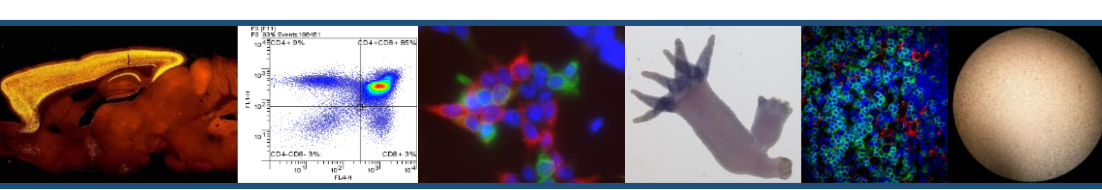
Life Science PhD Meeting Innsbruck 2019
Abstract Book



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Mission Statement

The Life Science PhD Meeting provides a platform for the whole Life Science community, from undergraduate students up to PIs, to share their knowledge, experience and critical thinking. Furthermore we want to encourage all students to present their research to train this important skill for international conferences.
We are proud to present excellent scientific work from numerous fields, which is only possible due to the huge variety of scientific interests of the groups represented in the meeting. Therefore the organizing committee would like to take the opportunity to thank the research programs making it possible to organize this meeting for all the Life Scientists in Innsbruck:

MUI:

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- SPIN
- SFB-F144

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- CMBI

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101 .... #61-69: Genetics and Genomics

110 .... #70-89: Immunity, Infectious Diseases (ID), Oncology and Clinical Medicine

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Program Thursday, April 25th 2019

09:30-12:00 M.01.470/490	Workshop "*From Research to Market: Creating your own business*"
12:00-12:35	Lunch break
12:35-12:45 M.EG.180	Welcome notes
12:45-13:30 M.EG.180	Plenary lecture: Patrick Eyers (University of Liverpool) *Understanding and responding to kinase-based drug-resistance in cancer cells*
13:30-14:00 M.EG.180	Flash talks Thursday session (2min presentations) Poster # 9, 10, 23, 25, 28, 30, 37, 40

	Short talks M.EG.180	Short talks L.EG.200
14:15-14:30	Talk#1: Miguel Lemos	Talk#4: Antonio Pérez Hansen
14:30-14:45	Talk#2: Buket Ucar	Talk#5: Verena Petzer
14:45-15:00	Talk#3: David Heimdörfer	Talk#6: Joachim Pfister

15:00-17:00 Aula / Foyer	Joint poster session with coffee: Poster #1-45 Please be at your poster if your abstract number is: even: 15:00-16:00 odd: 16:00-17:00

	Short talks M.EG.180	Short talks L.EG.200
17:00-17:15	Talk#7: Mia Kvåle Løvmo	Talk#11: Katharina Hutter
17:15-17:30	Talk#8: Manuel Berger	Talk#12: Athanasios Seretis
17:30-17:45	Talk#9: Monika Hunjadi	Talk#13: Mostafa Alilou
17:45-18:00	Talk#10: Luiz F. Garcia Souza	Talk#14: Felix Grabherr

18:15-19:00 M.EG.180	Plenary lecture: Ralf Amann (Universität Tübingen) *Next generation vaccines: Modifying immunity with novel orf virus vector concepts*
19:00–22:00	Crackers, cheese & wine at the posters

Program Friday, April 26th 2019

Time / Room	Program	
08:55-09:00 M.EG.180	Announcements	
09:00-09:45 M.EG.180	Plenary lecture: Andrea Pauli (IMP Vienna) *Small proteins with big roles – from coordinating cell migration to mediating species-specific fertilization*	
09:45-10:15 M.EG.180	Flash talks Friday session (2min presentations): Poster # 48, 50, 55, 58, 62, 67, 72, 73, 75, 76, 81	
10:15-10:45	Coffee break	
	Short talks M.EG.180	Short talks L.EG.200
10:45-11:00	Talk#15: Julia Freudenblum	Talk#20: Pierre Costé de Bagneaux
11:00-11:15	Talk#16: Ana Curinha	Talk#21: Lucia Zanetti
11:15-11:30	Talk#17: Manuel Haschka	Talk#22: Filippo Erli
11:30-11:45	Talk#18: Gerlinde Karbon	Talk#23: Sinead Rooney
11:45-12:00	Talk#19: Andreas Feichtner	Talk#24: Barbara Pinhero
12:00-13:00	Lunch break	
13:00-15:00 Aula / Foyer	Joint poster session with coffee: Poster #46-89 Please be at your poster if your abstract number is: even: 13:00-14:00	 odd: 14:00-15:00
15:00-17:00 M.EG.180	Award ceremony MCBO best paper award lecture: Mehmet Kaplan *Calcium influx and release cooperatively regulate AChR patterning and motor axon outgrowth during neuromuscular junction formation*	
	SPIN best paper award lecture: TBA	
	MCBO alumna talk: Marlies Meisel (University of Pittsburgh) *Host-microbe interactions in liver disease*	
	SPIN alumna talk: Letizia Marvaldi (Weizmann Institute of Science) *Neurotrophin-importin cross-talk in growth control of embryonic DRG neurons*	
	Best microscopy picture award	
	Poster/talk awards	
17:15-18:00 M.EG.180	Plenary lecture: Robert Messing (University of Texas at Austin) *Targeted and genomic approaches to drug discovery for alcohol use disorder*	
18:00-18:05	Closing remarks	
18:05	Buffet & Grand Finale (Party with DJane “Sandy im Getriebe”)	

Selected short talks

Lemos	Miguel	1	Combined anti-α-synuclein therapy for disease modification in multiple system atrophy
Ucar	Buket	2	Therapeutic Efficacy of Glial Cell-Derived Neurotrophic Factor Loaded Collagen Scaffolds in ex vivo organotypic brain slice Parkinson`s Disease Models
Heimdörfer	David	3	Function of non-coding RNAs in neurodegenerative and neurodevelopmental diseases
Pérez Hansen	Antonio	4	Analysis of hotspot regions of the genes involved in primary resistance (erg11 and fks1) in C. inconspicua, C. rugosa and C. ciferrii
Petzer	Verena	5	Non-Transferrin-Bound Iron in Hematopoietic Stem Cell Transplanted Patients Serves as Iron Source for Aspergillus fumigatus Outgrowth
Pfister	Joachim	6	Choose your label wisely: Hybrid imaging of Aspergillus fumigatus infections using 68Ga-labelled, fluorescent Siderophores
Kvåle Løvmo	Mia	7	Trapping and manipulation of biological samples with 3D ultrasound and optical tweezers
Berger	Manuel	8	A 3D Slicer plugin to improve impeded nasal breathing
Hunjadi	Monika	9	Matcha green tea enhances atherosclerosis in New Zealand White rabbits
Garcia-Souza	Luiz Felipe	10	Assessment of mitochondrial respiratory function in cryopreserved platelets
Hutter	Katharina	11	miR-26 regulates B cell expansion and differentiation at the pre-B cell stage
Seretis	Athanasios	12	Combination therapies to improve DC-based treatment of melanoma

Selected short talks

Alilou	Mostafa	13	Sesquiterpenes from the roots of Ferula hezarlalehzarica with anti-austerity activity on human pancreatic cancer cells
Grabherr	Felix	14	Western-diet-derived arachidonic acid induces epithelial ferroptosis which is a feature of Crohn's disease
Freudenblum	Julia	15	Tissue-specific, inducible gene modulation for studies of pancreatic islet morphogenesis
Curinha	Ana	16	Functional Analysis of RBM26 and RBM27
Haschka	Manuel	17	The dynamics of the apoptosis regulating BCL2 family during extended mitotic arrest
Karbon	Gerlinde	18	Understanding the role of the BCL-2 protein family in the generation and maintenance of genomic stability.
Feichtner	Andreas	19	The needles in the haystack: Identification of proliferation-relevant PKA substrates in colon cancer cells
Costé de Bagneaux	Pierre	20	The homozygous p.(Leu126Pro) variant in CACNB4 impairs nuclear targeting of the encoded β4b subunit of P/Q-type calcium channels and is associated with intellectual disability, epilepsy and cerebellar atrophy
Zanetti	Lucia	21	Contribution of bipolar cells to the pathology of congenital light blindness type 2
Erli	Filippo	22	Investigations on the role of mTOR signaling pathway in KOR-mediated aversion and antinociceptive activity in mice of HS665, a selective KOR full agonist
Rooney	Sinead	23	The role of inflammation in trait anxiety and its attenuation.
Pinheiro	Barbara	24	Mapping baseline presynaptic inputs to medial accumbens D1-medium spiny neurons using rabies monosynaptic tracing: focusing on insular cortex input to accumbens D1-MSNs

Combined anti-α-synuclein therapy for disease modification in multiple system atrophy

Multiple system atrophy (MSA) is a fatal neurodegenerative disorder characterized by Parkinsonism, cerebellar ataxia and autonomic dysfunction. The pathological hallmark of MSA is the presence of aggregated alpha-synuclein (α-Syn) within oligodendrocytes forming glial cytoplasmic inclusions (GCIs). The accumulation of pathological α-Syn in MSA brains is considered to contribute to neurodegeneration and therefore represents a promising target for disease-modifying therapies for MSA patients. Active immunotherapy allows stimulation of the immune system to produce antibodies against toxic α-Syn conformations and therefore provides a new therapeutic alternative. Recently, short immunogenic peptides (AFFITOPEs), carrying a sequence that mimics the original α-Syn epitope were generated to trigger potent α-Syn-specific antibody production without causing deleterious autoimmune responses. Also, previous experiments have shown that the aggregation inhibitor, Anle138b, reduces neurodegeneration and behavioural deficits in mouse models of α-Synucleinopathy and other proteinopathies.

Transgenic mice overexpressing α-Syn in oligodendrocytes under the proteolipid protein promoter (PLP-α-Syn mice), received either AFFITOPEs or Anle138b. A combination of both approaches was also tested to evaluate if the combined therapy is able to potentiate the effects of the single therapy. Motor behaviour was assessed. Brains and plasma samples were collected for neuropathological and immunological analysis.

We confirmed the efficacy of the single therapy with AFFITOPEs or Anle138b, observing motor improvement, rescue of dopaminergic neurons, reduction in α-Syn oligomers, decrease of GCIs density, and decreased levels of microglia activation. The combination of Anle138b+AFF2 was also beneficial showing reduced motor deficits, preservation of dopaminergic neurons in the substantia nigra, decrease of GCI density and microglia activation in treated PLP-aSyn mice, without further cumulative effect.

We conclude that both approaches show beneficial effects ameliorating the α-Syn pathology in MSA transgenic mice. Simultaneous application of Anle138b and AFF2 in PLP-α-Syn mice does not potentiate the effects of single drug therapy.

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Therapeutic Efficacy of Glial Cell-Derived Neurotrophic Factor Loaded Collagen Scaffolds in ex vivo organotypic brain slice Parkinson`s Disease Models

Parkinson´s disease (PD) affects 10 million people worldwide, yet there are no treatment option available to slow down or stop its progression. Glial cell line-derived neurotrophic factor (GDNF) is a potent trophic factor that supports the survival of dopaminergic neurons of the substantia nigra (SN), which degenerate in Parkinson´s disease (PD). However, application of GDNF to the brain constitutes a challenge and biomaterials such as collagen can present novel strategies to target therapeutics to the brain. In this study, we assessed the efficacy of collagen scaffolds loaded with GDNF on dopaminergic neuron survival in organotypic ex vivo slices: axotomy, rotenone and 6-hydroxydopamine (6-OHDA) models. We used organotypic brain vibrosections from wild type postnatal day 9-11 mice and evaluated the survival of tyrosine hydroxylase (TH) positive dopaminergic neurons by immunohistochemistry, as well as qRT-PCR and Western Blotting over 14 days. Collagen scaffolds were crosslinked with polyethyleneglycol, loaded with GDNF and directly placed onto organotypic brain slices. GDNF released from collagen scaffolds significantly protected dopaminergic SN neurons against axotomy and rotenone induced degeneration. Furthermore, GDNF loaded scaffolds had an overall trend for neuroprotection in 6-OHDA induced cell death. This approach holds the potential to be used as an injectable hydrogel system to address the need of targeted long-term growth factor delivery for preventing the progression of disease in the future. In conclusion, targeted slow release of GDNF from collagen scaffolds may provide a potent therapeutic tool for PD.

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Function of non-coding RNAs in neurodegenerative and neurodevelopmental diseases

Small non-protein-coding RNAs (ncRNAs) play important roles in the regulation of gene expression and have been implicated in various diseases of the central nervous system (CNS). To elucidate the functions of ncRNAs in CNS diseases, we have investigated several ncRNA species, including small nucleolar RNAs (snoRNAs), whose expression was found to be deregulated in a triple transgenic mouse model for Alzheimer’s disease (AD). While canonical snoRNAs modify ribosomal RNAs or small nuclear RNAs (i.e. by 2’-O methylation or pseudouridylation), the biological function of these disease-related snoRNAs has not been revealed. In addition, we examined human post-mortem brain samples of AD patients and investigated differential expression of snoRNAs and other ncRNAs by RNAseq analysis. In these studies, we identified differential expression of snoRNAs in brain samples of Alzheimer patients as well as differentially expressed tRNA fragments designated as 5'-tiRNAs. From the snoRNA class, differential expression of box C/D snoRNAs SNORD116 (HBII-85) and SNORD115 (HBII-52) have also been demonstrated in the Alzheimer mouse model by our lab . By investigating the biological functions of SNORD116, we were able to demonstrate that in an in vitro translation system this snoRNA is capable of up- or downregulating translation of capped or uncapped reporter mRNAs, respectively. Interestingly, a regulatory effect on translation was also found for identified 5’ tiRNAs. This effect was not observed with canonical snoRNAs or scrambled RNAs. We thus envision a model, in which these ncRNA species are able to fine tune protein synthesis. Thus, their deregulated expression might be directly linked to the etiology of CNS diseases such as AD.

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Analysis of hotspot regions of the genes involved in primary resistance (erg11 and fks1) in C. inconspicua, C. rugosa and C. ciferrii

Introduction: Our prior comprehensive susceptibility study and the literature indicate that C. inconpicua, C. rugosa and C. ciferrii have low levels of susceptibility to the commonly used antifungal drugs (azoles and echinocandins). However, since C. inconpicua, C. rugosa, and C. ciferri only represent a minor portion of all Candida infections, the molecular mechanism of their primary resistance is still unknown.

Objective: The objective of this study, is to characterize the amino-acid (AA) sequence of the hotspot regions of the genes involved in primary resistance against azoles (erg11) and echinocandins (fks1) in C. inconspicua, C. rugosa and C. ciferrii. We aim to identify amino acid changes that are linked with the limited activity of azole and echinocandin drugs.

Method: One representative strain per species was whole genome sequenced. Homologue genes of erg11 (lanosterol 14 alpha-demethylase) and fks1 (1 3-beta-glucan synthase) were identified by BLAST comparison with C. albicans genome. Primers were designed to amplify and sequence homologous “hotspot” regions in erg11 and fks1. Comprehensive amino acid alignments were generated for our collection of clinical isolates (n=202). Amino acid changes in the hotspot regions were correlated with resistance phenotypes and compared with literature.

Results and conclusion: All tested species had amino acid changes in at least one of the “hotspot” regions of erg11 and fks1 in comparison with C. albicans. Erg11 mutation at position D153E (erg11 HS1) present in C. ciferrii were previously associated with low azole susceptibility. The mutation H283N also in C. ciferrii has not been described yet, but other amino acid substitution in the same position (e.g., H283D, H283R– ERG11 HS2) was previously associated with fluconazole resistance. Mutations at position P649 (HS1) in the FKS1 gene were previously linked with a moderate increase in echinocandin resistance though the particular AA change present in our species haven’t been described before. The mutations occurring in FKS1 HS2 identified in this study have not yet been linked to echinocandin resistance.

The high species-specific AA substitutions points towards an evolutionary conserved intrinsic resistance, structural and functional studies are needed to evaluate the impact of these AAs on the enzyme.

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Non-Transferrin-Bound Iron in Hematopoietic Stem Cell Transplanted Patients Serves as Iron Source for Aspergillus fumigatus Outgrowth

Introduction: Patients undergoing hematopoietic stem cell transplantation (HSCT) are prone to develop non-transferrin-bound iron (NTBI). Despite improved antifungal prophylaxis regimens, invasive aspergillosis still represents a serious problem with mortality rates ranging up to 80% among HSCT patients. In fact, studies have reported an association between NTBI and therapy-related complications, including infections. Furthermore, the possible interaction of invasive aspergillosis and iron metabolism has been suggested. Thus, we specifically assessed the role of NTBI on the in vitro outgrowth of Aspergillus fumigatus (A. fumigatus) in serum samples of patients who underwent allogeneic HSCT.

Methods: Outgrowth of A.fumigatus in cultures containing 10% human plasma from a representative cohort of patients participating in the prospective ALLogeneic Iron inVEstigators (ALLIVE) trial, showing various NTBI-levels during the consecutive phases of HSCT, was explored. In addition, clinical A. fumigatus isolates, A. fumigatus mutant strains and iron scavenging agents were used to improve our fundamental understanding of the underlying molecular mechanisms.

Results: Our assay revealed that A. fumigatus outgrowth depends on the presence of NTBI (defined as NTBI ≥0.2 AU, OR = 2235, 95% CI [337-28638], p=4×10^-12) rather than total iron availability in all tested strains. In addition, if transferrin-saturation exceeded 75%, fungal outgrowth in vitro was dramatically increased (OR=4021, 95% CI [403-150999], p=8×10^-9). Correspondingly, addition of sufficient amounts of human apo-transferrin to eliminate NTBI prevented A. fumigatus outgrowth. While the iron chelators deferiprone and deferoxamine served as xenosiderophore for A. fumigatus, thus supporting fungal outgrowth, iron scavenged by deferasirox was not accessible, consequently limiting fungal outgrowth. Using A. fumigatus mutant strains with genetic defects in iron acquisition systems we could show that the fungal uptake of NTBI is dependent on siderophores.

Conclusion: Our findings highlight the relevance of NTBI in plasma of HSCT patients for A.fumigatus outgrowth in vitro. We propose that NTBI may serve as a potential prognostic biomarker as well as a therapeutic target in patients undergoing HSCT.

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Choose your label wisely: Hybrid imaging of Aspergillus fumigatus infections using 68Ga-labelled, fluorescent Siderophores

Aspergillus fumigatus (AF) is an ubiquitary spread fungi in our environment and can cause severe invasive aspergillosis in immunosuppressed patients, most prominently in the lung. A lethality rate of over 90% is due to a lack of accurate diagnostic methods, which makes it essential to find new ways to determine, as well as locate these infections. AF requires siderophores to be virulent in the human host, in particular Triacetylfusarinine C (TAFC) an iron complexing molecule which is recognized by a specific transporter (MirB). We aimed to modify the TAFC molecule by replacing one acetyl moiety with different optical dyes to obtain a fluorescent compound specific for AF infections. Additionally, we replaced the iron with a radioactive gallium-68 which allows to do imaging by Positron Emmission Tomography (PET), combined with optical imaging methods ("hybrid imaging").

[Fe]FusarinineC was obtained from genetically modified fungal cultures, which was the starting material to synthesise [Fe]Diacetylfusarinine C ([Fe]DAFC) where we coupled our fluorescent dyes. With these compounds live cell fluorescent microscopy images were done by using a confocal microscope in A. fumigatus and A. terreus cultures. After removal of iron, the conjugates could be radiolabelled with gallium-68 to determine LogD, protein binding and serum stability. Likewise uptake assays in iron free and iron containing AF-cultures were used to investigate recognition by MirB transporter. µPET/CT imaging complemented the characterisation of the DAFC-conjugates.

Modification of DAFC was achieved in high yields with a variety of different fluorescent dyes. Radiolabelling with gallium-68 resulted in a high molar radioactivity after 10 min at room temperature. Stability of the compounds were very high with some exemptions over time, which is likely caused by sensitivity of fluorescent dyes to the radioactive radiation. Uptake assays and fluorescence microscopy revealed different uptake patterns dependent on the dye used. Especially microscopy showed specific uptake in different compartments of the hyphae, which has been counterstained with commonly used fluorophores. µPET/CT studies in immunosuppressed Lewis-Rats, which were infected with AF in the lung, revealed delineation of the infection area with main excretion via the urinary or hepatobiliary tract, depending on the chemical structure and low retention in non-target areas.

Overall our work serves as a novel proof of concept of modifying TAFC to obtain hybrid imaging compounds for diagnostic applications in invasive fungal infections.

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Trapping and manipulation of biological samples with 3D ultrasound and optical tweezers

Many traditional microscopy techniques rely on the static fixation of a specimen. Non-invasive imaging of large, three-dimensional or highly sensitive, living samples is often difficult to achieve. Here, we present our efforts on combining 3D ultrasonic acoustic trapping with an optical tweezer. Guided by numerical simulations, we have designed a ceramic microfluidic chip with tailored ultrasonic transducers. The hybrid trap can levitate large, (sub-) millimeter sized samples in solution over an extended period of time in a contact-free, non-invasive and non-confining way. We exploit acoustic resonances in our sample chamber to create a patterned "trapping landscape", in which the optical tweezers allow for additional manipulation of the specimen. We demonstrate that even highly motile active swimmers can be trapped. This approach paves the way for patterning of cells or the actively controlled formation of clusters or spheroids in suspension or a gel pre-cursor. As the chip is designed to be compatible with conventional inverse microscopes, many established microscopy techniques can be implemented as well. Beyond these methods, with high-fidelity specimen control in place, advanced 3D-reconstruction methods via in-situ tomography are in reach.

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A 3D Slicer plugin to improve impeded nasal breathing

Purpose: A surgical intervention is not always successful in the improvment of the nasal breathing process, i.e. a nasal septum deviation. Therefore, a preoperative software tool to support the surgeon is developed.

Methods: A Laser Doppler Anemometry validated Lattice Boltzmann simulation is used to calculate the flow through the nasal cavity with an error smaller than 10 %. A programmed 3D Slicer plugin visualizes simulation results like the calculated resection volume in an overlay of the preoperative CT dataset. Within the graphical user interface the surgeon is able to select regions of interest for optimization. The software tool is applied on a patient with nasal septum deviation.

Results: A comparison of the simulation result with a postoperative CT dataset shows that with less resection volume the same or higher pressure drop reduction to simplify breathing can be achieved. The comparison of selected cross-sections shows that the difference of segmented post-CT and own optimization result is smaller than 20 %.

Conclusion: First optimization results are in good accordance with postoperative CT datasets. This 3D Slicer plugin will be validated with further patients..

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Matcha green tea enhances atherosclerosis in New Zealand White rabbits

Green tea consumption is associated with decreased risk for cardiovascular disease and stroke. Matcha is a special kind of powdered green tea, known for its use in Japanese tea ceremony. Due to its influence on lipoprotein parameters it was postulated to exert antiatherogenic effects. In this study we, therefore, investigated effects and underlying mechanisms of long term Matcha treatment in New Zealand White rabbits. After a pre-treatment phase based on a chow diet for 4 weeks, 10 female New Zealand White rabbits were fed a high fat diet for 20 weeks. Rabbits within the treatment group were additionally administered 1 % Matcha green tea during the whole experiment. Basic and lipid parameters were measured weekly. Additional measurements included cholesterol efflux capacity in vitro and in vivo, CE transfer, liver parameters as well as plaque formation using nuclear magnetic resonance (NMR) imaging technique and in situ atherosclerotic lesion area analysis. Long term treatment with Matcha led to increased arterial pulse wave velocity (PWV) as well as atherosclerotic lesion areas despite of a slight decrease of total cholesterol. However, HDL cholesterol concentration was lower and HDL size shifted towards smaller particles, with no changes in apolipoprotein AI, but higher uptake of radiolabeled cholesterol within the liver. Liver parameters were not altered. Long-term Matcha treatment of hypercholesterolemic rabbits seems to result in increased vascular stiffness and plaque formation despite of increased reverse cholesterol transport.

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Assessment of mitochondrial respiratory function in cryopreserved platelets

Platelets (PLTs) are potential powerful models for the diagnosis of mitochondrial dysfunction in several pathologies (e.g., diabetes, sepsis, Parkinson's), offering a minimally invasive approach in comparison to tissue biopsies. However, rapid isolation of PLTs and respiratory measurements are required to avoid post-blood harvesting stress and cellular activation followed by metabolic alterations. Cryopreservation, a promising strategy to store blood cells for long periods of time, has been used in PLTs successfully for the study of inflammatory properties. However, the impact on mitochondrial function remains unknown. The objective of our study was to optimize the cryopreservation of human PLTs for the measurement of mitochondrial respiration. Human blood samples were collected and isolated from healthy human volunteers via venous puncture. Different cryopreservation conditions were evaluated: PLTs were resuspended in autologous plasma and 10 mM EGTA with DMSO (1) 5%, (2) 10%. Samples were stored at -80 °C for one, two and four weeks. After defrosting, mitochondrial function of PLT was measured by High-Resolution FluoRespirometry using the Oroboros O2k and substrate-uncoupler-inhibitor-titration protocols. After 1- to 4-weeks of cryopreservation with 5% DMSO, mitochondrial respiration was decreased by 20%. Flux control ratios of cryopreserved cells were unchanged in comparison to freshly isolated PLTs. Cell viability of cryopreserved PLTs decreased by 20% based on a respirometric viability index. After 1-week of cryopreservation with 10% DMSO, electron transfer (ET)-capacity diminished by 26%. However, the differences in ET-capacity observed with cryopreservation in intact PLTs disappear after applying cell viability corrections. These results demonstrate that further optimisation of cryopreservation is required to prevent damage of PLTs. Modifications in the cryopreservation method (e.g., lowering DMSO concentrations further, reducing cell density) will be investigated in order to improve cell viability and mitochondrial respiratory function in PLTs. An optimum cryopreservation method offers a powerful tool in biomedical and diagnostic research by reducing resampling, allowing multiple measurements with a single sample and preserving sample quality during transport.

