MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

January 2017-December 2018

MUI-START Report

Servicecenter Forchung
Medical University of Innsbruck
January 2017-December 2018

Content

1 Background and aim of the programme

MUI-START is the follow-up programme of the MFI (Medizinische Forschungsfonds Innsbruck) which ended in 2011.

The MUI-START programme is devised as a start-up fund for young scientists with the aim to offer young scientists the opportunity of developing new project ideas, within the MUI research focuses, that could serve as basis for a successful subsequent application for external funding (e. g. FWF, ÖNB).

According to the present guidelines, eligible candidates must: 1) have a working contract with the Medical University of Innsbruck, 2) have completed their doctoral studies, and 3) be not older than 35 years by the application deadline. Fully justified career breaks can be taken into account (e. g. parental leave). Professors and PIs of third-party funded (FWF, OeNB, FFG and EU) projects are not eligible. Applicants' track records must be commensurate with their academic age. However, two peer-reviewed international publications as first author are compulsory**.**

The guidelines of the programme have been substantially modified over the years to adapt to the high standards applied by external funding agencies (e. g. FWF). Since 2016, proposals undergo a three-step evaluation procedure: 1) selection of proposals by the MUI-START jury, 2) international peer-review of the pre-selected proposals, and 3) hearing of the shortlisted applicants by the MUI-START jury. Final decisions are based on the reviewers' scores, as well as the outcome of the interviews.

Moreover, since the seventh call (2016) the submission of an external funding proposal before the end of the funding period has become compulsory for all MUI-START grantees. Failure to submit such an application results in the cancellation of the last quarter of the MUI-START grant budget.

2 Overview of MUI-START calls

The first MUI-START call was announced in the summer 2010 and supported 42% of the submitted proposals. Since then, the approval rates have been oscillating from year to year (Table 1) depending on both the available budget and the quality of the submitted proposals. At the moment the 10th call has been announced. Decisions are expected in autumn 2019.

Table 1. Overview of all MUI-START calls

Call	Proposals submitted	Proposals granted (Male/Female)	Funding rate	Total funding requested	Total funding granted
1st call	31	13 (7M/6F)	42%	€ 2.074.365,7	€ 667.054,80
2nd call	11	5 (2M/3F)	45%	€ 629.968,95	€ 173.171,00

3rd call	29	9 (4M/5F)	31%	€ 742.808,21	€ 240.000,00
4th call	28	14(11M/3F)	50%	€ 713.652,93	€ 323.484,66
5th call	31	12(4M/8F)	39%	€ 771.750,48	€ 260.826.60
6th call	28	8 (4M/4F)	28%	€ 711.035,41	€ 176.726,00
7th call	9	3 (1M/2F)	33%	€ 248.945,01	€ 85.000,00
8th call	15	7 (5M/2F)	47%	€ 365.189,29	€ 162.208,80
9th call	8	4 (1M/3F)	50%	192.576,23	€ 113.766,30

3 MUI-START jury members and reviewers

The MUI START jury members are professors and associate professors at the Medical University of Innsbruck working in both basic as well as in clinical research fields. The jury members are chosen according to their proven expertise in a specific field of research. The composition of the jury is not fixed, but changes as a result of the variety of topics covered by the proposals submitted to a particular call.

The following jury members helped in the selection of the projects presented in this report (5th, 6th and 7th MUI-START call). Their help and commitment is warmly acknowledged.

Univ.-Prof. Dr. Christine BANDTLOW	Neurobiochemistry Division
Univ.-Prof. Dr. Georg DECHANT	Institute of Neurosciences
Univ.-Prof. Dr. Francesco FERRAGUTI	Institute of Pharmacology
Univ.-Prof. Dr. Ludger HENGST	Biochemical Chemistry Division
o. Univ.-Prof. Dr. Werner JASCHKE	University Hospital for Radiology
Ao. Univ.-Prof. Dr. Alexandra LUSSER	Molecular Biology Division
Univ.-Prof. Dr. Gert MAYER	University Hospital for Internal Medicine IV
Univ.-Prof. Mag. Dr. Michael NOGLER	University Hospital for Orthopaedics
Univ.-Prof. Dr. Matthias SCHMUTH	University Hospital for Dermatology, Venerology and Allergology
Priv.-Doz. Dr. Patrizia STOITZNER	University Hospital for Dermatology, Venerology and Alergology

Ao. Univ.-Prof. Dr. Günter WEISS	University Hospital for Internal Medicine II
Ao. Univ.-Prof. Dr. Johann WILLEIT	University Hospital for Neurology
Univ.-Prof. Dr. Johannes ZSCHOCKE	Human Genetics Division

The tasks of the jury members comprise: 1) internal review of the proposals, 2) nomination of the reviewers at the suggestion of the MUI Research Office (SCF), and 3) presentation of proposals during the decision meeting.

The reviewers of the MUI-START projects are international experts active in their field of research. At least two reviews per proposal are necessary to support the jury members decision process.

4 MUI-START Symposium and project evaluation

By accepting the MUI-START grant, the recipients commit themselves to take part in the annual MUI-START Symposium organized by the MUI Research Office (SCF). This event represents an ideal occasion for the MUI-START jury members to assess the progress achieved by the grant holders in their respective projects. Additionally, the symposium promotes a mentoring effect for the grant holders who profit from the expertise and advice of the jury members.

During the last three MUI-START Symposiums a total of 18 projects were evaluated. The results of this evaluation are summarized below and in Fig. 1.

In more than 70% of the cases, the project development was considered good or very good. The PI qualifications and the research environment were considered good or very good in 94% of the evaluated projects. However, despite these positive results the jury members judged that only two thirds of the projects could translate into an external funding application. This discrepancy is due to the fact that several PIs quit the MUI earlier than expected or immediately after the end of the funding period. In few other cases, the jury members thought it would be difficult to find a suitable funding programme to submit a proposal.

Links: 5th MUI-START Symposium Programme
6th MUI-START Symposium Programme
7th MUI-START Symposium Programme

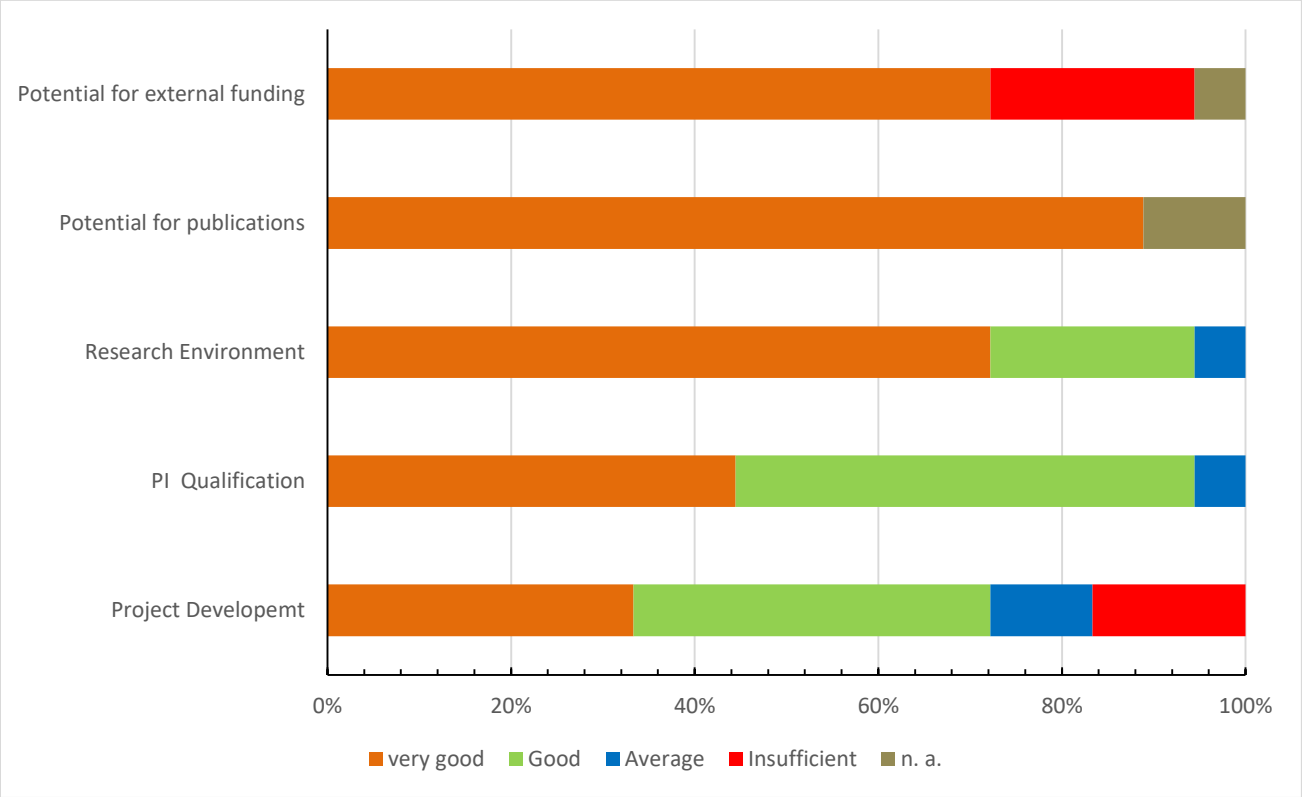

Fig. 1. Results of the evaluation of projects during the MUI-START Symposium. n.a. no answer.

5 External funding granted to MUI-START grant recipients

As stated in the first section of this report the aim of the programme is to help young scientists develop new project ideas that could serve as a basis for a subsequent application for third-party funding.

So far, (status 31.12.2018) 57 MUI-START funded projects have closed. Twelve PIs quit the MUI either before the planned end or immediately after the end of the project. Approximately 80 % (37 PIs) of the remaining PIs applied for external funding. Given the competitiveness of the current funding landscape, not all applications generated funding. Table 2 and Table 3 provide an overview of the funds acquired by the MUI-START grantees to date.

Table 2. FWF and ÖNB funded projects acquired by former MUI-START grant holders.

Applicant	Project Title	Funding Agency-Project Number	Duration (Months)	Funds Granted
Natasha Hermann-Kleiter	Orphan receptor NR2F6 as a negative regulator of T cell effector functions	FWF - P23537	36	€ 255.858,75
Natasha Hermann-Kleiter	NR2F6 governs immune defense against microbial pathogens	FWF - P 28694	36	€ 317.627,10

Galina Apostolova	Role of genome organizer Satb2 in adult brain function	FWF - P25014	36	€ 299.817,00
Galina Apostolova Co-PI	Function of special AT-rich sequence-Binding Protein 2 (SatB2)	FWF – SFB4416	48	€ 475.755,00
Birgit Frauscher	Motor activity during sleep in health and disease	FWF - KLI236	36	€ 203.609,70
Birgit Frauscher	REM sleep without atonia: early sign of neurodegeneration	ÖNB - 15127	30	€ 90.000
Martin Puhr	Functional significance of PIAS1 and BIRC5 in docetaxel resistant prostate cancer	FWF - P25639	30	€ 337.233,75
Markus Theurl	Catestatin for the treatment of myocardial ischemia	FWF - P26251	24	€ 262.731,00
Rupert Oberhuber	Evaluierung der Leberorganqualität vor Transplantation	ÖNB - 17287	36	€ 110.000,00
Manfred Nairz	Die Rolle von Innate Response Activator B Zellen bei Sepsis	FWF - J3486	24	€ 69.700,00
James Wood	Dopamine and NPY signaling in a fear extinction circuit	FWF - M1783	24	€ 157.380,00
Sebastian Herzog	Molecular regulation of the oncogenic miR-17-92 cluster	FWF - P 30194	48	€ 297.304,89
Sebastian Herzog	Unraveling miR-15 function in health and disease	FWF - P 30196	42	€ 364.019,25
Ramon Tasan	Role of the neurokinin B-expressing neurons in the bed nucleus of the stria terminalis	FWF - P 29952	36	€ 399.441,00
Johanna Gostner	Cellular reactions to low-dose volatile organic compounds (VOC) exposures	FFG - Bridge	36	€ 301.881,00
Stefan Coassin	Studying a *Lp*(a) mutation in human RNA and liver organoids	FWF - P 31458	42	€ 345.914,29
Bernhard Haubner	Investigating long non-coding RNA regulated pathways driving cardiac regeneration	FWF - I 4161	36	€ 206.892,00

Table 3. Additional funding acquired by former MUI-START grant holders.

