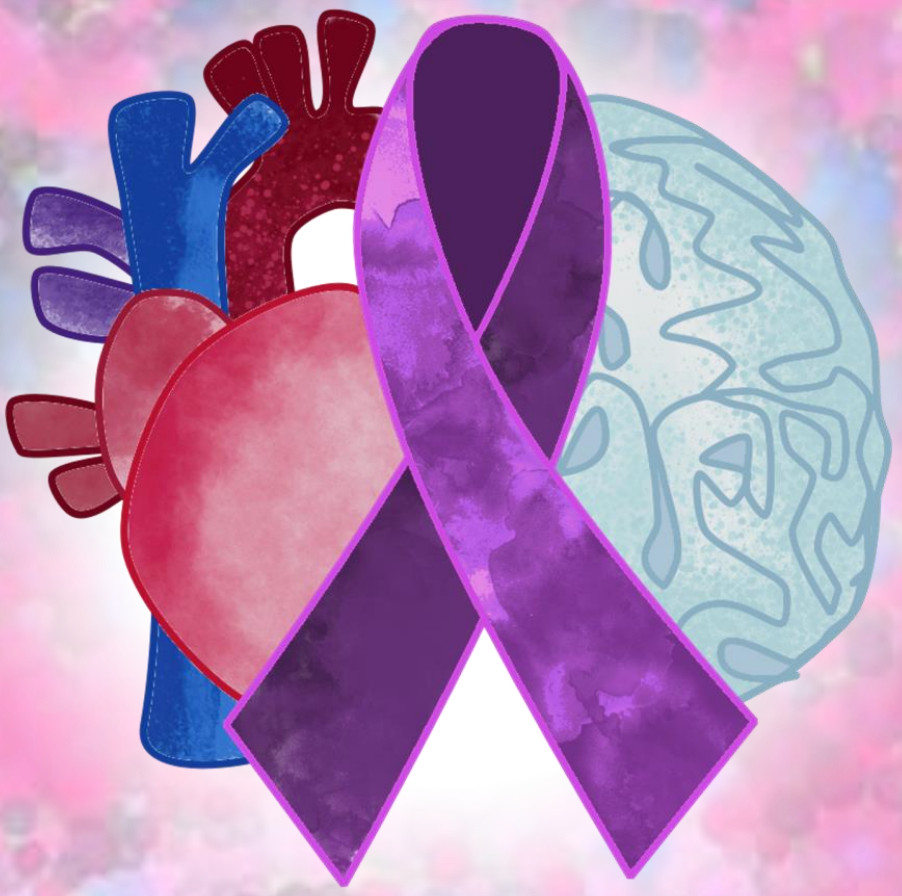
EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

DFG GRK 2381



CanCaN 2021

The 1st PhD Student Conference of the GRK 2381 on

Cancer, Cardiovascular diseases & Neurological disorders

17th – 19th Nov 2021

in Tübingen

Contents

About

Welcome

CanCaN 2021 - a conference on cancer, cardiovascular diseases, and neurological disorders – is hosted by the PhD students of the Research Training Group 2381 (GRK 2381: "cGMP: From Bedside to Bench") at the University of Tübingen. This event will give an opportunity to young scientists to learn from experts and discuss current research in a great variety of fields.

We are looking forward to meet you all at the Alte Aula in Tübingen or online!

Useful Information

All information, access to the live streams, and the poster gallery, as well as the program is available on our website:

event.fourwaves.com/cancan/

Talks will be held in the Alte Aula at Münzgasse 30, 72070 Tübingen.

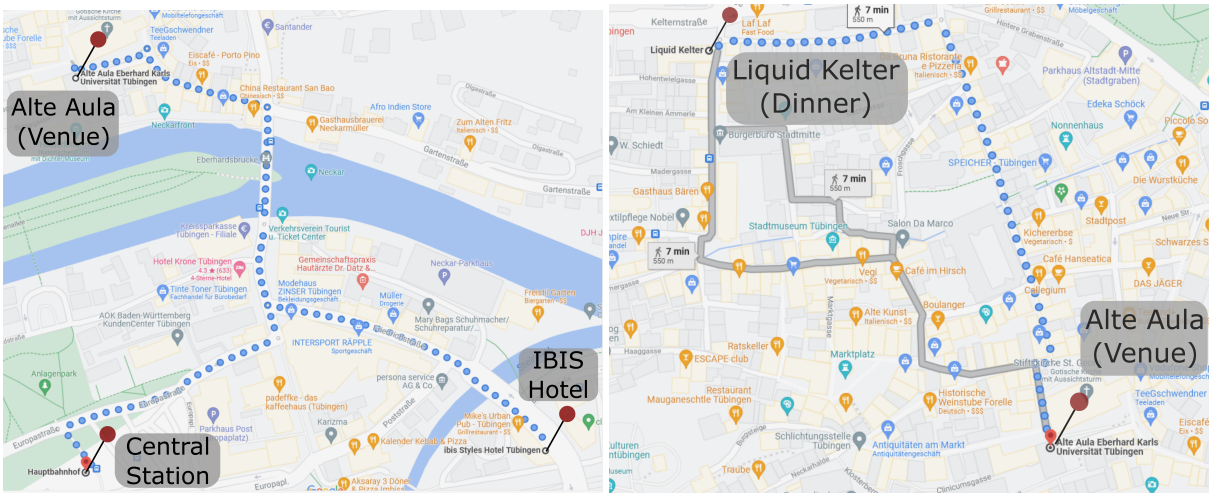
Coffee breaks and lunches will be provided at the Alte Aula.

Poster sessions will be held on Wednesday and Thursday afternoon.

Wi-Fi will be available during the conference via access to an eduroam network.

The conference dinner will be held at 7 pm on Thursday at the "Liquid Kelter" at Schmiedtorstraße 17, 72070 Tübingen.

Directions



GRK 2381 "cGMP: From Bedside to Bench"

In this Research Training Group ("Graduiertenkolleg" 2381, GRK 2381) entitled "cGMP: From Bedside to Bench", doctoral researchers will investigate the second messenger cyclic guanosine monophosphate (cGMP). cGMP is responsible for the transmission of signals in cells, and many drugs for the treatment of cardiovascular diseases target this signaling pathway. The latest findings suggest that cGMP-modulating drugs can be used even more widely. This will be investigated by the junior scientists of the GRK 2381.

uni-tuebingen.de/en/141767

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CanCaN 2021

17th- 19th November 2021, Tübingen

CanCaN 2021 – a conference on cancer, cardiovascular diseases, and neurological disorders – is hosted by the PhD students of the Research Training Group 2381 (**GRK 2381: "cGMP: From Bedside to Bench"**) at the University of Tübingen. This event will give an opportunity to young scientists to learn from experts and discuss current research in a great variety of fields.

We are looking forward to meet you all at the Alte Aula in Tübingen or online!

Wednesday 17.11.21

11:30 *Check-in*

12:30 **Welcome**

Session I: Cardiovascular Research
Chair: Melanie Cruz-Santos & Lena Birkenfeld

13:00 **Endothelial mechanosignalling roles in physiology and pathology**
- Stefan Offermanns

13:30 **Role of the extracellular matrix in vascular plasticity and atherosclerosis**
- Maria Zaldivia

14:00 **Illuminating cGMP signaling in cardiovascular cells for better cardiac and vascular protection**
- Viacheslav Nikolaev

14:30 **cGMP connected pathways in stroke**
- Dmitriy Atochin

15:00 *Coffee break*

Session II: Cancer & Immunology
Chair: Mariagiovanna Barresi & Jennifer Schulz

15:30 **cAMP: From Treg function to melanoma therapy**
- Christian Becker

16:00 **Understanding dynamic signaling networks in cancer - Paving the road for new targeted therapies**
- Monilola Olayioye

16:30 **Improving cancer immunotherapy by exercise**
- Dai Fukumura

17:00 **Poster Session I**

Thursday 18.11.21

9:00 *Check-in*

Session III: Neuroscience
Chair: Thomas Pham & Tamara Hussein

9:30 **Deciphering fear circuitry**
- Ingrid Ehrlich

10:00 **Cyclases and synapses**
- Christine Gee

10:30 **Nitric oxide release in the retina is controlled by a feedback loop that depends on cGMP signaling**
- Gregory Schwartz

11:00 *Coffee break*

Keynote Lecture I
Chair: Malte Roeßing & Timo Kopp

11:30 **sGC stimulators and sGC activators research and development @ Bayer: From bench to bedside**
- Peter Sandner

12:30 *Lunch break*

Session IV: Pharmacology & Biotechnology
Chair: Johanna Rodriguez & Aylin Balmes

13:30 **Lead discovery: A great TASK for small molecules**
- Thomas Müller

14:00 **Novel aspects of receptor pharmacology revealed by FRET-based biosensors**
- Moritz Bünemann

14:30 *Coffee break*

Session V: Pharmacology & Biotechnology
Chair: Philine Marchetta & Dila Calis

15:00 **Bioengineered reporters for monitoring alternative splicing and isoform expression - tools for interrogating signal transduction?**
- Gil Westmeyer

15:30 **The end of medicine and cGMP as we know it**
- Harald Schmidt

16:00 **Poster session II**

19:00 *Dinner at "Liquid Kelter"*

Friday 19.11.21

9:00 *Check-in*

Keynote Lecture II
Chair: Marcel Kremser & Valerie Dicenta

9:15 **Pericytes and myofibroblasts: Novel workplaces for NO-sensitive guanylyl cyclase**
- Andreas Friebe

10:15 *Coffee break*

Session VI: cGMP signaling
Chair: Dominic Gonschorek & Tom Schwerd-Kleine

10:45 **cGMP signaling in pain processing**
- Achim Schmidtko

11:15 **Multilevel functional neuroprotection of retinal neurons with the novel PKG inhibitor CN238**
- François Paquet-Durand

