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Universitätsklinikum
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IZKF Annual Report 2018

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IZKF Erlangen
Annual Report 2018

Interdisciplinary
Center for
Clinical Research

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IZKF Erlangen

Annual Report 2018

Editorial

The Interdisciplinary Center for Clinical Research Erlangen (IZKF) is the central structure of research development of the Faculty of Medicine of the Friedrich-Alexander-University Erlangen-Nürnberg (FAU). Its mission is to improve the overall quality of clinical research at the Faculty of Medicine, to stimulate interdisciplinary research, to advance the careers of young scientist and to foster the acquisition of extramural funds. This annual report is in compliance with our policy of transparency, an essential element for the high level of acceptance of this competitive intramural funding body.

Besides this printed report, IZKF's web-page is also an important element of rapid communication and transparency. Its new structure and design already developed in 2017 went online in early 2018 and received a very positive response from the community. We have also introduced some modifications to this printed annual report. To facilitate information retrieval, we slightly reorganised the way we present the information without changing the overall composition.

This year we also continued the consolidation of ELAN activities within IZKF. ELAN represents funding of pilot projects of up to one year duration and with a relative modest budget which are intended for development of promising ideas. All ongoing pilot projects are now presented in this annual report with an abstract, and we have also included an output analysis of previous years. We are delighted to see that ELAN activities are a valuable and successful contributor to overall scientific success by promoting projects and careers at an early stage.

As a major strategy, we envision a research path for young scientist with the continuous support of IZKF. Following a successful pilot project the next step for young researches usually is applying for a junior project. This programme provides 2.5 years funding with the expectation that after this period extramural funding is obtained. We have yearly calls for applications, and in 2018 we received 10 applications of which seven could be funded. This reliable, annual programme was initiated 10 years ago and has established itself as a cornerstone of IZKF activities. It has been an important starting point for the careers of many young scientists who are now research group leaders or even already obtained Faculty positions. Overall, a total of 75 scientists from all focal areas of the Faculty were or are being funded with an equal distribution between genders and with 43% of PIs trained as physicians and 57% as scientists. The great success of this programme is reflected in the overwhelming approval rate of extramural funding obtained by the PIs as follow-up of their IZKF projects as detailed in this report.

For established scientists IZKF has a separate funding programme, the advanced projects. The calls for these projects are every 3 years. The current funding programme with 31 projects is coming to its end. As in previous funding periods, almost all projects applied for extramural funding within the 30 months, enabling them to obtain additional 6 months of funding. This 30+6 months regulation has been instrumental in motivating PIs to more rapidly apply for extramural funding. The success rate of these applications is very high, confirming the rationale for this strategy. Not only applications of individual research grants arise from these projects but they also make a significant contribution to coordinated funding programmes, e.g. collaborative research centres and research units. Therefore it is reassuring that 83% of PIs from the University Hospital who are active in one of the DFG collaborative research centres at the University received IZKF funding, either currently or in the past. We hope to continue this success story with the new call for advanced projects announced in 2018. We will have a first review of applications in the summer of 2019 and look forward to the visit of our Scientific Advisory Board in the fall, which will then make the final selection. New projects will then start in 2020.

The overall idea of all IZKF programmes is to support projects so that they are successful in subsequently obtaining extramural funding. This motivated us to develop a new programme for a small but neglected group of researchers at the Faculty: Independent

scientists and professors without an own significant budget who are strongly dependent on extramural funding for financing their research. This group may have difficulties in maintaining research lines and their research groups when a research grant is not renewed promptly. Necessary revisions of these applications often need time and require additional resources for experimentation, which can represent an unsurmountable obstacle without funding. For this group of scientists we have now developed the bridging projects, a programme aiming at supporting the group in a time of hardship with the expectation that the revision can be completed in 6 months and that the application will then be successful allowing the work to be continued. This programme was recently launched, and the first scientist was already funded. We hope that this new programme conceived by deputy chairman Prof. Michael Wegner will be yet another IZKF success story.

In 2017 we established a formal Clinician-Scientist-Programme (CSP) according to DFG-recommendations. The CSP combines long established IZKF programmes for medical trainees e.g. laboratory rotations with new elements of structured education tailored to the needs of this group. The CSP has two lines of support, a basic programme for MDs starting their research careers and a more senior programme for those who already have their own research funding and have thus reached certain independence. In 2018 first participants were selected and both lines are now up and running. I am very glad that Profs. Christiane Zweier, Dimitrios Mougiakakos and Jürgen Winkler volunteered to coordinate the CSP.

Another important activity in 2018 was the preparation of the 7th International IZKF-Symposium in Kloster Banz on 27th-28th June 2019. The programme committee chose again "Translational Medicine" as the unifying theme as it perfectly summarises the overall goal of IZKF. Following the success of previous editions we will return to Bad Staffelstein, north of Erlangen, where the baroque cloister of Banz resides over the beautiful upper Main valley. In this ideal location we will have five scientific sessions with an impressive line-up of speakers from many countries sharing their newest findings. There will also be ample time for networking around the posters and for meeting old and new friends. I am thankful to the organising committee for developing this impressive programme and to the sponsors for making this meeting possible. Registration is already well advanced, but a few places are left in case you are tempted for a late registration.

2018 has also witnessed the consolidation of Life@FAU, the FAU Graduate School of Life Sciences founded in 2017. Life@FAU supports interdisciplinary graduate programmes in medicine and science at FAU and is supported by the Faculty of Medicine and the Department of Biology at the Faculty of Sciences. IZKF is one of the founders, and its Research Training Group is by far the biggest single contributor. Also the administrative office is run by the IZKF administrative offices team. We are delighted by the high acceptance rate of this programme and look forward to a continued strong development improving graduate education at our Faculty, both for natural scientists and physicians.

I would like to thank the Junior Scientists Committee headed by Prof. Christoph Becker for their efforts and welcome the newly elected members starting their terms in 2019.

I also want to take the opportunity to warmly congratulate our junior project leader Dr. Elisabeth Waldmann (Department of Medical Informatics, Biometry and Epidemiology) for obtaining a much sought-after Freigeist-Stipend from the Volkswagen Foundation which includes a sizable support of one million €. This stipend is awarded for young scientists engaging in high-risk projects for which we wish her all the best.

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

Prof. Dr. André Reis
Chairman

Imprint and Contents

IMPRINT

Annual Report 2018

Publisher

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

Editor

Dr. Katrin Faber
IZKF Administrative Office
Krankenhausstr. 12, 91054 Erlangen
phone: +49 9131 85 46841
fax: +49 9131 85 35903
katrin.faber@uk-erlangen.de

Cover

Anne Reichel
IZKF Administrative Office

Layout and setting

Bianca A. Meyerhöfer-Klee
IZKF Administrative Office

Photo credits

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Printing

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

Print run

350 copies

April 2019

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Scientific Reports Junior Groups/Projects

Scientific Reports Pilot Projects

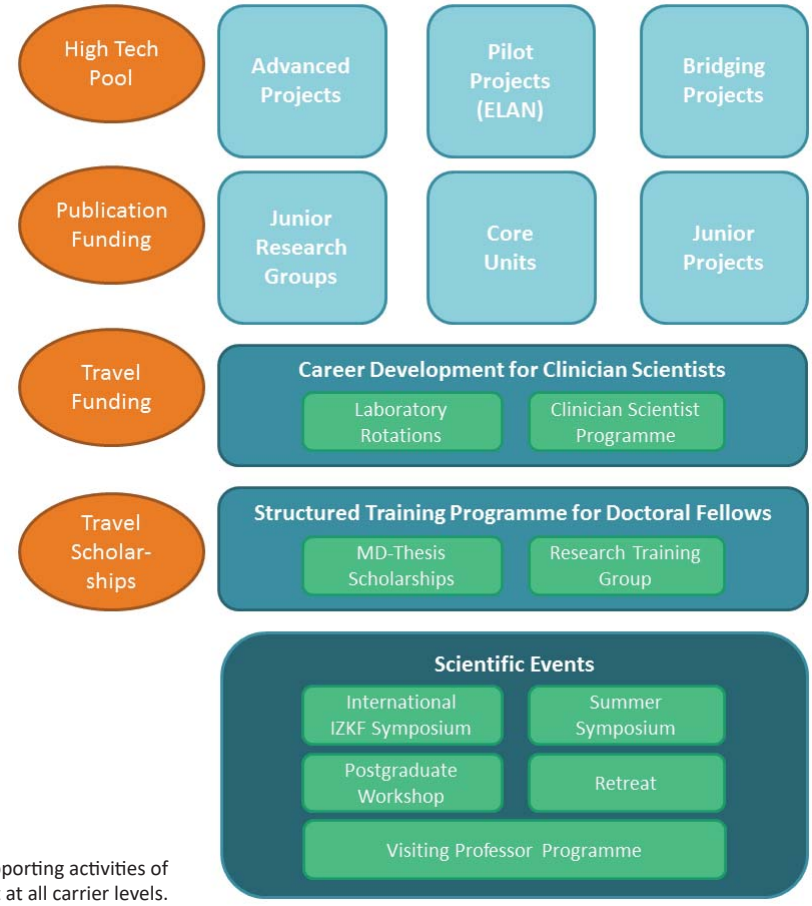
About us

FUNDING SCHEMES AND ACTIVITIES

Overview

The IZKF is the central structure of research development of the Faculty of Medicine. Its mission is to improve the overall quality of clinical research, to stimulate interdisciplinary research, to advance the careers of young scientists and to foster the acquisition of extramural funds. In order to achieve these goals, the IZKF supports projects in all research areas of the Faculty of Medicine on a strictly time-limited basis. The selection of projects is based exclusively on quality aspects. The various programmes are aimed at physicians and scientists at different stages of their scientific careers. Equipped with its own budget and its own management structures, the IZKF continuously develops its own funding programmes in line with the needs of the Faculty of Medicine. In addition, the Faculty of Medicine also uses the structures established in the IZKF for the allocation and management of funds and avoids the creation of parallel structures.

The IZKF has created more transparency about research activities in the various areas and strengthened cooperation between clinics and institutes, but also between different clinics. The IZKF enables research funding beyond budget boundaries and also supports risk projects. Young scientists from Germany and abroad can be attracted by the junior research groups.



Programmes and supporting activities of the IZKF for scientist at all carrier levels.

Advanced projects

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

scientists of the Faculty of Medicine based on a written proposal and public presentation. Decisions are reached after internal deliberation and are communicated immediately afterwards. Successful proposals are presented in full to the Scientific Advisory Board during their peer-review site visits. Negative funding decisions of the Board are binding. Projects must start within six months after acceptance. Over the years funding rates were about 30 - 40%. Proposals are accepted every two or three years.

LOM weighted 4-fold

- DFG incl. SFB
- BMBF
- Other Federal and State Ministries
- EU
- NIH-Grants

LOM weighted 2-fold

- Bill-Gates-Foundation
- DAAD
- Hertie-Foundation
- José-Carreras-Foundation
- Else-Kröner-Fresenius-Foundation
- Mildred-Scheel-Foundation
- German-Israelian-Foundation (GIF)
- Thyssen-Foundation
- Humboldt-Foundation
- German Foundation for Heart Research
- Volkswagen Foundation
- Wilhelm-Sander-Foundation
- Bayerische Forschungsstiftung/ Bayerische Landesstiftung
- State research associations

Funding agencies which allow the extension of projects according to the regulations of funds allocation in Bavaria (scientific performance criteria).

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

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

The last evaluation of the IZKF took place in autumn 2015. At this time, 31 new projects had already been accepted for funding 2 years after the previous evaluation, significantly more than in the previous evaluations. The call for projects originally planned for 2018 was therefore postponed to 2019 for financial reasons. At the end of 2018 the new call for projects took place. In the future, new projects are to be included in the funding every 3 years.

Staff	Single projects: graduate student or technical assistant (one position) Tandem projects: graduate student(s) and/or technical assistant (two positions)
Consumables	Single projects: EUR 15,000 p.a. Tandem projects: EUR 25,000 p.a.
Others	Participation in Travel, Publication and High Tech Pool
Duration	30 + 6 months

On the following pages you will find a list of all projects that were funded by the IZKF in 2018.

About us

Immunology and Infection

Project No.	Project title	Term	Applicant(s)	Institute
A63	Mechanisms of TNF-Mediated Control of Intracellular Pathogens in Mice and Man	01/07/2016-30/06/2019	Prof. Bogdan	Institute of Clinical Microbiology, Immunology and Hygiene
A64	The tyrosine-protein phosphatase SHP2 regulates TGFβ -dependent activation of JAK2/STAT3 in fibrotic diseases	01/02/2016-31/01/2019	Prof. Distler, Prof. Schett	Department of Medicine 3
A65	Tolerizing potential of human dendritic cell subpopulations	01/04/2016-31/03/2019	Prof. Dudziak	Department of Dermatology
A66	Genome wide CRISPR/Cas9 knockout for the identification of antiviral cellular restriction factors	01/07/2016-30/06/2019	Prof. Ensser	Institute of Clinical and Molecular Virology
A67	Analysis of the TRIM5alpha-mediated block to LINE-1 retroelements	01/02/2016-31/01/2019	Prof. Gramberg	Institute of Clinical and Molecular Virology
A68	Analysis of the role of the IL-23/Th17 axis during the control of antibody activity in rheumatoid arthritis	16/06/2016-15/06/2019	Prof. Krönke, Prof. Nimmerjahn	Department of Medicine 3, Division of Genetics
A69	Contribution of ATM kinase and the DNA-damage response in the innate immunity to infection	01/07/2016-30/06/2019	Prof. Lang	Institute of Clinical Microbiology, Immunology and Hygiene
A70	Novel targets for antiretroviral therapy - deubiquitinating enzymes regulate HIV-1 replication	01/07/2016-30/06/2019	Prof. Schubert	Institute of Clinical and Molecular Virology
A71	Viral modulation of the protein kinase ULK1	01/07/2016-31/12/2018	Prof. Stamminger	Institute of Clinical and Molecular Virology
A72	Targeted modulation of regulatory T cells and analyses of the underlying mechanisms	01/07/2016-30/06/2019	Prof. Steinkasserer	Department of Immune Modulation
A73	Checkpoint inhibitors as adjuvants for viral vaccines	01/07/2016-31/03/2019	Prof. Überla	Institute of Clinical and Molecular Virology
A74	The Role of Eosinophils in Allergic Bronchopulmonary Aspergillosis	01/06/2016-31/05/2019	Prof. Vöhringer, Prof. Krappmann	Department of Infection Biology, Institute of Clinical Microbiology, Immunology and Hygiene
A75	Role of MLKL-dependent programmed necrotic cell death in the pathogenesis of hepatitis	01/07/2016-30/06/2019	PD Dr. Dr. Günther, PD Dr. Dr. Wirtz	Department of Medicine 1

Oncology

Project No.	Project title	Term	Applicant(s)	Institute
D23	Influence of bone marrow adipocytes on the metastatic niche in experimental bone metastasis	01/01/2016-31/12/2018	Prof. Bozec	Department of Medicine 3
D24	Differentiation-associated Schwann cell transcription factors in melanoma– learning from embryogenesis	01/06/2016-31/05/2019	Prof. Bosserhoff, Prof. Wegner	Institute of Biochemistry
D25	Interaction of the EGFR- and the ZEB1-pathway in aggressive cancer types	01/05/2016-30/04/2019	Prof. Brabletz	Chair of Experimental Medicine I
D26	Identification of antigen specificity of tumor-infiltrating lymphocytes in triple-negative breast cancer	01/01/2016-31/12/2018	Prof. Mackensen, Prof. Fasching	Department of Medicine 5, Department of Obstetrics and Gynecology
D27	2-Hydroxyglutarate in Acute Myeloid Leukaemia: Novel Molecular Targets and Impact on Immune Escape	01/07/2016-30/06/2019	Prof. Mougiakakos	Department of Medicine 5

Project No.	Project title	Term	Applicant(s)	Institute
D28	SPARCL1 function in vessel maturation and metastasis of colorectal carcinoma	01/02/2016-31/01/2019	Prof. Stürzl, PD Dr. Naschberger	Department of Surgery
D29	Aging and senescence of the adaptive immune system in colorectal cancer	01/01/2016-31/12/2018	Prof. Waldner	Department of Medicine 1

Neurosciences

Project No.	Project title	Term	Applicant(s)	Institute
E19	Inhibitory neurotransmission in the cochlea: Glutamate and endocannabinoids	15/02/2016-14/02/2019	Prof. Enz	Institute of Biochemistry
E20	Identification of molecules, receptors and genes involved in chronic pruritus	01/05/2016-30/04/2019	Dr. Dr. Kremer, Prof. Zimmermann	Department of Medicine 1, Department of Anesthesiology
E21	Modulation of alpha-Synuclein pathology by FoxO-dependent pathways	01/05/2016-30/04/2019	Prof. Lie, Prof. Klucken	Institute of Biochemistry, Department of Molecular Neurology
E22	The role of Swiprosin-1/EFhd2 in resilience to drug addiction	01/03/2016-28/02/2019	Prof. Müller, Prof. Alzheimer, Prof. Dr. Mielenz	Department of Psychiatry and Psychotherapy, Institute of Physiology and Pathophysiology, Department of Molecular Immunology
E23	Identification and characterization of LOXL1 risk variants for pseudoexfoliation syndrome and glaucoma	01/01/2016-31/12/2018	Prof. Schlötzer-Schrehardt, Prof. Reis	Department of Ophtalmology, Institute of Human Genetics
E24	The role of alpha-synuclein during inflammatory demyelination and degeneration in the central nervous system	01/01/2016-31/12/2018	Prof. Winkler, Prof. Linker	Department of Molecular Neurology, Department of Neurology
E25	Modeling pain syndromes using human induced pluripotent stem cell-derived nociceptors	01/07/2016-30/06/2019	Prof. Winner, Prof. Schüttler	Department of Stem Cell Biology, Department of Anesthesiology
E26	Genetics and pathomechanisms of intellectual disability with microcephaly	01/03/2016-28/02/2019	Prof. Zweier	Institute of Human Genetics
E27	Lysophosphatidic acid-induced pruritus of cholestasis	01/03/2016-28/02/2019	Dr. Dr. Kremer, Prof. Fischer	Department of Medicine 1, Institute of Physiology and Pathophysiology

Renal and Vascular Research

Project No.	Project title	Term	Applicant(s)	Institute
F5	The Role of ANO1 in Polycystic Kidney Disease	01/07/2016-31/12/2018	PD Dr. Buchholz	Department of Medicine 4
F6	Renal afferent nerve activity - sympathoinhibitory or sympathoexcitatory?	01/07/2016-30/06/2019	Prof. Veelken, Prof. Amann	Department of Medicine 4, Department of Nephropathology

About us

The following table shows all institutions involved in the IZKF in 2018 and their association to the focal research areas of the Faculty:

Institute	Main Research Areas				
	Immunology and Infection	Oncology	Neurosciences	Renal and Vascular Research	Others
Chair of Experimental Medicine I		•			
Department of Anesthesiology			•		
Department of Dermatology	•				
Department of Immune Modulation	•				
Department of Infection Biology	•				
Department of Medicine 1	•	•	•		
Department of Medicine 3	•	•			
Department of Medicine 4				•	
Department of Medicine 5		•			
Department of Molecular Immunology			•		
Department of Neurology			•		
Department of Obstetrics and Gynaecology		•			
Department of Ophthalmology					
Department of Psychiatry and Psychotherapy			•		
Department of Stem Cell Biology			•		
Department of Surgery		•			
Division of Genetics	•				
Division of Molecular Neurology			•		
Division of Nephropathology				•	
Institute of Biochemistry		•	•		
Institute of Clinical and Molecular Virology	•				
Institute of Clinical Microbiology, Immunology, and Hygiene	•				
Institute of Human Genetics			•		
Institute of Physiology and Pathophysiology			•		

Pilot Projects (ELAN)

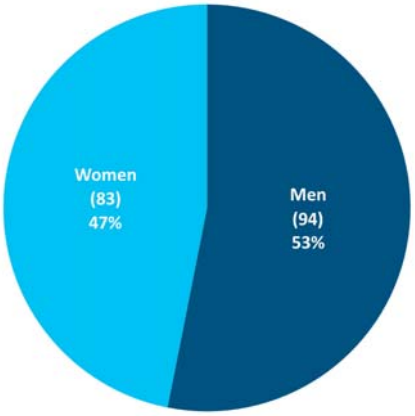
The aim of the ELAN programme is to support scientific projects at a very early stage and help prepare them for successful application for external funding (start-up projects), to support newly established working groups, to develop new innovative ideas (pilot projects) or act as interim funding if temporary gaps arise between individual extramural funding periods. Young scientists up to 38 years of age are supported with a maximum of € 50,000 for a period of up to 12 months. Since 2017, also applicants from the part of the Faculty administrated by the University have been approved for the programme, so that young scientists from the entire Faculty of Medicine can apply. In addition, newly appointed professors can submit their application regardless of age. A total of two ELAN projects can be applied for over the course of a scientific career, provided that a publication or a third-party funded project has arisen from the previous funding.

In 2017 the pilot project programme was newly integrated into IZKF. As a result, the nomenclature of the projects also changed. The projects now receive a P with a consecutive number as the project number. This replaces the old designation of the file numbers. Pilot projects are intended to support scientists at an early stage. In the reporting period of 2018, 26 proposals were discussed during the meetings of the ELAN-Commission. Thereof, 16 (62%) received funding. The approved projects cover nearly all the focal research areas of the Faculty of Medicine: oncology 4, immunology and infection 3, neurosciences 6 and others 3. In 2018, pilot project leaders from 16 different institutions were reviewed. In total, 9 (56%) of the successful applicants were men and 7 (44%) women. The median age was 33 for all project leaders. Women had a median age of 32 and men of 34 years.

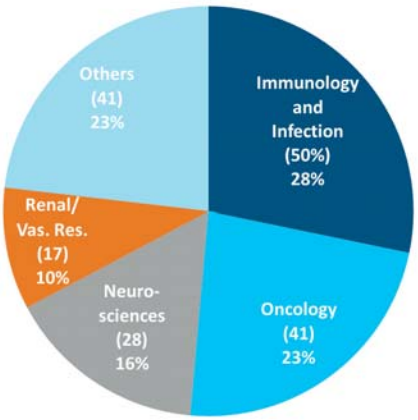
Applications for pilot projects can be submitted at any time. Since 2012 an electronic application using the ELAN-Tool is expected. The ELAN-Commission meets 5-6 times a year and selects projects for funding. The procedure provides for the participation of an external expert. Between 2012 and 2018 a total of 255 proposals for pilot projects were reviewed by the ELAN-Commission. Overall, 177 (69%) projects were granted for funding. Between 2012 and 2018 in total 83 women (47%) and 94 men (53%) applied successfully for pilot projects. The median age was at 34 years for all, 32 years for women and 34 years for men.

All focal research areas of the Faculty are represented; with immunology and infection (28%) and oncology (23%) being the most successful over the years. 59 (33%) of 177 projects were completed with a publication.

Staff	One position
Consumables	max. EUR 50,000
Others	Participation in Publication Pool
Duration	max. 12 months



Gender distribution of pilot project leaders between 2012 and 2018.



Distribution of pilot projects as per main research area between 2012 and 2018.

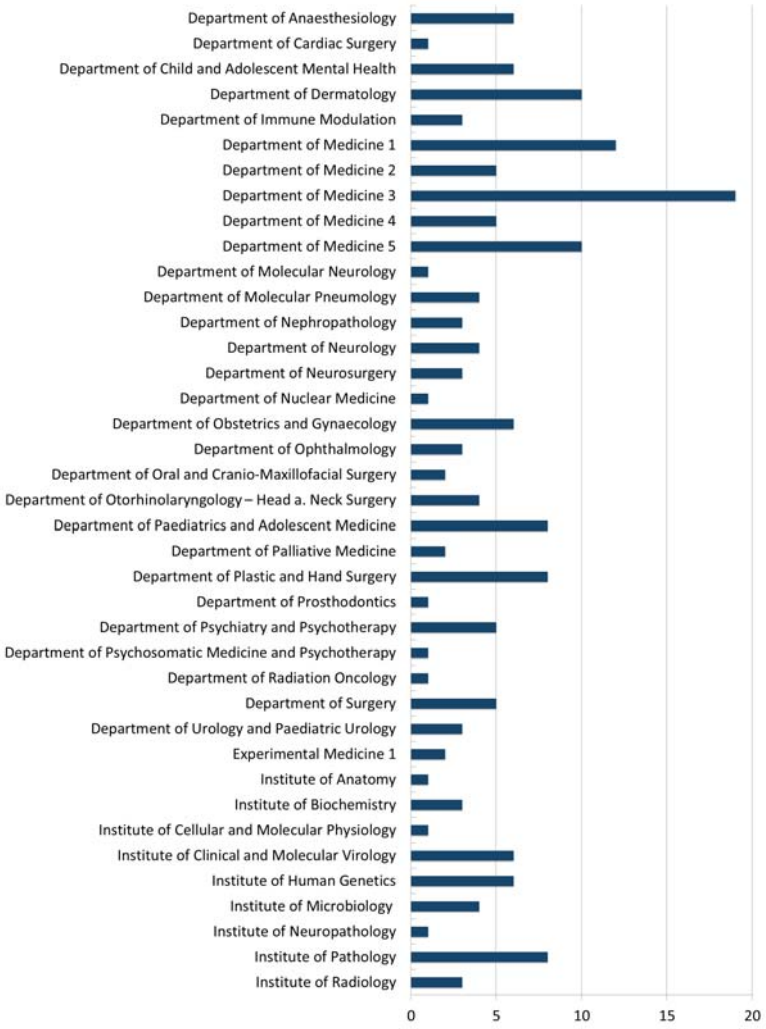
About us

In the table all projects are shown which have received funding or have been approved for funding in 2018:

Project No.	Project title	Term	Applicant(s)	Institute
without	Interleukin 9 is an essential factor for resolution of arthritis	01/04/2017-31/03/2018	Dr. Andreas Ramming	Department of Medicine 3
without	Measurements of kidney perfusion after transplantation by intraoperative fluorescence angiography	16/01/2017-15/01/2018	Dr. Ulrich Rother	Department of Surgery
without	Immunogenic cell death by nanoparticles	13/02/2017-16/04/2018	Dr. Christina Janko	Department of Oto-Rhino-Laryngology - Head and Neck Surgery
without	Automated sleep staging by EEG-pattern analysis	01/02/2017-31/01/2018	Dr. Maximilian Traxdorf	Department of Oto-Rhino-Laryngology - Head and Neck Surgery
P001	The function of microRNAs in dendritic cells	01/04/2017-31/03/2018	Dr. Xin Lai	Department of Dermatology
P002	Duotoxins against B-cell malignancies	01/05/2017-01/05/2018	Dr. Fabian Müller	Department of Medicine 5
P003	In vitro transformation model	13/02/2017-16/04/2018	Dr. Julienne Kathrin Münzner	Department of Medicine 5
P004	Trauma-related disorders in refugees from Syria	01/04/2017-31/03/2018	Dr. Eva Morawa	Department of Psychosomatic Medicine and Psychotherapy
P005	MRI of Collagen Gel Cartilage Repair	01/12/2017-30/11/2018	Dr. Milena Pachowsky	Department of Surgery
P006	Inhibition of JAKs in Systemic sclerosis	01/10/2017-31/10/2018	Dr. Yun Zhang	Department of Medicine 3
P007	Genome CRISPR/Cas9 in pancreatic cancer	01/08/2017-31/07/2018	Prof. Dr. Christian Pilarsky	Department of Surgery
P008	Gene-based vaccines for mucosal immunizations	01/12/2017-30/11/2018	Prof. Dr. Matthias Tenbusch	Institute of Clinical and Molecular Virology
P009	Influence of CD40 receptor blockade on B cells	13/12/2017-12/12/2018	Dr. Mandy Wahlbuhl-Becker	Department of Pediatric and Adolescent Medicine
P010	The FGFR1 amplicon in breast cancer	01/09/2017-31/08/2018	Dr. Ramona Erber	Institute of Pathology
P011	Tumor cachexia and muscle motor proteins	01/08/2017-31/07/2018	Dr. Raphaela Schwappacher	Department of Medicine 1
P012	RIPK4 in intestinal inflammation and cancer	01/01/2018-31/12/2018	Dr. Eva Martini	Department of Medicine 1
P013	Prenatal trauma, placenta and fetal HPA axis	15/08/2017-15/08/2018	Dr. Stefan Frey	Division of Child and Adolescent Mental Health
P014	Melanoma Metastasis to Steatotic Livers	01/11/2017-31/10/2018	Prof. Dr. Claus Hellerbrand	Institute of Biochemistry
P015	Bile acids as modulators of P2X4 receptor	01/05/2018-31/10/2018	Dr. Alexandr Ilyaskin	Institute of Physiology and Pathophysiology
P016	Laboratory detection of ultrasound microbubbles	01/02/2018-31/01/2019	Dr. Ferdinand Knieling	Department of Pediatric and Adolescent Medicine
P018	XCR1 as a marker for human crosspresenting DCs	01/03/2018-28/02/2019	Dr. Lukas Heger	Department of Dermatology
P019	The involvement of the enteric nervous system in the immunopathogenesis of multiple sclerosis	01/06/2018-30/04/2019	Prof. Dr. Stefanie Kürten	Institute of Anatomy
P020	Localisation of the EMT-transcription factor ZEB1	24/01/2018-23/01/2019	Dr. Rebecca Eccles	Chair of Experimental Medicine I
P021	Bone strength in rheumatic finger joints	01/06/2018-30/04/2019	Dr. Arnd Kleyer	Department of Medicine 3

Project No.	Project title	Term	Applicant(s)	Institute
P022	Vascularization and bone formation	01/08/2018-31/07/2019	Dr. Dominik Steiner	Department of Plastic and Hand Surgery
P023	DPP4 - a molecular target in fibrosis	01/06/2018-31/05/2019	Dr. Alina Soare	Department of Medicine 3
P024	The contribution of butyrophilins in the pathogenesis of Rheumatoid Arthritis	01/10/2018-31/03/2019	Dr. Kerstin Sarter-Zaiss	Department of Medicine 3
P025	Analysis of exosomal biomarkers for CRSwNP	01/08/2018-31/07/2019	Dr. Sarina Müller	Department of Otorhinolaryngology - Head and Neck Surgery
P026	On myelination processes in the cuprizone model	15/11/2018-15/11/2019	Prof. Dr. Frederik Laun	Institute of Radiology
P027	IFN-gamma and vasculature in CRC	01/07/2018-31/12/2019	Dr. Nathalie Britzen-Laurent	Department of Surgery
P028	3D organoid models for analysis of SOX11-CSS	01/10/2018-31/09/2019	Dr. Sören Turan,	Institute of Biochemistry
P029	Combined DNA-/RNA-transfection of T cells	01/11/2018-31/10/2019	Dr. Ugur Uslu	Department of Dermatology
P030	Ultrashort Echo Time MRI of Myelin at 7 T	01/01/2019-31/12/2019	Prof. Dr. Armin Nagel	Institute of Radiology
P031	Zwicker tone as a model for acute tinnitus	16/12/2018-16/06/2019	Dr. Achim Schilling	Department of Oto-Rhino-Laryngology - Head and Neck Surgery
P032	In vivo imaging of inflammation / bone remodeling	15/07/2018-14/07/2019	Dr. Christine Schauer	Department of Medicine 3
P033	YB-1 in molecular regulation of chondrogenesis	26/03/2019-25/03/2020	Dr. Ulrike Rotten-steiner-Brandl	Institute of Biochemistry
P034	Myeloid ZEB1 in colorectal cancer	16/01/2019-15/01/2020	Dr. Harald Schuhwerk	Chair of Experimental Medicine I
P035	Role of FBXO11 in intellectual disability	07/01/2019-07/01/2020	Dr. Anne Gregor	Department of Humangenetic
P036	Physicians' opinions on continuous sedation	12 months	Dr. Maria Heckel	Division of Palliative Medicine
P037	Prognostication in intracerebral hemorrhage	12 months	Dr. Jochen Sembill	Department of Neurology
P038	Molecular markers in stage T1 bladder cancer	01/04/2019-31/03/2020	Dr. Danijel Sikic	Department of Urology
P039	Bone characterization in early RA autoimmunity	12 months	Dr. David Simon	Department of Medicine 3
P040	FoxO-dependent mitophagy in stem cell function	12 months	Dr. Iris Schäffner	Institute of Biochemistry
P041	Polyploid cardiomyocytes for cardiac repair	12 months	Dr. Maria Leone	Department of Nephropathology
P042	Immunology of NMSC of the head and neck	12 months	Dr. Dr. Gesche Frohwitter	Department of Oral and Cranio-Maxillofacial Surgery
P043	The autotaxin-LPA axis in breast cancer	12 months	Dr. Annika Kengelbach-Weigand	Department of Plastic and Hand Surgery
P044	HIF-1a in IgA class switching	12 months	Dr. Xianyi Meng	Department of Medicine 3
P045	HSV-1 modulates the IL-6 signaling pathway in mDCs	12 months	Dr. Linda Grosche	Division of Immune Modulation

About us



Distribution of pilot projects among departments and institutes between 2012 and 2018 (absolute number of projects).

Bridging projects

As of 2019, the programme will allow independent scientists (usually with a permanent employment contract) to work in the field of research to bridge a precarious situation, including professors from the part of the Faculty administrated by the University. The prerequisite is a recently rejected application for third-party funding which has narrowly failed and which, after revision, can be submitted promptly. The application had to be filed to a donor, at least listed twice in LOM (scientific performance criteria), figure in the section Advanced projects.

Other third-party- or intramural funding must not currently exist, but in the past a corresponding external funding (at least listed twice in LOM) must already have been available. A repeated use of the programme is only possible if the previous funding was successful, i.e. the resubmitted application for third-party funding was finally granted. Applications can be submitted at any time and promptly after the precarious situation has occurred. The amount of funding is based on ELAN pro-

Staff and consumables	max. EUR 50,000 (the recruitment of new staff, especially of doctoral students is not intended)
Others	the use of central funds from the travel-, publication- and high-tech pool of the IZKF is not possible
Duration	about 6 months

jects, i.e. up to € 50,000 for a period of 6 months. The evaluation is to be carried out by the ELAN-Commission in a circulation procedure. A member of the ELAN-Commission coordinates the evaluation and integrates a member of the External Scientific Advisory Board of the IZKF as an external expert. There is no age limit. The programme started recently.

Junior Research Groups

Junior research groups offer an attractive career development opportunity for outstanding young scientists with a training in medicine or natural sciences and a strong background and reputation in one of the Faculties' main research fields. Over a period of 6 years, each junior research group receives funding for the group leader, one postdoctoral scientist and one graduate student, one technical assistant and consumables. From this position several previous junior research group leaders have been appointed to a professorship or have achieved other attractive positions. The groups operate independently but may be associated to individual clinics or institutes. For physicians a part time involvement in clinical activities is possible. Groups also have access to research funds allocated by the Faculty based on scientific performance criteria (LOM). At the end of 2018 there are two junior research groups. One group (N1) is housed in the Nikolaus Fiebiger Center for Molecular Medicine with its attractive scientific environment and diverse activities; the other (N2) is now located at the South-Campus in a new scientific building with the Optical Imaging Center Erlangen (OICE).

Until now, Dr. Dulin's group has been accommodated in the rooms of the OICE on the Kußmaul Campus. The high temperature fluctuations in the summer months posed a serious problem for obtaining scientifically valid results. In the new building, the group now has modern laboratories and offices with excellent equipment at its disposal.

Dr. Ceppi's group was able to acquire several external grants in addition to the IZKF grant. Recently, Dr. Ceppi was offered a tenured Associate Professor position from the Department of Molecular Biology and Biochemistry of the University of Southern Denmark in Odense. Dr. Ceppi accepted the appointment on a part-time basis while continuing his junior research group with reduced volume. The management board agreed to this solution.

Staff	Group leader Postdoctoral scientist Graduate student Technical assistant
Consumables	EUR 50,000 p.a.
Others	Participation in the allocation of funds based on performance criteria (LOM) Laboratory space Investment funds
Duration	6 years

Project No.	Project title	Term	Group leader
N1	Understanding the plasticity of cancer cells	01/08/2015 - 31/07/2021	Dr. Paolo Ceppi
N2	Physics and Medicine	01/09/2016 - 31/08/2022	Dr. David Dulin

About us

Core Units

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. Also, it allows students to be directly exposed to these modern developments.

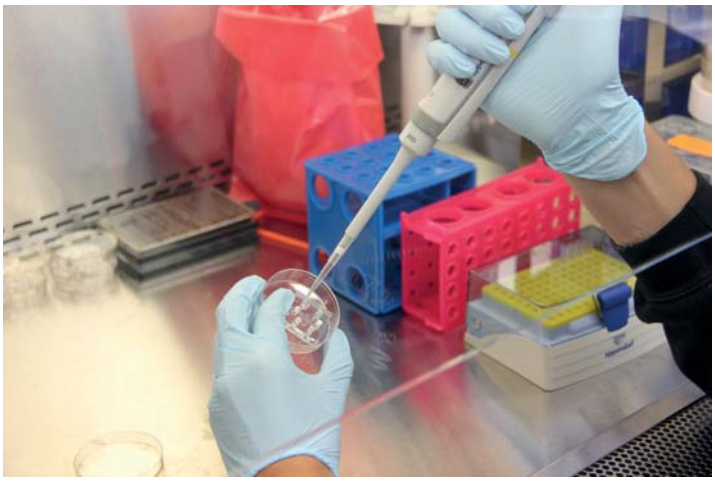
Core facilities are operated under the leadership of a scientific group with demonstrated excellence and interest in developing the methodology. In return for institutional support, it is expected that the operating group assists other groups with their know-how in accessing this technology. The support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

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

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

Next generation sequencing
Institute of Human Genetics, Prof. Dr. André Reis, Dr. Ekici
Cell sorting unit with immune monitoring
Department of Dermatology, Prof. Dr. Gerold Schuler Dep. of Molecular Immunology, Prof. Dr. Hans-Martin Jäck Division of Genetics, Prof. Dr. Thomas Winkler Department of Medicine 5, Prof. Dr. Andreas Mackensen Dep. of Transfusion Medicine, Prof. Dr. Holger Hackstein Sites: Nikolaus-Fiebiger Centre, Kussmaul Campus, Translational Research Center
Preclinical animal unit
Franz-Penzoldt Centre, Prof. Dr. Stephan von Hörsten
Small animal imaging - PIPE
Institute of Radiology, Prof. Dr. Michael Uder, Prof. Bäuerle

Core Units of the Faculty of Medicine currently in operation.



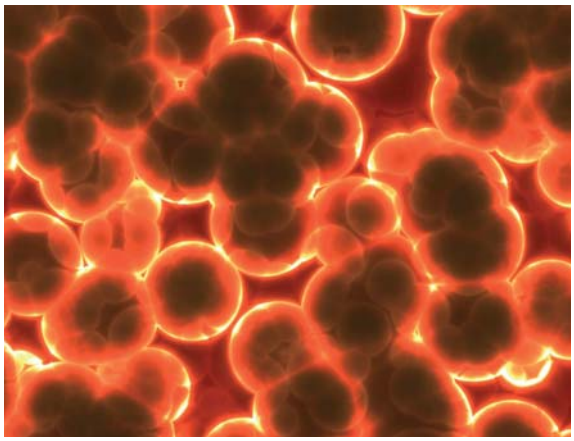
Junior Projects

For scientists starting their independent career, obtaining their first extramural research funding is an important step. To aid in this process, the IZKF offers starting grants to young postdoctoral physicians and scientists up to 35 years of age without previous significant external funding. Candidates should have a visible publication record and projects should be based on an original idea with first tangible results. Projects include a position for a technician or a graduate student and consumables for 30 months.

After this time it is expected that successful candidates submit an external grant application. If the application is filed within duration of the junior project, the spending period will be extended by another 6 months.

Staff	Technical assistant or Graduate student
Consumables	EUR 15,000 p.a.
Others	Participation in Travel, Publication and High Tech Pool; IZKF laboratory rotations for physicians
Duration	30 months

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

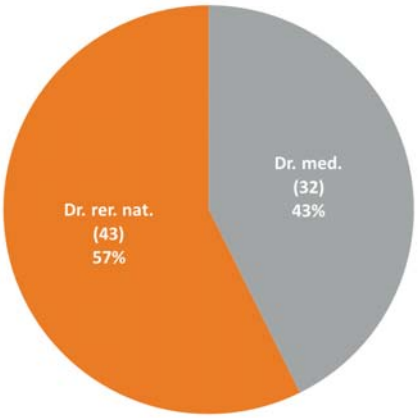


The first call for junior projects was in 2009. Proposals are accepted every year. Overall 75 junior projects were selected for funding between 2009 and 2018. In this period, 32 (43%) physicians received funding and 43 (57%) scientists. 21 (66%) of the physicians requested a laboratory rotation, thereof 7 (33%) were women and 14 (67%) men. Over the entire funding period, men and women were almost equally supported: 37 successful applicants were women and 38 men. The median age was at 32 at the time of application, for both women and men. All focal research areas of the Faculty are represented with immunology and infection (31%) and oncology (25%) being the most successful over the years. Overall candidates from 29 different institutions within the Faculty of Medicine were successful.

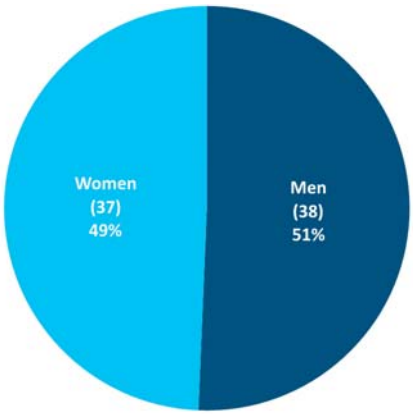
In 2018, 10 proposals were reviewed and 7 (70%) of them were selected for funding. The approved projects cover all of the focal research areas of the Faculty of Medicine. The successful applicants work in 6 different institutions within the Faculty of Medicine. In total, 2 (29%) are physicians and 5 (71%) scientists; 4 (57%) of the successful applicants are men and 3 (43%) are women. The median age was at 34 years. One of the 7 approved projects was not started for personal reasons.

About us

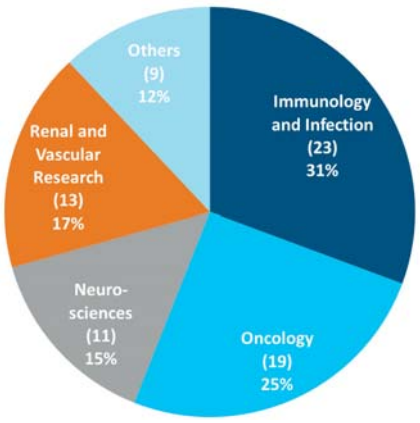
The following diagrammes show the distribution of junior projects per education, gender and main research areas over the period between 2009 and 2018.



Distribution of physicians (Dr. med.) and scientists (Dr. rer. nat.) between 2009 and 2018.



Gender distribution of junior projects between 2009 and 2018.



Distribution of junior projects as per main research area of the Faculty of Medicine between 2009 and 2018.

In the following tables all junior projects are shown which received funding or were approved for funding in 2018.

Immunology and Infection

Project No.	Project title	Term	Applicant	Institute
J45	Modulation of PRC2 activity by HCMV IE2	01/01/2015-30/06/2018	Dr. Reuter	Institute of Clinical and Molecular Virology
J50	Analysis of the role of IL-9 in the induction of Colitis-associated cancer (CAC)	16/10/2015-15/04/2018	Dr. Gerlach	Department of Medicine 1
J56	Epigenec reprogramming of macrophages	01/01/2017-30/06/2019	Dr. Palumbo-Zerr	Department of Medicine 3
J57	Herpesviruses and DUX4	01/01/2017-30/06/2019	Dr. Full	Institute of Clinical and Molecular Virology
J62	Mechanisms of neutrophil infiltration in rheumatoid arthritis	01/08/2017-31/01/2020	Dr. Grüneboom	Department of Medicine 3
J63	IL-3 in inflammatory bowel disease	01/12/2017-31/05/2020	Dr. Zundler	Department of Medicine 1
J69	Effect of HIV on pre-existing vaccine immunity	01/09/2018-28/02/2021	Dr. Nganou Makamdop	Institute of Clinical and Molecular Virology

Oncology

Project No.	Project title	Term	Applicant	Institute
J54	Analysis of alternative mechanisms of tumor rejection	01/11/2015 - 30/04/2018	Dr. Lehmann	Department of Dermatology
J55	The role of microRNA-188-5p dysregulation in hepato-cellular carcinoma development and progression	01/01/2016 - 30/06/2018	Dr. Dr. Dietrich	Institute of Biochemistry
J58	Counteracting Wnt signaling	01/09/2016-28/02/2019	Dr. Bernkopf	Chair of Experimental Medicine II
J59	Immunotoxin induced anti-tumor immunity	01/07/2018-31/12/2019	Dr. Müller	Department of Medicine 5
J67	Metabolic reprogramming of AML MDSCs	01/01/2018-30/06/2020	Dr. Dr. Jitschin	Department of Medicine 5
J68	Role of GATA4 in Intestinal Inflammation & Cancer	01/10/2017-31/03/2020	Dr. Patankar	Department of Medicine 1
J73	Intracellular signaling by SPARCL1 in colon cancer	16/09/2018-15/03/2021	Dr. Tenkerian	Department of Surgery

About us

Neurosciences

Project No.	Project title	Term	Applicant	Institute
J51	Inflammatory signature in Parkinson's disease	01/10/2015-31/03/2019	Dr. Marxreiter	Department of Molecular Neurology
J52	Modeling cortical dysfunction of SPG11 spastic paraplegia using patient-derived pluripotent stem cells	01/11/2015-30/04/2018	Dr. Regensburger	Department of Neurology
J53	Diffusion tensor imaging of the visual pathway in pseudoexfoliation glaucoma	03/08/2015-02/02/2018	Dr. Schmidt	Department of Neuroradiology
J66	ß subunits: adding pieces to the puzzle of pain	01/01/2018-30/06/2020	Dr. Eberhardt	Department of Anaesthesiology

Renal and Vascular Research

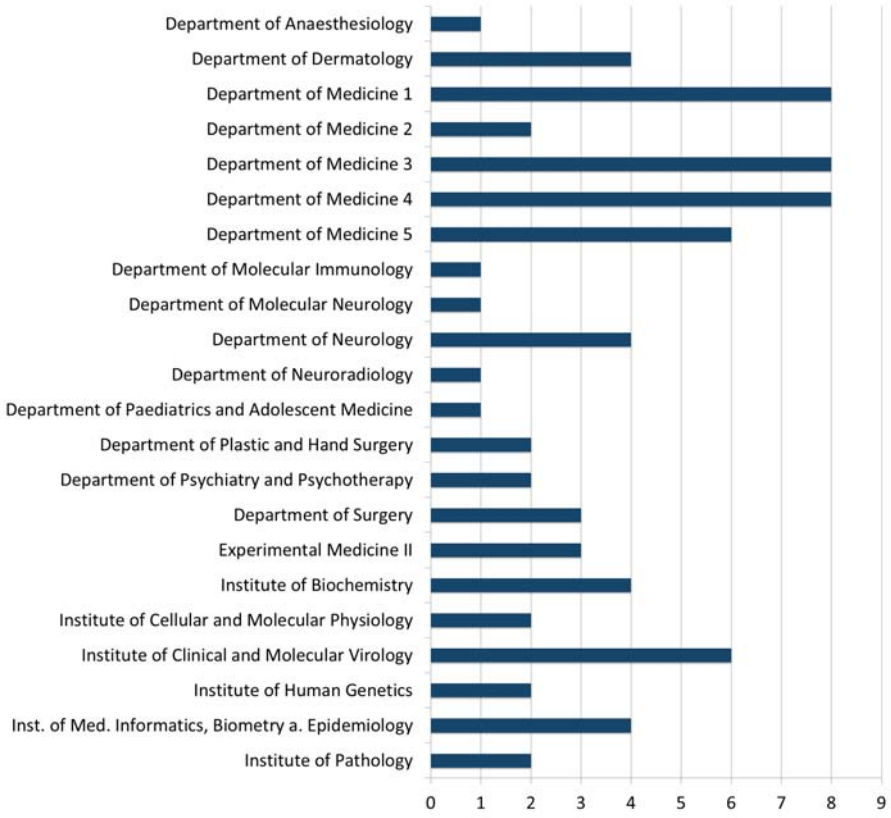
Project No.	Project title	Term	Applicant	Institute
J64	Nephroprotection by HIF-hydroxylase inhibitors	01/10/2017-30/09/2018	Dr. Grampp	Department of Medicine 4
J65	T-System Regulation by Glucocorticoids	01/11/2017-30/04/2020	Dr. Seidel	Institute of Cellular and Molecular Physiology
J70	Gene discovery in kidney disease	01/10/2018-31/03/2021	Dr. Jobst-Schwan	Department of Medicine 4
J71	P2Y2R-dependent cyst growth in ADPKD	01/01/2019-30/06/2021	Dr. Kraus	Department of Medicine 4

Molecular Medicine

Project No.	Project title	Term	Applicant	Institute
J60	The role of Hck/Lyn in Vesicles secretion	01/10/2016-30/04/2018	Dr. Lee	Department of Dermatology
J74	The role of CtBP1 in hippocampal and cortical neuroplasticity	01/02/2019-31/07/2021	Dr. Salar	Department of Psychiatry and Psychotherapy

Other methodologically oriented projects, informatics, statistics

Project No.	Project title	Term	Applicant	Institute
J61	Extending joint models in biomedical outcomes	01/01/2017-30/06/2019	Dr. Waldmann	Department of Medical Informatics, Biometry and Epidemiology
J75	Statistical Analysis of Infectious Disease Spread	16/10/2018-15/04/2021	Dr. Meyer	Department of Medical Informatics, Biometry and Epidemiology



Distribution of junior projects among departments and institutes between 2009 and 2018.

Career Development for Clinician Scientists

Release from clinical work for research

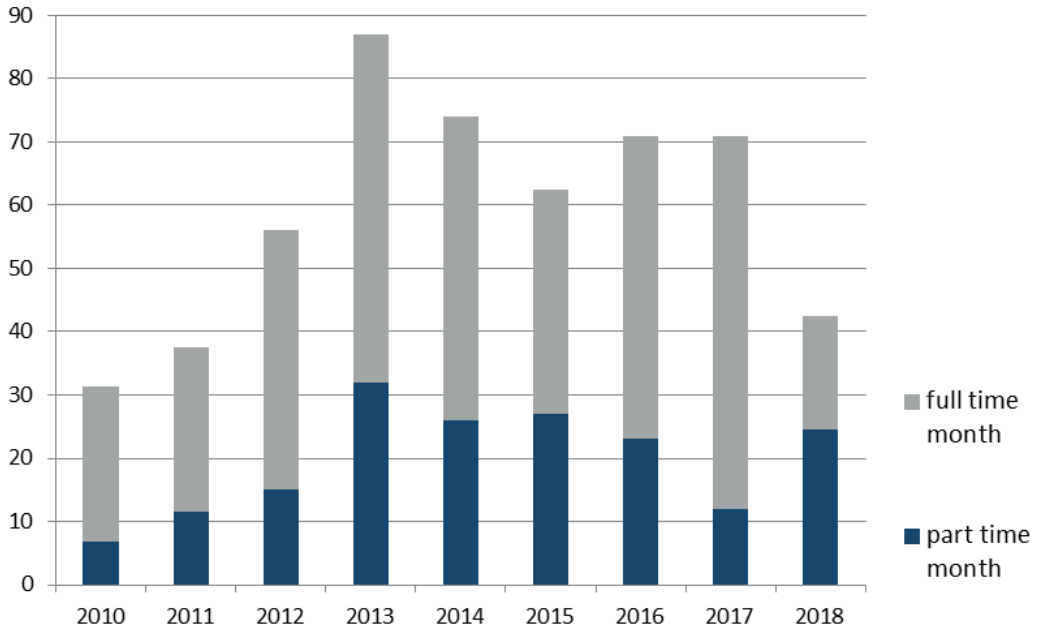
Access to protected research time is essential for young clinicians developing their projects. The laboratory rotation positions enable young scientists to fully devote themselves to a research project.

In the IZKF 6 rotation positions can be financed continuously. Since 2017, the IZKF has been offering physicians who apply for a rotation position in the first applicant programme the opportunity to apply for a rotation position for 12 months full-time or 24 months part-time directly as part of the project application. In 2018, the first applicant programme and the rotation programme were expanded into a Clinician Scientist Programme for the entire Faculty of Medicine. Since then, 4 of the total of 6 rotation positions of the IZKF have been reserved for first-time applicants and applicants in the Clinician Scientist Programme. The other 2 rotation positions can still be used flexibly. The positions are available for a period of 6 months full-time or 12 months part-time, an extension is not (any longer) possible. This makes it possible to support up to 4 rotation projects per year. Applications may be submitted on an ongoing basis. There is no age limit, but the planned rotation position must make a suitable contribution to the scientific development of the respective applicant.

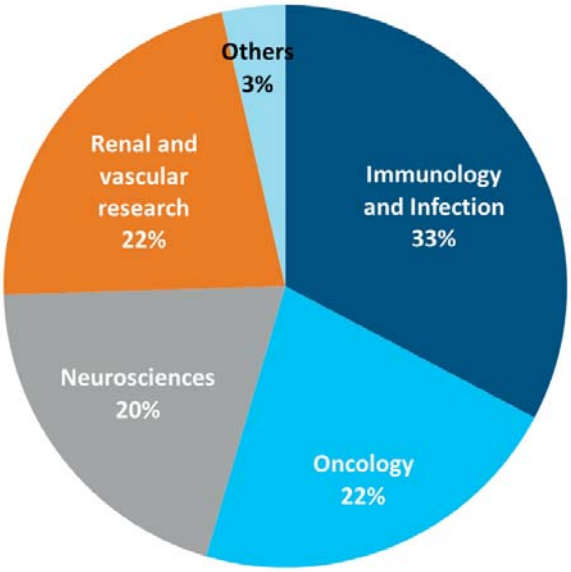
About us

Name	Institution	Funding period	Rotating scope
Rotations			
Dr. Lisa Meintker	Department of Medicine 5	07/2017 - 12/2017, 07/2018 - 10/2018	100% / 50%
Dr. Michael Übner	Department of Medicine 5	01/2018 - 12/2018	100% / 50%
Dr. Marcel Vetter	Department of Medicine 1	10/2017 - 09/2018	50%
Dr. Manuel Weber	Department of Oral and Cranio-Maxillofacial Surgery	06/2017 - 05/2018	50%
Rotations of Junior Project Leaders			
Dr. Esther Eberhardt	Department of Anaesthesiology	07/2018 - 07/2019	50%
Dr. Steffen Grampp	Department of Medicine 4	01/2018 - 04/2018, 07/2018 - 09/2018	50%
Dr. Regina Jitschin	Department of Medicine 5	01/2018 - 12/2018	50%
Dr. Tilman Jobst-Schwan	Department of Medicine 4	10/2018 - 09/2020	50%
Dr. Sebastian Zundler	Department of Medicine 1	03/2018 - 08/2018	100%
Rotations of Clinician Scientists			
Dr. Franz Marxreiter	Department of Molecular Neurology	09/2018 - 05/2019, 07/2019 - 09/2019	100%

Laboratory rotations running in 2018



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



Distribution of laboratory rotations as per main research area from 2009 until 2018.

The new Clinician Scientist Programme at the IZKF and the Medical Faculty

The Clinician Scientist Programme (CSP) is aimed at physicians who are in their specialist training, would like to conduct their own research project and to continue their scientific education within the framework of a structured training programme.

In 2016, the DFG hosted an event on „Clinician Scientists: Structured scientific qualification programmes for clinical researchers parallel to specialist training". 27 out of 37 medical faculties already have a CSP. An average of 20 participants takes part in the programme with a 6 or 12 months leave. The CSP includes professional as well as interdisciplinary further education, mentoring, retreats and monthly meetings. At the same time, the physicians conduct their own research project.

The aim of the CSP is to establish a new career path and promotion for Clinician Scientists and to create a structured scientific qualification programme for clinically researching physicians. The focus is also on strengthening translational research by creating time for scientific work and the preparation for habilitation.

The programme at the IZKF has a two-stage structure and is divided into a basic and an advanced module. The basic module lasts 2 years and requires a completed doctorate. The advanced module (duration 3 years) is aimed at physicians who have already successfully acquired a third-party funding- /IZKF- or first applicant project. The admission requirement for the advanced module is also fulfilled when having completed a post doctoral stay abroad of at least 2 years or with a successfully completed basic module. The leave of absence is 12 months full-time or equivalent part-time via rotation positions.

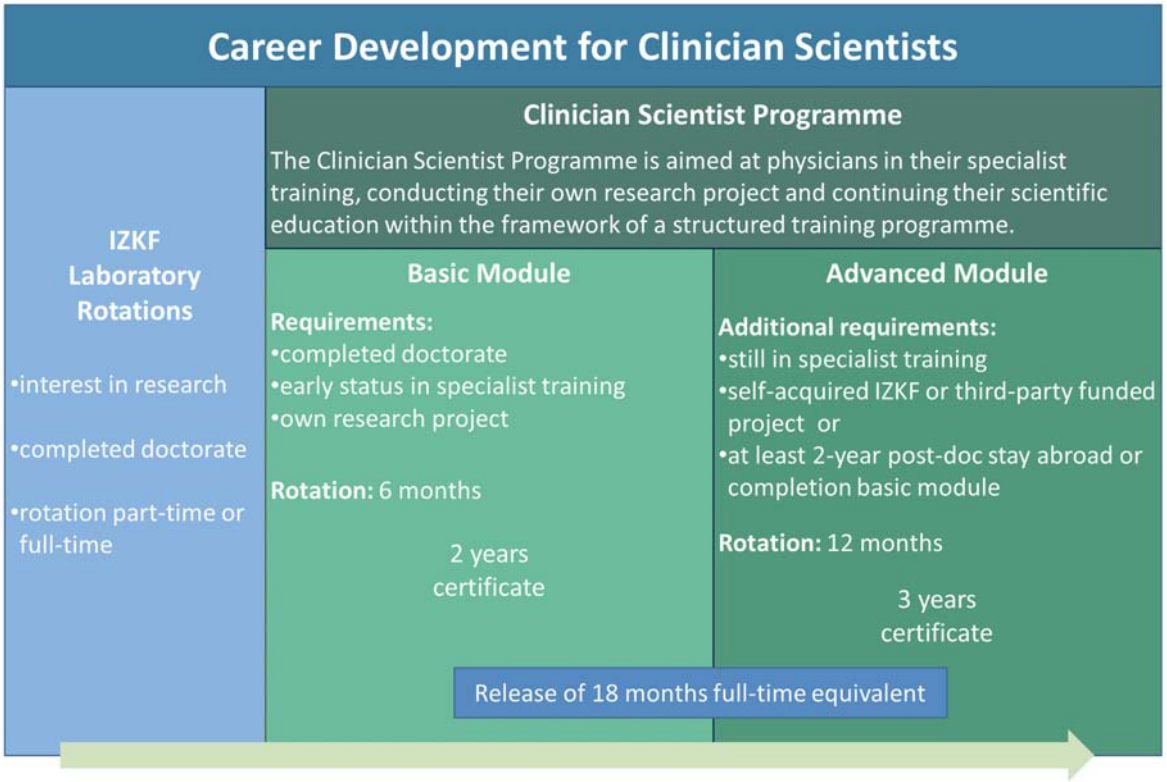
The deadline for the 2018 call for proposals was on 4th June 2018. All the participants selected by the IZKF call for proposals and other interested parties from the research networks participated in the kick-off event on 5th November 2018. The participants were informed about the structure and duration of the programme, admission requirements and planned contents of the CSP.

About us

The following physicians participate in the Clinician Scientist Programme:

Basic Module
- Dr. Ingo Ganzleben, Department of Medicine 1
- Dr. Harriet Morf, Department of Medicine 3
- Dr. David Simon, Department of Medicine 3
- Dr. Andrej Stoll, Department of Medicine 5

Advanced Module
- Dr. Esther Eberhardt, Department of Anaesthesiology
- Dr. Steffen Grampp, Department of Medicine 4
- Dr. Ferdinand Knieling, Department of Paediatrics and Adolescent
- Dr. Andreas E. Kremer, Department of Medicine 1
- Dr. Franz Marxreiter, Department of Molecular Neurology
- Dr. Markus Schüler, Department of Medicine 4
- Dr. Sebastian Zundler, Department of Medicine 1



Overview of career programmes for clinician scientists.