Applicant	Project Title	Funding Agency	Duration (Months)	Funds Granted
Wegene Borena	Genital HPV infection among	MFF - Nr.262	18	€ 13.728,00

	HIV - positive men in West Austria in the Austrian HIV Cohort Study			
Selma Tuzlak	Bcl-2 family	ÖAW - 23949	36	€ 105.000,00
Christian Ploner	MADI	Land Tirol	51	€ 73.466,98
Peter Lackner	The role of voltage gated Ca channels for neuroprotection in experimental subarachnoid hemorrhage	Land Tirol	24	€ 75.400,00
Rupert Oberhuber	Tetrahydrobiopterin as novel therapeutic strategy to improve outcome after the transplantation of organs from brain death donors	TWF-2013-1-17	24	€ 20.000,00
Sebastian Herzog	Systematic analysis of the miR-26 family in lymphocyte development and cancer	TWF-2014-1-17	24	€ 26.500,00
Michael Blatzer	Alternative regulatory circuits of secondary metabolite production in *Aspergillus terreus*	TWF-2016-1-1	24	€ 39.500,00
Beno Cardini	The impact of simvastatin on the ischemia reperfusion injury in the murine heart transplantation model	TWF-2016-1-6	18	€ 32.069, 00
Martin Bodner	Helena's many daughters - Massively parallel sequencing provides highest-resolution insights into the most common West Eurasian mtDNA control region haplotype	TWF-2016-1-2	24	€ 30.122,00
Martin Bodner	Helena's many daughters: massively parallel sequencing provides further insights into the most common West Eurasian mtDNA control region haplotypre at the highest resolution	DSF-2015-1-1	12	€ 5.000,00
Lourdes Rocamora	Role of Glucocorticoides on B cell development and function	TWF-2016-1-23	24	€ 37.390,00
Luca Fava	How do cells count their centrosomes? A mechanistic study	Armenise-Harvard Foundation	60	$1.000.000,0
Nina Clementi	What makes a Stop codon a Stop codon?	TWF-2017-1-3	24	€ 31.754,00
Elke Griesmaier	Beurteilung von Secretoneurin als Serum Biomarker der Hirnschädigung bei	TWF-2017-1-8	48	€ 18.400,00

	Frühgeborenen			
Manfred Nairz	Inhibition of p38 to combat bacterial infections	TWF-2018-1-4	24	€ 38.431,53
Judith Hagenbuchner	Targeting NOX2 to treat metabolic abnormalities in patients with Very-Long-/ and Long-Chain-3-Hydroxy Acyl CoA Dehydrogenase Deficiency	TWF-2018-1-5	24	€ 21.825,18
Theresa Hautz	Role of donor-derived leukocytes in the perfusate during normothermic machine perfusion of human liver allografts: Implication for organ function and outcome in liver transplantation	TWF-2018-1-14	36	€ 33.032,18
Romana Gerner	Siderophore-antibiotic conjugates as a selective strategy to combat gram-negative infections	Max-Kade Foundation	12	$ 57.000,00

6 Publications acknowledging the MUI-START programme

This section lists publications arising from the MUI-START projects that ended between January 2017 and December 2018. A list of publications of projects that finished before then can be found in the former MUI-START reports.

Bauer, I; Varadarajan, D; Pidroni, A; Gross, S; Vergeiner, S; Faber, B et al. (2016): A class 1 histone deacetylase with potential as an antifungal target. In: *mBio* 7 (6), e00831-16. DOI: 10.1128/mBio.00831-16.

Burgio, F; Delazer, M; Meneghello, F; Pertl, M-T; Semenza, C; Zamarian, L (2018): Cognitive training improves ratio processing and decision making in patients with mild cognitive impairment. In: *Journal of Alzheimer's Disease* 64 (4), S. 1213–1226. DOI: 10.3233/jad-180461.

Coassin, S; Erhart, G; Weissensteiner, H; Eca Guimarães de Araújo, M; Lamina, C; Schönherr, S et al. (2017): A novel but frequent variant in LPA KIV-2 is associated with a pronounced Lp(a) and cardiovascular risk reduction. In: *European heart journal* 38 (23), S. 1823–1831. DOI: 10.1093/eurheartj/ehx174.

Coassin, S; Schönherr, S; Weissensteiner, H; Erhart, G; Forer, L; Losso, JL et al. (2019): A comprehensive map of single-base polymorphisms in the hypervariable LPA kringle IV type 2 copy number variation region. In: *Journal of lipid research* 60 (1), S. 186–199. DOI: 10.1194/jlr.M090381.

Delazer, M; Zamarian, L; Frauscher, B; Mitterling, T; Stefani, A; Heidbreder, A; Högl, B (2016): Oxygen desaturation during night sleep affects decision-making in patients with obstructive sleep apnea. In: *Journal of Sleep Research* 25 (4), S. 395–403. DOI: 10.1111/jsr.12396.

Heim, B; Djamshidian, A; Heidbreder, A; Stefani, A; Zamarian, L; Pertl, M-T et al. (2016): Augmentation and impulsive behaviors in restless legs syndrome. In: *Neurology* 87 (1), S. 36. DOI: 10.1212/WNL.0000000000002803.

Oemer, G; Lackner, K; Muigg, K; Krumschnabel, G; Watschinger, K; Sailer, S et al. (2018): Molecular structural diversity of mitochondrial cardiolipins. In: *Proceedings of the National Academy of Sciences of the United States of America* 115 (16), S. 4158–4163. DOI: 10.1073/pnas.1719407115.

Pertl, M-T; Benke, T; Zamarian, L; Delazer, M (2015): Decision making and ratio processing in patients with mild cognitive impairment. In: *Journal of Alzheimer's Disease* 48 (3), S. 765–779. DOI: 10.3233/jad-150291.

Pertl, M-T; Benke, T; Zamarian, L; Delazer, M (2017): Effects of Healthy Aging and Mild Cognitive impairment on a real-life decision-making task. In: *Journal of Alzheimer's Disease* 58 (4), S. 1077–1087. DOI: 10.3233/jad-170119.

Pertl, M-T; Zamarian, L; Delazer, M (2017): Reasoning and mathematical skills contribute to normatively superior decision making under risk: evidence from the game of dice task. In: *Cognitive Processing* 18 (3), S. 249–260. DOI: 10.1007/s10339-017-0813-x.

Quarta, S; Mitrić, M; Kalpachidou, T; Mair, N; Schiefermeier-Mach, N; Andratsch, M et al. (2018): Impaired mechanical, heat, and cold nociception in a murine model of genetic TACE/ADAM17 knockdown. In: *The FASEB Journal* 33 (3), S. 4418–4431. DOI: 10.1096/fj.201801901R.

Weustenfeld, M; Eidelpes, R; Schmuth, M; Rizzo, WB.; Zschocke, J; Keller, MA (2019): Genotype and phenotype variability in Sjögren-Larsson syndrome. In: *Human Mutation* 40 (2), S. 177–186. DOI: 10.1002/humu.23679.

Zamarian, L; Delazer, M; Ehling, R; Pertl, M.-T; Bsteh, G; Wenter, J et al. (2019): Improvement of medical judgments by numerical training in patients with multiple sclerosis. In: *European Journal of Neurology* 26 (1), S. 106–112. DOI: 10.1111/ene.13778.

Zamarian, L; Berger, T; Pertl, M-T; Bsteh, G; Benke, T; Delazer, M (2016): Framing effects in multiple sclerosis: How patients may be misled by the way medical information is presented; In: *Zeitschrift für Neuropsychologie* 27(1): S 62.

Zamarian, L; Högl, B; Delazer, M; Hingerl, K; Gabelia, D; Mitterling, T et al. (2015): Authors response to "Deficits of attention and cognition in narcoleptic patients – is it hypocretin dependent?". In: *Sleep Medicine* 16 (8), S. 1025. DOI: 10.1016/j.sleep.2015.04.005.

Zamarian, L; Trinka, E; Kuchukhidze, G; Bodner, T; Unterberger, I; Luef, G; Delazer, M (2015): Intact information sampling in mesial temporal lobe epilepsy. In: *Neuropsychology* 29 (6), S. 998–1003. DOI: 10.1037/neu0000229.

Publications acknowledging the MUI-START programme that were not listed in the former MUI-START report (July 2015- December 2016)

Cardini, B; Oberhuber, R; Hein, SR.; Eiter, R; Hermann, M; Kofler, M et al. (2017): Mouse Model for Pancreas Transplantation Using a Modified Cuff Technique. In: *Journal of Visualized Experiments : JoVE* (130), S. 54998. DOI: 10.3791/54998.

Kummer, KK; Kalpachidou, T; Kress, M; Langeslag, M (2018): Signatures of Altered Gene Expression in Dorsal Root Ganglia of a Fabry Disease Mouse Model. In: *Frontiers in Molecular Neuroscience* 10, S. 449. DOI: 10.3389/fnmol.2017.00449.

Kummer, KK; Kalpachidou, T; Mitrić, M; Langeslag, M; Kress, M (2018): Altered Gene Expression in Prefrontal Cortex of a Fabry Disease Mouse Model. In: *Frontiers in Molecular Neuroscience* 11, S. 201. DOI: 10.3389/fnmol.2018.00201.

Loeschenberger, B; Niess, L; Würzner, R; Schwelberger, H; Eder, IE.; Puhr, M et al. (2018): Calcineurin inhibitor-induced complement system activation via ERK1/2 signalling is inhibited by SOCS-3 in human renal tubule cells. In: *European Journal of Immunology* 48 (2), S. 330–343. DOI: 10.1002/eji.201747135.

Namer, B; Ørstavik, K; Schmidt, R; Mair, N; Kleggetveit, IP; Zeidler, M et al. (2017): Changes in Ionic Conductance Signature of Nociceptive Neurons Underlying Fabry Disease Phenotype. In: *Frontiers in Neurology* 8, S. 335. DOI: 10.3389/fneur.2017.00335.

Rocamora-Reverte, L; Reichardt, HM; Villunger, A; Wiegers, GJ (2017): T-cell autonomous death induced by regeneration of inert glucocorticoid metabolites. In: *Cell Death & Disease* 8 (7), e2948-e2948. DOI: 10.1038/cddis.2017.344.

Schmidt, O; Weyer, Y; Fink, MJ.; Müller, M; Weys, S; Bindreither, M; Teis, D (2017): Regulation of Rab5 isoforms by transcriptional and post-transcriptional mechanisms in yeast. In: *FEBS letters* 591 (18), S. 2803–2815. DOI: 10.1002/1873-3468.12785.

Verius, M; Frank, F; Gizewski, E; Broessner, G (2018): Magnetic Resonance Spectroscopy Thermometry at 3 Tesla: Importance of Calibration Measurements. In: *Therapeutic Hypothermia and Temperature Management*. DOI: 10.1089/ther.2018.0027.

7 The MUI-START programme in numbers (effective December 2018)

★ **75** **Proposals granted / 57 Projects completed**

★ **39** **Male PIs / 36 Female PIs**

★ **76** **Publications acknowledging the MUI-START programme**

€ 1,9 Mio granted by the MUI START programme

€ 4,6 Mio funds acquired by MUI-START grant holders

Additionally, 49% of PIs of closed projects now have a permanent position at the MUI or at the Tirol Kliniken. Another 29% of PIs quit the MUI and got positions in other research institutions or at

pharmaceutical or high-tech companies. The remaining scientists are still working at the MUI as project collaborators or hold non-permanent positions.

8 MUI-START final reports

The principal investigators of the MUI-START funded projects are responsible for the content of their respective final reports.

I. Bauer - AN4022–A novel HDAC complex component as basis for a novel antifungal therapy

Division of Molecular Biology

5th Funding period

Project duration: 01.10.2014 – 30.04.2017

Summary

Aspergillus fumigatus is the most common airborne fungal pathogen infecting mainly immunocompromised patients. Difficulties in the diagnoses of invasive aspergillosis (IA) and an emerging resistance against commonly used antifungals result in high mortality. Therefore, the search for potential novel targets for treatment and/or prophylaxis of IA is one of the major aspects in fungal research.

Our lab has longstanding experience in studying fungal histone deacetylases (HDACs). To elucidate the function of enzymes in *Aspergillus nidulans*, a close but non-pathogenic relative of *A. fumigatus*, we have generated deletion strains of all four classical HDAC genes and found that one of them, *rpdA*, is essential for growth and development of the fungus. Comparing the primary structure of fungal RpdA-type enzymes to those of animals and plants, a clear C-terminal extension including an essential highly conserved acidic motif in RpdA-type enzymes was conspicuous. We were able to pinpoint the essential region to only 6 amino acids and could proof that these residues are indispensable for both, proper nuclear localization and sufficient catalytic activity of RpdA. Moreover, we could demonstrate that RpdA is also essential for other fungal species including *A. fumigatus*. Recently, these results were reported in mBio including an acknowledgement of the MUI-START funding. Both, the essentiality and the structural differences of fungal and mammalian enzymes, turn RpdA into a potential new target for antifungal therapy. Thus, this publication was also selected as research highlight in the field of uncovering new drugs and targets by Nature Reviews Microbiology.

It is well accepted that RpdA homologs like yeast Rpd3 or human HDAC1 exert their function in protein complexes and in baker's yeast for example, three Rpd3 complexes have been described. To analyze the composition of fungal RpdA-type complexes, we conducted tandem affinity purification (TAP) of RpdA coupled to mass spectrometry (MS) based protein identification. Indeed, we were able to identify most of the corresponding yeast homologs. In addition, however, we could also identify a previously uncharacterized conserved fungal protein, AN4022, as novel RpdA interactor, an interesting finding that could be confirmed subsequently by a vice-versa purification strategy. Orthologs of AN4022 are exclusively present in Eurotiomycetidae including a number of important fungal pathogens such as *A. fumigatus*, *Aspergillus terreus*, *Aspergillus flavus*, *Penicillium marneffei*, *Coccidioides immitis*, or *Histoplasma capsulatum*, indicating a unique function in this fungal taxon. Characterization of AN4022 and SntB, a second RpdA interacting protein conserved in the whole fungal kindom, was the aim of this project.