11:45 **Neuromodulation of visual transduction by bicarbonate**
- Clint Makino

12:15 **Poster prize ceremony**

12:30 **Conclusion**

12:45 *Lunch*

Poster List

#1 **Mechanosensitive cGMP signaling in vascular smooth muscle cells**
presented by Timo Kopp, *University of Tübingen*

#2 **Effect of NO-GC stimulators and activators in platelet biomechanics**
presented by Johanna G. Rodríguez, *University of Tübingen*

#3 **The cGMP signaling pathway in breast cancer cells**
presented by Mariagiovanna Barresi, *University of Tübingen*

#4 **GC-A/cGMP signaling is protective for preservation of auditory nerve function following acoustic overexposure and depends on limbic stress responses**
presented by Philine Marchetta, *University of Tübingen*

#5 **Therapeutic potential of the vascular NO/cGMP signaling pathway in atherosclerosis**
presented by Malte Roessing, *University of Tubingen*

#6 **Stress Receptors Link Auditory Periphery and Cognition via cGMP Signaling**
presented by Dila Calis, *University of Tübingen*

#7 **Identification of cGKI substrates mediating sensory axon bifurcation**
presented by Alexandra Böttcher, *University of Tübingen*

#8 **Measuring the stiffness of neuronal growth cones with scanning ion conductance microscopy**
presented by Aylin Balmes, *University of Tübingen*

#9 **NO-GC in the tumor microenvironment represents a potential drug target for melanoma treatment**
presented Jennifer Schulz, *University of Tübingen*

#10 **Role of NO/cGMP and eCB in visual signal processing in the inner retina**
presented by Tom Schwerd-Kleine, *University of Tübingen*

#11 **Platelet-derived PCSK9 is Associated with LDL Metabolism and Modulates Atherothrombotic Mechanisms in Coronary Artery Disease**
presented by Marcel Kremser, *University Hospital Tübingen*

#12 **Role of CXCR7 in platelet-dependent inflammationand functional recovery of ischemic myocardium**
presented by Valerie Dicenta, *University Hospital Tübingen*

#13 **Postsynaptic BK contributes critically to Schaffer collateral LTP and promotes hippocampus dependent memory formation**
presented by Thomas Pham and Tamara Hussein, *University of Tübingen*

#14 **Lack of cGKI in myofibroblasts amplifies cardiac fibrosis following Ang II infusion**
presented by Melanie Cruz Santos and Lena Birkenfeld, *University of Tübingen*

#15 **Systematic investigation of retinal ganglion cell modulation by NO-induced cGMP**
presented by Dominic Gonschorek, *University of Tübingen*

#16 **Investigating the role of cGMP signalling in hepatic stellate cells**
presented by Krithika Rajeeth, *University of Tübingen*

#17 **Investigation of the effect of nitric oxide on thrombus size and shape with scanning ion conductance microscopy (SICM)**
presented by Hendrik von Eysmondt, *University of Tübingen*

#18 **Role of mechanosensitive cGMP signaling in platelets and thrombus formation**
presented by Liubov Unger and Daniel Pinto Quintero, *University of Tübingen*

#19 **Can cGMP signaling recover fast auditory processing deficits related to an autism-like phenotype?**
presented by Morgan Hess, *University of Tübingen*

#20 **Patient-individual phenotypes of glioblastoma stem cells are conserved in culture and associate with radioresistance, brain infiltration and patient prognosis**
presented by Katrin Ganser, *University of Tübingen*

#21 **Combined IK channel targeting and glioblastoma irradiation in a syngeneic orthotopic mouse model**
presented by Nicolai Stransky, *University of Tübingen*

#22 **Development of a vascularized complex human 3D in vitro skin by combination of iPSC-derived skin organoids and vascular organoids to mimic a vascular network.**
presented by Amelie Reigl, *University of Würzburg*

#23 **De novo cytokine design**
presented by Mohammad ElGamacy, *Max Planck Institute for Developmental Biology*

#24 **The role of the sodium-activated potassium channel Slack in kainic acid and pilocarpine induced epilepsy models**
presented by David Skrabak, *University of Tübingen*

#25 **Protein design of growth factor inhibitors**
presented by Kateryna Maksymenko, *Max Planck for Developmental Biology*

#26 **IRAG2 interacts with IP3-receptor types 1, 2 and 3 and regulates intracellular calcium**
presented by Sally Prüschenk, *University of Regensburg*

#27 **Defining the localization of epicardial cell clusters in the heterogenous infarcted mouse epicardium**
presented by Ria Zalfen, *Heinrich-Heine-University Düsseldorf*

#28 **Quantitative secretome analysis in cultured cardiac fibroblasts isolated from the infarcted heart using SILAC labeling combined with Click-Chemistry**
presented by Jasmin Bahr, *Heinrich-Heine-University Düsseldorf*

#29 **Normoxic HIF1-α induction via A2BR activation in epicardial stromal cells and activated cardiac fibroblasts formed after myocardial infarction**
presented by Julia Steinhausen, *Heinrich-Heine-University Düsseldorf*

#30 **Monitoring metabolic changes by deuterium MRI in heart and BAT after I/R**
presented by Vera Flocke, *Heinrich-Heine University Düsseldorf*

#31 **Cellular localization of NO-GC in the murine heart and its role in angiotensin II-induced fibrosis**
presented by Lennart Kreutz, *Julius-Maximilians-Universität Würzburg*

Poster Abstracts

Poster Session I

Wednesday, November 17th: 5pm to 6pm
In this session, presenting authors of posters with odd ID numbers are kindly requested to be at their posters for discussion and evaluation by the Poster Prize Committee.

#1
Mechanosensitive cGMP signaling in vascular smooth muscle cells

Timo Kopp[1], ***Malte Roeßing***[1], ***Moritz Lehners***[1], ***Heiko Olbrich***[1], ***Remco TA Megens***[2,3,4], ***Robert Feil***[1], ***Susanne Feil***[1]

[1] Interfakultäres Institut für Biochemie, Universität Tübingen
[2] Institut für Prophylaxe und Epidemiologie der Kreislaufkrankheiten, Ludwig-Maximilians-Universität München
[3] Department of Biomedical Engineering, CARIM (Cardiovascular Research Institute Maastricht), Maastricht University, Maastricht, the Netherlands
[4] DZHK (German Center for Cardiovascular Research), Partner Site Munich Heart Alliance, Munich, Germany

Vascular tone is regulated by the balance between endogenous vasodilators and vasoconstrictors. Impaired vessel contraction/relaxation is frequently associated with changes in vascular stiffness and diseases like hypertension and aneurysm. A major mediator of vascular relaxation is nitric oxide (NO), which causes vasodilation by increasing the intracellular cyclic guanosine monophosphate (cGMP) level in vascular smooth muscle cells (VSMCs). Here we show that NO-induced cGMP production in VSMCs is mechanosensitive. VSMCs experience mechanical stress due to forces on the vessel wall (e.g., during contraction/relaxation or pulsatile blood flow). Our hypothesis is that mechanosensitive cGMP signaling is impaired in VSMCs of stiff diseased vessels. To visualize cGMP in real time, aortic VSMCs were isolated from mice that globally express the Förster/fluorescence resonance energy transfer (FRET)-based cGMP-sensor cGi500. VSMCs were exposed to the NO-donor DEA/NO, and cGMP signals were recorded in response to pressure puffs, locally applied via a glass capillary. Additionally, carotid arteries of cGMP sensor mice were mounted in an arteriograph, pressurized, exposed to DEA/NO and monitored for changes in cGMP. In response to NO and pressure puffs, primary VSMCs showed cGMP increases that correlated with the magnitude of the applied mechanical force. Similarly, mounted carotid arteries responded to NO under flow and pressure conditions with cGMP signals. These data demonstrate the presence of a mechanosensitive NO/cGMP pathway in murine VSMCs. Future studies are aimed at identifying the mechanosensor/mechanotransducer in the membrane that is linked to enhanced activation of NO-dependent cGMP production in VSMCs. Targeting the mechanosensitive cGMP signaling pathway might be a novel strategy for the treatment of vascular diseases with impaired smooth muscle relaxation.

#3

The cGMP signaling pathway in breast cancer cells

Mariagiovanna Barresi[1], ***Malte Roessing***[1], ***Hannes Schmidt***[1], ***Dai Fukumura***[2], ***Robert Feil***[1]

[1] Interfaculty Institute of Biochemistry, University of Tübingen, Tübingen, Germany ,
[2] Edwin L. Steele Laboratory, Department of Radiation and Oncology, Massachusetts General Hospital & Harvard Medical School, Boston, Massachusetts, USA

Breast cancer is the second most common cause of cancer mortality in women. Although new diagnostic tools and therapeutic strategies have led to a significant reduction in breast cancer related mortality, more effective and less toxic drugs are still needed. Recent studies have suggested that the cGMP signaling pathway may be aberrantly regulated in breast cancer, but cGMP's functional relevance during tumorigenesis is not completely understood. The aim of this study was to improve our understanding of cGMP signaling during breast cancer development and progression. Here, we show that several human and murine breast cancer cell lines exhibit different patterns of the cGMP signaling pathway expression and activity. Among the cell lines analyzed, the human Hs578T cells were characterized in detail. Protein expression of the components of the cGMP signaling pathway was detected via Western blot. Additionally, live imaging of the tumor cells after transfection with the Förster resonance energy transfer (FRET)-based cGMP biosensor cGi500 showed increased cGMP levels upon stimulation with C-type natriuretic peptide (CNP) and the nitric oxide (NO) donor DEA-NO. An in vitro cell growth assay showed that activation of the cGMP signaling pathway via stimulation with 8-Br-cGMP, led to inhibition of tumor cell growth in comparison to the control. This study provides evidence that the cGMP signaling pathway is present in various breast cancer cell lines and might play an inhibitory role in the growth of breast cancer cells. Thus, these findings suggest that this pathway could be a new target to improve therapies for breast cancer.

