6th Munich Medical Student

Science Conference 2022

„Science, more than ever“

Keynote Talk

"Violence against women and girls: a global overview“

Prof. Dr. Heidi Stöckl

MMS SCIENCECON

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10 am-5 pm

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Contents

Welcome message by the Dean of Research

Dear students,

due to their contribution of significant research findings Medical Faculties and University Hospitals have been increasingly perceived as important pillars of politics and society in the last two years. I am pleased to see the successful face-to-face continuation of the Munich Medical Student Science Conference MMS ScienceCon, which combines students' research projects from different fields, united by this year's conference theme "Science, more than ever". These projects show the broad range of basic, translational and clinical research and illustrate curiosity-driven research activities during both medical training and medical practice.

Research at the Medical Faculty and the University Hospital of LMU Munich covers a broad spectrum summarized by its mission statement "Biomedicine for Life and Quality of Life". Since last year, its six key areas - Molecular Biomedicine, Fighting Cancer, Inflammation and Infection, Vascular and Transplantation Medicine, Neuroscience, and Medicine for Society - are horizontally linked by the two methodological cross-sectional areas - Personalized Medicine and Digital Medicine.

The Dean's Office supports these areas by funding students' research projects as well as Medical and Clinician Scientist programs, which make it possible to follow your curiosity and link healthcare with first-rate science. In this context, please take a look at the overview of funding on the homepage of the Faculty of Medicine: https://forschungsportal.med.uni-muenchen.de/

I thank Dr Ruiß as well as the whole team organizing the Munich Medical Student Science Conference 2022, and I wish you many interesting contributions and discussions.

Prof. Dr. Stefan Endres
Dean of Research

Welcome message by the Associate Dean

„Aber Forschung braucht viel Zeit. Zeit zum Lesen der ständig neu erscheinenden Fachartikel, Zeit zum Nachdenken, Zeit zum Ausprobieren neuer Ideen.“ (1)

This quote comes from my teacher, the late Michael Frotscher, at the time professor of anatomy in Freiburg and then Hamburg.

There is certainly the danger that you might end up as the person described by Mary Wollstonecraft Shelley in her book ‚Frankenstein, or the Modern Prometheus‘. As summarized by Lorraine Daston, this scientist Viktor Frankenstein „vernachlässigt über seine Forschungen in Anatomie und Galvanismus nicht allein Freunde und Familie, seine Isolation verdirbt seinen ansonsten edlen Charakter, so dass er jegliche moralische Orientierung verliert.“ (2)

So take good care when striving for a career in science to strike your balance: avoid becoming too much like Viktor and engage fully in science. Apply and enjoy your creativity in life and in science. It is possible.

I am looking with great sympathy at your early steps in science and may learn later about your Victorian developments.

Enjoy the day!

Michael Meyer MD

Univ. Prof. of Molecular Neurophysiology
Associate Dean
Medical Faculty

1 https://www.gesundheitsindustrie-bw.de/fachbeitrag/aktuell/michael-frotscher-am-wichtigsten-ist-die-kreativitaetc

2 Lorraine Daston, Eine kurze Geschichte der wissenschaftlichen Aufmerksamkeit, Carl Friedrich von Siemens Stiftung, München 2001

Conference Timetable

Time	*Description*
09:30	**Participant Registration at the reception desk**
10:00	**Opening Ceremony** Welcome by ***Ayse Gertrude Yenicelik***
10:30	**Talk Session 1** 1) Loss of Two-Pore Channel 2 (TPC2) Reduces Migration of Melanoma Cells ***Sarah Bruchmann (AG Grimm)*** 2) Spatially resolved lipidomic analysis of the arrhythmic mouse brain by MALDI-IMS ***Kaela George (AG Lahiri)*** 3) Determination of nanobody binding sides of the mouse P2X7 receptor ***Emilie v. Poblotzki (AG Nicke)*** 4) An easy analysis of bulk RNA-Seq data using open access software packages ***Anna Litovskikh (AG Dietrich)*** 5) Growth of Tetrahymena thermophile ***Georg Scheffler*** 6) Direct neuronal reprogramming of astrocytes into cortical deep layer neurons ***Christina Schwenk (AG Götz und Masserdotti)***
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12:30

Talk Session 2

7) Stress coping strategies in German adolescents during the Covid-19 pandemic

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8) Role of Erlotinib and PP2 in cardiomyocyte cohesion and possible implication in arrhythmogenic cardiomyopathy

Philipp Menauer (AG Waschke)

9) Effects of repetitive neuromuscular magnetic stimulation targeting the upper trapezius muscles on pressure pain thresholds in children and adolescence with headache disorders

Amelie Amann (AG Bonfert)

10) Armoring anti-HER2 CAR-T cells with C-C-motive receptor 8 (CCR8) and a dominant negative TGF-b receptor (DNR) to enable efficacy in solid tumor models

Thaddäus Strzalkoski

11) Effects of expert eye-tracking videos with cued retrospective reporting on medical students' ECG interpretation skills

Aline Scherff

12) Influence of diabetes and chronic kidney disease on heart rate and blood pressure within a swine model. A summary of results obtained through monitoring using telemetry systems.

Raphael Rottenkolber (AG Merkus)

14:00	**Coffee Break** **combined with Poster Walk**
14:45	**Talk Session 3** 13) Intracellular distribution of calbindin D-28k in neurons – quantification by analysis of immunofluorescence images ***Aleksandra Jucha and Hannah Schmidt (AG Meyer)*** 14) Examining the possible interaction of calbindin-d28k and IMPA1 ***Leon Ebel (AG Meyer)*** 15) The Critical Micelle Concentration and its Influence on Preparation and Properties of siRNA Loaded Polyplexes ***Lamija Ibrahimpasic (Ag Merkel)*** 16) Investigation of TLR4-based resistance in SK-OV-3 cells through LPS treatment ***Erika Lynn Roberts (AG Merkel)***
15:45	**Keynote talk** Violence against women and girls: A global overview ***Prof. Heidi Stöckl***
16:45-17:00	**Awards and Closing Ceremony** Farewell by ***Prof. Dr. Michael Meyer***

Keynote by Prof. Dr. Heidi Stöckl

Violence against women and girls: a global overview

Violence against women and girls - in its multiple forms - is an important human rights abuse, and public health problem. There is a growing body of population-based evidence on the prevalence of different forms of violence, with global estimates suggesting that at least one in three women has experienced physical and/or sexual intimate partner violence or non-partner sexual violence in their lifetime, and that every third murdered woman is murdered by an intimate partner. Prevalence of intimate partner violence and non-partner violence varies widely across regions and population groups, highlighting important risk and protective factors that lead to an increase or decrease in intimate partner violence. Violence against women and girls is not only a public health issue in itself, it is also associated with a number of adverse health outcomes such as depression and anxiety, alcohol use, miscarriage, and injuries as well as unhealthy behaviours, including risky sexual behaviour. Violence against women and girls is preventable, with strong evidence on effective programming including in the health sector.

Tackling violence against women and girls is a policy priority for many governments and international governmental and non-governmental organisations seeking to improve the lives and health of women, children, families and men. This talk will provide an overview of the conceptualisation prevalence and measurement of violence against women and girls, its health impacts, risk and protective factors and summarise evidence and guidance on effective prevention strategies and health sector responses.