To generate deletion mutants, the sequences coding for AN4022 and SntB where replaced by a selectable marker gene and the resulting knock out strains were complemented by reintroduction of functional alleles of the corresponding genes. AN4022 and *sntB* mutants both were viable indicating no direct involvement of these proteins in the essential function of RpdA. Whereas AN4022 null mutants did not show alteration of growth or conidiation at standard growth conditions when

compared to wild type, Δ*sntB* mutants exhibited reduced growth and conidiation. Successful expression of Venus-tagged AN4022 and SntB allowed their localization within the nucleus, the preferential site of action of HDAC complexes.

Subsequently, deletion mutants were screened for their ability to grow on different nutrient sources and at various stress conditions. Whereas the tested nutrient sources for carbon and nitrogen did not alter growth compared to standard growth media, reduced development could be observed, when grown under oxidative stress (hydrogen peroxide), osmotic stress (1M sodium chloride), cell wall stress (calcofluor white), and at reduced or elevated temperature stress. The fact that fungi have to adapt to various stress conditions during host infection makes these results interesting and worth to be studied in more detail in the future.

During the vice-versa purification strategy with TAP-tagged AN4022 we, however, were able to identify an additional conserved protein. This novel component of the RpdA complex, AN8823 (ScrC), appears to be directly connected to AN4022 and indicates the presence of a fourth and independent RpdA complex in the taxon Eurotiomycetidae in addition to three complexes already known from yeast. Currently, Δ*scrC* and ΔAN4022/Δ*scrC* double mutants are being generated.

Another major part of the proposal was the analysis of gene expression in the mutants at a global level via next generation sequencing (RNAseq). Since we hypothesize that AN4022 is important for a quick adaptation to the host defense during infection, we decided to induce transcriptional response via specific stimuli in addition to steady state conditions. Our analysis includes a shift from iron deplete to iron replete conditions since a pilot study, done in a collaboration with the group of Hubertus Haas, had already indicated significant differential expression of hundreds of genes under these conditions. The results with the mutant strains will proof, whether the AN4022/ScrC/RpdA complex is involved in the response to iron shifts, however, results are still pending.

Besides their role as opportunistic pathogens, the production of a variety of small bioactive compounds, so called secondary metabolites, is another interesting aspect of filamentous fungi. Northern analysis of AN4022, *scrC*, and *sntB* mutant strains indicated an involvement of these proteins in proper regulation of the production of the fungal toxin sterigmatocystin in *A. nidulans*. To confirm these observations at product level, we have started a collaboration with the group of Dr. Ozgur Bayram from the Maynooth University Ireland, who is an expert in the analysis of sterigmatocystin production. This work is still in progress.

In the course of this project, we gained insight in the function of AN4022 and SntB, two novel RpdA complex members. The mutant strains generated in this project are valuable tools for further studies on these proteins. Pending results of the RNAseq analysis will, together with ScrC, another novel RpdA interacting protein, provide a solid basis for an application for third-party funding. A manuscript about the characterization of AN4022 is currently in preparation.

Publications issued by this project

Bauer, I; Varadarajan, D; Pidroni, A; Gross, S; Vergeiner, S; Faber, B et al. (2016): A class 1 histone deacetylase with potential as an antifungal target. In: *mBio* 7 (6), e00831-16. DOI: 10.1128/mBio.00831-16.

Bauer, I; Gross, S; Karahoda, B; Sarg, B; Kremser, L; Merschak, P; Lindner, H; Bayram, O; Graessle, S; Identification of a novel class 1 histone deacetylase complex in *Aspergillus nidulans*.

In preparation.

External funding

Application in planning, however, is depending on the successful publication of the project results (see above) to ensure fulfillment of the FWF formal criteria.

Miscellaneous

Under supervision of the applicant, Ms. Silke Gross, MMSc completed her master thesis in microbiology in the course of this project

A. Grams - Intracranial aneurysm as a hypertensive disease

Department of Neuroradiology

5th Funding period

Project duration: 01.10.2014 - 30.09.2017

Summary

Scientific background:

One main risk factor for the development of intracranial aneurysms and their rupture may be arterial hypertension. In theory, hemodynamic stress from arterial hypertension leads to a weakening of the vessel wall, which may result in the development of aneurysms. Another epiphenomenon of arterial hypertension displays arteriosclerosis, which can be easily quantified on computed tomography scans. In addition there is a known correlation between arterial hypertension and the presence of adrenal tumors. However, a correlation between ruptured intracranial aneurysms and adrenal tumors has not been investigated so far.

Objectives:

The aim of this study was to examine the relationship between the presence of ruptured intracranial aneurysms and supraaortal arteriosclerosis as a sequela of arterial hypertension as well as the presence of adrenal masses in these patients.

The following hypotheses were defined:

- There is a positive correlation between the presence of intracranial aneurysms and the amount of supraaortal vessel calcification
- There is a positive correlation between the presence of intracranial aneurysms and the presence of adrenal tumors

Methods:

In total 25 patients with aneurysmal subarachnoid hemorrhage were included (16 women, nine men; mean age 53 +/- 11 years), who agreed in a magnetic resonance imaging (MRI) examination of the upper abdomen, which was performed between six and 12 months after the subarachnoid hemorrhage. The MRI scans were screened for adrenal masses, and calcifying macroangiopathy of the intracranial vessels was quantified on computed tomography (CT) scans, which have been performed in the acute stage after subarachnoid hemorrhage. In addition 50 age and gender matched controls from a historical patient group, who did not suffer from aneurysmal SAH and who did receive a cerebral CT or a MRI of the upper abdomen, were included.

For statistical analysis mean values, standard deviations, Mann-Whitney U tests and Spearman´s correlations were calculated.

Results:

In the present population two of the subarachnoid hemorrhage patients and none of the controls displayed an adrenal tumor. Patients with ruptured intracranial aneurysms displayed a significantly higher amount of supraaortal arterial calcification than the controls (p=0.03). No correlation was found between gender and calcification.

Conclusion:

A positive connection between ruptured intracranial aneurysms and arterial calcification could be confirmed. A positive correlation between ruptured intracranial aneurysms and adrenal masses is difficult to prove with the present data, as only two patients displayed adrenal masses. The inclusion rate of patients so far was lower than expected; only 25 instead of 60 patients could be included, mostly due to the clinical condition or a missing consent.

Outlook:

In order to underline the present data a larger amount of patients are necessary. Especially patients with non-ruptured aneurysms should also be included and investigated in a further study.

Publications issued by this project

None yet, manuscript in preparation.

External funding

Miscellaneous

Supervision of Clinical PhD Student (Clinical Imaging Sciences) Fabian Steinkohl

Supervision of Medical Student (Diplomarbeit) Sarah Honold.

L. Zamarian - Decision making abilities in patients with multiple sclerosis – Assessment and training

Department of Neurology

5th Funding period

Project duration: 01.10.2014 – 30.09.2017

Summary

Background: Risk understanding is essential for participating in health care and making informed, advantageous decisions. Recent investigations have shown that patients with multiple sclerosis (MS) have some difficulties in making advantageous decisions [1,2]. Aims of this project were twofold: First, we aimed at investigating whether, relative to healthy controls, patients with MS gather less information before making a decision (pre-decisional stage) and make more irrational decisions (i.e., decisions against the evidence). In a second study, we investigated whether a targeted cognitive training improves medical judgements and performance on a framing task in both healthy people and patients with MS. The term framing effect [3] means that the way information is presented influences the individuals' preferences and decisions. Typically, people demonstrate a more favourable attitude towards a positively-framed information than towards a negatively-framed information [4]. A lower framing effect after training might indicate a lower influence by the information frame and higher reliance on analytical information processing, which is highly desirable in the health context.

Study 1 – Method: We recruited 50 patients with relapsing-remitting multiple sclerosis (RRMS) and 104 healthy controls. Participants performed a comprehensive neuropsychological background assessment and the Beads Task, which is a basic information-sampling task. Results: Groups significantly differed from each other with regard to age and education. When we took into consideration these differences in the analysis of performance on the Beads Task, we found no significant group effects, i.e. patients performed at the same level as controls. Age did not emerge as significant covariate, whereas education showed to affect how often people made irrational decisions but not how much evidence they collected before making a decision. In general, the lower the education, the higher the frequency of irrational decisions.

Study 2 - Method: 37 patients with RRMS and 73 healthy controls also participated in a training study. Groups were comparable with regard to age and education. In a controlled cross-over design, half of the participants underwent a week of numerical training followed by a week of control training (text comprehension); the other half had first the control training and then the numerical training. Before any intervention started (T1), participants performed a comprehensive neuropsychological background assessment and a framing task where they evaluated the success of fictive medications on a 7-point scale. Medications were described either in positive terms or in negative terms. Parallel forms of the framing task were used after each training week (T2, T3). Results: Both, patients and controls, showed a significant effect of training type. Indeed, the framing effect decreased after a week of

numerical training, while performance did not change following a week of control training. Regardless of the training type, patients were influenced more strongly than controls by the

frame of information. We also found that the framing effect of the whole sample correlated with both demographical variables and cognitive scores (logical reasoning, working memory, mental calculation, ratio processing), with a stronger framing effect being associated with older age, lower education, and lower cognitive functioning. However, only logical reasoning emerged as significant predictor of variance in the framing task.

General conclusions: Our results show that (1) pre-decisional information sampling as tested by the Beads Task is preserved in patients with RRMS; (2) however, patients with RRMS are more strongly influenced than healthy people by the way medical information is presented; (3) training alters the way medical information is evaluated; (4) patients with RRMS as well as healthy controls can profit from cognitive training; and (5) the type of training has specific effects. In light of these findings, we suggest particular caution in the communication of medical information to patients with MS. Also, a targeted cognitive training may enhance the evaluation of new information and reduce framing effects. This should favour informed and advantageous decision making in the health context.

Outlook: We are now extending the training study to different age ranges and other patient populations (e.g., older people with mild cognitive impairment, in collaboration with the University of Padua and San Camillo Hospital, Venice, Italy).

References: [1]Simioni S, et al. PLoS One. 2012;7(12):e50718. doi: 10.1371/journal.pone.0050718. [2]Farez MF, et al. BMJ Open. 2014; 4(7):e004918. [3]Tversky A, Kahneman D. Science 1981;211:453-8. [4]Moxey A, et al. J Gen Intern Med 2003;18:948–59.

Publications issued by this project

Burgio, F; Delazer, M; Meneghello, F; Pertl, M-T; Semenza, C; Zamarian, L (2018): Cognitive training improves ratio processing and decision making in patients with mild cognitive impairment. In: *Journal of Alzheimer's Disease* 64 (4), S. 1213–1226. DOI: 10.3233/jad-180461.

Delazer, M; Zamarian, L; Frauscher, B; Mitterling, T; Stefani, A; Heidbreder, A; Högl, B (2016): Oxygen desaturation during night sleep affects decision-making in patients with obstructive sleep apnea. In: *Journal of Sleep Research* 25 (4), S. 395–403. DOI: 10.1111/jsr.12396.

Heim, B; Djamshidian, A; Heidbreder, A; Stefani, A; Zamarian, L; Pertl, M-T et al. (2016): Augmentation and impulsive behaviors in restless legs syndrome. In: *Neurology* 87 (1), S. 36. DOI: 10.1212/WNL.0000000000002803.

Pertl, M-T; Benke, T; Zamarian, L; Delazer, M (2015): Decision making and ratio processing in patients with mild cognitive impairment. In: *Journal of Alzheimer's Disease* 48 (3), S. 765–779. DOI: 10.3233/jad-150291.

Pertl, M-T; Benke, T; Zamarian, L; Delazer, M (2017): Effects of Healthy Aging and Mild Cognitive impairment on a real-life decision-making task. In: *Journal of Alzheimer's Disease* 58 (4), S. 1077–1087. DOI: 10.3233/jad-170119.

Pertl, M-T; Zamarian, L; Delazer, M (2017): Reasoning and mathematical skills contribute to normatively superior decision making under risk: evidence from the game of dice task. In: *Cognitive Processing* 18 (3), S. 249–260. DOI: 10.1007/s10339-017-0813-x.

Zamarian, L.; Delazer, M.; Ehling, R.; Pertl, M.-T.; Bsteh, G.; Wenter, J. et al. (2019): Improvement of medical judgments by numerical training in patients with multiple sclerosis. In: *European Journal of Neurology* 26 (1), S. 106–112. DOI: 10.1111/ene.13778.

Zamarian, L; Berger, T; Pertl, M-T; Bsteh, G; Benke, T; Delazer, M (2016): Framing effects in multiple sclerosis: How patients may be misled by the way medical information is presented; In: *Zeitschrift für Neuropsychologie* 27(1): S 62.

