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Echocardiographic monitoring of cardiac dysfunction in T cell-driven autoimmune myocardial inflammation

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Introduction:
Myocarditis is an inflammatory heart disease that leads to myocardial remodeling and progression to inflammatory cardiomyopathy in 20-30% of patients. Thus, monitoring of myocardial function is crucial for a diagnosis and treatment of patients. Cardiac imaging based on echocardiographic strain analysis bears the potential for accurate diagnosis and outcome prediction in various cardiomyopathies. However, it is still unclear to what extent strain analysis can be used for the diagnosis of myocarditis and whether predictive parameters for progressive inflammatory cardiomyopathy can be defined.

Material and Methods:
We have used a mouse model of CD4+ T-cell-driven autoimmune myocardial inflammation followed by inflammatory cardiomyopathy and heart failure. To monitor the dynamics of cardiac dysfunction throughout the disease process and to assess the predictive potential of strain alterations high resolution ultrasound was performed. For the detailed mechanical analysis speckle tracking-based global strain was used. In addition, histopathologic analysis and quantification of collagen deposition are used to assess the degree of myocardial inflammation and fibrotic remodeling.

Results:
We found that the early phase of autoimmune myocardial inflammation was associated with increased myocardial deformation. Our analyses revealed that global circumferential strain (GCS) was strongly reduced in mice with severe acute myocarditis and rapid progression to heart failure. Likewise, mice with chronic inflammatory cardiomyopathy and staggered loss of cardiac function showed significantly reduced GCS.

Conclusions:
GCS is significantly impaired in the disease courses of inflammatory cardiomyopathy with heart failure and reduced systolic function. Further evaluation of regional alterations in myocardial strain and histopathological analyses will facilitate the establishment of specific strain parameters for risk stratification of disease progression during myocardial inflammation.

Immune-mediated improved tolerance to kidney and liver ischemia-reperfusion injury in female mice

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Introduction:
Previous experimental and clinical studies have highlighted sex-specific susceptibility to ischemia-reperfusion injury (IRI) in multiple organs. While sex hormones seem to be important, the precise underlying mechanisms still need to be uncovered.

Material and Methods:
IRI was modelled in mouse kidneys using unilateral nephrectomy, followed by 23min ischemia and reperfusion of the controlateral kidney, and in livers by applying 35min ischemia and reperfusion. Post-operative kidney and liver function was evaluated over time by transcutaneous assessment of FITC-Sinistrin clearance and by plasma transaminases levels, respectively. Histology, immunohistochemistry and Spatial total mRNA sequencing was carried out on paraffin-embedded tissue. qPCR was performed on RNA extracted from flash-frozen organ samples. Time-course CyTOF was applied on PBMCs isolated from whole blood.

Results:
Following IRI, female mice had significantly better renal and liver function. Renal histology showed increased tubulointerstitial lesions in males, together with significantly increased expression of IL-6 and TNF-alpha. Consistently, the secretion of pro-inflammatory cytokines was followed by a significantly higher increase of blood neutrophils in males at postoperative day 2. In male kidneys, this was associated with the infiltration of an immune cluster at the corticomedullary junction and downregulation of metabolism, proliferation and survival related genes in proximal tubules.

Conclusions:
Our data show an increased recruitment of neutrophils at the onset of IRI, particularly in male kidneys. IL-6 and TNF-alpha seem to be sex-specific key regulators of this early immune response. Therapeutic targeting of these pathways could translate into clinical trials to improve graft outcome.

Discovery of small molecules promoting the conversion of human α-cells into insulin-producers

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Introduction:

Regeneration of β-cells is the ultimate goal to cure Type 1 Diabetes. The reprogramming of human pancreatic α-cells into insulin-producers is a promising approach. This functional reprogramming can be induced in primary human α-cells through the expression, using adenoviral vectors, of the β-cell-specific transcription factors Pdx1 and MafA ("αPM"). Transplantation of such αPM cells completely restored glucose homeostasis in diabetic mice (Furuyama et al. 2019). Interestingly, the reprogramming efficiency of human α-cells increased after transplantation, suggesting the involvement of circulating factors in this process. Still, the translation of this vector-based gene therapy into the clinic remains risky. We therefore aim at discovering novel druggable targets to foster α-cell reprogramming without viruses.

