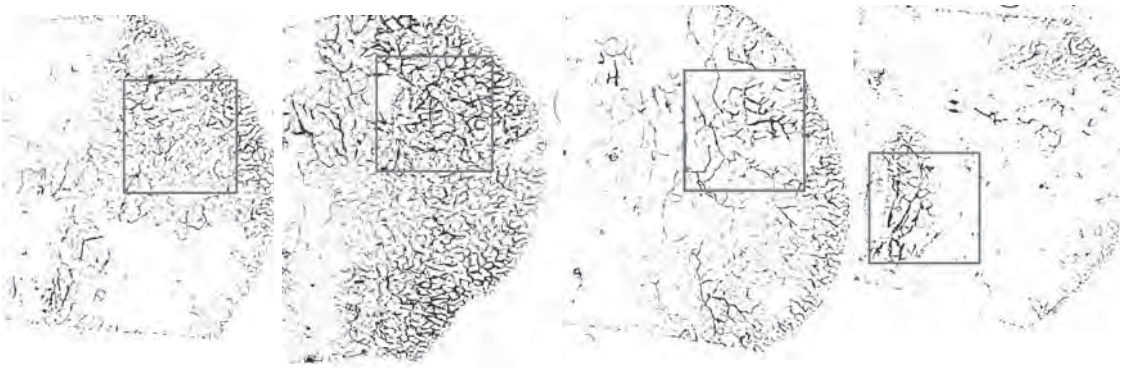
phone: + 9131 85 39300
e-mail: jens.titze@uk-erlangen.de



Left to right: Lymph capillary density from mice fed a low salt, high salt diet, or mice fed low or high salt diets treated with a drug to disrupt immune-cell driven changes in capillary density. The findings have identified a role of macrophages in blood pressure regulation via modification of the cutaneous lymph capillaries (http://blog.jci.org/2013/1084/june-3rd-titze-60113)

Prof. Dr. Titze

Invited lectures

Workshop on Landmark Discoveries in Hypertension, Kidney, and Cardiovascular Disease, High Blood Pressure Research Scientific Sessions, 2013/09/11, New Orleans, USA, "The skin as the Golden Hinde of sodium homeostasis. Dermal or renal regulation of body salt stores"

3rd Annual Cardiovascular Research Center Symposium on Lymphatics in Health and Disease, Yale School of Medicine & The North American Vascular Biology Organization, 2013/05/04, New Haven, USA, "Lymphatic control of blood pressure"

ISN World Congress of Nephrology 2013, 2013/06/02, Hong-Kong, China, "What's new in salt balance?" (http://www.theisn.org/index.php?option=com_k2&view=item&id=993:what-s-new-in-salt-balance&tmpl=component&print=1)

Kidney Week 2013 of the American Society of Nephrology, 2013/11/08 Atlanta, USA, "Evolving concepts in sodium homeostasis"

5th Annual Meeting of the Germany Society of Nephrology, 2013/10/06, Berlin, Germany, "Non-osmotic sodium reservoirs: insights from a mission to Mars"

Awards

Franz-Volhard-Prize of the German Society of Nephrology to Jens Titze, 2013/10/05, Berlin, Germany.

Mid-Career Award for Research Excellence, Council for High Blood Pressure Research of the American Heart Association to Jens Titze, 2013/09/13, New Orleans, USA.

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. Filed: October 4, 2012; Issued: April 18, 2013. Inventors: Jan Ruff, Jens Titze, Peter Linz, Thoralf Niendorf, Davide Santoro, Wolfgang Renz, Alexander Cavallaro, Michael Uder

Publications during funding period

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

Titze J (2013) Interstitial fluid homeostasis and pressure: News from the black box. Kidney Int 84: 869-71

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

Titze J, Machnik A (2010) Sodium sensing in the interstitium and relationship to hypertension. Curr Opin Nephrol Hypertens 19: 385-392

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

N3 - Progress Report

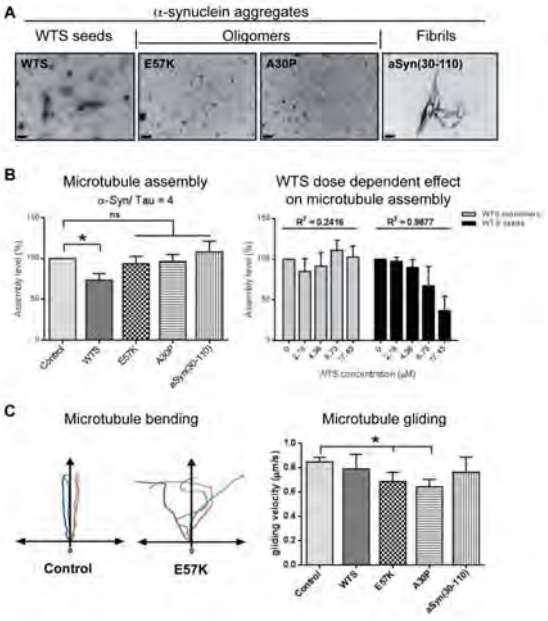
01.10.2010 - 30.09.2016

Modeling neurodegenerative diseases using stem cells

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

The overall goal of research in our laboratory is to model neurodegenerative diseases using stem cells and derivatives. Specifically, we investigate neurodegeneration and regeneration in synucleinopathies including Parkinson's disease (PD). Protein aggregation of mis-folded proteins is associated with several synucleinopathies. We are interested in studying the mechanism and functional consequences of oligomerization for neurite degeneration, axonal transport, and cellular membranes.

Deposits and aggregates of α-synuclein within neurites are a patholgical hallmark of multiple neurodegenerative diseases including dementia with Lewy bodies (DLB) and Parkinson's disease (PD). Pharmacological treatment cannot halt the progressive neuronal degeneration. While the destructive character of α-synuclein aggregation is evident and includes synaptic dysfunction and associated neurodegeneration, the underlying mechanisms and the cascade of events leading to α-synuclein-mediated toxicity and the relevance to human disease are still unclear. Furthermore, although α-synuclein oligomers are one of the toxic species of α-synuclein, their interaction with neuronal physiology is still not fully understood. Our recent data describe a structure-functional interaction of different α-synuclein species with the microtubule-based axonal transport machinery in a cell-free system and its consequences on human dopaminergic neurons.



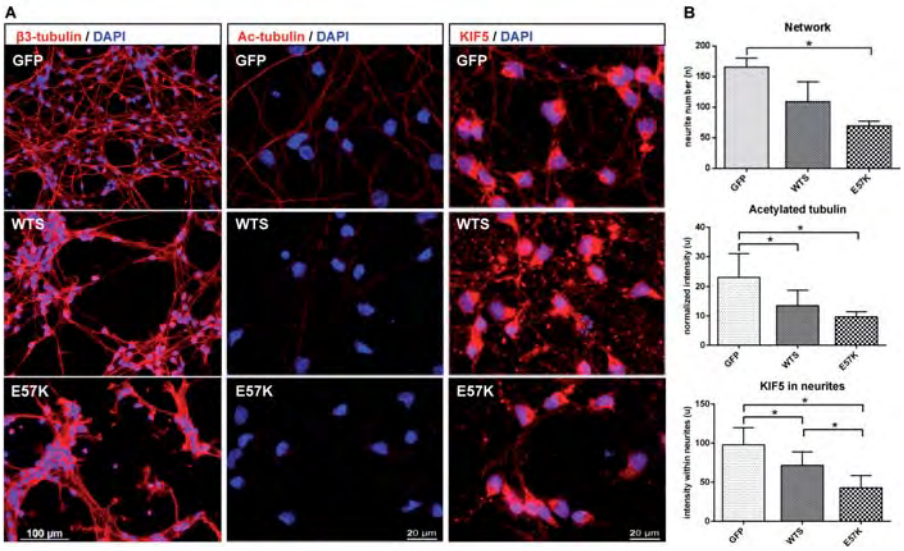
Interaction of a-synuclein aggregates with microtubule-kinesin system in vitro. (A) α-synuclein aggregates. (B) Tau-dependent microtubule assembly is inhibited by α-synuclein seeds. (C) α-synuclein oligomers inhibit kinesin-dependent microtubule gliding.

α-synuclein oligomers impair neuronal microtubule-kinesin interplay

We used recombinant α-synuclein proteins (wild type, single point mutants, and the fragment 30-110) to obtain different aggregate species in vitro. We evaluated the direct effect of these species on microtubule-based cytoskeleton in a cell-free system and in a human dopaminergic neuronal cell line. Our data show that wild type α-synuclein (WTS) monomers and aggregates bind to proteins required for microtubule-based anterograde axonal transport, such as kinesin, tubulin, microtubules, microtubule associated protein 2 (MAP2), and Tau. Interestingly, WTS aggregates composed of oligomers and short fibrils (WTS seeds) impaired microtubule polymerization in vitro promoted by axon-specific Tau protein in a dose-dependent manner. On the other side, α-synuclein oligomers decreased kinesin-microtubule motility in a model system of kinesin-driven transport in vitro. In a human dopaminergic neuronal cell line, the neurite network morphology was severely disrupted by mild overexpression of α-synuclein oligomers and less potently by a-synuclein seeds. Neurite morphology disruption in these cells correlated with a significant reduction of microtubule stability and impaired amounts of kinesin and kinesin-

Prof. Dr. Winner

dependent cargo in neurites. Thus, our study describes complex interactions of α-synuclein species with proteins involved in axonal transport and microtubule network. We propose a sequence of pathologic events in neurites induced by α-synuclein aggregation that involve impairment of microtubule-kinesin functionality microtubule network disruption by toxic soluble α-synuclein aggregates - oligomers and seeds. These alterations act together with other pathologic effects of α-synuclein oligomers, such as membrane thinning and leakage, and together represent critical early mechanisms of synucleinopathies. These results provide new intriguing insights into the pathomechanisms of very early stages of synucleinopathi*es.* (Prots et al., JBC 2013)



α-synuclein oligomers (E57K) and seeds (WTS) cause disruption of microtubule-kinesin cytoskeleton in neuronal cell line. (A) Staining and (B) quantification of neuritic network, acetylated tubulin and kinesin amount.

Contact:
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e-mail: beate.winner@med.uni-erlangen.de

Invited lectures

Seminar BioSysNet, Munich, 07.03.2013, I. Prots "Contribution of a-synuclein oligomers to axonal dysfunction"

12th Tom Wahlig Symposium, Dresden, Germany, 22.03.13, F. Perez-Branguli, „SPG11: A new member of synaptic family?"

Young Investigators Symposium of the Stem Cell Society Singapore. Biopolis, Singapore, 31.05.2013, S. Havlicek, „Modeling Autosomal Dominant Hereditary Spastic Paraplegia (SPG4) Using Human Induced Pluripotent Stem Cells"

2nd Eurogenesis meeting, Adult neurogenesis in physiology and disease, Bordeaux, Frankreich, 26.06.13, B. Winner, „Modeling synucleinopathies using stem cells"

Gage lab symposium, La Jolla, USA, 09.11.13, B. Winner, „Modeling hereditary spastic paraplegia using stem cells"

Awards

Steven Havlicek: IZKF Erlangen poster prize for the poster „Gene-dosage dependent neurite defects in a human induced pluripotent stem cell model of SPG4 related hereditary spastic paraplegia". 13th IZKF PhD workshop, 7.10.2013, Erlangen.

Publications during funding period

Havlicek S, Kohl Z, Mishra HK, Prots I, Eberhardt E, Denguir N, Wend H, Plötz S, Boyer L, Marchetto MC, Aigner S, Sticht H, Groemer TW, Hehr U, Lampert A, Schlötzer-Schrehardt U, Winkler J, Gage FH, Winner B (2013) Gene dosage dependent rescue of HSP neurite defects in SPG4 patients's neurons. Hum Mol Genet. 2013 Dec 30. [Epub ahead of print] PMID: 24381312

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

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

Junior Projects

year of application 2010 J12-J19

J20-J25 **year of application 2011**

year of application 2012 J26-J33

J20-J25 **year of application 2013**

Junior Projects

Project No.	Project leader	Project title	Start of project
J12	Waldner	The role of hypoxia inducible factor 1 during the pathogenesis of colitis-associated cancer	03/2011
J14	Kopp	$^{23}Na^{+}$-MRI in therapy refractory hypertension	04/2011
J15	Schmidt	Gap junctions and angiogenesis: A new mechanism in flow dependent vessel formation?	03/2011
J17	Dahlhaus	The relevance of differences in the human and murine Scal ortholog for the Fragile X Syndrome	02/2011
J18	Bruns	Function of macrophages in tumor stroma	02/2011
J19	Britzen-Laurent	Immune escape in colorectal carcinoma: role of the IFN-γ pathway	02/2011
J20	Fürnrohr	Functional analysis of the coding variant rs1143679 (R77H) in CD11b	11/2011
J21	Winkelmann	Function of KLF2 and 4 in B cell differentiation	01/2012
J22	Jochmann	The role of O-GlcNAc-modification in regulating KSHV gene expression and replication	11/2011
J23	Hackenbeck	The role of the hypoxia-inducible factor (HIF) and lysyl oxidases in renal fibrosis	11/2011
J24	Mougiakakos	Immune modulatory effects by oxidative stress in patients with chronic lymphatic leukemia	09/2011
J25	Boos	Establishing of an autonomous lymphatic vessel network	02/2012
J26	Kreß	Regulation of the tumor marker fascin by the oncoprotein tax of human T-cell lymphotropic virus type 1 (HTLV-1)	01/2012
J27	López Posadas	GGTase-I in intestinal epithelial cells	11/2012
J28	Leppkes	Pathomechanisms of inflammation dependent fibrogenesis	11/2012
J29	Beyer	Interaction of morphogene pathways in the development of fibrotic diseases	10/2012
J30	Thomas	Cytoplasmic functions of human cytomegalovirus pUL69	01/2013
J31	Schödel	Function of a novel, HIF-regulated transcript	02/2013
J32	Ben Abdallah	Neuropsychiatric symptoms of Parkinson's disease	09/2012
J33	Reiprich	Sox2 in the CNS: regulating myelination by microRNAs	02/2013
J34	Kremer	Indirect presentation of HLA class II restricted tumor antigens	08/2012
J35	Moskalev	lncRNA-directed epigenetic programming of HOX loci in GIST	12/2012
J36	Kremer	Identification of molecular signalling pathways in cholestatic pruritus	09/2013
J37	Bosch-Voskens	Adoptive cell therapy with ex-vivo expanded NK and γδT cells in metastatic melanoma.	07/2013
J38	Dietel	MCS-18 for the treatment of atherosclerosis	02/2014
J39	Dees	Hypermethylation of SOCS3 in fibrotic diseases	01/2014
J40	Ramming	PU.1 signalling in fibrotic diseases	01/2014
J41	Schauer	Resolution of inflammation in gout	12/2013
J42	Ferrazzi	Bayesian reverse engineering of developmental networks	04/2014

J12 - Final Report

13.03.2011 - 12.03.2013

The role of hypoxia inducible factor 1 during the pathogenesis of colitis-associated cancer

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

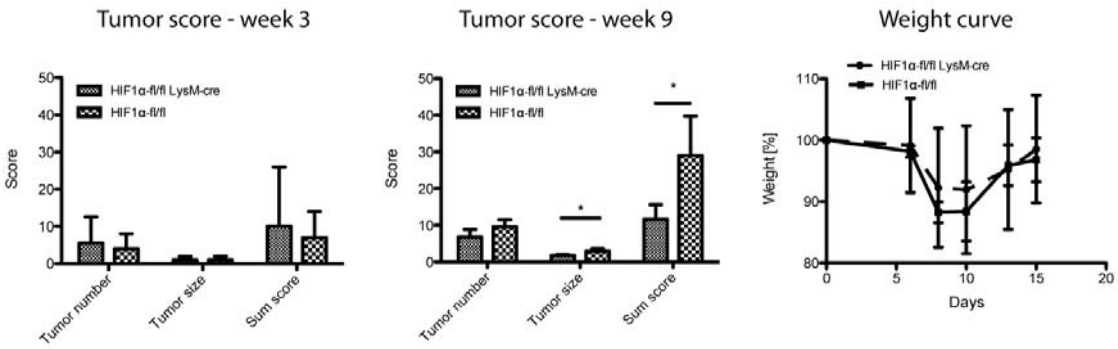
Hypoxia inducible factors (HIFs) are critical for the cellular response to hypoxia during inflammation and tumor development. This project aims at analyzing the cell specific role of HIF1 in the AOM+DSS model of colitis-associated cancer (CAC). Our results show that HIF1 activation in intestinal epithelial cells and myeloid cells is involved in the regulation of several important pathways that mediate tumor growth.

During recent years, hypoxia inducible factors (HIFs) have been identified as fundamental transcription factors regulating the adaption of cellular metabolism and angiogenesis following tissue hypoxia. Among the three known isoforms, HIF1 has been implicated in the pathogenesis of various types of human cancer. Besides tumor development, current data also propose an important role for HIF1 regulating immune cell function during acute and chronic inflammation.

Since chronic inflammation has been shown to pose an important risk factor for tumor development, as seen in inflammatory bowel disease, inflammation dependent HIF activation might contribute to tumor growth. This project analyzes the role of HIF isoforms during inflammation-associated tumor development using a murine model of colitis-associated cancer (CAC).

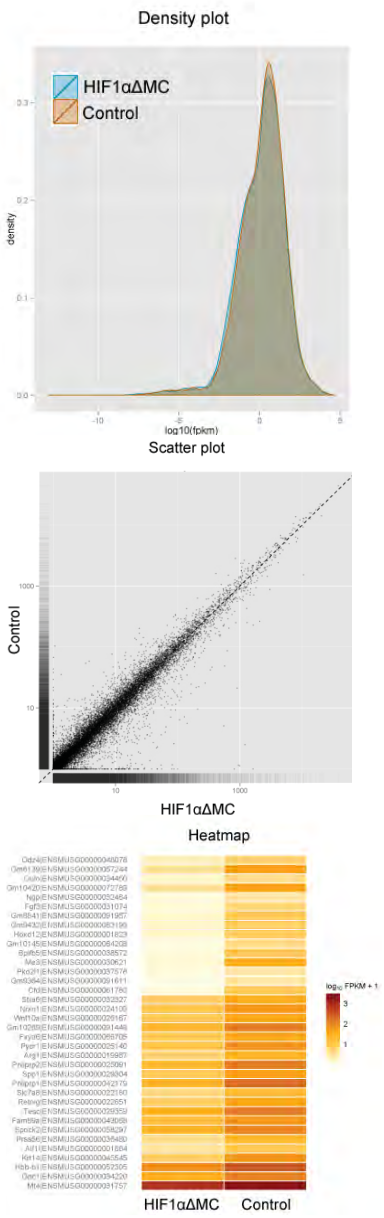
HIF1 accumulates in intestinal epithelial cells and myeloid cells during CAC development

To analyze HIF1 activation in murine CAC, wild type mice were exposed to azoxymethane (AOM) and dextran sodium sulfate (DSS). In comparison to control colon tissue, AOM+DSS induced tumors showed a marked accumulation of the HIF1alpha subunit in western blot analysis and an upregulation of HIF target genes upon qPCR. HIF1 accumulation could be located to intestinal epithelial cells and myeloid cells by immunohistochemistry.



Despite similar tumor initiation (wk 3), HIF1α-fl/fl – LysM-cre mice show reduced tumor growth at later time points (wk 9) in comparison to HIF1α-fl/fl mice upon endoscopy. Weight loss and intestinal inflammation are comparable between both groups.

Dr. Waldner



RNA-Seq of HIF1α–ΔMC tumors in comparison to control tumors. The heatmap on the right panel shows several genes downregulated in HIF1α–ΔMC tumors. These include genes involved in fibroblast growth factor and WNT signaling pathways.