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miR-26 regulates B cell expansion and differentiation at the pre-B cell stage

MicroRNAs (miRNAs) are short, non-coding RNAs of about 21-24 nucleotides length that regulate gene expression and thereby affect physiological as well as pathological processes such as cancer. Several miRNAs have been described to mediate pro-tumorigenic as well as tumor-suppressive functions. One miRNA family involved in cancerogenesis is the miR-26 family, which is aberrantly expressed in different types of cancer. However, little is known about the effect of altered miR-26 expression in the context of immune cell malignancies. Furthermore, despite its prominent expression in early lymphocytes, the role of miR-26 in hematopoiesis is still unknown. By combining in vitro and in vivo approaches we could demonstrate an important role for the miR-26 family in early B cell development. Upon overexpression of miR-26 in pre-B cells we could observe a block in differentiation into immature B cells. Furthermore, high miR-26 levels induced pre-B cell expansion and a partial inhibition of apoptosis upon IL-7 withdrawal, finally resulting in growth factor independency. We could identify the tumor-suppressor Pten as miR-26 target, being significantly downregulated upon induction of high miR-26 levels. Conversely, a functional knockdown of the miR-26 family by a competitive sponge construct enhanced B cell differentiation and induced a loss of pre-B cells over time. These observations are supported by findings in our miR-26 sponge mouse model. In analogy to our in vitro data, these mice displayed an increased number of immature B cells and a decrease in pre-B cells, indicating enhanced B cell differentiation. Together, these results clearly indicate the importance of the miR-26 family in early B cell development and suggest a pivotal role in regulating pre-B expansion versus differentiation. Furthermore, we suggest a potential oncogenic role of miR-26 in B cell leukemia by conferring a growth factor independent and anti-apoptotic pre-B cell phenotype.

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Combination therapies to improve DC-based treatment of melanoma

Dendritic cells (DC) are the most potent antigen-presenting cells of the immune system inducing CD4+ and CD8+ T cell responses. They have an unprecedented ability to capture and cross-present antigens and activate T cells via co-stimulatory receptor-upregulation and cytokine production. However, the tumor microenvironment actively suppresses anti-tumor immunity partly by suppressing DC activation and antigen accessibility. Previous clinical trials have established the safety of DC vaccination as antitumor therapy and shown promising clinical benefits. The aim of this project is to develop combination therapies, allowing control of melanoma growth by combining tumor-targeted therapy with BRAF inhibitor together with the direct delivery of tumor-associated antigens to DC via antibody-mediated uptake.

We observed that, BRAF kinase inhibition in two different melanoma mouse models, the transplantable D4M tumors and the inducible Tyr::CreER;BrafCA;Ptenlox/lox (BRAF/PTEN) mice, caused alterations in the composition of immune cell infiltrate. Interestingly, DC infiltrated tumors shortly after start of BRAF inhibitor treatment accompanied by an enhanced expression of the melanoma-associated antigens gp100 and trp2. After prolonged BRAF inhibitor exposure tumors develop resistance and tumor milieu shifts from inflammatory to immunosuppressive.

We have successfully cloned the gp100 and trp2 antigens into the skin DC targeting antibodies, anti-DEC-205 and Langerin. So far, we have produced and tested the DEC-205-hgp100 DC vaccine. Cross-presentation of the hgp100 antigen by DC after targeted delivery to antigen-specific CD8+ T cells, was confirmed by both in vitro and in vivo assays. The first experiments with combination of DEC-205-hgp100 and BRAF inhibitor showed the importance of DC activation for therapy success. Currently, we are optimizing DC vaccination during BRAF inhibition. Resistance development can be delayed by DC activation, still the DC vaccine needs further improvement

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Sesquiterpenes from the roots of Ferula hezarlalehzarica with anti-austerity activity on human pancreatic cancer cells

Pancreatic cancer is one of the most lethal types of cancer with a high occurrence rate particularly in Japan and the United States. With five-year survival rates lower than 5% and the absence of suitable treatment and detection options, the anti-austerity strategy for treatment of pancreatic cancer is one of the recently introduced methods which is targeting the tolerance of those cells to hypoxic and nutrient-deprived environments. Hence, compounds with the ability of reducing or eliminating the resistance of cells to a nutrient deprived medium can be introduced as lead compounds for treatment of pancreatic cancer. In our study, we isolated 15 sesquiterpenes from the roots of Ferula hezarlalehzarica for the first time. Structure elucidations were done using 1&2D NMR spectroscopy and MS. The absolute configuration of the compounds was determined by applying circular dichroism and quantum chemical calculation methods. The compounds were tested on the pancreatic cancer cell line Panc-1 using anti-austerity strategy. Our results revealed two highly active compounds (1 and 5) which showed to possess a PC50 value of 1.5 and 0.75 µM, respectively. Cells treated with 5 µM of the compounds showed changes in cell morphology displaying necrotic-type cell death. Furthermore, compounds 1 and 5 significantly inhibited the colony formation in soft agar at a concentration of 12.5 µM, by 50% and 90%, respectively. Our results show that the roots of F. hezarlalehzarica are a rich source of biologically active substances, which might be potential lead compounds for treatment of pancreatic cancer.

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Western-diet-derived arachidonic acid induces epithelial ferroptosis which is a feature of Crohn's disease

Introduction Ferroptosis is a cell death mechanism, which is defined by increased lipid peroxidation (LPO) and iron dependency. Glutathione peroxidase 4 (GPX4) is a key regulator of Ferroptosis. It was identified as a risk gene for Crohn's disease (CD) in a genome wide association study. So far, neither LPO nor ferroptosis in the intestine have been linked to CD.

Aim In this study intestinal epithelial cells from CD patients were analyzed for ferroptosis and LPO. Furthermore we analyzed the impact of GPX4-deficiency on inflammation occurring in intestinal epithelial cells (IEC).

Methods Biopsies collected from CD patients and healthy controls undergoing routine colonoscopy were used for IHC analysis. A second biopsy obtained from the same patients in the same procedure was used for isolating an epithelial cells enriched suspension which was further used for protein and RNA expression measurements. In vitro experiments were done using MODE-K cells, a murine small intestinal epithelial cell line. Prior to stimulating cells, GPX4 silencing was done for 24 hours. CrisprCas9 editing of MODE-K cells was used to obtain ACSL4 knockout cells. 4HNE staining was used to measure LPO in IHC, a BodipyC11 probe was utilized for LPO assessment by flow cytometry.

Results Lesional mucosa of CD patients showed diminished GPX4 activity and expression, when compared to healthy controls. Accordingly CD patients exhibited increased LPO. MODE-K cells silenced for GPX4 (siGPX4) underwent ferroptosis, indicated by increased cell death, which could be prevented by vitamin e and ferrostatin, but not by inhibitors of necrotic or apoptotic cell death and increased LPO. When stimulating siGPX4 cells with arachidonic acid (AA), these cells showed a markedly increased pro-inflammatory response. This AA induced inflammation was controlled by ACSL4 and lipoxygenases.

Conclusion A reduction of GPX4 activity, as observed in Crohn's disease, induces ferroptosis in IEC. Furthermore, it promotes an AA induced inflammation, which is controlled by ACSL4 and lipoxygenase-mediated LPO within IECs. Arachidonic acid is a polyunsaturated fatty acid largely contained within westernised diets. We conclude that ferroptosis is a feature of CD which could be triggered by compounds derived from the diet, such as fatty acids.

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Tissue-specific, inducible gene modulation for studies of pancreatic islet morphogenesis

During pancreas development, endocrine progenitors emerge as single cells from the duct epithelium and progressively coalesce to form mature islets. Due to the inaccessibility of the pancreas in most vertebrate model organisms, the molecular mediators involved in this process are poorly understood. Zebrafish is an in vivo system well suited for studies of islet cell assembly due to its transparency and the accessible location of the larval pancreas. Our group previously demonstrated that pharmacologic inhibition of PI3K and blockade of GPCR signaling by PTX expression impairs endocrine cell clustering. However, these approaches cause global pathway disruptions, making it difficult to dissect mechanisms acting cell-autonomously within islet cells. To further study islet morphogenesis, we are implementing cell-type specific, inducible approaches using the Gal4ER/UAS system. Endocrine cell specificity is achieved using driver lines containing a neurod promotor, which was developed for studies of the nervous system, but has not previously been validated for studies of endocrine pancreas. Firstly, we generated the neurod:memKate transgenic line, to characterize neurod-promoter driven gene expression in the developing and adult pancreas, and to provide a tool for highlighting cell morphology during islet formation. We then implemented this neurod promoter within the Gal4ER/UAS system, in which Gal4ER, activated following Tamoxifen administration, turns on expression of transgenes containing upstream UAS elements. Using these new tools, endocrine cell-specific fluorescent marker expression was detected in the primary islet as well as in the nervous system after an early tamoxifen treatment. Expression could also be activated in juvenile fish following immersion in tamoxifen-containing media, demonstrating a broad applicability of this inducible system for studies of pancreas and nervous system, in stages ranging from embryonic through adulthood. Using our previously established induction approach, in which islet morphogenesis consistently progresses during larval stages within a relatively short time frame, we can detect neurod:Gal4ER-dependent gene expression in induced secondary islet cells after a subsequent tamoxifen treatment. Furthermore, we are developing this system to elucidate morphogenetic mechanisms acting within endocrine cells during islet formation.

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Functional Analysis of RBM26 and RBM27

RBM26 and RBM27 are two poorly characterized proteins that have been identified in nuclear complexes likely involved in mRNA metabolism. Swm, their Drosophila homolog, is essential for cell proliferation and development. RBM26 depletion in zebrafish embryos showed a developmental delay and a ciliopathy phenotype, while no effect was observed in RBM26 mutants harbouring a loss of function allele. Similarly, acute depletion of RBM26 in human cell lines affected proliferation and ciliogenesis, while CRIPSR-CAS9-mediated RBM26 knockout cells did not show these phenotypes, suggesting that cells might be able to adapt. Interestingly, I could not obtain cells lacking both RBM26 and RBM27 and conditional RBM27 RNAi-mediated knockdown in RBM26 knockout cells revealed a strong effect on proliferation. Our results demonstrate that RBM26 and RBM27 co-localise with SC35, a protein known to be involved in transcriptional elongation and splicing, to nuclear speckles. RBM26 itself seems to be a cell cycle-regulated protein able to bind RNAs. Upon depletion of RBM26 or RBM27, proliferation is slightly reduced but double depletion causes a strong proliferation defect and a mitotic delay, which was found to be due to the activation of the spindle assembly checkpoint. RBM26 and RBM27 depletion caused a polyA+ RNA increases in the nucleus suggesting either a defect in degradation of aberrant mRNAs or an export failure. To understand the function of these proteins, I aim to perform a detail analysis of RBM26 domains. Knowing which domains are important for RBM26 localisation and function will allow me to have a better understanding about the molecular complex where RBM26 is involved in. Also, I plan to perform RNAsequencing analysis in order to define which mRNAs are enriched in the nucleus upon RBM26/27 depletion. The same experiment will allow me to have a better idea whether these two proteins play a role in degradation or export of mRNA. I aim to investigate in better detail putative protein interactors and RNA targets that might explain the mitotic delay upon RBM26/27 depletion. In Summary, RBM26 and RBM27 seem to control cell cycle progression by regulating processing, degradation or export of mRNAs important for progression through mitosis.

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The dynamics of the apoptosis regulating BCL2 family during extended mitotic arrest

Cell death during extended mitotic arrest is considered arguably most critical for the efficacy of microtubule-targeting agents (MTAs) in anticancer therapy. While the molecular machinery controlling mitotic arrest on MTA treatment, the spindle assembly checkpoint (SAC), appears well defined, the molecular components executing cell death, as well as factors connecting both networks remain poorly understood. We conducted a mini screen exploring systematically the contribution of individual BCL2 family proteins at single cell resolution to death on extended mitotic arrest, and demonstrated that NOXA leads to the degradation of MCL1, enabling BIM-dependent cell death. We could also show that NOXA accumulates in G2 phase and is – similar to MCL1 – degraded during mitotic arrest which lead us to hypothesize that MCL1 and NOXA could be codegraded. To better understand the cell cycle specific regulation of NOXA stability we set out to define the E3 Ubiquitin Ligase that controls NOXA – and by extension MCL1 – turnover. We found that knockdown or knockout of the mitochondria-resident E3 Ligase MARCH5 stabilized both NOXA and MCL1 during mitotic arrest. This was best observed when cell death execution was blocked since otherwise the depletion of MARCH5 strongly sensitized cells to mitotic death, leading to the degradation of MCL1 and to an unexpected accumulation of NOXA. Furthermore we could show that the cell death sensitization by MARCH5 depletion was mostly NOXA dependent. These results argue for a strong involvement of MARCH5 in the stability of both MCL1 and NOXA, therefore influencing the progression of extended mitotic arrest.

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Understanding the role of the BCL-2 protein family in the generation and maintenance of genomic stability.

Microtubule-targeting agents (MTAs) are standard of care for a number of human cancers. Through inhibition of the mitotic spindle, the so-called “spindle assembly checkpoint” (SAC) becomes activated and prevents the cells to progress through mitosis. SAC execution involves the assembly of the mitotic checkpoint complex (MCC) containing - besides other proteins - MAD2. MAD2 sequesters the co-activator of the Anaphase-promoting complex (APC), i.e., CDC20, thereby preventing mitotic exit. Cells kept in mitosis by MCC activation can either die, mainly via apoptosis which involves the BCL-2 protein family, or adapts to the MCC, which causes exit from mitosis in a process termed slippage. Such cells frequently miss-segregate their chromosomes and often fail cytokinesis, both leading to genetically instable cells that often die, yet by so far poorly defined mechanisms. Overexpression of MAD2 was shown to lead to extended mitotic timing, chromosome miss-segregation and aneuploidy as well as increased cell death. The latter response is thought to be induced to avoid chromosomal instability (CIN) that is otherwise linked to tumorigenesis. In our study, we make use of a mouse model with Tetracycline-inducible overexpression of HA-tagged MAD2 in combination with BCL-2 protein overexpression to explore the effects of the latter on chromosomal stability in vitro and cancer progression in vivo. We hypothesize that overexpression of BCL-2 will increase tolerance of MAD2 overexpression-induced CIN and aneuploidy leading to accelerated tumour development. First results on the impact of impaired apoptosis on CIN and aneuploidy using this mouse model and derived cell lines shall be discussed.

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The needles in the haystack: Identification of proliferation-relevant PKA substrates in colon cancer cells

Cellular membrane receptors sense and convert the vast array of extracellular input signals and transmit information through intracellular signaling circuits. Hereby, diverse scaffolding proteins interlink receptors and intracellular effectors to coordinate the spatiotemporal activation of enzymes such as kinases. Deregulation of G protein coupled receptor (GPCR) controlled kinase pathways contributes to the development and progression of cancer. One such example are activating mutations in the stimulating Gαs proteins which lead to constitutive downstream activation of the cAMP-dependent protein kinase A (PKA). We set out to determine the phospho-proteomic composition of macromolecular PKA complexes from a collection of Gαs mutated cancer cells and human glioblastoma biopsies. Using a substractive phospho-proteomic approach, we identified a multitude of proliferation relevant PKA substrates. From those, we selected druggable and cancer-implicated candidates. Currently, we are investigating two such enzymes, tripartite motive 33 (TRIM33) and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) respectively. TRIM33, which generally functions as transcription cofactor and as an ubiquitin ligase, was shown to act as a tumor supressor by counteracting Wnt/β-catenin signaling. We pinpointed PKA as the kinase responsible for the, by far, most frequently phosphorylated site within the enzyme, located in a critical nuclear localization sequence at its C-terminus. Currently we analyze the impact of TRIM33 phosphorylation on both, compartmentalization as well as its ubiquitin ligase activity. PFKFB3 is a key modulator of glycolysis and has recently been implicated in maintaining cancer cell metabolism. We confirmed that PFKFB3 is a PKA substrate and showed that PKA phosphorylation affects its conformation, possibly resulting in an altered activity. Additionally, we found that PFKFB3 inhibition by bioactive small molecules leads to significantly reduced proliferation in colon cancer cells. Last but not least, we currently analyze the impact of PFKFB3 inhibition on gene expression patterns, which might be relevant for PKA controlled reprogramming of the cancer cell.

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The homozygous p.(Leu126Pro) variant in CACNB4 impairs nuclear targeting of the encoded β4b subunit of P/Q-type calcium channels and is associated with intellectual disability, epilepsy and cerebellar atrophy

Voltage-gated calcium channels regulate fundamental cell functions like neurotransmission, pacemaking, and muscle contraction. In the brain, neurotransmission relies on CaV2.1 channels at most synapses, assisted by the auxiliary β subunit, which is essential for the channels to be functionally expressed at the membrane. β subunits consist of an SH3 domain, a GK domain, and a HOOK region connecting the two conserved domains and it has been shown that an hydrophobic pocket (α-binding pocket; ABP) within the GK domain interacts with the α1 subunit. However, the functions of the β subunit are not limited to membrane trafficking of the channel; they also modulate the channel gating properties and some β subunits engage in channel independent functions. For example, it has been the shown that β4b is targeted to the nucleus of CNS neurons and regulate transcription of multiple genes.

Using whole exome sequencing, we identified two siblings with severe intellectual and motor disabilities who carry the homozygous mutation p.(Leu126Pro) in the CACNB4 gene. In order to understand the functional consequences of this mutation leading to such a severe phenotype, we investigated the effect of the corresponding rat β4-L125P mutant in various in vitro models. Using electrophysiology, we showed that β4-L125P and the wildtype β4 are both capable of functionally incorporating the channel in the membrane, giving rise to almost identical currents properties. However, when expressed in myotubes and in neurons, the nuclear targeting of β4-L125P was abolished compared to the wildtype and its axonal trafficking and synapse targeting was also reduced compared to that of the wildtype. In addition, co-immunoprecipitation experiments showed that the ability of β4 to form a complex with the serine-threonine kinase TNIK was reduced by the p.Leu125Pro mutation. Altogether, our results indicate that the p.Leu126Pro mutation alters both channel and non-channel related functions of β4, possibly underlying the severe phenotype in the two siblings.

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Contribution of bipolar cells to the pathology of congenital light blindness type 2

Cav1.4 L-type calcium channels are predominantly expressed in the retinal photoreceptors (PR) and most likely also bipolar cells (BC) where they play a crucial role for light signal transmission. Mutations in the gene encoding Cav1.4 are associated to congenital stationary night blindness type 2 (CSNB2). In patients the Cav1.4 mutation I745T (IT) is associated with a severe CSNB2 phenotype. Our group previously reported that mice carrying the respective mutation serve as a good model for the functional phenotype in humans. Besides scotopic vision also photopic responses are severely effected in CSNB2 patients but the underlying mechanisms are still elusive. In this study we investigated the pronounced impairment in the cone-driven signal transmission using multielectrode array recordings and immunohistochemistry from IT compared to wild type retinas. We dissected ganglion cell (GCs) responses triggered by rods and cone pathways by means of scotopic and photopic light stimulation, respectively. We found that in the IT retina only 26% of the GCs responding to scotopic stimulation showed also a photopic answer indicative of a major impairment in cone light-path. This finding can be explained only by a defect in the cone-to-cone BC synapse. Cone BC-to-GCs transmission was intact, although cone BCs markers revealed sprouting of cone BC dendrites. Application of L-AP4, that typically prevent the transmission only form PR to ON BC, also ablated OFF GC responses in IT retinas in both scotopic and photopic light. These data confirmed the cone-to-cone BC impairment and suggested a flaw in the rod-to-cone pathway because in dim light rods communicate with cone through electrical coupling telodendrites. This hypothesis was supported by cone arrestin immunostainings in whole-mounted retinas which elicited irregular telodendrial contacts. Taken together we report that the strong reduction of the cone-driven signal in IT retinas is due to the impairment of the cones leaving cone BCs unaffected. These findings do not only help to better understand the role of Cav1.4 channels for retinal signaling but are also important for the development of future treatment options for CSNB2 patients.

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Investigations on the role of mTOR signaling pathway in KOR-mediated aversion and antinociceptive activity in mice of HS665, a selective KOR full agonist

Currently, all major opioids (e.g. morphine, oxycodone and fentanyl) used in clinical practice for management of moderate to severe pain are agonists at the mu-opioid receptor (MOR). They are frequently misused, resulting in increased opioid-related overdose deaths (the opioid crisis). Preclinical and clinical evidence has shown that analgesia also occurs upon activation of the kappa-opioid receptor (KOR) without the MOR-mediated adverse effects, such as respiratory depression and constipation. In addition to providing pain relief without the threat of overdose, activation of the KOR causes no physical dependence or abuse liability. However, KOR agonists induce dysphoria, psychotomimesis and sedation. Accumulated evidence indicates that KOR-mediated antinociception results from G protein-mediated signaling events, while alternative signaling pathways (i.e. β-arrestin2-mediated) may promote adverse effects (dysphoria). Recent phosphoproteomics analysis provided a systemic view of KOR in vivo signaling, revealing a novel mechanism of drug action, namely the activation of mechanistic Target of Rapamycin (mTOR) signaling pathway in mouse striatum upon KOR stimulation. Thus, modulation of the KOR emerges as a prominent avenue for the creation of safer non-addictive analgesics. Here, we present a thorough in vitro (binding and functional activity) and in vivo behavioral (nociception, aversion, and sedation/motor activity) study on HS665, a selective KOR full agonist. HS665 shows high affinity and selectivity for the human KOR expressed in CHO cells, is a potent and efficacious KOR agonist in CHO cells and mouse striatum and displays biased signaling towards G protein activation in vitro. In vivo, subcutaneous administration of HS665 produced effective, dose-dependent, KOR-mediated antinociception in mouse models of visceral pain (writhing assay) and inflammatory pain (formalin test). While no sedation/motor dysfunction (rotarod test) was observed in mice treated with HS665, significant aversion (conditioned place aversion) was caused by HS665. Furthermore, pre-treatment of mice with the mTOR inhibitor, temsirolimus, preserve antinociceptive effects of HS665, but did not abolish aversion, indicating that other mechanisms may be responsible for the drug action, as well as the complexity and ligand specific in vivo KOR signaling.

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The role of inflammation in trait anxiety and its attenuation.

Background: Inflammation has been identified as an important mechanism in stress-induced anxiety/depression, but less is known about changes in the inflammatory system in individuals vulnerable to develop such disorders. Enhanced trait anxiety is such a risk factor for developing anxiety disorders and depression. Such patient groups are often treatment-resistant to existing therapies including SSRIs, indicating that alternative targets, including anti-inflammatory approaches, should be considered.

Methods: In an inbred mouse model of high trait anxiety (HAB), we used immunohistochemistry and a multiplex immunoassay to reveal potential inflammatory imbalances, in brain and plasma respectively, compared to normal anxiety (NAB) mice. Chronic oral treatment with the antibiotic minocycline was used to assess the potential of an anti-inflammatory drug on hyperanxiety behavior in HABs.

Results: Our results show that inflammatory imbalances were particularly evident in the dentate gyrus (DG) of the hippocampus, as HAB compared to NAB exhibited considerably increased Iba1+, and phagocytically active (CD68+) microglia. In addition, HAB mice showed alterations in cytokine/chemokine levels in plasma, such as IL-2, IL-4, IL-23, IP-10, IFNγ and CCL2. Minocycline successfully reduced HAB hyperanxiety, which was associated with a decrease in the enhanced Iba1+ and CD68+ Iba1+ cell counts in the DG, and in cytokines in HAB plasma, specifically IL-2 and IL-23.

Conclusions: The present data suggest that disturbances in the inflammatory system can be observed in an animal model of high trait anxiety, without exposure to stress. Minocycline reduced hyperanxiety, likely by modulation of microglial migration and phagocytosis mechanisms. Thus, microglial inhibitors could serve as novel treatment approaches in treatment-resistant patients of anxiety disorders.

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Mapping baseline presynaptic inputs to medial accumbens D1-medium spiny neurons using rabies monosynaptic tracing: focusing on insular cortex input to accumbens D1-MSNs

The nucleus accumbens (Acb) is a major target for studies on the rewarding effects of drugs of abuse or physiological reinforcers since it mediates earlier-stage non-automatic drug-seeking behaviour. Our group had found that a history of dyadic social interaction conditioned place preference (CPP) profoundly inhibited cocaine CPP-associated activation of the medial Acb shell and core (the accumbens corridor) and other brain regions containing projection neurons to the Acb.

Since our group found that dopamine D1 receptor-expressing medium spiny neurons (D1-MSNs) seem to be more involved than D2-MSNs in mediating the rewarding properties of drugs of abuse vs. physiological reinforcers, our aim was to map the direct inputs to a genetically defined neural population, i.e., the D1-MSNs in the medial Acb corridor. Taking into account the extensive literature about the important role of the insular cortex in a many brain functions and in psychiatric and neurological disorders, we gave special attention to this area. In particular, the insular cortex is known to be involved in drug seeking and it is also thought to have a role in social behaviour, important features of our work.

To define the direct inputs to the D1-MSNs in the medial Acb corridor, we have combined the rabies virus mono-trans-synaptic tracing method with a specific Cre-expressing mouse line (Drd1a-Cre::TVA-lacZ). Several brain regions were identified with direct inputs to medial Acb D1-MSNs: infralimbic, prelimbic, piriform and insular cortex, the paraventricular nucleus of the thalamus, ventral hippocampus and basolateral amygdala.

To support our results we also injected a non-cell-type-specific retrograde tracer, i.e., the fluorescent cholera toxin B (CTB), into the same injection site. Robust retrograde labelling was found in many brain areas similar to the rabies virus tracing, an example being our area of focus, the insular cortex.

Thus, to further investigate the insular cortex projection to the medial Acb, we have injected the anterograde tracer Phaseolus vulgaris- leucoagglutinin (PHA-L) in the insular cortex and labeled fibers were detected in the medial accumbens. The comprehensive maps of direct inputs to Acb D1-MSNs, particularly the insula-accumbens pathway, will assist future functional dissection of the neuronetworks mediating motivated behavior.

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Poster abstracts

- Posters should stay up for the whole duration of the meeting and must be taken down immediately after the poster session on Friday.
- Poster presentations should last approximately 3min.
- Even poster numbers at the first hour of your assigned poster session
- Odd poster numbers at the second hour of your assigned poster session
- There will be poster prizes, so stay at your poster during your respective session!

The flash indicates that this poster will also be presented in a flashtalk on the same day.