Zamarian, L; Högl, B; Delazer, M; Hingerl, K; Gabelia, D; Mitterling, T et al. (2015): Authors response to “Deficits of attention and cognition in narcoleptic patients – is it hypocretin dependent?”. In: *Sleep Medicine* 16 (8), S. 1025. DOI: 10.1016/j.sleep.2015.04.005.

Zamarian, L; Trinka, E; Kuchukhidze, G; Bodner, T; Unterberger, I; Luef, G; Delazer, M (2015): Intact information sampling in mesial temporal lobe epilepsy. In: *Neuropsychology* 29 (6), S. 998–1003. DOI: 10.1037/neu0000229.

Delazer M, Pertl M-T, Berger T, Bsteh G, Benke T, Zamarian L. Reflection impulsivity in patients with multiple sclerosis. In preparation.

External funding

Application to EUREGIO - Project: NEURO-TRAIN – A neurocognitive approach to acquisition of arithmetic expertise.

Application to K-Regio - Project: Light4Future – zukünftige Lösungen für human-centric services

Miscellaneous

Zamarian L, Berger T, Pertl M-T, Bsteh G, Benke T, Delazer M. Framing effects in multiple sclerosis: How patients may be misled by the way medical information is presented. Annual Meeting of the Austrian Society of Neuropsychology, 03. October 2015. (Flash talk + Published abstract.)

Pertl M-T, Berger T, Benke T, Bsteh G, Ehling R, Glatzl S, Wenter J, Brenneis C, Delazer M, Zamarian L. Cognitive training improves performance in decision making under risk and ratio processing. Mid-Year Meeting of the International Neuropsychological Society, 06.-08. July 2016. (Oral presentation + Published abstract.)

Zamarian L, Pertl M-T, Berger T, Benke T, Bsteh G, Djamshidian A, Ehling R, Glatzl S, Wenter J, Brenneis C, Delazer M. Cognitive training improves performance in decision making under risk and ratio processing. XXXIV European Workshop on Cognitive Neuropsychology: An Interdisciplinary Approach (https://sites.google.com/site/ewcn2016/home), Bressanone, Italy, 24.-29. January 2016. (Poster presentation + Oral presentation).

Zamarian L. Cognitive training on ratio processing improves risk understanding. 6th Scientific Meeting of the Federation of the European Societies of Neuropsychology (FESN), Maastricht, the Netherlands, 13.-15. September 2017. (Oral presentation).

Pertl M-T, Delazer M, Berger T, Bsteh G, Ehling R, Wenter J, Glatzl S, Brenneis C, Benke T, Zamarian L. Medical judgements can improve following cognitive intervention – A study with healthy people and patients with multiple sclerosis. XXXVI European Workshop on Cognitive Neuropsychology: An Interdisciplinary Approach (https://sites.google.com/view/ewcn/home), Bressanone, Italy, 21.-26. January 2018. (Poster presentation).

Habilitation in Clinical Psychology, Medical University Innsbruck – Title: 'Neuropsychology of decision making, information sampling, and ratio processing'.

Supervision of the Thesis of Geske Lisa for the acquisition of the Master degree in Psychology, Leopold Franzens University Innsbruck – Title: 'Der Einfluss numerischer Trainingseffekte auf Entscheiden'.

S. Coassin - Application of third generation sequencing to the genotyping of the Lipoprotein(a) KIV-2 repeat

Division of Genetic Epidemiology

6th Funding period

Project duration: 01.09.2015 – 31.08.2017

Summary

Background: Lipoprotein(a) [Lp(a)] plasma concentrations are among of the most important, genetically determined risk factors for cardiovascular diseases (CVD), providing up to 2.5-fold increased CVD risk. The Lp(a) concentrations in the general population present a 1000-fold concentration range (0.2 to >200 mg/dL). More than 90% of the Lp(a) variance is explained by one single gene locus (*LPA*), which encodes the primary structural protein of the Lp(a) particle, named apo(a). This protein is highly polymorphic (300 - 800 kDa), due to a hypervariable copy number variation named kringle IV-type 2 (KIV-2), which encompasses two *LPA* exons and can be present in up to 40 copies *per allele*. Every repeat encompasses 5.6 kb on DNA, making the whole repeat region >200 kb in size and thus inaccessible to common sequencing.

The genetic regulation of the Lp(a) concentrations not yet fully understood. The number of KIV-2 repeats explains 30-70 % of the Lp(a) level variance, but nevertheless the individual Lp(a) levels can differ by >100-fold even within carriers of the same isoform. Recent genome-wide association studies for Lp(a) showed the existence of dozens of independent causal variants in the *LPA* locus. Some putative regulators have been identified (promoter polymorphisms, nonsense mutations, missense variants and intronic variants), but, with few exceptions, the causal link to Lp(a) has not been unraveled. All current reference datasets (1000 Genomes, ExAC, GnomAD) miss a consistent portion of the genetic variability in *LPA*, because to 70% of the LPA coding sequence is located within the KIV-2 repeat, which is not accessible to common genome sequencing, because the detection of one variant KIV-2 repeat in up to 80 KIV-2 repeats requires a mutation detection sensitivity of 1.2%. Only recently, 2nd and 3rd generation sequencing technologies allow detecting mutations at such low levels, if backed by strong bioinformatics and comprehensive wet lab validation. Moreover, “Nanopore sequencing” now trivializes multi-kb sequencing reads and promises to allow haplotyping the KIV-2 by single molecule sequencing. This would be a major step to include this region into the haplotype maps used for association studies and causal variant mapping.

Objective: Establish mutation screening in the LPA KIV-2 region by using new sequencing technologies and develop the necessary bioinformatics to lay the basis for future projects.

Results: The project developed into a RNA and DNA branch. **We successfully established Nanopore sequencing at our institute,** but the high error rate of third generation sequencing precluded direct mutation detection in transcript data without a reference. We thus selected n=127 samples from our in-house biobank with >20,000 Lp(a) phenotyped individuals. To enrich for clinically relevant, functional variation, 80 samples were selected based on a “discordant phenotype approach”, where the observed Lp(a) concentrations

strongly differed from the concentration, which would be expected based on the observed Lp(a) isoform. For mutation screening, we adapted a "batch amplification approach" (i.e. amplification of all KIV-2 simultaneously using primers which bind in conserved sites in every repeat, *Noureen et al, PLOS ONE 10:e0121582, 2015*) to ultra-deep next generation sequencing (NGS) and, using recombinant standards, **developed a dedicated bioinformatic pipeline capable to detect KIV-2 mutations at 1% level.** Technically, this revealed an **unexpected, but large impact of the polymerase used for PCR amplification** in the detection of a specific KIV-2 haplotype ("KIV2B haplotype") and a **detrimental impact of the widely used BAQ NGS algorithm on the detection sensitivity in KIV-2 data**. Both observations are novel (*manuscripts in preparation*). Overall, **we observed 521 variants** with widely varying population frequencies and mutation levels (a proxy for the percentage of KIV-2 repeats carrying a variant). **This indicates a large genetic variability in the KIV-2 region, suggesting that previous reports of a high conservation in the KIV-2 were likely biased by insufficient sensitivity.** We also pinpointed the genetic variant underlying the "*KIV-2 DraIII"* polymorphism.

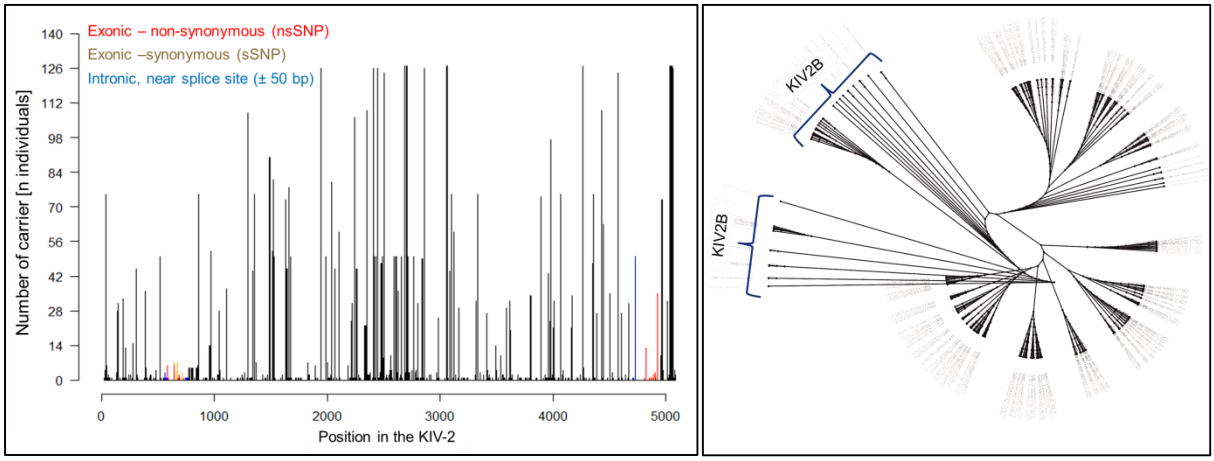

Figure 1: Left: Number of carriers for each variant (vertical lines). The colors indicate non-synonymous (red), synonymous (brown), near splice site (blue) and intronic (black) variants. Right: Unrooted tree (using MUSCLE alignment and MrBayes) of the haplotypes generated by INC-Seq. Reads from the KIV2B haplotype (defined by marker positions determined in the DNA sequencing) can be clearly differentiated.

One variant (*LPA* KIV-2 4925G>A) was followed up in the KORA F4 population (n=2,892) by applying for the first time a castPCR assay for large scale genotyping in the KIV-2. **4925G>A was associated with an Lp(a) reduction by 70% in the so-called "low molecular weight Lp(a)" group, which is otherwise associated with genetically increased Lp(a). It explains 19.3% of Lp(a) variance** in the population and thus appears as an important player in determining the large variability of Lp(a) levels *within* the same isoform group. Minigene assays showed impaired splice site recognition.

Finally, the DNA variation map was used to explore the applicability of "intra-molecular ligated consensus sequencing" (Inc-Seq; *Li et al, Gigascience 5:34, 2016*) to haplotyping in the KIV-2. Indeed, **we could clearly differentiate the previously known KIV-2B haplotype from the rest** by applying a phylogenetic approach to the sequencing data. This represents an important proof-of-principle for the feasibility of KIV-2 haplotyping using Nanopore sequencing.

Conclusion: The large amount of novel variants provide several new causal candidates regulating Lp(a), as shown by the strong effect of 4925G>A. Together with our bioinformatic pipeline, this lays the basis for future projects investigating this white spot in the genome.

Outlook: This project generated fruitful cooperations on the campus (Visceral, Transplant and Thoracic Surgery: Assoc. Prof. Dr. M. Maglione/Univ. Prof. Dr. D. Öfner-Velano; Internal Medicine II: PD Dr. Armin Finkenstedt/Assoc. Prof. Dr. H. Zoller; Cell Biology: Univ. Prof. Dr. L. A. Huber; Division of Genomics and RNomics: Univ. Prof. A. Hüttenhofer). We are working to establish a novel organoid cell culture model from liver samples of Lp(a) phenotyped individuals to generate novel model for LPA research and characterization of 4925G>A.

Publications issued by this project

Coassin, S; Erhart, G; Weissensteiner, H; Eca Guimarães de Araújo, M; Lamina, C; Schönherr, S et al. (2017): A novel but frequent variant in LPA KIV-2 is associated with a pronounced Lp(a) and cardiovascular risk reduction. In: *European heart journal* 38 (23), S. 1823–1831. DOI: 10.1093/eurheartj/ehx174.

Coassin, S; Schönherr, S; Weissensteiner, H; Erhart, G; Forer, L; Losso, J L et al. (2019): A comprehensive map of single-base polymorphisms in the hypervariable LPA kringle IV type 2 copy number variation region. In: *Journal of lipid research* 60 (1), S. 186–199. DOI: 10.1194/jlr.M090381.

External funding

Untersuchung einer Lp(a) Mutation mittels RNA und Organoide (FWF P 31458). Amount granted: € 345.914,29.

A second FWF proposal is in preparation.

Miscellaneous

Prizes

Sanofi-Aventis Award 2017

Annual Meeting of the Austrian Atherosclerosis Society - Best Presentation Award 2017

Congress presentations – National

Coassin S: „A novel highly frequent variant in the LPA KIV-2 is associated with a pronounced Lp(a) and cardiovascular risk reduction", **24th Annual Meeting – Austria Atherosclerosis Society AAS**, St. Gilgen am Wolfgangsee, Mai 2017

Congress presentations – International

Coassin S: "A novel but highly frequent variant in the LPA KIV-2 repeat is associated with a pronounced Lp(a) and cardiovascular risk reduction", **40th Meeting of the European Lipoprotein Club**, Tutzing, (D), September 2017

Coassin S: “A frequent splice site variation in the repetitive kringle IV type 2 of the LPA gene substantially lowers Lp(a) concentrations in the general population”, **7th Workshop of Genetic Epidemiology,** Grainau (D), April 2017

Students

Jamie Lee Losso, Bachelor thesis in Biomedical Science (FH Gesundheit); 01/2016 – 04/2016

Silvia Di Maio, Master thesis in biology (international student from the University of Milan); ongoing

A. Gratl - Neuroprotective potential of tetrahydrobiopterin in spinal cord ischemia using a rat model

Department of Vascular Surgery

6th Funding period

Project duration: 01.09.2015 – 31.07.2018

Summary

Background: Spinal cord ischemia (SCI) is a devastating complication in thoracoabdominal aortic aneurysm surgery. There is an urgent need to identify neuroprotective substances in order to in prevent this fatal complication.