Structured Training Programmes for doctoral fellows at the IZKF

Life@FAU

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

Research Training Group	Registered participants
SFB 1181	35
GRK 2162	26
GRK 1962	23
TRR 130	11
GRK 1660	25
TRR 221	6
TRR 224	0
TRR 225	12
IZKF	74 + 27 MD
others	2

Research Training Groups participating in Life@FAU, indicating the number of participants (as of 31st December 2018).

MD-Thesis Scholarships

This programme was initiated to arouse interest for science in motivated medical students early on in their career. Medical students are supported in performing an experimental thesis in association with the IZKF or externally funded projects. It is expected that they spend a significant time in a laboratory. Up to 25 grants are available for medical students with outstanding performance and commitment in studies. The participants have to work full-time in the laboratory over a period of 9 months, whereby a scholarship is offered during their research activity of 8 continuous months. Furthermore, the doctoral students have to complete defined training modules during their studies. Training modules like guest speaker seminars, soft skill courses and the continuous supervision by a Doctoral Committee should continue throughout and until completion of the doctorate. Every participant of the MD-Thesis Scholarship Programme automatically becomes a member of the IZKF Research Training Group and the Graduate School of Life Sciences at FAU (Life@FAU) inaugurated in October 2017. Thus, the doctoral students can benefit from a structured, interdisciplinary training programme.

In 2018, a total of 27 medical doctoral students from 17 institutions were funded. Due to the fact that some scholarships granted in 2017 ended in 2018, the number of funded doctoral students is higher than the number of scholarships available. Overall, 22 applications for the MD-Thesis scholarship programme have been received in 2018. The Junior Scientists Committee approved 21 applications (95%), 11 (52%) of the successful applicants were females and 10 (48%) males. The median age was at 23 years.

Since its inception in 2007, the IZKF supported a total of 156 medical students with a scholarship. Medical students often initiate experimental work on their doctoral thesis during their studies. They will finish the thesis, though, only several years after they graduate. By the end of 2018, 51 (33%) students had already completed their doctoral thesis. Interestingly, 22 students (43%) obtained the highest degree possible, summa cum laude. This compares very favourably to the average 5% of all MD-Theses presented and is testimony to the excellent quality of MD-Theses performed within this programme.

About us

MD-Thesis Scholarships 2018

Department of Medicine 1
- Greif, Vicky (12/2017 - 07/2018)
- Osterloh, Justus (10/2017 – 03/2018)
- Schmid, Jonas (10/2017 – 03/2018)
- Schramm, Sebastian (04/2018 – 11/2018)
- Said, Perau (01/2018 – 08/2018)
- Stark, Markus (07/2018 – 02/2019)

Department of Medicine 3
- Müller, Dorothea (04/2018 – 11/2018)
- Taubmann, Jule (01/2018 – 08/2018)

Department of Medicine 4
- Gaupp, Charlotte (04/2018 – 09/2018)
- Scholz, Julia (10/2017 – 05/2018)

Department of Medicine 5
- Richter, Silja (10/2018 – 05/2019)
- Rottmar, Tanja (04/2018 – 11/2018)

Department of Nephropathology
- Miedl, Markus (10/2017 – 05/2018)
- Rümmele, David (10/2017 – 03/2018)

Department of Neuropathology
- Hoffmann, Lucas (12/2017 - 07/2018)
- Schowalter, Mirjam (04/2018 - 11/2018)

Others
- Eitler, David, Department of Otorhinolaryngology – Head and Neck Surgery (10/2017 - 05/2018)
- Hug, Karsten, Department of Medicine 2 (10/2017 - 05/2018)
- Mäurer, Hannah, Institute of Anatomy (02/2018 - 09/2018)
- Mittag, Nora, Institute of Biochemistry (08/2017 - 03/2018)
- Rau, Ludwig, Chair of Experimental Medicine I (10/2018 - 05/2019)
- Ritter, Maximilian, Department of Surgery (10/2017 - 05/2018)
- Ronicke, Moritz, Department of Dermatology (10/2018 - 05/2019)
- Rösel, Nadine, Department of Psychiatry and Psychotherapy (10/2017 - 05/2018)
- Schustereder, Magdalena, Institute of Clinical Microbiology, Immunology, and Hygiene (04/2018 - 11/2018)
- Staudner, Tobias, Institute of Cellular and Molecular Physiology (04/2018 - 09/2018)
- Waldmann, Dennis, Institute of Physiology and Pathophysiology (04/2018 - 11/2018)

Research Training Group

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

Aims of the IZKF Research Training Group include fostering networking and scientific self-organisation, methodological competence and soft skills as well as offering insights into other scientific fields and career opportunities. A structured seminar programme, courses in basic methods, in scientific writing and presentation are organised by the IZKF. In addition, the participants of the graduate school have to attend guest speaker seminars and to participate in the annual internal retreat. Participation in external congresses and in seminars organised by the doctoral students are mandatory.

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

Currently, the IZKF Research Training Group is now divided into four areas: the 2 already existing fields of neuroscience (Neuro) as well as immunology/infection/ renal and vascular research (Ink). An additional Oncology and DigIT group was set up.

Course “Intellectual Property Rights” given on 24th May 2018.

Courses given in 2018

The following soft skill- and statistic courses were given in 2018:

- Scientific Writing: An introduction (foundation level), Dr. Deborah Bennett, 7th-9th March 2018, 19th-21th September 2018
- Scientific Writing: English for research publication (advanced level), Dr. Deborah Bennett, 23th-27th July 2018, 8th-12th October 2018
- Presentation Skills, Dr. Deborah Bennett, 18th-20th July 2018, 26th-28th September 2018
- Intellectual Property Rights, Prof. Dr. Christian Pilarsky, 24th May 2018
- Entrepreneurship, Prof. Dr. Christian Pilarsky, 25th May 2018
- Career Development in Academia, Dr. Stefanie Herberger, 4th April 2018
- Microscopy course on sample preparation for two channel confocal fixed sample and spinning disc live cell imaging, Dr. Ralf Palmisano, 3rd-6th September 2018
- Biostatistics, Dr. Matthias Englbrecht, 16th-17th March 2018, 28th-29th September 2018

About us

Events of the IZKF Erlangen

International IZKF Symposium Kloster Banz

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

After 2016 the symposium will take place again in 2019 on the topic „Translational Medicine“. The proven concept of sessions with 2 external speakers and 2 speakers from the FAU was again implemented. New is the separate poster session on Friday with an oral presentation of the winning posters together with the winner of the IZKF Publication Prize. The IZKF Publication Prize is awarded regularly during the IZKF Symposium. It is aimed at all young scientists of the Medical Faculty up to 38 years of age at the time of publication. For the Prize 2019, the publication must have been published as first author in the period from 1st January 2016 – 31st December 2018. Additionally, the scientific work that led to the publication must have been carried out in Erlangen.

Conference hall at the IZKF symposium.

Summer Symposium/ Harald zur Hausen-lecture hall

The Summer Symposium regularly takes place in the years in which no IZKF-Symposium at Kloster Banz is held. In July 2017, the IZKF organised a one-day summer symposium “Research Highlights at IZKF”. Selected current and former IZKF project leaders and IZKF junior research group leaders presented research results achieved in connection with IZKF funding. The summer symposium was held on July 21st in the newly inaugurated Harald zur Hausen-lecture hall of the Faculty of Medicine. After an attractive programme, a networking party took place in the Nikolaus-Fiebiger Center where advanced and junior project leaders exchanged ideas about their research. The next Summer Symposium is planned for 2020.

IZKF Postgraduate Workshop 2018.

Postgraduate Workshop/Hörsaalzentrum

Every two years, the Junior Scientists Committee organises the IZKF Postgraduate Workshop. The Postgraduate Workshop alternates with the IZKF International Symposium at Kloster Banz und the IZKF-Summer Symposium. At the IZKF Postgraduate Workshop, lectures are held by internationally recognized speakers on a timely topic. The focus of the workshop is on a poster session in which all members of the graduate school are expected to present their projects. Two poster prizes are awarded.

On Wednesday, October 17th in 2018, the IZKF Postgraduate Workshop took place. During the poster session in the foyer, the doctoral students of the IZKF Graduate School presented their scientific work with a poster. The three best posters were awarded a prize by the Junior Scientists Committee.

- Benjamin **Häberle,** Institute of Biochemistry

SoxC factors are essential for early neuroblast/immature neuronal survival during adult neurogenesis

- Nora **Feldker,** Experimental Medicine I

Genome-wide cooperation of the EMT inducer ZEB1 with YAP and AP-1 factors

- Anna Katharina **Koller,** Pharmacy

Physical investigations on the compatibility of amiodarone and milrinone with frequently used drugs at intensive care units

In addition to the poster session, two internationally recognised speakers talked on timely topics: Prof. Jochen Guck (TU Dresden), who gave a lecture entitled “Feeling for cell function” and Prof. Ewa Dąbrowska (Department of English Language and Applied Linguistics, Alexander von Humboldt Professor of Language and Cognition at the FAU), who talked about “Implicit and explicit processing in typical first language development”.

About us

Retreat

In 2018 the retreat for the Jour fixes „Immunology and Infection“ and „Oncology“ took place for the first time. From June 5th to 6th, the group of doctoral students travelled to the Fraunhofer Research Campus in Waischenfeld to present their own research projects in two parallel lecture series. Within five sessions, the medical - and scientific doctoral students from the IZKF but also from associated projects were able to present their field of research to the other participants. All the lectures lasted 15 minutes, followed by a brief discussion. Interesting approaches from the fields of virology, microbiology and biochemistry (among others) were the main topics for the lecture series immunology and infection. Research topics from oncology, molecular medicine and biochemistry, among others, were presented in the oncology lecture series. Furthermore, the retreat offered the opportunity to network and exchange ideas within the framework of a joint evening event. In addition to the 34 doctoral students, some of the IZKF project leaders and Professor Becker (spokesman for the Junior Scientists Committee) also took part in the event.

Neuro Retreat in Maroldsweisach 2018.

An annual retreat for the doctoral students of the IZKF Research Training Group is offered every year. The next retreat will take place in 2019 at the Fraunhofer Research Campus, the event is scheduled for October 1st and 2nd.

In addition to the retreat „Immunology and Infection“ and „Oncology“, a Neuro Retreat has also been taking place for several years now.

The venue for the Neuro Retreat in 2018 was the Seminarzentrum Wasmuthausen in Maroldsweisach. The 13 advanced and newly started doctoral students worked very effectively in a scientific but also friendly environment. Each participant gave a presentation and/or a ‚breakout‘ session where different topics and other issues concerning lab life in general were approached. On the first day all doctoral fellows introduced themselves in a pitch talking about the ‘coolest thing’ in their doctoral project, e.g. state of the art techniques, genius approaches, etc. . The second day began with the method talks in which 4 doctoral fellows presented the most unique technique used in their respective projects. After that, the second session concentrated on the trouble shooting - the challenges the students experience during their doctoral studies. The project talks comprised the third session and the fourth session ended with the guest speaker, Dr. Seda Salar, who gave a broader overview about careers in science. The last and fifth session ended the retreat. Here, the students came up with a worthwhile research topic and prepared a short presentation in order to convince the audience. The daily presentations, but also the constructive criticism enhanced the participant’s scientific way of thinking and gave the opportunity to discuss lab-related issues.

The retreats together with the monthly seminars provide an excellent opportunity to get to know colleagues from all corners of the graduate school.

Special Programmes

Special programmes provide additional funding for IZKF projects but not all programmes are available to all funding lines of IZKF.

High Tech Pool

IZKF actively encourages the use of modern "omics" technologies in the projects, such as those provided by the Core Unit Next Generation Sequencing. Since these experiments are generally expensive and consumables within IZKF advanced and junior projects are restricted, additional support is necessary. Costs for consumables can therefore be supported upon request with up to € 10,000 per project, provided that the project itself contributes at least 30% of the total sum, if the total exceeds € 5,000. This programme is available for advanced and junior projects but not for ELAN- and bridging projects.

Travel Funding

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

This programme is also available for successful applicants for MD-Thesis scholarships and laboratory rotations (up to 3 months after the end of the funding period), but not for pilot projects.

Publication Funding

The publication of results obtained in IZKF projects in scientific journals is actively supported. It is expected that the IZKF funding of the project is acknowledged. The IZKF covers publication costs up to € 1,500. If the total costs are more than € 3,000 a financial contribution of € 2,000 is given by the IZKF. This programme is also available for successful applicants for MD-Thesis scholarships, laboratory rotations and pilot projects (up to 12 months after the end of the funding period, respectively).

Travel Scholarships

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

Visiting Professor Programme

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

IZKF Visiting Professor Programme

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

FAU Visiting Professor Programme

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

About us

Scientist	Institute	Lecture title
Dr. Dannielle Engle	Cold Spring Harbor Laboratory, New York, USA	Aberrant Glycosylation Promotes Pancreatic Pathology and Yields Novel Biomarkers
Dr. Hervé Tiriac	Cold Spring Harbor Laboratory, New York, USA	Organoid Profiling Parallels Therapeutic Response in Pancreatic Cancer Patients
Prof. Stephen Kent	Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne, Australia	Immunity to HIV - role of ADCC
Dr. Anna-Rachel Gallagher	Department of Internal Medicine, Section of Nephrology, Yale University School of Medicine, USA	The role of beta-catenin in ADPKD: new insights into an old topic
Dr. Heidi Seibold	Institute for Medical Information Processing, Biometry, and Epidemiology, University of Munich, Germany	Practical steps towards reproducible research and open science

IZKF Visiting Professor Programme

Scientist	Institute	Lecture title
Prof. Paul A. Welling	Department of Physiology, University of Maryland, USA	Protecting Potassium at all Cost, Molecules to Man
Prof. Hongjun Song	Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, USA	Epitranscriptomic regulation in the nervous system
Prof. Guo-li Ming	Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, USA	Brain-Region-Specific Organoids for Modeling Neurodevelopment and Disease
Prof. Melda Kunduk	Department of Communication Sciences & Disorders, Louisiana State University, USA	Objective quantification of swallowing events from modified barium swallow studies
Prof. Andrew Biankin	Wolfson Wohl Cancer Research Centre, University of Glasgow, Scotland	New avenues in pancreatic cancer treatment
Prof. Lawrence G. Palmer	Department of Physiology and Biophysics, Weill Cornell Medical College, USA	Mechanisms of ENaC regulation by aldosterone in the kidney
Prof. Sebastian Jessberger	Laboratory of Neural Plasticity, Brain Research Institute, University of Zurich, Switzerland	Elucidating the molecular and cellular dynamics of neurogenesis in the adult hippocampus
Prof. Francesca Peri	Institute of Molecular Life Sciences, University of Zurich, Switzerland	The brain under surveillance: the role neuronal-microglial interactions in the development and repair of the CNS
Prof. Denis Jabaudon-Gandet	Department of Basic Neurosciences, University of Geneva, Switzerland	Dynamic control of neuronal diversity in the developing necortex
Prof. Hans van Bokhoven	Department of Human Genetics, Radboud University, Netherlands	Converging molecular and cellular pathways across neurodevelopmental disorders
Prof. Fred H. Gage	Laboratory of Genetics, Salk Institute for Biological Studies, USA	Early life experience and structural variation of neural genomes
Prof. Eyal Gottlieb	Technion - Israel Institute of Technology, Israel	Identifying and exploiting cancer‘s metabolic liabilities

FAU Visiting Professor Programme

GOVERNANCE AND STATISTICS

The IZKF in Numbers

31 Advanced Projects

13 Immunology and Infection
7 Oncology
9 Neurosciences
2 Renal and Vascular Research

11 tandem projects between departments and institutes
6 projects completed in 2018
46 project leaders

2 Junior Research Groups

37 Institutions with running projects 2018

20 Junior Projects

6 Immunology and Infection
6 Oncology
4 Neurosciences
2 Renal and Vascular Research
1 Molecular Medicine
1 Others

thereof **6** projects completed in 2018

35 Pilot Projects

16 Newly granted in 2018
19 Projects completed in 2018
7 Projects approved in 2018, starting 2019

90 Employees of the IZKF

63 Doctoral fellows, Post-Docs and laboratory rotations
27 Non-scientists

About us

3 patents

97 Ongoing Scientific Theses in 2018

7 Master theses
74 Doctoral theses
6 Habilitations
10 Laboratory rotations

20 awards

2 Appointments of IZKF project leaders to W2/ W3 - positions

241 Members of Life@FAU 2018

35 SFB 1181
26 GRK 2162
23 GRK 1962
11 TRR 130
25 GRK 1660
6 TRR 221
12 TRR 225
101 IZKF - RTG
27 Dr. med.
74 Dr. rer. nat.
2 participants outside RTG

6,304 K€ total expenditures in 2018

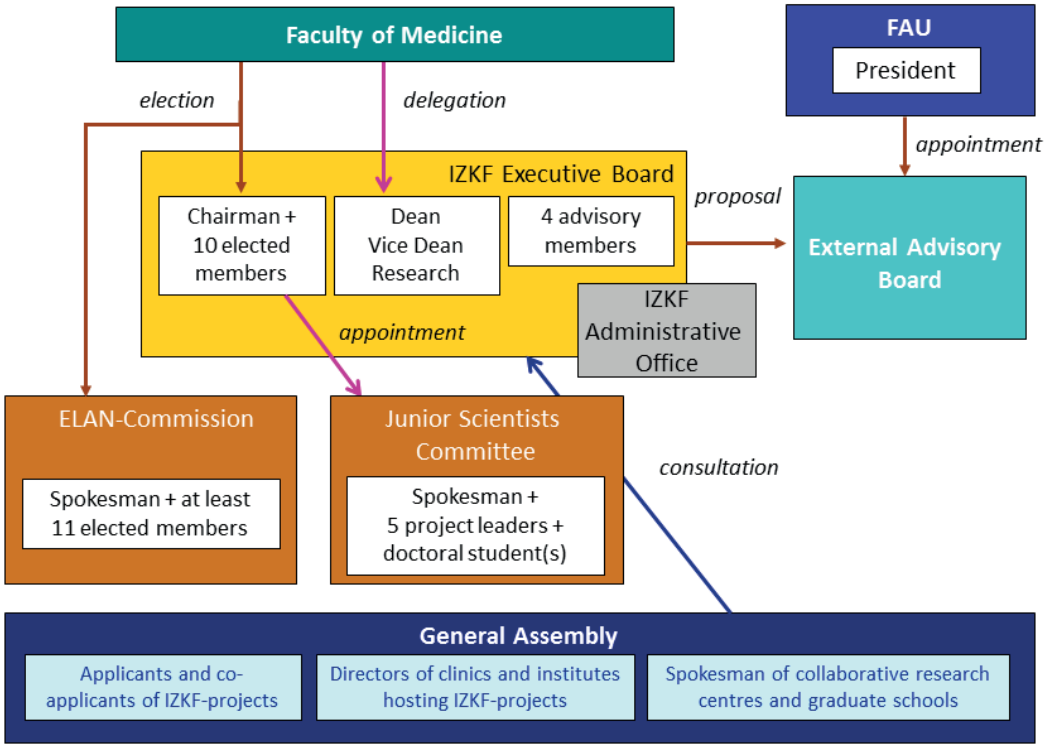
78 Publications

Cumulative Impact Factor **480.173**
Average Impact Factor per publication **6.163**
Average publications per project **1,5**
13 publication with an IF more than 10

Structure of the IZKF

The IZKF is a self-organised structure within the Faculty of Medicine. The IZKF has a set of written rules and regulations approved by the Faculty of Medicine. All rules and regulations are continuously reviewed and revised, if necessary. The Statutes of the IZKF regulate the status, tasks and objectives of the IZKF as well as the competence and composition of the committees. The Rules of Procedure specify the application procedure, the funding and duration of the projects as well as the decision-making process between the Chairman, the Management Board and the External Advisory Board. Finally, the Advisory Board regulations regulate the IZKF's cooperation with the Advisory Board in detail. All regulations are available at the IZKF Homepage.

Governing bodies include the General Assembly, the Management Board, the Junior Scientists Committee, the ELAN-Commission and the External Scientific Advisory Board (SAB).



Governance of the IZKF.

About us

The Management Board is the general steering commission of the IZKF. It is responsible for developing the scientific programme, controlling the financial framework and allocating resources to projects as well as ensuring that results are reported. It is composed of up to 13 members with voting rights, up to 11 elected by the Faculty of Medicine for a three year period and two ex-officio members from the Faculty of Medicine as well as four advisory members from the University Hospital and the University. Five annual meetings are held and decisions are taken by simple qualified majority. Elected members include the Chairman who is responsible for daily operations with the support of the Administrative Office. Re-election is possible for all members of the Board.

Programmes and the financial framework are reviewed and approved by the External Scientific Advisory Board. This body meets on site every two or three years to oversee the general development of the IZKF and the proposed projects.

The Board consists of at least 10 internationally recognized scientists (16 as of 31st December 2018) from universities and research institutes led by an elected chairperson, who is appointed for a period of 4 years with a maximum term of office of 8 years. The members of the External Scientific Advisory Board are appointed by the University President, upon the proposal of the Management Board for a period of six years. The maximum term of office in the External Scientific Advisory Board is usually 12, as an exception 13 years.

Since the merger of the IZKF and the ELAN Fund under the umbrella of the IZKF, the ELAN-Commission has been a part of the IZKF body. It is responsible for reviewing pilot projects and assists in the selection of advanced and junior projects. It consists of the spokesman for pilot projects (ELAN) and at least 11 further members all elected by the Faculty of Medicine for a period of three years. A re-election is possible. The chairperson of the ELAN-Commission is an elected member of the IZKF Board.

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 Research Training Group. In addition, it participates in the internal review process for project funding and for laboratory rotations.

It is composed of the spokesman (who also belongs to the IZKF-Management Board) for promotion of young researchers elected by the Faculty of Medicine, five project leaders, three from advanced projects, one from junior projects and one of the junior research group leaders and at least one, but generally two representatives from the doctoral students, who will be chosen from among the Jour Fixe speakers. The members are appointed for a period of 3 years by the Board of the IZKF. The maximum term of office is 6 years.

The most recent committee of the IZKF is the Clinician Scientist Programme Commission (CSP-Commission). This three-member commission, which is made up of research-active physicians, accompanies the Clinician Scientist Programme of the IZKF in terms of organisation and content and makes recommendations regarding the admission of new applicants to the Clinician Scientist Programme. The members of the CSP-Commission are appointed by the IZKF Management Board for a period of 3 years. One of the members of the commission shall be a member of the Management Board. The IZKF Management Board also appoints the Chairman of the CSP-Commission.

The IZKF-Administrative Office is responsible for the administrative and financial management of the IZKF. Its main focus lies on the assistance of the chairman, the Management Board and the Junior Scientists Committee, the preparation and follow-up of IZKF Committee meetings, handling of IZKF application procedures, internal communication (ongoing, website, annual report, informative events for new project leaders), account management and financial controlling as well as the organisation of events. The IZKF-Administrative Office is an integral part of the Research Funding Department of the Finance Department of the University Hospital.

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

Staff and bodies

In 2018 some changes in the committees were experienced. Prof. Dr. Sendtner (former Vice-Chair) was appointed as Chairman of the External Scientific Advisory Board from 1st January 2018 on. Some members of the Scientific Advisory Board have rotationally completed their term of office. Prof. Linker accepted a call to Regensburg and therefore resigned from the ELAN-Commission. Dr. Bosch-Voskens and Prof. Schulze retired from the Junior Scientists Committee due to the expiration of their term of office. New in office are Prof. Katharina Zimmermann and Dr. Christiane Krystelle Nganou Makamdop. There were no changes within the Management Board in 2018. The Clinician Scientist Programme Commission was appointed as a new body.

Junior Scientists Committee (from left to right): Dr. Ceppi, Prof. Schulze, Prof. Becker, Prof. Bozec, I. Stolzer

About us

Management Board

Chairman

Prof. Dr. André Reis, Institute of Human Genetics

Deputy Chairman

Prof. Dr. Michael Wegner, Institute of Biochemistry

Members

Prof. Dr. Christoph Becker, Department of Medicine 1
Prof. Dr. Christian Bogdan, Institute of Clinical Microbiology, Immunology and Hygiene
Prof. Dr. Anja Bosserhoff, Institute of Biochemistry
Prof. Dr. Thomas Brabletz, Chair of Experimental Medicine I
Prof. Dr. Johann Helmut Brandstätter, Division of Animal Physiology
Prof. Dr. Dr. Raymund Horch, Department of Plastic and Hand Surgery
Prof. Dr. Andreas Mackensen, Department of Medicine 5
Prof. Dr. Dr. Jürgen Schüttler, Dean of the Faculty of Medicine, Department of Anaesthesiology
Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

Consultative Members

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

Prof. Dr. Reis

Prof. Dr. Wegner

Prof. Dr. Becker

Prof. Dr. Bogdan

Prof. Dr. Bosserhoff

Prof. Dr. Brabletz

Prof. Dr. Brandstätter

Prof. Dr. Dr. Horch

Prof. Dr. Mackensen

Prof. Dr. Dr. Schüttler

Prof. Dr. Winkler

Prof. Dr. Hornegger

Zens

Prof. Dr. Dr. Iro

Dr. Bender

Current members of the Management Board

About us

External Scientific Advisory Board

Chairman

Prof. Dr. Michael Sendtner (chairman since 1st January 2018 Chairman),
University Hospital Würzburg - Institute for Clinical Neurobiology

Members

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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


Prof. Dr. Sendtner


Prof. Dr. Busch


Prof. Dr. Hengel


Prof. Dr. Kalinke


Prof. Dr. Kamradt


Prof. Dr. Katschinski


Prof. Dr. Kuhlmann


Prof. Dr. Moch


Prof. Dr. Pavenstädt


Prof. Dr. Prinz


Prof. Dr. Rieß


Prof. Dr. Schulz


Prof. Dr. Seufferlein


Prof. Dr. Siebert


Prof. Dr. Sorokin


Prof. Dr. Tiegs

External Scientific Advisory Board
(as of 31st December 2018)

About us

ELAN-Commission

Spokesman for pilot projects (ELAN)

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

Members

Prof. Dr. Tobias Bäuerle, Institute of Radiology
Prof. Dr. Jürgen Behrens, Chair of Experimental Medicine II
Prof. Dr. Robert Cesnjevar, Department of Paediatric Cardiac Surgery
Prof. Dr. Yesim Erim, Department of Psychosomatic Medicine and Psychotherapy
Prof. Dr. Peter A. Fasching, Department of Obstetrics and Gynaecology
Prof. Dr. Martin Fromm, Chair of Clinical Pharmacology and Clinical Toxicology
Prof. Dr. Ralf Linker, Department of Neurology (till 30/09/2018)
Prof. Dr. Gerhard Krönke, Department of Medicine 3
Prof. Dr. Christian Pilarsky, Department of Surgery
Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation
Prof. Dr. Regina Trollmann, Department of Paediatrics and Adolescent Medicine
Prof. Dr. Klaus Überla, Institute of Clinical and Molecular Virology
Prof. Dr. Beate Winner, Department of Stem Cell Biology

Prof. Dr. Brabletz

Prof. Dr. Bäuerle

Prof. Dr. Behrens

Prof. Dr. Cesnjevar

Prof. Dr. Erim

Prof. Dr. Fasching

Prof. Dr. Fromm

Prof. Dr. Krönke

Prof. Dr. Pilarsky

Prof. Dr. Steinkasserer

Prof. Dr. Trollmann

Prof. Dr. Überla

Prof. Dr. Winner

Current members of the ELAN Commission

About us

Junior Scientists Committee


Prof. Dr. Becker


Prof. Dr. Bozec


Dr. Ceppi


Prof. Dr. Engel


Häberle


Dr. Nganou Makamdop


Iris Stolzer


Prof. Dr. Zimmermann

Current members of the Junior Scientists Committee

Spokesman for career development programmes

Prof. Dr. Christoph Becker, Department of Medicine 1

Members

Prof. Dr. Caroline Bosch-Voskens, Department of Dermatology (till 31/12/2018)

Prof. Dr. Aline Bozec, Department of Medicine 3

Dr. Paolo Ceppi, IZKF Junior Research Group 1

Prof. Dr. Felix Engel, Department of Nephropathology

Benjamin Häberle, Institute of Biochemistry

Dr. Christiane Krystelle Nganou Makamdop, Institute of Clinical and Molecular Virology (since 01/0172019)

Iris Stolzer, Department of Medicine 1

Prof. Dr. Schulze, Department of Oto-Rhino-Laryngology - Head and Neck Surgery (till 31/12/2018)

Prof. Dr. Katharina Zimmermann, Department of Anaesthesiology (since 01/01/2019)

Clinician Scientist Programme Commission


Prof. Dr. Mougiakakos


Prof. Dr. Zweier


Prof. Dr. Winkler

Current members of the CSP-Commission

Spokesman for Clinician Scientist Programme

Prof. Dr. Christiane Zweier, Department of Humangenetics

Members

Prof. Dr. Dimitrios Mougiakakos, Department of Medicine 5

Prof. Dr. Jürgen Winkler, Department of Molecular Neurology

Administrative Office


Meyerhöfer-Klee


Dr. Faber


Neufang


Reichel

Current staff of the Administrative Office

Manager

Dr. Katrin Faber

IZKF Administration

Anne Reichel

Kathrin Neufang

Bianca Meyerhöfer-Klee

About us

General Assembly

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

Surname	Name
Full	Florian
Gefeller	Olaf
Gramberg	Thomas
Grampp	Steffen
Grützmann	Robert
Günther	Claudia
Häberle	Benjamin
Hartmann	Arndt
Heger	Lukas
Hellerbrand	Claus
Hilgers	Karl F.
Horch	Raymund
Iro	Heinrich
Jäck	Hans-Martin
Jitschin	Regina
Jobst-Schwan	Tilman
Kleyer	Arnd
Klingberg	Anika
Klucken	Jochen
Knieling	Ferdinand
Korbmacher	Christoph
Kornhuber	Johannes
Krappmann	Sven
Kremer	Andreas
Krönke	Gerhard
Kruse	Friedrich E.
Kürten	Stefanie
Lang	Roland
Leppkes	Moritz
Lie	Dieter Chichung
Lutzny-Geier	Gloria
Mackensen	Andreas

Surname	Name
Martini	Eva
Marxreiter	Franz
Meyer	Sebastian
Mielenz	Dirk
Moll	Gunther
Mougiakakos	Dimitrios
Müller	Christian
Müller	Sarina
Nganou Makamdop	Christiane Krystelle
Naschberger	Elisabeth
Neurath	Markus
Nimmerjahn	Falk
Nitschke	Lars
Pachowsky	Milena
Palumbo-Zerr	Katrin
Patankar	Jay
Petter	Michaela
Rascher	Wolfgang
Reis	André
Sarter-Zaiss	Kerstin
Schauer	Christine
Schett	Georg
Schlötzer-Schrehardt	Ursula
Schöpe	Isabella
Schubert	Ulrich
Schuler	Gerold
Schulze	Holger
Schüttler	Jürgen
Schwab	Stefan
Seidel	Thomas
Soare	Aline
Stamminger	Thomas
Steiner	Dominik
Steinkasserer	Alexander
Stürzl	Michael
Tenbusch	Matthias
Tenkerian	Clara
Turan	Sören
Überla	Klaus
Uslu	Ugur
Veelken	Roland
Vöhringer	David
Wahlbuhl-Becker	Mandy
Waldmann	Elisabeth
Waldner	Maximilian
Wegner	Michael
Winkler	Jürgen
Winner	Beate
Wirtz	Stefan
Zimmermann	Katharina
Zweier	Christiane
Zundler	Sebastian

General Assembly of the IZKF (as of 14th November 2018)

About us

Organisation of the IZKF Research Training Group

All members regularly participate in the Jour Fixe (JF) once a month. Due to the broad thematic range of the doctoral theses at the IZKF, several Jour Fixes are held, which are at the moment

- Immunology, infection, kidney and circulatory research (Ink)
- Neurology (Neuro)
- Onkology (Onko) and
- DigIT

In 2018, the two JF T(h)INK - Tumor Research, Infection Research and Immunology, Kidney and Circulatory Research and Neurosciences - which is closely linked to the Interdisciplinary Centre for Neurosciences and the ICN School, were joined by the two new JF Oncology and DigIT. The JF Onko represents an alternative for doctoral fellows with an oncological focus who have been active in the T(h)INK-JF up to now.

Each JF is supervised by one to two spokespersons from the ranks of doctoral students, who are elected from among the participants for a period of 2-3 years as a rule. Usually, a new election takes place at the end of the doctoral thesis of the respective spokesperson. In addition to the spokespersons, each established JF has a scientific head from the ranks of appointed professors.

Jour Fixe Ink (Immunology, Infection, Renal and Vascular Research; former T(h)ink)

Scientific Head
Prof. Becker, Department of Medicine 1

Spokespersons
Iris Stolzer, Department of Medicine 1
Axel Dietschmann, Department of Infection Biology

At the Jour Fixe INK, doctoral fellows working in the areas of immunology, infection, renal and vascular research will present the progress and results of their respective doctoral projects. The seminar is held in English and takes place once a month. It promotes both the transfer of knowledge between doctoral fellows in the different fields and the presentation and discussion skills in front of an audience.

Jour Fixe Neuro

Scientific Head
Prof. Dieter Chichung Lie, Institute of Biochemistry

Spokespersons
Benjamin Häberle, Institute of Biochemistry
Judith Stemick, Department of Molecular Neurology

The neuroscientific doctoral fellows of the FAU Erlangen-Nuremberg meet monthly for the Jour Fixe „Neuroscience“, at which the doctoral fellows discuss new methods and technologies in addition to their respective doctoral projects. The programme of the Jour Fixe is solely organised by the doctoral students. Current speakers of the Jour Fixe are Mr. Benjamin Häberle, Ms. Marie-Theres Wittmann, Ms. Judith Stemmick and Mr. Georgios Kogias.

Jour Fixe Onko

Scientific Head
Prof. Anja Bosserhoff, Institute of Biochemistry

Spokespersons
Tatjana Seitz, Institute of Biochemistry
Valerie Fritz, Institute of Biochemistry

In the Oncology Jour Fixe, doctoral fellows focusing on research in different fields of oncology discuss ongoing work as well as new approaches. Every participant presents her/his own project once a year in the form of a progress report. Currently 45 doctoral fellows participate in this seminar with topics ranging from basic research in various cancer entities to clinical studies and targeted therapies.

Jour Fixe DigIT

Scientific Head
Prof. Olaf Gefeller, Institute of Medical Informatics, Biometry and Epidemiology

Spokespersons
Colin Griesbach, Institute of Medical Informatics, Biometry and Epidemiology

The JF DigIT is aimed at doctoral students with a data-analytical methodical approach. All participating institutions assign their self-conception to life sciences on the basis of their research orientation, even if in some doctoral projects there are clear references to other fields of science such as mathematics/statistics, computer science, physics and electrical engineering.

Financial Report 2018

Since 2004, the IZKF has been fully supported by intramural funds. The main financial contribution is given by the Faculty of Medicine. Additional contributions are received from the FAU.

Part of the expenditures of 2018 were financed from residual funds of the previous years.

Revenues	
Support of the Medical Faculty	5,470 K€
Support of the University	364 K€
Participation fee courses	1 k€
Contribution of IZKF for junior research groups	- 33 K€
Total revenues 2018	**5,802 K€**
Expenditures	
Advanced projects	3,246 K€
Pilot projects	766 K€
Career development	1,995 K€
thereof junior research groups	502 K€
thereof junior projects	968 K€
thereof laboratory rotations	377 K€
thereof MD-thesis scholarships	121 K€
thereof research training groups	27 K€
Central projects	132 K€
Administration	165 K€
Total expenditures 2018	**6,304 K€**

Revenues and expenditures 2018

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 the 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 53 scientific projects were actively running: 31 advanced projects, 20 junior projects and 2 junior research groups. In addition, 6 junior projects started their work in 2018 (4) or in the beginning of 2019 (2). These 53 funded scientific projects published 78 original articles in 2018 resulting in an average of 1.47 publications per project. The cumulative impact factor (IF) was 480.173, averaging 6.163 per publication. The high quality of many of these publications is reflected in 13 publications with an IF of more than 10. Being part of the IZKF allows intensive networking and direct access to collaborations, which can be seen in 9 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 the IZKF advanced and junior projects is reflected in 7 master theses, 74 doctoral theses and 6 habilitations that were in progress or finalised in 2018. A total of 68 project leaders and 63 employed scientists are involved in 51 scientific projects (advanced and junior) funded by the IZKF.

Some IZKF project leaders were able to achieve outstanding results. 20 prizes were awarded to IZKF project leaders. Two professorships were offered, one of them was accepted.

In many instances funding by the IZKF starts at an early phase of the project, thus it must be considered as a high risk funding programme. It is nevertheless reassuring that most of the projects are successful and many of them are continued after the termination of intramural funding. To support this with figures, results of a detailed survey of acquired third-party funding by the IZKF-projects are given on the next pages.

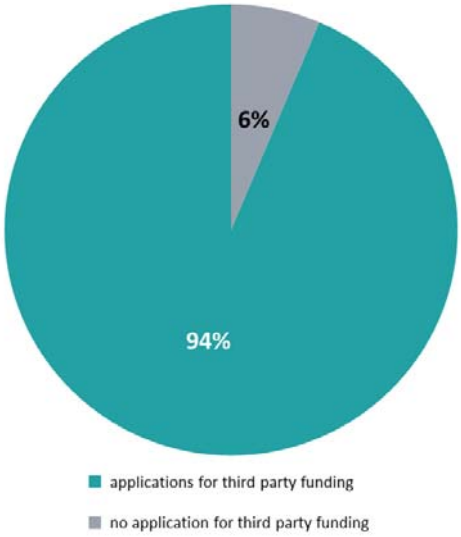
Beginning with the funding period of 2010-2013, grants were awarded for a period of 30 months with an extension by 6 months, if these projects are submitted for external funding. Within the funding period of 2013-2016 all projects submitted third party funds applications and therefore received the 6 months funding extension. Of the 31 projects from the 2016-2019 funding period, 28 (90%) have so far applied for project extensions.

When considering the last two funding periods (2010-2016), 47 projects were funded by the IZKF of

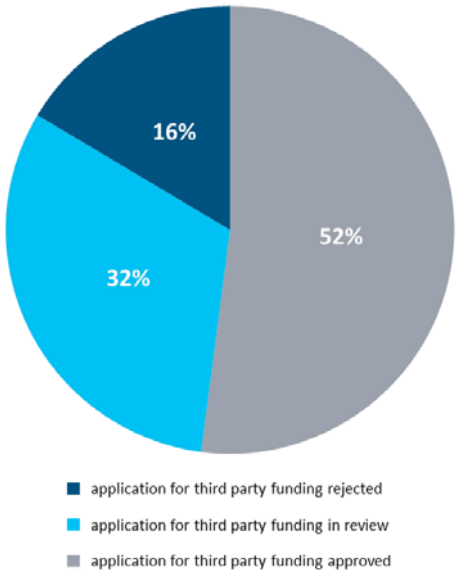
which 44 (94%) submitted third party funds applications. 35 of these projects (80%) were granted extramural funding and only 9 (20%) were not funded. This impressive success is also reflected by the fact that IZKF funding resulted in the acquisition of more extramural funds than were originally spent.

29 (94%) of the 31 projects from the 2016-2019 funding period, which has not yet been completed, have already submitted an application to an external funding institution and applied for project extension.

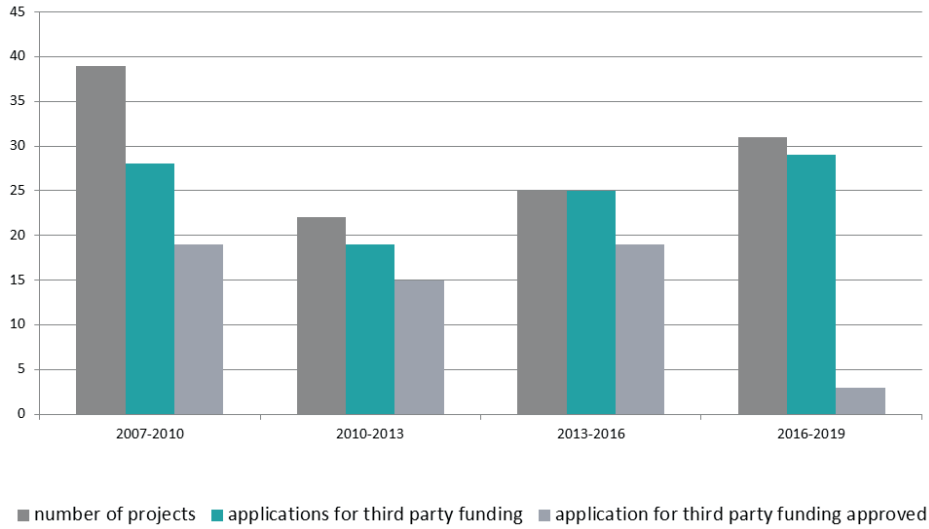
Similarly, the junior projects lead to a high number of extramural funding applications with a very high success rate. This development has been stable over the entire duration of the programme.



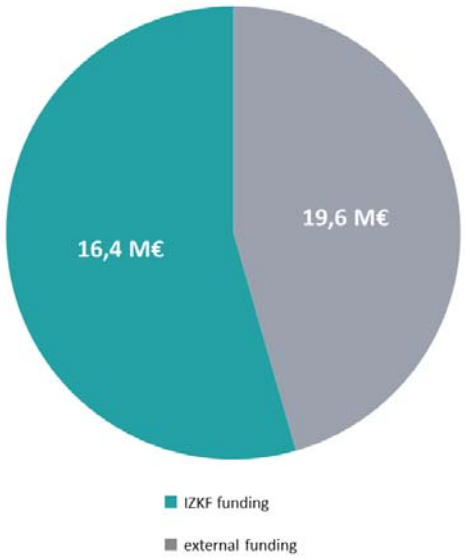
Applications for third-party funding submitted by advanced projects between 2010 and 2018.



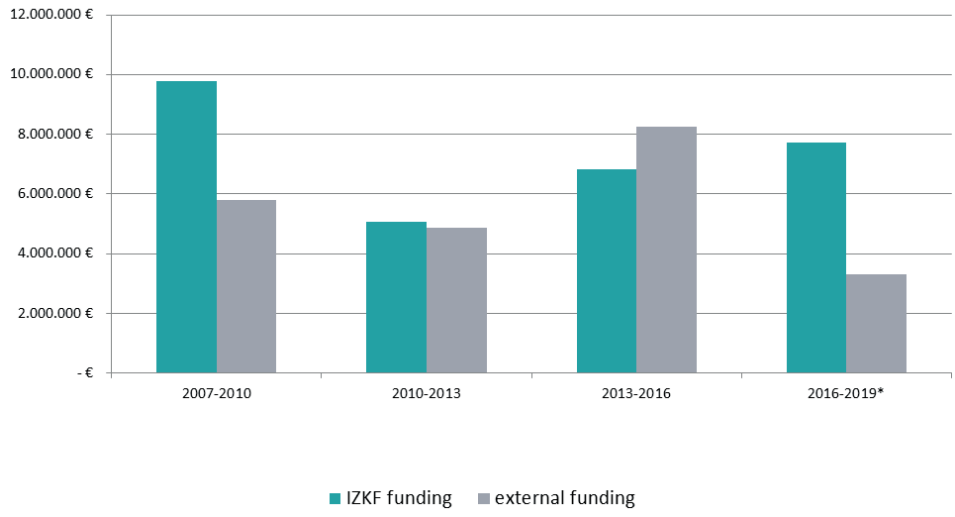
Approved applications for third-party funding of advanced projects between 2010 and 2018.



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



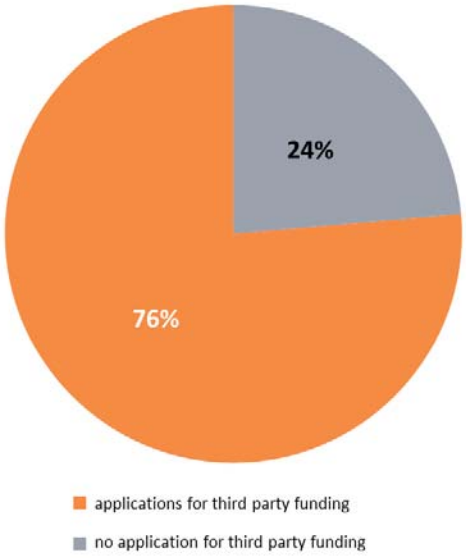
External funding received from advanced projects between 2010 and 2018.



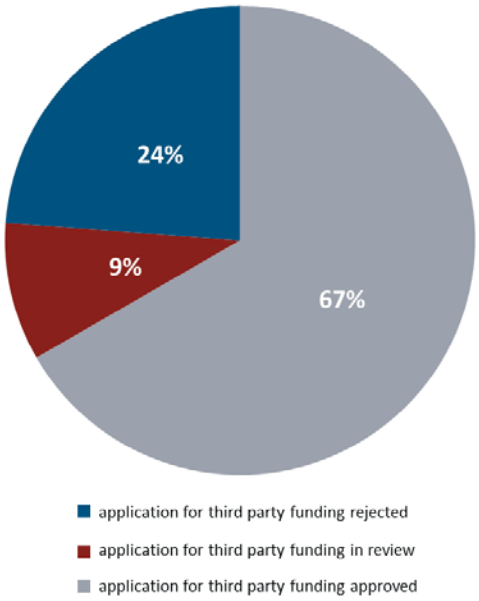
External funding received from advanced projects between 2007 and 2018.

About us

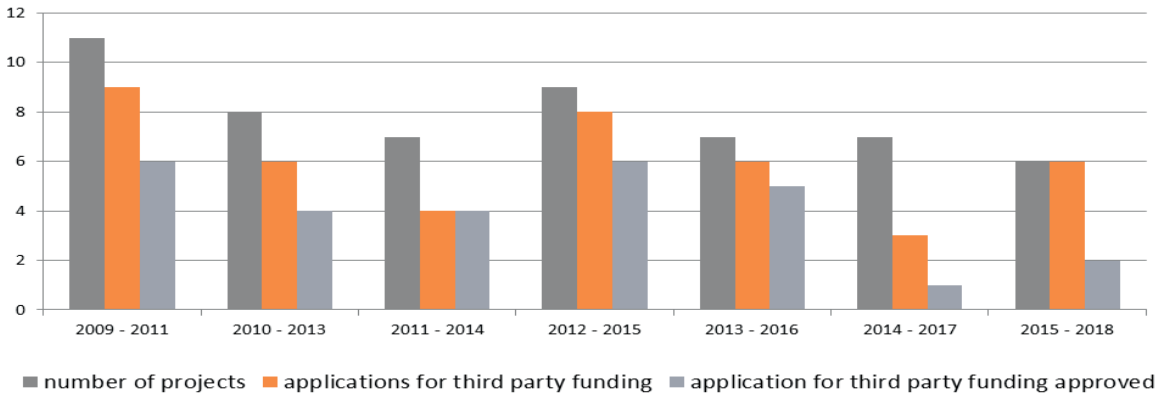
Acquisition of third-party funding by junior projects



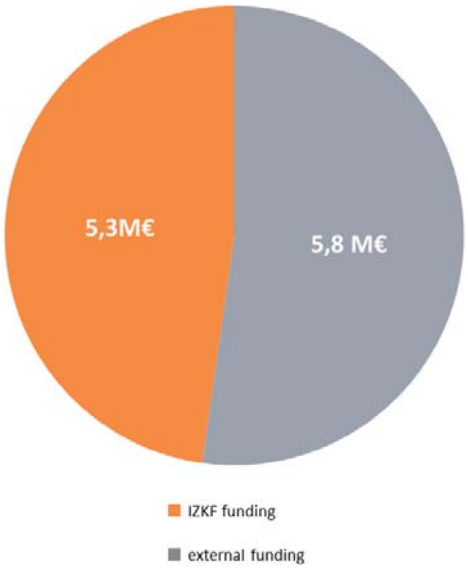
Applications for third-party funding submitted by junior projects (projects initiated between 2009 and 2015).



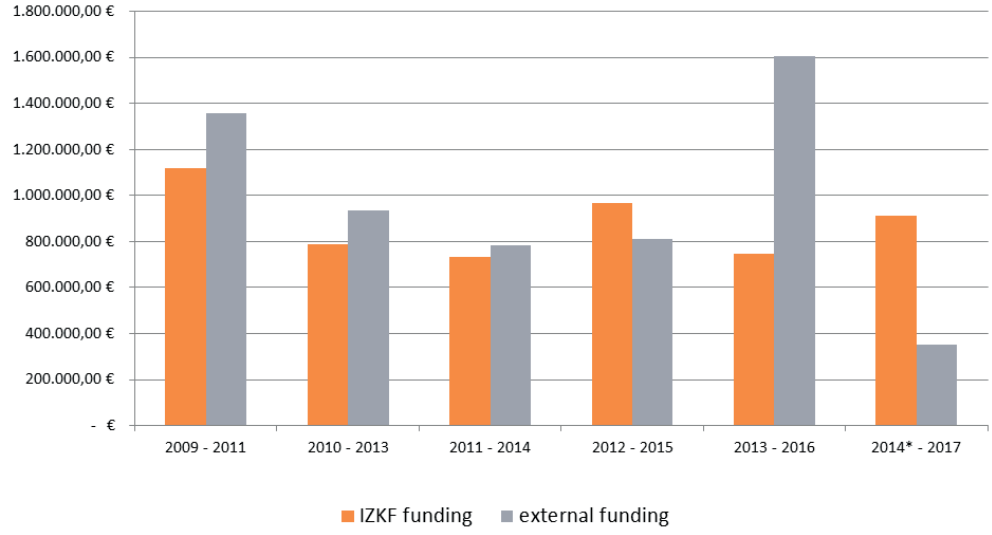
Approved applications for third-party funding of junior projects (projects initiated between 2009 and 2015).



Success-rate of junior projects. Initiated 2009-2015.

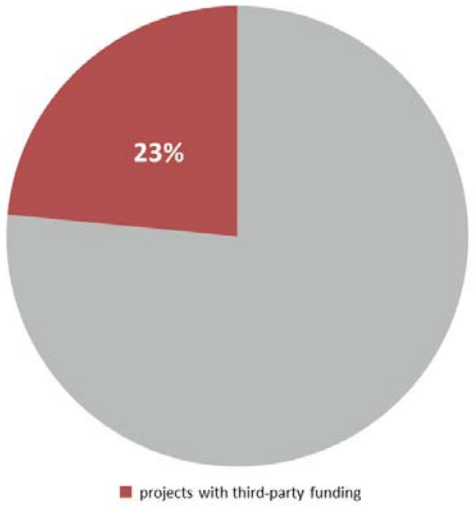


External funding received from advanced projects between 2010 and 2018.

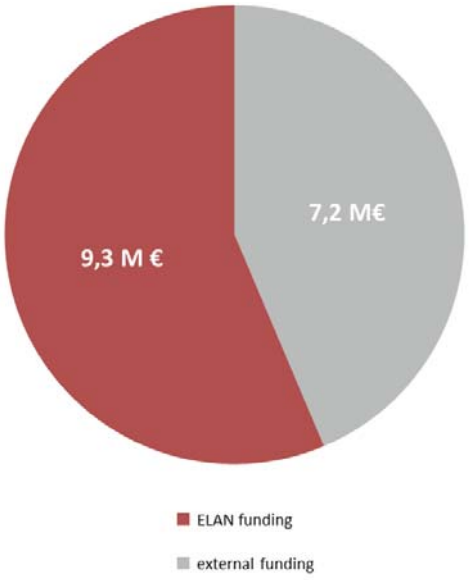


External funding received from advanced projects between 2007 and 2018.

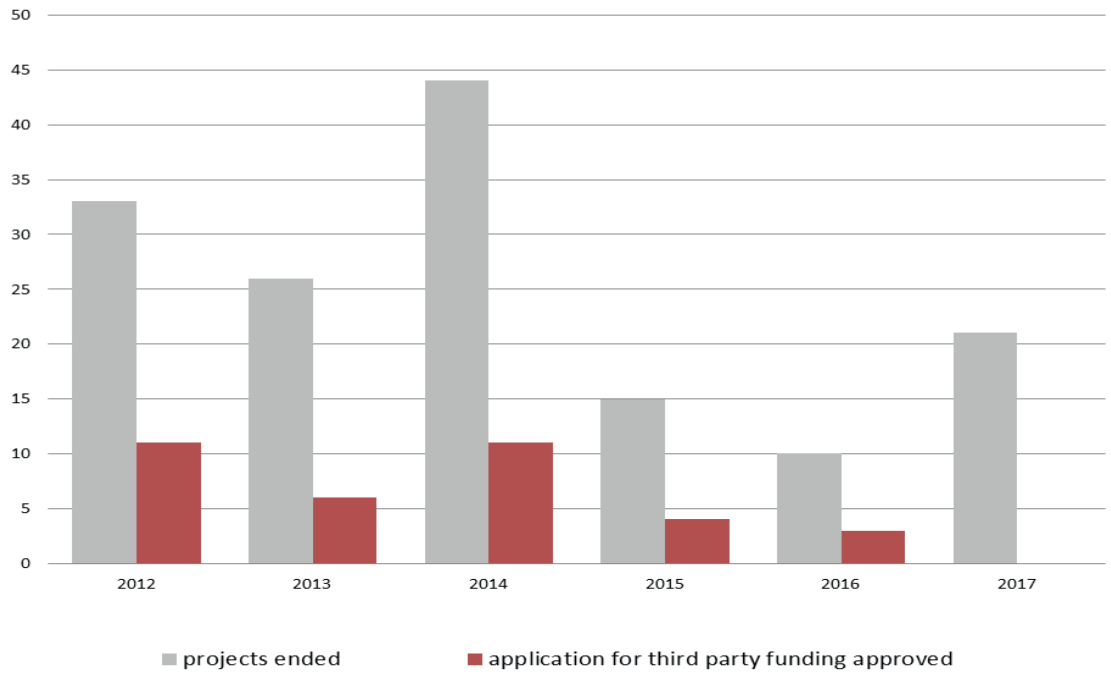
Acquisition of third-party funding by pilot projects



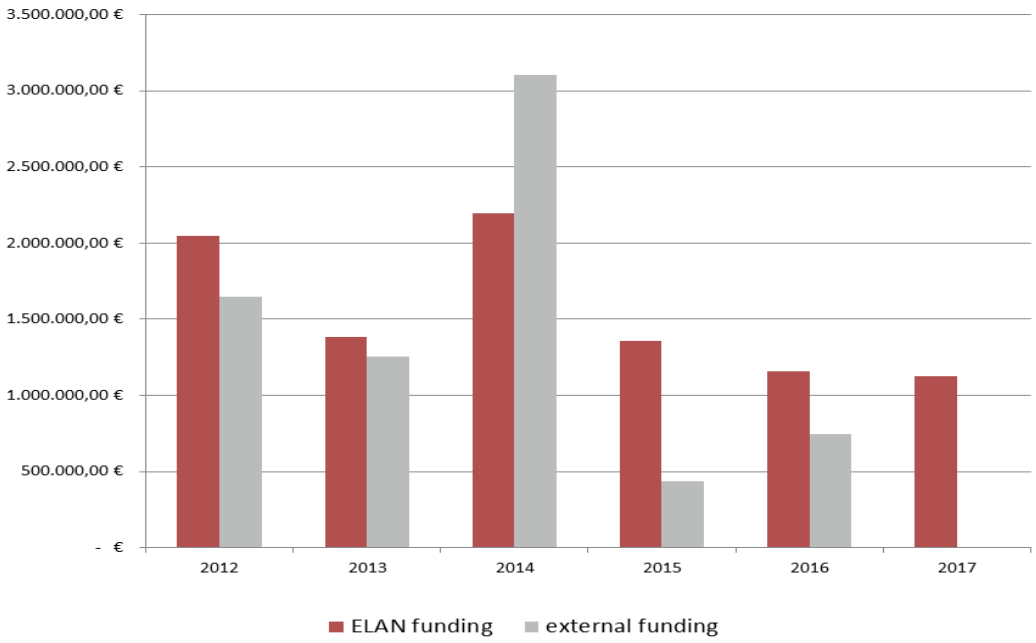
Pilot projects with third-party funding (projects completed between 2012 and 2017).



External funding received from all pilot projects completed between 2012 and 2017.



Success-rate of pilot projects. Further applications of projects, initiated in 2017, are planned.



External funding from pilot projects completed between 2012 and 2017.

Scientific Reports

Scientific Reports Advanced Projects

Scientific Reports Junior Groups/Projects

Scientific Reports Pilot Projects

A63 - Progress Report

01/07/2016 - 30/06/2019

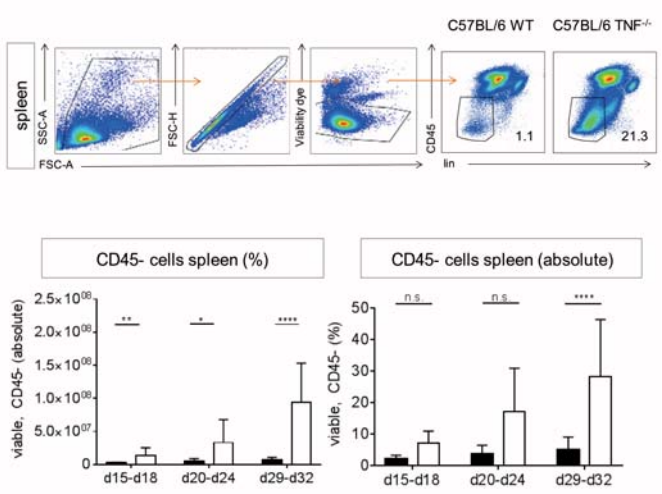
Mechanisms of TNF-Mediated Control of Intracellular Pathogens in Mice and Man

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

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

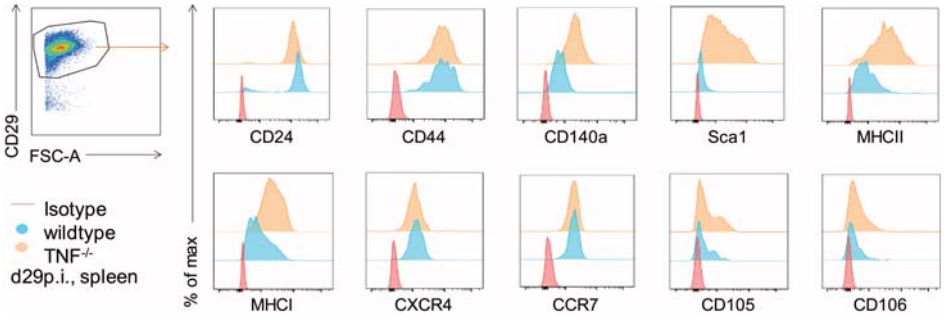
Tumor necrosis factor (TNF)-deficient C57BL/6 mice fail to control a local cutaneous infection with the intracellular pathogen Leishmania (L.) major and develop progressive and fatal visceral disease, despite a fully preserved Th1 immune response and unaltered expression of type 2 nitric oxide (NO) synthase (NOS2). NOS2 metabolizes L-arginine to citrulline and NO, which extert antimicrobial and immunoregulatory effects. The activity of NOS2 can be antagonized by arginase (Arg1) that converts the same substrate L-arginine into urea and ornithine. Previous studies in our group revealed that the lack of TNF results in hyperexpression of Arg1, which led to an impaired production of NO at the sites of infection. To further address the functional role of Arg1 expression in $TNF^{-/-}$ mice, we generated C57BL/6 mice deficient for TNF and for Arg1 in hematopoietic and endothelial cells. Surprisingly, $TNF^{-/-}Tie2Cre^{+/-}Arg1^{fl/fl}$ mice still developed progressive cutaneous leishmaniasis comparable to $TNF^{-/-}$ mice, although the production of NO was restored in the infected tissues. These results indicate that mechanisms other than the upregulation of Arg1 contribute to the non-healing course of infection in L. major-infected $TNF^{-/-}$ mice.