- Abstracts are sorted by category:

  #1-19: Biochemistry and Cell Biology

  #20-45: Pharmacology and Neuroscience

  #46-52: Analytics

  #53-60: Developmental Biology and Ageing

  #61-69: Genetics and Genomics

  #70-89: Immunity, ID, Oncology and Clinical Medicine

Poster session Thursday

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Garipi	Enis	3	Phenotyping mitochondrial metabolism in oral and oesophageal cancer: respiratory capacity in cancer cell lines and human oesophagus biopsies
Hoppe	Katharina	4	High-resolution profiling of PKA controlled TAF15:RNA interactions by iCLIP
Kahlhofer	Jennifer	5	Molecular mechanism of nutrient transporter regulation in human cells
Kalchschmid	Christina	6	Competitive antiestrogens - coactivator binding site inhibitors or receptor degraders? Biological investigations of homo- and heterodimeric estrogen receptor ligands
Lackner	Katharina	7	Alkylglycerol monooxygenase knockout mouse: A novel tool to study the physiology of an ether lipid-metabolizing enzyme
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Nothdurfter	Daniel	11	3D Bioprinting of microfluidic devices for investigation of tumor angiogenesis
Oliva	Regina	12	Effect of nuclear magnetic resonance on the circadian clock and the hypoxia signaling pathway
Pavel	Petra	13	Alterations in lipid metabolism in the epidermis of Flaky tail (Ft/Ft) mice, a model of atopic dermatitis
Schuler	Fabian	14	Checkpoint kinase 1 is essential for establishing fetal and maintaining adult haematopoiesis
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Poster session Thursday

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Weyer	Yannick	18	The Dsc complex mediates post-ER organelle-associated degradation (pERAD) of a transmembrane protein at cytoplasmic proteasomes
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Ablinger	Cornelia	20	Characterization of the synaptogenic potential of the retinal calcium channel α2δ isoform
Auer	Theresa	21	Functional Characterization of Novel Bumetanide Derivatives for Epilepsy Treatment
Bergmeister	Lisa	22	Structure-function relationship of mitochondria in neuronal diseases
Comeras	Lucas	23	Hunger promotes fear extinction through activation of the amygdala by the paraventricular thalamus
Dumitrascuta	Maria	24	Injectable peptide hydrogels as controlled-release system for opioid peptides and their application for pain treatment
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Fernandez	Monica	26	Structure modeling of CaV1.1 reveals functional trans-domain interactions involved in voltage-sensing
Fontebasso	Veronica	27	The neuropeptidergic PACAP/PAC1 receptor system modulates behavioral and neuroendocrine stress reactions of rats within different forebrain areas
Fritz	Eva Maria	28	How to starve fear: Exploring dopamine and ghrelin as novel targets in a rodent model of refractory anxiety disorders
Heigl	Thomas	29	Proof of concept study for an AAV-mediated supplementation therapy for congenital-stationary night blindness type 2
Hofer	Nadja	30	Novel CACNA1D mutation identified in patient with a severe neurodevelopmental disorder of unknown cause induces severe gating changes in a splice variant dependent manner

Poster session Thursday

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Wanner	Marina	89	Blood lactate levels in relation to 18F-Fluor-desoxyglucose Positron Emission Tomography (PET) results in tumor patients and controls

Engineering chimera constructs to crystallize a complex protein structure

Ras GTPase activating protein (RasGAP) negatively regulates Ras/MAPK signaling pathway, which is one of the most crucial processes in human cells. Neurofibromin is a 320-kDa RasGAP that interacts with Ras via its GAP related domain. It has been demonstrated that EVH1 domain of Spred1 protein plays an important role in membrane localization of neurofibromin where Ras is anchored. In order to get a detailed view of these critical protein interactions and determine the 3-dimensional structure of Ras/GRD of neurofibromin/EVH1 domain of Spred1, obtaining crystal is a fundamental step. Although formation diffracting crystals is a major challenge in biomolecular crystallography and may take ages of experimental trial particularly if the protein domain under study contains inserted domains of much larger size than its own, isolation of such domains may be challenging. Despite substantial efforts, we were unable to obtain diffraction-quality crystals from a purified protein complex of interest, which might be due to the conformational flexibility of some regions of participating protein components in this complex. We used to sequence and structure comparing approaches to engineer chimera constructs of a two domain signal regulatory module that functions as a membrane recruitment platform. Our study allows selection of promising constructs although protein expression and crystallization tests are yet to be performed.

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Cellular succinate transport and mitochondrial respiratory function in prostate cancer.

Succinate dehydrogenase (SDH, mitochondrial Complex II) links the oxidation of succinate to fumarate and reduction of FAD to FADH2 in the tricarboxylic acid (TCA) cycle. Further electron transfer (ET) proceeds from FADH2 to ubiquinone in the ET system. Changes in ET capacity through the succinate pathway affect TCA cycle function and cell respiration. Succinate can accumulate in the mitochondria and be transported to the cytosol playing a role in stabilization of hypoxia inducible factor 1α, finally enabling tumour progression and metastasis. Succinate uptake is enhanced in various cancer cells and its mitochondrial utilisation is increased in permeabilized prostate cancer cells. To decipher the pathophysiological role of succinate in prostate cancer, we tested the utilization of external succinate by mitochondria in terms of succinate pathway capacity and kinetic properties in prostate cancer and control cell lines. Respiration in RWPE-1 (noncancerous), LNCaP (lymph node metastasis) and DU145 (brain metastasis) cells was measured using High-Resolution FluoRespirometry (O2k, Oroboros Instruments) and substrate-uncoupler-inhibitor titration (SUIT) protocols developed specifically for the study. To assess succinate utilization in intact cells independent of a plasma membrane succinate transporter, we applied novel plasma membrane-permeable succinate prodrugs (pS). In LNCaP cells, transport of external succinate is enhanced through the plasma membrane as compared to the other cell lines, while pS exerted similar effects in all cell lines, suggesting an important regulatory role of the transport mechanism. Furthermore, kinetic measurements demonstrated that in LNCaP cells, mitochondria utilize succinate with higher affinity than control cells, underlining its (patho)physiological role. Moreover, our results confirm that a lower extracellular pH can stimulate succinate utilization by mitochondria in LNCaP cells, possibly due to its interaction with the transporter, as previously described. Our results indicate a “succinate-phenotype” in LNCaP, with enhanced transport and utilization. As such, succinate is a potential mitochondrial metabolic biomarker in prostate cancer cells. We propose a model in which succinate does not only play a role in signalling but has a central role in the maintenance of mitochondrial respiration as a fuel substrate.

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Phenotyping mitochondrial metabolism in oral and oesophageal cancer: respiratory capacity in cancer cell lines and human oesophagus biopsies

In 2018, 18 million people worldwide were diagnosed with cancer, with an incidence of 5.5% and a 5-year survival rate of 18-30% for oral and oesophageal cancer (OOC). Two of the main causes for the devastating influence of OOC are the lack of convenient biological markers for its diagnose and effective treatment options. Metabolism of cancer cells is highly adaptable with great plasticity of metabolic pathways, providing key characteristics for survival and spreading. In the last decade, alterations of the pH in the cancer microenvironment and its intracellular regulation emerged as a modulator of cell metabolism. Therefore, we want to address the influence of extracellular and intracellular pH (pHe and pHi) on the metabolism of human oral cell lines (HOK/NOK; DOK and UPCI-SCC090) using High-Resolution FluoRespirometry (O2k, Oroboros Instruments). The contribution of the glycerol 3-phosphate pathway to mitochondrial respiration was compromised at high pHi in UPCI-SCC090 cells. Glycerol 3-phosphate is a crosslink between penthose phosphate and glycolytic pathways. Therefore, pHi and glycerophosphate dehydrogenase presents a possible target for cancer treatment. In addition, we study the mitochondrial metabolic fingerprint of human biopsies obtained during diagnostic endoscopy in patients suspected for Barrett's oesophagus to correlate it with pathohistological reports. This blinded study is ongoing, aimed at finding metabolic biomarkers for the different stages of cancer development such as inflammation, metaplasia, dysplasia and adenocarcinoma.

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High-resolution profiling of PKA controlled TAF15:RNA interactions by iCLIP

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the loss of motor neurons in the brain and spinal cord, leading to muscle weakness, paralysis, and death. During the last years several genetic mutations in ALS patients have been unveiled and most of them comprise genes encoding RNA-binding proteins (RBPs). One of them is the TATA-box binding protein associated factor 15 (TAF15), a member of the FET family. Under normal conditions TAF15 is located in the nucleus controlling transcription, RNA maturation, and RNA splicing. But in ALS patients, TAF15 shows aberrant cytoplasmic localization. However, until now the molecular mechanisms of TAF15 dysfunction in this compartment are still elusive. In a phospho-proteomic screen for cAMP-dependent protein kinase A (PKA) and its interactors we have identified a physical link between PKA and TAF15. We confirmed that TAF15 is a novel PKA substrate, which shows dynamic phosphorylation in one of its RNA-binding modules. Our hypothesis is that PKA activities might have an impact on deregulated RNA-binding functions of mutated TAF15. Our aim is to identify the TAF15-bound RNA species which are directly regulated by PKA phosphorylation. Therefore, we performed the individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) experiments. Using this method it is possible to specify the RNA targets which are bound to TAF15 at nucleotide resolution. We present a detailed workflow how to analyse phosphorylation controlled RNA binders. We assume that the cAMP-PKA signalling axis may interfere with TAF15:RNA complexes which contribute to ALS progression.

This work was supported by the Alfonso Martín Escudero Foundation, the Tirolean Science Fund TWF (AP712003), Tyrolian Cancer Grant and the Austrian Science fund FWF (P22608, P27606, P30441, and SFB-F44).

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Molecular mechanism of nutrient transporter regulation in human cells

Proliferating cells increase nutrient acquisition to fuel anabolic processes for biomass formation. Conversely, differentiated or quiescent cells adjust their nutrient uptake to maintain metabolic homeostasis for survival. A key strategy by which cells reconfigure nutrient uptake across the plasma membrane is by selectively adding or removing nutrient transporters. How cells control nutrient uptake to increase biomass or to preserve homeostasis is not understood.

The goal of this project is to precisely define the molecular mechanisms that control nutrient transporter abundance at the plasma membrane during proliferation and upon entry into quiescence.

Using hTERT RPE1 (human telomerase reverse transcriptase retinal pigmented epithelial) cells, we show that serum withdrawal caused cell cycle arrest and concomitantly induced the selective downregulation by lysosomal degradation of the glutamine transporter SLC1A5/ASCT2 and the neutral amino acid transporter SLC7A5/LAT1 in the non-transformed hTERT RPE1 cells. Other transporters were not affected or even upregulated under similar conditions because the amino acid transporter SLC38A2 is upregulated upon amino acid starvation. Upon re-addition of serum, SLC1A5 and SLC7A5 protein levels increased again. One hypothesis is that the regulation of nutrient transporter abundance might be coupled to cell growth regulation. To analyze the roles of SLC1A5 and SLC7A5 directly, I transformed hTERT RPE1 cells with a construct expressing H-RASV12. The transformed cells expressed higher protein levels of SLC1A5 and were partly “resistant” to starvation-induced endocytosis. Similarly, SLC1A5 were less efficiently degraded in cancer cell lines HeLa and A549. These results might provide first ideas how SLC1A5 and SLC7A5 are upregulated in cancer cells. On my poster I will describe our current working hypothesis and delineate how we proceed with the project.

Overall, the result of my project will provide a better molecular understanding of how cells proliferating cells and quiescent cells control their nutrient transporter repertoire to adjust nutrient uptake accordingly.

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Competitive antiestrogens - coactivator binding site inhibitors or receptor degraders? Biological investigations of homo- and heterodimeric estrogen receptor ligands

Background: The estrogen receptor alpha (ERα) plays an essential role in the treatment of hormone- dependent breast cancer. Selective ER modulators, like tamoxifen, were the first representatives of endocrine breast cancer therapeutics. They can act either as agonists or antagonists. Pure antiestrogens that antagonize and partially downregulate the ERα are also well established. Both, however, have to cope with limitations due to resistances, side effects or their poor pharmacodynamic properties. New approaches to inhibit the estrogen signalling such as bivalent ligand binding, both inter- and intramolecular, have been investigated. Blocking the coactivator binding site (CBS) as well as the ligand binding domain enables to prevent the dimerization and consequently the estrogen activity. The compounds may further provoke degradation of the receptor as an improvement of the clinical profile.

Aims and methods: Guided by structure-based ligand design and assisted by molecular modelling, bivalent ligands tethered by varying spacers have been synthesized and tested for their biological attributes. This study presents the evaluation of the cytotoxicity, the antiestrogenic properties, the binding affinity to the ER and its downregulation caused by the newly synthesized compounds. The determination of cell growth inhibition was assessed in ERα-positive MCF-7 breast cancer cells and the fibroblast-like cell line COS-7 via crystal violet staining. The inhibition of the transactivation was evaluated applying a luciferase based assay in U2OS cells transiently transfected with pSG5-ERα or pSG5-ERβ correspondingly, p(ERE)2-luc+ and pRenilla-CMV. To examine the relative binding affinity a TR-FRET ERalpha competitive binding assay was conducted. Downregulation of the ER was defined by In-Cell Western technology.

Results: The bivalent ligands featured binding affinities in the low nanomolar range. The spacer length and the chemical scaffold, which blocked the CBS, influenced the extent of cytotoxic potential, the ER downregulation and the transactivation interference.

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Alkylglycerol monooxygenase knockout mouse: A novel tool to study the physiology of an ether lipid-metabolizing enzyme

Alkylglycerol monooxygenase (Agmo) is a highly hydrophobic integral membrane enzyme dependent on the vitamin-like cofactor tetrahydrobiopterin. It is the only enzyme so far described capable of cleaving the saturated ether bond of alkylglycerols and lyso-alkylglycerophospholipids generating free fatty acids and glycerol derivatives. Our laboratory discovered the Agmo gene in 2010. Recent publications show a role of Agmo in lipid metabolism of murine RAW264.7 macrophage-like cells pointing to a potential implication in immunology together with other reports on genetic predisposition of humans for leishmaniosis and tuberculosis. Nevertheless, the physiological and pathophysiological role of Agmo is far from being completely understood. In order to study Agmo physiology, we have established an Agmo knockout mouse in C57bl/6J mice starting from EUCOMM stem cells (Germany), which harbor a tm1A Agmo conditional knockout first allele. In this knockout first allele expression of the reporter lacZ is linked to the Agmo promoter via a strong splice acceptor side after exon 1 and insertion of this cassette disrupts translation of a functional Agmo protein. For the characterization of this mouse model we analyzed Agmo activity and Agmo gene expression in eleven tissues. This was performed by a sensitive Agmo activity assay and Taqman technology based quantitative PCR, respectively, in male and female mice with two of these knockout alleles (homozygous knockout mouse) and comparing them to littermates harboring one modified allele (heterozygous knockout mouse) or no modified allele (wild type control). In parallel, we also analyzed lacZ gene expression and performed lacZ stainings in histological sections of selected tissues. Taken together, we could generate an Agmo knockout mouse model and present our first results on this novel and unprecedented tool to study Agmo physiology *in vivo*.

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Anti-inflammatory effects of secondary metabolites from soil algae extracts

A novel cell-based reporter assay was developed for primary screening of soil algae extracts with anti-inflammatory potential. The spontaneously immortalized human keratinocyte cell line HaCaT has been used to generate a stable NF-κB reporter cell line by retroviral transduction. Activity of the transcription factor NF-κB has been utilized as a representative marker of the inflammatory state of cells due to its key role in various inflammatory processes within all human cells. The assay concept is based on induction of an inflammatory response in the NF-κB reporter cell line and screening for anti-inflammatory soil algae extracts. To screen for anti-inflammatory activities in a "receptor independent" approach, cells were treated with non-cytotoxic concentrations of algae extracts either prior to UVB irradiation ("pre-treatment") or afterwards ("post-treatment").

Using the "receptor mediated" approach, cells were treated with extracts after an inflammatory response was induced with TNFα. Possible hits have led to reduced reporter activity in this primary screening, suggesting their potential inhibitory effect on NF-κB. These hits were validated using a concentration gradient to verify the dose dependency of metabolites in soil-algae.

In total 98 extracts were tested, several of them had a pronounced inhibitory effect on the reporter activity, either in the "receptor independent"- or "receptor mediated"-approach, while the dose dependency showed an increase of their effect with higher concentrations of extracts.

Those hits are presently investigated more in secondary screenings to verify their anti-inflammatory potential in physiologically highly relevant 3D human epidermis models. In an ongoing NF-κB pathway analysis, the precise mode of action will be established to investigate direct or indirect effect of secondary metabolites from soil-algae. Cells are treated with algae extracts followed by TNFα treatment and analyzed for degradation/resynthesis of IκBα using immunoblotting. This allows us to discriminate whether the point of action for inhibition is upstream or downstream of the IKK complex. Further studies are designed to more specifically address molecular and signaling details on how algae extracts interfere with the NF-κB pathway.

For characterization of anti-inflammatory metabolites extracts will be fractionated to simplify identification of active compounds by analytical analysis with HPLC/GC-MS. In conclusion, the established inflammatory-response assay is a cost-effective and reliable screening tool to identify algae metabolites capable to identify potent agents, which could be used for further investigations and anti-inflammatory compound development.

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The LAMTOR-complex is necessary for keeping Brown Adipose Tissue Homeostasis

The LAMTOR complex is anchored to late endosomal/lysosomal membranes. It is known to regulate mTORC1 signaling in an amino acid and cholesterol dependent manner and MAPK signaling. Both signaling pathways play a crucial role in cellular homeostasis.

Deletions of LAMTOR components are early embryonic-lethal in mice, but conditional knockouts allow the study of the complex. Microarray and proteomic studies in LAMTOR2 -/- mouse embryonic fibroblasts (MEF) and macrophages point to a regulation of lipid metabolism including lipid synthesis, uptake, transport and degradation. Furthermore, the LAMTOR complex seems to play an important role in adipocyte differentiation since LAMTOR2 -/- MEFs are deficient in adipogenesis. The LAMTOR complex was deleted specifically in adipose tissue using an AdipoqCRE-trangenic mouse line (LT2 AKO). Metabolic and biochemical studies of the brown and white adipose tissue were performed at 30°C, room temperature and 5 °C as well as in fasting and refeeding conditions. The LT2 AKO mice show an accumulation of lipids e.g. triglycerides in the blood, brown adipose tissue (BAT) and liver on chow diet. Although thermogenesis genes are downregulated in BAT of LT2 AKO mice, LT2 AKO mice can keep their body temperature upon cold treatment and the observed phenotype in BAT and blood is reversed.

The mTORC1, AMPK and ERK signaling pathways are especially under fasting and refeeding conditions uncoupled in BAT of LT2 AKO mice. In consequence, the Insulin signaling pathway cannot be switched off sufficiently leading to a higher lipid and glucose uptake into the tissue. This results in the observed lipid accumulation phenotype. Additionally, lipolysis is reduced which further enhances the phenotype. In summary, an adipose tissue specific knock out of LAMTOR2 disrupts a couple of signaling pathways keeping BAT homeostasis leading to effects on the whole body lipid metabolism.

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Analysis of the Impact of α-Synuclein Expression on Protein Dynamics in Yeast

Parkinson disease is the second most common neurodegenerative disorder. Curative treatment does not exist to date and the pathological mechanism remains unclear. Inclusions of aggregated, misfolded α-synuclein (αSyn) are the histological hallmarks of the disease. These accumulations indicate a significant perturbation of protein homeostasis. In turn, the aggregation tendency of αSyn depends on post-translational modifications (PTM). Several of them are conserved from the budding yeast Saccharomyces cerevisiae to humans. In this study, the impact of αSyn expression on protein turnover in yeast was investigated adapting a tool termed tandem fluorescent timer (tFT). The tFT reports on protein degradation by changing its color over time. 4044 yeast strains were simultaneously screened for proteins with altered stability. In parallel, the tool was exploited to monitor the effect of PTM on αSyn turnover. This study provides the basis for systematic characterization of proteome homeostasis upon αSyn expression by using the tFT.

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3D Bioprinting of microfluidic devices for investigation of tumor angiogenesis

3D-cell cultures systems provide better and more complex cell-cell and cell-matrix interactions than conventional 2D-cell cultures and the use of these techniques reduces, refines and replaces animal testing in medical research. Robotic 3D bio-printing adds an additional level of complexity, as also the specific 3D structure of tissues can be mimicked including micro-vessels for nutrient and oxygen supply. The 3D Bioprinting Lab at MUI is the first and until now the only research laboratory in Austria that focuses on this rapidly evolving field of additive manufacturing. By combining different technologies such as custom-designed 3D-printed mini-bioreactors, 3D extrusion and microjet printing we are developing tools for the cultivation of tumor avatars and perfusable macroscopic tissue models to study tumor angiogenesis. These systems will open novel avenues for in-vitro drug testing in precision medicine.

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Effect of nuclear magnetic resonance on the circadian clock and the hypoxia signaling pathway

Nuclear magnetic resonance (NMR) therapy is used for the treatment of musculoskeletal disorders and was originally derived from the magnetic resonance imaging (MRI) technology which is known as a diagnostic tool. Therefore NMR bases on the physical principle of MRI but differs in magnetic field intensity (NMR therapy: 0.4-3 mT compared to MRI: 0.2-5.0 T) as well as frequency (NMR therapy: 17-130 kHz compared to MRI: 10-200 MHz). Our previous studies in zebrafish revealed that NMR significantly affects key genes of the circadian clock and the hypoxia signaling pathway, which are reciprocally regulated and important targets for several diseases such as osteoarthritis. To address the specific effects of NMR also in mammals, we analyzed its impact on these pathways in mouse embryonic fibroblasts. Our results indicate that specific genes of the circadian clock are selectively affected by NMR, thus we also analyzed dosis dependent effects on protein levels of these genes. Taken together NMR therapy affects the cellular clock and the tightly intertwined hypoxia signaling pathway in zebrafish and mouse.

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Alterations in lipid metabolism in the epidermis of Flaky tail (Ft/Ft) mice, a model of atopic dermatitis

Atopic dermatitis (AD) is a chronic and relapsing skin disorder, characterized by epidermal hyperplasia and inflammation. Several defects in the lipid composition of the stratum corneum barrier have been described in AD. Observed changes include alterations in ceramide subfractions, with reduced levels of very long (VL)- and ultra-long-chain fatty acids (ULCFAs). Peroxisomes are involved in the oxidation of complex lipids, including eicosanoids, VL- and ULCFAs. However, their function in metabolism has not yet been studied in the skin. We hypothetised that in AD skin, keratinocytes shift their metabolism to fatty acid oxidation, presumably by using structural lipids as a substrate to provide energy for cutaneous barrier renewal. This may trigger reactive oxygen species (ROS) production and in turn sustain inflammation. High-pressure liquid chromatography analysis showed increased levels of shorter chain fatty acids in ceramides of the epidermis of Ft/Ft mice, when compared to control mice. In contrast, levels of lignoceric- and cerotic acid – both are exclusively oxidized in peroxisomes – are significantly decreased in the epidermis of our mouse model of AD. Moreover, Western blot analysis shows increased protein level of PEX14 – a marker of peroxisomal abundance - in Ft/Ft mouse epidermis. In line with this, microarray analysis revealed increased expression of genes involved in fatty acid metabolism and antioxidant response in the epidermis of Ft/Ft mice, when compared to control mice. Immunefluorescence staining showed elevated protein levels of peroxisomal acyl-coenzyme A oxidase 1 (ACOX1) - that catalyzes the first and rate limiting step of peroxisomal beta oxidation of VLCFAs – and FABP5 (Fatty acid binding protein 5) – involved in lipid shuttling - in the upper layers of the epidermis of our mouse model of AD. Total triglyceride content of the epidermis was decreased in the Ft/Ft mice, indicating an increased fatty acid mobilization form storage pools. Intracellular ATP levels were elevated in samples obtained from Ft/Ft mice, when compared to controls. Our results show that in atopic skin, beta-oxidation of fatty acids in peroxisomes might be triggered to provide cells of the outer epidermal sheet with energy in the form of ATP. This may contribute to lipid abnormalities observed in AD.

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Checkpoint kinase 1 is essential for establishing fetal and maintaining adult haematopoiesis

Checkpoint kinase 1 is critical for S-phase fidelity and preventing premature mitotic entry in the presence of DNA damage. Tumour cells have developed a strong dependence on CHK1 for survival and hence this kinase has developed into a popular drug-target. Chk1-deficiency in mice results in blastocyst death due to G2/M checkpoint-failure showing that it is an essential gene and may be difficult to target therapeutically without side-effects. Here, we show that chemical inhibition of CHK1 kills murine hematopoietic stem and progenitor cells (HSPCs) as well as human CD34+ HSPCs by the induction of BCL2-regulated apoptosis. Moreover, Chk1 is essential for HSPC survival and definite hematopoiesis in the embryo. Cell death in apoptosis-competent HSPCs is independent of p53 but requires the BH3-only proteins BIM and NOXA. Remarkably though, cell death inhibition in HSPCs cannot restore blood formation as HSPCs lacking CHK1 accumulate DNA damage and stop dividing. Conditional deletion of Chk1 in hematopoietic cells of adult mice leads to rapid counter-selection of blood cells retaining CHK1, documenting its essential role in maintaining functional hematopoiesis. Our findings establish a previously unrecognized role for CHK1 in establishing and maintaining hematopoiesis; they also suggest adverse effects of therapeutic CHK1-inhibtion, particularly under conditions forcing stem cells out of dormancy, such as chemotherapy-induced myelosuppression.

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Induction of ferroptosis by iron(II) salene complexes as a novel approach in cancer treatment

Transition metal complexes have a long history in anticancer treatment. Platinum complexes are well established with Cisplatin being the most prominent representative. However, due to side effects and the occurrence of resistances, efforts are made to develop drugs exerting a different mode of action and containing other metals besides platinum. Regarding the latter, iron complexes were found as promising compounds. As salene complexes were previously reported to have strong cytotoxic effects, a series of [diarylsalene]iron(II) complexes was synthesized. The structural variation of the aryl moiety covered both fluorination and methoxylation of the meso- or d,l-configurated compounds, respectively. The newly synthesized complexes were investigated for their activity against different leukemic (HL-60, K-562, LAMA-84) and breast cancer (MCF-7, MDA-MB-231) cell lines. Moreover, the participation of necroptosis and ferroptosis in their mode of action was examined on the example of MDA-MB-231 cells. Ferroptosis is an iron-dependent form of non-apoptotic cell-death. It is characterized by the accumulation of lipid-based reactive oxygen species. All the tested iron salene complexes showed a concentration-dependent attenuation of the metabolic activity and the proliferation. Interestingly, the fluorinated complexes showed stronger antimetabolic and antiproliferative effects than the methoxylated derivatives. The antimetabolic activity of the compounds on MDA-MB-231 cells was completely revoked upon concomitant administration either of the ferroptosis inhibitor Ferrostatin-1 alone or in combination with the necroptosis inhibitor Necrostatin-1. Conspicuously, the cells treated with the d,l-configurated complexes mainly induced cell-death by ferroptosis, as Ferrostatin-1 itself abrogated the antimetabolic activity, while the effect of Necrostatin-1 was negligible. In contrast, the meso-configurated compounds caused ferroptosis as well as necroptosis since both inhibitors were essential to revoke the antimetabolic effect. Ferroptosis exerted by iron(II) salene complexes represents an auspicious perspective for the future development of anticancer active metallodrugs, especially because several tumor cells are susceptible to ferroptosis.