#5

Therapeutic potential of the vascular NO/cGMP signaling pathway in atherosclerosis

Malte Roessing[1], ***Moritz Lehners***[1], ***Maria Teresa Kristina Zaldivia***[1], ***Mariagiovanna Barresi***[1], ***Peter Sandner***[2,3], ***Robert Feil***[1], ***Susanne Feil***[1]

[1] Interfaculty Institute for Biochemistry, University of Tubingen, Tubingen, Germany ,
[2] Bayer AG, Cardiovascular Research, Pharma Research Center, Wuppertal, Germany ,
[3] Department of Pharmacology, Hannover Medical School, Hannover, Germany

Vascular smooth muscle cells (VSMCs) are key players in atherosclerosis. During the formation of atherosclerotic plaques, VSMCs can migrate, expand clonally and transdifferentiate. However, neither the specific role of VSMCs nor the molecular mechanisms underlying growth and phenotypic modulation are fully understood. The NO/cGMP signaling pathway has been shown to be an important regulator in vascular biology. It is well known that activation of the NO-sensitive guanylyl cyclase (NO-GC) in VSMCs leads to an increase of intracellular cGMP levels inducing vasodilation. Our hypothesis is that stimulation of the NO/cGMP signaling pathway promotes phenotypic modulation of VSMCs in atherosclerosis. In the present study, NO-GC-stimulating drugs (e.g. Vericiguat) were used to modulate the activity of this pathway in vitro and ex vivo. We visualized cGMP signals in real time in isolated primary VSMCs and aortae of transgenic mice ubiquitously expressing the FRET-based cGMP sensor cGi500. The growth effects of drugs were monitored in real time in VSMCs of wildtype animals. FRET microscopy of primary VSMCs and aortae showed a concentration dependent cGMP generation in response to the NO donor DEA/NO or NO-GC stimulating drugs. The combination of NO donor and drugs led to a potentiation of the cGMP signals. Interestingly, co-treatment with DEA/NO and NO-GC stimulators also enhanced VSMC growth in comparison to DEA/NO or drug alone. These results show that NO-GC stimulators trigger cGMP signals in VSMCs that lead to enhanced cell growth. The aim of further studies is to analyze the effects of NO-GC-activating drugs on VSMC phenotypic modulation and plaque composition in a mouse model of atherosclerosis. This will allow us to elucidate the therapeutic potential of the vascular NO/cGMP signaling pathway for the treatment of vascular diseases like atherosclerosis.

#7

Identification of cGKI substrates mediating sensory axon bifurcation

Alexandra Böttcher[1], ***Boris Macek***[2], ***Ana Velic***[2], ***Robert M. Blanton***[3], ***Robert Feil***[1], ***Hannes Schmidt***[1]

[1] Interfaculty Institute of Biochemistry, University of Tübingen, Tübingen, Germany ,
[2] Proteome Center Tuebingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany ,
[3] Molecular Cardiology Research Institute and Division of Cardiology, Tufts Medical Center, Boston, Massachusetts, USA

Neuronal circuitry in the adult nervous system is shaped by axonal branching during embryonic and early postnatal development. Neurons of dorsal root ganglia (DRG) are a useful tool to study these processes, as they exhibit both principal modes of axonal branching growth cone splitting (bifurcation) and interstitial branching in a highly conserved and time-dependent manner. Our group could dissect a cGMP-dependent signaling cascade controlling axon bifurcation in embryonic sensory neurons: the cGMP-dependent protein kinase type I (cGKI) is activated after guanylyl cyclase B (GC-B) produces cGMP upon binding of its extracellular ligand C-type natriuretic peptide (CNP). Loss of any of these critical components leads to a bifurcation error. Our aim is to further elucidate this signaling pathway by characterizing phosphorylation substrates of cGKI that are involved in the process of growth cone splitting. Using mass spectrometric analysis, a murine melanoma cell line and embryonic DRGs were screened for phosphopeptides after stimulation of cGKI activity. Thus, several candidate proteins were identified, which will be further validated as substrates of cGKI. An analogue sensitive mutant of cGKI will be applied to confirm the ability of the kinase to phosphorylate the candidate proteins. Furthermore, the phosphorylation sites that have been determined will be validated using mutants containing an alanine substitution of the putative phosphorylated serine residue. The interaction of candidate proteins with full length cGKI or specifically its leucine zipper domain will be characterized with pull down assays. The functional role of candidate proteins in sensory axon bifurcation will be analyzed by DiI-labeling of DRG neurons in spinal cord whole mount preparations of respective mouse mutants. Thus discovering further elements of the cGMP-dependent signaling cascade controlling bifurcation in sensory neurons during embryonic development should facilitate a better mechanistic understanding of axonal branching.

#9

NO-GC in the tumor microenvironment represents a potential drug target for melanoma treatment

Daniel Stehle[1]**,** ***Jennifer Schulz***[1]**,** ***Susanne Feil***[1]**,** ***Dai Fukumura***[2]**,** ***Robert Feil***[1]

[1] University of Tübingen, Interfaculty of Biochemistry (IFIB), Tübingen, Germany ,
[2] Edwin L. Steele Laboratory, Department of Radiation and Oncology, Massachusetts General Hospital & Harvard Medical School, Boston, Massachusetts, USA

Question

Melanoma is a dangerous type of skin cancer with a 5-year survival rate of only 27 % in its end-stage. One reason for this low survival is that many late-stage melanoma patients do not or only partially respond to the existing therapies, demonstrating the need for novel treatment options. The cGMP signaling pathway is an important player in many physiological processes including vasodilation. We hypothesize that the cGMP pathway also regulates the tumor vasculature and could provide an additional target for co-therapy approaches of melanoma.

Methods

We have generated mice that express the FRET-based cGMP biosensor cGi500 selectively in distinct cell types. We performed real-time in situ cGMP imaging using alpha smooth muscle actin (a-SMA)-specific cGMP sensor mice. Additionally, we analyzed the role of cGMP in tumor pericytes and endothelial cells with classical immunostaining.

Results

Endothelial NO synthase (eNOS), a generator of the endogenous cGMP-increasing signaling molecule NO, was found to be expressed in the endothelium of tumor blood vessels. The canonical NO receptor, NO-sensitive guanylyl cyclase (NO-GC), was specifically detected in tumor pericytes. In line with these results, real-time cGMP imaging confirmed the capacity of tumor pericytes to generate cGMP in response to NO. Furthermore, the pharmacological NO-GC stimulator riociguat potentiated the NO-induced cGMP generation in these cells.

Conclusions

Together, these data demonstrate the presence of a functional NO/NO-GC/cGMP signaling cascade in the melanoma microenvironment, involving NO generation by endothelial cells and NO-induced cGMP generation in pericytes. Although the downstream effects of cGMP in tumor pericytes are yet to be determined, pharmacological NO-GC stimulators like riociguat display a powerful and novel tool to specifically modulate cGMP signaling in the melanoma vasculature.

#11

Platelet-derived PCSK9 is Associated with LDL Metabolism and Modulates Atherothrombotic Mechanisms in Coronary Artery Disease

Marcel Kremser[1], ***Álvaro Petersen-Uribe***[1], ***Anne-Katrin Rohlfing***[1], ***Tatsiana Castor***[1], ***Kyra Kolb***[1], ***Valerie Dicenta***[1], ***Frederic Emschermann***[1], ***Bo Li***[1], ***Oliver Borst***[1], ***Dominik Rath***[1], ***Karin Anne Lydia Müller***[1], ***Meinrad Paul Gawaz***[1]

[1] Department of Cardiology and Angiology, University Hospital Tübingen, Eberhard Karls University Tübingen, Tübingen, Germany

Background

Platelets play a significant role in atherothrombosis. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is critically involved in regulation of LDL metabolism and interacts with platelet function. The effect of PCSK9 in platelet function is poorly understood. The authors sought to characterize platelet as a major source of PCSK9 and its role in atherothrombosis.

Methods

In a large cohort of patients with coronary artery disease (CAD), platelet count, platelet reactivity and platelet-derived PCSK9 release were analyzed. The role of platelet-PCSK9 on platelet and monocyte function was investigated in vitro.

Results

Platelet count and hyper-reactivity correlated with plasma LDL in CAD. The circulating platelets express on their surface and release substantial amounts of PCSK9. Release of PCSK9 augmented platelet-dependent thrombosis, monocyte migration and differentiation into macrophages/foam cells. Platelets and PCSK9 accumulated in tissue derived from atherosclerotic carotid arteries in areas of macrophages. PCSK9 inhibition reduced platelet activation and platelet-dependent thrombo-inflammation.

Conclusion

The authors identified platelets as a major source of PCSK9 in CAD and may have an impact on LDL metabolism. Further, platelet-derived PCSK9 contributes to atherothrombosis, and inhibition of PCSK9 attenuates thrombo-inflammation which may contribute to the reported beneficial clinical effects.

#13

Postsynaptic BK contributes critically to Schaffer collateral LTP and promotes hippocampus dependent memory formation

Thomas Pham[1], ***Helmut Bischof***[1], ***Tamara Hussein***[1], ***Stefanie Simonsig***[1], ***Daniel Kalina***[1], ***Rebekka Ehinger***[1], ***Peter Ruth***[1], ***Robert Lukowski***[1], ***Lucas Matt***[1]

[1] Department of Pharmacology, Toxicology and Clinical Pharmacy, Institute of Pharmacy, University of Tübingen

Objectives: Impaired synaptic plasticity, the bidirectional modulation of synaptic transmission strength is an often-neglected cause of cognitive impairment occuring in Alzheimer's disease, autism, and schizophrenia. Even though mutations of the large-conductance Ca2+- and voltage-activated potassium channel (BK) have already been correlated with cognitive impairment, BK's role in synaptic plasticity remains elusive. Because global BK knockout mice (BK-/-) suffer from cerebellar ataxia to the extent that they are unable to conduct behavior-based memory tasks, we used CA1 pyramidal neuron-specific BK conditional knockout (CA1BKfl/-) and littermate control mice to study BK's role in hippocampus-dependent memory formation and synaptic plasticity.