HIF1 activation in myeloid cells promotes colitis-associated tumor growth

Conditional knockout mice for HIF1α in myeloid cells (HIF1α-ΔMC) were generated by crossing HIF1α-fl/fl and LysM-cre mice. In comparison to control mice, tumor growth was attenuated in HIF1α-ΔMC mice despite similar tumor numbers in the AOM+DSS model.

To identify HIF target genes and molecular pathways involved in myeloid cell dependent tumor growth, we performed next-generation sequencing for a profiling of the transcriptome in pooled HIF1α-ΔMC vs. HIF1α-fl/fl tumors (RNA-Seq). Expression analysis revealed a down-regulation of several cancer-related signaling pathways in HIF1α-ΔMC tumors including WNT, MAPK/ERK and sonic hedgehog pathways.

Subsequent experiments will involve the functional analysis of deregulated soluble factors released by myeloid cells in dependence of HIF1 activation in the AOM+DSS model.

The opposing role of HIF1 and HIF2 in intestinal epithelial cells during colitis-associated tumor development

To evaluate the role of HIF1 in comparison to HIF2 in intestinal epithelial cells (IECs) during CAC development, we crossed HIF1α-fl/fl and HIF2α-fl/fl mice with Villin-cre mice (HIF1αΔIEC and HIF2αΔIEC mice) and exposed these mice to the AOM+DSS protocol. Interestingly, HIF1αΔIEC showed reduced tumor growth whereas tumor development was aggravated in HIF2αΔEC mice. These data propose an opposing role for HIF1 and HIF2 in IECs during CAC development.

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Invited lectures

Cesar-Annual Meeting 2013, 29.6.2013, Tübingen, Präklinische Bildgebung in der onkologischen Forschung und deren Translation

Publications during funding period

none

J14 - Final Report

01.04.2011 - 31.03.2013

$^{23}Na^{+}$-MRI in therapy refractory hypertension

Dr. Christoph Kopp, Department of Medicine 4 – Nephrology and Hypertension

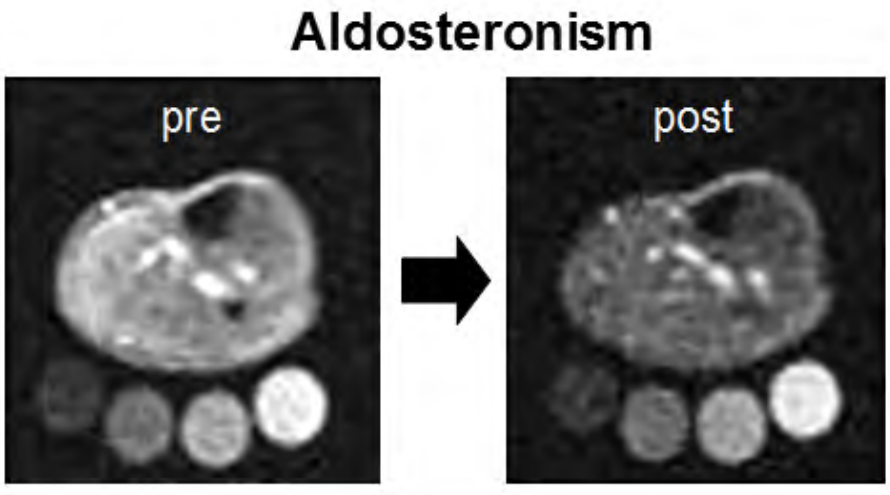
A high Na+ diet leads to Na^{+} accumulation in tissue and high blood pressure in animals. However, nothing is known about tissue Na+ storage in humans. To non-invasively visualize Na^{+} reservoirs in humans we established ^{23}Na-MRI. Using this new methodology we could demonstrate that patients with refractory hypertension and aldosteronism are tissue Na^{+} overloaded, which can be reversed upon treatment. Aging was associated with tissue Na^{+} accumulation and increased prevalence of hypertension.

Introduction

High dietary Na^{+} intake is associated with hypertension. It is believed that a renal impaired salt-/water excretion capability leads to fluid and Na^{+} retention and hypertension. This assumption was based on the paradigm that Na^{+} accumulation is inevitably followed by water retention to keep iso-osmolarity. However, in animals fed a high salt diet, we could find water-free Na^{+} accumulation in tissue, which was associated with elevated blood pressure. Human balance studies confirmed that an increase in body Na+ is not necessarily paralleled by water retention. As these studies were indirect approaches the question still remained, whether Na^{+} storages exist in human and if they are relevant in blood pressure regulation. To address this question, we implemented ^{23}Na-MRI to visualize tissue Na+ distribution in humans.

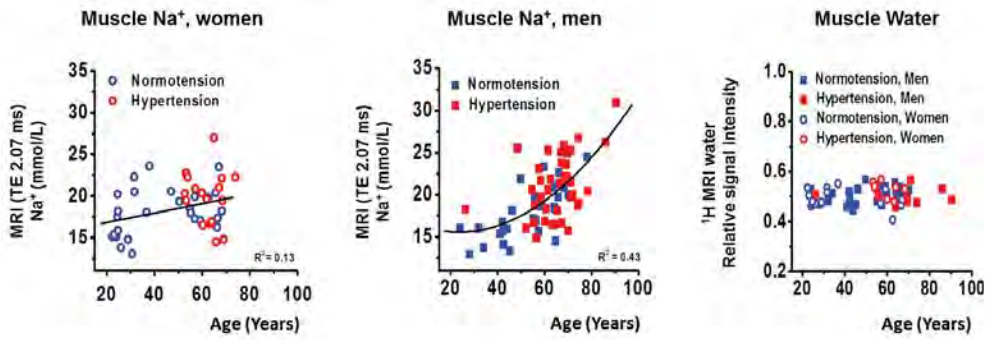
Tissue Na+ accumulation and hypertension

We first focused on patients with secondary hypertension due to aldosteronism. We investigated tissue Na^{+} by ^{23}Na-MRI on the level of the calf before and after removal of an adrenal gland or spironolactone treatment. Patients with aldosteronism showed a 29% higher muscle Na+ content than age-machted controls, which was reversed upon treatment. To further characterize the link between hypertension and tissue Na+ distribution, we investigated 56 healthy men and women and 57 men and women with essential hypertension. In this cohort refractory hypertensive patients (≥3 antihypertensive drugs) showed significant increased tissue Na+ content compared to age-matched controls.



^{23}Na-MRI of the lower leg from a representative patient with aldosteromism before and after adenoma removal. Calibration solutions containing 10-40 mmol/l of NaCl are arranged below the calf.

Dr. Kopp



Muscle Na+ detected by ^{23}Na-MRI in women (left) and men (middle) with or without the diagnosis of hypertension. Using 1H-MRI an additional analysis of muscle water content was performed in this study group (right). Muscle Na+ increased with age.

Age, gender and tissue Na+ distribution

Ages in our cohort ranged from 22 to 90 years. Systolic blood pressure increased with age in this cohort. Increasing age was paralleled by skin Na^+ storage in men and women; however skin Na^+ content remained lower in women. Age-dependant elevation of Na^+ was also found in muscle in men but not in women. This muscle Na^+ accumulation did not lead to water retention, as estimated by 1H-MRI.

States of pathological tissue Na^+ distribution

According to the principal of iso-osmolarity, changes in serum Na^+ as found in dysnatremias should be followed by a proportional difference in tissue Na^+. Surprisingly, in a patient with hypernatremia, undergoing ^{23}Na-MRI measurements before and after normalization of serum Na+, we found unexpected large shifts of Na+ inside and outside of muscle. These shifts were related to changes in serum aldosterone levels.

Conclusion

Using ^{23}Na-MRI we found an accumulation of tissue Na^+ with age and in patients with refractory hypertension and aldosteronism. Besides, there was a gender difference in tissue Na^+ distribution and muscle Na^+ was increased without parallel water retention, indicating water-free Na+ storage. In hypernatremia internal tissue Na^+ shifts occurred, that could not be predicted by serum Na^+ measurements. In future we want to elucidate, if the observed tissue Na+ overload is an independent risk factor for cardiovascular morbidity and mortality.

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Publications during funding period

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(3): 635-40.

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 K, Morukov B, Luft FC, Titze J (2013) Long-term space flight simulation reveals infradian rhythmicity in human Na+ balance. Cell Metab. 17(1): 125-31.

Kopp C, Linz P, Hammon M, Schöfl 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(12): 1343-4.

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(1): 167-72.

J15 - Final Report

01.03.2011 - 14.03.2013

Gap junctions and angiogenesis: A new mechanism in flow dependent vessel formation?

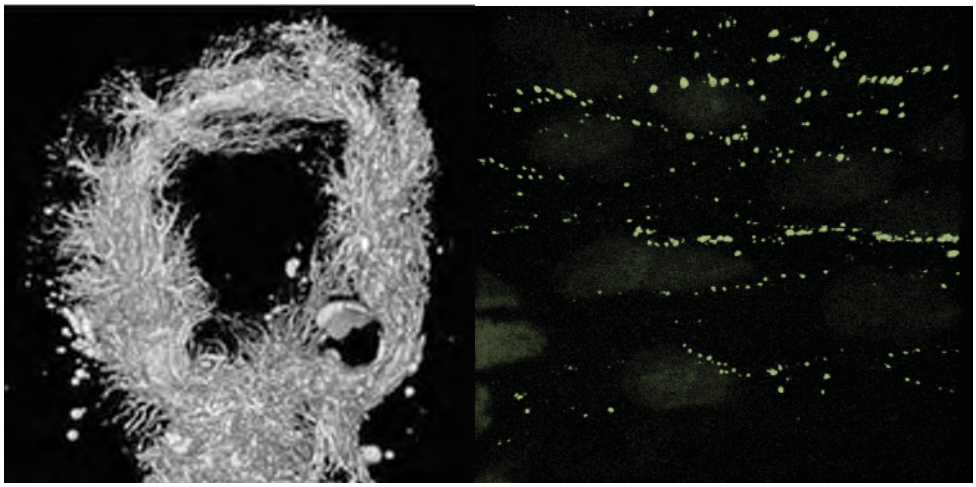
Dr. Volker Schmidt, Department of Plastic and Hand Surgery (till 31.10.2012)
Berufsgenossenschaftliche Unfallklinik Ludwigshafen (from 01.11.2012)

We hypothesize that hemodynamic changes affect vascular connexion (Cx) expression and thereby may subsequently contribute to angiogenesis. To test this hypothesis, blood flow was characterized in a rat angioinductive AV loop model and expression of Cx was assessed in a vein used as interpositioned vessel. Further, we investigated the role of VEGF and radiation on vessel formation and proposed a quantitative evaluation method to provide a precise tool for the analysis of angiogenesis.

The Cx gene family comprises 20 members in the mouse genome which are named according to their molecular weight. In human and rodents four Cxs are expressed in the cardiovascular system, namely Cx37, Cx40, Cx43 and Cx45. A recent study revealed, that Cx43 is of special importance in uterine angiogenesis during pregnancy, as its specific deletion impairs development of new blood vessels in mice, resulting in the arrest of embryo growth.

In the present study a rat AV loop model was used in which a venous graft from the femoral vein was placed between the contralateral femoral artery and vein. The AV loop was then encased in an isolated subcutaneous chamber filled with a fibrin matrix. After 15 days, a large network of new vessels was observed within the 3-dimensional construct by means of micro-CT which arose from the venous graft and was perfused as evidenced by staining following ink injection.

Measurement of blood flow by transit time flow probes revealed that the interposition of a venous graft between artery and vein introduced substantial higher flow through the grafted vessels (~ 4.5-fold) and also exposed the venous graft towards a drastically increased pulsatile flow that even exceeded arterial pulsatility. During time of angiogenesis, flow through the graft decreased and attained at day 15 levels that were observed in non-treated vessels. The substantial decrease of flow reflects a pronounced increase of the resistance along the grafted



Micro-CT analyses following microfil© perfusion revealed strong luminal neovascularisation within the fibrin filled chamber arising from the venous graft.

Immunostaining detects endothelial cell specific Cx43 expression (dots) within the graft after hemodynamic stimulation.

Dr. Schmidt

vessel. Along the loop the flow amplitude decreased and thus was significantly lower at its exit than at the entry suggesting that at least part of the flow perfuses a microcirculatory bed.

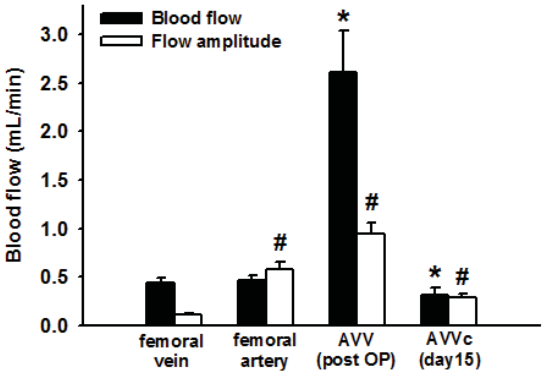
Most interestingly, Cx43 expression appears to be specifically enhanced in endothelial cells, while it is detected only marginally at physiologic conditions in this vein. In contrast, Cx37 expression was unaffected and the expression of Cx40 was significantly reduced within the venous graft after 5 days of AV-loop hemodynamic exposure.

Systemic application of the VEGF inhibitor bevacizumab as well as irradiation of the arteriovenous graft prior surgery attenuated the total amount of newly formed vessel. However, combination of radiation and VEFG inhibition does not induce a synergistic negative effect on neovascularisation.

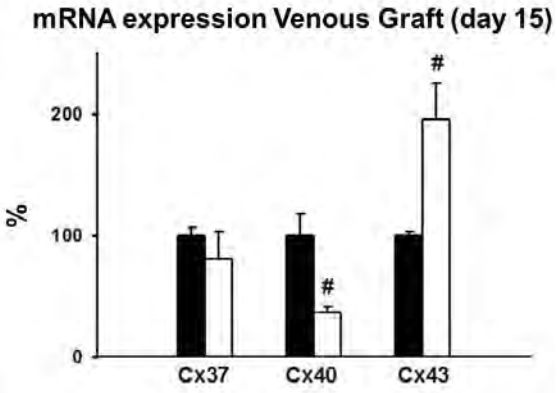
In summary, we present evidence that Cx43 expression is strongly upregulated in a grafted vein exposed to large oscillatory flow and demonstrate a phenotypic change of a vessel in vivo with

respect to Cx expression. The subsequently ensuing angiogenesis that is observed independent of angiogenic factors may be a consequence of this phenotypic change. This observation may

have implications in diseases in which angiogenesis is a therapeutical measure. Further we emphasized the role of VEGF for the AV loop associated vessel formation and negate the hypothesis that its inhibition may have a negative synergistic effect on neovascularisation in combination with radiation.



Median blood flow and median flow amplitude (cardiac-cycle dependent flow difference) are measured using microcirculation flow probes.



Venous Cx43 mRNA expression is highly induced by hemodynamic changes.

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Invited lectures

35. Jahrestagung der deutschsprachigen Arbeitsgemeinschaft für Mikrochirurgie der Gefäße und Nerven (DAM), 21.-23.11.2013 Deidesheim, „Hoher Blutfluss, Connexine und Vascular Endothelial Growth Factor (VEFG) vermitteln die intrinsische Vaskularisation von in vivo gezüchteten Lappenplastiken“

Publications during funding period

Schmidt VJ, Hilgert JG, Covi JM, Weis C, Wietbrock JO, de Wit C, Horch RE, Kneser U (2013), High flow conditions increase connexin43 expression in a rat arteriovenous and angioinductive loop model. PLoS One. 2013 Nov 13;8(11)

Schmidt VJ, Jobs A, von Maltzahn J, Wörsdörfer P, Willecke K, de Wit C (2012) Connexin45 is expressed in vascular smooth muscle but its function remains elusive. PLoS One. 2012;7(7)

J17 - Final Report

15.02.2011 - 14.02.2013

The relevance of differences in the human and murine Scal ortholog for the Fragile X Syndrome

Dr. Regina Dahlhaus, Institute of Biochemistry

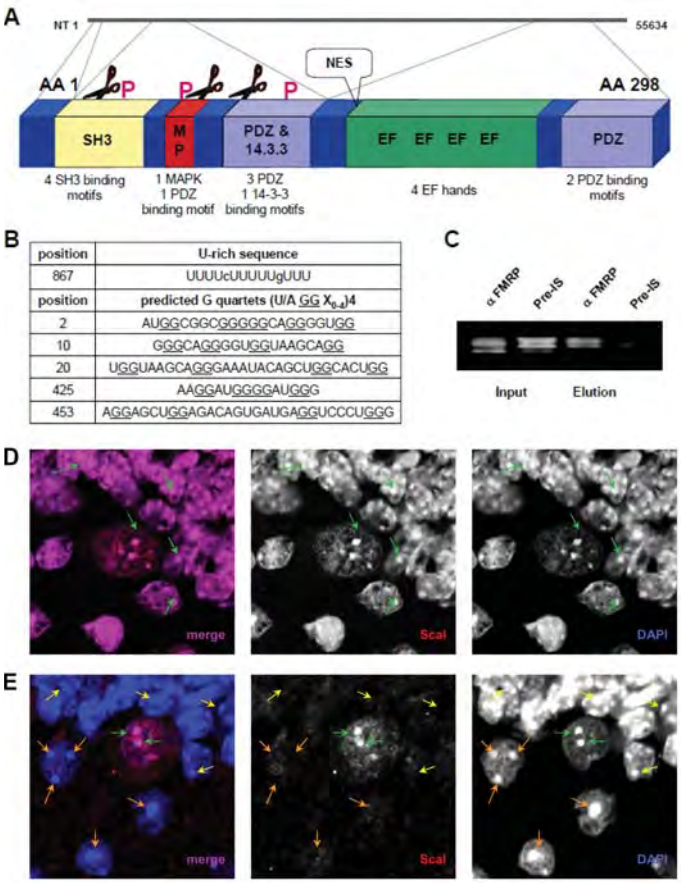
Mental retardation and autistic behaviours characterise the Fragile X Syndrome. Caused by a loss of the mRNA binding protein FMRP, vast misregulation of the mRNA metabolism is underlying the disease. Accordingly, Scal, a novel Ca-sensor protein whose mRNA contains several FMRP recognition motifs, is shown to be expressionally regulated by FMRP. Interestingly, the murine isoform of Scal has several different orthologs in human, suggesting that the diversification is relevant to human evolution.

Adaptor proteins are of special interest with regard to the regulation of signalling pathways. A protein that contains several binding interfaces as they are required for the constitution of protein complexes and the cross-linkage of cellular processes is Scal.

Though being a small protein of 33kDa only, Scal still contains several consensus sequences for protein-protein interactions as well as a nuclear export signal and some phosphorylation and proteolytic cleavage sites. In addition, the protein is marked by the presence of 4 EF hands, which characterize Scal as a calcium sensor protein.

Scal is derived from an above average sized gene which consists of 3 normal dimensioned exons and 2 large introns of several kb in size. As extensive introns increase the transcription time, vast introns are typically not observed in highly transcribed genes, suggesting that the Scal-mRNA is of rather low abundance. Rare mRNAs are not so exceptional in the mammalian brain and typically encode proteins with restricted expression patterns. Indeed, immunofluorescene labelling revealed a highly confined localisation of Scal in the murine brain.