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Molecular mechanism of ESCRT-III assembly during reverse membrane budding

The endosomal sorting complexes required for transport (ESCRT) drive the formation of multivesicular bodies (MVB), membrane abscission at the end of cytokinesis, HIV budding, nuclear envelope closure and repair of holes in the plasma membrane. Common denominators of this complex molecular machinery, in those processes are the AAA ATPase Vps4 and the ESCRT-III complex. ESCRT-III is a hetero-oligomer consisting of the four proteins Vps20, Snf7, Vps24 and Vps2 and its assembly is essential for the recruitment of the Vps4 complex. By quantitative fluorescence lattice light-sheet microscopy, we have shown that ESCRT-III subunits polymerize rapidly on yeast endosomes, together with the recruitment of at least two Vps4 hexamers. During their 3-45 second lifetimes, the ESCRT-III assemblies accumulated 75-200 Snf7 and 15-50 Vps24 molecules. Productive budding events required at least two additional Vps4 hexamers. Membrane budding was associated with continuous, stochastic exchange of Vps4 and ESCRT-III components, rather than steady growth of fixed assemblies, and depended on Vps4 ATPase activity. An all-or-none step led to final release of ESCRT-III and Vps4. We propose a model in which multiple Vps4 hexamers (four or more) draw together several ESCRT-III filaments. This process induces cargo crowding and inward membrane buckling, followed by constriction of the nascent bud neck and ultimately ILV generation by vesicle fission. Tomographic electron microscopy demonstrated that acute disruption of Vps4 recruitment stalled membrane budding. How individual ESCRT-III filaments are formed and how Vps24 and Vps2 bind to each other and to the Snf7 filament is poorly understood. Our goal is to understand how Vps24, Vps2 and Snf7 bind to each other and therefore, how the of the ESCRT-III filament is organized. We will use purified ESCRT components to reconstitute the ESCRT machinery on artificial liposomes for analyzing budding reactions in vitro, and combine this with yeast genetics and biochemical assays, to understand recruitment of the ESCRT machinery.

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The role of CaV1.3 voltage gated calcium channel on pancreatic cell mass

Calcium influx through the High Voltage-gated Calcium Channels (HVCC) is crucial for hormone release from all cells of the pancreatic island of Langerhans. Expression profiling, electrophysiology recordings, and pharmacological experiments demonstrated that pancreatic islet cells express five of the ten HVCC isoforms. Although from all HVCC isoforms the CaV1.3 mRNA levels are the highest, previous functional evidence using two independently generated knock-out mouse models showed a minor contribution of CaV1.3 channels to pancreatic β-cell insulin release and only one CaV1.3-/- mouse showed reduced β–cell mass due to impaired postnatal β-cell neogenesis. Here we investigated the effect of CaV1.3 deletion on postnatal survival of insulin-secreting β-cells and glucagon-secreting α-cells. TUNEL staining of pancreatic slices showed that in 1 day old mice the β-cell apoptosis was not changed by CaV1.3 deletion. Nevertheless, the α-cell apoptosis was significantly higher (p=0.007) in CaV1.3-/- mice (6,33 ± 0,83%) compared to WT controls (4,86 ± 0,43%). Surprisingly, the pancreatic islets of the 14 days old mice showed a dramatic increase in cell apoptosis following CaV1.3 genetic ablation. In CaV1.3-/- pancreatic islets 21,07 ± 1,15 % of β-cells were TUNEL staining positive compared to only 3,42 ± 0,92% in WT. CaV1.3 deletion also increased the pancreatic α-cell apoptosis from 4,60 ± 0,59% in WT to 10,73 ± 2,84% in CaV1.3-/-mice. The 3 month old CaV1.3-/- mice did not show any change in islet cell apoptosis, β-cell mass, or islet morphology compared to WT controls. This suggests that an increased islet cell neogenesis must compensate for the higher apoptosis observed in younger animals. Current experiments are on the way to quantify the proliferation rate in pancreatic islets of WT and CaV1.3-/- mice. Our results show that CaV1.3 L-type calcium channel plays a critical role in pancreatic islet cell survival in a cell specific and age-dependent manner.

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The Dsc complex mediates post-ER organelle-associated degradation (pERAD) of a transmembrane protein at cytoplasmic proteasomes

Two largely independent systems operate in eukaryotic cells to ensure homeostasis of the membrane proteome. In the endoplasmic reticulum (ER) membrane proteins are extracted by the ER-associated degradation machinery (ERAD) and subsequently degraded by cytoplasmic proteasomes. Failure of ERAD activates an evolutionary conserved transcriptional backup system to restore membrane proteostasis termed the ‘unfolded protein response’ (UPR).

In most post-ER organelles (Golgi, plasma membrane, endo-/ lysosomes) membrane proteins are transported in vesicles to endosomes. Here, the multivesicular body (MVB) pathway mediates their selective degradation in lysosomes through the combined action of five endosomal sorting complexes required for transport (ESCRTs). Whether other membrane protein degradation systems or stress responses form a backup for impaired function of the MVB pathway is unknown.

We hypothesized that important backup systems of the MVB pathway might genetically interact with mutants of the ESCRT machinery (i.e. vps4Δ) - in analogy to ERAD and the UPR, where deletion of both pathways is synthetically lethal. We performed a synthetic genetic array screen in budding yeast that yielded 119 candidate genes. Among other hits, we found mutants of the transmembrane ubiquitin ligase complex ‘defective in SREBP cleavage’ (Dsc) and demonstrate that it is a central player in pERAD, a putative novel proteasome-dependent membrane protein degradation system in post-ER organelles with similarities to ERAD. We find that pERAD regulates sphingolipid homeostasis by mediating degradation of the regulatory protein Orm2 following its phosphorylation by the TORC2-Ypk1 protein kinase cascade and migration to post-ER organelles.

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Metabolic regulation of nutrient transport in eukaryotic cells

Nutrient uptake fuels cellular metabolism and thereby promotes cell growth and survival. Little is known about the molecular mechanisms that control nutrient transport across the plasma membrane in response to nutrient availability. We have discovered that changes in amino acid and nitrogen availability selectively triggers endocytosis and lysosomal degradation of over 30 different nutrient transporters in Saccharomyces cerevisiae (Müller et al. eLife 2015). We refer to this process as starvation induced endocytosis which promotes cellular adaptation and ensures entry into quiescence and cell survival during nutrient limitation. To characterize the regulatory networks that couple nutrient availability to nutrient transporter endocytosis we use the methionine transporter Mup1 as a model because it is removed from the cell surface, both in response to nutrient excess and starvation. Mup1 fused pHluorin was introduced into the yeast nonessential knockout collection (5133 genes). The capability of 4962 mutants to sort MUP1-pHluorin to the vacuole where the fluorescence of pHluorin is quenched was analysed by 96-well fluorescence-activated cell sorting (FACS). Potential hits were re-assayed by live cell epifluorescence microscopy. Today I present the initial characterization of the most promising hits from the screen.

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Characterization of the synaptogenic potential of the retinal calcium channel α2δ isoform

Voltage-gated Ca2+ channels (VGCC) play important roles in the central nervous system (CNS). The auxiliary α2δ subunits of these high voltage-activated calcium channels modulate membrane trafficking, current properties, synaptic transmission and synapse formation. Four genes (Cacna2d1-4) encode for α2δ subunits (α2δ-1 to α2δ-4) out of which three isoforms are expressed in the brain. The fourth isoform, α2δ-4 is specifically expressed in photoreceptor cells of the retina. In photoreceptor cells, α2δ-4 has an attributed role in establishing and maintaining the specialized ribbon synapse and mutations of α2δ-4 cause structural and functional abnormalities. By employing an α2δ triple knock-out neuronal culture system and reintroducing any of the three brain α2δ subunits, our group was able to show that α2δ subunits have an essential but highly redundant role in glutamatergic synapse formation and their aberrant expression can account for alterations in synaptic wiring and the formation of mismatched synapses. Here we aimed to compare the subcellular localization and the synaptogenic potential of the retinal L-type with the brain non-L-type α2δ subunits. Using hippocampal neurons we characterized the neuronal surface localization of α2δ-4 and the synapse composition under α2δ-4 overexpression. Comparison of α2δ-4 with the other neuronal α2δ isoforms will reveal whether all α2δ subunits share redundant functions or whether their roles are defined by the cell type and/or calcium channel complex.

To this end we used cultured hippocampal wildtype neurons as expression system and performed high resolution immunofluorescence analysis. Using a 2HA-tagged α2δ-4 construct we were able to show that α2δ-4 is expressed at the neuronal surface, specifically in axons, dendrites and importantly at presynaptic terminals. Intriguingly, and in contrast to the other α2δ isoforms it is predominantly located in the perisynaptic membrane around a central synapsin cluster. In contrast to α2δ-2, overexpression of α2δ-4 did not affect pre- and postsynaptic differentiation and not induce mismatched synapse formation.

In the next step we will test whether expression of α2δ-4 can rescue the defect in synapse formation in our α2δ subunit triple knock out model. This will ultimately reveal the synaptic potential of α2δ-4 and contribute to our understanding of basic neuronal processes. At present the perisynaptic localization of α2δ-4 as well as the absence of mismatched synapses suggest a cell type- or channel complex- specific mechanism of α2δ-subunits.

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Functional Characterization of Novel Bumetanide Derivatives for Epilepsy Treatment

An impaired cellular chloride homeostasis was reported in temporal lobe epilepsy (TLE) and is seen as key factor for the frequent resistance of seizures to GABA mimetic drugs. Therefore, bumetanide, an antagonist to Na K Cl cotransporters (NKCCs), gained interest as potential therapeutic option for patients with therapy resistant TLE. Despite encouraging results in preclinical studies, its strong diuretic effect and poor penetration across the blood brain barrier limit the utility of bumetanide for chronic epilepsy treatment. The aim of this study was to assess the antiepileptic and anxiolytic potential of three novel bumetanide derivatives (CSTS1, CSTS2, CSTS3) with improved pharmacological profiles using in vivo mouse models.

The threshold of acute seizures was not altered by any of the derivatives alone, but CSTS2 potentiated the anticonvulsant effect of the established antiepileptic drug phenobarbital (PB), which acts as GABA mimetic. In the intra hippocampal kainic acid mouse model of TLE inter-ictal spikes, spike trains and hippocampal paroxysmal discharges (HPDs), which serve as model of therapy resistant focal seizures, were assessed by 4 channel in vivo EEG recording. CSTS2 ± PB, bumetanide ± PB and PB alone induced a moderate reduction of HPDs but not spike trains. By contrast, CSTS1, although inactive in the acute seizure model, applied alone and even stronger when combined with PB suppressed spike trains as well as HPDs dose dependently without affecting inter-ictal spikes. The major advantage of CSTS1, however, is that compared to the fast onset and short lasting effect of the other tested compounds higher CSTS1 doses achieved a long lasting reduction of epileptiform activity. Testing all three derivatives in paradigms related to anxiety (open-field, elevated plus maze, light-dark test) did not reveal significant behavioural alterations.

Taken together, our data demonstrate the potential of improved bumetanide derivatives for the treatment of therapy resistant TLE, in particular in combinatorial drug regimes with a GABA mimetic drug. In further work effects of long term treatment need to be investigated.

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Structure-function relationship of mitochondria in neuronal diseases

The involvement of changes in mitochondrial functions and morphology in neuronal diseases like temporal lobe epilepsy (TLE) receives growing attention. Mitochondrial alterations have been observed after epileptic seizures and during epileptogenesis. Additionally, important aspects in the pathogenesis of epilepsy are increased oxidative stress and mitochondrial dysfunctions. During an epileptic seizure hypoxia/ischemia can occur, which is thought to be involved in the neurodegenerative effects of epilepsy. However, different studies demonstrated that hypoxic preconditioning (HPC) can be neuroprotective, can decrease seizure susceptibility and severity. In this study we will focus on the effects of the neuropeptide enkephalin (Enk) and its primary receptor the delta opioid receptor (DOR) on functional and dynamical alterations of mitochondria in HPC. Recently in our lab it was shown that Enk has an influence on mitochondrial respiration. Complex I and II linked respiration where both decreased in naive and epileptic Enk knockout (KO) mice compared to wild type mice. Additionally, regardless of the proconvulsive effects of Enk, KO mice displayed less dynamic hippocampal mitochondrial respiration during epileptogenesis and increased granule cell dispersion 3 weeks after status epilepticus. This indicates a Janus-headed role of Enk in epilepsy. The binding of Enk to DOR is known to elicit neuroprotective effects. It is thought that one of the neuroprotective effects of DOR activation is due to adaptations of mitochondrial respiration. In line with this HPC is suggested to act via DOR.

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Hunger promotes fear extinction through activation of the amygdala by the paraventricular thalamus

Dys-regulation of fear, anxiety and related behavioral disturbances are hallmarks of anxiety disorders, while eating disorders are often linked to anxiety and depression. However, how feeding affects anxiety- or fear-related processes is still unknown. Here we aimed at investigating this interaction and the underlying neuronal circuitries.

Fear was investigated by Pavlovian fear conditioning, in which an initially neutral stimulus, such as a tone (CS), is repetitively paired with an unconditioned stimulus (foot shock, US). The resulting fear memory is characterized by increased freezing behavior to the CS. Importantly, repetitive exposure to the CS in the absence of a US, gradually reduces the acquired fear response, a phenomenon called fear extinction. To identify the involved neuronal ensembles, we performed immunohistochemistry against the immediate early gene c-Fos, a marker of neuronal activity. We further used Tettag, a molecular system that allows the tagging of neurons that were activated only during a specific time window. By this approach we determined whether the same neurons were activated by hunger and fear extinction. Finally, we used neuronal tract tracing to identify the projection targets of hunger- and fear-activated neurons. In fasted mice fear extinction was facilitated when compared to non-fasted controls. Fasting during extinction increased activation of several brain areas, including different amygdala nuclei, which are central for fear processing. We identified a population of neurons in the basolateral amygdala that was activated by both, hunger and fear extinction. Interestingly, the arcuate nucleus of the hypothalamus, an important center for energy homeostasis, has no direct connection to the BLA. By using tract tracing experiments we identified a population of calretenin positive neurons in the paraventricular thalamus that were activated by hunger and directly project to the BLA.

Thus, our experiments outline a neuronal circuit that explains how hunger may modify fear extinction. Calretinin neurons of the paraventricular thalamus may serve as relay station connecting homeostatic signaling from the hypothalamus to extinction-related neurons of the BLA.

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Injectable peptide hydrogels as controlled-release system for opioid peptides and their application for pain treatment

The central goal in the management of severe and chronic pain is analgesia of adequate efficacy and duration. Nowadays opioid-based pain pharmacotherapy offers unsatisfactory long-term solutions, due to serious side effects associated with the chronic administration of opioid drugs, such as morphine, oxycodone and fentanyl, which are mostly applied via oral or parenteral routes. One major focus is the pursuit for opioid-based therapies that can maintain consistent circulatory drug levels, pain relief with improved safety profile, and better patient compliance. Injectable peptide hydrogels represent highly attractive extended-release (ER) systems due to biocompatibility and biodegradability. Consequently, lower dosage and frequency of administration are possible, resulting in improved drug efficacy while lowering the risk of side effects. In this study, we report on the in vitro and in vivo evaluation of two different ER formulations: (i) the analgesic drug is encapsulated within the hydrogel network ('co-formulation'), and (ii) the analgesic pharmacophore is covalently linked to the hydrogelator, resulting in an analgesic hydrogel conjugate ('biogel'). Competition radioligand binding, [35S]GTPγS functional and proteolytic stability assays were used for in vitro characterization. In vivo studies included nociception assessment in a model of thermal nociception and in vivo stability (SPECT/CT imaging) after subcutaneous (s.c.) administration in mice. Peptidomimetic opioid ligands bound with high affinity, were potent and fully efficacious to the mu-opioid receptor in vitro. In vivo, hydrogelators showed a slow degradation profile (SPECT/CT imaging) in mice, with around 20% of the hydrogel still present 72 hours after s.c. injection. Antinociceptive efficacy with prolonged action up to 96 hours was also demonstrated for both ER formulations of active mu-opioid peptides and hydrogelators and biogels after s.c. administration to mice. In conclusion, these results establish the potential of such peptide-based hydrogels as efficient systems for the controlled-delivery of opioids, and opens an avenue for new strategies to treat chronic pain.

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Metabolism of the developing brain in Schizophrenia

Schizophrenia (SCZ) is a devastating psychiatric disorder that appears in late adolescence with a prevalence of 0.5-1% worldwide. SCZ is characterized by a loss of contact with reality and a disruption of thought, perception, mood and movement. Studies on monozygotic (MZ) twins have revealed that, although sharing the same genome, around 50% of SCZ patients have a non-affected twin, suggesting an environmental contribution to the disease. Investigations on the metabolome in plasma of SCZ patients have shown that aberrations in biosynthetic pathways may contribute to the mechanisms of the disease. Cellular reprogramming of patients' cells into induced pluripotent stem (iPS) cells or induced neurons (iN) provide a promising cell source to study the patient specific metabolome and connectivity of neurons in SCZ. Using the iPS cell-based approach, diminished neuronal connectivity, decreased neurite number, PSD95-protein levels and glutamate receptor expression were revealed in neurons differentiated from iPS cells. However, a sub-type specific detailed analysis of the metabolome is lacking.

Here, we aim to perform a multi-omics analysis including RNAseq and metabolic analyses of iPS-derived cortical neurons of SCZ patients and controls to gain a comprehensive unbiased insight into the metabolic components of the developing SCZ brain. For that, we apply cortical neuronal differentiation protocols in 2D adherent culture and 3D organoids. Pilot experiments with undifferentiated neural stem cells and iN will be shown using bioinformatic analysis of metabolites of a targeted metabolomics approach. This analysis reveals substantial differences between proliferating neural stem cells and directly converted neurons, particularly in their lipid components. Additionally, structural dysorganization of the developing SCZ organoids were investigated in cortical 3D models and compared to non-affected MZ. Sox2 and Pax6 positive ventricular zone like structures emerge in immature organoids (40 days in vitro). Tbr1 accumulate outside these ventricular zones like structures but expression of Ctip2 is only found in SCZ positive organoids. Two out of three more mature organoids (100 days in vitro) expresse NeuN positive cells. Seven percent of these cells were positive for Satb2 but without cortical layering.

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Structure modeling of CaV1.1 reveals functional trans-domain interactions involved in voltage-sensing

Voltage-gated calcium channels (CaV) consist of four homologous but non-identical repeats (I, II, III, IV), each containing a separate voltage-sensing domain (VSD) arranged around the common channel pore. Within each VSD the positive gating charges (R1, R2, R3, R4) in the transmembrane helix S4 sequentially interact with negative counter-charges in helices S2 and S3 to support the movement of the gating charges across the electrical field of the membrane and thus to activate or deactivate the channel. Previously we identified an interaction between R1 and R2 with aspartate 1196 (D4) in S3 of the VSD-IV of CaV1.1 that is critical for modulation of voltage-sensitivity and current-density by alternative splicing in the IVS3-S4 linker. Using molecular structure modeling we now identified an additional interaction with a glutamate (E216) in S5 of VSD-I that participates in the R1/R2-D4 interaction of VSD-IV. Charge-neutralization (E216Q) or substitution with alanine (E216A) in CaV1.1e caused a 7mV and 16mV, respectively, right-shift of voltage-dependence of activation and a 30 % reduction in current density. This effect is specific to the splice variant lacking exon 29 and is quantitatively in the same range as previously observed when R1, R2, or D4 were mutated; indicating that this trans-domain interaction between repeats I and IV participates in the voltage-dependent gating and its modulation by alternative splicing. Structure models of CaV1.1 in the activated and pre-open state show that the four VSD differ greatly regarding their intra-domain interactions, consistent with their specific contributions to CaV1.1 gating properties. The newly discovered inter-domain interaction is unique for VSD-I and IV. Together in silico structure modelling of this pseudo-tetrameric ion channel revealed hitherto not appreciated differences between the VSDs and a functional inter-domain interaction.

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The neuropeptidergic PACAP/PAC1 receptor system modulates behavioral and neuroendocrine stress reactions of rats within different forebrain areas

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a neuropeptide with neurotransmitter/neuromodulator properties that has been implicated in the regulation of emotional processes such as stress and anxiety reactions. However, despite the evidence of an implication of PACAP in stress mechanisms, there has been no direct functional evidence for an action of endogenous PACAP in distinct forebrain area on stress responses under ethologically relevant conditions. The aim of the present study was to investigate the role of the PACAP/PAC1 receptor system on neuroendocrine and behavioral stress reactions. We administered a PACAP agonist (PACAP-38) ICV and bilaterally into the PVN or LS of male Sprague-Dawley rats (200-250g) and tested animals in a stress behavioral task such as the modified forced swim test with simultaneous stress hormone measurements in plasma samples. Furthermore, the results obtained were complemented by another behavioral test, the splash test, as measure of motivational behaviour. Moreover, we compared c-Fos expression as a marker of neural activation after central PACAP administration. In addition, we also investigated whether different stress paradigms caused changes in the expression of PACAP38 and its most prominent receptor type the PAC1 receptor in several limbic brain areas. We found that ICV administration of PACAP-38 increased c-Fos expression in PVN and LS. Moreover, ICV as well local administration of PACAP-38 in these brain areas increased the immobility time and reduced active coping behavior during the forced swim stress exposure. Furthermore, administration of PACAP-38 into the LS and PVN significantly increased ACTH stress response without changing basal ACTH levels. Moreover, we found that PACAP38 injection into the LS induced a lack of motivational behavior in response to pleasurable stimuli. Finally, we demostrate that PACAP38 expression was differentially altered in the LS as well as in other brain areas in a stressor-dependent manner. Thus, our data show that the PACAP/PAC1 receptor system is implicated in stress regulation that are mediated within distinct forebrain areas such as PVN and LS. (Funded by the FWF - P28146-B21)

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How to starve fear: Exploring dopamine and ghrelin as novel targets in a rodent model of refractory anxiety disorders

Anxiety and trauma-related disorders constitute the largest group of psychiatric illness in modern western societies, but about 40% of patients do not show long-term benefit from currently available treatments. First-line psychotherapeutic interventions that aim at the extinction of fear fail in patients with an impaired ability to form new, fear-inhibitory memories, leaving them with refractory symptoms and a high risk of relapse. This treatment resistance is well modeled in the 129S1/SvImJ (S1) mouse strain, which exhibits deficient fear extinction following Pavlovian fear conditioning. Previously, our group has reported that enhancing dopaminergic signaling by L-DOPA treatment can rescue the high-fear phenotype of S1 mice. In order to elucidate the involved neurocircuitry we now optogenetically targeted the main dopaminergic input into the infralimbic cortex (IL), a key region in the fear network. Our preliminary findings suggest that selective inhibition of projections from the ventral tegmental area (VTA) to the IL retards extinction learning in usually extinction-competent C57BL/6J mice. To forge new therapeutic paths, we are also exploring non-pharmacological measures to enhance dopaminergic signaling. We utilized a short-term fasting paradigm to stimulate release of the hunger hormone ghrelin, the receptors of which can be found on cell bodies in the VTA, promoting mesocorticolimbic dopamine release. Indeed, we found that in S1 mice fasting increases ghrelin levels and supports the formation of extinction memories and protects from a later reinstatement of fear. Intriguingly, the fasted group also displayed higher expression of Zif268, a marker for neuronal activity, in layer 5/6 of the IL, which receives the majority of dopaminergic VTA projections. As ghrelin itself has also been implicated in various psychiatric disorders we are currently establishing the hormone/receptor profile of our S1 mouse model using ELISA and qPCR. Overall, our data further strengthens the notion of the IL as a critical player in the fear extinction network, mediating dopamine’s extinction-promoting effects. We propose enhancing dopaminergic signaling (pharmacologically or non-pharmacologically) as a powerful strategy to augment existing psychotherapeutic interventions in order to overcome treatment resistance and tackle high relapse rates in patients with refractory anxiety disorders.

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Proof of concept study for an AAV-mediated supplementation therapy for congenital-stationary night blindness type 2

Cav1.4 L-type calcium channels (LTCC) are key players in the retinal signal transduction machinery. They are predominantly expressed at the synaptic photoreceptor terminals and responsible for glutamate release at the ribbon synapse. Human mutations in the CACNA1F gene that encodes Cav1.4 channels lead to congenital stationary night blindness type 2 (CSNB2) in patients. The phenotype includes low visual acuity, involuntary eye movement, myopia, nystagmus and variable levels of night blindness. CSNB2 is only clearly diagnosed by electroretinography. The majority of CSNB2 mutations are loss-of function mutations, where supplementation therapy is one option to reconstitute a functional channel in retinal neurons. Retinal clinical trials have shown that the use of recombinant adeno-associated viruses (rAAVs) as a vector for gene transduction is promising (e.g. NCT01208389). Because the CACNA1F coding sequence exceeds the capacity of AAV vectors, we took advantage of split-inteins, which have the ability to splice separately expressed proteins together in a scarless manner. Therefore, we split the Cav1.4 α1 subunit in the II-III loop region at the position at the position of the amino acid 842 and tagged each half with an optimized version of split-intein from the bacterium Nostoc punctiforme. Western blot analysis of membrane bound proteins showed the successful reconstitution of the Cav1.4 channel in transfected tsA-201 cells. In whole-cell patch-clamp recordings, we confirmed that the reconstituted Cav1.4 LTCCs showed comparable activation and inactivation properties when compared to wild type full-length control in tsA-201 cells. rAAVs carrying the two halves of Cav1.4 driven by a CMV promoter are already available and will be investigated to transduce neuronal cell lines and retinal explants. Retinal explant cultures from adult c57bl6 mice are therefore currently established for the proof-of-concept. Together, these approaches will help to evaluate the pharmaco-therapeutic potential of retinal LTCCs and allow to develop viral based therapies for Cav1.4-mediated retinal diseases. Funding: FWF CavX P7400-024-013, ITN-Switchboard 674901, FWF P29359, the LFUI and CMBI.

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Novel CACNA1D mutation identified in patient with a severe neurodevelopmental disorder of unknown cause induces severe gating changes in a splice variant dependent manner

Background: Low voltage-activated Cav1.3 L-type Ca2+-channels are key regulators of neuronal excitability controlling neuronal development. Recently, large-scale genetic analysis revealed germline de-novo missense mutations in their pore-forming α1-subunit (CACNA1D gene) in eight patients associated with a broad neurodevelopmental disease spectrum. Functional characterization revealed severe gating changes compatible with a gain-of-channel-function. Here we investigated if similar gating changes are observed in a de-novo CACNA1D mutation (IIS4-S5 linker, S652L) which could explain symptoms in a patient diagnosed with a severe neurodevelopmental disorder of unknown cause. Since Cav1.3 channels are subject to extensive splicing we also tested if this mutation stabilizes even more negative activation voltages not only in a C-terminal long WTL (ex8a/ex42) but also in different C-terminally short splice variants WTS (ex8a/ex43s) or WTS-b (ex8b/ex11/ex43s).