Methods: CA1 pyramidal neuron-specific BK knockout mice were created using Cre/loxP mediated recombination of floxed BK alleles. Fear behavior and locomotor performance were evaluated in open-field and beam-walk tests, respectively. Hippocampus-dependent memory acquisition, retrieval, and flexibility were tested in the Morris Water Maze (MWM). CA1 to CA3 long-term potentiation (LTP) and AMPA receptor (AMPAR) phosphorylation levels after chemically induced LTP (cLTP) were assessed in acute hippocampal slices. A genetically encoded potassium ion indicator (GEPII) and the Ca2+ sensitive fluorescent dye (FURA-2 AM) were used to monitor intracellular [K+] and [Ca2+] dynamics during cLTP induction in dissociated hippocampal neuronal cultures.

Results: Western blot and immunofluorescence demonstrate specific and effective BK depletion in the hippocampal CA1 region. In open-field and beam-walk, CA1BKfl/- fear behavior and locomotor properties did not differ from littermate controls, as expected. In the MWM, CA1BKfl/- exhibit increased latency to reach the platform, as they did not develop a target-oriented search strategy, but randomly swim around, both indicating impaired hippocampus-dependent memory formation in vivo. Deficient learning could further be confirmed in vitro by electrical and chemical induction of LTP in CA1BKfl/-, as this failed to elicit LTP and AMPAR phosphorylation at S845-GluA1, a surrogate parameter of LTP, respectively. In dissociated hippocampal neuronal cultures, GEPIIs unveiled strong BK-mediated K+efflux provoked by the cLTP stimulus. Consistent with substantially decreased intracellular [K+], FURA-2 based measurements detect an increased frequency in neuronal Ca2+ oscillations sensitive to AP5 and Nifedipine in the presence of BK.

Conclusion: Our data suggest that BK acts as a negative feedback modulator of NMDA receptor- and voltage-gated Ca2+channel-mediated Ca2+ influx during LTP. We propose that BK-mediated K+ efflux during LTP induction, enhances and sustains neuronal Ca2+ oscillations to allow effective formation of synaptic plasticity in hippocampal neurons. Our findings suggest BK modulation as a therapeutic option to prevent or even treat impaired cognitive performance in neurodegenerative disorders.

#15

Systematic investigation of retinal ganglion cell modulation by NO-induced cGMP

Dominic Gonschorek[1,2,3], ***Tom Schwerd-Kleine***[1,2,3], ***Zhijian Zhao***[2], ***Timm Schubert***[1,2], ***Thomas Euler***[1,2,3,4]

[1] Centre for Integrative Neuroscience, University of Tübingen,
[2] Institute for Ophthalmic Research, University of Tübingen,
[3] GRK 2381 'cGMP: From Benchside to Bed', University of Tübingen,
[4] Bernstein Center for Computational Neuroscience, University of Tübingen

In the outer retina, the function of cGMP as a second messenger in the photoreceptors is well understood. However, there is no comprehensive view of its function and contribution to signal processing within the inner retina, despite its widespread presence. In the inner retina, cGMP levels can be upregulated by soluble guanylate cyclase (sGC/NO-GC) upon activation by the neuromodulator nitric oxide (NO), which is naturally released by a subgroup of amacrine cells (nNOS-2 ACs).

In this project, we aim to gain a systematic and comprehensive view of the effects of NO-induced cGMP modulation on retinal ganglion cells (RGCs) in order to understand how the output signal to the brain is shaped and which possible circuits are involved in the signal processing. To this end, we express the synthetic calcium indicator OGB-1 in mouse RGCs via electroporation and use two-photon microscopy to record their activity, while simultaneously presenting different visual stimuli. To measure and identify the modulatory effects of RGC responses, we add the NO-donor DETA/NO via bath application. To systematically investigate the modulatory effects in different functional RGC types, we developed a RGC type classifier.

We found that repeated recordings of the same retinal fields can cause changes in the responses of some RGC types, even without drug application, making it difficult to separate modulatory features induced by NO and time-dependent systematic changes occurring by sequential recordings. Therefore, we have three questions to address: (1) What are the systematic time-dependent changes, and what are their underlying cause? (2) Which cell types are robust in their responses over time and which cell types change? (3) Which cell types are modulated by NO and how?

To address the question of systematic time-dependent changes, we developed an auto-encoder with adversarial training (RAVE(+)) to remove the inter-experimental variability; by inspecting this variability we expect a better understanding of these changes. In parallel, we have started to analyse the time- and drug-dependent changes at cell type-level. For instance, we identified two similar cell types that show very different behaviour: Type 31 RGCs (Off suppressed 1) changed their responses time-dependent and independent of drug application, whereas type 32 RGCs (Off suppressed 2) were stable, displaying no substantial time-dependent response changes, but were strongly modulated by NO application. This suggests that time-dependent response changes are type-specific and that NO acts differentially on RGC types. A next step will be to measure if NO-evoked effects coincide with changes in cGMP levels in these RGC types. Taken together, with the tool (RAVE(+)) to remove and inspect systematic, time-dependent changes in RGC activity at hand, we are now better prepared to systematically investigate neuromodulatory effects of NO-induced cGMP in the inner retina.

#17

Investigation of the effect of nitric oxide on thrombus size and shape with scanning ion conductance microscopy (SICM)

Hendrik von Eysmondt[1], ***Liubov Unger***[2], ***Daniel Pinto Quintero***[2], ***Frank Regler***[2], ***Susanne Feil***[2], ***Robert Feil***[2], ***Tilman E. Schäffer***[1]

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Thrombosis, i.e. the platelet-facilitated formation of blood clots or thrombi at the site of injury is necessary for proper hemostasis. In certain conditions, however, like in cardiovascular diseases such as acute coronary syndrome or ischemic stroke, anticoagulants are administered to inhibit thrombosis. Current anticoagulants inhibit thrombosis by severely disrupting platelet activity, potentially leading to internal bleeding. Therefore, endogenous mechanisms inhibiting thrombosis are investigated as a promising alternative. It is currently known that a shear-dependent NO-cGMP-cGKI cascade in platelets acts as a self-regulatory brake of thrombosis [1]. Nitric oxide (NO) released by the endothelium activates the NO-sensitive soluble guanylat cyclase (sGC) in platelets. This increases the cyclic guanosine monophosphate (cGMP) levels and subsequently increases activity of the cGMP-dependent protein kinase I (cGKI), ultimately resulting in a decreased thrombotic activity without fully disrupting thrombosis. However, this effect of the NO-cGMP-cGKI cascade on thrombosis has not yet been investigated at the level of individual thrombi.

Scanning ion conductance microscopy (SICM) uses a nanopipette as a probe to scan a surface in electrolyte solution. An ion current is passed through the pipette and the dependence of the ion current on the probe-sample distance is used for non-contact imaging of the topography of 3D objects on the sub-micrometer scale. Diethylamine nonoate (DEA-NO) is a NO-donor, that mimics the effect of NO released by the endothelium. The drug Riociguat (BAY 63-2521) increases the sensitivity of sGC to NO and stimulates sGC at the same time, independently of NO, thus increasing cGMP-levels and proposedly decreasing thrombotic activity and thus thrombus size. In this work, we quantified the effect of these drugs on thrombus shape parameters like area, volume, height, aspect ratio and circularity using SICM.

#19

Can cGMP signaling recover fast auditory processing deficits related to an autism-like phenotype?

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Brain-derived neurotrophic factor (Bdnf) is critical for the development of sensory systems1. When deleted under the Pax2 promoter, effectively deleting Bdnf from Pax2-derived GABAergic interneurons, mice maintain normal hearing at threshold level; however, their fast auditory processing is profoundly impaired, likely through impaired inhibitory shaping of peripheral and brainstem neurons2. Interestingly, in response to the missed shaping of the peripheral neurons, central brain functions are also affected. BdnfPax2 knock-out mice show a behavioral phenotype consisting of reduced social activity, impaired communication, increased anxiety, impaired learning, and stereotypic self-grooming2. Both the behavioral and the hearing phenotypes are consistent with characteristic symptoms of autism spectrum disorder (ASD)3,4. In addition, preliminary data indicates that this phenotype is linked with altered cGMP signaling (GC-A). As ASD symptoms have been shown to be ameliorated by environmental enrichment, we tested the effect of acute environmental sound enrichment (80 dB SPL, 40 minutes) on the hearing and behavior of BdnfPax2 knock-out mice. Surprisingly, it was found that sound enrichment could almost completely restore the fast auditory processing deficits in BdnfPax2 knock-out mice. We further aim to test the effect of sound enrichment in conjunction with cGMP modulators as a treatment approach to target both the hearing and behavioral phenotype.

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#21

Combined IK channel targeting and glioblastoma irradiation in a syngeneic orthotopic mouse model

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Introduction Glioblastoma, the most common malignant primary tumor of the brain in adults, remains a therapeutic challenge with a median survival of only around 1.75 years1. Inhibition of KCa3.1 channels is an attractive new therapeutic approach, as it both exerts synergistic effects with irradiation2 and temozolomide chemotherapy3, two cornerstones of standard therapy4.

Methods We established the syngeneic orthotopic SMA-560/VMDk glioma mouse model to study the effects of combined glioblastoma irradiation (5x 4 Gy) and systemic KCa3.1 channel inhibition with TRAM-34 (120 mg/kg b.w.). We studied the overall survival of the animals between the four different treatment arms (control, irradiation, TRAM-34 or irradiation + TRAM-34) and the effects on tumor morphology as well as immune cell composition, both in the tumor microenvironment and in the blood.

