Life Science PhD Meeting

Innsbruck
April 2022

Abstract Book

MEDIZINISCHE UNIVERSITÄT INNSBRUCK

universität innsbruck

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 Medical University of Innsbruck and the Leopold-Franz-University, as well as the research programs making it possible to organize this meeting for all the Life Scientists in Innsbruck:

- MCBD
- CBD
- CMBI
- ARDRE
- HOROS
- SPIN
- CavX
- Clinical PhD programs

Abstract book designed by Sadegh Rahimi and Lisa Bergmeister

Background of the first and last pages are edited from "new7ducks / Freepik"

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Mitochondrial integrity and function partially recover during long-term normothermic perfusion of the liver

BACKGROUND: Long-term normothermic machine perfusion (NMP) of the liver could represent a novel platform with the potential for organ modification, regeneration, and repair. As a prerequisite, detailed insights into the cellular metabolism and bioenergetic processes during the preservation are required.

METHODS: Attempting to delineate the consequences of long-term organ procurement on mitochondrial integrity and function, a porcine model of 7-day liver NMP was applied. Liver biopsies were obtained before the initiation of perfusion, as well as on day 1, 5, 6 and 7, and analyzed by high-resolution respirometry (HRR; O2k, Oroboros Instruments, Innsbruck, Austria). Tissue biopsies were homogenized, and the succinate-linked respiration was measured at 37°C in MiR05-Kit medium in the absence and presence of ADP. The outer mitochondrial membrane was assessed by cytochrome c addition.

RESULTS: OXPHOS capacity showed a continuous decline in throughout perfusion (day 0: 49.1 ± 24.4, day 1: 40.4 ± 23.7, day 7: 28.3 ± 6.3 pmol s-1 mg wet weight-1). Comparison of mitochondrial efficacy of ATP production before initiation of NMP and after 24 hours revealed an initial decline (P-L coupling efficiency on day 0: 0.84 ± 0.05 vs. day 1: 0.75 ± 0.10; p = 0.016). Importantly, ATP production efficiency recovered on day 5 (0.81 ± 0.03; p= 0.188) remaining stable on day 6 (0.8 ± 0.04; p= 0.125) and 7 (0.82 ± 0.04; p= 0,437). Potential damage to the outer mitochondrial membrane was assessed by analysis of the cytochrome c control factor. A discrete elevation was observed after 24 hours (day 0: 0.21 ± 0.11 vs day 1: 0.33 ± 0.06; p<0.05). However, recovery from this initial damage was indicated by lowered levels after day 5 (0.19 ± 0.07; p= 0.625) of perfusion, remaining unaltered until day seven (day 7: 0.2 ± 0.07; p=0.593).

CONCLUSION: Our data indicate that, mitochondrial integrity and function, can be maintained stable during NMP of several days. A time-dependent decrease of mass-specific respiration is counterbalanced by stable ATP production efficiency. Importantly, a discrete impairment after 24 hours reveals to recover in the long-term perfusion setting.

Funding:

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Dysglycemia and Reperfusion Failure in Patients With ST-Segment Elevation Myocardial Infarction

Background: Failed myocardial tissue reperfusion due to microvascular injury despite successful culprit lesion percutaneous coronary intervention (PCI) is associated with poor clinical outcome in patients with ST-elevation myocardial infarction (STEMI). A possible influence of dysglycaemia on myocardial reperfusion injury is unclear.

Objectives: To investigate the association between glycaemic status and microvascular injury determined by magnetic resonance imaging in STEMI patients.

Methods: This prospective observational cohort study included 260 consecutive STEMI patients undergoing primary PCI between 2016 and 2019. Peripheral venous blood samples for glucose and HbA1c measurements were drawn on admission. Primary microvascular injury endpoint was defined as presence of intramyocardial haemorrhage (IMH) assessed by magnetic resonance T2* mapping 4 (interquartile range [IQR]:2-5) days after PCI.

Results: HbA1c (odds ratio[OR]:1.73 [95%CI:1.24-2.40];p=0.001), pre-diagnosis of diabetes (OR:2.63 [95%CI:1.18-5.90];p=0.02) and glucose concentration (OR:1.01 [95%CI:1.00-1.01];p=0.01) significantly predicted IMH, which was present in 90 (35%) patients. Of these three parameters, only HbA1c remained significantly associated with IMH (OR:2.05 [95% CI:1.09-3.85];p=0.02) after adjusting for total ischemic time, culprit lesion location, pre- and post-interventional TIMI flow and peak biomarker concentrations (troponin, N-terminal pro-B-type natriuretic peptide, C-reactive protein). The rate of IMH was 24% in patients with HbA1c <5.7%, 43% in patients with HbA1c ≥5.7 to 6.4% and 59% in patients with HbA1c ≥6.5 % (p<0.001). After exclusion of patients with diabetes (pre- and newly diagnosed, n=34), HbA1c ≥5.7% remained predictive of IMH in both univariable (OR:2.42 [95% CI:1.36-4.30];p=0.003) and multivariable analysis (OR:3.68 [95% CI:1.64-8.21];p=0.002).

Conclusions: In STEMI patients undergoing primary PCI, admission HbA1c was independently associated with reperfusion injury as determined by IMH. These findings suggest that IMH could represent the underlying pathophysiological link between dysglycaemia and adverse outcomes following STEMI.

Funding:

P. Fink, MD, 1; M. Reindl, MD, PhD, 1; I. Lechner, MD, 1; C. Tiller, MD, 1; M. Holzknecht, MD, 1; A. Mayr, MD, 2; M. Theurl, MD, PhD, 1; G. Klug, MD, 1; C. Brenner, MD, 1; A. Bauer, MD, 1; B. Metzler MD, 1; S. J. Reinstadler, MD, PhD, 1*

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Fluid retention, peripheral edema and cardiac volume overload in ultra-distance cyclists – an observational pilot-study

Objectives: Ultra-distance cyclists expose themselves to extreme physical challenges, the health consequences of which are mostly unknown. As ultracycling events often route through remote areas without access to continuous medical support, accidents and severe physical dysfunction can be fatal. We aimed to elucidate in detail the physical effects of ultra-distance cycling on electrolyte and fluid balance.

Methods: Thirteen experienced ultracyclists (8 male, 5 female) completed a long-distance bicycle ride over six consecutive days. Laboratory and bioelectrical impedance analyses were performed on days 0, 4, 6 of the ride and after one day of recovery. Echocardiography was performed on days 0, 6 and after recovery. Throughout the entire ride, participants tracked liquid intake and self-measurements of body part circumferences in a custom mobile application. Continuous self-testing of urinary electrolytes was performed using a point-of-care testing device. Swelling symptoms were documented photographically.

Results: Participants covered a mean distance of 1,205 km and 19,417 vertical metres in 59.14 hours effective ride time and exhibited varying degrees of fluid retention with signs of peripheral edema and cardiac volume overload. As compared with baseline values, on day 6 a mean increase of plasma volume (+18.9 +/- 10.7 %), total body water (44.89 +/- 7.43 L vs. 46.08 +/- 6.86 L), right ankle circumference (22.02 +/- 1.25 cm vs. 22.92 +/- 1.37 cm), NT-proBNP (34.46 +/- 18.87 ng/L vs. 332.46 +/- 217.06 ng/L), right atrial volume (54.21 +/- 11.25 mL vs. 63.84 +/- 13.18 mL) and right ventricle volume (71.09 +/- 13.98 mL vs. 79.71 +/- 15.23 mL) were observed, respectively. After 24 hours of resting, changes were mostly resolved.

Conclusion: Ultra-endurance cycling induces transient signs of volume retention with peripheral edema and cardiac volume overload.

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Seroprevalence, waning, and correlates of anti-SARS-CoV-2 IgG antibodies in Tyrol, Austria: Large-scale study of 35,193 blood donors conducted between June 2020 and September 2021

Background

There is uncertainty about the seroprevalence of anti-SARS-CoV-2 antibodies in the general population of Austria, and about the extent to which antibodies elicited by vaccination or infection wane over time.

Aim

To estimate seroprevalence, waning, and correlates of anti-SARS-CoV-2 IgG antibodies in the Federal State of Tyrol, Austria.

Methods

We conducted a seroepidemiological study between June 2020 and September 2021, enrolling blood donors aged 18-70 years across Tyrol, Austria (participation rate 84.0%). We analysed serum samples for antibodies against spike or nucleocapsid proteins of SARS-CoV-2 with Abbott SARS-CoV-2 IgG assays.

Results

We performed 47,363 serological tests among 35,193 individuals (median age 43.1 years [IQR: 29.3-53.7], 45.3% women, 10.0% with prior SARS-CoV-2 infection). Seroprevalence increased from 3.4% (95% CI: 2.8-4.2%) in June 2020 to 82.7% (95% CI: 81.4-83.8%) in September 2021, largely due to vaccination. Anti-spike IgG seroprevalence was 99.6% (99.4-99.7%) among fully vaccinated individuals, 90.4% (88.8-91.7%) among unvaccinated with prior infection, and 11.5% (10.8-12.3%) among unvaccinated without known prior infection. Anti-spike IgG levels were reduced by 44.0% (34.9-51.7%) at 5-6 months compared to 0-3 months after infection. In fully vaccinated individuals, they decreased by 31.7% (29.4-33.9%) per month. In multivariable adjusted analyses, both seropositivity among unvaccinated and antibody levels among fully vaccinated individuals were higher at young age (<25 years), higher with a known prior infection, and lower in current smokers.

Conclusion

Seroprevalence in Tyrol increased to 82.7% in September 2021, with the bulk of seropositivity stemming from vaccination. Antibody levels substantially and gradually declined after vaccination or infection.

Funding: The study was supported by the Federal State of Tyrol and the Tirol Kliniken GmbH.

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CARDIOVASCULAR HEALTH PROFILES AND THE IMPACT OF BEING BORN PRETERM IN ADOLESCENCE – PRELIMINARY DATA FROM THE EVA-TYROL STUDY

Background: Cardiovascular disease (CVD) is the number one cause of death worldwide. Studies suggest that first vessel alterations may occur early in life due to fetal and postnatal factors. Previous data showed an unfavorable cardiovascular risk profile of former preterm infants in adolescence and adulthood. Early adoption of lifestyle has positive effects on cardiovascular health (CVH) and is essential for the prevention of CVD. The aim of this study is to assess whether CVH profiles are related to prematurity in a cohort of healthy adolescents.

Methods: In this cross-sectional study, 14- to 19-year-old adolescents in North Tyrol, Austria and South Tyrol, Italy were prospectively enrolled. CVH determinants (smoking, body mass index, physical activity, dietary patterns, systolic and diastolic blood pressure, total cholesterol and fasting blood glucose) were assessed and analyzed for preterm- and term-born adolescent subgroups separately. Ideal CVH according to the guidelines of the American Heart Association are defined as never smoked a cigarette, 4-5 points in the Dietary Approach to Stop Hypertension-Score, a BMI < 85th percentile and ≥ 60 minutes of physical activity per day. Blood pressure values after 10 minutes at rest < 90th percentile, fasting blood glucose < 100mg/dl and total cholesterol levels < 170 mg/dl were considered as ideal. Complete data for gestational age was available in 1491 adolescents.

Results: A total number of 123 participants (8.2%) was born preterm. Mean gestational age was 34 weeks, mean age at examination 16.3 years (55.3% female). Systolic blood pressure and diastolic blood pressure were significantly higher in the preterm group than in the term group ($p<0.05$). Only in less than 2% of study participants all CVH determinants were in an ideal range. There were no significant differences between both groups regarding all other CVH metrics.

Conclusion: Our results demonstrate the impact of prematurity on blood pressure in adolescence. The low prevalence of ideal CVH in adolescence highlights the need for early health intervention in preterm- as well as term-born individuals.

Funding: Competence Centers for Excellent Technologies (COMET) of the Austrian Research Promotion Agency FFG: "Research Center of Excellence in Vascular Ageing—Tyrol, VASCage" (K project number 843536), VASCage (Centre for Promoting Vascular Health in the Ageing Community, project number 868624) of the Austrian Research Promotion Agency FFG (COMET program)

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Molecular regulation of the oncogenic miR-17-92 cluster

MicroRNAs are a class of small, non-coding RNAs that posttranscriptionally regulate gene expression 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, 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, during their biogenesis or by 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. Interestingly, 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. Of note, it is mostly unclear how these imbalanced expression patterns are established, thereby warranting an in-depth analysis.

To generate a comprehensive understanding of the molecular mechanisms that regulate miR-17-92 on the level of the cluster as well as on the level of the individual miRNAs, we have established a fluorescence-based genome-wide CRISPR/Cas9 screen. Using this platform, we have identified and validated 15 candidate genes whose deletion has a statistically significant impact on individual or groups of cluster miRNAs. The most interesting candidate is a gene involved in pre-spliceosome-assembly. We describe this gene in a new context, namely during miRNA maturation. At the moment we are assessing the exact level of regulation during miRNA biogenesis.

Funding: FWF, ÖAW DOC Fellowship

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Uterus transplantation - a new hope for transgender women?

The upcoming field of uterus transplantation opens the meaningful discussion of whether a uterus could be transplanted into a transgender female to fulfil their wish for an own family or an entire female body perception. Since the first successful uterus transplantation in 2014, many authors have discussed the hypothetical possibility of uterus implantation in a male-born person. Among others, the anatomical obstacles were discussed. Nevertheless, more information on the anatomical situation is needed. Therefore, we conducted an anatomical study investigating the exact venous situation of the internal iliac vein and the uterine veins concerning the anatomical implications. We analysed 33 internal iliac vein samples of cadaveric donors. We measured the length of the internal iliac vein until the first join with a uterine vein to elucidate if an anastomosis would always be possible. Moreover, we evaluated the number of uterine veins and their conformation to assess the complexity of a possible end-to-side uterine-internal iliac anastomosis.

Until the first uterine conduct, the internal iliac vein showed a mean length of 38.07 mm (± 3.71 mm) with no significant differences between the right and left sides. The uterine veins number varied from 1 to 7 with no significance between the sides. The classical 'plexus uterinus' described in most textbooks was found in 9 of 33 situations.

We can conclude that a uterine-internal iliac anastomosis is not always possible from this data. Nevertheless, a suitable length is given for a possible end-to-side internal iliac venous anastomosis even in genetically male recipients.

Funding:

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1: Univ.-clininc for visceral-, transplantation- and thorax surgery

2: Institute of Clinical and Functional Anatomy

miR-17~92 promotes lung maturation during embryonic development

miRNAs serve essential roles for developmental timing, cell fate transitions and cell fitness during embryonic development and tissue homeostasis in the adult. At least 30 microRNAs (miRNAs) are essential for normal mouse embryogenesis, among which miR-17~92 is involved in heart and lung development. The miR-17~92 cluster codes for six miRNAs that can be grouped into four different families based on their nucleotide sequence. Targeted deletion of the miR-17~92 cluster in mice caused perinatal lethality associated with lung hypoplasia. The pro-apoptotic BCL-2 interacting mediator of cell death (BIM) was identified as a potential critical miR-17~92 target gene. BIM acts as a cellular stress sentinel, initiating mitochondrial apoptosis. Using a novel engineered mouse model, our lab could show that inhibiting the binding of miR-17~92 miRNAs to the BIM mRNA 3' untranslated region partially phenocopies the lung developmental defect of miR-17~92 deficient mice. Neonates from both genotypes display hypoplastic and immature lungs, with moderately increased BIM mRNA and protein levels. Furthermore, more differentiated epithelial cell types, such as secretory Club cells and surfactant producing AT2 cells, were found diminished. In contrast, epithelial stem/progenitor cells appear enriched, as indicated by an increased propensity to form lung organoids.

Altogether, our data indicate that repression of BIM by miR-17~92 miRNAs prevents accidental cell death during terminal epithelial differentiation. In a broader context, BIM-mediated apoptosis of differentiating epithelial cells in the developing lung may cause immature lung phenotypes in humans. Current research efforts aim at elucidating the molecular pathways that drive this cell death during normal development, and whether our novel finding may serve as therapeutic entry point to boost lung epithelial maturation in embryonic and neonatal lungs, or for the treatment of lung pathologies in adults.

Funding: FWF P 32755-B , TWF F.16958/5-2019

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The association of allergic asthma and carotid intima-media thickness in adolescence – Data of the prospective Early Vascular Ageing (EVA)-Tyrol cohort study

Background: In recent years, there has been increasing evidence that asthma is associated with atherosclerosis and cardiovascular disease. However, data in children and adolescents are scarce and conflicting. We aimed to assess the impact of asthma with and without an allergic component on the carotid intima-media thickness in a large pediatric population.

Methods: The community-based Early Vascular Ageing-Tyrol cohort study was performed between May 2015 and July 2018 in North, East (Austria) and South Tyrol (Italy) and recruited youngster aged 14 years and above. Medical examinations included anthropometric measurements, fasting blood analysis, measurement of the carotid intima-media thickness by high-resolution ultrasound, and a physician guided interview.

Results: The mean age of the 1506 participants was 17.8 years (standard deviation 0.90). 851 (56.5%) participants were female. 22 subjects had a physician diagnosis of non-allergic asthma, 268 had inhalative allergies confirmed by a positive radio-allergo-sorbent-test and/or prick test, and 58 had allergic asthma. Compared to healthy controls, participants with non-allergic asthma (411.7 vs. 411.7 micrometer; p = 0.932) or inhalative allergy (420.0 vs. 411.7 micrometer; p = 0.118) did not have significantly higher carotid intima-media thickness (cIMT). However, participants with allergic asthma had significantly higher cIMT (430.8 vs. 411.7 micrometer; p = 0.004) compared to those without and this association remained significant after multivariable adjustment for established cardiovascular risk factors.

Conclusion: Allergic asthma in the youth is associated with an increased carotid intima-media thickness. Physicians should therefore be aware of allergic asthma as a potential cardiovascular risk factor in children and adolescents.

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REPROGRAMMING AND REJUVENATION TRAJECTORIES DURING THE DIRECT CONVERSION OF HUMAN FIBROBLASTS INTO INDUCED NEURAL STEM CELLS ANALYZED BY SINGLE-CELL RNA SEQUENCING

Mammalian tissue regeneration, particularly in the central nervous system, is limited. Comparative analysis with regeneration in fish and axolotl indicates evolutionary conserved transcriptional programs, that are not well understood in the human brain. Cellular reprogramming of human somatic cells into induced pluripotent stem cells (iPSCs) provides new opportunities to analyze regeneration trajectories. During reprogramming cells get epigenetically rejuvenated, whereas direct conversion of adult dermal fibroblasts (ADFs) into induced neurons (iNs) circumvents the rejuvenation and maintains the majority of epigenetic ageing marks. Moreover, the direct conversion into induced neural stem cells (iNSCs) provides a novel system for studying human neural regeneration. However, the transcriptional programs underlying iNSC-type rejuvenation are poorly investigated. In this study we aim to assess the reprogramming trajectories during the conversion of ADFs into iNSCs. For this purpose, we employed a lentiviral cell barcoding method, CellTagging, to label cells with barcodes at multiple stages during direct conversion. Subsequently, we collected multiple samples along the iNSC conversion in order to analyze the transcriptome and epigenome on a single-cell level by multi-omics. Single-cell iNSC RNA sequencing data revealed the generation of a highly homogeneous cell population, enriched for bona fide NSC markers, like Nestin and PAX6, and the absence of pluripotency-associated markers OCT4, cMYC and KLF4, indicating successful conversion into iNSC. Analysis of markers for the anterior/posterior and ventral/dorsal axis of the neural tube revealed a hindbrain, dorsal cellular identity. Moreover, we were able to reconstruct lineage trees through bioinformatics analysis of the integrated barcoded cell tags. In the next steps of our study, we will perform high resolution lineage analysis by analyzing the multi-omics data at several time points of conversion and we will identify the key genes affecting the conversion efficiency. Ultimately, we expect our study to provide deep insights into transcriptional programs regulating neural ageing and regeneration.

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TET enzymes ensure physiological antibody-mediated immunity

Aberrant epigenetic patterns associate with pathologies of the immune system. The most common epigenetic modification in eukaryotic genomes is methylation of cytosine (5mC). TET enzymes (TET1-3), of which TET2 and TET3 are expressed throughout the immune system, catalyze the oxidation of 5mC in DNA. The resulting 5mC variants support chromatin accessibility and transcriptional activity by facilitating cytosine demethylation, and serving as epigenetic marks on their own.

We and others have shown that TET2 and TET3 establish and adapt cell identity programs during lineage specification of immune cells. Thus, TET deficiency facilitates the appearance of pathologies. In human leukemias and lymphomas, TET2 loss-of-function mutations are recurrently found, and TET3 gene variants associate with autoimmunity. In mice, TET2 loss promotes inflammation and boosts oncogene-driven lymphomas and leukemias. However, the order of molecular and cellular changes upon TET2 and/or TET3 loss-of-function remain ill-defined.

Thus, we employ cell culture systems and pre-clinical mouse models to elucidate the pathophysiological mechanisms that underlie TET loss-of-function-driven pathologies originating from B lymphocytes.

Upon B lymphocyte-specific combined deletion of TET2 and TET3, our lab could establish that these two TET's synergize in stabilizing DNA methylation patterns as well as transcriptional programs guarding cell identity while B lymphocytes transit through developmental stages, allowing the generation of functional B lymphocytes. Combined loss of TET2 and TET3 specifically in antigen-activated B lymphocytes, leaving B lymphocyte development unperturbed, produced immunodeficiency in combination with autoimmunity, revealing key roles for antibody-mediated immunity. Prolonged TET deficiency targeted to antigen-activated B lymphocytes caused a manifestation of these phenotypes, resulting in pronounced lymphoproliferation that affected also TET-proficient immune cells such as T lymphocytes.

Ultimately, our work aims to identify vulnerabilities of TET loss-of-function, elucidating therapeutic entry points for pathologies originating from B lymphocytes and other immune cells.

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Supernumerary centrosomes in immune cells

Centrosomes are primarily known for their unique ability to nucleate and organize microtubules, regulating key cellular processes that define shape, polarity and spindle assembly during mitosis. Cycling cells commonly carry one centrosome that is formed by a pair of centrioles, which are duplicated exactly once during the cell cycle. Aberrantly generated "extra" centrosomes, however are associated with genomic instability, possibly due to increased abnormalities in spindle-pole organization. Notably, centrosome amplification is sufficient to drive spontaneous tumorigenesis in murine cancer models. Despite this obvious pathophysiological role of extra centrosomes, recent evidence suggests numerous physiological functions, such as in mononucleated dendritic cells where they guide locomotion and trafficking of effector cytokines. However, centrosome amplification has not been described for other immune cells.

We found that certain subtypes of developing and antigen-activated mouse B lymphocytes, best known for their ability to produce and secrete antibodies, display extra centrosomes in vivo. On the one hand it is tempting to speculate that this feature may be causal for the enhanced likelihood of exactly these B cell subsets to transform into leukemias or lymphomas. To address this hypothesis, we are currently studying whether forced overduplication of centrosomes by PLK4 overexpression alters tumorigenesis in a MYC-driven lymphoma model. We found that PLK4 overexpression in developing B cells is strongly selected against and therefore tumor latency was not affected. On the other hand, supernumerary centrosomes in B cells may serve non-mitotic functions in signaling, secretion or migration processes. Our data suggests that supernumerary centrosomes are required for optimal antibody-mediated immune responses. Understanding why and how centrosome amplification can affect B cell pathology and function will be a first step towards elucidating their diverse roles beyond faithful chromosome segregation, and shed light on novel therapeutic opportunities for the treatment of B cell pathologies.

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Deletion of tumor necrosis factor in C57BL/6N mice leads to altered macrophage iron homeostasis and promotion of Salmonella Typhimurium multiplication

Tumor necrosis factor alpha (TNF) is a key regulator in the control of infection with the intracellular bacterium Salmonella Typhi, which is partly referred to the induction of nitric oxide (NO) by inducible NO synthase (iNOS). Apart from direct anti-microbial effects, NO induces nuclear factor erythroid 2-related factor 2 (NRF2) expression which promotes the transcription of the cellular iron exporter ferroportin-1 (FPN1), making the essential nutrient iron less accessible for intracellular microbes and thereby resulting in bacteriostatic effects. Herein, we investigated how genetic deletion of TNF affects the immune control of Salmonella infection.

To study the impact of TNF deletion on Salmonella infection in vitro, bone marrow-derived macrophages (BMDM) from TNF-/- knock-out and C57BL/6N wild-type mice were infected with Salmonella Typhimurium (S.tm) which is the correlate to the human pathogen Salmonella typhi. TNF-/- BMDM showed a significantly worse S.tm killing capacity. By analysing changes in iron homeostasis through quantitative real-time PCR (qRT-PCR), Western blotting and FACS analysis, we found an increase in the expression of hepcidin in infected TNF-/- macrophages, resulting in proteolytic degradation of FPN-1 and cellular iron retention, a situation which promotes the growth of S.tm within macrophages. TNF-/- mice infected with S.tm had a poorer infection outcome, which was paralleled by increased interleukin-6 (IL-6) levels, upregulation of hepcidin transcription and reduced FPN-1 expression.

Thus, deletion of TNF leads to subtle changes of iron metabolism resulting in iron retention via hepcidin mediated blockade of iron export thereby increasing iron access of intramacrophage S.tm and subsequently promoting their proliferation.

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Therapeutic Biomarkers for Neuroinflammation

Objective: Neuroinflammation has been identified as an important factor in autoimmune diseases of the central nervous system, including rare neurological diseases like aquaporin-4 antibody (AQP4-Ab) associated neuromyelitis optica spectrum disorders (NMOSD) and myelin oligodendrocyte glycoprotein-antibody associated diseases (MOGAD). Previous studies highlight an up-regulation of Th17 and Th2 associated cytokines in NMOSD and MOGAD, which differs from multiple sclerosis (MS). Of particular interest is IL-6, which was found to be a central mediator in the pathogenesis of NMOSD and MOGAD. Therefore, drugs targeting the IL-6 receptor were developed and shown effective in clinical trials.

Methods: To investigate further disease-specific altered biomarkers, we analysed 65 cytokines, chemokines and related molecules at baseline in blood samples of 40 AQP4-Ab-positive NMOSD patients, 40 MOGAD patients and 54 MS patients as a control group.

To assess the stability of these 65 analytes and their change during progression or recovery, we measured follow-up samples after 12 months. Therefore, Procartaplex multiplex immunoassays were used, based on the Luminex xMAP technology, which enables the simultaneous detection and quantification of multiple secreted analytes.

Results: We found 40 significantly altered cytokines/chemokines of which a majority was up-regulated in NMOSD and MOGAD compared to MS.

In detail, especially cytokines associated with the Th17-pathway were shown to be elevated in MOGAD and a difference in cytokine/chemokine levels of children compared to adults with MOGAD was found. Importantly, the majority of measured analytes did not significantly change during the 12 months follow-up period.

Discussion: These results not only highlight an association of distinct serum cytokine/chemokine profiles in NMOSD and MOGAD, which differ from MS, but even more strikingly show the stability of these cytokines/chemokines over time. Therefore, we emphasize that our results yield a basis for the establishment of novel diagnostic biomarkers or even more for the development of new therapies.

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Identifying tumor suppressor functions of TET2

Remodeling of the epigenetic landscape via DNA cytosine methylation drives cell lineage specification, establishing and maintaining stable gene expression programs that uniquely define given cell types. During tumorigenesis the epigenome becomes corrupted, disturbing lineage-specific gene expression and cell identity. TET2, an enzyme that contributes to the erasure of cytosine methylation via oxidation of the methyl group, is among the most frequent somatically mutated genes in hematological tumors, commonly identified as loss-of-function versions. Similar to the situation in humans, TET2 loss on its own is only weakly tumorigenic in mice, but rather facilitates leukemic transformation driven by prototypic oncogenes such as FLT3ITD or AML-ETO. However, TET2 LOF is not limited to leukemias.

TET2 mutated diffuse large B cell lymphomas, the major B cell lymphoma class, are common and display a distinct gene expression profile. Using in vitro culture systems and pre-clinical mouse models, we establish that TET2 loss indeed fosters the formation of B cell lymphomas driven by the MYC oncoprotein. Thus, (1) TET2 loss specifically in B cells facilitates MYC-mediated transformation in a dose dependent manner, and (2) TET2 loss early on in hematopoietic stem cells potentiates the transformative potential later on in B cells.

Currently, we seek to understand how TET2 loss conspires with MYC to overcome the barriers that maintain cell identity and functionality. Our data suggests that TET2 may exert tumor suppressor functions by controlling the expression of DNA repair genes, or via direct functions of oxidized cytosine species. In addition, TET2-deficient lymphomas select for high expression of the pro-survival BCLXL protein, providing protection from MYC-driven apoptosis.

Altogether, the impact of TET2 loss-of-function on DNA repair and apoptosis mechanisms creates mechanistic understanding of how TET2 deficiency nudges transformation and reveals testable therapeutic vulnerabilities.

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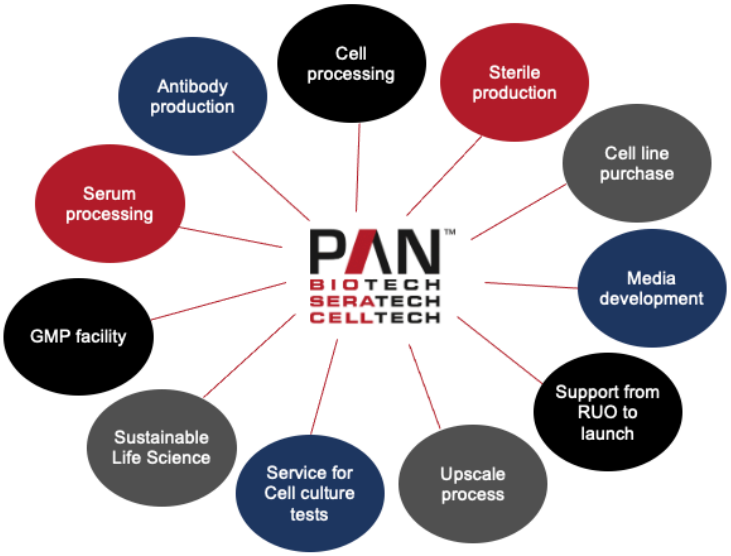

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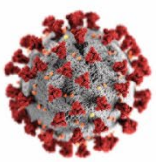

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Loss of autism-associated α2δ-3 affects synaptic protein expression, presynaptic function, neuronal excitability, and mouse behavior

In neurons, alpha2delta subunits of voltage-gated calcium channels (VGCC) serve as modulators, regulating synaptic functions independently from their channel specific role. Moreover, the human genes encoding for α2δ subunits have been linked to a variety of neurological disorders. Particularly CACNA2D3, encoding for α2δ-3, has been strongly associated with autism spectrum disorders.

Thus, we hypothesize that α2δ-3 knockout mice may serve a potential model for studying autism. We further suggest that defects in specific synapses or synaptic connections can explain the autism-like phenotype.

To test this, we examined brain structure in Nissl-stained sections and analysed synaptic protein expression. Moreover, we tested synaptic functions in cultured hippocampal neurons using presynaptic calcium imaging and analysed neuronal excitability in slice electrophysiology. Furthermore, behavioural phenotypes were assessed using open field and forced swim testing.

Initial behavioural characterisation revealed a mild anxiogenic phenotype together with increased active coping in knockout mice compared to littermate controls. Structural brain analysis in Nissl-stained sections illustrated no gross morphological changes in α2δ-3 knockout brains, but showed a slightly reduced brain volume. Interestingly, α2δ-3 over-expression in hippocampal neurons reduced the size of presynaptic boutons, suggesting a presynaptic role of α2δ-3. Hence, we tested synaptic function by imaging presynaptic calcium signals. Knockout of α2δ-3 resulted in a reduction of presynaptic calcium transients after stimulation with 1 action potential (AP), 3 APs, and 10 APs. Biochemical analysis of whole brain and synaptosomal lysates demonstrated a strong reduction of synaptic protein expression in α2δ-3 knockout brains, most strikingly of striatal NMDAR2B. Therefore, we analysed the intrinsic excitability of striatal neurons in slice electrophysiology, revealing a hypo-excitability in α2δ-3 knockout brains.

Taking together, α2δ-3 deficiency results in mild reduction of brain volume, causes changes of synaptic protein expression and interferes with proper presynaptic function and neuronal excitability. Furthermore, knockout mice display a mild anxiogenic phenotype and reduced passive coping behavior, observations that are in line with previous descriptions of autism mouse models. To ultimately assess the suitability of α2δ-3 knockout mice as a model for autism, we will test for signs of autism-like behaviour, and examine striatal synaptic plasticity, synaptic ultrastructure and wiring.

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CaV1.3 L-type Ca2+ channel modulates pancreatic β-cell electrical activity and survival.

Pancreatic β-cells express several high voltage-gated Ca2+ channel isoforms critical for insulin release, cell differentiation, and survival. RNAseq and qPCR analyses demonstrated that CaV1.3 L-type Ca2+ channel is highly expressed in pancreatic islets of both mice and men. Moreover, genetic polymorphisms leading to loss-of-function associate with increased susceptibility for diabetes while CaV1.3 gain-of-function mutations cause hyperinsulinaemic hypoglycemia in humans. Nevertheless, functional evidence for the physiological role of CaV1.3 is contradictory and largely unknown. Here we show that CaV1.3 deletion led to a 6-fold increase in DNA damage and a 3-fold decreased proliferation markers in pancreatic β-cells of 14-days old mice, while adult mice were largely unaffected. However, β-cell mass was reduced by ~20% in both young and old mice. Functionally, CaV1.3 deletion led to similar effects in both ages. Voltage-clamp recordings in β-cells of 14-days old mice showed a ~20% reduction in whole-cell Ca2+ influx (WT Ipeak = -19.8±1.0 pA/pF, CaV1.3-/- Ipeak = -14.8±0.6 pA/pF) accompanied by slower activation and inactivation kinetics as well as a ~5mV rightwards shift of the voltage-dependence of activation (WT V1/2 = -7.7±0.8 mV, CaV1.3-/- V1/2 = -2.3±1.1 mV). Moreover, current-clamp recordings showed that CaV1.3 deletion delayed the glucose-induced action potential (AP) onset, reduced AP firing frequency (in 7.5mM glucose WT= 4.3Hz, CaV1.3-/-= 2.1Hz) and AP-train frequency (in 7.5mM glucose inter-train interval WT= 49.3±9.6 sec, CaV1.3-/-= 120.3±25.5 sec). AP-clamp experiments indicated that these effects were caused by reduced pace-making current at the beginning of an AP-train as well as during repetitive stimulation due to reduced voltage-dependent facilitation. Therefore, our data demonstrate that the CaV1.3 channel is important for postnatal β-cell survival and proliferation, contributes to Ca2+ influx, is required for the initiation of glucose-induced electrical activity and thereby modulates insulin release both at low and high glucose concentrations.

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Molecular mechanisms responsible for the sexual dimorphism in pancreatic β-cell insulin release

In humans, Type 2 Diabetes Mellitus (T2DM) has a higher incidence in males compared to females, a phenotype recapitulated by many rodent models. While the sex difference in insulin sensitivity partially accounts for this phenomenon, hitherto uncharacterized differences in pancreatic β-cell insulin release strongly contribute. Here we show that stepwise increase in extracellular glucose concentration (2, 5, 7.5, 10, 15, 20 mM) induced electrical activity in β-cells of both sexes with similar glucose sensitivity (Female EC50 =9.45±0.15 mM, Male EC50 =9.42±0.16 mM). However, female β-cells resting membrane potential (RMP) and inter-spike potential (IP) were significantly higher compared to males (e.g.@15mM glucose: Male RMP= -82.7±6.3, IP= -74.3±6.8 mV, Female RMP= -50.0±7.1, IP= -41.2±7.3 mV). Females also showed higher frequency of trains of action potential (AP) (@10mM glucose: Male F= 1.13±0.15 trains/min, Female F= 1,78±0.25 trains/min) and longer AP-burst duration (e.g.@10mM glucose: Male 241±30.8ms, Female 419±60.2ms). The higher RMP in females reduced the voltage-gated calcium channel (CaV) availability by ~60%. This explains the paradoxical observation that despite identical CaV expression levels and higher electrical activity, the islet Ca2+ transients were smaller in females compared to males. Interestingly, the different RMP is not caused by altered KATP, TASK, or TALK K+ currents. However, Stromatoxin-1-sensitive Kv2.1 K+ current amplitude was almost double in males (IK = 130.93±7.05pA/pF) compared to females (IK = 75.85±11.3pA/pF) when measured at +80mV and the addition of Stromatoxin-1 to male islets was able to depolarize the cell ~20mV. Our results are in agreement with previous findings showing that Kv2.1 genetic deletion or pharmacological block leads to higher insulin release and β-cell survival. Therefore, we propose the sex-specific expression of Kv2.1 to be the mechanism underlying the observed sexual dimorphism in insulin release and the incidence of T2DM.

Funding: FWF P31434, DOC30-B30

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Complement activation by MOG and AQP4 antibodies

Autoantibody associated demyelinating diseases of the central nervous system (CNS) are rare diseases with often severe clinical manifestations. Autoantibodies against myelin oligodendrocyte glycoprotein (MOG), which is expressed on the outermost layer of myelin sheaths and on oligodendrocytes, are found in MOG-antibody associated disease (MOGAD). Autoantibodies targeting aquaporin 4 (AQP4), a water channel expressed on the endfeet of astrocytes, are found in the majority of patients with neuromyelitis optica spectrum disorder (NMOSD). Both, MOG and AQP4 antibodies can induce complement dependent cellular cytotoxicity (CDC), leading to the formation of the terminal complement complex (TCC) and consecutive cell damage. Whereas CDC is a main pathological hallmark in NMOSD, the role of complement activation in MOGAD is less clear. Furthermore, we have recently found differences in antibody binding to the six major MOG isoforms. In this study, we aimed to compare complement activation of rodent and human antibodies to different MOG and AQP4 isoforms. For that purpose, we investigated the lactate dehydrogenase release of HEK293 cells transfected with different MOG isoforms or AQP4 M23 to compare the amount of complement induced cell damage caused by autoantibodies. To visualize complement activation, we also stained the TCC complex, using a C9 neoantigen specific antibody, and complement component 3. AQP4 antibodies showed stronger complement activation compared to MOG antibodies, but in both cases, complement activation was dependent on the antibody titers. Furthermore, immunostaining showed deposition of complement system products on the cell surfaces after incubation with patient sera together with active complement. To summarize, with our analysis of complement activation by MOG and AQP4 antibodies and the comparison of different MOG isoforms we aim to further elucidate the contribution of CDC to the pathogenicity of MOG and AQP4 antibodies.