Tetrahydrobiopterin (BH4) is one of four essential cofactors of nitric oxide synthase (NOS). It is known to play a protective role in ischemia-reperfusion injury (IRI) following solid organ transplantation. IRI of transplanted organs results in reduced BH4 tissue levels with associated uncoupling of the constitutive NOS isoforms. The resulting oxidative injury has been shown to be deleterious in murine pancreas transplantation as well as in a murine aortic transplantation model. Of note, pre-treatment with BH4 of the donor mice resulted in the former model in preventing the occurrence of a lethal microcirculatory derangement of the transplanted pancreas, (Maglione et al. Am J Transplant. 2010 Oct;10(10):2231-40) and in the latter model in a significantly decreased severity of chronic rejection (Oberhuber et al. Scie Rep. 2016 Nov;24;6:37917).

With the pre-existing knowledge about the beneficial effects of BH4, the aim of this study was to evaluate the neuroprotective potential of BH4 in SCI using a rat model. A blinded observer evaluated neurological outcome. The BBB-Score (0-21; 0 = no hind limb movement; 21 = normal hind limb function) (Basso et al. J Neurotrauma. 1995 Feb;12(1):1-21) was compiled two hours after surgery (day 0) and 7 days after surgery (day 7). Tissue was retrieved for further histopathological analysis after sacrificing animals 7 days after the procedure. Hematoxylin and eosin (H.E.) staining were performed to calculate the mean number of neurons out of 5 transverse sections of the thoracic spinal cord, and TUNEL assays were performed to evaluate viability of these neurons by a blinded observer.

Results: The ability of BH4 to pass the blood brain barrier was demonstrated by administration of different dosages of BH4 and measurements of BH4 tissue level from the serum and several organs including the cerebrum and the spinal cord after different time intervals between administration and tissue recovery. A dosage of 50 mg per kg after a time interval of 15 minutes showed the highest concentration in the spinal cord and was therefore selected for the following experiments. SCI was induced by balloon occlusion of the descending aorta. Based on the model described by Hwang et al, a 2F Fogarty catheter was inserted via the left femoral artery and aortic occlusion was achieved by balloon inflation. After 10 minutes of aortic occlusion, the balloon was deflated and blood supply was restored (Hwang et al. J Neurosurg Spine. 2015 Apr;22(4):432- 8). After acquisition of the technical skills needed to perform the surgical procedure, the model turned out to be associated with an unexpected high intra- and perioperative mortality, which was in contrast to reported results from Hwang and co-workers. Beside of technical failures, hemodynamic and embolic

complications were accountable for occurring deaths of animals resulting in the need of adapting the initially planned treatment groups. Animals were finally grouped into a sham-group (n=12), a control group (n=12; i.m. injection of saline 15 minutes before induction of SCI) and a treatment group (n=11; i.m. injection of 50 mg/kg BH4 15 minutes before induction of SCI). Within the control group, 3 out of 12 animals (25%) survived until day 7, within the treatment group 4 out of 11 (36%) animals reached the end point of day 7. Animals within the sham-group showed BBB-scores of 21 ± 0 at day 0 and 7, the control group shown a BBB score of 15.3 ± 2.1 at day 0 and 18.3 ± 3.8 at day 7, the treatment group showed a BBB score of 18.3 ± 3.4 at day 0 and 21 ± 0 at day 7. The observed improved neurological outcome was significant two hours after the procedure (day 0) ($p=0.014$), at day 7 the benefit was not statistically significant anymore ($p=0.205$). Results from neurological evaluation are shown in figure 1. Regarding results from H.E. staining, comparison of the mean counted neurons in 5 transverse sections from thoracic spinal cords of the sham group (n=4; 17.93 ± 6.13 neurons) with either animals of the control group (n=3; 14.56 ± 3.95 neurons) or animals of the treatment group (n=4; 13.77 ± 4.17 neurons), showed a significant decrease of vital neurons when SCI was induced. (sham vs. control $p=0.002$; sham vs. treatment $p<0.001$). However, there was no difference between the control and the treatment group (control vs. treatment $p=0.329$). TUNEL assays performed to count vital neurons (5 transverse sections of thoracic spinal cords for each animal) showed a significant decrease of vital neurons in the control group (n=3; 11.89 ± 4.04 vital neurons) compared to the sham group (n=4; 15.48 ± 7.1 vital neurons; $p=0.003$). Of note, the difference in the mean vital neuron count between the treatment group (n=4; 13.28 ± 4.77 vital neurons) and the sham group (n=4; 15.48 ± 7.1 vital neurons) showed no significance ($p=0.49$). When comparing the control group with the treatment group, pre-treatment with BH4 revealed an increased number of vital neurons without, however, reaching statistical significance ($p=0.117$). Results from histopathological evaluations are visualized in Figure 2.

Conclusion: We were able to establish the model of induction of SCI in a rat model in our laboratory. Results seem to be at first ambiguous. On the one hand, the evaluation of the BBB score clearly indicates a protective effect of BH4 confirming our hypothesis, however, on the other hand the high incidence of postoperative complications severely compromises the interpretation of study results. The final resulting sample size of animals surviving day 7 post SCI does not allow any meaningful conclusions and necessitates interpretation with caution. The high peri- and postoperative mortality of SCI animal models, which was confirmed by another research group (Oberhuber A. University of Münster, Germany – personal communication) might have resulted in the loss of significance in the TUNEL staining, and highlights the necessity to adapt the postoperative observation point in the used model.

Outlook:The obtained results in the early post-ischemic period clearly indicate the neuroprotective potential of BH4. The high postoperative mortality urges optimization of the model in terms of shortening the postoperative observation. Further grant applications aimed at gaining mechanistic insights at the basis of the protective effect are in progress.

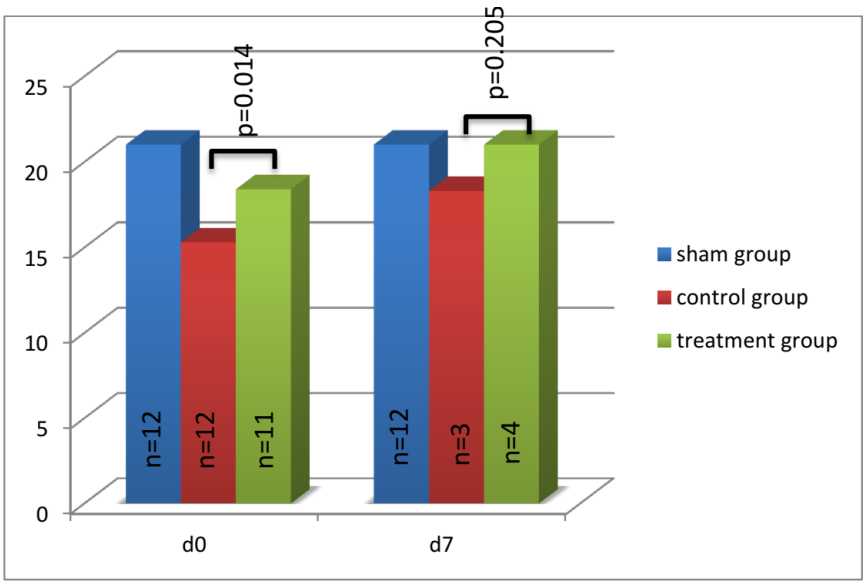

Figure 1 – BBB Scores; p<0.05 indicates statistical significance

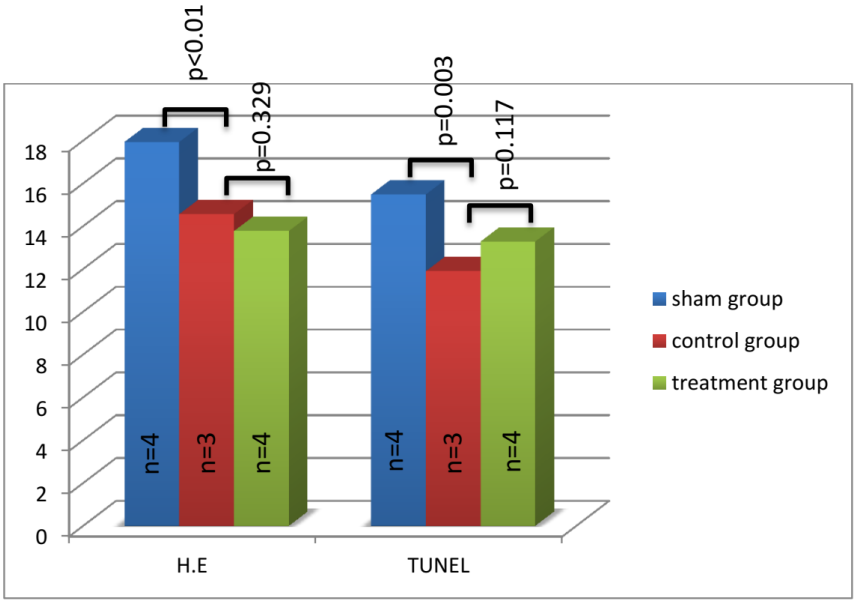

Figure 2 – Numbers of counted neurons; p<0.05 indicates statistical significance;

Publications issued by this project

A manuscript for further submission for publication is currently in progress. Finalisation and submission is expected by end of 2018 with publication within the first half of 2019.

External funding

Further grant applications are in progress and will be finished within the first half of 2019.

Miscellaneous

Katharina Mascherbauer, a student within her 4th year at Medical University of Innsbruck, was involved within the project and is currently working on her diploma thesis. Finalization of the thesis is expected by the first half of 2019.

Presentation of data at the national and international meetings is planned for autumn 2019.

S. Quarta - ADAM: The involvement and mechanisms of ADAM17 in neuropathic pain

Division of Physiology

6th Funding period

Project duration: 01.09.2015 – 31.08.2017

Summary

Several cytokines and growth factors are active when retained on the cell surface and restrict their biological effects to a specific microenvironment. In addition, soluble receptors upon shedding from the cell of origin can regulate the activity of their ligands or antagonize their effect on the same cells as decoy receptors lacking signaling activity. The tumor necrosis factor-α converting enzyme (TACE/ADAM17) is a protease and member of the ADAM (A Disintegrin And Metalloproteinase) family. It sheds a group of over fifty different membrane-anchored proteins and factors, like TNFα, TNF receptor (TNFR), Fractalkine, IL-6R, EGFR, Notch and molecules engaged in macrophages migration, like L-Selectin. In previous studies, we found that IL-6 cytokine family and the specific receptors contribute to the induction of pain and hypersensitivity associated with inflammation, neuropathy or cancer in mice and rats. Due to its proteolytic function, ADAM17 is involved in development and in the modulation of the immune system while altered ADAM17 function leads to cancer and inflammatory disorders. The link of ADAM17 to pro-inflammatory cytokines and chemokines like TNFα, IL-6 and Fractalkine, which are strongly associated with pathological pain disorders, points towards a role for ADAM17 in the development of neuropathic pain. However, ADAM17 involvement in the generation and maintenance of pathological pain is largely unknown.

Aims*:* The overall aim of the project was to understand how ADAM17 contributes to neuropathic pain and the activation of microglia after injury. The project aimed also to clarify which ADAM17 targets could be important for microglia response in neuropathic pain. To address these queries we used a battery of *in vitro* and *in vivo* experiments for phenotyping adult hypomorphic *Adam17*$^{ex/ex}$ mice. The level of ADAM17 mRNA expression is barely detectable in these mice due to an insertion of a new exon within the *Adam17* gene which modifies the gene and induces a translational stop in 95% of the cells.