Results While more than 75% of the animals from the control or TRAM-34 treatment arm reached the endpoint after around 35 days, animals from the irradiation arm only lived slightly longer. However, more than 80% of the animals from the combined irradiation + TRAM-34 treatment arm were still alive 80 days after tumor cell injection. The main difference of tumor morphology in HE stained brain slices was that 80% of the animals from the irradiation arm developed multiple satellite tumors in the brain, distant from the primary tumor site, compared to 17% of the control animals ($p = 0.036$, Chi-square test) and 0 % of animals treated with combined irradiation and TRAM-34 ($p = 0.006$, Chi-square test). We could not identify negative effects of any treatment on the blood count or on the infiltration of CD3+ T cells, CD8+ cytotoxic T cells or FOXP3+ regulatory T cells.

Conclusion Irradiation of glioblastoma may induce a hypermigration of tumor cells, which may be inhibited by concomitant TRAM-34 treatment. This may add to the rationale of inhibiting KCa3.1 in glioblastoma.

#23
De novo cytokine design

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Cytokine receptors stimulate biological responses in diverse cell types, such signalling is activated through cytokine-induced homo- or hetero-dimeric arrangements (or higher oligomers in some cases) of the cognate receptor subunits. While widely deployed to treat haematopoietic disorders, the use of cytokines has expanded to treat clinical indications such as immune disorders, viral infections, and cancer. Clinical deployment of recombinant forms of many native cytokines has been hampered by three major drawbacks, namely: molecular instability, poor receptor- and tissue-specificity, and their limited signalling scope. Our research aims to address these problems by de novo design of novel receptor modulators with tailored biophysical and pharmacological properties through a three-pronged approach.

1) Idealised cytokines with novel structure: All clinically approved cytokine therapies are minimally engineered recombinant forms of their native counterparts. These therapeutic proteins possess irregular structures that are post-translationally modified and suffer poor thermal and proteolytic stabilities. These drawbacks lead to their costly production and pharmacokinetic limitations. By creating cytokine receptor agonists with simpler topologies and idealised structures, we could successfully create hyper-stable cytokine-mimics that are active in vivo. Such agonists possess diverse folds, are free of post-translational modifications, and exhibit remarkable thermal and proteolytic stabilities. We further our initial work on creating G-CSFR agonists, by designing a set of idealised agonists of the TPO, IL11, and FLT3 receptors.

2) Homing cytokines: The functional redunancy of a cytokine across different tissues and the pleiotropic signalling effect of some cytokines across different receptor types hampers many potential cytokine therapies from reaching the clinic, e.g. due to pro-inflammatory or off-target effects, narrowing the therapeutic dose windows of such therapies. Overcoming these two properties (i.e. redundancy and pleiotropy) by designing surface antigen-targetted cytokines can thus unlock novel therapies with minimal off-target activity. Encoding structural motifs on our designs to target unique surface antigens would simultaneously increase the potency and specificity of our cytokines by increasing their local concentrations around the target cells.

3) Novokines; cytokines with novel structure and function: Natural cytokines dimerise and activate 40 cognate pairs of cytokine receptor subunits. These cognate receptor dimers stimulate a unique pattern of different secondary messengers intracellularly, which in turn regulate a set of target genes. Chimeric receptor binders that dimerise non-native receptor pairs have been shown to result in unique and novel signalling patterns, and novel cellular responses. The space of 1600 possible dimers is vastly unexplored, providing a novel means of controlling cells by an extracellular modulator. Moreover, the non-cognate receptor dimerisers have the additional advantage of only activating cell types or stages that simultanously express the pre-defined receptor pair, aiding in specific targetting. We are presently developing single-domain "novokines" that can cross-activate non-cognate cytokine receptor pairs in an all-or-none fashion.

#25

Protein design of growth factor inhibitors

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Growth factors are signaling molecules coordinating the complex functionality of multicellular organisms during development and homeostasis. Since aberrant expression of growth factors can cause diverse disorders such as cancer, autoimmune and cardiovascular diseases, growth factors and their receptors are central targets for therapeutic modulation. One of the options to manipulate signaling interactions is to use protein-based binders that are highly specific and able to target various molecular surfaces. Here, we present two different strategies of computational protein design to obtain inhibitors against growth factors which are key modulators of tumor progression. The first approach requires the structure of a native growth factor:growth factor receptor complex and aims to re-engineer a natural binding domain to make it more soluble, more stable, or more affine. In contrast, the second approach relies only on the structure of a target epitope and takes advantage of new software for massive-scale docking of a target site against a protein structure database to select the high shape complementary scaffolds. Adopting the first approach, we designed an inhibitor of epidermal growth factor (EGF) using a single domain of EGF receptor as a template. Biophysical measurements revealed that the designed inhibitor is solubly expressed, stable, and binds EGF with a submicromolar affinity (i.e. 10-fold stronger than a native domain). Using the second strategy, we designed inhibitors of vascular endothelial growth factor (VEGF) based on two different scaffolds. The binding affinities of the designs to VEGF range from nano- to micromolar levels. X-ray structure determination of one of the candidates showed atomic-level agreement with the design model. Moreover, the best designs showed the ability to inhibit proliferation of VEGF-dependent cells. Thus, our results demonstrate the feasibility of the rational and generalizable approaches to design high-affinity protein binders against predefined conformational motifs.

#27

Defining the localization of epicardial cell clusters in the heterogenous infarcted mouse epicardium

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The epicardium of the adult heart consists of a rather quiescent cellular monolayer. After myocardial infarction (MI) the epicardium becomes activated by upregulating embryonic genes and epicardial cells form a multi-cell layer termed epicardial stromal cells (EpiSCs). EpiSCs play an important role in cardiac healing/repair by secreting paracrine factors that can stimulate cardiomyocyte growth and angiogenesis. Still, little is known about cell heterogeneity and molecular identifiers within the epicardial layer of the post MI heart. The present study characterized the localization of EpiSC populations by RNA in situ hybridization, which were identified within the activated epicardium by scRNA-Seq.

#29

Normoxic HIF1-α induction via A2BR activation in epicardial stromal cells and activated cardiac fibroblasts formed after myocardial infarction

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Introduction: Myocardial infarction (MI) induces the activation of cardiac fibroblasts (aCF) and the de-novo formation of epicardial stromal cells (EpiSC). Both cell types are known to play a crucial role in the post MI healing process by secretion of paracrine factors. In a recent single cell transciptomics study of our group, EpiSC were found to express hypoxia-inducible factor 1 alpha (HIF1-α) and various glycolytic enzymes1 suggesting that the epicardium may be a hypoxic niche. Tissue hypoxia is also well known to stimulate the extracellular formation of adenosine.

Methods: This study investigated the crosstalk between A2B receptor (A2BR) activation and HIF1-α in cultured EpiSCs, isolated from rat hearts 5 days after MI according to a protocol recently reported by us2. Similar studies were also conducted in aCFs, isolated from mouse hearts 7 days after MI. Oxygen consumption and metabolic switches of the cells were analyzed by using Extracellular Flux Technology (Seahorse XFe96).

Results: Using the A2BR-selective agonist BAY 60-6583 we found that even under normoxic conditions, A2BR activation significantly increased HIF1-α mRNA expression in EpiSCs and aCFs. In addition, this activation was associated with a significant decrease in mitochondrial oxygen consumption. In aCFs, normoxic A2BR activation similarly induced a HIF1-α-associated metabolic switch towards glycolysis. Both, the upregulation of HIF1-α mRNA together with the glycolytic switch and decreased oxygen consumption, were also observed in cardiac fibroblasts from healthy mouse hearts.

Conclusion: In summary, even under normoxic conditions, extracellular adenosine can induce HIF1-α in EpiSCs and aCFs, via A2BR activation. The resulting metabolic switch towards glycolysis may be cardioprotective and discloses a novel aspect in HIF1-α regulation.

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#31

Cellular localization of NO-GC in the murine heart and its role in angiotensin II-induced fibrosis

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NO-sensitive guanylyl cyclase (NO-GC) regulates many different physiological functions through generation of cGMP and is also involved in the pathophysiology of several diseases. Vericiguat, an NO-GC stimulator, was shown to exert positive effects in heart failure patients by reducing hospitalizations and overall mortality (VICTORIA trial). However, little is known on the individual cardiac cell types that express NO-GC and the role of the enzyme in cardiac fibrosis and heart failure .

Using immunohistochemistry, we investigated the cellular expression of NO-GC in the murine heart. We also performed tdTomato-based lineage tracing using Cre recombinase under the control of the platelet-derived growth factor receptor ß (PDGFRß) promoter. With this system, we also generated a pericyte-specific NO-GC knockout (PDGFRß-GCKO). We used angiotensin II-releasing mini-pumps to induce cardiac hypertrophy. In addition, basic cardiac parameters were determined.

In the heart, NO-GC is mainly expressed in cardiac pericytes, a PDGFRß-expressing cell type involved in the formation and function of capillaries as well as in cardiac remodeling after injury. In addition, NO-GC expression was identified in smooth muscle cells, but in neither endothelial cells nor fibroblasts. Angiotensin II-induced cardiac hypertrophy was paralleled by the development of fibrotic lesions, which were positive for NO-GC and PDGFRß immunosignals as well as PDGFRß-tdTomato-expressing cells. Pericyte-specific deletion of NO-GC (PDGFRß-GCKO) revealed reduced cardiac contractility after angiotensin II treatment. Taken together, our preliminary experiments indicate that NO-GC in cardiac pericytes plays a role under pathophysiological conditions regarding fibrotic scarring and contractility.

Poster Session II

Thursday, November 18th: 4pm to 5pm
In this session, presenting authors of posters with even ID numbers are kindly requested to be at their posters for discussion and evaluation by the Poster Prize Committee.