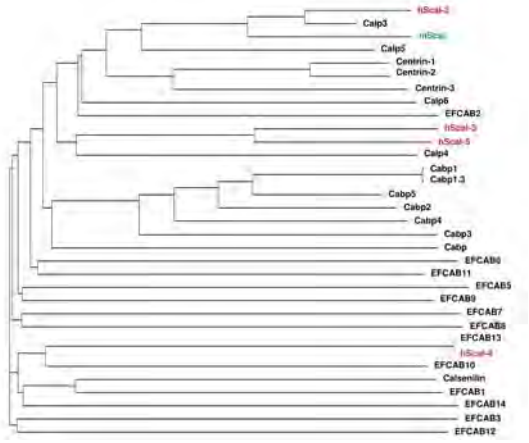
On the RNA level, a number of FMRP recognition motifs, which are located in the 5' and 3' UTR, can be identified. Since re-



position	U-rich sequence
867	UUUUcUUUUUgUUU
position	predicted G quartets (U/A GG X_{0-4})4
2	AUGGCGGCGGGGGCAGGGGUGG
10	GGGCAGGGGUGGUAAGCAGG
20	UGGUAAGCAGGGAAAUACAGCUGGCACUGG
425	AAGGAUGGGGAUGGG
453	AGGAGCUGGAGACAGUGAUGAGGUCCCUGGG

The pictures illustrate the structure of Scal (A), the interaction of its transcript with FMRP (B,C) as well as the subsequent loss of colocalisation of Scal and DAPI in brain slices from adult FMR1-/- mice (E) when compared to wildtype animals (D).

Dr. Dahlhaus



The phylogenetic tree shows several families of human Calcium sensor proteins, which display a significant similarity with Scal.

gulatory elements found in the 5‘ UTR of a transcript are likely to function in translation regulation, while those located in the 3‘ UTR are usually dedicated to RNA transport, the presence of such elements in both UTRs of the Scal transcript suggests a role of FMRP in the transport and translation of the mRNA.

Histological studies conducted in FMR1-/-mice, a model for FXS, showed that the localisation of Scal changes significantly when FMRP is lost. While the intense expression in neuronal fibres or the lateral cerebellar nucleus present in wildtype mice is maintained in FMR1-/- animals, nuclei throughout the brain display not only a decreased amount of Scal, but also region dependent variations in the degree of colocalisation with chromatin. These findings suggest that FMRP regulated mRNA transport and translation are important for an appropriate expression of Scal and that altered nuclear functions of Scal are involved in FXS.

Interestingly, the human and the murine isoform of Scal differ significantly, suggesting that novel and varying functions evolved from mouse to man. Moreover, while there is only one isoform present in mouse, four were found in man thus far. Sequence analysis revealed that in humans, Centrins, Calsenilin, EF hand containing Calcium binding proteins (EFCABs), Calmodulin-like proteins (Calps) and Calcium binding proteins (Cabps) show the highest degree of similarity with Scal, however, except for Centrins and Cabps, the different protein families are not clearly distinguished from each other, but rather occur in heterogeneous groups. These findings imply that the outlined Ca-sensor proteins quickly diverged from a common ancestor and/or developed convergently during human evolution.

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Invited lectures

GRS: Excitatory Synapses & Brain Function, 9.6.2013, Les Diablerets, „The novel protein Mate provides first evidence for a role of nuclear speckles in the pathology of the Fragile X Syndrome“

Publications during funding period

none

J18 - Final Report

01.02.2011 - 31.01.2013

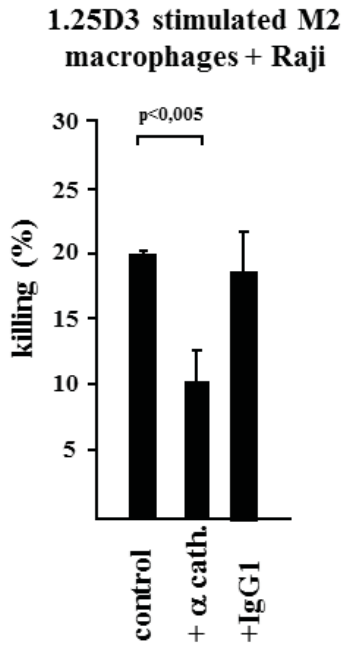
Function of macrophages in tumor stroma

Dr. Heiko Bruns, Department of Medicine 5 – Hematology and Oncology

Although macrophages are in principle directly cytotoxic against tumor cells, tumor associated macrophages (TAMs) regularly fail to exert cytotoxic functions. The underlying mechanism responsible for this loss of function remains unclear. Here we show that the therapeutic activation of the vitamin D pathway may restore tumoricidal effector mechanisms of TAMs

M1 macrophages kill lymphoma cells in vitro.

It remains unexplained which effector mechanisms are operative in human tumoricidal macrophages and are compromised in human TAMs. We approached this question using a well-characterized human macrophage model representing two extremes in the spectrum of the macrophage polarization, and measured cytotoxic activity of in vitro generated M1 or M2 macrophages against several BL cell lines. When cultured with M1 macrophages significant cytotoxicity was detected, after 48 hours and at E/T ratios of 5:1, for all lymphoma cell lines tested. In contrast, M2 macrophages were unable to effectively kill BL cells over a broad range of E/T ratios.

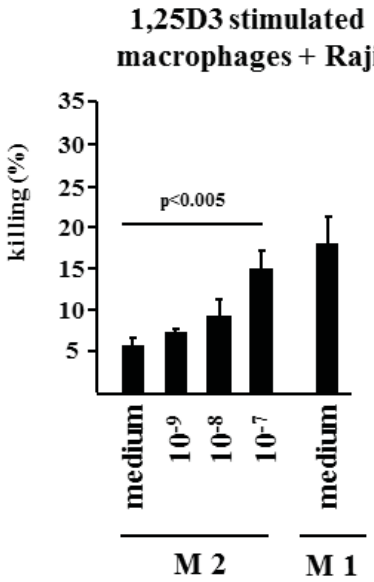


Vitamin D triggered cytotoxicity by M2 macrophages is mediated by cathelicidin

M1 macrophages kill lymphoma cells by secretion of cathelicidin.

Given that vitamin D plays a key role in regulating effector functions of human macrophages and M2 macrophages failed to kill BL cells, we reasoned that the decisive effector molecule in M1 and M2 macrophages is regulated by this pathway. We therefore analyzed vitamin D-dependent effector molecules differently expressed in M1 and M2 macrophages. M2 macrophages showed significantly decreased mRNA levels and intracellular protein expression of cathelicidin, compared to M1 macrophages, even in the presence of sufficient 25D levels. We detected increasing amounts of cathelicidin in the supernatant of M1 macrophages after co-culture with BL Raji target cells. In contrast, we found only a minimal expression of cathelicidin in the supernatant of M2 macrophages co-cultured with BL Raji cells. To confirm the requirement of cathelicidin for the macrophage mediated tumour killing, we investigated the cytotoxicity of M1 macrophages in the presence of a neutralizing anti-cathelicidin monoclonal antibody or an isotype control mAb. The cytotoxicity of M1 macrophages was markedly inhibited in the presence of the anti-cathelicidin antibody, while isotype control treatment had no effect.

Dr. Bruns



Vitamin D triggers killing of Burkitt's lymphoma cells by M2 macrophages

Vitamin D triggers killing of Burkitt's lymphoma cells by M2 macrophages

Because cathelicidin is up-regulated by the bioactive form of vitamin D, 1,25D3, we hypothesized that addition of 1,25D3 would increase the cytotoxicity of M2 macrophages against lymphoma cells. Activation of macrophages with 1,25D3 or Bxl-628 (a vitamin D receptor agonist) enhanced gene expression of cathelicidin and increases the cytotoxicity of M2 macrophages against BL cells reflecting the levels of cathelicidin expression. In summary, these experiments identify cathelicidin as an important effector molecule in tumoricidal human macrophages. Furthermore, these findings strongly indicate that treatment of TAMs with vitamin D increase the cathelicidin production, thereby triggering their tumoricidal activity and promoting antitumor immunity.

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Invited lectures

7th Cellular Therapy Symposium, 14.03. 2013, Erlangen, „Vitamin D triggers killing of B-cell lymphoma cells by macrophages".

Jahrestagung der Deutschen Gesellschaft für Hämatologie und Onkologie (DGHO), 21.10.2013, Wien, Human macrophages kill Burkitt Lymphoma cells via vitamin D mediated induction of cathelicidin

Jahrestagung der Deutschen Gesellschaft für Hämatologie und Onkologie (DGHO), 20.10.2013, Wien, Transdifferenziation of malignant B-cells into macrophages in a murine model of Burkitt´s lymphoma

Awards

Abstract Achievement Award, Annual Meeting of the American Society of Hematology (ASH) New Orleans, USA, 7.12.201313 – 10.12.2013

Publications during funding period

none

J19 - Final Report

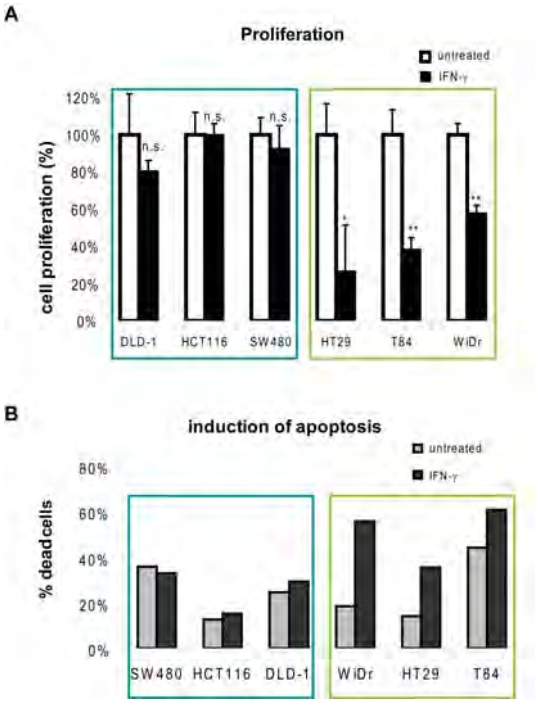
15.02.2011 - 14.02.2013

Immune escape in colorectal carcinoma: role of the IFN-γ pathway

Dr. Nathalie Britzen-Laurent, Department of Surgery

The presence of a Th1 adaptive immune response in colorectal carcinoma (CRC) correlates with improved survival of patients. An important mediator of this response is IFN-γ, which exerts direct and indirect tumor suppressive effects. Our previous results suggested that CRC cells escape anti-tumor immunity by becoming resistant to IFN-γ. In the present project we have characterized the response to IFN-γ in CRC cell lines and investigated the status of the IFN-γ response pathway in CRC tumor samples.

The presence of a Th1 adaptive immune response correlates with improved clinical outcome and survival of patients with colorectal carcinoma (CRC). The Th1 adaptive immune response is mediated in particular by IFN-γ, which exerts anti-proliferative, anti-apoptotic and pro-immunogenic effects on tumor cells. We previously identified the IFN-γ-induced guanylate-binding protein-1 (GBP-1) as a marker of the Th1 immune response in CRC. Our preliminary data suggested a loss of responsiveness to IFN-γ in CRC tumor cells, notably characterized by a loss of GBP-1 expression. The aims of the present project were to 1) characterize the consequences of the loss of GBP-1 in terms of tumorigenic activities of the CRC tumor cells, 2) to identify the defects involved in the impairment of the IFN-γ signaling pathway and 3) to investigate the IFN-γ response pathway in CRC tumor samples.

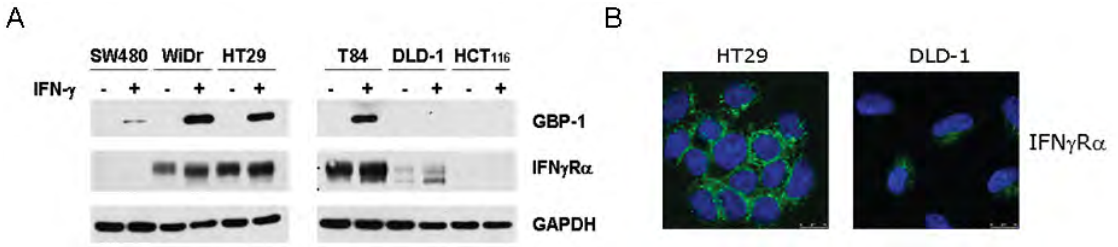


A. Ratio of proliferating cells between IFN-γ-treated and untreated CRC cell lines. B. Effects of IFN-γ treatment on apoptosis in CRC cell lines. WiDr, HT29 and T84 cell lines (green frame) are sensitive to the anti-proliferative and pro-apoptotic effects of IFN-γ, whereas DLD-1, HCT116 and SW480 cell lines (blue frame) are resistant.

Characterization of the IFN-γ response in CRC cell lines

The phenotypic characterization of six CRC cell lines after treatment with IFN-γ (DLD-1, WiDR, SW480, HCT116, HT29 and T84) included the expression of IFN-γ target genes, the inhibition of proliferation and the induction of apoptosis. We could show that 3 of these cell lines (WiDr, HT29 and T84) are sensitive to treatment with IFN-γ while DLD-1, SW480 and HCT116 cells are resistant. Furthermore, we showed that GBP-1 mediates the anti-tumorigenic effects of IFN-γ in CRC cell lines. More precisely, GBP-1 was found to be necessary and sufficient to inhibit the proliferation, migration, invasion and anchorage-independent growth of CRC cells. In addition, GBP-1 was necessary for the IFN-γ-induced apoptosis and inhibited tumor formation in immunodeficient mice. This means that the loss of GBP-1 expression represents a growth advantage for the tumor cells.

Dr. Britzen-Laurent



A. Expression of IFNγRα in CRC cell lines. B. Localization of IFNγRα in HT29 and DLD-1 cells. In DLD-1 cells, the receptor is mislocalized in the perinuclear area. C. The detection of IFNγRα in human CRCs by immunohistochemistry revealed different levels of expression in tumor cells.

Identification of defects in the IFN-γ response pathway

In order to identified the potential defects of the IFN-γ response pathway in the resistant cells, the expression of genes involved in the IFN-γ response pathway or of target genes has been evaluated at the RNA level using quantitative RT-PCR. In HCT116 and SW480, a down-regulation of the expression of the IFN-γ receptor α chain (IFNGR1) was observed and confirmed at the protein level whereas the presence of a truncated form of the receptor with abnormal subcellular localization was detected in DLD-1 cells. The expression of other downstream genes of the pathway (STAT1, IRF1) and target genes such as GBP1, CASP1, B2M or OAS1 was also down-regulated.

Investigation of the IFN-γ pathway in situ

The detection of IFNGR1 in paraffin-embedded CRC tissues by immunohistochemistry has been established. The expression of IFNGR1 has been subsequently analyzed in 73 CRC tumor sections. In 34% of the cases, IFNGR1 expression was negative or low. Subsequently, the expression of IFNGR1 has been investigated in a tissue microarray from 388 CRC patients at different stages of the disease. The results are currently under evaluation and will be statistically analyzed in order to evaluate the correlation of IFNGR1 expression with clinical parameters such as 5-year disease-related survival or the development of metastasis.

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Publications during funding period

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(1):153-62

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

J20 - Final Report

01.11.2011 - 15.08.2013

Functional analysis of the coding variant rs1143679 (R77H) in CD11b

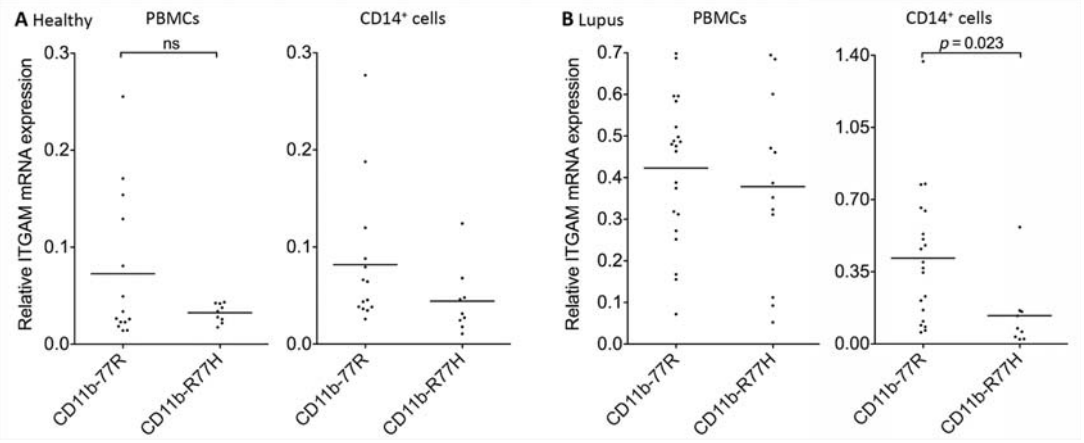
Dr. Barbara Fürnrohr, Department of Medicine 3 – Rheumatology and Immunology

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease of unknown origin, potentially involving almost every organ. The genetic variant rs1143679 of ITGAM encoding the R77H exchange in CD11b is significantly associated with SLE. CD11b is expressed on phagocytes, functioning as an adhesion molecule and a phagocytic receptor. CR3 ligation is likely to be important in immune complex and apoptotic cell clearance, both of which may contribute to the pathogenesis of SLE.

ITGAM encodes the integrin, αM (CD11b), a single-pass type I membrane protein predominantly expressed on monocytes, neutrophils, eosinophils and natural killer cells as well as on B and T cell subpopulations. Together with its β2 chain (CD18) integrin αM forms the functionally active heterodimer integrin αM/β2 (complement receptor 3 (CR3)/Mac1), a receptor for iC3b, fibrinogen, factor X and ICAM1. CR3 mediates adhesion, phagocytosis and immune signal transduction, which are all known to be affected and at least partially impaired in SLE. The rs1143679 polymorphism encodes an amino acid exchange at position 77 from arginine to histidine (R77H). This coding variant has considerable impact on the clinical outcome of patients with SLE, as the risk allele strongly correlates with renal, discoid and immunological manifestations in lupus patients. Recently, we could demonstrate that ex vivo monocytes from healthy individuals homozygous for CD11b-77H have reduced adhesive and phagocytic capacities and that CR3-ligation abolishes cross-regulation and inhibition of Toll-like receptor 7/8 signalling.

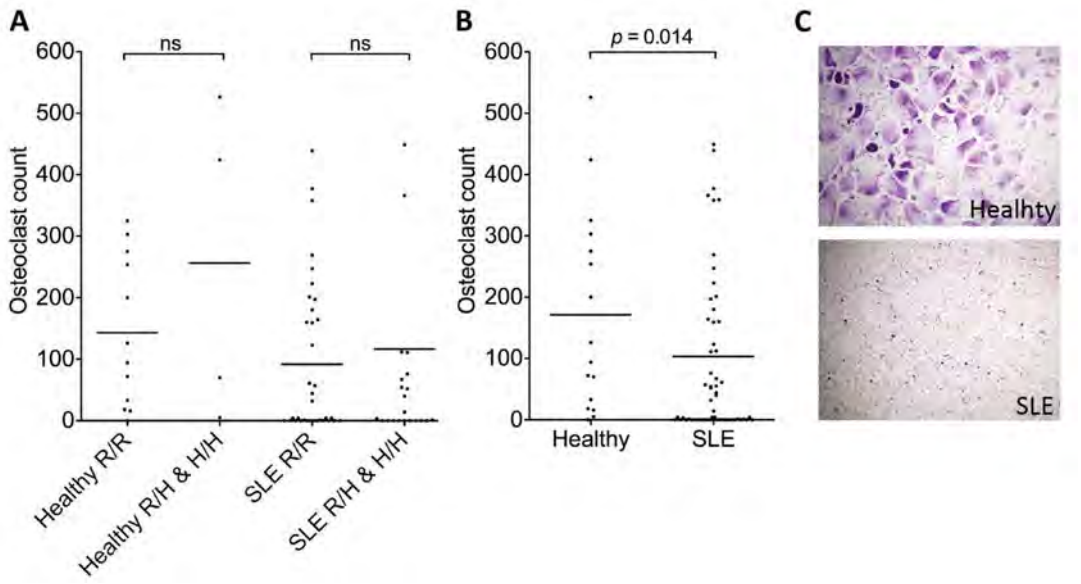
CD11b expression in healthy individuals and SLE patients

Apart from adhesive and phagocytic phenotypes, we have analysed CD11b cell-surface expression in homozygous and heterozygous healthy volunteers and SLE patients by flow cytometry and qPCR of peripheral blood mononuclear cells (PBMCs) and isolated CD14+-cells. We found no genotype-specific difference in CD11b expression in healthy individuals, however, in heterozygous patients with SLE the expression of ITGAM mRNA was significantly reduced in monocytes after normalisation to four different housekeeping genes.