Results: Immunohistochemical analysis of DRG and spinal cord from wt (a*dam17*$^{+/+}$) mice revealed that ADAM17 protein is predominantly expressed by neurons, rather than glia cells. *Adam17* mRNA was detected in DRG neuronal culture from wt mice, on the contrary it was barely detectable in hypomorphic *adam17*$^{ex/ex}$ mice. Despite the comparable morphology of the a*dam17*$^{ex/ex}$ DRG with the wt, the percentage of the nociceptive IB4^{+}-neurons was found significantly decreased in a*dam17*$^{ex/ex}$ DRG compared to a*dam17*$^{+/+}$, while the number of neurons belonging to the other two subpopulation, CGRP^{+}- and NF200^{+}-neurons, was similar between the genotypes. We found that *adam17*$^{ex/ex}$ mice had similar skin innervation pattern and epidermis thickness, and the number of free nerve endings per µm^2 was comparable to wt mice. *In vivo*, a*dam17*$^{ex/ex}$ mice were hyposensitive to noxious stimuli compared to wt animals: a*dam17*$^{ex/ex}$ mice showed higher mechanical Von Frey thresholds and heat and cold sensitivity were reduced. Interestingly, electrophysiological properties of DRG neurons or either C-fibers of a*dam17*$^{ex/ex}$ mice were unaltered. In models of post-operative and

neuropathic pain a*dam17*$^{ex/ex}$ mice were protected from the severe effect of the peripheral injury and higher mechanical thresholds were observed compared to wt. It is known that neuropathic pain leads to alterations of the somatosensory system and triggers immune responses. In the nerve, immune cells become activated and migrate toward the lesion site to trigger the recovery while, in the spinal dorsal horn, microglia respond to the injury by changing their morphology and moving from a surveillance state to an activated state. Surprisingly, a*dam17* mRNA expression was not changed after pain induction and the overall microglia activation in the dorsal horn of a*dam17*$^{ex/ex}$ mice at 3, 7 and 28 days post-lesion was comparable to controls. In line with these results, levels of the phosphorylated p38, which increase in activated microglia, were comparable to the one observed in the spinal cord from wt mice. We investigated also ADAM17 substrates involvement in microglia activation after lesion. Some of the originally selected targets (CX3CL1, Notch1, NRG1 and EGFR) were not clearly detectable due to their high molecular weight and different antibodies were tested. Preliminary experiments did not show changes in the target protein expression levels after injury in the spinal cord of a*dam17*$^{ex/ex}$ mice compared to controls. These unexpected results could be due to the variable down-regulation of ADAM17 observed in the hypomorphic line. Thus, the investigator was hosted for one month by the Heppenstall's laboratory at the EMBL (Monterotondo, Rome) to learn the principles of the CRISPR/Cas9 system, which could be useful in future to robustly generate at first *in vitro* cell cultures with a null mutation for *Adam17* since global depletion results in a lethal phenotype.

Conclusions: Our data support the hypothesis that ADAM17 contributes to pain associated with tissue injury, however the mechanisms implicated in acute nociception and hypersensitivity to painful stimuli could be associated with a central sensitization rather than changes at the peripheral afferents or in microglia activation.

Short Outlook: These studies have been performed using a hypomorphic line which is characterized by a certain variability in a*dam17*$^{ex/ex}$ down-regulation, thus it will be useful to develop at first an *in vitro* model using the CRISPR/Cas9 system to study possible modifications in signaling pathways linked to the lack of ADAM17, and as well a future *in vivo* application. Further investigation is required in order to reveal the central mechanisms associated with the involvement of ADAM17 in acute hypersensitivity to painful stimuli.

Publications issued by this project

Quarta, S; Mitrić, M; Kalpachidou, T; Mair, N; Schiefermeier-Mach, N; Andratsch, M et al. (2018): Impaired mechanical, heat, and cold nociception in a murine model of genetic TACE/ADAM17 knockdown. In: *The FASEB Journal* 33 (3), S. 4418–4431. DOI: 10.1096/fj.201801901R.

External funding

Miscellaneous

Congress presentations:

16th World Congress on Pain, Yokohama, Japan (2016). “Involvement of ADAM17 in acute nociception and hypersensitivity to painful stimuli.” Quarta S., Mitric M., Mair N., Andratsch M., Schiefermeier N., Langeslag M., Rose-John S., Kress M.

Research stay:

26/04/2017 – 28/05/2017: EMBL Monterotondo, Rome in Prof. Paul Heppenstall laboratory

R. Stanika - Role of the endogenous L-type calcium channel $Ca_V1.3$ in dendritic spine morphogenesis

Divison of Physiology

6th Funding period

Project duration: 01.09.2015 – 31.01.2018

Summary

Background and objectives. Neurons express multiple types of voltage-gated calcium channels (VGCC). VGCC are key transformers of extracellular signal input into up- and down-regulated signalling pathways. Neuronal L-type calcium channels are mainly encoded by $Ca_V1.2$ (α_1C) and $Ca_V1.3$ (α_1D) pore-forming subunits. They are localized in neuronal cell bodies and dendrites in both synaptic and extrasynaptic positions. For $Ca_V1.3$ channels in neurons three functionally diverse C-terminal splice variants exist, a long ($Cav1.3_L$) and two short ($Cav1.3_{42A}$, $Cav1.3_{43S}$) variants. We could recently demonstrate that $Ca_V1.3$ channels are critical modulators of dendritic spine postsynaptic stability. Here we studied dendritic spine modulation by $Ca_V1.3$ channels in hippocampal cultures from $Ca_V1.3$ knockout mice and analyzed the consequences on postsynaptic AMPA receptor distribution, CaMKII autophosphorylation, and synaptic activity.

Results. Reconstituting $Ca_V1.3$ knockout neurons with full-length $Ca_V1.3_L$ resulted in a slightly increased spine density and a larger fraction of mature mushroom-like spine. Consistent with our previous findings deletion of the C-terminal PDZ-binding sequence ($Ca_V1.3_{\Delta ITTL}$) significantly increased spine size and induced the formation of more filopodia-like spines (Fig. 1).

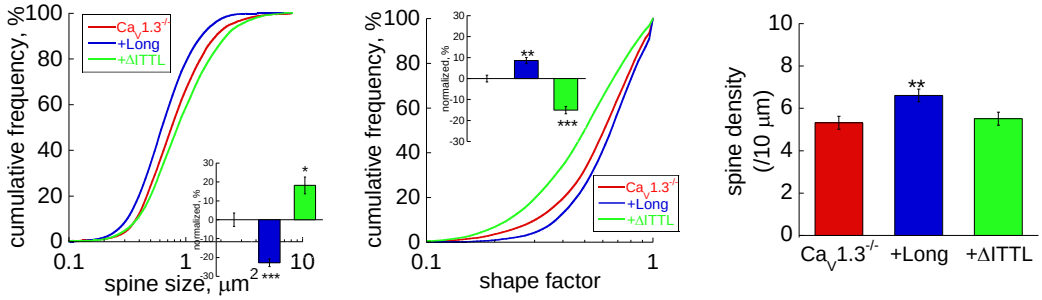

Fig. 1. Quantitative analysis of dendritic spine morphology. Statistics: ANOVA with Holm-Sidak posthoc analysis, *p<0.05, **p<0.01, ***p<0.001. Data from 3 culture preparations, 30 cells and between 450 and 570 spines were analyzed in each condition

Dendritic spine stabilization by $Ca_V1.3_L$ was associated with increased spine and decreased dendritic AMPA-Rs. In contrast, spine elongation induced by $Ca_V1.3_{\Delta ITTL}$ was paralleled by decreased spine and increased dendritic AMPA-Rs. Overall AMPA-R cluster intensity was significantly larger in neurons expressing $Ca_V1.3_{\Delta ITTL}$ compared to $Ca_V1.3_L$ and control ($Ca_V1.3^{-/-}$) (Fig. 2).

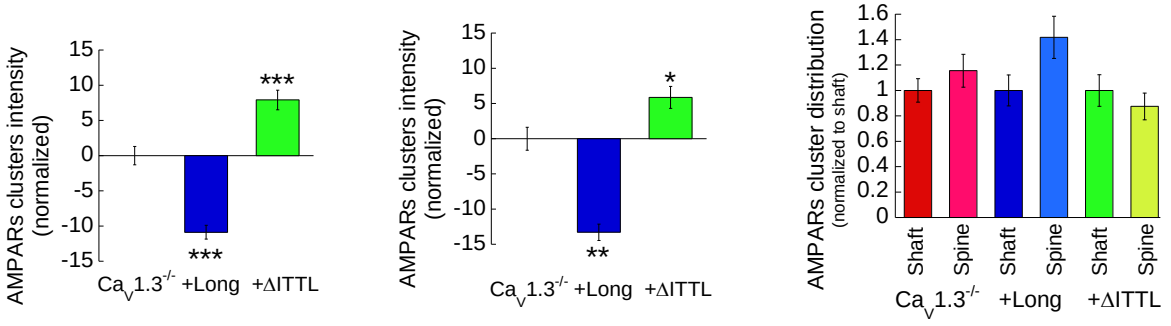

Fig. 2. Quantification of AMPA-Rs cluster intensity and distribution of clusters between dendritic shaft and spines. Statistics: ANOVA with Holm-Sidak posthoc analysis, ***p<0.001, **p<0.01, *p<0.05 Data from 2 culture preparations, 20 cells per condition.

Reconstitution of $Ca_V1.3^{-/-}$ neurons with $Ca_V1.3_L$ and $Ca_V1.3_{\Delta ITTL}$ both reduced amplitude of miniature excitatory postsynaptic currents (mEPSC), but did not alter CaMKII autophosphorylation at T286.
δ-catenin is a brain specific member of the adherens junction complex with an PDZ-domain, that localizes to the post-synaptic compartments and regulates the maintenance of dendrites and dendritic spines in mature cortex. Stabilization of spines by $Ca_V1.3_L$ is accompanied by increased expression of δ-catenin in spines (Fig. 3).

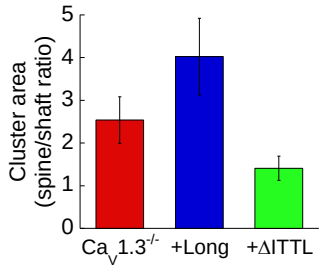

Fig. 3. Quantification of δ-catenin cluster distribution between dendritic shafts and spines. Data from 2 culture preparations, 10 cells per condition. Statistics: Kruskal-Wallis ANOVA with Dunn's posthoc analysis, $H_2 = 5.6$, $p = 0.062$.

Summary and Outlook. Taken together, $Ca_V1.3$ channels regulate dendritic spine, postsynaptic AMPA-receptor abundance and dendritic distribution of δ-catenin depending on the presence of the C-terminal PDZ-binding sequence (ITTL). Whether this affects evoked synaptic transmission will be tested in future experiments.

Publications issued by this project

Manuscripts for publication are in preparation.

External funding

FWF application has been submitted.

Miscellaneous

Results of the research project were presented on the following meetings:

- Society for Neuroscience (Washington, DC, USA 2017)
- Joint Meeting of Austrian Neuroscience Excellence Networks (Alpbach, Austria 2018)
- 3rd European Calcium Channel Conference (Alpbach, Austria 2018)

F. Messner - Mechanical stress as a trigger of skin rejection in composite tissue allotransplantation

University Hospital for Visceral, Transplant and Thoracic Surgery

6th Funding period

Project duration: 01.10.2015 – 30.09.2018

Summary:

Background: In four hand transplanted patients (Austria, Belgium, Italy and the United States) a novel type of skin rejection referred to as „atypical“ rejection has been observed. All patients experienced previous to the rejection some form of mechanical or thermal stress (intense physical hand therapy, intense manual work, hot water). Besides differences in macroscopic features also histologic findings differed from classical rejection.

Objective: The aim of the study is to investigate skin irritation and its effect on skin alterations/rejection after limb transplantation in a rat model.

Methods: Syngeneic and allogeneic orthotopic hind limb transplantations have been performed using male Lewis (recipient/donor) and Brown Norway (donor) rats. Immunosuppressive therapy consisted of anti-lymphocyte serum (ALS, 0,5ml) and tacrolimus (Prograf®) which was individually adjusted and tapered (final dose of 0,1-0,2mg/kg KG) to prevent primary alloimmune response. Mechanical irritation was applied to the planta pedis of the transplanted hind limb graft using a specifically designed mechanical stimulation device.). Mechanical skin irritation was applied to the planta pedis of the transplanted limb using a mechanical irritation device. Irritation was performed for 10 days, four times/day for 10 minutes, applying 5 Newton force. Skin biopsies were taken immediately after the last stimulation and after a five days' observational period. Samples were assessed histopathologically and protein expression was measured using luminex technology.

Results: Allogeneic transplanted + irritated animals displayed significant aggravated macroscopic skin alterations (Fig. 1) compared to naïve irritated ($p<0.0001$) and syngeneic transplanted + irritated controls ($p= 0.0023$). Overall, histopathology showed a trend towards higher rejection/inflammation grades in allogeneic irritated animals than in syngeneic (mean rejection grade 2.3±0.95 vs. 1.7±0.81; ns.). After 10 days of irritation, minor skin alterations in syngeneic transplanted animals recovered quickly, however, in allogeneic transplanted animals macroscopic features were more pronounced ($p<0.0001$) and improved only little over the following five days of observation without irritation (Fig. 2). In allogeneic transplanted + irritated animals IL-1b and INF-y levels were up-regulated compared to irritated controls (Fig. 3).