Heidi Stöckl is a Professor of Public Health Evaluation at the Ludwig-Maximilians-University Munich, Germany. Prior to Joining LMU, she has been a professor of Social Epidemiology at the Department for Global Health and Development at the London School of Hygiene and Tropical Medicine. Prior to that she has completed her DPhil in Evidence-based Social Intervention at the University of Oxford, Nuffield College, conducting the first study on intimate partner violence during pregnancy in Germany. Heidi Stöckl also holds an MSc in Sociology from the University of Oxford and a Diploma in Political Science from the Free University Berlin. Heidi Stöckl is currently holding an ERC Starting Grant to investigate the risk and protective factors and consequences of intimate partner violence through a mixed-methods longitudinal study in Mwanza, Tanzania.

Abstracts in the order of presentation

1) Loss of Two-Pore Channel 2 (TPC2) Reduces Migration of Melanoma Cells

Sarah Bruchmann[1], Carla Abrahamian[1], Christian Grimm[1]

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Aim: Melanoma is a malignant tumor of melanocytes, neural-crest derived cells, that produce the pigment melanin. A high mutation rate, metastatic potential and rising incident rates stress the importance of a more detailed understanding of tumorigenesis, proliferation and migration of this type of cancer. In recent years, endolysosomal cation channels have emerged as potential targets for cancer, specifically, therapy has been established: Two-pore channels (TPC2), encoded by *MCOLN1* and *TPCN2* genes, respectively[1].

TPC2 is a non-selective cation channel, which resides in late endosomes, lysosomes, and melanosomes and regulates many endolysosomal trafficking processes. This channel has been found to play an essential role in proliferation, migration, and invasion of melanoma cells[2].

This study aims to further confirm this role, focusing on the migratory phenotype of TPC2 knockout (KO) melanoma cells *in vitro*.

Methods: TPC2-KO was created using CRISPR-Cas9 gene editing in the SK-MEL-5 melanoma cell line. The KO model was confirmed via different methods, including standard polymerase chain reaction (PCR), agarose gel electrophoresis, qPCR for measuring gene expression, and sanger sequencing. Migration wound healing assay was then performed.

Results: The wound scratch assay revealed a significant difference in the recovered area of wildtype (WT) vs knock-out, monitored over 24- and 48-hours, confirming a reduced migration rate of the TPC2-KO melanoma cells, as compared to the WT.

Conclusion: Our findings provide evidence that TPC2 is a key player in melanoma migration, suggesting this channel as an important target for melanoma therapy.

References:

2

[1]Netcharoensirisuk, Abrahamian, Tang, et al. (2021). Flavonoids increase melanin production and reduce proliferation, migration and invasion of melanoma cells by blocking endolysosomal/melanosomal TPC2
[2]Abrahamian, Grimm (2021). Endolysosomal cation Channels and MITF in Melanocytes and Melanoma

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2) Spatially resolved lipidomic analysis of the arrhythmic mouse brain by MALDI-IMS

Kaela George, Frederike Schäfer, Shibojyoti Lahiri

Imhof Group, Protein Core Unit, Biomedical Center, Ludwig-Maximilians University Munich

Life on earth requires the adaptation to daily changing light conditions. To synchronize their physiological functions to the changing environmental conditions, most organisms developed an internal clock. This circadian rhythm influences a variety of functions ranging from behavior down to molecular pathways. While its disruption can generate a wide spectrum of pathologies, exact mechanisms of action are still not fully understood. Especially molecular alterations in the brain are largely unknown. Here, lipids are of particular interest due to their crucial role in neuronal functionality. The complexity of the brain necessitates the consideration of regional specificity in the analysis of molecular changes. MALDI Imaging Mass Spectrometry (MALDI-IMS) is a technique that provides the advantage of detailed *in situ* tissue composition analysis with corresponding spatial information Therefore, this project aimed at identifying changes in regional lipid composition upon circadian disruption using MALDI-IMS. To model a disturbance of the internal clock, *Cryptochrome 1/2* deficient mice were used. Lack of these core clock proteins induces arrhythmicity and manifests in behavioral abnormalities. While similar lipid clustering according to brain regions were observed, the two genotypes displayed varying compositions of these clusters. Additionally, individual lipid species showed dynamic variations across the circadian day, affecting lipid classes like glycerophospho-ethanolamines or glycerophosphoserines. Lipid clusters were found to be adequate markers for the differentiation of functional brain areas and display unique compositional changes in the healthy and diseased states. These findings highlight once more the importance of regional analysis in general and demonstrate the importance of neuronal lipid species in particular as they show significant regional differences. Current work seeks to further link brain lipid composition with manifestation of disease and ageing which could provide new medical treatment approaches.

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3) Determination of nanobody binding sides of the mouse P2X7 receptor

Emilie v. Poblotzki

The P2X7 receptor is a trimeric ATP gated non-specific cation channel. It is often present on immune cells and function as a “danger signal detector”. It has an important role in inflammatory processes. P2X7 activation helps to eliminate bacteria and initiates the release of different interleukins. After the activation it can make the plasma membrane permeable for large cations.

Nanobodies are made of the variable region of heavy chain only antibodies. Camelids produce heavy chain only antibodies used to make nanobodies. Nanobodies are more soluble and their smaller size allows binding into clefts that are inaccessible to normal antibodies. Nanobodies have a high potential in medicine. For example, a combination of nanobodies is used in a therapy to treat covid19 disease.

The aim of the thesis was to find the nanobody binding sides on the P2X7 receptor. Because the investigate nanobody binds only to the mouse P2X7 receptor not on the human or rat receptor, the amino acid sequences were compared and the differing residues were substituted by the human residues in the mouse receptor.

Oocytes from *Xenopus laevis* were used to express the receptors. After injection of the respective in vitro-synthesized RNA, the receptor currents were recorded by two-electrode voltage clamp. Based on the analysis of various mutants and chimeras, amino acids that reduced or prevented nanobody binding were then combined and tested again. Several amino acid residues that contribute to the nanobody binding site could be identified.

Outlook: A mouse mutant combining all point mutations that are showing some binding effects will be generated and for the final proof of concept a reverse human receptor with introduced mouse residues will be made. In addition, structural analysis by cryo electron microscopy is planned.