RNA and cDNA was isolated from PBMCs and monocytes of healthy donors (A) and SLE patients (B). Expression of ITGAM mRNA in cells from wild-type (CD11b-77R) and heterozygous (CD11b-R77H) volunteers was normalised to four housekeeping genes.

Dr. Fürnrohr



CD14+-cells were negative selected and differentiated to osteoclasts in the presence of M-CSF, RANKL and TGFβ for 13 days. No genotype specific effect was observed (A), however, in SLE patients osteoclastogenesis seems to be impaired (B & C).

Osteoclast differentiation in patients with SLE

Results from others provide evidence that differentiation of CD14+-cells into osteoclasts is likely to be a CD11b-dependent process, as they found impaired osteoclastogenesis in cells with low CD11b expression or in presence of an anti-CD11b-blocking antibody. Since we observed reduced expression of CD11b in monocytes of SLE patients, we hypothesised that osteoclastogenesis might also be affected.

To investigate osteoclastogenesis in patients with SLE we have isolated untouched CD14+-cells from PBMCs by negative selection and differentiated these cells into osteoclasts. After 13 days osteoclasts were stained for the histochemical marker tartrate-resistant acid-phosphatase and nuclei as well as stained osteoclasts were counted. We found no genotype-specific difference concerning the osteoclast count in both healthy individuals and SLE patients. However, in a certain proportion of SLE patients the osteoclastogenesis seemed to be markedly impaired, since in some SLE patients hardly any osteoclasts could be detected. Interestingly, in SLE patients with very low osteoclast count, serum level of interferon-α was significantly elevated. Clinical and medicinal parameters are currently analysed, which might shed further light on this observed differentiation defect.

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Invited lectures

Seminarvortrag am Department für Genetische Epidemiologie der Medizinischen Universität Innsbruck, 21.03.2013, Innsbruck, „Functional analysis of a Lupus associated coding variant in complement receptor 3“

Publications during funding period

Rhodes B1 , Fürnrohr BG1, Roberts AL, Tzircotis G, Schett G, Spector TD, Vyse TJ (2012)

The rs1143679 (R77H) lupus associated variant of ITGAM (CD11b) impairs complement receptor 3 mediated functions in human monocytes. Ann Rheum Dis. 2012 Dec;71(12): 2028-34. (1 equal authors contribution)

J21 - Final Report

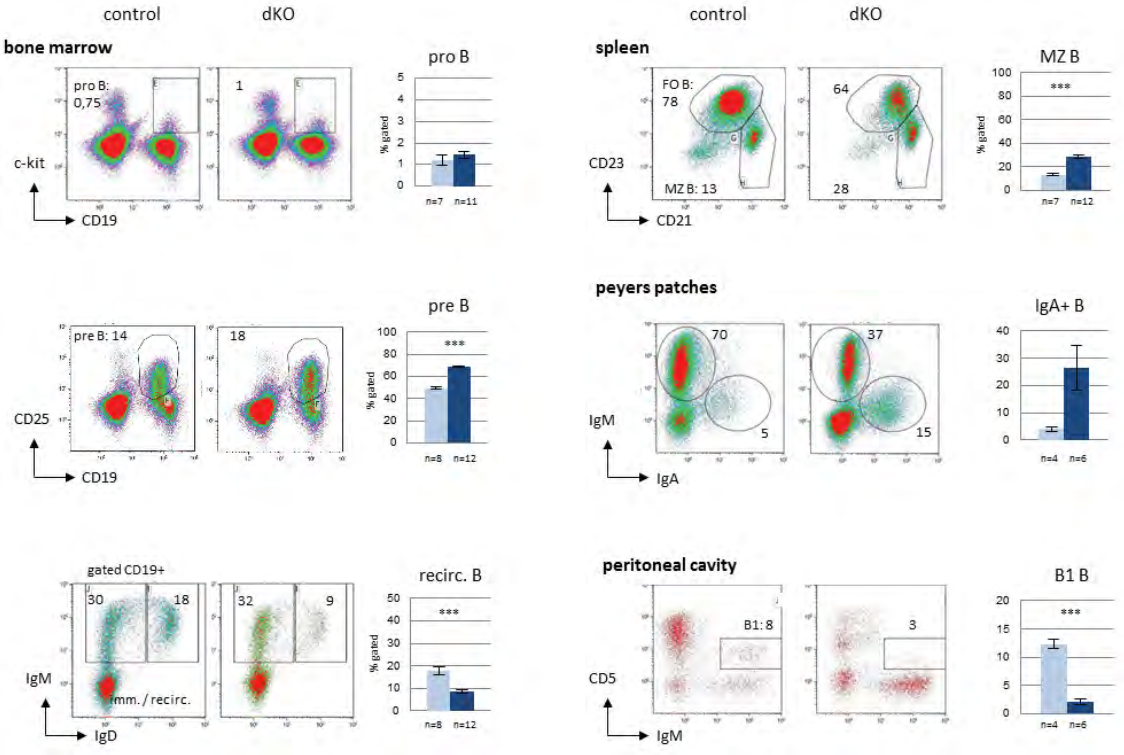
01.01.2012 - 31.12.2013

Function of KLF2 and 4 in B cell differentiation

Dr. Rebecca Winkelmann, Department of Molecular Immunology

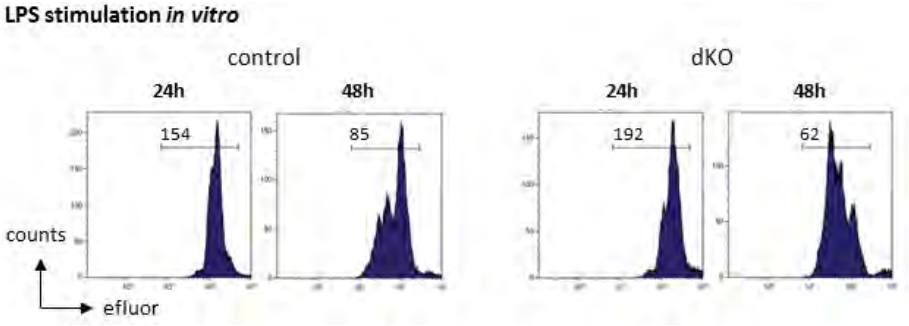
Deletion of KLF2 or KLF4, well established cell cycle regulators, shows no impact on proliferation of B cells, which could be explained by a redundant function of these factors. Analyses of KLF2 and KLF4 double-deficient mice revealed an aggravated KLF2-KO phenotype meaning significantly more pre B cells, IgA+ cells additional to more MZ B cells, less recirculating B cells and less B1 cells. Furthermore dKO mature B cells proliferate faster than their wildtype counterparts after LPS stimulation.

The closely related transcription factors Krüppel-like factor 2 (KLF2) and Krüppel-like factor 4 (KLF4) are well established regulators of proliferation and differentiation in many cell types. Ectopic expression of these two factors induces cell cycle inhibition in vitro by induction of p21 and repression of c-myc. In B cells KLF2 and KLF4 are both expressed simultaneously after pre B cell receptor surface presentation. Upon antigen contact both factors are downregulated and are re-expressed in plasma and memory cells. However B cell specific deletion of KLF2 or KLF4 alone does not influence proliferation or survival of



FACS analyses of B cell subsets in different lymphatic organs. Frequencies of pro B, pre B, immature and mature B, FOB, MZB, IgA+, IgM+ cells and B1 cells are shown. Exemplary density plots of control (left) and dKO (middle) mice are shown as well as statistical analyses as bar graphs (right).

Dr. Winkelmann



Efluor mean fluorescence after 24 and 48h LPS culture of CD43- splenic B cells.

B or T cells which could be explained by a redundant function of these two factors. Therefore this project focused on elucidating the importance of KLF2 and KLF4 in B cell development and B cell activation using B cell specific KLF2/KLF4 double deficient animals (dKO). To achieve this aim, KLF2 floxed animals were mated with KLF4 floxed animals and after that crossed to the mb1 cre strain, to delete KLF2 and KLF4 specifically in B cells.

If KLF2 and KLF4 as negative cell cycle regulators are important for the termination of pre B cell expansion, we would expect an increase in the number of pre B cells in the bone marrow after deletion of both factors. Indeed analyses of dKO mice showed a significant increase of the pre B cell compartment (CD19+/CD25+), whereas recirculating (IgD+/IgM+) B cells were diminished in the bone marrow. Furthermore marginal zone (MZ) B cells (CD21+/CD23low) in the spleen as well as IgA+ cells in peyers patches are dramatically increased and B1 cells (IgM+/CD5+) in the peritoneal cavity are reduced.

Both factors are downregulated after activation of B cells. Therefore these factors could be important for keeping B cells in a quiescent, non proliferating state. Deletion of both factors in mature B cells should therefore result in an enhanced proliferation and activation capacity in vitro. Indeed, stimulation experiments with LPS showed that dKO cells proliferate faster in comparison to control cells as measured by the loss of efluor fluorescence dye.

Taken together analyses showed that the deletion of both Krüppel-like factors 2 and 4 simultaneously leads to additional phenotypes compared to single knockouts. It could be assumed that missing both factors, redundant KLF2 and KLF4 functions cannot be maintained any more leading to more proliferational effects in dKO mice as expected from known functions and target genes like p21 and c-myc. In the future the clinical consequences like establishment of an autoimmune phenotype and/or the occurrence of leukemia or lymphomas could be interesting to analyze after deletion of both factors.

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Publications during funding period

none

J22 - Final Report

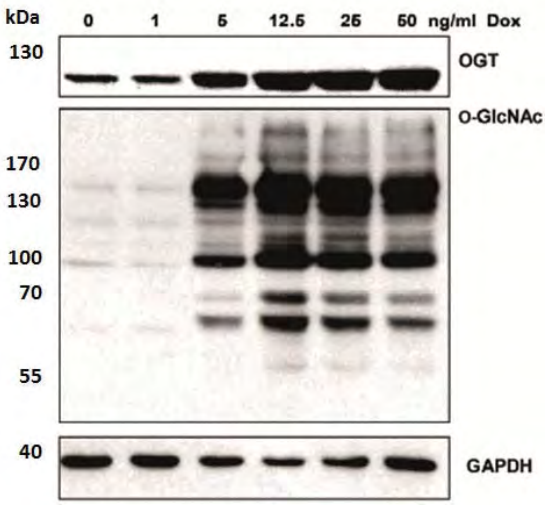
01.11.2011 - 31.10.2013

The role of O-GlcNAc-modification in regulating KSHV gene expression and replication

Dr. Ramona Jochmann, Department of Surgery

O-GlcNAcylation is an inducible, highly dynamic and reversible post-translational modification that regulates the function of many nuclear and cytoplasmic proteins. In previous studies (Jochmann et al, Glycobiology) we showed that increased O-GlcNAcylation reduces the replication efficiency of Kaposi's sarcoma-associated herpesvirus (KSHV) in a dose-dependent manner. This project aimed to elucidate the role of O-GlcNAc in KSHV propagation and in the development of Kaposi's sarcoma (KS).

In eukaryotic cells, myriad proteins are post-translationally modified with the monosaccharide N-acetyl-D-glucosamine (GlcNAc), added in an O-glycosidic linkage to serine and threonine residues. O-GlcNAc modification is mediated by O-GlcNAc transferase (OGT), and occurs in response to extracellular stimuli and glucose metabolism.



Induction of OGT in the stably transfected inducible OGT expressing cell lines depends on the concentration of doxycycline
Inducible HEK293/TO/nc-OGT cell lines were treated with different concentrations of doxycycline. O-GlcNAc staining mirrors the activity of induced OGT. GAPDH was used as a loading control.

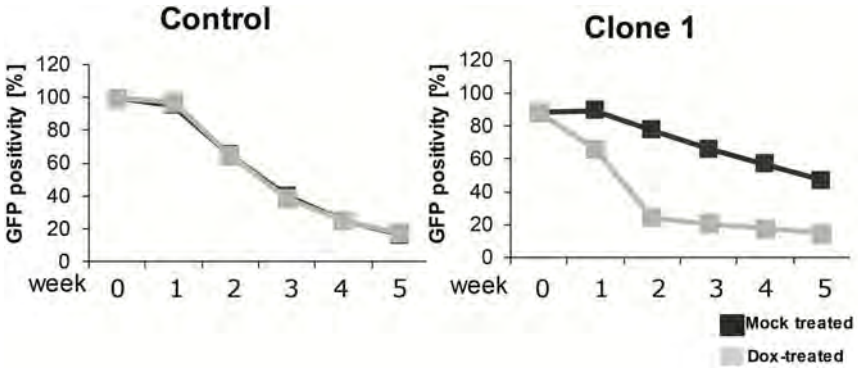
OGT-overexpression leads to a higher transcription of several cellular and viral genes

A correlation of KSHV-infection and increased O-GlcNAc levels was shown in KS-tumors and confirmed in immortalized blood (BEC-TI) and lymphatic endothelial cells (LEC-TI). To investigate the influence of O-GlcNAc on the transcriptome, a model cell line (HEK293) stably transfected with a doxycycline inducible OGT was generated, characterized and used for microarray analysis. The results of the microarrays show an increase in transcription of several histone cluster 1 family members and of the OGT antagonist MGEA5 in KSHV infected and non-infected cells upon OGT overexpression. Further, an increase of four lytic KSHV-genes was observed, namely ORF64, K2, ORF2 and ORF69. The increase of replication dependent histones upon a higher O-GlcNAc level could be an anti-viral response of the cell and may be important to inhibit KSHV propagation.

Increased O-GlcNAcylation blocks KSHV propagation

The cell lines for inducible OGT expression were used in order to investigate the impact of OGT on replication and propagation of KSHV. Under lytic conditions, the induction of OGT resulted in the inhibition of KSHV replication. In long-term culture of latently infected cells OGT induction resulted in a gradual and significantly more rapid loss of KSHV as compared to control cells where OGT expression was not induced. Both findings suggest that O-GlcNAcylation has a significant impact on the KSHV live cycle. This is in agreement with previous data showing that most KSHV proteins involved in viral DNA synthesis and replication are O-GlcNAc-modified (Jochmann et al, Glycobiology, 2013).

Dr. Jochmann



OGT overexpression decreases KSHV propagation.
FACS analysis of KSHV-infected inducible OGT-expressing cells treated either with PBS or doxycycline over a period of 5 weeks. Control contains the empty vector system.

O-GlcNAc prediction servers are not sensitive enough to predict modified sites

The performance of the two major prediction programs for O-GlcNAcylation (YinOYang 1.2 server and OGlcNAcScan) were compared using 1100 experimentally validated O-GlcNAc sites which were not used in the initial establishing procedure of the prediction programs. This approach indicated that both programs are suggesting more than 56% false negative O-GlcNAc-sites (Jochmann et al, BBA 2013). This is far below the prediction sensitivity of other prediction tools (e.g. for phosphorylation, > 70%). Further analyses indicated that the low sensitivity of the O-GlcNAc programs may be based on the fact that O-GlcNAcylation may not target at a defined amino acid within a conserved consensus motif. Instead, O-GlcNAcylation may be more promiscuous, occurring at different amino acids within a variety of different target sequences.

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Publications during funding period

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. 1844(2):416-421

Jochmann R, Pfannstiel J, Chudasama P, Kuhn E, Konrad A, Stürzl M (2013) O-GlcNAc transferase inhibits KSHV propagation and modifies replication relevant viral proteins as detected by systematic O-GlcNAcylation analysis. Glycobiology 23: 1114-30

J23 - Final Report

15.11.2011 - 11.08.2013

The role of the hypoxia-inducible factor (HIF) and lysyl oxidases in renal fibrosis

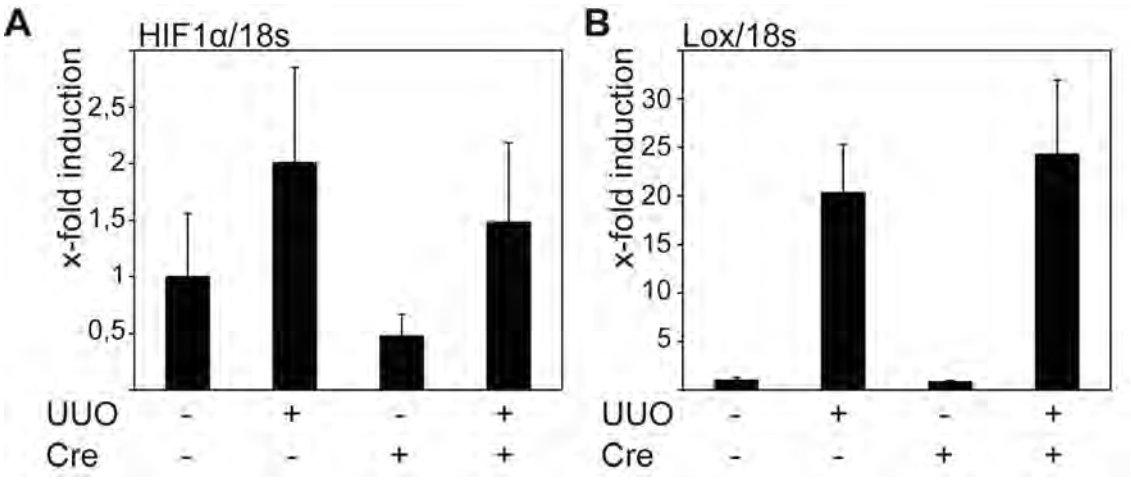
Dr. Thomas Hackenbeck, Department of Medicine 4 – Nephrology and Hypertension

A regular feature of Chronic kidney disease (CKD) is the development of kidney fibrosis driving disease progression towards reduced kidney function. Hypoxia may be an important stimulus for these processes. By stabilisation of the Hypoxia-inducible transcription factor (HIF), hypoxia could be at least in part responsible for the fibrotic phenotype. The aim of this study was to characterise this context in detail focussing on the HIF target genes Lox and LoxL2.

Many different diseases like diabetes mellitus, hypertension or nephritis lead to a progressive decline in kidney function resulting in the development of CKD. A common feature of CKD is the development of morphological changes leading to interstitial fibrosis and tubular atrophy (IF/TA), a reduction of capillaries and thereby insufficient oxygen supply to the organ. On molecular level, reduced oxygen levels (hypoxia) lead to the stabilisation of the Hypoxia-inducible transcription factor HIF and to the activation of several HIF target genes like Lox and LoxL2. Both may contribute to the development of the fibrotic phenotype in CKD. These enzymes are secreted copper-dependent amine oxidases, mediating the covalent crosslinking of collagens and/or elastins in the extracellular matrix (ECM).