Unlike to self-healing C57BL/6 wild-type (WT) mice L. major-infected $TNF^{-/-}$ mice succumbed to visceral disease with high splenic parasite loads. We therefore focused on this organ and performed RNASeq analysis of L. major-infected $TNF^{-/-}$ vs. WT mice at two different time points of infection to discover "novel" TNF-regulated antimicrobial effectors (cooperation Dr. Ekici). Based on the RNASeq data we detected a significant expansion of a non-hematopoietic, CD45-negative cell compartment in the spleen of infected $TNF^{-/-}$ mice. Flow cytometry analyses revealed that these CD45-negative cells neither represented endothelial cells nor fibroblastic reticular cells or hematopoietic stem cells. Instead, the marker profile of this cell population (positive: CD29, CD44, CD24, CD140a, CXCR4, CCR7, Sca1, MHCII, MHCI; negative: e.g. CD3, CD19, CD11b, c-kit) was reminiscent of mesenchymal stem cells (MSCs). The MSC-like cells were also present in spleens of L. major-infected WT mice, but to a much lower extent. Additionally, the expression of Sca1, MHC class I and II on MSCs was stronger in mice lacking TNF as compared to WT mice, indicating an



Flow cytometric analysis of CD45-negative cells in spleens of L. major infected $TNF^{-/-}$ vs WT control mice. Gating strategy, percentage and absolute cell numbers of the CD45-negative cells during infection are given.

Prof. Dr. Bogdan



Surface marker profile of CD45- and lineage-marker-negative cells in the spleen of L. major infected TNF$^{-/-}$ mice.

increased activation status of the TNF$^{-/-}$ MSCs. The functional relevance of this high number of MSC-like cells in TNF$^{-/-}$ mice is currently investigated.

Moreover, we continue to analyse the regulation of Arg1 by TNF in humans. So far, we have been unable to detect definite changes in the expression Arg1 in peripheral blood mononuclear cells and neutrophils from patients with rheumatoid arthritis before and during treatment with TNF-antagonists. In an unbiased approach, RNASeq of whole blood samples of these patients is currently performed to detect genes regulated by the blockade of TNF.

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

Invited lectures

10th International Conference on the Biology, Chemistry and Therapeutic Applications of Nitric Oxide, 16-20.09.2018, Oxford, United Kingdom, Nitric oxide and arginase 1 in acute and latent cutaneous leishmaniasis (plenary lecture) (Bogdan)

32nd Annual Conference of the European Macrophage and Dendritic Cell Society (EMDS), 26-29.09.2018, Verona, Italy, Resolution of cutaneous leishmaniasis and persistence of Leishmania parasites in the absence of arginase 1 (plenary talk) (Bogdan)

1st International Leishmaniasis Conference, 28-31.10.2018, Caprichia/Lissabon, Portugal, Arginine metabolism and tumor necrosis factor in leishmaniasis (plenary lecture) (Bogdan)

Publications during funding period

Schleicher U, Liese J, Justies N, Mischke T, Haeberlein S, Sebald H, Kalinke U, Weiss S, Bogdan C (2018) Type I Interferon Signaling Is Required for CpG-Oligodesoxynucleotide-Induced Control of Leishmania major, but Not for Spontaneous Cure of Subcutaneous Primary or Secondary L. major Infection. Frontiers in Immunology 9: 79

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

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

Schleicher U, Paduch K, Debus A, Obermeyer S, König T, Kling JC, Ribechini E, Dudziak D, Mougiakakos D, Murray PJ, Ostuni R, Körner H, Bogdan C (2016) TNF-meditated restriction of arginase 1 expression in myeloid cells triggers type 2 NO synthase activity at the site of infection. Cell Reports 15(5): 1062-1075

A64 - Progress Report

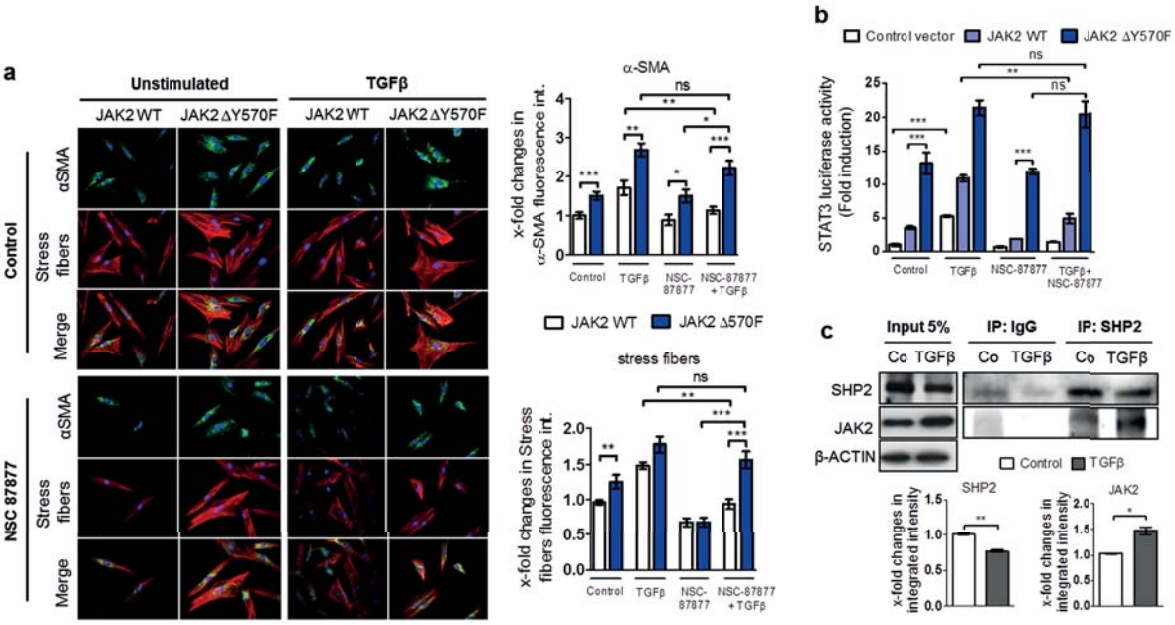
01/02/2016 - 31/01/2019

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

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

SHP2 is a ubiquitously expressed non-receptor tyrosine phosphatase with important regulatory effects on receptor tyrosine kinase-, cytokine- and G-protein coupled receptor signaling. Altered SHP2 activity due to mutations of the PTPN11 gene have been found in Noonan syndrome, juvenile myelomonocytic leukemia, and several types of human malignancies. We provide first evidence that SHP2 might also play a central role in the pathogenesis of fibrotic diseases such as Systemic Sclerosis (SSc) by inhibiting TGF-ß mediated activation of JAK2 / STAT3 signaling.

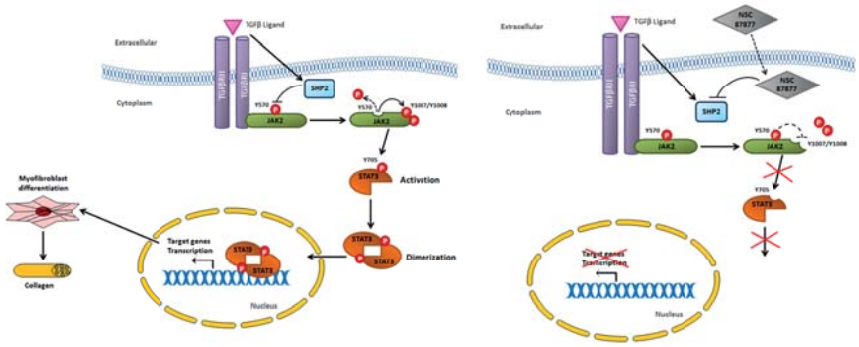
Uncontrolled activation of TGFβ signaling is a common denominator of fibrotic tissue remodeling. Here we characterize the tyrosine phosphatase SHP2 as a molecular checkpoint for TGFβ-induced JAK2/STAT3 signaling and as a potential target for the treatment of fibrosis. TGFβ stimulates the phosphatase activity of SHP2, although this effect is in part counterbalanced by inhibitory effects on SHP2 expression. Stimulation with TGFβ promotes recruitment of SHP2 to JAK2 in fibroblasts with subsequent dephosphorylation of JAK2 at Y570 and activation of STAT3. The effects of SHP2 on STAT3 activation translate into major regulatory effects of SHP2 on fibroblast activation and tissue fibrosis. Genetic or pharmacologic inactivation of SHP2 promotes accumulation of JAK2 phosphorylated at Y570, reduces JAK2/STAT3 signaling, inhibits TGFβ-induced fibroblast activation and ameliorates dermal and pulmonary fibrosis. In sum-



Overexpression of JAK2ΔY570F prevents the inhibitory effects of SHP2 inhibitors on TGFβ-induced fibroblast activation. a: Representative images of immunofluorescence stainings for α-SMA and stress fiber and respective quantifications. b: STAT3 reporter Assay. c: Co-immunoprecipitation and quantifications of endogenous JAK2 with endogenous SHP2 in human fibroblasts stimulated with TGFβ.

Prof. Dr. Distler

Prof. Dr. Schett



Schematic summary of the role of SHP2 in TGFβ-induced fibroblast activation and tissue fibrosis.

mary, we characterize SHP2 as a positive regulator of TGFβ-dependent activation of JAK2/STAT3 signaling. Genetic or pharmacologic inactivation of SHP2 inhibits JAK2/ STAT3 signaling, reduces fibroblast activation and ameliorates experimental fibrosis in several complementary models. Given the availability of potent SHP2 inhibitors, SHP2 might be a potential novel target for the treatment of fibrosis.

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

Invited lectures

Annual Meeting of the European League Against Rheumatism, June 2018, Madrid, Spain, Future perspectives on the treatment of fibrosis

Annual Meeting of the European League Against Rheumatism, June 2018, Madrid, Spain, The stromal link to inflammation

Publications during funding period

Zehender A, Huang J, Györfi AH, Matei AE, Trinh-Minh T, Xu X, Li YN, Chen CW, Dees C, Beyer C, Gelse K, Zhang ZY, Bergmann C, Ramming A, Birchmeier W, Distler O, Schett G, Distler JHW (2018) The tyrosine phosphatase SHP2 controls TGFβ-induced STAT3 signaling to regulate fibroblast activation and fibrosis. Nat Commun. 14. 9: 3259

Zhang Y, Pötter S, Chen CW, Liang R, Gelse K, Ludolph I, Horch RE, Distler O, Schett G, Distler JHW*, Dees C* (2018) Poly(ADP-ribose) polymerase-1 regulates fibroblast activation in systemic sclerosis. Ann Rheum Dis. 77: 744-751 [Epub ahead of print]
* contributed equally

Soare A, Weber S, Maul L, Rauber S, Gheorghiu AM, Luber M, Houssni I, Kleyer A, von Pickardt G, Gado M, Simon D, Rech J, Schett G, Distler JHW, Ramming A (2018) Cutting Edge: Homeostasis of Innate Lymphoid Cells Is Imbalanced in Psoriatic Arthritis. J Immunol. 15.200: 1249-1254

Matei AE, Beyer C, Györfi AH, Soare A, Chen CW, Dees C, Bergmann C, Ramming A, Friebe A, Hofmann F, Distler O, Schett G, Distler JHW (2018) Protein kinases G are essential downstream mediators of the antifibrotic effects of sGC stimulators. Ann Rheum Dis. 2018 Jan 8 [Epub ahead of print]

Huang J, Maier C, Zhang Y, Soare A, Dees C, Beyer C, Harre U, Chen C-W, Distler O, Schett G, Wollin L, Distler JH (2017) Nintedanib macrophage activation and ameliorates vascular and fibrotic manifestations in the Fra2 mouse model of systemic sclerosis. Ann Rheum Dis 76: 1941–1948

Rauber S, Luber M, Weber S, Maul L, Soare A, Wohlfahrt T, Lin N-Y, Dietel K, Bozec A, Herrmann M, Kaplan MH, Weigmann B, Zaiss M, Fearon U, Veale DJ, Canete JD, Distler O, Rivallese F, Pitzalis C, Neurath MF, McKenzie ANJ, Wirtz S, Schett G, Distler JH, Ramming A (2017) Resolution of inflammation by interleukin 9 producing type 2 innate lymphoid cells. Nat Med. 23: 938-944

Zhang Y, Liang R, Chen C-W, Mallano T, Dees C, Distler A, Reich A, Bergmann C, Ramming A, Gelse K, Milenz D, Distler O, Schett G, Distler JH (2017) JAK1-dependent transphosphorylation of JAK2 limits the anti-fibrotic effects of selective JAK2 inhibitors upon long-term treatment. Ann Rheum Dis 76: 1467-1475

Maier C, Ramming A, Bergmann C, Weinkam R, Kittan N, Schett G, Distler JH, Beyer C (2017) Inhibition of phosphodiesterase 4 (PDE4) reduces dermal fibrosis by interfering with the release of interleukin-6 from M2 macrophages. Ann Rheum Dis 76: 1133–1141

Chen CW, Beyer C, Liu J, Maier C, Li C, Trinh-Minh T, Xu X, Cole SH, Hsieh MH, Ng N, Althage A, Meeusen S, Pan S, Svensson EC, Seidel HM, Schett G, Gergely P, Harris JL, Distler JH (2017) Pharmacological inhibition of porcupine induces regression of experimental skin fibrosis by targeting Wnt signalling. Ann Rheum Dis 76: 773–778

A65 - Progress Report

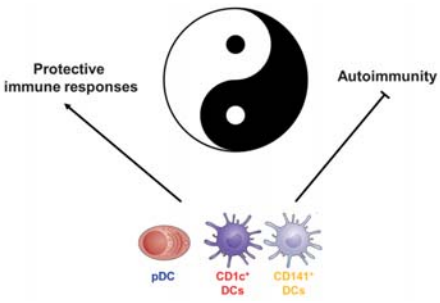
01/04/2016 - 31/03/2019

Tolerizing potential of human dendritic cell subpopulations

Prof. Dr. Diana Dudziak, Department of Dermatology

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

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



Human DC subpopulations and their role in tolerance and immunity.

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

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

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

Thymic DCs might be influenced by either tissue factors or differential activity of regulatory components (e.g. transcriptions factors). Investigating the expression of NFκB and NFκB regulating factors we found only slight differences between thymic and blood DCs. Therefore, we measured the phosphorylation of signaling molecules upon stimulation with TLR ligands of sorted blood and thymic DCs. Notably, we found a differential DC specific phosphorylation profile in that NFκB p65 and p38 MAPK were stronger and longer phosphorylated in R848-stimulated blood DCs compared to thymic DCs. Those data are of specific importance, as we needed more evidence about potential regulatory mechanisms in thymic

Prof. Dr. Dudziak

DCs. Based on our findings, we have sorted non-activated and TLR-activated DC subsets and performed Nanostring analyses. Epigenetic profiling, including ATAC sequencing combined with RNA-Seq analyses will be performed next.

Contact:
Prof. Dr. Dudziak
phone: +49 9131 85 39346
e-mail: diana.dudziak@uk-erlangen.de

Invited lectures

GRK Retreat 1962, 23.11.2018, Thurnau, Understanding DC subset functions for the development of future cancer therapies

ESMRC2018, 02. - 03.11.2018, Essen, workshop chair in 'Microbiota, Pathogen Recognition and Vaccination I and II'

SFB 1054 Symposium, 12.10.2018, Munich, Understanding DC subset functions for the development of future cancer therapies

Annual Meeting of the Autumn School of Immunology, 08.10.2018, Merseburg, How dendritic cells (interact and) activate T cells

European Congress of Immunology (ECI), 03.09.2018, workshop chair in 'Early T cells'

DC2018, 10.06.2018, Aachen, representative of the German Society of Germany (AKDC), teaching course for dendritic cells, Dendritic Cell Subsets in Mouse and Man

Antibody Engineering & Therapeutics Europe, 07.06.2018, Amsterdam, Antibody-mediated antigen delivery to antigen presenting cells via Fc receptors

Tumor Immunology meets Oncology, XIV (TIMO), 26.05.2018, Halle, Human dendritic cell subpopulations in steady state and inflammation

AAI, 07.05.2018, Austin, USA, representative of the German Society of Germany for the introduction of the study group dendritic cells (AKDC), Human lymphoid organ dendritic cell identity

Awards

Coordinator and speaker of the Emerging Fields Initiative of the Friedrich-Alexander-University Erlangen-Nürnberg 'Integrative Big Data Modeling' for the development of novel therapeutic approaches for breast cancer - BIG-THERA'

Elected member of the executive board of the German Society of Immunology (DGfI)

Speaker and organizer of the annual meeting of the 'Dendritic Cell Study Group (AKDC)', Budenheim (near Mainz)

Publications during funding period

Dudeck J, Froebel J, Kotrba J, Lehmann CHK, Dudziak D, Speier S, Nedospasov SA, Schraven B, Dudeck A (2018) Engulfment of mast cell secretory granules on skin inflammation boosts dendritic cell migration and priming efficiency. J Allergy Clin Immunol. doi: 10.1016/j.jaci.2018.08.052. [Epub ahead of print]

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Messlinger H, Sebald H, Heger L, Dudziak D, Bogdan C, Schleicher U (2018) Monocyte-Derived Signals Activate Human Natural Killer Cells in Response to Leishmania Parasites. Front Immunol. 9: 24

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

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

Gerstner S, Köhler W, Heidkamp G, Purbojo A, Uchida S, Ekici AB, Heger L, Luetke-Eversloh M, Schubert R, Bader P, Klingebiel T, Koehl U, Mackensen A, Romagnani C, Cesnjevar R, Dudziak D, Ullrich E (2016) Specific phenotype and function of CD56-expressing innate immune cell subsets in human thymus. J Leukoc Biol. 100(6): 1297-1310

Heidkamp GF, Sander J, Lehmann CH, Heger L, Eissing N, Baranska A, Lühr JJ, Hoffmann A, Reimer K, Lux A, Söder S, Hartmann A, Zenk J, Ulas T, McGovern N, Alexiou C, Spriewald B, Mackensen A, Schuler G, Schauf B, Forster A, Repp R, Fasching PA, Purbojo A, Cesnjevar R, Ullrich E, Ginhoux F, Schlitzer A, Nimmerjahn F, Schultze JL, Dudziak D (2016) Human lymphoid organ dendritic cell identity is predominantly dictated by ontogeny, not tissue microenvironment. Science Immunol 1(6): 1-17

A66 - Progress Report

01/07/2016 - 30/06/2019

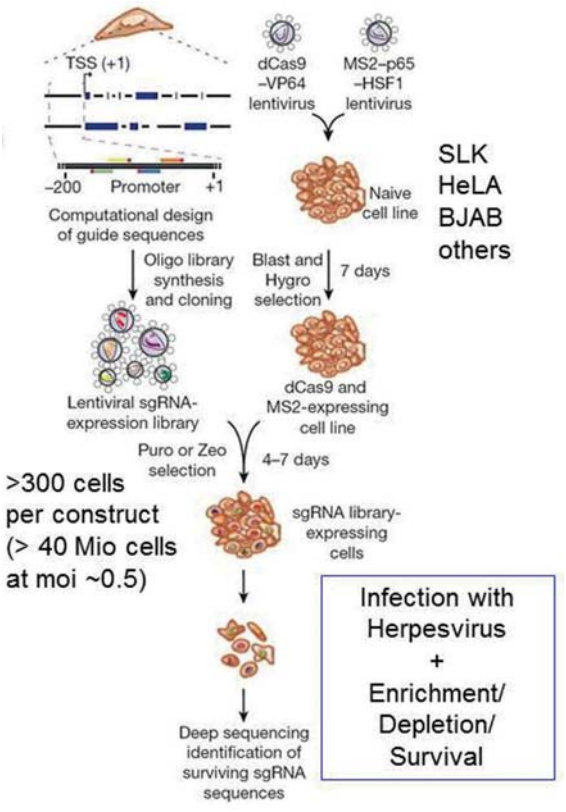
Genome wide CRISPR/Cas9 knockout for the identification of antiviral cellular restriction factors

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

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

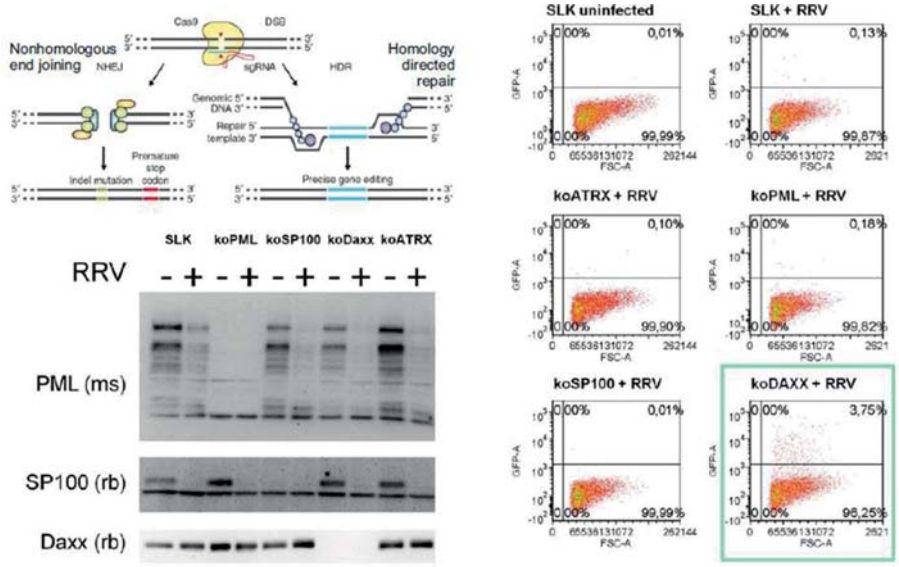
Viruses, like other intracellular parasites, must evade the actions of the host cell's innate immune response, and often devote a substantial portion of their coding capacity to counteract these cellular restriction factors. The systematic and unbiased approach at identifying cellular restriction factors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

After performing first genome wide screening experiments on KSHV infected SLK target cells, we found the initial screens were limited by the FACS-sorting rate of large epithelial cells, such as SLK(Caki) or HeLa, which allowed to separate ~1500 cells per second, and would have resulted in sort times exceeding 20 hours for a typical sample of 50-100 million cells. Therefore, we constructed recombinant KSHV Bac16, Bac16RGB and HVS, expressing a murine H-2Kk surface marker that allows magnetic (pre-) sorting of virus infected cells. This can then be followed by FACS sorting of GFP and GFP/BFP populations. The synergistic activation mediator (SAM)



Synergistic activation mediator (SAM) system (modified from Konerman, 2015).

Prof. Dr. Ensser



Confirmation of RRV restriction.

system was functionally verified and established as a complementary approach to validate the sgRNA ko-screen. Its genome-wide sgRNA2.0 library of >70.000 plasmids is now available and the components of the SAM system, NLS-dCas9-VP64 and helper transactivator MS2-p65-HSF1 were tested in model cell lines (e.g. SLK(Caki), HeLa). Furthermore, an improved version of the genome-wide sgRNA library in the pLentiCRISPRv2 vector was obtained and amplified. This “Brunello-Library” consisting of 4 sgRNA per cellular gene and 1000 non-targeting control sgRNAs (total 77.441 constructs) is more efficient due to its optimized algorithm for sgRNA design, resulting in a lower false discovery rate (FDR), and the lower complexity requires 1/3 less cells to be transduced, and consequently, less sorting time for selection of enriched or depleted cells. In summary, the two complementary screens will help us to identify targets with increased confidence via the respective opposite ranking in knockout vs. SAM screens, ensuring that we can focus on relevant genes. The Brunello Library has also been used to screen for factors restricting CMV reactivation in undifferentiated monocytes, and to search for factors restricting replication and entry of RRV, the model virus of KSHV, in infected human cells.

Contact:
Prof. Dr. Ensser
phone: +49 9131 85 22104
e-mail: armin.ensser@fau.de

Publications during funding period

Ensser A, Grosskopf AK, Matz-Rensing K, Roos C, Hahn AS (2018) Isolation and sequence analysis of a novel rhesus macaque foamy virus isolate with a serotype-1-like env. Arch Virol 163: 2507-2512

Grosskopf AK, Ensser A, Neipel F, Jungnickl D, Schlagowski S, Desrosiers RC, Hahn AS (2018) A conserved Eph family receptor-binding motif on the gH/gL complex of Kaposi’s sarcoma-associated herpesvirus and rhesus monkey rhadinovirus. PLoS Pathog 14: e1006912

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A67 - Progress Report

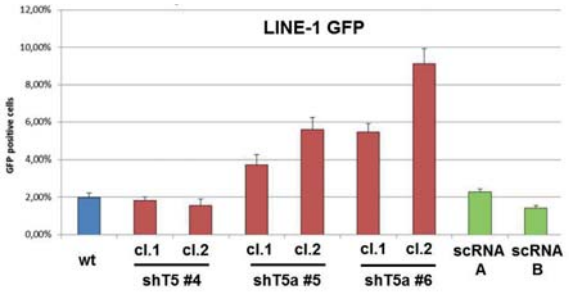
01/02/2016 - 31/01/2019

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

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

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

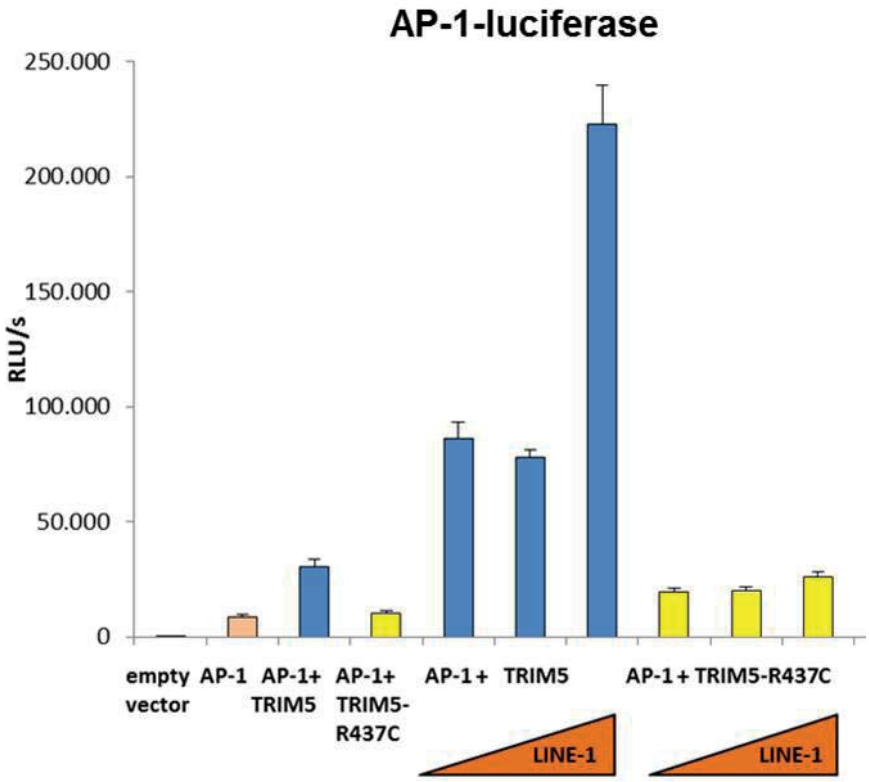
Initially, we demonstrated that the retroviral restriction factor human TRIM5α reduces the retrotransposition frequencies of LINE-1 reporter elements. In addition, we were able to establish stable TRIM5a knockdown cells and found that the knockdown of endogenous TRIM5α enhanced LINE-1 retrotransposition. By establishing a digital droplet PCR protocol we saw that the number of LINE-1 integrates is significantly reduced in the presence of TRIM5α. To identify regions within TRIM5α important for LINE-1 restriction, we analyzed naturally occurring TRIM5α SNPs and TRIM5α deletion mutants in LINE-GFP reporter assays. We identified a single SNP located within the SPRY domain of TRIM5α, which directly interacts with retroviral capsids, that causes a complete loss of LINE-1 restriction. To confirm the importance of the SPRY domain and to determine the role of the B-Box domain, which is important for multimerization of TRIM5α, in LINE-1 restriction, we analyzed the retrotransposition efficiency of LINE-GFP in the presence of various SAMHD1 mutants. We were able to confirm the importance of the SPRY domain for LINE-1 restriction and found that changing critical amino acids within the B-box strongly reduced TRIM5α activity against LINE-1. This suggests that, similar to HIV restriction, multimerization of TRIM5α via its B-Box is necessary for a direct interaction of TRIM5a with LINE-1 via its SPRY domain. In addition, we found that TRIM5α directly interacts with LINE-1 ribonucleoprotein complexes upon coexpression and both proteins, TRIM5α and LINE-1 ORF1p, colocalize in the cytoplasm suggesting a direct interaction of LINE-1 ribonucleoprotein particles and TRIM5α in the cytoplasm.



Endogenous TRIM5α restricts LINE-1. 293T cells stably expressing shRNAs (#4, #5, #6) targeting TRIM5α or scrambled shRNAs (scRNA A, B) were transiently transfected with a LINE-GFP reporter plasmid. Retrotransposition events were quantified 5 days later by flow cytometry.

Since the interaction of TRIM5a with retroviral capsids has been shown to induce AP-1 and NfkB signaling, we asked whether the TRIM5α-mediated induction of AP-1 and NF-kB might also play a role in LINE-1 restriction. Indeed, we found that the replication of LINE-1 GFP is potently restricted by overexpression of constitutively active mutants of the AP-1 signaling pathway. In AP-1 and NfKb-luciferase reporter assays, TRIM5 expression led to NF-kB and AP-1 activation. Interestingly, the TRIM5α-mediated AP-1 signal in the presence of LINE-1 was enhanced, suggesting that TRIM5α induces inhibitory AP-1 signaling upon binding to LINE-1. Conclusively, in LINE-1 promoter reporter assays, we found that the induction of AP-1 negatively regulates LINE-1 promoter activity. Together, our results suggest that multimeric TRIM5α senses LINE-1 protein complexes in the cytoplasm and downregulates LINE-1 promoter activity in a negative feedback loop by activating AP-1

Prof. Dr. Gramberg



TRIM5α induces AP-1 signaling in the presence of LINE-1. 293T cells were transfected with plasmids encoding an AP-1-dependent luciferase, TRIM5a wt or inactive mutant R437C, and LINE-1. AP-1 activity was quantified 2 days later by luciferase assay.

signaling. Therefore, we show that TRIM5α is sensing and limiting LINE-1 retrotransposition and is thereby contributing to genome integrity.

Contact:
Prof. Dr. Gramberg
phone: +49 9131 85 36481
e-mail: thomas.gramberg@fau.de

Invited lectures

GfV Workshop Immunobiology of viral Infections, 27.09.2018, Tauberbischofsheim, "Analysis of the TRIM5α-mediated block to LINE-1 retroelements"

Publications during funding period

none

A68 - Progress Report

16/06/2016 - 15/06/2019

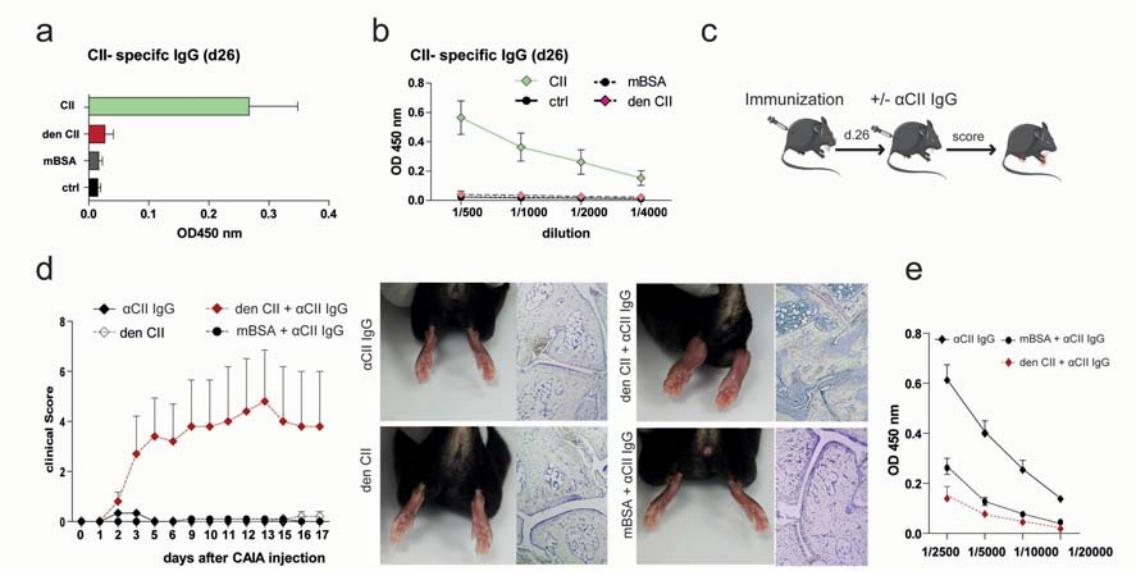
Analysis of the role of the IL-23/Th17 axis during the control of antibody activity in rheumatoid arthritis

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

In our project we are analyzing the role of the IL-23/TH17 axis during the pathogenesis of rheumatoid arthritis (RA). We have shown that TH17 cells control of the intrinsic inflammatory activity of autoantibodies during onset of autoimmune arthritis via regulation of the expression of glycosyltransferases in plasma cells. Recent data additionally show that TH17 cells also control the onset of arthritis by licensing the tissue for autoantibody-induced inflammation.

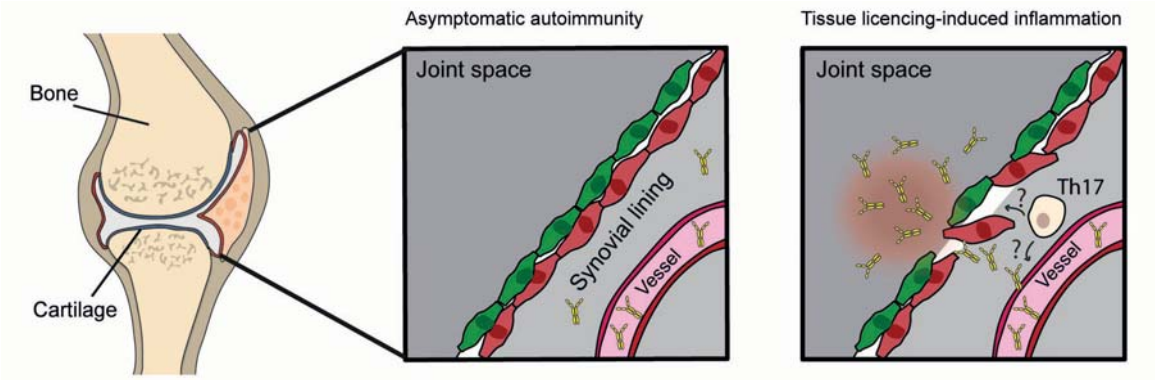
We previously identified the IL-23/TH17 axis as important modulator of the inflammatory activity of autoantibodies during rheumatoid arthritis (RA). TH17 cells critically contributed to the initial production of pro-inflammatory and arthritogenic IgG before onset of inflammation. Here, we identified TH17 cells in secondary lymphatic organs that displayed a T follicular helper cell phenotype and entered germinal centers where they regulated the glycosyltransferase expression in newly differentiating plasma cells. This IL-23-dependent pathway determined the glycosylation profile of newly-produced autoantibodies.

Additional data show that collagen II (CII)-specific autoreactive TH17 cells also essentially control the onset of arthritis that develops upon passive transfer of CII-specific arthritogenic autoantibodies. Although



a, b) CII-specific IgG after immunization with CII, denatured CII (den CII) or mBSA. c) Experimental setting d) Collagen-induced arthritis (CIA) after immunization with den CII and mBSA in combination with transfer of αCII IgG. e) Titers of CII-specific IgG on day 42 after initial immunization.

Prof. Dr. Krönke Prof. Dr. Nimmerjahn



IL-23-dependent T cells license the joint for autoantibody-mediated inflammation via yet to be identified effector mechanisms.

underlying molecular mechanisms remain elusive, our preliminary findings suggest that autoreactive T cells "license" the joint for autoantibody-induced inflammation and that T cells and autoantibodies act in a synergistic and antigen-specific manner. These findings thus strongly suggest a role of specific T cell subsets in regulating the trafficking and activity of autoantibodies to and within the joint.

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

Awards

Mosaic of Autoimmunity (MAI) Award at the 11th International Congress of Autoimmunity, Gerhard Krönke, May 2018, Lisbon, Portugal

Theodor Naegeli Award from the Theodor Naegeli foundation, Gerhard Krönke, April 2018, Basel, Switzerland

Publications during funding period

Scholtysek C, Ipseiz N, Böhm C, Krishnacoumar B, Stenzel M, Czerwinski T, Palumbo-Zerr K, Rothe T, Weidner D, Klej A, Stoll C, Distler J, Tuckermann J, Herrmann M, Fabry B, Goldmann WH, Schett G, Krönke G (2018) NR4A1 Regulates Motility of Osteoclast Precursors and Serves as Target for the Modulation of Systemic Bone Turnover. J Bone Miner Res. 33(11): 2035-2047

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

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A69 - Progress Report

01/07/2016 - 30/06/2019

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

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

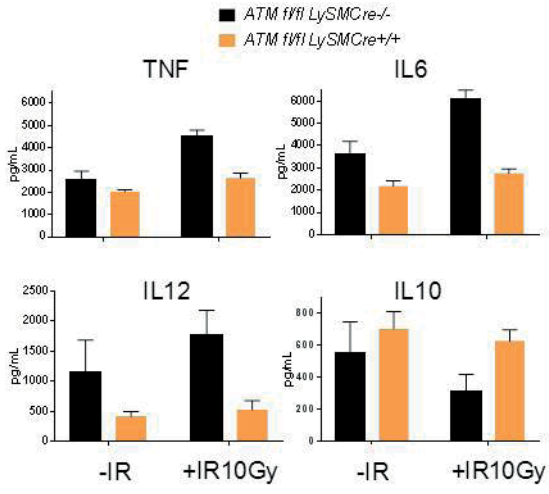
The DNA-damage response (DDR) requires the kinase ATM and is essential for the integrity of the host genome. We observed activation of the ATM kinase pathway in Toll-like receptor (TLR)-stimulated macrophages and a modulation of the inflammatory response by ATM-inhibition. Here, we carry out detailed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

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

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

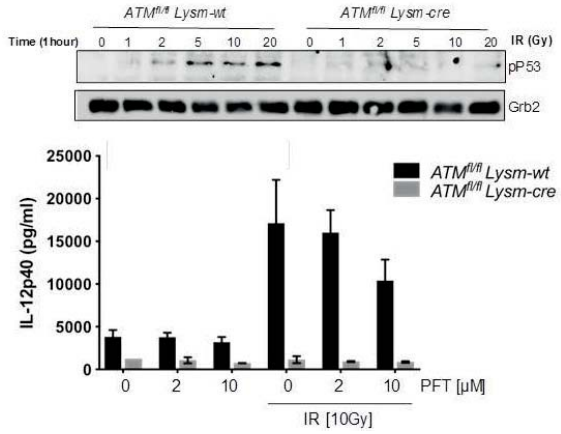
The transcription factor p53 is a canonical downstream target of ATM kinase. In irradiated macrophages, we found strong and dose-dependent phosphorylation of p53 after one hour, which was completely dependent on ATM. IR per se induced a moderate and transient upregulation of mRNA encoding IL-6 and TNF, with a peak between 1.5–3 hours. Pharmacological inhibitors of p53 and NFκB activation, but not of MAPK signaling, abrogated the IR-induced cytokine expression. To further dissect the mechanisms of cross-regulation between DNA-damage response and TLR signaling in macrophages, we plan to employ conditional knockout mice for p53 and components of the NFκB pathway.

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



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

Prof. Dr. Lang



ATM-dependent p53 mediates IR-induced increase in cytokine production. (top) BMDM were irradiated with the indicated doses. Phosphorylation of p53 was determined after 1 h by Western blot. (bottom) Macrophages were treated as indicated with increasing doses of the p53 inhibitor Pifithrin-α.

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

Contact:
Prof. Dr. Lang
phone: +49 9131 85 22979
e-mail: roland.lang@uk-erlangen.de

Invited lectures

DGfI Autumn School Current Concepts in Immunology, October 8, 2018, Merseburg, Germany: Ontogeny and function of macrophages

Workshop on Frontiers in Phosphatase Research and Drug Discovery, October 23-25, 2018, Tokyo, Japan: Regulation of innate immunity by DUSP family members

Publications during funding period

none

A70 - Progress Report

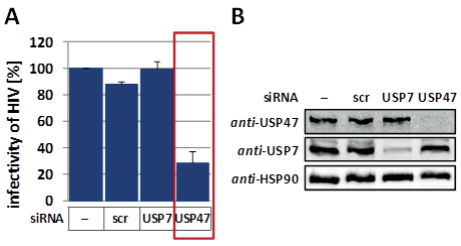
01/07/2016 - 30/06/2019

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

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

Based on our finding that certain deubiquitinating enzymes play an essential role in HIV-1 replication we have been investigating the role of regulatory proteins in the interaction of HIV-1 with the ubiquitin proteasome system. While Vpr is involved in HIV-associated fat metabolism diseases, Vpu directs the polyubiquitination of certain host cell-receptors. The small p6 protein regulates membrane association and polyubiquitination of Gag and is specifically degraded by the insulin-degrading enzyme.

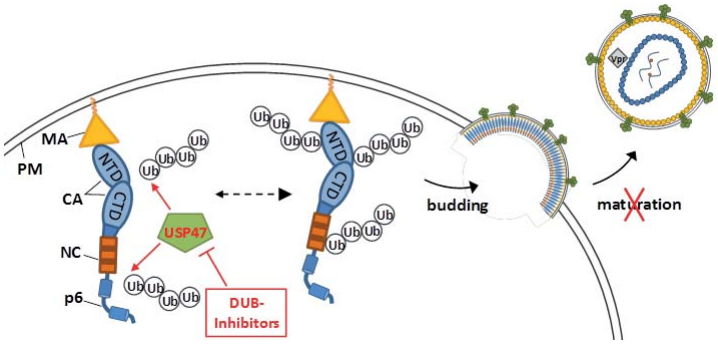
For the first time we were able to demonstrate that deubiquitinating enzymes (DUBs) are involved in HIV-1 replication by regulating the Gag processing and thus virus infectivity. As only certain DUB-inhibitors (DIs), which specifically inhibit USP47, have anti-retroviral activity, we hypothesized that the DUB candidate USP47 plays a significant role in HIV-1 replication. By performing loss of function analysis we could indeed confirm that USP47 is crucial for the maintenance of the infectivity of HIV-1. Furthermore, we could show that ex vivo treatment of lymphoid tissue with USP47-specific DIs, resulting in a complete block of virus replication. Thereby, virtually no toxicity was detected even at the highest concentrations used for treatment. Most strikingly, combinatory treatment of lymphoid tissue with DIs and proteasome inhibitors in a concentration range, where the inhibitors alone had no influence on virus replication, revealed a synergistic antiretroviral activity of inhibitors that act on both components of the ubiquitin proteasome system (UPS), proteasomes and DUBs.



Knockdown of USP47 selectively interferes with HIV-1 replication. (A) Infectivity of HIV-1 after siRNA mediated knockdown of USP7 and USP47. (B) Determination of knockdown-efficiency via western blot using USP7- and USP47-specific antibodies.

To further unravel the role of the UPS in HIV-1 replication we have been investigating the interaction of small HIV-1 proteins with the UPS. Currently, it is not clear of whether the ion channel activity of the regulatory HIV-1 Vpu protein is involved in the degradation of host cell receptors, like tetherin or CD4. However, we were able to demonstrate that this activity is conserved throughout the evolution of HIV-1 and its ancestor SIVcpz. Regarding the HIV-1 regulatory protein Vpr we could show, together with the group of Dr. Balasubramanyam, Houston, USA, that Vpr, by interacting with the cellular DBB1, DCAF and Cul4A-ligase complex plays an important role in HIV-associated fatty liver diseases. Currently we are investigating the role of certain DUBs in these processes.

Prof. Dr. Schubert



Hypothetical model: influence of DIs on HIV-1 replication. Specific DUBs play an important role in the HIV-1 replication as they regulate ubiquitination and processing of Gag. Consequently, DUB-inhibitors interfere with the maturation of Gag.

The 52 aa HIV-1 p6 Gag protein does not only regulate the late steps in virus replication but also the polyubiquitination of Gag. Now we could demonstrate that the interaction of Gag with the plasma membrane and its subsequent polyubiquitination and access to DUBs, as well as the 26S proteasome, is regulated by the charge distribution within p6. Furthermore, we found that the p6 represents the first known viral substrate of the ubiquitously expressed cytosolic metalloendopeptidase insulin degrading enzyme (IDE). Thereby, p6 is approximately 100-fold more efficiently degraded by IDE than its eponymous substrate insulin. This phenomenon could be considered as one explanation for the significantly higher risk for type II diabetes in HIV-1 carriers. In addition, we were able to show that the degradation of p6 by the IDE is regulated by its N-terminus. This phenomenon is specific for the pandemic HIV-1 group M isolates. Until now it is unclear if certain DUBs are involved in the IDE-mediated degradation of p6, a topic which we will further investigate.

Contact:
Prof. Dr. Schubert
phone: +49 9131 85 26478
e-mail: ulrich.schubert@fau.de

Publications during funding period

Schmalen A, Karius-Fischer J, Rauch P, Setz C, Korn K, Henklein P, Fossen T, Schubert U (2018) The N-Terminus of the HIV-1 p6 Gag Protein Regulates Susceptibility to Degradation by IDE. Viruses 10(12): 710

Agarwal N, Iyer D, Gabbi C, Saha P, Patel SG, Mo Q, Chang B, Goswami B, Schubert U, Kopp JB, Lewis DE, Balasubramanyam A (2017) HIV-1 viral protein R (Vpr) induces fatty liver in mice via LXRα and PPARα dysregulation: implications for HIV-specific pathogenesis of NAFLD. Nature Scientific Reports 17.7(1): 13362

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

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

A71 - Final Report

01/07/2016 - 31/12/2018

Viral modulation of the protein kinase ULK1

Prof. Dr. Thomas Stamminger, Institute of Clinical and Molecular Virology (till 31/12/2017),
Institute of Virology, Ulm University Medical Center (from 01/01/2018)

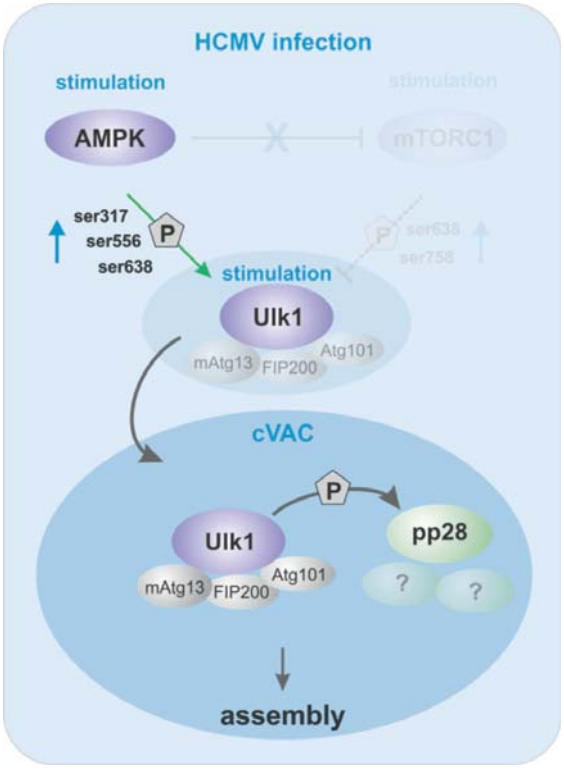
The cellular factor Ulk1 is an important protein kinase which regulates both autophagy and innate immunity. We observed that Ulk1 and other components of the autophagy machinery are strongly upregulated after infection with human cytomegalovirus. Our results suggest that HCMV utilizes specific autophagy proteins for efficient viral particle release. Inhibition of Ulk1 kinase activity interferes with HCMV particle release and may thus constitute a novel antiviral principle.

Ulk1 and other components of the Ulk1 complex are upregulated at late times during HCMV infection

We observed that HCMV infection of primary human fibroblasts results in a strong upregulation of Ulk1 as well as of other components of the Ulk1 complex (FIP200 and ATG13) at late times of infection. This correlated with a hyperphosphorylation of Ulk1 at serines 317, 556, 638 and 758. Since Ulk1 is phosphorylated via AMPK and mTOR we performed infection experiments in the presence of the inhibitors compound C (specific for AMPK) and mTorin1 or rapamycin (specific for mTOR). The most important result of these experiments was the observation that compound C was able to abrogate the HCMV-mediated induction of Ulk1 indicating that AMPK might represent the key regulator of Ulk1 during HCMV infection.

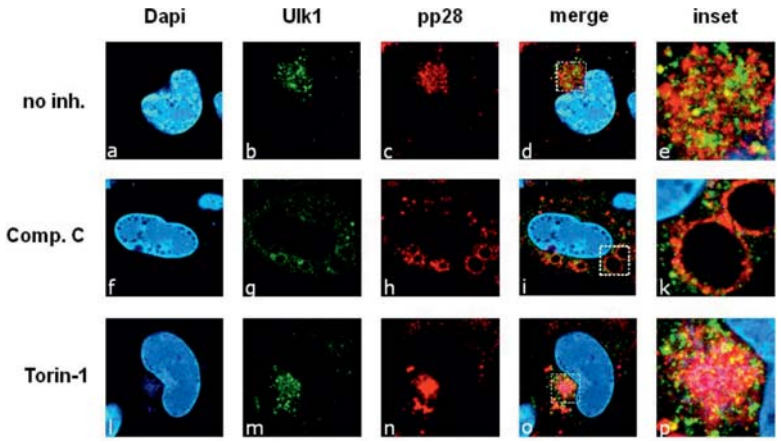
Efficient viral replication depends on Ulk1 and the Ulk1 kinase activity

To characterize the role of Ulk1 for HCMV replication we first generated primary human fibroblasts with an shRNA-mediated stable knockdown of Ulk1. Multistep growth curve analysis revealed a severe replication defect of HCMV in these cells. In addition, to corroborate these results, cell lines with a doxycycline (dox)-inducible knockdown were established which exhibited a nearly complete depletion of Ulk1 within 72 h of induction. Furthermore, protein kinase inhibitors were used to define the relevance of Ulk1 phosphorylation, mediated by AMPK, as well as Ulk1 kinase activity for HCMV replication. In the presence of the AMPK inhibitor compound C, which was added to infected cells at 48 hpi, we observed a significant decline in the release of viral particles while viral early or late gene expression were not affected. Similar results were obtained using SBI-0206965, which specifically interferes with Ulk1 kinase activity. In summary, these results indicate that the Ulk1 kinase is necessary for efficient cytomegalovirus particle release from infected cells.



Model summarizing the role of Ulk1 during HCMV infection: Ulk1 which is phosphorylated via AMPK, contributes to efficient HCMV assembly by targeting pp28.

Prof. Dr. Stamminger



Ulk1 and pp28 colocalize in the cytoplasmic virion assembly complex (panel d) which is disrupted upon treatment with the AMPK inhibitor compound C (panel i).

Mechanism exerted by Ulk1 to modulate HCMV particle release

In order to clarify the mechanism how Ulk1 modulates HCMV particle release we searched for viral phosphorylation targets. In an in vitro kinase assay several viral proteins were tested for Ulk1-mediated phosphorylation revealing strong signals for the tegument protein pp28 of HCMV. Addition of SBI-0206965 to the reaction abrogated pp28 phosphorylation in a dose-dependent manner confirming the specificity of the reaction. Importantly, pp28 has been described as a viral protein playing an essential role for the process of secondary envelopment. In co-localization experiments we detected a recruitment of Ulk1 to the cVAC (cytoplasmic virion assembly complex) which, however, could only be observed with a specific monoclonal antibody against Ulk1. Furthermore, addition of compound C to infected cells resulted in a disruption of the cVAC and the accumulation of multivesicular bodies. Altogether these results suggest that Ulk1 may affect the secondary envelopment of HCMV particles.

Contact:
Prof. Dr. Stamminger
phone: + 49 731 500 65 100
e-mail: thomas.stamminger@uniklinik-ulm.de

Invited lectures

The 23rd Glasgow Virology Workshop, 10.02.2018, Glasgow, UK, The Human Cytomegalovirus IE1 Protein: An Offender of PML Nuclear Bodies

CMV-Symposium „Von Grundlagen zu Translation“, 23.02.2018, Tübingen, IE bei HCMV – von den Anfängen bis zur Funktion

Eröffnungsvortrag zum Semesterauftakt, Medizinische Fakultät Ulm, 15.10.2018, Ulm, Ist gegen Viruserkrankungen kein Kraut gewachsen?

7th International Workshop on CMV and Immunosenescence, 10.11.2018, Waldthausen Castle, Mainz, The vGPCR pUS27 of HCMV induces the expression of chemoattractant cytokines and is restrained by PDZ proteins regulating epithelial polarity

Awards

Berufung zum Leiter des Konsiliarlabors für Cytomegalievirus durch das Bundesministerium für Gesundheit, Thomas Stamminger, 29.11.2018, Berlin

Publications during funding period

Reuter N, Reichel A, Stilp A-C, Scherer M, Stamminger T (2018) SUMOylation of IE2p86 is required for efficient autorepression of the human cytomegalovirus major immediate-early promoter. J Gen Virol 99: 369-378

Hahn F, Fröhlich T, Frank T, Bertzbach L D, Kohrt S, Kaufer B B, Stamminger T, Tsogoeva S B, Marschall M (2018) Artesunate-derived monomeric, dimeric and trimeric experimental drugs – their unique mechanistic basis and pronounced antiherpesviral activity. Antiviral Res 152: 104-110

Reichel A, Stilp A C, Scherer M, Reuter N, Lukassen S, Kasmapour B, Schreiner S, Cicin-Sain L, Winterpacht A, Stamminger T (2018) Chromatin-remodeling factor SPOC1 acts as a cellular restriction factor against human cytomegalovirus by repressing the major immediate early promoter. J Virol 92: e00342-18

A72 - Progress Report

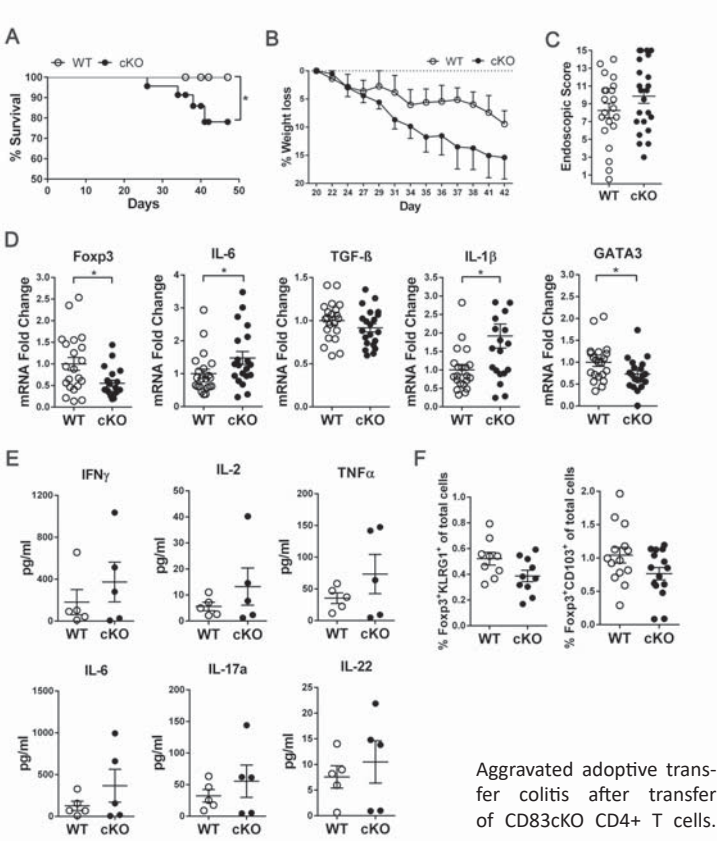
01/07/2016 - 30/06/2019

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

Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation

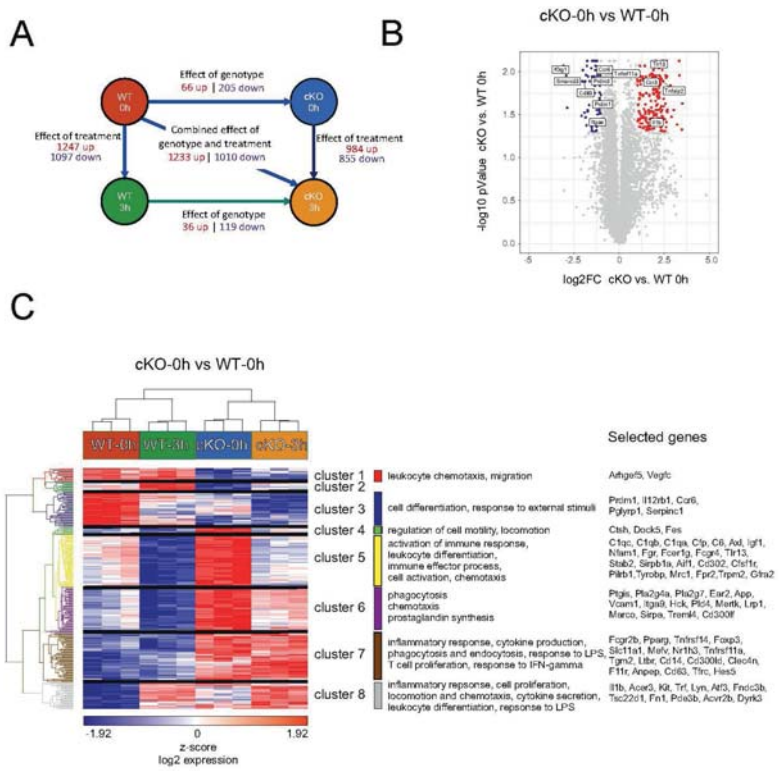
Regulatory T cells (Tregs) are crucial players to maintain immune homeostasis, to establish tolerance mechanisms and to prevent autoimmunity. Previously we showed that activated murine as well as human Tregs express the cell surface molecule CD83, indicating that this molecule is functionally important. Within the last year we elucidated the biological function of CD83 expression on Tregs using our Treg-specific CD83 conditional knockout (CKO) animals.

Upon activation, Tregs are transferred into an effector state expressing transcripts essential for their suppressive activity, migration and survival. However, how different intrinsic and environmental factors control differentiation is not completely understood. In order to investigate the specific role of CD83 exclusively on Tregs we generated conditional KO mice (Foxp3Cre CD83flox/flox) using the Cre-loxP system. Using these mice, we present for the first time data showing that Treg intrinsic expression of CD83 is essential for Treg differentiation upon activation. Interestingly, mice with Treg intrinsic CD83 deficiency are characterized by a pro-inflammatory phenotype. Furthermore, the loss of CD83 expression by Tregs leads to the downregulation of Treg specific differentiation markers and the induction of an inflammatory profile. Next, for in vivo analyses we used the EAE model, which is the best animal model to study the early inflammatory phase of multiple sclerosis (MS). These data revealed that animals, which do not express CD83 on their Tregs, show (i) an earlier and highly increased disease onset and (ii) a prolonged paralyses, indicating that the resolution of inflammation is critically impaired in CD83 CKO mice. These data clearly demonstrate that Treg specific CD83 CKO animals have a functional phenotype, supporting our hypothesis that CD83 is of critical importance for regulatory T cells. To further analyze these in vivo findings, we raised the question whether cKO Tregs can be equally expanded as WT Tregs upon activation in vitro. Thus, naïve CD4+CD25+CD62L+ T cells were cultured in the presence of IL-2 and anti-CD3/CD28 expansion beads and after 10 days, cKO Tregs showed equal ex-



Aggravated adoptive transfer colitis after transfer of CD83cKO CD4+ T cells.

Prof. Dr. Steinkasserer



CD83 deletion on Tregs leads to highly differential gene expression.
(A) Up- and downregulated genes. (B) Volcano plot comparing unstimulated cKO T cells versus WT Tregs (C) Hierarchical clustering.

pansion rates as WT Tregs. However, on mRNA level we detected increased IFNγ levels in cKO Treg cells and a downregulation of GATA3 expression levels. Thus, we conclude that anti-CD3/CD28 and IL-2 stimulated cKO Tregs can be equally activated and expanded, however, cKO derived Tregs showed an altered pro-inflammatory cytokine pattern. To analyze if the suppressive capacity of cKO Tregs is impaired using an additional in vivo model, total CD4+ T cells from WT or cKO mice were isolated and transferred into RAG1-/- mice. Strikingly, a strongly increased mortality rate in RAG1-/- mice, and an increased weight loss was observed. In addition an increased clinical severity score with higher inflammation was observed in cKO cell transferred animals compared to WT controls. Analysis of mesenteric lymph nodes showed a significant lower infiltration of Tregs, whereas pro-inflammatory cytokine production was significantly increased. In contrast, significantly reduced GATA3 expression levels were detected in cKO animals. Flow cytometric analyses of mLNs revealed reduced KLRG1+ and CD103+ expression levels among the Foxp3+ T cell population in cKO cell transferred mice. This indicates a reduced number of terminal differentiated Tregs in the gastrointestinal tract of RAG1-/- mice after adoptive CD4+ T cell transfer from CD83cKO mice. Altogether, CD83 expression in Treg cells is an essential factor for the development and function of effector Treg cells upon activation. Since Treg cells play a crucial role in the maintenance of immune tolerance and thus prevention of autoimmune disorders, our findings are also clinically relevant.