#2 Effect of NO-GC stimulators and activators in platelet biomechanics

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Impaired nitric oxide signalling is widely recognized as a hallmark of cardiovascular diseases. Soluble guanylyl cyclase NO-GC stimulators potentiate the NO-mediated cGMP signalling pathway acting synergistically with nitric oxide and amplifying the nitric oxide physiological signal. On the other hand, NO-GC activators do not depend on nitric oxide bioavailability to generate cGMP. cGMP generation activates the cGMP-dependent protein kinase PKG. Vasodilator-stimulated phosphoprotein VASP is phosphorylated by PKG. It is hypothesized that an increase in pVASP may change the platelet cytoskeleton and will lead to F-actin polymerization inhibition. Thus, in order to understand the role of NO-GC in platelets, Riociguat (NO-GC stimulator) and Cinaciguat (NO-GC activator) were tested in human platelets. Platelet biomechanical changes (stiffness) were measured with Scanning Ion Conductance Microscopy (SICM). F-actin and P-selectin quantification were measured with confocal microscopy in a fibrinogen coated petri-dish to emulate biological conditions. The results suggest that stimulation with Riociguat and Cinaciguat lead to decreased expression of F-actin and P-selectin. F-actin polymerization inhibition can be linked with a decreased platelet stiffness and changes in platelet morphology. Morphological changes in circularity were also detected using a neural network based analysis. These data demonstrate the role of NO-GC-cGMP pathway in platelet cytoskeleton and platelet inhibition1. This research will help to understand the mechanistic role of NO-GC-cGMP in platelets and the endothelial-platelet crosstalk in relation to an increased risk of cardiovascular pathologies.

Keywords: Platelet, NO-GC stimulator, NO-GC activator, F-actin, P-selectin, Stiffness.

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#4 GC-A/cGMP signaling is protective for preservation of auditory nerve function following acoustic overexposure and depends on limbic stress responses

Philine Marchetta[1], ***Dorit Möhrle***[1], ***Mirko Jaumann***[1], ***Wibke Singer***[1], ***Marlies Knipper***[1], ***Lukas Rüttiger***[1]

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In the inner ear, cyclic guanosine monophosphate (cGMP) signaling has been described to facilitate otoprotection after noise exposure on peripheral and central hearing, what was observed through phosphodiesterase 5 (PDE5) inhibition (Jaumann et al. 2012). We could show that these protective effects may be related to the cGMP generator guanylyl cyclase A (GC-A), as mice with a genetic disruption of GC-A, display a greater vulnerability in hearing function. In detail, GC-A knockout mice exhibited elevated hearing thresholds over age, inner hair cell (IHC) synapse impairments and reduced amplitudes of auditory brainstem responses (ABR) that progressed with age and with acoustic trauma, when compared to GC-A wildtype littermates, which was not the case for outer hair cell (OHC) function (Marchetta et al. 2020). Thus, the augmentation of natriuretic peptide/GC-A/cGMP signaling likely has potential to overcome hidden- and noise-induced hearing loss, as well as presbycusis (Marchetta et al. 2021). We asked if the assumed increase in cGMP levels through treatment with PDE5 inhibitors on peripheral auditory function might be influenced through effects on the HPA-axis or altered corticosterone (CORT) levels. We analyzed IHC ribbon synapses after acoustic overstimulation and found that an increased trauma-induced loss in the number of ribbons per IHC was linked with increasing CORT levels. This finding was counteracted by PDE5 inhibitor treatment, suggesting that hearing function and IHC ribbon synapse integrity after noise exposure may be influenced by CORT in a cGMP-dependent way (Rüttiger et al. submitted). Indeed, we could show, that changes in the extrahypothalamic stress control, induced through tamoxifen-induced single or double deletion of central stress receptors influenced basal auditory nerve function (Marchetta et al. submitted; Rüttiger et al. submitted). Thereby we conclude that proper cGMP signaling mediated extrahypothalamic control of stress-induced top-down signaling is critical for physiological auditory nerve function.

#6

Stress Receptors Link Auditory Periphery and Cognition via cGMP Signaling

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Glucocorticoids and its receptors mineralocorticoid (MR) and glucocorticoid (GR) receptors affect hearing function in opposing way: elevated corticosterone levels have negative influence on hearing, while they are most common treatment for hearing disorders. The mechanistic explanation for the link between stress mechanisms (HPA-axis) and hearing function is unclear. When stress receptors are centrally deleted under the promoter CaMKIIa, the basal auditory nerve functions are differentially altered which appears to be a top-down mechanism. On the other hand, cGMP signaling cascade has been suggested to have protective effect in auditory pathway against stressful events such as aging, and acoustic trauma (see Poster Philine Marchetta) shown by the deletion of guanylyl cyclase A (GC-A). Here we wanted to investigate GC-A/cGMP signaling cascade by the inhibition of PDE9A, as the molecular mechanism which could link central stress response and auditory periphery.

#8

Measuring the stiffness of neuronal growth cones with scanning ion conductance microscopy

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Dorsal root ganglion (DRG) neurons have previously been used as a system to study axonal branching, an important process in neuronal circuit formation. Studies showed that a cyclic guanosine monophosphate (cGMP) signaling cascade involving c-type natriuretic peptide (CNP) regulates the bifurcation of DRG axons [1,2]. At the end of extending axons, highly motile structures called growth cones can be found.

It was recently demonstrated that nanoscale dynamic structural changes in live neurons can be visualized using scanning ion conductance microscopy (SICM) [3]. SICM can not only image sample topography but also sample stiffness with high spatial and temporal resolution [4]. There is no direct mechanical contact between the probe and the sample during SICM imaging, making it a very suitable technique to study fragile samples such as neurons [5]. Studies investigating the stiffness of growth cones with SICM have not been performed yet.

The aim of this study is to investigate the influence of the cGMP signaling cascade on the stiffness of the growth cones of DRG neurons using SICM. First results indicate that the presence of cGMP reduces the stiffness of the growth cones.

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#10

Role of NO/cGMP and eCB in visual signal processing in the inner retina

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We aim at studying the role of nitric oxide/cyclic guanosine monophosphate (NO/cGMP) and endocannabinoid (eCB) signaling in visual signal processing in the inner retina. To this end, we introduce different biosensors into mouse retinal tissue and present light stimuli that can be used for functional cell type classification. Then, we record the light responses of known types of bipolar cells (BC) or retinal ganglion cells (RGCs) using a two-photon microscope while modulating either NO/cGMP or eCB signaling pathways with pharmacological agents.

#12

Role of CXCR7 in platelet-dependent inflammationand functional recovery of ischemic myocardium

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Background: Platelet activation plays a critical role in thrombosis and thrombo-inflammation. Inhibition of platelet activation is a cornerstone in treatment of acute organ ischemia, however with an increased risk of bleeding. Strategies to limit platelet-mediated thrombosis and inflammation with favorable bleeding complications are warranted. We hypothesized that modulation of the platelet chemokine receptor CXCR7 both interferes with thrombosis and thrombo-inflammation and modulates organ injury following ischemia/reperfusion.

Methods: We generated a megakaryocyte/platelet-specific knock-out mouse (PF4-Cre-CXCR7flox/flox) and characterized platelet function in vitro and in vivo. Further, we performed ischemia/reperfusion experiments (transient LAD-ligation) in mice to assess the effect of genetic CXCR7 deficiency in platelets on tissue inflammation and injury in ischemic myocardium. Tissue inflammation and injury was characterized by gross pathology, histochemistry and immunophenotyping of infiltrating inflammatory cells. In addition, we characterized the effects of CXCR7 activation on platelet function.

Results: We found that expression of CXCR7 on platelets in patients with symptomatic CAD is associated with clinical prognosis. Genetic deficiency of platelet CXCR7 promotes platelet activation (a-granula release, aggregation, intracellular Ca2+, and platelet-mediated thrombus formation ex vivo and in vivo). Further, loss of platelet CXCR7 enhances tissue injury in ischemic myocardium and aggravates tissue inflammation and systemic thrombo-inflammation. Activation of platelet-CXCR7 via specific CXCR7 agonists inhibits platelet activation and thrombus formation and attenuates tissue injury in ischemic myocardium.

Conclusions: We demonstrate that the platelet chemokine receptor CXCR7 is a critical regulator of platelet activation, thrombus formation and organ injury following ischemia/reperfusion. Thus, targeting platelet CXCR7 may be an attractive strategy to control thrombus formation and thrombo-inflammation in the early phase of acute organ ischemia.

#14

Lack of cGKI in myofibroblasts amplifies cardiac fibrosis following Ang II infusion

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Background: Cardiac fibrosis is defined by excessive deposition of extracellular matrix (ECM) proteins produced by cardiac myofibroblasts (CMF) and it contributes to the adverse cardiac remodelling that leads to heart failure (HF) and death. Angiotensin II (Ang II) stimulates cardiac fibroblasts (CF) to undergo a phenotype change to CMFs and its actions have been implicated in hypertensive heart disease and in symptomatic HF. CMFs are characterized by an increased expression of cell surface receptors and production of ECM proteins as well as matricellular proteins such as periostin (Postn), necessary to adapt to the function CMFs perform i.e., the maintenance of the structural integrity of the heart. However, a persistent activation of CMFs results in massive ECM protein deposition and thereby in myocardial fibrosis, ventricular stiffness, pump dysfunction and eventually in HF. Preclinical studies attributed anti-fibrotic effects to cardiac NO-cGMP-cGKI pathway activation, while the role of this cascade in CMF in vivo remains elusive so far.