Material and Methods:

We are combining our unique model of αPM with novel bioinformatic approaches to identify potent reprogramming inducers. Specifically, we are performing a small-molecule screening to unravel effectors downstream of Pdx1 and MafA to find key druggable targets. Furthermore, using state-of-the-art computational approaches, we plan to discover the circulating factors that enhance αPM reprogramming *in vivo*.

Results:

Our preliminary drug screening suggests that the activation of Notch, FoxO1, and Hedgehog pathways, while maintaining Wnt signaling inactive, might be required to stabilize αPM reprogramming. Furthermore, through our bioinformatic pipeline, we have so far identified one *in vivo* circulating factor that enhances insulin gene expression in αPM.

Conclusions:

The combination of both approaches will allow us to design a cocktail of small molecules to pharmacologically promote α-cell reprogramming without the use of viral transduction.

Opposite effects in fractional donor-derived cell-free DNA from urine and plasma in kidney recipients? Results from a pilot study

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Introduction:
Limited sensitivity and specificity of current blood- and urine-based biomarkers can delay the suspicion of a kidney allograft injury as well as the biopsy needed for diagnosis confirmation. Timely detection of allograft injury would be of the essence. Donor-derived cell-free DNA (dd-cfDNA) in plasma has been shown to indicate kidney allograft injury. Our aim was to perform a pilot investigation of urinary dd-cfDNA as a potential new, non-invasive biomarker for allograft monitoring in kidney recipients.

Material and Methods:
Blood and urine samples from kidney transplant recipients (n = 93) were obtained at regular clinic visits. Droplet digital PCR was performed using mismatched HLA alleles between recipient and donor to determine the absolute (cp/mL) and relative (%dd-cfDNA) quantities of dd-cfDNA. Adjusting for dependency of dd-cfDNA on time after transplantation, correlations with clinical and biochemical parameters were assessed.

Results:
In samples from patients with biopsy-proven allograft injury compared to samples from stable recipients, showed significantly increased dd-cfDNA cp/mL ($p = 0.002$) and %dd-cfDNA ($p = 0.032$) in plasma, whereas the %dd-cfDNA in urine was significantly decreased ($p = 0.016$). Similarly, recipients of deceased donor organs showed increased %dd-cfDNA values in plasma compared to living donor recipients ($p = 0.049$), whereas the opposite was seen in urine ($p = 0.026$).

Conclusions:
Interestingly, our pilot study on dd-cfDNA in kidney allograft recipients identified contrary tendencies of urinary %dd-cfDNA compared to %dd-cfDNA in plasma. Dd-cfDNA measured in urine, which as a sample material originated from direct filtration through the kidneys, could therefore give additional insight into the condition of the allograft.

References:

- "Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: Results from a prospective observational study", Oellerich M. et al, 2019, American Journal of Transplantation
- "A Urine Score for Non-Invasive Accurate Diagnosis and Prediction of Kidney Transplant Rejection", Yang J.Y.C. et al., 2020, Science Translational Medicine

Assisted Suicide in Patients with Colorectal Cancer; Swiss Data from a 20-year Experience (1999-2018)

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Introduction:
The legalization of assisted suicide (AS) is one of the most debated topics in the field of medical ethics worldwide. There is limited data on the long-term development and trends of patients who underwent AS due to colorectal cancer (CRC).

Patients and Methods:
By using data of the Swiss Federal Statistical Office over a 20-year period from 1999-2018, our analysis is based on:
- All death cases: *n*=1´261´923: median age at death: 82 years (♀/♂ in 1999: 84 years/76 years, in 2018: 86 years/80 years.)
- All cancer-related death cases: *n*=323´610 (♂: 55%/♀: 45%); 25.6% of all death cases; median age at death: 74 years.
- All CRC-related death cases: *n*=32´979 (♂: 55%/♀: 45%); 10.2% of all cancer deaths, and 2.6% of all death cases; (1999-2003, *n*=8´112; 2004-2008, *n*=8´061; 2009-2013, *n*=8´384; 2014-2018, *n*=8´422); median age at death: 77 years.
- All AS death cases: *n*=8´738 (♀: 57%/♂: 43%): median age at death: 78 years.
For every 5-year sub period, the number of AS cases have roughly doubled compared to the respective preceding 5-year period (1999-2003, *n*=582; 2004-2008, *n*=1´161; 2009-2013, *n*=2´175; 2014-2018, *n*=4´820).