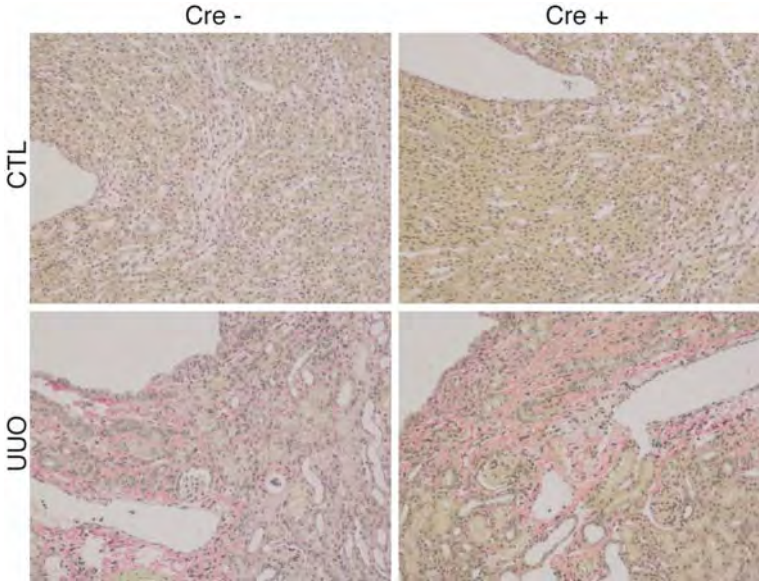
Lysyl oxidases in the UUO and adenine model of kidney fibrosis

To analyse the effect of lysyl oxidases during the development of kidney fibrosis, we successfully established two different animal models in our laboratory. On the one hand C57Bl/6 mice were treated with the purine nucleobase adenine ("adenine diet", 0.2% w/w food). On the other hand, the method of unilateral uretic obstruction (UUO) was performed. We could demonstrate, that both animal models lead to a clear induction of kidney fibrosis with impaired kidney function accompanied by the upregulation of different fibrotic and kidney injury markers on mRNA and protein level. Interestingly, the expression of the lysyl oxidases Lox and LoxL2 was strongly increased in adenine and UUO suggesting that both proteins



HIF-1α (A) and Lox (B) expression (RT-PCR) in Ksp-Cre/HIF1αLoxP mice after unilateral uretic obstruction (UUO; 7d after operation). Cre: Cre-recombinase.

Dr. Hackenbeck



Sirius Red (total collagen) staining of kidneys from Ksp-Cre/HIF1αLoxP mice after unilateral uretic obstruction (UUO; 7d after operation) as a marker of kidney fibrosis. Cre: Cre-recombinase; CTL: Contralateral ("unoperated") kidney.

may play an important role in the development of fibrosis. Nevertheless, treatment of the animals in both experiments with ß-APN (ß-aminopropionitrile), an irreversible inhibitor of enzymatic Lox activity, was not able to reduce the fibrotic phenotype. We therefore concluded, that the inhibition of enzymatic activity of lysyl oxidases alone is not sufficient to inhibit the development of fibrosis.

Development of fibrosis in HIF knockout mice

We generated mice with renal distal tubular knockout of HIF-1α by crossing animals expressing Cre-recombinase under the control of a kidney specific promoter (Ksp-Cre, Cadherin 16) with HIF-1α floxed mice (HIF-1αLoxP). Ksp-Cre/HIF1αLoxP mice showed strong distal tubular expression of the Cre-recombinase and a significant reduction of HIF-1α expression in total kidney extracts after RT-PCR analysis. Next, we performed unilateral ureter obstruction and the fibrosis model of adenine with our Ksp-Cre/HIF1αLoxP mice. Again, both animal models show a strong development of kidney fibrosis and upregulation of several kidney injury markers. The induction of HIF-1α mRNA was reduced significantly in the presence of Cre-recombinase demonstrating the strong effect of our promoter. Nevertheless, the knockdown of HIF was not able to reduce the fibrotic phenotype in both animal models or even to reduce the induction of the expression of Lox or LoxL2.

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Publications during funding period

Schietke RE*, Hackenbeck T*, Tran M, Günther R, Klanke B, Warnecke CL, Knaup KX, Shukla D, Rosenberger C, Koesters R, Bachmann S, Betz P, Schley G, Schödel J, Willam C, Winkler T, Amann K, Eckardt KU, Maxwell P, Wiesener MS. (2012) Renal tubular HIF-2α expression requires VHL inactivation and causes fibrosis and cysts. PLoS One 7(1):e31034. *shared first authorship

J24 - Final Report

15.09.2011 - 14.09.2013

Immune modulatory effects by oxidative stress in patients with chronic lymphatic leukemia

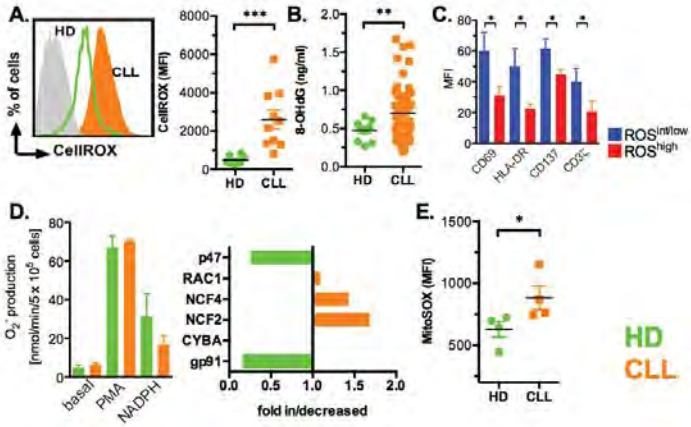
PD Dr. Dimitrios Mougiakakos, Department of Medicine 5 – Hematology and Oncology

Oxidative stress is present in cancer patients. It is detrimental for immune cells thus contributing to a phenomenon known as „tumor-immune-escape". Chronic lymphatic leukemia (CLL) represents the leukemia with the highest incidence. Since an increasing number of immune-based therapies is emerging in CLL we are interested in (A) studying whether oxidative stress contributes to the CLL-associated immunodefects and in (B) identifying its underlying molecular mechanisms in order to target them.

CLL is mostly B cell-derived and represents the leukemia with the highest incidence among adults. Already at early disease stages patients display immunodeficiences, which as yet remain to be fully understood. In order to (A) boost intrinsic immune responses as well as to (B) increase the efficacy of immune-based approaches it is necessary to antagonize such immune escape pathways. In this context oxidative stress represents a potential target. This metabolic condition describes the accumulation of so-called reactive oxygen species (ROS) that exert various cytopathic effects. Effector cells of the immune system are susceptible towards ROS-mediated effects and combining antioxidants with immunostimulation is already approved for the treatment of acute myeloid leukemias. The role of oxidative stress in the CLL-associated immunosuppression and its cellular source remain elusive.

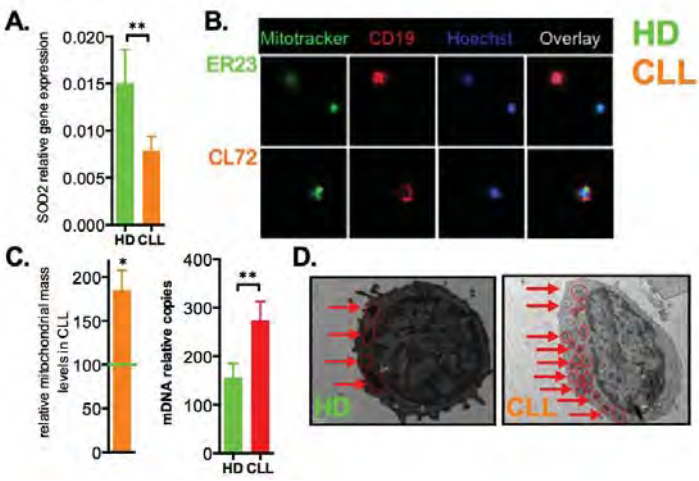
Oxidative stress and T cell alterations in CLL Patients

We have banked material from 100 patients without prior treatment as chemotherapy could affect cellular metabolism. The analysed CLL cells displayed increased ROS levels as compared to healthy B cells. Furthermore, we measured in patients' and healthy controls' sera established surrogate markers for ROS-burden namely DNA-oxidation products (=8-OHdG). As anticipated, higher levels of oxidized biomolecules were prevalent in CLL patients corroborating the notion of systemic oxidative stress in CLL. Higher concentrations of oxidized products (ROShigh patients) were associated with CD4+ T cells of a less activated phenotype, reduced CD3ζ-chain expression, and less circulating terminally differentiated effector memory cells. The precise pathway that evokes oxidative stress in cancer-cells often remains vague. Malignant cells can generate large amounts of ROS through an increased expression and activation of NADPH oxidases (NOX). We measured the superoxi-



(A) Cellular ROS levels (B) 8-OdHG serum levels. (C) Expression of T-cell activation markers in ROSint/low and ROShigh patients. (D) ROS production by NOX, NOX subunits and mitochondrial ROS. *, p<0.05; **, p<0.005; ***, p<0.001.

PD Dr. Mougiakakos



(A) SOD2 expression. (B) Mitochondrial mass (green). (C) Mitochondrial mass (CLL/B cell) and relative mitochondrial DNA copy number. (D) Electron-microscopy of mitochondria (red arrows and circles). *, p<0.05.

de anion production by NOX based on the extracellular reduction of cytochrome c. However, superoxide anion levels were similar in B and CLL cells before and after the addition of NOX substrates and the induction of respiratory burst by PMA. None of the NOX subunits was significantly higher expressed in CLL cells as compared to regular B cells. Based on these data NOX can most likely be excluded as the source for abundant ROS in CLL-cells. In fact, using a fluoroprobe that selectively detects mitochondrial ROS, we found significantly elevated levels in CLL cells.

Mitochondrial metabolism in CLL cells

Mitochondrial SOD2 plays a decisive role in metabolizing superoxide to hydrogen peroxide, which is then converted by catalase to water. The observed reduced SOD2 expression in CLL cells could therefore contribute to the increased mitochondrial ROS. Applying various methodologies we observed increased mitochondrial mass in CLL cells, which by itself lead to an enhanced generation of ROS and which led to an increased respiratory rate.

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Invited lectures

Annual Meeting of the Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie (DGHO), 2013, Wien, Aberrant myeloid cells in patients with CLL.

Lecture course at the National Center for Tumor Diseases (NCT), 2013, Heidelberg, Immune regulation by oxidative stress.

Lecture course at the Cancer Centre Karolinska (CCK), 2013, Stockholm, Oxidative stress in cancer.

Awards

Research award of the German Stem Cell Transplantation Working Party (DAG-KBT), PD Dr. med. Dimitrios Mougiakakos, 28.06.2013 Berlin Ria Freifrau von Fritsch Award for Cancer Research, PD Dr. med. Dimitrios Mougiakakos, 13.07.2013 Erlangen

Publications during funding period

Jitschin R*, Mougiakakos D*, Von Bahr L, Völkl S, Moll G, Ringden O, Kiessling R, Linder S, Le Blanc K. Alterations in the cellular immune compartment of patients treated with third-party mesenchymal stromal cells following allogeneic hematopoietic stem cell transplantation. Stem Cell 2013; 31(8):1715-25.

Mougiakakos D*, Jitschin R*, von Bahr L, Poschke I, Gary R, Sundberg B, Gerbitz A, Ljungman P, Le Blanc K. Immunosuppressive CD14+HLA-DRlow/negIDO+ myeloid cells in patients following allogeneic hematopoietic stem cell transplantation. Leukemia 2012;27(2):377-88.

Mougiakakos D*, Okita R*, Ando T, Dürr C, Gadiot J, Ichikawa J, Zeiser R, Blank C, Johansson CC, Kiessling R. High expression of GCLC is associated with malignant melanoma of low oxidative phenotype and predicts a better prognosis. Journal of Molecular Medicine 2012; 90(8):935-44.

Le Blanc K and Mougiakakos D. Multipotent Mesenchymal Stromal Cells and the Innate Immune System. Nature Review Immunology; 2012;12:383-96.

J25 - Progress Report

01.02.2012 - 31.01.2014

Establishing of an autonomous lymphatic vessel network

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

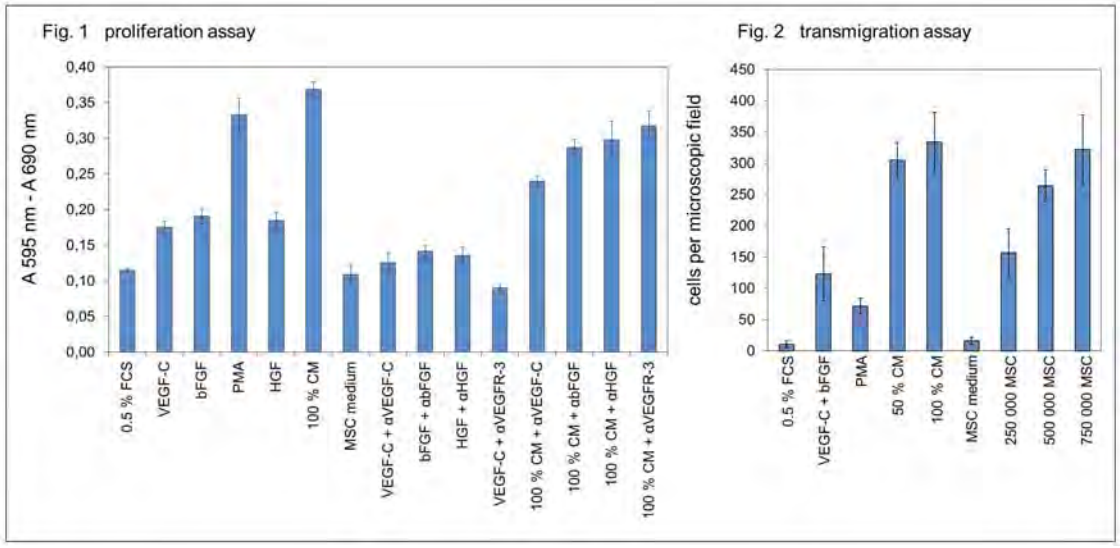
Molecular mechanisms of lymphangiogenesis are still rarely explored. This project addresses the interaction between mesenchymal stem cells (MSC) and lymphatic endothelial cells (LEC) by an in vitro part and aims at identifying novel paracrine factors. In the following in vivo part the arteriovenous (AV)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

Backround

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.

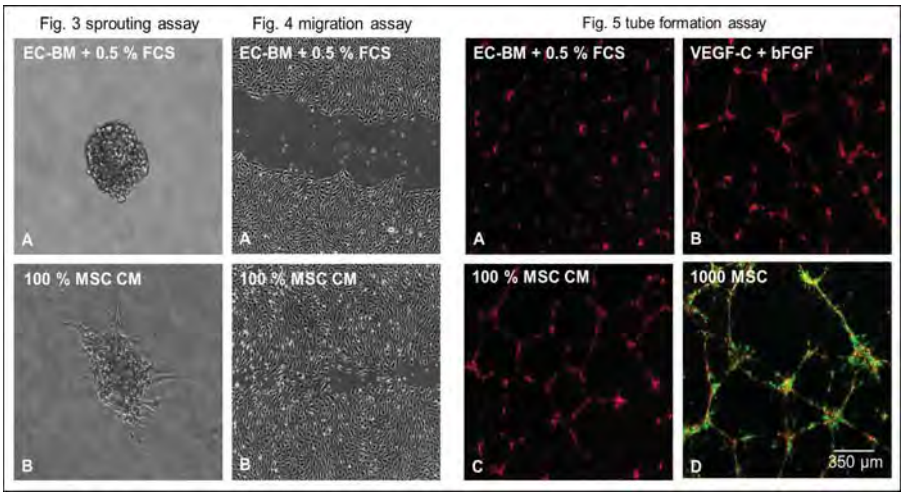
In vitro evaluation of interaction of LEC and MSC

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: 100ng/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



LEC proliferation/transmigration. 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.

Dr. Boos



LEC were stimulated with different media. Migration and sprouting were stimulated by MSC secreted factors. LEC tube formation assay. MSC secreted LEC stimulating factors and enhanced LEC tube formation directly. LEC labeled red / MSC green.

medium and also addition 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. In both assays LEC were stimulated to migrate by MSC secreted factors in a higher extent than by VEGF-C + bFGF. LEC spheroids were embedded in collagen 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. After establishment of the model the focus will lie on loss-of-function and gain-of-function experiments to get deeper insights into the lymphangiogenic processes.

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Publications during funding period

none

J26 - Final Report

01.01.2012 - 31.12.2013

Regulation of the tumor marker fascin by the oncoprotein tax of human T-cell lymphotropic virus type 1 (HTLV-1)

Dr. Andrea K. Kreß, Institute of Clinical and Molecular Virology

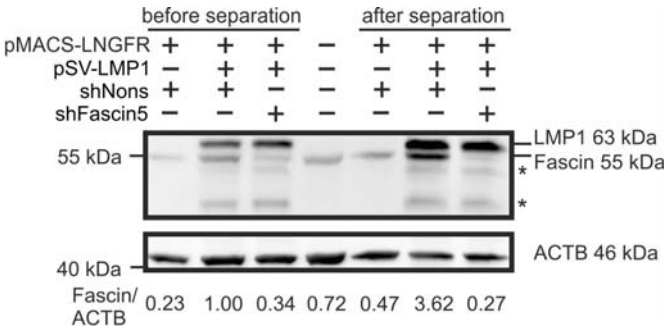
The actin-bundling protein Fascin (FSCN1) is a tumor marker that is highly expressed in many types of cancer. Fascin is important for metastasis and it is also upregulated in certain types of virally-induced lymphomas. We found that two viral oncoproteins are potent inducers of Fascin, Tax-1 of Human T-lymphotropic virus type 1 (HTLV-1), and LMP1 encoded by Epstein-Barr virus. In this project, we analyzed the regulation of Fascin making use of these viral oncoproteins.

Tax-1-mediated transactivation of the Fascin promoter is not sufficient for transcriptional induction of Fascin.

In our previous work, we found that the viral Tax-1 oncoprotein induces Fascin dependent on NF-kB signals. However, the transcriptional control region was unknown. For this purpose, promoter studies were performed. We found a defined Tax-1-responsive region (TRR) within the Fascin-promoter, which is surrounded by large parts that could not be activated by Tax-1. Transactivation of the TRR was dependent on Tax-1 mediated NF-kB activation. However, the TRR was not sufficient to control Fascin transcription as it was also responsive to the closely-related protein Tax-2, which does not induce Fascin mRNA. In line with the expression profile, chromatin-immunoprecipitations revealed a decrease of inhibiting histone modifications near the TRR at the Fascin promoter upon expression of Tax-1. Thus, the TRR is necessary, but not sufficient for Fascin gene expression.

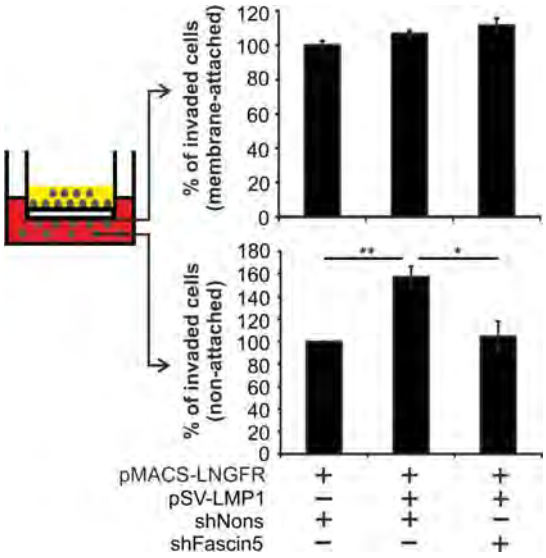
LMP1-mediated upregulation of Fascin depends on NF-κB and contributes to invasive migration

Fascin expression has been detected in B lymphocytes that are freshly-infected with Epstein-Barr-virus (EBV), however, both the inducers and the mechanisms of Fascin upregulation were still unclear. We could show that the EBV-encoded oncoprotein latent membrane protein 1 (LMP1), a potent regulator of cellular signaling and transformation, is sufficient to induce both Fascin mRNA and protein in lymphocytes. Fascin expression is mainly regulated by LMP1 via the C-terminal activation region 2 (CTAR2), which is important for LMP1-mediated induction of the canonical NF-κB signaling pathway. Block of canonical NF-κB signaling using a chemical inhibitor of IkB kinase b (IKK-ß) or cotransfection of a dominant-negative inhibitor of IκBα (NFKBIA) reduced not only expression of p100, a classical target of the canonical NF-kB-pathway, but also LMP1-induced Fascin expression. Furthermore, chemical inhibition of IKKß



LMP1-mediated Fascin induction is repressible. Jurkat cells were enriched using LNGFR-specific magnetic beads and immunoblots were performed. Numbers indicate the expression of Fascin normalized on ß-Actin (ACTB). shFascin5, small hairpin RNA targeting Fascin; shNons, control.