References:

1. „Full-Length P2X(7) Structures Reveal How Palmitoylation Prevents Channel Desensitization.", McCarthy AE, Yoshioka C, Mansoor SE
2. „Electrophysiological recording from Xenopus oocytes“, Stühmer W.
3. “Nanobodies that block gating of the P2X7 ion channel ameliorate inflammation“ Danquah W, Meyer-Schwesinger C, Rissiek B, Pinto C, Serracant-Prat A, Amadi M, Iacenda D, Knop JH, Hammel A, Bergmann P, Schwarz N, Assunção J, Rotthier W, Haag F, Tolosa E, Bannas P, Boué-Grabot E, Magnus T, Laeremans T, Stortelers C, Koch-Nolte F.
4. “P2X7 Interactions and Signaling - Making Head or Tail of It.” Kopp R, Krautloher A, Ramírez-Fernández A, Nicke A. Front Mol Neurosci. 2019 Aug

5. Max Planck Institute: https://www.mpg.de/17264487/0722nanobodies

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4) An easy analysis of bulk RNA-Seq data using open access software packages

Anna Litovskikh, Philipp Alt, Suhasini Rajan, Lena Schaller, Fabienne Geiger and Alexander Dietrich

Walther-Straub-Institute of Pharmacology and Toxicology, LMU-Munich, Germany

Purpose/Background: Impact of different triggers may disrupt cell integrity of the lung epithelium. A well-regulated wound healing response by transforming-growth-factor β1 (TGF-β1), which induces fibroblast-to-myofibroblast differentiation restores the tissue. However, if this process is dysregulated, excess proliferation of myofibroblasts with accumulation of extracellular matrix (ECM) produces lung fibrosis with a severe reduction in gas exchange in patients[1].

Recent reports indicate a role of TRPV4 channels in fibroblasts and the lung epithelium. To identify candidate genes of TRPV4-regulated pathways in these tissues, cells from human biopsies as well as from mice with or without a defective *trpv4* gene were isolated. Selected fibroblasts were incubated with TGF-β1 or solvent. RNA was isolated and sent out for a transcriptomic analysis to IMGM laboratories (Martinsried, Germany). Tools from open access software packages were evaluated for an efficient analysis of bulk raw RNA-Seq data.

Methods: First, a decoy-aware transcriptome index was generated for the latest version of GRCm39 Mus Musculus reference assembly. Raw RNA-seq data were quantified in a Salmon mapping-based mode with selective alignment. Obtained transcript quantification data were summarized at the gene level with the tximeta R package. Differential gene expression analysis was performed using the DESeq2 R package and the top differentially expressed genes between WT and TRPV4-/- cells as well as samples with and without TGF-β1 were plotted on heatmaps and volcano plots using the gplots and ggplot2 R packages.

We also performed a knockout validation for protein samples from TRPV4-/- mice via Western Blot analysis with a specific TRPV4 antibody.

Results: An analysis with open access software packages identified several promising gene candidates in TRPV4-regulated pathways in fibroblasts and epithelial cells, which need to be verified in biochemical assays in the future.

Conclusion: Open access software packages are suitable to identify differentially regulated genes in fibroblasts and epithelial cells.

References:
[1]Wilson MS, Wynn TA. Pulmonary fibrosis: pathogenesis, etiology and regulation. *Mucosal Immunol*. 2009;2(2):103-121. doi:10.1038/mi.2008.85

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5) Growth of Tetrahymena thermophila

Georg Scheffler

Purpose: Research needs model organisms. The unicellular eukaryote ciliate *Tetrahymena thermophila* is used as a model organism in cellular and molecular biology[1], and toxicology[2]. The aim of this project was to investigate the effect of media and iron concentration changes on the growth of two strains of *T. thermophila* (B2086.1 and CU428.2). The effects of iron concentration changes will be important for experiments with toxins that serve as iron chelators.

Methods: A standard media3 consists of peptone from casein and was changed to peptone from soy. A standard iron concentration amounts to 33µM FeCl33 and was reduced by 25% and 50%. The cell concentration of samples (12 overall) with different combinations of media types, iron concentrations and cell lines was measured by counting with the Neubauer chamber every 24 hours for 3 days. Two experiments have been done consisting each of 3 repetitions of this procedure with different colonies to prove biologic replicability and collect enough data. The statistical analysis was done with generalized linear models in Flexplot4, a package in R.

Results: The first experiment failed, while the second succeeded. The media type change and reduction of the iron concentrations have an effect on the cell concentration respectively the growth of T. thermophila and the two variables show interaction. The media with a combination of the peptone from casein and an iron concentration of 33µM FeCl3 showed the highest growth. The change of the peptone seems to have a bigger effect on the cell concentration than the reduction of the iron concentration.

Conclusion: When choosing the media composition for experiments with T. thermophila, the components of the media have to be chosen wisely and set in consideration of each other. In addition the peptone source could affect experiments where the iron concentration is changed due to toxins or bacteria.

References:

1. Cassidy-Hanley DM. Tetrahymena in the Laboratory: Strain Resources, Methods for Culture, Maintenance, and Storage. *Methods Cell Biol*. 2012;109:237. doi:10.1016/B978-0-12-385967-9.00008-6
2. Chasapis CT. Preliminary results from structural systems biology approach in Tetrahymena thermophila reveal novel perspectives for this toxicological model. *Arch Microbiol*. 2019;201(1):51-59. doi:10.1007/S00203-018-1571-6/TABLES/3
3. Cassidy-Hanley D, Bowen J, Lee JH, et al. Germline and somatic transformation of mating Tetrahymena thermophila by particle bombardment. *Genetics*. 1997;146(1):135-147. doi:10.1093/GENETICS/146.1.135
4. Fife D. Flexplot: Graphically-Based Data Analysis. *Psychol Methods*. Published online 2021:1-20. doi:10.1037/MET0000424

6) Direct neuronal reprogramming of astrocytes into cortical deep layer neurons

Christina Schwenk[1], Giacomo Masserdotti[1,2] and Magdalena Götz[1,2]

[1]Physiological Genomics, Biomedical Center, Ludwig-Maximilians-University; [2]Institute of Stem Cell Research, Helmholtz Center Munich

Neurodegenerative diseases are an increasing health problem in ageing society. In most cases, neurodegenerative diseases are sporadic, while familiar cases have been associated to specific disease-causing genes. For instance, Huntington's disease is caused by the expansion of polyglutamine (polyQ) repeats within the Huntingtin gene[1]. This affects primarily striatal neurons, but also deep layer neurons in the cortical gray matter, leading to movement disorder and cognitive disfunction. While attempts to replace striatal neurons are ongoing, a novel strategy to delay the onset of HD disease and improve patients' lifespan would replace lost cortical deep layer neurons with functional ones.

Direct neuronal reprogramming is a very promising avenue in cell-based therapeutic strategies[2]: it aims at converting differentiated cells into functional neurons without a proliferative and pluripotent intermediate step[3]. Astrocytes are an interesting cell source for direct neuronal conversion, as they are widespread in the nervous system and retain developmentally established patterning cues[3].

The research lab of Prof. Goetz has recently started to evaluate whether combinations of transcription factors could reprogram primary cultures of astrocytes into deep layer neurons. Candidate TFs Lmo4 and Sox5 were selected based on the literature[4] and co-expressed with the main reprogramming factors – Neurogenin2 (Ngn2) or Ascl1[3]. During my internship, I analysed the reprogramming efficiency following the expression of different combinations of factors by counting the

proportion of cells with a neuronal morphology and expressing the neuronal marker β3-tubulin over the co-transduced astrocytes.

Preliminary results suggest that Ngn2-Lmo4 co-expression improves reprogramming efficiency compared to the expression of Ngn2. Conversely, the co-expression of Ascl1 and Lmo4 does not increase efficiency compared to Ascl1 alone. Remarkably, Sox5 expression dramatically reduces either Ngn2 or Ascl1-mediated direct reprogramming.