Results:
The most common underlying condition for AS was cancer: *n*=3´580 (♂: 51%/♀: 49%), 41.0% of all AS cases. CRC was the second most common subtype (*n=387*; ♂: 52%/♀: 48%; 10.8% of all cancer- related AS cases).
The most common subtype was lung cancer (*n*=508, 14.2%), the third most common subtype was breast cancer (*n*=386, 10.8%), followed by prostate (*n*=367, 10.3%), and pancreatic cancer (*n*=270, 7.5%). During the observation period, the number of CRC-related AS rose significantly: when analyzing four 5- year periods (1999-2003, 2004-2008, 2009-2013, 2014-2018), the number of AS cases approximately doubled over each period compared to the respective preceding one (*n*=22; *n*=49; *n*=99; *n*=217).
The median age of patients with CRC-related AS (74 years) was younger than that of all CRC-related deaths (77 years). In both groups, the median age at which CRC-related death took place remained relatively stable over time.
The ratio of CRC-related AS in relationship with all CRC-related deaths increased from 0.3% at the beginning of the study period (1999-2003) to 2.8% from 2014-2018. In this most recent study period (2014- 2018), the CRC ratio was similar compared to that of three of the other four major cancer subtypes: 3.0%, breast cancer (♀ only); 2.9%,

prostate cancer; 2.4%, pancreatic cancer; 2.8%, all other cancer types. Lung cancer showed a slightly lower ratio (1.7%).

Conclusions:
During the 20-year study period, the proportion of individuals who chose CRC-related AS has increased nine-fold. However, AS among CRC cancer patients remains rare and represents only approximately 3% of all CRC-related deaths.

Clinical sensitivity and specificity of a high-throughput microfluidic nano-immunoassay combined with capillary blood microsampling for the identification of anti-SARS-CoV-2 Spike IgG serostatus

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Introduction:
We evaluate the diagnostic performance of dried blood microsampling combined with a newly developed high-throughput microfluidic nano-immunoassay (NIA) for the identification of anti-SARS-CoV-2 Spike IgG seropositivity.

Material and Methods:
We conducted a serological study among 192 individuals with documented prior SARS-CoV-2 infection and 44 SARS-CoV-2 negative individuals. Participants with prior SARS-CoV-2 infection had a long interval of 11 months since their qRT-PCR positive test. Serum was obtained after venipuncture and tested with an automated electrochemiluminescence anti-SARS-CoV-2 S total Ig reference assay, a commercial ELISA anti-S1 IgG assay, and the index test NIA. 109 participants from the positive cohort and 44 participants from the negative cohort also participated in capillary blood collection using three microsampling devices: Mitra, repurposed glucose test strips, and HemaXis. Samples were dried, shipped by regular mail, extracted, and measured with NIA.

Results:
Using serum samples, we achieve a clinical sensitivity of 98·33% and specificity of 97·62% on NIA, affirming the high performance of NIA in participants 11 months post infection. Combining microsampling with NIA, we obtain a clinical sensitivity of 95·05% using Mitra, 61·11% using glucose test strips, 83·16% using HemaXis, and 91·49% for HemaXis after automated extraction, without any drop in specificity.

Conclusions:
High sensitivity and specificity in anti-SARS-CoV-2 serological testing was demonstrated when testing micro-volume capillary dried blood samples using NIA, which is expected to facilitate its use in large-scale studies using home-based sampling or samples collected in the field.

Nitric oxide synthase activity required for beneficial effects of hypothermic oxygenated perfusion in cardiac grafts obtained with DCD

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Introduction:

Cardiac donation after circulatory death (DCD) is a promising option to increase graft availability. We previously reported that graft preservation with hypothermic oxygenated perfusion (HOPE) between cardioplegic flush and normothermic reperfusion improves graft recovery as compared to cold static storage (CSS), the current clinical standard. We investigated the role of preserved nitric oxide synthase (NOS) activity during HOPE on its beneficial effects.

Material and Methods:

Using a rat model of DCD, hearts underwent in-situ ischemia (21 minutes), were explanted for a cold storage period (30 minutes), and then reperfused under normothermic conditions (60 minutes) with left ventricular loading. Three cold storage conditions were compared: CSS, HOPE, and HOPE with L-NAME (NOS inhibitor). To evaluate potential confounding effects of high coronary flow (CF) during early reperfusion in HOPE hearts, bradykinin (BK) was administered to normalize CF to HOPE levels in two additional groups (CSS and HOPE with L-NAME).