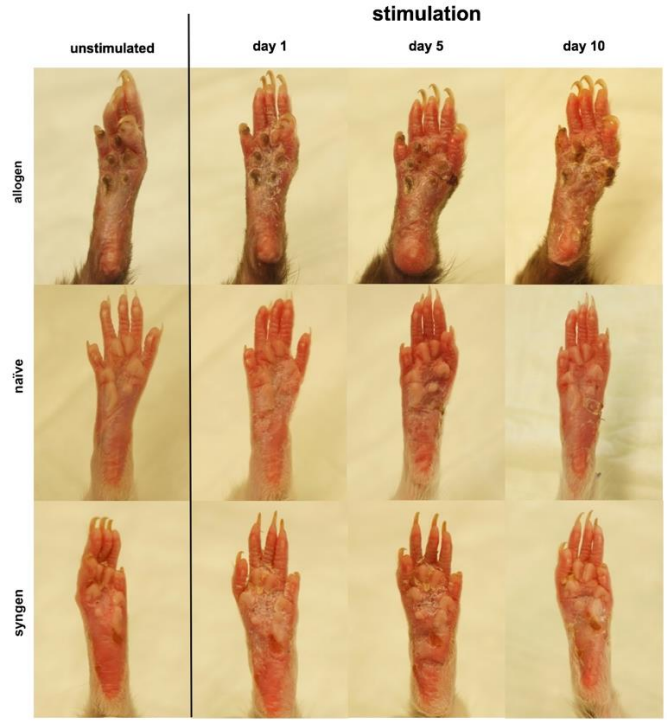

Figure 1. Macroscopic rejection grading (MRG) before (left panel labeled with unstimulated) and after 1, 5 and 10 days of standardized mechanical stimulation, respectively. In the top row (allogen), images of the allogeneic transplanted group are displayed. Before standardized stimulation, animals lacked macroscopic and histologic signs of acute rejection. Upon stimulation, erythema, dryness and scaling of the skin developed immediately after the first stimulation (day 1). Marospcoic changes aggravated over the stimulation period with excessive crust formation and edema of the forefoot (day 5 and 10). Naïve stimulated animals (second row, naïve) displayed only minor changes including light erythema, dryness and scaling of the directly irritated plantar skin. Syngeneic transplanted animals (bottom row, syngen) showed more pronounced scaling and eventually some crust formation but changes were strictly limited to the challenged area.

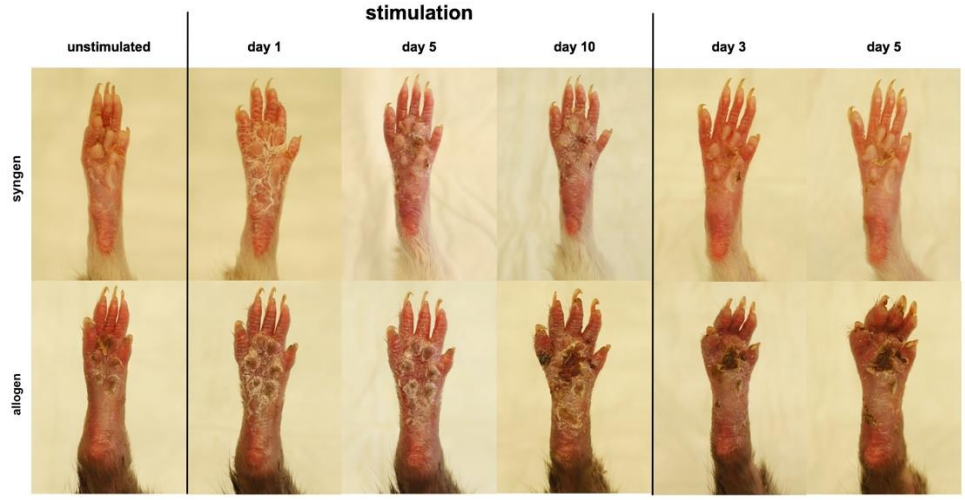

Figure 2. Dynamics of macroscopic alterations provoked by standardized mechanical stimulation of the planta pedis in syngeneic (top row, syngen) and allogeneic transplanted rat hind limbs (bottom row, allogen). Before initiating mechanical stimulation, hindlimbs did not display macroscopic or histologic features of acute rejection (unstimulated). Then, the planta pedia of the transplanted hindlimbs was stimulated over 10 days using a standardized stimulation apparatus. Both groups developed erythema, dryness, scaling and crust formation however, alterations were more pronounced in the allogeneic setting. After completion of stimulation, animals were daily inspected and assessed for their macroscopic rejection grades. Skin alterations decreased quickly in syngeneic animals and almost no changes could be observed after 3 and no changes could be seen after 5 days of observation. Contrarily, allogeneic transplanted animals did only improve very slowly. Whereas crust formation and scaling markedly decreased, swelling and erythema was still present even after 5 days of observation.

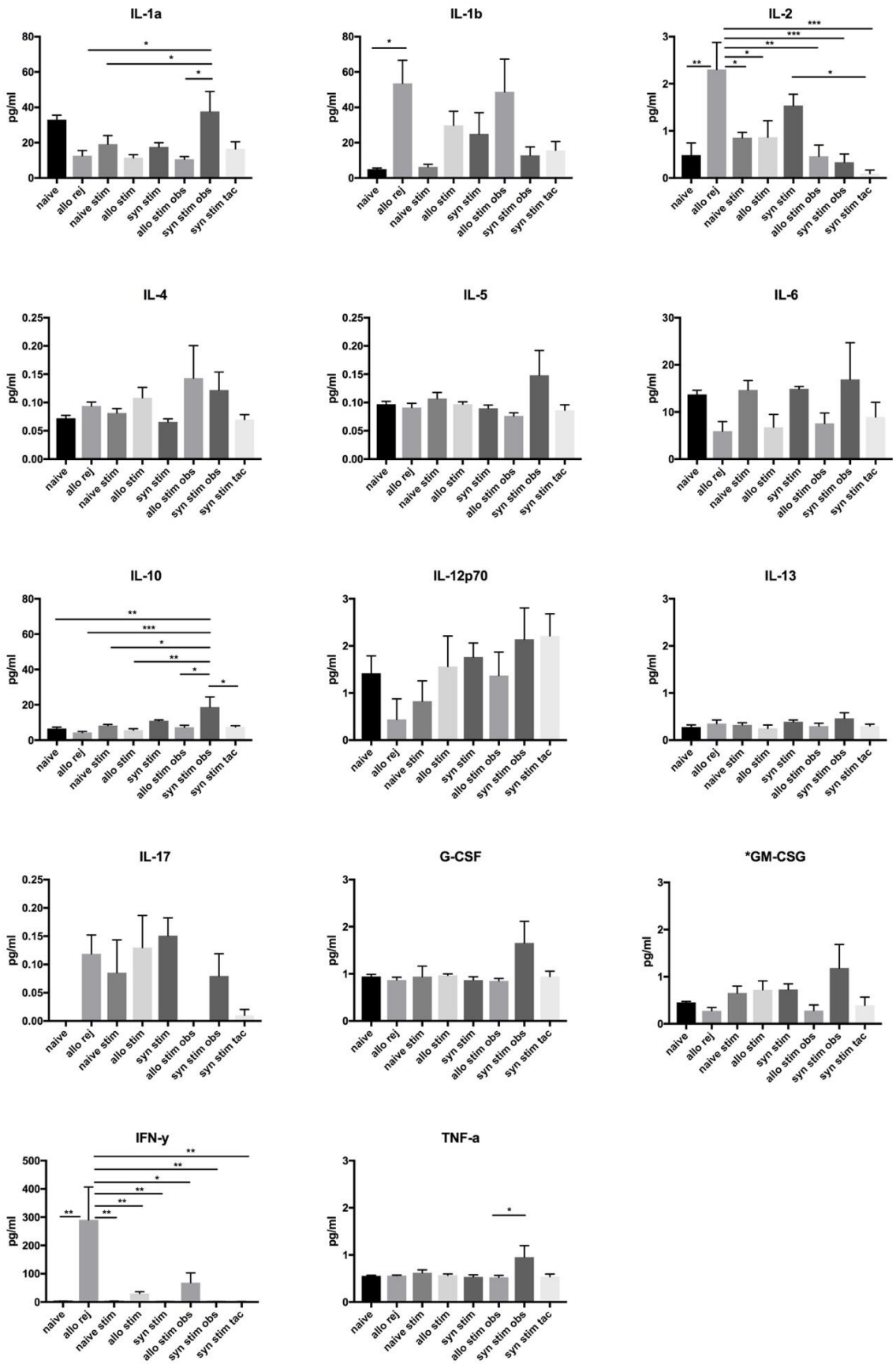
IL-1a
IL-1b
IL-2
IL-4
IL-5
IL-6
IL-10
IL-12p70
IL-13
IL-17
G-CSF
*GM-CSG
IFN-y
TNF-a
pg/ml
naive
allo rej
naive stim
allo stim
syn stim
allo stim obs
syn stim obs
syn stim tac

Figure 3. Th Complete 14-Plex Rat ProcartaPlex™ Panel for Luminex analysis of plantar skin after 10 days of standardized mechanical irritation.

Conclusion: Standardized mechanical skin irritation in vascularized composite allotransplantation can trigger localized skin alterations consistent with rejection. Our findings hence indicate that disproportionate external stimuli are able to trigger alloimmune activation and thus localized “atypical” rejection in this setting.

Difficulties: Despite conducting a pilot study to identify adequate stimulation settings, we experienced quite some difficulties to find the pressure, duration and frequency of stimulation, which allows for generation of externally triggered alterations. With the final settings we were able to apply external mechanical irritation without causing substantial damage (laceration, bleeding, etc.) with the tooth brush itself. In addition, we added a taper regimen for immunosuppression in order to better mimic the clinical setting, where lowest possible doses of immunosuppression are desirable to minimize associated side effects.

Outlook: We are currently preparing our manuscript for publication. We anticipate to submit our manuscript to the American Journal of Transplantation, which is one of the highest ranked journal in this particular area.

Publications issued by this project

Manuscript is currently in preparation.

External funding

None.

Miscellaneous

Congress presentations:

- ISVCA Salzburg, Austria
- Austrotransplant 2018
- PSRC Meeting 2019, Baltimore, MD (accepted)
- ATC 2019, Boston, MA (accepted)
- ESOT 2019, Copenhagen (submitted)

Supervision of diploma students:

- Anna Fischer
- Elias Runggaldier
- Sebastian Eiter

M. Keller - Nutrition dependent reconfiguration of the mitochondrial cardiolipidome in ageing mice

Division of Human Genetics

7th Funding period

Project duration: 01.11.2016 – 31.10.2018

Summary

We were able to successfully conduct this project by addressing the hypothesis and developing our experiments plans along the results obtained.

Despite its pivotal role in establishing life enabling biomembranes, the regulatory origins of the structural diversity of polar lipid species are so far largely elusive. This diversity starts at the level of the different lipid classes, but is to a great extent shaped by the corresponding fatty acyl side-chain substitutions, generating complex membrane compositions of hundreds to thousands of lipid species (Harayama and Riezman 2018). Mitochondrial membranes are no exception, since their strongly folded nature and the high protein content generate special requirements on the underlying lipid constitutions. In contrast to other organelles mitochondria additionally contain cardiolipins (CL), dimeric phospholipids that are predominantly found in the inner membrane (IMM), where they make up ~20% of the lipid content (Daum 1985).

CLs have a unique conical structure with four glycerol bridged fatty acyl side chains. These properties allow them to be functionally involved in a manifold of mitochondrial tasks including transmembrane protein complex stabilisation (Pfeiffer et al. 2003), proton gradient buffering (Haines and Dencher 2002), mtDNA protection (Luévano-Martínez et al. 2015), cristae formation (Ikon and Ryan 2017), as well as apoptosis (Paradies et al. 2014) and mitophagy (Shen et al. 2017). With four side chains and up to five chiral centers CLs are combinatorially the most diverse phospholipid class. However, their fatty acyl arrangement is not stochastically distributed, but introduced by a post-synthetic remodelling processes (Claypool and Koehler 2012). This leads for example in heart tissue to only one dominating CL species, the tetralinoeyl-cardiolipin (Xu et al. 2003), whereas for example in brain an almost inextricable multiplicity of CLs was found. Although the functional implications of these different cardiolipin states are not yet studied in detail, we could e.g. recently show an elevated linoleic acyl content is linked to a higher efficiency of mitochondrial respiration (Oemer et al. 2018).

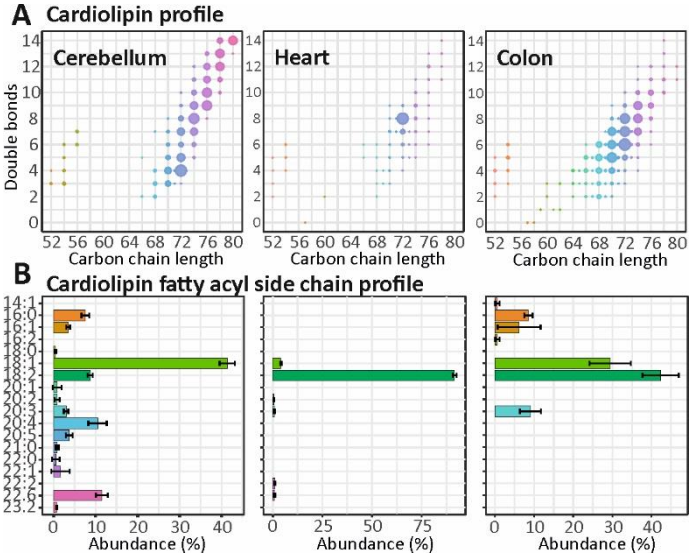

Figure 1: Diversity of cardiolipin composition in mouse tissues. (A) Cardiolipin profile plots: Each CL is characterized by its carbon chain length and number of double bonds. The size is corresponding to its relative abundance. Each plot is normalized to 1. (B) Corresponding fatty acyl side chain profiles of cardiolipins.