Dr. Kreß



Knockdown of Fascin reduces invasion of LMP1-expressing cells through extracellular matrix. Upper panel: % of invaded cells that are attached to the bottom of the membrane. Lower panel: % of invaded cells that are not attached and have migrated to the lower compartment.

reduced both Fascin mRNA and protein levels in EBV-transformed lymphoblastoid cell lines, indicating that canonical NF-κB signaling is required for LMP1-mediated regulation of Fascin both in transfected and transformed lymphocytes. Fascin contributed to LMP1-mediated enhancement of invasive migration through extracellular matrix. Upon enrichment of LMP1-expressing cells by magnetic separation, invasion assays were performed. While LMP1 enhanced the number of invaded cells, functional knockdown of Fascin using small hairpin RNAs resulted in a significant reduction of invaded, non-attached cells. Thus, our data show that LMP1-mediated upregulation of Fascin depends on NF-kB and contributes to invasive migration of LMP1-expressing lymphocytes.

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Invited lectures

Workshop, 17.01.2013, Department of Oncology and Surgical Science, University of Padova, Italy, Rerouting of Fascin – rerouting of HTLV-1?

Seminar, 22.04.2013, Institute for Virology and Immunobiology, Julius-Maximilians-Universität Würzburg, Modulation of the host cell by HTLV-1/Tax.

Publications during funding period

none

J27 - Progress Report

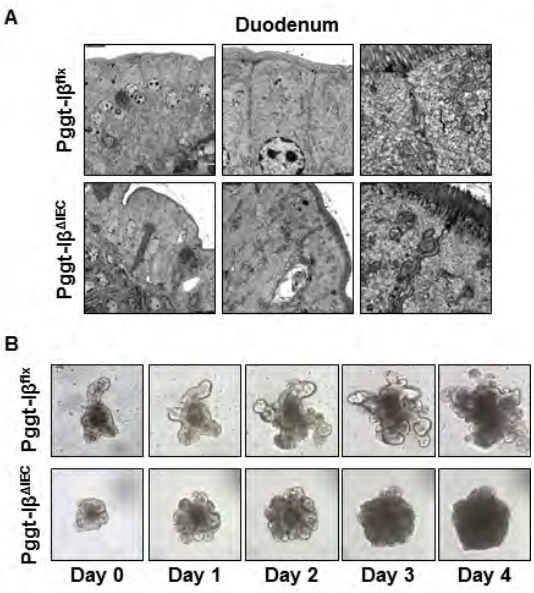
01.11.2012 - 31.10.2014

GGTase-I in intestinal epithelial cells

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

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

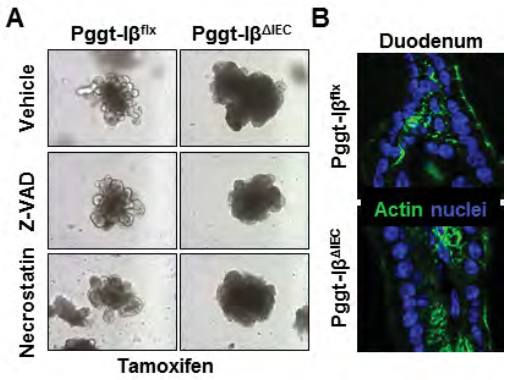
Deletion of GGTase-Iβ (one of the main enzyme responsible for prenylation) in IEC leads to embryonic lethality (before day 14.5). Mice with an inducible deletion of GGTase-I in intestinal epithelial cells (IECs) were generated by crossing floxed PggtIβ mice with Villin-CreERT2 mice. Inhibition of prenylation in IECs due to deletion of GGTase-Iβ gene led to a severe enteric disease, resulting in the death of the animals. 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. Breakdown of intestinal homeostasis is intrinsic of IEC since epithelial architecture is also destroyed in in vitro organoid culture. Organoids developed from small intestine crypts from Pggt-IβΔIEC were treated with tamoxifen, leading to GGTase-Iβ deletion and inhibition of prenylation. Consequently, organoid lacking GGTase-Iβ expression started to die on day 3 (after tamoxifen), while PggtIβflx derived organoids were unaffected.



Breakdown of intestinal architecture due to abrogation of geranylageranylation in IEC. (A) Electron microscopy pictures of duodenum from tamoxifen injected Pggt-IβΔIEC mice. (B) Organoid culture from Pggt-IβΔIEC mice and treated with tamoxifen.

Gene expression as well as proteomic assay clearly demonstrated the dramatic impact of geranylagernalyation in IECs biology, since a significant number of genes and proteins showed an altered expression due to abrogation of prenylation in IECs (725 genes, and 322 proteins, respectively; fold change ≥ 2, and P ≤0,05). Pathway and network analysis using the platform Ingenuity 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 as well as cell death as key mediators. Abolition of microbiota by antibiotic treatment was not able to rescue survival of mice lacking GGTase-I expression in IECs. In the case of cell death pathways, death of RIP3K-/- Pggt-IβΔIEC and Casp8ΔIEC Pggt-IβΔIEC mice clearly demonstrated that induction of necroptosis or apoptosis did not represent a primary requirement of impaired barrier function in the described context but might rather occur as a secondary effect. According to this conclusion, inhibition of apoptosis or necroptosis by Z-VAD or Necrostatin-1

López Posadas, PhD.



Mechanism underlying the phenotype of Pggt-IβΔIEC mice. (A) Organoid culture from Pggt-IβΔIEC mice treated with apoptosis or necroptosis inhibitors. (B) iActin staining (green) in duodenum from tamoyifen injected Pggt-IβΔIEC mice.

respectively, was not able to overcome the inability of GGTase-Iβ deficient IEC to maintain stable organoid organization in vitro. However, our data strongly support cytoskeleton rearrangement as main consequence of abrogation of geranylageranylation in IECs. As a confirmation of cytoskeleton rearrangement in the absence of GGTase-I, we were able to show altered actin fibers disposition and myosin-IIA complexes formation in GGTase-I deficient IECs. This fact was associated with a dysfunction of Rho-A pathway after GGTase-I deletion, since IECs showed decreased levels of activated GTP-bound Rho-A, translocation of Rho-A from membrane to cytosol, and altered activation of downstream signalling pathways (decreased phosphorylation of MLC-2).

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Awards

Annual Meeting of the Deutsche Gesellschaft für Verdauungs- und Stoffwechselerkrankungen (DGVS)/Viszeralmedizin 2014; 11-14.09.2013. Nürnberg (Germany): The submitted abstract „Postranslational protein prenylation as a crucial factor for intestinal epithelium integrity and homeostasis“ by Rocío López Posadas, Martin O. Bergö, Christoph Becker, Fermín Sánchez de Medina, Markus Neurath and Imke Atreya, was presented orally and awarded (Abstractpreis fur gastroenterologische Beiträge).

Publications during funding period

none

J28 - Progress 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 a central mediator of chronic inflammatory disorders. By using transgenic and vector-based approaches I have shown that the forced expression of IL-17A leads to a marked infiltration of neutrophils into the pancreas and to the development of chronic pancreatitis. In the past year I have tested the functional role of different cell types and IL-17A related cytokine synergies in this model. Furthermore I addressed a potential role of IL-17A in human chronic pancreatitis.

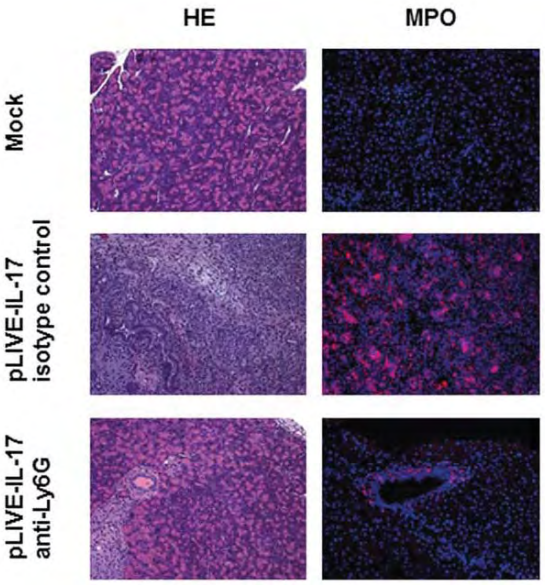
Pathogenic cell types in IL-17A induced pancreatitis

The development of an IL-17A expression vector which leads to a stable systemic expression of IL-17A (pLIVE, Dr. Wirtz) opened the possibility to analyse the role of specific cell types and molecular mediators in the pathogenesis of IL-17 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 complete destruction of the exocrine pancreas and consecutive tissue fibrosis. Flow cytometry shows an enhanced granulopoesis in the bone marrow as well as an increased mobilization into the spleen. I have shown, that vector injection into RAG1-deficient mice also leads to a marked pancreatitis indistinguishable from WT mice. This shows that neither T- nor B-cells are required in the pathogenesis of IL-17 induced pancreatitis. To analyse the role of RORγt dependent innate lymphoid cells (ILC) in this model, I have made use of RAG-/- RORγt-/- deficient mice. These mice also suffered from IL-17 induced pancreatitis, demonstrating that IL-17A induces pancreatitis independently from RORγt-expressing ILC populations.

The depletion of neutrophil granulocytes using anti-Ly6G antibodies prevented the development of IL-17 induced pancreatitis thereby implementing neutrophil granulocytes as the main pathogenic cell type in this model.

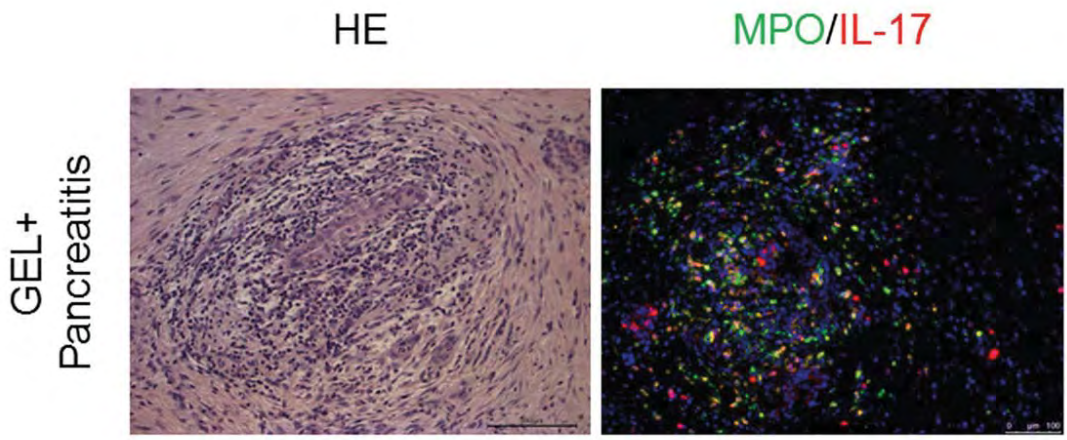
IL-17A in cytokine networks

It has been proposed that IL-17A itself might show only weak biological activity and may reach its full potential only in synergistic effects with other pro-inflammatory mediators such as IL-1β, TNFα or IL-6. Indeed, my in vitro studies support a synergy between IL-1β and IL-17A in increasing the expression of cxcl1 and cxcl5. The in vivo analysis using IL1R1-/-, TNFR1+2-/- and IL-6-/- mice showed that these cytokines and/or their receptor mediated signaling are dispensable in the pathogenesis of IL-17A induced pancreatitis. This highlights the role of IL-17A as a strong proinflammatory cytokine acting independently of synergies with other proinflammatory mediators.



HE Stains (left) and immunohistochemical MPO stains (right) of murine pancreas is shown highlighting the development of pancreatitis after pLIVE mediated forced expression of IL-17. Neutrophil depletion abrogates IL-17 induced pancreatitis development.

Dr. Leppkes



HE stain (left) and immunohistochemical double stain for MPO (green) and IL-17 (red) is shown, demonstrating the marked expression of IL-17A in granulocytic epithelial lesions (GEL) of human autoimmune pancreatitis type 2

IL-17A in human chronic pancreatitis:

In a cooperation with Prof. Klöppel (Munich) and Dr. Detlefsen (Odense, Denmark) I have initiated immunohistochemical studies of human chronic pancreatitis samples in order to assess the role of IL-17A in this disorder. In samples classified as autoimmune pancreatitis type 2 we have been able to show a marked infiltration of neutrophil granulocytes into the pancreas leading to the pathognomonic hallmark of granulocyte epithelial lesions. As demonstrated by a MPO/IL-17A double stain we could show that neutrophils themselves represent the main source of IL-17A in this context. The massive infiltration of an IL-17A producing cell points to a pathogenic role of this cytokine in disease development. It is currently our goal to underline these findings in a larger study population including pancreatitis samples of different etiologies.

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Awards

Dr. M. Leppkes, Boehringer-Ingelheim Stiftungspreis, Mainz, 26.6.2013

Publications during funding period

none

J29 - Progress Report

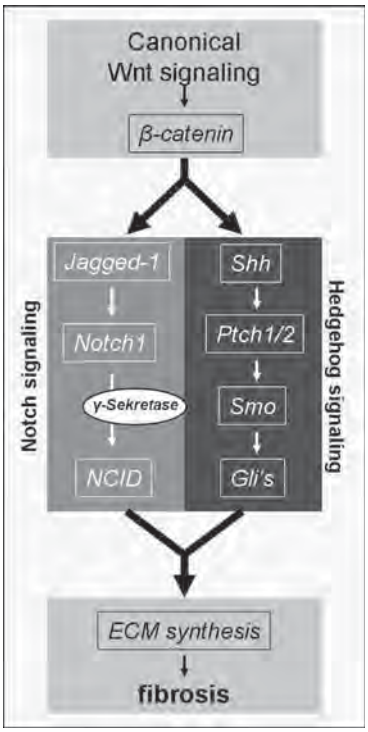
01.10.2012 - 30.09.2014

Interaction of morphogen pathways in the development of fibrotic diseases

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

Our project studies the crosstalk of the pro-fibrotic, morphogen pathways Wnt, Hedgehog and Notch in the development of fibrosis. Using pharmacological and genetic approaches, we investigate synergistic effects of these three pathways in different experimental models of fibrosis. Thus, our project may path the way for clinical studies with combined blockade of several morphogen pathways for 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. Based on preliminary findings, we postulate a hierarchy of morphogen pathways in fibrosis in which Wnt signaling stimulates Hedgehog and Notch signaling to transmit pro-fibrotic effects. So far, we have generated the following important results:



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.

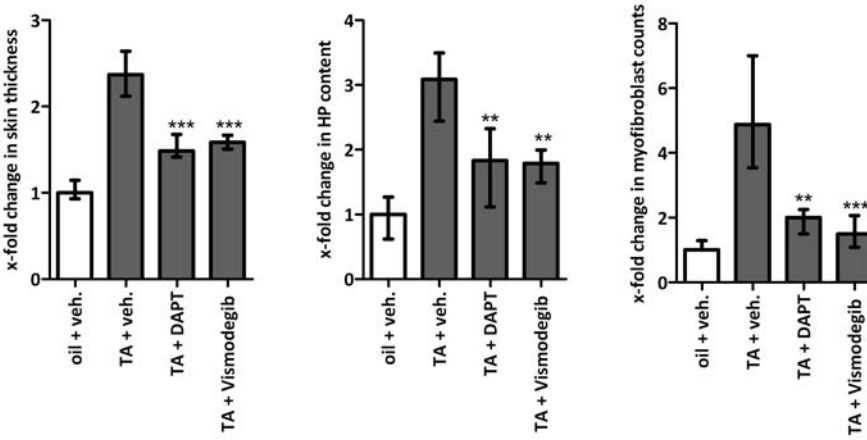
Pharmacological blockade of Wnt signaling inhibits experimental fibrosis.

In the model of bleomycin-induced dermal fibrosis and in a model of TGF-β-driven fibrosis, we demonstrated 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. These data support the crucial role of morphogen pathways, in particular Wnt signaling, in fibrosis and highlight their therapeutic potential for fibrotic diseases.

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; Cre-ER 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).

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.

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 deserves further experimental investigation.

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

Mice overexpressing a constitutively active form of β-catenin in fibroblasts develop spontaneous dermal fibrosis. In these animals, 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. Targeted, combined blockade of morphogen pathways may be superior in efficacy and tolerability compared to inhibition of a single morphogen pathway.

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Awards

Exchange programme of the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) (12/2012)

Scholarship for the International EUREKA Certificate Course for Translational Medicine (05/2013)

Scholarship for the Entrepreneurs in Clinical Academia Course at the INSEAD business school (09/2013)

Publications during funding period

Beyer C, Distler JH (2013) Morphogen pathways in systemic sclerosis. Curr Rheumatol Rep. Jan 2013;15(1): 299

Beyer C, Reichert H, Akan H, Mallano T, Schramm A, Dees C, Palumbo-Zerr K, Lin NY, Distler A, Gelse K, Varga J, Distler O, Schett G, Distler JH (2013) Blockade of canonical Wnt signalling ameliorates experimental dermal fibrosis. Ann Rheum Dis. Apr 17 2013

Beyer C, Skapenko A, Distler A, Dees C, Reichert H, Munoz L, Leipe J, Schulze-Koops H, Distler O, Schett G, Distler JH (2013) Activation of pregnane X receptor inhibits experimental dermal fibrosis. Ann Rheum Dis. Jan 4 2013

Dees C, Schlottmann I, Funke R, Distler A, Palumbo-Zerr K, Zerr P, Lin NY, Beyer C, Distler O, Schett G, Distler JH (2013) The Wnt antagonists DKK1 and SFRP1 are downregulated by promoter hypermethylation in systemic sclerosis. Ann Rheum Dis. May 22 2013

Distler A, Ziemer C, Beyer C, Lin NY, Chen CW, Palumbo-Zerr K, Dees C, Weidemann A, Distler O, Schett G, Distler JH (2013) Inactivation of evenness interrupted (EVI) reduces experimental fibrosis by combined inhibition of canonical and non-canonical Wnt signalling. Ann Rheum Dis. Nov 20 2013

J30 - Progress Report

01.01.2013 - 31.12.2014

Cytoplasmic functions of human cytomegalovirus pUL69

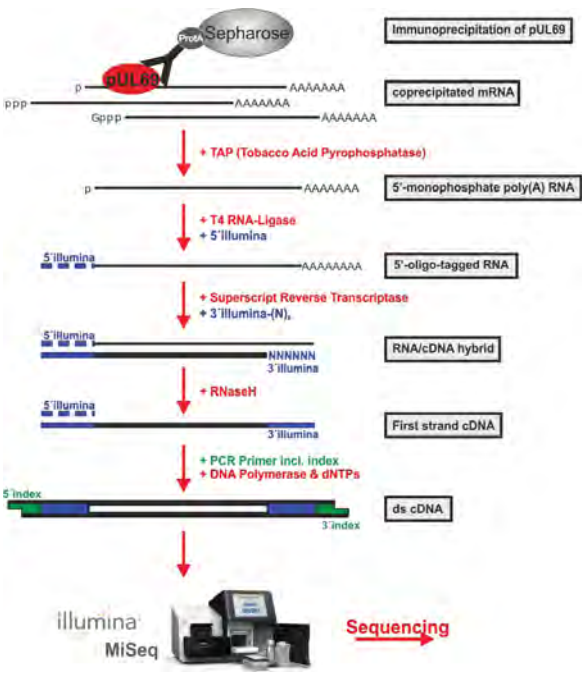
Dr. Marco Thomas, Institute of Clinical and Molecular Virology

Human cytomegalovirus encodes for the multifunctional regulatory protein pUL69 that has an important role for viral mRNA export and a so far uncharacterized cytoplasmic function. Here we reconfirm the published interaction of pUL69 with cytoplasmic PABPC1 or eIF4A1 and identified additional translation factors as novel pUL69-interactors. RNA-immunoprecipitation analyses demonstrated RNA binding specificity of pUL69 in vivo and lead to the identification of various pUL69-associated transcripts.