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Sex-related Differences in Multiple System Atrophy

Background

Multiple system atrophy (MSA) is a rare neurodegenerative disorder of the α-synucleinopathies that is generally considered to affect both sexes equally without any influence on survival. However, recent findings suggest that female MSA individuals may have a survival benefit due to less severe autonomic failure.

Methods

Here we investigated sex-related differences in a well-characterized, retrospective cohort including probable MSA OR possible MSA with ≥3 years disease duration and ≥12 months of follow-up treated at the Department of Neurology, Innsbruck Medical University, between 1998 to 2020. Demographics, comorbidities, and disease specifics were analyzed using SPSS V25.0.

Results

Seventy-three female (51%) and 71 male (49%) MSA individuals were included. Median age at disease onset was 57 [49; 62] years in women and 59 [52; 66] years in men (p=0.059).

Prompted by orthostatic intolerance (14 vs. 4%; p=0.044), a disease onset with autonomic failure has been reported more frequently in men (29 vs. 19%; p=0.186). At the last available follow-up, orthostatic hypotension (85 vs. 66%; p=0.009), severe blood pressure falls ≥30/15 mmHg (71 vs. 51%; p=0.021), supine hypertension (53 vs. 33%; p=0.023), and a history of syncope (50 vs. 30%; p=0.014) were more common in men. Male MSA individuals also suffered more cardiovascular comorbidities (48 vs. 30%; p=0.029).

In contrast, female MSA showed a longer disease duration (64 [48; 86] vs. 53 [42; 68] months; p=0.045) with higher motor impairment as measured by the Hoehn & Yahr scale (p=0.043) and more prevalent depression (81 vs. 45%; p<0.001).

Sexual dysfunction was present in 82% of women and all men (p=0.006). Neurogenic bladder disturbances, motor symptoms, follow-up time, and age at last available follow-up were comparable in both sexes.

Conclusion

High blood pressure volatility arising from cardiovascular autonomic failure has been associated with increased morbidity, mortality, and poor prognosis in the α-synucleinopathies. Early-onset, more frequent, and severe cardiovascular autonomic failure may underlie worse survival of male MSA individuals.

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The role of enkephalin in hypoxic preconditioning

Background: Hypoxic preconditioning (HPC) is the application of mild transient hypoxia which preconditions the brain to a following more severe hypoxic insult as it occurs during epileptic seizures. HPC was shown to decrease seizure susceptibility and severity as well as neuronal damage in the hippocampus. The delta opioid receptor (DOR) and its primary endogenous ligands, the neuropeptides met- and leu-enkephalin (Enk) are thought to be involved in the neuroprotective actions of HPC. Recently, we showed that Enk influences mitochondrial respiration that may contribute to the neuroprotective effects of the Enk/DOR system. The present study aims at investigating the effects of the Enk/DOR system on structural and functional alterations of mitochondria in HPC.

Methods: Wild type (WT) and met-enkephalin knockout (met-Enk KO) mice were exposed to hypoxia for 7 h (9-11 % O2). Subsequently, we determined the seizure threshold (infusion of GABAA receptor antagonist pentylenetetrazol), analyzed mitochondrial function (high-resolution respirometry) and dynamics (real-time qPCR of key genes).

Results: In WT mice after HPC we observed an elevated seizure threshold, improved mitochondrial reserve capacity and increased mitochondrial fusion. In addition, our results suggest mitochondrial biogenesis after HPC in WT mice. Met-Enk KO mice displayed an increased seizure threshold and increased mitochondrial fusion already at naïve state but no changes upon HPC.

Conclusion: The observed mitochondrial alterations after HPC in WT mice could explain improved neuronal survival and increased seizure threshold. Enhanced mitochondrial reserve capacity improves energy supply in situations with increased energy demand, like epileptic seizures. Elevated mitochondrial fusion among other things is associated with neuronal survival and improved Ca2+ storage capacities. Which together support increased stress resistance and, thereby, neuronal survival. However, the precise role of met-Enk in HPC is unclear but we observed adaptive mechanisms in WT mice upon hypoxia which are absent in met-Enk KO mice.

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Towards overcoming the gender gap in epilepsy research – a female temporal lobe epilepsy mouse model for screening of antiepileptic effects

Introduction

With a prevalence of 0.5 – 1%, epilepsy represents one of the most common neurological diseases affecting men and women likewise. The medical management of female epilepsy patients is complicated by interactions between sex hormones, seizure control and antiepileptic drugs as well as special needs regarding pregnancy. Despite these challenges, previous studies have mainly focused on males, leaving a gender gap in epilepsy research which needs to be urgently addressed.

Aim

Our aim was to establish a female equivalent of the intrahippocampal kainic acid (KA) mouse model of temporal lobe epilepsy, which is one of the most frequently studied models in males, and subsequently screen for antiepileptic effects of different treatments.

Methods

After injecting KA unilaterally into the hippocampus of female mice, we monitored the development of epileptiform activity in in-vivo EEG recordings. Subsequently, we tested different concentrations of commonly prescribed anti-epileptic drugs (AEDs) as well as the solvent dimethyl sulfoxide (DMSO) for potential antiepileptic effects in comparison to male mice. In addition, we analyzed post-mortem Nissl-stained brain sections regarding typical neuropathological alterations of the hippocampus.

Results

Female KA mice replicated EEG features and neuropathological changes which have been extensively described in males. Likewise, the females proved to be refractory to widely used AEDs, but showed significantly reduced epileptiform activity after the benzodiazepine Diazepam. DMSO, frequently used as a solvent for antiepileptic drug candidates with limited water solubility, resulted in significant short-term attenuation of epileptiform activity in both females and males.

Conclusions

Having successfully established the intrahippocampal kainic acid model of temporal lobe epilepsy in female mice, we have laid a foundation for future research addressing gender issues.

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Involvement of parvalbumin-positive GABAergic neurons in basal forebrain modulation in a mouse model of neuropathic pain

Chronic neuropathic pain is a public health issue that affects ~5 % of the European population and is caused by a lesion or disease of the somatosensory system. The central mechanisms underlying the chronification of pain are not yet fully understood. The medial prefrontal cortex receives input from basal forebrain (BF) cholinergic neurons that is altered after nerve injury. Parvalbumin (PV)-positive GABAergic neurons are of special importance for this regulation, as those neurons produce widespread inhibition.

To elucidate the role of PV neurons in the pain-associated changes of BF cholinergic neurons, we investigated the electrophysiological properties of BF-PV neurons. PV neurons were selectively labelled and acute brain slices were prepared one week after spared nerve injury (SNI).

After SNI, BF-PV neurons demonstrated decreased firing rates in response to depolarizing current injections compared to sham controls. Consistently, the area under the curve (AUC) of the action potential frequency against injected currents was reduced in SNI mice. As postsynaptic transmission can influence the observed decrease in BF-PV neuron excitability, we recorded spontaneous inhibitory (sIPSC) and excitatory (sEPSC) postsynaptic currents. Neurons recorded from brain slices obtained from SNI treated mice demonstrated a reduced frequency of sEPSCs, suggesting a reduction of excitatory inputs.

Our findings suggest that alterations in cholinergic synaptic transmission in neuropathic pain may be caused by reduced inhibitory inputs mediated by local BF-PV neurons. In order to elucidate the direct nature of this PV-to-ChAT signaling, we will perform patch-clamp recordings from ChAT neurons in response to optogenetic activation of BF-PV neurons.

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Liver fat content is associated with intima-media thickness of the aorta in adolescents

Background: The formation of atherosclerosis often initiates during childhood and adolescence and depends on risk factor exposure. Cardiovascular disease (CVD) is the leading cause of death in metabolic associated fatty liver disease (MAFLD) - the most common liver disease in adolescents. It often remains undetected but would be amenable to treatment including lifestyle interventions. We aimed to assess the association between liver fat content and intima-media thickness of the aorta (aIMT) in adolescents.

Methods: In 485 adolescents from the general population of Tyrol we carefully assessed liver fat content, aIMT and cardiovascular risk factors. Liver fat content was measured by means of the controlled attenuation parameter using FibroScan® and the aIMT was assessed by high-resolution ultrasound. A linear regression model with multivariable adjustment for potential confounders was employed to investigate the association between liver fat content and aIMT.

Results: The study participants were on average 17.0 years old (SD 1.4), 43.9% were female and 45.2% were apprentices. Liver fat content significantly predicted aIMT when adjusting for age, sex, education, BMI, systolic blood pressure, LDL-cholesterol, HbA1c, physical activity, alcohol consumption and smoking status (given as difference in aIMT for a 10 dB/m higher liver fat content: 3.3 µm, 95% CI: 0.5-6.1).

Conclusion: Liver fat content was significantly associated with aIMT in adolescents of the general population even after adjustment for traditional cardiovascular risk factors. Hence, early detection and treatment of MAFLD has the potential to decrease the risk of future CVD.

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The role of Hippocampal VIP-expressing interneurons in the Pathophysiology of Temporal Lobe Epilepsy

Aim:

In the hippocampus, two groups of VIP-expressing interneurons, containing CCK-expressing basket cells and/or interneuron-selective interneurons (ISIs) control GABAergic transmission and pyramidal cell activity. The aim of the current study is to assess the possible role of VIP-expressing interneurons in the pathophysiology of epilepsy.

Methods:

We permanently inhibited GABA release selectively from VIP interneurons of the ventral subiculum by injecting a viral vector expressing tetanus toxin light chain (TeLC) in male epileptic as well as nonepileptic VIP-cre mice. Mice were then subjected to telemetric EEG recording for 4 weeks. In addition, spontaneous alternation Y-maze and novel location recognition tests were conducted to evaluate spatial memory and learning, respectively.

Results:

In non-epileptic mice, injection of TeLC and GFP did not cause development of seizures in both groups. In addition, behavioral tests addressing anxiety, memory, and navigation showed no differences between groups. In epileptic animals, the average number of spontaneous seizures per day as well as the average time spent in seizure per day showed a significant reduction in TeLC in comparison to GFP group. Surprisingly, we observed a clear connection between gender and the severity of status epilepticus and the animal's response to vector injection.

Conclusions:

ISIs mainly target other interneurons; therefore, silencing them would increase inhibition in pyramidal cells. However, the outcome of the silencing of VIP-interneurons in epileptic mice is highly dependent on the status of the network before silencing. To prove, we need further assessment by triple immunohistochemistry labeling for GFP, CCK, and VIP, which is currently ongoing in our lab.

Funding: This study is funded by the FWF (Austrian Science Fund; grant numberP 31777-B26)

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Impact of cancer-associated stromal cell heterogeneity on the development of castration resistant prostate cancer

Stromal fibroblasts and mural cells are well established as key contributors to the development, progression and therapy resistance of prostate cancer (PCa), the second leading cause of male cancer-related death in Western nations. Whilst localised PCa is often curative, a subset recur for which androgen deprivation therapy remains a standard-of-care approach. Despite initial response, nearly all patients progress to lethal castration resistant prostate cancer (CRPC). Consequently, the necessity for new therapeutic strategies combined with increasing awareness of the fundamental role of the tumour microenvironment (TME) has led to considerable interest in targeting the stromal component of disease. However, the TME displays considerable functionally heterogeneity with some types of cancer-associated stromal cells (CASCs) mediating tumour-supportive effects, whereas others display tumour-restrictive properties. In view of this functional heterogeneity, a better characterisation of CASC subtypes is needed in order to specifically target onco-promoting stromal entities.

This study therefore aims to identify, characterise and isolate CASC subtypes present in the PCa microenvironment and evaluate their functional relevance with respect to the development of castration resistance. Initial studies have thus focused on optimising a rapid tissue dissociation protocol for maximal recovery of stromal cell populations from patient-matched benign and cancerous prostate biopsy cores. The resulting protocol yields a reproducibly high number of single viable cells and CD90+ mesenchymal cells. Based on in-house single cell RNA sequencing data from digested PCa tissue, cell surface marker panels were designed to subsequently detect, isolate and validate putative CASC subtypes from dissociated tissue specimens via flow cytometry and quantitative real time PCR. These new markers are currently under investigation to assess their CASC subtype specificity. It is hoped that these marker panels and optimised dissociation strategy will provide a means to better characterise the prostate TME at the cellular level and, in the long term, evaluate the contribution of distinct CASC subtypes to PCa pathophysiology and therapy resistance.

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Impaired spindle assembly checkpoint function impairs B cell survival but facilitates their MYC-driven transformation

One mechanism leading to genomic instability is the emergence of aneuploid cells as a consequence of errors during mitosis. Chromosomal instability (CIN) that precedes aneuploidy is prevented by the mitotic spindle assembly checkpoint (SAC) that blocks cell cycle progression in the presence of unattached kinetochores by inhibiting the anaphase promoting complex (APC). Deficiencies within the SAC frequently leads to cell death in healthy cells. On the contrary, in human cancer expression of SAC components is often altered, thought to contribute to CIN, accelerated tumor progression and unfavorable clinical prognosis. However, in B-cell malignancies the impact of CIN on tumorigenesis is less clear, as in some tumor types CIN and deregulated expression of SAC components associates with better clinical prognosis.

To investigate the impact of CIN in B-cell development and B-cell transformation, two mouse models where created where two key-components of the SAC, mitotic arrest defective protein 2 Like 1 (MAD2L1) or monopolar spindle kinase 1 (MPS1), were conditionally deleted or mutated, respectively, using floxed alleles in combination with Mb1-Cre. Full SAC deficiency achieved by loss of Mad2l1 was found incompatible with normal B cell development and also precluded MYC-driven lymphomagenesis of early B progenitor cells. Residual SAC proficiency exerted by a truncated mutant of MPS1, however, led to impaired B cell development due to increased mitochondrial cell death rates while accelerating MYC driven transformation. Remarkably though, impaired SAC proficiency due to MPS1 truncation per se was insufficient to lead to spontaneous B cell transformation. Our findings are in line with observations that SAC proteins have never been found deleted in cancer while their deregulated expression levels or activity can accelerate malignant disease, at least in the context of aberrant oncogene activation.

Funding:

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Aspergillus fumigatus at the crossroad between 5-fluorocytosine metabolic activation and resistance

Annually more than 1.5 million people die from fungal diseases. A major proportion is caused by invasive and chronic mold infections, predominantly by the most prevalent airborne mold pathogen Aspergillus fumigatus.

Currently only 3 major classes of antifungal acting agents are used to treat Aspergillus infections: azoles, echinocandins and polyenes. A fourth class, nucleobase analogs, with its only member 5-flucytosine (5FC) is barely used for the treatment of aspergillosis. 5FC represents a prodrug and requires intracellular, pyrimidine salvage-mediated metabolization into toxic RNA and DNA nucleotides to inhibit fungal growth. Previous work has shown that 5FC is highly efficient against this fungus at pH5 in comparison to neutral pH, where the antifungal activity of 5FC is insignificant.

In this work we functionally characterized pyrimidine salvage enzymes in A. fumigatus involved in the metabolization of 5FC as well as its derivatives 5-fluorouracil and 5-fluorouridine and assessed the role of individual genes in resistance to the respective molecules. By quantifying the intra and extracellular amounts of 5FC, 5FU and 5FUR in mutants lacking individual salvage activities, we further obtained biochemical evidence confirming the enzymes involved in 5FC metabolization. Moreover, we determined that resistance against 5FC and its derivatives may be strongly promoted by efflux pump driven fluoropyrimidine detoxification.

Taken together, this work aims to acquire a comprehensive understanding on the genetic and molecular factors contributing to the antifungal activity of 5FC against A. fumigatus.

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Characterization of the EGAD-Ubiquitin ligase complex reveals unexpected flexibility in protein degradation

Ubiquitin-dependent protein degradation pathways and their associated stress responses are essential to maintain the integrity of eukaryotic cells. Endoplasmic reticulum (ER) associated degradation (ERAD) selectively ubiquitinates and extracts transmembrane proteins from the ER and targets them for proteasomal degradation. Ubiquitinated membrane proteins in post-ER compartments (at the plasma membrane, the Golgi, on lysosomes and on endosomes) are typically sorted by the endosomal sorting complexes required for transported (ESCRT) along the MVB pathway into lysosomes for degradation.

We have recently identified an additional post-ER membrane protein degradation pathway in Saccharomyces cerevisiae called Endosome and Golgi Associated Degradation (EGAD). At the Golgi, the membrane embedded Dsc-E3 ubiquitin ligase complex targets the integral membrane protein Orm2, a protein described as a negative regulator of sphingolipid synthesis, for degradation in a proteasome-dependent manner. So far, the Dsc complex has only been described to initiate the degradation of a few transmembrane proteins from post-ER compartments in an ESCRT- and lysosome-dependent manner. Hence, EGAD constitutes a novel proteasome-dependent route for the degradation of transmembrane proteins from post-ER compartments.

The overall aim of this project is to understand how orphaned transmembrane proteins, that have escaped their natural localization are detected and specifically targeted for proteasomal- or lysosomal-dependent degradation to restore cellular proteostasis. I will describe novel substrates of the Dsc complex and demonstrate that protein degradation pathways are much more flexible than previously anticipated.

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FAHD-Proteins as potentially druggable onco-targets

Most cancer cells divide rapidly, implying a higher use of energy resources compared to most non-cancerous cells. The accelerated energy metabolism of cancer cells is mostly based on enhanced glycolysis in the cytosol and increased glutaminolysis in the mitochondria. Targeting glutamine metabolism appears to be a promising strategy to combat triple negative breast cancer, but the underlying mechanisms of carcinogenesis of triple-receptor negative breast cancer cells (TNBC) are not understood. The mitochondrial enzyme fumarylacetoacetate hydrolase domain-containing protein 1 (FAHD1) is up-regulated in breast cancer tissues. Acting as an oxaloacetate decarboxylase, FAHD1 has been proposed to be a regulator of TCA cycle flux. Here we show that FAHD1 plays a distinct role in triple-receptor negative breast cancer cells and is indispensable for the survival of BT-20 cells, representing the basal breast cancer cell type. A lentiviral knock-down of FAHD1 (FAHD1-KD) in the luminal and basal breast cancer cell lines MCF-7 and BT-20, respectively, results in mitochondrial impairment based on lower succinate-dehydrogenase (mitochondrial complex II) activity. In luminal MCF-7, this leads to reduced proliferation of cancer cells cultured in medium containing only glutamine as main carbon source. Of interest, this is accompanied by attenuated protein levels of the enzyme glutaminase (GLS). Furthermore, basal BT-20 cells do not tolerate FAHD1 withdrawal, resulting in significant morphological changes and increased cell death. These findings demonstrate that FAHD1 is crucial for the functionality of complex II in breast cancer cells and that FAHD1 acts as an indirect regulator of mitochondrial glutaminolysis. Therapeutic options could emerge for glutamine-dependent tumors with high sensitivity to shifts in carbon metabolism.

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Poster abstracts

- Posters should stay up for the whole session of the day and must be taken down at the end of the day.
- Poster presentations should last approximately 3 min.
- Odd poster numbers at Thursday
- Even poster numbers at Friday
- There will be poster prizes, so stay at your poster during your respective session!

Abstracts are sorted by category:

#1-7: Analytics, Diagnostics and 3Rs
#8-21: Biochemistry & Cell Biology
#22-34: Developmental Biology and Ageing
#36-42: Genetics & Genomics
#35, 43-65: Immunology, ID, Oncology
#66-96: Pharmacology and Neuroscience
#97-104: Clinical Oncology
#105-127: Clinical Cardiology
#128-149: Clinical Neuroscience

Poster session

Thursday: Odd numbers 13:45-15:45 **Friday: Even numbers 13:45-15:45**

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Michael	Zwerger	1	Isolation of Mycosporine-like Amino Acids by FCPC
Tobias	Rainer	2	Online protein digestion for LC-MS enabled by 3D printed enzyme reactors
Yannick	Scharll	3	Comparison of a robotic and patient-mounted device for CT-guided needle placement: a phantom study
Christoph	Schwabl	4	Monosodium Urate Crystal Deposition in Coronary Artery Plaque by 128-Slice Dual-Energy Computed Tomography: An Ex Vivo Phantom and In Vivo Study
Julia	Abram	5	Flow-controlled ventilation versus pressure-controlled ventilation in thoracic surgery requiring one-lung ventilation – a randomized, controlled, single-center trial
Mia	Kvaale Loevmo	6	Controlled orientation and sustained rotation of biological samples in a sono-optical microfluidic device
Simon	Moser	7	Label free tomographic imaging of biological samples in a sono-optical microfluidic device
Sinead	Schwabl	8	The role of Endosome and Golgi Associated Degradation (EGAD) in cellular quality control
Mario	Aguiar	9	Siderophore uptake in Aspergillus fumigatus
Isabel	Singer	10	Protein interactions and metabolic signaling at the lysosome
Ilaria	Dorigatti	11	Exploring the role of ferroptosis in B cell leukemia
Rezan	Amjadi	12	Towards the structural basis of NDPK-C inhibition
Andrea	Medici	13	Mitochondrial dysfunction in ACM pathogenesis
Annie	Yap	14	Ambient availability of amino acids, proteins, and iron impacts copper resistance of Aspergillus fumigatus

Poster session

Thursday: Odd numbers 13:45-15:45 **Friday: Even numbers 13:45-15:45**

Presenting Author		#	Abstract title
Andreas	Feichtner	15	Systematic identification of druggable PKA substrates involved in colon cancer progression
Jana	Unterhauser	16	Epitope Masking by Polyphenols in the Major Apple Allergen Mal d 1
Valentina	Kugler	17	Deciphering of CDK complex formation and kinase conformation dynamics
Patrick	Knoll	18	Cell-penetrating peptide decorated charge converting nanocarriers: Key to overcome the polycation dilemma
Selina	Schwaighofer	19	Impact of kinase activation and inactivation on mitochondrial functions
Anant	Kakar	20	The membrane activity of a Temporin B peptide analog enables rapid cell killing in C. albicans
Jakob	Fleischmann	21	Impact of receptor signalling pathways on GTPase and kinase interactions and conformations
Ines	Martic	22	THE ROLE OF MELANOCYTES IN SKIN PIGMENTATION, SENESCENCE, AND SKIN AGING INDUCED BY EXPOSURE TO ENVIRONMENTAL STRESSORS
Marc	Sathianathan	23	Establishing a differentiation protocol that produces in-vitro beta-like cells
Anna Lena	Huber	24	Changes in Systemic Levels of Vascular Endothelial Growth Factor After Intravitreal Injection of Aflibercept or Brolucizumab for Neovascular Age-Related Macular Degeneration.
Camille	Brucker	25	Characterization of weight-loss target genes in human adipose stem/progenitor cells
Nicki	Marami-Zonouz	26	Infrared thermography as a non-destructive tool for the diagnosis of seed viability
Elisa	Gabassi	27	Progerin-mediated ageing in human brain cerebral organoids reveals specific patterns of DNA damage
Melanie	Zott	28	Regulatory role of Mnx1 in beta-cell differentiation and maturation

Poster session

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Anna Sophie	Smaling	29	Utilizing CRISPR guided cytidine deaminase for PKM isoform switching in AD patient-derived iNs
Moses	Kitilit	30	Role of Myc associating factor x (MAX) in Neoblast maintenance in flatworms
Alessandro	Pennati	31	Hmx gene conservation identifies the evolutionary origin of vertebrate cranial ganglia
Jessica	Lagerwall	32	Multi-omics-driven investigation to determine factors governing fate-determination of induced human neurons
Lucia	Zhou Yang	33	Microcarrier-based 3D cultures of age-equivalent human induced neurons for aging and neurodegenerative disease research
Julianne	Beirute-Herrera	34	Characterization of human-specific regulators of neurodevelopment
Gabriell	Knoll	35	HLA-DR+ CD8+ regulatory T cells inhibit T cell proliferation via direct cell-to-cell contact and are more sensitive for cell death
Kamila	Nykiel	36	Characterizing the m5C-dependent RNA-protein interactome
Silke	Huber	37	Changes in Systemic Levels of Vascular Endothelial Growth Factor After Intravitreal Injection of Aflibercept or Brolucizumab for Neovascular Age-Related Macular Degeneration.
Chiara	Ellensohn	38	Generating auxotrophic mutants in Trichoderma atroviride mediated by preassembled CRISPR/Cas9 ribonucleoprotein complexes
Claudia	Steinacher	39	Neurotrophins in developing human inner ear
Nico	Wahl	40	SATB2 organizes the 3D genome architecture of cognition in cortical neurons
Rosalie	Dittrich	41	Cre-Controlled CRISPR mutagenesis of voltage-gated Calcium Channel Cav1.2 in pancreatic exocrine cells
Isabel	Delazer	42	5-methylcytosine in mRNA: a regulator of transcript stability?

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Lukas	Lanser	44	Neopterin predicts disease severity in hospitalized patients with COVID-19
Lukas	Buchwinkler	45	Antibody Response to mRNA Vaccines against SARS-CoV-2 with Chronic Kidney Disease, Hemodialysis, and after Kidney Transplantation
Alexander	Hoffmann	46	Baseline iron status and presence of anaemia determine the course of systemic Salmonella infection following oral iron supplementation in mice
Thomas	Zöggeler	47	Immunological memory and affinity maturation after vaccination in patients with propionic acidemia.
Gerlinde	Karbon	48	BCL2 family proteins as effectors of the mitotic spindle assembly checkpoint
Lennart	Seizer	49	Real-Life Temporal Relations Between Urinary IL-6 and sTNF-R55 Levels with Specific and Nonspecific Symptoms in an SLE Patient
Verena	Harpf	50	The role of complement in a murine model of disseminated mucormycosis
Sára	Kellnerová	51	Characterization of monoclonal antibodies against different forms of the A subunit of Shiga toxin 2a
Margot	Fodor	52	Single-cell RNA sequencing reveals immune cell dynamics in normothermic machine perfusion of the liver
Chiara	Andolfi	53	Key components of the Mediator complex and their functional roles in prostate cancer
Elena	Brunner	54	Targeting a myofibroblastic prostate cancer-associated fibroblast subtype through pharmacological inhibition of NADPH oxidase 4
Jasper	Van Goubergen	55	Identification of functional single nucleotide polymorphisms in cryptic exon 3 of the androgen receptor gene
Florian	Hornsteiner	56	Investigating the role of dendritic cells in tumor-targeted therapy mediated anti-tumor immunity

Poster session

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Sarah	Danklmaier	58	Prediction and validation of C57BL/6J MHC-class I epitopes of the oncolytic virus VSV-GP
Katharina	Rosam	59	Y129F and V293A in the sterol 14-α-demethylase-F5 paralogue confer resistance to short-tailed azoles in Mucor circinelloides
Giulia	Bertacchi	60	HIV-1 Trans Infection via TNTs Is Impeded by Targeting C5aR
Viktoria	Zaderer	61	Adding new dimensions to viral entry on mucosal sites
Silvia	Eller	62	Overcoming Drug Resistance in BRAF-mutated Melanoma
Tina	Rauchenwald	63	The effect of macrophage inflammation on human adipose-derived stem cells and its role in human wound healing“
Ines	Schoberleitner	64	Immunoreactivity of Surface Topography on Human Foreign Body Response to Silicone Breast Implants
Gabriel	Diem	65	“Complement opsonization of HIV-1 promotes sustained survival regulated by C3a and C5a in human dendritic cells”
Mehmet	Mahsum Kaplan	66	Counteractive and cooperative actions of muscle β-catenin and CaV1.1 during early neuromuscular synapse formation
Maryam	Sadeghi	67	Automatic localization of Histological mouse brain slices in a 3D reference atlas using a deep learning
Ferenc	Török	68	The novel de novo variant F747S is associated with a new phenotype in CACNA1D channelopathies
Nikolas	Hörmann	69	Synthesis and biological evaluation of subtype-selective heterodimeric estrogen receptor antagonists for the treatment of estrogen receptor-positive and tamoxifen-resistant breast cancer
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Poster session

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Andrea	Cucchiaro	74	Interaction of Zeise's Salt amino acid derivatives with sulfur-donor compounds of biological interest
Matthias	Ganglberger	75	A truncation mutation in Cav1.4 L-type calcium channels primarily affects the retinal rod pathway
Thomas	Heigl	76	Congenital stationary night-blindness type 2: targeting the mouse retina for future gene therapies
Wietske	Tuinte	77	STAC proteins inhibit calcium and voltage dependent inactivation in L-type Ca channels
Dhwani	Korde	78	Spreading of P301S aggregated tau investigated in organotypic mouse brain slice cultures
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Isolation of Mycosporine-like Amino Acids by FCPC

Isolation of Mycosporine-like Amino Acids by FCPC

Due to their exposed habitats, marine red algae have developed unique strategies to protect themselves from environmental stressors like UV radiation. One is the synthesis of specific metabolites, namely mycosporine-like amino acids (MAAs). These substances are very polar, small molecules with extremely high UV-absorbance, which renders them optimal sunscreen compounds. In the here presented study a novel approach for the economic isolation of shinorine and porphyra-334, two of the most abundant MAAs, is described. The technique was developed based on fast centrifugal partition chromatography (FCPC) using an aqueous two-phase system comprising water, ethanol, ammonium sulfate and methanol in ascending mode. In approximately 90 minutes both compounds could be separated and obtained in good yield. As a second purification step, solid phase extraction (SPE) was employed, finally resulting in highly pure compounds as determined by TLC, NMR and LC-MS. The required purification time was much shorter compared to standard techniques like semi-preparative HPLC. Using this approach, 15.7 mg shinorine as well as 36.2 mg porphyra-334 were obtained from 4 g of crude Porphyra sp. (Nori) extract.

Funding:

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Online protein digestion for LC-MS enabled by 3D printed enzyme reactors

Immobilized enzyme reactors (IMER), i.e., surface immobilized proteases are powerful tools for performing online digestion of proteins or proteomes. Implementing online enzymatic digestion in a bottom-up LC-MS setup comes with several advantages, e.g., miniaturization potential, reduced liquid handling in a true online fashion and drastically reduced experiment time. However, given their high cost, IMERs are not widely employed in proteomics, apart from special experiments such as H/D exchange mass spectrometry. Using high resolution stereolithography (SLA) 3D printing, we developed highly effective microscale enzyme reactors (360 um i.d. x 30 mm). The immobilized protease enables protein digestion in a few minutes rather than using overnight protocols. Coupled to LC-MS, this setup facilitates peptide mapping of recombinant proteins within 20 minutes. Several aspects of using 3D printed devices in conjunction with mass spectrometry were addressed, e.g., pressure resistance and durability of the IMER as well as the mitigation of sample contamination by residual photopolymer resin and the identification of leachables by tandem mass spectrometry. We aim at the development of comprehensive MS protocols for H/D epitope mapping and proteomic research using our in-house produced protease chip. Our protocol allows for the fast production of tailored analytical devices employing high resolution addictive manufacturing. Photopolymer based 3D printing will greatly contribute to future advancements in analytical chemistry and microfluidics.

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Comparison of a robotic and patient-mounted device for CT-guided needle placement: a phantom study

Background: Robotic-based guidance systems are becoming increasingly capable to assist in needle placement during interventional procedures. Despite the technical advances, less sophisticated low-cost guidance devices promise to enhance puncture accuracy compared to the traditional freehand technique.

Purpose: To compare the in vitro accuracy and feasibility of two different aiming devices for computed tomography (CT)-guided punctures.

Material and methods: A total of 560 CT-guided punctures were performed by using either a robotic (Perfint Healthcare: Maxio) or a novel low-cost patient-mounted system (Medical Templates AG: Puncture Cube System [PCS]) for the placement of Kirschner wires in a plexiglas phantom with different slice thicknesses. Needle placement accuracy as well as procedural time were assessed. The euclidean (ED) and normal distances (ND) were calculated at the entry and target point.

Results: Using the robotic device, the ND at the target for 1.25 mm, 2.5 mm, 3.75 mm and 5 mm slice thickness were 1.28 mm (SD +/- 0.79), 1.25 mm (SD +/- 0.81), 1.35 mm (SD +/- 1.00) and 1.35 mm (SD +/- 1.03). Using the PCS, the ND at the target for 1 mm, 3 mm and 5 mm slices were 3.84 mm (SD±1.75), 4.41 mm (SD +/- 2.31) and 4.41 mm (SD +/- 2.11), respectively. With all comparable slice thicknesses, the robotic device was significantly more accurate compared to the low-cost device. Needle placement with the PCS resulted in lower intervention time (mean, 158.83 s vs. 225.67 s).

Conclusion: Although the robotic device provided more accurate results, both guidance systems showed acceptable results and may be helpful for interventions in difficult anatomical regions and for those requiring complex multi-angle trajectories.

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Monosodium Urate Crystal Deposition in Coronary Artery Plaque by 128-Slice Dual-Energy Computed Tomography: An Ex Vivo Phantom and In Vivo Study

Objective: Monosodium uric acid (MSU) crystals may accumulate in the coronary plaque. The objective was to assess whether dual-energy computed tomography (DECT) allows for detection of MSU in coronary plaque.

Methods: Patients were examined with 128-slice DECT applying a cardiac electrocardiogram-gated and peripheral extremity protocol. Patients were divided into 3 groups: gout (tophi >1 cm in peripheral joints), hyperuricemia (>6.5 mg/dL serum uric acid), and controls. The groups were matched for cardiovascular risk factors. Monosodium uric acid-positive (+) and calcified plaque were distinguished, and the coronary artery calcium score was calculated. Ex vivo phantom: MSU solutions were diluted in different NaCL solutions (5%/10%/15%/20%/25%). Coronary artery models with 2 different plaque types (MSU+ and calcified) were created.

Results: A total of 96 patients were included (37 with gout, 33 with hyperuricemia, and 26 controls). Monosodium uric acid-positive plaques were found more often in patients with gout as compared with controls (91.9% vs 0.38%; $P < 0.0001$), and the number of plaques was higher ($P < 0.0001$). Of 102 MSU+ plaques, 26.7% were only MSU+ and 74.2% were mixed MSU+/calcified. Monosodium uric acid-positive plaque had mean 232.3 Hounsfield units (range, 213-264). Coronary artery calcium score was higher in patients with gout as compared with controls (659.1 vs 112.4 Agatston score; $P < 0.001$). Patients with gout had more MSU+ plaques as compared with patients with hyperuricemia (91.6% vs 2.9%; $P < 0.0001$), and coronary artery calcium score was higher (659.1 vs 254 Agatston score; $P < 0.001$), but there was no difference between patients with hyperuricemia and controls. Ex vivo phantom study: MSU crystals were detected by DECT in solutions with a concentration of 15% or greater MSU and could be distinguished from calcified.

Conclusions: Coronary MSU+ plaques can be detected by DECT in patients with gout.

Funding:

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Flow-controlled ventilation versus pressure-controlled ventilation in thoracic surgery requiring one-lung ventilation – a randomized, controlled, single-center trial

Background and Goal of the Study

Flow-controlled ventilation (FCV) establishes a continuous gas flow during the whole ventilation cycle. Coupled with direct intra-tracheal pressure measurement precise determination of dynamic compliance is feasible and ventilator settings can be adjusted accordingly to achieve the highest dynamic compliance as a personalized approach. Aim of this randomized trial was to investigate the effect of compliance-guided FCV in terms of gas exchange compared to current standard pressure-controlled ventilation (PCV) during one-lung ventilation (OLV).

Materials and Methods

Overall 46 patients were randomized to receive FCV or PCV for the duration of general anaesthesia. FCV was established with compliance titrated end-expiratory pressure (PEEP) and peak pressure, flow adjusted to achieve normocapnia during total lung ventilation (TLV) and mild permissive hypercapnia during OLV. In the control group PCV was established with compliance titrated PEEP, peak pressure set to achieve a tidal volume of 6-8 ml/kg predicted body weight (PBW) during TLV and 4-6 ml/kg PBW during OLV, respiratory rate set to maintain normocapnia during TLV and mild permissive hypercapnia during OLV. The primary outcome parameter was defined as oxygenation (paO2/FiO2) at 30 minutes after OLV initiation.

Results and Discussion

43 patients were included into final analysis and the primary outcome parameter paO2/FiO2 was significantly higher in the FCV group (n=21) compared to control (n=22) (187 vs 136, MD 39 (95% CI 1 to 75); p=0.047) after 30 minutes of OLV (Timepoint T4). Additionally, the required respiratory minute volume (MV) to obtain similar paCO2 levels was significantly lower in FCV (3.0 vs 4.5, MD -1.3 (95% CI -1.9 to -0.8) l/min; p<0.001), which indicates improved CO2-removal.

Conclusion

In this randomized trial flow-controlled ventilation was found to be superior to current standard pressure-controlled ventilation after 30 minutes of OLV in terms of oxygenation and CO2-removal.

Funding:

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Controlled orientation and sustained rotation of biological samples in a sono-optical microfluidic device

3D cell culture models, as organoids and cell spheroids, has the potential to yield more accurate results in biomedical research compared to traditional 2D cell culture. These extended cell cluster models could bridge the gap to in-vivo models, as they incorporate 3D cell-cell interactions, which is crucial for cell response and more representative of tissues. 3D cell culture has become a valuable tool in oncology and development studies, and as they can be made from a patient's own cells, they bring us towards personalized medicine with the potential to speed up drug screening as well as reducing the need for animal models. Platforms for both assembly and non-invasive long-term monitoring of 3D cell models are of great interest. We have developed a sono-optical microfluidic device suitable for non-contact manipulation and imaging of such biological samples in liquid suspension. Combining optical tweezers with acoustic trapping in one platform allows us to trap and manipulate sub-millimeter sized biological samples in a contact-less and flexible manner. The acoustic radiation forces levitate and trap the sample and steerable holographic optical tweezers give us an additional means of manipulation. We have implemented 3D acoustic trapping, with three independent MHz transducers in three orthogonal directions; two side-transducers and one transparent top-transducer facilitating optical access for optical trapping and imaging. By tuning the relative strengths of the ultrasound transducers, we can transiently reorient the sample by means of the acoustic radiation torque, arising from pressure gradients. We can also induce sustained rotations of samples by means of the acoustic viscous torque, arising from the shear forces at the viscous boundary layer around the particle, or alternatively, in combination with the optical tweezers. This allows us to gain access to multiple viewing angles of the object. We also have adapted our 'sono-optical' trapping platform to support high-NA imaging.