In a recent study funded by this project we investigated the mitochondrial lipid species compositions in mammals, which is so far of largely unexplored mechanistic origin. We utilized our established methods to analyse and subsequently mathematically model cardiolipin molecular species in 15 different male and female mouse tissues and identified strong tissue specificity in the side chain structural variability (Fig 1).

Furthermore, using MALDI-imaging of tissue sections (collaboration with Prof. Thomas Müller, University of Innsbruck) we could show that distinct structural variability patterns are spatially organized within the same tissues. It is clear that fatty acyl availability in combination with transporter and enzyme specificities, gene expression, and physicochemical effects are involved in establishing functional/natural compositions. Nevertheless, simple linear correlative models as well as gene-set enrichment analysis fail to explain total cardiolipin abundance as well as their structural variability on basis of an integrative analysis of mRNA expression levels and our lipidomics data.

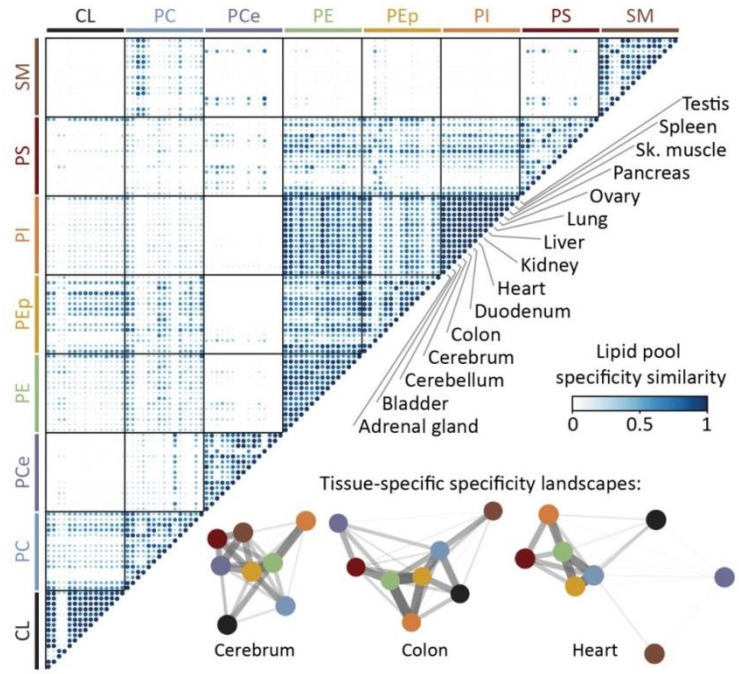

Figure 2: Tissue-specific lipid pool specificity correlations. Lipid species profiles for all major phospholipid classes (9 most abundant classes shown here) were analysed in 15 mouse tissues with our lipidomics platform. Pairwise lipid pool similarities between all profiles and tissue were calculated by determining (Fischer distance measure, 1=high similarity) and are shown as correlation matrix. This allows generating tissue-specific specificity landscapes that depict to which extend individual lipid pools share side chain specificity

Interestingly however, our bioinformatics analyses allowed us to find a differential specificity coupling of cardiolipins with other lipid pools in a tissue-dependent manner (Fig. 2). While in a series of tissues such as liver, colon, and pancreas, the side chain specificity of cardiolipins is closely linked to phosphatidylcholines (PC) and/or phosphatidylethanolamine plasmalogens (PEp), such a coupling is largely missing in heart and brain regions (indicated by distance network plots in Fig. 2).

Further, we have collected experimental evidence that these effects are linked to the balance of nutritional lipids, physical activity, as well as the age of mice. This suggests that the cardiolipin remodeling process in especially these tissues is able to redirect the side chain specificity away from the compositions of their potential acyl-donors. Interestingly we find that especially in brain regions an additional factor comes into play. In many experiments, we could observe linoleic acid to be one of the preferred fatty acyls of CL, which can only be found in minute amounts in the phospholipidome of the brain and therewith opens up the available structural diversity space accessible for CL species (manuscript under preparation). This illustrates the importance of generating equally comprehensive datasets in a model system in which cardiolipin-unrelated differences can be excluded (as being a major goal of this project), in order to elucidate the regulatory basis for these differences.

Publications issued by this project

Weustenfeld, M; Eidelpes, R; Schmuth, M; Rizzo, WB.; Zschocke, J; Keller, MA. (2019): Genotype and phenotype variability in Sjögren-Larsson syndrome. In: *Human Mutation* 40 (2), S. 177–186. DOI: 10.1002/humu.23679.

Oemer, G; Lackner, K; Muigg, K; Krumschnabel, G; Watschinger, K; Sailer, S et al. (2018): Molecular structural diversity of mitochondrial cardiolipins. In: *Proceedings of the National Academy of Sciences of the United States of America* 115 (16), S. 4158–4163. DOI: 10.1073/pnas.1719407115.

Manuscripts under preparation/submitted/under preparation:

Koch J, Oemer G, Lackner K, Edenhofer ML, Gebert R, Wohlfahrter Y, Werner ER, Zschocke J, Keller MA**.** Fatty acyl specificity of cardiolipin remodeling in living cells **(**under preparation)

Oemer G, Koch J, Edenhofer ML, Alam MT, Watschinger K, Sailer S, Zschocke J, Werner ER, Keller MA. Cardiolipin map of the mouse (under preparation)

Huber A, Oemer G, Malanovic N, Lohner K, Kovács L, Salvenmoser W, Zschocke J, Keller MA* and Marx F. Membrane sphingolipids regulate the fitness and antifungal protein susceptibility of Neurospora crassa. Frontiers in Microbiology (in revision).

External funding

- FWF Start Programme; 09/2017- rejected
- OeNB Jubiläumsfonds; 03/2017; rejected
- FWF Start Programme; 09/2018; pending
- BSF Grant Programme; 10/2018; pending

- FFG Bridge 1; 11/2018; pending
- FWF Stand-Alone project; 12/2018; pending

Under preparation for 03/2019: 1x TWF and 1x OeNB Jubiläumsfonds

Miscellaneous

Invited talks:

18.-21. July 2018	Invited Talk, 13th Conference on Mitochondrial Physiology and MitoEAGLE WG and MC Meeting, 2018, Jurmala, Latvia. “The structural molecular diversity of mitochondrial cardiolipins”
18.-21. July 2018	Invited Talk, 9th International Scientific, Medical & Family Conference, Clearwater, USA. “The structural molecular diversity of cardiolipins”
20.-21. March 2017	Invited Talk, Deutsches Diabetes Zentrum (DDZ) Düsseldorf. “Tissue specificity of mitochondrial membrane phospholipids: The cardiolipin map of the mouse”

Supervision:

- Master theses (5): Gregor Oemer, Jakob Koch, Marie Luise-Edenhofer, Katharina Lackner, Katharina Muigg
- Diploma thesis (1): Maximilian Weustenfeld
- Bachelor theses (2): Rita Gebert, Maria Tettamanti

R. Gerner - Gut microbiota, novel biomarkers and potential therapeutic implications in gastrointestinal acute graft-versus-host disease

University Hospital for Internal Medicine I (Gastroenteroloy, Hepatology and Endocrinology)

7th Funding period

Project duration: 01.11.2016 – 31.10.2018

Summary

Acute graft−versus−host disease (aGVHD), the major complication of allogeneic hematopoietic stem cell transplantation (allo−HSCT), displays a multi−organ inflammatory syndrome. Alloantigen−activated donor T cells generate robust immune responses resulting in tissue damage of skin, liver and gut. Despite exciting discoveries in the pathophysiology of GVHD in recent years, this complication remains a major limiting factor of successful allo−HSCT. Approximately 50% of the patients are refractory to the standard treatment with corticosteroids, resulting in poor outcomes after second-line therapy irrespective of the treatment agent. Here we demonstrate that targeting the NAD metabolism offers a novel and powerful approach in the treatment of acute GVHD and additionally exerts strong anti-tumor activity.

Metabolic reprogramming is a hallmark of activated T cells and cancer cells to meet the bioenergetic demands for specific immune responses and/or rapid cell expansion. While biosynthetic requirements of naïve T cells are minimal and can be mainly supplied by mitochondrial respiration, activated T cells strongly rely on additional energy sources such as aerobic glycolysis, similar to metabolism in cancer cells. Nicotinamide-adenine-dinucleotide (NAD) is an essential co-enzyme in energy metabolism and furthermore is a substrate for NAD-dependent enzymes, thus regulating important biological events. In mammals, the enzyme Nicotinamide-phosphoribosyl-transferase (Nampt) catalyzes the rate-limiting step in the NAD salvage pathway and counteracts NAD depletion in times of high NAD turnover.

We found that Nampt is highly elevated in serum and intestinal T cells of patients with acute GVHD. Likewise, Nampt was strongly induced in gut T cells during the course of experimental GVHD in mice. Nampt inhibition with the small-molecule inhibitor Fk866 in fully MHC−mismatched murine GVHD models effectively protected mice from GVHD-mediated pathology, which was attributable to differential effects of NAD depletion in different T cell subsets. Fk866 disrupted the mitochondrial metabolism of effector T cells and selectively induced apoptosis in alloreactive T cells during GVHD. Simultaneously, Fk866 enhanced FoxP3 expression in regulatory T cells (Treg) through blockage of the NAD-dependent histone-deacetylase Sirtuin-1, which translated into increased suppressive properties of Tregs.

To substantiate our findings in mice, we investigated the anti−proliferative capacity of Fk866 on leukocytes from treatment-naïve GVHD patients in vitro. Following T cell receptor stimulation, cells were cultured in the presence or absence of the standard immunosuppressant dexamethasone (DXA) and compared to Fk866, showing that Fk866's anti-proliferative capacity was superior to DXA. Since Fk866−mediated NAD depletion also affected the biology of murine Tregs, we wondered if this observation could be recapitulated in human Treg cells. Human naive CD4+ T cells were differentiated towards

FoxP3−expressing iTreg cells in the presence or absence of Fk866. In agreement with our findings in mice, Nampt inhibition resulted in increased FoxP3 and decreased SIRT1 mRNA expression.

Immunosuppression, however, weakens graft-versus-leukemia effects. In our work we demonstrate, that Nampt inhibition strongly abrogates leukemia cell expansion in a mouse leukemia and graft-versus-leukemia model, which further supports that tumor cells and T cells critically rely on NAD metabolism.

In summary, we show that Nampt and the NAD immuno−metabolism are implicated in experimental and human aGVHD, which can be exploited to selectively modulate immune responses without increasing leukemic relapse. Nampt blockage might display a metabolic checkpoint, achieving reasonable cellular selectivity based on the context of its employment. As such, targeting NAD metabolism by FK866 represents a promising approach to interfere with the altered bioenergetics of T cells and cancer cells while beneficially modulating immune responses during aGVHD.

Publications issued by this project

A manuscript entitled “Targeting NAD immunometabolism limits severe graft−versus−host disease and has potent anti−leukemic activity”, has been submitted to Blood and is currently in revision. The MUI-START funding has been acknowledged and the research office (Servicecenter-Forschung) will be notified upon acceptance.

External funding

The applicant has received a MAX KADE fellowship (57,000 USD, USA Fellowships of the Max Kade Foundation New York) in 2018 and is currently employed as a postdoctoral fellow at the University of California San Diego (UCSD).

Miscellaneous

The MUI-START program allowed the applicant to establish the herein presented data together with our PhD student Dr. Sophie Macheiner, who is co-first author on the submitted manuscript. Four additional PhD students (advisors: Prof. Herbert Tilg, Prof. A. Moschen or Dr. T. Adolph) significantly contributed to this work and are co-authors on the manuscript. Part of the translational studies have been conducted by Christina Schwabegger within the framework of a bachelor thesis to obtain her degree (Bachelor of Science in Health Studies). Due to Christina’s excellent performance during her bachelor studies, she has been hired by our Department director as a biomedical analyst in our laboratory.

Research investigating mechanisms involved in the pathophysiology and treatment of GVHD is mainly conducted in excellent centers in Germany and the US. Together with Prof. D. Nachbaur from the Department of Oncology and Hematology, the applicant has initiated a bio-repository for clinical samples from patients undergoing allogeneic hematopoietic stem cell transplantation. This bio-bank is currently utilized to test for innovative hypotheses, which are further investigated in *in-vitro* studies and mouse models. This grant therefore significantly contributed to establish the applicant’s own research program, which sets the basis to internationally compete in this exciting field.