Methods: Mutant (S652L) and wild-type (WT) Cav1.3 α1 subunits were co-expressed together with β3 and α2δ-1 subunits in tsA-201 cells and calcium or barium currents (15 mM) were measured using the whole-cell patch-clamp technique.

Results: Mutation S652L dramatically shifted the voltage-dependence of Cav1.3 activation and steady-state inactivation to more negative potentials (~20 mV) with pronounced faster inactivation kinetics. These changes are diagnostic for a disease-causing phenotype. Furthermore, we discovered that splicing outside the C-terminus (ex8b and ex11) contributes to the stabilization of even more negative potentials compared to WTS in WTS-b. Introduction of mutation S652L in various splice variants exerts gating changes in a splice variant dependent manner, in the case of S652LS-b shifting the voltage-dependence of activation and inactivation to even more hyperpolarized potentials (up to 33 mV) compared to WTL.

Conclusion: By demonstrating severe gating changes induced by this mutation we propose that mutation S652L explains the symptoms in this patient with a severe developmental disorder. Our work confirms CACNA1D as a causative disease gene for the development of a broad range of neurodevelopmental disorders. Pathological channel properties are affected by alternative splicing and may therefore alter neuronal functions depending on resting membrane potentials, firing patterns and splice-variant expression. Patients carrying such mutations may benefit from treatment with already available L-type Ca2+ channel blockers, such as nimodipine.

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Presynaptic differentiation at the neuromuscular synapse is orchestrated by postsynaptic calcium

Proper development of the synapses requires reciprocal communication between presynaptic neurons and their postsynaptic target cells. At the neuromuscular junction (NMJ), nerve-induced regulation of the postsynaptic specialization is well-studied, whereas retrograde mechanisms by which the muscle controls the presynaptic differentiation are still poorly understood. We have recently shown that skeletal muscle L-type calcium channel-driven calcium signaling determines where the neuromuscular junctions are formed by regulating AChR clustering and motor axon outgrowth. Here, we report our unexpected discovery that CaV1.1-driven calcium signaling is required also for the retrograde regulation of the presynaptic differentiation at the NMJ. In mice lacking CaV1.1 expression or activity-dependent calcium signals, motor nerves defasciculated and displayed aberrant navigation during early NMJ development. Motor axon branches failed to recognize their postsynaptic target structures, leading to randomly localized motor axon endings. Moreover, synaptic vesicles and active zones failed to correctly accumulate at the nerve terminals facing the postsynaptic structures, suggesting that naïve neurites fail to transform into specialized nerve terminals in the absence of postsynaptic activity-dependent calcium signaling. Although previous studies revealed that each of these characteristics of presynaptic differentiation (defasciculation, navigation, target-recognition and nerve terminal specialization) is regulated by distinct retrograde mechanisms, our data here indicate that, in the absence of postsynaptic calcium, all of these steps fail to occur. Thus, postsynaptic calcium appears to be the master orchestrator of presynaptic differentiation at the neuromuscular synapse.

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Knockout of Cav1.3 channels delays photoreceptor degeneration in rd10 mutant mice, a model of Retinitis Pigmentosa

Retinitis Pigmentosa (RP) is a genetically heterogeneous degenerative retinal disease that is characterized by gradual dysfunction and death of photoreceptors (PRs), first rods and later cones, and progressive blindness. Retinal degeneration 10 (rd10) mice mimic the phenotype of typical human RP. Rd10 mice carry a mutation in the rod-phosphodiesterase 6 gene that causes cGMP accumulation and consequential increase of calcium influx in the outer segments, triggering cell death. Cyclic nucleotide-gated channels mediate the major calcium influx in the outer segment, but L-type Ca2+ channels (LTCC) are dominant in the axon terminal. Previous studies suggest that pharmacological intervention in LTCCs can rescue PRs function in paradigms of RP caused by Ca2+ overflow, even if not all the literature agrees. To answer the question whether LTCCs play a role in the PR apoptotic process, we established a new mouse model crossing rd10 with Cav1.3 KO mice. The latter do not show any major alteration in retinal architecture. When we recorded ganglion cells (GCs) light responses using multielectrode array (MEA) in whole-mounted rd10 and rd10/Cav1.3KO retinas GCs showed similar response to light stimuli at different light level. No significant difference between the mouse strains was observed at P21 (beginning of PR degeneration), P24 (peak of rod degeneration) or P45 (peak of cone degeneration). On contrary, cell counting in the outer nuclear layer (ONL), performed on retinal sections of P45 mice, showed a significant higher survival rate of PRs in the periphery of rd10/Cav1.3KO retinas (mean number of cell rows per 335-450µm sections: 2.46±0.1 in rd10; 4.01±0.1 in rd10/Cav1.3KO, ***p<0.001 ANOVA). Cone arrestin staining also revealed a significantly higher number of cones in the center of rd10/Cav1.3KO retinas (cone number means per 100µm sections: 8±0.4 in rd10; 12±0.4 in rd10/Cav1.3, ***p<0.001, ANOVA). Data from the periphery were not indicative as the degeneration wave has not yet reached peripheral cones at the corresponding age. These results show that Cav1.3 contributes to the death of PRs, however, its absence might not be sufficient to restore proper retinal function. Funding: ITN-Switchboard 674901 to AK, ES, FWF P26881, P29359 to AK, LFUI and CMBI.

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Radiolabelled DOTA-MGS5: A new theranostic probe suitable to target cholecystokinin-2 receptors overexpressed in medullary thyroid carcinoma and other tumours

Introduction Minigastrin (MG) analogues, known for their high potential to target cholecystokinin-2 receptor (CCK2R) expressing tumours, like medullary thyroid carcinoma, show limited applicability caused by high kidney uptake or low enzymatic stability. Recently we have reported on a highly promising new approach to overcome these limitations. By designing a library of new MG analogues we could identify specific amino acid substitutions retaining the receptor affinity of the C-terminal receptor binding sequence (Trp-Met-Asp-Phe-NH2).

Materials & Methods Based on the peptide sequence DGlu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH2 different peptide-derivatives were synthesized introducing the substitution of Met with N-Me-Nle or Phg and of Phe with N-Me-Phe or Nal-1. The binding affinity of the peptides and the cell uptake after radiolabelling with indium-111 was investigated in A431 human epidermoid carcinoma cells transfected with human CCK2R and mock-transfected cells. For derivatives with retained receptor affinity and cell uptake, stability studies were performed in rat tissue homogenates (in vitro) and in BALB/c mice (in vivo) followed by small animal single-photon emission computer tomography (NanoSPECT/CT) and ex vivo biodistribution studies in tumour bearing mice.

Results DOTA-MGS1, DOTA-MGS4 and DOTA-MGS5 showed high CCK2R affinity in the low nanomolar range combined with internalisation when labelled with In-111 (≥25% for DOTA-MGS1 and DOTA-MGS4; >50% DOTA-MGS5; 2 h incubhighation), for DOTA-MGS2 and DOTA-MGS3 lower affinity (IC50 ~100 nM) and internalisation values (<5% after 2 h) were observed. Stability in rat tissue homogenates was in the order of DOTA-MGS4, >DOTA-MGS5, >DOTA-MGS1. The in vivo stability in the blood of BALB/c mice (10 minutes post injection) was highest for 111In-DOTA-MGS5 (82.7 ±3.3%) followed by 111In-DOTA-MGS4 (79.0 ±0.6%) and 111In-DOTA-MGS1 (<1%). Biodistribution studies revealed a moderate tumour uptake for 111In-DOTA-MGS4 and 111In-DOTA-MGS1 (1-10% IA/g) allowing high contrast microSPECT/CT. For 111In-DOTA-MGS5 a highly improved tumour uptake with values of 23.5±1.3% IA/g at 4 h p.i. was observed resulting in extraordinary imaging performance and favourable tumour-to-organ ratios.

Conclusion The applied concept aiming to improve tumour targeting of MG analogues by applying substitutions in the receptor-specific C-terminal sequence is highly promising. Especially DOTA-MGS5 shows high potential for improved diagnostic and therapeutic nuclear medicine applications in patients with CCK2R expressing tumours.

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Functional Neuroanatomy of Prodynorphin

DYN and KOPr are abundantly expressed throughout limbic brain areas and were shown to be involved in stress-induced behavioural alterations, including increased aversion, dysphoria, and anxiety. In line with this, the DYN/KOPr system is implicated in the pathophysiology of depression and addiction. Understanding the highly complex organization of the DYN/KOPr system is a prerequisite for potential therapeutic intervention.

To gain deeper insight into the functional neuroanatomy of the DYN/KOPr system, we implemented independent, yet complementary strategies based on restricted PDYN knock-out or PDYN re-expression within the extended amygdala. Such mice were tested in paradigms related to anxiety and stress-coping behaviour, and cocaine-induced conditioned place preference.

Stress-induced reinstatement of the conditioned place-preference was observed in wild-type animals and several control groups. By contrast, no reinstatement was observed in animals deficient for PDYN expressed in the central amygdala, the bed nucleus of the stria terminalis or NKB-expressing neurons. Still, these animals re-expressed place preference upon the cocaine challenge. Interestingly, no differences in trait anxiety or stress coping behaviour was observed by applying standard tests.

Our findings suggest critical involvement of specific populations of dynorphinergic neurons in stress-induced relapse of drug abuse.

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Epitope specificity of MOG antibodies

Myelin oligodendrocyte glycoprotein (MOG) is uniquely expressed in oligodendrocytes and represents a minor component of the myelin sheets in the mammalian central nervous system (CNS). Despite intensive research its biological function is still not clear. An autoantibody response against MOG is found in demyelinating diseases of the CNS including neuromyelitis spectrum disorder. Animal studies showed that MOG elicits an encephalitogenic T and B cell response in rodents and that MOG antibodies (MOG-Ab) can mediate demyelination.

The pathological role of human MOG-Ab and their epitope specificity remain an important open scientific question. Our preliminary data demonstrate distinct binding patterns of human autoantibodies to different MOG isoforms: those recognizing epitopes located on all isoforms and those only binding to some isoforms. This isolated binding pattern could be explained by conformational epitopes formed of MOG and other proteins or glycolipids. We therefore aim to identify possible interaction partners of MOG and to further investigate the pathophysiological role of MOG-Ab in order to identify pathogenic epitopes. First, MOG-Ab with known MOG isoform binding patterns will be purified from patient samples. Those autoantibodies will be used to identify and characterize MOG interaction partners. Furthermore, we plan to analyze protein-antibody complexes by mass spectroscopy to make an explorative search for possible new interaction candidates and to validate our findings on human brain tissue. Finally, we will analyze MOG-Ab complex internalization, alterations of the cytoskeleton, Ca^{2+}-signaling and activation of the complement system.

Taken together we propose to gain new insights into the pathophysiological role of MOG-Ab and to solve the question whether pathogenicity of human MOG-Ab is indeed dependent on their epitope binding pattern. Furthermore, the identification of MOG interaction partners could also extend our knowledge of its biological function.

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Tracing the neuronal circuits of Neurokinin B neurons in the extended amygdala and their role in fear processing

Behavioral correlates of fear and anxiety reflect survival-relevant adaptations to potentially dangerous environments. However, inappropriate or excessive fear and anxiety may lead to disease, constituting a major burden for affected individuals and society. Identification of specific neuronal processes that cause aversive internal emotional states and direct behavioral responses may serve as an important basis for further translational research. The bed nucleus of the stria terminalis (BNST), a forebrain structure and part of the extended amygdala, has gained considerable attention as an important brain region for human stress-related psychiatric diseases. The BNST is also a rich source of neuropeptides, which act as modulatory neurotransmitters to fundamentally shape neuronal responses subserving distinct behavioral repertoires. For instance, Neurokinin B (NKB), a member of the tachykinin family of neuropeptides, has an important role in the consolidation of learned fear, however, its precise role in the BNST and in particular its function in specific subnulcei and/or distinct projections of the extended amygdala is only beginning to emerge. Thus, we aimed at elucidating the neuronal circuitry of BNST NKB neurons and their role in emotional processing. By combining immunohistochemical and neuronal tract tracing techniques, we mapped the expression and propagation of the elaborated axonal network of BNST NKB neurons. In mice, viral vector mediated chemogenetic activation of NKB neurons during behavioral challenge resulted in increased fear expression, while promoting fear extinction learning. Our next step is to further characterize the projection targets of these neuronal ensembles as well as their neurochemical, electrophysiological and fear-associated activation properties.

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Development and optimization of an AAV based gene therapy in rodent models of temporal lobe epilepsy

Introduction Current pharmacotherapies for epilepsy cause severe side effects and are ineffective in over 30% of patients diagnosed with temporal lobe epilepsy (TLE). Neuropeptide receptors are considered modulators of neuronal activity and may represent valuable novel drug targets to treat epilepsies. In recent years, our lab provided proof of principle that permanent over-expressing of preprodynorphin (pDyn) in the epileptogenic focus reduces seizures in a mouse model of TLE. However, many promising therapeutic strategies proved efficient in mouse models but failed to translate to other species. Therefore, the first aim of this study is to further validate the AAV-based expression of pDyn as gene therapy in TLE. During pDyn processing a multitude of neuropeptides with different pharmacological profiles are produced; since this variability could be a disadvantage, the second aim is to reduce it and to optimize the AAV product sequence to maximize kappa opioid receptor (KOR) affinity and selectivity.

Methods Self-sustained status epilepticus (SSSE) was induced in rats through lateral amygdala electrical stimulation. Focal seizure-like abnormalities in both hippocampi were analyzed using EEG before and after AAV based pDyn expressing (AAV-pDyn) vector infusion. In silico (FlexPepDock Rosetta docking and GROMACS molecular dynamics simulation) and in vitro (phosphoproteomics) and in vivo (pentylenetetrazole mouse model) methods were used to screen for optimized gene product.

Results SSSE-induced EEG abnormalities observed in ipsi- or contralateral hippocampi were almost completely suppressed within 1 month after AAV-pDyn infusion. Modified pDyn derived peptides displayed altered potency and efficacy in restoring the reduced seizure threshold of pDyn KO mice, suggesting potential for optimized therapy vectors. A protocol combining docking and MD simulations seems a reliable source of new modifications to the gene product sequence.

Conclusions Replenishing pDyn in the epileptogenic focus reduces EEG abnormalities in mice and rats. Further optimization of the gene product starting from an in silico screening may lead to a more efficient and safer therapy for TLE.

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Electrophysiological approach to study subtype specificity of L-type calcium channel blockers

Voltage-dependent L-type calcium channels (LTCCs) are necessary for several important physiological processes in the body. One characteristic unique to LTCCs is their high sensitivity for 1,4-dihydropyridines (DHPs), which act as calcium channel inhibitors by binding to the pore-forming α1 subunit. This trait has made DHPs not only an essential tool in analyzing L-type calcium currents in vitro, but also for clinically approved pharmaceuticals. However, DHP blockers are considered nonselective for LTCCs because they inhibit the L-type isoforms (Cav1.2, Cav1.3 and Cav1.4) with similar affinity. CaV1.3 and CaV1.4 members of the LTCC family feature various functions supported by corresponding channelopathies. Mutations in CACNA1D, the gene that codes for the CaV1.3 α1 subunit, have been implicated with deafness, cardiac dysfunction and neuropsychiatric diseases. Mutations in CACNA1F, the gene coding for the CaV1.4 α1 subunit, cause several forms of human retinal diseases. Thus, there is great interest in identifying novel pharmaceutical approaches, which specifically target CaV1.3 or CaV1.4, because CaV1.2 is more sensitive to DHPs and overmedication to target CaV1.3 or CaV1.4 can lead to severe cardiovascular side effects. Since there is a stable cell line for CaV1.3 already available in our institute (Genbank# EU363339), we developed a stable cell line for CaV1.4 using the Flp-In T-REx system. Both cell lines express the α1 subunit together with auxiliary β3 and α2δ-1 subunits. First, by employing the patch clamp technique in the whole-cell configuration, we characterized the newly generated CaV1.4 cell line, and compared both channel physiology and pharmacology (e.g. Isradipine, a DHP) with previous data available in literature. We also showed that the CaV1.4 stable cell line has comparable inactivation and activation gating properties to HEK cells transiently transfected with the same subunit composition. We then focused on LTCC blockers that have been shown to be beneficial in retinal degeneration diseases, both DHPs (e.g. Nilvadipine) and benzothiazepines (e.g. D-cis-Diltiazem). We studied the pharmacological profile through drug perfusion at different concentrations and compared the IC50 values. This approach will help us to assess their potential for pharmacological intervention in retinal LTCC diseases, mainly involving CaV1.4. Funding: ITN-Switchboard 674901, FWF P29359, the LFUI and CMBI.

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Expression of Toll-like receptors in the mouse intrahippocampal kainic acid (KA) model of temporal lobe epilepsy.

Epilepsy is one of the most common neurological disorders, and approximately one-third of the patients are resistant to anti-epileptic drugs. Various studies demonstrate a link between inflammation and epileptogenesis. Toll-like receptors (TLRs) play essential roles in generating innate immune responses, and their activation culminates in proinflammatory cytokine production. In this context, the contribution of TLR4 and IL-1R to inflammation has been largely studied. However, the role of other TLRs has remained concealed. Previous studies have shown increased expression of TLR2 and HMGB1 (High Mobility Group Box 1) in human epilepsy associated with cortical malformations. Additionally, it has been shown that TLR2 and HMGB1 interact. Therefore, HMGB1 might be a ligand for TLR2 in epilepsy. We speculate that other TLRs might also play a role in epileptogenesis due to their well-known involvement in inflammation.

The goal of the current study is to investigate the expression of TLRs in the mouse intrahippocampal kainic acid (KA) model of temporal lobe epilepsy.

Methods: Adult male mice were implanted with telemetric EEG transmitters and injected Intrahippocampally with 7 ng (acute model) or 200 ng (chronic model) of KA and perfused at different time intervals after KA-injection (4, 12, and 24 hours, 4 days, 3 weeks, and 7 weeks). Brain sections were analyzed by immunohistochemistry.

Results: In the chronic model, TLR2 is expressed in neurons already 4 hours after kainate-induced status epilepticus and in microglia after 4 days. TLR2 is expressed in the ipsilateral hippocampus 7 weeks after KA-induced status epilepticus. In the acute model, the injection of a TLR2 agonist 30 minutes before KA significantly increased the numbers of KA-induced seizures.

Using double fluorescence immunohistochemistry, we also saw an expression of TLR5 in astrocytes 7 weeks after KA-induced status epilepticus.

Conclusion: The results suggest an involvement of TLRs in epileptogenesis. We will further investigate TLR-expression with radioactive in situ hybridization in order to confirm our results. Lastly, we will antagonize TLR2 with small-molecules in order to deepen our understanding of their role in epilepsy since these receptors might be a valid target for antiepileptic therapy.

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Region and cell-type specific contributions of mGlu5 receptors to social behavior and anxiety

A large body of evidence has implicated an altered signaling or expression of the metabotropic glutamate 5 (mGlu5) receptor in the pathology of several neuropsychiatric disorders, including autism, anxiety disorders and schizophrenia. In particular, enhanced mGlu5 receptor signaling has been suggested to underlie impaired social behavior, a symptom shared by these disorders. Recent research suggests that systemic deviations in any direction of mGlu5 receptor function may lead to social dysfunction. Whereas germ line deletion of the mGlu5 receptor gene leads to a phenotype showing remarkable analogies with Williams syndrome, characterized by a marked prosocial behavior and enhanced anxiety, increased mGlu5 receptor expression and signaling underlies obsessive-compulsive behavioral abnormalities.

So far, it remains unclear how mGlu5 receptors contribute to the expression of social preference and anxiety and particularly which neural circuits underlying these behaviors are preferentially affected by activity of these receptors. Our work aims to elucidate whether mGlu5 receptor ablation in a cell-type specific manner can affect social and anxiety-like behavior.

Our preliminary data suggests that mGlu5 receptors in the ventral hippocampus modulate novelty-induced locomotion and have divergent effects on social recognition and anxiety. Our findings corroborate the view of a complex role played by mGlu5 receptors in social behavior and anxiety and questions systemic pharmacological treatment strategies aimed at alleviating social dysfunction and pathological anxiety e.g. in neurodevelopmental disorders such as autism

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Plasticity of Amygdala Intercalated Cell Microcircuits in Fear Learning

The amygdala plays a crucial role in attaching emotional significance to environmental cues. Its intercalated cell masses (ITC) are tight clusters of GABAergic neurons, which are distributed around the basolateral amygdala complex (BLA), and appear to be involved in the acquisition as well as extinction of conditioned fear responses. As their ablation results in a deficit of the expression of fear extinction, ITC have been the subject of intense investigations. The aim of our study is to characterize neuron subtypes and plasticity properties of pre- and postsynaptic partners of ITC neurons within the medial paracapsular cluster (mpITC). To address the question whether changes in AMPA and NMDA distribution and density correlate with functional synaptic changes observed during different fear states, a detergent-digested freeze-fracture replica labelling approach was used. By investigating the spatial distribution as well as density of ionotropic glutamatergic receptors from thalamic inputs within postsynaptic specializations, we show that indeed functional changes in the A/N ratio are attributable to changes in the expression pattern of AMPA and NMDA receptors. Furthermore, by combining whole-cell patch-clamp recordings and biocytin labelling with full anatomical reconstruction and analysis of synaptic contacts, we confirm the presence of heterogeneous mpITC projection subtypes. Our data suggest that projections to the amygdalostriatal transition zone (AStria) and, hence, potentially to the striatum, might be a major pathway of the mpITC, which is most likely implicated in a much more complex microcircuit than originally proposed. Together, our results further a circuit-based understanding of how ITC activity can contribute to high and low fear states. Funded by D-A-CH I2215 grant from the FWF (F.F.) and DFG (I.E.)

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Voltage-gated calcium channels in mouse retinal rod bipolar cells

Retinal bipolar cells collect photoreceptor signals in the outer retina and relay the signals to the inner retinal neurons. They modulate their transmitter release through the influx of calcium via L-type calcium channels (LTCC). However, the LTCC subtype is highly debated. We therefore aimed to identify the channels underlying synaptic transmission from rod bipolar cells (RBC) in murine retina.

We collected RBCs by FACS-sorting to isolate cell type-specific mRNA and used qPCR to determine the expression levels of different pore-forming and auxiliary Ca channel subunits. We determined LTCC transcript variants that we found in the qPCR via end-point RT-PCR experiments from RBC-enriched samples and through screening of publically available RNAseq datasets. Finally, we examined the contribution of Cav1.4 to RBC calcium currents in whole-cell patch-clamp recordings from RBCs in acute retina slice preparations of wild-type and Cav1.4 knock-out mouse retinas.

In the FACS-sorted RBC we found expression of Cav1.4, Cav1.3 and a truncated transcript of Cav1.1 which had the highest copy numbers of the three LTCC genes. These pore-forming subunit transcripts were accompanied by β2 and α2δ-4. The RNAseq data-screen verified the expression of all annotated Cav1.4 exons and of a splice variant of Cav1.3 with exons 8a, Δ11, 31b and 32, and also confirmed the absence of the first 15 exons from Cav1.1 transcripts. This retinal Cav1.1 transcript showed two novel exons at the N-terminus that we also confirmed in our RT-PCR experiments. Though there was substantial transcript of Cav1.4 in RBCs, we did not find a strong impact on VGCC currents or biophysical properties when comparing WT and Cav1.4 KO RBCs.

Our qPCR experiments suggested the relevance of Cav1.4 in RBC synaptic transmission. However, our recordings showed that VGCC currents persisted when Cav1.4 channels were knocked out. Hence one of the other two LTCC expressed might take over. To date it has not been demonstrated, that truncated LTCCs can form functional channels, so it remains to be elucidated whether the retinal Cav1.1 transcript might be the first to do so. Furthermore, our findings are of importance for the development of gene-therapeutic approaches in Cav1.4-related retinal diseases.

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Cav2.3 Ca2+ channel activity during SN DA activity patterns: role of accessory β-subunits

Background and Aim: Voltage-gated Ca2+ channels transform membrane depolarizations into intracellular Ca2+ signals and are strongly associated with CNS disorders. More recently there is emerging evidence that Cav2.3 Ca2+ channels contribute to the high vulnerability of substantia nigra dopamine (SN DA) neurons in Parkinson's disease. We therefore biophysically characterize the activity of this channel subtype during SN DA regular pacemaking (2.5 Hz) and burst firing patterns.

Methods: Cav2.3 stably expressing cell lines were generated using the Flp-In T-REx system. Channel properties were investigated by whole-cell patch-clamp using 2 and 15 mM Ca2+ or Ba2+ as charge carriers. The activity of Cav2.3 during SN DA firing patterns was determined by measuring Cav2.3 currents while applying typical SN DA regular action potential waveforms (2.5 Hz) or burst firing protocols as command voltages (recorded from mouse brain slices). To test effects of β-subunits on Cav2.3 gating tsA-201 cells were transfected with Cav2.3e-α1, α2δ1, eGFP and either β2a, C3S/C4S-β2a (a non-palmitoylated mutant), β2d or β3.

Results: During regular SN DA pacemaking firing patterns, the residual Cav2.3 current during prolonged activity was small (~3%) when β3 subunits were part of the channel complex. However, Cav2.3 currents increased about 8-fold after recovery from inactivation during burst firing-induced pauses. Co-transfecting β2a, C3S/C4S-β2a or β2d in comparison to β3 revealed a pronounced shift of the steady-state inactivation curve to more positive voltages (β2a: ~35mV; C3S/C4S-β2a and β2d: ~20mV). Inactivation kinetics were dramatically slower with β2a and C3S/C4S-β2a but faster with β2d whereas the voltage-dependence of activation did not change.

Conclusion: We were able to establish a Cav2.3 stably expressing cell line containing all auxiliary subunits with the expected biophysical properties and high current amplitudes. When using regular SN DA pacemaking protocols we were only able to show a minor contribution of Cav2.3 channels due to their pronounced inactivation properties stabilized by β3. However, we hypothesize that the observed strong inhibition of inactivation by β2a-subunits leads to a higher contribution of Cav2.3 current during continuous pacemaking activity. This hypothesis is currently tested.

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Dissecting the functions of multiple interactions of STAC3 in skeletal muscle excitation-contraction (EC) coupling

The adaptor protein STAC3, previously identified as an essential component of the EC coupling machinery, was reported to have three distinct functions. (1) It facilitates the membrane expression of CaV1.1. (2) It is crucial for the function of CaV1.1 as a voltage sensor and as a calcium channel. (3) It is essential for the conformational coupling between CaV1.1 and the RyR1. We and others have previously identified two distinct interactions that STAC3 establishes with CaV1.1, one between the SH3-1 domain of STAC3 and the II-III loop of CaV1.1, and one between the C1-linker region of STAC3 and the proximal C-terminus of CaV1.1. However, which of these interactions is responsible for each function is still elusive.