The next step will be to investigate whether Ngn2-Lmo4 combination generates functional cells that resemble layer 5 neurons, or if other combinations/more factors will be needed to obtain specific neuronal subtypes.

References:
[1]Caron NS, Wright GEB, Hayden MR. Huntington Disease. 1998 Oct 23 [updated 2020 Jun 11]. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Mirzaa GM, Amemiya A, editors. GeneReviews® Seattle (WA): University of Washington, Seattle; 1993–2022.
[2]Barker RA, Götz M, Parmar M. New approaches for brain repair-from rescue to reprogramming. Nature. 2018 May;557(7705):329-334. doi: 10.1038/s41586-018-0087-1. Epub 2018 May 16.
[3]Bocchi R, Masserdotti G, Götz M. Direct neuronal reprogramming: Fast forward from new concepts toward therapeutic approaches. Neuron. 2022 Feb 2;110(3):366-393. doi: 10.1016/j.neuron.2021.11.023. Epub 2021 Dec 18.
[4]Lai T, Jabaudon D, Molyneaux BJ, Azim E, Arlotta P, Menezes JR, Macklis JD. SOX5 controls the sequential generation of distinct corticofugal neuron subtypes. Neuron. 2008 Jan 24;57(2):232-47. doi: 10.1016/j.neuron.2007.12.023.

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7) Stress coping strategies in German adolescents during the Covid-19 pandemic

Mariia Bashaeva, Belinda Platt

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Purpose: Covid-19 pandemic had a great outcome on the life of people of all ages[1],[2], but maybe even more drastically on growing up adolescents who was exposed to a limitation in contact with peers and stress due to the changing life routine[3],[4],[5]. The aim of the study was to investigate the factors that positively or negatively affected mental well-being of adolescents during the pandemic as well as obtain an overview of recommendations from study participants to other adolescents in the same condition.

Methods: Data was collected from 195 adolescents in Germany aged from 12 to 18 via free text questions in an online survey essay, and was then analyzed using the Kuckartz qualitative content analysis methodology[6].

Results: A total of 62 different positive factors, 94 negative factors and 65 recommendations were observed. Talking to other people, maintaining hobbies, doing sport and contacting friends appeared to be the most common factors that improved mental wellbeing of the study participants with 40%, 35%, 28% and 26% of the participants mentioning them. Maintaining interests and talking to others were also mentioned prominently as recommendations (19% and 14%), together with positive thinking (15%). School stress (21%) and lack of day structure (16%) were often mentioned among the negative effectors.

Conclusion: Although the analysis was completed by several persons and results were discussed for multiple times, the results should be still considered under such limitations as semantic and grammatical misunderstandings. The results can be used to understand the tendency of coping with described stressors among the adolescents in Germany and therefore to develop a strategy of supporting young people living under difficult circumstances of lockdown and social isolation.

References:

[1] Budimir, Sanja et al. "Coping strategies and mental health during COVID-19 lockdown." *Journal of mental health (Abingdon, England)* vol. 30,2 (2021): 156-163. doi:10.1080/09638237.2021.1875412

[2] Brooks, Samantha K et al. "The psychological impact of quarantine and how to reduce it: rapid review of the evidence." *Lancet (London, England)* vol. 395,10227 (2020): 912-920. doi:10.1016/S0140-6736(20)30460-8

[3] Jones, Elizabeth A K et al. "Impact of COVID-19 on Mental Health in Adolescents: A Systematic Review." *International journal of environmental research and public health* vol. 18,5 2470. 3 Mar. 2021, doi:10.3390/ijerph18052470

[4] Singh, Shweta et al. "Impact of COVID-19 and lockdown on mental health of children and adolescents: A narrative review with recommendations." *Psychiatry research* vol. 293 (2020): 113429. doi:10.1016/j.psychres.2020.113429

[5] Samji, Hasina et al. "Review: Mental health impacts of the COVID-19 pandemic on children and youth - a systematic review." *Child and adolescent mental health*, 10.1111/camh.12501. 28 Aug. 2021, doi:10.1111/camh.12501

[6] Kuckartz U. Qualitative Inhaltsanalyse. Methoden, Praxis, Computerunterstützung. 4th ed. Weinheim, Basel:Beltz Juventa; 2018

8) Role of Erlotinib and PP2 in cardiomyocyte cohesion and possible implication in arrhythmogenic cardiomyopathy

Philipp Menauer, Maria Shoykhet, Jens Waschke, Sunil Yeruva

Institute of Vegetative Anatomy – Ludwig Maximilian University Munich

Aim: Arrhythmogenic cardiomyopathy (AC) is a congenital heart disease symptomized by loss of cardiomyocytes, adipogenesis, fibrosis and arrhythmias. More than 50% of AC patients are diagnosed with mutations in genes coding for desmosomal proteins such as desmoglein-2 (DSG2), desmocollin-2 (DSC2), desmoplakin (DP), plakoglobin (PG) or plakophilin2 (PKP2). Apart from implanting a cardioverter-defibrillator and β-blocker therapy, there are only symptomatic therapies, such as avoiding endurance or competitive exercises.[1] Molecular mechanisms that affect the desmosomes of the cardiomyocytes are partially understood. Therefore, further elucidation of molecular mechanisms regulating desmosome function might provide new therapeutics for treating AC.[2]

Methods: Wild-type murine cardiac slices were treated with EGFR-inhibitor erlotinib and SRC-inhibitor PP2. Immunostaining and confocal microscopy were perfomed to detect changes in the desmosomal protein localization in the intercalated discs (ICDs) between the cardiomyocytes.

Results: Immunostaining of cardiac slices showed that inhibition of EGFR or SRC led to a higher staining width of DP and DSG2 in the ICD of wild-type mice.

Conclusion: Inhibition of erlotinib or SRC leads to an increase of DP and DSG2 in the ICD which stabilizes desmosome integrity and thereby cardiomyocyte cohesion. Future directions utilizing the same approach in living animals might provide new treatment options for AC.

References:

[1] Corrado, D., Basso, C. & Judge, D. P. Arrhythmogenic Cardiomyopathy. Circ Res 121, 784-802, doi:10.1161/CIRCRESAHA.117.309345 (2017)

[2] Shoykhet, M. *et al.* EGFR inhibition stabilizes cardiomyocyte cohesion in a murine model for arrhythmogenic cardiomyopathy (2022) in progress of publication

9) Effects of repetitive neuromuscular magnetic stimulation targeting the upper trapezius muscles on pressure pain thresholds in children and adolescence with headache disorders

A. Amann[1], C. Börner[1,2,3], J. Staisch[1], A. Hauser[1], M. Lang[1], M. Frohnmüller[1], I. Hannibal[1], K. Huß[1], S. Kruse[1], B. Klose[1], M. F. Lechner[1], N. Sollmann[2,3,4], M. N. Landgraf[1], F. Heinen[1], M. V. Bonfert[1]

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[4]Department of Diagnostic and Interventional Radiology, University Hospital Ulm, Ulm, Germany

Purpose: Primary headache disorders as well as post-traumatic headache are frequent in children and adolescents [1, 2]. Repetitive neuromuscular magnetic stimulation (rNMS) targeting the upper trapezius muscles (UTM) is a non-invasive, non-pharmacological treatment approach [3-5]. To assess muscular effects of rNMS, this study assessed pressure pain thresholds (PPT) measured above the UTM during a rNMS intervention in children and adolescence with headache disorders.