Contact:
Prof. Dr. Steinkasserer
phone: +49 9131 85 36725
e-mail: alexander.steinkasserer@uk-erlangen.de

Invited lectures

Research Seminar at the “EURAC”, 08.02.2018, Bozen, “Resolution of pathogenic inflammatory responses in autoimmunity”

Research lecture at the “32nd EMDS Annual Conference”, 28.09.2018, Verona, “Virus induced immune escape mechanisms and how the DC empire strikes back“

Publications during funding period

Döbbeler M, Koenig C, Krzyzak L, Seitz C, Wild A, Ulas T, Baßler K, Kopelyanskiy D, Butterhof A, Kuhnt C, Kreiser S, Stich L, Zinser E, Knippertz I, Wirtz S, Riegel C, Hoffmann P, Edinger M, Nitschke L, Winkler T, Schultze JL, Steinkasserer A, Lechmann M (2018) CD83 expression is essential for Treg cell differentiation and stability. JCI insight, 3(11). pii: 99712. doi: 10.1172/jci.insight.99712

A73 - Progress Report

01/07/2016 - 31/03/2019

Checkpoint inhibitors as adjuvants for viral vaccines

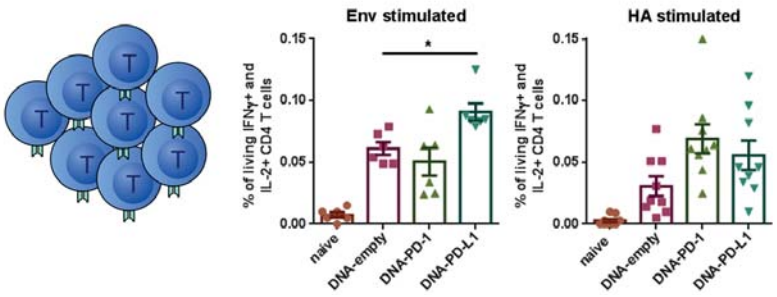
Prof. Dr. Klaus Überla, Institute of Clinical and Molecular Virology

Checkpoint inhibitors show great promise in improving immune control of cancer. How these antibodies affect the immune response to vaccines is unknown. Using a mouse model, we locally blocked immune checkpoints by co-expression of soluble PD-1 and PD-L1 ectodomains during DNA immunizations against HIV-1 Env and Influenza HA. This resulted in a significant upregulation of antigen-specific CD4 T cells secreting IFNγ and IL-2 and higher antibody titers.

Monoclonal antibodies targeting co-inhibitory molecules of the immune system show promising results in tumor therapy. These checkpoint inhibitors counteract immuno-suppressive signals from tumors and therefore improve control of tumor growth by the immune system. By blocking immune checkpoints via co-expression of PD-1 and PD-L1 ectodomains we modulated antigen-specific T cell and antibody responses induced by DNA-based vaccines.

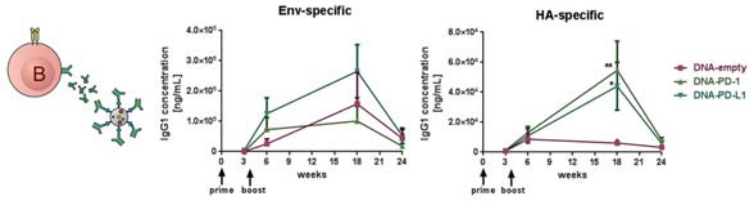
Effect of checkpoint inhibition on vaccine-induced CD4+ T cell responses

Blocking of PD-1 by co-administration of DNA encoding the ectodomain of PD-L1 induced a significant upregulation of Env-specific IFN-producing CD4+ T cells in comparison to immunization with Env-DNA only. As the HIV Env antigen induces an unusual IgG subtype response, we additionally used DNA vaccines encoding the Influenza HA and NP antigens. In contrast to the HIV Env immunization, PD-1 and to a lower extent PD-L1 encoding DNA applied together with the anti-Influenza DNA vaccine significantly increased the percentage of HA-specific CD4+ T cell expressing IFNγ and IL-2.



CD4+ T cell response after co-expression of the ectodomains of PD-1 and PD-L1. Env- and HA-specific IFNγ and IL2-producing CD4+ T cells after immune checkpoint blockade by co-expression of soluble immune checkpoints two weeks after intramuscular DNA immunization as determined by intracellular cytokine staining.

Prof. Dr. Überla



IgG1 antibody levels after blocking of immune checkpoints. Env- and HA-specific IgG1 antibody concentrations after prime-boost DNA immunization and checkpoint blockade by co-expression of soluble PD-1 and PD-L1 over a time-course of 24 weeks as determined by a quantitative serum-ELISA.

Modulation of antibody responses after checkpoint blockade

After HIV-1 or Influenza DNA prime-booster immunization regiments, we monitored the antigen-specific antibody responses over a time-course of 24 weeks. In contrast to the induction of antigen-specific T cell responses, co-expression of PD-L1 resulted only in slightly increased Env antibody titers. Interestingly, after both PD-1 and PD-L1 ectodomain co-expression HA antibody titers were significantly increased up to 14 weeks after the last immunization. The avidity of the antibody response seemed not to be affected by checkpoint inhibition.

Together, these data indicate that checkpoint inhibitors can modulate antigen-specific T helper cell and antibody responses after vaccination. Whether the induced responses lead to better protection after virus challenge remains to be elucidated.

Contact:
Prof. Dr. Überla
phone: +49 9131 85 23563
e-mail: klaus.ueberla@fau.de

Publications during funding period

none

A74 - Progress Report

01/06/2016 - 31/05/2019

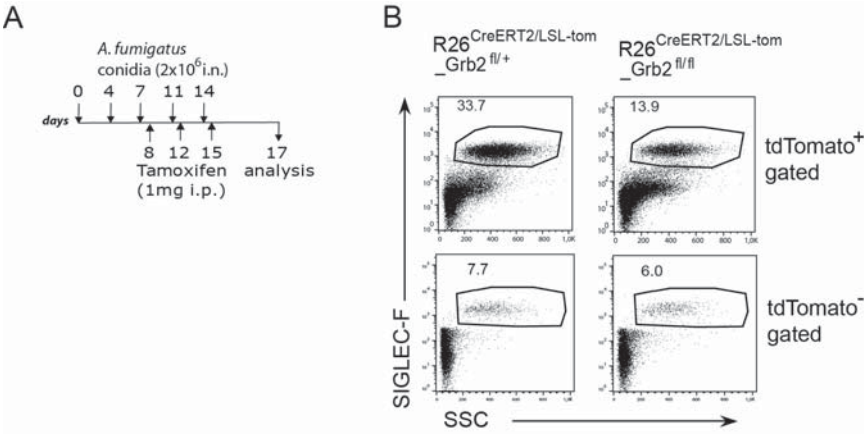
The Role of Eosinophils in Allergic Bronchopulmonary Aspergillosis

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

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

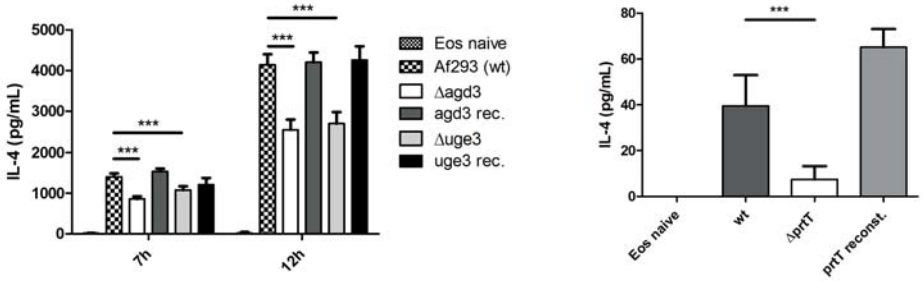
Using a mouse model with repetitive intranasal application of live or heat-inactivated A. fumigatus conidia, we could show that only live conidia elicited eosinophilia, expression of eosinophil-recruiting chemokines including eotaxins (CCL11 and CCL24), Th2 polarization, goblet cell hyperplasia and differentiation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

We further investigated the role of the adaptor protein Grb2 in eosinophils for establishment of lung eosinophilia in the ABPA model. Grb2 mediates signaling downstream of the IL-5 receptor but was also reported to induce apoptosis so that the overall effects of Grb2 function in eosinophils remained unclear. Here, we used conditional Grb2-deficient mice crossed to R26CreERT2/LSL-tom mice so that by administration of tamoxifen Grb2 is deleted in tdTomato+ cells. These mice were subjected to the ABPA model and we observed that Grb2 was requi-



The signaling adaptor Grb2 is required for lung eosinophilia in the ABPA model. A) experimental setup. B) Dot plots show the eosinophils (Siglec-F+SSChi) in the lung on day 17 after first intranasal A. fumigatus administration. Data are published in Eur J Immunol (2018) 48:1786.

Prof. Dr. Vöhringer Prof. Dr. Krappmann



Analysis of IL-4 secretion from eosinophils stimulated with different genetically modified strains of A. fumigatus. A) IL-4 concentrations of co-culture supernatants. Eosinophils were incubated with conidida of different fungal strains impaired in GAG formation with an MOI of 1:5 (E:T) for 7 and 12 h. B) IL-4 concentration of eosinophil culture, stimulated with culture supernatants of A. fumigatus wt, ΔprtT and reconstituted prtT strains for 24 h.**

red for accumulation of eosinophils in the lung. These results were published last year in the European Journal of Immunology (Willebrand et al. Eur J Immunol 48:1786).

To investigate fungal determinants of the host-pathogen interaction, in vitro experiments with different A. fumigatus mutant strains were performed. Previous studies could already show that fungal cell wall carbohydrate structures are involved in the interaction with different immune cell types. We could show that the recently discovered fungal exopolysaccharide galactosaminogalactan (GAG), which is responsible for adhesion processes, also influences the activation of eosinophils: A. fumigatus strains lacking GAG or that exhibit an impaired deacetylation of GAG adhere less to eosinophils. Furthermore, the release of IL-4 is significantly reduced after co-cultivating eosinophils with these mutant strains compared to co-cultures with the corresponding wild-type strain. Whether this effect is based on direct recognition of GAG or an indirect effect due to impaired adherence and thus a reduced pattern recognition receptor binding of other PAMPs than GAG needs to be explored in further experiments. Moreover, we became interested whether secreted proteases contribute to the eosinophil-fungus interaction. To address this issue, eosinophils were stimulated with culture supernatants of wild-type A. fumigatus and a strain which is deficient in the synthesis of extracellular proteases (ΔprtT). Whereas the supernatant of the wild type strain induced secretion of IL-4, this effect could not be observed for the knock-out strain, which indicates that also secreted fungal compounds, e.g. extracellular proteases, are able to activate eosinophils.

To identify novel fungal determinants in the eosinophil-fungus interaction, an A. fumigatus transcription factor (TF) deletion library is tested in an ongoing screening approach. Here we analyze the fungal susceptibility towards the antimicrobial activity of eosinophils as well as the influence of the TF-encoding gene deletion on the capability to activate eosinophils. In further hit picking experiments potential TF candidates will be analyzed in more detail with respect to their influence on ABPA pathogenesis.

End of last year we submitted a DFG grant application based on our preliminary results from this IZKF-funded project to continue our research on the role of eosinophils in ABPA for the next three years.

Contact:
Prof. Dr. Vöhringer
phone: +49 9131 85 32735
e-mail: david.voehringer@uk-erlangen.de

Prof. Dr. Krappmann
phone: +49 9131 85 22580
e-mail: sven.krappmann@uk-erlangen.de

Publications during funding period

Willebrand R, Dietschmann A, Nitschke L, Krappmann S, Voehringer D (2018) Murine eosinophil development and allergic lung eosinophilia are largely dependent on the signaling adaptor GRB2. Eur J Immunol. 48: 1786-1795

A75 - Progress Report

01/07/2016 - 30/06/2019

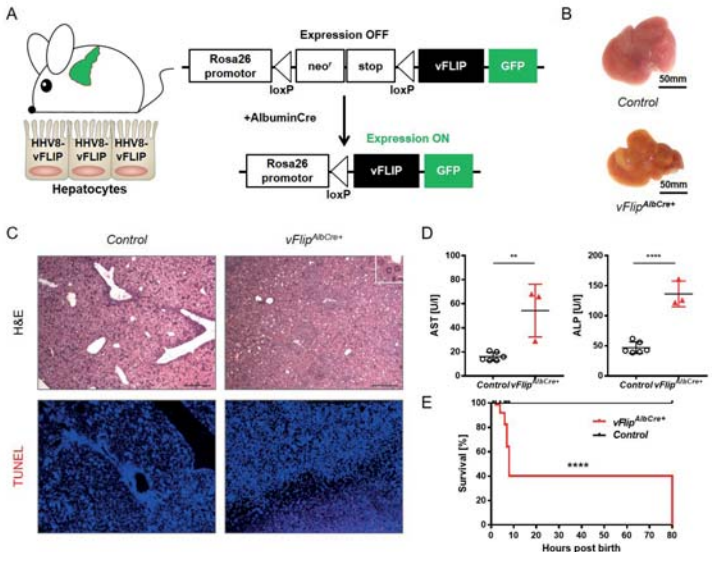
Role of MLKL-dependent programmed necrotic cell death in the pathogenesis of hepatitis

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

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

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

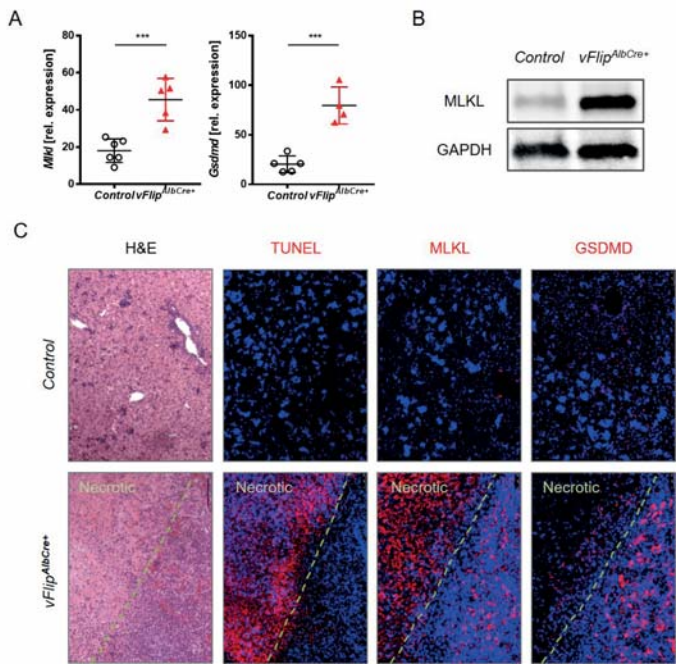
To evaluate the contribution of MLKL to hepatocellular necrosis-induced liver dysfunction we developed a new mouse model characterized by acute, cell death mediated liver failure. Thereby, we took advantage of the fact that several viruses produce distinct effector proteins interfering with the host-cell death. In this process, caspase-8 represents a decisive 'checkpoint' in anti-viral response due to its function as potential apoptosis initiator. Therefore, many viruses like the human herpesvirus 8 (HHV8), also known as Kaposi sarcoma, produce proteins directly inhibiting caspase-8 activation. To elucidate the impact of a single viral protein – with the potential to interfere with the host-cell death response – on liver homeostasis in vivo, we generated mice constitutively expressing vFLIP in hepatocytes. Surprisingly, we uncovered that this single viral protein is sufficient to cause severe liver injury exhibiting histopathological characteristics of acute viral hepatitis and acute-on-chronic liver failure including yellowish discoloration of the liver and extended areas of necrosis and extensive loss of hepatocytes. Accordingly, hepatocel-



Transgenic expression of vFLIP induces massive hepatocellular death (A) Mice expressing vFLIP in hepatocytes. (B) Livers 80h post birth. (C) H&E and TUNEL stained liver cross sections. (D) Aspartate transaminase (AST) and Alkaline Phosphatase (ALP) serum levels. (E) Survival analysis.

PD Dr. Dr. Günther

PD Dr. Dr. Wirtz



vFLIP expression triggers non-apoptotic regulated necrosis in hepatocytes (A) mRNA expression in livers of control and vFlipAlbCre+ mice (B) Western Blot analysis with liver lysates. (C) H&E TUNEL, MLKL and GSDMD staining of neonatal liver cross-sections.

lular necrosis was accompanied by elevated serum aminotransaminase levels and culminated finally in severe liver dysfunction and perinatal lethality of vFlipAlbCre+ animals. Furthermore, we found that hepatocellular necrosis was associated with strong upregulation of key mediators of regulated necrosis such as Mlkl and Gsdmd, which both induce membrane breakdown and a necrotic cell death. Interestingly, immunohistochemistry revealed that MLKL staining was more pronounced in necrotic areas than in adjacent cells, while GSDMD, an effector protein of pyroptosis, was particularly increased in close proximity to necrotic areas. Importantly, these mice not only displayed severe hepatocellular death but also loss of intrahepatic bile ducts (IHBD) and impaired and defective bile acid production and transport, as well as lipid peroxidation with a direct effect on the liver-gut axis.

Contact:
PD Dr. Dr. Claudia Günther
phone: +49 9131 85 35909
e-mail: c.guenther@uk-erlangen.de

Invited lectures

Stefan Wirtz, EAACI, 27.05.2018, München, Regulation of group 2 innate lymphoid cells via IL-27 and interferon signalling

Awards

Thiersch Award, Claudia Günther, 04.11.2018, FAU Erlangen-Nürnberg

Publications during funding period

Ruder B, Murtadak V, Stürzl M, Wirtz S, Distler U, Tenzer S, Mahapatro M, Greten FR, Hu Y, Neurath MF, Cesarman E, Ballon G*, Günther C*, Becker C* (2018) Chronic intestinal inflammation in mice expressing viral Flip in epithelial cells. Mucosal Immunol 11(6): 1621-1629 * These authors equally contributed

Hefele M, Stolzer I, Ruder B, He GW, Mahapatro M, Wirtz S, Neurath MF, Günther C (2018) Intestinal epithelial Caspase-8 signaling is essential to prevent necroptosis during Salmonella Typhimurium induced enteritis. Mucosal Immunol 11: 1191-1202

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

He GW*, Günther C*, Kremer AE, Thonn V, Amann K, Poremba C, Neurath MF, Wirtz S, Becker C (2016) PGAM5-mediated programmed necrosis of hepatocytes drives acute liver injury. Gut 66: 716-723* These authors equally contributed

D23 - Final Report

01/01/2016 - 31/12/2018

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

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

The project aims to determine the effect of bone marrow adipocytes in the melanoma bone metastatic niche using a high-fat diet fed mouse model (Chen G et al. 2016). We found that tdTomato expressing B16 cells show a superior staining index when compared to GFP and that tdTomato expressing B16 cells can be quantified using in-vivo imaging following intra-tibial injection. In addition, we discovered that HFD feeding regulate NK cell numbers in the bone marrow of mice injected with B16 cells.

tdTomato offers superior staining index and signal strenght compared to GFP when stably transfected into B16 cells.

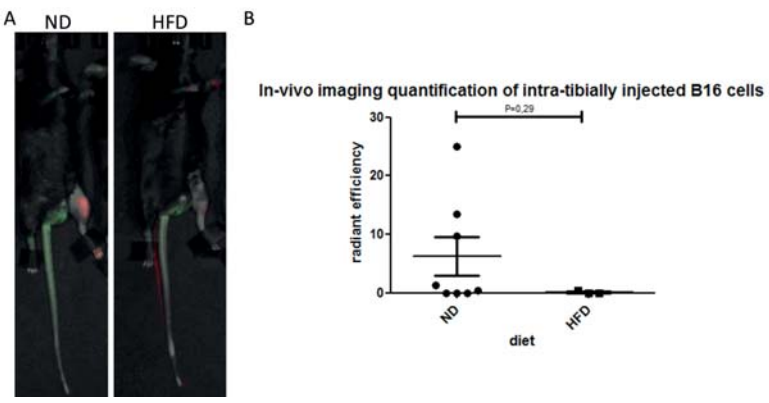
Flow cytometric comparisons of cells stably transfected with either eGFP of tdTomato showed that the cells transfected with tdTomato showed a greatly increased signal strenght and a significantly improved separation between positive and negative populations in-vitro.

B16 expressing tdTomato are identifiable after intra-tibial injection using in-vivo fluorescence imaging.

We found that, in contrast to previous experiments using eGFP expressing B16 cells, when B16 cells were stably transfected with tdTomato the cells were detectable using in-vivo imaging following intra-tibial injection.

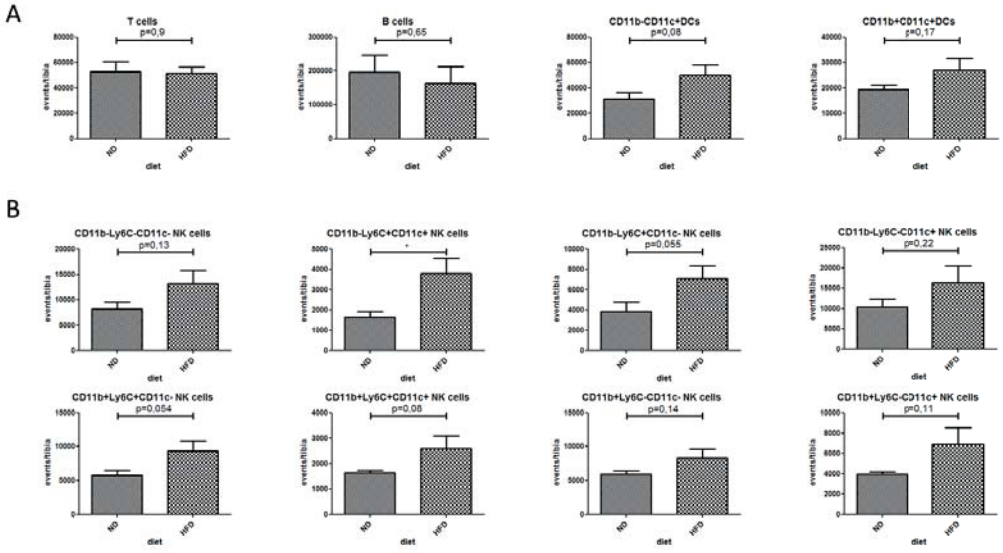
High-fat diet fed mice show decreased amounts of NK cells in the bone marrow niche.

Flow cytometry analysis of the bone marrow tumor niche revealed that the mice fed with high-fat diet have an increased amount of NK cells present in the tumor niche. No differences in the number of B and T cells were observed in the bone marrow tumor niche suggesting that the adaptive immune system is not involved in our model.



(A) In vivo imaging of B16 cells (red) and background (green). (B) Graph of tumor volume excluding statistically significant (p=<0,05) outliers.

Prof. Dr. Bozec



(A-B) Numbers of B, T, NK, and Dendritic cells (DCs) in the bone tumor niche excluding mice without tumor incidence (<100 GFP+ events).

Contact:
Prof. Dr. Bozec
phone: +49 9131 85 29002
e-mail: aline.bozec@uk-erlangen.de

Invited lectures

Keystone meeting, 13.06.2018-18.03.2018, Snowbird Resort USA, High-Fat Diet and Microbiota Control the Bone Marrow Niche and Hematopoietic Stem Cell Differentiation

Osteologie meeting, 08.03.2018-10.03.2018, Dresden, Introduction of osteoimmunology

Publications during funding period

Luo Y, Grotsch B, Hannemann N, Jimenez M, Ipseiz N, Uluckan O, Lin N, Schett G, Wagner E, Bozec A (2018) Fra-2 expression in osteoblasts regulates systemic inflammation and lung injury through osteopontin. Molecular and Cellular Biology. doi:10.1128/MCB.00022-18

Hannemann N, Jordan J, Paul S, Reid S, Baenkler HW, Sonnewald S, Bäuerle T, Vera J, Schett G, Bozec A (2017) The AP-1 Transcription Factor c-Jun Promotes Arthritis by Regulating Cyclooxygenase-2 and Arginase-1 Expression in Macrophages. J. Immunol. 198: 3605–3614

Chen G, Luo Y, Eriksson D, Meng X, Qian C, Bäuerle T, Chen X, Schett G, Bozec A (2016) High fat diet increases melanoma cell growth in the bone marrow by inducing osteopontin and interleukin 6. Oncotarget 7: 26653–26669

D24 - Progress Report

01/06/2016 - 31/05/2019

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

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

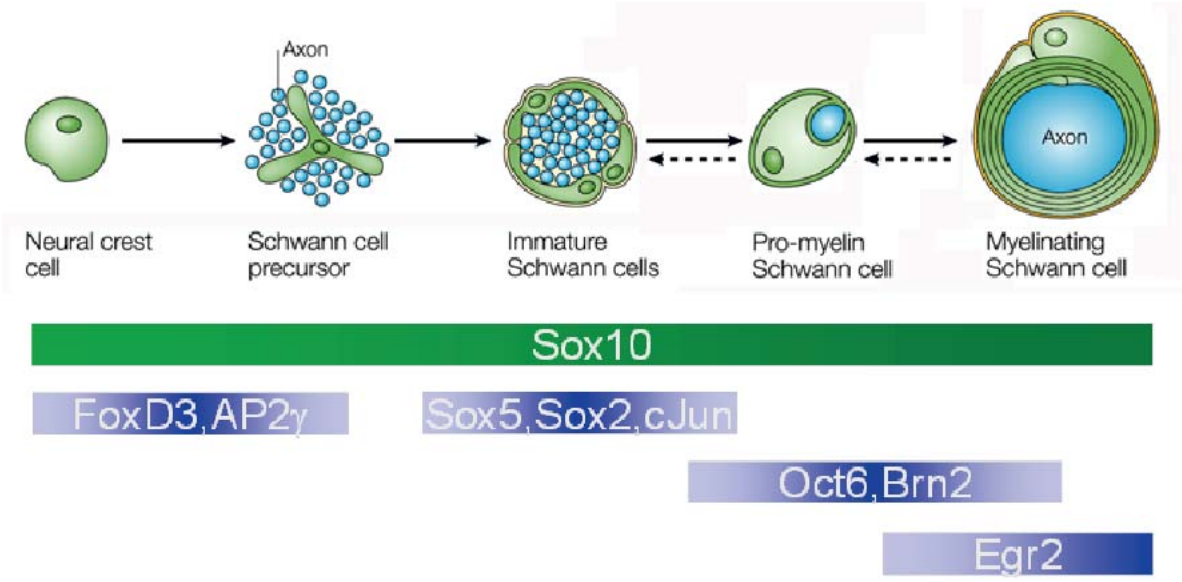
Melanoma is an aggressively disseminating cancer with continuously rising incidence. Melanoma cells derive from melanocytes, which originate from the neural crest and display characteristics of cells of the nervous system. Interestingly, Schwann cells, nervous system cells derived from the neural crest,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

Aims in this project:

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

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

At the beginning of the project we started to determine, which of the transcription factors that are important in Schwann cells during development and in the adult differentiated cells, are deregulated in melanoma development or progression. We were able to define several Schwann cell transcription factors as strongly deregulated in melanoma cell lines and in tissue material compared to melanocytes including TFAP2C and EGR2.



Transcription factors involved in differentiation of Schwann cells.

Prof. Dr. Bosserhoff Prof. Dr. Wegner

In subsequent studies, we have focused on the transcription factor EGR2, which is most strongly deregulated, and analyse its specific impact on melanoma in detail. EGR2 is one of the key drivers of myelination in Schwann cells, where it is induced in a Sox10-dependent manner early during terminal differentiation and then regulates (in cooperation with Sox10) target genes that code for structural proteins of the myelin sheaths or for proteins involved in lipid metabolism. However, regarding melanoma few data are available so far. Upon EGR2 downregulation via siRNA, melanoma cells reduced their rate of proliferation and their migratory potential. In clonogenic assays, the number and size of colonies was significantly altered upon knock-down of EGR2. These findings point towards a possible role of EGR2 in melanoma progression. To get an initial idea whether EGR2 influences lipid metabolism in melanoma cells as it does in Schwann cells, we analyzed the triglyceride content in melanoma cells. Upon treatment with siRNA against EGR2, the triglyceride amount was substantially reduced in three independent melanoma cell lines supporting the assumption that EGR2 is at least in part responsible for the high triglyceride levels in melanoma. An important task will now be the definition of target genes of EGR2 in melanoma and Schwann cell lines. Here, we are following up on the idea of an impact on myelination of these factors. We are planning to generate melanoma and Schwann cell lines in which EGR2 is deleted by CRISPR/Cas9. The resulting transcription factor-deficient cell lines will be compared in their expression profile to the original ones and between melanoma and Schwann cell line.

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

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

Contact:

Prof. Dr. Bosserhoff
phone: +49 9131 85 24190
e-mail: anja.bosserhoff@fau.de

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

Invited lectures

Hinterzartener Kreis Meeting, Cadenabbia, Italy, 11.5.2018, Non-coding RNAs and Cancer (AB)

Society of Melanoma Research Meeting, Manchester, Great Britain, 26.10.2018, Wild-type KRAS as a novel player in melanoma (AB)

Gordon Research Conference on Myelin, Ventura, CA, USA, 18.03.-23.03.2018, Myelination in early childhood (MW)

Publications during funding period

none

D25 - Progress Report

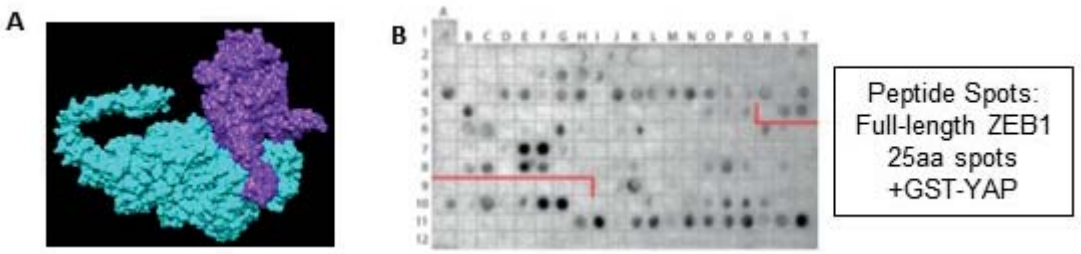
01/05/2016 - 30/04/2019

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

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

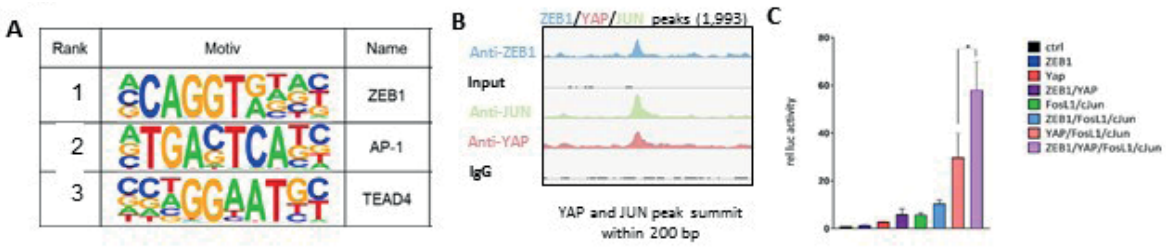
The EMT-program provides cancer cells with motility, invasiveness and stem cell features. A major EMT inducer is the transcriptional ZEB1. However, many of the underlying molecular mechanisms of its tumor promoting effects are unknown. To clarify the versatile functions of ZEB1, we validate, verify and map interactions with novel interaction partners identified by MassSpec and ChIP-Seq analyses. We further investigate their relevance for Zeb1 function and cancer progression.

We could show that under certain conditions - e.g. in an oncogenic context of cancer cells – the oncogenic factor ZEB1 can switch from a transcriptional repressor of epithelial genes to a transcriptional activator of tumor promoting genes. We proposed novel nuclear interaction partners and previously exemplified this by showing interaction with YAP1, a main effector of Hippo signalling, to activate a specific common target gene set. In order to identify additional coactivators of ZEB1, we had performed Co-IPs from nuclear extracts of aggressive cancer cells, coupled to mass-spec and proteomic analyses and had detected about 20 unknown nuclear co-factors of ZEB1. Among the top 5 identified putative co-factors of ZEB1 were the nuclear EGFR and STAT3, AP-1 factors and YAP1. In the first year of funding, we confirm the nuclear interactions of EGFR and Stat 3 with Zeb1 by applying CoIPs and proximity ligation assays in tumor cells and IL6-activated fibroblasts. In parallel we started analyses assessing a functional cooperation to activate a common target genes set. In addition we further mapped the detected interaction of ZEB1 with the Hippo-Pathway effector YAP1, e.g. by applying an in silico modelling approach (in cooperation with Dr. Viji Mahadevan, Bangalore, India). Their functional interaction was also confirmed by detection of the TEAD-motif (TEAD=YAP1 DNA-binding partner) as one of the top3 DNA binding motifs in a ZEB1 ChIP-Seq.Furthermore a second unbiased strategy was applied to identify functional cooperation partners of Zeb1 in promoting tumor progression. To this end we analysed Zeb1 ChIP-Seq-data sets (performed partially with support of the IZKF high tech pool) allowing the identification candidate interaction partners on tumor cell enhancers and promoters. Motif searches on Zeb1 ChIP Seq peaks revealed a strong overlap of with binding sites for the transcription factors ZEB1, AP-1 (Jun/Fos) and Tead (confirming the interaction with the Tead partner YAP1). Direct comparison of ChIP Seq peaks for Zeb1, Jun and Yap1 revealed a strong overlap in target enhancers and promoters. Their functional cooperation to commonly activate tumor promoting genes was further validated, e.g. in reporter assays.



(A) In silico medelling of the 3D interaction between ZEB1 (green) and YAP1 (lilac) (B) Validation of the proposed interaction domains and further characterization the interactions motifs by peptide spots.

Prof. Dr. Brabletz



(A) Zeb1 ChIP-Seq peaks enrich for AP-1 (Jun/Fos) and Tead/Yap motives. (B) Strongly overlapping peaks after ChIP-Seqs for Zeb1, Jun and Yap1 on the promoter of a putative, tumor-promoting target genes. (C) Functional validation of the interaction of all three factors on the target gene promoter using reporter assays.

Contact:
Prof. Dr. Brabletz
phone: +49 9131 85 29104
e-mail: thomas.brabletz@fau.de

Invited lectures

Jubiläumssymposium - 275 Jahre FAU, 19.01.2018, Erlangen, Germany, Mechanisms of Tumor Metastasis

Jahrestagung des Deutschen Pankreasclub, 26.01.2018, Universitätsklinikum Ulm, Germany, Cellular plasticity in cancer: driving force and therapeutic target

ADELIH congress „The Origins of Cancer: once upon a cell", 30.03.2018, Inst. Curie, Paris, France, Cellular plasticity in cancer: driving force and therapeutic target

Annual meeting of the „Hinterzarten Circle on Cancer Research" of the DFG: „From Molecular Mechanisms to Cancer Therapy", 11.05.2018, Cadenabbia, Italy, Cellular plasticity in cancer: driving force and therapeutic target

Jahrestagung der Deutschen Gesellschaft für Pathologie, 25.05.2018, Berlin, Germany, Cellular plasticity in cancer: driving force and therapeutic target

Jahrestagung der Deutschen Gesellschaft für Pathologie, 26.05.2018, Berlin, Germany, EMT and tumor evolution

Cambridge Seminars in Oncology, 05.06.2018, Univ. Cambridge, United Kingdom, Cellular plasticity in cancer: driving force and therapeutic target

International Congress of the Metastasis Research Society (MRS), 03.08.2018, Princeton University, Princeton, USA, Cellular plasticity in cancer: driving force and therapeutic target

Chirurgische Forschungstage, 06.09.2018, Universitätsklinikum Erlangen, Germany, Cellular plasticity in cancer: driving force and therapeutic target

Cancer and Stem Cells 2018 Symposium, 14.09.2018, The European Cancer Stem Cell Research Institute, Cardiff, United Kingdom, Cellular plasticity in cancer: driving force and therapeutic target

DGZ International Meeting, 18.09.2018, Universität Leipzig, Germany, Cellular plasticity in cancer: driving force and therapeutic target

Nobel Conference 2018, Epithelial-Mesenchymal Plasticity in Cancer Metastasis, 14.12.2018, Karolinska Institute, Stockholm, Sweden, Cellular plasticity in cancer: driving force and therapeutic target

Awards

German Cancer Award 2018, Thomas Brabletz, 22.02.2018 in Berlin

Poster Award of the IZKF Erlangen 2018, Nora Feldker, 17.10.2018 in Erlangen

Publications during funding period

none

D26 - Final Report

01/01/2016 - 31/12/2018

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

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

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

Breast cancer is the most common tumor in women with an annual incidence of 75,000 women in Germany. The tumor is classified based on expression of hormone receptors (estrogen or progesterone-receptor) and HER2. About 15-20% of breast cancer do neither express HER2 nor hormone receptors [triple-negative breast cancer (TNBC)]. 15-30% strongly express HER2. These entities are biologically more aggressive as compared to hormone-receptor positive tumors. The lack of surface expression of hormone receptors and HER2 in TNBC has an additional negative impact on therapeutic options. Interestingly, several studies have shown that the density of the T-lymphocyte infiltrate in the primary tumor has a strong positive prognostic value. These data indicate immunogenicity of the tumor and suggest that T-cell based immunotherapy could be a promising therapeutic approach. To allow highly potent cellular immunotherapy, it would be desirable to identify tumor-specific antigens as e.g. in tumor specific mutations.

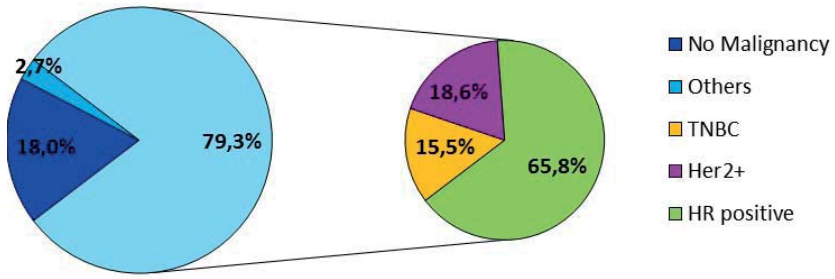
We therefore aimed to identify the targets of tumor-infiltrating T-cells in TNBC and HER2+ breast cancer with special emphasis being placed on tumor-specific mutations.

Characterization of tumor-infiltrating immune cells

We so far collected and analyzed the infiltrating immune cells of more than 400 patients. 50 (15,5%) were derived from patients with initial diagnosis of TNBC, while 60 (18,6%) were HER2+ tumors.

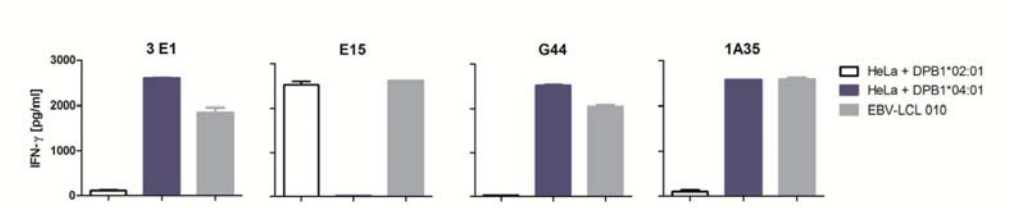
Expansion of tumor-infiltrating T-lymphocytes

T-lymphocytes in biopsies derived from TNBC and HER2+ were expanded in vitro to average cell number of around 50-60 million T-lymphocytes within 2-3 weeks.



Frequencies of malignancy and subtypes of breast cancer in lump biopsies.

Prof. Dr. Mackensen Prof. Dr. Fasching



Reactivity of T-cell clones 3E1, E15, G44 and 1A35 against tumor specific mutant peptide (P28) loaded on HeLa cells transduced with the indicated HLA molecules and autologous EBV-LCL as tested by INF-γ ELISA.

Whole genome sequencing of tumor and reference DNA

So far whole genome sequencing has been performed for reference and tumor DNA of 3 patients. Bioinformatics revealed 28, 26, and 65 potential neoeptiopes based on tumor specific, missense mutations within the coding region and analyzed for binding to the patient's HLA type.

Identification of neoantigen specific T-cell clones

We analyzed T-cell reactivities of 3 patients by co-culturing expanded TILs with peptide loaded autologous dendritic cells and sorting for activated T-cells. In one patient we could not find any peptide specific T-cells. In another patient, we found 4 peptide reactive T-cell clones. All four clones were CD4 T-cells reactive against the same tumor-specific peptide (P28) presented in two different HLA-DP molecules. The wildtype counterpart was not recognized. Full length mutated antigen was processed and presented if retrovirally transduced in autologous EBV-LCL or breast cancer cell line MCF-7. In the third patient we found 3 CD8 T-cell clones recognizing a tumor-specific peptide (P13) in HLA-A*11 and one CD4 T-cell clone reactive against peptide P35.

In conclusion, we successfully identified tumor-specific T-cell clones within the population of tumor-infiltrating lymphocytes in TNBC and HER2+ breast cancer. This gives the opportunity to treat these patients with personalized immunotherapy either by vaccination or adoptive T-cell transfer.

Contact:
Prof. Dr. Mackensen
phone: +49 9131 85 35954
e-mail: andreas.mackensen@uk-erlangen.de

Prof. Dr. Fasching
phone: +49 9131 85 33553
e-mail: peter.fasching@uk-erlangen.de

Publications during funding period

Würfel F, Erber R, Huebner H, Hein A, Lux MP, Jud S, Kremer A, Kranich H, Mackensen A, Häberle L, Hack CC, Rauh C, Wunderle M, Gaß P, Rabizadeh S, Brandl AL, Langemann H, Volz B, Nabieva N, Schulz-Wendtland R, Dudziak D, Beckmann MW, Hartmann A, Fasching PA, Rübner M (2018) TILGen: A Program to Investigate Immune Targets in Breast Cancer Patients - First Results on the Influence of Tumor-Infiltrating Lymphocytes. Breast Care (Basel) 13:8-14

Erber R, Hartmann A, Beckmann MW, Mackensen A, Kremer A, Reimann H, Hübner H, Hein A, Lux MP, Jud S, Häberle L, Gaß P, Volz B, Schulz-Wendtland R, Rübner M, Fasching PA (2018) TILGen study-immunological targets in patients with breast cancer: Influence of tumor-infiltrating lymphocytes. Pathologe. Nov 8. doi: 10.1007/s00292-018-0526-7 [Epub ahead of print]

D27 - Progress Report

01/07/2016 - 30/06/2019

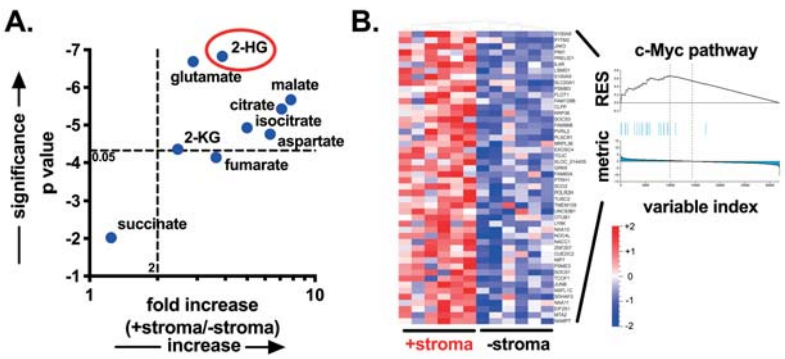
2-Hydroxyglutarate in Acute Myeloid Leukaemia: Novel Molecular Targets and Impact on Immune Escape

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

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

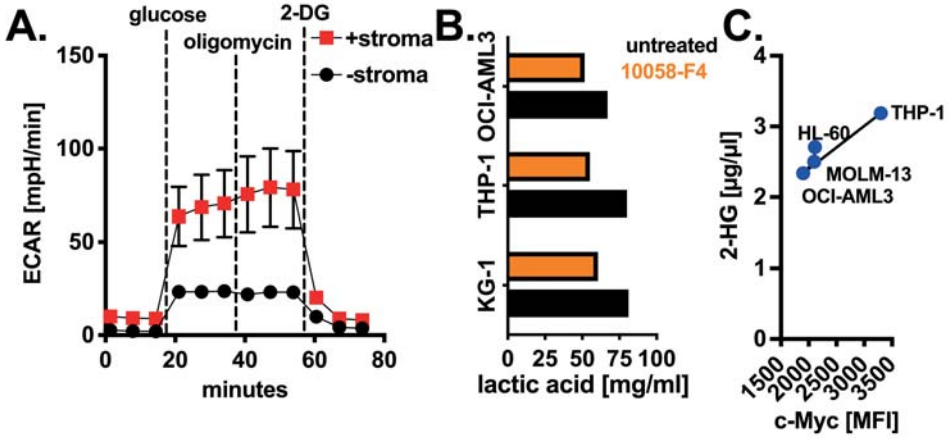
Our findings using mass spectrometry show an increased 2-HG production in patient-derived AML-cells carrying wild-type IDH1/2 after being cultured in presence of human bone marrow-derived stromal cells. We could recapitulate those observations when testing AML cell lines that are negative for IDH mutations. Co-culturing AML-cells isolated from different patients with stromal cells led to marked alterations of their gene expression profile. Among the significantly upregulated genes we found several candidates belonging to the c-Myc signaling pathway. In line, c-Myc protein levels were also significantly increased. Similar to observations from other disease models 2-HG accumulation was associated with a HIF1a stabilization and an increase of intracellular ROS levels, which (together with the c-Myc upregulation) could contribute as a so-called "pseudohypoxic response" to the marked glycolytic skewing. The role of c-Myc for such metabolic transition is further underscored by experiments revealing a reduced lactic acid production (indicative for aerobic glycolysis) when treating (c-Myc+) AML cell lines with the pharmacological c-Myc inhibitor 10058-F4. In fact, our data suggest that the cells' c-Myc levels might correlate with the intracellular content of 2-HG in the tested AML cell lines and in analogy to previous observations from studies in breast cancer and multiple myeloma.

Accumulation of cancer-associated metabolites such as ROS or lactic acid has been previously linked to immune modulation. Therefore, we were interested whether 2-HG holds potential immune regulatory properties. We found that exogenously applied 2-HG was readily taken up by T-cells. While viability, proliferation, and IFNγ production were mainly un-



AML-cells were cultured +/- stromal cells. (A) Metabolites were quantified by mass spectrometry (n=6). (B) Microarray analyses were performed and top upregulated genes in AML-cells in presence of stromal cells are shown as a heat map together with a GSEA enrichment plot for the c-Myc pathway.

Prof. Dr. Mougiakakos



(A) Extracellular acidification rate (ECAR, Seahorse Flux Analyzer) was measured in AML-cells +/- stromal cells (n=5). (B) C-Myc+ AML cells were treated with the c-Myc inhibitor 10058-F4 and lactic acid measured. (C) C-Myc expression and 2-HG concentrations were assessed in AML-cells. **, p<0.01.

affected by 2-HG, bioenergetics of activated T-cells shifted away from aerobic glycolysis towards respiration. This is at least partly explained by the observed 2-HG-triggered HIF1a destabilization and despite the upregulation of genes linked to adaptation towards tissue hypoxia. In line with previous findings that HIF1a-dependent (aerobic) glycolysis orchestrates differentiation of Th17 cells, we found a reduced Th17 polarization in presence of 2-HG together with lower levels of circulating Th17 cells in AML patients. These results were in parts recently published (Böttcher M. et al., 2018). Furthermore, various tolerogenic cell subsets including TRegs and MDSCs are overrepresented in cancer patients (including AML) thus contributing to immune evasion. In fact, metabolites such as lactic acid or ROS have been shown to create a local permissive environment that promotes induction and/or survival of such immune regulators. We observed a significant 2-HG-triggered reduction of HLA-DR levels on CD14+ monocytes resembling a CD14+HLA-DRlo MDSC-like phenotype, which is abundantly found in AML patients. Overall, our data indicates that 2-HG might be involved in the promotion of immune regulatory cells of lymphoid and myeloid origin.

Contact:
Prof. Dr. Mougiakakos
phone: +49 9131 85 43172
e-mail: dimitrios.mougiakakos@uk-erlangen.de

Invited lectures

SFB850 Seminar, 04.05.2018, Freiburg, Stroma cells: an immune metabolic hub?

GvH/GvL Meeting, 08.03.2018, Regensburg, Impact of ROS on GvL

Publications during funding period

Böttcher M, Renner K, Berger R, Mentz K, Thomas S, Cadenas EZ, Dettmer K, Oefner PJ, Mackensen A, Kreutz M, Mougiakakos D (2018) D-2-hydroxyglutarate interferes with HIF-1a stability skewing T-cell metabolism towards oxidative phosphorylation and impairing Th17 polarization. OncoImmunology 7(7): e1445454

D28 - Progress Report

01/02/2016 - 31/01/2019

SPARCL1 function in vessel maturation and metastasis of colorectal carcinoma

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

In previous work we demonstrated tumor microenvironment (TME)-dependent heterogeneity of tumor endothelial cells (TECs) in colorectal carcinoma (CRC) and identified SPARCL1 as an important regulatory molecule of TME-associated vessel homeostasis and vascular-derived inhibition of tumor growth. Here we investigated the functions and underlying mechanisms of SPARCL1 in physiological angiogenesis.

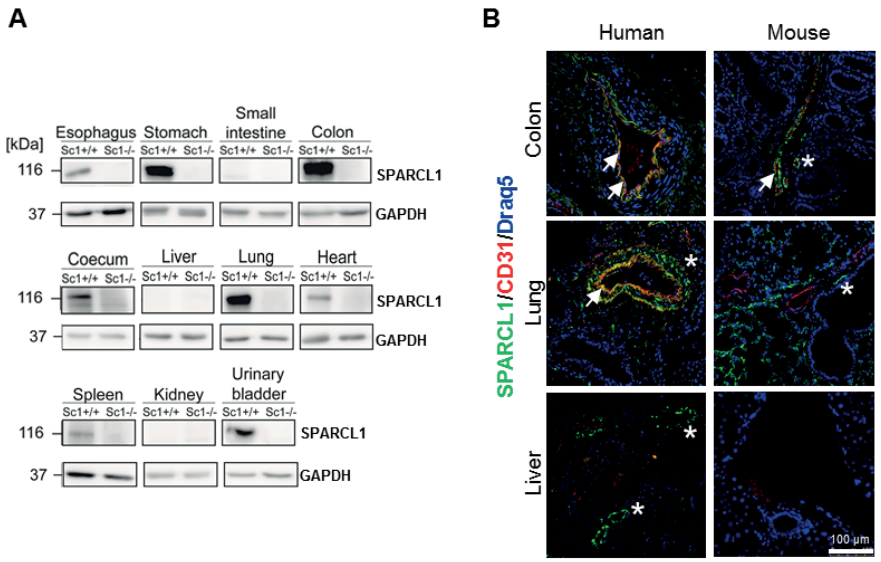
The specific aims of the project are

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

Wild type recombinant human SPARCL1 (hSPARCL1) has been successfully cloned and was purified from supernatants of human eukaryotic cells. The protein showed similar anti-angiogenic activity compared to commercially recombinant hSPARCL1. Subdomains of hSPARCL1 were cloned and purified and are presently used for structure-function analysis of the anti-angiogenic activity of hSPARCL1. CD105/endoglin was identified as a cellular receptor of hSPARCL1 in HeLa cells.

Aim 2: Mapping of SPARCL1 expression between different species

For subsequent mechanistic studies in mice, SPARCL1 expression was systematically analyzed in different human and mouse tissues, both, by western blot and at the single cell level by immunofluorescence analyses. Murine SPARCL1 (mSPARCL1) was most strongly expressed in the lung, colon and stomach, intermediately in esophagus, coecum, heart, spleen, urinary bladder and absent in the liver, kidney and small intestine. Immunofluorescence analyses in humane tissues showed coherently with the results obtained in mice a strong expression of hSPARCL1 in in stomach, large intestine and lung. Interestingly,



SPARCL1 is expressed in a species- and cell type dependent manner. (A) SPARCL1 expression in SPARCL1 wt (Sc1+/+) and KO (Sc1-/-) mice by western blot. (B) SPARCL1 (green) and CD31 (vessels) double IHC of human and mouse tissues.


Prof. Dr. Stürzl


PD Dr. Naschberger

SPARCL1 expression in mouse tissues was mostly associated with mural cells whereas in human tissues it was expressed in both, mural and endothelial cells. These analyses revealed that SPARLC1 expression is strongly associated with vascular cell types and exhibits organ specific variations.

Aim 3: Impact of SPARCL1 in physiological angiogenesis

In order to analyze the impact of SPARCL1 on angiogenesis/vessel maturation in vivo the metatarsal angiogenesis assay (cooperation Ramming/Wohlfahrt, Med3) has been established. Metatarsal bones from embryos of wild type SPARCL1 animals were explanted at E18.5 and cultivated under conditions allowing outgrowth of endothelial sprouts with a supporting feeder layer. An in vivo inhibition of vessel sprouting by addition of recombinant mSPARCL1 was detected. Moreover, metatarsals from SPARCL1-ko mice showed altered vessel morphology with dilated and fused vessels characteristically associated with reduced vessel maturation and increased permeability. In accordance with this, an increased vessel permeability to FITC-dextran was detected in the colon of SPARLC1 ko mice. These results suggested that SPARCL1 regulates physiological vessel function.

Aim 4: Impact of SPARCL1 on prognosis and therapy response of patients with CRC

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

Contact:
PD Dr. Naschberger
phone: +49 9131 85 39524
e-mail: elisabeth.naschberger@uk-erlangen.de

Publications during funding period

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D29 - Final Report

01/01/2016 - 31/12/2018

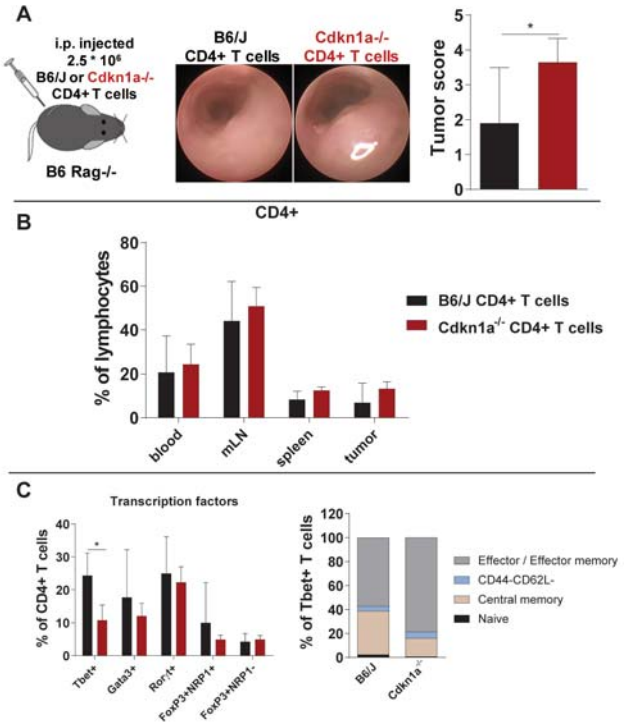
Aging and senescence of the adaptive immune system in colorectal cancer

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

An aging-related accumulation of genomic damage during lifetime has been described as one of the risk factors for colorectal cancer (CRC) development. Furthermore, aging is known to influence the immune system as well, as tumor-infiltrated T cells with short telomeres can lead to a worse prognosis in CRC. This project aims to evaluate the functional role of aging and cellular senescence in immune cells in the response against CRC.

Aging is defined as a time-dependent loss of cellular and molecular function that can lead to disorders such as cardiovascular diseases, Alzheimer and cancer. During lifetime, homeostasis can be affected by the accumulation of genomic alterations such as loss of telomeres, oxidative stresses or DNA damage. Upon structural and functional changes, DNA damage response (DDR) induces either apoptosis via p53 activation or cellular senescence via p53/p21 or p16INK4a/pRB pathways. Besides its role in aging and cancer, an increasing amount of data additionally suggests a role of cellular senescence in adaptive immunity as well, affecting the number and function of these cells. For example, the infiltration of tumor tissue with T cells with short telomeres has been associated with a poor prognosis in various types of cancer including colorectal cancer. Nevertheless, the functional relevance of an aged immune system on CRC development have not been evaluated so far.

The goal of this project is to understand the role of aging and cellular senescence in CD4+ T cells in the response against CRC. We have previously showed our initial results on aging and cellular senescence using TERC-/- and Cdkn1a-/- mice. Here, we present our latest results on the importance of the p21 pathway in CD4+ T cells in CRC.



Role of p21 in CD4+ T cells during CRC development. (A) CRC model and endoscopic tumor assessment after orthotopic MC38 model. Flow cytometry analysis of (A) CD4+ T cells in blood, mLN, spleen and tumor microenvironment and (B) transcription factors and cell maturation in the anti-tumor response.

Prof. Dr. Waldner

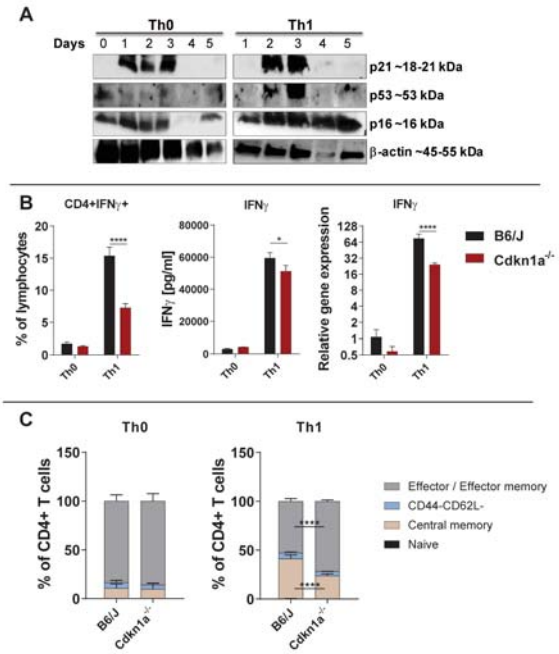
p21 deficiency in CD4+ T cells leads to increased tumor growth

In order to study the role p21 deletion in CD4+ T cells during CRC development, we exposed Rag-/- mice to the MC38 orthotopic model and reconstituted their immune system with MACS-isolated CD4+ T cells from the spleens of either B6/J or Cdkn1a-/- mice. Endoscopic evaluation revealed increased tumor growth in mice reconstituted with p21-deficient CD4+ T cells. Flow cytometry data revealed no differences in the frequencies of CD4+ T cells in blood, mLN, spleen or tumor microenvironment. However, we noticed a decreased infiltration of p21-lacking Tbet+ CD4+ T cells in the tumor microenvironment. Furthermore, p21 deletion in CD4+ T cells affected T cell maturation, leading to increased frequencies of effector/effector memory Tbet+ T cells, but less central memory cells.

p21 loss in CD4+ T cells affects in vitro T cell polarization

Since p21-deficiency in CD4+ T cells seems to affect the anti-tumor response by inducing less Tbet+ cells, we further investigated when p53/p21 and p16 pathways are activated during Th1 polarization in wild-type CD4+ T cells. We observed that these pathways are activated at day 2 during T cell polarization, revealing the crucial role of p21 in inducing Th1 T cells. Next, we differentiated in vitro CD4+ T cells isolated from the spleens of B6/J or Cdkn1a-/-. p21-deficient CD4+T cells polarize less into IFNγ producing cells, as shown by flow cytometry, ELISA and qPCR data. Moreover, loss of p21 affects CD4+ T cell maturation as well, leading to increased frequencies effector/effector memory and a reduced frequency of central memory cells.

In summary, our data show that p21 deficiency in CD4+ T cells leads to an impaired Th1 response against CRC by altering T cells maturation. Our further steps will involve understanding the molecular mechanisms leading to p21 activation in CD4+ T cells in CRC.



In vitro characterization of p21-deficient T cell polarization. (A) Western blots showing p53/p21 and p16 pathway activation during T cell differentiation. (B) Th1 polarization of p21-/- CD4+ T cells as shown by FACS, ELISA and qPCR. (C) T cell maturation status in as shown by flow cytometry data.