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Label free tomographic imaging of biological samples in a sono-optical microfluidic device

Optical Diffraction Tomography is a powerful tool to retrieve the three-dimensional refractive index distribution of micrometer-sized objects in a label free manner. To gain access to multiple viewing angles for tomographic imaging, rotating the object or scanning the illumination angle is necessary. Both modalities complement each other: Illumination scanning allows to precisely control the viewing angle, but is inherently limited due to the limited NA of the optical microscope, leading to a lower resolution along the axial direction. Rotating the object enables access to more information about the object leading to isotropic resolution. In a sono-optical device, we can continuously rotate and reorient sub-millimeter sized particles. However, the motion undergone by the object in an acoustic trap is not known precisely. We extended a commercial microscope by an angle scanning system to combine both modalities, which allows us to scan the illumination angle, and rotate the object simultaneously. We believe this to be a promising avenue to infer the motion and structure of 3D cell culture models simultaneously and to resolve them isotropically. Classical Optical Diffraction Tomography is based on the single-scattering approximation, which is valid for single cells which scatter light only weakly. To overcome this limit we implemented more sophisticated propagation methods to model the light-matter interaction more accurately in a gradient based optimization framework with sparse regularization.

Funding:

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The role of Endosome and Golgi Associated Degradation (EGAD) in cellular quality control

To maintain cellular integrity, quality control networks detect and selectively degrade proteins that mis-fold, cannot integrate into protein complexes, fail to target to the correct organelles or are destined for degradation by regulatory mechanisms. In eukaryotic cells, three selective pathways are known to degrade membrane proteins. At the endoplasmic reticulum (ER), membrane proteins are ubiquitinated and retro-translocated into the cytoplasm for proteasomal degradation by the ER associated degradation (ERAD) pathway. Upon export from the ER, most ubiquitinated membrane proteins are sorted by the endosomal sorting complexes required for transport (ESCRT) into the lumen of lysosomes for degradation. We have recently identified in budding yeast, S. cerevisiae, a third pathway that selectively extracts membrane proteins at Golgi and endosomes for degradation by cytosolic proteasomes. One endogenous substrate of this endosome and Golgi - associated degradation pathway (EGAD) is the ER - resident membrane protein Orm2, a negative regulator of sphingolipid biosynthesis. Phosphorylation of Orm2 regulates ER export to Golgi and endosomes, where Orm2 is then poly-ubiquitinated by the membrane embedded 'Defective in SREBP cleavage' (Dsc) ubiquitin ligase complex. Ubiquitination of Orm2 and the function of the AAA-ATPase Cdc48/VCP are essential for its subsequent proteasomal degradation. This is an example for regulated degradation of an EGAD substrate protein.

During my PhD, I aim to delineate more generally how the EGAD pathway is embedded in cellular quality control networks. To address this question, I first conducted SAturated Transposon Analysis in Yeast (SATAY). These genetic screens identified several mutants in protein sorting pathways between the ER and the Golgi that required the EGAD pathway for survival. Further analysis indicated that in these mutants the EGAD pathway reduced the accumulation of membrane proteins and thereby helped to maintain the integrity and architecture of post-ER organelles. Currently I combine yeast genetics, quantitative proteomics, biochemical approaches, and live cell imaging to characterize on a mechanistic level how EGAD contributes to cellular quality control. The results of my research project will provide a detailed conceptual framework for the role of the EGAD pathway in eukaryotic post-ER quality control processes.

Funding:

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Siderophore uptake in Aspergillus fumigatus

Siderophore-mediated acquisition of iron has been shown to be indispensable for virulence of several fungal pathogens, the siderophore transporter Sit1 was found to mediate uptake of the novel antifungal drug VL-2397, and siderophores were shown to be useful as biomarker as well as for imaging of fungal infections. However, siderophore uptake in filamentous fungi is poorly characterized. The opportunistic human pathogen Aspergillus fumigatus possesses five putative siderophore transporters. Here, we demonstrate that the siderophore transporters Sit1 and Sit2 have overlapping as well as unique substrate specificities. With respect to ferrichrome-type siderophores, utilization of ferrirhodin and ferrirubin depended exclusively on Sit2, use of ferrichrome A depended mainly on Sit1, and utilization of ferrichrome, ferricrocin, and ferrichrysin was mediated by both transporters. Moreover, both Sit1 and Sit2 mediated use of the coprogen-type siderophores coprogen and coprogen B, while only Sit1 transported the bacterial ferrioxamine-type xenosiderophores ferrioxamines B, G and E. Neither Sit1 nor Sit2 were important for utilization of the endogenous siderophores fusarinine C and triacetylfusarinine C. Furthermore, A. fumigatus was found to lack utilization of the xenosiderophores schizokinen, basidiochrome, rhizoferrin, ornibactin, rhodotorulic acid and enterobactin. Taken together, this study characterized siderophore use by A. fumigatus and substrate characteristics of Sit1 and Sit2.

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Protein interactions and metabolic signaling at the lysosome

In recent years, the view of lysosomes as a cellular garbage disposal system has been extended by data underlying its importance in orchestrating cellular metabolism. Lysosomes are crucial for cell growth, proliferation, differentiation and cell-type specific processes, rendering proper lysosomal function indispensable for cellular homeostasis.

Lysosomes harbor a complex nutrient sensing machinery that integrates information about extra- and intracellular nutrient availability and activates corresponding signaling pathways, causing changes in the cell's metabolic program.

The LAMTOR [late endosomal/lysosomal adaptor and MAPK (mitogen-activated protein kinase) and mTOR (mechanistic target of Rapamycin) activator] complex plays a central role in these processes by recruiting and/or activating AMPK (AMP-activated protein kinase), MAPK and mTOR on the lysosomal surface.

In order to regulate these processes, LAMTOR associates with a number of partners including the Rag-GTPases, SLC38A9, the lysosomal v-ATPase, MEK, BORC, AXIN, LKB1, and many more.

We know that some of these associations are mutually exclusive, whereas others occur under the same physiological conditions. Nonetheless, how these interactions are regulated remains largely unclear.

The aim of this project was to extend the functional characterization of LAMTOR's associations to its many partners, with a special focus on the regulatory mechanisms defining the interplay between the different signaling cascades. In addition, this project included the analysis of known phosphorylation sites present in the N-terminus of LAMTOR1, both in terms of the triggered changes in the interactome of the complex, as well as signaling downstream of LAMTOR.

I found that phosphorylation of LAMTOR1 has a broad range of effects on cells, including the regulation of binding to the Rags and BORC, as well as regulation of the AMPK and MAPK signaling cascades.

Additionally, I established a so far unknown role of specific LAMTOR1 phosphorylation in the regulation of lysosomal size and distribution.

Using recombinant LAMTOR-complex, I could confirm in in-vitro phosphorylations followed by Mass-Spectrometry, that AMPK, MAPK and CDK2 phosphorylate LAMTOR1 at S63, S45, and S27, respectively. Present work focuses on determining the effects of the aforementioned phosphorylation-sites in-vivo.

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Exploring the role of ferroptosis in B cell leukemia

Ferroptosis is an iron-dependent cell death mode, genetically, biochemically and morphologically distinct from other forms of regulated cell death. Excessive peroxidation of polyunsaturated fatty acyl moieties prompts cellular membrane dysfunction, ultimately causing ferroptotic cell death. Upon accumulation of lipid reactive oxygen species (ROS), glutathione peroxidase 4 (GPX4)-mediated detoxification of lipid peroxides is a central mechanism to prevent ferroptosis. In the recent years, small molecule inhibitors of the GPX4 pathway have been developed with the aim to induce ferroptosis, both in vitro and in vivo. Thus, ferroptosis-inducing drugs may represent novel therapeutic options to treat malignancies, in particular cancers that have developed resistance to standard therapies which commonly induce apoptotic cell death.

To assess whether established tumor drivers originate differential ferroptosis sensitivity in established leukemia cells, we expressed the MYC or v-ABL oncoproteins or a constitutive active version of RAS, or deleted the p53 tumor suppressor gene in BaF3 leukemia B cells. To test whether acquired apoptosis resistance would impact ferroptosis susceptibility, we blocked mitochondrial apoptosis by overexpression of BCL2 or by combined deletion of BAX and BAK.

Our data settle that specifically MYC- and v-ABL-driven cellular reprogramming enhance mitochondrial fitness, reduce lipid ROS accumulation and foster the survival of a small fraction of cells in culture during pharmacological ferroptosis induction. Mechanistically, deregulation of the GPX4-driven detoxification system is not the cause for cellular persistence. Despite partially preventing ferroptotic cell death and lipid ROS accumulation, combined elimination of BAX and BAK did not foster the outgrowth of persisting cells upon ferroptosis induction.

Altogether, our data suggest that pharmacologic induction of ferroptosis may be a promising strategy for the treatment of therapy-resistant B cell leukemias. However, personalized therapy approaches will be required to select for patients that may benefit from such a strategy.

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Towards the structural basis of NDPK-C inhibition

The Nucleoside diphosphate kinase (NDPK) family comprises several members including NDPK-A, -B, and -C [1]. It has been shown that these member of the class I subfamily is particularly enriched at the plasma membrane of patients suffering from end-stage chronic heart failure (HF)[2].

NDPKs catalyze the transfer of the γ-phosphate from a nucleoside triphosphate donor, mainly ATP, to a nucleoside diphosphate acceptor via a ping-pong mechanism. NDPK isoforms oligomerize to trimers which can form hexamers with them self or other isoforms. NDPK C plays an essential role in the complex formation of NDPK isoforms with G-proteins and allow for their GPCR-independent activation. By switching its prevalence from Gαs to Gαi, NDPK-C may thus contribute to lower cAMP levels and the related contractile dysfunction in HF [2]. By screening a small molecule library, a novel compound was identified which binds to NDPK-C and inhibits its enzymatic activity. However, the composition of the binding for this inhibitor and the inhibiting mechanism remain to be detected.

We apply biophysical methods including X-ray crystallography in order to see with atomic resolution how NDPK-C activity can be suppressed by the recently identified inhibitor.

Funding:

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Mitochondrial dysfunction in ACM pathogenesis

Arrhythmogenic Cardiomyopathy (ACM) is a genetically transmitted heart disease characterized by the progressive loss of myocardium and its substitution with fibro-fatty tissue. Reactive Oxygen Species (ROS) are produced during normal mitochondrial activity as waste, but apart from their potentially dangerous effects at high concentrations they can act as signalling molecules for a number of metabolic pathways such as lipogenesis and fibrogenesis, the main hallmarks of ACM. Recently, in the process of ACM a link between and mitochondrial dysfunction has also been suggested.

Primary human ventricular cardiac stromal cells (CStC) from from patients and normal controls are being used as disease model. Ongoing experiments address the role of mitochondrial events (morphology, ROS production, OXPHOS) in the process of lipid accumulation and probe into a possible role of Wnt signaling involvement downstream of ROS.

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Ambient availability of amino acids, proteins, and iron impacts copper resistance of Aspergillus fumigatus

The transition metals iron and copper are required by virtually all organisms but are toxic in excess. Acquisition of both metals, as well as resistance to copper excess have previously been shown to be important for virulence of the most common airborne human mold pathogen, Aspergillus fumigatus. Here we demonstrate that ambient availability of amino acids and proteins increases copper resistance of A. fumigatus wild type and particularly of the ΔcrpA mutant that lacks export-mediated copper detoxification. The highest protecting activity was found for L-histidine followed by L-asparagine, L-aspartate, L-serine, L-threonine, and L-tyrosine. Other amino acids and proteins displayed also significant but lower protection. The protecting activity of non-proteinogenic D-histidine, L-histidine-mediated growth inhibition in the absence of high-affinity copper uptake, determination of cellular metal contents, and expression analysis of copper-regulated genes suggested that histidine inhibits low-affinity but not high-affinity copper acquisition by extracellular copper complexation. An increase of the cellular copper content was found to be accompanied by an increase of the iron content and, in agreement, iron starvation increased copper susceptibility, which underlines the importance of cellular metal balancing. Due to the role of iron and copper in nutritional immunity, these findings are likely to play a role in the host niche.

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Systematic identification of druggable PKA substrates involved in colon cancer progression

Cellular membrane receptors sense and convert the vast array of extracellular input signals and transmit information through tightly regulated intracellular kinase signaling circuits. Deregulation of G protein coupled receptor (GPCR) controlled kinase pathways contributes to the development and progression of cancer. Examples are activating mutations in the AC-stimulatory Gαs proteins (GNAS), which occur in 4,2% of all tumors. These lead to constitutive downstream activation of the cAMP-dependent protein kinase A (PKA) pathway. In order to identify druggable PKA-effector proteins, we determined 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 and selected two druggable and cancer-implicated candidates for closer examination, namely the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) and Tripartite motif 28 (TRIM28) respectively. PFKFB3 is a key modulator of glycolysis, implicated in maintaining cancer cell metabolism. We showed that nuclear PFKFB3 acts as a PKA substrate. Moreover, small molecule mediated inhibition of PFKFB3 reduced proliferation of Gαs-mutated colon cancer cells. Using PFKFB3 activity reporter (KinCon) we tracked kinase conformation alterations upon PKA activation and inhibition. Further, besides quantitative metabolite analyses of the cellular glycolytic flux, we revealed a nuclear function of PFKFB3. Using the RNAseq technology TUCseq we recorded immediate transcriptome changes upon PFKFB3 inhibition. Thus, we gained evidence for a possible link of PFKFB3 to p53 function in the studied colon cancer cell setting. TRIM28, the second novel PKA substrate, supports tumor progession through ubiquitination of the tumor suppressor p53. We investigated changes in protein stability of known anti-oncogenic TRIM28 ubiquitination substrates upon kinase activation. Currently, we explore a polypharmacology approach by inhibiting nuclear TRIM28 and PFKFB3 functions which may hamper proliferation of selected colon cancer cells.

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Epitope Masking by Polyphenols in the Major Apple Allergen Mal d 1

Polyphenols are known for their potential to reduce the allergenicity of food allergens. It was proposed in the literature that polyphenol binding changes the three-dimensional fold of proteins, therefore leading to the loss of conformational epitopes and reduced recognition of the antibody.

We investigated the effect of polyphenols on the apple allergen Mal d 1 and examined if the structure of the protein is affected by the binding of chlorogenic acid.

Mass spectrometry experiments revealed a cysteine residue on the surface of the protein to bind covalently to previously oxidised chlorogenic acid.

NMR spectra of Mal d 1 modified by chlorogenic acid showed that fold and stability of the protein were not significantly altered by covalent binding of chlorogenic acid, and the modification does not interfere with ligand binding to the internal pocket of the protein.

Interestingly, the modified cysteine residue lies near a presumed IgE binding epitope, with the bound polyphenol partially covering the epitope. This could hinder IgE binding and therefore lead to reduced allergenicity of the modified protein.

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Deciphering of CDK complex formation and kinase conformation dynamics

Kinase directed protein phosphorylation is frequently studied due to their involvement in a plethora of cellular processes like cell cycle control, protein synthesis, signal transduction and proliferation. Therefore, it is not surprising that deregulated kinase activities often occur in diseases such as cancer, multiple sclerosis, Psoriasis, Parkinson and Alzheimer. The search for drugs led to the identification of small molecules which target mutant kinase activities. To increase the efficacy of new drugs, it is necessary to learn more about the drug:kinase binding mode, its impact on the full-length kinase structure and the effect on cellular kinase complexes. The cyclin dependent kinases 4 and 6 (CDK4 and CDK6) are key regulators of the cell cycle. The CyclinD-CDK4/6 complex is responsible for the initial phosphorylation of the retinoblastoma protein (Rb) which leads to cell cycle progression and subsequently to cell division. Selective inhibition has emerged as a therapeutic strategy to counteract CDK4/6 upregulated activities. Currently, there are three different FDA-approved CDK4/6 specific inhibitors (CDK4/6i) in clinical use for breast cancer. Thus, knowledge about the effect of CDK4/6i on CDK complexes is critical to determine drug efficacies and unveil off-target effects. First, we used a PPI reporter system to investigate CDK4/6 interaction dynamics with Cyclin D and the CDK inhibitor proteins p16INK4a. We show that mutations in CDKs and in p16INK4a lead to decreased complex formation. Furthermore, the same reporter system was utilized to track modes of CDK4 and CDK6 PPI dynamics. Second, our previously established Kinase Conformation (KinCon) reporter was used determine conformation changes of the full-length kinases upon exposure to CDK4/6i. So far, we were not able to track significant conformational changes upon inhibitor treatment using the wild-type and mutated KinCon reporters. Third, we applied the same reporter system to quantify changes of the thermal stability of proteins upon CDK4/6i binding. For these experiments we established a variation of the Cellular Thermal Shift Assay (CETSA) that uses bioluminescence as a readout. Indeed, we were able to show that inhibitor binding thermally stabilizes the kinases. In the future we aim to investigate combinations of patient mutations and PPI of CDK4/6 in the presence of a third interaction partner.

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Cell-penetrating peptide decorated charge converting nanocarriers: Key to overcome the polycation dilemma

In this study, nanostructured lipid carriers (NLC) were decorated with a polycationic cell-penetrating peptide (CPP) and coated with polyphosphates for charge conversion. These charge-converting nanoparticles combine the benefits of negatively charged nanocarriers that overcome the mucus barrier with the advantages of positively charged nanocarriers exhibiting high cellular uptake capacity after cleavage of polyphosphates by the cell-membrane bound intestinal alkaline phosphatase (IAP). The CPP stearyl-nona-L-arginine (R9SA) was generated by solid-phase synthesis. The polycationic R9SA-NLC were formed by solvent diffusion method and coated with polyphosphates. The nanocarriers were investigated for size, polydispersity index, zeta potential and charge conversion in presence of IAP. Phosphate release after polyphosphate cleavage was determined after incubation with IAP and on Caco-2 cells. Cytocompatibility and cellular uptake were evaluated on Caco-2 cells. In addition, nanocarriers were characterized for their endosomal escape properties using erythrocytes. NLC were formed in a size range between 146 nm and 152 nm and a polydispersity index of ~0.2. R9SA-NLC showed a high positive zeta potential of 43.2 mV. Incubation of polyphosphate (PP) coated PP-R9SA-NLC with IAP exhibit a charge conversion from -41.8 mV to 6.4 mV (Δ48.2 mV). After four hours of incubation with IAP, phosphate release reached a plateau, demonstrating a faster polyphosphate cleavage than on Caco-2 cells incubated for the same time. Nanocarriers indicated cytocompatibility over 24 hours. Cellular uptake of R9SA-NLC and PP-R9SA-NLC was enhanced 15.6- and 13.2-fold, respectively, compared to control NLC. The erythrocytes interaction study showed increased endosomal escape properties for R9SA-NLC and for PP-R9SA-NLC when incubated with IAP. The polyphosphate coating of CPP-decorated NLC led to a promising design of a charge-converting drug delivery system that can overcome the polycation dilemma.

Funding:

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Impact of kinase activation and inactivation on mitochondrial functions

Mitochondria are a highly evolved system for coordinating energy production and distribution. Changes in the parameters of the mitochondrion can impact a variety of biosynthetic pathways and may lead to cell proliferation. Mitochondrial dysfunction has been linked to diseases, as defective mitochondria have been implicated in neurodegenerative disorders. In cancer, the mitochondrial metabolism is highly activated to supply energy for new building blocks of tumor cells. Using an array of different techniques we are characterizing the mitochondrial respiratory properties of distinct cancer cell lines of purified live mitochondria.

With HRR real time analysis of oxygen consumption and oxygen flux was performed. We combined kinase inhibition experiments, to inhibit specific kinases and to analyze the impact of inhibition on mitochondrial respiration states with mitochondrial purification experiments. First, we show that differences in media composition (e.g glucose content) has an influence on mitochondrial function of cancer cells. Second, we show enrichment of the PINK1 kinase in purified mitochondria. PINK1 is a kinase that controls mitochondrial activity and integrity. It is upstream of Parkin, a protein which is directly involved in Parkinson's disease. Using the KinCon biosensor platform we have generated a PINK1 reporter to screen for possible modulators of PD mutated PINK1. Third, we show that general kinase inhibitors has an impact on isolated mitochondria.

Moreover, we utilized the KinCon reporter platform to generate pseudokinase conformation reporters. We are analyzing the complex formation of of STRADα, a LKB1 kinase activity enhancing pseudokinase. It has been shown that LKB1 activation is not mediated by phosphorylation but rather by complex formation of the trimer. Since loss of function mutations in LKB1 have been linked to disease, we are investigating mutations in two complex binding partners. Thus we set up a kinase conformation reporter system for LKB1, and STRADα on their impact on the conformation of the corresponding proteins and thereby their binding ability. We show that upon complex formation of the proteins the conformation of LKB1 and STRADα changes. Currently we validate the impact of the conformation on downstream signaling and perform proliferation studies.

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The membrane activity of a Temporin B peptide analog enables rapid cell killing in C. albicans

Temporin B (TB) is a 13 amino acid long, cationic peptide secreted by the granular glands of the European frog Rana temporaria. We could recently show that the modified TB analog TB_KKG6K inhibited the growth of the human opportunistic pathogenic yeast C. albicans at low micromolar concentrations. It was effective against sessile and planktonic C. albicans, acting rapidly in a fungicidal manner, and exhibited neither hemolytic nor cytotoxic activity in mammalian cells in vitro. The current study aimed to shed insights into its mechanism of action, with a focus on its yeast cell membrane activity. Taking advantage of different fluorescent dye-based techniques, we could prove that it strongly interacts with the C. albicans cell membrane. The evaluation of the transmembrane electrical potential with 3,3'-dipropylthiacarbocyanine iodide [DiSC3(5)] showed that this peptide undergoes electrostatic interactions with the cell membrane, inducing its depolarization. Fluorescence microscopy revealed that it rapidly diffuses into C. albicans cells, and its ability to permeabilize the cell membrane during cellular uptake was confirmed by the entry and subsequent fluorescence of Sytox Green, a compound unable to permeate live cells with intact cell membranes. Furthermore, studies on model membrane systems using artificial lipid vesicles with different lipid compositions containing the fluorescent dyes 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt (ANTS) and p-xylene-bis-pyridinium bromide (DPX) revealed that the TB_KKG6K has a preference towards anionic lipid constituents and induces membrane disruption and subsequent ANTS/DPX leakage in vesicles composed of negatively charged phospholipids. Taken together, these results prove that the TB_KKG6K is membrane active and disrupts C. albicans membrane integrity, explaining the previously observed rapid, fungicidal mode of action. Its membrane activity also has important implications in the context of fungal resistance development, as membrane active molecules are hypothesized to hamper this process. In conclusion, the TB_KKG6K has great potential as a future anti-Candida therapeutic.

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Impact of receptor signalling pathways on GTPase and kinase interactions and conformations

The Mitogen-Activated-Protein-Kinase (MAPK) pathway is a major regulator of growth-factor induced proliferation. Central players of this pathway are the proteins RAS, RAF, and MEK. These signaling molecules are frequently mutated and constitutively activated in a large variety of cancers, rendering them central targets for pharmaceutical intervention. The search for bioactive small molecule inhibitors for deregulated RAS remains challenging. In contrast, for RAF and MEK several inhibitors found the way into the clinic. Nevertheless, further functional studies on the molecular details of kinase functions are needed to cope with the onset of drug resistance. We recently developed an intramolecular protein conformation reporter system, in order to track conformation changes of the kinases RAF and MEK (=KinCon) and interactions with RAS variants. In addition, we subjected our kinase reporter platform to perturbation studies of wt and mutated MEK-KinCon reporter. Upon drug exposure we tracked dynamic structure states of the MEK1-KinCon reporter. More importantly, our data indicates that the MEK activation state is crucial for MEK:inhibitor binding. We extended this working hypothesis to RAF kinase dynamics. In the presence of permanently active HRAS-G12V we demonstrated an increase of wt BRAF activation upon BRAF inhibitor treatment. Overall, our data shows that the kinase conformation reporter system, originally established for BRAF, can be extended to the measurement of MEK1 dynamics and MEK1:drug interactions. Finally, we hope to correlate the respective enzyme activities with alternating protein-protein interactions of the full-length MEK1 and RAF proteins. Looking to the future, we plan to extend our reporter system also to other kinases.

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THE ROLE OF MELANOCYTES IN SKIN PIGMENTATION, SENESCENCE, AND SKIN AGING INDUCED BY EXPOSURE TO ENVIRONMENTAL STRESSORS

Introduction: Extrinsic aging of human skin is mainly a result of exposition to environmental factors such as sunlight, air pollution, and cigarette smoke. Melanocytes become less active and senescent with aging and environmental factors can lead to premature aging and pigmentation disorders. However, the mechanisms involved under exposition of melanocytes to environmental stressors are not fully understood.

Material & Methods: We treat melanocytes with UV (UVA+UVB) or urban particulate matter (UPM) or a combination of these two stressors (UV+UPM), in order to understand how these environmental stressors affect melanocytes, but also the skin as a whole by ex vivo experiments. To investigate morphological and physiological parameters we examined proliferation, senescence status, apoptosis, pigmentation, and DNA damage.

Results: Preliminary results using UV or UPM alone as well as the combination of both stresses have demonstrated that melanocytes respond diversely to each different type of stress in terms of senescence markers, cell survival and pigmentation. Accordingly, all three types of treatments induced different changes in ex vivo skin biopsies, indicating that an understanding of the underlying molecular processes activated in response to these treatments are vital to estimate the impact of exposure to such environmental factors on the progression of skin aging and health in general.

Conclusion: Taken together, this new experimental setup will allow us to perform further research on mechanisms of extrinsic skin aging, including the role of melanocytes in this process and could give rise to the development of new therapeutical targets for pigmentation disorders and premature skin aging.

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Establishing a differentiation protocol that produces in-vitro beta-like cells

The directed differentiation of human pluripotent stem cells into functional beta-like cells that produce insulin is of great interest as a replacement therapy for diabetes patients. Moreover, such approaches offers unique options to elucidate molecular regulators of beta cell development and function. In order to study activities of conserved Diabetes associated genes, our lab recently started establishing a protocol for efficient in-vitro

beta-cell differentiation (in collaborate with Prof. Matthias Hebrok from UCSF). This protocol is optimized to the human stem cell line MEL-INSGFP/w, in which an INS:GFP reporter system allows separation of beta-cells simply by FACS .

So far, procedures have been established to progress MEL-INSGFP/w into GFP-positive cells beta-cell precursors and to monitor differentiation progress and efficiency by using flow cytometry and immunofluorescence staining. Further, we have developed an optimized procedure for efficient CRISPR/CAS9 based genome editing in MEL-INSGFP/w cells.

Having implemented this protocol, we currently opt for optimisation and increasing the yields of the subsequent stages of the differentiation as weil as to account for possible intra-cell line specific diversions. Ultimately, this protocol allows us to generate data on human specific pancreas development and offers the possibility to investigate potential genes of interest related to beta-cell formation. Recent results including cultivation methods, stage specific immunofluorescence, FACS data and a primary characterization of novel knockout and knock-in reporter lines for the neonatal Diabetes gene MNX1 will be presented.

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Changes in Systemic Levels of Vascular Endothelial Growth Factor After Intravitreal Injection of Aflibercept or Brolucizumab for Neovascular Age-Related Macular Degeneration.

Purpose

To analyze and compare the effects of intravitreal brolucizumab vs. aflibercept on systemic vascular endothelial growth factor (VEGF)-A levels in patients with neovascular age-related macular degeneration.

Methods

In this prospective interventional case series study, brolucizumab (6.0 mg/50 µL) or aflibercept (2.0 mg/50 µL) was injected intravitreally in 30 patients each. Blood samples were drawn at baseline and 7 and 28 days after the first injection. Systemic VEGF-A levels were measured using enzyme-linked immunosorbent assay. Thirty healthy individuals served as controls.

Results

The median baseline systemic VEGF-A levels in the brolucizumab, aflibercept, and control groups were 10.8 (8.0-13.2), 12.0 (8.0-18.5), and 10.0 (8.0-15.1) pg/ml, respectively ($p=0.315$). In the brolucizumab group, VEGF-A levels significantly decreased to 8.0 (8.0-11.5) pg/ml on day 7 ($p=0.0254$) and to 8.0 (8.0-8.0) pg/ml on day 28 ($p<0.001$). In the aflibercept group, VEGF-A levels significantly decreased to 8.0 (8.0-8.0) pg/ml on day 7 ($p<0.001$) but returned to baseline level, 12.5 (8.5-14.6) pg/ml, on day 28 ($p=0.120$). VEGF-A levels were significantly different between the treatment groups after 28 days ($p<0.001$).

Conclusion

Intravitreal brolucizumab resulted in a sustained reduction of systemic VEGF-A levels until 28 days post-treatment, which raises concerns regarding its safety and long-term effects.

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Characterization of weight-loss target genes in human adipose stem/progenitor cells

Human adipose stem/progenitor cells (hASCs) are crucial for adipose tissue homeostasis and regeneration. A better understanding of these cells contributes to elucidate the role of adipose tissue in metabolic diseases as obesity, and ageing. The most impressive anti-ageing intervention known so far in animal models is caloric restriction (CR) performed without malnutrition. Beneficial effects of CR in normal weight people and of weight loss (WL) interventions in obese humans have also been shown. These interventions lead to a reprogramming of adipose tissue physiology, increased health span and postponement of the onset of age-related diseases. The effects of WL on adipose tissue are so far mainly studied in adipocytes; however, its impact on ASCs is less well understood. Therefore, to better understand WL effects on ASCs, a microarray analysis was performed by our lab comparing global gene expression pattern in ASCs of formerly obese-, normal-weight and obese donors. This screening brings out the gene Sushi Domain Containing 2 (SUSD2) to be upregulated after WL in ASCs from formerly obese people. The aim of this project is to better characterize the role of SUSD2 in ASCs in loss- and gain-of-function experiments.

Funding:

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Infrared thermography as a non-destructive tool for the diagnosis of seed viability

Seed viability is the ability of a seed to survive a period of quiescence, and upon water uptake, germinate and grow into a seedling. Seed viability is affected by environmental factors during development on the mother plant. In seed banks, orthodox (i.e., desiccation tolerant) seeds can be stored long-term in order to preserve plant genetic resources. Highly viable and high-quality seeds are key to agriculture and food production. However, seeds inevitably lose viability over time, impacting negatively on their agricultural use and in some cases, directly on human health. In this PhD project, we aim to further advance a previously reported method for the noninvasive diagnosis of seed viability through infrared thermography (IRT). We report on a comparison of non-aged control seeds, dead seed and seed lots with 50% total germination (TG), subjected to controlled deterioration. Thermal fingerprints of viable seeds and dead seeds are being compared, to separate seeds of different viability prior to germination. As a marker of ageing, the glutathione half-cell reduction potential has been used. Glutathione is the major low-molecular-weight (LMW) antioxidant in orthodox seeds conferring protection from oxidative damage during storage. The study provides further insights into the biochemical mechanisms of seed ageing, linking invasive and non-invasive diagnosis of seed viability.

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Progerin-mediated ageing in human brain cerebral organoids reveals specific patterns of DNA damage

Nowadays it is possible to model numerous features of human diseases by induced pluripotent stem cells (iPSCs), however, the recapitulation of ageing in vitro has several limitations. This is mainly due to the reprogramming process where reprogrammed cells lose age-related signatures. Recently, it has been shown that cells overexpressing Progerin, a protein associated with the Hutchinson-Gilford progeria syndrome, show diverse hallmarks of ageing. However, the ability to induce ageing in complex 3D organoid models remained to be investigated.

In this study, we have generated iPSCs that overexpress Progerin in a doxycycline (dox)-inducible manner and used them to establish a 3D human model of aged cerebral organoids. We show that GFP-T2A-Progerin transgenic iPSCs can give rise to brain organoids that look morphologically homogeneous. Administration of dox results in widespread expression of GFP in d60 and d90 organoids (up to 90% of the cells as judged by flow cytometry), whereas vehicle-treated cells remained GFP-negative. Interestingly, GFP expression was even detectable after long-term culture (up to d90) indicative of resistance toward transgene silencing. Immunostaining revealed that the majority of GFP-positive cells express Progerin. Progerin expression gives rise to various well-established hallmarks of ageing. We detected a strong reduction of heterochromatin as marked by H3K9me3 immunostaining. Moreover, at the level of age-induced DNA damage, we observed a marked increase in dsDNA breaks (γH2Ax) and oxidative damage as shown by 8oxoG staining. Notably, γH2Ax signals are highly abundant in the neural precursor regions, whereas 8oxoG appears more evident in the mature neuronal regions of the organoids.

We plan to comprehensively characterize the ageing phenotype of our aged brain model to understand the impact of ageing on mitochondrial stress, DNA methylation status, and transcriptome. Finally, the Progerin system will be employed in patient-specific iPSCs to study the contribution of ageing to the pathophysiology of Parkinson's disease.

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Regulatory role of Mnx1 in beta-cell differentiation and maturation

The neonatal Diabetes factor Mnx1 is a key regulator of fate-determination and –maintenance of insulin producing beta-cells. Despite its essential role in beta-cell formation, Mnx1 function at molecular levels is still widely unknown.

To gain a better understanding of Mnx1 function we generated different mnx1-mutant and -transgenic zebrafish lines and started exploring molecular activities by a combination of FACS, RNA-sequencing and detailed expression approaches.

Our studies revealed the presence of a previously missed delta-cell sub-population in zebrafish and they showed that in mnx1 mutants the majority of beta-cell-precursors trans-differentiate into this novel delta-like cell type. In addition we found that adult mnx1 mutants display a severe increase in islet cell mass with ins+/sst1.1 bihormonal cells being the dominating cell type. Importantly, mnx1-mutants display massive hyperglycemia and therefor the ectopic cell mass likely reflects secondary consequences of cellular mechanisms that aim to compensate the lack of insulin. Whether the ectopic cells are only generated by trans-differentiation or in addition by proliferation of already differentiated cells is center of currently ongoing experiments.

Our analyses also revealed an unexpected difference in pancreatic phenotypes between a mutant with an ATG-covering 31-bp deletion in exon 1 as compared to the a more severe homeodomain lacking mutant. Our ongoing studies provide strong evidence for expression of a shorter Mnx1 variant in the ATG-mutants by using the second ATG for translation initiation. The data suggests that two different Mnx1 protein variants with slightly different functions are expressed in zebrafish.

Taken together, our data demonstrate that Mnx1 activities are much more conserved as expected and underlies the crucial role of Mnx1 in beta-cell fate decision and maturation.

Funding: FWF

Melanie Zott, Sonja Töchterle, Dirk Meyer

Utilizing CRISPR guided cytidine deaminase for PKM isoform switching in AD patient-derived iNs

Alzheimer's disease (AD) is a neurodegenerative disease that mostly affects the elderly. Despite over a century of research, fundamental knowledge about disease mechanisms causing AD is lacking. We study the underlying mechanisms in induced neurons (iNs), directly converted from patient-derived fibroblasts. Bypassing the pluripotent state, iNs retain age-related signatures. We previously showed that AD iNs exhibit a metabolic switch comparable to the Warburg effect in cancer. In essence, AD neurons switch from oxidative phosphorylation to aerobic glycolysis despite the presence of oxygen and functional mitochondria. Our data suggests that this phenotype is driven by an isoform switch of pyruvate kinase M (PKM) from PKM1 to the cancer-associated PKM2 isoform. While PKM1 expression is essential for maintaining energy production through oxidative phosphorylation in post-mitotic neurons, PKM2 drives energy production via aerobic glycolysis in proliferative cells. The isoform switch to PKM2 is associated with the fate-loss phenotype in AD iNs, which results in the re-gain of apoptosis competence. In this project, we aim to revert the AD-related phenotype by inducing the PKM isoform switch towards PKM2 using regularly interspaced short palindromic repeats (CRISPR) based cytidine deamination. By supporting their metabolic identity, we hope to make the neurons more robust against AD associated neurotoxic changes.

Funding:

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Role of Myc associating factor x (MAX) in Neoblast maintenance in flatworms

Myc is a widely studied transcription factor of the family basic helix loop oncoprotein, its upregulation occurs when it dimerizes with Myc associating factor x (Max). The downstream effect in normal cells includes cell proliferation as well as prevention of cell differentiation. However, in flatworms Myc is conspicuously missing.

Flatworms are known for their powerful regenerative capacity and their pluripotent stem cells(neoblasts), but this takes place in absence of the key driving protein Myc. Here, we want to understand the role of the understudied Max and whether it is involved in neoblast proliferation and the regenerative activity in absence of its usual binding partner Myc.

Using the small flatworm Macrostomum lignano as an animal model due to its ease to handle and rear, we have successfully synthesized the complementary DNA. From this, we are focusing on sequencing the gene and later performing Max knockdown. Based on the outcome, we will expand the scope and try and understand the binding partners which replace Myc.

From the flatworm transcriptomic data, we have already assembled, Myc was not identified, but Max was; in the case of Max, it only looks like a normal Max in some catenulids, but not in rhabditophorans, where it deviates from the norm.

Funding:

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Hmx gene conservation identifies the evolutionary origin of vertebrate cranial ganglia

Vertebrates perceive external stimuli through sensory systems serviced by cranial sensory ganglia (CSG), whose neurons predominantly arise from cranial placodes; however, understanding the evolutionary origin of placodes and CSGs is hampered by the gulf between living lineages and difficulty in assigning homology between cell types and structures. We used the Hmx gene family to address this question showing that Hmx is a constitutive component of vertebrate CSG development. Through differential RNA seq and gain and loss of function we also showed that Hmx in the tunicate Ciona is necessary and sufficient to drive the differentiation program of Bipolar Tail Neurons (BTNs), cells previously thought neural crest homologs. Using Ciona and lamprey transgenesis we demonstrate that a unique, tandemly duplicated enhancer pair regulate Hmx in the stem-vertebrate lineage. Strikingly, we also show robust vertebrate Hmx enhancer function in Ciona, demonstrating that deep conservation of the upstream regulatory network spans the evolutionary origin of vertebrates. These experiments demonstrate regulatory and functional conservation between Ciona and vertebrate Hmx, and point to BTNs as CSG homologs.