Methods: 20 patients suffering from episodic migraine, tension-type headache or post-traumatic headache and involvement of the neck muscles (14.10±2.69 years, 60% females) were included in this analysis. Patients received rNMS targeted to the UTM bilaterally in 6 sessions (15min/side, frequency: 20Hz, 7s ON-time, 10s OFF-time; Zimmer MedizinSysteme GmbH, Neu-Ulm, Germany). PPT above four reference points (bilaterally at 1/3 and 2/3 of the distance C7-acromion) were manually measured using an algometer. PPT were compared before the first rNMS session (pre1), before the last rNMS session (pre6), and at 3-month follow-up examination (FU).

Results: When comparing PPT before the first and last rNMS sessions, PPT significantly increased (pre1: left lateral: 2.00±1.37, left medial: 1.96±1.27, right lateral: 1.94±1.37, right medial: 1.83±1.26; pre6: left lateral: 3.28±2.21, left medial: 3.17±2.06, right lateral: 3.24±2.25, right medial: 3.17±2.06) (left lateral: p=.002, left medial: p=.002, right lateral: p=.003, right medial: p=.001). From the last rNMS session to FU, the increase in PPT sustained (FU: left lateral: 2.87±2.11, left medial: 2.95±2.11, right lateral: 2.81±2.04, right medial: 2.95±2.11) (left lateral: p=.047, left medial: p=.012, right lateral: p=.028, right medial: p=.002).

Conclusion: rNMS treatment leads to a PPT increase in pediatric patients with headache disorders. Based on the model of neuromodulation within the trigemino-cervical complex, rNMS may affect nociceptive processing at central levels via neuromodulation.

References:

1. Vos, T., et al., *Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019.* The Lancet, 2020. **396**(10258): p. 1204-1222.
2. Albers, L., et al., *Headache in school children: is the prevalence increasing?* Curr Pain Headache Rep, 2015. **19**(3): p. 4.
3. Sollmann, N., et al., *Magnetic stimulation of the upper trapezius muscles in patients with migraine - A pilot study.* Eur J Paediatr Neurol, 2016. **20**(6): p. 888-897.
4. Renner, T., et al., *Alleviation of migraine symptoms by application of repetitive peripheral magnetic stimulation to myofascial trigger points of neck and shoulder muscles - A randomized trial.* Sci Rep, 2020. **10**(1): p. 5954.
5. Renner, T., et al., *Repetitive Peripheral Magnetic Stimulation (rPMS) in Subjects With Migraine-Setup Presentation and Effects on Skeletal Musculature.* Front Neurol, 2019. **10**: p. 738.

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10) Armoring anti-HER2 CAR-T cells with C-C-motive receptor 8 (CCR8) and a dominant negative TGF-b receptor (DNR) to enable efficacy in solid tumor models

Thaddäus Strzalkoski

Purpose: Although chimeric antigen receptor (CAR) modified-T cells have shown great efficacy in treating hematological malignancies, CAR T cells have yet to demonstrate their value in solid oncology. Here, CAR T cells frequently are prevented access to tumor tissue and face profound suppression at the tumor site. To overcome this issue, our group could previously demonstrate that arming CAR T cells with C-C-motive-receptor 8 for improved tumor-directed migration and a dominant-negative receptor for TGF-β for resistance to suppression enabled activity in pancreatic cancer models. The value of this approach for other entities was however unclear. We now investigated the potential of this combination for treatment of HER2 positive cancer models in conjunction with a HER2-targeted CAR.

Methods: We isolated primary murine and human T cells and transduced activated T cells with virus from previously generated virus producing cell lines. For functional assays, impedance-based killing assays were used. Phenotype, activation and exhaustion were monitored with FACS readouts. IFNγ, CCL1 and Granzyme B

production was assessed with ELISA. In vivo, we measured survival and tumor growth of mice that were subcutaneously injected with tumor cells and treated with CAR-T cells carrying either CCR8, DNR or both receptors. To look at chemokine expression in tumor material, mRNA was isolated from tumor material and RT-qPCR was performed.

Results: We found that expression of CCR8 can redirect CAR-T cells to the tumor and a DNR can prevent immunosuppression of CAR-T cells in the tumor microenvironment. The improved functionality of CAR-CCR8-DNR T cells compared to CAR-T cells against the HER2 antigen could be demonstrated in vitro and in vivo in human HER2+ tumor models.

Conclusion: Equipping CAR T cells with CCR8 and DNR emerges as a disease spanning strategy to enable cellular therapies and warrants further preclinical and perhaps clinical development.

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11) Effects of expert eye-tracking videos with cued retrospective reporting on medical students' ECG interpretation skills

Aline Scherff

Purpose: Teaching ECG interpretation skills currently relies on personal experience or schemas, with frequently poor student outcomes. It is unknown whether ECG teaching strategies are consistent with actual expert viewing patterns during ECG interpretation. This study investigated the effects of making a cardiology expert's gaze interpreting ECGs visible – through eye-tracking videos with cued retrospective reporting (CRR) – on medical students' ECG interpretation skills gain.

Methods: The study consisted of (I) a material development phase for eye-tracking video generation and CRR audio commentary creation, and (II) a student ECG learning intervention phase. Initially, eye-tracking recordings of an expert cardiologist's gaze while silently interpreting 15 ECGs were collected. The expert then watched the video with their own gaze and retrospectively verbally explained their visualized gaze. This led to the creation of a 9-minute video simultaneously showing expert gaze and audio explanations. The video was used as additional learning stimulus (intervention, INT n = 47) during an ECG learning session (training as usual, TAU n = 44) aimed at medical students' ECG interpretation skills gain (0-100%).

Results: Results showed overall improvement of medical students' ECG interpretation skills on the derived outcome measures (M = 4.80-15.97%, SD = 9.02-

10.29%). A small initial advantage of the CRR teaching method signifying greater knowledge gain of INT vs. TAU was observed (ΔM = 1.25-2.19%). In addition, typical student performance factors that may inform future teaching approaches were evaluated for their effect on post-learning ECG knowledge scores and successfully predicted a large proportion of the variance (R^2 = .44-.63). Greater gains after an only 9-minute intervention is a promising finding. Participant feedback suggested that pausing videos at crucial points instead of a continuous loop could further improve the usefulness of CRR.

Conclusion: Expert eye-tracking videos with CRR audio commentary bear potential to improve medical students' ECG interpretation skills.

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12) Influence of diabetes and chronic kidney disease on heart rate and blood pressure within a swine model.

A summary of results obtained through monitoring using telemetry systems.

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Purpose: Chronic kidney disease (CKD) is a common disease in the population, moreover, the prevalence of diabetes has been increasing for a long time [1]. In this context, it is of great importance how the heart and, consequently, the blood pressure and heart rate develop in both clinical pictures since both conditions can lead to cardiovascular diseases. [2] This paper will give an overview of how blood pressure and heart rate evolve within a transgenic diabetes swine model.