Methods: We studied a Tamoxifen- (TAM) induced CMF-specific conditional cGKI knockout (PostnCre-cGKIfl/fl = cKO) mouse model and littermate control (PostnCre-cGKI+/+ = CTR) siblings to explore the putative function of cGMP-cGKI signaling in CMF in Ang II induced cardiac remodelling. CMF-specific Cre recombination was assessed in parallel by employing a ROSATg/+ reporter mouse strain. For the induction of cardiac hypertrophy and fibrosis, osmotic minipumps releasing Ang II for 28 days were implanted subcutaneously. Cardiac hypertrophy, cardiomyocyte cross sectional areas as well as cardiac fibrosis were evaluated by histological staining's of heart slices. Cardiac performance was investigated by non-invasive echocardiography in parasternal long-axis in M-mode to determine cardiac function (ejection fraction (EF)), fractional shortening (FS), and wall as well as chamber dimensions) and in B-mode to quantitatively evaluate myocardial deformation (strain, strain rate) using speckle tracking analysis.

Results: The PostnCre-based recombination system enables sensitive and specific detection of CMF in ROSATg/+ reporter mice treated with Ang II for 28 days. By employing the same treatment protocol we demonstrate that fibrotic regions lack cGKI expression in cKO hearts. Heart weight to tibia length ratio, a surrogate parameter for cardiac hypertrophy, increased upon the prolonged Ang II exposure to similar levels in CTR and cKO mice. Although the extent of cardiac hypertrophy was identical in both genotypes, myocardial fibrosis and cardiomyocyte cross sectional areas were significantly increased in the absence of cGKI in CMF. Consistent with this adverse remodelling phenotype, cKO mice exhibited a significant structure-related distortion of global cardiac function and muscle deformation versus CTR mice, confirmed by a worsened EF, FS, reduced longitudinal peak strain, and strain rate.

Conclusion and Outlook: Our data suggest that cGMP/cGKI signaling in CMF attenuates the Ang II-induced pro-fibrotic responses of the heart in vivo. To further validate these data, we aim to gain insights into the molecular and cellular impact of cGMP/cGKI on CMF function(s). In vivo, we will explore whether the potential of cGMP pathway activation to treat or prevent Ang II-induced cardiac dysfunction(s) and adverse remodelling events depends on cGKI in CMF.

#16

Investigating the role of cGMP signalling in hepatic stellate cells

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Hepatic stellate cells (HSCs) are pericytes of the liver located in the space of Disse between sinusoidal endothelial cells and hepatocytes. They play an important role in liver physiology and pathophysiology. In a healthy liver, HSCs are quiescent and contain numerous lipid droplets. Interestingly, HSCs express proteins of the cGMP pathway like the nitric oxide (NO)-sensitive guanylyl cyclase (NO-GC) and the cGMP-dependent protein kinase type I. Under pathological conditions, HSCs are activated and can change their phenotype to proliferative, fibrogenic, myofibroblast-like cells. This transformation of HSCs has been associated with the progression of liver diseases like fibrosis, cirrhosis and hepatocellular carcinoma. Our hypothesis is that the cGMP signalling pathway in HSCs plays an important role in liver homeostasis. In the present study, we investigated the role of the cGMP signalling pathway in primary murine HSCs. As a model for activated HSCs, cells were analysed after 8 days in culture and compared to quiescent cells, which were cultured for 1 day and contained lipid droplets. To visualize cGMP production in live cells in real time, we used HSCs from transgenic cGMP-sensor mice. The NO-donor DEA/NO, a stimulator of NO-GC, triggered cGMP production in quiescent as well as activated HSCs. The small-molecule NO-GC stimulator Riociguat enhanced the NO-induced cGMP increases. C-type natriuretic peptide (CNP), which activates the particulate guanylyl cyclase B (GC-B) triggered the generation of cGMP only in activated cells (e.g., 8 days in culture), which had lost their lipid droplets. Western blot analysis confirmed the presence of GC-B in activated but not quiescent HSCs. These data indicate that changes in the activation state of HSCs are associated with changes in the cGMP signalling pathway, which could play an important role in the development of liver disease. Pharmacological manipulation of this pathway in HSCs may represent a novel strategy for the treatment of liver disease.

Key words: Hepatic stellate cells, cGMP signalling, cGKI, NO-GC, CNP, Riociguat

#18

Role of mechanosensitive cGMP signaling in platelets and thrombus formation

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Thrombotic events remain one of the main causes of death worldwide. There are several antithrombotic therapies available, but most of them bear the risk of internal bleeding. An important endogenous inhibitor of platelet aggregation is nitric oxide (NO). Recent work indicated that NO drives the generation of cGMP in platelets via the NO-sensitive guanylyl cyclase (NO-GC) in a shear-dependent manner, also referred to as mechanosensitive cGMP signaling (mechano-cGMP) [1]. However, the functional and therapeutic relevance of mechano-cGMP for hemostasis and thrombosis is not fully understood. Our aim is to investigate the molecular mechanism behind flow-regulated cGMP signals in platelets as well as the feasibility of its pharmacological modulation. Using co-immunoprecipitation followed by liquid chromatography online-coupled to mass spectrometry (LC-MS), we identified a cGMP signaling complex at the human platelet membrane consisting of NO-GC, cGMP-dependent protein kinase I, and several integrins. Blocking integrin αIIbβ3 led to an attenuation of mechano-cGMP as measured by real-time imaging of cGMP signals in platelet thrombi ex vivo. Thrombosis was analyzed ex vivo via a flow chamber assay and in vivo via a laser-induced thrombosis model in arteries of the cremaster muscle. Pharmacological activation of mechano-cGMP with the NO-GC stimulator Riociguat led to an inhibition of thrombus growth ex vivo and in vivo. Taken together, we propose that integrins play a key role in mechanosensitive cGMP signaling and that pharmacological targeting of mechano-cGMP in platelets with Riociguat might prove to be a valuable strategy to limit thrombosis while evading the life-threatening side effect of bleeding.

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#20

Patient-individual phenotypes of glioblastoma stem cells are conserved in culture and associate with radioresistance, brain infiltration and patient prognosis

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Introduction. Identification of prognostic or predictive molecular markers in glioblastoma resection specimens may lead to strategies for therapy stratification and personalized treatment planning.

Methods. Here, we analyzed in primary glioblastoma stem cell (pGSC) cultures the mRNA abundances of 7 stem cell (MSI1, Notch1, nestin, Sox2, Oct4, FABP7, ALDH1A3), and 3 radioresistance or invasion markers (CXCR4, IKCa, BKCa). From these abundances, an mRNA signature was deduced which describes the mesenchymal-to-proneural expression profile of an individual GSC culture. To assess its functional significance, we associated the GSC mRNA signature with the clonogenic survival after irradiation with 4 Gy and the fibrin matrix invasion of the GSC cells. In addition, we compared the molecular pGSC mRNA signature with the tumor recurrence pattern and the overall survival of the glioblastoma patients from whom the pGSC cultures were derived.

Results. As a result, the molecular pGSC mRNA signature correlated positively with the pGSC radioresistance and matrix invasion capability in vitro. Moreover, patients with a mesenchymal (> median) mRNA signature in their pGSC cultures exhibited predominantly a multifocal tumor recurrence and a significant (univariate log rank test) shorter overall survival than patients with proneural ($\leq$ median mRNA signature) pGSCs. The tumors of the latter recurred predominately unifocally.

Conclusions. We conclude that our pGSC cultures induce/select those cell subpopulations of the heterogeneous brain tumor that determine disease progression and therapy outcome. In addition, we further postulate a clinically relevant prognostic/predictive value for the 10 mRNAs-based mesenchymal-to-proneural signature of the GSC subpopulations in glioblastoma.

#22

Development of a vascularized complex human 3D in vitro skin by combination of iPSC-derived skin organoids and vascular organoids to mimic a vascular network.

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Human skin organoids derived from induced pluripotent stem cells (iPSC) provide unique opportunities for the study of mechanisms of morphogenesis and diseases to complement animal studies. Our current in vitro skin model consists of the two main cell types of skin, i.e., epidermal keratinocytes and dermal fibroblasts, extracted from the foreskin of human donors. Both cell types are combined with a collagen scaffold to mimic dermis and epidermis and cultivated in an air-liquid interface. These three-dimensional (3D) full-thickness skin equivalents (FTSE) are suitable as simple pharmacological test systems.

To improve our in vitro skin model, we use self-organized skin organoids, which create complex tissue via cell-cell interaction. These organoids mimic complex structures like hair follicles, neuronal networks and other cell types (e.g., adipocytes, chondrocytes) which can improve our current skin model.

However, iPSC-derived skin organoids lack a functional vasculature. To address this drawback, we used a second iPSC-derived vascular organoid to introduce a vascular network into the in vitro skin model. We could show that endothelial cells (CD31+) and smooth muscle cells (SMMHC+) form tube-like structures growing into the FTSE. Cells of the vascular organoid express NO-GC, the main target for NO which plays an important role during angiogenesis and arteriogenesis. Vascular organoids represent a perfect tool to study morphogenesis of human vasculature and serve as in vitro test systems for disease modelling and drug screening.

We already embedded vascular organoids into FTSE to generate vascularized FTSE. In parallel, skin organoids are generated to be embedded into the vascularised FTSE to form a vascularized complex human 3D in vitro skin. In summary, human in vitro 3D vascularized skin will shed light on the role of NO/cGMP signalling in angiogenesis and will help to develop cGMP-modulating drugs to treat skin defects and improve skin regeneration.

#24

The role of the sodium-activated potassium channel Slack in kainic acid and pilocarpine induced epilepsy models

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The rare epilepsy disorders Epilepsy of Infancy with Focal Migrating Seizures (EIFMS) and Autosomal Dominant Nocturnal Frontal Lobe Epilepsy (ADNFLE) are characterized by early onset of severe seizures and are refractory to anti-epileptic drugs. Both disorders are associated with mutations in KCNT1, the gene encoding the sodium-activated potassium channel Slack. To date approx. 60 pathogenic mutations are known, most of which cause a gain-of-function (GOF), and thereby an increased K+ current amplitude. While GOF was shown to result in overexcited nervous tissue, also a loss-of-function Slack variant is linked to epilepsies and increased neuronal vulnerability in vivo and in vitro.