Results:

Cardiac recovery was significantly improved in HOPE vs. CSS hearts, as determined by cardiac output, left ventricular work, contraction and relaxation rates, as well as CF ($p<0.05$). Strikingly, the addition of L-NAME during HOPE largely abolished its beneficial effects, even when early reperfusion CF was normalized to HOPE levels.

Conclusion:

HOPE provides superior preservation of ventricular and vascular function compared to the current clinical standard. Importantly, preservation of NOS activity during the cold storage period is required for HOPE's protective effects. The application of HOPE prior to normothermic machine perfusion is a promising approach to optimize graft recovery in DCD cardiac grafts.

Exploration of function and regulation of an alternative mRNA transcript of the *EPO* gene

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Introduction:
Erythropoietin (Epo) is the main physiological regulator of erythropoiesis. The protein is produced from a gene with 5 exons and 4 introns. Fully transcribed, it gives rise to the functional protein via the so-called P1 mRNA transcript. It is known that there is a second alternative Epo mRNA transcript, called P2. Its origin lies in a highly conserved DNA region in intron 1 of the *EPO* gene. The putative promotor of the P2 mRNA holds 3 GATA boxes as locations for possible binding of transcription factors. This work aims to contribute to the understanding of the physiological function and regulation of the P2 mRNA transcript.

Material and Methods:
To study the effects of the P2 transcript, we created hepatoma Hep3B P2 knock-out cell lines using CRISPR/Cas9 engineering. Subsequently, we quantified the effects on Epo P1 mRNA and P2 mRNA under normoxia by RT-qPCR and $\Delta\Delta$Ct-analysis.

Results:
The CRISPR/Cas9-mediated targeting strategies gave rise to 55 clones. 3 of them were sequenced and analyzed in detail. Removal of GATA boxes in intron 1 of the *EPO* gene leads to downregulated P2 expression by 3.6-fold ($P < 0.001$). At the same time, expression of the P1 transcript starting in Exon 1 was decreased by 5-fold ($P < 0.001$).

Conclusions:
We suggest that GATA boxes in intron 1 have a regulatory function toward the P2 expression. Additionally, we propose that P2 expression is necessary for P1 expression to function properly. Hence, P2 could have a regulatory function regarding P1 on RNA level, since we know that P2 does not give rise to a functional protein. Further experiments are needed to strengthen this hypothesis and explain a possible mechanism of action.

Precision Cut Lung Slices as an ex vivo Model to study Respiratory Diseases

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Introduction:
Conditions including infections, sepsis, and pneumonia may lead to acute lung injury (ALI) and eventually to Acute Respiratory Distress Syndrome (ARDS). ARDS contributes to major healthcare burden. ARDS patients have increased levels of heme in the blood and alveolar spaces, and elevated systemic heme levels have been associated with unfavorable clinical outcomes in sepsis related ARDS (Janz DR.J Intensive Care. 2015 Jun 17;3:20). A major limiting factor in studying ARDS is the lack of suitable experimental models. Therefore, we have developed an advanced ex vivo model of heme-induced acute lung injury using human Precision Cut Lung Slices (PCLS).

Material and Methods:
PCLS were obtained from 11 human donors undergoing lung surgery for various indications. PCLS were cultured and stimulated with 100 µM heme for 24h. Viability was assessed by colorimetric XTT assay at different heme concentrations. Inflammation was evaluated by cytokine secretion measurements (ELISA).

Results:
Heme-treated PCLS had a dose-dependent reduction of viability compared to untreated controls starting at 100µM. Pro-inflammatory markers and injury signals were observed after heme stimulation. Inflammatory cytokines such as IL-6, TNF-α and IL-8 were significantly elevated in supernatants from PCLS treated with heme compared to untreated PCLS.

Conclusions:
Heme induces inflammation in human PCLS and is cytotoxic at higher concentrations. Our model of human PCLS will be further developed as a disease model for ALI to better understand the mechanism of heme-induced lung injury during ARDS.

Loss of S1P Lyase Expression in Human Podocytes Causes a Reduction in Nephrin Expression That Involves PKCδ Activation

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Introduction:
Sphingosine 1-phosphate (S1P) lyase (SPL, *Sgpl1*) is an enzyme that irreversibly degrades the bioactive lipid, S1P. Biallelic mutations in the human *Sglp1* gene lead to a severe form of steroid-resistant nephrotic syndrome. We hypothesized that the S1P metabolism plays a key role in the development of the nephrotic syndrome by interfering with the production of the core protein of the kidney filtration barrier, nephrin.