Contact:
Prof. Dr. Waldner
phone: +49 9131 85 45025
e-mail: maximilian.waldner@uk-erlangen.de

Invited lectures

JEDIS (Jena-Davis-Alliance for Excellence in Biophotonics) Summer School 2018, 12.09.2018, Jena, Biophotonic detection of gastrointestinal cancer - Breaking the barrier of conventional endoscopy

Publications during funding period

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

E19 - Final Report

15/02/2016 - 14/02/2019

Inhibitory neurotransmission in the cochlea: Glutamate and endocannabinoids

Prof. Dr. Ralf Enz, Institute of Biochemistry

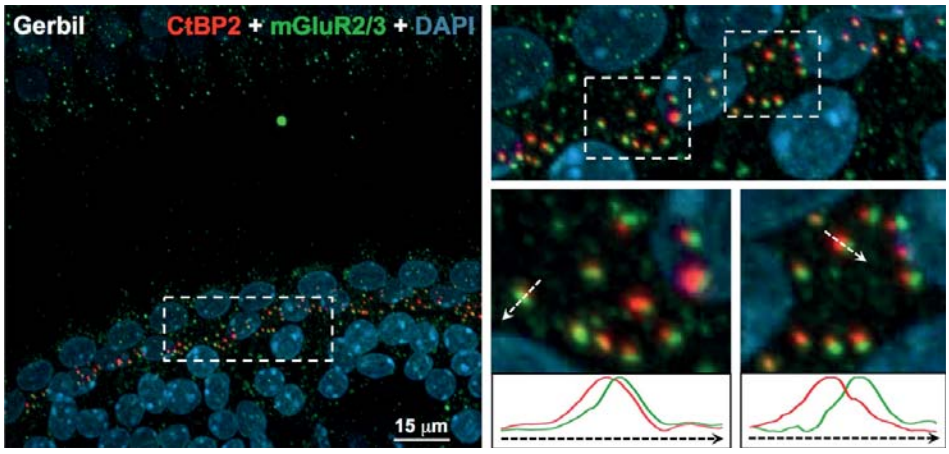
Glutamate and endocannabinoid receptors can regulate activity and survival of sensory neurons via inhibitory feedback loops. While inhibitory circuits of photoreceptors in the retina are well described, corresponding protective mechanisms in hair cells of the cochlea are largely unknown. Since sensory organs need a tailor-made regulation of their signal transduction pathways, this project investigates receptor expression in hair cells and elucidates their regulation by interacting proteins.

Introduction

Neurotransmitter receptors represent major determinants that control neuronal signal transduction at synapses. There, the receptors interact with multiple proteins, such as enzymes and scaffolds that regulate their trafficking, localization, ligand affinity, desensitization behaviour and surface concentration. In this way, receptors and regulatory proteins assemble into synaptic signal complexes.

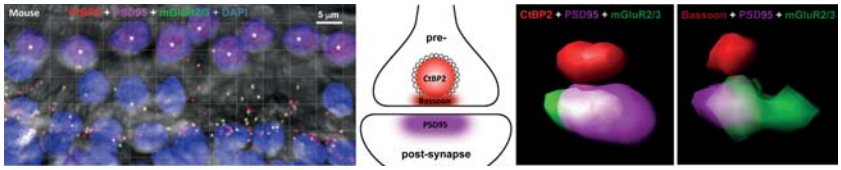
Receptor guided inhibitory feedback loops are important factors for activity and survival of sensory neurons, as well as for protection against noxious stimuli. G-protein coupled metabotropic glutamate receptors (mGluRs) expressed at pre- or post-synaptic sites can invert the activity of the excitatory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

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



Staining of cochlear wholemounts for mGluR2 and mGluR3 (green) showed puncta in close vicinity to presynaptic ribbons (red). This is best seen in the magnification of the boxed regions. Fluorescence profiles were measured along the arrows and signal intensities are compared in the graphs.

Prof. Dr. Enz



(left) Cochlear wholemounts were stained for mGluR2/3, pre- and post-synaptic markers of inner hair cell synapses, as indicated in the sketch. (right) 3D-reconstructions show a clear post-synaptic localisation of the receptors. Inner hair cell nuclei are marked with asterisks.

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

In contrast to mGluR1 and mGluR5, expression and localization of mGluR2, mGluR3, mGluR4, mGluR7a, mGluR7b, mGluR8a, mGluR8b and CB receptors in the inner ear is largely unknown. Here, we detected transcripts for all receptor types in the mouse cochlea. Furthermore, cochlear wholemounts of gerbil or mouse incubated with antibodies specific for mGluR2/3, mGluR4, mGluR8 or CB2 showed punctate signals, indicating synaptic localization of these receptors. Signals for mGluR2/3 were present at ribbon synapses of inner hair cells. Using combinations of confocal microscopy, super resolution STED microscopy and 3D reconstructions, we found a co-localization of mGluR2/3 with post-synaptic (PSD95), but not with pre-synaptic (CTBP2, Bassoon) markers at inner hair cell ribbon synapses.

How are cochlear mGluR2/3 regulated?

Given the post-synaptic expression of mGluR2/3 at inner hair cell ribbon synapses, we searched for intracellular proteins that bind to and thereby regulate receptor function. Yeast 2-hybrid screens using intracellular C-termini of mGluR2 or mGluR3 as baits for a cochlear cDNA-library yielded several hundred potential interaction partners that were clustered in functional groups, representing proteins involved in post-translational modifications, trafficking, cell adhesion or of the cytoskeleton. Of these, pull-down assays showed robust and reproducible interaction of 3 proteins with the receptors´ C-termini. The molecular and functional characterization of these protein-protein interactions is on-going.

Contact:
Prof. Dr. Enz
phone: +49 9131 85 24185
e-mail: ralf.enz@fau.de

Publications during funding period

none

E20 - Progress Report

01/05/2016 - 30/04/2019

Identification of molecules, receptors and genes involved in chronic pruritus

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

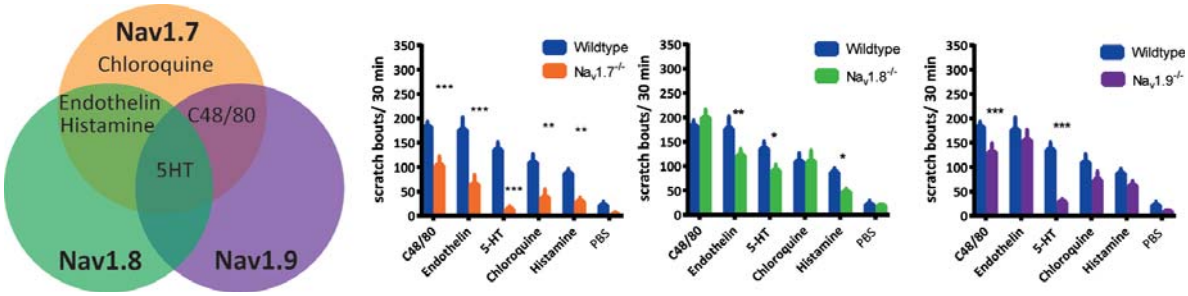
Chronic pruritus is a distressing symptom accompanying many dermatological and systemic disorders. The three aims of this project are to (I) identify pruritogens in plasma of patients suffering from chronic pruritus, to (II) characterize the specific voltage-gated sodium channel (NaV) subtypes that generate and propagate the action potentials in itch pathways, and, (III) to identify and characterize novel gene products that predispose or protect from itch by quantifying phenotypic differences in scratch behavior in inbred mouse strains.

Identification of pruritogens in plasma of patients suffering from chronic pruritus

Several members of the Mas-gene related receptor (MRG) family are selectively activated by pruritogens leading to activation of primary sensory neurons. To elucidate a potential contribution to itch signaling in chronic pruritus in patients, we established two screening methodologies to screen patient serum and pharmaceutical drugs for MRG receptor activation. Thereby, we identified novel agonists of MRGX2 which lead to mast cell degranulation and potentially itch.

Identification of specific Na_V channel subtypes required for itch signaling

Three Na_V channel subtypes, Na_V 1.7, Na_V1.8 and Na_V 1.9, are restricted to the peripheral sensory system and play essential roles in the generation and transmission of action potentials. They have been suggested as potential drug targets for itch. Since the precise contribution of these three different subtypes in itch signaling of different acute stimuli remains elusive, we used murine knockout models and assessed the scratch behavior upon intradermal injection of 10 different pruritogens. DRGs isolated from knockout and wildtype mice showed an equal depolarization capacity upon stimulation with different pruritogens measured by calcium imaging and exhibited only minor neurophysiological differences. Scratching behavior after intradermal injection of the pruritogens in the nuchal fold differed in the three knockout mice as compared to wildtype mice. Noticeably, the deletion of single NaV channels led to highly variable deficits in scratching behavior suggesting an unexpectedly high complexity of itch signaling. Nonetheless, Na_V1.7 was essential for all strong pruritogens and seemingly functions as the threshold channel and is a key channel for the effects of all potent pruritogens. Na_V 1.9 and Na_V1.8 probably function as amplifiers for some pruritogens or as high-threshold backup for long-lasting depolarizations, respectively.



Intradermal injection of pruritogens caused variably reduced scratching in Na_V1.7-/-,1.8-/- and 1.9-/- mice showing a diversity in Na_V-dependent itch signalling (N=10-12). The results also are summarized in a Venn-diagram.


Dr. Dr. Kremer


Prof. Dr. Zimmermann

Identification of novel genes that modulate pruritus severity in mice

Individual and ethnical differences in the experience of pruritus are recognized challenges in the treatment of pruritus. To investigate heritable differences in the sensitivity to pruritogens we used an automated scratch assay and evaluated scratch behavior in 20 inbred mouse strains subsequent to intradermal injection of 10 commonly known pruritogens. We found a large influence of the genetic background with numerous strains being highly sensitive and other strains with resistance to some or all pruritogens. The trait values served to perform haplotype-based computational genetic mapping (HBCGM) and led to the identification of genes with single-nucleotide polymorphisms and high correlation with the trait value variability. Subsequent pathway analysis, literature and expression database searches led to the selection of 35 candidate genes. Some of them will be subject of further molecular biology, electrophysiology and behavioral studies.

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

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

Invited lectures

14th Expert summit on viral hepatitis, 23.02.2018, Frankfurt, IgG4 assoziierte Erkrankungen – multiple Facetten

PBC in Motion, 10.03.2018, London, Managing symptoms and other manifestions in PBC

5. Bonner Hepatogastroenterologisches Forum, 28.04.2018, Bonn, Problemsymtom Pruritus – wen, wie, womit behandeln?

Falk Gastro Forum, 05.05.2018, Erlangen, Update: Therapie der cholestatischen Lebererkrankungen

Clinics and Science in Hepatology, 19.05.2018, Birmingham, Case-based challenges: autoimmune cholestatic liver diseases

Hamburger Lebertage, 25.05.2018, Hamburg, Erhöhte Leberwerte bei Schwangerschaft - wann ein Alarmsignal?

China Lecture Tour, June 2018, Lanzhou, Shenyang, u.a., China, NASH: the new epidemic in hepatology

Münsteraner Pruritussymposium, 08.09.2018, Münster, Neues in der Therapie von Pruritus bei Lebererkrankungen

Publications during funding period

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

*contributed equally

E21 - Progress Report

01/05/2016 - 30/04/2019

Modulation of alpha-Synuclein pathology by FoxO-dependent pathways

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

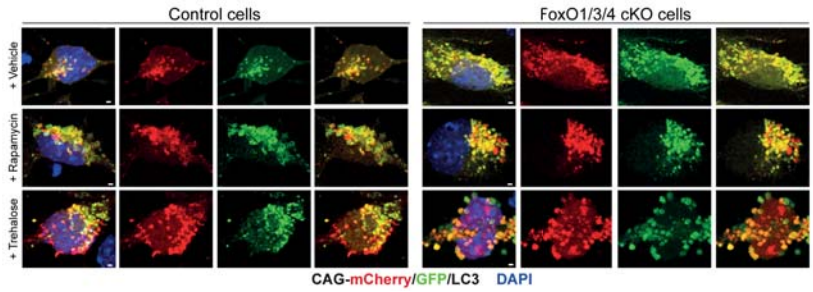
Dysregulation of autophagy, the central cellular self-clearance mechanism, is impaired in synucleinopathies including Parkinson´s disease and has been implicated in the cell-to-cell transfer of aSyn potentially leading to disease progression. This project addresses the currently unresolved question of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

FoxO transcription factors potently modulate autophagy in neural cells

Autophagy is a highly conserved catabolic pathway with emerging functions in human neurodevelopmental and neurodegenerative diseases. We now demonstrated that conditional deletion of the Forkhead Box O transcription factors FoxO1, FoxO3, and FoxO4 in neurons, strongly impaired autophagic flux in developing neurons. Impaired autophagic flux was associated with altered synaptic integration and survival in neurons. Strikingly, pharmacological induction of autophagy was sufficient to correct abnormal dendrite and spine development of FoxO-deficient neurons. Collectively, these findings uncover a novel link between FoxO transcription factors, autophagic flux, and maturation of developing neurons. Future work will analyze to what extent FoxO-dependent autophagy is involved in the pathophysiology of neurodevelopmental and neurodegenerative diseases.

Autophagy induction by running as a protective factor in synucleinopathies

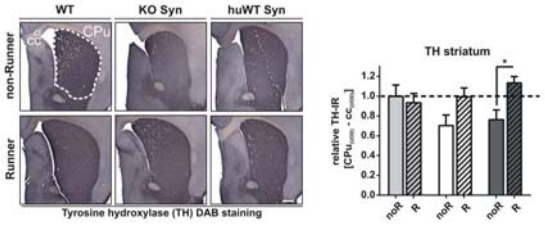
The goal addressed in the 2018th phase of the project was to understand the effect of autophagy induction by physical exercise on synucleionpathies in the brain. By combining running intervention in alpha-synuclein transgenic mice, we both addressed changes in phenotype (gait and postural control patterns) and neuropathology patterns. Physical exercise improves gait in PD patients and motor function in rodent lesion models. Moreover, exercise is considered neuroprotective and ALP induction has been reported, e.g. in human skeletal muscle, rodent peripheral and cerebral tissues. 4 weeks treadmill exercise intervention in adult human alpha-synuclein expressing mice revealed that at baseline, alpha-synuclein mouse models exhibited irregular and less active gait, with impaired dynamic postural control, compared to wild type mice. Treadmill exercise particularly improved speed and stride length, while increasing dual diagonal versus three-paw body support in both the alpha-synuclein knockout and transgenic mice. Biochemical analyses showed higher striatal tyrosine hydroxylase immuno-reactivity and reduced higherorder alpha-synuclein species in the cerebral cortex. However, no significant cerebral ALP induction was measured. This was an important finding



Autophagolysosomes (red, no green) are absent in FoxO-deficient neural stem cells indicating impaired autophagolysomal flux. Rapamycin or Trehalose enhance autophagolysomal flux.

Prof. Dr. Lie

Prof. Dr. Klucken



Treadmill training (running – R) in a-Synuclein mouse models (KO or human WT tg) restored reduced striatal TH-levels in non-runners hWT Syn mice "noR".

since physical exercise was able to induce systemic autophagy and protect from the neurodegenerative phenotype, however, without affecting cerebral autophagy. The work is in press in Behavioural Brain Research, 2019.

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

Prof. Dr. Klucken
phone: +49 9131 85 34127
e-mail: jochen.klucken@uk-erlangen.de

Invited lectures

Abcam Conference „Adult Neurogenesis", 02-04 May 2018, Schäffner I and Lie DC, Autophagy-Dependent Control of Neurogenesis

Publications during funding period

Minakaki G, Canneva F, Chevessier F, Bode F, Menges S, Timotius IK, Kalinichenko LS, Meixner H, Müller CP, Eskofier BM, Casadei N, Riess O, Schröder R, Winkler J, Xiang W, von Hörsten S, and Klucken J (2018) Treadmill exercise intervention improves gait and postural control in alpha-Synuclein mouse models without inducing cerebral autophagy. Behavioural Brain Research, doi: 10.1016/j.bbr.2018.11.035

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Balta EA, Wittmann MT, Jung M, Sock E, Haeberle BM, Heim B, von Zweydorf F, Heppt J, von Wittgenstein J, Gloeckner CJ, Lie DC* (2018) Phosphorylation Modulates the Subcellular Localization of SOX11. Frontiers in Molecular Neuroscience 11: 211 (*corresponding authors)

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Kavyanifar A, Turan S, Lie DC (2018) SoxC transcription factors: multifunctional regulators of neurodevelopment. Cell and Tissue Research 371(1): 91-103

Minakaki G, Menges S, Kittel A, Emmanouilidou E, Schaeffner I, Barkovits K, Bergmann A, Rockenstein E, Adame A, Marxreiter F, Mollenhauer B, Galasko D, Buzás EI, Schlötzer-Schrehardt U, Marcus K, Xiang W, Lie DC, Vekrellis K, Masliah E, Winkler J, Klucken J (2018) Autophagy inhibition promotes alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophago/exosome-like phenotype. Autophagy 14(1): 98-119

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E22 - Progress Report

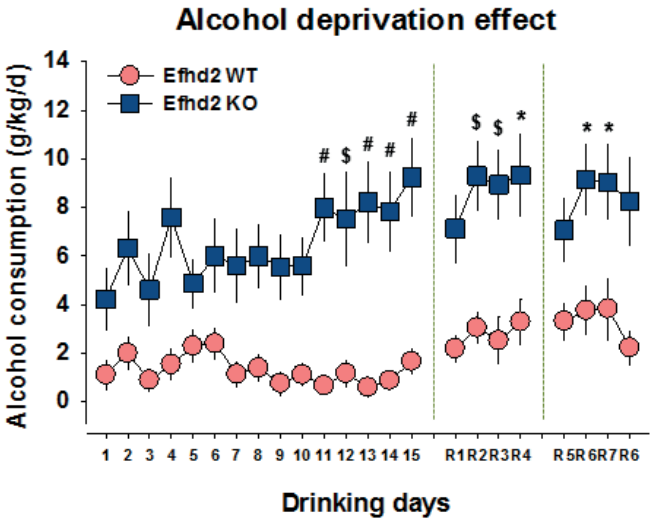
01/03/2016 - 28/02/2019

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

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

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

In many societies, the majority of adults regularly consume alcohol. However, only a small proportion develops alcohol addiction. Individuals at risk often show a high sensation-seeking/ low anxiety behavioural phenotype. Here we asked which role EFhd2 (Swiprosin-1) plays in the control of alcohol addiction-associated behaviours. EFhd2 knock out (KO) mice drink more alcohol than controls and spontaneously escalate their consumption. This coincided with a sensation-seeking and low anxiety phenotype. A reversal of the behavioural phenotype with β-carboline, an anxiogenic inverse benzodiazepine receptor agonist, normalized alcohol preference in EFhd2 KO mice, demonstrating an EFHd2-driven relationship between personality traits and alcohol preference. These findings were confirmed in a human sample where we observed a positive association of the EFHD2 SNP rs112146896 with lifetime drinking and a negative association with anxiety in healthy adolescents. The lack of EFhd2 reduced extracellular dopamine levels in the brain, but enhanced responses to alcohol. In confirmation, gene expression analysis revealed reduced tyrosine hydroxylase expression and the regulation of genes involved in cortex development, Eomes and Pax6, in EFhd2 KO cortices. These findings were corroborated in Xenopus tadpoles by EFhd2 knockdown. Magnetic resonance imaging (MRI) in mice showed that a lack of EFhd2 reduces cortical volume in adults. Moreover, human MRI confirmed the negative association between lifetime alcohol drinking and superior frontal gyrus volume. These findings showed that EFhd2 is a conserved resilience factor against alcohol consumption and its escalation, working through Pax6/Eomes. Reduced EFhd2 function induces high-risk personality traits of sensation seeking/ low anxiety associated with enhanced alcohol consumption which may be related to cortex function. In a parallel study we found that EFhd2 also controls the establishment of the conditioned rewarding effects of cocaine and methamphetamine, two psychostimulant type drugs. EFhd2 is also here required to control the drug-induced activation of monoaminergic signalling in the brain as

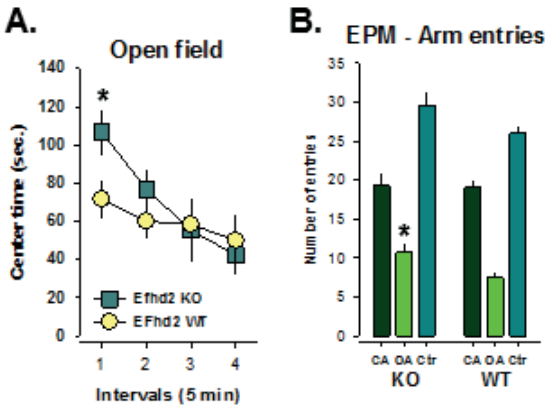


The lack of Swirprosin-1/EFhd2 in mice leads to enhanced consumption of alcohol in a free-choice drinking paradigm and spontaneous escalation of consumption.


Prof. Dr. Müller


Prof. Dr. Alzheimer


Prof. Dr. Mielenz



EFhd2 knock out mice display a sensation seeking/ low anxiety behavioural phenotype in (A) the open field test and (B) the elevated plus maze.

a functional marker for the rewarding effects of the drugs. These findings support the view that EFhd2 may not only provide resilience for alcohol addiction, but for drug addiction in general.

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

Invited lectures

DFG training grant RTG1962, 09.01.2018, FAU Erlangen-Nürnberg, Erlangen, The role of Swiprosin-1/EFhd2 in drug addiction and neurodegeneration (Mielenz)

Institute for Experimental Muskuloskeletal Medicine, Series of Lectures, Wilhems-Universität Münster, 15.05.2018, Cytoskeletal control of antibody affinity maturation and plasma cell differentiation (Mielenz)

Experimental Neonatology, Series of Lectures, Universität Köln, 02.08.2018, Assessing mitochondrial metabolism in B cells (Mielenz)

BSRT Symposium, 28-30.11.2018, Publishing negative results, Charité Berlin, Berlin (Mielenz)

Awards

Forschungspreis des Norddeutschen Suchtforschungsverbundes e.V., Christian P. Müller, 18.04.2018, Hannover

Publications during funding period

Satoh T, Banaschewski T, Bokde ALW, Bromberg U, Büchel C, Quinlan EB, Floor H, Frouin V, Garavan H, Gowland P, Heinz A, Ittermann B, Martinot JL, Paillère Martinot ML, Artiges E, Lemaitre H, Nees F, Orfanos DP, Paus T, Poustka L, Millenet S, Fröhner JH, Smolka MN, Walter H, Whelan R, Bakalkin G, Liu Y, Desrivières S, Elliott P, Eulenburg V, Levy D, Crews F, Schumann G (2018) TANK controls alcohol drinking and cortical emotion pathways. Cerebral Cortex doi: 10.1093/cercor/bhy341

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Müller CP (2018) Animal models of psychoactive drug use and addiction – present problems and future needs for translational approaches. Behavioural Brain Research 352: 109-115

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Huber SE, Lenz B, Kornhuber J, Müller CP (2017) Prenatal androgen-receptor activity has organizational morphological effects in mice, PLOS One 12(11): e0188752

E23 - Final Report

01/01/2016 - 31/12/2018

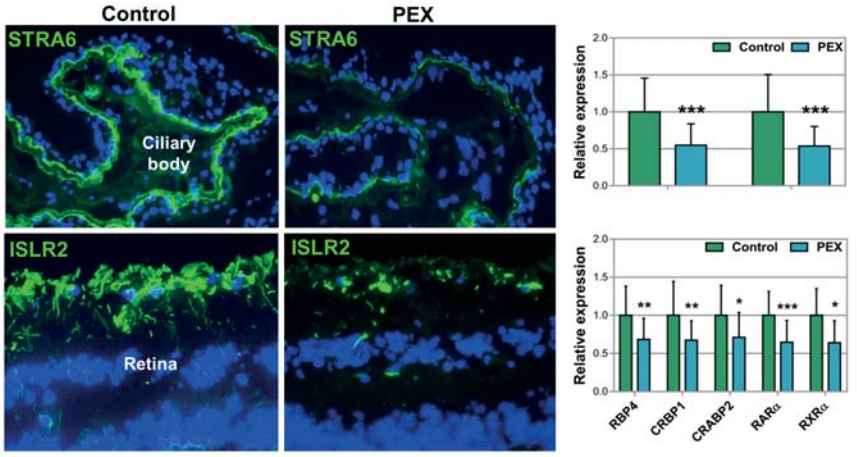
Identification and characterization of LOXL1 risk variants for pseudoexfoliation syndrome and glaucoma

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

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

Initially, we searched for sequence variants influencing transcriptional output of LOXL1. In a genome-wide association scan on German, Italian, and Japanese patients, we identified a four-component polymorphic locus spanning introns 1 and 2 of LOXL1 with enhancer-like chromatin features. We found that the rs11638944:C>G transversion exerts a cis-acting effect on the expression levels of LOXL1 by differential binding of the transcription factor RXRα and by enhancing splicing of an alternative LOXL1 transcript associated with nonsense-mediated decay (Pasutto et al. 2017). In a follow-up study, we further showed that "alternative splicing coupled to nonsense-mediated decay" represents a dynamic mode of adapting LOXL1 expression to PEX-associated environmental and nutritional cues (Berner et al. 2017).

In a joint effort with the International PEX Genetics Consortium, we participated in a large-scale genome-wide association study on >10,000 PEX cases and >100,000 controls from 24 countries. We identified five new loci, underlined by POMP, TMEM136, AGPAT1, RBMS3 and SEMA6A, associated with PEX. Protein and mRNA expression levels of POMP and TMEM136 were significantly reduced in ocular tissues of PEX patients compared to age-matched controls (Aung et al. 2017). Through deep resequencing we further identified a spectrum of rare alleles at LOXL1 predicted to affect protein function. Functional assays on the p.Y407F variant showed an enhanced binding of protein variants harboring the protective p.407F allele to fibulin-4, a major component of fibrillar PEX aggregates.

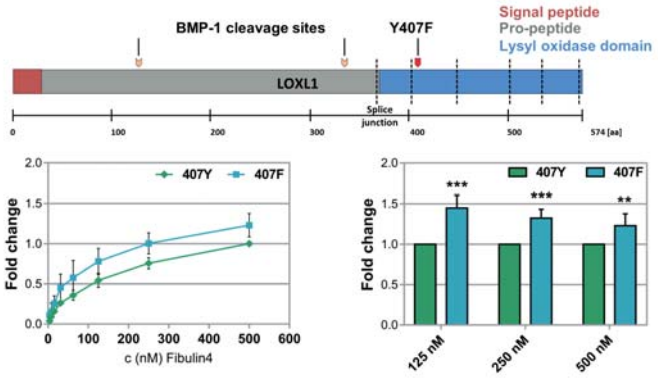


Reduced mRNA and protein expression levels of STRA6, together with other components of the retinoic acid pathway, and ISLR2 (green fluorescence) in ocular tissues (ciliary body, retina) of PEX eyes compared with normal human donor eyes.

Our deep resequencing effort revealed a common noncoding variant, rs7173049:A>G downstream of LOXL1 consistently associated with a decrease in PEX risk in multiple populations. Using CRISPR/Cas9 genome editing, we provided evidence for a functional

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

enhancer-like regulatory activity of the genomic region surrounding rs7173049 influencing expression levels of STRA6 and ISLR2. We further showed that the rs7173049 genotype correlates with tissue expression levels of STRA6 and ISLR2 and that both genes are downregulated in PEX tissues together with key components of the retinoic acid receptor-mediated signaling pathway. These data indicate that dysregulation of STRA6 and impaired retinoid metabolism are involved in the pathophysiology of PEX syndrome and that the variant rs7173049-G, which represents the first common variant at the LOXL1 locus consistently associated with PEX in all populations worldwide, mediates a protective effect through upregulation of STRA6 in ocular tissues (Berner et al., under revision).



Localization of the rare variant p.Y407F in LOXL1.Functional analysis shows enhanced binding of LOXL1 harboring the protective allele T to fibulin-4 compared with the A allele.

Finally, we have initiated a pilot study on transcriptome analysis of ocular tissues derived from PEX and control patients in cooperation with the Core Unit Next Generation Sequencing. Statistical analysis revealed about 50 differentially expressed genes (DEGs) in iris and 500 in lens capsule specimens with some overlap between the two tissue types. Pathway analyses and functional clustering of the DEG datasets revealed several biological pathways, including extracellular matrix organization, inflammatory response, and calcium signaling, relevant to PEX pathogenesis, which will be further analyzed in follow-up projects.

Contact:
Prof. Dr. Schlötzer-Schrehardt
phone: +49 9131 85 44433
e-mail: ursula.schloetzer-schrehardt@uk-erlangen.de

Prof. Dr. Reis
phone: +49 9131 85 22318
e-mail: andre.reis@uk-erlangen.de

Invited lectures

World Ophthalmology Congress, 16-19.06.2018, Barcelona, Spain, The dual role of LOXL1 in exfoliation syndrome/glaucoma
Glaucoma Research Society Meeting, 29.08.-01.09.2018, Parma, Italy, Pseudoexfoliation syndrome genomics: Contributions of common and rare variants

36th Congress of the ESCRS, 22-26.09.2018, Vienna, Austria, What is pseudoexfoliation?

Awards

Membership German National Academy of Sciences Leopoldina, U. Schlötzer-Schrehardt, July 2018

Membership Glaucoma Research Society, U. Schlötzer-Schrehardt, September 2018

Publications during funding period

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

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

Berner D, Zenkel M, Pasutto F, Hoja U, Liravi P, Gusek-Schneider GC, Kruse FE, Schödel J, Reis A, Schlötzer-Schrehardt U (2017) Post-transcriptional Regulation of LOXL1 Expression Via Alternative Splicing and Nonsense-Mediated mRNA Decay as an Adaptive Stress Response. Invest Ophthalmol Vis Sci. 58: 5930-5940

[*authors contributed equally]

E24 - Final Report

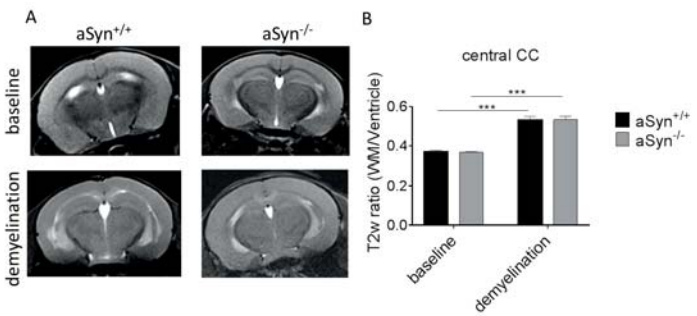
01/01/2016 - 31/12/2018

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

Prof. Dr. Jürgen Winkler, Department of Molecular Neurology
Prof. Dr. Ralf Linker, Department of Neurology (till 30/09/2018)

Demyelination and inflammation are hallmarks of neurodegenerative disorders such as Parkinson's disease or multiple system atrophy which present neuropathological alpha-synuclein (aSyn) aggregations. Previously, increased aSyn levels were also reported in lesions of multiple sclerosis patients. Recent findings showing impaired maturation and myelination of aSyn overexpressing primary oligodendrocytes provide evidence for a detrimental role of aSyn aggregates in myelin homeostasis. Therefore, the aim of this project is to study the role of aSyn in myelination processes in the context of multiple sclerosis.

Despite their different entity as neuroinflammatory and –degenerative disease, respectively, multiple sclerosis (MS) and multiple system atrophy (MSA) are characterized by similar features such as myelin loss and activation of immune responses in central and peripheral tissues. Neuropathologically, both diseases present aSyn aggregation, however, the specific contribution of these protein accumulations to degenerative and regenerative processes as well as inflammation remains to be elucidated. Therefore, our project aims at investigating the role of aSyn during inflammatory demyelination and regulation in the context of MS. For this purpose, we employ two different animal models: the experimental autoimmune encephalomyelitis (EAE) model, a chronic model of MS reflecting inflammation and demyelination in the central nervous system (CNS) and the Cuprizone model, a model of acute demyelination and remyelination.



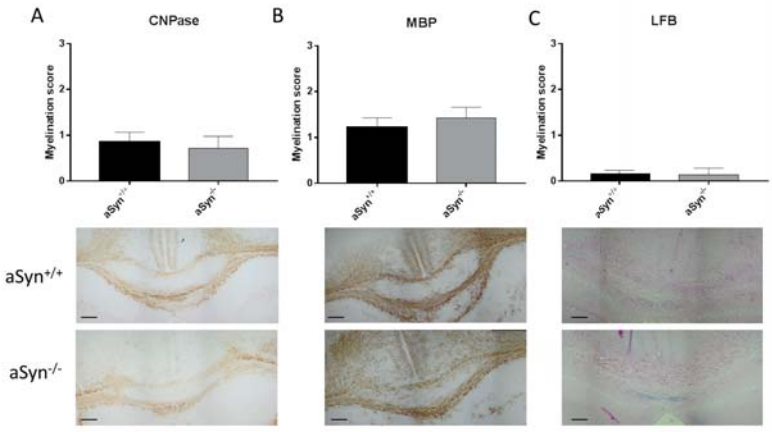
In vivo MRI of aSyn$^{+/+}$ and aSyn$^{-/-}$ mice. (A) Representative T2w images. (B) Quantification of signal intensities revealed similar demyelination of the central corpus callosum (CC) after 5 weeks of CPZ treatment in aSyn$^{+/+}$ and aSyn$^{-/-}$ mice (***p<0.001, n=5-7 per group).

Role of aSyn in acute inflammatory processes and chronic demyelination

In the first years, our project focused on the interaction of aSyn and inflammatory processes. In vitro experiments revealed that aSyn is able to modulate microglial immune responses by uptake of aSyn fibrils (Hoffmann et al., 2016). Furthermore, we observed physiological aSyn expression by different immune cell subsets such as CD4- and CD11b/c-positive cells. To analyze the functional role of endogenous aSyn in neuroinflammation, we induced EAE in wildtype (aSyn$^{+/+}$) and aSyn-knockout (aSyn$^{-/-}$) mice. Our results suggest that in the acute phase of EAE endogenous aSyn acts as a new regulator of Th1 responses in neuroinflammation (Ettle, Kuhbandner et al., 2016). To further elucidate the role of aSyn in inflammatory demyelination in the chronic phase of EAE, we then monitored aSyn$^{+/+}$ and aSyn$^{-/-}$ mice for 8 weeks after EAE induction. Interestingly, aSyn$^{-/-}$ mice showed an ameliorated disease course compared to aSyn$^{+/+}$ mice. At the peak of motor dysfunction, these mice exhibited mild gait ataxia, while the control group (aSyn$^{+/+}$) suffered from moderate paralysis of the hind limbs. Furthermore, aSyn$^{-/-}$ mice showed fast recovery and displayed a difference of 1 score point to aSyn$^{+/+}$ mice at the end of the observation period. Histological analysis of spinal cord cross sections revealed a significantly lower number of CD3-positive cells and less severe axonal damage assessed by Bielschowsky silver staining in spinal cord lesions of aSyn$^{-/-}$ mice.


Prof. Dr. Winkler


Prof. Dr. Linker



Myelin expression analysis of the CC of aSyn$^{+/+}$ and aSyn$^{-/-}$ mice after 5 weeks of CPZ treatment using the different myelin markers: (A) CNPase, (B) MBP and (C) Luxol Fast Blue staining (LFB) (n= 5-7 per group, scale bar 200 µm).

Impact of aSyn deficiency on myelination processes

In the following, we focused on the impact of aSyn deficiency in the Cuprizone (CPZ) model, an acute de- and remyelination model lacking peripheral immunological processes. First, aSyn$^{-/-}$ mice and controls (aSyn$^{+/+}$) were fed a diet containing 0.2% CPZ for 5 weeks. At this time point, MRI in vivo measurements revealed severe demyelination in the corpus callosum (CC) due to CPZ treatment, however, no differences in demyelination was observed between both groups. In line with these results, histological analysis of the CC by staining for different myelin markers such as CNPase and MBP showed a similar demyelination pattern in aSyn$^{+/+}$ and aSyn$^{-/-}$ mice.

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

Prof. Dr. Linker
phone: +49 941 941 3001
e-mail: ralf.linker@klinik.uni-regensburg.de

Invited lectures

Gordon Research Conference, 18-23.02.2018, Ventura, United States, Role of AngII in neuroinflammation

DGN Jahreskongress, 30.10.2018, Berlin, Therapie der fortgeschrittenen MS (Ralf Linker)

DGN Jahreskongress, 31.10.2018, Berlin, Hoffnung und Hype neuroprotektiver Therapie (Ralf Linker)

DGN Jahreskongress, 01.11.2018, Berlin, Immuntherapie und Neuroprotektion bei der MS (Ralf Linker)

DFG SFB 1009 Meeting Autoimmunity Breaks Barriers, 29.10.2018, Münster, The role of salt in neuroinflammation (Ralf Linker)

Awards

ECTRiMS Award for the best oral presentation of a young scientific investigator, Kristina Kuhbandner, 12.10.2018, Berlin

ECTRiMS Travel grant, Kristina Kuhbandner, 10.10.2018, Berlin

Novartis MS Research Day 2018 Posterpreis, Alana Hoffmann, 26.01.2018, Berlin

Publications during funding period

Hoffmann A, Ettle B, Battis K, Reiprich S, Schlachetzki JCM, Masliah E, Wegner M, Kuhlmann T, Riemenschneider MJ, Winkler J (2018) Oligodendroglial A-synucleinopathy driven neuroinflammation in multiple system atrophy. Brain Pathology; doi:10.1111/bpa.12678

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

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E25 - Progress Report

01/07/2016 - 30/06/2019

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

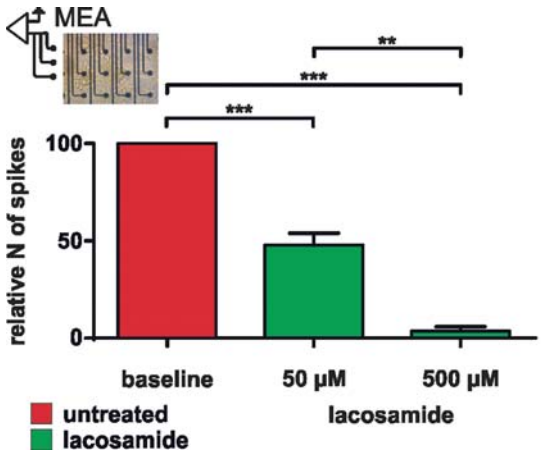
Prof. Dr. Beate Winner, Department of Stem Cell Biology
Prof. Dr. Dr. Jürgen Schüttler, Department of Anesthesiology

Our project aims to use patient-derived nociceptors to model the neuropathic pain syndrome small fiber neuropathy. We found that increased spontaneous activity of patient's C-fibers was mirrored by hyper-excitability of patient-derived nociceptors and could be reverted by the FDA approved anti-epileptic drug lacosamide. Based on these in-vitro findings we could predict an effective treatment in an individualized therapeutic approach for a patient suffering from refractory neuropathic pain.

Small fiber neuropathy (SFN) can manifest as chronic neuropathic pain syndrome characterized by severe burning pain in the extremities with limited therapeutic options. Using a fibroblast reprogramming approach we generated human induced pluripotent stem cells (hiPSCs) from a 69-year old Caucasian patient suffering from SFN with refractory neuropathic pain for over ten years. These patient-derived hiPSCs were differentiated into sensory neurons that we characterized with molecular biology and electrophysiological methods. We found that increased spontaneous activity of the patient's C-fibers investigated with microneurography recordings (26,3 % compared to 11,8 % in healthy age matched controls) was mirrored by in-vitro findings in patient-derived nociceptors. These showed increased spontaneous activity measured with patch-clamp technique in current-clamp mode and increased number of spikes when grown on multielectrode array (MEA) plates.

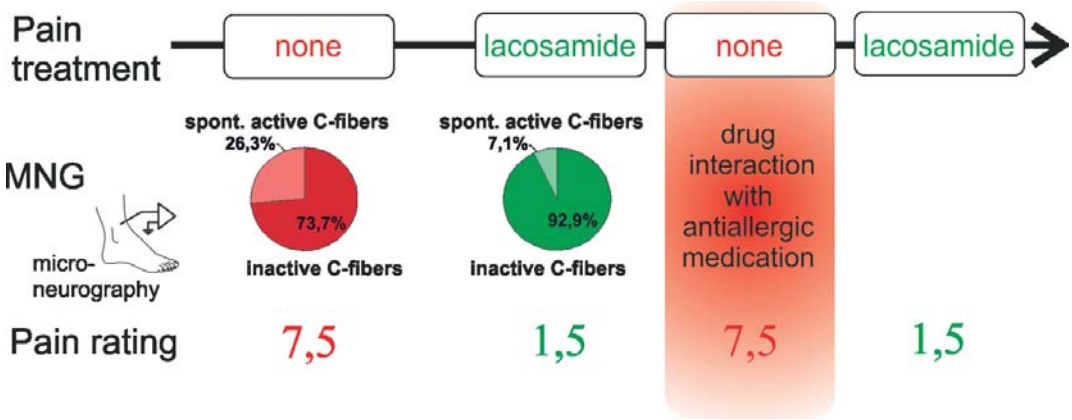
In MEA recordings, the FDA approved antiepileptic drug lacosamide (500 µM) strongly reduced the number of spikes in patient derived nociceptors but not in control groups. This finding indicates that pathological hyperactivity can be reduced by lacosamide but general action potential generation is not affected. At plasma equivalent concentrations of 50 µM lacosamide was still effective on patient-derived sensory neurons.

Based on this preclinical prediction the patient started off-label treatment with lacosamide 50 mg orally in the evening when pain was strongest. Within five days pain ratings on numeric rating scale (0 no pain, 10 worst imaginable pain) decreased from 7,5 to 1,5. Simultaneously, spontaneous activity of C-fibers in microneurography recordings was diminished to 7,1 %. This proves that lacosamide is also effective in the peripheral nervous system. When the patient interrupted medication due to increased sedating side effects by combining lacosamide with antiallergic medication during hay fever season, this interruption of lacosamide treatment led to reoccurrence of severe pain. After continuing lacosamide pain levels again decreased to NRS 1,5 and the effect was still preserved after 6 month of treatment.



Lacosamide reduces activity of patient-derived nociceptors in MEA recordings and is still effective in plasma equivalent concentrations (50 µM). Number of spikes were normalized to activity under control condition before drug application.

Prof. Dr. Winner Prof. Dr. Schüttler



Treatment with lacosamide alleviated pain assessed on numeric rating scale and reduced spontaneous activity of patient's C-fibers in microneurography recordings.

In summary our findings make a mere placebo effect of lacosamide unlikely and provide evidence for an individualized translational therapeutic approach based upon patient-derived sensory neurons. We could also show that patient-derived nociceptors are not only suitable for monogenetic disorders but also for more complex sporadic or polygenic conditions because of the preserved genetic background of the patient.

Contact:
Prof. Dr. Winner
phone: +49 9131 85 39301
e-mail: beate.winner@fau.de

Publications during funding period

Namer B, Schmidt D, Eberhardt E, Maroni M, Dorfmeister E, Kleggetveit IP, Kaluza L, Meents J, Gerlach A, Lin Z, Winterpacht A, Dragicevic E, Kohl Z, Schüttler J, Kurth I, Warncke T, Jorum E, Winner B, Lampert A (2018) Pain relief in a neuropathy patient by lacosamide: Proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors. EBioMedicine 39: 401–408

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Popp B, Krumbiegel M, Grosch J, Sommer A, Uebe S, Kohl Z, Plötz S, Farrell M, Trautmann U, Kraus C, Eckici A, Asodollahi R, Regensburger M, Günther K, Rauch A, Edenhofer F, Winkler J, Winner B, Reis A (2018) Need for high-resolution Genetic Analysis in iPSC: Results and Lessons from the ForIPS Consortium. Scientific reports 8: 17201

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E26 - Progress Report

01/03/2016 - 28/02/2019

Genetics and pathomechanisms of intellectual disability with microcephaly

Prof. Dr. Dr. Christiane Zweier, Institute of Human Genetics

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

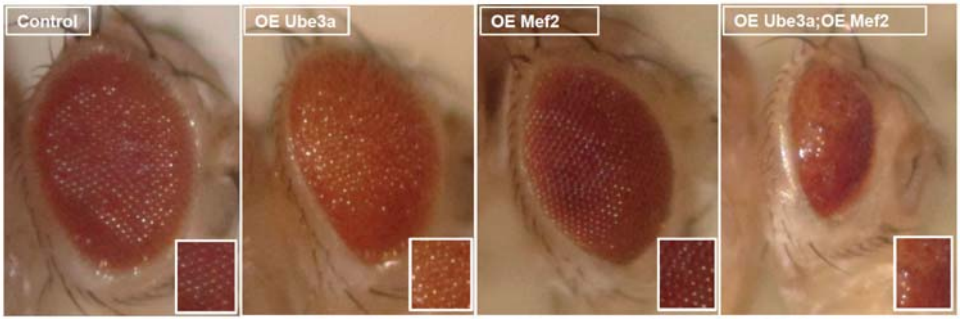
We selected five genes (*TCF4, MEF2C, UBE3A, ZEB2* and *ATRX*) implicated in clinically overlapping, syndromic forms of severe ID with epilepsy and postnatal microcephaly that are often considered as close differential diagnoses. Most of these genes are involved in transcriptional regulation. By using genome-wide transcriptome analysis and by using *Drosophila melanogaster* as a model to screen for genetic interactions, we identified commonly deregulated target genes involved in neurodevelopment and specific genetic interactions, e.g. between Ube3a and Mef2. These molecular commonalities might contribute to the clinically overlapping features of the investigated disorders.

Genetic Interaction screen in *Drosophila* demonstrates functional links between ID genes

We assessed potential genetic interactions of orthologues of the five ID genes in Drosophila melanogaster. We used the UAS/GAL4 system to induce knockdown or overexpression of each single gene and in pairwise combinations ubiquitously and in various specific tissues (eye, wing, pan-neuronal, glia). Modification of a phenotype resulting from deregulation of a single gene A by simultaneous deregulation of a second gene B indicates genetic interaction between gene A and B. Several parameters such as lethality, wing and eye morphology, neuromuscular junction morphology and bang sensitivity and climbing behaviour were assessed. We found evidence for genetic interaction between several of these genes, most stringently between Ube3a and Mef2: pairwise knockdown and pairwise overexpression as well as a combination of *Ube3a*-overexpression and *Mef2*-knockdown resulted in either more severe or milder eye phenotypes compared to single knockdown or overexpression of any of the two genes.

Transcriptome analysis identifies commonly deregulated target genes

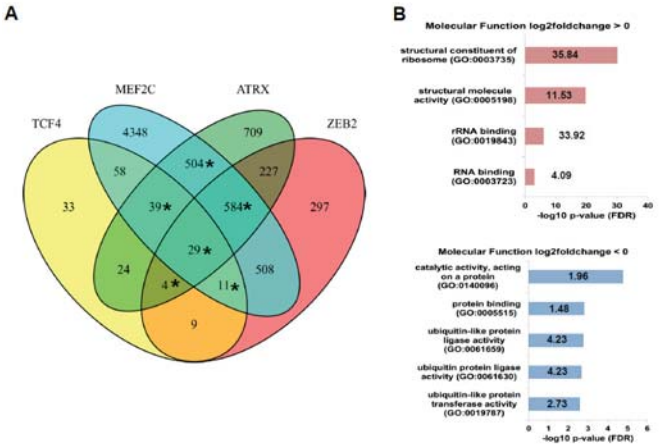
We performed transcriptome analysis on RNA from patient blood samples to investigate possible common transcriptional targets of the four transcriptional regulators TCF4, ZEB2 (3 individuals, each),



Genetic Interaction. While overexpression of *Ube3a* or *Mef2* alone causes a mild eye phenotype (rough eye or no bristles), respectively, simultaneous overexpression of both genes results in a severely reduced eye size and dissolved ommatidia structure.

Prof. Dr. Dr. Zweier

MEF2C, and ATRX (1 individual, each). In all patient groups, markedly more genes were down-regulated than up-regulated (TCF4: 132 vs. 75, ZEB2: 1163 vs. 506, MEF2C: 3779 vs. 2311, ATRX: 1505 vs. 615), indicating a shared role as transcriptional activators for all four proteins. Pairwise comparison of deregulated genes in the four patient groups revealed significant overlap, indicating common transcriptional targets. Moreover, we found significant enrichment of known ID genes among the deregulated genes, indicating central roles of the four proteins in neurodevelopment. 667 genes were commonly deregulated in at least three of the four patient groups. These genes were enriched for gene ontology terms such as ribosomal and RNA-related functions (up-regulated genes) and catalytic activity ubiquitin ligase activity (down-regulated genes).



Transcriptome Analysis. A: Overlap of deregulated genes in patients with mutations in one of the four tested genes. B: Gene Ontology Term enrichment in commonly deregulated genes (marked by * in A).

Contact:
Prof. Dr. Dr. Zweier
phone: +49 9131 85 29113
e-mail: christiane.zweier@uk-erlangen.de

Invited lectures

Syndromclub, 09.11.2018, Salzburg, Austria, Dysmorphologie und Syndromologie in Zeiten der Exom-Sequenzierung

Institute of Human Genetics, 24.04.2018, Göttingen, Neurodevelopmental disorders: genes and beyond

Publications during funding period

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E27 - Progress Report

01/03/2016 - 28/02/2019

Lysophosphatidic acid-induced pruritus of cholestasis

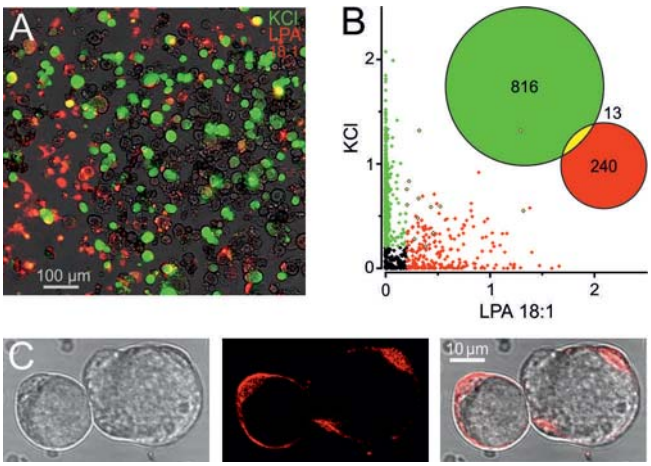
Dr. Dr. Andreas Kremer, Department of Medicine 1 - Gastroenterology, Pneumology and Endocrinology
Prof. Dr. Michael Fischer, Institute of Physiology and Pathophysiology (till 31/08/2016)

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

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

LPA and known agonists were sequentially applied to dissociated mouse dorsal root ganglia (DRG). LPA-activated cells differ from cells activated by potassium chloride (KCl), used as positive control for neurons. Cells responsive to both LPA and KCl were rare, as could be shown by comparing the respective calcium time courses and the inverse correlation between these responses ($r = -0.37$, $p < 0.001$, $n = 1237$). The subsequent application of LPA 1 µM, several establishes TRP-channel agonists and KCl 60 mM showed two distinct response patterns. Cells were activated either by LPA or by neuronal receptor agonists. The activation pattern was analyzed regarding the cell phenotype: Only 1.6% (13 of 829) of all cells reacting to LPA 18:1 were neurons based on their phenotype and response to KCl. Responses to potassium were larger in neurons compared to SGCs but LPA differentiates the two populations more clearly. The phenotype of LPA-activated cells in DRGs matched those of satellite glia cells, the cells were small and found in a halo-like shape around neurons. In stained DRGs sections we observerd a co-localization for LPAR1 and the glial marker glutamine synthetase while the LPAR1 expression in neurons is marginal at best.

LPA activated more hTRPV1-transfected HEK293t cells compared to untransfected controls exceeding a threshold of 0.2 ratio increase (25% vs 1% in hTRPV1, $p < 0.001$, Chi-square test). As the magnitude of the response was only 11% of capsaicin, this indicates a clearly detectable but limited activation compared to the gold standard agonist. The activation of DRG cells from TRPV1-deficient mice were similar to wild type controls. Experiments in calcium-free conditions show that the LPA-induced increase in cytoplasmatic calcium is derived from the endoplasmatic reticulum. In combination with LPA receptor expression results from DRGs and Schwann cells, pharmacological results indicate a signaling pathway through LPAR1. This result is supported by the elimination of LPA responses using the LPAR1 and LPAR3 antagonist Ki16425.

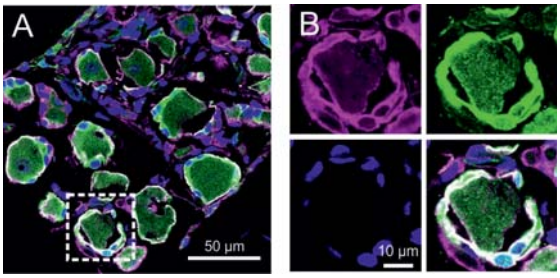


LPA 18:1 activates satellite glia cells but only 1.6% of sensory neurons. A) Overlay of transmission image (gray-scaled) and the response to LPA (red) and to KCl (green). B) Scatterplot of the ratio increase for every cell for LPA and KCl. C) Confocal image of satellite glia cells responding to LPA.

Dr. Dr. Kremer Prof. Dr. Fischer

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

LPA was applied intradermally by insertion of LPA-loaded heat-inactivated cowhage spicules in healthy volunteers. Control applications included histamine, capsaicin and the vehicle solution. The pain and itch intensities were quantified using a numeric rating scale. LPA applied into the skin using cowhage spicules induced a mild itch sensation compared to vehicle control (mean ± SEM; 1.4 ± 0.4 vs. 0.3 ± 0.2; $p<0.001$) lasting for several minutes. In contrast, intradermal injection of LPA caused a dose-dependent burning pain which occurred delayed compared to capsaicin. LPA hardly induced any flare reaction but a sensitization to heat. Responses to cold, mechanical and electrical stimuli remained unaltered.



LPAR1 staining co-localizes with glial markers. Mouse DRGs were stained for LPAR1 (magenta), glutamine synthase (GS, green) and Hoechst 33342 (blue). A) The merged image shows a substantial co-localization of LPAR1 and glutamine synthase. B) Enlargement marked of cell.

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

Invited lectures

14th Expert summit on viral hepatitis, 23.02.2018, Frankfurt, IgG4 assoziierte Erkrankungen – multiple Facetten

PBC in Motion, 10.03.2018, London, Managing symptoms and other manifestions in PBC

5. Bonner Hepatogastroenterologisches Forum, 28.04.2018, Bonn, Problemsymtom Pruritus – wen, wie, womit behandeln?

Falk Gastro Forum, 05.05.2018, Erlangen, Update: Therapie der cholestatischen Lebererkrankungen

Clinics and Science in Hepatology, 19.05.2018, Birmingham, Case-based challenges: autoimmune cholestatic liver diseases

Hamburger Lebertage, 25.05.2018, Hamburg, Erhöhte Leberwerte bei Schwangerschaft - wann ein Alarmsignal?

China Lecture Tour, June 2018, Lanzhou, Shenyang, u.a., China, NASH: the new epidemic in hepatology

Münsteraner Pruritussymposium, 08.09.2018, Münster, Neues in der Therapie von Pruritus bei Lebererkrankungen

Publications during funding period

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*contributed equally

F5 - Final Report

01/07/2016 - 31/12/2018

The Role of ANO1 in Polycystic Kidney Disease

PD Dr. Björn Buchholz, Department of Medicine 4

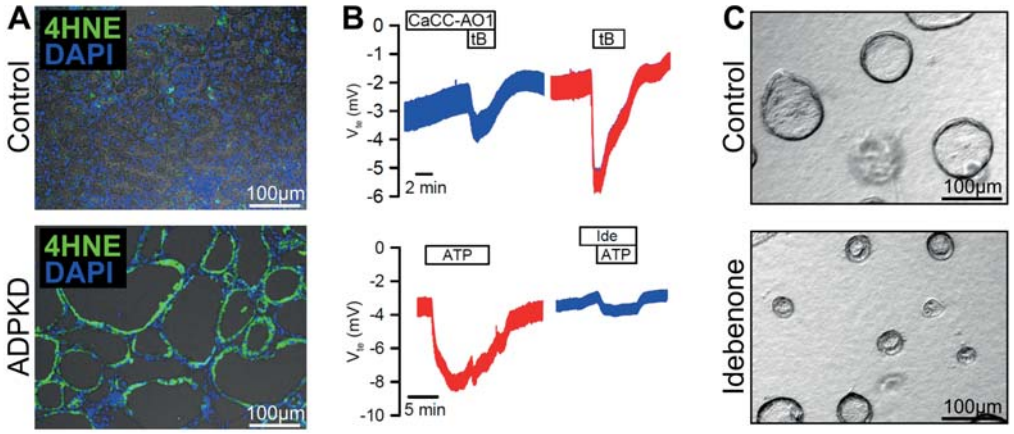
Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a frequent renal disorder which is characterized by continuous secretion-dependent growth of multiple cysts in both kidneys often resulting in end stage renal disease. We have shown that the calcium-activated chloride channel anoctamin 1 (ANO1)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

ANO1 promotes cyst growth in an ADPKD mouse model

We have established an ADPKD mouse model in collaboration with Prof. Peters (Dept. of Human Genetics, Leiden). This mouse model is characterized by an inducible tubule-specific deletion of the PKD1-gene ($KSPCreER^{T2}$;$PKD1^{lox;lox}$), the main affected gene in human ADPKD. Deletion of PKD1 by application of tamoxifen at postnatal day 20 resulted in significant polycystic kidney disease within 9 weeks. In contrast, double knockout of PKD1 and ANO1 significantly ameliorated the renal cystic phenotype (unpublished data). In addition, we have tested several ANO1 inhibitors in our established in vitro cyst model for their efficacy and toxicity and found 2 candidates which we are also planning to apply in our in vivo model.

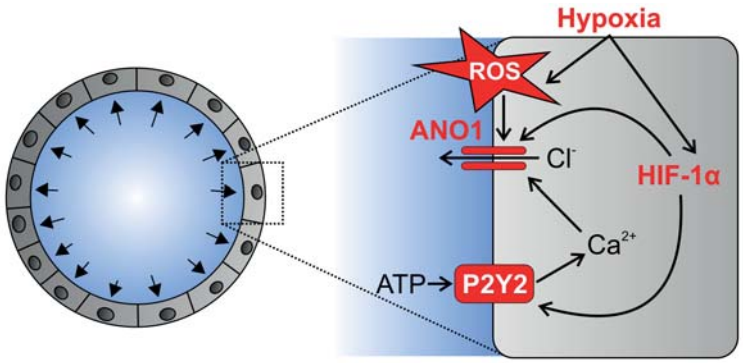
HIF-1α and P2Y2R mediate ANO1-dependent chloride secretion and cyst expansion

Since ANO1-dependent chloride conductance is activated by cytosolic increase of calcium, we tested for the underlying mechanisms. We found that ATP, which highly accumulates in the cyst fluid, leads to activation of ANO1. In addition, we could show that the Gq-coupled purinergic receptor P2Y2R mediates ANO1-dependent chloride secretion and in vitro cyst expansion. Furthermore, we identified P2Y2R as a target gene of the hypoxia inducible transcription factor HIF-1α which is in line with our previous findings that showed that HIF-1α promotes secretion-dependent cyst enlargement in vitro. Recently, we could show that HIF-1α is highly expressed in cyst-lining cells. In line with these findings, tubular deletion of HIF-1α in our ADPKD mouse model significantly



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

PD Dr. Buchholz



Cysts grow continuously in ADPKD which leads to hypoxia. Hypoxia activates HIF-1α which induces P2Y2R. ATP stimulates P2Y2R which results in Ca^{2+}-activated Cl^- Secretion. Both, ROS and HIF-1α lead to activation of the Ca^{2+}-activated Cl^- channel ANO1.

attenuated cyst growth, whereas pharmacological induction of HIF-1α resulted in a deleterious aggravation of the cystic phenotype which could be rescued by genetic deletion of HIF-1α. This is of further clinical interest since several drugs that result in HIF-induction are currently under investigation for their application as erythropoiesis-stimulating agents.