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. While pUL69 non-specifically binds to several RNAs in vitro, RNA-immunoprecipitation (RIP) analyses demonstrated selective RNA-binding in vivo (Toth et al., 2006) and HCMV-recombinants encoding RNA-binding deficient pUL69 revealed a replication defect compared to wild type HCMV (Zielke & Thomas et al., 2011). Besides its important function for viral mRNA export, HCMV pUL69 seems to have additional, so far uncharacterized functions within the cytoplasm.

Therefore we set out to analyze the mechanism of pUL69´s mRNA binding specificity and aim to unravel its impact on protein translation.

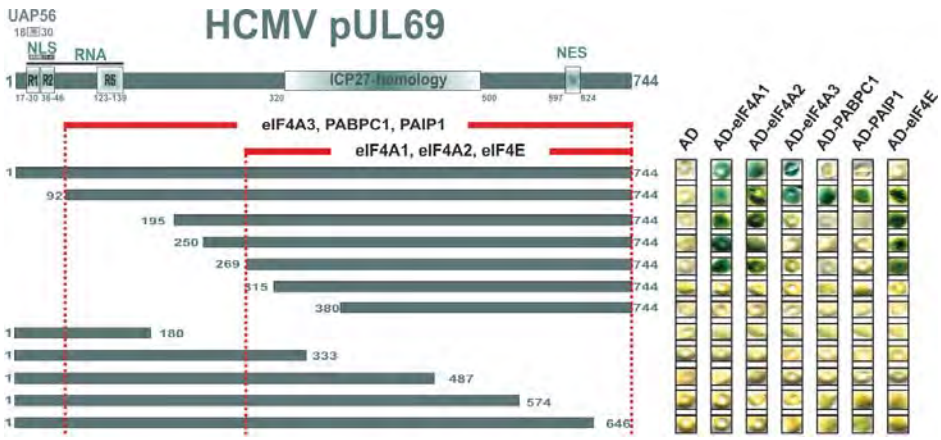


Identification of transcripts associated with pUL69 in vivo by RIP-Seq. HCMV pUL69 is precipitated from infected cells and coprecipitated mRNAs are reversely transcribed. Thereby generated cDNAs are subjected to next generation sequencing.

Identification of mRNAs associated with pUL69 in vivo

In order to identify cellular and viral mRNAs associated with pUL69, we generated cDNAs of transcripts coprecipitated by pUL69 from HCMV-infected HFF cells and tested a subset of HCMV-encoded mRNAs for their association with pUL69 during infection. These RIP-experiments identified the viral mRNAs encoded by the open reading frames UL44, UL65, UL82 and UL83 and the cellular mRNAs encoding CDC42 or containing a TULP-like sequence as pUL69-associated mRNAs. These targets were reconfirmed by RIP-analyses from transiently transfected HEK293T cells, thereby underlining the specificity of the RNA selection strategy and further excluding that this association was dependent on viral cofactors. We therefore suggest that pUL69 targets cellular and viral mRNAs via pUL69 response element(s) and this association is of in vivo relevance for HCMV replication.

Dr. Thomas



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

Recently HCMV pUL69 was proposed to exert a function in translation, as it interacts with the cytoplasmic translation factors PABPC1 or eIF4A1 (Aoyagi et al., 2010). In our current study protein-protein interactions were determined by yeast two-hybrid analysis. 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.

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 nuclear export into the cytoplasm where pUL69 likely regulates their translation.

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Invited lectures

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

Publications during funding period

none

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

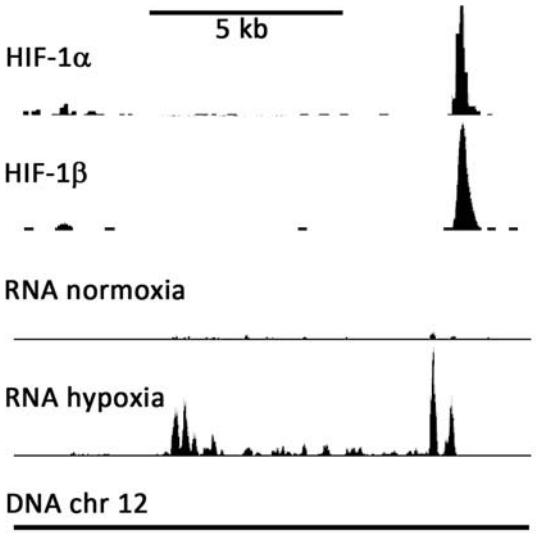
Adaptation of cells and whole organisms to reduced oxygen conditions (hypoxia) is essential for survival. Hypoxia inducible transcription factors (HIF) are crucially involved in hypoxic gene regulation. HIFs induce a variety of RNA species including non-coding RNAs. We have identified a novel hypoxia 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 programme that aims to increase oxygen supply and optimise metabolism. The role of long non-coding RNAs in hypoxic gene regulation or a potential crosstalk with the HIF response have not been addressed to date.

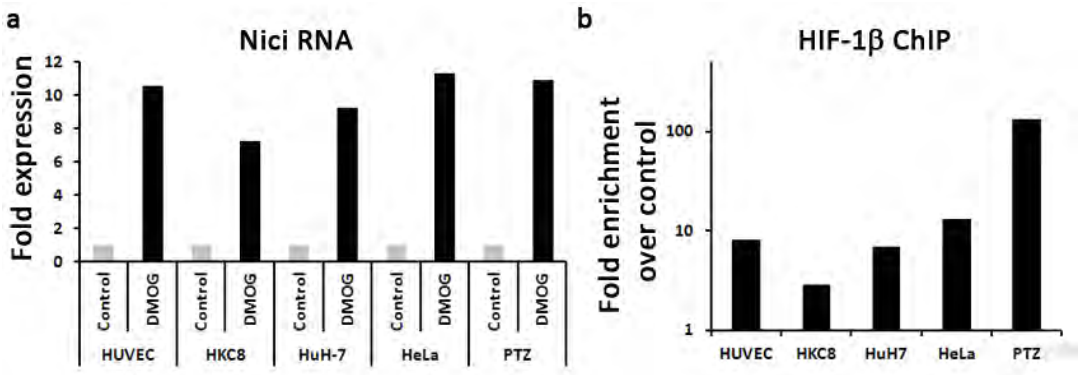
Nici – a novel long non-coding RNA regulated via the HIF-pathway:

In previous work, we described a set of high stringency HIF DNA-binding sites identified by chromatin immunoprecipitation coupled to high-throughput sequencing (ChIP-seq) in MCF-7 breast cancer cells. Using RNA-sequencing 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.



HIF-1α and HIF-1β ChIP-seq signals at the intergenic Nici locus on chromosome 12 in MCF-7 cells. Corresponding RNA-seq signals indicate the upregulation of a transcript close to the HIF-binding site in hypoxia.

Dr. Dr. Schödel



a) Relative expression levels of the Nici transcript in different cell lines exposed to vehicle or the hypoxia mimetic DMOG (dimethyloxallylglycine) as measured by qPCR. b) HIF-1β DNA-binding to the Nici locus across the same panel of cell lines stimulated with DMOG. Values are normalised to normoxic values.

Nici is ubiquitously induced via the HIF-pathway:

During the first months of the funding period we focussed on testing whether expression and regulation of the transcript is present in other cell types. Using a variety of human cell lines derived from different tissue origins we could show that Nici is ubiquitously induced by hypoxia or pharmacological stabilisation of HIF. In addition, Hif-DNA-binding to the hypoxia responsive elements is conserved across the cell lines. Comparing expression levels in normal renal tissue versus renal cancer tissue, in which HIFs are frequently stabilised by the loss of von Hippel Lindau tumour suppressor protein, we found a strong upregulation of the transcript levels in tumours. These findings strengthen the hypothesis that Nici commonly contributes to the hypoxic response in human cells. In order to understand the functional role of Nici and the consequences of its upregulation in hypoxia we are currently testing effects of manipulating Nici expression levels on neighbouring genes and general features of cell survival and metabolism.

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Invited lectures

Jahrestagung der Deutschen Gesellschaft für Nephrologie, 08.10.2013, Berlin, „Die Rolle von hypoxia inducible factor in der Tumorgenese“

Publications during funding period

Choudhry H*, Schödel J*, Oikonomopoulos S, Camps C, Grampp S, Harris AL, Ratcliffe PJ, Ragoussis J, Mole DR (2013) Extensive regulation of the non-coding transcriptome by hypoxia: role of HIF in releasing paused RNApol2. EMBO rep, epub ahead of print 20.12.2013.

*these authors contributed equally.

J32 - Progress Report

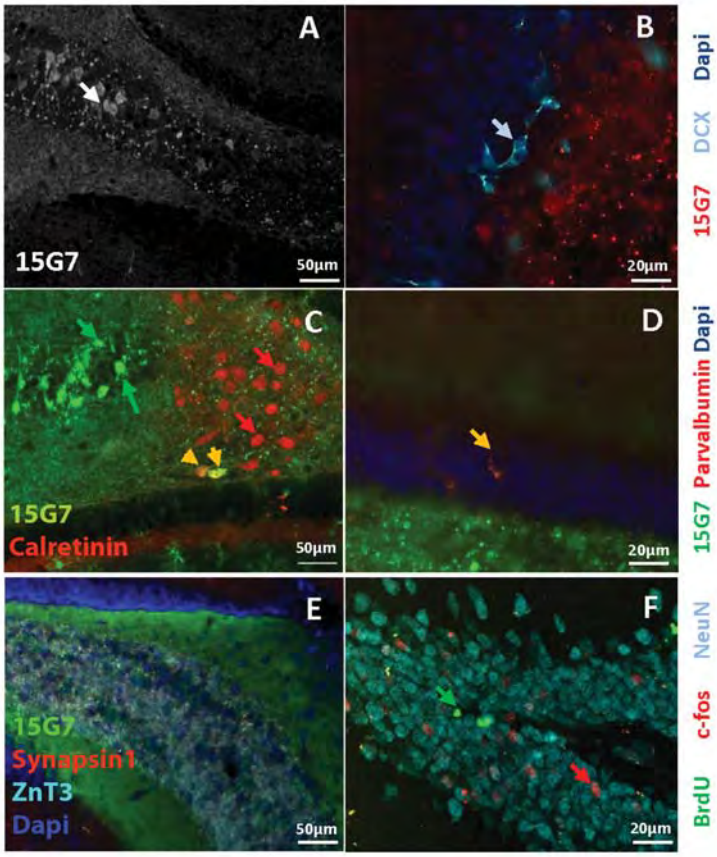
01.09.2012 - 30.08.2014

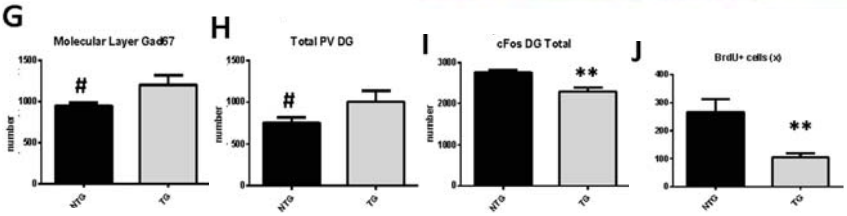
Neuropsychiatric symptoms of Parkinson's disease

Dr. Nada Ben Abdallah, Department of Molecular Neurology

The aetiology of neuropsychiatric symptoms of cognitive and affective nature in Parkinson's disease (PD) is not well understood, but accumulation of alpha-synuclein in hippocampal circuits, particularly within the presynaptic compartment, is a plausible mechanism. We hypothesize that alpha-synuclein neuronal and presynaptic accumulation entails cognitive and mood deficits due to interference with the developmentally- and postnatally-born hippocampal cell populations.

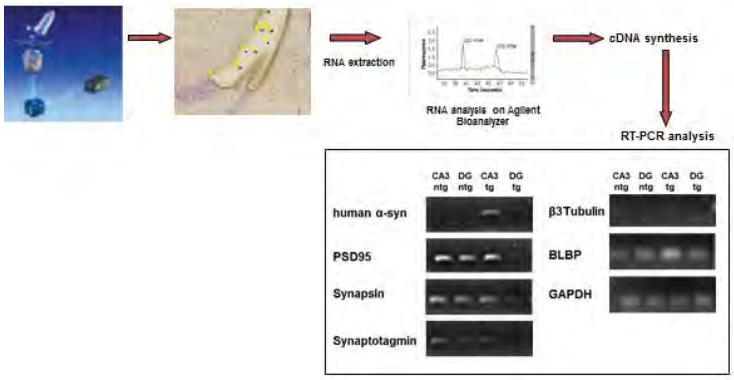
Non-motor symptoms like anxiety and cognitive deficits often occur early before the onset of PD motor symptoms. These deficits may be associated with aggregation of abnormally phosphorylated alpha-synuclein (α-syn) within the hippocampus. The proposed sites of initial α-syn aggregation include α-syn-enriched presynaptic terminals, leading to dendritic degeneration in both human PD brains and PD transgenic models. Adult hippocampal neurogenesis, which accounts responsible for distinct hippocampal functions like cognition and anxiety, is also severely altered in PD. Specific toxicity of α-syn in postmitotic dentate neurons has been reported in PD animal models. It is now known that synapse formation and maintenance are active processes required for the survival of DG neurons. The overall hypothesis of the present proposal is to investigate whether there is a direct





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



cDNA was synthetized from isolated RNA of dentate gyrus and CA3 subregions. Using RT-PCR we qualitatively analyzed postsynaptic gene PSD95 and presynaptic genes Synapsin 1 and synaptotagmin and found reduction in all three markers in the TG dentate.

link between DG neurogenesis decline, presynaptic deficits, and neuropsychiatric symptoms observed in transgenic PD models. We used transgenic (TG) mice overexpressing human wildtype α-syn under the PDGF-ß promoter leading to a neuronal expression.

We found significant reduction in newborn cell survival and neuronal differentiation in TG mice compared to controls. Alpha-synuclein overexpression was strongly induced within neurons of the hippocampus in all its subregions, namely the dentate gyrus, hilus, cornus ammonis (CA1-3) and the subiculum. More precisely, we detected the transgene in GABAergic interneurons as well as in 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.

Although the mossy fibers volume remained unchanged in TG mice compared to control littermates even at different ages.

When exposed to an open field with enriched environment during 15 minutes, all mice explored exhaustively the open field. This exploration induced increased cfos expression within all hippocampal subregions. Absolute numbers and density of cfos-expressing cells were however significantly smaller in TG mice, suggesting a possible functional alteration.

Initial results from analysis on hippocampal mRNA using the laser microdissection method suggest a specific reduction in presynaptic markers like synaptotagmin and synapsin-1 as well as the postsynaptic density protein PSD95 within the dentate gyrus of TG mice.

Further structural and molecular analyses are in progress to comprehensively characterize this phenotype. In addition to these ongoing investigations of hippocampal alterations in association with α-syn overexpression, our next step is to correlate structural modifications with behavioural changes associated with cognition and anxiety.

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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 myelinating cells of the central nervous system, the transcription factor Sox2 is continuously expressed and its deletion interferes with differentiation. Besides a direct influence on myelin gene expression, Sox2 promotes differentiation in part by negatively controling miR145 and thereby preventing this microRNA 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.

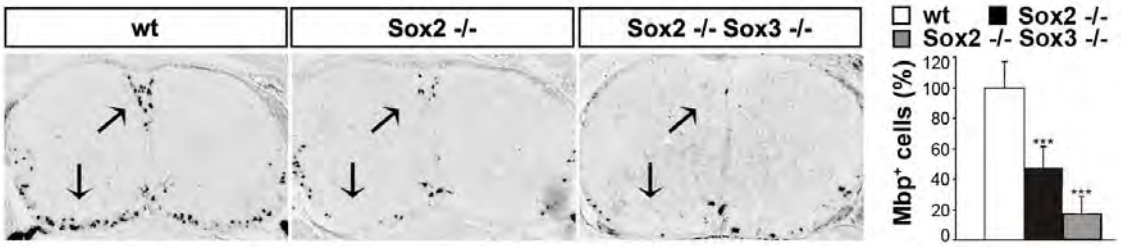
Oligodendrocytes as the myelinating glia of the central nervous system provide electrical isolation and nutritional support for neurons. During development of the mouse spinal cord, the transcription factor Sox2 is expressed from neural precursor cells through oligodendrocyte progenitors into early differentiating oligodendrocytes. Since maintenance of progenitor characteristics has been the major function ascribed to Sox2 we aimed to analyze its possible role in differentiating cells.

Sox2 and Sox3 influence terminal differentiation of oligodendrocytes.

Genetic ablation of Sox2 in oligodendrocyte progenitors in the mouse model did not affect expansion or distribution of oligodendroglia at pre-differentiation stages. 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 observed only 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.

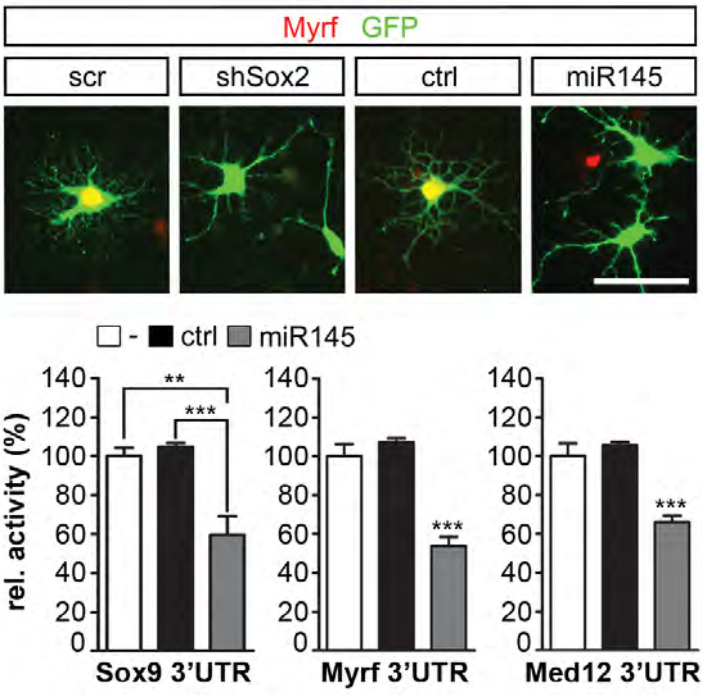
miR145 negatively regulates expression of factors required for oligodendrocyte differentiation.

Post-translational regulation is typically achieved by microRNAs. With miR145 we 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. 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.



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

Dr. Reiprich



Knockdown of Sox2 and overexpression of miR145 inhibit Myrf expression (red) in primary oligodendrocytes which is a sign of impaired differentiation. miR145 targets the 3'UTR of Sox9, Myrf or Med12 in reporter gene assays shown by repressed expression.

This adds another aspect to the functions of Sox2 beyond its well-characterized role as a stem cell factor.

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Publications during funding period

none

J34 - Progress Report

15.08.2012 - 14.08.2014

Indirect presentation of HLA class II restricted tumor antigens

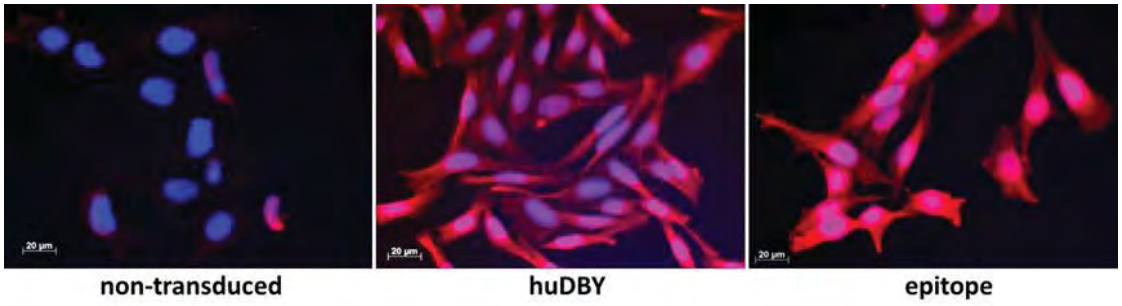
Dr. Anita Kremer, Department of Medicine 5 – Hematology and Oncology

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.