Material and Methods:
We used an immortalized human podocytes cell line to better understand the mechanism underlying nephrotic syndrome in patients. A stable SPL-kd was generated by the lentiviral shRNA transduction characterized by reduced SPL mRNA and protein levels and increased S1P levels with LCMS.

Results:
SPL-kd leads to the downregulation of the nephrin protein and mRNA expression, as well as the Wilms tumor suppressor gene 1 (WT1), which is a key transcription factor regulating nephrin expression. Mechanistically, SPL-kd resulted in increased total cellular protein kinase C (PKC) activity, while the stable downregulation of PKCδ revealed increased nephrin expression. Furthermore, the pro-inflammatory cytokine, interleukin 6 (IL-6), also reduced WT1 and nephrin expression. In addition, IL-6 caused increased PKCδ Thr^{505} phosphorylation, suggesting enzyme activation.

Discussion: Our data demonstrate that nephrin is a critical factor downregulated by the loss of SPL, which may directly cause podocyte foot process effacement as observed in mice and humans, leading to albuminuria, a hallmark of nephrotic syndrome. Furthermore, our in vitro data suggest that PKCδ could represent a new possible pharmacological target for the treatment of a nephrotic syndrome induced by SPL mutations.

SHP2 targeting enhances the therapeutic efficacy of JAK inhibition in myeloproliferative neoplasms

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Introduction:

Myeloproliferative neoplasms (MPN) are hematologic malignancies characterized by mutations in JAK2, CALR or MPL, which result in constitutive JAK2 signaling and subsequent hyperactivation of JAK-STAT, MAPK and PI3K pathways. JAK2 inhibitors like ruxolitinib inhibit the JAK-STAT axis, but MAPK pathway signaling remains activated in vivo. Prior studies confirmed the relevance of MAPK signaling in MPN, but the connection between JAK2 and the MAPK pathway remains unclear. Different proteins including SHP2 have been implicated in this connection, but its role in MPN has not been fully investigated.

Material and Methods:

SHP2 was depleted by shRNA in Ba/F3 EPOR cells expressing V617F mutant or wildtype Jak2. Pharmacologic SHP2/JAK2 inhibition was evaluated in Ba/F3 cells and MPN mouse models.

Results:

shRNA-induced or pharmacologic SHP2 targeting reduced activation of MAPK pathway kinases ERK1/2 and RSK and expression of MAPK downstream effector DUSP6. Signaling effects were most pronounced after combined JAK2/SHP2 targeting. Genetic and pharmacologic SHP2 targeting sensitized cells to ruxolitinib, reflected by significantly reduced IC_{50} compared to ruxolitinib as single agent. More moderate effects were observed in JAK2 wildtype cells. In a Jak2V617F mutant mouse model, SHP2/JAK2 inhibition corrected phenotypic features including splenomegaly, erythrocytosis and leukocytosis. In a MPLW515L mutant mouse model, JAK2/SHP2 inhibition normalized splenomegaly and leukocyte counts and reduced MPN infiltration of the spleen and extramedullary hematopoiesis in the liver.

Discussion/Conclusions:

Our findings suggest a relevant role of SHP2 in MPN. Future studies will detail the mechanistic role of SHP2 in MPN and consolidate the therapeutic potential of combined JAK2/SHP2 inhibition.

Early Minimally Invasive image-guided eNdoscopic Evacuation of iNTracerebral haemorrhage: A pilot trial (EMINENT-ICH Pilot Trial)

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Introduction:
Intracerebral haemorrhage is the most devastating form of stroke with mortality rates over 50%. Currently, no sufficiently effective treatment is available. Endoscopic surgery (ES) seems to improve survival and functional outcome rates. We present results of our pilot trial assessing feasibility and safety of ES for ICH.

Patients and Methods:
Patients at the University Hospital Basel with a hematoma volume between 20ml and 100ml between July 2021 and January 2023 were enrolled. Endoscopic evacuation was performed within 24 hours after bleeding onset. Co-primary outcomes were 1) good functional outcome, defined as mRS ≤3 at 6 months and 2) adequate hematoma removal to below 15mL. Secondary outcomes were mortality and morbidity rates.

Results:
Ten Patients (median age 72.5 years [IQR 68.25-79.75], 70% male) were enrolled. Favourable outcome after 6 months was achieved in 83% (5/6) of the patients with completed follow-up while favourable outcome at the last registered visit was achieved in 70% (7/10). Satisfactory hematoma evacuation was achieved in 70% (7/10) with a median evacuation percentage of 69.5% [IQR 60-93%]. Two patients (20%) died from pneumonia and re-bleeding respectively while one patient died due to a glioblastoma. Four patients experienced a total of five complications, one re-bleeding, three pneumonias and one seizure. The median duration of surgery was 92 minutes [IQR 78-108].