Using the CRISPR/Cas9 method, we generated a double STAC3/CaV1.1 KO skeletal muscle cell line. As previously reported in mouse KO myotubes, in the newly generated cell line CaV1.1 currents are negligible and EC coupling fails unless STAC3 expression is rescued.

In order to determine which CaV1.1/STAC3 interaction is responsible for each function of STAC3, we are going to reconstitute STAC3 KO myotubes with STAC3 fragments that contain the domain responsible for that particular interaction. Ongoing recordings of CaV1.1 currents in the presence of either STAC3 fragment indicate that the interaction one between the C1-linker region of STAC3 and the proximal C-terminus of CaV1.1 is essential for the functionality of CaV1.1 as a calcium channel, whereas the one between the SH3-1 domain of STAC3 and the II-III loop of CaV1.1 is dispensable.

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A chemogenetic approach for attenuation of neuronal excitability in temporal lobe epilepsy

With a prevalence of 0.5 – 1 %, epilepsy is one of the most frequent neurological diseases affecting people of all ages. Considering that a third of all epilepsy patients and even 80 % of patients with temporal lobe epilepsy (TLE) do not achieve seizure-freedom with the currently available pharmacotherapies, there is an urgent need for the development of novel treatment strategies.

Gene therapy targeted to the epileptogenic zone has shown encouraging results. Recent evidence provided by our lab demonstrated that gene therapy with adeno-associated viral (AAV) vectors expressing neuropeptides with anticonvulsant effects in the epileptogenic focus can successfully reduce seizures in a model of drug-resistant TLE.

A novel gene therapy approach to achieve targeted and temporally limited suppression of neuronal excitability relies on DREADDs (designer receptors exclusively activated by designer drugs). These genetically engineered G protein-coupled receptors (GPCR), which can be activated solely by otherwise inert drug-like small molecules but not by endogenous ligands, are promising novel tools to modulate neuronal activity. Using the kainic acid mouse model of TLE, we will employ virally expressed KORD (kappa-opioid receptor DREADD), which inhibits neuronal activity upon activation by the pharmacologically inert compound Salvinorin B (SALB). This strategy offers the prospect of suppressing neuronal excitability in a seizure focus 'on demand'.

After unilateral intrahippocampal injection of kainic acid we will inject an AAV 6 vector construct encoding the KORD under the control of a hSyn promoter into the epileptogenic focus in the hippocampus. Subsequently we will evaluate the ability of the KORD to attenuate seizure activity following systemic administration of SALB by analyzing in-vivo EEG recordings regarding ipsilateral hippocampal paroxysmal discharges (hpds) and generalized seizures

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The molecular complexity of (N-Terminal) Pro-B-Type natriuretic peptide forms

N-terminal pro–B-type natriuretic peptide (NT-proBNP) and its C-terminal physiologically active counterpart, B-type natriuretic peptide (BNP), have become well-accepted heart failure (HF) biomarkers and were implemented into the guidelines for HF-management more than 10 years ago. Both forms, as well as their unprocessed precursor proBNP circulate as various N- and C-terminally truncated and O-glycosylated forms in severely ill HF patients. As commercial antibody-derived immunoassays show different affinities to the varying molecular forms and thereby do not reflect their entirety and impede comparisons between platforms, understanding the structural attributes of the molecular heterogeneity in the diverse settings of HF displays a critical prerequisite for improved design of assays as well as their clinical application. We demonstrate a variable distribution of human endogenous (NT-) proBNP forms in different patients. Materials and Methods: Heparinized plasma samples of HF patients were purified by Immunopurification using a biotin-conjugated polyclonal antibody directed against amino acids 1–21 of (NT-) proBNP bound to streptavidin-coated magnetic microparticles. Eluates were partially deglycosylated with an exoglycosidase cocktail and subsequently proteolytically digested. Samples were analyzed by nanoflow liquid chromatography electrospray ionization mass spectrometry (nano-LC ESI-MS) on a Q-Exactive HF (Thermo Fisher Scientific).

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DAPPER - AI for histology classification of H&E-stained Whole Slide Images

The impact of Artificial Intelligence on healthcare domain is exponentially increasing. In particular, Deep Learning breakthrough in computer vision tasks is offering new insights on clinic-pathological features of medical images. In our study, we introduce the DAPPER framework to evaluate deep learning models and classifiers applied to digital pathology, as a hallmark of reproducibility, which is a top priority need in healthcare domain. DAPPER is indeed framed into a rigorous Data Analysis Plan originally developed in the FDA's MAQC project and designed to analyze causes of variability in predictive biomarkers. We applied the DAPPER framework on models trained to identify tissue/organ of origin of 787 H&E stained Whole Slide Images (WSI) publicly available at the Genotype-Tissue Expression (GTEx) portal. From each WSI, we extracted up to 100 informative patches of size 512x512, creating the benchmark dataset HINT (Histological Imaging - Newsy Tiles) composed of 53 000 histological tiles. We worked with four sub-datasets of HINT (5, 10, 20 or 30 classes) and tested our pipeline on the KIMIA Path24 dataset for identification of slide of origin (24 classes). Moreover, we compared our results on a test set with an expert pathologist, both at tile-level and WSI-level: DAPPER outperforms the pathologist in both cases, with an improvement of more than 20% at tile-level. We analyzed accuracy and feature stability of three different deep learning architectures (VGG, ResNet and Inception) as feature extractors and classifiers (a fully connected multilayer, Support Vector Machine and random Forests), also demonstrating the need for diagnostic tests (e.g., random labels) to identify selection bias and risks for reproducibility. The DAPPER software, including its deep learning pipeline and the HINT dataset, is released as a basis for standardization and validation initiatives in AI for digital pathology.

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Quantification of Anidulafungin and Micafungin in Wound Secretion

Introduction: Anidulafungin and micafungin are echinocandins recommended for the treatment of invasive Candida infections because they are highly effective in patients with candidaemia. However, their efficacy in soft tissue and wound infection is largely unknown. Achievement of fungicidal concentrations at the target site is crucial for clinical response. Objectives: Assessment of anidulafungin and micafungin penetration into wound secretion of adult critically ill patients.

Methods: Wound secretion was collected via drainages inserted for therapeutic purpose. Samples were taken once or, when available, before as well as 1, 4, 8, 12, 18, and 24 hours after start of infusion. Blood samples were drawn simultaneously. From patients treated with V.A.C.® (Vacuum Assisted Closure®) therapy, wound secretion was taken once when the collection canister was changed. In the canisters, wound secretion was adsorbed to a gel from which anidulafungin and micafungin had to be extracted. Anidulafungin and micafungin were quantified by high performance liquid chromatography (HPLC) and UV detection at 306 nm after pre-purification by solid phase extraction.

Results: The concentrations of anidulafungin and micafungin were examined in wound secretion samples of fifteen patients. Multiple wound secretion samples were obtained from seven patients. Single samples were taken from four patients via drains and from four patients on V.A.C.® therapy. Anidulafungin concentrations of < 0.01 - 1.94 mg/L were measured in wound secretion, whereas simultaneous anidulafungin plasma concentrations amounted to 0.97 - 9.41 mg/L. The micafungin levels were 0.01 - 1.98 mg/L in wound secretion and 0.40 - 7.43 mg/L in the respective plasma samples. The time to the peak concentration (Tmax) in wound secretion was 8 - 24 hours for anidulafungin and 1 - 24 hours for micafungin.

Conclusion: Both anidulafungin and micafungin reached lower concentrations in wound secretion than in plasma samples collected at the same time. Minimal inhibitory concentrations of some relevant Candida species might equal or even exceed anidulafungin and micafungin levels in wound secretion.

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Characterization of highly modified Histone-Peptides and their effect on Electron Capture Dissociation

Introduction: Posttranslational modifications (PTMs) of proteins play an important role in many biochemical processes, including protein activity, turnover rates, localization within the cell, interaction with other proteins or small molecules, and ultimately, human disease. Mass spectrometry (MS) is ideally suited for the detailed characterization of modified proteins such as histones as it can identify, localize, and potentially quantify all PTMs. However, quantitation of PTMs by MS requires a solid understanding of how they affect ionization by electrospray ionization (ESI) and fragmentation by electron capture dissociation (ECD).

Methods: Experiments were performed on a 7 Tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an ESI source and a hollow dispenser cathode for ECD. Model peptides (26 aa) were partially acetylated by N-Acetylsuccinimide (NAS) at pH ~7 at 90˚C for 10 min. The highly modified peptides were electrosprayed from 1-2.5 µM solutions in 1:1 CH3OH/H2O with 1 vol% acetic acid at a pH ~3.

Preliminary data: Various histone H3 peptide forms, i.e., peptides with the same canonical amino acid sequence but different methylation sites and extent, including mono-, di-, and trimethylation, were studied by ESI and ECD. For this propose, model peptide (26 aa) ions were isolated at charge states 3+, 4+, 5+ and 6+ and dissociated by ECD. Lysine trimethylation (fixed charge) was found to have an effect on the site-specific yield at charge state 3+ whereas higher charge states of the different modified peptides showed no significant differences in ionization and fragment ion yields. To eliminate possible interferences of other lysine residues that could mask individual effects of methylation, the N-terminus and the lysine side chains were acetylated at their primary amines by NAS. Peptides with different numbers of acetylated side chains in combination with the lysine trimethylation (fixed charge) revealed an significant effect on Electron Capture Dissociation.

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Dual-targeting Agents Towards αvβ3 Integrin and Folate Receptors for Molecular Imaging

Folate-based radiopharmaceuticals have been used for targeting the folate receptor (FR) positive malignant tissue (e.g. ovarian and brain cancer) due to their high affinities to FR. Cyclic Arg-Gly-Asp (c(RGD)) peptides show high affinity to αvβ3 integrin receptors which are overexpressed during tumor-induced angiogenesis, an essential mechanism for tumor growth and metastasis. Therefore c(RGD) peptides are recognized as targeting vector for tumor-induced angiogenesis imaging. Click chemistry, a method for highly selective reaction under mild condition, has been used intensively for bioconjugations in recent years. Here we present the introduction of multiple types of targeting vectors, folate and c(RGD), to one chelator fusarinine C (FSC) in a “one-pot” using orthogonal “click” reactions. All heterobivalent compounds were then evaluated for dual-targeting of FR and αvβ3 integrin.

FSC derivatives with folate and c(RGDfK) motifs were synthesized in a three-step one-pot synthesis. Starting with [Fe]FSC functionalized with clickable moieties, alkyne and maleimide, thiol-maleimide click reaction with c(RGDfK)-(PEG)4-SH was performed, followed by Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC) with folate-(PEG)3-N3 and subsequent iron removal. Stability, binding affinity (IC50), distribution coefficient (logD) and protein binding were investigated. Internalization assays were performed in FR-positive cancer cells (KB) and human melanoma αvβ3-positive cells (M21).

Orthogonal click reactions, thiol-maleimide and CuAAC, were successfully applied for a one-pot synthesis of heterobivalent conjugates. Products were obtained in moderate yields and could be radiolabeled with [68Ga] in quantitative radiochemical yields (> 99 % RCY). All conjugates revealed high hydrophilicity (logD = -3.46 to -3.83) and low protein binding. A dimeric folate derivative showed higher specific uptake in KB cells than monomeric folate conjugates. A dimeric c(RGDfK) conjugate showed higher affinity and uptake on M21 cells as compared to two monomeric c(RGDfK) conjugates. Biodistribution studies and microPET/CT imaging in tumor xenografted mice are ongoing.

This study shows the possibility of applying orthogonal click reactions for introducing different targeting vectors to one chelator scaffold in a one-pot synthesis with high selectivity and without purification steps. This finding may stimulate new strategies for the design of dual-targeting agents not only for diagnostic but also for therapeutic and theranostic applications.

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Role of Corticosteroids on Immune Cells within a 3D lung model

Background: During fungal infection of the lung, there are two major immune responses: these include phagocytosis by resident macrophages and non-oxidative killing of conidia. This renders healthy individuals relatively resistant to invasive fungal disease. However, when it comes to immunocompromised hosts, colonization by opportunistic respiratory fungi is promoted due to lack of immune cells thus making treatment and management of fungal infections challenging. Aspergillus fumigatus is the most prevalent pathogenic fungus affecting both upper and lower respiratory tracts. Therefore, understanding the mechanisms by which the pathogen interacts with the host is essential. There have been several reports alleging that high-dose corticosteroid treatment is a major predisposing factor for the acquisition and severity of invasive Aspergillosis. Therefore, it is necessary to delineate the mechanisms of action of corticosteroids and fungal infections in detail in order to improve diagnostic and therapeutic approaches.

Methods: Monocultures of various immune cells will be generated and exposed to various concentrations of Dexamethasone (low to high dose). They will then be infected with PolyAb-exposed and -non-exposed DsRed-labelled A. fumigatus conidia +/- Dexamethasone and analyzed for fungal growth, cytokine and surface marker expression. In addition, immune cells will be co-cultured with respiratory epithelial cells in air-liquid interphase medium (3D lung model), infected as described above and observed for pathogen-specific effects Culture of DsRed-labelled Aspergillus fumigatus will be done on Aspergillus Complete Medium. Conidia will be harvested and used to infect the cell cultures. Confocal microscopy will be applied to study phagocytic uptake and other cell functions and cell viability will be assessed. FACS analysis will confirm cell types via their surface markers. For biomarker evaluation, supernatants will be analyzed by ELISA or luminex assay and real-time PCR will quantify fungal burden and gene expression.

Descriptive Summary: During the project period we plan to study immune cell characteristics in the presence of corticosteroids during fungal exposure and elucidate the specific role that these drugs play when it comes to fungal colonization, management of disease burden and cell functions. The 3D lung cell model will be employed in order to simulate a condition similar to the in vivo setting.

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Audio and 3D-Visual guidance for optimal placement of an Auditory Brainstem Implant with magnetic navigation and maximum clinical application accuracy

This work presents a dynamic 4D (audio and 3D-visual) feedback system for positional guidance in real-time during surgical placement of an Auditory Brainstem Implant.

At present, dummy and simultaneous preoperative measurements, usually including the CT and/or MRI scans of the patient's head, are used to determine the optimal position for an Auditory Brainstem Implant (ABI) on the nucleus cochlear. When found, the optimal position is marked by the surgeon and in the next phase the surgeon tries to locate the optimal position in the patient´s head and place the implant.

With current technology there are no quantifiable methods for storing the optimal implant position. Among other things, brainstem implants do not always provide satisfying and predictable results in hearing perception. Therefore, the aim is to equip the surgeon with a new navigation system based on magnetic navigation and maximum clinical application accuracy; to provide intuitive audio and 3D-visual guidance for positioning the implant; to store the optimal position of the implant in a database and to reevaluate its optimal position.

The navigation software will be platform-independent and developed in the C++ programming language. The basis for this software is Rhinospider Technology developed by the University Clinic for ENT at the Medical University of Innsbruck. One of the goals is to get the certification of this software as a medical device. This project would be paving the path for intraoperative support during ABI procedures.

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Development of a novel mitotic inhibitor targeting the NOXA-MCL1-BIM axis

Caspase-2 is activated in response to extended mitotic arrest and is an essential mediator of mitotic cell death and cytokinesis failure-induced arrest. We took advantage of this property to identify novel mitosis targeting agents. Using a fluorescence-based reporter of caspase-2 activation to screen a library of drug-like molecules, we identified 372 as a potent inducer of mitotic arrest and cell death. 372 acts through inhibition of bipolar spindle formation, inducing proteasome-dependent MCL-1 degradation, p53 activation, and Bax/Bak-dependent apoptosis in a pathway which involves NOXA. 372 is active against a range of tumor cell lines as well as primary leukemic bone marrow progenitors. A back-screen against 133 other antitumor agents revealed that 372 synergizes with BCR-ABL inhibiton, and is able to deplete both wild type and p53 mutant BCR-ABL+ cells.

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Cultivation of Human Brain-Like Tissue Models in a 3D-Printed Minibioreactor

Standard three-dimensional (3D) tissue-like in vitro cultivation involves agitation-mediating devices such as orbital shakers or stirred-tank bioreactors, which facilitate oxygen and nutrient uptake and help forming compact spheroids. However, these devices do not support parallelization while allowing minimal condition testing only. Here were show a customized, 3D-printed minibioreactor that can be utilized for brain-like tissue cultivation in up to 48 (12-well format) or 96 (24-well format) conditions in parallel due to its stackable setup. We find that our minibioreactor in combination with a modified cultivation protocol for cerebral organoids is beneficial for standardized growth, shape and nutrient supply. These optimized conditions result in the formation of brain-like tissues with an overall more physiological, non-spheroid morphology as compared to standard agitation cultivation. Moreover, we show that human neuroblastoma-derived spheroids profited from cultivation in the minibioreactor judged by an average 2-fold increase in diameter. We anticipate our optimized procedure to be of high value for cost-efficient and robust realization of in vitro 3D models of brain and tumor development needed to parallelize drug screening at small scale.

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Chronic treatment with five vascular risk factors causes cerebral amyloid angiopathy but no Alzheimer pathology in C57BL6 mice

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder and the most common form of dementia coming along with cerebral amyloid angiopathy (CAA) in more than 70% of all cases. However, CAA occurs also in pure form without AD pathology. Vascular life style risk factors such as obesity, hypertension, hypercholesterolemia, diabetes, stress or an old age play an important role in the progression of CAA. So far, no animal model for sporadic CAA has been reported, thus the aim of the present study was to create and characterize a new mouse model for sporadic CAA by treatment with different vascular risk factors. Healthy C57BL6 mice were treated with lifestyle vascular risk factors for 35 or 56 weeks: lipopolysaccharide, social stress, streptozotozin, high cholesterol diet and copper in the drinking water. Four behavioral tests (black-white box, classical maze, cheeseboard maze and plus-maze discriminative avoidance task) showed impaired learning, memory and executive functions as well as anxiety with increased age. The treated animals exhibited increased plasma levels of cortisol, insulin, interleukin-1ß, glucose and cholesterol, confirming the effectiveness of the treatment. Confocal microscopy analysis displayed severe vessel damage already after 35 weeks of treatment. IgG positive staining points to a severe blood-brain barrier (BBB) disruption and furthermore, cerebral bleedings were observed in a much higher amount in the treatment group. Importantly, inclusions of beta-amyloid in the vessels indicated the development of CAA, but no deposition of beta-amyloid plaques and tau pathology in the brains were seen. Taken together, we characterized a novel sporadic CAA mouse model, which offers a strategy to study the progression of the disease and therapeutic and diagnostic interventions.

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The role of CD24 in adipose stem/progenitor cells

Adipose derived stem/progenitor cells (ASCs) are essential for adipose tissue regeneration und homeostasis. These cells can be isolated from the stromal vascular fraction (SVF) of adipose tissue which is a heterogeneous cell population that consists of pericytes, endothelial cells, and several other cell types besides ASCs. Cell surface proteins can be employed as markers to define ASCs. Freshly isolated ASCs can be defined as cell surface DLK1-/CD34+/CD24+/CD45-/CD31-. The glycophasphatidylinositol-linked cell surface receptor CD24, that is expressed by various stem cell populations and thought to play a role in cell proliferation and differentiation, is an interesting candidate for a functional cell surface marker of ASCs. However, the functions of CD24 in ASCs remain to be elucidated. To analyse the role of CD24 in ASCs ex vivo we isolated the SVF of human subcutaneous white adipose tissue and sorted cs-DLK1-/CD34+/CD24+ and cs-DLK1-/CD34+/CD24- subpopulations. We show that only a small proportion of freshly isolated ASCs express CD24 and present data on the functional characterization of the CD24+ and CD24- ASC subpopulations.

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CD8+ HLA-DR+ regulatory T cells in old age

Background: Regulatory T cells are a self-check subpopulation of T cells and are one of the most progressing T cell lines in task of research programs. Their main task is to prevent the immune system of excessive reactions. Besides their importance in the field of autoimmunity correlating with age and cancer causing disabilities, this cell type plays also an important role in the research of the so called subclinical uncontrolled processes referred to as ``inflammaging``. Although CD8+ regulatory T cells have first been described to show immunosuppressive properties, it took quite a time to characterize a distinct phenotype whereas CD4+ regulatory T cells were intensively studied meanwhile. Recently, CD8+ HLA-DR+ T cells were reported to have immunoregulatory properties and as such, could play an important role in modulating the immune balance. Methods: Similar to my Master thesis project we will use several FACS staining and sorting techniques, with and without stimulation of PBMCs and later also with BMMCs and using ELISA to check for CMV-titers. We will also include a Microarray and validate selected genes with qPCR as well as suppressive assays.

Results: We were able to prove this cell line as Regulatory T cells through my master thesis and checked their suppressive activity also in the old age where they show lower suppressive impact correlating with increased number of expression of this cell line. We also checked for the expression of the classical checkpoint inhibitory markers CTLA-4, PD-1, TIM-3 and LAG-3 where they showed decreased numbers in old age. At least we found out, that persons without CMV-infection show increased numbers of these mentioned molecules.

Conclusions: With my upcoming PhD I aim for a more detailed characterization of this cell line and what cell functions and what cell types correlating with age can get influenced by the activity of CD8+ HLA-DR+ Regulatory T cells. Therefore I hope to contribute to a better understanding of the immune system leading to a healthier aging.

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The Role of p27Kip1 in Erythroid Cell Proliferation and Differentiation.

Erythropoiesis is a complex process during which hematopoietic stem cells (HSC) differentiate and give rise to mature erythrocytes. The earliest committed progenitors of the erythroid lineage are the burst forming unit-erythroid cells, which differentiate into colony-forming unit-erythroid (CFU-E) progenitors. The CFU-E progenitors divide rapidly and they are highly dependent on the presence of Erythropoietin (Epo). Binding of Epo to its receptor, EpoR, triggers multiple signal transduction pathways through the activation of JAK2, which regulate proliferation, survival and differentiation of erythroid progenitors. Activation of EpoR is essential for erythropoiesis and the receptor is not only highly expressed in the CFU-E progenitor cells but remains expressed during the late stages of erythroid differentiation. Erythroid differentiation is associated with accumulation of the cyclin-dependent kinase inhibitor p27Kip1, but little is known about its role and regulation during erythroid proliferation. We previously observed that phosphorylation of p27Kip1 on tyrosine residue 88 (Y88) by a number of tyrosine kinases, including JAK2 and Lyn, impairs its CDK-inhibitory capacity and can initiate its ubiquitin-dependent degradation. In this project, we investigate the role of p27Kip1 in response to Epo stimulation in vivo and in vitro during erythropoiesis. In UT-7/Epo cells, we observed that JAK2 phosphorylates p27Kip1 on tyrosine residue 88 upon Epo stimulation. Endogenous p27Kip1 co-immunoprecipitated with EpoR in UT-7/Epo cells. Surprisingly, using bacterially expressed recombinant proteins, we found that p27Kip1 can directly bind to the EpoR in vitro even in the absence of JAK2. To further elucidate the role of p27Kip1 in EpoR signaling, we monitored the stages of erythropoiesis in the knock-in mouse model, p27Y88F/Y88F C57BL/6. We found that the knock-in mice have a significantly lower number of CFU-E colonies related to the wild type littermates, indicating a defect in proliferation in the early stages of erythropoiesis. Furthermore, FACS analysis with the use of the erythroid differentiation markers CD71 and Ter-119 revealed that p27Y88F/Y88F mice displayed an enhanced erythroid differentiation in comparison to the wild type.

Our study suggests that p27Kip1 has a role in early stages of erythropoiesis and additional studies are needed to uncover the molecular mechanism underlying this phenotype.

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CRISPR/Cas9 mediated modifications in the locus of diabetes-related gene MNX1 alter expression profile of its splice variants

Modification of complex loci on human genome creates technically difficult situations. Particularly studying functions of lineage specific genes and transcription factors involved in tissue specification possesses great challenges. Due to the presence of splice variants, essential domains within the protein and sequence complexity (repetitive elements and GC content), design of gene targeting applications require better understanding of the regulation on the gene of interest. In our project, we designed specific genome editing strategies to modify the diabetes-associated MNX1 gene in order to investigate its functions in pancreatic beta-cell formation. Different constructs were crafted for modification of the locus by CRISPR/Cas9 genome editing to generate knock-ins with tagged MNX1 or conditional knock-outs to study its loss-of-function at specific stages of beta-cell differentiation in human stem cell models. Surprisingly, we observed that integration of large foreign fragments into the N-terminal region and the intronic areas severely interfered with gene expression, whereas a C-terminal modification did not lead to a remarkable change in the transcript levels. In addition, levels of splice variants also varied depending on integration area and the construct. In the light of the results presented in this work, we hope to establish a better understanding of gene structure and improved genome editing strategies.

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Linking Ciona larval settlement to ageing

Ciona intestinalis is an invertebrate model organism with a close vicinity to the vertebrates, and tissue resemblance of their tadpole larvae. Interestingly, the settlement of free-swimming Ciona larvae triggers aging related events like tissue resorbtion and apoptosis during their metamorphosis to the adult. However, the question of how larval settling triggers ageing related tissue remodelling at a molecular level is not well known yet. Because Ciona is well established to answer diverse questions of cell biology at molecular and even at gene regulatory network level, we are examining such intruiging links. To better understand the event of attachment, we first figured out the cellular structure of the settlement organs (papillae) and the factors that may influence the settlement. Interestingly, we found that L-DOPA, normally stored in supporting test cells is actively taken up via the papillae during settlement. The removal of test cells perturbs the tissue remodelling events suggesting that papillar DOPA could play a role in coordinating this process. As a known neurotransmitter precursor, L-DOPA is clinically used in the management of Parkinson's disease to improve the event of neurodegeneration. In addition, recent attention of ageing research considers a role of RAS-MAPK-ERK-ETS signalling, since blocking the ERK-ETS signalling pathway was associated with longevity in Drosophila and other organism. Remarkably, the pharmacological ERK inhibitor U0126, well described to block neural induction in Ciona also blocks larval settlement. We have found an ETS repressor factor, ERFa, that is expressed in neural and papillar precursors and may therefore influence ERK signaling at metamorphosis. In preliminary experiments we have used the palp lineage specific overexpression of Ci-ERF and ETS¬-VP16, and in both cases could significantly influence the larval papillae formation required for triggering metamorphosis events. Therefore, the effect of ERF repression in the process of larval settlement and the regulation of age related processes will be further investigated. Finally, from our investigation of L-DOPA and ERF, Ciona can be further developed to a model for age related effects in the human with a long-term goal to design tissue targeted anti-ageing compounds.