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

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

Contact:
PD Dr. Buchholz
phone: +49 9131 85 39002
e-mail: bjoern.buchholz@uk-erlangen.de

Publications during funding period

Schreiber R*, Buchholz B*, Kraus A, Schley G, Scholz J, Ousingsawat J, and Karl Kunzelmann (2019) Lipid peroxidation drives renal cyst growth in vitro through activation of TMEM16A. Journal of the American Society of Nephrology. doi: 10.1681/ASN.2018010039. *equal contribution

Kraus A, Peters DJM, Klanke B, Weidemann A, Willam C, Schley G, Kunzelmann K, Eckardt K-U, and Buchholz B (2018) HIF-1α promotes cyst progression in a mouse model of autosomal dominant polycystic kidney disease. Kidney International. doi: 10.1016/j.kint.2018.06.008

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F6 - Final Report

01/07/2016 - 31/12/2018

Renal afferent nerve activity - sympathoinhibitory or sympathoexcitatory?

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

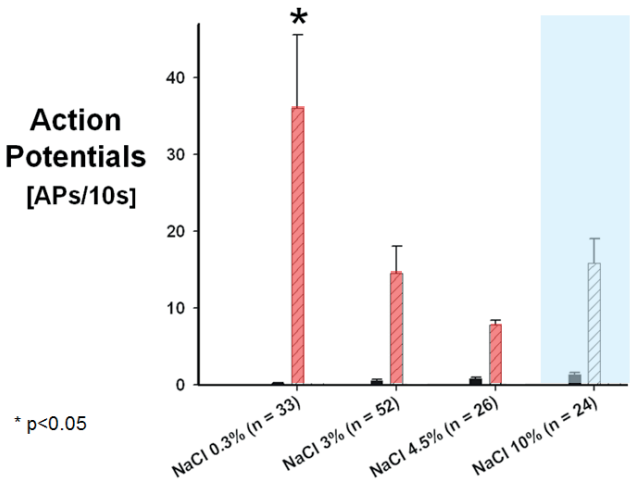
All our experimental activities during the time of the project supported our main hypothesis hat afferent nerves from the kidneys are important cardiovascular regulators in health and disease. With the projects of the recent months we wanted to improve our understanding of afferent nerve units with respect to the kidney and beyond.

Na^+ – further analysis of its effects on action potential production.

We had seen that intrarenal sodium injection in quite small amounts decreased renal sympathetic nerve activity in vivo while stimulating tonic highly active renal neurons in culture. Further analysis revealed that the superperfusion of cultured neurons with a solution of quite low sodium (0.3 %) elicited the highest production of action potentials that decreased in response to higher NaCl concentrations. These data could also suggest that chronic intrarenal increases of interstitial NaCl might eventually affect the sensitivity of afferent renal nerves units.

Renal afferent nerve units – autostimulation by secreted proinflammatory substances?

Release of the proinflammatory peptides SP and CGRP from afferent nerves influence local inflammation. Hence we wanted to test the hypothesis that SP and CGRP also influence action potential production in cultured neurons with afferent axons from the kidney. However, neither the mere addition of SP nor CGRP could increase action potential generation in renal neurons. Proton stimulation (pH 6) of TRPV1 is known to increase action potential production in highly active neurons. The co-stimulation of renal neurons with protons (pH 6) and SP increased



Responses to NaCl superfusion of renal neurons. Highly active neurons showed a significant increase of action potentials to NaCl.


Prof. Dr. Veelken


Prof. Dr. Amann

the number of action potentials in these neurons compared to a co-stimulation with CGRP, that was ineffective. Hence, SP in contrast to CGRP facilitated action potential production in highly active neurons. SP might increase the sensitivity of afferent renal nerve pathways in renal inflammation thus influencing renal sympathetic nerve control.

Decreased activity of afferent nerve units – a common pathophysiological feature ?

In rat models with hypertension and/or renal disease the responsiveness of afferent renal nerve units was in all our experiments shifted from units with highly active neurons to units with neurons of low activity. Likewise, in afferent vagal nerve activity in congestive heart failure (CHF) a decreased activity was described by others. Hence, we tested the hypothesis that in CHF the vagal afferent nerve pathway consists of a decreased number of highly active sensory neurons. We used rats with nephropathy as a renal model for comparison. In CHF rats, the number of harvested cardiac neurons with highly active response pattern did not differ from controls, but highly active cardiac neurons from CHF rats exhibited an increased production of action potentials. However, in nephropathic rats, the number of neurons with a highly active response pattern decreased significantly, but action potential production was unaltered. Hence, in congestive heart failure vagal afferent neurons increase their sensitivity possibly due to impaired intracardiac receptors whereas in renal disease the responsiveness of the afferent pathway seemed to be impaired as a whole.

Contact:
Prof. Dr. Veelken
phone: +49 9131 85 39002
e-mail: roland.veelken@uk-erlangen.de

Prof. Dr. Amann
phone: +49 9131 85 22291
e-mail: kerstin.amann@uk-erlangen.de

Invited lectures

Council on Hypertension 2018, Prof. Dr. med. Roland Veelken, 06.-09.09.2018, Chicago, Illinois, USA, Inhibition Of Renal Sympathetic Nerve Activity by intrarenal Salt

International Society of Hypertension 2018, Martin Hindermann, 20.-24.09.2018, Beijing, China, Intrarenal NaCl Boli Cause Longlasting Inhibition Of Renal Sympathetic Nerve Activity (RSNA)

Jahrestagung der deutschen Gesellschaft für Nephrologie, Martin Hindermann, 27.-30.09.2018, Berlin, Intrarenales Bradykinin in niedrigen Dosen führt zu einer monophasischen Sympathoinhibition

Publications during funding period

none

Junior Research Group 1

Dr. Paolo Ceppi

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

Biographical Sketch

The Junior Group Leader Dr. Paolo Ceppi started his appointment at the Interdisciplinary Center for Clinical Research (IZKF), Friedrich-Alexander University Erlangen-Nürnberg in Erlangen on August 1st, 2015.

Below is a list of the previous research appointments:

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

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

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

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

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

From the left: Sabine Marschall, Heike Wagner, Beatrice Parma, Paolo Ceppi, Annemarie Schwab, Paradesi Naidu Gollavilli, Aarif Siddiqui

Research Focus

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

Background and Rationale: Despite the progresses made in the last years with the development of novel molecularly targeted agents, cancer is still a very deadly disease. This could be attributable in part to the fact that only a minority of selected patients benefit from the novel compounds (such as those targeting oncogenic drivers like EGFR, BRAF, HER2 and many others), while poor therapeutical options are available for the vast majority of the patients in which a targetable driving oncogenic mutation is undetermined. Moreover, the pathway redundancy and the very frequent occurrence of mutations limit the efficacy of these novel drugs even in initially responding patients. There is therefore an urgent need for the identification of novel fundamental mechanisms of cancer biology and of relevant determinants of chemoresistance in order to develop more effective drugs and therapeutic strategies.

The discovery of epithelial-to-mesenchymal transition (EMT), cancer stem cells (CSCs) and of their functional association and interdependence represent some of the most promising advances in the last two decades of cancer research. CSCs are defined as a subpopulation of undifferentiated cancer cells with stem-like features responsible for tumors' heterogeneity and for some of the most lethal features of cancers: tumorigenicity, metastatic spread, relapse and chemoresistance. The inter-conversion between CSCs and non-CSCs has been recently reported and the EMT clearly functionally involved. The EMT is a de-differentiation process frequently observed in cancers with increased invasive potential and drug resistance. A recently emerging concept is that the plasticity of cancers is greater than what initially hypothesized, and therefore a better understanding of the mechanisms behind the inter-conversion of cancer cells between differentiation stages may have many therapeutic implications. Moreover, cancers, and the CSC population in particular, are highly dependent on aerobic glycolysis, which they use as a major pathway for biosynthesis. The enhanced rate of glycolysis occurs largely because of the increased demand of a transformed cell for macromolecule components (the so-called Warburg effect). The connection between increased glycolytic rate, EMT and CSCs has recently started to emerge in the literature, but the molecular determinants involved are still undefined.

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

Third-party funding

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

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

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

Paolo Ceppi, DFG Research Grant, Deciphering and targeting the metabolic control of lung cancer de-differentiation, 2019-2022

N1 - Progress Report

01/08/2015 - 31/07/2021

Understanding the plasticity of cancer cells

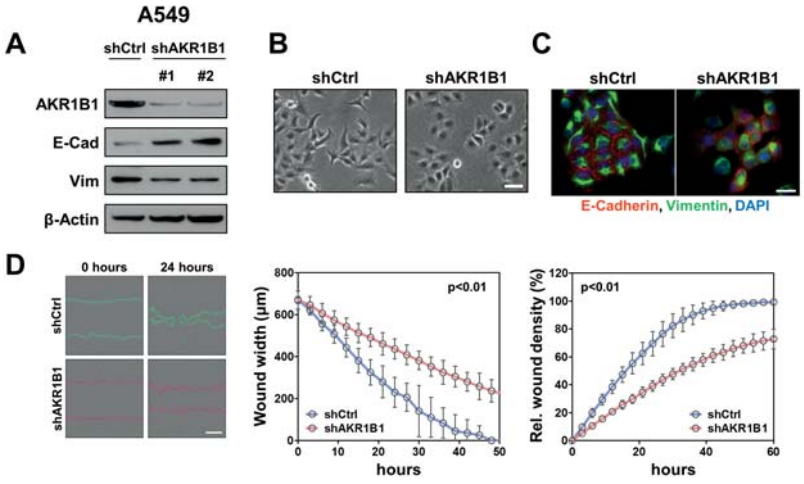
Dr. Paolo Ceppi, IZKF - Junior Research Group 1

The group focuses on the identification of novel fundamental mechanisms of cancer biology using several cell and molecular biology techniques, mouse models, high-throughput approaches and the analysis of human samples. We aim at discovering novel genes and molecular pathways that regulate the plasticity and the aggressiveness of cancer cells and at studying the association between cancer differentiation and sensitivity to chemotherapy, with a special attention on metabolism genes. The final goal is the development of more effective drugs and therapeutic strategies.

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

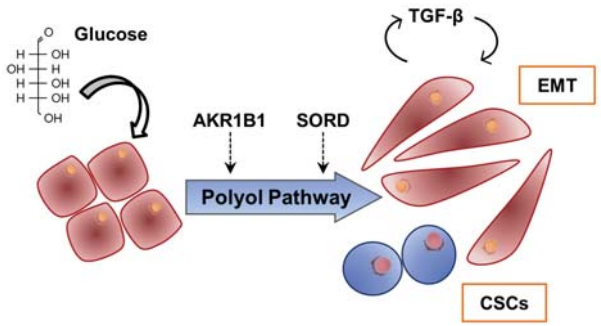
1) The role of polyol pathway in linking glucose metabolism to the aggressiveness of cancer cells (Schwab et al. Cancer Research 2018).

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



AKR1B1 suppression inhibits EMT in cancer cells. A) Western blot analysis, B) morphological appearance, C) immunofluorescence of E-Cadherin and Vimentin expression, and D) Incucyte migration assay in A549 infected with shRNA targeting AKR1B1 compared to scrambled-infected cells.

Dr. Ceppi



Scheme of the proposed role of polyol pathway genes in EMT and CSCs: excess glucose is partly metabolized in the PP supporting EMT via TGF-β autocrine stimulation.

2) The role of the nucleotide metabolism enzyme thymidylate synthase in the de-differentiation of triple-negative breast cancer (Siddiqui et al. Submitted).

Summary: Cancer cells frequently boost nucleotide metabolism (NM) to support their increased proliferation, but the consequences of elevated NM on tumor de-differentiation are mostly unexplored. Here, we identified a role for thymidylate synthase (TS), a NM enzyme and established drug target, in cancer cell de-differentiation and investigated its clinical significance in breast cancer (BC). In vitro, TS knockdown increased the population of CD24+ differentiated cells, and attenuated migration and sphere-formation. RNA-seq profiling indicated a repression of epithelial-to-mesenchymal transition (EMT) signature genes upon TS knockdown, and TS-deficient cells showed an increased ability to invade and metastasize in vivo, consistent with the occurrence of a partial EMT phenotype. Mechanistically, TS enzymatic activity was found essential for the maintenance of the EMT/stem-like state by fueling a dihydropyrimidine dehydrogenase – dependent pyrimidine catabolism. In patient tissues, TS levels were found significantly higher in poorly differentiated and in triple negative BC, and strongly correlated with worse prognosis. The present study provides the rationale to study in-depth the role of NM at the crossroads of proliferation and differentiation, and depicts new avenues for the design of novel drug combinations for the treatment of BC.

Contact:
Dr. Ceppi
phone: +49 9131 85 39300
e-mail: paolo.ceppi@uk-erlangen.de

Invited lectures

Lecture series of the Department of Molecular Medicine, University of Pavia, 25.10.2018, Pavia, Italy, Metabolic pathways as regulators of EMT

5th Annual Meeting of the International Society of Cancer Metabolism, Metabolic Adaptations and Targets in Cancer, 18.10.2018, Bratislava, Slovakia, Polyol pathway connects glucose metabolism with the aggressiveness of cancer cells

Cancer Research & Targeted Therapy 2018 Conference, 07.08.2018, London, UK, Polyol pathway as a novel therapeutic target for aggressive tumors

Lecture series of the Cancer and Stem Cell Biology Department of Duke NUS, 23.04.2018, Singapore, Metabolic pathways as regulators of EMT

Publications during funding period

Krumbholz M, Woessmann W, Zierk J, Seniuk D, Ceppi P, Zimmermann M, Singh V, Metzler M, Damm-Welk C (2018) Characterization and diagnostic application of genomic NPM-ALK fusion sequences in anaplastic large-cell lymphoma. Oncotarget 9(41): 26543-26555

Schwab A, Siddiqui A, Vazakidou ME, Napoli F, Böttcher M, Menchicchi B, Raza U, Saatci Ö, Krebs AM, Ferrazzi F, Rapa I, Dettmer-Wilde K, Waldner MJ, Ekici AB, Rasheed SAK, Mougiakakos D, Oefner PJ, Sahin Ö, Volante M, Greten FR, Brabletz T, Ceppi P (2018) Polyol pathway links glucose metabolism to the aggressiveness of cancer cells. Cancer Research 78: 1604-1618

Rasheed SAK, Leong HS, Lakshmanan M, Raju A, Dadlani D, Chong FT, Rajarethinam R, Skanthakumar T, Tan EY, Hwang JSK, Lim KH, Tan DS, Ceppi P, Wang M, Tergaonkar V, Casey PJ, Iyer G (2018) GNA13 expression promotes drug resistance and tumor-initiating phenotypes in solid tumors. Oncogene 37(10): 1340-1353

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

Junior Research Group 2

Dr. David Dulin

Contact:
phone: +49 9131 85 70347
e-mail: david.dulin@uk-erlangen.de

Biographical Sketch

Since September 2016, Dr. Dulin has started the "Physics and Medicine" IZKF Junior Research group N2 at Erlangen, aiming at studying viral and cellular transcription and replication at the single-molecule level using biophysical techniques.

Before starting his lab, Dr. Dulin graduated his Bachelor in physics and mathematics at the University of Bordeaux (France) in 2004 and his Master "Laser, Matter and Nanoscience" in 2006.
Between 2006 and 2009, he was a PhD candidate in the Laboratory Charles Fabry of the Institut d'Optique (Paris) in the group of Prof. A. Aspect and under the supervision of Prof. N. Westbrook. There, he worked at establishing a new biophysics lab, with a focus on bacterial ribosome translation kinetics using single-molecule fluorescence microscopy.
He then moved to a first postdoctoral position in the lab of Prof. N. Dekker at TU Delft (The Netherlands), where he stayed until August 2014. There, he developed new magnetic tweezers approaches for high throughput and high-resolution study of polymerases and helicases kinetics. In particular, he studied the mechanism of misincorporation and antiviral nucleotide analogue incorporation by viral polymerase.
He then moved to the University of Oxford (UK) for a second postdoctoral position, where he studied bacterial transcription initiation dynamics using single-molecule FRET in the lab of Prof. A. Kapanidis, until being appointed in Erlangen.

From the left: D. Dulin, M. Seifert, M. Spermann, F. Stal-Papini, E. Ostrofet

Research Focus

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

1- RNA virus replication mechanism

RNA viruses represent an important class of human and animal pathogens. They are responsible of numerous pandemics worldwide, with an important economical and societal cost. Our lab is interested in discovering how RNA viruses replicate their genome, and whether there are conserved mechanism that could be targeted by antiviral drugs. In particular, our lab focuses its research on the flavivirus genus, the most resurgent and emergent family of virus. Members of this family, e.g. Dengue, West Nile and Zika, are responsible for hundreds of million of viral infections every years, with little or none therapeutic options. One key target for drug development is the replication machinery of these viruses. However, little is known concerning the mechanisms of genome replication in flavivirus, limiting the potential development of drugs. Using magnetic tweezers and single-molecule FRET, we aim at understanding how the viral proteins that form the flavivirus replicase are recruited and how they work in synergy during viral genome replication.

2- Human mitochondrial transcription

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

N2 - Progress Report

01/09/2016 - 31/08/2022

Physics and Medicine

Dr. David Dulin, IZKF - Junior Research Group 2

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

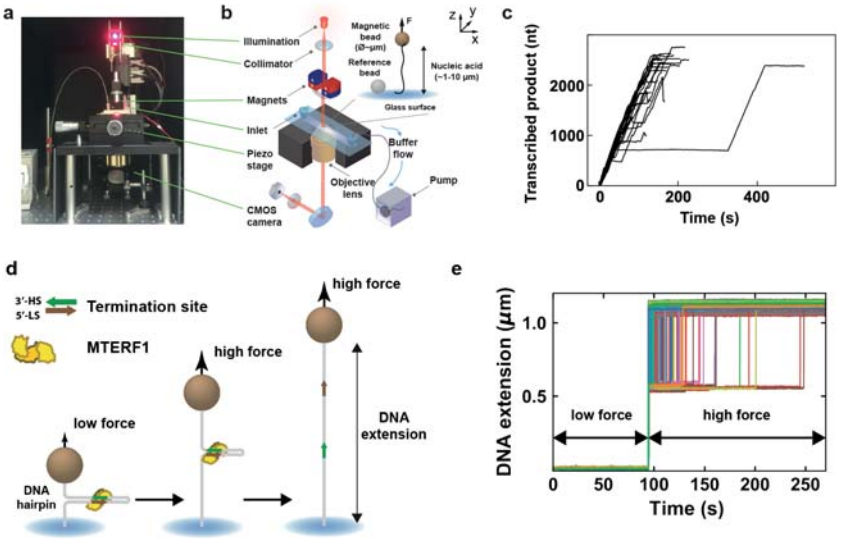
In September 2016, the Junior Research Group N2 has started a new research activity in Erlangen focused on the study of molecular processes with high-end microscopy. During the first six months, we have established a fully functional molecular biology lab to synthesize the nucleic acids scaffolds used in magnetic tweezers experiments. We have now a microscopy lab with two magnetic tweezers apparatuses and a molecular biology lab to prepare nucleic acids scaffolds. In addition, the lab has grown with new members: Dr. Flavia Stal-Papini (research assistant, October 2016), Eugen Ostrofet (PhD candidates, May 2017) and Mona Seifert (PhD candidates, January 2018). A new PhD candidate, Ibrahim Obulqasim, and a new postdoc, Dr. Subhas Chandra Bera, will join us in 2019. Finally, the lab is moving to a new location in February: the Interdisciplinary Centre for Nanostructured Films (IZNF), Cauerstrasse 3 (south campus of the FAU at Erlangen).

To perform single-molecule magnetic tweezers experiments, it is necessary to design and synthesize specific DNA scaffolds. Using standard molecular biology techniques, we produce DNA or RNA scaffold with a high yield and a high purity. We have now established protocols to synthesize DNA and RNA hairpins, linear DNA and RNA for the different experiments we perform in our lab. We will soon submit an article that describes our new developments (Project Leader: Dr. Flavia Stal Papini). Magnetic tweezers are a force and torque spectroscopy technique, i.e. one uses them to apply force and torque on nucleic acids. However, a complete force calibration needs to be performed first, which necessitates DNA scaffolds of a defined length. Following the fabrication of the appropriate DNA scaffold, we have developed a new approach to perform such calibration, which has recently been published in Scientific Reports (Project leader: E. Ostrofet).

In addition, we have developed a simple and efficient temperature control system to maintain our experiments at a constant temperature from room temperature to ~50°C, which may be applicable to other high end microscopes. We used this assay to study the temperature dependence of poliovirus, human rhinovirus C and Φ6 RdRp's viral polymerase in vitro replication activity, and we are now writing a publication about it. Being now able to work at the optimum growth temperature for a given virus, we are now investigating the sequence dependent elongation kinetics of several RNA viruses (Project leader: M. Seifert).

In another project, we study the directionality and the mechanism of transcription termination in human mitochondria. In mitochondria, transcription is terminated in a directional manner by a protein coined MTERF1, which specifically binds to the termination site. To investigate how is transcription termination directionality, we have developed a DNA hairpin based approach where we can open the hairpin from either direction. We have observed that strand displacement is enough to explain the directionality of transcription termination by MTERF1 (Project leader: E. Ostrofet). Interestingly, this stra-

Dr. Dulin



(a) Magnetic tweezers set up and (b) its schematic description. (c) Traces of individual human rhinovirus C polymerase from a single experiment. (d) MTERF1-DNA interaction study using a force jump experiment on a DNA hairpin. (e) Experimental observations from the experiment described in (d): MTERF1 blocks the opening of the DNA hairpin.

tegy for transcription termination is shared with Pol I, potentially presenting a conserved mechanism for termination induced by a DNA bound protein.

Finally, we are building a new high-end microscope (TIRF) to perform high spatiotemporal resolution fluorescence microscopy measurements at the single molecule level in order to study cellular transcription and viral replication.

Contact:
Dr. Dulin
phone: +49 9131 85 70347
e-mail: david.dulin@uk-erlangen.de

Invited lectures

GFV2018 Annual Meeting of the German Society for Virology, March 2018, Würzburg, Germany, Use of highly multiplexed single-molecule magnetic tweezers to characterise drug incorporation by Polio virus RNA-dependent RNA polymerases

Single Molecule Biophysics meeting, January 2019, Aspen, CO, USA, Temperature dependent kinetic study of RNA-dependent RNA polymerases using high-throughput magnetic tweezers

Publications during funding period

Ostrofet E, Stal Papini F, Dulin D (2018) Correction-free force calibration for magnetic tweezers experiments. Scientific Reports 8: 15920

Dulin D, Bauer DLV, Malinen AM, Bakermans JJW, Kaller M, Morichaud Z, Petushkov I, Depken M, Brodolin K, Kulbachinskiy A and Kapanidis AN (2018) Pausing controls branching between productive and non-productive pathways during initial transcription in bacteria. Nature Communications 9: 1478

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

Modulation of PRC2 activity by HCMV IE2

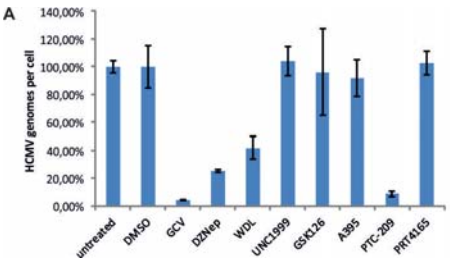
Dr. Nina Reuter, Institute of Clinical and Molecular Virology

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

Role of PRC1/2 during lytic HCMV replication

To address the relevance of PRC1/2 activity for the productive life cycle of HCMV, we dissected the levels of PRC1/2 core components following HCMV infection. This revealed that all major PRC1 and 2 factors are massively upregulated following infection and recruited into viral replication compartments (VRCs; sites of viral DNA amplification) as infection progresses. Interestingly, however, the repressive histone marks instituted by PRC1/2 turned out to be specifically excluded from these sites suggesting a role of both complexes in viral DNA synthesis independent of their repressor activity. Indeed, using primary human foreskin fibroblast (HFF) knockdown cells in which individual PRC1/2 core factors were depleted by expression of respective shRNAs, we could show that PcG proteins are required for an efficient HCMV genome amplification. This is in accordance with recent reports from literature that identified a novel role of PcG factors in regulating the normal progression of cellular DNA replication. Since PRC1/2 have emerged as promising drug targets in cancer therapy, we were able to test a series of diverse PRC1/2 inhibitory substances. All of these agents have in common that they inhibit the enzymatic activity of the targeted repressor complex. Importantly, some substances additionally have the capacity to induce a destabilization of the respective complex, which is known to go along with a downregulation of certain PRC core components. Intriguingly, only substances which negatively affected complex stability like DZNep, Wedelolactone (WDL) or PTC-209 were able to compromise HCMV genome synthesis, while inhibition of PRC1/2's enzymatic activity alone (UNC1999, GSK126, A395 and PRT4165) had no effect. Taken together, this leads to the overall assumption that regulation of DNA amplification by PRC1/2, which is poorly defined yet, occurs in an enzymatic-independent manner (non-canonical mode of action).

A

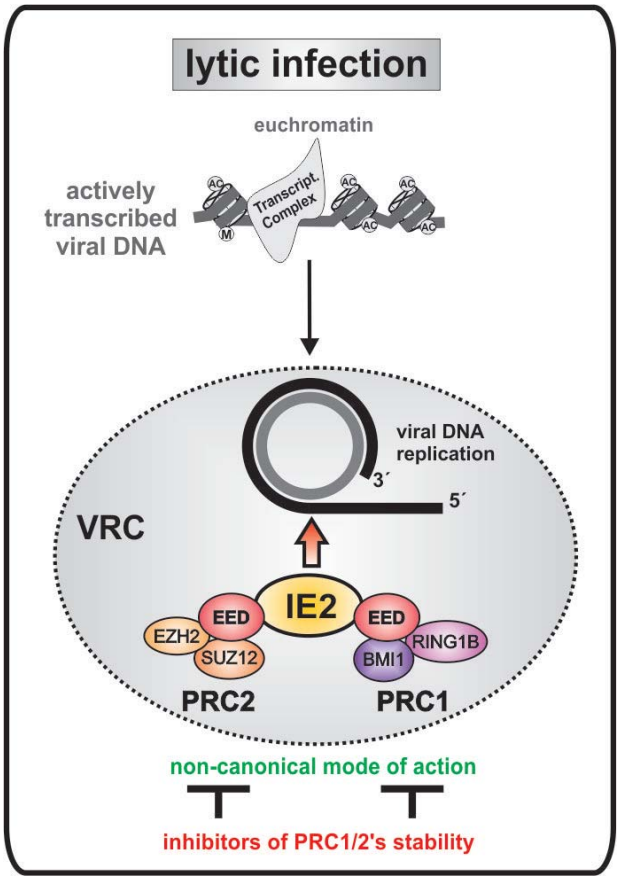


B

Substance	Target	Inhibition of		
		Enzymatic activity	Complex stability	HCMV DNA replication
DZNep	PRC2	+	+	+
WDL	PRC2	+	+	+
UNC1999	PRC2	+	-	-
GSK126	PRC2	+	-	-
A395	PRC2	+	-	-
PTC-209	PRC1	+	+	+
PRT4165	PRC1	+	-	-

Enzymatic-independent contribution of PRC1/2 to HCMV DNA synthesis. (A) Quantification of viral genome copies following treatment of infected HFFs with indicated inhibitors. (C) List of PRC1/2 inhibitors and their effect on HCMV DNA replication.

Dr. Reuter



Role of PRC1/2 for lytic HCMV infection. PRC1/2 are recruited by the HCMV IE2 protein into VRCs for efficient genome synthesis. Both PRCs function in a non-canonical manner as they can only be inhibited by compounds that target complex stability.

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

In former studies, we discovered an interaction between the HCMV transactivator protein IE2 and the PcG protein EED, which is a shared component of both complexes, PRC1 and 2. This suggests that HCMV has the capacity to regulate PRC1/2 activity for its own benefit. To test this theory, we generated recombinant viruses lacking the EED interaction interface within IE2. Multi-step growth curve analysis revealed a severe growth defect of the EED interaction-deficient IE2 mutants in comparison to wildtype (wt) HCMV. In accordance with our hypothesis, we observed an impaired intracellular accumulation of newly synthesized viral DNA in case of the mutant viruses which resulted from an incomplete relocalization of PcG proteins into VRCs when compared to wt HCMV. In summary, we identified a novel interaction between IE2 and EED, which contributes to the recruitment of PcG proteins into VRCs for efficient HCMV DNA replication.

Contact:
Dr. Reuter
phone: +49 9131 85 22017
e-mail: nina.reuter@uk-erlangen.de

Publications during funding period

Reuter N, Reichel A, Stilp AC, Scherer M, Stamminger T (2018) SUMOylation of IE2p86 is required for efficient autorepression of the human cytomegalovirus major immediate-early promoter. J. Gen. Virol. 99: 369–378

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Scherer M, Otto V, Stump JD, Klingl S, Müller R, Reuter N, Muller YA, Sticht H, Stamminger T (2015) Characterization of recombinant human cytomegaloviruses encoding IE1 mutants L174P and 1-382 reveals that viral targeting of PML bodies perturbs both intrinsic and innate immune responses. J. Virol. 90(3): 1190-205

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

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

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

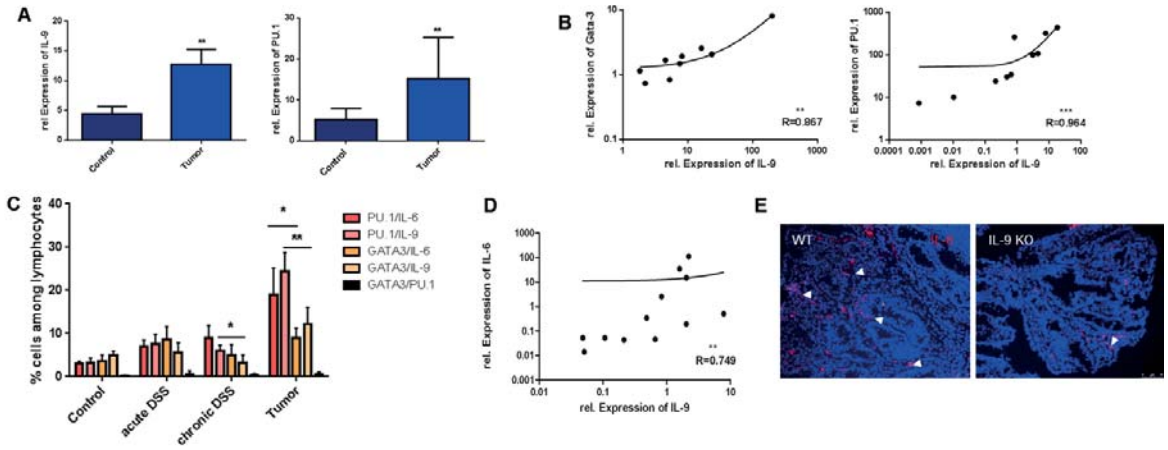
Inflammatory bowel disease (IBD) is combined with an elevated risk for developing colitis-associated colorectal cancer (CAC). Especially T cells are critical mediators playing important roles in the development of inflammation and cancer. Here, we found significant expansion of IL-9-expressing Th9 cells in CAC whereas IL-9 deficiency led to less tumor growth. The increased presence of PU.1-expressing T cells in tumorigenic tissue illustrates the involvement of Th9 cells in the carcinogenesis.

Mucosal PU.1+ T cells producing IL-9 are induced in colitis-associated neoplasias

Recently, a group of interleukin 9 (IL-9)-producing CD4+ T cells, termed Th9 cells, has been characterized with both tumour-inhibiting as well as tumour-promoting effects. In the model of AOM/DSS we found an up regulation of the proinflammatory cytokine IL-9 and the Th9 cell related transcription factor PU.1 suggesting a protumorigenic role for Th9 cells. Additionally, IL-9 levels correlated with the expression of the Th2 cell transcription factor GATA3 and the Th9 cell transcription factor PU.1. As Th9 cells can develop from Th2, we next checked the contribution of both cell types in CAC. Indeed we found both T cell subtypes present in the development from acute colitis to chronic colitis to CAC formation, but in tumor tissue the number of Th9 cells excelled Th2 cells suggesting that Th9 cells are the main drivers in the emergence of colitis associated neoplasias.

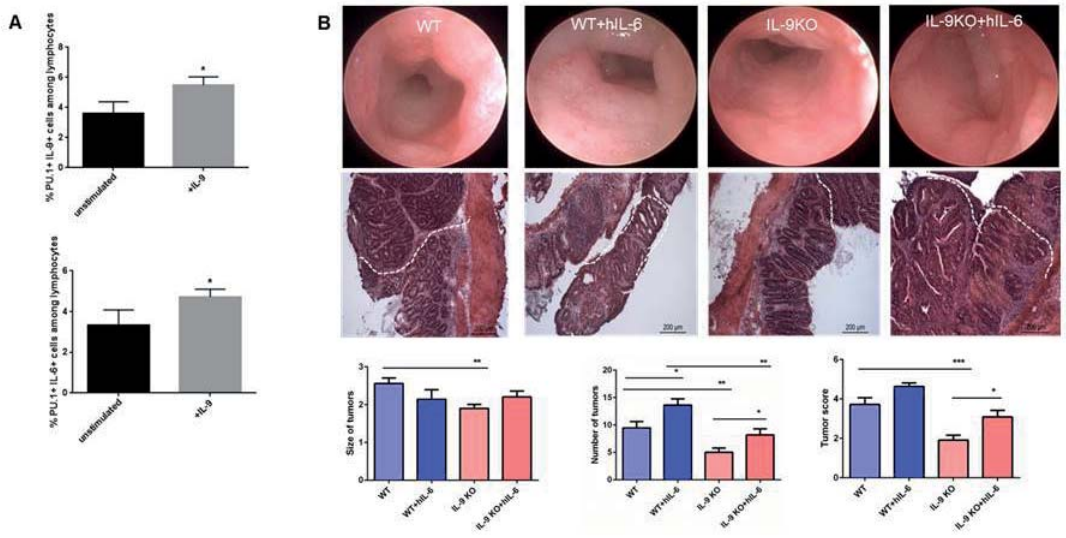
Increased IL-9 levels lead to higher levels of the pro-inflammatory cytokine IL-6

Interestingly, IL-6-expressing T cells rose during the different stages of tumor development from control tissue to tumor tissue suggesting that the pro-inflammatory cytokine IL-6 is secreted during colitis to tumor development. As IL-6 is a protumorigenic cytokine that is highly elevated in colitis-associated neoplasias we further checked the correlation between IL-6 and IL-9 mRNA levels in CAC tumors of wildtype mice. Indeed IL-6 and IL-9 expression positively correlated in AOM/DSS tumor tissue. Further-



(A) Up regulation of IL-9 and PU.1 expression in CAC. (B) In CAC IL-9, GATA3 and PU.1 expression correlated. (C) Higher numbers of PU.1+ T cells in the tumor tissue were present producing IL-9 and IL-6. (D) Correlation of IL-6 and IL-9 expression in tumor tissue (E) More IL-6+ cells were detectable in the tumor tissue of wildtype mice.

Dr. Gerlach



(A) Stimulation of LPMCs with recombinant IL-9 led to an induction of PU.1+IL-6 expressing T cells. (B) Abrogation of the tumor-promoting effect of IL-9 KO upon administration of hIL-6 in the experimental AOM/DSS model.

more, higher numbers of IL-6+ cells were evident in the lamina propria of tumor tissue from wildtype mice than in tumor tissue from IL-9 KO mice. Along with that we found that Th9 cells under IL-9 stimulation were induced to produce more IL-9 as an autocrine mechanism and certain amounts of IL-6 as well. These findings confirmed that IL-6 levels where elevated by IL-9. Therefore we analysed in a next set of experiment the influence of IL-6 on tumor development in IL-9-deficient animals. Consequently, we treated IL-9 KO mice during the experimental tumor model with hyper IL-6, a protein consisting of the soluble IL-6 receptor and the protein IL-6 itself. Strikingly, IL-9 deficient mice given hyper IL-6 showed normal induction and development of colorectal tumors comparable with wild-type mice as shown in the miniendoscopic analysis and H&E stainings. Administration of hyper IL-6 reconstituted the phenotype of IL-9 KO mice in the AOM/DSS model and restored tumor induction to levels observed in wildtype. Taken together, IL-9 appears to play a crucial role in inflammation-associated neoplasias by favoring production of proinflammatory cytokines and inflammation-mediated tumor growth. These findings identify IL-9 as a potential link between mucosal inflammation and cancer.

Contact:
Dr. Gerlach
phone: +49 9131 85 35891
e-mail: katharina.gerlach@uk-erlangen.de

Awards

Falk Symposium 210, Poster of distinction, Katharina Gerlach, April 2018, Lisbon, Portugal

Publications during funding period

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J51 - Progress Report

01/10/2015 - 31/03/2019

Neurosciences

Inflammatory signature in Parkinson´s disease

Dr. Franz Marxreiter, Department of Molecular Neurology

The project aims to address the contribution of inflammatory processes in the course of Parkinson's Disease (PD). During the funding period, we assessed the contribution of the innate immune system (specifically monocytes), and the adaptive immune system (specifically T-lymphocytes) in PD. Furthermore, we analyzed whether altered gut microbiota contribute to Parkinson's disease (PD). Here, we provide an overview of the results obtained during the funding period.

1. Innate Immune System (Monocytes)

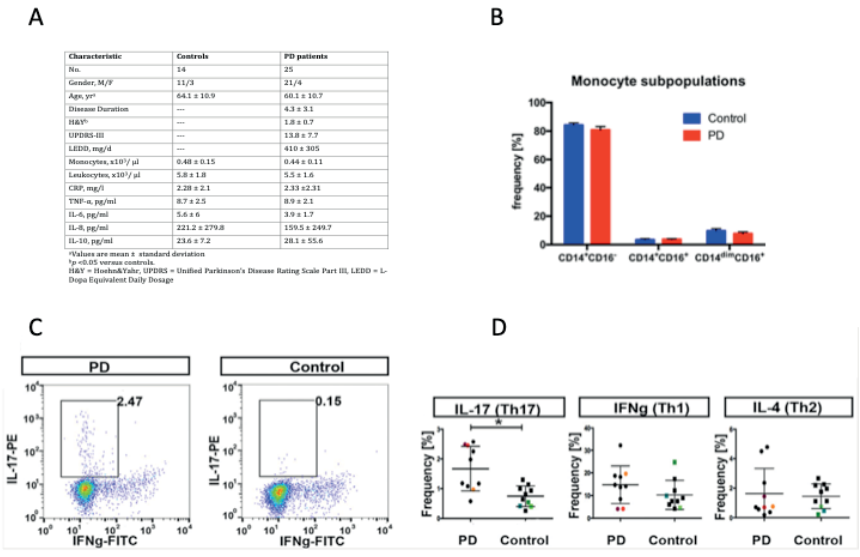
Since previous studies suggested that an activation of the innate immune system may contribute to PD pathology (Hirsch & Hunot, 2009), we initially addressed the role of monocytes in PD. Monocytes have a critical role as effectors and regulators of the innate immune system. Therefore, we aimed to explore monocyte status as potential biomarker and/or regulator for PD. We were able to identify a distinct gene expression profile that seperates PD from controls (Schlachetzki et al., 2018). Yet, the systemic cytokine profile and the monocytic composition were not altered in our PD cohort.

2. Adaptive Immune System (Th-17/Treg axis)

More recently T-cells directed against alpha-synuclein, the protein aggregating in PD, linked PD pathology to the adaptive immune system (Sulzer et al., 2017). We assessed circulating T cells in PD and observed higher Th17 frequencies as well as elevated production of IL-17 by CD4+ T cells compared to controls, and were able to show, that these PD derived Th-17 lympocytes are drivers of PD associated neurodegeneration (Sommer et al., 2018).

3. Gastrointestinal microbiome in PD

Currently, we are to analyzing the gastrointestinal microbiome in a large Bavarian cohort of PD patients. Increasing evidence suggests that altered gut microbiota may contribute to or trigger the pathological process of alpha-synuclein aggregation in the enteric nervous system (ENS) (Hopfner et al., 2017; Sampson, Debelius, Thron, Cell, 2016, n.d.; Scheperjans et al., 2014).

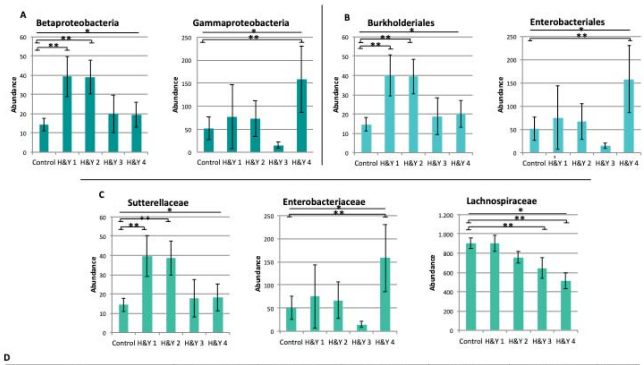


Characteristic	Controls	PD patients
No.	14	25
Gender, M/F	11/3	21/4
Age, yr[a]	64.1 ± 10.9	60.1 ± 10.7
Disease Duration	---	4.3 ± 3.1
H&Y[b]	---	1.8 ± 0.7
UPDRS-III	---	13.8 ± 7.7
LEDD, mg/d	---	410 ± 305
Monocytes, x10³/ µl	0.48 ± 0.15	0.44 ± 0.11
Leukocytes, x10³/ µl	5.8 ± 1.8	5.5 ± 1.6
CRP, mg/l	2.28 ± 2.1	2.33 ±2.31
TNF-α, pg/ml	8.7 ± 2.5	8.9 ± 2.1
IL-6, pg/ml	5.6 ± 6	3.9 ± 1.7
IL-8, pg/ml	221.2 ± 279.8	159.5 ± 249.7
IL-10, pg/ml	23.6 ± 7.2	28.1 ± 55.6

[a]Values are mean ± standard deviation
[b]p <0.05 versus controls.
H&Y = Hoehn&Yahr, UPDRS = Unified Parkinson's Disease Rating Scale Part III, LEDD = L-Dopa Equivalent Daily Dosage

A-B) The cytokine profile and the monocytic composition is not altered in PD (Schlachetzki et al., 2018) C-D) Analysis of circulating T cells of PD patients revealed increased frequencies of IL-17-producing CD4+ T cells (Th17 cells). Data are shown as means ± SD. *p < 0.05 (Sommer et al., 2018).

Dr. Marxreiter



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Genus	Control	H&Y 1	H&Y 2	H&Y 3	H&Y 4	P-value
Fusicatenibacter	79.00 ± 12.32	72.87 ± 13.09	70.00 ± 16.88	34.08 ± 9.82 ^	14.8 ± 4.31 ^	< 0.001
Citrobacter	1.19 ± 0.59	62.93 ± 62.22	3.0 ± 2.82	1.83 ± 1.49	46.50 ± 32.08 ^	0.014
Faecalibacterium	263.16 ± 26.80	210.07 ± 35.23	220.97 ± 24.95	233.83 ± 71.97	103.20 ± 22.12 ^	0.022
Gemmiger	179.68 ± 23.99	209.87 ± 49.15	128.24 ± 31.65 ^	112.50 ± 32.33	138,00 ± 28.95	0.022
Lachnospiraceae incertae sedis	273.26 ± 31.23	279.27 ± 40.59	173.39 ± 21.31	196.17 ± 59.27	152.40 ± 40.88	0.022

Differences in abundances between ctrls and H&Y stages for bacterial classes (A), orders (B) and families (C) (* = p < 0.05; ** = p< 0.0125). D) shows abundances in reads (mean ±SE) for bacterial genera (^ = vs. control p < 0.0125) in different H&Y stages.

Our goal was to evaluate

(1) whether gut microbiota are altered in a Bavarian PD cohort and

(2) whether altered gut microbiota are present already early in the course of PD.

This observational study consisted of 102 participants, comparing 71 PD patients to 31 controls. The gastrointestinal microbiome was analyzed by high-throughput sequencing (Illumina MiSeq) of the V3 and V4 regions of bacterial 16S ribosomal RNA gene.

We did not observe differences in alpha- or beta diversity between controls and PD patients, nor did we observe differences in alpha- or beta-diversity between controls and Hoehn and Yahr (H+Y) stages 1-4. Abundancies of distinct bacterial classes, orders, families, and genera differ between PD patients and healthy controls. Furthermore, distinct bacteria may be altered at early disease stages, whereas others may be altered at more advanced disease stages. On family level, Sutterellaceae were increased in early disease stages, while Enterobacteriaceae, being increased and Lachnospiraceae, being reduced at late disease stages.

Contact:
Dr. Marxreiter
phone: +49 9131 85 46928
e-mail: franz.marxreiter@uk-erlangen.de

Invited lectures

Monatstreffen der Regionalgruppe Coburg der Deutschen Parkinson Gesellschaft, 27.9.2018, Coburg, Parkinson und Darm

PARKINSONAKADEMIE, 17.11.20178, Würzburg, Parkinson und Darm

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J52 - Final Report

01/11/2015 - 30/04/2018

Neurosciences

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

Dr. Martin Regensburger, Department of Neurology

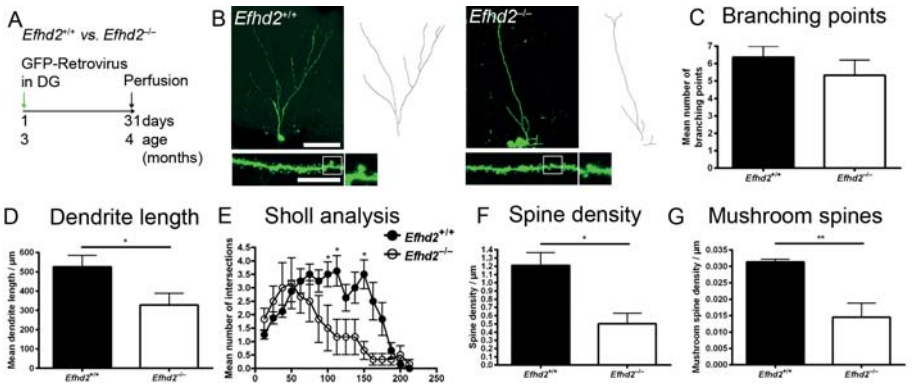
Characterizing neurodegeneration using neural stem cell based models is the overall goal of my project. To this end, we have identified a deficit of survival and spine formation of adult hippocampal newborn neurons in a transgenic mouse model of oligomeric alpha-synuclein and in an EFhd2 knockout mouse model. Moreover, we are studying neurodegeneration in induced pluripotent stem cell derived alpha motor neurons as a model of neuropathy in SPG11 hereditary spastic paraplegia.

Oligomeric species of alpha-synuclein impairs neuronal integration

Based on emerging knowledge about toxicity of the oligomeric species of alpha-synuclein in the pathogenesis of Parkinson's disease, we have analyzed newborn neurons in transgenic mouse models of alpha-synuclein. Overexpression of human wildtype alpha-synuclein merely showed a reduction of the mushroom spine type in newborn neurons. Overexpression of an alpha-synuclein mutant with predominant oligomeric conformation, however, resulted in a significant reduction of all spines. Interestingly, these changes were already evident at an age before overt neurodegeneration takes place in these models. Thus, alterations in dendritic spines precede neuronal loss in these models and are useful to study early pathology in alpha-synucleinopathies in the future.

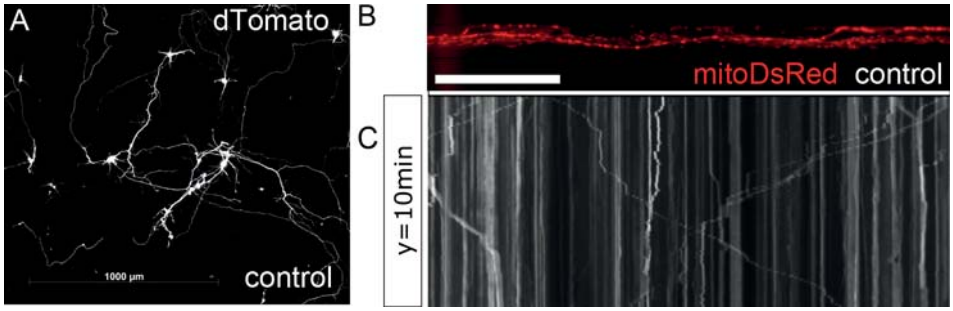
Role of Swiprosin-1/EFhd2 in the adult hippocampal neurogenic niche

Previous studies indicated a dysregulation of EFhd2 in human Alzheimer's disease brains. EFhd2 is highly expressed in the adult hippocampal neurogenic niche and has been implicated in neurodegenerative diseases. We observed a severe reduction of the survival of adult newborn neurons in Efhd2 knockouts. Dendrite morphology of newborn neurons was compromised in full EFhd2 knockouts, but not upon cell autonomous EFhd2 deletion. These data connect EFhd2 to impaired synaptic plasticity and identify a role of EFhd2 in neuronal survival and synaptic integration in the adult hippocampus. Future studies will focus on the role of EFhd2 in Parkinson's disease.



Newborn neurons in EFhd2 knockouts (A-B) show compromized dendrite morphology (C-E) and reduced spine and mushroom spine density (F-G).

Dr. Regensburger



(A) Morphological neurite analysis of motor neurons. (B-C) Analysis of mitochondrial movement using kymographs.

Axonal function in SPG11

Mutations in SPG11 are the most frequent cause of complicated autosomal-recessive hereditary spastic paraplegia. A subset of patients develops severe axonal motor neuropathy. As a disease model, we have set up the differentiation of SPG11-patient induced pluripotent stem cells (iPSC) into motor neuron progenitors (MNP) and alpha motor neurons (aMN). Survival and proliferation of MNP and aMN were not impaired in SPG11-derived cells. Future studies will focus on the axonal compartment in SPG11 aMN.

Conclusion

Within this project, different stem cell models of neurodegeneration were established and will deepen the understanding of underlying pathways. Moreover, patient-specific models of motor neuron degeneration in SPG11 hereditary spastic paraplegia serve as a platform to test different therapeutic approaches.

Contact:
Dr. Regensburger
phone: +49 9131 85 44798
e-mail: martin.regensburger@uk-erlangen.de

Publications during funding period

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J53 - Final Report

03/08/2015 - 02/02/2018

Neurosciences

Diffusion tensor imaging of the visual pathway in pseudoexfoliation glaucoma

Dr. Manuel Schmidt, Department of Neuroradiology

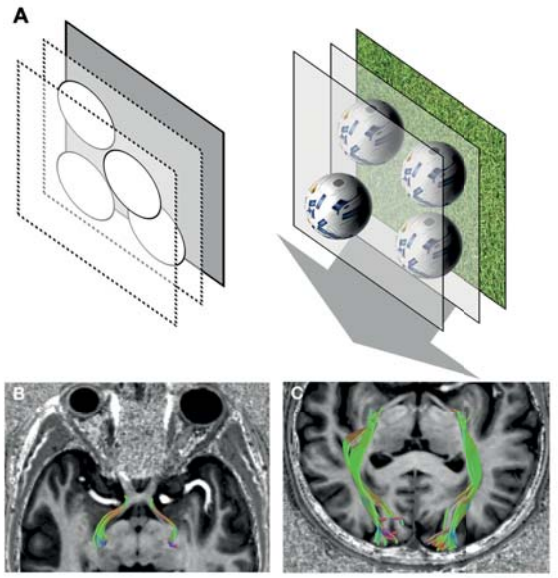
Pseudoexfoliation syndrome (PEX) is an aging-related systemic disorder of the extracellular matrix. Some patients with PEX develop glaucoma (PEXG). There is a strong genetic component. However, genetic testing is not suitable to identify those with PEX at increased risk for developing secondary glaucoma. Aim of this project is to explore the role of injury of the central visual pathway in affected patients with functional and structural MR-imaging.

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

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

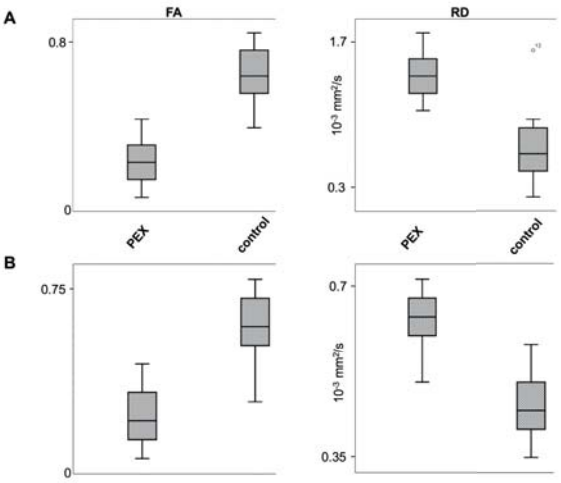
One important clinical feature of PEXG is an impaired stereoacuity resulting in depth perception deficits.

We examined the stereopsis performance in PEXG patients using a dynamic stereo test that provides a moving stereoscopic stimulus on a background with grass texture. The visual targets consisted of four spheres with the same soccer ball texture. Three of those virtual soccer balls were located on the screen plane; one had an enlarged disparity and appeared in front of the screen plane. In this configuration, the balls move out of the screen towards the observer by continuously enlarging only their disparities. The subject's task was to detect the leading ball as fast as possible. Stereoacuity - measured in arcsec as a discrimination of two points - is impaired in PEXG compared to controls (322.2 vs 29.3, p=0.01). With distinct disparity cells sensitive to binocular disparity, located in the primary visual cortex and extrastriate areas representing the neuronal basis of stereovision, injury of the central visual pathway is suggestive in PEXG.



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

Dr. Schmidt



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

Decreased fractional anisotropy and corresponding increased radial diffusivity of the optic tract (A) and the optic radiation (B) as a marker of neuronal degradation.
(C) Analysis of two common SNPs of the LOXL1 gene. The high-risk haplotype GG is significantly more frequent in PEXG.

Indeed, PEXG patients show rarefaction of the optic radiation on diffusivity maps. We discovered markedly reduced fractional anisotropy in the optic radiation (right: 0.648 vs. 0.547, p = 0.002; left: 0.638 vs. 0.554, p= 0.003) of PEXG patients suggesting axonal damage.

The structural imaging methods developed within the scope of this project have already found their way into other scientific projects regarding neurodegenerative diseases (Encephalomyelitis disseminata, Parkinson's disease).

We continue our work within the scope of a project funded by the Deutsche Forschungsgemeinschaft. In this project, we aim to develop and validate (ex vivo and in vivo) a knew MR-imaging contrast called chemical exchange saturation transfer (CEST-imaging) that allows the presence of low-concentration proteins (in the range of µM to mM) to be imaged indirectly. CEST imaging could serve as an elegant method to explore protein deposits in neuronal tissues and their role regarding axonal degradation.

Contact:
Dr. Schmidt
phone: +49 9131 85 44821
e-mail: manuel.schmidt@uk-erlangen.de

Publications during funding period

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J54 - Final Report

01/11/2015 - 30/04/2018

Oncology

Analysis of alternative mechanisms of tumor rejection

Dr. Christian Lehmann, Department of Dermatology

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

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

Investigation of new targeting-antibodies in a murine melanoma model

Previously, we mainly focused on targeting the two major classical DC subsets (CD8⁺ and CD8⁻ DCs), which mainly induce CD8⁺ or CD4⁺ T cell responses, respectively. As we hypothesized that a concomitant induction of both kinds of T cells could be beneficial, we widened our spectra to our recently cloned targeting antibodies specific for FcγRIIB, FcγRIV, and FcγRIIB/III. In particular, αFcγRIV-Ova was able to induce strong CD4⁺ and CD8⁺ T cell responses simultaneously. In the naïve system this was demonstrated by the induction of IFNγ and IL2 in CD8+ and CD4+ T cells as well as the killing of SIINFEKL-loaded target cells in vivo.

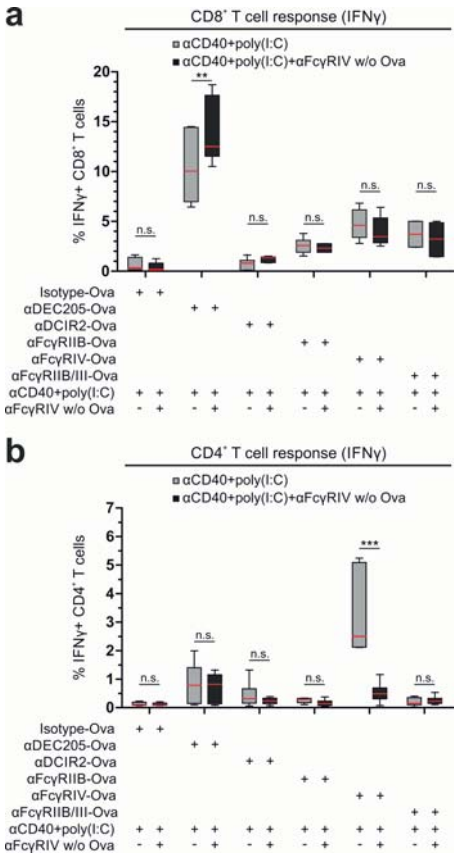
Introduction of the MCA101-Ova tumor model and antigen targeting to αFcγRIV

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

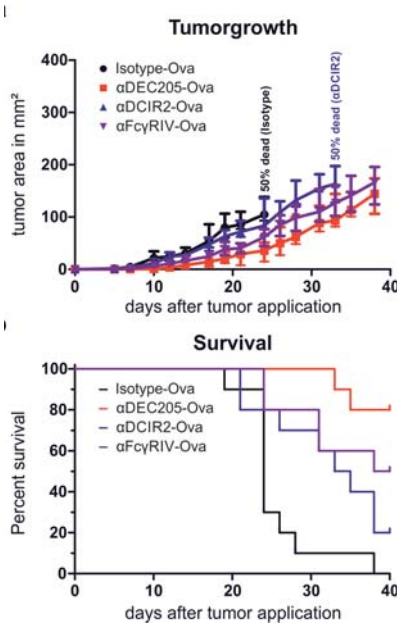
The first experiments with B16F10 revealed that there is only a very short time for intervention, as the cells grow very aggressive. Therefore, we started to use MCA101-Ova cells, a well-established fibrosarcoma model. By preventive treatment of naïve mice with αDEC205-Ova, αDCIR2-Ova, αFcγRIV-Ova (+αCD40/pIC),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

For future experiments, we were also able to generate sublines of our previously described melanoma cell line B16F10-Ova by single cell sorting. These lines differ not only in their level of Ova expression, but also in the expression of several key molecules, such as MHC-I and II as well as the checkpoint inhi-

Dr. Lehmann



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



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

bitor target PDL1. Therefore, this closely related cell lines are ideal tools to study new approaches for tumor therapies in vivo.

Contact:
Dr. Lehmann
phone: +49 9131 85 29321
e-mail: christian.lehmann@uk-erlangen.de

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J55 - Final Report

01/01/2016 - 30/06/2018

Oncology

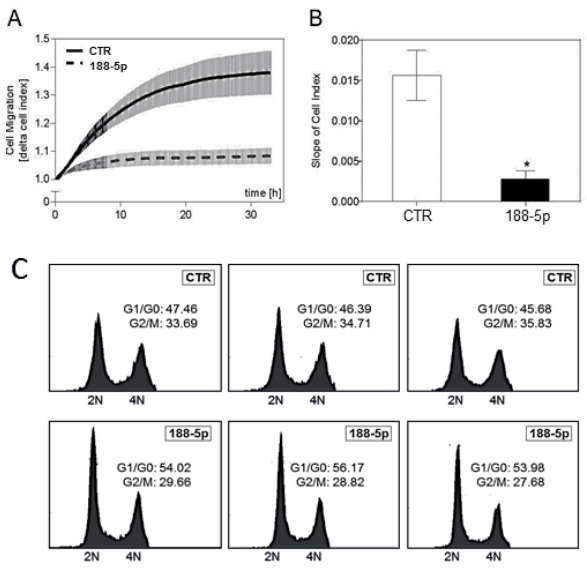
The role of microRNA-188-5p dysregulation in hepatocellular carcinoma development and progression

Dr. Dr. Peter Dietrich, Institute of Biochemistry

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

Background

The molecular landscape underlying hepatocellular carcinoma (HCC) is known to be decisively shaped by the regulatory functions of microRNAs (miRs). Based on our previous findings that downregulation of microRNA-188-5p contributes to the aggressive phenotype of activated synovial fibroblasts in rheumatoid arthritis (Ruedel and Dietrich et al., 2015), we hypothesized that miR-188-5p could also affect HCC. The aim of this study was to analyze the function of miR-188-5p and to reveal novel potential diagnostic and therapeutic target genes for this microRNA as well as other microRNAs (including miR-622 which had also been identified to potentially affect HCC) in liver cancer.