As Slack crucially modulates neuronal excitability, we investigated its role during epileptic seizures using wildtype (WT) and global Slack knock-out (KO) mice in kainic acid (KA) and pilocarpine (Pilo) induced acute epilepsy models. Compared to WT, 12-week-old adult and 4-week-old juvenile KO mice display increased seizure scores and mortality after both KA and Pilo application. This indicates a neuroprotective role of Slack during epileptic seizures in vivo. Strikingly, 24 h after seizures, GFAP, BDNF and GluA1/2 mRNA levels are equal between KA treated WT and KO, suggesting that genotype-specific effects are limited to acute epilepsy. Hippocampal slice cultures of WT and KO mice were exposed to KA and cell death was quantified by propidium iodide uptake. Compared to WT, sclerosis-like neuronal cell loss was significantly increased in the CA3 region of Slack KO, confirming in vivo findings obtained from KA challenged mouse brains. These findings were further verified in dissociated primary hippocampal neurons by recording real time Ca2+ dynamics during KA exposure using Fura-2AM. At low KA concentrations KO neurons exhibited more Ca2+ influx compared to WT. This changes in Ca2+ dynamics cannot be modulated by the AMPA or NMDA receptor blockers NBQX and AP5, suggesting dysregulation of another Ca2+ source or alteration of intrinsic membrane properties to underly the KA-induced Slack-specific effect. Ongoing experiments use genetically encoded biosensors to monitor K+ dynamics in real-time during epileptic neuronal activity.

We propose that Slack dependent K+ efflux crucially modulates neuronal activity during epileptiform high frequency firing of glutamatergic and cholinergic neurons. By limiting firing rate via prolonged after hyperpolarization, Slack channel modulation might help to specifically cure otherwise untreatable epilepsy syndromes.

#26

IRAG2 interacts with IP3-receptor types 1, 2 and 3 and regulates intracellular calcium

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Background: The inositol 1,4,5-triphosphate receptor-associated 2 (IRAG2), also known as lymphoid-restricted membrane protein (LRMP) or Jaw1, is a type II membrane protein that is localized to the cytoplasmic face of the endoplasmic reticulum (ER) and shares a homology of 44% with the inositol 1,4,5-triphosphate receptor-associated cGMP kinase substrate 1 (IRAG1), especially in its coiled-coil domain. As IRAG1 interacts with IP3-receptors (IP3R) via its coiled-coil domain and modulates Ca2+ release from intracellular stores, we investigated if IRAG2 has similar interaction partners like IRAG1. Moreover, the aim of our work was to study if IRAG2 also modulates intracellular Ca2+ release.

Methods: We investigated expression and localization of IRAG2 in the pancreas via X-Gal-staining, using IRAG2-KO mice, that express β-galactosidase as a reporter for IRAG2. Via co-immunoprecipitation interaction of IRAG2 with different IP3-recepors in the pancreas was analyzed. Expression of different IP3-receptor subtypes was determined and quantified. Furthermore, we analyzed Ca2+ signaling in WT and IRAG2-KO pancreatic acinar cells by Fura-2-AM ratiometry using carbachol as stimulating agent.

Results: IRAG2 was expressed in the pancreas and X-Gal-staining showed localization of IRAG2 in pancreatic acinar cells. Co-immunoprecipitation revealed interaction of IRAG2 with IP3-receptor subtypes 1, 2 and 3 in the pancreas. We detected a higher expression of IP3R3 in pancreata from IRAG2-KO mice compared to WT mice, whereas expression of IP3R1 and IP3R2 was not altered between WT and IRAG2-KO. IRAG2-KO cells revealed lower basal, unstimulated intracellular Ca2+-levels than WT cells, though carbachol induced Ca2+-release normalized to basal release is higher in IRAG2-KO cells.

Conclusion: Our data suggest that IRAG2 modulates intracellular Ca2+ signaling and increases basal Ca2+ release in pancreatic acinar cells. Loss of IRAG2 and the resulting altered Ca2+ release could therefore modify secretion of digestive enzymes in the exocrine pancreas, that play a critical role in development of pancreatic diseases.

#28

Quantitative secretome analysis in cultured cardiac fibroblasts isolated from the infarcted heart using SILAC labeling combined with Click-Chemistry

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Introduction

Cardiac repair after myocardial infarction (MI) starts right after reperfusion and involves cardiac fibroblasts (CF) as key players in cardiac remodeling. In the early phase, CF secrete proinflammatory proteins, cytokines and proteases which degrade the damaged tissue. Thereafter, cardiac fibroblasts are activated (aCF) and proliferate producing collagen and other extracellular matrix proteins that promote scar formation [1]. In a recent single-cell mRNA sequencing study our group has shown that activated cardiac fibroblasts (5 days post MI) express numerous paracrine factors which potentially may influence inflammation and myocardial contraction [2]. In the present study we explored the secretome of cultured CF and aCF using sensitive and specific mass spectrometry methods.

Methods

We used a recently published technique that combines stable isotope (SILAC) labeling and Click-Chemistry [3] which enables the quantitative secretome analysis of newly synthesized proteins of CF and aCF in-vitro. Cardiac fibroblasts were isolated 5 days after MI (50 min ischemia followed by reperfusion) or 5 days after sham surgery (control). As SILAC uses the metabolic incorporation of "heavy" or "intermediate" 13C- or 15N-labeled amino acids into proteins followed by mass spectrometry (MS), we compared the secretome of CF and aCF obtained after MI with sham surgery. In brief: cultured CF and aCF were depleted of arginine (Arg), lysine (Lys) and methionine (Met) for 1h. Thereafter, cells were labeled with stable isotopes: either heavy arginine and lysine isotopes or intermediate isotopes plus azidohomoalanine AHA for 6h. The azide group of AHA allows binding of these proteins to a biotin resin (CLICK reaction) so all other proteins and contaminants can be removed by washing.

Results

We identified 84 proteins secreted from cardiac fibroblasts obtained 5 days post MI. Biologically interesting is the significant increase in Lysyl Oxidase Like 3 (Loxl3) compared to CF of sham-operated hearts. Loxl3 oxidates lysyl and hydroxyl residues from collagen and plays a crucial role in epithelial-mesenchymal transition and matrix crosslinking after MI [4]. In addition we found a higher abundance of collagens such as Col1a1 and proinflammatory proteins such as Chemokine (C-C motif) ligand 9 (CCL9). Furthermore we identified several proteins which play a role in cell signaling and migration e.g. Microfibril Associated Protein 5 (Mfap5), Sushi Repeat Containing

Protein X-linked 2 (Srpx2) and Tyrosine-protein Kinase Receptor UFO (Axl) which regulates cell migration as well as cell proliferation [5].

Conclusion

Quantitative secretome analysis of CF and aCF formed after MI is possible using a combination of SILAC labeling and Click-Chemistry. Loxl3 was found to be significantly increased after MI other proteins such as CCL9, Mfap5, Srpx2 and Axl showed higher abundance. These proteins may be functionally relevant targets in future investigations.

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#30

Monitoring metabolic changes by deuterium MRI in heart and BAT after I/R

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Introduction:

Brown adipose tissue (BAT) has long been known only for its thermogenic capacity. New studies suggest that it may also have an influence on cardiovascular disease. Acute myocardial infarction (AMI) leads to massive sympathetic activation of BAT, which in turn could impact on cardiac outcome after MI. In the present study, we investigated the consequences of BAT activation on lipid and glucose homeostasis using a non-invasive magnetic resonance imaging (MRI) approach to simultaneously assess BAT and heart metabolism in vivo1,2. To this end, we employed deuterium metabolic imaging as novel, non-invasive method for monitoring the metabolic pathways of deuterated substrates3.

Methods:

For BAT activation, mice received an i.p. injection of a β3-specific agonist [1µg/g], which was verified by T2 mapping after 1 h. after 1 h. In separate experiments, mice were subjected to deuterium metabolic imaging (DMI) using a dedicated 25mm-^{2}H/1H resonator. After anatomical localization, field map-based shimming followed by manual adjustment was performed to optimize magnetic field homogeneity in the region of interest. 2H-MR spectra were acquired over the entire thorax to resolve organ-specific glucose metabolism. Exponential weighting was applied, resulting in a 10 Hz line broadening, and chemical shifts were referenced to the resonance frequency of water at 4.7 ppm. After acquisition of baseline spectra, mice received an i.p. bolus injection of 2 mg/g [6,6-2H2]glucose, followed by monitoring of deuterated metabolites for 60 min.

Results and Discussion:

We acquired spatially resolved 2H MR spectra to evaluate the turnover/metabolic rate of deuterated glucose tracer in the heart and in the BAT simultaneously. In a first step, the BAT was pharmacologically activated by using a β3-agonist. The baseline spectra showed a high signal at 4.7 ppm caused by the natural abundance of 2H in water4. Bolus injection of [6,6-2H2] glucose leads to an increase of the corresponding 2H signal of glucose at 3.8 ppm in both tissues. As compared to baseline spectra, the BAT showed increased lactate levels at 1.3 ppm in parallel to an enhanced glucose consumption, which indicates a specific activation of this tissue. To verify whether ischemia and reperfusion (I/R) results in a similar response, we subjected mice 1 day before and one day after I/R to this protocol. One day after induction of myocardial infarction, there was indeed again a strong rise of the lactate signal and an enhanced glucose consumption in the heart and even higher in the BAT as well. These results indicate increased glucose flux by glycolysis after I/R in both tissues.

Conclusion

Our results demonstrate that 2H-MRI can be successfully used for simultaneous in vivo monitoring of metabolic alterations in multiple organs. Enhanced glucose flux through glycolysis can be detected in BAT, specifically induced by the β3-agonist, and in heart and BAT after I/R, whereby

even stronger changes were detected in the BAT.

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