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Sit1 and Sit2 mediate utilization of ferrichrome-type and ferrioxamine-type siderophores in Aspergillus fumigatus

Introduction: Iron is an essential micronutrient. The mold Aspergillus fumigatus employs two high affinity iron uptake systems, reductive iron assimilation (RIA) and siderophore-mediated iron acquisition. Siderophores are low-molecular mass ferric iron-specific chelators. A. fumigatus secretes two fusarinine-type siderophores for iron uptake, triacetylfusarinine C (TAFC) and fusarinine C (FsC), and employs two ferrichrome-type siderophores for intracellular handling of iron, ferricrocin (FC) and hydroxyferricrocin (HFC). Siderophore biosynthesis has been shown to be essential for virulence of this opportunistic human pathogen and to enable imaging of fungal infections. A. fumigatus possesses five putative siderophore transporters, which are transcriptionally repressed by iron indicating a role in iron homeostasis.

Results: To characterize siderophore uptake in A. fumigatus, we generated mutants lacking the putative siderophore transporters Sit1 and Sit2, respectively, in a genetic background avoiding interference with endogenous siderophores and RIA. This allows characterization of siderophore uptake by growth studies. Lack of either Sit1 or Sit2 did not affect utilization of FC and the fungal xenosiderophore coprogen, while combined lack of Sit1 and Sit2 dramatically decreased their utilization. Lack of Sit1 blocked utilization of ferrioxamines B, G and E – xenosiderophores produced by Streptomycetes, and decreased utilization of the fungal ferrichrome-type siderophore ferrichrome A. Lack of Sit2 significantly decreased utilization of the ferrichrome-type xenosiderophores ferrirhodin and ferrirubin, which are produced by other Aspergillus species. In contrast, Sit1, Sit2 or both did not affect utilization of FsC or TAFC. Notably, ferrichrome A and coprogen were only poorly utilized and ferrioxamines did not support growth and sporulation to the same extent as FC or TAFC.

Conclusions: This study reveals that A. fumigatus is able to utilize a wide spectrum of xenosiderophores, although with different efficiency. Furthermore, we identified substrate specificity differences of Sit2 and Sit2 transporters, even within ferrichrome-type siderophores.

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Fishing for complements: a study on periodontal Ehlers-Danlos syndrome

Introduction: Periodontal Ehlers–Danlos syndrome (pEDS) is a connective-tissue disorder characterized by early severe periodontitis and various joint and skin manifestations. Other EDS types are caused by mutations in collagens or protein-modifying enzymes. In contrast, periodontal type is caused by heterozygous missense or in-frame insertion/deletion mutations in C1R or C1S, indicating a previously unknown connection between the inflammatory complement pathway and connective tissue homeostasis. pEDS is autosomal dominant and involves gain-of-function effects; loss-of-function variants in C1R/C1S are asymptomatic when heterozygous and can cause a lupus-like phenotype when homozygous.

Methods/Results: In-vitro overexpression of all known C1r variants in HEK293T cells revealed that all pEDS mutations retain enzymatic function in the complement cascade but show domain-specific abnormalities of intracellular processing and secretion. Unlike C1r wild type, mutations in the CUB1 and CCP1 domains of C1r result in intracellular retention of the N-terminal interaction domains. Mutations in the CUB2 domain cause secretion of aggregated N-terminal fragments, whereas CCP2 domain mutations induce a new cleavage site. Importantly, the C-terminal catalytic fragment from all C1r mutants was secreted and enzymatically active in the supernatant. Western blot analysis of patient-derived skin and gingival fibroblasts confirmed aberrant activation and secretion of mutated C1r even in the presence of C1s and C1 inhibitor. C1s activation was confirmed by in-vitro complement activation assays.

Conclusion: pEDS is caused by gain-of-function mutations that cause abnormal activation of complement 1 independent of microbial triggers. We hypothesize that secreted catalytic fragments cleave extracellular matrix proteins with adverse consequences on connective tissue homeostasis

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Mitochondrial-derived tRNAs: Novel regulators of gene expression?

Computational analysis of next-generation sequencing data revealed the presence of mitochondrial genome-derived tRNAs within 187 nuclear genomic positions, most of which were intronic regions. Although some of these mitochondrial-like tRNAs (mtl-tRNAs) displayed point mutations compared to their mitochondrial counterparts, the canonical secondary structure of tRNAs was maintained, pointing to an evolutionary conserved function of intron-encoded mtl-tRNAs. As one of several potential models, we have investigated the possibility whether mtl-tRNAs influence splicing efficiency, thereby acting by similar mechanisms as reported for splicing regulatory elements in nuclear introns. Indeed, introduction of mtl-tRNAs into the intron of an eGFP splicing reporter construct resulted in an increase in respective mRNA abundance. When introducing different numbers of mtl-tRNAs into the intron of the eGFP splicing reporter construct, a copy number-dependent increase in mRNA abundance was observed, as known for bona fide splicing enhancer sequences. Introducing mtl-tRNAs into the eGFP intron, containing a less efficient 5'-splice site resulted in an increase of mRNA abundance by about 20-fold. In a different splicing reporter construct, exhibiting a more sophisticated 3' splice site, the introduction of the same five mtl-tRNAs only marginally increased mRNA abundance, due to a higher endogenous efficiency of splicing in the wildtype construct. Whereas, pre-mRNA abundance was reduced by over 50%, thus indicating a splicing-related effect of intronic mtl-tRNAs on the increase in host gene expression. Single, different mtl-tRNAs each resulted in an increase in mRNA abundance in the eGFP reporter. The deletion of the T-stem of mtl-tRNA Tyrosine as well as the scrambling of the whole mtl-tRNA reversed this effect. Interestingly, despite the significant effects of mtl-tRNAs on splicing of host genes, no fully processed mtl-tRNAs were observed by northern blotting, pointing to a rapid turnover or degradation of mtl-tRNAs. By a pull-down of proteins binding to a biotinylated transcript containing mtl-tRNAs and subsequent protein gel analysis as well as MS analysis, we were able to identify several potential trans-acting protein factors potentially involved in mtl-tRNA-dependent increase in splicing efficiency. Our data demonstrates that mtl-tRNAs present in nuclear introns might represent a novel post-transcriptional mechanism to regulate gene expression of nuclear genes. .

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Properties of small, cysteine-rich and cationic antimicrobial proteins of fungal origin

The increasing incidence of fatal microbial infections due to the development of resistance against licensed antibiotics raises a strong demand for new antimicrobial treatment strategies. Small cysteine-rich and cationic antimicrobial proteins (AMPs) - secreted by filamentous Ascomycetes - are interesting candidates for developing novel antifungal compounds. Many studies have been published dealing with AMPs, giving us insight into their structural and functional properties. The aim of this study is to highlight the features of AMPs and to emphasize the potential of these bio-molecules for future antifungal drug development. AMPs of fungal origin are secreted into the culture supernatant of easily fermentable molds and can be purified via one-step chromatography. By the use of a Penicillium chrysogenum-based expression system, we could increase the protein yields up to 50 mg per litre supernatant. This is beneficial for large-scale-protein-production. AMPs possess a highly stable disulphide-bond mediated, compact β-fold structure which gives them a high stability under extreme pH, high temperature and against proteolysis. In vitro susceptibility tests demonstrated that AMPs effectively inhibit the growth of human pathogenic filamentous fungi such as Aspergillus fumigatus or Trichophyton rubrum in micromolar concentrations. Antimicrobial potency was also observed against the opportunistic human pathogen Candida spp. and some have been shown to possess antiviral potential. Additionally, many AMPs have been tested to exhibit no cytotoxic effect on mammalian cell lines in vitro. However, variations in the antifungal spectrum and the antifungal mode of action have been observed for the structurally very similar and related antifungal proteins, e.g. for PAF and PAFB produced by P. chrysogenum. Localization studies using fluorescence-labelled proteins indicated that interaction with the cell envelope of sensitive fungi and strictly regulated protein uptake are required for the toxicity. This lets us assume, that the presence and activity of host interaction molecules determine the susceptibility of fungal species towards AMPs. As these targets remain elusive, receptor identification would be of great relevance for the generation of new antifungal therapeutics. Taken together our findings indicate the enormous antifungal potential of AMPs and the importance of further research focusing on antifungal mechanism and application.

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Molecular regulation of the oncogenic miR-17-92 cluster

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that posttranscriptionally regulate gene expression by sequence-specific repression of almost all mRNAs in mammals. In consequence, every cellular pathway is fine-tuned by miRNAs, establishing them as an essential layer of gene regulation. However, since miRNA research in the last decade has mainly focused on the downstream events of the miRNA, i.e. the identification of actively repressed mRNA targets, the upstream regulatory networks that modulate miRNA activity have been largely neglected. Thus, little is known about the processes that specifically regulate miRNA activity, such as on the transcriptional level or during their biogenesis or miRNA decay/turnover. The polycistronic miR-17-92 cluster, frequently overexpressed or amplified in cancer, is transcribed as one long primary transcript that is then processed into six individual miRNAs. In the beginning, this genetic organization suggested that all members of the cluster functionally cooperate and drive tumor progression synergistically. However, recent data demonstrate that the cluster is not oncogenic per se, but rather encodes tumor-promoting as well as tumor-suppressing functions. In consequence, it appears that an imbalanced expression of the cluster members, with a shift towards the oncogenic components, is crucial to drive tumor cell formation in both mouse and men. Of note, it is mostly unclear how these imbalanced expression patterns are established, thereby warranting an in-depth analysis. We have performed a set of genome-wide CRISPR/Cas9 loss-of-function screens to generate a comprehensive understanding of the molecular mechanisms that regulate miR-17-92. The genome-wide screens generated a list of about 200 novel genes that have not been associated with the regulation of miR-17-92 cluster before. While still ongoing, the validation of these hits already confirmed a clear regulatory role for 9 of those genes (out of 34 tested), i.e. deletion of these genes has a statistically significant impact on individual or groups of cluster miRNAs. We aim to characterize the exact molecular function of confirmed novel regulators using a set of biochemical, genetic and molecular tools. In the future, this knowledge may open doors towards innovative therapeutic approaches in miR-17-92 associated cancer entities

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Sex-/Gender and Diversity Categories as Cross-Cutting Issues in Resarch – Guidelines for Grant Application and Teaching

Sex/Gender and Diversity – Background: The requirement to consider the sex/gender dimension and diversity categories in science and research is introduced in national and European legislation as well as in policies of funding organizations. Meanwhile it is obligatory to consider both sexes in research and innovation content in grant application. The differentiation of the sexes as well as for example the consideration of the diversity category of age not only are a matter of legislation, they are also a sign of scientific quality and reliability of studies. Talking about gender normally is connected to the two levels of equality of women and men and gender balance in team building or leadership. The third level to be considered is the integration of sex/gender in the research content itself. Beside the dimension of gender the second important category to be considered in medical research is age. Sex/Gender and Diversity – Why does it matter? The evidence that sex and gender differences and diversity categories matter in health and interact is constantly increasing. Not only biological differences as hormones, genes, immunological differences play an important part, but also sociocultural differences like nutrition, profession, financial background or role understanding. The interaction of the two dimensions sex and gender and additional influences like for example age, education or the financial background of patients make it even more complex. If the aim of health research is to find adequate answers and therapies for patients, it is necessary to develop health offers that take gender and diversity into account. This means to integrate these aspects in the design of research projects from the beginning. At the level of basic research sex factors might be in the foreground as research is done with cells and mice, later on in the research chain, gender factors gain considerable more importance. Sex/Gender and Diversity – Information and tools: The introduction of sex/gender during all phases of projects requires methodological competence. The poster presents the guidelines for research and teaching developed at the Medical University of Innsbruck for scientists and clinicians.

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Barth Syndrome and the complexity of mitochondrial cardiolipin

Barth Syndrome is a rare x-linked disorder that is characterized by mitochondrial dysfunction leading to severe cardiomyopathy and muscle weakness. The affected TAZ gene encodes the CoA-independent transacylase tafazzin that remodels the side chains of cardiolipins (CL). This phospholipid is almost exclusively found in mitochondrial membranes and is functionally involved in cristae formation, supercomplex assembly, ROS scavenging and apoptotic signaling. CL has a unique dimeric structure with four, instead of two, fatty acyl side chains rendering CL the most diverse lipid class. We developed a novel lipidomics method to detect and quantify each single CL species in biological samples and furthermore, predict its side chain composition by mathematical modeling. Additionally, we generated TAZ-knock-out cell lines by CRISPR/Cas9 and thereby established a well working model for Barth Syndrome patients. We were already able to gain new insights in the complex metabolism of CL such as the preferential depletion of polyunsaturated fatty acids in tafazzin-deficient cells, suggesting an oxidative-driven side chain cleavage. In addition, we were able to alter the CL composition by cell culture media supplementation in a targeted manner. This cellular model system was used to performed high-resolution respirometry and observed a shift in the OXPHOS flux control in respect to NADH-depended pathways and a simultaneous increase of the biochemical complex I activity. These data suggest a partial re-routing of the central carbon metabolic flux in metabolically active TAZ-deficient cells to compensate for the perturbation of phospholipids in the inner mitochondrial membrane.

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Conservation and Divergence of the Hedgehog-transducer Smoothened

Hedgehog signalling is an important pathway involved in development and disease. It controls cell specification, proliferation and migration in embryogenesis and in the development and progression of cancer. A defining feature of vertebrate Hedgehog signalling is the compartimentalization of the pathway components to the primary cilium. Defects in ciliary transport and ciliogenesis lead to impaired Hedgehog signalling and key components like the transmembrane receptor Smoothened need to be at the cilium to be able to function. This importance of primary cilia in Hedgehog signalling has been thought to have evolved relatively recently, as it has been found only in vertebrates, whereas primary cilia are not involved in Drosophila melanogaster Hedgehog signalling. To investigate whether the role of primary cilia in Hedgehog signalling is limited to vertebrates, we isolated the Smoothened homologue from the sea anemone Nematostella vectensis, a very basal animal from the Cnidarian clade which has split off from other Eumetazoans about 650 million years ago. We show that despite extensive sequence divergence, Nematostella vectensis Smoothened localizes to cilia in Nematostella, zebrafish and mammalian cells and is able to partially compensate for Smoothened loss of function in zebrafish. This indicates that the involvement of primary cilia is a basal feature in eumetazoan Hedgehog signalling.

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Role of RNA cytosine methyltransferase Nsun3 in mouse embryonic stem cell differentiation

First epigenetic modifications of both DNA and RNA were already described more than 60 years ago with the DNA modification, 5-methyl-2'-deoxycytidine (m5dC), as the best understood one to date. In contrast to DNA methylation, the study of RNA methylation was more challenging due to the lack of proper techniques. In case of 5-methylcytidine, the establishment of methods, such as bisulphite sequencing for RNA, made it possible to study RNA methylation at nucleotide resolution. m5C modifications in RNA can be installed by any of the seven proteins of the Nol1/Nop2/SUN domain (NSUN) family as well as the DNA methyltransferase family member DNMT2. Our previous data suggested that RNA methylation in mitochondria might be higher than in the cytoplasm. In a search for mitochondria-specific RNA cytosine methyltransferases (RCMTs), we targeted Nsun3, which was unstudied at that time and showed close sequence similarity to the mitochondrial enzyme Nsun4. Using CRISPR/Cas9 we introduced mutations in the presumptive catalytic domain of Nsun3 in embryonic stem cells (ESC) to study Nsun3's function. We show that Nsun3 methylates mitochondrial tRNAMet at position C34 in the anticodon loop. Nsun3 inactivation results in substantial impairment of mitochondrial translation and transcription and affects cellular glycolysis and respiration rates. Nsun3 mutant cells also exhibit compromised ESC differentiation. Together, these results suggest an important role for mitochondrial Nsun3 in ESCs even though these cells largely rely on glycolysis rather than oxidative phosphorylation for generating energy.

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Targeting the antioxidant systems to overcome therapy resistance in breast cancer

In the last decades, huge improvements in treatments for patients, diagnosed with breast cancer, have been achieved. Despite the development of novel techniques and schedules of treatments, the problems of primary and acquired therapy resistances (TR) are still not solved. It is currently accepted that an intra-tumoral subpopulation of cancer stem cells (CSCs) is responsible for TR and disease progression in cancer patients. Therefore, the malignant tumors could be cured by eliminating all CSCs in parallel to the tumor bulk. Despite intensive investigation of the molecular signaling underlying intrinsic and acquired CSC TR, it is still poorly understood how CSCs survive different treatment approaches (e.g. apoptosis inducers, DNA damaging agents, radiation therapy etc.). CSC are characterized by an upregulation of their antioxidant systems and cope better with oxidative stress compared to the tumor bulk. Recently a new targetable regulated cell death ferroptosis was described (Dixon et al. 2012), which directly targets the antioxidant systems. In this project, the main aim is to elucidate resistance mechanisms of CSC to radiation therapy (RT) which is widely used in management of breast cancer. Nearly all breast cancer patients receive RT either in curative or palliative settings. The combination of ferroptosis induction and radiation therapy (reactive oxygen species are the main drivers of radiation induced cell death) is a promising approach to combat TR breast cancer. To investigate mechanisms of primary and acquired TR two different in vitro models were established: Breast carcinoma cell lines with increased invasive abilities (INV cells) and breast carcinoma cells with acquired radiation resistance (RR cells). Parental cell lines representing the major molecular subtypes of breast cancer: MDA-MB-231 (triple negative), T47D (hormone dependent) and Au565 (Her2 positive) are used in this study. Invasive potential of parental, INV and RR cells was validated by the Cell Biolabs CytoSelectTM Laminin and Collagen I Cell Invasion Assay Kits (Cell Biolabs, Inc., San Diego, USA). In order to identify possible molecular pathways involved in therapy-resistance, quantitative proteomics using TMT labelling was performed. Dysregulation of the molecules of interest were confirmed in parental, INV and RR cells using FACS and Western blot analysis. Breast cancer cell sensitivity to ionizing radiation was investigated by FACS analysis after Nicoletti staining and clonogenic survival assay. Recent experiments showed that ferroptosis inducers in combination with RT were more effective in killing of INV cells compared to parental counterparts, and RR cells were equally responsive as parental cells. Those results suggest that CSC rich tumors could be targeted by ferroptosis inducers in combination with ionizing radiation in order to improve therapy outcome of patients diagnosed with aggressive breast cancer types or relapsed disease after treatments.

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The Role of the PIDDosome in Cancer

Aneuploidy is a common feature of cancer cells. One cause of this unequally distribution of chromosomes are supernummery centrosomes. During Mitosis this results in the formation of multipolar spindles which increase the chance of chromosome misssegregation. The PIDDosome has been described to be activated in rersponse to extra centrosomes to limit genomic instability by inducing a p53-mediated cell cycle arrest or apoptosis. Hence, in consequence PIDDosome activation should protect cells from malignant transformation. Yet, it is still unclear whether the PIDDosome can act as a first barrier for cancerogenesis in vivo in response to centrosome amplification. To investigate this in more detail, genetically modified mice overexpressing polo like kinase 4 (PLK4), the master regulator of centriol biogenesis, in a tissue-specific manner, will be used as a trigger for PIDDosome-dependent and p53-mediated cell cycle arrest or apoptosis. Incrossing PLKOE-mice with mice deficient for components of the PIDDosome should result in a system to study the relevance of the PIDDosome in supressing cancer development. This will be investigated in the context of colon cancer and B-cell lymphomagenesis. Therefore we expect mice overexpressing PLK4 and lacking components of the PIDDosome to develope earlier cancer onset.

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Regulation of Arginase1 and Inducible Nitric Oxide Synthase in Salmonella Typhimurium Infected Bone Marrow Derived Macrophages

Background: Interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) are potent inducers of inducible nitric oxide (NO) synthase (iNOS) which is central for the control of Salmonella enterica serovar Typhimurium (S. Typhimurium) by macrophages. Two types of macrophages, inflammatory M1 macrophages expressing iNOS and anti-inflammatory M2 macrophages expressing Arginase1 (Arg1), are involved in the immune response against S. Typhimurium. High expression of Arg1, which cleaves L-arginine, the substrate of iNOS, impairs host control of infection with intracellular microbes. Herein, we investigated the protective effects of TNFα and IFNγ on iNOS and Arg1 expression in macrophages and how this impacts on the immune control of Salmonella infection in vitro.

Methods: Bone marrow derived macrophages (BMDMs) generated from C57BL/6 mice upon M-CSF stimulation for six days were infected with S. Typhimurium for one hour. Cells were then stimulated with either Interleukin-4 (IL-4), IFNγ, or TNFα and combinations thereof for various time points. mRNA expression of Arg1 and iNOS was analyzed by qRT - PCR. Furthermore, bacterial load of infected BMDMs was analyzed by plating lysates on LB-agar plates for determining colony forming units.

Results: We observed that IL-4 is a potent inducer of Arg1 expression in a time dependent manner, which is more pronounced in infected than in uninfected BMDMs. Accordingly, inducible Arg1 expression was significantly suppressed by IFNγ whereas TNFα had only little effect in S. Typhimurium infected BMDMs. In a comparable fashion IFNγ was the most potent inducer of iNOS expression as compared to TNFα, and IL-4 blocked the latter effect more potently. Surprisingly, high iNOS expression did not translate into improved control of intracellular Salmonella proliferation and IL-4 stimulation even resulted in reduction of bacterial numbers.

Conclusions: Our data underline the inhibitory effects of IFNγ and TNFα on the IL-4 dependent Arg1 expression, leading to an enhanced generation of NO by iNOS in vitro. Ongoing experiments aim to investigate how these cytokines influence the transcriptional and epigenetic regulation of iNOS and Arg1, how genetic deletion of Arg1 impacts on immune control of Salmonella in vivo and how pharmacological modification of Arg1 expression affects disease outcome in Salmonella septicemia.

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Effects of dietary and genetic iron overload on mitochondrial function and consecutive metabolic pathways

Background: Iron is an essential co-factor for many metabolic processes, and mitochondria are the main sites of iron utilization as they need iron for mitochondrial respiration. Iron deficiency negatively affects cellular energy metabolism and iron overload increases the production of reactive oxygen species (ROS) through the Fenton reaction. Therefore, the consequences of dietary and genetic iron overload on metabolic circuits and mitochondrial function were investigated in wildtype and Hfe-/- mice.

Methods: 10 weeks-old male C57/BL6N wildtype mice and Hfe-/- mice were fed either with a low iron (>10 mg iron/kg) or a high iron diet (25 g iron/kg) for two weeks. Mitochondrial respiration was measured in liver homogenate using High-Resolution Respirometry (Oroboros Instruments, Innsbruck). Moreover, different molecular biological methods were applied for the determination of the tissue iron content, protein and mitochondrial gene expression.

Results: Mitochondrial respiration in liver homogenate revealed no differences between the genotypes as well as the different diets. Tissue iron distribution was different according to genotype and diet exhibiting higher amounts of iron in mice fed the high iron diet and the Hfe-/- genotype seen especially in duodenum, liver and spleen. Furthermore, the expression of proteins involved in iron and mitochondrial metabolism in the liver confirmed these differences, as ferritin expression was increased in mice with iron overload. Moreover, the low iron diet led to an elevated expression of the transferrin receptor in the wildtype mice compared to the expression in the Hfe-/- knockout mice fed a low iron diet which was the same as in the mice fed a high iron diet. However, the expression of the marker protein for mitochondrial biogenesis, PGC1α, was the same in all groups. Mitochondrial gene expression exhibited differences between iron loading and iron deficiency that was independent of genotypes. In iron overload hepcidin expression is increased compared to all other groups.

Conclusion: The results showed that a knockout of the hemochromatosis gene and a diet rich in iron altered mitochondrial iron metabolism and function. This might be an explanation for the fatigue that is a common symptom in iron overload and iron deficiency patients.

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NMR structural and dynamic investigation of hazelnut allergens

In large parts of Europe, Northern America and China people are suffering from allergies after consuming certain kinds of fruits and vegetables. Typical allergic symptoms range from scratching and itching of the throat to severe symptoms like rhino conjunctivitis and anaphylaxis. For hazelnuts, apples, plums, and peaches, these allergic reactions can result from initial sensitization to the birch pollen allergen Bet v 1 and subsequent development of so-called cross-allergic reactions. These cross-reactive fruits contain proteins that are similar in their three-dimensional structure to the sensitizing protein Bet v 1 in birch pollen. It is thus necessary and critical to determine the three-dimensional structures of these proteins at high resolution to understand the cross-reactivity on a molecular basis. Apart from apples (80%), hazelnuts (60%) are the most frequent cross-reactive plant food source. Experimental structural data for the four known hazelnut allergen isoforms Cor a 1.0401, Cor a 1.0402, Cor a 1.0403, and Cor a 1.0404 are, however, not available to date. We have determined NMR solution structures of the first three isoforms and a structural model of the last isoform. All of them show the highly conserved PR-10 fold consisting of seven beta-strands, which are interrupted by two short alpha-helices and closed by a long C-terminal alpha-helix. In comparison to the stable isoforms Cor a 1.0401-03 the isoform Cor a 1.0404 tends to be slightly unstable and partly unfolds over time. Using NMR relaxation experiments we show that the three stable isoforms Cor a 1.0401-03 show similar dynamics on the micro- to millisecond time scale but on a higher level than other food allergens, such as the major apple protein Mal d 1.

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The role of p300 in human tumor-initiating prostate cancer cells & chemotherapy resistance

Prostate cancer (PCa) is one of the most frequently diagnosed tumors in men. Therapies for advanced PCa predominantly target the androgen receptor (AR) and include chemotherapeutics like docetaxel, which is a microtubule inhibitor. However, these treatments inevitably lead to the development of castration resistance within a few years. Therefore, new therapy options are needed. The histone acetyltransferase p300 is a well-known coactivator of the AR and has already been correlated to PCa progression. Due to its multifunctional role p300 may serve as a promising new therapeutic target. This study aimed to determine whether p300 plays a role in chemotherapy resistance, in particular in tumor-initiating cells. Clonogenic Assays were performed to determine colony formation efficiency and colony types of PCa cell lines PC3 and DU145. p300 knockdown was achieved by doxycycline inducible shRNA expression in stably transduced cells. Expression of p300 and c-Myc was measured by qPCR or Western blotting in absence or presence of docetaxel. Expression of stem cell genes Nanog, OCT4, SOX2 and ALDH1 as well as downstream targets Cyclin A and CDK2 were determined by qPCR or Western Blotting. Cell migration was analysed by wound scratch assays. In parental cells p300 knockdown did not influence the number of colonies but decreased the tumor-initiating capacities of colonies. In particular, the CD24-/CD44high population and mRNA levels of several stem cell genes were decreased. Docetaxel treatment for 72 hours resulted in elevated expression of p300 and its downstream target c-Myc at mRNA and protein level. In line with these observations, docetaxel-resistant cells displayed increased p300 and c-Myc protein expression. Furthermore, p300 knockdown in docetaxel-resistant cells decreased the clonogenic potential. Downregulation of p300 also decreased cell migration and affected several downstream targets including c-Myc, Cyclin A and CDK2. Interestingly, also ALDH1 mRNA expression was decreased upon p300 knockdown. Taken together, we showed that docetaxel treatment increases p300 expression and docetaxel-resistant cells show an elevated p300 expression. In contrast to parental cells, docetaxel-resistant cells showed a reduced clonogenic potential and cell migration upon p300 knockdown. Based on our findings, we suggest that p300 may plays a role in development of chemotherapy resistance.