Methods: To illustrate this, we needed an animal model that has a comparable disease progression to human, for which a swine model is ideally suited. In this transgenic pig model, diabetes was induced via a genetic C94Y substitution. One wild type and one diabetic animal were instrumented with a telemetry device to monitor vital signs [3]. Furthermore, the diabetic pig then developed CKD via an embolization using microspheres. It can be assumed that both the heart rate and blood pressure of the wildtype, produce stable vital signs, while the modified pig should show a higher blood pressure and an increased heart rate [4].

Results: The model did not provide the expected results; the diabetic pig had a blood pressure of 91.6 mmHg and a heart rate of 91 bpm at the average of 10 weeks of

telemetric monitoring. In contrast, the wild type had a blood pressure of 101 mmHg and a heart rate of 96 bpm. One reason for this could be that it was not checked that the animals sleep during the measurement at 23 o'clock. So, in the future it would be better to make the measurement at a time when the animals are safely asleep, for example at 3 o'clock or 4 o'clock in the morning.

Conclusion: The procedure should be repeated, improved, and the N number should be extended to more experimental pigs. Then it could be checked whether the results of this work can be disproved and results closer to the physiological expectation can be obtained.

Resources:

1. Glovaci, D., W. Fan, and N.D. Wong, *Epidemiology of Diabetes Mellitus and Cardiovascular Disease.* Curr Cardiol Rep, 2019. **21**(4): p. 21.
2. Vallianou, N.G., et al., *Chronic Kidney Disease and Cardiovascular Disease: Is there Any Relationship?* Curr Cardiol Rev, 2019. **15**(1): p. 55-63.
3. Renner, S., et al., *Permanent neonatal diabetes in INS(C94Y) transgenic pigs.* Diabetes, 2013. **62**(5): p. 1505-11.
4. Reed, R., et al., *Accuracy of an oscillometric blood pressure monitor in anesthetized pigs.* Lab Anim, 2018. **52**(5): p. 490-496.

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13) Intracellular distribution of calbindin D-28k in neurons – quantification by analysis of immunofluorescence images

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Purpose: Calbindin D-28k (CB) belongs to the family of EF-hand calcium-binding proteins and is widely expressed throughout the nervous system but has also been found in many other organs including gut and kidney. [1,2] Evidence suggests that CB has different functions. As a calcium sensor, CB translates calcium concentrations in signalling cascades by interaction with target proteins [3,4]. In neurons, CB is supposed to serve as a calcium buffer [5,6] whereas in kidney it may act as a ferry for cytosolic calcium and thus allow transcellular calcium transport [7,8]. CB is believed to be principally located in the cytosol but nuclear localization has been demonstrated as well.[9] The aim of this investigation was to quantify the intracellular distribution of CB in neurons by means of indirect immunofluorescence and to asses if this is an appropriate method for quantification of CB.

Methods: Neuronal progenitor cells were differentiated in neurons that express CB. Besides, neurons that do not express CB physiologically were transfected with expression plasmids that were encoding CB and a second protein which allowed colocalization analysis. In a third approach, CB cDNA was tagged with a nuclear localization sequence (NLS). The intracellular CB of all these cells was visualized by indirect immunofluorescence and then quantified by using an image processing program.

Results: In HN10e cells, the intensity of CB in the cytosol was greater than in the nucleus, with the quotient CB cytosol/CB nucleus amounting to 1,33 or 3,19, depending on the method of analysis. In NE4C cells, the CB staining was equally or more intense in the cytosol than in the nucleus, with the quotient CB cytosol/CB nucleus at 0,99 or 1,56. The CB modified with the NLS did not localize primarily to the nucleus, contrary to our expectations.

Conclusion: The results of this investigation confirm the assumption that the intracellular distribution of CB is principally cytosolic. However, the chosen method turned out not to be reliable enough for an exact quantification.

References:

[1] Christakos S., Gabrielides C., Rhoten WB. (1989). Vitamin D-dependent calcium binding proteins: chemistry, distribution, functional considerations, and molecular biology. Endocr Rev 10: 3–26

[2] Gross M., Kumar R. (1990). Physiology and biochemistry of vitamin D-dependent calcium binding proteins. Am J Physiol 28: F195–F209

[3] Morgan D. W., Welton A. F., Heick A. E. and Christakos S. (1986). Specific in vitro activation of Ca2+ Mg-ATPase by vitamin D-dependent rat renal calcium-binding protein (calbindin-D28k). Biochem. biophys. Res. Commun. 138, 547–553

[4] Reisner P. D., Christakos S. and Vanaman T. C. (1992). In vitro enzyme activation with calbindin-D28k, the vitamin D-dependent 28k˜Da calcium-binding protein. Fedn Eur. biochem. Socs Lett. 297, 127–131

[5] Baimbridge K. G., Miller J. J. and Parkes C. O. (1982). Calcium binding protein distribution in rat brain. Brain Res. 239, 1519–1523

[6] Mattson M. P., Rychlik B., Chu C. and Christakos S. (1991). Evidence for calcium-reducing and excito-protective roles for the calcium binding protein calbindin-D28k in cultured hippocampal neurons. Neuron 6, 41–51

[7] Feher J.J., Fullmer C.S., Wasserman R.H. (1992). Role of facilitated diffusion of calcium by calbindin in intestinal calcium absorption. Am J Physiol 262:C517–C526

[8] Wasserman R.H., Fullmer C.S. (1995). Vitamin D and intestinal calcium transport: facts, speculations and hypotheses. J Nutr 125:1971S–1979S

[9] German D.C., Ng M.C., Liang C.L., McMahon A., Iacopino A.M (1997). Calbindin-D-28k in nerve cell nuclei. Neuroscience; 81: 735–743

14) Examining the possible interaction of calbindin-d28k and IMPA1

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Purpose: Calbindin d28k is a calcium-binding protein expressed in a variety of tissues, like various subpopulations of neurons, epithelial and endocrine cells. Besides its assumed function as calcium-buffering protein, several studies indicate that there is an interaction between calbindin and the enzyme inositol monophosphatase 1 (IMPA1)[1]. This is of significant interest, as the inhibition of IMPA1 is used to treat bipolar disorders. Since the current inhibition by lithium contains certain risks for the patient, the discovery of alternative ways of inhibition could provide new treatments.

Our aim is to verify the interaction of calbindin and IMPA1 in a cell culture model not used yet, which might help to further analyze the physiological relevance of the interaction.

Methods: As we used a cell line generated from mouse hippocampal neurons, in contrast to previous studies targeting human postmortem brain specimens, we first wanted to make sure, the cells we used contain IMPA1. This was managed by immunostaining the cells with rabbit polyclonal antibodies to IMPA1 to carry out an indirect immunofluorescence. We continued with a co-immunoprecipitation followed by a western blot to look for the interaction of calbindin d28k and IMPA1. For that, the cells were transfected with a Calb α-expression plasmid and a control plasmid.

Based on earlier studies[2] and to avoid possible fundamental problems of the transfection approach, we are currently working on an immunoprecipitation using a cerebellum of a mouse.