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

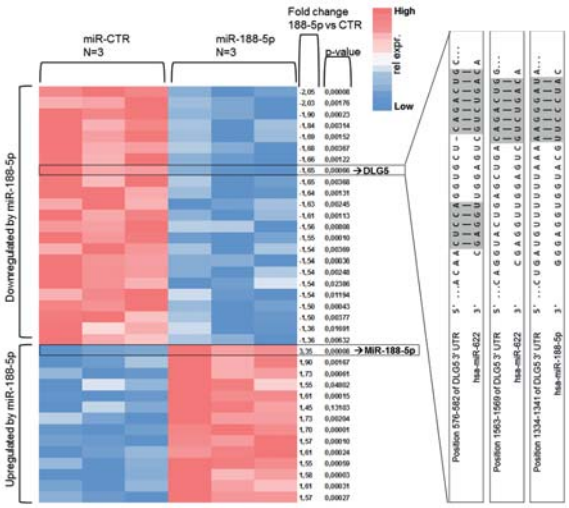
Results

MiR-188-5p was strongly downregulated in HCC and re-expression of miR-188-5p exerted marked tumor-suppressive effects (reduced migration, proliferation, clonogenicity and induction of a G0/G1 cell cycle arrest) in HCC. RNA-expression arrays revealed a list of potential novel target genes for miR-188-5p that are unknown in HCC including Krueppel-like factor 12 (KLF12), Interleukin-6 signal transducer (IL6ST) and Discs large MAGUK scaffold protein 5 (DLG5). MiR-188-5p-mediated regulation of these target genes was confirmed in vitro. Using tissue patient samples, KLF12, IL6ST and DLG5 were found to be elevated in HCC as compared to non-tumorous liver tissues. IL6ST knockdown significantly altered HCC cell migratory behavior. Moreover, functional analysis using si-RNA-Pool-mediated gene knockdown identified KLF12 as a novel therapeutic target for HCC. Next to miR-188-5p, we identified miR-622 as a crucial novel regulator of Kirsten rat sarcoma (KRAS) that drives progression and chemoresistance to sorafenib in HCC in vitro and in vivo.

Conclusions/Outlook

By combining global gene expression analysis with a systematic in silico and experimental screening process, we identified several novel target genes of the tumorsuppressive miR-188-5p in HCC. KLF12

Dr. Dr. Dietrich



cDNA array analysis comparing control-transfected (miR-CTR) and miR-188-5p-transfected HCC cells (Hep3B). Significantly downregulated genes with 3'UTR binding sites for the miR-188-5p (e.g. DLG5) will be further analyzed.

and IL6ST were identified as important modulators of oncogenic functions in HCC cells and could serve as molecular targets for HCC therapy in the future. Furthermore, we revealed that a further microRNA – miR-622 – strongly contributes to chemoresistance and tumor progression in HCC.

Contact:
Dr. Dr. Dietrich
phone: +49 9131 85 29384
e-mail: peter.dietrich@fau.de

Invited lectures

Gastroenterologie – Von der Forschung zur Praxis, 13.12.2018, Bauchzentrum, Inselspital Bern, Schweiz, Chronische Lebererkrankung und Leberkrebs – Molekulare Mechanismen und therapeutische Möglichkeiten

Institutsseminar DKFZ Heidelberg, 23.07.2018, Heidelberg, DKFZ, Institut für Pathologie, Function of microRNAs and novel microRNA-target genes in liver cancer

siTOOLs Biotech Seminar – Cancer and Inflammation, 20.09.2018, Nikolaus-Fiebiger-Zentrum, FAU Erlangen, Beyond mutations: Function of microRNA-target gene-networks in liver cancer

Awards

Preis der Deutschen Leberstiftung 2018, Dr. Dr. Peter Dietrich, 29.06.2018, Hannover

Patents/ Licenses during funding period

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

Publications during funding period

Dietrich P, Gaza A, Wormser L, Fritz V, Hellerbrand C, Bosserhoff AK (2019) Neuroblastoma RAS viral oncogene homolog (NRAS) is a novel prognostic marker and contributes to sorafenib resistance in hepatocellular carcinoma. Neoplasia 21(3): 257-268

Schmidt KM, Dietrich P, Hackl C, Guenzle J, Bronsert P, Wagner C, Fichtner-Feigl S, Schlitt HJ, Geissler EK, Hellerbrand C, Lang SA (2018) Inhibition of mTORC2/RICTOR Impairs Melanoma Hepatic Metastasis. Neoplasia 20(12): 1198-1208

Stieglitz D, Lamm S, Braig S, Feuerer L, Kuphal S, Dietrich P, Arndt S, Echtenacher B, Hellerbrand C, Karrer S, Bosserhoff AK (2018) BMP6-induced modulation of the tumor micro-milieu. Oncogene 38: 609-621

Dietrich P, Kuphal S, Spruss T, Hellerbrand C, Bosserhoff AK (2018) MicroRNA-622 is a novel mediator of tumorigenicity in melanoma by targeting Kirsten rat sarcoma. Pigment Cell Melanoma Res. 31(5): 614-629

Dietrich P, Fritz V, Koch A, Hartmann A, Bosserhoff A, Hellerbrand C (2018) Wildtype Kirsten rat sarcoma is a novel microRNA-622-regulated therapeutic target for hepatocellular carcinoma and contributes to sorafenib-resistance. Gut. 67(7): 1328-1341

Dietrich P, Freese K, Mahli A, Thasler WE, Hellerbrand C, Bosserhoff AK (2017) Combined effects of PLK1 and RAS in hepatocellular carcinoma reveal rigosertib as promising novel therapeutic „dual-hit" option. Oncotarget. 9(3): 3605-3618

Dietrich P, Kuphal S, Spruss T, Hellerbrand C, Bosserhoff A (2018) Wildtype KRAS is a Novel Therapeutic Target for Melanoma Contributing to Primary and Acquired Resistance to BRAF Inhibition. Oncogene. 37(7): 897-911

Mahli A, Saugspier M, Koch A, Sommer J, Dietrich P, Lee S, Thasler R, Schulze-Luehrmann J, Luehrmann A, Thasler WE, Müller M, Bosserhoff A, Hellerbrand C (2018) ERK activation and autophagy impairment are central mediators of irinotecan-induced steatohepatitis. Gut. 67(4): 746-756

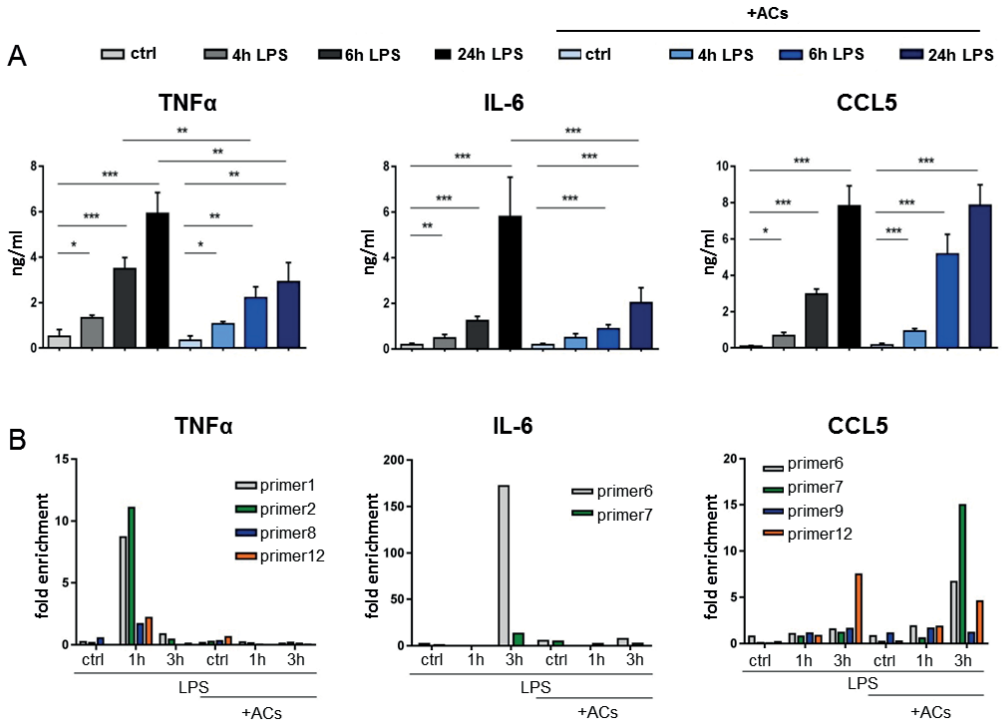
Epigenetic reprogramming of macrophages

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

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

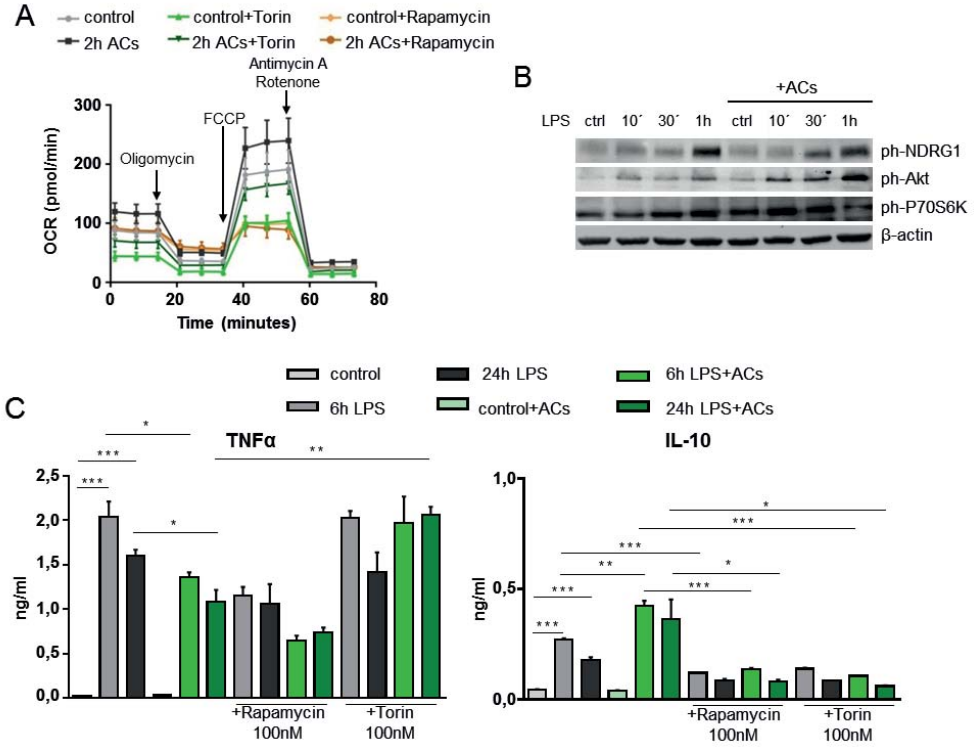
Differential regulation of cytokines upon phagocytosis of ACs

Our results show that uptake of apoptotic cells (ACs) results in the differential regulation of the expression of LPS-induced cytokines in macrophages. Whereas we observe an inhibition of pro-inflammatory cytokines such as TNFα and IL-6, several other cytokines such as CCL5 are not blocked. These differential changes in cytokine expression correlate with changes in histone (H3K27) acetylation at the respective promoters.



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

Dr. Palumbo-Zerr



(A) Seahorse analysis of oxygen consumption rate (OCR). (B) Western blot analysis of mTOR downstream targets. (C) ELISA-based analysis of TNFα and IL-10 cytokine release; Rapamycin (mTORC1 inhibitor); Torin (mTORC1+2 inhibitor).

mTOR regulates metabolic changes upon ACs engulfment

Moreover, we determined metabolic changes within the macrophage and detected a drastic increase in oxidative phosphorylation in macrophages that ingested ACs. Interestingly, these metabolic changes were linked to activation of the mTOR pathway as inhibition of mTOR by Torin and Rapamycin blocked the increase in oxygen consumption rate (OCR) in macrophages after uptake of ACs. Increased mTOR activity has been reported to upregulate oxidative phosphorylation. In accordance, mTOR signaling and its downstream targets were shown to be activated by stimulation with LPS, but to an even higher degree by AC engulfment. Inhibition of mTOR by Rapamycin or Torin reversed the anti-inflammatory effects of ACs on the expression of LPS-induced cytokines, such as TNFα and IL-10 in macrophages. Together these data suggest a fundamental immunometabolic reprogramming of macrophages during the clearance of ACs. Future research will focus on the interconnection between the observed changes and the responsible molecular pathways to identify novel targets for the treatment of chronic inflammatory diseases.

Contact:
Dr. Palumbo-Zerr
phone: +49 9131 85 39379
e-mail: katrin.palumbo@uk-erlangen.de

Publications during funding period

none

Herpesviruses and DUX4

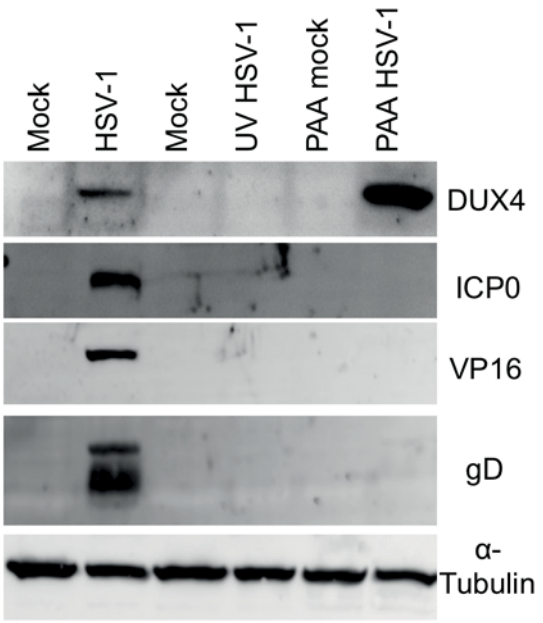
Dr. Florian Full, Institute of Clinical and Molecular Virology

The embryonic transcription factor DUX4 causes Facioscapulohumeral Muscular Dystrophy (FSHD) and is the master regulator of zygotic gene activation (ZGA). We show that DUX4 and hundreds of its target genes are actively induced by herpesviruses mimicking ZGA. We are currently elucidating the molecular mechanism of how DUX4 and DUX4-induced genes affect herpesviral replication and their impact on herpesviral disease.

Herpesviral infection is the cause of significant morbidity and mortality in humans worldwide, especially in immunocompromised individuals. Furthermore, two of the eight human herpesviruses, Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV), are classified as human carcinogens.

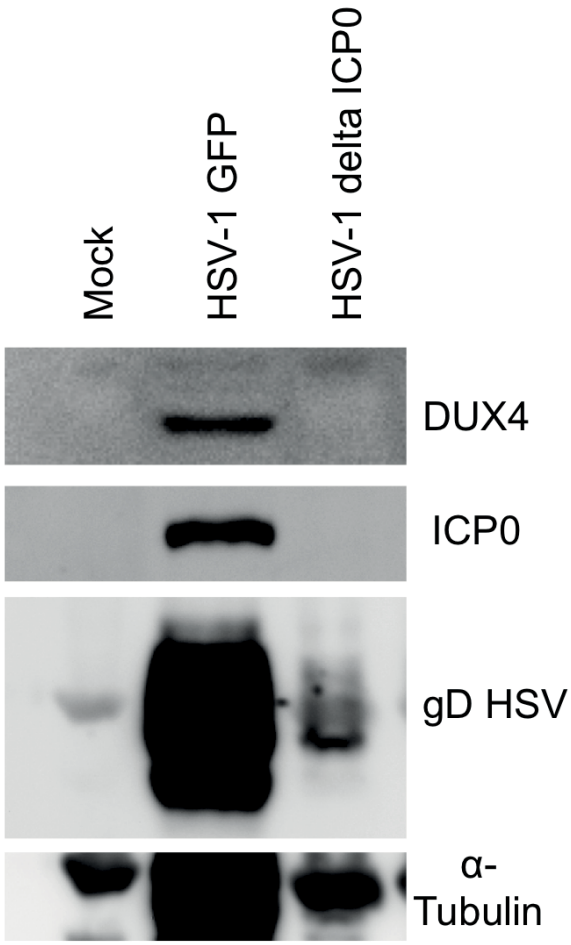
DUX4 is a transcription factor and regulator of zygotic gene activation (ZGA) during early embryogenesis. Zygotic gene activation is crucial for maternal to zygotic transition at the 2-cell stage in order to overcome epigenetic silencing of genes and enable transcription from the zygotic genome. Moreover, aberrant expression of DUX4 in adult muscle cells is the cause of the genetic disorder Facioscapulohumeral Muscular Dystrophy (FSHD). Using RNA-Seq experiments we identified DUX4 as a transcription factor that is activated upon lytic replication of Herpes simplex virus (HSV), but not of adenoviruses, negative strand RNA viruses or positive strand RNA viruses. Further experiments also confirmed expression of DUX4 upon lytic replication of Human Cytomegalovirus (HCMV) and lytic reactivation of Kaposi's sarcoma associated herpesvirus (KSHV).

The DUX4 transcript observed upon herpesviral infection is identical to the DUX4 transcript found in ZGA. We demonstrate that DUX4 expression upon herpesviral replication leads to the induction of hundreds of DUX4 target genes, such as several members of the TRIM, PRAMEF and ZSCAN protein families. Most DUX4 target genes activated during ZGA are also induced upon herpesviral infection. Moreover we could show that DUX4 expression is



Herpesviral DUX4 induction is dependent on immediate early genes but not early / late genes since it cannot be blocked by the viral polymerase inhibitor phosphonoacetic acid (PAA).

Dr. Full



DUX4 induction by HSV-1 is dependent on immediate early protein ICP-0.

a direct consequence of herpesviral gene expression, as it can be stimulated by overexpression of herpesviral immediate early proteins, indicating active induction of ZGA genes by herpesviral infection. Our results indicate that infection with viruses from alpha-, beta- and gamma-herpesvirus subfamilies induces a DUX4-dependent germline specific transcriptional program mimicking ZGA. Most ZGA genes are exclusively expressed during early embryonic development; consequently almost no information about their function is available. We are currently investigating the function of several DUX4 target genes and want to elucidate how DUX4 and DUX4-induced genes affect herpesviral replication. Our hypothesis is that herpesviruses exploit DUX4 function in order to overcome silencing of their genome and facilitate viral gene expression, similar to DUX4 function in ZGA.

Contact:
Dr. Full
phone: +49 9131 85 26494
e-mail: florian.full@uk-erlangen.de

Awards

Travel Award, International Herpesvirus Workshop 2018 (IHW 2018), Dr. Florian Full, July 2018, Vancouver, BC, Canada

Publications during funding period

none

J58 - Progress Report

01/09/2016 - 28/02/2019

Oncology

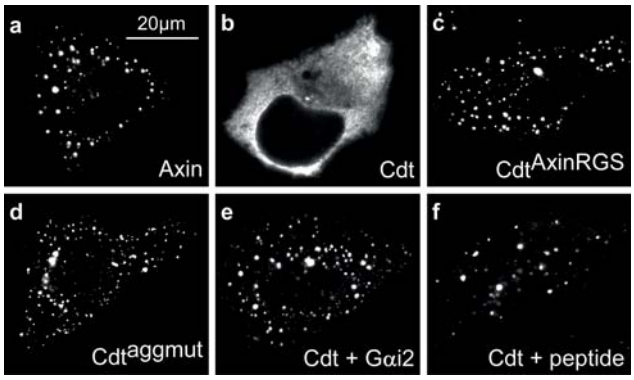
Counteracting Wnt signaling

Dr. Dominic Bernkopf, Chair of Experimental Medicine II

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

An aggregon in the conductin RGS domain prevents polymerization

We could previously show by functional sequence comparison between axin and conductin through domain swapping that the axin regulator of G-protein signaling (RGS) domain is permissive for polymerization whereas the conductin RGS domain prevents polymerization. Performing in silico analysis using the TANGO algorithm, we identified an aggregon in the conductin RGS domain which is absent from axin. An aggregation-preventing point mutation triggered conductin polymerization, as seen by the formation of microscopically-visible spherical structures called “puncta”, suggesting that this newly identified aggregon blocks polymerization and accounts for the striking difference in localization between axin (puncta) and conductin (diffuse).

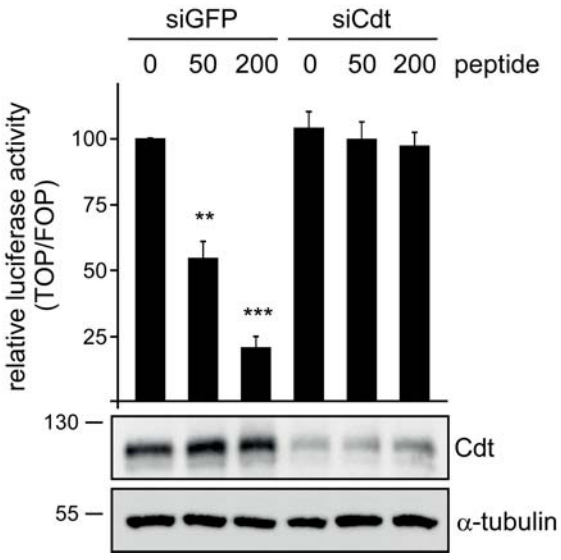


Induction of conductin polymerization. Fluorescence-based detection of axin (a), conductin (Cdt) (b), a Cdt mutant containing the axin RGS domain (c), a Cdt point mutant with inactive aggregon (d), Cdt co-expressed with Gαi2 (e) or the peptide (f).

Conductin polymerization can be triggered by masking the aggregon

Importantly, conductin polymerization cannot only be induced by mutation of the aggregon. Also co-expression of Gα subunits triggered polymerization of transiently expressed conductin. The conductin RGS domain shows high homology to RGS domains in GTPase-activating proteins which bind to Gα subunits of trimeric G-proteins. Out of four tested Gα proteins (Gαo, Gαi1, Gαi2, Gαi3), Gαi2 showed the strongest induction of conductin polymerization and the strongest interaction with the conductin RGS domain in GST-pulldown assays suggesting that polymerization is induced by Gα binding. In line, weakening the Gαi2-RGS interaction by introducing a published mutation in Gαi2 (G184S) significantly reduced induction of conductin polymerization. Moreover, treatment of cells with AlF4-, which strengthens the Gα-RGS interaction, increased induction of conductin polymerization by Gαi2. Interestingly, Gαi2 binds the conductin RGS domain in close proximity to the identified aggregon, and we believe that the observed polymerization is triggered by masking the aggregon. In line, also co-expression of small conductin fragments which contain the aggregon and can thereby interact with the RGS domain triggered polymerization of full length conductin. Strikingly, a small peptide containing the aggregon was sufficient to induce conductin polymerization, probably by masking the aggregon in conductin.

Dr. Bernkopf



The peptide which induces conductin (Cdt) polymerization inhibited a reporter to measure the transcriptional activity of β-catenin (TOP/FOP) in colorectal cancer cells, and this inhibition was rescued by siRNA-mediated knockdown of conductin.

Triggering conductin polymerization inhibits Wnt/β-catenin signaling

Of note, triggering polymerization enhanced conductin-mediated β-catenin degradation and inhibition of β-catenin-dependent transcription in colorectal cancer cells. Enhanced inhibition of Wnt signaling was observed irrespective of how conductin polymerization was triggered, i.e. aggregon mutation, co-expression of Gαi2 or the peptide.

Our data reveal an aggregon in the conductin RGS domain which allows regulating conductin polymerization and consequent inhibition of Wnt signaling. Physiologically, Gαi2 signaling might inhibit Wnt signaling via this mechanism. Therapeutically, the identified peptide holds potential to inhibit Wnt signaling in colorectal cancer.

Contact:
Dr. Bernkopf
phone: +49 9131 85 29115
e-mail: dominic.bernkopf@fau.de

Publications during funding period

Bernkopf DB, Daum G, Bruckner M, and Behrens J (2018) Sulforaphane inhibits growth and blocks Wnt/beta-catenin signaling of colorectal cancer cells. Oncotarget 9: 33982-33994

Bernkopf DB, and Behrens J (2018) Feedback regulation of mitochondrial homeostasis via Wnt/beta-catenin signaling. Mol Cell Oncol 5: e1458015

Bernkopf DB, Jalal K, Bruckner M, Knaup KX, Gentzel M, Schambony A, Behrens J (2018) Pgam5 released from damaged mitochondria induces mitochondrial biogenesis via Wnt signaling. J Cell Biol 217: 1383-1394

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

J59 - Progress Report

01/07/2017 - 31/12/2019

Oncology

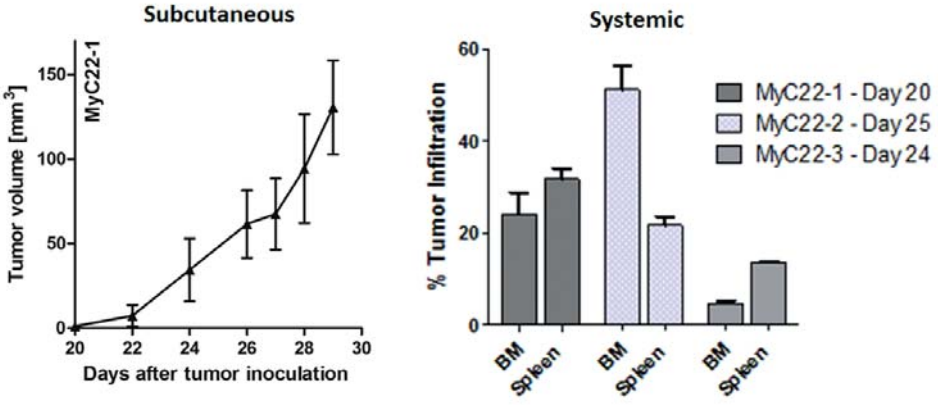
Immunotoxin induced anti-tumor immunity

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

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

The goal of this project is to establish an immune competent lymphoma mouse model that facilitates the testing of Moxetumomab pasudotox (Moxe) and its effects on the murine immune system. Because Moxetumomab only binds human CD22, we generated a chimeric protein (h/mCD22) consisting of the intracellular domains of the murine and the extracellular domains of the human CD22. We hyposezised that Moxe binds the human parts on the cell surface and that the intracellular murine parts ensure correct transport of Moxe through the various intracellular compartments.

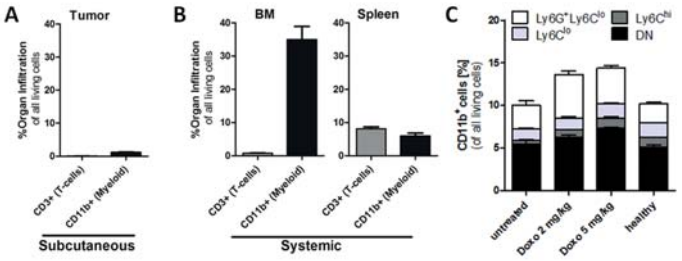
The murine lymphoma cell line 291PC was transduced with the h/mCD22 using murine lentiviruses. Single clones of the highest expressing cells, named 291PC$^{h/mCD22}$, were specifically killed by Moxe in a dose dependent manner supporting that the chimeric protein efficiently transports Moxe and thus, sensitizes the murine cells to a drug which exclusively targets human CD22. 291PC$^{h/mCD22}$, however, failed to stably engraft in mice.



NSG mice were injected with indicated h/mCD22 positive primary murine lymphoma cells either (A) subcutaneously or (B) intravenously tumor burden determined by caliper (A) or by flow cytometry (B).

Dr. Müller

By cross-breeding BL/6$^{\lambda\text{-myc}}$ mice which spontaneously develop aggressive B-cell lymphomas and BL6 mice which carry the h/mCD22 as transgene, thus expressing h/mCD22 in all their B cells, we generated BL/6$^{\lambda\text{-myc / h/mCD22}}$ mice. These mice spontaneously developed B-cell lymphoma which highly expressed the h/mCD22. Three h/mCD22 positive lymphoma cells from distinct mice, termed MyC22-1 through 3, were extracted and serially transplanted into recipient BL/6$^{\text{h/mCD22}}$ mice. Next, we characterized growth rates in vivo, responses to Moxe treatment, and characterized the lymphoma infiltrating immune cells of the three primary murine lymphoma models. Distinct from human myc-driven lymphoma, subcutaneously growing tumors were sparcely infiltrated by T-cells or myeloid cells by less than 0,5% and less than 1%, respectively. But when the tumor cells were injected systemically, the immune cell infiltration of tumor bearing bone marrow, spleen, and lymph nodes much more closely resembled the high rate of immune infiltration found in men. As for the human disease, we identified a substantial number of myeloid cells. We used doxorubicin, a known modulator of lymphoma infiltrating suppressive myeloid cells, to test effects on the host immune system. Treating lymphoma bearing mice led to a 1.5-fold increase of tumor-infiltrating myeloid cells. These new tumor-infiltrating myeloid cells furthermore showed a change of phenotype with an increase of granulocytic cells and an increase of double-negative, immature myeloid cells. Whether these cells, similar to doxorubicin induced myeloid cells in solid tumor models are immune activating is currently under investigation.



Tumor infiltrating CD3-pos T-cells or CD11b-pos myeloid cells were measured in subcutaneous (A) or systemic tumors (B) and the CD11b-pos myeloid cells (C) were further detailed using indicated markers after systemic treatment with Doxorubicin.

During the remaining funding period we will use the newly established and characterized immune competent lymphoma models to determine the functional state of tumor-associated myeloid cells at steady state and after treatment with Moxetumomab pasudotox *in vivo*.

Contact:
Dr. Müller
phone: +49 9131 85 39542
e-mail: fabian.mueller@uk-erlangen.de

Patents/ Licenses during funding period

PCT/EP2018/085503 „Novel antibody based targeted therapy for the treatment of autoimmune diseases and cancer“

Publications during funding period

Müller F, Cunningham T, Beers R, Bera TK, Wayne AS, Pastan I (2018) Domain II of Pseudomonas Exotoxin Is Critical for Efficacy of Bolus Doses in a Xenograft Model of Acute Lymphoblastic Leukemia. Toxins, 10(5), 210

Müller F, Cunningham T, Stookey s, Tai C-H, Burkett S, Jailwala P, Stetler Stevenson M, Cam MC, Wayne AS, Pastan I (2018) 5-Azacytidine prevents relapse and produces long-term complete remissions in leukemia xenografts treated with Moxetumomab pasudotox. Proceedings of the National Academy of Sciences 115 (8): E1867-E1875

J60 - Final Report

01/10/2016 - 30/04/2018

Molecular Medicine

The role of Hck/Lyn in Vesicles secretion

Dr. Jung-Hyun Lee, Department of Dermatology (till 30.04.2018)

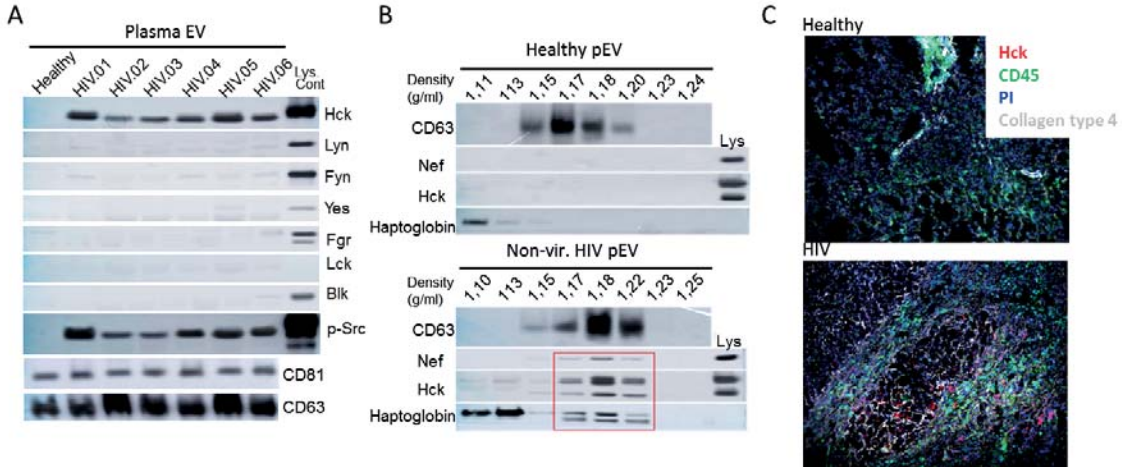
Research of the last years revealed that ADAM proteases-containing EV contributed to the HIV pathogenesis. In the present project we aim to understand and elucidate the underlying molecular and cellular mechanism of the secretion of EV. We discovered that EV secretion was governed by the tyrosine kinases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

Extracellular vesicles (EV) are secreted membrane vesicles which carry effector functions from one cell to the other, in short-range but also over long distances. Mediating these effector functions are micro-RNAs and proteins, including cytokines and proteases.

We recently reported that the number of ADAM17-containing plasma EV (pEV) was strongly upregulated in HIV infection. Their number did not decline during therapy and their ADAM17 content correlated inversely with CD4 T cell counts. The viral Nef protein of HIV caused the uploading of ADAM17 into EV and one of the well documented effects of Nef is the activation of the tyrosine kinase Hck by interaction of its PxxP motif with the Hck SH3 domain. While Hck seemed required for disease progression, the molecular role of Hck in the viral life cycle remained unclear.

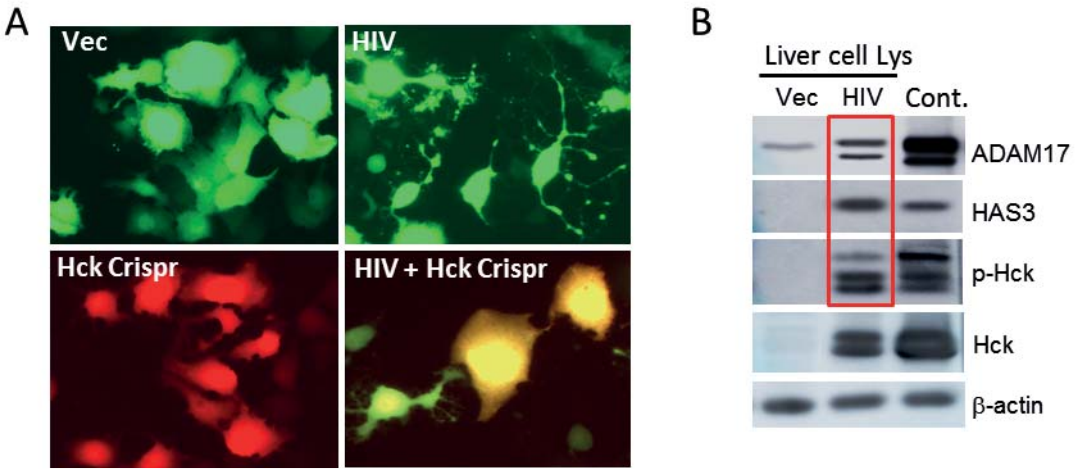
The activated Hck is detected in pEV and liver tissue of HIV patients

In vitro, the tyrosine kinase was essential for the uploading of ADAM17 into EV and was also present in EV in large amounts. We therefore speculated that pEV from patients may harbor a tyrosine kinase. Purified pEV from 6 non-viremic patients and 1 healthy control were blotted for 7 tyrosine kinases. Hck was identified in all HIV samples but not in controls. In addition, a phopho-Src (p-Src) was detected, suggesting that the pEV-associated tyrosine kinase was



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

Dr. Lee



(A) Florescent microscopy analysis of indicated constructs expressed liver cells. (B)Western blot analysis of indicated constructs transfected liver cells. (C) Florescent microscopy analysis of Has3-GFP expressed liver.

activated. Interestingly, we detected haptoglobin, an acute phase protein mainly secreted by liver cells in HIV pEV. This liver-specific factor was found in sucrose gradient fractions of HIV pEV along with activated Hck and Nef, but not in respective fractions of healthy control pEV. These data suggested that liver could be one of cellular source of EV secretion in HIV infection. To confirm this idea, we analyzed liver tissue from a HIV-infected non-viremic individual who died of liver cirrhosis-associated complications. Staining of liver samples showed that strong increased Hck expression was detected in HIV patient liver, but not in healthy donor liver. These results suggested that Hck activation was potentially connected to the generation and/or function of HIV pEV and liver as the likely cellular origin of HIV pEV.

Hck mediates the cytoskeleton reorganization for vesicular secretion

We noticed that HIV infection induced membrane protrusions, similar as described Hck function for F-actin reorganization to form protrusions of the plasma membrane in a Cdc42- and Rac-dependent manner. To analyze this effect we co-transfected GFP. Transfection of HIV proviral construct induced the membrane protrusions, while Hck knockdown (Hck Crispr/cas9-RFP) blocked this function. We hypothesized that one of regulatory protein might be related to this induction of membrane protrusions by Hck activation. Recent studies showed that Hyaluronan acid synthase 3(HAS3) was related to the regulation of cytoskeleton reorganization through induction of cell protrusions. In line with these findings, translation of HAS3 was induced by HIV infection along with activated ADAM17 and Hck. In addition, overexpression of Has3 induced the membrane protrusions. We concluded that HIV infection increased the secretion of ADAM proteases-containing EV through Hck activation, which induced a plasma membrane reorganization that possibly allowed vesicular secretion.

Contact:
Prof. Dr. Baur
phone: + 49 9131 85 45813
e-mail: andreas.baur@uk-erlangen.de

Publications during funding period

none

J61 - Progress Report

01/01/2017 - 30/06/2019

Others

Extending joint models in biomedical outcomes

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

Biomedical studies often aim at two goals: prediction the development over time, or risks of events. I.e. variables of interest are collected repeatedly or data on times of events is reported. In many cases those two outcomes are related and collected alongside each other, analysis however is done separately. Models connecting the two structures are called joint models. This project aims at extending them in terms of variable selection and beyond the mean modelling.

Adaptive Step Lengths for more belanced models

One of the types of algorithms mainly used in this project is the so called gradient boosting. This is an iterative algorithm, for which, in most cases the number of steps is a good tuning parameter. This means in order to find out, which is the result that best predicts our dependent variable, it is sufficient to find out how many steps the algorithm takes. The algorithm updates the parameters step by step usually using a fixed step-length, which prevents the algorithm from overfitting and selecting too many variables. In our case however, the algorithm has to jump between different types of dependent variables (the longitudinal measurement and the time of event) and decide which sub-predictor has to be updated as well as which of the variables in the sub-predictor is the one to update. This leads to complications in balance and finding the best stopping criterion. Thus we changed the algorithm towards a step-length based criterion, in which the step-length vary depending on the current state of the algorithm. This allows us to focus on two details of the process: a better balance between the choice for the longitudinal and the time-to-event relevant as well as the joint part of the model, but also the question on when does an update not contribute to the improvement of the model at all, i.e. when can we actually stop updating. While this concept works for low dimensional and very simple settings, many questions remain for the more complicated setups and we plan on investigating this further in the course of the project.

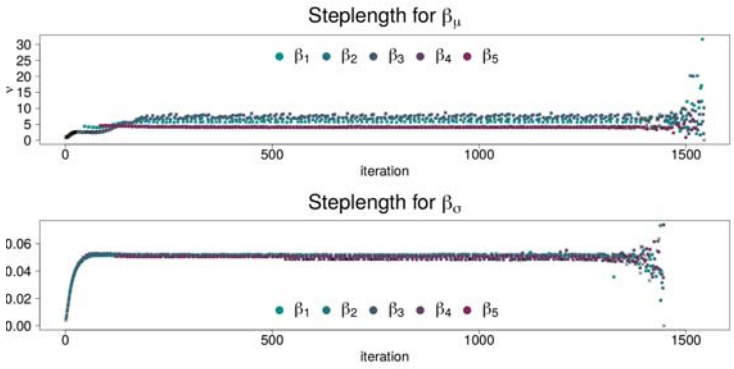


Figure showing the different step-length for a model with two sub-predictors and five covariates each with three variables being informative, two not. Crosses indicate the iterations in which the sub-predictor was not chosen to be updated.

Dr. Waldmann

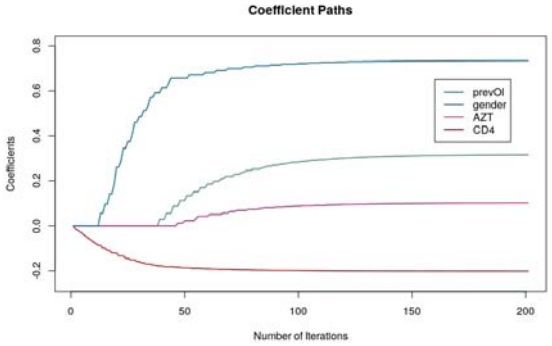


Figure showing the coefficient paths for the survival analysis of AIDS patients achieved by likelihood-based boosting. The path proceeding below the zero line indicates a negative association between CD4 cells and risk for death

Likelihood-based Boosting Techniques for improved performance in survival analysis

So far the project's research focused on making joint models feasible for variable selection and high dimensional data by using the advantages offered by gradient boosting techniques. These optimization methods are well established in longitudinal analysis but demand further developments for time-to-event data. Since we intend to more and more expand the model with respect to survival analysis, we make use of a different boosting approach, the so called likelihood-based boosting. This method takes the updating scheme gradient boosting implicitly offers and produces it artificially for well-known optimization methods like Newton algorithms. Hence we gain the advantages of both the component-wise updating process and well performing optimization algorithms. Initial approaches already proved to work and make it now possible to boost the association parameter, which quantifies the relation between the longitudinal and the survival submodel, along with other baseline covariates in survival analysis instead of treating it as a nuisance parameter like gradient boosting. So far the approach focuses solely on survival analysis where the longitudinal model is estimated in prior and incorporated as a covariate into the survival model. However the path is levelled for extending the algorithm to a complete analysis of both the longitudinal and the survival submodel as well as developing different stopping criteria as alternatives to cross validation in order to reduce computational effort.

Contact:
Dr. Waldmann
phone: +49 9131 85 22722
e-mail: elisabeth.waldmann@fau.de

Invited lectures

CMStatistics 2018, 14.12.2018, Università di Pisa, Italy, Joint modelling approaches to survival analysis via likelihood-based boosting techniques (Colin Griesbach)

Publications during funding period

Waldmann E (2018) Quantile Regression: A short story on how and why. Statistical Modelling 18(3–4): 203–218

Mechanisms of neutrophil infiltration in rheumatoid arthritis

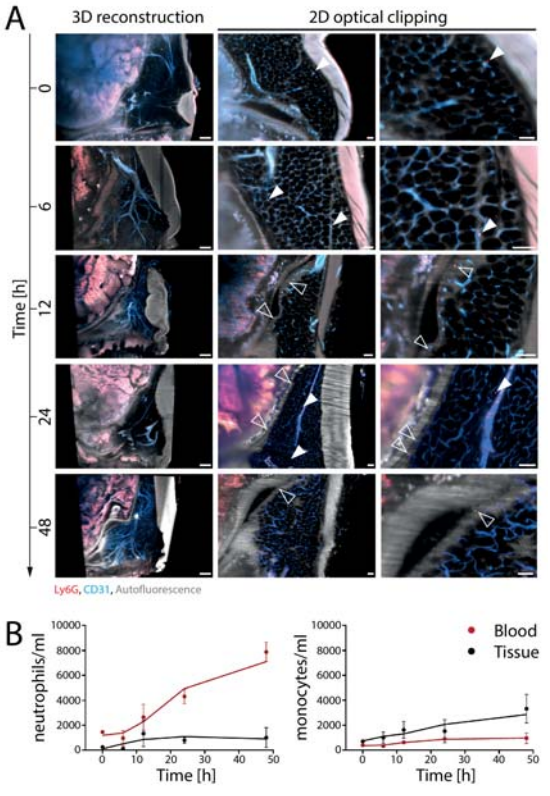
Dr. Anika Grüneboom, Department of Medicine 3 – Rheumatology and Immunology

Neutrophil granulocytes play a key role in innate immunity, but their contribution during propagation of chronic inflammation is incompletely understood. Although neutrophils are highly abundant in the inflamed joints of RA patients, their role in rheumatoid arthritis, one of the most common autoimmune diseases, is poorly understood. By using newly available mouse lines and highly advanced imaging techniques we aim to elucidate the role of neutrophils in the K/BxN serum transfer induced arthritis (STA) in vivo.

Neutrophil granulocyte infiltration is a biphasic process in K/BxN STA

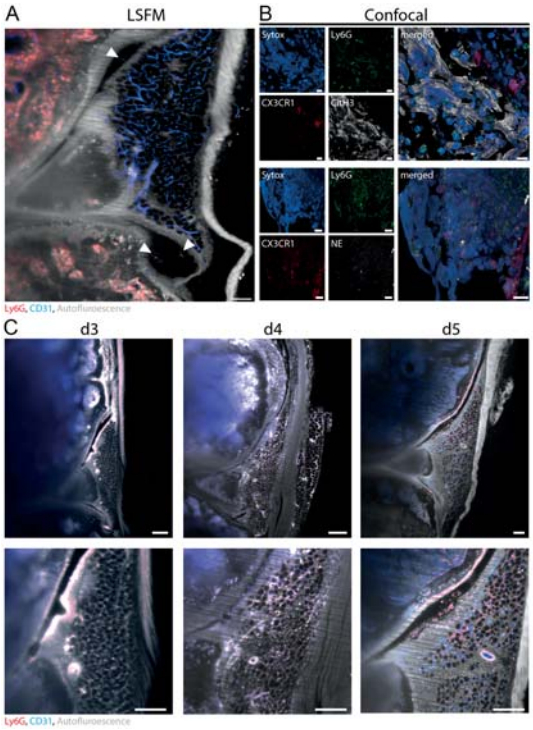
To define the timelines and severity of neutrophil infiltration into the synovial joint flow cytometric analysis of the neutrophil recruitment into the general blood circulation and into the inflamed synovial tissue were performed. These analyses proved a constantly increasing recruitment of neutrophils from the bone marrow to the peripheral circulation over the first four days after disease induction. In contrast the synovial tissue was moderately infiltrated within the first 12 hours after RA induction and then sustained the level of infiltrating cells. The flow cytometric analysis did not allow any conclusions regarding the disposition of the neutrophils in the joint. We were wondering why the increase neutrophil numbers in the synovial tissue did not correlate with the numbers detected in the blood circulation. Thus we continued with imaging approaches to answer this question.

Light-sheet fluorescence microscopy (LSFM) enables the visualization and analysis of large biological samples and whole murine organs in 3D. This method was used here to study the infiltration mechanisms and migration patterns of neutrophils into the inflamed synovial tissue. The neutrophil specific expression of the fluorescent protein tdTomato in Catchu-pIVM-red mice combined with antibody-staining of the vasculature allowed the simultaneous visualization and analysis of these structures. Imaging analysis exhibited low numbers of patrolling neutrophils in the synovial vascularization. In case of K/BxN STA a fast recruitment and extravasation of neutrophils about 6 hours after induction of arthritis could be observed. Already 12 hours after induction of arthritis neutrophils migrated through the entire synovial tissue towards the synovial cavity. Under healthy conditions this joint area is immune privileged but was infiltrated by neutrophils about 24 hours after induction of RA.



Short term recruitment of PMNs in STA (A) PMNs (Ly6G, red) are patrolling the synovial tissue (ST) and enter the cavity about 48h after STA induction. Scalebars, 500µm, 50µm, and 20µm.
(B) Numbers of PMNs constantly increase in the peripheral circulation but remain static in flamed tissue

Dr. Grüneboom



Biphasic recruitment of PMNs (A) 3D reconstructions of infiltrated joints. Scalebar = 100 µm. (B) PMNs (Ly6G, green) infiltrate the joint in higher numbers than monocytes (Cx3cr1, red). Scalebars, 10 µm. (C) At day 3-5 of RA PMNs preferentially remain in the ST. Scalbars, 100 µm and 50 µm.

To further analyze the infiltrates we preprocessed the LSFM-analyzed knee joints for histological approaches. Confocal laser scanning microscopy enables a higher optical magnification than LSFM and was used for characterizing the neutrophil infiltrates. Based on this technique we could find viable neutrophils in the synovial fat pad but exclusively apoptotic or extracellular nets (NETs)-forming neutrophils in the cavity.

These optical findings helped to interpret the flow cytometric observations of constant neutrophil numbers in the synovial cavity upon 12 hours of induction of arthritis. In the used flow cytometric analysis living cells were quantified while dead neutrophils or NET formation could not be quantified. Thus, we hypothesized a constantly increasing influx of neutrophils into the synovial cavity at the onset of K/BxN serum transfer induced arthritis.

To further elucidate the infiltration mechanism of neutrophils we performed imaging analysis of later timepoints after arthritis induction. Surprisingly the generated LSFM data indicated a change in neutrophil recruitment and infiltration during ongoing pathogenesis. Three days after K/BxN STA neutrophils started to remain in the synovial tissue after extravasation instead of infiltrating the synovial cavity. At later timepoints, namely day 4 and 5 after onset of RA, neutrophils seemed to accumulate in the synovial fat pad while no equal increase of NET formation in the synovial cavity could be observed. These results might hint to a biphasic recruitment and infiltration mechanism of neutrophils at the initial phase of K/BxN STA.

In the first phase neutrophils directly migrate towards the synovial tissue to enter the synovial cavity and perform NET formation. At later timepoints they preferentially stay in the synovial fat pad. To confirm this hypothesis we are planning to perform intravital two-photon laser scanning microscopy (TPLSM) of the inflamed synovial tissue. This method will allow analyzing neutrophil migration patterns, like velocity and directionality, in vivo in the living animals. Additionally we are planning to sort and sequence infiltrated neutrophils at specific timepoints for identifying functional differences of neutrophils during ongoing pathogenesis.

Contact:
Dr. Grüneboom
phone: + 49 9131 85 43216
e-mail: anika.grueneboom@uk-erlangen.de

Invited lectures

Tissue Clearing and Light Sheet Microscopy Workshop, LaVision BioTec GmbH 05.-07.09.2017, Stockholm Title (Talk): Optical clearing and fluorescence preservation via Ethyl cinnamate Practical lecture: Hands-on-Training for optical clearing via ECi

Tissue Clearing and Light Sheet Microscopy Workshop, LaVision BioTec GmbH 05.12.2017, Frankfurt am Main Title (Talk): Optical clearing and fluorescence preservation via Ethyl Cinnamate

UltraMicroscope User Meeting 2018 20.-22.03.2018, Essen Practical lecture: Hands-on-Training for optical clearing via ECi

Publications during funding period

none

J63 - Progress Report

01/12/2017 - 31/05/2020

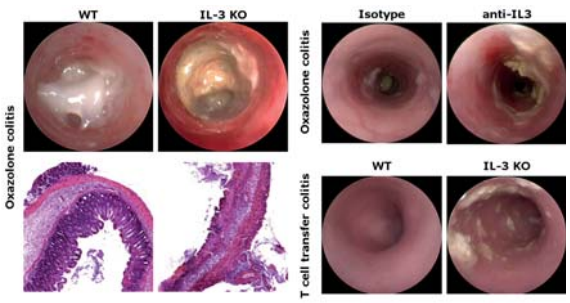
IL-3 in inflammatory bowel disease

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

The aim of this project is to elucidate the role of interleukin-3 (IL-3) in the pathogenesis of inflammatory bowel diseases (IBD). The data generated in the first year of funding show that IL-3 is clearly upregulated in active ulcerative colitis. In vivo, deficiency or depletion of IL-3 results in protection from experimental colitis. RNA sequencing data and preliminary in vitro findings suggest that IL-3 is an important driver of anti-inflammatory Tr1 cells secreting IL-10.

Inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) arise from a complex interplay of environmental and host factors, eventually resulting in undercontrolled activation of the intestinal immune system. Multiple cytokines have been shown to play a key role in this process, but the contribution of IL-3 is so far unclear. Thus, our goal is to explore the function of IL-3 in IBD taking advantage in patient samples and experimental in vivo models.

Using colonic samples from a large cohort of IBD and control patients, we could show that IL-3 mRNA is clearly upregulated in patients with UC compared to patients with CD. Moreover, in UC, the expression of IL-3 was higher in tissue with active compared with inactive inflammation. Preliminary data suggest that T cells are the main source of IL-3 in the colonic mucosa.



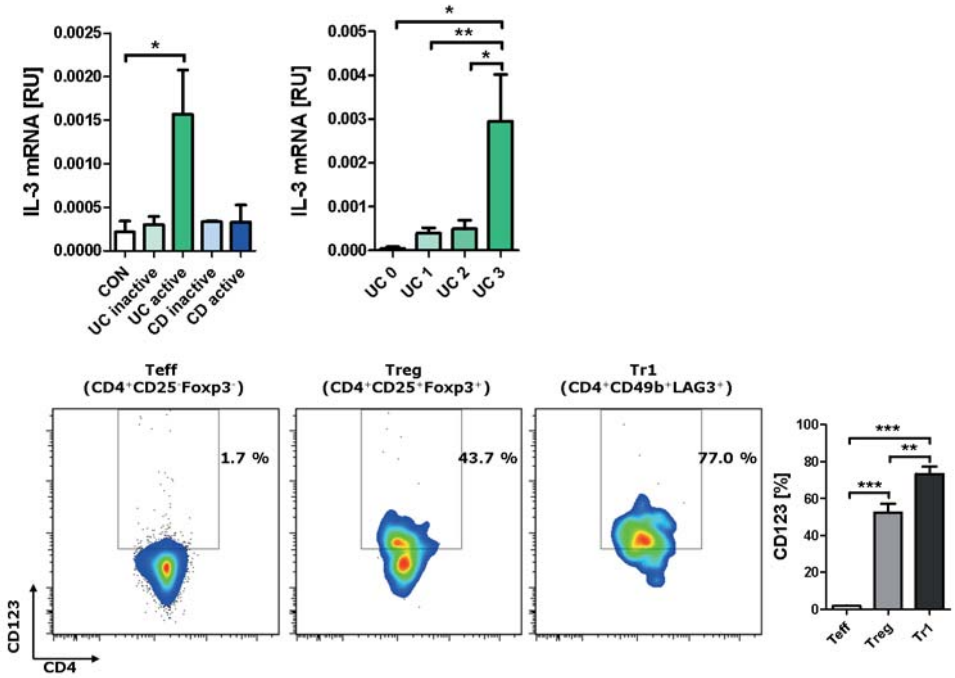
In acute oxazolone colitis, IL-3 deficiency (left panels) and IL-3 depletion with neutralizing antibodies (upper right panels) leads to aggravated disease as does transfer of IL-3-deficient T cells in T cell transfer colitis (lower right panels).

Taking advantage of IL-3$^{-/-}$ mice, we studied the role of IL-3 in oxazolone colitis, an experimental colitis model sharing several features with UC. The course of colitis was aggravated in mice deficient for IL-3, suggesting a protective role of IL-3. This was consistent with a severer disease course in wildtype mice treated with anti-IL-3 antibodies compared to mice treated with isotype control antibodies. Moreover, upon transfer of CD4^{+} T cells from IL-3$^{-/-}$ and IL-3$^{+/+}$ mice to Rag1$^{-/-}$ mice, T cell transfer colitis was more pronounced in mice that received IL-3$^{-/-}$ cells. In the meantime, we have also developed IL3R$^{-/-}$ mice, which we will now use for similar colitis experiments.

In search of the mechanism underlying these observations, we performed RNA sequencing with CD4^{+} T cells from IL-3$^{-/-}$ vs. IL-3$^{+/+}$ mice with oxazolone colitis. Our analyses identified 1740 differentially regulated genes, many of which are associated with antigen presentation and T cell phenotype. In particular, markers and factors determining a Tr1 phenotype were downregulated in IL-3$^{-/-}$ mice. Consistently, the IL3R was expressed on Tr1 cells and IL-3 drove IL-10 secretion in vitro.

Taken together, our so far data suggest an important protective role of IL-3 in ulcerative colitis that is mediated by Tr1 cells. In our ongoing experiments, we therefore aim to further shape the underlying immunologic concept with the help of new genetic models and detailed mechanistic investigations.

Dr. Zundler



The expression of IL-3 mRNA is increased in active UC (upper panels). CD49b+LAG3+ Tr1 cells from the peripheral blood express show substantial expression of the IL3 receptor CD123 (lower panels).

Contact:
Dr. Zundler
phone: + 49 9131 85 45022
e-mail: sebastian.zundler@uk-erlangen.de

Publications during funding period

Becker E, Schramm S, Binder M, Allner C, Wiendl M, Neufert C, Atreya I, Neurath M, Zundler S (2018) Dynamic Adhesion Assay for the Functional Analysis of Anti-adhesion Therapies in Inflammatory Bowel Diseases. J Vis Exp; doi:10.3791/58210.

Binder M, Becker E, Wiendl M, Schleier L, Fuchs F, Leppkes M, Atreya R, Neufert C, Atreya I, Neurath M, Zundler S (2018) Similar Inhibition of Dynamic Adhesion of Lymphocytes From IBD Patients to MAdCAM-1 by Vedolizumab and Etrolizumab-s. Inflamm Bowel Dis 24:1237-1250.

J64 - Progress Report

01/10/2017 - 31/08/2020

Renal and Vascular Research

Nephroprotection by HIF-hydroxylase inhibitors

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

In Acute Kidney Injury (AKI) restricted blood flow and reduced oxygen supply lead to tissue hypoxia and ultimately to cell death. There is evidence from rodent models of AKI that the pre-conditional stabilization of hypoxia-inducible factors (HIFs) in renal tubular epithelial cells leads to an improved kidney function. However, the underlying mechanisms of the HIF response and its regulation in AKI are poorly understood. It is still unclear whether these effects can be translated into human disease.

Background

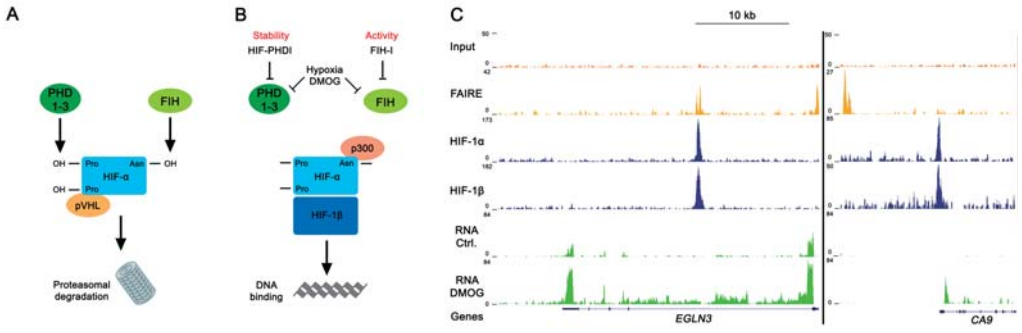
HIFs are transcription factors regulated by prolyl-hydroxylases (PHD) and factor inhibiting HIF (FIH1) with PHDs regulating HIF-stability and FIH1 regulates HIF-activity. New PHD inhibitors (HIF-PHDI) have been developed for the treatment of chronic anemia in patients with chronic kidney disease. Through stabilization of HIF-2α they induce erythropoietin (EPO) in kidney cells. However, the new generation of HIF-PHDI does not only stabilize HIF-2α and induce EPO expression in the kidney, but potentially leads to the induction of HIF (HIF-1a and HIF-2a) in most of the cells of the organism mimicking a state of acute hypoxia with potentially unselective target gene induction. Furthermore these compounds only inhibit the PHD axis of HIF regulating enzymes, but not FIH1. A potential additional protective HIF-effect by FIH1 inhibition e.g. in the setting of AKI has not been tested so far. This work will test both approaches (PHD- and FIH1-inhibition) in human renal tubular cells and in AKI animal models. AKI provides an attractive setting for a potential short time use of HIF-PHDIs.

Characterization of the tubular HIF response hPTC

The first part of this project was to characterize the HIF response in primary human tubular cells (hPETC). Chromatin immunoprecipitation (ChIP) and formaldehyde assisted isolation of regulatory elements (FAIRE) were used to identify HIF-binding sites and open chromatin under pan-hydroxylase inhibition. We identified 49000 sites of open chromatin and 600 HIF binding sites in hPTEC which significantly overlapped with open chromatin. RNA-seq revealed about 1300 hypoxic regulated genes in hPETC which are significantly associated with HIF-binding sites.

FIH1 inhibition increases the hypoxic gene induction

The new generation of selective HIF-PHDI showed a reduced induction of HIF target genes in comparison to the pan-hydroxylase inhibitor dimethyloxalylglycine (DMOG). Pharmacobiological studies revealed that these compounds only inhibit PHD enzymes. In order to address the relevance of an additional FIH1-inhibition for HIF target gene induction we used a specific FIH1-inhibitor, DM-NOFD. The results



Schematic overview of the HIF-Pathway in A) normoxic and B) hypoxic conditions. C) FAIRE-, HIF-ChIP- and RNA-seq tracks reveal robust HIF-binding to the EGLN3 and CA9 gene loci and increased expression of both genes.