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Multi-omics-driven investigation to determine factors governing fate-determination of induced human neurons

Recent developments in the realm of multi-omic data integration have allowed for an improved insight and comprehension into the pathogenic mechanisms of diseases. Indeed, concomitantly considering distinct data types has been shown to improve the capacity to reliably identify molecular disease signatures. While Alzheimer's disease (AD) has a distinguishable molecular signature considering an induced neuronal cell model, the patient fibroblasts from which the induced neuronal cells are derived neglect to possess any signature (and if so, to a negligible degree). Thus, this project seeks to disentangle the molecular signature in AD patient fibroblasts using a Bayesian probabilistic framework for unsupervised integration of multi-omics data sets. Specifically, transcriptomic, methylation, chromatin accessibility and metabolomic data are analyzed to provide multi-level insight into the variability between AD patient and control fibroblasts.

Funding:

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Microcarrier-based 3D cultures of age-equivalent human induced neurons for aging and neurodegenerative disease research

Progressive aging is a central driver for the development of neurodegenerative diseases, such as sporadic Alzheimer's disease (AD). Despite extensive research, many mechanistic links between aging and disease remain incompletely understood, and new model systems that account for human aging as a factor are desirable. Direct conversion of human fibroblasts to induced neurons (iNs) has become an established technique in aging research, as iNs preserve donor-specific epigenetic features and other cellular hallmarks of aging. While patient-specific iN models have provided unique insights into the interface between aging and disease, more authentic three-dimensional (3D) models that include neuron-glia interactions remain desirable. Stem cell-derived 3D cerebral organoids have been extensively explored in the recent years but have been shown to be largely rejuvenated and merely reflect fetal stages of human brain development. Here, we have set out to establish an age-equivalent multicellular model for the adult brain based on patient-specific iNs. For this, we have tested different synthetic 3D microcarriers as culture substrates because, unlike for organoids, the adult-like postmitotic state of iNs does not allow for self-organization via progenitor cell proliferation and differentiation. We have identified a type of specific custom microcarrier that serve as highly suitable substrates for the attachment of old donor-derived iNs and enable prolonged iN cultures in combination with astrocytes and other glial cells. Our data indicates that combining the benefits of 3D cultures with the aging factor results in a reliable, stable, and more complex model for the aging human brain in the dish.

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Characterization of human-specific regulators of neurodevelopment

Subtle genomic (1.2%) and epigenomic (3.5%) differences between humans and non-human-primates (NHP) are enough to translate into remarkable cognitive differences. Human brains have higher complexity, connectivity and proliferative capacities that facilitate neocortical expansion. Nonetheless, the study of the genetic modulation behind these differences has been challenged by ethical, legal, and methodological limitations.

This project attempts to surpass these limitations by using state-of-the-art technologies like neural stem cells (NSCs) derived from human induced pluripotent stem cells (hiPSCs), gene editing, and single cell RNA sequencing. Specifically, the aim is to characterize the function of human-specific genes responsible for the proliferation and differentiation of hNSCs. For example, among the human-specific genes replicated in the literature, we found ANKRD20A2, ARHGAP11B, and NOTCH2NL. The last one been associated with the expansion and neuronal output of cortical progenitors though Delta/Notch regulation. Not surprisingly, some of the genes of interest are associated with aging and neurodevelopmental conditions like autism spectrum disorder and Schizophrenia. Candidate genes like these will be knocked down in hNSCs with Clustered Regularly Interspaced Short Palindromic Repeats interference (CRISPRi), a gene editing variation that represses gene expression with the binding of dCas9-KRAB to the Transcription Start Site of the gene targeted. As a proof of principle, dCas9-KRAB hiPSCs were transduced with gRNAs targeting SOX2, OCT4, and ARHGAP11B, leading to an interruption of proliferation, and mesodermal differentiation.

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The findings of this project will not only contribute to unravel the mechanisms behind the specializations of the human brain, but can have implications in disease modelling, regenerative medicine, and aging processes.

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HLA-DR+ CD8+ regulatory T cells inhibit T cell proliferation via direct cell-to-cell contact and are more sensitive for cell death

Regulatory T cells (Tregs) prevent excessive immune reactions by modulating T cells and other immune cells and thereby maintain tolerance to self-antigens and regulate immune responses to pathogens. Different T cell subsets are known for holding regulatory capacities, such as producing anti-inflammatory cytokines or inhibiting the proliferation via checkpoint inhibitory molecules. Among these Tregs is a newly described HLA-DR expressing CD8+ T cell population, which suppresses T cell proliferation. Co-cultivation experiments with peripheral blood mononuclear cells (PBMC) and sorted CD8+ HLA-DR+ Tregs demonstrated that the suppressive capacity is cell-to-cell contact dependent and requires checkpoint inhibitory molecule signalling, for example via CTLA-4. As seen for some CD4+ Treg populations, CD8+ HLA-DR+ Treg increase in numbers with aging, while losing suppressive capacity and checkpoint inhibitory molecules. In co-cultures of PBMCs and CD8+ HLA-DR+ Treg, apoptosis is induced in CD8+ T cells to a greater extent than in other cell types, particularly in young adults. Interestingly, the CD8+ HLA-DR+ Tregs themselves are also highly susceptible to apoptosis, more than their HLA-DR- counterparts and CD4+ T cells.

However, little is known about the exact mechanisms and interactions of the cell types involved. Therefore, we extended the co-culture experiments from PBMCs towards isolated T cells. As isolated T cells do not sufficiently respond to the classical stimulation via anti-CD3 and anti-CD28 antibodies, we utilized a new stimulation protocol named TransAct, which mimics antigen-presenting cells. This induces similar proliferation levels in isolated T cells as seen in T cells within PBMC cultures via anti-CD3 and anti-CD28 stimulation. This proliferation system is now tested in suppression assays to investigate the CD8+ HLA-DR+ T cell regulation network in more detail. We hypothesize that HLA-DR+ CD8+ Tregs do not mediate their suppressive activity directly towards T cells, but need a third yet still unknown cell type for their function.

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Characterizing the m5C-dependent RNA-protein interactome

Background: The list of RNA Binding Proteins (RBPs) that affect the fate and function of cells has been growing since the development of RNA interactome capture (RIC) and other physicochemical methods (e.g. XRNAX, OOPS, PTex). The modification of bases and/or ribose in RNA, specifically in mRNA, contributes to that proteome portrait. However, little is known about the reader proteins of specific RNA modifications. According to CLIPdb, several RBPs overlap with identified m5Cs. Our aim is to identify and characterize readers of the 5-methylcytosine (m5C) modification.

Approach: We perform differential analysis of RNA-protein complexes isolated from wild-type mouse embryonic stem cells (ESCs) and ESCs mutant for the mRNA cytosine methyltransferases Nsun2, Nsun6, and DNMT2 (3KO). To this end, we use in vivo UV crosslinking of RNA-protein complexes followed by TRIzol extraction. Complexes are purified from interphase and mRNA is enriched before mass spectrometry analysis of RNA-associated proteins. For quantification, we either use SILAC metabolic labeling or iTRAQ postextraction labeling.

Results: A preliminary proteome analysis confirmed the suitability of the method for the enrichment of mRNA-associated proteins. First comparative data suggest distinct differences in the RNA-interactome of wildtype and 3KO cells.

Funding: FWF

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Genomic and phenotypic analysis of Linezolid-resistant Staphylococcus epidermidis in a tertiary hospital in Innsbruck, Austria

In recent years, whole genome sequencing has become a useful and WHO-recommended tool for monitoring the spread and dynamics of resistance mechanisms in Gram-positive bacteria. We investigated the genetic resistance mechanisms and phenotypes of linezolid-resistant Staphylococcus epidermidis (LRSE) recovered from a cohort of patients receiving or not receiving linezolid in our tertiary hospital in Innsbruck, Austria

LRSE isolates (n=129) and linezolid-susceptible Staphylococcus epidermidis (LSSE) isolates (n=17) from our biobank with known EUCAST minimal inhibitory concentration (MIC) breakpoints were genetically and phenotypically analyzed for resistance mechanisms, sequence type, level of resistance, and influence of linezolid administration to the patient.

The most common resistance mechanism was identified as the point mutation G2603T in the 23S rRNA (n=91), followed by the presence of plasmid-derived cfr (n=30). Antimicrobial resistance markers were not found in susceptible isolates (n=17). The majority of LRSE isolates were ST2 strains, followed by ST5 strains, according to multilocus sequence typing. The overall level of linezolid resistance in LRSE isolates was high, with the majority of isolates having a MIC of 256 mg/L (n=84). Furthermore, we found that when the cfr gene was present, the level of resistance was significantly higher. Linezolid use up to one year before pathogen isolation had no effect on the resistance mechanism.

Within our hospital, we found that a specific point mutation of the 23S rRNA is the main linezolid resistance mechanism of Staphylococcus epidermidis. Furthermore, the study found that isolates with the cfr gene had higher levels of linezolid resistance. Monitoring plasmid-derived cfr in LRSE could be a critical step in reducing linezolid resistance and its spread to more pathogenic Gram-positive bacteria.

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Generating auxotrophic mutants in Trichoderma atroviride mediated by preassembled CRISPR/Cas9 ribonucleoprotein complexes

Trichoderma atroviride is a filamentous soil fungus that is a well-known mycoparasite applied for protecting plants against fungal pathogens (biocontrol agent). To facilitate genetic manipulation in this species, like multiple gene deletions and retransformations, expanding the genetic toolbox is indispensable to understand the genes involved in this mycoparasitic lifestyle. Here we describe the generation of mutants with an auxotrophic marker in this species using in vitro assembled CRISPR/Cas9 ribonucleoprotein (RNP) complexes, which were then transformed. The gen pyr4, encoding the essential OMP decarboxylase involved in the pyrimidine biosynthetic pathway, was targeted and disrupted. Mutants showed phenotypically resistance to 5-fluorotic acid and no growth in the absence of uridine and uracil. Sequencing confirmed insertions and deletions (INDELs) precisely at the targeted pyr4 gene locus induced by Cas9 generated double-strand breaks. For confirmation, that auxotrophy was due to a truncation in the pyr4 locus a functional pyr4 gene from a related fungus was retransformed, turning mutants back to prototrophy. Thus, the uracil auxotrophic mutants can be used as a genetic tool for gene replacement. This is one of the first reports using preassembled CRISPR/Cas9 ribonucleoprotein complexes in the fungal species T. atroviride for gene manipulation without the use of laborious cloning techniques.

Funding:

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Neurotrophins in developing human inner ear

Today, around 180 genes and factors are known to be involved in non-syndromic sensory human hearing loss. One this factors are Neurotrophins that are play a role in regulation of survival, development, function and plasticity of neurons. The mammalian neurotrophin BDNF, NT-3 and its associated factors play a role in supporting of afferent sensory neurons, innervation, survival and sprouting. The known receptors for BDNF and NT-3 are tropomyosin related kinase (Trk). The family of Trk receptors include the ligands TrkA (NTRK1), TrkB (NTRK2), TrkC and p75 neurotrophin receptor (p75NTR) witch interact specific with BDNF or NGF proteins. A lack in synthesis or secretion of neurotrophins cause in a reduced development of spiral ganglion neurons during gestation or a complete degeneration during adulthood. This loss of spiral ganglion neurons are associated with severe deafness syndromes.

Our laboratory showed that BDNF expression in the humen fetus inner ear is decreasing from GW09 to GW12, without a spatio-temporal gradient from apical to basal turn. For TrkB and TrkC it was possible to examined that the expression from GW10 onwards to GW12 with a later upregulation in sensory epithelium.

In this study, BDNF and its associated factors will be analysed on development human inner ear tissue between gestational weeks 12 to 19. We will determine the protein and RNA expression level with immunohistochemical (DAP-MAP) and fluorescence (FISH) staining, real time PCR, InSitu hybridisation and Next-Generation Sequencing.

The aim of this research is to identify the gene expression pattern of brain-derived neurotrophic factor (BDNF) and its associated receptors in the gestational weeks 12 to 19 of human foetal inner ear. To understand the initiation of hair cell formation, completion of organ of Corti formation, beginning of vestibular functionality and initiation of hearing.

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SATB2 organizes the 3D genome architecture of cognition in cortical neurons

The homeodomain protein SATB2 is expressed in pyramidal neurons of cortex and hippocampus. These cells are required to perform complex cognitive functions such as comprehension, perception, and planning. Common variation in SATB2 is associated with human cognitive ability, whereas de novo protein-damaging mutations cause an autosomal dominant disorder, characterized by severe intellectual disability and speech deficiency. Previously, we have shown that SATB2 determines L-LTP and long-term memory in the adult forebrain. Since SATB2 forms homodimeric or tetrameric DNA-binding complexes with the potential to influence chromosomal looping, we explored the role of SATB2 in spatial genome organization in synaptically active primary cortical neurons from Satb2 KO vs control mice. We identified strong effects of SATB2 on 3D genome conformation at all hierarchical levels. On whole-chromosome level, loss of SATB2 caused a global decrease in chromatin compaction, reflected by decreased interaction frequency between loci at all distance scales. SATB2 deletion caused multiple A/B compartment changes (strength and A/B switches), TAD boundary alterations, as well as gain and loss of a large number of chromatin loops. SATB2 binding sites were enriched at the anchors of the differential promoter–centered but not non-promoter-centered loops. This indicates that some SATB2 effects are indirect and likely mediated by interactions between DNA and SATB2 co-binding factor(s). SATB2-dependent chromatin looping was tightly correlated with chromatin accessibility changes: anchors of chromatin loops lost upon SATB2 deletion overlapped with regions of reduced accessibility, whereas anchors of gained loops in Satb2 KO neurons overlapped with regions of increased chromatin accessibility. In order to test the relevance of SATB2-dependent 3D genome changes, we defined separate gene sets, composed of genes affected by SATB2 deletion at compartment, TAD boundary and promoter-centered chromatin loop level. We found that at all architectural levels these gene sets were enriched for cognition-related GO terms, associated with synaptic function, learning and memory as well as neuronal morphology. Gene sets linked to SATB2-driven 3D chromatin alterations were specific for the individual hierarchical architectural levels with little overlap between them. All gene sets combined, SATB2 affected the spatial organization of almost half of the genes comprising the enriched cognition-related GO categories. Furthermore, SATB2-directed 3D genome folding correlated well at all levels with SATB2-dependent gene regulation. Our data establish SATB2 as a 3D genome organizer in cortical neurons, exerting strong effects on spatial genome conformation that are highly tailored to the regulation of cognition-related gene regulatory networks.

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Cre-Controlled CRISPR mutagenesis of voltage-gated Calcium Channel Cav1.2 in pancreatic exocrine cells

Assessing the response of pancreatic islet cells to glucose stimulation is important for understanding the tightly regulated release of hormones, including insulin.

Previous studies identified voltage-dependent calcium channels (Cav) as key components of the signaling cascade mediating glucose induced insulin secretion. Taking advantage of a Cav1.2 mutant line in the zebrafish model organism (Danio rerio), we previously collected evidence which suggests an important role of the α1 subunit Cav1.2 not only in insulin-releasing beta cells, but also in somatostatin-releasing delta cells: Altered glucose levels and regulation of beta-cell activity not only speak in favor of the relevance of Cav1.2 functions in non-beta cells, but also the necessity of intra-islet communication to maintain glucose homeostasis.

However, the currently available global Cav1.2 mutant line provides very limited options for defining cell-autonomous Cav1.2 requirements. The extent to which distorted islet cell activity might stem from other Cav1.2-impaired tissue associated with pancreas function (e.g., neurovascular supply) cannot be answered with this approach.

Therefore, we are currently working on a cell-specific conditional Cav1.2 gene inactivation in zebrafish. CreER-controlled CRISPR (3C) mutagenesis allows for a cell-type specific and temporally (heat-shock driven) controlled inactivation of the Cav1.2 gene (in cooperation with TU Dresden). This way, we will be able to analyze novel contributions of Cav1.2 in, e.g., delta-cell and alpha-specific Ca2+ dynamics on the one hand, and their impact on intra-islet communication on the other hand. Together with in vivo Ca2+ imaging of GCaMP6s expressing fish, these data will add to a better understanding of non-cell-autonomous functions of Cav1.2 in pancreatic beta-cell regulation, and, hence, glucose homeostasis.

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5-methylcytosine in mRNA: a regulator of transcript stability?

That RNA is a target of post-transcriptional modifications was discovered about 70 years ago. To date, about 160 different RNA modifications were identified, most of them were found to occur in abundant RNA such as rRNA and tRNA. By contrast, only 5 internal modification types have been observed in mRNA – one being 5-methylcytosine (m5C). The methylation of cytosines is carried out by 7 members of the NOL1/NOP2/sun (Nsun) domain family and the DNA methyltransferase homologue Dnmt2. However, only Nsun2 and Nsun6 were reported to be responsible for targeting mRNA. The functional relevance of m5C in mRNA is still poorly understood.

Because in mammalian mRNAs, an enrichment of m5C in the 3'UTR is observed, we are interested to determine if m5C affects mRNA stability. To this end, we generated Nsun2-knock-out mouse embryonic stem cells by CRISPR/Cas9 technology and studied mRNA expression dynamics by metabolic labeling and TUC-seq analysis in wildtype and Nsun2-KO cells. The results of these analyses will be discussed.

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The NLRP3 Inflammasome and Orthodontic Tooth Movement

The NACHT (nucleotide-binding oligomerization), LRR (leucine-rich repeat), and PYD (pyrin) domains-containing protein 3 (NLRP3) inflammasome plays a crucial role in the progression of inflammatory and adaptive immune responses throughout the body. Studies proved that NLRP3 is associated with the pathogenesis and progression of several oral diseases, including periodontitis. The main function of the NLRP3 inflammasome is processing pro-inflammatory cytokines like IL-1 β.

Orthodontics is a specialty in the field of dentistry, dedicated to the investigation and practice of correcting misaligned teeth and jaws. Orthodontic forces cause the release of active biological agents generating a complex multifactorial sequence of biological events, leading to inflammation and further bone remodeling that enables orthodontic tooth movement (OTM). Key regulators of inflammation and tissue turnover include secreted molecules like the Receptor Activator of NF-κB ligand (RANKL), osteoprotegerin (OPG), several transcription factors, and cytokines (i.e., IL-1β), prostaglandins, tissue necrosis factors, and proteases. As inflammation is a mandatory condition in bone remodeling due to orthodontic appliances, NLRP3 might take up an important function during OTM.

In this work, we hypothesize that the application of orthodontic forces triggers similar microbiological processes in the bone as in periodontitis, since both proceed based on inflammation.

Our study will investigate the role of NLRP3 in OTM and shed light on the processes involved in bone transformation upon orthodontic treatment.

Therefore, experimental OTM will be induced in wild type (WT) and NLRP3-depleted (NLRP3 -/-) mice using Ni-Ti coil- springs that were fixed between an upper first molar and the upper incisors. After 7, 14, and 21 days of OTM, the jaws will be removed and examined by micro-computed tomography (µCT), decalcified histology, and immunohistochemistry.

Inflammation during tooth movement needs to be well controlled, as dysregulated inflammatory reaction leads to tissue destruction, manifested in orthodontic induced root resorption or periodontal disease.

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Neopterin predicts disease severity in hospitalized patients with COVID-19

Introduction: Infections with the pandemic virus SARS-CoV-2 can result in severe disease requiring intensive medical treatment. Early recognition of disease severity and prediction of complications is essential for risk stratification of hospitalized patients. Therefore, we evaluated the predictive value of circulating inflammatory markers, especially neopterin, in patients with COVID-19.

Methodology: In this retrospective analysis of 377 consecutive patients (61.0% men, median age 68 years) with PCR-confirmed SARS-CoV-2 infection who needed hospitalization at the Innsbruck University Hospital between February and December 2020. Clinical outcomes during hospitalization including intensive care unit (ICU) admission, mechanical ventilation and death as well as demographics, clinical and laboratory parameters were collected.

Results: Elevated neopterin levels upon admission were significantly associated with a higher WHO score ($r_s = 0.479$, $p<0.001$) and temperature ($r_s = 0.287$, $p<0.001$) as well as lower SpO2 ($r_s = -0.425$, $p<0.001$) with consequently higher oxygen requirements ($r_s = 0.356$, $p<0.001$), and also with longer in-hospital stays ($r_s = 0.313$, $p<0.001$) and a higher risk for death, ICU admission and need for mechanical ventilation. Patients with neopterin levels > 45nmol/L had a more than nine-fold higher risk to die during hospital stay (OR 9.793 [95%CI 3.401–28.196], $p<0.001$, see Figure) and in patients under the age of 70 years six-fold higher risk for ICU admission (OR 5.973 [95%CI 2.870–12.433], $p<0.001$) and need of mechanical ventilation (OR 6.857 [95%CI 2.684–17.517], $p<0.001$) when compared to patients with neopterin levels ≤ 45nmol/L. This was independent of sex, age and oxygen saturation in multivariate logistic regression analysis and also true when correcting neopterin levels for renal function by calculating a neopterin/eGFR ratio.

Conclusions: Neopterin is a reliable marker for severity of infection with SARS-CoV-2 and for predicting complications and outcome and thus may help to improve the clinical management of patients.

Funding:

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Antibody Response to mRNA Vaccines against SARS-CoV-2 with Chronic Kidney Disease, Hemodialysis, and after Kidney Transplantation

Most trials on mRNA vaccines against SARS-CoV-2 did not include patients with chronic kidney disease (CKD), hemodialysis (HD) patients, or kidney transplant recipients (KTR). However, those patients have a higher risk for a severe course of COVID-19 disease and mortality. Available literature has demonstrated a reduced efficacy of mRNA vaccines in HD patients and KTR, while data on CKD patients is scarce. Additionally, factors associated with non-response are poorly understood and not well characterized. We assessed antibody (AB) response (n = 582, 160 CKD patients, 206 patients on HD, 216 KTR) after the administration of two doses of a mRNA-vaccine with either BNT162b2 or mRNA-1273. AB measurements were carried out after a median of 91 days after first vaccinations, demonstrating non-response in 12.5% of CKD patients, 12.1% of HD patients, and 50% of KTR. AB titers were significantly higher in CKD patients than in HD patients or KTR. Factors associated with non-response were treatment with rituximab in CKD patients, the use of calcineurin inhibitors in HD patients and older age, and the use of BNT162b2, mycophenolic acid, or glucocorticoids and lower hemoglobin levels in KTR. This study contributes to the understanding of the extent and conditions that predispose for non-response in patients with impaired kidney function.

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Baseline iron status and presence of anaemia determine the course of systemic Salmonella infection following oral iron supplementation in mice

Background: Iron deficiency anaemia (IDA) is a major health concern. However, preventive iron supplementation in regions with high burden of infectious diseases resulted in an increase of infection related morbidity and mortality.

Methods: We fed male C57BL/6N mice with either an iron deficient or an iron adequate diet. Next, they received oral iron supplementation or placebo followed by intraperitoneal infection with Salmonella Typhimurium (S.Tm).

Findings: We found that mice with IDA had a poorer clinical outcome than mice on an iron adequate diet. Interestingly, iron supplementation of IDA mice resulted in higher bacterial burden in organs and shortened survival. Increased transferrin saturation and non-transferrin bound iron in the circulation together with low expression of ferroportin facilitated the access of the pathogen to iron and promoted bacterial growth. Anaemia, independent of iron supplementation, was correlated with reduced neutrophil counts and cytotoxic T cells. With iron supplementation, anaemia additionally correlated with increased splenic levels of the cytokine IL-10, which is suggestive for a weakened immune control to S.Tm infection.

Interpretation: Supplementing iron to anaemic mice worsens the clinical course of bacterial infection. This can be traced back to increased iron delivery to bacteria along with an impaired anti-microbial immune response. Our findings may have important implications for iron supplementation strategies in areas with high endemic burden of infections, putting those individuals, who potentially profit most from iron supplementation for anaemia, at the highest risk for infections.

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Immunological memory and affinity maturation after vaccination in patients with propionic acidemia.

Former studies recommended routine childhood immunization in patients with propionic acidemia (PA); however, the literature presents insufficient data on the response to vaccines, notably specific IgG concentrations and avidity maturation, after measles, mumps, rubella (MMR), and diphtheria/tetanus (DiphtTe) vaccinations in this population. In patients with PA, cellular and humoral changes of the immune system (e.g. a decreased CD4+ T cell count, with a reversal of CD4/CD8 T cell ratio, a deficient gamma-globulin fraction, and in one case a decreased lymphocyte blastogenesis) were documented in literature. Former reports detected pancytopenias accompaning febrile infections in PA patients.

We analyzed vaccine-specific IgG concentrations and avidity maturation after MMR and DiphtTe vaccinations in 10 patients with PA. Compared to gender and age matched controls, all 10 had protective IgG concentrations for at least one tested antigen, and in 6/10 high relative avidity indices for measles and rubella were measured.

In summary, the present study revealed a sufficient immune outcome and memory function of the immune system in patients with PA after booster vaccinations with ongoing age.

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BCL2 family proteins as effectors of the mitotic spindle assembly checkpoint

Interfering with the mitotic spindle using microtubule-damaging agents is widely used to treat highly proliferating cancer cells. Activating the spindle assembly checkpoint (SAC) induces mitotic arrest by inhibition of the anaphase-promoting complex (APC) via the mitotic checkpoint complex (MCC) that includes the protein MAD2. Preventing mitotic exit can end in mitotic cell death. However, adaptation to the mitotic checkpoint by a process called "slippage" can lead to premature exit from mitosis, mis-segregation of chromosomes and chromosomal instability. Here we are using a mouse model allowing Dox-inducible MAD2 overexpression to investigate the impact of the BCL2 protein family on mitotic arrest and subsequent cell death in vivo. MAD2 overexpression can lead to CIN, increased cell death and tumorigenesis. We observed attrition of hematopoiesis and severe gastrointestinal tissue damage upon MAD2 overexpression causing rapid death of the animals. Deleting pro-apoptotic proteins BIM & NOXA, which are involved in regulating mitotic cell death, can rescue these effects, suggesting roles in tissue homeostasis upon mitotic errors.

Funding:

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Real-Life Temporal Relations Between Urinary IL-6 and sTNF-R55 Levels with Specific and Nonspecific Symptoms in an SLE Patient

This integrative single-case study investigated the temporal relations between interleukin-6 (IL-6) and 55kD soluble tumor necrosis factor receptor type 1 (sTNF-R55) with specific and unspecific symptoms in a 52-year-old woman diagnosed with systemic lupus erythematosus (SLE). The patient collected her entire urine for 56 days in 12h-intervals to determine sTNF-R55/creatinine, IL6/creatinine and protein/creatinine levels (ELISA, HPLC). Additionally, twice a day, she took notes on oral ulceration and facial rash; answered questionnaires (VAS) on fatigue, weakness, and joint pain; and measured body temperature orally. Time series analysis consisted of ARIMA modeling and cross-correlational analyses. Thereby, several significant lagged correlations between urinary sTNF-R55, urinary IL-6 and SLE symptoms in both directions of effect were identified. Specifically, increased sTNF-R55 concentrations preceded decreased urinary protein levels by 36-48h ($r = -.213$; $p = .024$) and, in the opposite direction of effect, increased protein levels preceded increased sTNF-R55 concentrations by 24-36h ($r = +.202$; $p = .033$). In addition, increased urinary sTNF-R55 levels preceded increased oral ulcers by 36-48h ($r = +.277$; $p = .003$) and, conversely, increased oral ulceration preceded decreased sTNF-R55 levels by 36-48 h ($r = -.313$; $p = .001$). IL-6 concentrations showed a counterregulatory behavior, when cross-correlated with SLE symptoms: increased urinary IL-6 concentrations preceded increased urinary protein levels by 36-48h ($r = +.225$; $p = .017$) and, in the opposite direction of effect, increased urinary protein preceded urinary IL-6 decreases by 12-24h ($r = -.322$; $p < .001$). Moreover, urinary IL-6 increases co-occurred with increased oral ulceration ($r = +.186$; $p = .049$); and after 48-60 h, showed a strong tendency to precede oral ulceration decreases ($r = -.170$; $p = .072$). This study gathered first, preliminary evidence of real-life feedback loops between cytokines and SLE symptoms, with counterregualtory activity of sTNF-R55 and IL-6.

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The role of complement in a murine model of disseminated mucormycosis

Introduction: Mucormycetes, a rather heterogeneous group of fungi, induce a life-threatening disease called mucormycosis. The prevalence of this disease, which shows high morbidity and mortality, increased within the last decade. Main risk factors for mucormycosis are immune deficiencies. An important link between innate and adaptive immunity is the complement system, which also provides several crucial functions in first-line defense against non-self-structures like fungi.

To enlighten the responsibility of complement in the defense against mucormycosis, our objectives were comparing the role of different parts of complement in a murine model of disseminated mucormycosis and studying the relevance of complement for pathogenesis.

Material/Methods: Complement C3- or C6-deficient mice were intravenously challenged with Lichtheimia corymbifera (LC), Lichtheimia ramosa (LR), Rhizopus arrhizus (RO), Rhizopus microsporus (RM), Rhizomucor pusillus (RmP) or Mucor circinelloides (M). Survival, clinical status, and immunological parameters were monitored for 14 days and compared to that of immunocompetent (wt) or neutropenic mice. Additionally, serum from healthy wt mice was analyzed for capacity to opsonize the fungi.

Results: When intravenously infected with M or RO, there is no difference between immune deficient and wt mice. Complement deficiencies represent a risk factor for a lethal outcome in LC, LR, RM, and RmP. LC and RM lead to higher mortality in complement deficient mice, compared to neutropenic. There is no significant difference between the lethality of C3- and C6-deficient mice in intravenous infections with LC, M, RO, and RM. C3-deficient mice exhibited higher mortality than C6-deficient mice when infected with LR. The opposite was the case in RmP infections.

Conclusion: Complement plays an important role in the murine model of disseminated mucormycosis. Mortality of the complement deficient animals varies between the species. Further investigations are needed to fully understand the immunopathogenesis of mucormycosis and help to fight the high morbidity and mortality of this disease.

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Characterization of monoclonal antibodies against different forms of the A subunit of Shiga toxin 2a

Introduction: Infection with enterohemorrhagic Escherichia coli (EHEC) can give rise to the development of hemolytic uremic syndrome (HUS), which represents the major cause of acute kidney injury in children. Shiga toxin 2a (Stx2a) is one of EHEC´s main virulence factors relevant for the pathogenesis of the disease. Recently it has been discovered that the biological properties and biding abilities of Stx2a, including its interaction with complement proteins, are determined by the structure of its A subunit. As a result, the generation of specific monoclonal antibodies, capable of distinguishing between the different forms of the toxin, is critical for a better understanding of the structure of circulating Stx2a during infection, as well as for determining its impact on eHUS pathogenesis and the development of new therapeutics.

Methods: Mouse monoclonal antibodies against the different forms of the A subunit of Stx2a were produced using hybridoma technology after immunizing mice with short peptides from the A subunit of Stx2a. Subsequently, the antibodies were selected and characterized by Western blots and Enzyme-linked immunosorbent assays (ELISAs).

Results: Monoclonal antibodies capable of recognizing distinct forms of the A subunit of Stx2a were generated. Nonetheless, the antibodies primarily recognized just the denatured forms of the toxin as consequence of using short peptides as immunogens for the mice.

Conclusion: In order to better understand the biological properties of the toxin and its impact on eHUS pathogenesis, it is important to characterize its circulating forms throughout the different stages of EHEC infection. In the present study, specific monoclonal antibodies capable of discriminating between different denatured forms of the A subunit of Stx2a were generated. Nonetheless, new attempts to generate antibodies capable of differentiating the diverse native forms are currently in progress.

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Single-cell RNA sequencing reveals immune cell dynamics in normothermic machine perfusion of the liver

Single-cell RNA sequencing is a novel technology enabling the identification of both the cell composition and the gene profile. We applied this technology to provide a first comprehensive single cell atlas and its dynamic change of human livers perfused ex vivo. Furthermore, passenger leukocytes and the inflammatory profile were quantified and characterized in the perfusate of human donor livers during normothermic machine perfusion (NMP). Building on a human liver cell atlas integrating 56.560 cells our study enlightens an abundance of CXCR2+ neutrophils which increased, while tissue-resident neutrophils decreased during NMP. Concordantly, a massive efflux of passenger leukocytes with predominance of neutrophil granulocytes was observed in the perfusate in the course of NMP. A pro-inflammatory profile, where neutrophils constitute the primary source of inflammatory messengers during ex vivo perfusion (i.e. SDF-1a, IL-6, IL-8 and GRO-α), was identified. These findings serve as the groundwork for advancement in the field of ex vivo perfusion and organ transplantation.

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Key components of the Mediator complex and their functional roles in prostate cancer

Prostate cancer (PCa) is the fifth leading cause of cancer-related death among men. Androgen receptor (AR) targeting therapies such as androgen deprivation therapy (ADT) and the second-generation AR inhibitor enzalutamide significantly prolong the survival of patients suffering from advanced metastatic PCa. However, the cancer cells ultimately adapt by developing a large variety of poorly understood resistance mechanisms, such as AR reactivation. Among these, the alteration of AR co-activators can promote its function as a transcription factor.

In this context, the role of the mediator complex in the regulation of AR activity has started to emerge.

The mediator complex is a large multi-subunit protein that plays a major role in regulating gene expression. In particular, MED12 subunit was observed to be overexpressed in mCRPC and involved in the proliferation of prostate cancer cell lines.

Therefore, our aim is to study the role of MED12 in PCa promotion and acquirement of enzalutamide resistance.

Analysis of publicly available CRISPR and RNAi databases revealed that AR dependent PCa cell lines are highly sensitive to MED12 inhibition. In addition, mRNA expression of MED12 was elevated in AR dependent PCa cell lines (LNCaP, VCaP, 22Rv1) compared to AR negative PC3 cells. RNAseq transcriptome analysis of enzalutamide resistant PCa cell lines developed in our laboratory showed that they retain a similarly high MED12 expression levels to their enzalutamide sensitive counterparts. To study the therapeutic opportunity of MED12 inhibition in enzalutamide resistant cells, we are currently setting up a CRISPR interference system to obtain stable cell lines with inducible downregulation of MED12.

Our future aim will be to analyse the impact of MED12 downregulation on enzalutamide sensitive and resistant PCa cell transcriptome and on their response to enzalutamide treatment.

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Targeting a myofibroblastic prostate cancer-associated fibroblast subtype through pharmacological inhibition of NADPH oxidase 4

Recent developments in single cell sequencing have revealed significant heterogeneity in the stromal tumor microenvironment. It is now widely accepted that there are different cancer-associated fibroblast (CAF) subtypes with different functions and effects on tumor progression. Since CAFs are generally considered an emerging therapeutic target, it is crucial to identify those subtypes that are tumor-promoting and to characterize their driver pathways.

In this study we describe a population of CAFs in prostate cancer (PCa) that express elevated levels of NADPH oxidase 4 (Nox4) and localize adjacent to tumor foci. We previously showed that Nox4 is essential for TGFβ-mediated differentiation to a myofibroblastic CAF phenotype and that its increased expression is associated with biochemical relapse and reduced survival. Thus, this study aims to investigate whether pharmacological Nox4 inhibition can be used as an adjuvant therapeutic approach and which molecular pathways are regulated by Nox4 in the prostate tumor microenvironment.

Experiments using GKT831, a small molecule Nox1/Nox4 inhibitor with promising effects in clinical trials for fibrotic diseases, so far support its use as a therapeutic target. Most importantly, Nox4 inhibition reduced CAF marker expression in primary prostate CAFs and attenuated the expression of PSA, the key clinical biomarker of PCa progression, in ex vivo cultured human PCa tissue. The translational application of Nox4 inhibition is currently under investigation in vivo using CAF-PCa cell xenografts.

To elucidate the molecular mechanisms regulated by Nox4 we performed integrative bioinformatics and functional assays, which revealed that Nox4 regulates CAF adhesion and migration. In addition, Nox4 regulated genes were associated with a YAP signature and the transcription factor TEAD1, a major cofactor of YAP. Ongoing experiments suggest that during adhesion Nox4 signals via oxidation of SHP2, a redox-sensitive phosphatase that has previously been implicated in regulating YAP transcriptional activity.

Previous studies have shown that adhesion and mechanotransduction pathways are commonly altered in myofibroblastic CAFs and that they support the formation of a tumor-promoting microenvironment. Our data suggest that Nox4 acts as a central regulator of these key oncogenic pathways and thus represents a promising therapeutic target.

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Identification of functional single nucleotide polymorphisms in cryptic exon 3 of the androgen receptor gene

Castration resistant prostate cancer (CRPCa) involves the upregulation of androgen receptor variants (AR-Vs), of which AR-V7 is clinically the most relevant. By lacking the ligand-binding domain, AR-V7 is constitutively active and may therefore bypass AR signaling inhibitors. Treating CRPCa involves improved AR signaling inhibitors or taxane-based chemotherapy. However, the optimal treating sequence may differ from patient to patient. The presence of AR-V7 in the nucleus of circulating PCa cells, which is associated with AR targeted therapy resistance, is used as a therapy-guiding biomarker. This stratification has a high predictive power and specificity, but its sensitivity is unsatisfying. A PCR/sequencing based test would improve the sensitivity of this biomarker, but it requires a better understanding of AR-V7's functional regulation. Therefore, we aim here to analyze single nucleotide polymorphisms (SNPs) in the 3' UTR of AR-V7 that might have an impact on AR-V7's expression and/or functional activity.

SNP rs5918762 was selected as it is in linkage disequilibrium with several PCa tagSNPs (rs5919393, rs5919432). Using an in silico approach, this SNP is predicted to destroy/create a binding site for the splicing machinery involved protein SRSF9. Disrupting this SNP region via NHEJ resulted respectively in an increased and decreased expression of AR-FL and AR-V7 mRNA in 22RV1 cells (C allele). Additionally, analysis of the SU2C dataset (CRPC patients) correlated SRSF9 expression with AR-V7 expression. Subsequently, SRSF9 knock out (KO) resulted in reduced AR-V7 mRNA expression.

Our data suggests that SNP rs5918762T/C is located in a region important for CE3 inclusion during AR splicing, which might involve SRSF9. Further validation of these results is in need and encompasses splicing assays by an AR-V7 mini-gene assay, protein-RNA interaction assays and an analysis of a lack of effect in cell lines expressing the T allele.

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Investigating the role of dendritic cells in tumor-targeted therapy mediated anti-tumor immunity

Melanoma belongs to the 10 most common cancer types and has a high mutational load with driver mutations affecting genes regulating critical signaling pathways involved in proliferation and cell growth. Half of the melanoma patients carry a specific point mutation affecting BRAF, which constitutively activates the MAPK pathway. Targeted therapy using inhibitors specific for mutant BRAF (BRAFi) elicts high response rates in melanoma patients. However, patients frequently relapse within the first year of treatment due to the development of therapy resistance.