In our previous results we have observed that some, but not all, intracellular antigens are able to activate CD4+ T-cells by intercellular transfer to HLA class II positive cells. These antigens were subsequently processed and presented on the cell surface of previously antigen negative bystander cells. One of these antigens was the Y-chromosome encoded protein DBY. We also had indications that binding of cytosolic antigens to the chaperone hsc70 and subsequent invagination in the intraluminal vesicles of the late endosome – a process called microautophagy - leads to secretion of these antigens. To further analyze this process of intercellular antigen transfer and to investigate the role of microautophagy, 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 binding sites.

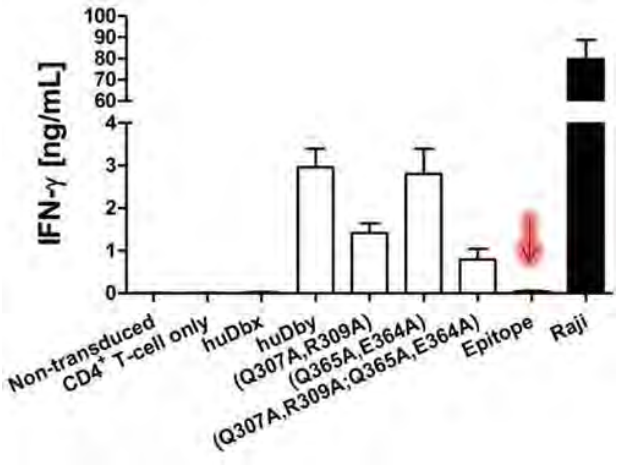
We verified expression of our transgenes by flow cytometric quantification of marker gene expression on the cell surface, western blot analyses, and immunofluorescence. To confirm the capability of our antigens to be processed, presented and recognized on the cell surface we additionally transduced them into HLA class II positive EBV-transformed B-cells. By this, 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.

In our in vitro model of indirect antigen presentation, we co-cultured antigen transduced HLA class II negative HeLa cells with antigen negative, HLA class II positive EBV-transformed B-cells for 5 days. Subsequently, we removed the B-cells from the culture and incubated them overnight with the DBY-specific CD4+ T-cell clone. Thereby, 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



Untransduced HeLa cells and HeLa cells transduced with wildtype DBY or DBY epitope were analyzed by immunofluorescence for expression of the transgene.

Dr. Kremer



Indirect presentation of wildtpye and mutated constructs of DBY. Mean release of IFN-γ by the DBY specific CD4+ T-cell clone is depicted. The male B-cell line Raji was used as positive control.

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, upon co-culture of HeLa cells expressing the DBY epitope with our antigen presenting cells we did not observe any T-cell activation at all. This points to an additional regulatory element outside the T-cell epitope influencing the intercellular transfer of DBY.

To further analyze these intriguing observations, we are currently establishing cell lines with additional truncated DBY constructs. Furthermore, to test the in vivo relevance we have generated the murine homologues of our DBY constructs and are currently establishing a mouse model to investigate tumor rejection as a function of antigen composition.

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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 - Progress Report

01.12.2012 - 30.11.2014

lncRNA-directed epigenetic programming of HOX loci in GIST

Dr. Evgeny Moskalev, Institute of Pathology

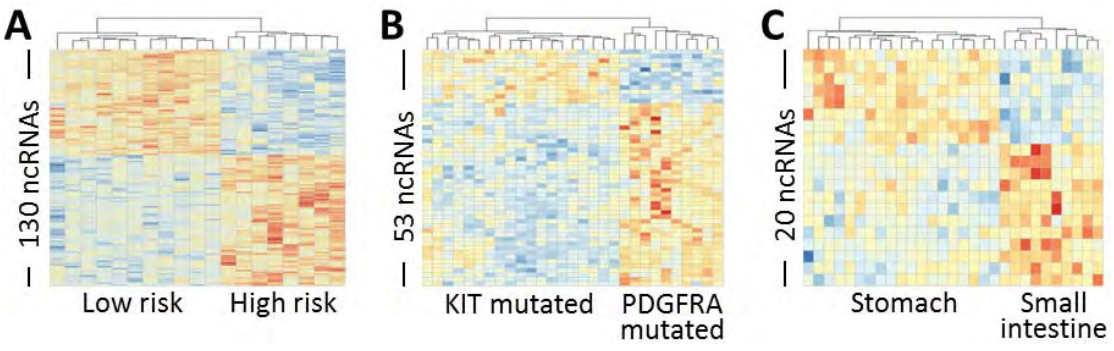
A large group of lncRNAs consists of structurally and functionally distinct transcripts, with some species contributing to carcinogenesis. To reveal functional contribution, we performed expression profiling of lncRNAs in GISTs and identified transcripts specific for different clinico-pathological groups. To investigate if aberrations of DNA methylation can be determined by lncRNAs, we generated a knock-down of HOTAIR in GIST cells and analyse potential alterations of DNA methylation patterns.

As opposed to well characterised protein-coding transcripts that are coded by 1-2% of the genome, functions of much more abundant classes of non-coding RNAs just begin to be elucidated. A prominent part of the non-coding transcriptome – long non-coding RNAs (lncRNAs) – is increasingly recognised as key regulators of diverse cellular processes. Few characterised lncRNA species are involved in maintaining pluripotency, embryonic development, orchestration of epigenetic programmes, nuclear organisation and other core processes. While clear indications exist for the role of certain lincRNA species in carcinogenesis, this field remains largely unexplored to date.

We aim to investigate the functional role of lncRNAs with a particular focus on epigenetic deregulation by using gastrointestinal stromal tumours (GISTs) as a model. Our earlier large-scale DNA methylation analysis of GISTs has revealed distinct DNA methylation patterns according to anatomical localisation, genotype, and mitotic counts. Identification of differential DNA methylation within HOX clusters and earlier reports on multiple non-coding RNAs transcribed from these loci suggested the contribution of such transcripts to both malignant behaviour and epigenetic deregulation in GIST.

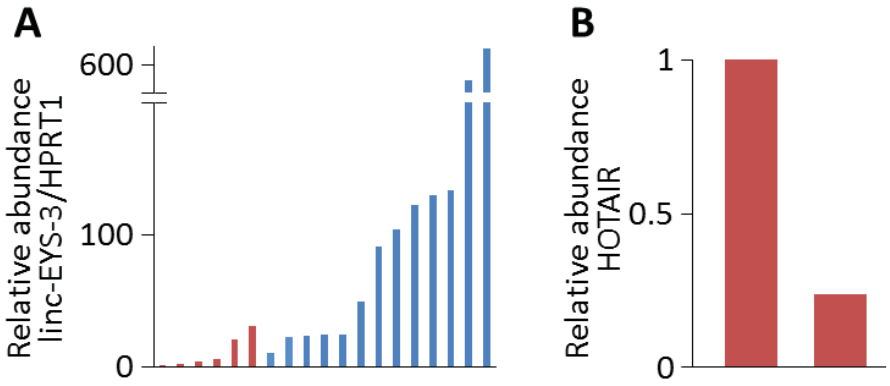
Many novel lncRNAs and other ncRNA classes are specific for GISTs of different clinico-pathological groups

By using genome wide expression profiling we characterised GISTs of (1) low vs. high risk, (2) KIT vs. PDGFRA mutations, (3) stomach vs. small intestinal location. Differentially expressed transcripts were revealed between all the groups that included lncRNAs, sn- and snoRNA species. We next focused on GISTs of low and high risk and performed validation of differentially expressed transcripts that are of



Expression pattern of most prominent ncRNA species that reflect different aggressive behaviour of gastric GISTs (A), aberrant constitutive signalling from KIT or PDGFRA receptors (B) or associate with anatomic location (stomach vs. small intestine, C).

Dr. Moskalev



(A) Novel lincRNA species that significantly discriminates gastric GISTs of low (red) and high (blue) risk. (B) Representative stable knock-down of HOX antisense intergenic RNA (HOTAIR) in cell line GIST T1 for evaluation of subsequent epigenetic reprogramming and functional consequences.

both mechanistic and biomarker interest for the disease. Differential expression of seven lincRNAs and eight sn-/snoRNAs was confirmed by using qPCR. Analysis of a large independent sample set as well as functional analysis of most prominent transcripts is in progress, which will show biomarker potential and provide mechanistic insights into their function.

LncRNAs as modulators of the methylome of cancer cells

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 address the HOX antisense intergenic RNA (HOTAIR), which is capable of targeting repressive histone marks to specific genomic sites. To ascertain if up-regulation of HOTAIR in GIST may be responsible for specific aberrations of DNA methylation, too, we first screened and selected GIST cell lines with high levels of endogenous HOTAIR expression: GIST 48b, GIST882, GISTT1 and GIST430. We next generated by means of lentiviral transduction a GISTT1 cell line with stable knock-down of HOTAIR. Analysis of the methylome in cells upon HOTAIR knock-down is in progress, which may reveal RNA-directed aberrations of DNA methylation in cancer.

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Invited lectures

Keystone Symposium Noncoding RNAs in Development and Cancer, 20-25.01.2013, Vancouver, Long noncoding RNA HOTAIR is associated with distinct clinicopathological features of gastrointestinal stromal tumours (GISTs)

Gordon Research Conference on Cancer Genetics and Epigenetics, 21-26.04.2013, Lucca (Barga), Global deregulation of long intervening non-coding RNAs is a feature of gastrointestinal stromal tumours (GIST)

36 Annual Meeting of the Molecular Biology Society of Japan, 3-6.12.2013, Kobe, Expression of different classes of noncoding RNAs is associated with distinct clinico-pathological features of gastrointestinal stromal tumours (GISTs)

Publications during funding period

none

Newly started Projects

J36 01.09.2013 - 31.08.2015

Identification of molecular signalling pathways in cholestatic pruritus


Dr. Kremer

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

Pruritus is a common and often agonizing symptom of various hepatobiliary disorders. 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.

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J37 01.07.2013 - 30.06.2015

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


Dr. Bosch-Voskens

Dr. Caroline Bosch-Voskens, Department of Dermatology

Anti-CTLA-4 therapy underscores the relevance of stimulating T cells to treat melanoma. Unfortunately, melanomas can escape T cell attack in many ways, notably by down-regulation of HLA class I molecules. NK and γδT cells kill tumor cells in a HLA-unrestricted fashion and as such, ideally supplement T cells. In this study, we will establish pre-clinical adoptive transfer protocols with NK and γδT cells in order to boost innate immunity and to overcome tumor cell escape due to HLA loss.

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J38 01.02.2014 - 31.01.2016

MCS-18 for the treatment of atherosclerosis


Dr. Dietel

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

Progression of atherosclerosis is driven by an inflammatory process, in which dendritic cells are involved. Our preliminary work has shown that the preventive application of the natural substance MCS-18, which inhibits DC maturation, prevents the formation of plaques in the early stage of atherosclerosis. In the proposed study we intend to investigate both the potential mechanisms of the antiatherogenic effects and the impact of MCS-18 on advanced atherosclerotic plaques.

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J39 01.01.2014 - 31.12.2015

Hypermethylation of SOCS3 in fibrotic diseases


Dr. Dees

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

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

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Newly started Projects

J40 01.01.2014 - 31.12.2015

PU.1 signalling in fibrotic diseases


Dr. Ramming

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

PU.1 is a transcription factor epigenetically inactivated in the “young” immune system. The rather complex role of PU.1 necessitates a tight physiologic control of its expression during the maturation of the immune system. Interestingly, wound healing in PU.1 deficient mice appears scar-free as in the embryo suggesting a fundamental but still unknown role in fibrotic diseases. This project aims to investigate PU.1-signaling as a new therapeutic strategy in fibrotic diseases.

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J41 01.12.2013 - 30.11.2015

Resolution of inflammation in gout


Dr. Schauer

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 cytokines.

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J42 24 months

Bayesian reverse engineering of developmental networks


Dr. Ferrazzi

Dr. Fulvia Ferrazzi, Institute of Human Genetics

The proposal 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 onto congenital heart disease, cardiac stem cell differentiation and regeneration.

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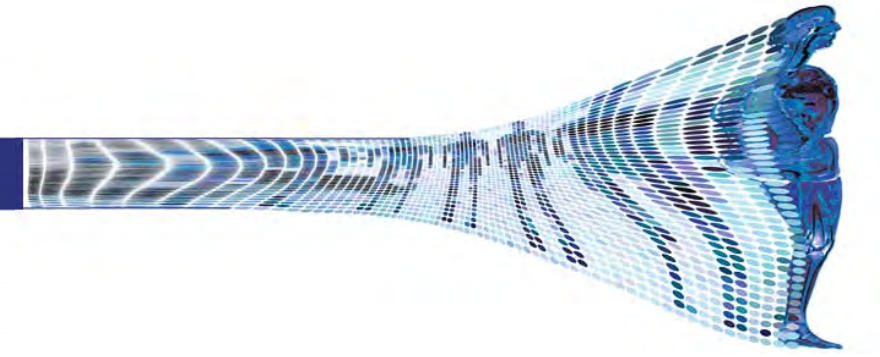
Core Facilities and Supporting Activities



Z4 - Final Report

01.01.2009 - 31.12.2013

Core Unit DNA-Extraction plattform (Biobank)

Prof. Dr. André Reis, Institute of Human Genetics
Dr. Cornelia Kraus, Institute of Human Genetics

Genetic and genomic studies for complex traits and clinical studies require high quality DNA-samples of probands as well as a rigorous and reliable handling and tracking of large numbers of samples. Starting in 2009 the core facility Z4 "DNA-Extraktionsplattform (Biobank)" was established offering quality controlled DNA-extraction from blood samples and other body fluids, their handling and aliquoting as well as and their long-term archiving. After conclusion of the IZKF funding period the core facility will continue to offer its services through user fees.

A reliable and standardized DNA extraction process yielding high quality DNA is a prerequisite for successful downstream genetic research applications, e.g. genotyping, microarray applications, next-generation sequencing or quantitative PCR analysis. From 2009 till 2013, the core unit offered automated DNA extraction services from whole EDTA-blood, including small (400 µl), medium (1-5 mL) and large (5-10 mL) volumes. DNA extraction from cell lines, tissue, buccal swabs, saliva and mouse tails is also available. All procedures were performed under SOPs.

Upon arrival of specimens, the patient's identifier (specific for each clinic) was recorded in an electronic database and each sample was labeled with an individual laboratory bar code. This allowed tracking of samples during the whole extraction process. After extraction each individual sample was subject to quality assessment by gel electrophoresis and OD measurement. The final product was quantified by measuring the OD 280/260 with an Infinite 200 NanoQuant reader and results were recorded electronically. DNAs were then stored under appropriate conditions until they were returned to the referring scientist. Average yield was 150-250 µg of DNA from 5-8 mL whole blood with a quality score = OD ratio 260/280 > 1.9.

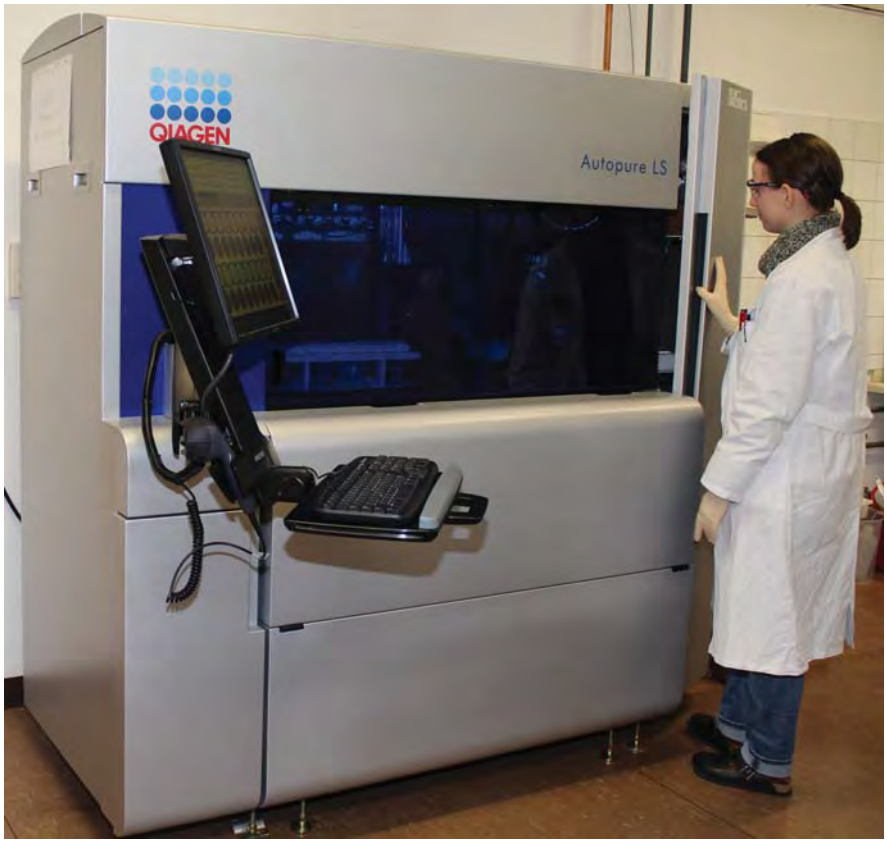
Upon request, normalization of samples was performed to ensure a uniform concentration of all samples as well as aliquoting of samples in 2D-barcoded containers. This allowed arraying samples in individual configurations depending on specific experimental requirements ("cherry picking"). The in-house robotic liquid handler Biomek FX with integrated barcode reader enabled sample tracking throughout the complete process. Probe-IDs were linked to phenotypic and clinical project data through STARLIMS software. This allowed tracking of samples also from within the project.

Initially, the system relied on an automatic DNA extraction platform from Chemagen (chemagic Magnetic separation module I). To attend the increasing demand especially from oncological projects the capacity of the Core Facility was expanded with an additional platform Autopure LS (Qiagen) purchased in August 2012 and co-funded by the Erlangen University Comprehensive Cancer Center. Both platforms yielded high quality DNA samples suitable for all downstream applications such as PCR, SNP genotyping, microarray applications and next-generation sequencing. The lab was generally able to successfully process samples despite heterogeneous age and prior storage conditions with more than 99.3% of samples extracted at high quality and with adequate DNA-quantity.

In 2013, the Core Facility extracted 8,733 study samples (compared to 8,075 in 2012), which represents a 10% increase at an already very high level and an increment of more than 37% compared to 2011 (6,372 samples) and more than a duplication compared to 2010 (4,079 samples). In 2013, 76% of all samples were received from 8 different university clinics from Erlangen, compared to 79% and 55% in 2011 and 2010, respectively. Altogether, samples were collected by 28 different studies of various sizes ranging from 2 to 2,856 samples. Since its inception in 2009 the core unit extracted a total of


Prof. Reis


Dr. Kraus



High-throughput DNA-extraction platform (Autopure LS, Qiagen) for extraction of nucleic acids from various sample materials and volumes.

30,516 samples for more than 100 different studies. In summary, the work of the Core Facility was highly successful clearly exceeding initial expectations and critically supporting numerous studies. After conclusion of the IZKF funding period the core facility will continue to offer its services through user fees.

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Publications during funding period

Given the service character of the core unit no co-authorship was requested for the service.

Index of Names

IMPRINT

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