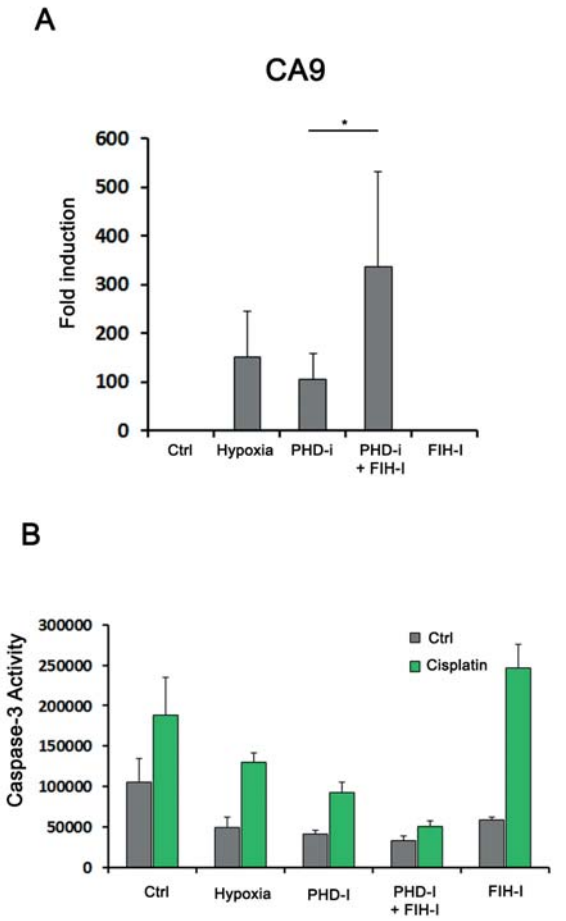
Dr. Grampp

showed an increased induction of HIF target genes in hPTEC when both HIF regulation enzymes, PHD and FIH1 were inhibited.

Preconditional HIF-Stabilization protects hPETC in an in vitro AKI model

Preconditional HIF-stabilization has a nephroprotective effect in rodent models of AKI. To explore this effect in human PETC, we used an in vitro cisplatin AKI model. Results of Caspase-3-Apoptose assays indicated a cell-protective effect when HIF was stabilized prior to cisplatin incubation. Additional inhibition of FIH1 displayed the strongest reduction of apoptosis in hPETC. Taken together these experiments indicate a promising nephroprotective potential of combined PDH and FIH1 inhibition.

Further experiments will focus on the underlying molecular mechanisms and candidate pathways as well as if these effects can be translated into in vivo AKI models.



A CA9 mRNA expression in hPTC. Increased target gene induction was observed with combined PHD and FIH1 inhibition. B Caspase-3 assay of cisplatin in vitro AKI model revealed a cell-protective effect after combined PHD and FIH inhibition

Contact:
Dr. Grampp
phone: + 49 9131 8540112
e-mail: steffen.grampp@uk-erlangen.de

Publications during funding period

none

J65 - Progress Report

01/11/2017 - 30/04/2020

Renal and Vascular Research

T-System Regulation by Glucocorticoids

Dr. Thomas Seidel, Institute of Cellular and Molecular Physiology

The transverse tubular system (t-system), a specialized system of membrane invaginations in cardiac myocytes, facilitates cardiac excitation-contraction coupling. In heart failure, the t-system undergoes severe remodeling, which impairs cardiac contraction and prevents recovery. In this project we investigate mechanisms underlying t-system remodeling in heart failure with the ultimate goal to identify strategies for preventing and reversing heart failure.

Dexamethasone prevents t-system loss and improves junctional coupling via the glucocorticoid receptor

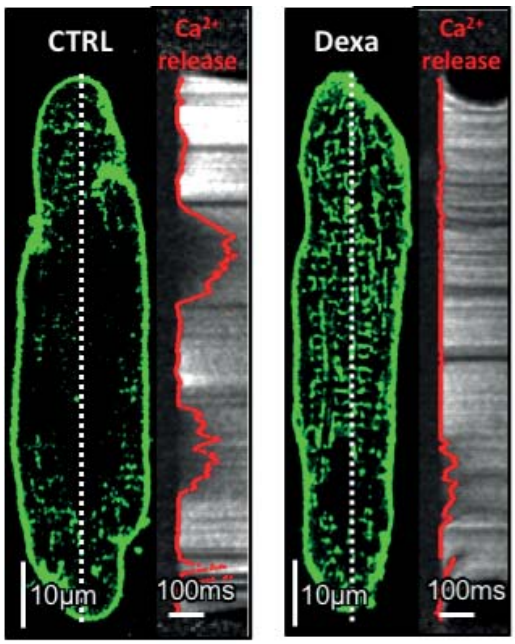
Culturing isolated cardiomyocytes is a common model of t-system loss and remodeling. We discovered that dexamethasone and corticosterone prevent t-system remodeling in cell culture. By application of spironolactone and mifepristone in combination with dexamethasone, we identified the glucocorticoid receptor (GR) as the major mediator of these effects. By co-immunostaining of L-type calcium channels (LCC) and ryanodine receptors (RyR), we found that the spatial coupling between LCC and RyR clusters as well as the intracellular distribution and density of LCC clusters was improved in dexamethasone-treated cells.

Dexamethasone improves excitation-contraction coupling

Line scan imaging with a confocal microscope of living cardiomyocytes loaded with a calcium indicator revealed that dexamethasone not only prevented structural remodeling of the t-system, but also enhanced excitation-contraction coupling. Synchrony of intracellular calcium release was significantly higher when compared with control cells.

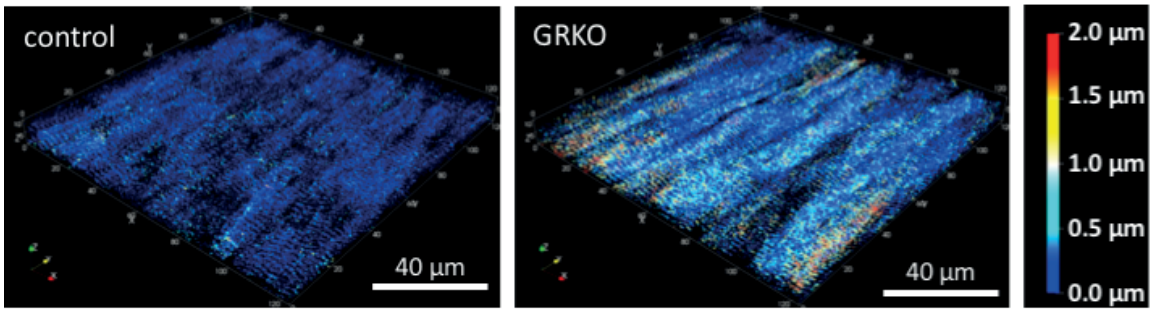
Junctional coupling is decreased in glucocorticoid receptor knockout mice

To assess if glucocorticoid receptor signaling is important for t-system maintenance in vivo, we investigated hearts obtained from cardiac-specific glucocorticoid receptor knockout (GRKO) mice and compared them to control hearts. GRKO led to significantly increased RyR-LCC distances, indicating t-system remodeling and impaired junctional integrity. These results were confirmed by 3D STED microscopy and suggest that GR signaling is important for t-system structure in vivo. In accordance with these findings, GRKO mice develop signs of heart failure and reduced cardiac contractility.



T-System with surface membrane (green) and Ca2+ release times (red) determined by line scans (dotted line) in adult rat cardiomyocytes treated with either vehicle (CTRL) or 1 µM dexamethasone (Dexa). Dexa improves t-system and Ca2+ release synchrony.

Dr. Seidel



Color map of distances between ryanodine receptors and L-type Ca2+ channels in cardiac tissue from control or cardiac glucocorticoid receptor knockout (GRKO) mice. Increased distances in GRKO indicate impaired junctional coupling and t-system loss.

GR effect on the t-system does not involve upregulation of BIN1, JPH2 or Cav3

We then asked if maintenance of the t-system is associated with higher expression of t-system associated proteins, such as BIN1, Cav3 and JPH2. Using qPCR and Western blotting, we did not find any significant increases in mRNA or protein levels of these proteins in dexamethasone-treated cells. In contrast, we detected a slight downregulation of BIN1 mRNA in response to dexamethasone. These results were in agreement with quantitative analyses of confocal microscopic images of immuno-stained cardiomyocytes. When compared with freshly isolated cells, both dexamethasone-treated and control cells exhibited increased protein levels of BIN1, which may indicate a compensatory effect or activation of fetal gene programs in response to cell culture, since BIN1 has been suggested to drive the perinatal development of t- tubules. In summary, however, the preserving effects of dexamethasone on t-system structure and EC coupling do not seem to be mediated by direct regulation of BIN1, JPH2 or Cav3.

Contact:
Dr. Seidel
phone: + 49 9131 85 22303
e-mail: thomas.seidel@fau.de

Publications during funding period

none

β subunits: adding pieces to the puzzle of pain

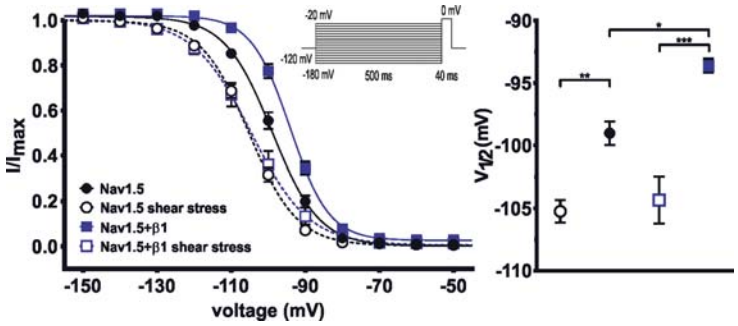
Dr. Esther Eberhardt, Department of Anaesthesiology

Chronic pain is a common health problem for which therapy remains often unsatisfactory. Rare mutations in voltage-gated sodium channels (Navs) have helped our understanding of the pathophysiology of pain. The aim of this study is to use human induced pluripotent stem cells from patients with rare variants in Nav accessory proteins to elucidate the contribution of these β subunits to cellular excitability and to obtain insights in more complex polygenetic pathomechanisms of pain.

Voltage-gated sodium channels are important for generation and propagation of action potentials in excitable cells. Their expression and gating is further modulated by regulatory proteins (β subunits). Mutations of these accessory subunits have been linked to arrhythmias and epilepsy syndromes and have recently also been found in patients suffering from the chronic pain syndrome erythromelalgia.

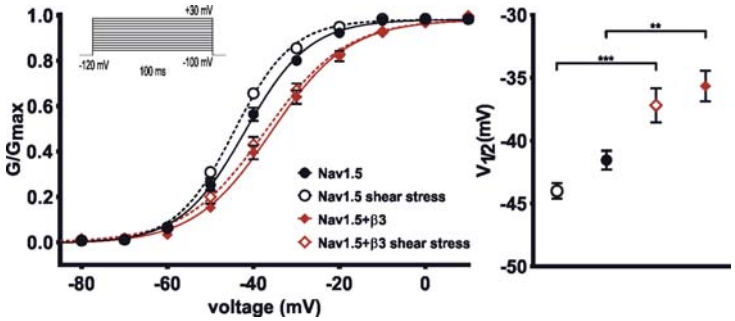
We obtained skin biopsies of two erythromelalgia patients carrying mutations in β1 and β3 respectively which both show increased spontaneous activity of their C-fibers in microneurography recordings indicating a pathology in small sensory neurons. Using a fibroblast reprogramming approach, we successfully generated human induced pluripotent stem cells (hiPSCs) from both patients which we differentiate into patient-derived sensory neurons and currently characterize with electrophysiological methods to investigate pathological firing behavior compared to age matched healthy controls.

Recently it has been shown that the β1 subunit stabilizes the nociceptive sodium channel Nav1.7 against mechanical stress. The cardiac sodium channel Nav1.5 is also mechanosensitive and associates with β1 and β3. As mechanical forces are particularly relevant for cardiomyocytes during the cardiac cycle, we asked how the coexpression of β1 or β3 affects mechanosensitivity of heterologously expressed Nav1.5.



Effects of β1 on inactivation of Nav1.5 are abolished by mechanical stress. Left panel: inactivation of Nav1.5 in dependence of shear stress and expression of the β1 subunit. Right panel: statistical comparison of the midpoint of inactivation ($V_{1/2}$).

Dr. Eberhardt



β3 induces a resistance of Nav1.5 against mechanical stress. Left panel: activation of Nav1.5 in dependence of shear stress and expression of the β3 subunit. Right panel: statistical comparison of the midpoint of activation ($V_{1/2}$).

In patch-clamp experiments in voltage-clamp mode we found that shear stress induced by a standardized gravity-driven perfusion system led to hyperpolarization of activation and inactivation of Nav1.5 consistent with previously published studies. Coexpression of β1 with Nav1.5 did not affect activation but induced a depolarizing shift in channel inactivation. Shear stress abolished this effect and led to a comparable hyperpolarization of activation and inactivation as observed in the absence of β1. The β3 subunit led to a depolarization of Nav1.5 activation and inactivation. These effects were preserved for activation under mechanical stress and coexpression of β3 prevented mechano-induced hyperpolarization of inactivation.

Our data suggest that in contrast to the nociceptive sodium channel Nav1.7 the cardiac isoform Nav1.5 remains mechanosensitive despite expression of β1. However, expression of β3 seems to cause a resistance of Nav1.5 against shear stress and β3-induced effects on gating mostly remain. β1 and β3 could therefore be involved in the mechano-electrical feedback in cardiomyocytes and their antiarrhythmogenic effects could partly be due to differential regulation of the mechanosensitivity of Nav1.5.

Contact:
Dr. Eberhardt
phone: + 49 9131 85 42465
e-mail: esther.eberhardt@uk-erlangen.de

Publications during funding period

Namer B, Schmidt D, Eberhardt E, Maroni M, Dorfmeister E, Kleggetveit IP, Kaluza L, Meents J, Gerlach A, Lin Z, Winterpacht A, Dragicevic E, Kohl Z, Schüttler J, Kurth I, Warncke T, Jorum E, Winner B, Lampert A (2018) Pain relief in a neuropathy patient by lacosamide: Proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors. EBioMedicine epub ahead of print, doi:10.1016/j.ebiom.2018.11.042

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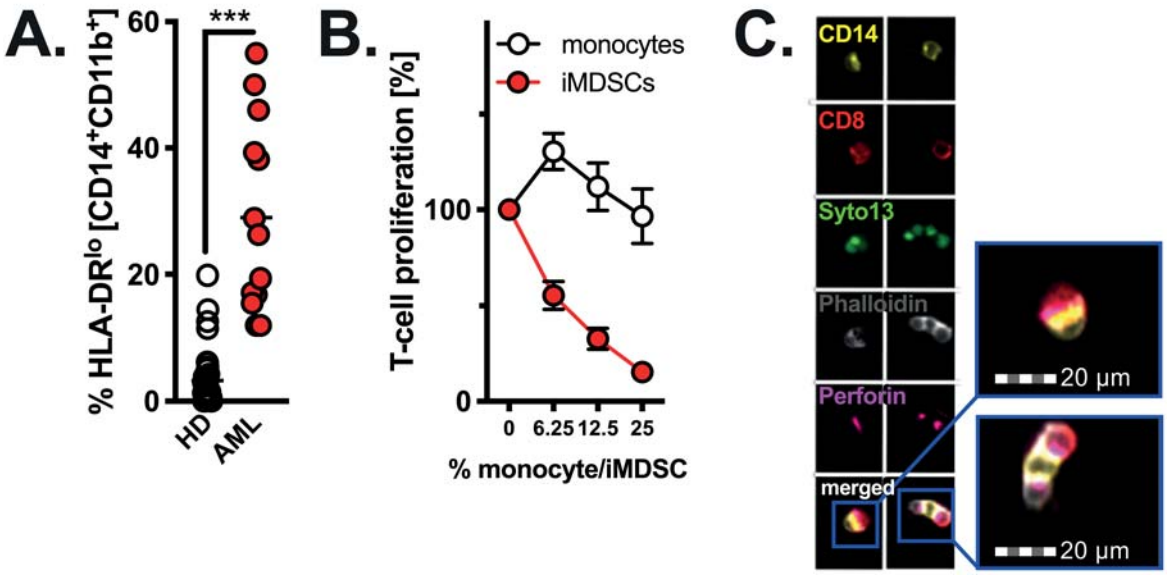
Metabolic reprogramming of AML MDSCs

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

Acute myeloid leukemia (AML) is the most common acute leukemia amongst adults. Emerging evidence suggests that immune alterations favor leukemogenesis and relapse. Myeloid derived suppressor cells (MDSCs) have gained momentum as mediators of immune escape. We aim to decipher interconnections between metabolic reprogramming and MDSC abundance and to unravel the role of AML-derived exosomes in this context. Understanding those mechanisms is key for improving immune-based therapeutic approaches.

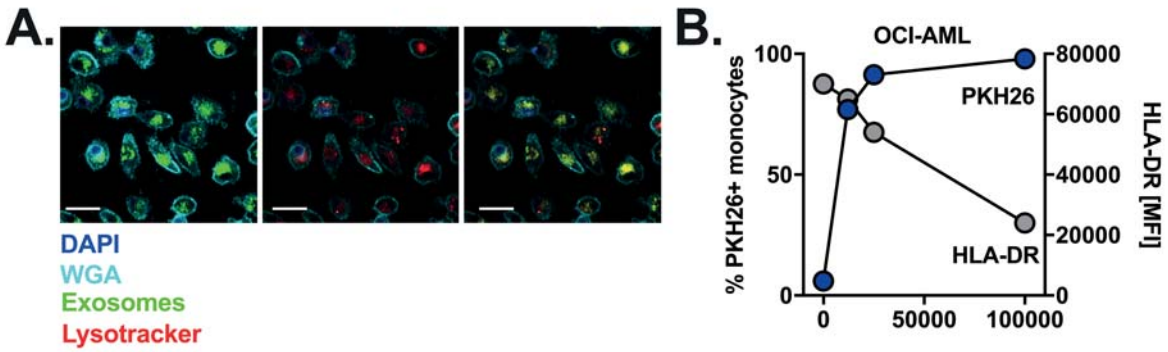
MDSCs are a heterogeneous cell population morphologically resembling either monocytes or granulocytes and sharing some key features including myeloid origin, aberrant (immature) phenotype, and immunosuppressive activity. Increasing evidence suggests that accumulating MDSCs are involved in hampering anti-tumor immune responses and immune-based therapies. In this study we sought to investigate whether the CD33/CD3-bispecific BiTE® antibody construct (AMG 330) with documented activity against AML blasts can also target (exosome-induced) AML-MDSCs by redirecting and activating T-cells.

First, we assessed the presence of circulating $CD14^+$ cells that co-express CD33 but lack HLA-DR expression ($HLA\text{-}DR^{lo}$) in patients with newly diagnosed AML. These monocytic cells represent one of the best-defined human MDSC subsets. Frequency of CD14+HLA-DRlo cells was significantly increased in untreated AML patients. Purified $CD14^+HLA\text{-}DR^{lo}$ cells suppressed in vitro T-cell proliferation in a concentration-dependent manner allowing us their denomination as MDSCs. Primary AML blasts and human AML cell lines (THP, OCI-AML, and HL-60) (and their respective exosomes) induced HLA-DRlo cells



(A) CD14+HLA-DRlo cells in AML patients and healthy controls. (B) T cell-suppressive activity of monocytes and AML-induced MDSCs was evaluated in co-cultures with autologous T-cells using FACS. (C) FACS-imaging of CD8+ T-cells (red) conjugated with CD14+CD33+ MDSCs (yellow). ***, p<0.001.

Dr. Dr. Jitschin



: (A) OCI-AML-derived exosomes are taken up by healthy control-derived monocytes as assessed by confocal microscopy. Nucleus, membranes, and lysosomes are visualized by DAPI, WGA, and lysotracker respectively. (B) Uptake of PKH26-labeled exosomes and HLA-DR expression correlate inversely.

from healthy donor-derived monocytes that were T-cell suppressive and expressed the immune regulatory indoleamine-2,3-dioxygenase (IDO).

Using ex vivo co-culturing models of primary AML blasts/AML cell lines (=target cells) and T-cells (=effector cells) we observed AMG 330-triggered antibody construct-dependent cell-mediated cytotoxicity/ADCC. Short-term (three to six days) treatment of AML patient-derived peripheral blood mononuclear cells (PBMCs) with 10 or 100 pM AMG 330 led to T-cell activation. MDSC levels in AML-PBMCs had no effect on AMG 330-mediated T-cell (and bystander cell) activation, which was further corroborated in experiments where the total $CD14^+$ cell compartment (comprising AML-MDSCs) was removed prior AMG 330 application.

However, we did observe an impact of MDSCs on BiTE® mediated T-cell proliferation, which has been shown as highly relevant for clinical activity of BiTE® antibody construct. Here we found an 1,68-fold enhanced (p=0.001) T-cell expansion in AML-PBMCs treated with 100 pM AMG 330 following $CD14^+$ cell depletion. Given the importance of T-cell proliferation for clinical activity this finding warrants further exploration.

Taken together, our results suggest that anti-CD33/CD3 bispecific BiTE® antibody constructs may achieve anti-leukemic efficacy not only through direct T cell mediated cytotoxicity against AML blasts but also through circumventing immune evasion via MDSCs targeting. Although therapeutic targeting of MDSCs in patients has not yet been successfully accomplished, bystander killing of $CD33^+$ MDSCs via anti-CD33/CD3-bispecific BiTE® antibody constructs could represent a very promising approach to increase the anti-leukemic T-cell response in AML patients and to reverse immune evasion.

Contact:
Dr. Dr. Jitschin
phone: + 49 9131 85 43113
e-mail: regina.jitschin@uk-erlangen.de

Publications during funding period

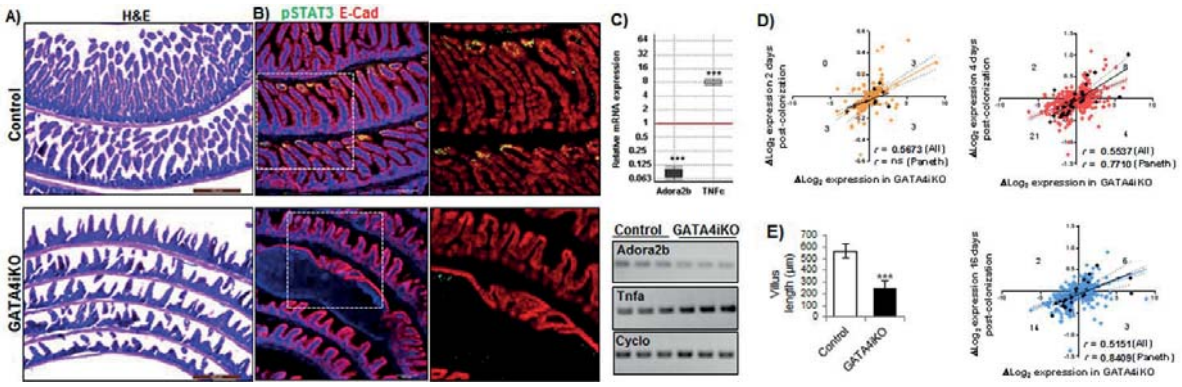
Jitschin R, Saul D, Braun M, Tohumeken S, Völkl S, Kischel R, Lutteropp M, Dos Santos C, Mackensen A, Mougiakakos D (2018) CD33/CD3-bispecific T-cell engaging (BiTE®) antibody construct targets monocytic AML myeloid-derived suppressor cells. Journal of Immunotherapy of Cancer 6(1): 116

Role of GATA4 in Intestinal Inflammation & Cancer

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

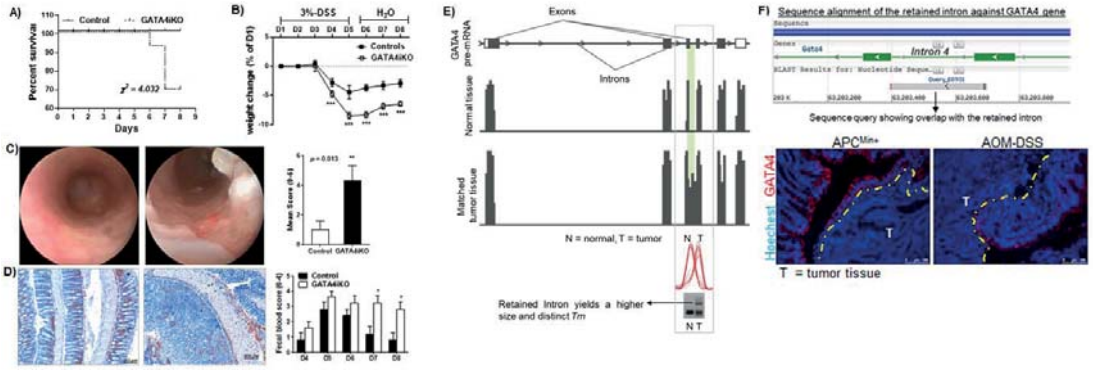
One of the key endodermal transcription factors involved in intestinal differentiation is GATA4 with an unrecognized role in intestinal pathologies. Our previous work and recently published literature indicates that GATA4 might regulate epithelial inflammatory transcription and could be adversely affected in cancer. Intron retention (IR) is a novel post-transcriptional modification of mRNA that frequently affects tumor suppressor genes. We have now identified GATA4 as a key regulator of intestinal microbiota composition, epithelial STAT3 response and a novel target of IR.

We hypothesized in Objective 1 an interaction between INF-STAT signaling and GATA4. We have now shown that GATA4iKO mice have a complete repression in epithelial STAT3 phosphorylation in a NSAID-induced model of small intestinal inflammation. The reduced phosphorylation of STAT3 was associated with lower mRNA expression of epithelial STAT3 target Adora2b, which is also a well-known IBD risk gene. The NSAID-induced injury was also more severe in the GATA4iKO group compared with controls with the average villus length reduced by 313µm. These data indicate that epithelial STAT3 is under regulation of GATA4 and if GATA4 is absent, the phosphorylation of STAT3 and the regulation of critical STAT3 downstream target genes such as Adora2b are adversely affected. It is known that epithelial STAT3 phosphorylation is regulated by the sensing of gut microbiota by specific T cells in the gut. To test whether the absence of STAT3 phosphorylaiton could stem from altered gut microbiota, we next measured the levels of various microbial species in the intestines of control and GATA4iKO mice. Our analysis has revealed that the jejunum GATA4iKO mice had a significantly different composition than control mice (p=0.02, perMANOVA). The jejunum of GATA4iKO mice is depleted in several families, including several known mucus-associated groups (Verrucomicrobiaceae, Deferribacteraceae, and Desulfovibrionaceae), indicating that the normal mucus-associated microbiota is disrupted in these mice. These data indicate that epithelial GATA4 is a critical regulator of the normal microbial dynamic in the gut. For our second objective, we aimed at outlining the role of GATA4 in intestinal wound healing and resolution in vivo. For this, we took advantage of the DSS induced intestinal injury model. We found that GATA4iKO



(A) H&E (B) pSTAT3, E Cadherin IF staining in jejunal swiss-rolls of control & GATA4iKO (C) transcript levels in GATA4iKO vs control jejunum
(D) Expression correlation plots between GATA4iKO & germfree mice colonized with commensal bacteria.

Dr. Patankar



A) Survival & (B) body weight on DSS (C) endoscopic presentation and score (D) H&E and fecal blood score (E) Exon scanning qPCR of tumor and normal tissues showing Intron Retention (F) sequence alignment and GATA4 staining in tumor tissues.

mice have a significant reduction in survival on 3.5% DSS. When DSS is removed to allow for recovery, GATA4iKO mice show a delay in the normal course of recovery with higher fecal blood loss ($p<0.05$, t-test) and a significantly higher endoscopic damage score ($p = 0.013$, t-test). This was accompanied with a higher infiltration of F4/80 positive macrophages in the damaged area. These results support our hypothesis that epithelial GATA4 is crucial for intestinal recovery and repair. Our third objective links the homeostatic functions of GATA4 to its putative role in intestina carcinogenesis. Here, we have identified that intron 4 of the GATA4 gene is affected by a specific intron retention (IR) event in two colorectal cancer mouse models and have followed it up in patient samples where we have identified IR in of a corresponding non-conserved intron, intron 6, of the human GATA4 gene in colorectal cancer samples. Both of these introns introduce premature stop codons, however, the transcript stability is not affected. Future experiments will investigate the coding potential of the GATA4 transcript in these cancer tissues.

Contact:
Dr. Patankar
phone: + 49 9131 85 35791
e-mail: jay.patankar@uk-erlangen.de

Publications during funding period

none

Newly started Projects

J69 01/09/2018 - 28/02/2021 Immunology and Infection

Effect of HIV on pre-existing vaccine immunity


Dr. Nganou Makamdop

Dr. Christiane Krystelle Nganou Makamdop, Institute of Clinical and Molecular Virology

Disruption of immune homeostasis can impair antigen-specific responses and vaccine-induced immunity. Despite treatment, persistent inflammation in HIV deteriorates the immune system. This research project will investigate the effect of HIV on pre-existing vaccine-induced immunity. By means of polychromatic FACS and RNA-seq of antigen-specific T cells, we will dissect T cell responses to measles and tetanus; helping towards a needed rationale for immunization guidelines in HIV infected persons.

e-mail: krystelle.nganou@uk-erlangen.de

J70 01/10/2018 - 31/03/2021 Renal and Vascular Research

Gene discovery in kidney disease


Dr. Jobst-Schwan

Dr. Tilman Jobst-Schwan, Department of Medicine 4 - Nephrology and Hypertension

The genetic background of chronic kidney disease in adults is poorly investigated. This study aims to identify novel candidate genes by Whole Exome Sequencing in adult patients with chronic kidney disease. Transcriptome analysis of patient specific primary cells will be employed to functionally characterize these candidate genes. CRISPR/Cas9 based zebrafish experiments will transfer the in vitro findings in an animal disease model.

e-mail: tilman.jobst-schwan@uk-erlangen.de

J71 01/01/2019 - 30/06/2021 Renal and Vascular Research

P2Y2R-dependent cyst growth in ADPKD

Dr. Kraus

Dr. Andre Kraus, Department of Internal Medicine 4 - Nephrology and Hypertension

The aim of our project is to descramble the role of the purinergic receptor P2Y2R in the context of Ca^{2+}-dependent Cl^--secretion as a main course of cyst growth in ADPKD. We will use our novel PKD1-deficient 3D cyst model and micropuncture cysts to analyse the effects of luminal ATP as well as the P2 inhibitor Suramin. In addition, we will test for the impact of P2Y2R knockout on the cystic burden in our PKD1 knockout mouse model and determine the therapeutic potential of Suramin *in vivo.*

e-mail: andre.kraus@uk-erlangen.de

J73 16/09/2018 - 15/03/2021 Oncology

Intracellular signaling by SPARCL1 in colon cancer

Dr. Tenkerian

Dr. Clara Tenkerian, Department of Surgery

The tumor microenvironment (TME) plays a pivotal role in tumorigenesis, prognosis and therapy. SPARCL1 is a vascular derived anti-tumorigenic factor that counteracts CRC tumorigenesis in a TME-dependent manner. Preliminary results indicate that SPARCL1 regulates ERK phosphorylation and subcellular localization in endothelial and CRC cells. This project aims to elucidate the signaling pathways by which SPARCL1 transmits its anti-proliferative and anti-angiogenic functions, mainly focusing on ERK.

e-mail: clara.tenkerian@uk-erlangen.de

Newly started Projects

J74 01/02/2019 - 31/07/2021 Molecular Medicine

The role of CtBP1 in hippocampal and cortical neuroplasticity

Dr. Seda Salar, Department of Psychiatry and Psychotherapy

Dr. Salar

The pathways leading to physiological and maladaptive neuroplasticity are overlapping and strictly controlled by transcriptional regulators such as CtBP1. Following neuronal activity, CtBP1 translocates from nuclei and enables transcription of a number of genes that are involved in memory formation and also seizure generation. Using CtBP1 knock-out mice model, we aim to identify candidate pathways regulating hyperexcitability and develop new intervention mechanisms against these alterations.

e-mail: seda.salar@uk-erlangen.de

J75 16/10/2018 - 15/04/2021 Others

Statistical Analysis of Infectious Disease Spread

Dr. Meyer

Dr. Sebastian Meyer, Department of Medical Informatics, Biometry and Epidemiology

Epidemic models are used to analyse and forecast the spread of infectious diseases. Specialized regression methods for surveillance data allow us, for example, to evaluate socio-demographic and environmental factors. The aim of this project is to extend such statistical modelling frameworks for two types of applications: multivariate time series of proportions and spatio-temporal point patterns. All methodological developments are accompanied by implementations in open-source software.

e-mail: seb.meyer@fau.de

P001 01/04/2017 - 31/03/2018 Immunology and Infection

The function of microRNAs in dendritic cells

Dr. Xin Lai, Department of Dermatology

Dendritic cells (DCs) play an important role in the immune system by presenting antigens to B and T cells. MicroRNAs (miRNAs) are small, non-coding RNAs that are linked with the immune system through post-transcriptional regulation of gene expression. Alterations in miRNA expression can affect the development of DCs as well as their immune function. In this project, we integrate experiments with computational biology to identify miRNAs regulating the DCs' immunogenicity.

e-mail: xin.lai@uk-erlangen.de

P002 01/05/2017 - 30/04/2018 Oncology

Duotoxins against B-cell malignancies

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

CD22-targeted antibody therapeutics such as Moxetumomab pasudotox or Pinatuzumab vedotin show impressive efficacy in patients with B-cell malignancies. However, more than 50% of patients are rapidly refractory and improved therapies are urgently needed. Based on the strong synergy between paclitaxel and Pseudomonas exotoxin (PE), we are developing a new CD22-targeted drug that combines PE with the paclitaxel-like MMAE on one antibody molecule..

e-mail: fabian.mueller@uk-erlangen.de

P003 01/05/2017 - 30/04/2018 Oncology

In vitro transformation model

Dr. Julienne Kathrin Münzner, Department of Pathology

Rhabdoid sarcomatoid (colon) carcinomas represent a rare type of highly aggressive tumors with early metastasis that are associated with a very poor prognosis of patients. Due to their low frequency the pathogenesis of this type of cancer is largely unknown. Hence, our study aims to establish a reliable in vivo model of rhabdoid sarcomatoid colon carcinomas using transformed intestinal epithelial cells and the CAM assay in order to better understand the development of this type of tumor.

e-mail: julienne.muenzner@uk-erlangen.de

P004 01/04/2017 - 31/03/2018 Others

Trauma-related disorders in refugees from Syria

Dr. Eva Morawa, Department of Psychosomatic Medicine and Psychotherapy

Study I examines mental disorders (PTSD, depression, anxiety) and the association with protective factors (sense of coherence, social support), health-related quality of life, acculturation styles, perceived discrimination and subjective illness concepts in refugees from Syria with residence permit. In addition, the activity of the HPA axis will be analyzed by means of steroid hormone profiles.

e-mail: eva.morawa@uk-erlangen.de

P005 01/12/2017 - 30/11/2018 Others

MRI of Collagen Gel Cartilage Repair

Dr. Milena Pachowsky, Department of Surgery

Collagen gel is a novel surgical treatment for cartilage defects. It is a promising option to prevent osteoarthritis. This project aims to visualize the advantages of this technique by high-field MR imaging. In combination with clinical outcome assessment this study will establish an postoperative follow-up algorithm. Addionally, the concept can be applied for other cartilage repair techniques and will lead to a better understanding of cartilage tissue.

e-mail: milena.pachowsky@uk-erlangen.de

P006 01/10/2017 - 31/10/2018 Immunology and Infection

Inhibition of JAKs in Systemic sclerosis

Dr. Yun Zhang, Department of Medicine 3 – Rheumatology and Immunology

Janus-kinase 2 (JAK2) has recently been described as a novel downstream mediator of the pro-fibrotic effects of TGFbeta. We aim to evaluate combined JAK1/JAK2 inhibition and co-treatment with an HSP90 inhibitor as strategies to overcome resistance of JAK2 inhibition during chronic treatment of SSc, meanwhile compare the different treatment strategies with the monotherapy with the JAK2 inhibitor in murine sclerodermatous chronic graft-versus-host disease and in adTBR-induced dermal fibrosis.

e-mail: yun.zhang@uk-erlangen.de

P007 01/08/2017 - 31/07/2018 Oncology

Genome CRISPR/Cas9 in pancreatic cancer

Prof. Dr. Christian Pilarsky, Department of Surgery

The objective of this project is to express the GeCKo libraries of sgRNAs in different cell lines and to investigate which sgRNAs confer chemoresistance against a combination of gemcitabine and paclitaxel. We will then evaluate the identified candidates using a CRISPR/Cas9 based single gene approach. This analysis will also include the identification of genes which are needed for the maintenance of cell proliferation in different cell types.

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

P008 01/12/2017 - 30/11/2018 Immunology and Infection

Gene-based vaccines for mucosal immunizations

Prof. Dr. Matthias Tenbusch, Institute of Clinical and Molecular Virology

The objective of this study is to develop a gene-based vaccination strategy providing efficient and long-term protection against respiratory tract infections by Influenza or respiratory syncytial virus. The induction of mucosal and systemic immune responses will be analyzed after heterologous prime-boost immunizations. Of special interest is the interplay of tissue-resident memory and circulating memory T-cells and their contribution to protection.

e-mail: matthias.tenbusch@uk-erlangen.de

P009 13/12/2017 - 12/12/2018 Immunology and Infection

Influence of CD40 receptor blockade on B cells

Dr. Mandy Wahlbuhl-Becker, Department of Pediatric and Adolescent Medicine

Regulatory B cells (Breg) play a crucial role in modulating the immune response and subsets among them possess immunoregulatory properties. Subpopulation of Bregs will be studied with/out CD40 blockade using a non-depleting antibody. Based on these findings further therapeutic approaches involving CD40 pathway blockade to prolong allograft survival and understanding autoimmune diseases might be identified and transferred to clinic.

e-mail: mandy.wahlbuhl-becker@uk-erlangen.de

P010 01/09/2017 - 31/08/2018 Oncology

The FGFR1 amplicon in breast cancer

Dr. Ramona Erber, Institute of Pathology

The FGFR1 amplicon is associated with Luminal B like breast cancer and worse prognosis. In order to clarify whether FGFR1 or another gene belonging to the 8p11.2-p12 amplicon is the driver gene, the mRNA expression of these amplicon genes will be analysed in n=523 breast cancer samples and correlated with clinicopathological parameters and outcome. Additionally, the PAM50 expression profile will be investigated in order to compare the gene expression profile of the amplicon 8p11.2-p12 in different intrinsic molecular subtypes.

e-mail: ramona.erber@uk-erlangen.de

P011 01/08/2017 - 31/08/2018 Oncology

Tumor cachexia and muscle motor proteins

Dr. Raphaela Schwappacher, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

With our pilot study we want to gain knowledge about muscular dysfunctions in tumor patients. We will analyze the effects of physical exercise on key components of single muscle fibers using biomechanical and ultrastructural analysis. We hope that our data on muscle fiber composition and quality might contribute to the development of targeted therapies counteracting cachexia. Stabilizing and maintaining the muscle status can significantly improve prognosis and quality of life of cancer patients.

e-mail: raphaela.schwappacher@uk-erlangen.de

P012 01/01/2018 - 31/12/2018 Oncology

RIPK4 in intestinal inflammation and cancer

Dr. Eva Martini, Department of Medicine 1 – Gastroenterology, Pneumology and Endocrinology

Preliminary data show an important role of RIPK4 during gut inflammation and colorectal cancer development. The functional role of RIPK4 in the gut and the molecular signaling pathway, so far, has not been investigated. We intent to verify our preliminary data during intestinal inflammation and colorectal cancer development.
AIM1: Elucidate the role of RIPK4 during gut inflammation.
AIM2: Elucidate RIPK4 dependent signalling pathways.
AIM3: Is RIPK4 a potential therapeutic target?

e-mail: eva.martini@uk-erlangen.de

P013 15/08/2017 - 14/08/2018 Neurosciences

Prenatal trauma, placenta and fetal HPA axis

Dr. Stefan Frey, Division of Child and Adolescent Mental Health

In order to understand the trauma-induced changes we intend to investigate maternal, placental and embryonal changes in the HPA-axis system. We will measure maternal and amniotic CORT concentrations on the GD18, examine the Hsd11b isotype 1 and 2 expression and protein concentrations in placental tissue to assess CORT exposure of the respective pups after a traumatic event. We will perform the expression analysis of the HPA-axis key genes in placenta and several brain regions of pups.

e-mail: stefan.frey@uk-erlangen.de

P014 01/11/2017 - 31/10/2018 Oncology

Melanoma Metastasis to Steatotic Livers

Prof. Dr. Claus Hellerbrand, Institute of Biochemistry

Obesity increases the risk of different types of cancer including melanoma and worsens the prognosis. The majority of obese individuals also have significant hepatic steatosis („fatty liver“) and this condition is increasingly recognized to promote development and progression of primary liver cancer. In this project, we analyze whether (obesity-induced) hepatic steatosis affects the homing and survival of cancer cells and intend to identify underlying molecular mechanisms.

e-mail: claus.hellerbrand@fau.de

P015 01/05/2018 - 31/10/2018 Others

Bile acids as modulators of P2X4 receptor

Dr. Alexandr Ilyaskin, Institute of Physiology and Pathophysiology

In the preliminary work we have demonstrated for the first time that human ionotropic ATP-activated P2X4 receptor can be modulated by several bile acids. Moreover, computer simulations suggest that the specific region in the vicinity of the P2X4 channel pore may play a critical role in the interaction of the receptor with bile acids. Thus, the main focus of the proposed project is the detailed investigation of the molecular mechanisms involved in the modulation of P2X4 receptor by bile acids. .

e-mail: alexandr.ilyaskin@fau.de

P016 01/02/2018 - 31/01/2019 Others

Laboratory detection of ultrasound microbubbles

Dr. Ferdinand Knieling, Department of Pediatric and Adolescent Medicine

Contrast Enhanced Sonography (CEUS) is an established diagnostic procedure in adults. It is highly accurate, easy to use, free of ionizing radiation and does not require anesthesia / sedation in children. The aim of this project is the establishment of a laboratory methodology for the detection of the used microbubbles. In addition to analytical parameters, the preanalytical conditions are investigated.

e-mail: ferdinand.knieling@uk-erlangen.de

P018 01/03/2018 - 28/02/2019 Oncology

XCR1 as a marker for human crosspresenting DCs

Dr. Lukas Heger, Department of Dermatology

DCs have an important role in der regulation of immune responses. Recent studies in mice suggest that crosspresentation is a specialized function of XCR1+ cDC1. Also in humans different DC subsets can be differentiated based on surface markers, which show transcriptional homologies to murine DC subsets. Therefore, this proposal aims to analyze whether XCR1 is a marker for human crosspresenting DCs and whether these polarize naïve T cells mainly into Th1 cells due to enhanced IL-12 secretion.

e-mail: lukas.heger@uk-erlangen.de

P019 01/06/2018 - 30/04/2019 Others

The involvement of the enteric nervous system in the immunopathogenesis of multiple sclerosis

Prof. Dr. Stefanie Kürten, Institute of Anatomy

The proposal evolves around the central aim to provide a precise morphological analysis of enteric nervous system (ENS) pathology in MP4-induced experimental autoimmune encephalomyelitis (EAE) as a mouse model of multiple sclerosis (MS). It can be further subdivided into two main objectives. While objective 1 will determine the extent of submucous plexus pathology, objective 2 aims to identify the exact neuronal target population(s) of ENS-reactive autoantibodies in MP4-induced EAE.

e-mail: stefanie.kuerten@fau.de

P020 24/01/2018 - 23/01/2019 Oncology

Localisation of the EMT-transcription factor ZEB1

Dr. Rebecca Eccles, Chair of Experimental Medicine I

ZEB1 is a major driver of cancer metastasis, the leading cause of cancer-associated death, as it couples the activation of cellular motility with stemness and survival properties. As a transcription factor ZEB1 is normally found in the nucleus. However, we have observed cytosolic ZEB1 in a variety of cancer types, and the presence of this form seems to correlate with better patient prognosis. We therefore plan to explore the role cytosolic ZEB1 plays in cancer and metastasis.

e-mail: rebecca.eccles@fau.de

P021 01/06/2018 - 30/04/2019 Immunology and Infection

Bone strength in rheumatic finger joints

Dr. Arnd Kleyer, Department of Medicine 3 – Rheumatology and Immunology

The aim of this study was to develop a FEA model for the Metacarpo-phalangeal (MCP) joints imaged with HR-pQCT to understand the impact of changes in bone density and microarchitecture due to inflammation on bone strength of the MCP head. Segmented MCP heads were divided into trabecular and cortical regions and modeled by FEA (Faim v8.0, Numerics88 Solutions Ltd., Canada). Failure load was based on 2% critical volume at 0.007 strain. To transfer the force of 100 N into the joint, a polymethylmethacrylat (PMMA) cap was added at the distal end of the MCP head. Bone stiffness ranged from 4.4 - 6.8 kN/mm and failure load from 540 N to 780 N. Displacement decreases in the proximal direction.

e-mail: arnd.kleyer@uk-erlangen.de

P022 01/08/2018 - 31/07/2019 Others

Vascularization and bone formation

Dr. Dominik Steiner, Department of Plastic and Hand Surgery

The broad use of bioartificial bone tissue is limited by the insufficient integration into the host vasculature. One strategy to improve vascularization is the generation of an arteriovenous fistula (AV loop). The goal of this project is to establish a cell-loaded hydrogel matrix for the reconstruction of large bone defects. Human ADSCs and HUVECs will be encapsulated in a hydrogel matrix, vascularization will be performed by means of an AV loop and BMP-2 will enhance bone formation.

e-mail: dominik.steiner@uk-erlangen.de

P023 01/06/2018 - 31/05/2019 Immunology and Infection

DPP4 - a molecular target in fibrosis

Dr. Alina Soare, Department of Medicine 3 – Rheumatology and Immunology

Fibrotic diseases can be considered as a consequence of persistent, exaggerated and uncontrolled tissue repair processes. Systemic sclerosis (SSc) is associated with a high mortality and effective anti-fibrotic therapies are still lacking. Dipeptidyl-peptidase-4 (DPP4) has been recently shown to identify a distinct dermal lineage of fibroblasts involved in scar formation. We have demonstrated that DPP4-positive cells are increased not only in experimental fibrosis, but also in skin biopsies from SSc patients as compared to healthy volunteers. DPP4-inhibitors showed potent anti-fibrotic effects in bleomycin-induced skin fibrosis. However, the specific role of DPP4-positive fibroblasts in fibrotic diseases is unknown. We aim in this study to characterize the phenotype of DPP4-positive fibroblasts and assess the potential pro-fibrotic properties of DPP4-positive fibroblasts. Further we plan to test DPP4-inhibitors in mouse models of fibrotic diseases resembling, different subtypes and stages of other fibrotic diseases such as chronic graft-versus-host disease mouse model and models of pulmonary fibrosis. These results may have direct clinical implications as DPP4-inhibitors are already in clinical use for diabetes.

e-mail: alina.soare@uk-erlangen.de

P024 01/10/2018 - 31/03/2019 Immunology and Infection

The contribution of butyrophilins in the pathogenesis of Rheumatoid Arthritis

Dr. Kerstin Sarter-Zaiss, Department of Medicine 3 – Rheumatology and Immunology

The aim of this project is to analyze the role of the co-stimulatory molecule Btn2a2 in the development and / or pathology of RA and to lay the foundations for future research to understand the underlying mechanisms of action of Btn2a2 during the dissolution of inflammation decrypt.

e-mail: kerstin.sarter-zaiss@uk-erlangen.de

P025 01/08/2018 - 31/07/2019 Others

Analysis of exosomal biomarkers for CRSwNP

Dr. Sarina Müller, Department of Otorhinolaryngology - Head and Neck Surgery

Exosomes are spherical 30-150nm vesicles which are secreted by virtually all cell types in virually all body fluids. For this reason, exosomal proteins are an innovative and non-invasive way for the analysis of biomarkers. We could identify Pappalysin-A (PAPP-A) in own preliminary studies as a promising biomarker for chronic rhinosinusitis with nasal polyps (CRSwNP). The objective of this project is the detailed analysis of Pappalysin-A and its evaluation as a potential biomarker for CRSwNP.

e-mail: sarina.mueller@uk-erlangen.de

P026 15/11/2018 - 14/11/2019 Neurosciences

On myelination processes in the cuprizone model

Prof. Dr. Frederik Laun, Institute of Radiology

The aim of this work is gaining knowledge about the ability of quantitative susceptibility mapping (QSM) to monitor de- and remyelination processes. QSM is a rather novel magnetic resonance imaging (MRI) approach that reveals the magnetic susceptibility of tissue, which, e.g., indicates the presence of myelin. Cuprizone mouse models shall be used, which allow monitoring de- and remyelination. Cuprizone is a copper chelator that induces a demyelination process.

e-mail: frederik.laun@uk-erlangen.de

P027 01/07/2018 - 31/12/2019 Oncology

IFN-gamma and vasculature in CRC

Dr. Nathalie Britzen-Laurent, Department of Surgery

The microenvironment is critical for tumor growth and regulates both angiogenesis and the anti-tumor immune response. We have previously shown that IFN-gamma, a mediator of the anti-tumor immune response can also influence the vasculature by inhibiting angiogenesis and inducing vessel permeability in vitro and in vivo. Here, we will investigate the vascular effects of IFN-gamma and their influence on tumor development using a chemically-induced colon carcinogenesis mouse model.

e-mail: nathalie.britzen-laurent@uk-erlangen.de

P028 01/10/2018 - 31/09/2019 Neurosciences

3D organoid models for analysis of SOX11-CSS

Dr. Sören Turan, Institute of Biochemistry

Coffin-Siris syndrom (CSS) is a neurodevelopmental disorder with cardinal features of intellectual disability and microcephaly. Mutations of transcription factor SOX11 were described as a genetic cause of CSS. In order to allow a better understanding of the pathophysiological consequences of SOX11 haploinsufficiency during human cortex development, this proposal aims to generate and analyse a SOX11+/- cerebral organoid model.

e-mail: soeren.turan@fau.de

P029 01/11/2018 - 31/10/2019 Oncology

Combined DNA-/RNA-transfection of T cells

Dr. Ugur Uslu, Department of Dermatology

Tumor cells can by-pass T-cell recognition, which is a challenge in adoptive T-cell therapy. To increase the pressure on the tumor, we want to generate T cells expressing two additional tumor-specific receptors by combining stable DNA- and transient RNA-based receptor transfer. The latter of the two receptors shall have a “boost” effect in the first days of therapy by induction of direct tumor-cell killing and T-cell proliferation, leading to an effective tumor cell lysis.

e-mail: ugur.uslu@uk-erlangen.de

P030 01/01/2019 - 31/12/2019 Neuroschiences, Others

Ultrashort Echo Time MRI of Myelin at 7 T

Prof. Dr. Armin Nagel, Institute of Radiology

The aim of this project is to implement and to evaluate an ultra-short echo time (UTE) pulse sequence for clinical and preclinical research at 7 Tesla, which enables non-invasive mapping of the myelin content. Image artifacts - that are common in UTE imaging - will be analyzed and corrected. The newly implemented UTE imaging technique will be applied in two proof-of-concept studies, including a mouse model of multiple sclerosis and multiple sclerosis patients.

e-mail: armin.nagel@uk-erlangen.de

P031 16/12/2018 - 15/06/2019 Neurosciences

Zwicker tone as a model for acute tinnitus

Dr. Achim Schilling, Department of Oto-Rhino-Laryngology - Head and Neck Surgery

The main goal of the here proposed project is the identification of the cortical representation of a Zwicker tone as a model for tinnitus. Thus, we plan to carry out MEG and EEG measurements in cooperation with PD Dr. Rampp, to compare the neuronal representation of silence, pure-tones and Zwicker tones. As we expect neuronal mechanisms within the dorsal cochlear nucleus as cause for acute tinnitus, we consider the cortical representation of Zwicker tones and pure tones to be similar.

e-mail: achim.schilling@uk-erlangen.de

P032 15/07/2018 - 14/07/2019 Immunology and Infection

In vivo imaging of inflammation / bone remodeling

Dr. Christine Schauer, Department of Medicine 3 – Rheumatology and Immunology

Abnormal neo-ossification in humans with SpA and gout are common, but there are only few suitable animal models. Conventional methods require a high number of animals or can only insufficiently distinguish between inflammatory edematous swelling and/or increased bone thickness. The aim of this application is to establish a mouse model of gouty enthesitis in vivo by means of a non-invasive combination of MRI and PET/CT, in which live animals can longitudinally be examined.

e-mail: christine.schorn@uk-erlangen.de

P033 26/03/2019 - 25/03/2020 Others

YB-1 in molecular regulation of chondrogenesis

Dr. Ulrike Rottensteiner-Brandl, Institute of Biochemistry

YB-1 is expressed in chondrocytes during late embryonic stages and after birth, suggesting a crucial role of YB-1 in chondrogenesis. Little is known about signaling cascades linked to YB-1 mediating its action. The goal of this study is to investigate YB-1-dependent pathways during chondrogenesis. Particular interest will be paid to MIA-dependent signaling (upstream) and target genes involved in transition to hypertrophy and dedifferentiation (downstream).

e-mail: ulrike.rottensteiner@fau.de

P034 16/01/2019 - 15/01/2020 Oncology

Myeloid ZEB1 in colorectal cancer

Dr. Harald Schuhwerk, Chair of Experimental Medicine I

In colorectal cancer (CRC), the transcription factor ZEB1 is upregulated in tumor cells and tumor-associated macrophages. As only its tumor-promoting role in tumor cells is known, we are analyzing myeloid-specific ZEB1 knockout mice. Our preliminary data suggest that ZEB1 plays a role in macrophage polarization, intestinal inflammation and CRC growth. Here, we will explore novel functions of ZEB1 in immune homeostasis, macrophage plasticity, immune-modulation in CRC and colitis-associated CRC.

e-mail: harald.schuhwerk@fau.de

P035 07/01/2019 - 06/01/2020 Neurosciences

Role of FBXO11 in intellectual disability

Dr. Anne Gregor, Institute of Human Genetics

Recently we identified de novo variants in FBXO11, encoding a subunit of an E3-ubiquitin ligase complex, as causative for a neurodevelopmental disorder (NDD). The goal of this grant is to characterize the role of FBXO11 in NDDs. With the model organism Drosophila melanogaster anatomical studies of synapses and behavioral assays will be performed. Additionally effects of patient mutations will be tested in cell-based assays and target proteins of FBXO11 will be identified using AP-MS.

e-mail: anne.gregor@uk-erlangen.de

P036 01/04/2019 - 31/03/2020 Others

Physicians' opinions on continuous sedation

Dr. Maria Heckel, Division of Palliative Medicine

Physicians' practice and opinions regarding continuous sedation until death are to be collected internationally. The German subproject aims to gain a comprehensive overview of the opinions of German palliative physicians by online survey and to link them to their professional background and experiences. The crosscultural comparison might contribute to an internationally binding definition and a more uniform treatment practice.

e-mail: maria.heckel@uk-erlangen.de

P037 12 months Neurosciences

Prognostication in intracerebral hemorrhage

Dr. Jochen Sembill, Department of Neurology

Prognostication in intracerebral hemorrhage (ICH) is biased by self-fulfilling prophecy. We will 1) validate the max-ICH Score, pooling patient data from i) single-center study from Massachusetts General Hospital (Harvard), ii) single-center UKER study, iii) multicenter RETRACE study. We will 2) conduct a prospective multicentre study with randomized controlled prognostic score usage to evaluate physician's prognostic variability & accuracy, optimal prognostic timing, improved outcome measures.

e-mail: jochen.sembill@fau.de

P038 01/04/2019 - 31/03/2020 Oncology

Molecular markers in stage T1 bladder cancer

Dr. Danijel Sikic, Department of Urology

Previous studies demonstrated a prognostic relevance of several molecular markers in stage T1 bladder cancer. These might optimize risk stratification and decision making with regard to immediate cystectomy or bladder sparing approach. However, these findings have not been validated yet.

The goal of the current study is to validate the association of the mRNA expression of these molecular markers with clinical and survival data in a new cohort consisting of stage T1 bladder cancer.

e-mail: danijel.sikic@uk-erlangen.de

P039 12 months Immunology and Infection

Bone characterization in early RA autoimmunity

Dr. David Simon, Department of Medicine 3 – Rheumatology and Immunology

To better understand the influence of the early phase of autoimmunity of rheumatoid arthritis (RA) on joint structure, longitudinal observations of pre-RA patients are necessary. High-resolution CT is used to investigate how bone density and structure and biomechanical properties of pre-RA patients develop over time, what influence different biomarker profiles have and what bone characteristics patients developing clinical RA have.

e-mail: david.simon@uk-erlangen.de

P040 12 months Neurosciences

FoxO-dependent mitophagy in stem cell function

Dr. Iris Schäffner, Institute of Biochemistry

Mitochondrial function is crucial for maintenance of the adult neural stem/progenitor cell (NSPC) pool. I found that loss of FoxO transcription factors leads to hyperproliferation and depletion of NSPCs and impairs autophagy-lysosome pathway activity. Moreover, loss of FoxOs is associated with mitochondrial dysfunction. I propose to investigate mitochondria as targets of FoxO-dependent autophago-lysosomal degradation, to establish a FoxO-mitophagy axis in the control of adult NSPC function.

e-mail: iris.schaeffner@fau.de

P041 12 months Renal and Vascular Research

Polyploid cardiomyocytes for cardiac repair

Dr. Maria Leone, Department of Nephropathology

Humans are incapable to regenerate their heart. Cardiac injury results in cardiomyocyte loss due to hypoxia and a changed mechanical micro-environment. Here we propose to determine the potential of polyploid cardiomyocytes, the majority in the adult heart, to contribute to heart repair. We propose to clarify if polyploid cardiomyocytes can be induced to proliferate or whether diploid and polyploid cardiomyocytes differ in regards to stress resistance, cell size, and mechanical properties.

e-mail: marina.leone@uk-erlangen.de

P042 12 months Oncology

Immunology of NMSC of the head and neck

Dr. Dr. Gesche Frohwitter, Department of Oral and Cranio-Maxillofacial Surgery

The facial skin is most frequently affected by non-melanoma skin cancer (NMSC). However, the immunological profile of these tumors is still poorly understood. The anticipated ELAN project aims to address this problem by immunohistochemical investigations and may anticipate the establishment of an immunoscore which supplements the TNM classification in prognostic information and therapeutic decision making.

e-mail: gesche.frohwitter@uk-erlangen.de

P043 12 months Oncology

The autotaxin-LPA axis in breast cancer

Dr. Annika Kengelbach-Weigand, Department of Plastic and Hand Surgery

Breast cancer is the most common cancer in women worldwide. It is hypothesized that in a vicious cycle autotaxin (ATX) secreted by fat tissue influences breast cancer cells in behavior and leads to secretion of inflammatory cytokines which in turn stimulate ATX secretion of fat tissue. Radiotherapy could lead to an amplification of this effect. It is the aim of this study to evaluate the significance of the ATX/LPA-axis and the effect of radiotherapy in different breast cancer subtypes.

e-mail: annika.kengelbach-weigand@uk-erlangen.de

P044 12 months Immunology and Infection

HIF-1a in IgA class switching

Dr. Xianyi Meng, Department of Medicine 3 – Rheumatology and Immunology

Germinal center (GC) has been described to contain hypoxic regions linked to B cell class switching. In this project, we will delineate molecular mechanism between HIF-1a-dependent glycolysis and epigenetic modification on IgA class switching region. By studying the IgA response following the C. rodentium infection, we aim to identify the link between the HIF-1a-dependent glycolytic metabolic shift and IgA class switching during microbial infection.

e-mail: xianyi.meng@uk-erlangen.de

P045 12 months Immunology and Infection

HSV-1 modulates the IL-6 signaling pathway in mDCs

Dr. Linda Grosche, Division of Immune Modulation

The focus of the present project is the investigation of Herpes simplex virus type-1 (HSV-1)-mediated modulations of the IL-6 signaling pathway in mature dendritic cells (mDCs). In particular, the underlying molecular mechanisms of reduced IL-6R?, gp130 and STAT3 expression will be analyzed on directly-infected versus uninfected bystander mDCs. Moreover, we will elucidate whether non-infectious L-particles, released from HSV-1-infected cells, are essential/sufficient to induce these modulations.

e-mail: linda.grosche@uk-erlangen.de

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