Results: The immunofluorescence clearly proved the existence of IMPA1 in the used cell line. Although technically immunoprecipitation of calbindin was efficient, not even traces of immunoprecipitated IMPA1 were detected.

Conclusion: We were not able to prove an interaction of calbindin d28k and IMPA1 in our experimental model. Whether we can find an interaction in a physiological context, i.e., mouse cerebellum, is yet to be demonstrated after the completion of our experiment in the upcoming weeks.

References:

[1] Alon Shamir, Naama Elhadad (Rosolio) et al. *Interaction of calbindin D28k and inositol monophosphatase in human postmortem cortex: possible implications for bipolar disorder*, 2005

[2] Schmidt H, Schwaller B, Eilers J. *Calbindin D28k targets myo-inositol monophosphatase in spines and dendrites of cerebellar Purkinje neurons*, DOI: 10.1073/pnas.0407855102, 2005

15) The Critical Micelle Concentration and its Influence on Preparation and Properties of siRNA Loaded Polyplexes

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Purpose: Therapeutic nucleic acids such as siRNA, its stability and life within the circulation system are a very interesting research topic. siRNA is a promising tool, utilized as therapeutic agent against various diseases.[1] Critical micelle concentration is one of, if not the most important factor, when speaking about the application of the amphiphilic polymers. Cationic polymers are seen as a promising system to their electrostatic interaction with siRNA which results in higher stability within the circulation system. Ideally, the siRNA delivery vector must find their way to escape from the endosomes/lysosomes compartment after entering inside the target cell to efficiently release the loaded siRNA in the cytosolic compartment.[2]

Methods: This study examines physical characteristics of tree different polymer systems using dynamic light scattering method, critical micelle concentration studies and SYBR Gold assay. Although the micelles become labile upon dilution, their stability can be improved trough including hydrophobic interactions. During this research, some of the samples were produced using microfluidics.

Results: The limited stability of nucleic acids in the blood system and thus the typically short circulation times represent major obstacles which limit their application in clinical practice. As a result, it is necessary to encapsulate such therapeutic nucleic acids into suitable carrier systems. Cationic polymers are seen as a promising platform as they show great chemical tunability and excellent loading capacities *via* electrostatic interaction with siRNA.

Conclusion: The results have shown that depending on the polymer type, polyplex characteristics can be highly depended on the concentration during the fabrication process. Further tests are necessary to assess the behavior of micelleplexes in different physiological environments.

References:

1 Nikam RR, Gore KR. Journey of siRNA: Clinical Developments and Targeted Delivery. Nucleic Acid Ther. 2018 Aug;28(4):209-224. doi: 10.1089/nat.2017.0715. Epub 2018 Mar 27. PMID: 29584585.

2 Zhu J, Qiao M, Wang Q, Ye Y, Ba S, Ma J, Hu H, Zhao X, Chen D. Dual-responsive polyplexes with enhanced disassembly and endosomal escape for efficient delivery of siRNA. Biomaterials. 2018 Apr;162:47-59. doi: 10.1016/j.biomaterials.2018.01.042. Epub 2018 Feb 3. PMID: 29432988.

16) Investigation of TLR4-based resistance in SK-OV-3 cells through LPS treatment

Erika Lynn Roberts

Purpose: Resistance often occurs in high grade serous ovarian cancer leading to a decreased overall survival rate within 10 years. Resistance is hypothesised to be caused through TLR4-pathway activation leading to NF-kB up-regulation and increased migratory and pro-survival proteins. Paclitaxel (PTX), an anticancer agent, was revealed as a potential agonist of TLR4. Knowing this, SK-OV-3 cell were treated for various exposure times with a positive control, Lipopolysaccharid (LPS), to test for up-regulation of NF-kB. Additionally, TLR4 receptor presence was tested using flow cytometry.

Methods: Treated samples were run through SDS-Page and Western Blot. Biological duplicates were treated with 10µl/ml LPS concentration. In the first assay, 2h and 4h exposure times were investigated. In the second, 1h, 2h, 4h, 6h, 24h, and 48h were used. The targeted proteins were NF-kB and ß-Actin.

Use of a BCA Bradford Assay determined protein concentration before blotting.

Flow Cytometry was used with TLR4 (CD284) Alexa Fluor 488 labeled antibody in two concentrations (2µg vs 5µg).

Results: The BCA Bradford assay showed proper concentrations with a strong standard curve.

In the first Blot, LPS+ and LPS- showed detectable NF-kB for all time points yet LPS+ showed enhanced activation. In the second, 4h and 6h showed activation, yet these results are non-conclusive.
The x-median of cells treated with antibody was not any stronger than that of the isotype. This suggests no receptor expression- the study requires further establishment.

Conclusion: Most presented data is minimal or non-conclusive, yet the hope of understanding TLR4-based resistance mechanisms in ovarian cancer is compelling. This understanding would invite the opportunity to develop chemotherapy co-formulations which work against resistance.

Poster:

1) Evaluation of mTOR inhibitor Vistusertib in comparison to Rapamycin in the RIST molecular-targeted regimen in MycN-amplified neuroblastoma cell lines

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PURPOSE: Neuroblastoma (NB) is the most common extracranial solid tumor of childhood in which the amplification of the MycN oncogene correlates with high-risk disease. Despite intensive treatment regimens the outcome for patients with relapsed or treatment refractory high-risk NB remains poor and novel treatment strategies are urgently needed. The RIST therapy represents a novel multimodal treatment design for high-risk NB and is based on metronomically combining molecular-targeted inhibitors (Rapaymcin and Dasatinib) with conventional cytostatics (Irinotecan and Temozolomid). The phase II prospective randomized RIST clinical trial has been completed and the encouraging results will be published soon. With the expectation to improve the RIST therapy, we tested the novel and more potent ATP-competitive $mTORC_{1/2}$ inhibitor Vistusertib as substitute for the allosteric $mTORC_1$ inhibitor Rapamycin.

METHODS: The applied methods included MTT-based cell viability assays, immunoblot analyses and fluorescence-based cell cycle analyses.

RESULTS: By determining the IC_{50} values in three MycN-amplified neuroblastoma cell lines, we demonstrated that nanomolar concentrations of Vistusertib effectively reduced the cell viability compared to higher IC_{50} values of Rapamycin in the micromolar range. Vistusertib treatment at IC_{50} values inhibited the mTOR signaling pathway with reduced phosphorylation of S6K1 and 4E-BP1, and furthermore induced a G_1 phase cell cycle arrest but not apoptotic activity. Combination treatment of Vistusertib plus Dasatinib inhibited the cell viability significantly more compared to single treatments. Substitution of Rapamycin with Vistusertib in the multimodal VIST regimen showed that cell viability was significantly reduced in all three NB cell lines tested.

CONCLUSION: Taken together, our results suggest that Vistusertib acts at much lower drug concentrations as effective as Rapamycin in the RIST protocol. Recent clinical studies applying Vistusertib indicate an acceptable toxicity profile and prolonged stability of disease. Therefore, Vistusertib represents a valid option to be evaluated in prospective clinical trials for relapsed or treatment-refractory high-risk neuroblastoma.