Tumor-targeted therapy modulates the tumor immune microenvironment and induces anti-tumor immune responses. The functional role of dendritic cells (DC) in anti-tumor responses by BRAFi remains elusive. As DC are crucial to initiate T and NK cell responses, we want to address which DC subsets are involved in immune modulation during treatment using the transplantable D4M.3A BRAFV600E mutant melanoma mouse model. We hypothesize that tumor-targeted therapy boosts T cell responses by improving DC function.

In order to understand the complexity and functionality of these cells, we designed a multicolor flow cytometry panel to clearly discriminate the DC compartment from other myeloid cells. This panel provides deep insights into the different DC subsets, including cDC1, cDC2 and plasmacytoid DC (pDC). Using this optimized panel, we can show that treatment with BRAFi decreases immunosuppressive myeloid cells in the tumor microenvironment. Furthermore, this targeted therapy recruits activated DC to the inflammatory tumor milieu, which subsequently migrate in an increased frequency to the tumor-draining lymph nodes. In addition, by using Zbtb46-GFP mice, a DC-specific reporter mouse strain, we identified a novel CD64+ DC population in tumor-draining lymph nodes. The function of this cell population will be investigated in more detail to gain insights about their relevance during anti-tumor immunity.

Further characterization of tumor-infiltrating as well as migratory DC subsets in the draining lymph nodes will give us valuable insights whether alterations in DC function contribute to resistance development.

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MIO: MicroRNA target analysis system for Immuno-Oncology.

MicroRNAs have been shown to be able to modulate the tumor microenvironment and the immune response and hence could be interesting biomarkers and therapeutic targets in immuno-oncology, however, dedicated analysis tools are missing. Here we present the user-friendly web platform MIO and the Python toolkit miopy integrating various methods for visualization and analysis of provided public or custom bulk microRNA and gene expression data. We include regularized regression and survival analysis and provide information of forty microRNA target prediction tools as well as a collection of curated immune related gene signatures and processed TCGA data. The integration of several machine learning methods enable the selection of prognostic and predictive microRNAs and gene interaction network biomarkers. Using the microRNA correlation tool from MIO we found that miR-200 family members (miR-200a, miR-200b, miR-200c, miR-429, miR-141) are potentially targeting PD-L1 (CD274) in the available lung adenocarcinoma dataset (TCGA-LUAD). We have tested our MIO tool through analyzing the TCGA combined colon and rectal cancer cohort (TCGA-CRC) including 572 patients with primary tumor and another cohort of 105 colon cancer patients (CPTAC-2) for validation. Based on the TCGA-CRC cohort we performed a feature selection procedure in order to obtain the 25 most informative microRNAs for separation of microsatellite instability high (MSI-H) tumors, indicating patients who benefit from immunotherapy such as PD-1 targeting antibodies. In the TCGA-CRC training set the MSI groups could be separated with an area under receiver operating characteristics (ROC) curve of 0.894 and mean accuracy over 10-fold cross validation of 0.96, using the microRNA-MSI predicted signature. In summary, MIO will help cancer researchers and oncologists to identify microRNAs and their target genes as potential biomarkers or candidates for cancer immunotherapy.

Availability and Implementation: https://mio.icbi.at, https://github.com/icbi-lab/mio, https://github.com/icbi-lab/miopy

Funding:

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Prediction and validation of C57BL/6J MHC-class I epitopes of the oncolytic virus VSV-GP

In addition to the direct killing of cancer cells, oncolytic viruses also lead to the activation of the immune system, which might help in anti-tumor immunity. However, anti-viral T cells against the oncolytic virus are induced as well, and these activated T cells may represent the dominant T cell pool. Upon oncolytic virus treatment, the anti-tumor and anti-viral specific immune response cannot be discriminated. Hence, the aim of this study was to identify the anti-viral T cells raised by VSV-GP virotherapy in preclinical C57BL/6J mouse models. An adapted bioinformatics viral epitope prediction approach was used to identify the VSV-GP epitopes to which the mouse anti-viral T cells react. The tools netMHCpan, MHCflurry and netMHCstabPan, which are usually used in neoepitope identification, were applied for the prediction. Based on different scorings, including the binding strength, prediction score and predicted stability, these predicted viral epitopes were ranked, and the top ranked epitope candidates were selected. Using ELISpot, we could identify which viral epitopes presented on mouse MHC-I alleles H2-Db and H2-Kb induce T cell activation, measured by IFN-γ secretion. Taken together, these results identifying the VSV-GP T cell epitopes enable monitoring of the full repertoire of anti-viral T cells upon VSV-GP oncolytic virotherapy in mouse models. Assessing anti VSV-GP immunity could contribute to preclinical development of novel variants of VSV-GP, and could aid in monitoring vector immunity raised by VSV-based vaccines, including the FDA-approved VSV-EBOV vaccine.

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Y129F and V293A in the sterol 14-α-demethylase-F5 paralogue confer resistance to short-tailed azoles in Mucor circinelloides

Background:

Resulting of the latest Covid-19 pandemic, mucormycosis cases increased especially in India and Brazil. The disease is challenging and treatment options remain limited as mucormycetes are intrinsically resistant to short-tailed azoles, leaving only amphotericin-B, posaconazole, and isavuconazole. The mechanism is suspected to be mediated by two amino acid (AA) exchanges (Y129F, V293A) in the sterol-14-α-demethylase (SDM) paralog McSDM-F5. We tested the hypothesis in a heterologous model to a gain deeper understanding of the substitutions' impact within the ligand-binding pocket of SDM.

Methods:

Using a modified, hypersensitive S. cerevisiae model, overexpression of McSDM-F1, McSDM-F5 with their cognate cytochrome-P450-reductase (CPR) was performed at PDR5/PDR15 loci, respectively. The impact of the two AA exchanges was tested by inverting the wildtype AAs of each paralogue. Resistance profiles were conducted using EUCAST guidelines for amphotericin-B, voriconazole, fluconazole, isavuconazole, posaconazole, and itraconazole. For protein expression analysis, growth kinetics and SDS-PAGE plus western blots were performed. Ergosterol and precursors, as well as toxic intermediates, were identified using GC-MS with/without azole treatment. Protein structures were calculated in silico for ligand interaction studies.

Results:

McSDM-F1, McSDM-F5 were successfully expressed with the co-enzyme CPR. SDM-F1 paralogue showed MIC values of 0.3 mg/L (voriconazole) and 14.5 mg/L (fluconazole), but SDM-F5 displayed higher levels (voriconazole: 4 mg/L and fluconazole: 128 mg/L). Inverted wild-type AAs in the SDM-F5 present susceptible phenotypes, but changed AAs of SDM-F1 were unfunctional. Protein expression relative to the ScERG11 control, was higher for the SDM-F5 variant (177%), than for the SDM-F1 (90%). Regarding ergosterol content, SDM-F1 was higher affected under voriconazole treatment than SDM-F5. With in silico calculations, specific binding sites could be shown for lanosterol and azoles.

Conclusions:

Summarizing, wild-type and inverted versions of McSDM were successfully expressed in the yeast model. Resistance profiling, protein expression, and sterol analysis verified McSDM F5 and its AA substitutions as the molecular mechanism of short-tailed azole resistance in M. circinelloides.

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HIV-1 Trans Infection via TNTs Is Impeded by Targeting C5aR

Nonadjacent immune cells communicate through a complex network of tunneling nanotubes (TNTs). TNTs can be hijacked by HIV-1, allowing it to spread between connected cells. Dendritic cells (DCs) are among the first cells to encounter HIV-1 at mucosal sites, but they are usually efficiently infected only at low levels. However, HIV-1 was demonstrated to productively infect DCs when the virus was complement-opsonized (HIV-C). Such HIV-C-exposed DCs mediated an improved antiviral and T-cell stimulatory capacity. The role of TNTs in combination with complement in enhancing DC infection with HIV-C remains to be addressed. To this aim, we evaluated TNT formation on the surface of DCs or DC/CD4+ T-cell co-cultures incubated with non- or complement- opsonized HIV-1 (HIV, HIV-C) and the role of TNTs or locally produced complement in the infection process using either two different TNT or anaphylatoxin receptor antagonists. We found that HIV-C significantly increased the formation of TNTs between DCs or DC/CD4+ T-cell co-cultures compared to HIV-exposed DCs or co-cultures. While augmented TNT formation in DCs promoted productive infection, as was previously observed, a significant reduction in productive infection was observed in DC/CD4+ T-cell co-cultures, indicating antiviral activity in this setting. As expected, TNT inhibitors significantly decreased infection of HIV-C-loaded-DCs as well as HIV- and HIV-C-infected-DC/CD4+ T-cell co-cultures. Moreover, antagonizing C5aR significantly inhibited TNT formation in DCs as well as DC/CD4+ T-cell co-cultures and lowered the already decreased productive infection in co-cultures. Thus, local complement mobilization via DC stimulation of complement receptors plays a pivotal role in TNT formation, and our findings herein might offer an exciting opportunity for novel therapeutic approaches to inhibit trans infection via C5aR targeting.

Funding:

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Adding new dimensions to viral entry on mucosal sites

One of the key points for a better understanding of host-pathogen interactions - such as virus entry into the human system - happens at the outer barriers of our body. Here, epithelial sites function as physical and chemical obstacles that prevent or exacerbate external pathogens to enter human body.

In particular, during the last years, the optimization of complex human models to examine such interactions in vitro gained more attention due to the obvious low translatability rates between mice and man and due to increasing interest in personalized settings. For example, novel three-dimensional (3D) models allow completely animal-free design of human tissues in vitro and thus provide a very good, standardized basis to study host-pathogen interactions. To improve in

vivo-like situations, it is aspired to include a further component into such models by adding cellular and humoral immune components.

Herein, interactions between host cells and viruses within a three-dimensional respiratory system were evaluated. Since the respiratory model that was optimized and so was accepted by the NIH as mucosal model, the first encounter not only between SARS-CoV-2 and the human lung epithelium but also very first HIV/barrier interactions were analyzed. Moreover, different antiviral substances and their effects on the mucosal barrier were investigated.

Data revealed that some of the tested antiviral compounds could rescue epithelial integrity during viral infection and, too, decrease inflammatory reactions as well as productive infection. Since dendritic cells (DCs) are among the first immune cells encountering pathogens after these are able to cross the barriers, the antiviral compounds were further tested on their efficiency using HIV-exposed DCs.

Interestingly, some of these compounds turned out to work as adjuvant for DC function during HIV-1 infection. In summary, a complex insight into very first host-pathogen interactions at native mucosal barriers was established.

Funding:

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Overcoming Drug Resistance in BRAF-mutated Melanoma

Cancer development requires metabolic adaptations as one of the established hallmarks of cancer. Changes in intracellular reactive oxygen species (ROS) are a consequence of the transformation process and increasing ROS to toxic levels is part of current cancer therapies including irradiation and drug treatment. However, to exploit this strategy for a broader guided treatment approach a better understanding of physiological and non-physiological ROS effects and feasible approaches to generate a redox stress appropriately tailored to kill a specific tumor are required. We previously have identified a deficiency in mitochondrial ROS production in cells transformed by mutant BRAF, an alteration observed in the majority of melanoma and fractions of other tumors. Subsequent work highlighted a possible link to the protein p66Shc that is directly involved in the generation of mitochondrial ROS. We dissected the signaling pathways in p66Shc activation and identified phosphorylation of serine 36 on p66Shc by the kinases JNK1/2 as the most critical step. We also reported a defect in the activation of JNK1/2 in melanoma cell lines expressing BRAFV600E preventing p66Shc activation, ROS production and cell death. Moreover, lowering p66Shc expression enhanced proliferation of BRAFV600E mutant cells supporting a possible tumor suppressor function for p66Shc. Ongoing work addresses the role of p66Shc as a possible target to overcome BRAF inhibitor resistance in melanoma.

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The effect of macrophage inflammation on human adipose-derived stem cells and its role in human wound healing“

Introduction:

After skin injury, inflammation is initiated. During wound healing, activated cells of the immune system such as macrophages (MQ) dominate the inflammatory microenvironment by releasing signaling molecules to interact with a subset of different cells. Recent studies have shown that adipocytes and adipose-derived stem cells (ASC) are involved in skin repair and may exert positive effects on skin regeneration. Therefore, this study investigates the interaction of differentially activated macrophages and ASCs and its role in human wound healing.

Materials and Methods:

Human granulation tissue was gained from chronic wounds and analyzed by microscopy and FACS. Additionally, an in-vitro macrophage polarization model based on the acute monocytic leukemia (AML) cell line THP1 was established to generate conditioned media resembling a pro-inflammatory (macrophages activated by IFNG/LPS = MQ(IFNG/LPS)) and an anti-inflammatory (macrophages activated by IL4/IL13 = MQ(IL4/IL13)) microenvironment. Human ASCs were exposed to conditioned media (CM) from differentially activated macrophages (MQ) for 72 hours. Subsequently, ASC physiology was phenotypically and molecularly assessed using microscopy, viability assays, quantitative real-time-PCR, immunoblotting and FACS. CM of THP1 cells served as control.

Results:

Macrophages and ASCs were found in close proximity in human chronic wounds. In-vitro experiments indicated that pro-inflammatory and anti-inflammatory macrophages exerted different effects on ASC properties. Pro-inflammatory MQ(IFNG/LPS)-CM induced expression of pro-inflammatory cytokines secreted by ASCs. Cytoskeleton changes with an increase in focal adhesion and stress fiber formation resembling a myofibroblast-like phenotype were detected. Inhibition of pro-inflammatory effects were observed after addition of the IL1B inhibitor IL1-RA to MQ(IFNG/LPS)-CM suggesting that observed effects involve IL1B.

Conclusions:

ASC physiology is affected by differentially activated macrophages. In chronic wounds, an inflammatory microenvironment may cause resident stem cells to show a myofibroblast-like phenotype promoting wound contraction. Vice versa, Inhibition by IL1-RA may present a future treatment option for excessive wound healing with hypertrophic scarring.

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Immunoreactivity of Surface Topography on Human Foreign Body Response to Silicone Breast Implants

Silicone mammary implants (SMI) have been used for aesthetic as well as reconstructive indications for more than 50 years. However, the most common local complication is still capsular contracture. The etiology of this exaggerated capsule formation is multifactorial but primarily induced by immune mechanisms towards the foreign material silicone. Risk factors that were identified included specific implant topographies (the use of smooth (vs. textured) implants that can influence wound healing and the immune response. Moreover, recent evidence pointed out to the role of microbiota leading through biofilm formation to inflammatory processes, thus stimulating fibrosis.

We hypothesize, that novel nano textured SMI surface causes less inflammatory immune response in the early postop phase (first week after implantation) as well as during the early process of capsule formation (6-8 months after implantation) than normal textured SMI.

To study this question in human, a total of 25-30 patients will receive will receive either the nano-textured or the routinely used textured SMI, randomized to the left or right breast after bilateral prophylactic NSME (nipple-sparing mastectomy) or therapeutic NSME / SSME (skin-sparing mastectomy) due to ductal carcinoma in situ (DCIS). Systemic and local immune cell profile and function will be compared by flow cytometry, immunohistochemistry, multiplex lectin sorbent assay and gene expression, the protein surface adsorption - by mass spectrometric analysis. To study the effects of topography on biofilm formation, we will apply NextGen microbiome profiling of microbiome abundance in capsular tissue.

SMI are designed to be nontoxic and non-immunogenic while keeping an active role in host response which –we know- plays an active role in fibrogenesis. We expect better immune biocompatibility of nano textured devices with minor capsule formation, which leads long-term to lower revision rates and therefore better aesthetic outcome and higher patient satisfaction in aesthetic and reconstructive breast surgery.

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I. Schoberleitner (1), C.Brunner (2), D. Egle (2), C. Tripp (3), B. Zelger (4), K. Faserl (5), J. Pallua (6), A. Brunner (5), B. Amort (6), T.Rauchenwald (1), A. Augustin (1), P. Stoitzner (3), D. Daniaux(3),T. Bauer (1), W. Ulmer (7), D. Wolfram (1)

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"Complement opsonization of HIV-1 promotes sustained survival regulated by C3a and C5a in human dendritic cells"

HIV has been a central subject of research since it has been first described in 1983. The primary focus of most studies since then has revolved around the development of neutralizing antibodies and disruption of the latency phase. While these aspects certainly are very important to design new vaccine candidates and therapies, the role of the innate immunity and especially the complement system is often not taken into account. The innate immune system is traditionally known as a system of serum proteins that immobilize, tag, directly lyse pathogens, recruit immune cells and boost the immune response via various signals. Recent work has shown that immune cells like dendritic cells and T-cells are also capable of producing, cleaving and secreting the complement factors C3 and C5 upon stimulation. This local secretion of cellular complement has been described to play an important role in sustaining T cell homeostasis, differentiation and activation. Beyond its immune-related functions, cellular and intracellular complement might also influence other factors in basic cellular physiology.

In this work, we show that complement opsonized HIV leads to increased survival as a result of the stabilization of the anti-apoptotic Bcl-2 family member Mcl-1, via phosphorylation at threonine 163. This effect is mediated by the anaphylatoxin C5a and the interaction with its receptor (C5aR), as upon inhibition, this stabilization is impeded. The pathways controlling this phosphorylation event is controlled by MAP kinase- and to a lesser extent by the protein kinase C pathway. Inhibition of either leads to a reduction in phospho Mcl1 (Thr163) with the strongest effect when blocking both. The presence of C5a requires the assembly of complement to form C3/C5 convertases, or other intracellular mechanisms of C3/C5 processing. We could show that acute HIV infection induces expression of C3 and all necessary complement components to form active convertases.

Funding:

Gabriel Diem

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Counteractive and cooperative actions of muscle β-catenin and CaV1.1 during early neuromuscular synapse formation

During neuromuscular synaptogenesis motor nerves project their axons towards the central muscle region where postsynaptic acetylcholine receptors (AChRs) are clustered, establishing the stereotypical central innervation pattern. This precise spatio-temporal organization of neuromuscular junctions (NMJ) requires a delicate interplay between anterograde and retrograde mechanisms between nerve and muscle. We have recently shown that activity-induced calcium signaling initiated by skeletal muscle L-type calcium channel CaV1.1 is a key controller of neuromuscular patterning, correct guidance of the axons to their target territory and of the differentiation of nerve terminals. However, how muscle calcium signaling interacts with downstream effectors to govern these processes remained unknown. Muscle β-catenin was previously identified as a retrograde regulator of motor axon fasciculation and nerve terminal development. Here we show that coordinated functions of CaV1.1 and β-catenin are required for proper neuromuscular synaptogenesis. By analyzing NMJ formation in the diaphragm of mice lacking CaV1.1, muscle β-catenin, or both, we found that the role of CaV1.1 is to determine the innervation territory, while β-catenin determines the degree of nerve branching so that their opposite but complementary roles induce sufficient nerve branching specifically in the muscle center. On the other hand, in the double knockouts AChR clustering and synapse formation are severely perturbed, indicating a cooperativity of CaV1.1 and β-catenin in these processes. Furthermore, by using a β-catenin reporter mouse line, Western blot, and qRT-PCR analysis, we show that CaV1.1 does not directly regulate β-catenin expression but has opposite effects on the activity of transcriptional partners of β-catenin, TCF/Lef and YAP. While CaV1.1 promotes TCF/Lef-dependent transcription, it suppresses YAP expression, phosphorylation and transcriptional activity. All together, these data show that CaV1.1 and β-catenin cooperate in various ways to regulate distinct aspects of the NMJ formation. And we identified possible signaling mechanisms by which CaV1.1 controls gene expression in the regulation of neuromuscular synapse formation.

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Automatic localization of Histological mouse brain slices in a 3D reference atlas using a deep learning

The study of the anatomical organization of neural circuits in the brain using histological mouse brain sections is a prominent area of research in the neuroscience field. Accurate quantitative and comparative analysis of anatomical data require precise mapping of brain sections to a common reference atlas. The existing methods rely either on using 2D coronal atlases or 3D reconstruction prior to registration. The problem with the former is that atlases are not always a good match, since they do not account for the slicing angle. The drawback of the latter is that 3D to 3D registration methods are not only computationally expensive, but also require a full set of consecutive slices, which are not always available. In this study, a deep learning based approach is proposed to automatically detect the position and angle of individual mouse brain sections in the 3D reference atlas. The novel method is implemented as a pipeline consisting of 3 blocks of CNN regression models which detect the slicing angle and the position of the section in the anterior-posterior(AP) axis. The proposed method not only generates matching 2D atlases by taking the slicing angle into account but is also considerably faster and more robust to histological artifacts, compared to 3D brain volume to 3D atlas registration approaches. We have shown that predictions of our method are comparable to a neuroscientist expert.

Funding:

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The novel de novo variant F747S is associated with a new phenotype in CACNA1D channelopathies

Background: Germline gain-of-function missense variants in the pore-forming Cav1.3 α1-subunit (CACNA1D gene) confer high risk for severe neurodevelopmental disorder with or without endocrine symptoms. Here we report a four weeks old newborn with the novel de novo missense variant F747S with massive jittering as a new phenotype, in addition to symptoms previously reported for CACNA1D mutations including developmental delay, elevated aldosterone level, and high blood pressure. Our goal was to test for its potential gain-of-function phenotype using functional studies in combination with homology modeling to confirm the pathogenicity of this variant. We also determined the sensitivity for the brain-permeable dihydropyridine L-type channel inhibitor isradipine because DHPs could be used for symptomatic treatment.

Methods: Wild-type and F747S Cav1.3 α1-subunits were heterologously expressed in HEK-293T cells together with accessory β2a or β3, and α2δ-1 subunits and screened for pathogenic gating changes and altered drug sensitivity using whole-cell patch-clamp (15 mM Ca2+ as charge carrier).

Results: With both co-expressed β-subunits the F747S variant significantly shifted the voltage dependence of activation (23-29 mV, n>10) and channel availability (~20 mV, n>8) to more negative voltages enhancing channel activity at subthreshold membrane potentials. It significantly slowed the time course of current inactivation as well as current deactivation predicting increased inward current during action potential repolarization. With both co-expressed β-subunits F747S increased sensitivity to isradipine up to 2-4-fold compared to wild-type.

In silico modelling and molecular dynamics simulations predicted the stabilization of the activated channel state by formation of an additional interdomain hydrogen bond interaction between residues S747 (IIS6) and N1145 (IIIS6) in mutant channels.

Conclusion: Our data confirm the pathogenicity of the F747S variant by demonstrating typical gating changes allowing a channel gain-of-function and provide evidence for an even increased DHP-sensitivity suggesting isradipine as a potential symptomatic off-label treatment of this patient.

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Synthesis and biological evaluation of subtype-selective heterodimeric estrogen receptor antagonists for the treatment of estrogen receptor-positive and tamoxifen-resistant breast cancer

More than 75% of mammary carcinomas are estrogen receptor positive (ER+) and one-third of breast cancer patients will develop recurrent cancer within 15 years of endocrine treatment. Notably, tumor growth in a hormone-refractory state still relies on the interaction between ER and upregulated co-activators. Here, we synthesized a series of heterodimeric ER ligands, that simultaneously target the primary ligand binding domain (LBD) and the allosteric co-activator binding site (CABS), that may not be involved in the development of endocrine therapy resistance. Blocking this co-activator interaction in a more direct manner may provide an effective treatment option for resistant breast cancer. Therefore, a constant benzoxepine-based acrylic acid was respectively connected to pyrimidine - or benzimidazole co-activator binding inhibitors (CBIs) via flexible alkyl spacers of specific lengths, based on molecular modeling studies. The use of a benzoxepine scaffold was intended to prevent the formation of E/Z-isomers at its stilbene core during synthesis and to shift the overall selectivity towards the ERα subtype. All compounds showed higher activity than the acrylic acid precursor and inhibited estradiol stimulated transcriptional activity in the nanomolar range, preferentially at ERα (IC50 = 18.2 – 461.4 nM) vs. ERβ (IC50 = 61.5 – 2255 nM). They demonstrated strong antiproliferative effects in MFC-7 - (IC50 = 65.9 – 304.4 nM) and TamR cell lines (IC50 = 88.9 – 986.6 nM). These findings demonstrate the relevance of blocking the CABS as mode of action and provide a promising perspective for the treatment of ER-positive and tamoxifen-resistant breast cancer with bifunctional ER ligands.

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CALCIUM CURRENT MODULATION BY THE GAMMA1 SUBUNIT DEPENDS ON ALTERNATIVE SPLICING OF CAV1.1

The skeletal muscle voltage-gated calcium channel (CaV1.1) primarily functions as voltage sensor for excitation-contraction coupling. Conversely, its channel function is limited by multiple mechanisms within the pore-forming alpha1S subunit and the auxiliary alpha2delta-1 and gamma1 subunits. Particularly, developmentally regulated alternative splicing of exon 29, which inserts 19 amino acids in the extracellular IVS3-S4 loop of CaV1.1a, greatly reduces the current density and shifts the voltage-dependence of activation to positive potentials outside the physiological range. We generated a new HEK293-cell line stably expressing alpha2delta-1, beta3, and STAC3. When the adult (CaV1.1a) and the embryonic (CaV1.1e) splice variants were

expressed in these cells, the difference in the voltage-dependence of activation observed in muscle cells was reproduced, but not the reduced current density of CaV1.1a. Only when we further co-expressed the gamma1 subunit, the current density of CaV1.1a, but not of CaV1.1e, was reduced by >50 %. In addition, gamma1 caused a shift of the voltage-dependence of inactivation to negative voltages in both variants. Thus, the current-reducingeffect of gamma1, but not its effect on inactivation, is specifically dependent on the inclusion of exon 29 in CaV1.1a.

Molecular structure modeling revealed several direct ionic interactions between oppositely charged residues in the IVS3-S4 loop and the gamma1 subunit. However, substitution of these residues by alanine, individually or in combination, did not abolish the gamma1-dependent reduction of current density, suggesting that structural rearrangements of CaV1.1a induced by inclusion of exon 29 allosterically empower the gamma1 subunit to exert its inhibitory action on CaV1.1 calcium currents.

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Investigations on the axial ligand exchange reaction of iron(III)salophene complexes

It has been shown that imbalance between reactive oxygen species (ROS) and antioxidant activity may result in increased ROS level and cell death. Therefore, a number of the currently investigated chemotherapeutic drugs such as Erastin were designed to kill cancer cells by inducing the generation of ROS. Erastin is also capable to stimulate ferroptosis, a process which cause toxic lipid oxidation. The development of this new cytotoxic pathway led to the search for antitumor agents, with this mode of action. Although it was postulated that only Fe(II) ions initiate the formation of lipid-ROS, we demonstrated that tumor cells treated with the chlorido[N,N'-disalicylidene-1,2-phenylenediamine]iron(III) complex [Chlorido(Fe(III)Salophene] show mainly ferroptosis, but also apoptosis and necroptosis.

In order to study the relevance of the chlorido ligand on the biological activity, various derivatives with nitrato, thiocyanato, acetato, pyridine, 2-bromopyridine, 2-fluoropyridine, imidazole, 1-methylimidazol and oxo for the dimeric species as axial ligands were synthesized. The influence on the redox behavior was analyzed via cyclic voltammetry in different solvents, e.g., dichloromethane, acetonitrile, dimethylformamid and dimethylsulfoxid. The voltammograms reveal that these ligands significantly influence the redox potential of the Fe(II/III) redox pair in the non-dissociative solvent dichloromethane. However, in dimethylsulfoxid, the standard potential remained unchanged in comparison to [Chlorido(Fe(III)Salophene]. These findings lead to the conclusion that dimethylsulfoxid, a solvent commonly used for cell based assays, exchanges the axial ligand. Consequently, the derivatives dissolved in dimethylsulfoxid, showed identical antiproliferative properties. Interestingly, voltammograms of two Fe(III)Salophene monomers briged by an oxo ligand show different behavior in dissociative solvents.

Funding:

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Linkage of imbalances in iron metabolism and Parkinson's disease: dopamine and its impact on intracellular iron trafficking

Parkinson's disease (PD) is a frequent neurodegenerative disease primarily characterized by the degradation of dopaminergic neurons. Among the pathological hallmarks are accumulation of alpha synuclein-enriched Lewy body inclusions, neuroinflammation, mitochondrial dysfunction and oxidative stress. In addition to idiopathic, environmental or infection-associated PD cases, 5-10% of the cases have a genetic origin (e.g. SNCA, PRKN, PINK1 genes). The death of dopaminergic neurons primarily in the substantia nigra (SN) results in loss of dopamine (DA)-mediated neurotransmission causing the primary motor symptoms (tremor at rest, bradykinesia, postural instability) of PD.

Iron is involved in many cellular processes such as mitochondrial respiration, DNA synthesis, and oxygen transport, and both iron deficiency and iron overload can lead to disease. There is increasing evidence for disturbed iron metabolism in PD, in particular iron overload in dopaminergic neurons of the SN, although the precise mechanism is unresolved. Based on a recently established link between dopamine and intracellular iron content in bone marrow-derived macrophages, resulting in increased oxidative stress in mitochondria, we investigate this relationship in human SH-SY5Y neuroblastoma cells and induced pluripotent stem cell (iPSC)-derived neurons from PD patients with α-synuclein (SNCA) mutations. The treatment of SH-SY5Y cells with dopamine, iron, and dopamine combined with iron showed an increased expression of genes involved in iron metabolism such as transferrin receptor 1 (TfR1) and ferroportin-1 (FPN-1). In addition, dopamine might lead to an increased import of iron into the mitochondria by Mitoferrin-1 (Mfrn1) and Mitoferrin-2 (Mfrn2). In the iPSC-derived neurons, we found a defective mitochondrial iron handling being suggestive for higher mitochondrial iron levels in PD patients. This condition is linked to oxidative/nitrosative stress and impaired mitochondrial respiration. These alterations might be prevented/reversed by the treatment with dopamine indicating that this defective pathway is linked to an impaired dopamine availability in these cells.

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Defining the roles of VSD I, II & III of CaV1.1 in regulating calcium currents and EC-coupling

In skeletal muscle CaV1.1 functions as voltage-sensor for excitation contraction coupling (ECC). Upon membrane depolarization CaV1.1 activates the opening of RyR1, leading to calcium release from the sarcoplasmic reticulum and consequently to muscle contraction. Upon intense depolarization, CaV1.1 can also elicit a voltage-dependent calcium current (ICa); however, with kinetics and voltage-dependence of activation distinct from those of ECC. These characteristic activation properties relate to the distinct roles of the four voltage sensing domains (VSD I-IV) of CaV1.1. Each VSD comprises four transmembrane helices (S1-S4); S4 contains positive gating charges, which move outward upon membrane depolarization. The specific properties of this motion depend on interactions of the S4 gating charges with negative countercharges in the surrounding helices. Recently, our team identified critical countercharges in VSDs I and IV, which regulate the typical slow activation kinetics and right-shifted voltage-dependence of CaV1.1 activation, respectively. In contrast, based on the literature, VSDs II and III and the cytoplasmic II-III loop between them, may be involved in ECC. Using structure-guided mutagenesis and VSD chimeras together with patch-clamp analysis and fluorescence calcium recordings in dysgenic (CaV1.1-null) myotubes, we examined the involvement of individual VSDs in channel gating and/or ECC. The same VSD I countercharges involved in ICa-modulation, were found to contribute to ECC as well. Instead, neutralization of analogous countercharges in VSDs II and III altered neither the current properties nor ECC. However, transferring the long extracellular S3-S4 loop from VSD IV to VSD II, left-shifted the voltage-dependence of activation of both ICa and ECC. This, for the first time, demonstrates the involvement of VSDs I and II in both CaV1.1 functions. Whether VSD III also contributes to the activation of ECC is currently under investigation.

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Interaction of Zeise's Salt amino acid derivatives with sulfur-donor compounds of biological interest

The aqueous instability of some platinum organometallic complexes is a major challenge in platinum-based drug development and limits their use for biological purposes. Moreover, platinum exhibits a high affinity for sulfur-containing proteins and peptides, which results in the unspecific metalation of these biomolecules and consequently in a lower amount of platinum-based drug being available to exert a medicinal effect. Amino acids as chelating agents have been shown to stabilize such platinum complexes in the conditions mentioned above. Interestingly these kinds of molecules have not been tested yet for their biological activity. Here we present the synthesis of three Zeise-type platinum complexes and their reactions with sulfur-donor compounds of biological relevance. DMSO, methionine, and substance P were chosen as sulfur-containing species relevant for the in vitro assays. The platinum organometallic complexes were incubated with the selected substance in the appropriate solvent and followed by NMR spectroscopy and mass spectrometry over 24 or 72 hours. Platinum complexes with amino acid ligands seem to better retain the ethylene moiety in the presence of a sulfur-donor substance compared to the trichlorido-analogues. The obtained results suggest that amino acids chelated to platinum can improve the stability and the bioavailability of these kinds of compounds.

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A truncation mutation in Cav1.4 L-type calcium channels primarily affects the retinal rod pathway

L-type calcium channels (LTCC) are the main regulator for cellular events, like excitation-contraction coupling, transmitter release and hormone secretion. In the retina, Cav1.4 LTCCs channels are predominantly expressed at the synaptic terminals of photoreceptors in the outer plexiform layer (OPL) and required for tonic glutamate release. In patients, mutations in the CACNA1F gene, which encodes Cav1.4 channels can cause congenital stationary night blindness type 2 (CSNB2). Symptoms like abnormal visual acuity, myopia, nystagmus, strabismus and variable levels of night blindness are linked to this X-linked retinal disorder.

In this study we investigated a Cav1.4 C-terminal truncation mutation, which is caused by exchanging the base pair cytosine to thymine at position 5446 in the human CACNA1F gene and results in a stop codon at position Arg 1827 (Cav1.4 RX). Heterologously expressed Cav1.4-RX channels showed calcium-dependent inactivation, which is normally not present in this LTCC isoform. The aim of the current study is to examine morphological and functional changes in mice carrying the truncation mutation (Cav1.4 RX) in comparison to the gain-of-function mutation I756T (Cav1.4 IT) and WT retinas by using immunohistochemical and functional (multielectrode array, MEA) analyses.

Initial immunohistochemical studies showed significant changes in the morphology of Cav1.4-RX retinas. In addition to changes in the presynaptic ribbon structure, we also found neurite sprouting of second-order neurons similar to Cav1.4 IT. However, cones seemed to be largely unaffected; a finding that is in contrast to Cav1.4 IT retinas. This difference is also seen in MEA recordings in which ganglion cell responses were mainly affected under scotopic conditions suggesting that the truncation primarily affects the rod pathway. This observation might be explained by differences in the protein composition in rod and cone photoreceptor terminals. Further investigations will include also a proteomic approach needed to fully understand the molecular mechanism of the distal C-terminal part of Cav1.4 channels.

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Congenital stationary night-blindness type 2: targeting the mouse retina for future gene therapies

Congenital stationary night blindness type 2 (CSNB2) is a non-blinding X-linked retinal disease caused by mutations in the CACNA1F gene, which encodes Cav1.4 voltage-gated L-type calcium channels. CSNB2 is reliably diagnosed through electroretinography as patients' symptoms are diverse and can manifest in variable levels of night-blindness but also photophobia or low visual acuity. Since no therapy is currently available, we aim to apply gene supplementation therapy by utilizing adeno-associated viral (AAV) vectors which have proven to be highly efficient and safe in delivering genetic information into retinal cells.

Given the small size of AAVs, we consider a two-vector approach to transport the full coding sequence of Cav1.4 into mouse retinas. We therefore employed split-inteins for protein trans-splicing to reconstitute Cav1.4 channel halves in-vitro. To achieve this, the Cav1.4 coding sequence was separated in the II-III loop and intein sequences were attached. In tsA-201 cells, Cav1.4 channels successfully reconstituted and were functional as indicated by immunohistochemistry, western blot and whole-cell patch-clamp analysis.

Some nonsense mutations result in truncated channels, which lack the distal part of the intracellular C-terminus, leading to changes in voltage- and calcium-dependent activation and inactivation properties. This can be attributed to the lack of C-terminal modulation, in which normally the distal forms an electrostatic interaction with a proximal part of the Cav1.4 C-terminus. We have previously shown that co-expression of truncated Cav1.4 with the last 122 amino acids rescued biophysical wild type channel properties in vitro. Hence, we generated an AAV2/2-quadYF vector for ex- and in-vivo expression of N-terminally tagged C122 in the retina. Both, transduction of retinal explant cultures as well as subretinal injections (N = 2 and 3 respectively) indicated successful infection of retinal photoreceptors in wild type mice.

Future experiments aim to show expression of reconstituted Cav1.4 channels or the C-terminal peptide at photoreceptor terminals.

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STAC proteins inhibit calcium and voltage dependent inactivation in L-type Ca channels

Recently, we demonstrated that calcium-dependent inactivation (CDI) of L-type calcium channels (LTCC), an important negative feedback mechanism in calcium signaling, is inhibited by STAC proteins. This could be demonstrated for CaV1.2, CaV1.3 and CaV1.4, but not for the skeletal muscle channel CaV1.1, as it requires STAC3 for its functional expression. Interestingly, CaV1.1 currents show negligible inactivation, which could be either an intrinsic property of the channel or the result of an inhibitory effect of STAC3 on the inactivation of CaV1.1. In order to discriminate between these two possibilities, we inserted a triple mutation in the linker region of STAC3 (ETLAAA). In fact, the analogous mutation in the paralog STAC2 was shown to abolish the inhibitory effect on the CDI of CaV1.3. In patch clamp electrophysiology experiments in myotubes, we found that STAC3-ETLAAA coexpression results in dramatically faster kinetics of activation and inactivation of CaV1.1 currents, suggesting that STAC3 plays a role in determining the slow CaV1.1 currents kinetics. To determine if STAC3 does indeed slow down the CDI of CaV1.1 currents, we found that that CaV1.1 displays negligible CDI, which is not affected by STAC3, and that STAC3 inhibits the voltage dependent inactivation (VDI) of CaV1.1 currents. To further investigate the effect of STAC proteins on VDI, we did experiments on other LTCC. We found that STAC proteins inhibit the VDI of CaV1.2 and CaV1.3. Using the ETLAAA mutant, we could demonstrate that the inhibition of VDI relies on the same STAC linker region as CDI. Experiments co-expressing CaV1.3 with β2a with or without STAC3 revealed that STAC proteins inhibit VDI using a different mechanism compared to membrane tethered β subunits (β2a, β2e). Together, our results suggest that STAC3 slows down the kinetics of activation and inactivation of CaV1.1 and that STAC proteins inhibit not only the CDI, but also the VDI of LTCC currents.