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Distinct serum and cerebrospinal fluid cytokine and chemokine profiles in autoantibody-associated demyelinating diseases

Background: Demyelinating diseases of the central nervous system (CNS) associated with autoantibodies against aquaporin-4 (AQP4-Ab) and myelin oligodendrocyte glycoprotein (MOG-Ab) are mediated by different immunopathological mechanisms compared to multiple sclerosis (MS). Objective: To evaluate cytokine and chemokine profiles in paired serum and cerebrospinal fluid (CSF) samples of patients in the acute phase of AQP4-Ab or MOG-Ab-associated demyelinating diseases compared to MS and anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis.

Methods: Serum and CSF cytokine and chemokine levels were analysed using Procartaplex Multiplex Immunoassays. First, we analysed a panel of 32 cytokines and chemokines in a discovery cohort (9 AQP4-Ab, 9 MOG-Ab, 8 encephalitis, 10 MS). Significantly dysregulated cytokines and chemokines were validated in a second cohort (11 AQP4-Ab, 18 MOG-Ab, 18 encephalitis, 33 MS).

Results: We found 11 significantly altered cytokines and chemokines in CSF and serum samples in the discovery group (APRIL, Fractalkine=CX3CL1, GRO-α, IL-1RA, IL-6, IL-8=CXCL8, IL-10, IL-21, IP-10=CXCL10, MIG=CXCL9, MIP-1ß=CCL4). Most of these cytokines and chemokines were up-regulated in AQP4-Ab or MOG-Ab seropositive patients compared to MS. We confirmed these results for CSF IL-6 and serum IL-8, GRO-α, APRIL and MIP-1β in the validation set. Receiver operating characteristic analysis revealed increased levels of CSF IL-6, serum IL-8 and GRO-α in most patients with autoantibody-associated neurological diseases.

Conclusion: This study suggests that distinctive CSF and serum cytokine and chemokine profiles are associated with autoantibody-mediated demyelinating diseases, but not with MS. Our results might be helpful to establish novel diagnostic biomarkers for these syndromes.

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Synthesis of 2 arylquinoline derivatives as selective estrogen receptor β agonists for the treatment of breast cancer

Estrogen receptors (ERs) belong to the class of nuclear receptors. The ERs comprise of the two subtypes ER α and ER β. Both are contrary in tumor development and progression. Activation of ER α promotes the proliferation, while the ER β inhibits tumor formation by leading to a G2 cell cycle arrest. Therefore, the development of compounds binding selectively to ER β is a promising approach for the treatment of breast cancer. In addition, the side effects caused by stimulation of the ER α can be circumvented following this strategy. Previous studies showed that 2-aryltetrahydroquinoline derivatives had auspicious cytotoxicity towards breast cancer cell lines. Moreover, halogenation of the phenyl moiety exhibited a beneficial influence on both cytotoxicity and ER β selectivity. Besides, 2-arylquinolines with superior selectivity for ER β were described in literature. Thus, the synthesis of halogenated 2-arylquinolines as selective ER β agonists analogous to the 2-aryltetrahydroquinoline derivatives is aimed. Starting from different halogen substituted anisoles, aldehydes were synthesized following a procedure with four conventional reactions. Then, the aldehydes formed imines upon reaction with p-anisidine. Preparation of the protected 2-arylquinolines was performed using cyclopentene or cyclohexene within an inverse electron-demand Diels-Alder reaction. The final ether cleavage yielded the 2-arylquinoline derivatives. The reaction steps are purposed to be optimized regarding the occurrence of byproducts and increasing the yields. Finally, the newly synthesized 2-arylquinoline derivatives will be investigated for their biological profile concerning cytotoxicity and ER β selectivity.

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Metabolic characterization of CD8+ T cell subsets during differentiation

T cells feature a remarkable versatility of metabolic activities during their differentiation from naïve to memory and effector cells and accordingly, alterations of T cell metabolism affect differentiation, proliferation and immune function. We have previously shown that impaired acquisition of an effector phenotype by tumor-infiltrating CD8+ memory T cells is associated with less favorable clinical outcomes in colorectal cancer patients. Thus, reprogramming of T cell metabolism to modulate the differentiation process is our strategy to overcome the dysfunctional states of tumor-infiltrating T cells frequently encountered in colorectal cancer. To this end, we characterized the metabolic phenotypes adopted by primary human naïve CD8+ T cells during their differentiation in vitro into stem cell memory, central memory and effector memory subsets. We employed different techniques to investigate T cell subset metabolomes and associated signaling pathways. Measurements of the extracellular acidification and oxygen consumption rates showed a differential activation of glycolysis and oxidative phosphorylation in different T cell populations, and RNA sequencing experiments revealed a global regulation of metabolic pathways on a transcriptional level. In order to obtain a direct account of central metabolic pathway activities, we conducted 13C labeling experiments and quantified the incorporation of 13C isotopes into metabolites by high-resolution mass spectrometry. The obtained labeling patterns confirmed the distinct preferential pathway usage in the investigated T cell subsets and indicated a demand for various biosynthetic processes to support proliferation and cytotoxicity. Our comprehensive characterization of the metabolic programs of CD8+ T cell subsets will thus enable the development of procedures for ex vivo or in vivo metabolic reprogramming of T cells to enhance antitumor immunity.

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Associations of circulating levels of dickkopf-1 and sclerostin with cardiovascular events: Results from the prospective Bruneck Study

Background and Aims: Dickkopf-1 and sclerostin have been implicated in vascular calcification and atherogenesis. We aimed to quantify the association between circulating levels and incident cardiovascular disease (CVD) in the general population.

Methods: Circulating serum levels were measured in 707 participants of the prospective population-based Bruneck Study (2000 examination). Incident CVD events were recorded that occurred between 2000 and 2016. The combined CVD endpoint subsumed ischemic and hemorrhagic stroke, myocardial infarction, angina pectoris, transient ischemic attack, peripheral vascular disease, or revascularization procedures. Cox regression was used to estimate hazard ratios adjusted for age, sex, systolic blood pressure, C-reactive protein, triglycerides, total cholesterol, high-density lipoprotein cholesterol, body mass index, estimated glomerular filtration rate, smoking, diabetes mellitus, previous CVD, and intake of platelet aggregation inhibitors.

Results: Over a median 15.6 years of follow-up, 181 incident CVD events were recorded. Mean circulating level was 44.5 pmol/L for dickkopf-1 (SD 14.7) and 47.1 pmol/L for sclerostin (SD 17.5). The hazard ratios for CVD risk per one standard deviation higher circulating level were 1.21 (95% confidence interval: 1.05-1.40; P=0.009) for dickkopf-1 and 0.91 (0.78-1.07; P=0.266) for sclerostin. The association of dickkopf-1 was primarily driven by a 1.44-fold risk for stroke (1.15-1.80; P=0.002), whereas no increase in risk was observed for myocardial infarction (1.04; 0.77-1.42; P=0.780) or for other CVD events (1.12; 0.88-1.43; P=0.355).

Conclusions: Elevated circulating levels of dickkopf-1 were associated with a higher risk for incident CVD in the general population, particularly for stroke. The underlying mechanistic role of dickkopf-1 in CVD deserves future investigation.

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Dissecting the role of the PIDDosome in hepatocellular carcinoma

The PIDDosome is an activation platform for Caspase-2 additionally containing the proteins PIDD1 and RAIDD. The PIDDosome functions as a sensor for supernumerary centrosomes which can occur upon cytokinesis failure or endoreduplication. In such polyploid cells, the PIDDosome activates Caspase-2, starting a signaling cascade which triggers cell cycle arrest via cleavage of Mdm2, p53 stabilization and p21 induction. Failed cell division and centrosome amplification promote genomic instability which is considered to be a hallmark of cancer. In the liver, however, polyploidization of hepatocytes is part of normal organogenesis devoid of malignant transformation.

Previously, we could show that the PIDDosome has a key role in regulation of hepatocyte polyploidyzation in vivo during liver development and regeneration. To investigate the consequences of PIDDosome deficiency on tumor development we used a carcinogen-driven hepatocellular carcinoma mouse model. Surprisingly, we found that PIDDosome knockout mice exhibit significantly fewer tumors than wildtype mice which was also confirmed by histopathological analysis. To further assess how PIDDosome deficiency protects against tumor development, we analyzed the ploidy state of tumors and corresponding healthy parenchyma. We could show that tumors mainly arise from diploid cells, indicating that the PIDDosome indirectly affects tumorigenesis by regulating polyploidization during liver development. Moreover, samples of human HCC patients will be analyzed with regards to the ploidy state and activation of the PIDDosome pathway to reveal the relevance of polyploidy and the centrosome-PIDDosome axis in human liver tumorigenesis.

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Polyunsaturated fatty acids fuel intestinal inflammation in GPX4 deficient hosts

Introduction Crohn's disease (CD) is a chronic remittent inflammatory condition of the gastrointestinal tract that arises from a deranged interplay of the immune system, microbiota and unknown environmental factors in genetically susceptible hosts. A single nucleotide polymorphism next to glutathione peroxidase 4 (GPX4) was associated with the development of CD in a GWAS study. Reduced GPX4 activity leads to a form of regulated cell death termed ferroptosis that is characterised by an iron dependent accumulation of lipidperoxides. The prime substrates for lipidperoxidation are polyunsaturated fatty acids (PUFAs) in cellular membranes, one of which is arachidonic acid (AA). The increased consumption of PUFAs during the last decades is paralleled with an increase in inflammatory bowel disease. However, the impact of increased PUFA/AA uptake on the development of IBD remains unknown.

Materials and Methods In this study we aimed to investigate the influence of AA on intestinal epithelial cells with reduced GPX4 expression evoked by siRNA silencing. For in vivo analysis we crossed Gpx4flox/flox mice with Villin-Cre+/- mice to obtain Gpx4flox/wt;Villin-Cre+/- (Gpx4+/-IEC) mice. We investigated an inflammatory phenotype in Gpx4-deficient murine MODE-K small intestinal epithelial cells (IEC) upon PUFA treatment.

Results Gpx4 silenced IECs showed increased lipid peroxidation and cell death which was aggravated by PUFA stimulation. Furthermore siGpx4 IECs produced IL-6 and the IL-8 homologue CXCL1 upon PUFA stimulation. Cytokine production and LPO were governed by ferric iron. Moreover, Gpx4+/-IEC mice fed a PUFA enriched western style diet developed a small intestinal inflammation while wildtype mice were unaffected. Similar, Gpx4+/-IEC mice that were orally challenged with AA and ferric maltol developed a neutrophilic inflammation. Treatment with -tocopherol protected Gpx4+/-IEC mice from PUFA induced small intestinal inflammation.

Conclusion Our data shows that PUFAs promote lipid peroxidation and cytokine release in GPX4-deficient IECs which trigger small intestinal inflammation in mice with reduced GPX4 levels in the intestinal epithelium. As such, we identified an environmental trigger in a Western diet that instigates intestinal inflammation in susceptible hosts.

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Alterations of cell metabolism after ferroptosis induction in invasive and radioresistant model of BC cell lines

Introduction: Breast cancer (BC) is still one of the most common causes of cancer-related mortalities among women worldwide. However, despite the development and use of novel and effective therapeutic schedules, tumor relapse can still occur. BC recurrences can occur either from carcinoma cells with constitutive or aquired treatment resistance and metastatic spread. Nearly all BC patients receive radiotherapy after surgery, but it is assumed that BC cells recovered after their exposure to ionizing radiation could contribute to the recurrence development.

It was recently shown that carcinoma cells are affected in the iron-dependent non-apoptotic cell death by lipid peroxidation (ferroptosis) and it is generally accepted that carcinoma cells with aggressive behaviour are more susceptible for ferroptosis. Since cell metabolism could also contribute to ferroptosis-associated cell death, but it is still unclear whether cell susceptibility to ferroptosis depends on metabolic activities of BC cells, this study aims to elucidate the role of cell metabolism after ferroptosis induction in BC cells with increased invasive abilities or radiation resistance.

Methods: Triple negative, hormone-positive, and HER2-positive BC cell lines (MDA-MB-231, T47D and Au565, respectively) were used in this study. To obtain BC cells with increased invasive abilities, carcinoma cells were allowed to migrate through the uncoated membrane toward 10% FCS containing medium.BC cells with radiation resistance (RR cells) were received after exposure to repetitive irradiation at a total dose of 100 Gy. Ferroptosis was induced after cell treatment with ferroptosis inducers FIN56 or erastin, either alone or in combination with RT at 24, 48 and 72 hours. Lipid ROS and lipid peroxidation after ferroptosis induction were detected, respectively, by FACS analysis using MDA assay. Cell metabolism upon treatment was evaluated by using Seahorse metabolic analyser.

Results: RR BC cells also possessed ferroptosis-resistant phenotype, meanwhile invasive BC cell lines were more sensitive to ferroptosis inducers. Combination of ferroptosis inducers and ionizing radiation was more effective in radioresistant and invasive BC cells in comparison with the treatment using irradiation or ferroptosis inducers alone. Pro-ferroptotic agents markedly changed the metabolic activities of all investigated cells. Conclusions: Radioresistant and invasive BC cells can effectively be eliminated by the use of ferroptosis inducers either alone or in combination with ionizing radiation.

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Xenoantigen-dependent complement-mediated neutralization of LCMV glycoprotein pseudotyped VSV in human serum

Neutralization by antibodies and complement limits the effective dose and thus the therapeutic efficacy of oncolytic viruses after systemic application. We and others previously showed that pseudotyping of oncolytic rhabdoviruses like the maraba virus and the vesicular stomatitis virus (VSV) with the lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) results in only a weak induction of neutralizing antibodies. Moreover, LCMV-GP-pseudotyped VSV (VSV-GP) was significantly more stable in normal human serum (NHS) than VSV. Here, we demonstrate that depending on the cell line used for virus production, VSV-GP showed different complement sensitivities in non-immune NHS. The NHS-mediated titer reduction of VSV-GP was dependent on the activation of the classical complement pathway mainly by natural IgM antibodies against xenoantigens like galactose-α-(1,3)-galactose (α-Gal) or N-glycolylneuraminic acid (Neu5Gc) expressed on non-human production cell lines. VSV-GP produced on human cell lines was stable in NHS. However, VSV-GP generated in transduced human cells expressing α-Gal became sensitive for NHS. Furthermore, GP-specific antibodies induced complement-mediated neutralization of VSV-GP independent of the producer cell line, suggesting that complement regulatory proteins potentially acquired by the virus during the budding process are not sufficient to rescue the virus from antibody-dependent complement-mediated lysis. Thus, our study points to the importance of a careful selection of cell lines for viral vector production for clinical use.

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Perturbation biology of colorectal cancer organoids reveals patient-specific signaling rewiring and interference with immunity

Colorectal cancer (CRC), a cancer with 1.4 million new cases diagnosed annually worldwide, is refractory to immunotherapy (with the exception of a minority of tumors with microsatellite instability). This is somehow paradoxical as CRC is a cancer for which we have shown that it is under immunological control and that tumor infiltrating lymphocytes represent a strong independent predictor of survival. Based on our previous work showing that the immunophenotypes are determined by the genotypes, we hypothesized that mutations are rewiring signaling pathways and thereby modulate the recognition of tumor cells by T cells.

In order to investigate rewiring of signaling networks and their interference with immunity for individual patients, we developed an experimental-computational framework using perturbation experiments with patient-derived tumor organoids and comprehensive multidimensional molecular and cellular profiling. A biobank of CRC organoids was generated from histologically verified tumor samples, normal tissue, and liver metastases obtained from CRC patients (n=22). Comprehensive characterization of the organoids (exome sequencing, RNA sequencing and proteomics) and of the tumors (multiplexed immunofluorescence for 6 immune cell types) was carried out and the resulting data used to prioritize perturbation experiments. Organoids were then perturbed with kinase inhibitors (MEKi, PI3Ki, mTORi, TBK1i, IKKi, BRAFi, and TAKi) and large-scale phosphoproteomic profiling using data-independent acquisition (SWATH-MS) was carried out. Integration of independent datasets of mutations, transcriptional changes, and phosphoproteomics activities revealed patient-specific signaling rewiring and interference with actionable pathways, suggesting possible pharmacological modulation by approved targeted agents to induce immunogenic effects.

We show for the first time that systematic and comprehensive analysis of the signaling rewiring can provide a mechanistic rationale for immunotherapy-based combination regimens in CRC. This work is an important step towards the development of a precision immuno-oncology platform that integrates tumor organoids with high-throughput and high-content data, and machine learning for making therapeutic recommendations for individual patients.

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Rescue of skin dendritic cells is key for the resuscitation of the anti-tumor immune responses

Immunotherapy of cancer by checkpoint blockade has led to significant outcomes for a large portion of melanoma patients. However, in patients whose tumors are poorly infiltrated by effector T cells the results are not encouraging. Therefore, combination approaches that would enhance pre-existing anti-tumor immunity and would reset the patients' immunological status are necessary. A loss of skin dendritic cells (DCs) has previously been reported for primary melanoma lesions. In our study we used the tg(Grm1)EPv mouse model. In this model, the metabotropic glutamate receptor-1 is ectopically expressed in melanocytes. This alteration that has also been observed in 40% of melanoma patient samples, leads to an uncontrollable proliferation and resistance to apoptosis in melanocytes. We here show that tumor growth in this mouse model results in a loss of the dermal DCs from the skin, with the CD11b+ DCs (cDC2) being the main subset affected. We hypothesized that this DC loss is due to receptor-mediated apoptosis within the tumor microenvironment (TME). Indeed we found an upregulation of FasL and of TNFα with tumor growth. By administering an agonistic anti-Fas antibody (ab) and TNFα intradermally in WT mice, we found that the cDC2 subset is susceptible to receptor-mediated apoptosis. In addition we found that skin DC loss in this mouse model is accompanied with a concomitant inhibition of both CD4+ and CD8+ T cells within the tumor. We examined the expression of inhibitory ligands within the TME and we found that there is a significant increase of PD-L1 and galectin-9. Treatment with anti-PD-1 and anti-TIM-3 abs was not sufficient to inhibit tumor growth. In order to enhance responsiveness to checkpoint blockade we administered Flt3L systemically and anti-CD40/polyI:C intratumorally. This DC boost approach resulted in increased numbers of cDC2 in the tumor and in the tumor-draining lymph nodes (tdLN). By combining this approach with checkpoint blockade, both CD4+ and CD8+ T cells were able to produce more cytotoxic cytokines and tumor growth was kept under control.

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The combination of oncolytic virotherapy with DC-based immunotherapy is highly effective in a mouse melanoma model

Background: Immunotherapy against cancer has proven to be highly effective, however for many cancer types patients do not respond or develop resistance. Combination therapies have increased response rates considerably. Here, we studied if local inflammation induced by an oncolytic virus enhances the therapeutic efficacy of a dendritic cell (DC)-based cancer vaccine. Since the complement system acts as regulator of inflammation and other immune defense mechanisms, we also aimed to investigate the influence of complement activation on the therapeutic outcome. As complement is thought to be involved in tumor immunesurveillance, we extended our investigations for the role of complement in oncolytic virotherapy and DC vaccination.

Methods: We used VSV-GP, a chimeric vesicular stomatitis virus (VSV) pseudotyped with the glycoprotein of the lymphocytic choriomeningitis virus, a promising new oncolytic virus candidate. Taking advantage of the B16-OVA melanoma model we evaluated the therapeutic efficacy of the combination of VSV-GP with OVA peptide-loaded DC Vaccine (DCVacc) in wild type (wt) and complement-deficient (C3) mice.

Results: We show that the combination of VSV-GP virotherapy with DC-based cancer vaccine significantly enhanced the survival of tumor bearing mice over the single agent therapies in both, wt and C3-dificient mice. Although, both DCVacc and DCVacc/VSV-GP treatments induced comparable levels of OVA-specific CD8 T cell responses, tumor growth was best controlled by the combination therapy. In wt mice the strong therapeutic effect of the DCVacc/VSV-GP combination treatment was associated with high numbers of tumor infiltrating T cells and the relative reduction of regulatory T cells in treated and contra-lateral non-treated tumors. Accordingly, depletion of CD8 T cells but not NK cells abrogated the therapeutic effect of DCVacc/VSV-GP supporting the crucial role of CD8 T cells for the improved therapeutic outcome in the combination therapy. Interestingly, therapeutic efficacy was significantly reduced for all treatments in complement-deficient mice.

Conclusion: Taken together, we demonstrated that VSV dramatically increases the efficacy of a DC-vaccine by reprogramming the tumor microenvironment rather than by inducing additional vaccine antigen-specific CD8 T cells. Furthermore, our data suggest a supporting role of complement for the therapeutic outcome of DC vaccination and oncolytic virus treatment in B16-OVA melanoma model.

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Ferroptosis inducers in combination with radiotherapy: have we found a therapeutic option for radioresistant HNSCCs?

Introduction: Radiation therapy is an effective therapeutic approach in the management of head and neck squamous cell carcinomas (HNSCC). However, approximately 40% of HNSCC patients experience a tumor relapse after treatment. Unfortunately, re-irradiation of the previously treated tumors is not possible in a majority of cases. Therefore, the new therapeutic approaches and schedules are needed to use them in the treatment of the recurrent and radioresistant HNSCCs. Since radioresistant carcinoma cells are characterized by the diminished ROS production and affected lipid metabolism, our research team has assumed that ferroptosis inducers can reveal an efficacy in the killing of radioresistant cells.

Materials and Methods: Radiation resistant IRR cells have been derived from parental FaDu, SCC25, and CAL27 HNSCC cells after their repetitive exposure to ionizing radiation ten times every two weeks at a single dose of 10 Gy with a total dose of 100 Gy. Protein profilings of parental and IRR cells were determined using gel-based nano-LC mass spectrometry. Ferroptosis inducers FIN56 and erastin were applied at the dosis of 100 nM and 5 μM, respectively. Cell death development was determined at 24 h, 48 h, and 72 h after treatment of the investigated HNSCC with either FIN56 or erastin alone or with their combination. Human gingival fibroblasts (HGF) have also been treated with ferroptosis inducers in order to know whether non-malignant cells are sensitive to ferroptosis inducers.

Results: FaDu-IRR, SCC25-IRR, and CAL27-IRR cells demonstrated a pronounced radiation resistance compared to their parental counterparts. Proteomics data have shown that radiation resistance of IRR cells can be associated with upregulation of proteins involved in pro-survival pathways. Thus, a number of ferroptosis-related proteins (GPX4, SLC family members, CARS, etc.) are dysregulated in IRR HNSCC cells compared to parental cells. Although all investigated parental and IRR carcinoma cells were differently sensitive to FIN56 or erastin, all of the investigated HNSCC cells showed pronounced cell response to combination treatment using FIN56 and erastin. HGFs demonstrated very limited cell death development to FIN56 or erastin, or their combination.

Conclusion: Received results open the new perspectives in the treatment of the relapsed radioresistant HNSCCs.

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Increased carotid intima-media thickness predicts incidence of carotid plaque: Meta-analysis of 6 studies involving 8,599 participants

Background and Aims: Carotid intima-media thickness and carotid plaque are well-established markers of subclinical atherosclerosis, but their relationship with each other is not fully understood. We therefore aimed to systematically review and combine available epidemiological evidence from prospective population-based studies on the relationship of carotid intima-media thickness with incident carotid plaque.

Methods: Two independent reviewers systematically sought PubMed for relevant studies. Study-specific risk ratios for incident carotid plaque comparing individuals in the top versus the bottom quartile of baseline carotid intima-media thickness were combined using random-effects meta-analysis.

Results: We identified and analyzed six relevant studies that involved a total of 8,599 participants. The pooled risk ratio for incident carotid plaque was 1.84 (95% confidence interval: 1.52-2.24; I^2=16.1%) using random-effects meta-analysis. Sensitivity analysis using fixed-effect meta-analysis yielded an overall risk-ratio of 1.79 (95% confidence interval: 1.54-2.08). This result was robust across various study-level characteristics including average age, proportion of female participants, and duration of follow-up (all P>0.05). There was no evidence for publication bias as assessed by Egger's test (P=0.11).

Conclusions: In general population studies, higher values of carotid intima-media thickness were associated with a higher risk of developing carotid plaque

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Blood lactate levels in relation to 18F-Fluor-desoxyglucose Positron Emission Tomography (PET) results in tumor patients and controls

The question of cancer cell metabolism has been influenced since almost 100 years by the model advanced by Otto Heinrich Warburg on glucose metabolism. New experimental data suggest that lactate might be an important player. The aim of this clinical study was to evaluate blood lactate levels in oncological settings. Clinical data from the Nuclear Medicine therapy ward (n=230) was analyzed retrospectively. This group of tumor patients included those who can show a positive 18F-FDG PET scan (neuroendocrine and thyroid cancer) as well as those that are known to be 18F-FDG-PET negative (prostate cancer). An additional control group of non-oncological disease from the Thyroid Out-patient unit was also evaluated (n=110). Blood lactate levels were determined at the Central Laboratory of the hospital. Radionuclide imaging procedures were done according to each tumor entity: 131 Iodine for thyroid carcinoma, 68Ga-PSMA for prostate carcinoma, 68Ga-DOTA TOC for neuroendocrine tumors and 18F-FDG when loss of differentiation was suspected. Blood lactate levels allowed no differentiation of tumor staging even when 18F-FDG was positive. Normal controls presented similar blood lactate levels. The theory of tumors been dependent on lactate for metabolism cannot be confirmed on an in-vivo setting even in situations where glycolytic tumor activity can be seen in 18F-FDG scans. Lactate levels were also non-indicative of tumor progression when other imaging modalities were considered. We cannot discard that the Warburg effect is valid only for the tumor micro-environment. Nuclear Medicine imaging retains it valid place in diagnosis and staging.

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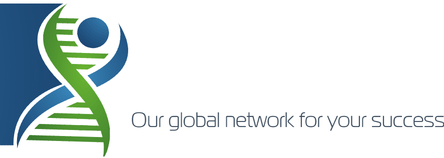

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