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2) The impact of itaconate on human osteoclastogenesis

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Purpose: Healthy bone structure requires a fine balance between bone formation (osteoblasts) and bone resorption (osteoclasts). Alterations in the number or activity of osteoclasts play a crucial role in bone loss, which is a distinctive clinical feature of several diseases including rheumatoid arthritis (RA) (1). It was demonstrated that osteoclasts undergo certain metabolic changes during their differentiation process (2). However, the precise link between development and function of osteoclasts, and their cellular metabolism is unclear. One specific metabolite derived from a TCA cycle intermediate, itaconate, may play an important role in this matter. While recent studies indicate an anti-osteoclastogenic effect for itaconate in murine models (3), its role in human osteoclasts remains unknown. In this study, we investigated the energy metabolism of human osteoclasts and the role of 4-octyl-itaconate (4-OI), a cell-permeable derivative of itaconate, in the differentiation of osteoclast progenitors, derived from the blood of patients with RA and healthy donors.

Methods: Human osteoclasts were generated *in vitro* from peripheral blood mononuclear cells and stimulated with 4-OI. For the quantification of osteoclasts, tartrate-resistant acid phosphatase staining was used, along with quantitative real-time PCR to determine the mRNA expression levels of osteoclast markers and metabolic genes. Extracellular flux assays were performed to investigate the mitochondrial and glycolytic activity during the osteoclast differentiation, using a Seahorse Analyser.

Results: We demonstrated that 4-OI inhibits osteoclast differentiation in patients with RA and healthy controls. We found that human osteoclasts undergo a metabolic switch towards glycolysis during their differentiation and RA-patients show enhanced glycolytic gene expression. Furthermore, we showed that 4-OI limits the glycolytic activity of osteoclasts.

Conclusion: Our findings reveal a crucial role for itaconate in human osteoclastogenesis, paving the way for a new therapeutic approach in the future against pathological bone loss and bone destruction from the cellular metabolic point of view.

References:

1. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003;423(6937):337-42.

2. Lemma S, Sboarina M, Porporato PE, Zini N, Sonveaux P, Di Pompo G, et al. Energy metabolism in osteoclast formation and activity. Int J Biochem Cell Biol. 2016;79:168-80.
3. Sun X, Zhang B, Pan X, Huang H, Xie Z, Ma Y, et al. Octyl itaconate inhibits osteoclastogenesis by suppressing Hrd1 and activating Nrf2 signaling. FASEB J. 2019;33(11):12929-40.

3) Temporal changes in intracellular calcium concentration in presence of Calbindin D-28k in hippocampal HN10e cells

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PURPOSE/AIM: Calbindin D-28k (CB) is a member of the EF-hand calcium-binding protein family and mainly expressed in neurons. It comes with various functions like calcium transporting, binding, buffering as well as sensing. Since calcium is an essential signaling molecule, the aim of our research is to understand how CB shapes the intracellular calcium free levels in HN10e cells, especially temporally by using a CB-GFP plasmid. Furthermore, we attempt to investigate the process of this modulation and respectively its physiological and pathophysiological relevance.

METHODS: First of all, we constructed a plasmid called pEGFP-Calb1 to fuse both CB and GFP using the vector pEGFP-N3. For a precise determination whether the desired sequence was achieved it was sent for DNA sequencing and we also performed a Western blot analysis. Then we could start with the transfection in HN10e cells (poly-L-lysine coated) on chamber coverslips (15 µ-slide by ibidi) in standard imaging buffer and labeled them with the calcium indicator X-rhod-1. For the fluorescence microscopy we used a Zeiss LSM 710. To induce an increase in the calcium concentration we used potassium chloride, glutamate and ionomycin (each 100 µl) in various quantities. To record the results, regions of interest were selected and we performed time series of 120 seconds and a snap before and after.

RESULTS: By adding the abovementioned substances to cause concentration differences during the recordings (measured by fluorescence), the microscope generates correspondent graphs, representing the intensity depending on time. The analysis of the latter revealed that even in non-transfected cells (control) there were intercellular deviations, which hindered the accurate comparison. Also, there weren't any strong distinctions between the transfected and non-transfected cells.

CONCLUSION: Concluding, there was no concrete evidence for a significant shaping of the intracellular calcium concentration in the presence of CB or at least not a repeatable effect.

Nevertheless, the project provides a solid basis for further research.

References:

1. Schmidt H. Three functional facets of calbindin D-28k. Front Mol Neurosci. 2012 Mar 15;5:25. doi: 10.3389/fnmol.2012.00025. PMID: 22435048; PMCID: PMC3304297.

2. Turnbull CI, Looi K, Mangum JE, Meyer M, Sayer RJ, Hubbard MJ. Calbindin independence of calcium transport in developing teeth contradicts the calcium ferry dogma. J Biol Chem. 2004 Dec 31;279(53):55850-4. doi: 10.1074/jbc.M409299200. Epub 2004 Oct 19. PMID: 15494408.
3. Schwaller B, Meyer M, Schiffmann S. 'New' functions for 'old' proteins: the role of the calcium-binding proteins calbindin D-28k, calretinin and parvalbumin, in cerebellar physiology. Studies with knockout mice. Cerebellum. 2002 Dec;1(4):241-58. doi: 10.1080/147342202320883551. PMID: 12879963.
4. Hara M, Wang X, Kawamura T, Bindokas VP, Dizon RF, Alcoser SY, Magnuson MA, Bell GI. Transgenic mice with green fluorescent protein-labeled pancreatic beta -cells. Am J Physiol Endocrinol Metab. 2003 Jan;284(1):E177-83. doi: 10.1152/ajpendo.00321.2002. Epub 2002 Sep 17. PMID: 12388130.
5. Cabantous S, Terwilliger TC, Waldo GS. Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein. Nat Biotechnol. 2005 Jan;23(1):102-7. doi: 10.1038/nbt1044. Epub 2004 Dec 5. PMID: 15580262.

Acknowledgements

„Science, more than ever“ is the motto of our this year`s conference. The corona pandemic impressively showed us and the whole world how important science and research are and will be in the future. Thousands of scientists have been working on the new corona virus since its emergence trying to elucidate the entrance routes, the RNA sequence, the spreading mode and other facts that are necessary to efficiently combat the virus and ultimately end the pandemic. Researchers of different fields, from academic as well as the industry, have joined forces, enabling that more than twenty corona vaccines could have been developed in amazingly short time and more than 350 vaccine projects are still in the pipeline. Some involved scientists have become the new heroes of our time, being more popular than politicians or rock stars. Of course the Corona pandemic is not the only example showing that scientific research is important but definitely one of the most obvious.

The Munich Medical Student Science Conference MMS ScienceCon combines students' research projects from different fields. I am delighted to see that so many medical students took the opportunity to get own research experience helping them to receive a deeper understanding of diseases. It also might inspire one or the other for an academic career.

I therefore want to thank Prof. Michael Meyer, the Associate Dean, for believing in our students and for keeping the Undergraduate research opportunity (Forschungsmodul) and the MMS ScienceCon alive. I want to thank the Dean of Research, Prof. Stefan Endres for supporting the conference format since its beginning. Furthermore, I am very grateful to Prof. Heidi Stöckl for agreeing to be our keynote speaker.

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Romana Ruiß

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