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Spreading of P301S aggregated tau investigated in organotypic mouse brain slice cultures

Background: Neurofibrillary tangles composed of hyperphosphorylated tau protein aggregates are a key neuropathological feature of Alzheimer's disease (AD). Tau pathology is reported to spread throughout the brain in a prion-like fashion through connected brain regions. However, the details of the underlying mechanisms and general spreading characteristics are incompletely understood. The present study aims to examine the spreading of tau proteins using organotypic slice cultures.

Methods: Coronal hippocampal organotypic brain slices (170 µm) were prepared from postnatal (day 8-10) C57BL6 wild-type mice. Collagen hydrogels loaded with different tau proteins (full-length tau, K18 PHF tau, Δ306-311 tau, and P301S aggregated tau) were applied to slices and the spread of tau was assessed by immunohistochemistry after 8 weeks of culturing period. Western Blot and release experiments were performed to support the data.

Results: Collagen hydrogels prove to be an effective protein delivery system subject to natural degradation in 14 days and release tau proteins up to 8 weeks. From all four tested tau proteins, only P301S aggregated tau loaded in collagen hydrogels was detectable by Western blotting. Slices with collagen hydrogels loaded with un- and hyperphosphorylated P301S aggregated tau demonstrate significant spreading to the ventral parts of the hippocampal slices compared to empty collagen hydrogels after 8 weeks. Moreover, the spread of P301S aggregated tau occurs in a time-dependent manner.

Discussion: We illustrate that the spreading of tau can be investigated in organotypic slice cultures using collagen hydrogels to achieve a localised application and slow release of tau proteins. P301S aggregated tau significantly spreads to the ventral areas of the slices, suggesting that the disease-relevant aggregated tau form possesses more spreading potential compared to the other tau proteins. Thus, the results offer a novel insight, which sheds more light on tau spreading in AD.

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Molecular mechanisms of disease-causing mutations in the first voltage-sensing domain of CaV1.1

Voltage-gated calcium channels (CaV) control most activity-dependent functions of excitable cells. However, the molecular mechanisms by which different CaV`s respond to membrane depolarization with specific gating properties remain unknown. Voltage-dependent gating of CaV channels is determined by four distinct voltage-sensing domains (VSD I-IV) linked to a common channel pore. Each VSD consists of four transmembrane helices (S1-S4) containing four to five positive gating charges. These arginines and lysines interact with negative countercharges in the helices S2 and S3, thus facilitating the outward movement of the S4 helix upon depolarization. We hypothesize that these state-dependent interactions, which greatly differ between the four VSDs, determine the specific gating properties of CaV channels. Naturally occurring mutations of the innermost gating charge R4 (R174W) and its putative countercharge E100(K) located in the VSD I S2 helix are disease-causing. Thus, we investigated the roles of R4 and E100 in voltage-sensing and in the specific contributions of VSD I to channel gating and/or excitation-contraction (EC) coupling. We combined site-directed mutagenesis and patch clamp analysis in dysgenic (CaV1.1-null) myotubes with molecular dynamics (MD) simulations to study the effect of charge-neutralizing and charge-reversing mutations of E100 and R174. The mutation R174W previously had been shown to severely right shift voltage dependence and abolish Ca2+ currents, without affecting EC coupling. Our data demonstrate that neutralizing or reversing the countercharge E100 (E100A/E100K) lead to a left-shift in voltage-dependence of current activation. The differential effects of mutating each of the putative interaction partners are discussed, considering their specific interactions upon voltage-sensor motion, using MD simulations.

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Effect of enriched environment on hyperanxiety and neuroinflammatory dysbalance

Neuroinflammation is discussed to play a role in specific subgroups of different psychiatric disorders. We have previously shown that a mouse model of trait-anxiety (HAB) displays enhanced microglial-density and phagocytic-activity in key regions of anxiety-circuits in comparison to normal anxiety controls (NAB). Using minocycline we provided causal evidence that reducing microglia activation within the dentate gyrus (DG) attenuated enhanced-anxiety in HABs. Besides pharmacological interventions; "positive-stimuli", which have the advantage of exerting no or negligible side-effects, have been shown to attenuate inflammation in humans. Therefore, in the current study, we investigated whether environmental-enrichment (EE) as a "positive intervention" would be sufficient to modulate inflammation in hgh anxiety HABs. . We now show for the first time that EE can also attenuate enhanced-anxiety when presented during adulthood, complimenting our previous observations of such EE effects in early development. Using immunohistochemistry, we found that EE-induced anxiolysis was associated with attenuation of enhanced microglial-density in the DG and dentate gyrus (DG) and medial-prefrontal cortex. Furthermore, EE also reduced phagocytic-activity of microglia within the DG. Hence, successful attenuation of trait-anxiety by EE was associated with normalization of part of the identified neuro-inflammatory imbalances. Together with our previous pharmacological findings, these results indicate that beneficial environmental cues can partly mimic anti-inflammatory effects of minocycline in individuals predisposed to trait-anxiety. Recently, we also found sex differences in microglial-expression, microglial-morphology as well as synaptic-pruning pathways in the DG of HABs compared to NABs and we are currently investigating whether and how these contribute to EE-induced anxiolytic-like effect.

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Organotypic Mouse Brain Slices coupled with Microcontact Prints to study Aspects of Neurodegenerative Diseases

Microcontact printing allows to pattern biomolecules onto substrates capturing the spatial complexity of the extracellular environment. The proteins are printed onto semipermeable 0.4 µm pore membranes as 30 µm wide stripes using a novel "stamp system" which utilizes the biomaterial collagen. The microcontact prints were coupled with organotypic brain slices of postnatal day 10 wildtype mice (C57BL6), cultured for 2-6 weeks and immunohistochemically characterized. In a recent publication, we successfully microcontact printed NGF embedded in collagen onto membranes and cholinergic neurons of organotypic brain slices were found to grow along the NGF microcontact prints (~24 cholinergic processes/section with a length of ~300 µm). Also, we observed endothelial cells migrating out of the brain slice following the printed stripes (loaded with VEGF, FGF-2, Ang-1, PDGF-AA, PDGF-BB) and starting to form vessels. Furthermore, we showed that microglia are activated in the brain slices and migrated along the printed stripes (containing MCP-1, GM-CSF, M-CSF, SCF, tau, beta-amyloid and fluorescent microbeads), as amoeboid cells and in highly differentiated forms. Our experimental setup also allows to perform treatments during culture, such as oxygenglucose deprivation or acidosis to study the effects of ischemia. Both treatments induced microglial ramification when investigating the migratory capacity of microglia upon ischemic events. In conclusion, we provide a novel innovative microcontact printing technique on semipermeable membranes which can be coupled to organotypic brain slices. Collagen was used as loading substance and allows the microcontact printing of nearly any protein of interest. Microcontact prints coupled to organotypic brain slices could build the base for a future "triple model" to simultaneously study neurodegenerative, cerebrovascular and neuroinflammatory aspects observed in neurodegenerative diseases in vitro.

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Role of the VIP/VPAC1/2 system in the rodent brain: Implication for the regulation of stress and anxiety reactions

Vasoactive intestinal polypeptide (VIP) is a 28-amino-acid long peptide isolated first from porcine duodenum. This peptide was also found in the neural tissue, both in the peripheral and central nervous system (CNS). Notably, within the CNS, VIP and its cognate VPAC receptors have been strongly localized in stress and anxiety-related brain areas such as amygdala, lateral septum (LS), bed nucleus of stria terminalis (BNST) and some hypothalamic structures. Although of these neuroanatomical findings, the role of VIP in stress and anxiety is not well characterized. Accordingly, our first aim was to examine whether the exposure to emotional stressors leads to alterations in the expression of VIP and VPAC1/2 receptors in in selected brain areas of the stress/anxiety circuitry. Consequently, by using quantitative real - time PCR analysis, gene expression levels of brain VIP and VPAC1/2 receptors were compared in Sprague-Dawley rats exposed to either repeated swim stress or chronic variable stress with respective unstressed control groups. Our results show that both stress paradigms induced a significant change in VIP, VPAC1 and VPAC2 mRNA transcript levels in distinct stress-related brain areas (e.g. LS, BNST) compared to control animals. Our second aim was to investigate the effects of central VIP infusions on anxiety-related behaviors of rodents. Therefore, C57Bl6/J male mice were tested in the light/dark or elevated plus-maze test 15 minutes after a single intracerebroventricular injection of either VIP (0.1 or 1 µg in 0.5 µL) or vehicle (artificial cerebrospinal fluid). We found that VIP-injected animals show a reduction of time spent in aversive zones of the behavioral tests performed compared to vehicle-injected controls, suggesting an anxiogenic-like effect. Taken together, these data implicate an stress-induced upregulation of the central VIP/VPAC receptor system and highlight a crucial role of VIP signaling in the regulation of stress and anxiety functions.

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Dihydropyridines acting at T-type calcium channels

Dihydropyridine (DHPs) calcium-channel blockers are widely used to treat hypertension and thought to selectively act at L-type channels (LTCCs). DHP molecules preferentially bind to inactivated channel states, thus their IC50 gets lower with increased availability of inactivated channel states at more depolarized membrane potentials (voltage-dependent block).

An extensive literature survey revealed that there also are some DHPs reported as active at low-voltage-activated T-type channels (TTCCs) with less pronounced voltage-dependence than at LTCCs. The TTCC subfamily comprises Cav3.1, Cav3.2 and Cav3.3 α1-subunits, encoded by genes CACNA1G, CACNA1H and CACNA1I, respectively, with unique physiological, biophysical and pharmacological properties. In LTCCs, DHPs bind to a fenestration formed by repeats II and IV. To investigate the binding of DHPs to TTCCs, we have generated hCav3.1 and hCav3.2 homology models and thoroughly analysed the corresponding site in these channels. Molecular Docking calculations resulted in DHP binding poses similar to the binding mode observed for nifedipine bound to rabbit Cav1.1 (PDB accession code 6JP5), suggesting that DHPs can also bind to this site in TTCC. This is independent from the bulkiness of the DHP side chain substitution, however, our results predict that DHPs which have a bulky substituent at the DHP-ring are able to extend towards the channel pore region, potentially explaining why these compounds act in a less voltage-dependent manner.

Additionally, we hypothesize that DHPs might show higher affinity for Cav3.2 over Cav3.1 (and Cav3.3) due to the presence of a Serine residue within their DHP - binding site, which forms an H-bond with the DHP - amino group fundamental for their activity, and which is not conserved in Cav3.1 and Cav3.3.

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Machine learning model to predict the biophysical effects of point mutations in VGCC

Structure models in combination with machine learning approaches allow functional characterizations of poorly documented mutations. Nowadays, emerging new mutants and their available electrophysiological data capture functional alterations in high resolution, while studies elucidating voltage gating mechanism with atomistic details enable better understanding of biophysics behind. In this study, we optimize an existing prediction model, by extra training with external mutations from literature and by translating insights on channel dynamics into additional structural-function relationships. Our prediction model reflecting functioning mechanism of α1 subunites treat target proteins as isolated molecular machines. Different from direct experimental measurements, it is independent of additional protein-protein interactions (such as phosphorylation by kinase), complex regulations by auxiliary subunits and influences of dizzying voltage-gated calcium channel (VGCC) related pathways. Accordingly, map of functional annotations guided by point mutations resulted records pure structural influences on channel functioning, hence provides good reference control for experimental electrophysiologic measurements. Resulting differences allow to identify unique changes of voltage-current regulation in distinct domains, highlight characteristic roles of different variants to aid design of variant-specific ligands and suggest interesting sites for external regulations for future researches.

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Novel Photosensitizers from Fungi for Photodynamic Antimicrobial Therapy

Under the perspective of the global antibiotic crisis and the lack of innovative drugs to fight multidrug-resistant microorganisms our group developed a high-throughput screening (HTS) method to find and test novel photosensitizers (PS) for the photodynamic antimicrobial therapy (PACT). In this fresh approach, multidrug-resistant MOs can be killed through the synergistic effect of light and a chromophore by non-specific multi target damage through reactive oxygen species. Utilizing the ancient evolutionary battle between bacteria and fungi we isolate new PS from Ascomycota, Basidiomycota, and lichen, following a bioactivity guided approach. Our PACT experiments are based on the protocol of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), allowing the examination and comparison of potential PSs under different wavelengths, light doses, and preincubation times. Here, we report the efficient PACT killing of gram-positive bacteria (Staphylococcus aureus), gram-negative bacteria (Escherichia coli), and several yeasts, including multiple strains of Candida auris and C. albicans with active concentrations varied between 0.1 µg/mL and 3 µg/mL of our most potent PSs.

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Two-Step isolation of Protolichesterinic acid and Lichesterinic acid

Paraconic acids are a group of secondary metabolites mainly found in lichens and some species of fungi. Within this compound class, lichesterinic acid and protolichesterinic acid are of special interest due to their inhibitory activity of 5-lipoxygenase and 12-lipoxygenase. Comparable to saturated fatty acids, these two compounds show a very poor UV-light absorption, which makes them hard to detect by thin layer chromatography (TLC) using UV detection or HPLC with UV detection. Moreover, due to their very similar structure their separation is rather challenging. In the present project, we developed a two-step isolation protocol of lichesterinic acid and protolichesterinic acid by size exclusion chromatography followed by fast centrifugal separation chromatography from the commercially available lichen Cetraria islandica. In parallel, analytical methods were developed to detect these compounds by means of TLC and liquid chromatography hyphenated to a mass spectrometer or evaporative light-scattering detector. The presented workflow enables the isolation of lichesterinic acid with a purity about 96% (recovery 20.71%) and protolichesterinic acid with a purity about 95% (recovery 32.87%).

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Melatonin directly targets microglia to maintain the anti-inflammatory response to low concentrations of β-amyloid

Background. Melatonin is a hormone that exerts neuroprotective activity against neuroinflammation, a feature common to several neurodegenerative conditions including Alzheimer's Disease. A particular role in its anti-inflammatory actions seems to be associated with the up-regulation of Silent Information Regulator 1 (SIRT1), which is involved in enhancing microglial anti-inflammatory functions in response to β-amyloid protein (Aβ42) oligomers.

Methods: The human microglial cell HMC3 cells were exposed to low concentrations of Aβ42 oligomers (200 nM) and cellular extracts were analyzed by Real Time PCR (qRT-PCR), western blot (WB), immunocytochemistry, ELISA and enzymatic activity assays.

Results. Aβ42 rapidly (6 h) increased the expression of BDNF and anti-inflammatory markers, IL-4 and IL-13, as revealed by qRT-PCR. This event was accompanied by increased intracellular BDNF content and release as shown by WB and ELISA; these effects were sensitive to selective SIRT1 inhibitor EX527 (5 μM). Aβ42, through the activation of pAMPK signaling, induced early (6 h) expression of SIRT1, that translocated to the nuclear compartment and increased its activity as shown by WB, immunocytochemical analysis and activity assay. In contrast, after prolonged exposure (72 h), Aβ42 significantly increased mRNA expression of pro-inflammatory markers, TNFα and IL-1β and nuclear localization of NF-kB, coincident with return to basal levels of BDNF and SIRT1. Melatonin (1 μM), added in combination with Aβ42, kept high BDNF levels and reduced NF-kB nuclear translocation in a SIRT1-dependent manner.

Conclusions. In response to low concentrations of Aβ42, microglia polarize towards an anti-inflammatory phenotype, an effect mediated by the pAMPK/SIRT-1 pathway. Melatonin, through SIRT1 activation, is able to sustain this phenotype at later times, when the switch into a pro-inflammatory phenotype has already occurred. Our data confirm melatonin as a potential pharmaceutical agent for modulation of microglia function.

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PATIENT-DERIVED STEM CELLS TO STUDY THE PATHOLOGY OF AUTISM SPECTRUM DISORDERS-RELATED VOLTAGE-GATED CALCIUM CHANNEL GAIN-OF-FUNCTION MUTATIONS

Voltage-gated calcium channels (VGCCs) are involved in many physiological processes and are highly expressed in human cardiac, endocrine and brain tissues. In addition, increasing evidence emerges that VGCC are key modulators of early neurodevelopment. VGCC gain-of-function mutations, as observed in the Cav1.3 encoding CACNA1D gene, have been linked to a range of neurological pathologies, including Autism Spectrum Disorders (ASD). One such mutation affects the Cav1.3 L271 residue, which is highly conserved among VGCC pore-forming α1-subunits. Electrophysiological studies in tSA-201 cells overexpressing Cav1.3 L271H indicate that this mutation induces channel gain of function by lowering the voltage dependency of channel activation and inactivation, thereby permitting increased subthreshold inward Ca2+ currents. However, currently no functional studies are available on how this mutation affects early neurodevelopment or the physiology of disease-relevant human neurons.
Here, we describe the generation of an induced pluripotent stem cell (iPSC)-line, carrying the heterozygous Cav1.3 L271H mutation, through reprogramming of peripheral blood mononuclear cells (PBMC) obtained from a patient diagnosed with a severe neurodevelopmental disorder. By employing Sendai virus OSKM vectors, we have generated stable iPSC lines expressing pluripotency markers. The obtained Cav1.3-mutant lines can be differentiated into all three germ layers and show a normal karyotype. Additionally, a neural progenitor cell (NPC) line, expressing NPC markers SOX2, NESTIN and PAX6, has been generated. We demonstrate that these NPCs express Cav1.3, as confirmed by RT-qPCR, and that these cells can readily be used for in vitro differentiation into neurons typically associated with abnormal Cav1.3 activity, such as dopaminergic neurons.

We will present a comprehensive analysis including immunostainings, electrophysiological recordings and calcium imaging aimed to investigate how the Cav1.3 L271H mutation interferes with neural differentiation and neuronal function. Overall, this study will broaden our knowledge regarding the role of Cav1.3 channels during neurodevelopment and their pathogenic role in CACNA1D channelopathies, thereby paving the way for novel therapeutic strategies for affected individuals.

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Cellular Uptake study of new platin complexes generation

Cisplatin, has been used as anticancer drugs since 1978 and remains the drug of choice in the therapy of about 40% of human tumours to date. Despite its high efficacy, the side effects are severe and many research groups are trying to develop new derivatives with optimized pharmacological properties.

This project aims to synthesize Zeise´s salt complexes as novel anticancer agents. Potassium trichlorido[η2-((prop-2-en/but-3-en)-1-yl)-2-acetoxybenzoate]platinates (Prop-AAS-PtCl3 and But-AAS-PtCl3) are stable enough to be used as anti-tumor agents. Several biological data have been collected for these compounds, however, cell accumulation studies explaining the diverse cytotoxicity are missing. Therefore, an established high resolution continuum-source atomic absorption spectrometry (HR CS AAS) was improved, especially the optimization of pyrolysis and atomization stages during the analysis.

Additionally, various modifiers were used, such as HNO3 at various concentrations also in combination with Triton X. The method was validated using Cisplatin and Prop-AAS-PtCl3 and But-AAS-PtCl3 derivatives. With the improved method, cell uptake could be correlated with IC50 values obtained in MTT assays. High cytotoxicity agrees with higher cellular uptake, whereas inactive Zeise´s derivatives show insufficient cellular platinum levels.

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Grazia Larosa, Alexander Weininger, Jessica Sagasser

Molecular candidates in the nucleus accumbens shell involved in the protective effect of social interaction when available as an alternative to cocaine

Social interaction, when available in a distinct context from the one associated with drug consumption, is able to eliminate preference for cocaine and prevents against cocaine relapse, as shown in a conditioned place preference (CPP) paradigm. However, it is not known how this shielding effect is mediated.

In a first study, we investigated the involvement of an intracellular pathway, the calcium/calmodulin-dependent protein kinase II (CaMKII), in the nucleus accumbens (NAc), in the expression of reward-related learning of cocaine versus social interaction reward. We observed an increased αCaMKII expression in the NAc of rats that expressed social interaction CPP. In another experiment, we inhibited CaMKII in the NAc shell (NAcSh) or core before testing rats for CPP expression in a concurrent model where social interaction was available in a different context to the one associated with cocaine. Whereas vehicle infusions led to equal preference for both stimuli, inhibition of CaMKII in the NAcSh, but not in the core, shifted the rats' preference toward the cocaine-associated context. This suggests that social interaction reward engages NAcSh CaMKII.

In a second study, we explored corticotropin-releasing factor (CRF) system modulation of social interaction reward. In a cocaine CPP, (ICV) CRF-infused rats displayed increased preference for cocaine, an effect reversed in rats that received the CRF receptor antagonist, α-helical CRF. Importantly, when social interaction was available alternatively to cocaine, the CRF-induced increase in cocaine preference was completely reversed to the level of rats that received cocaine and α-helical CRF. This was paralleled by a decrease in NAcSh p38 MAPK expression as well as a decrease in the number of incorrect cephalocaudal grooming transitions - evaluated as stress markers.

These results suggest that social interaction positive effects against cocaine abuse are mediated by rewarding effects via CaMKII and anti-stress effects via p38 MAPK, in the NAcSh.

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Towards a Novel 3D Model of the Blood-Brain Barrier

Rare neurological autoimmune diseases like aquaporin 4 (AQP4) seropositive neuromyelitis optica spectrum disorder (NMOSD) or myelin oligodendrocyte glycoprotein (MOG) antibody associated disease (MOGAD) are associated with autoantibodies targeting neuronal or glial antigens. Under physiological conditions, the highly selective blood brain barrier (BBB) prevents those antibodies from gaining access into the central nervous system (CNS). However, after immune-mediated activation, the BBB becomes permeable, thus facilitating the penetration of autoantibodies into the CNS. The underlying mechanisms are still not fully understood and human tissue culture models are urgently needed. Here, we report a novel 3D printed model of the BBB to investigate the migration of autoantibodies through the BBB and their pathogenic effects on glial cells. Specifically, a cell-laden hydrogel is 3D inkjet printed onto a laser cutter-fabricated chip containing either human astrocytoma cells (U373) expressing AQP4 or human oligodendrocytoma cells (MO3.13) expressing myelin oligodendrocyte glycoprotein (MOG). To resemble the BBB, microchannels coated with Geltrex as a basement membrane substituent and a layer of human umbilical vein endothelial cells (HUVEC), were introduced. Hydrogel composition and antigen expression of cells were optimized to reach good cell viability up to 8-12 days. Tightness was tested by dextran- and antibody-diffusion assays and observed under a fluorescence microscope. Dextran-diffusion assays showed tight HUVEC layer formation as most of the dextran remained inside the channels. Diffusion assays with fluorescent-labeled antibodies showed spreading into the hydrogel and specific binding of the target cells after insertion into uncoated channels. This promising model of the BBB could help elucidating pathological mechanisms and BBB function in neurological autoimmune diseases such as NMOSD and MOGAD. Further optimizations and experiments will include cell and extra cellular matrix component refinements, implementation of a perfusion system, introduction of other antibodies and cytokines and investigating the pathogenicity of autoantibodies to astroglial, oligodendroglial and neuronal antigens.

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Peripheral sensory neurons involved in regulating postoperative cognitive performance

Inflammatory reactions are related to mental disorder and neuroinflammatory components, especially in the medial prefrontal cortex (mPFC). Post-operative cognitive deficits (POCD), particularly of memory, attention and mental flexibility, develop in approximately one third of patients after surgery and are maintained in ~12% after three months. This project aims to contribute to a better understanding of POCD pathogenesis and elucidate the pathways and mechanisms of the interactions between the peripheral nervous system and the brain. Interleukin 6 (IL-6), its receptor (IL-6R), and the IL-6 signal transducer gp130 (IL-6ST) are known regulators of the inflammatory responses, which have been found involved in enteric neuron activation as well as gut-brain communication and cognitive performance. Therefore, to identify the role of sensory neurons expressing gp130 in the development of postoperative cognitive dysfunctions, we used a transgenic mouse model in which IL6ST is conditionally depleted in sensory neurons expressing the voltage-gated sodium channel Nav1.8 (SNS-gp130-/-). Mice of both sexes, alongside littermate controls (gp130fl/fl) were subjected to the established spared nerve injury model (SNI) for neuropathic pain. Cognitive and emotional performance were investigated 14 days after peripheral nerve injury through the assessment of mice behaviors using the open field test, novel object recognition test, marble burying test, behavior box test and the forced swim test. Preliminary results suggest differences between the two genotypes as well as sex-specific alterations. In addition, we will characterize transcriptomic signatures of relevant brain regions affected by the intervention and perform electrophysiology measurements to reach the overall aim and gain new insights into how surgical interventions affect brain function.

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Autism-associated A749G de novo mutation in Cav1.3 pore-forming subunit alters dendritic spine morphology

Voltage-gated L-type Ca2+ channels Cav1.2 and Cav1.3 play an important role in neuronal development by regulating dendritic refinement and synaptic connectivity. In mice, Cav1.3 knockout decreases rates of adult hippocampal neurogenesis and reduces dendritic complexity. Moreover, Cav1.3 short splice variants increase dendritic Ca2+-currents in cultured neurons and induce aberrant dendritic spine elongation. Previously, we showed that de novo missense mutations in the Cav1.3 α1-subunit confer a high risk for neurodevelopmental disorders by causing increased Ca2+-influx at subthreshold potentials compatible with a gain-of-function phenotype. However, how these pathogenic Cav1.3 variants affect synaptic structure and mainly dendritic spine morphology remains unclear.

Here we investigated the effect of the Cav1.3 α1 (CACNA1D) A749G mutation, found in a patient with autism spectrum disorder and intellectual disability, on the dendritic spine morphology and density. We expressed HA-tagged and untagged Cav1.3 WT and mutant channels in cultured hippocampal neurons and evaluated spine morphology parameters. Compared to wild-type, expression of the HA-tagged and untagged A749G α1-subunits induced a significant increase in dendritic spine and fiber length but had no significant effect on spine density. Consequently, the proportions of thin and filopodia-like spines were higher in A749G transfected neurons compared to WT. Additionally, we observed a reduction in spine shape factor with the A749G mutant, which indicates an elongation of the entire population of dendritic spines. We currently investigate if the mutation also affects channel surface expression and if similar changes are observed in Golgi-Cox-stained neurons from a knock-in mouse model carrying the A749G mutation.

Our findings demonstrate aberrant spine morphology caused by the autism-linked Cav1.3 mutation A749G. This suggests that neurodevelopmental deficits of Cav1.3 gain-of-function mutations are caused by altered local calcium signaling leading to changes in spine structure and likely synapse stability. Our results provide further proof that CACNA1D is a high-risk gene for neurodevelopmental disorders.

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Consequences of alpha2delta subunit mutations linked to brain disorders on neuronal calcium channel trafficking and synapse composition

The roles of auxiliary alpha2delta subunits of voltage-gated calcium channels in modulating membrane expression and calcium current properties are widely recognized. In addition, recent literature suggests an important role of alpha2delta proteins in synapse formation and differentiation. Therefore, it is not surprising that alpha2delta genes have been linked to neurological and neuropsychiatric disorders, emphasizing their importance in brain connectivity.

Here we aimed to investigate human mutations in alpha2delta proteins by addressing their synaptic functions, besides their role as channel subunit, to shed light on the underlying pathophysiological mechanisms.

We characterized two mutations, the autism-associated mutation p.Arg351Thr in alpha2delta-1 (CACNA2D1) and the epilepsy-related mutation p.Arg596Pro in alpha2delta-2 (CACNA2D2) , cloned into mouse cDNA, by employing primary hippocampal neurons as homologous expression system. To this end we quantified plasma membrane trafficking and analyzed potential consequences on synaptic composition. To determine potential effects on the biophysical channel properties, we performed electrophysiological recordings after heterologous expression of either Cav2.1 or Cav1.3 together with the mutated alpha2delta and auxiliary beta subunits.

Live-cell labelling of cultured hippocampal neurons transfected with 2HA-tagged alpha2delta subunits revealed a strong reduction in membrane and synaptic targeting of both mutants. However, only neurons transfected with alpha2delta-2_p.Arg596Pro showed a significantly reduced mismatched localization of postsynaptic GABAARs opposite glutamatergic nerve terminals, a previously identified synaptogenic function of alpha2delta-1 and alpha2delta-2 splices lacking exon 23. Similarly, preliminary electrophysiological analysis indicated that current density of Cav2.1 was not compromised by alpha2delta-1_p.Arg351Thr, contrary to co-expression alpha2delta-2_p.Arg596Pro, which resulted in a strongly reduced current density.

Structural homology modeling predicts an important role of Arg351 and Arg596 in stabilizing the interaction of alpha2 peptide with alpha1 subunit as well as with delta peptide. Whether this destabilization affects alpha2delta rescue potential of the severe synaptic phenotype in alpha2delta triple-knockout model will next be investigated.

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Exploring behavioral deficits and hippocampal alterations in a mouse model of Fabry disease

Accumulation of glycosphingolipids in internal organs and the nervous systems is a main characteristic of Fabry disease (FD). This lysosomal storage disorder is caused by a defective x-chromosomal α-galactosidase A (αGal) gene, leading to dysfunctions in the metabolism of neutral glycosphingolipids, mainly globotriaosylceramide (Gb3). FD patients develop neurological symptoms such as cognitive deficits with older age which may be caused by Gb3 accumulating in diverse regions of the central nervous system. We therefore explored Gb3 accumulation and behavior during the progression of FD in a murine transgenic α-Gal A-/0 (Gla-KO) model of FD.

To determine the effect of Gb3 accumulations in brains of FD mice, we performed immunohistochemistry using anti-Cd77 for Gb3, anti-Cd31 for blood vessel (endothelial cells), and anti-Tmem119 for microglial morphology. Behavioral differences were assessed in Gla-KO and wild type (wt) mice using the open field test for general motor behavior and anxiety, the Rotarod test for sensorimotor coordination, as well as the Barnes maze for spatial memory assessment.

We found Gb3 accumulation in the hippocampal dentate gyrus, a brain area that is involved in the spontaneous exploration of novel environments. Gla-KO mice showed decreased thickness of blood vessels and alteration of microglia morphology characterized by a smaller number of intersections of microglial processes. Overall, Gla-KO mice showed deficits in exploratory behavior and duration until task completion in the Barnes maze test. No alterations were observed in the Rotarod test.

To summarize, Gb3 accumulation in the hippocampal dentate gyrus of Gla-KO mice was associated with a deficit in exploration of new environments but not sensorimotor coordination, suggesting that region-specific structural alterations might be causally involved in cognitive deficits in FD. These findings might link Gb3 accumulation to neuroimmune and vascular changes as a potential cause of dementia developing in FD patients at older ages.

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NOVEL INSIGHT INTO THE DEVELOPMENT OF INFLAMMATORY HEAT HYPERALGESIA: ROLE OF THE MITOGEN- AND STRESS-ACTIVATED KINASE 1 SIGNALLING IN PRIMARY SENSORY NEURONS

Recent studies estimate that up to 50 % of people experience long-term inflammatory pain in their life. Approved and currently used "symptom based" analgesic drugs show low efficacy and half of the treated patients still report unacceptable pain levels during treatment. This results in physical and mental comorbidities leading to a reduced quality of life. Thus, a better understanding of the mechanisms involved in nociceptive processing is needed to develop effective mechanism-based treatments for inflammatory pain. In the current project we explore molecular pathways involved in the development of heat hypersensitivity in primary sensory neurons associated with inflammation of peripheral tissues stressing the focus on the mitogen-and stress activated kinase 1 (MSK1).

Since the capsaicin-sensitive TRPV1 ion channel is the most relevant transducer of noxious heat stimuli, we performed patch-clamp recordings in cultured mouse dorsal root ganglion neurons exposed to capsaicin. The effect of inflammatory mediators and the role of MSK1 in the modification of the channel was assessed.

Cells were exposed to repeated 3-second applications of capsaicin (250 nM) with 3-minute intervals. After the 3rd capsaicin application, a conditioning stimulus with inflammatory soup (containing Bradykinin, Prostaglandin E2, Norepinephrine and Histamine, each at 1 muM final concentration) was applied. Inflammatory soup application increased the capsaicin-induced excitatory inward currents in MSK1 knockout as well as in littermate control mice.

These results indicate that the TRPV1 channel was not modified by MSK1 knockout in this short-term inflammatory in-vitro pain model. To provide further mechanistic insights, expression and phosphorylation of MSK1 and downstream targets will be addressed in ongoing experiments.

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Machine learning in ENT oncology

In this project we will use Radiomics methodologies in order of helping and monitoring ENT oncology. Neck lymph nodes are major negative predictors of head and neck squamous cell carcinoma, hence evaluating their Radiomics features is very useful for clinical prediction.

Extracted features from segmented computer tomography scans will be fed into feature selection algorithms, which will allow to classify the lymph nodes.

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Sara Naccour

Gender differences in applied tidal volume with compliance titrated flow-controlled ventilation during cardiac surgery – a subgroup analysis of a randomized clinical trial

Background

Flow-controlled ventilation (FCV) provides a continuous gas flow and coupled with direct tracheal pressure measurement a precise determination of dynamic compliance is feasible. Accordingly, not only positive end-expiratory pressure (PEEP), but also peak pressure can be titrated to achieve the highest dynamic compliance. This personalized ventilation approach leads to an automatic adaption of the applied tidal volume to the functionally available lung tissue within individual lung mechanic limits, which may differ between female and male patients and represents the rationale for this sub-group analysis.

Materials and Methods

A sub-group analysis of 24 patients randomized to receive flow-controlled ventilation in cardiac surgery without ventilation during the cardiopulmonary bypass period was performed. Ventilation was established with compliance titrated PEEP and peak pressure settings and the flow adjusted to maintain normocapnia at an I:E ratio of 1:1.

Results

Whereas in women (n=6) and men (n=18) PEEP and peak pressure settings were similar after compliance guided titration, the resulting tidal volume was significantly lower in female patients (8.6 vs 9.9, 95% CI -2.3 to -0.2 ml/kg PBW; p=0.029) compared to male individuals. Concomitantly, female patients had a significantly lower compliance (49.3 vs 70.3, 95% CI -33.1 to -8.8 ml/cmH2O; p=0.003) compared to men. Gas exchange parameters were comparable in either gender.

Conclusion

Female patients were found to receive lower tidal volumes after compliance guided pressure settings with FCV compared to men during cardiac surgery. This finding may indicate that the functionally available lung volume in women is lower and thus using predicted body weight (PBW) does not adequately comply with sex related differences. This supports the use of a personalized ventilation strategy with FCV.

Funding:

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LCBiome- Deciphering the host-microbiome crosstalk in lung cancer

Background: Within the tumor microenvironment (TME) of lung cancer, the local microbiota is believed to tightly interact with the heterogenous cell populations of the host, particularly the immune cell compartment, potentially modulating (anti-tumor) immune reactivity. Both the tumor microbiota composition and the mechanisms behind this host-microbiome interaction is poorly understood.

Aim: To in-depth map the microbiome composition together with the TME (of both the tumor tissue and the respective bronchoalveolar space) on a genomic level and to compare this to healthy tissue. This approach will help to identify potential bacterial patterns that directly influence the TME.

Methods: We will use a multi-omic approach by applying next generation sequencing techniques to analyze both tumor and healthy lung tissue as well as bronchoalveolar lavage fluid in 5 patients with early stage non-small cell lung cancer. The analyses include single cell RNA sequencing (host TME), whole genome sequencing (microbiota) and whole exome sequencing (tumor antigenicity). These in-depth data will be combined by bioinformatic analysis to decipher a potential host-microbe crosstalk in non-small cell lung cancer (NSCLC).

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Effect of recombinant von Willebrand factor concentrate on platelets in critically ill patients with and without sepsis: an ex-vivo approach

Background:

Critically ill patients present a variety of coagulation disorders. Primary haemostasis in septic patients presents such a form of an altered status with higher levels of ultra-large von Willebrand factor multimers (ULVWM), which is "stickier" than its smaller cleavage products, and dysfunctional platelets. When critically ill patients develop severe cardiac and/or pulmonary dysfunction, mechanical circulatory support (MCS) devices such as extracorporeal membrane oxygenation (ECMO) or Impella (a special heart pump) may be necessary to prevent more serious injury or death. The use of these systems is known to impair platelet function and cause a loss in high-molecular-weight (HMW) von Willebrand factor (vWF) multimers leading to a bleeding disorder called acquired von Willebrand syndrome (avWS). This disease presents one reason for the high bleeding rates in patients on MCS, where application of vWF concentrate with a high ULVWM content may pose an appropriate treatment strategy. However, vWF concentrate's effect on platelet function, especially in critically ill patients with an altered inflammatory response, is not known.

Material & Methods:

Healthy persons and critically ill patients are investigated. Blood from healthy persons is drawn at a single time point. The investigated groups of critically ill patients are: patients without sepsis and ECMO, patients with sepsis and ECMO, patients with Impella. Blood from patients of these groups is drawn directly before start of, 24 hours after start of, and 24 hours after stop of MCS. Restoration of platelet function and the effect on platelet characteristics of ex-vivo-added recombinant vWF concentrate (containing a higher proportion of ULVWM) are then analysed at each time point and compared. Multiplate® (including RISTOtest), PFA-100® and fluorescence activated cell sorting (FACS; e.g., expression of CD41/61, CD62P, CD42b, platelet-bound vWF) are performed apart from platelet count and volume.

Status:

Awaiting ethic committee's approval.

Funding:

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Sequential liver and hematopoietic stem cell transplantation in a child with erythropoietic protoporphyria: a case report.

Background

Erythropoietic protoporphyria (EPP) is a rare genetic photodermatosis resulting from a deficiency of ferrochelatase, an enzyme of the heme-biosynthesis pathway. This leads to increased production of protoporphyrin, which accumulates in skin and liver endothelium. In some cases, this results in progressive liver disease requiring liver transplantation. As phototoxicity may exacerbate EPP, filtered light is required during all procedures including liver transplantation. Hematopoietic stem cell transplantation is the eventual curative therapy.

Case presentation

We report a case of a 7-year-old girl with EPP and liver cirrhosis who successfully underwent a sequential liver and hematopoietic stem cell transplantation. Primary stem cell transplantation was no option because of hepatic toxicity of conditioning regimen. The preparation for liver transplantation included avoidance of exposure to non-filtered light in every step of the way. To avoid phototoxic injury during liver transplantation special light filters blocking the harmful wavelength of light (320-470 nm) were used. Measures to avoid regular light exposure were kept in place for two weeks. This became relevant upon an intervention for acute bleeding on day 8. Otherwise, the surgical postoperative course was uneventful. Prolonged hyperbilirubinemia was attributed to the persistent protoporphyrin injury to the new liver. Ongoing protoporphyrin lowering therapy with regular therapeutic plasma exchange was conducted until allogenic stem cell transplant was eventually performed 10 weeks later.

Conclusion

Sequential liver and hematopoietic stem cell transplantation is a curative treatment option in selected patients with end-stage liver disease due to EPP. Perioperative management includes use of special light filters to prevent phototoxicity and protoporphyrin lowering therapy with regular therapeutic plasma exchange.

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Intrapancreatic Presence of Malassezia spp. And Pancreatic Ductal Adenocarcinoma development – A Pilot Study

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is associated with an extremely poor prognosis. Hence, there is an urgent need to identify new diagnostic markers and therapeutic targets. Recent findings suggest a critical role of the genus Malassezia ssp. in PDAC tumor progression.

Methods: We retrospectively analyzed formalin-fixed paraffin-embedded pancreatic specimens extracting fungal DNA using QIAamp DNA FFPE Tissue (Qiagen). PCR analysis was performed using both primers for the internal transcribed spacer (ITS) of the nuclear ribosomal DNA of Malassezia ssp., as well as primers specific for different Malassezia species. Results were compared with demographic data, histologic features, and patients´ risk factors.

Results: 19 specimens were analyzed, including 10 PDAC, 5 intraductal papillary mucinous neoplasms (IPMN), 2 chronic pancreatitis, 1 autoimmune pancreatitis and 1 serous cystic neoplasm. Specimens included tumoral as well as peritumoral tissue. Male to female ratio was 11:8. The median age was 63 (range 30-81). No significant correlation was identified between sex and malignant entities. Also, the distribution of risk factors like smoke and alcohol did not differ significantly between malignant and benign samples. No gender specific differences were observed regarding fungal colonization of the samples and none of the considered risk factors were significantly associated with positivity for Malassezia. In all the 10 samples derived from malignant diseases positivity for Malassezia species was identified. In contrast, only 4 benign samples were positive (p=0.006). Among the positive benign samples 3 of them were precursor lesions, 1 IPMN, 1 chronic pancreatitis and 1 pseudotumor by autoimmune pancreatitis. Of note, there was no correlation between preoperative invasive interventions and the presence of Malassezia spp.

Conclusion: We identified the presence of Malassezia spp. in the pancreatic tissue with a predominance in samples of PDAC as well as its precursor lesions. These findings open new scenarios for feature diagnostic as well as therapeutic interventions.

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Are there gender differences in the distribution of dendritic cells and T cells within the skin tumor microenvironment?

Immunophenotyping of tumor infiltrating immune cells become important for diagnostic and treatment decision for skin cancer patients. Since significant gender differences in the incidence, progression and response to cancer therapy have been widely described in the literature, we investigated the microenvironment of different skin cancer types such as actinic keratosis, squamous cell carcinoma, basal cell carcinoma, and melanoma and focused on gender differences. The presence and localization of dendritic cells (Langerin, CD1a+) and T cells (CD3+) within the tumor microenvironment were analyzed using immunofluorescence. For this purpose, we retrieved FFPE blocks of different skin tumor types from the archives of the Department of Dermatology, Medical University Innsbruck, and performed immunofluorescence stainings with antibodies against CD1a and CD3.

We found CD1a+ dendritic cells ($p=.0035$) and CD3+ T cells ($p=.0093$) in different locations, without significant difference between men and women. Higher levels of CD1a+ dendritic cells than CD3+ T cells were detected within the tumor and the epidermis ($p<.001$), in both men and women. Most CD3+ T cells were in close vicinity to the tumor margin.

Our next aim is to establish multiplex stainings of the immune cells in the tumor microenvironment, that enables the simultaneous detection of multiple immune cell markers (CD1a, CD3, CD56, CD45). In addition, we want to compare these data with FACS data obtained from the same tumor entity.

Funding:

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Comprehensive mapping of tumor microenvironment in surgically resectable non- small cell lung cancer (NSCLC) treated with immune checkpoint inhibitors and anti- angiogenic treatment in an window of opportunity trial (INN WOP-1)

Background: Cancer cells are tightly embedded within a multi-facetted TME and are in constant interaction with surrounding stromal cells. The INN WOP 1 study, which is the basis of this PhD project, is an investigator-initiated experimental trial evaluating neo-adjuvant immune checkpoint blockade (pembrolizumab) and anti-angiogenic therapy (lenvatinib) in surgically resectable, non-metastatic NSCLC. Tumor tissue will be analysed before and after the neoadjuvant treatment to gain insights in the TME under therapy pressure. Primary endpoint of the clinical trial is the rate of major pathological response.

Methods: We generated single cell suspensions for in-depth single cell analysis such as scRNA-seq (BD Rhapsody) as well as high dimensional flow cytometry (HD-FC with FACSymphony) from diagnostic NSCLC biopsies and resected tumor samples after neo-adjuvant combinational therapy. In parallel we obtained blood samples and stool samples at different time points to perform HD-FC to characterize circulating immune cells as well as gut microbiota. Multiplex cytokine analysis panels are planned from freezed samples sequentially.

Preliminary Results: Generating single cell suspension from diagnostic lung biopsies and surgical samples for HD-FC and scRNAseq has been successfully established, which is a crucial step of the feasibility of the project. We identified stromal cell clusters such as multi-lineaged immune cells (T and B cells, NK cells, myeloid cells), endothelial cells, fibroblasts and epithelial/alveolar cells by flowcytometry. ScRNAseq has been successfully performed in three patients sequentially from biopsy cores and surgical samples, sequencing data by scRNAseq is still being processed and pending. Results will be eventually presented at the meeting.

Aims and Outlook: The techniques used in the project and the crucial feasibility for biopsy cores are fully established. The clinical trial is ongoing primarily in the screening phase and currently recruiting patients. It is planned to include approximately 30 patients and further monitor disease kinetics via circulating tumor DNA.

Funding:

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Systemic sclerosis-associated interstitial lung disease: a study update from the COLIPRIS-registry

Introduction: Interstitial lung disease (ILD) is a frequent organ manifestation of systemic connective tissue diseases, especially systemic sclerosis, with up to 50 % of patients affected. Current evidence suggests that patients with a specific antibody profile are at higher risk for ILD development. Specifically, the presence of Scl-70, RNA polymerase III, and Ro52 antibodies have been associated with increased ILD prevalence and disease severity.

Methods: This is a prospective observational study of systemic sclerosis patients aiming to gather clinical examination, laboratory testing, lung function parameters, and radiologic findings at baseline, six and twelve months to characterize the study cohort, identify the prevalence of ILD, and determine risk factors of ILD development. Long-term follow-up analyses are planned for three, five, and ten years after study entry.

Results: Currently, 64 patients with systemic sclerosis are included in the analysis, 57 (89%) being female and 7 (11%) being male with a mean age of 49 (☒4) years at study entry. The mean disease duration at study entry was 124 (☒25) months. Raynaud's phenomenon was the most frequent symptom with 60 (94%) patients affected, while telangiectasia and digital ulcers were less frequent with 25 (40%) and 8 (13%) patients affected. 25 (39%) presented with the diffuse cutaneous subtype, while 39 (61%) presented with the limited cutaneous subtype. High-resolution computed tomography of the chest showed signs of ILD in 34 (53%) of patients, occurring more frequently in Scl-70 positive patients compared to CENP-B/A positive patients (86% vs. 33%, $p < .05$). Forced vital capacity and diffusion capacity for carbon monoxide did not differ between the groups.

Discussion: Systemic sclerosis-associated ILD depends on antibody profile. Lung function parameters currently used for screening could not predict ILD development. Follow-up analyses including inflammatory and pro-fibrotic markers and their relationship to occurrence and severity of ILD and antibody profile in the COLIPRIS-registry are yet to be completed and awaited to characterize systemic sclerosis-associated ILD better.

Funding:

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Preliminary data on a fully automated left ventricular late gadolinium enhancement detection by a convolutional neuronal network in chronic myocardial infarction

Aim: To compare a fully automated segmentation of left ventricular late gadolinium enhancement (LGE) as evaluated by a convolutional neuronal network (CNN) with manual segmentation in chronic myocardial infarction.

Methods: Cardiac magnetic resonance imaging including two-dimensional LGE imaging was performed in 191 patients on a 1.5 T clinical scanner 12 months after ST-elevation myocardial infarction. LGE images were presented to a trained CNN for automated determination of left ventricular myocardium and consequently absolute LGE volume. Manual LGE segmentation according to the +5-SD method was used as reference standard. Image quality was assessed according to a 3-point Likert scale (2 = perfect image quality, 1 = some artifacts witout impaired LGE delineation, 0 = strong artifacts with impaired LGE delineation). Regression and Bland-Altman analysis were performed.

Results: In 191 included patients (182 male, mean age 57 years) LGE volume was 9.7 [IQR 3.6 to 16.2] ml according to manual segmentation and 8.3 [3.2 to 17.6] ml according to CNN segmentation. Bland-Altman analysis showed little average difference (-0.5 ml, $p=0.257$), however, limits of agreement ranged from -18.4 ml to 17.5 ml. Linear correlation was fair (0.57, $p<0.001$). Subgroup analysis according to image quality showed comparable performance of CNN segmentation in all three groups.

Conclusion: Our fully automated LGE segmentation based on a CNN in two-dimensional data sets provides measurements with little average difference compared to very time-consuming manual segmentations. However, dispersion is substantially and limits the current application of this approach on a per-patient basis. Image quality does not affect CNN performance.

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Deep learning analysis of vascular imaging biomarker in vascular diseases

The accurate segmentation of cervical arteries from computer tomography (CT) images is a difficult challenge in radiology. Its automation, however, will allow for a quantitative analysis of arterial geometrical structure for the use in large cohort patient studies. Although convolutional neural networks have achieved state-of-the-art results for numerous segmentation tasks in medical imaging, the large memory requirements for processing 3D CT angiography images as well as a lack of manually annotated training data prevent straightforward application. We present a method to extract the region of interest (ROI) using intensity based gaussian windowing to highlight the arteries. Then, the ROI is extracted by using Radon transforms to find the parallelepiped that covers most of the remaining pixel intensity. Convolutional neural networks are used to acquire the segmentation masks.

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Association between inflammation and left ventricular thrombus formation following ST-elevation myocardial infarction

Background: Current evidence suggests a link between the inflammatory state and left ventricular thrombus (LVT) formation following ST-elevation myocardial infarction (STEMI). However, a comprehensive study investigating the association between inflammatory biomarkers and LVT diagnosed by cardiac magnetic resonance (CMR) is lacking.

Methods: We studied 309 patients with acute STEMI treated with primary percutaneous coronary intervention (pPCI) from the prospective MARINA-STEMI cohort study. Concentrations of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), white blood cell count (WBCc), fibrinogen and D-dimer were measured two days after STEMI. Infarct characteristics and presence of LVT were assessed with the use of contrast-enhanced CMR at a median of 4 (interquartile range [IQR] 3-5) days after pPCI.

Results: In total, 309 STEMI patients (18% female) with a median age of 57 (IQR 52-65) years were included. An LVT was observed in 8% (n=24) of the overall cohort and in 15% of patients with an anterior STEMI. Hs-CRP (OR:2.16, 95% CI:1.54-3.02,p<0.001), IL-6 (OR:2.38, 95% CI:1.48-3.81,p<0.001) and fibrinogen levels (OR:2.05, 95% CI:1.40-3.00,p<0.001) were significantly associated with presence of LVT. Among all assessed inflammatory biomarkers, only hs-CRP was independently associated with LVT after adjustment for markers of inflammation and CMR parameters (OR:1.77, 95% CI:1.21-2.59, p=0.004).

Conclusion: In patients with STEMI treated with pPCI, inflammatory markers (hs-CRP, IL-6 and fibrinogen) are associated with the presence of LVT. However, only hs-CRP was independently associated with the occurrence of LVT, highlighting the key role of CRP as clinical risk marker for LVT formation in STEMI patients treated with pPCI.

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CRITIC - ComoRbidITies In Copd Study

Background

Chronic obstructive pulmonary disease (COPD) is a multifaceted disease which has major global health impact. Numerous factors can favour the disease, especially comorbidities can have a decisive influence on the course of the disease as they contribute significantly to morbidity and mortality. To date, the interactions between comorbidities and COPD are poorly understood, but are thought to ultimately contribute to different phenotypes of COPD.

Aim and Objective

Within this study we aim on establishing a prospective COPD registry at the Department for Internal Medicine II at the Medical University of Innsbruck in order to record the respective comorbidities and their influence on exacerbation frequency, severity and genesis, morbidity, mortality and quality of life in COPD patients.

Methods

This is a prospective, non-interventional, monocentric observational study. COPD patients will be included during clinical routine at the pulmonary outpatient department. Laboratory values, findings from lung function tests, imaging, as well as all examination findings collected during routine examinations will be recorded. The prospective registry is expected to run until 30.09.2030.

Hypothesis

• Comorbidities of COPD substantially influence the course of the disease, as well as the quality of life of patients.

• COPD can be sub-divided into different phenotypes based on comorbidities.

• Each phenotype requires a different therapeutic approach and screening algorithm.

Conclusion

The identification of distinct COPD phenotypes could lead to a more individualised treatment approach, ultimately resulting in improved prognosis, management and quality of life of COPD patients.

Funding:

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Increased cardiovascular risk in formerly preterm infants: A review of literature.

Background: Preterm birth accounts for approximately 11% of all livebirths globally. Due to improvements in perinatal care, more than 95% of these infants now survive into adulthood. Research has indicated a robust association between prematurity and increased cardiovascular risk factors and cardiovascular mortality. While the innate adverse effects of prematurity on these outcomes have been demonstrated, therapeutic strategies on the mitigation of these concerning developments are lacking.

Aim: The aim of the present review is to summarize and highlight the evidence of a heightened cardiovascular risk profile in formerly preterm infants.

Discussion: A wide range of population-based studies shows an increased cardiovascular risk in formerly preterm-born adults. This is highlighted in a heightened risk of developing hypertension as well diabetes mellitus type I and II, all of which are known to be independent risk factors for cardiovascular events. This association if further aggravated by an inverse correlation between gestational age at birth and future cardiovascular risk. While this evidence has been known for some time now, effective treatment to alleviate this worrisome trend is lacking.

Funding: This study is supported by VASCage – Research Centre on Vascular Ageing and Stroke. As a COMET centre VASCage is funded within the COMET program - Competence Centres for Excellent Technologies by the Austrian Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology, the Austrian Ministry for Digital and Economic Affairs and the federal states Tyrol, Salzburg, and Vienna.

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Airway epithelial cells differentially adapt their iron metabolism to infection with Klebsiella pneumoniae and Escherichia coli in vitro

Background

Pneumonia is often elicited by bacteria and can be associated with a severe clinical course, respiratory failure and the need for mechanical ventilation. In the alveolus, type-2-alveolar-epithelial-cells (AECII) contribute to innate immune functions. We hypothesized that AECIIs actively adapt cellular iron homeostasis to restrict this essential nutrient from invading pathogens – a defense strategy termed 'nutritional immunity', hitherto mainly demonstrated for myeloid cells.

Methods

We established an in-vitro infection model using the human AECII-like cell line A549. We infected cells with Klebsiella pneumoniae (K. pneumoniae) and Escherichia coli (E. coli), two gram-negative bacteria with different modes of infection and frequent causes of hospital-acquired pneumonia. We followed the entry and intracellular growth of these gram-negative bacteria, and analyzed differential gene expression and protein levels of key inflammatory and iron metabolism molecules.

Results

Both, K. pneumoniae and E. coli, are able to invade A549 cells, whereas only K. pneumoniae is capable of proliferating intracellularly. After peak bacterial burden, the number of intracellular pathogens declines, suggesting that epithelial cells initiate anti-microbial immune effector pathways to combat bacterial proliferation. The extracellular pathogen E. coli induces an iron retention phenotype in AECII, mainly characterized by the downregulation of the pivotal iron exporter ferroportin, the upregulation of the iron importer transferrin-receptor-1 and corresponding induction of the iron storage protein ferritin. In contrast, cells infected with the facultative intracellular bacterium K. pneumoniae exhibit an iron export phenotype indicated by ferroportin upregulation. This differential regulation of iron homeostasis and the pathogen-specific inflammatory reaction of AECII is likely mediated by oxidative stress.

Conclusion

AECII derived A549 cells, show pathogen-specific innate immune functions and adapt their iron handling in response to infection. The differential regulation of iron transporters depends on the preferential intra- or extracellular localization of the pathogen and likely aims at limiting bacterial iron availability.

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Gender-specific differences 12-months after SARS-CoV-2 infection

Background: Sex and gender are important drivers of morbidity and mortality in COVID-19. We aimed at tracking the gender-specific 12-month kinetics of cardiopulmonary recovery after disease.

Methods: In this longitudinal, multicentre observational study COVID-19 patients prospectively underwent clinical evaluation, laboratory testing, pulmonary function analysis, echocardiography and thoracic low-dose computed tomography (CT) at four time points (6 weeks, 3, 6 and 12 months) after disease onset. Six-minute walking distance (6MWD) and quality of life were cross-sectionally assessed at 12 months.

Results: 108 of 145 included patients (74.5%) completed the 12-months follow-up. Male participants constituted the majority with 59.3% (64/108) and 40.7% (44/108) were female. Hospitalization rates during acute COVID-19 were significantly higher among men (50.9% vs. 24%). Yet, women showed a significantly higher burden of persisting symptoms at the 12 month visit (median number of persistent symptoms: 2 vs. 1). Notably, significantly decreased quality of life was more frequent in women. Interestingly, pulmonary function analysis, CT imaging and results of the 6MWD showed no significant gender-specific differences.

Conclusion: Within this observational study we confirm that men are at a higher risk for severe COVID-19, whereas women tend to have a protracted course of recovery reflected by higher burden of persistent symptoms and life quality impairment.

Funding:

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Effects of COVID-19 protective measures on the ophthalmological patient examination with an emphasis on gender-specific differences

Objective: In response to the COVID-19 pandemic, strict hygiene and containment measures have been instituted in the clinical ophthalmological examination to prevent virus transmission. The aim of this study is to assess the effects of these protective measures on the quality of the examination with an emphasis on gender-specific differences.

Methods and analysis: An online survey was sent to ophthalmologists in 10 countries. The collected data included demographics, place of work, current professional status, COVID-19 protective measures and their impact on the quality of the examination. Descriptive statistics were used to analyse the data. Fisher's exact test was used to analyse gender differences.

Results: A total of 120 responses were collected. 54.0% of the respondents identified as female and 43.4% as male. Over 75% agreed that protective measures made the examination conditions more difficult. The major problems were fogging of the lenses (87.6%) or slit lamp oculars (69.9%), reduced operability of the slit lamp due to protective barriers (60.2%) and time delay due to disinfection measures (68.1%). Significantly more women than men reported that they used filtering face piece (FFP2) instead of surgical masks (p=0.02). More male participants reported that they removed their mask to prevent fogging (p=0.01). 31% of all participants felt that the COVID-19 protective measures reduced the overall quality of slit lamp examination and 43.4% reported a reduced quality of fundoscopic examination.

Conclusion: COVID-19 related safety measures reduce the feasibility of the clinical ophthalmological examination. Practicable solutions are required to maintain good examination quality without compromising personal safety.

Funding:

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Immunologic response in bacterial sepsis is different from that in COVID-19 sepsis

Background

Critically ill COVID-19 patients fulfill the current SEPSIS-3 criteria. However, there might be substantial differences in phenotype and the pattern of inflammatory parameters compared to bacterial sepsis. This retrospective pilot study was conducted to investigate differences between severe COVID-19 and bacterial sepsis defined by SEPSIS-3 criteria.

Methods

For this purpose, we evaluated patients diagnosed with bacterial sepsis according to SEPSIS-3, treated at the medical ICU of the University Hospital Innsbruck from September 2018 to October 2020, and compared them to patients with severe COVID-19 from the second wave (August 2020 – April 2021). Both cohorts were matched in a 1:2 ratio (bacterial: COVID-19 - 2nd wave) by age, sex, Simplified Acute Physiology Score III (SAPS III) and invasive mechanical ventilation. Since there was a significant difference in the use of corticosteroids between the first and second wave, we included patients from the first wave (March 2020 – July 2020) in an additional control cohort.

In all patients, we determined maximum levels of C-reactive protein (CRP), interleukin-6 (IL-6), procalcitonin (PCT), ferritin, arterial lactate and minimal lymphocyte count within 48h after ICU admission.

Results

Serum IL-6, as well as PCT and CRP levels were dramatically higher in bacterial sepsis compared to severe COVID-19. Steroid effects may not explain our findings between bacterial sepsis and second wave of COVID-19, because COVID-19 patients, treated during first wave, showed similar results, despite considerably less frequent use (14 %) of glucocorticoids. Ferritin levels showed no significant differences between bacterial sepsis and the second wave of COVID-19.

Conclusion

Despite COVID-19 patients meeting SEPSIS-3 criteria, phenotypes of dysregulated host response following infection by bacteria or SARS-CoV-2 appear to be substantially different. Our pilot study questions the classification of severe COVID-19 as sepsis.

Funding:

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International variability of renal and cardiovascular outcomes and mortality in patients with type 2 diabetes mellitus in Europe

Type 2 diabetes and its complications represent a huge burden to public health. With this prospective, observational cohort study, we aimed to estimate and to compare the incidence rate (IR) of renal and cardiovascular outcomes and all-cause mortality in patients with type 2 diabetes in different European countries.

The renal endpoint was a composite of a sustained decline in estimated GFR of at least 40 %, a sustained increase in albuminuria of at least 30 % including a transition in albuminuria class, progression to kidney failure with replacement therapy, or death from renal causes. The cardiovascular endpoint was a composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke.

3131 participants from four European countries (Austria, Hungary, The Netherlands, and Scotland) with a median follow-up time of 4.4 years were included. IRs were adjusted for several risk factors including sex, age, estimated GFR, albuminuria, glycated haemoglobin, blood pressure and duration of type 2 diabetes.

Across countries, the adjusted IR for the renal endpoint was significantly higher in Hungary and Austria, the adjusted IR for the cardiovascular endpoint was significantly higher in Scotland and Austria. All-cause mortality was significantly higher in Scotland compared to all other countries.

Our findings show how the longitudinal outcome of patients with type 2 diabetes varies significantly across European countries even after accounting for the distribution of underlying risk factors.

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Gender Differences in Aortic Stenosis: A Phase-Contrast Cardiac MRI Study

Objectives. Aortic stenosis (AS) is the most common valvular heart disease in Europe. To date, diagnosis, classification and treatment of this disease are not gender-sensitive; however, due to distinct differences in the natural history of AS, further investigations of differences between men and women in AS patients are needed. Thus, the aim of our study was to detect gender differences in AS, especially concerning flow-patterns, via phase-contrast cardiac magnetic resonance imaging (PC-CMR).

Methods. Fifty patients with moderate to severe AS (21 women, 42% vs. 29 men, 58%) and a median age of 72 years (interquartile range (IQR): 66-77) underwent transthoracic echocardiography (TTE), cardiac catheterization (CC) and CMR. Aortic valve area (AVA) and stroke volume (SV) were determined in all modalities, with CMR yielding a geometrical AVA via cine-planimetry and a functional AVA via PC-CMR, which was also used to examine flow-patterns. Additionally, computed tomography was performed in 35 patients (70%) to assess valvular calcium load.

Results. Geometrical AVA showed no difference between women and men (0.91cm^2, IQR: 0.58-1.13 vs. 0.96cm^2, IQR: 0.78-1.23, p=0.133). However, functional AVA differed significantly between sexes in all three modalities (TTE, CC, PC-CMR, all p<0.04). In men, no significant intermethodical biases in functional AVA-measurements between different modalities were found (all p>0.2); however, in women the particular measurements differed significantly from each other (all p<0.04). Regarding flow-patterns, momentary flowrate showed marked sex differences depending on momentary degree of opening (at 50%, 75% and 90% of maximum AVA, all p<0.001), with men showing higher flowrates with increasing opening area. Contrary, in women momentary flowrate did not differ between 75% and 90% of maximum AVA (p=0.149). Valvular calcium load was significantly higher in men (calcium-score: 1606 (IQR: 959-2647) vs. 809 (IQR: 285-1489) in woman, p=0.008).

Conclusion. Whilst geometrical AVA did not differ between sexes, functional AVA showed marked differences between men and women in all modalities. What is more, inter-methodical biases were negligible in men, but not in women. Lastly, significant sex differences in calcium load and flow-patterns highlight the different natural history of AS between men and women.

Funding:

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Screening for chronic obstructive pulmonary disease in symptomatic patients after exclusion of significant coronary artery disease

Background

Both, coronary artery disease (CAD) and chronic obstructive pulmonary disease (COPD) contribute significantly to global morbidity and mortality. Due to overlapping symptoms and a similar risk profile the diagnostic differentiation between CAD and COPD remains challenging.

Aim and Objective

Therefore, the aim of our study is to assess the prevalence of COPD in patients with chest pain and/or dyspnoea after prior CAD exclusion by invasive coronary angiography (ICA). Furthermore, we aim to evaluate if current CAD risk stratification models are able to differentiate between CAD and COPD.

Methods

This is a prospective, monocentric, non-randomised, open clinical trial, including all symptomatic patients without known structural cardiac or pulmonary diseases and with prior CAD exclusion by ICA. After inclusion, detailed symptoms, medical history and current medication are obtained from all patients. Subsequently, a potential underlying pulmonary disease is investigated by performing pulmonary function tests. Furthermore, after 3 month a telephone follow-up is performed obtaining changes in symptoms, medical history and medication.

Hypothesis

• Screening for chronic obstructive pulmonary disease is often neglected in routine clinical practice due to overlapping symptoms and similar risk factors to CAD.

• Routine spirometry after invasive CAD exclusion would significantly contribute to early detection of chronic obstructive pulmonary disease and would have a major impact on prognosis and symptom relief

• Current CAD risk stratification models are not able to adequately distinguish between cardiac and pulmonary symptoms in symptomatic patients.

Conclusion

Early diagnosis is crucial for the prognosis of COPD, therefore COPD must be considered as differential diagnosis in the assessment of symptomatic patients with dyspnoea and/or chest pain. Further, improved diagnostic screening tools could significantly contribute to a better differentiation between pulmonary and cardiac diseases.

Funding:

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Stroke Card Long-term Follow-Up

Long-term outcome of a pragmatic trial of multifaceted intervention (STROKE-CARD care) to reduce cardiovascular risk and improve quality-of-life after ischaemic stroke and transient ischaemic attack

Background: The Stroke-Card Trial was a prospective, multicentre, block-randomised open pragmatic trial performed between 2014 and 2019 with blinded outcome assessment comparing Stroke-Card Care to usual post-stroke-patient care. Stroke-Card Care is a multifaceted post-stroke disease management program with the objective of reducing recurrent cardiovascular events and improving quality of life. The Stroke Card Trial showed significant reduction of cardiovascular risk and improved health-related quality of life and functional outcome in the intervention group. We started the Stroke-Card Long-Term Follow-up Trial to evaluate long-term effects of Stroke-Card Care compared to Standard Care.

Methods: The study is a long-term follow up of a randomized controlled trial (STROKE-CARD: NCT02156778). All patients from the STROKE-CARD trial (n=2149) will be contacted by phone or mail for possible enrollment in the study. The study visit will be scheduled 3-6 years after the stroke/TIA event. The co-primary endpoint is the composite of major recurrent cardiovascular events (nonfatal stroke, nonfatal myocardial infarction, and vascular death) from hospital discharge until the long-term follow-up visit and health-related quality of life measured with the European Quality of Life-5 Dimensions (EQ-5D-3L) at the final visit. Secondary endpoints include overall mortality, long-term functional outcome, and target-level achievement in risk factor management. We will perform a blinded outcome assessment for all events documented within the long-term follow-up.

Results: Data acquisition is not yet completed. Up to date 800 participants performed an outpatient-department study visit. 152 participants not able to attend a study visit at the department had telephone-based follow-up interviews.

Discussion: The trial is ongoing. The Results of this study will provide evidence if benefits of the Stroke Card-Care sustain in the long-term outcome.

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Histological validation of cardiac 99mTc-DPD uptake in patients with cardiac transthyretin amyloidosis

Background: Cardiac transthyretin (ATTR) amyloidosis is a fatal disease caused by the extracellular deposition of misfolded ATTR protein in the myocardium. 99mTc-DPD scintigraphy is a key tool for non-invasive diagnosis of cardiac ATTR amyloidosis. However, its value as a disease monitoring tool has not been systematically assessed.

Aim: This single-center observational study aimed to compare the extent of histological amyloid infiltration on endomyocardial biopsy (EMB) with the quantification of cardiac 99mTc-DPD uptake (planar, SPECT/CT).

Methods: 25 patients with cardiac ATTR amyloidosis were enrolled. Patients were included in case of (1) EMB-proven ATTR amyloidosis and (2) availability of 99mTc-DPD scintigraphy (reference activity: 550 MBq). Visual interpretation using the Perugini score, quantitative analysis of cardiac 99mTc-DPD uptake by planar scintigraphy and SPECT/CT using regions of interest (ROI) were performed, and heart to whole-body ratio (H/WB) was measured. Histological amyloid load was quantified as percentage of the analysed myocardial tissue using Sulfated Alcyan Blue staining and the Fiji-ImageJ programme. Pearson's correlation and Bland-Altman plots were used for correlation analysis and assessment of agreement.

Results: ATTR patients had a median age of 77 [73-80] years and were predominantly male (84 %). An abnormal Perugini score (i.e. 2 or 3) was present in 24 patients (96 %), whereas 1 patient was assigned Perugini score 1 (4 %). Increased cardiac tracer uptake was documented in all patients, both on 99mTc-DPD planar scintigraphy [ROImean 129 ± 37] and SPECT/CT [ROImean 369 ± 142]. Histologic amyloid burden on EMB was 32 ± 19 % on average. It significantly correlated with Perugini score (p=0.018), cardiac 99mTc-DPD uptake (planar: r=0.54 p=0.006, SPECT/CT: r=0.48 p=0.018) and H/WB (r=0.41 p=0.046).

Conclusion: We have demonstrated a good correlation between histological amyloid infiltration on EMB and cardiac 99mTc-DPD uptake, illustrating the potential of 99mTc-DPD scintigraphy to yield reliable quantitative information on cardiac amyloid burden.

Funding:

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Characteristics and outcome of critically ill COVID-19 patients in Tyrol

INTRODUCTION

With the beginning of the pandemic in Tyrol (Austria) a register study was established, in order to observe characteristics and outcome of critically ill COVID-19 patients at a regional level.

OBJECTIVES

The aim of this study was to identify important differences in baseline characteristics, resource use and outcome in critically ill COVID-19 patients in Tyrol.

METHODS

This multicenter prospective register study was conducted in 12 ICUs (from 8 hospitals in Tyrol, Austria) and included all patients with a SARS-CoV-2 infection confirmed by polymerase chain reaction. Patients were divided in two groups according to their survival status at hospital discharge. Hospital mortality for different age groups (<40, 40<60, 60<80, >80) was analyzed with a Kaplan Meier survival analysis and differences were assessed by the log-rank test.

RESULTS

Since the onset of the COVID-19 pandemic 943 patients were included in this prospective register study. The overall median age was 66 years (IQR 56-75). Patients who died in the hospital were significantly older (75 vs. 63) than patients who were discharged alive. The male-to female ratio was 68.5% to 31.5%. The most common comorbidities were hypertension, cardiovascular disease, obesity and diabetes mellitus. Hypertension, cardiovascular disease, renal comorbidities and chronic obstructive pulmonary disease were more frequent in hospital non-survivors. Hospital non-survivors were significantly more often mechanically ventilated and had higher SOFA and SAPS-3 scores at ICU admission. The time from symptom onset to ICU and hospital admission was significantly shorter in hospital non-survivors. The overall ICU and hospital mortality was 24.3% and 27.5% respectively. Older age was associated with impaired hospital survival in a Kaplan-meier survival analysis.

DISCUSSION

Several risk factors for poor outcome in critically ill COVID-19 patients have been identified in various cohort studies around the world. While the most common comorbidities, especially hypertension and cardiovascular disease, were more common in non-survivors in our study, age appears to be the most important determinant of hospital survival.

Funding: Tyrolean Government

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Gender aspects in heart transplantation

Background

Gender has an important impact on the outcome after heart transplantation. Previous studies discussed different risk factors controversially. The aim of this investigation is to describe pre- and intraoperative differences between female and male heart recipients and their impact on outcome after heart transplantation.

Methods

The study population comprised 498 patients undergoing heart transplantation between 1993 and 2021. The patient cohort was divided into two groups according their gender. 100 female and 398 male patients were transplanted in this period. Pre- and intraoperative differences were calculated using Chi2 Test and student t-test. In order to analyze the impact of gender mismatch between donor and recipient, a Kaplan Meier survival analysis was performed including four groups: A female recipient and female donor, B female recipient and male donor, C male recipient and male donor, D male recipient and female donor.

Results

Female heart transplant recipients were younger (52 years [39.3-60.8] vs. 57 years [47-62], p=0.001), had a lower mean pulmonary artery pressure (28 mmHg [21-35] vs. 28 mmHg [21-35], p=0.011), a higher pulmonary resistance (3 R units [2-4] vs. 2.6 R units [1.8-3.7], p=0.032) and were more frequently cytomegalovirus serology positive (68 (69%) vs. 221 (57%), p=0.024). The organ donor for female heart recipients were mainly female (63 (64%) vs. 91 (24%), p<0.001). Intraoperatively, aortic cross clamp time were shorter in female patients (74 min [65-84] vs. 85 [73-105], p<0.001), but more red blood units were needed (3 [1-5] vs. 2 [0-4], p=0.017). There was no impact of gender on primary graft dysfunction, perioperative graft rejection, length of stay, postoperative kidney function, postoperative stroke, wound infection or perioperative mortality.

Gender mismatch had also no impact on long term survival (log rank= 0.112) as shown in Figure 1.

Conclusion

Despite some preoperative differences between male and female heart transplant recipients, gender do not have an impact on long term survival in our patient cohort.

Funding:

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Gender differences in HerzMobil Tirol – a multidimensional disease management programme for patients after acute heart failure

Aims

Heart failure is a major health threat to women and men. The efficacy of disease management programmes in improving the outcome of heart failure patients remains uncertain and may vary across health systems. Our objective was to evaluate the gender difference of the outcome of a disease management program such as HerzMobil Tirol (HMT) in patients following AHF.

Methods and Results

The non-randomized study included 497 patients with acute HF (AHF) that were managed in HMT (n = 240) or contemporaneously in usual care (UC, n = 257) after discharge from hospital from 2016 to 2019. The primary endpoint was time to HF hospitalization and all-cause mortality within twelve months. Multivariable Cox proportional hazard models were used to assess the effectiveness. Patient characteristics were comparable in the groups.

In the analysis, 153 women and 344 men were considered. The primary endpoint occurred in 21 women (30%) in HMT, 34 women (41%) in UC, 47 men (27.6%) in HMT and 85 men (48,9%) in UC.

Overall patients, there were no significant difference between men and women (HR 1.13 (0.82–1.55); p=0.45). Subgroup analyses revealed that HMT management of men after AHF was associated was associated with a 52%- (HR 0.48 (0.34–0.69); p<0.001) reduction in the primary endpoint. In contrast, the reduction in the primary endpoint was not significant in women (HR 0.69 (0.40–1.19); p=0.181).

Conclusion

A multidimensional post-discharge disease management programme, like HerzMobil Tirol is feasible and effective in the period after acute heart failure for women and men. Our results suggest that the programme may be associated with a more pronounced reduction of 12-months HF-readmissions and all-cause mortality in men than in women.

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Telecovid-Tirol: Remote monitoring of Covid-19 positive high-risk patients in domestic isolation: experience from two waves of disease

Background: For almost two years, the Covid-19 pandemic has posed a challenge to healthcare systems. The telemedical care program Tele-Covid-Tirol, which had been reinstalled during the current Covid-19 wave, proved its worth in monitoring high-risk patients in home isolation.

Methods: Covid-19-positive high-risk patients (age > 65 years and/or severe comorbidities) from the greater Innsbruck area are fitted with a Cosinuss® Home Monitoring System. The monitoring team (25 medical students under the supervision of 6 physicians) provides continuous monitoring of vital signs (24/7). If a predefined risk score is exceeded, the patient is contacted by telephone. The combination of the clinical condition and the risk score determines the further course of action: (a) watchful waiting, (b) notification of the primary care physician, or (c) referral to our center for therapy optimization.

Results: The program was started in December 2020. After 6 months, the program was temporarily paused. During this first period, 48 patients (age 74.5 IQR: [60-81]; 37.5% male) were monitored. At the end of November 2021, the program was reactivated and is still running. Since the start of the second period, 68 patients (age 73.5 IQR: [68.3-79.8]; 44.1% male) participated in the program. Comparing the patient populations of the two periods, a significant decrease in hospitalizations (29.2% versus 7.4%; $p<0.005$) was observed in the second period. 60.2 % of the patients in the second period were immunized with at least two dosages of Covid-19 vaccines before infection. Four out of five (80%) of hospitalized patients were not vaccinated.

Conclusion: The striking decrease in hospitalization rate in the second monitor phase is probably a consequence of higher vaccination rates in the population. It is also possible that the currently predominant Covid-19 subtype B.1.1.529 (Omikron) is associated with a more favorable disease course. A comprehensive analysis is planned after completion of Tele-Covid-Tirol.

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Evaluation of the immune status in elderly BNT162b2 vaccinees against SARS-CoV-2 WT and Delta variant

Objectives: In scope of the ongoing SARS-CoV-2 pandemic elderly persons turned out to be among the most vulnerable individuals. Therefore, we aimed to characterize humoral and cellular immune responses of >70 years old participants before, after first and second administration of the mRNA-based SARS-CoV-2 vaccine BNT162b2. Results were furthermore compared with immune responses of persons <60 years old.

Methods: SARS-CoV-2-specific T cell responses were analyzed by IFN☒ELISpot using peripheral blood mononuclear cells of 45 elderly and 40 younger vaccinees. In addition, serum specific immunoglobulin G antibody (IgG) titers against SARS-CoV-2 spike protein as well as neutralization capacity were determined against SARS-CoV-2 wild-type (WT) as well as Delta variant (B.1.617.2).

Results: Our results clearly showed a significant elevation of serum IgG titers, induction of virus-specific T cells as well as neutralization ability against SARS-CoV-2 WT and Delta variant after first and second vaccination with BNT162b2. In particular the importance of the second immunization was highlighted by the remarkable increase of cellular and humoral immune response. Interestingly no significant differences between the groups >70 years and <60 years were found regarding assessed immune responses.

Conclusion: Our investigation reveals a comprehensive picture of humoral and cellular immune response for two different age cohorts vaccinated twice with BNT162b2. After second immunization data clearly show a significant elevation of IgG and neutralizing antibody titers as well as T cell response. In both cohorts, strong correlation of SARS-CoV-2 WT-specific IgG and neutralizing antibodies was determined. Moreover, our data show an imparity of neutralization efficiency between WT and Delta variant, thus, pointing out the relevance of an additional booster for better protection against emerging variants.

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IgG/IgA neutralizing activity induced by three COVID-19 vaccines against variants of concerns

Abstract PhD Life Science day

IgG/IgA neutralizing activity induced by three COVID-19 vaccines against variants of concerns

Background: Amidst 2021, first COVID-19 vaccine rollout started aiming to tackle the ongoing SARS-CoV-2 pandemic, but at the same time new variants emerged, bearing mutations in the viral spike protein. Only a few studies directly compared vaccine-elicited virus-specific antibodies and their neutralization capacity against the circulating variants.

Objectives: In this study, we assessed SARS-CoV-2 Spike-specific antibodies from serum samples of vaccinated as well as convalescent cohorts. Vaccinated cohorts received two doses of a mRNA vaccine (BNT162b2 or mRNA-1272) or a vector-based vaccine (ChAdOx1). The neutralization capacity was determined as half-maximal neutralization titers against SARS-CoV-2.

Methods: Serum samples were collected at least 3 weeks after the second vaccine dose or post positive PCR test from 107 vaccinated or convalescent individuals and analyzed for anti-SARS-CoV-2 Spike-Immunoglobulins G and A as well as RBD-specific total Ig. Moreover, neutralization assays were performed using SARS-CoV-2 wild type and the variants B.1.1.7 (Alpha), B.1.1.7 E484K (Alpha E484K), B.1.351 (Beta) and B1.617.2 (Delta).

Results: We found a robust IgG response in most participants, however the highest antibody titers were detected in mRNA vaccine recipient. In regards to serum IgA titers, differences between mRNA vaccines and the convalescent or vector-based vaccine were even more pronounced with the highest levels also found in mRNA vaccine recipients. Remarkably, the three tested vaccines elicited effective neutralization against all VOC and wild type, with mRNA-1273 reaching the highest neutralization, although some individuals showed low or no neutralization especially against Alpha E484K and Delta variants.

Conclusion: Here, we show that mRNA-1273 vaccinated individuals had the highest neutralization abilities compared to BNT162B2 and ChAdOx1 vaccinees. Interestingly, the convalescent cohort demonstrated the most heterogeneous range of antibody titers and neutralization capacity. Finally, we observed a significant, positive correlation between antibodies titers and NT50 values for all cohorts.

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Dark-Blood Delayed Enhancement (FIDDLE) Cardiac Magnetic Resonance optimizes discrimination of myocardial scar borders after ST-elevation myocardial infarction

Background: "Bright-blood" late gadolinium enhancement (LGE) cardiac magnetic resonance (CMR) sequence represents the reference standard for imaging of myocardial infarction, both in clinical practice and in randomized trials. However, standard LGE-CMR may limit the differentiation of bright subendocardial scars to the bright blood pool whereas the recently introduced dark-blood "Flow-Independent Dark-Blood Delayed Enhancement" (FIDDLE) sequence holds the promise to overcome this specific limitation.

Purpose: To prospectively compare subjective and objective image quality of the FIDDLE sequence with standard "bright blood" LGE.

Methods: The conventional "bright blood" LGE and FIDDLE sequence were performed in 109 patients (60.4 ± 9.7, 19% women) within 7 days (n=33) as well as 4 months (n=38) and 12 months (n=38) after acute ST-elevation myocardial infarction (STEMI). Subjective image quality including confidence in scar segmentation and blood pool bordering was each rated on a 4-point Likert scale. Objective CMR image quality was assessed by the contrast-to-noise ratio (CNR) [(signal intensity (SI)scar lesion – SIblood/standard devation (SD)blood)].

Results: Overall median image quality was excellent for FIDDLE and the standard "bright blood" LGE sequence (p=0.996). Qualitative analysis revealed a significantly higher confidence in scar segmentation as well as in blood pool bordering for FIDDLE as compared to the standard LGE sequence (all p=0.001). The signal intensity ratios of left and right ventricular blood pool were comparable for FIDDLE and bright blood LGE. CNR of scar lesion versus left ventricular (LV) blood pool was higher for FIDDLE (8.9%) compared to conventional LGE sequence (2.0%) (p=0.001).

Conclusion: In comparison to the standard LGE technique, dark-blood FIDDLE CMR significantly optimizes the discrimination between myocardial scar boundary after STEMI and adjacent blood pool, as assessed both subjectively and objectively.

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Augmented Reality – The Future of Spine Surgery?

Background: Augmented reality (AR) is an emerging technology that allows digital images to be superimposed onto the user's view of the real-world environment. In minimally invasive spine surgery (MISS), the narrowed working corridor makes visualization of relevant surgical anatomy challenging. With AR, relevant surgical anatomy can be highlighted and merged on intraoperative CT (iCT) with integrated 3-dimensional navigation (3D NAV) which when registered with a surgical microscope can be projected into the surgeon's field of view.

Methods: The prospective case study was conducted on data collected from patients who underwent elective spinal surgery between August 2019 and October 2019. Prior to surgery, we reviewed the patient's preoperative MRI or CT scans and used a "smart brush" function to identify and "paint" anatomy relevant to the procedure being performed. This preoperative image metadata was then fused to the iCT (AIRO, Mobius Imaging, Massachusetts, USA; BrainLAB, Munich, Germany). The "painted anatomy" was then visualized on the 3D navigation monitor and in the microscope as an overlay.

Results: We included 13 patients with intradural and extradural spinal pathology (e.g. intradural tumors, extradural tumors, cysts, disc herniations, spondylolisthesis). The average time required to initialize the AR was 15.6 minutes (1.2 minutes - 31.2 minutes). The average total effective radiation dose was 12.8 mSv (5.1 – 21.45 mSv) per scan for each patient. The radiation dose is comparable to cases without AR according to the literature. This was done in parallel with other activities in the OR and did not add to overall procedure time.

Conclusion: The continual, passive projection of surgical anatomy into the microscopic field of view facilitates seamless anatomic orientation, rapid identification of surgical landmarks and precise targeting of pathology. Our preliminary experience suggests that AR has the potential to improve surgical safety and efficiency and should be investigated with further study.

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Chasing Shadows: Cytologically detected Shadow Cells in the Cerebrospinal Fluid of Patients with Multiple Sclerosis

Background: Pathophysiology of multiple sclerosis (MS) is dominated by both inflammation and neurodegeneration. A correlation between inflammation and regulation of apoptosis has been suggested previously. Shadow cells in the cerebrospinal fluid (CSF) are considered apoptotic cells.

Objective: To assess the occurrence of shadow cells in MS patients in comparison to other neurological diseases (OND).

Methods: We conducted cytological examination of CSF in 114 MS patients and 125 patients with OND, who had diagnostic lumbar puncture at the Department of Neurology, Medical University of Innsbruck, with time to laboratory processing ≤0.5 hours, showed a CSF white blood cell (WBC) count ≤50/µl and a red blood cell (RBC) count ≤500/µl. Shadow cells were counted by two blinded, independent, experienced investigators, using a standardized approach on microscopic slides.

Results: The number of shadow cells did not statistically significantly differ between patients with MS patients (median: 12, IQR: 0-85) and OND (median 6, IQR: 0-94; p=0.106). Multivariate regression analysis including age, sex, time to laboratory processing, CSF WBC and RBC count, CSF/serum glucose ratio, CSF/serum albumin quotient and disease group as independent variables, identified WBC count as significant predictor of shadow cells (⊠[ln WBC count]=0.73, p<10-9), whereas the disease group had no impact (p=0.466).

Conclusions: Occurrence of shadow cells in the CSF seems to depend on the extent of inflammatory cells rather than MS disease-specific mechanisms.

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Sex-specific differences in tumor volume and survival in newly diagnosed glioblastoma patients treated with dendritic cell-based immunotherapy

Introduction: Gliomas exhibit male:female incidence ratios of up to 2:1. In addition, male patients show a poorer response on tumor therapy, which may lead to a shorter progression free survival (PFS) and overall survival (OS). Differences in the immune system caused by distinct sex hormones may lead to an additional dissimilarity in the response to immunotherapy. In this post-hoc analysis we compared PFS and OS between male and female newly diagnosed glioblastoma patients and determined if there is a difference in tumor volume, edema volume and central necrosis volume.

Methods: 51 male (m) and 28 female (f) patients with newly diagnosed glioblastoma (GB) enrolled in a multicenter randomized phase II trial receiving standard of care (SOC, n= 44) or SOC + Audencel vaccine (n= 35) were included. Tumor volumes, volumes of central necrosis and volumes of tumor edema were calculated by semiautomatic segmentation. Two dimensional and volumetric measurements were applied on mRANO and Vol-mRANO criteria. To detect differences in PFS and OS as well as in tumor-, necrosis- and edema volumes among male and female patients Mann-Whitney U test was used.

Results: There was no statistical significant difference in median PFS (mRANO m: 7.3 months, f: 9.5 months, p= 0.198; Vol-mRANO m: 7.7 months, f: 9.5 months, p= 0.292) and OS (m: 16.1 months, f: 20.0 months, p= 0.117. Median tumor volume (m: 18.6 mm3, f: 17.4 mm3, p= 0.246), median necrosis volume (m: 10.8 mm3, f: 9.9 mm3, p= 0.375) and median edema volume (m: 58.9 mm3, f: 42.3 mm3, p= 0.211) was also not statistical significantly different.

Conclusion: When comparing PFS and OS in patients treated with dendritic cell-based immunotherapy, no significant sex-specific differences were observed. Median tumor volume, necrosis volume and edema volume was similar between male and female patients.

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Sex-related differences in hospital course and functional outcome in patients with non-traumatic subarachnoid hemorrhage.

Background: Female gender is a recognized risk factor for the occurrence of aneurysmal subarachnoid hemorrhage (SAH), although, only a few reports have focused on SAH-related complications and functional outcome based on sex differences.

Objective: To investigate complication rates in SAH patients based on sex differences and to study the associations with 3-months functional outcome.

Materials and methods: All consecutive SAH patients admitted between 2011 and 2021, who gave their informed consent, were included. We estimated sex differences in non-traumatic SAH for hospital course and functional outcome. Functional outcome was assessed at 3-month follow up defining poor functional outcome as a modified Rankin Scale of 3-6.

Results: Among 411 consecutive SAH patients 61% (n =250/411) were women and were older (median age 59 [IQR 49-69] vs. 53 [IQR 47-63], respectively, p = 0.001). Hunt & Hess grade on admission (OR = 1.20 , 95% CI 1.06-1.37, p = 0.005) and Modified Fisher Grading Scale (OR = 1.84, 95% CI 1.18-2.86, p = 0.007) differed significantly, having higher scores in women than in men. Women also had a higher Early Brain Edema Score (OR = 1.64, 95% CI 1.01-2.66, p = 0.046) and were more likely to have early neuroworsening (OR = 1.84, 95% CI 1.17-2.92, p = 0.009) compared with men. We found significant differences in SAH-related complications as acute hydrocephalus (37% vs. 53%) and delayed cerebral ischemia (DCI) (11% vs. 20%) beeing more comon in women. In univariate anlysis poor fuctional outcome in women was found (OR = 1.66, 95% CI 1.09-2.53, p = 0.018),however, multivariate analysis revealed no significant difference between groups.

Conclusion: Women outnumbered men among SAH patients and had increased global disease severity. Despite higher rates of SAH-related complications like acute hydrocephalus and DCI, no significant relationship between sex and poor functional outcome was observed.

Funding:

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Measurement of the intracochlear hypothermia distribution utilizing tympanic cavity hypothermic rinsing technique in a Cochlea hypothermia model

Importance:

Cochlea implants can cause severe trauma leading to intra-cochlear apoptosis, fibrosis and eventually to residual hearing loss. Previous research indicates that mild hypothermia can reduce inner ear inflammation and hearing loss.

Design:

Human temporal bones were prepared following standard mastoidectomy and posterior tympanotomy. Applying a retrocochlear approach leaving the mastoidectomy intact temperature probes were placed in the basal turn (n=4), the middle turn (n=2), the helicotrema and the modiolus. Temperature probe positions were visualized, using µCT imaging and manually segmented using Amira® 7.6. Through the posterior tympanotomy the tympanic cavity was rinsed at 37° Celsius as control group, at room temperature (in the range between 22 and 24° Celsius) and at 4° C. The data was statistically evaluated using pairwise t-tests with Bonferroni correction.

Participants:

In this cadaver study three temporal bones provided by the department of anatomy were included.

Intervention:

Each temperature model was rinsed for 20 minutes at the different tested temperatures in 0.5 second intervals. At least 5 repetitions were performed.

Results:

Steady state conditions achieved in all three different temperature ranges (4 °C, 22-24 °C and 37 °C) were compared in periods between 150 and 300 seconds. The inner ear cooled down within the initial 150 seconds. Temperature probe placed at basal turn, the helicotrema and middle turn detected statistically significant fall in temperature levels following cooling rinse. Temperature rinse at 4° C indicated significant comparable temperature variations. No changes where all curves during the temperature remained stable at 37° C rinses.

Conclusion and Relevance:

Therapeutic hypothermia is achieved with standard surgical irrigation fluid. Further, a temperature gradients along the cochlear is reported. Surgical irrigation rinsing of 120 seconds result in a therapeutic local hypothermia throughout the cochlea. This otoprotective surgical procedure can be easily realized in clinical practice.

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PRIMARY HEADACHE DISORDERS IN ADOLESCENTS IN NORTH- AND SOUTH-TYROL: FINDINGS OF THE EVA-TYROL-STUDY

Introduction: Assessment of the prevalence of primary headache disorders, associated risk factors and use of acute/preventive medication in a representative large sample of adolescents.

Methods: Within the EVA-Tyrol project, a community-based non-randomized controlled cross-sectional study, data was collected from adolescents aged 14-19 years from 45 sites across North-, East- and South Tyrol. Characteristics of headaches and information about the use and the category of acute medication were collected by trained headache specialists in structured face-to-face interviews. Headaches were classified in line with the latest ICHD-3 diagnostic criteria.

Results: Of 1923 participants 930 (48.4%) reported having headaches. Female to male ratio was 2:1. Migraine, tension-type headache and other headache were diagnosed in 10%, 30.2% and 8.2% respectively. Infrequent headaches were reported by 22.8%, episodic headaches by 65.5% and chronic headaches by 11.9% of the adolescents. Medication overuse was diagnosed in 3.4%, increasing up to 21.7% in participants with chronic headache. The use of preventative medication was not reported by any adolescent. Sleep disturbances ($p<0.05$), regular alcohol consumption ($p<0.05$), low physical activity ($p<0.01$) and high screen time exposure ($p<0.01$) were associated with an increased risk of headaches. Only 1.9% with chronic headaches reported poor health, whereas 63.0% and 11.0% of those within the highest headache frequency category still rated their health as good and excellent, respectively. Conclusion: We report high prevalence of primary headache disorders and medication overuse in a large community-based sample of teenagers. Promoting health education in teenagers and encouraging public awareness, including that of health care providers is pivotal.

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Gender differences in loneliness during the Covid-19 pandemic– A follow-up study in the general population of Tyrol, AustriaGender differences in loneliness during the Covid-19 pandemic– A follow-up study in the general population of Tyrol, Austria

Background: The Covid-19 pandemic and related measures represent an enormous burden on mental health. Lockdown forces many people into quarantine and social isolation, which can increase loneliness. Loneliness, in turn, can lead to psychological distress. The aim of this study was to investigate longitudinal changes of loneliness over the course of the pandemic with a special focus on gender differences.

Methods: Residents of Tyrol (≥18a) completed an online survey on sociodemographic data and loneliness by using the Three-Item Loneliness Scale (TILS). The baseline assessment took place from June 26th to August 20th, 2020, the follow-up survey was conducted from November 30th, 2020 to January 24th, 2021. Electronic data collection was performed using the Computer-based Health Evaluation System (CHES).

Results: Of the 961 baseline participants, 384 took part in the follow-up survey. At baseline, 27.9% of participants experienced moderate and 25.5% severe loneliness. A significantly higher risk to be affected from severe loneliness was observed in women (26.9% vs. 12.7%, p= <0.001). Overall, the prevalence of loneliness did not change over time. However, at follow-up, the number of those experiencing moderate loneliness had increased significantly (27.9% vs. 40.8%, p= <0.001). In contrast, severe loneliness was detected in significantly less people at follow-up (25.5% vs. 20.3%, p= 0.043). Changes in loneliness to the worse were observed in men (mean increase 0.39, Z=2.94, p=0.003, Wilcoxon matched-pairs test), but not in women (mean change -0.12, p>0.1). This effect was small in comparison to the initial difference in TILS total score between male and female participants (mean TILS scores of 4.76 and 5.34 at baseline in men and women, respectively, difference of 0.58, p=0.009).

Conclusion: These findings indicate that a subset of the general population consistently suffers from loneliness. Among women, severe loneliness was particularly prevalent at the onset of the pandemic and remained unchanged thereafter. By contrast, severe loneliness was detected less often among men at the onset of the pandemic and increased significantly thereafter. In light of these findings, there is an urge to develop sex-specific programs focussing on a reduction of loneliness, e.g. internet-based behavioural therapy concepts with the aim of increasing well-being.

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Neurological long-term outcome after COVID-19 infection

Since the beginning of the COVID-19 pandemic more then four hundred million people have been infected with the novel SARS-CoV-2 virus, whilst almost six million patients have died due to the infection and affiliated complications of this disease. Today, more than 200 different acute and long-term symptoms of a COVID-19 infection have been described; next to pulmonary and other internal manifestations typical neurological manifestations include headache, loss of taste and smell, encephalopathy, stroke and neurocognitive dysfunctions. The knowledge of prevalence and course of these symptoms during the acute phase and after convalescence is still in development. The aim of this study is to describe acute neurological symptoms and their course over time during and after COVID-19 infection together with neurocognitive deficits, quality of life and MRI brain scans, which will be asserted at 3 and 12 months after the acute phase.

Funding: FWF

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Sex differences in social framework in the elderly: Results from the population-based Bruneck Study

Introduction:

The aim of the study was to investigate differences between female and male individuals regarding utilization of social services, social support, or desired living situation when requiring assistance.

Methods:

Information on social framework was obtained using a standardized questionnaire and interviews of individuals which participated in the 2017 survey of the Bruneck Cohort II, a population - based cohort study which recruited individuals over 67 years of age living in the area of Bruneck, Italy. Differences between groups were assessed with chi2 tests, and $P \leq 0.05$ was considered statistically significant.

Results:

Of 1036 subjects, a statistically significant larger proportion of women were living alone at home, when compared to men (40.7% vs. 13.0%, $P<0.001$). There was no significant difference between female and male participants regarding utilization of social services (e.g. home care or home-delivered meals; 12.2% of women vs. 11.1% in men, $P=0.569$). Of 1023 subjects, the majority (85.9%) would obtain support by family members, whereas 3.6% would not receive support, with a greater proportion in women (5.1%) than in men (1.9%) ($P=0.006$). In addition, we observed no differences in the proportion of women and men regarding the desired living situation when requiring assistance (all $P>0.05$). In detail, 11.6% had willingness for an assisted living situation, 14.2% preferred a retirement home, and 74.2% preferred to stay at their own homes.

Conclusions:

This cross-sectional population-based study showed greater proportions of women living alone and women reporting not to receive support from family members than men, but otherwise no statistically significant sex differences in utilization of social services, social support, or desired living situation.

Funding:

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MR-Spectroscopy: Investigating neurochemical changes in brain metabolism in migraineurs before and after CGRP-Antibody treatment – a randomized, controlled, open-label trial.

Background: Imaging techniques have revealed important aspects of the underlying pathophysiological mechanisms of migraine, suggesting abnormal energy metabolism and increased cerebral hyperexcitability as triggers for a migraine attack. Since only a small percentage of monoclonal CGRP antibodies crosses the blood-brain barrier, their main site of action outside the blood-brain barrier is discussed. It is uncertain whether they lead to central effects through their action outside the blood-brain barrier or exert direct central effects.

Objective: To investigate whether neurochemical, structural, and functional changes in the migraine brain are associated with CGRP-antibody treatment.

Methods: This prospective, randomised, controlled, open-label study will enrol 38 patients diagnosed with episodic migraine (w/o aura) according to ICHD-3 criteria. All participants will undergo an initially stratified 1H-, 31P- MR-Spectroscopy and resting-state fMRI interictally. Half of the participants (n=19) will receive CGRP mAB treatment (Fremanezumab 225mg monthly) after the first scan for three months according to local standard guidelines for CGRP mAB treatment. MR-spectroscopy and resting-state fMRI will be repeated after the treatment. Controls will be measured in an identical setting at the same time points but without CGRP mAB treatment.

Future aspects: Investigating the effects of CGRP mAB on the metabolism and its association to functional connectivity in the migraine brain provides in-depth knowledge about the mechanism of action of the CGRP antibody and permits individualized treatment.

Funding:

V Filippi (1), R Steiger (2,3), F Frank (1), K Kaltseis (1), A Grams (2), E Gizewski (2), V Beliveau (1,3), G Broessner (1)

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Decision-making in neurocritical care patients in the early course of the disease: satisfaction of family members

Background: Neurocritical care provides specialized treatment to critically ill patients by covering the broad scope of life-threatening primary or secondary neurological disease. The presence of neurocritical care units (NICU) is associated with lower mortality. Nevertheless, in-hospital death rate remains high at 12.4% worldwide. While decision-making is a crucial step in neurocritical care patients in the early course of the disease, scarce data on family satisfaction with decision-making and the use of shared decision-making items exist.

Methods: In this single-center survey at a NICU, family satisfaction with decision-making and care of patients admitted to the NICU (Department of Neurology, Medical University of Innsbruck) is assessed. One representative family member is required to take the questionnaire 'Family Satisfaction in the Intensive Care Unit' (FS-ICU) within 72 hours of admission to the NICU. Additionally, a 12-item questionnaire on the decision-making process will be used. The participant is asked whether a legal guardian exists and, if so, the participant is the legal guardian and the preferred decision-making approach. In a follow-up four weeks after discharge from the NICU or the death of the patient, the participating family member will be surveyed by mail using again the FS-ICU and the questions on whether a legal guardian exists. Additionally, the 'decision regret scale' will be used to assess the satisfaction with decisions for the neurocritical care patient.

Objective: Primary objective is to assess the family satisfaction of neurocritical care patients with decision-making. Secondary objectives are to assess the family satisfaction of neurocritical care patients with care, family member's perception of the use of shared decision-making items in the early course of the disease and the satisfaction of the family members with the decisions made for the neurocritical care patient.

Funding: None.

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Recovery after High-Risk TIA and Ischemic Stroke

Background: Stroke is globally one of the leading causes of disability and the second most frequent cause of death.

Survivors of stroke and transient ischemic attack (TIA) are vulnerable to further strokes, with the highest risk immediately after the incident. Therefore, it is clearly of great health economic concern to further refine care and reintegration into a life worth living after stroke. Of particular interest are the detection of complications after stroke, the assessment of the patients demand for caregiving, rehabilitation and welfare state assistance.

Methods: This project will be a prospective observational substudy of the Stroke-Card Registry Study (NCT04582825). The target population will be patients in the Stroke-Card Registry Study who fulfill the inclusion criteria. Patients are tested during their inpatient stay and at 3, 6, and 12 months in an outpatient format. Study patients are not subjected to experimental therapies, and the procedures performed are well established in routine day-to-day clinical practice.

Key physical status questionnaires such as the modified Rankin Scale (mRS), Singer score, Barthel Index (BI), and NIHSS will be used to characterize recovery during the study period. This observational study will be evaluated with descriptive statistics.

Objecitve: Our aim is to analyze the dynamics of recovery after stroke and TIA one year after the index event, to refine recovery after the index event, and to elucidate factors that negatively affect recovery. Furthermore we want to investigate the effects of the already gained and implemented information gained from the Stroke-Card trial on our post-stroke and post-TIA population and detect subgroups who benefit the most and demonstrate the principle that a patient-centered follow-up program leads to improved outcomes.

Funding: VASCage – Research Centre on Vascular Ageing and Stroke

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Augmenting microscopic navigated surgery with knowledge

The study of 3D object segmentation is one of the most challenging areas of research in computer vision especially in medical video analysis. The aim of my work is to identify 3D anatomical ear-nose-throat (ENT) structures in live video streams provided by a high-precision stereo-microscopic navigation system. The goal of 3D anatomical detection is to demonstrate structures in the external ear lobe and the ossicular chain in order to create automatic segmentation of anatomical structures and to augment microscopic navigated surgery for ENT surgeons. In addition, the oriented 3-dimensional bounding boxes around the mentioned areas in the 3D real world will be estimated. Most of the existing methods for the 3D object detection, are based on supervised machine learning techniques, considering that accurate 3D ground truth is provided in the training dataset. For this purpose, a manually created preliminary atlas of ENT structures created from both CT and MR data will serve as ground truth to extract the anatomic structures form the live video stream using computer vision and (un-) supervised machine learning approaches. As a proposed method, Convolutional Neural Network (CNN) will be applied to the trained datasets due to recent works which have been shown bright performances in various applications such as image classification, object detection, and semantic segmentation. Due to the lack of a large-scale training dataset, transfer learning approach and data augmentation techniques will be checked using different deep learning algorithms.

Funding: FWF fund

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A deep learning pipeline for the automated segmentation of posterior limb of internalcapsule in preterm neonates

Segmentation of specific brain tissue from MRI volumes is of great significance for brain disease diagnosis, progression assessment and monitoring of neurological conditions. Manual segmentation is time-consuming, laborious, and subjective, which significantly amplifies the need for automated processes. The active development in the field of deep learning, especially convolutional neural networks (CNNs), over the last decades and the associated performance improvements have increased the demand for CNN-based methods in practice in order to provide consistent measurements and quantitative analyses. In this paper, we present an efficient deep learning approach for the segmentation of brain tissue. More specifically, we address the problem of segmentation of the posterior limb of the internal capsule (PLIC) in preterm neonates. To this end, we propose a CNN-based pipeline comprised of slice-selection modules and a multi-view segmentation model, which exploits the 3D information contained in the MRI volumes to improve segmentation performance. One special feature of the proposed method is its ability of identifying one desired slice out of the whole image volume, which is relevant for paediatricians in terms of prognosis. In order to increase computational efficiency, we apply a strategy that automatically reduces the information contained in the MRI volumes to its relevant parts. Finally, we conduct an expert rating alongside standard evaluation metrics, such as dice score, to evaluate the performance of the proposed framework.

We demonstrate the benefit of the multi-view technique by comparing it with its single-view counterparts, which reveals that the proposed method strikes a good balance between exploiting the available image information and reducing the required computing power compared to 3D segmentation networks. Standard evaluation metrics as well as expert-based assessment confirm the good performance of the proposed framework, with the latter being more relevant in terms of clinical applicability. It could be demonstrated that the proposed deep learning pipeline is able to compete with the expert in terms of accuracy.

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Cognitive dysfunction one year after COVID-19: evidence from Eye Tracking

Introduction

Increasing evidence suggests persistent neurological, neuropsychiatric, and neuropsychological symptoms after coronavirus disease (COVID-19). Aim

To assess frontal lobe functions twelve months after COVID-19 using eye tracking. Methods

We recruited 55 patients who recovered from COVID-19 and 23 age/gender-matched healthy controls (HC). Patients were further divided into those who required hospital admission (n=38) and those who were managed as outpatients (n=17). Cognition was tested in all participants using Montreal Cognitive Assessment (MoCA) and fatigue was assessed in patients using the Fatigue Assessment Scale (FAS). Eye tracking assessment included an overlap pro-saccade, an anti-saccade, and a dual-task anti-saccade paradigm.

Results

There were no demographic differences between HC and patients (all $p>0.1$). Post-COVID-19 patients made more directional errors in the anti-saccade task ($p<0.001$), in the dual-task anti-saccade ($p=0.043$), and more anticipatory errors in the pro-saccade task ($p=0.002$) compared to HC. Furthermore, inpatients made more directional errors in the anti-saccade and dual-task anti-saccade ($p<0.03$) compared to outpatients and HC. Saccadic performance did not correlate with MoCA nor with FAS. Conclusions

We found impaired inhibitory cortical control in post-COVID-19-patients. The association between disease severity and its sequelae may contribute to a better understanding of post-COVID-19 cognitive function.

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Post-Stroke Osteopathy: Current status and outlook

Due to no preliminary data is available yet this abstract describes the current status of the Pilot Study "Post-Stroke Osteopathy", discusses perils and gives an outlook on the project.

Background: Patients who survive an ischemic stroke and those with a transient ischemic attack (TIA) are at a higher risk of fractures (and falls). It is assumed that this is caused through a bone altering process initiated by the stroke itself. This process does not only occur on the stroke affected site of the body, which suggests that a systemic mechanism leads to structural alterations in the bones.

Methods: A high-resolution peripheral quantitative Computer Tomography imaging (HR-pQCT) will be performed of the distal radius and distal tibia right and left site on four different time points. Blood samples are drawn on five time points.

Current status and outlook: So far 92 patients have been included, 30 (32,6%) women and 62 (67,4%) men. With all of them a HR-pQCT has been performed at baseline. 48 patients completed the three and 17 patients additionally the six months visit, each including a HR-pQCT and blood sampling. The surplus of men in our population can be lead back to the exclusion of patients with a current intake of bone altering medication, which affects more women and the lack of women in the ground population. A major limitation is the narrow entrance to the facility of the HR-pQCT, which excludes patients with moderate or severe immobilization. This has to be discussed in future projects. Currently we would like to extend the recruiting period to include more patients and expand our investigations to the aspects of Sarcopenia and Frailty in this population. Furthermore an automated assessment of the HR-pQCT data regarding bone quality based on machine learning will be developed.

Funding: VASCage GmbH – Austria

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Sex Differences in the Association of Peripheral inflammation, Panss Scores and Sex in Schizophrenia

There is a growing literature regarding a relationship between neuroinflammation and schizophrenia, however, little is currently known about a potential sex-specific relationship between psychopathology when starting antipsychotic monotherapy and its change over one month and peripheral inflammation.

The sample consisted of 116 (52.6% male) patients with schizophrenia who were started on monotherapy with a second-generation antipsychotic. Sociodemographic and clinical data were collected at baseline. Psychopathology was rated at baseline and after 2 and 4 weeks of treatment using the Positive and Negative Syndrome Scale (PANSS). Blood samples (full blood count, CRP) were taken at the same points in time. Besides CRP, the integrative immune inflammation markers neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and systemic immune-inflammation index were considered.

Data of 116 cases were available for baseline analysis and of 57 cases at weeks 2 and 4. PANSS (sub)scores decreased significantly from baseline to consecutive follow-ups, and the two sexes did not differ in this regard. Similarly, no significant sex differences were found in CRP levels, NLR, MLR, and SSI. The results of Spearman rank correlation revealed no statistically significant association between PANSS (sub)scores, neuroinflammation markers, and sex at any time of investigation.

In the light of these findings, the two sexes do not differ in regards of changes in psychopathology and inflammatory biomarkers.

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Biomarkers in Parkinson's Disease

Parkinson's disease (PD) was first described by its' eponym James Parkinson in an essay on "the shaking palsy" more than 200 years ago. Since then, extensive research on this movement disorder has resulted in a better understanding of the clinical and pathophysiological aspects of PD. The clinical diagnosis of Parkinson's disease is defined by the presence of bradykinesia with at least one additional cardinal motor feature (rigidity and/or rest tremor) together with supporting and exclusionary criteria. However, the clinical phenotype of PD shows huge heterogeneity and is accompanied by a broad range of non-motor symptoms as well. As neuronal dysfunction is thought to begin already years before the occurrence of motor symptoms in Parkinson's disease, validated biomarkers are needed for the detection of prodromal stages of this movement disorder and to improve diagnostic accuracy. The aim of this PhD-Thesis will be to evaluate imaging biomarkers in Parkinson's disease and their applicability in clinical routine, particularly we will focus on imaging of the nigrosome 1.

A loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) is a well known histopathological hallmark of Parkinson's disease. The nigrosome 1 (N1) is a cluster of high concentrations of neuromelanin located in the dorsal medial aspect of the tail of the SNpc and was studied to be the territory mostly affected by the degeneration of neuromelanin-rich dopaminergic neurons. In recent years, susceptibility-weighted imaging (SWI) MRI studies made use of the disappearance of the N1 sign as a potential imaging biomarker in PD. In the present PhD project, we will evaluate the utility of the N1 sign for clinical practice by a blinded analysis of SWI sequences from PD patients and controls obtained from 1.5 Tesla vs. 3 Tesla MRI images.

Funding:

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Comparison of seizure quality in electroconvulsive therapy (ECT) after ASTI (anaesthesia to intervention time interval) or Narcotrend - a prospective randomized trial.

Major depression affects millions of people worldwide. For patients who do not respond to psychotherapy and antidepressive therapy the electroconvulsive therapy (ECT) may be an effective and safe treatment option. State of the art ECT treatment is performed under general anaesthesia with muscle relaxation to protect from seizure-related injuries. The quality of seizure correlates with the therapy effectiveness of depression, therefore it is crucial to improve seizure quality. The depth of anaesthesia is a relevant factor that influences the seizure quality: Light anaesthesia is associated with better ictal characteristics, while deep anaesthesia might impair seizure quality and treatment success. Nowadays, there are monitoring devices e.g. electroencephalogram (EEG), like the Narcotrend (NCT), to optimize the depth of anaesthesia by prolonged time interval from anaesthesia to the electrical stimulation (ASTI). Aim of this study was to evaluate a NCT-guided ECT management compared to an ECT management based on an ASTI.

This prospective, randomised trial started on 01.03.2022 and is still ongoing. Patients scheduled for ECT with a score >18 on the Montgomery-Asberg Depression Rating Scale (MADRS) were included in the study. Every patient was randomly assigned either into the NCT group, or into the ASTI group. NCT analyses the EEG and shows a classification on a scale from 1 (very deep narcosis) to 100 (fully awake). In the NCT group the seizure was induced, when the patient reached a NCT value between 41 and 64. In the ASTI group the seizure was induced four minutes after the start of administration of Thiopental. Seizure quality was assessed based three levels of seizure quality. These levels of seizure quality were our primary study endpoints.

An analysis of the data will be performed in the next few months once all patients are included and the results will be presented as part of the poster.

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Psychological distress in mental ill individuals: the mediating role of resilience and extraversion during the COVID-19 Pandemic in Tyrol and South Tyrol

Introduction:

Psychological distress increased at the beginning of the covid-19 pandemic in the general population worldwide. However, few studies investigating the impact on the mental health in mental ill individuals during the pandemic are available. The aim of this study was to investigate the affection of the long-term consequences on psychological distress of people with a mental illness compared to the general population in Tyrol and South Tyrol, as well as the assumed protective role of resilience and extraversion.

Participants and Methods:

178 people with a mental illness and 481 healthy controls from the general population participated in this online survey after the first wave of the pandemic and five months thereafter, during the second lockdown in Italy and Austria. Next to the assessment of sociodemographic and COVID-19-related variables the Brief-Symptom-Checklist, the Resilience Scaled, and the Big Five Inventory were used to assess psychological distress, resilience, and extraversion. Mediation analysis was used to investigate the role of resilience and extraversion in the context of sex related differences in psychological distress.

Results:

Psychological distress of people with a mental illness was at least 20% higher compared to the general population. Women were more burdened than men, while the burden in men increased slightly from the first to the second time point. Resilience could be identified as a strong coping mechanism that could mediate the relationship between patients/controls and psychological distress. Extraversion could be identified as a significant mediator between the patients/control group and psychological distress at the second time, although upon closer inspection, extraversion had a mediation effect only in the men's group.

Conclusion:

People with a mental illness are particularly affected by the measures and sequelae of the COVID-19 pandemic. Our findings indicate that they may have special needs during the pandemic and that there is a need for tailored prevention and mitigation strategies.

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Telemedical follow-up in patients after decompressive spine surgery – a retrospective, single center analysis

Introduction: During the COVID-19 pandemic telemedicine became an indispensable tool in health care. In our institution patients received a telemedical follow-up after lumbar and cervical decompressive spinal surgery to reduce patient load in our ambulance and minimize risk of infection. However, could be the implementation of telemedicine in the daily routine in spine surgery in a post-COVID era a reasonable measure?

Methods: In this retrospective analysis we used the clinical registry to identify patients who underwent cervical and lumbar decompression or sequestrectomy. 1343 patients were operated from 01. Feb 2020 to 11. Jan 2022. In total, 479 patients, who received a telemedical follow-up, were included into the study. Resident doctors called patients on average 6 weeks after surgery and checked with the help of a 10-point questionnaire their postoperative state. Demographic analysis, bivariate analysis and Cox regression were performed for statistical analysis.

Results: 139 (29%) of the included 479 patients needed an in-person visit despite of a telemedical follow-up. The main cause for a personal visit after telemedical follow-up were radiculopathy (HR 2.46, $p<0.001$) and pain in back or neck (HR 1.69, $p=0.011$). Also wound-healing disorders (HR 2.27, $p=0.005$) and subjective impairment of Quality of Life (HR 2.70, $p<0.001$) were significant reasons. However, sex ($p=0.503$), age ($p=0.755$) or multilevel decompression ($p=0,596$) showed no significant correlation with re-presentation. Recidive surgery was performed in 43 (9%) patients due to a recidive prolapse, wound-healing disorder or consequences of a duralaceration.

Conclusion: Telemedical follow-up showed a benefit in reducing the in-person visits after decompressive spine surgery. 70% of the patients included in this retrospective analysis did not require an in-person visit in our ambulance. Despite the potential advantages to telemedicine in spine surgery to optimize patient´s health care and satisfaction further research and development is needed.

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