Conclusions:
ES seems feasible, safe and leads to improved favourable outcome. Adequate hematoma removal is achievable. Based on this pilot trial a national multicentre RCT comparing ES to best medical treatment is planned (NCT05681988).

Rac1 GTPase as a regulator of the oscillation dynamics in the mouse presomitic mesoderm

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Introduction:

Presomitic Mesoderm (PSM) is an embryonic tissue primarily organized along the main anterior-posterior(A/P) axis of vertebrates and periodically forms somites at its anterior end. The tissue is characterized by the existence of gene transcription waves sweeping through it from its posterior to the anterior end. In order for the waves to be established and propagated along the PSM tissue, individual cells undergo sequentially in time and space coordinated gene expression oscillations of a subset of their genes, which are called cyclic genes. Beyond the directionality of the waves, the polarity of the PSM along the A/P axis is visible at the level of gene expression gradients (Wnt3a, Fgf8) and cell behavior (cell motility), which peak at the posterior end. The periodicity of wave generation and propagation is characteristic for the different vertebrae species and is called the segmentation clock.

Material and Methods

In this system, we sought to apply chemical perturbations on signaling pathways associated with cell motility, directionality, polarity and mechanosensitivity. The analysis of the effect on oscillation dynamics was done by incorporating a two-dimensional explant culture model derived from the posterior most part of the mouse E9.5 embryo PSM and a reaggregation assay, where we dissociated first and then re-mixed PSM cells in vitro. Time series imaging was performed in an LSM710 confocal microscope and image analysis in Fiji with an automated macro script. The period was measured with the RSSA package in R. Somites were quantified after overnight (21.5h) incubation of whole mouse E10.5 embryo tails and tails were then processed for either RNA extraction or in situ hybridization.

Results:

The chemical screen on 2D explant cultures revealed that Rac1 small GTPase inhibitors can increase the periodicity of gene expression oscillations almost two-fold compared to control samples, while this effect is also concentration dependent. When induced six hours after culture start, Rac1 inhibition does not only change the periodicity but also the oscillation phase and amplitude. Additionally, we observed that after overnight incubation of embryo tails with the inhibitor, a clear phenotype of increased somite sizes and decreased somite numbers appears. In order to find the mechanism through which Rac1 exerts its actions on the PSM, we performed whole mount in situ hybridization and quantitative PCR experiments for downstream Wnt, Notch and FGF targets in both the 2D explants as well as the whole tail cultures. We found that inhibition of Rac1 in the PSM causes a downregulation of downstream Wnt targets like Msgn and Bra. Reversely, we reported an upregulation of Hes5, which is a core component of the segmentation clock and downstream Notch target. Investigating also the effect on the somitic tissue, Rac1 inhibition appears to change the transcriptome of certain somitic markers as reported also previously.

Conclusions:
All in all, we managed to standardize the experimental process of the screen and automate the analysis of time series. This analysis led to the identification of Rac1 small GTPase as a potent regulator of the segmentation clock. Rac1 inhibition manifests a clear phenotype in somite formation. Our next steps will focus on shedding more light in the interplay between Rac1 and the central regulators of somitogenesis as well as its role on somitic patterning.

mTOR signaling in sensory hair cells of the inner ear

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Introduction:
Both hearing and balance rely on highly specialized sensory hair cells (HCs). HC loss is mostly permanent and current therapies for the treatment of hearing loss are limited to prosthetic devices. To design future therapies for hearing or vestibular disorders, we depend on understanding molecular mechanisms involved in sensory HC function, loss, and survival. The mammalian target of rapamycin (mTOR) kinase forms part of two structurally and functionally distinct multiprotein complexes, mTOR complex (mTORC1) 1 and mTORC2. Each of these complexes signals via its own signaling pathway, regulating numerous cellular processes. The role of each mTOR complex in sensory HCs remains incompletely understood. RAPTOR and RICTOR are both defining and essential subunits of mTORC1 and mTORC2, respectively.

Material and Methods:
In order to decipher the role of mTORC1 and mTORC2 in sensory HCs, we generated HC-specific *Raptor* and *Rictor* knockout (HC-RicKO and HC-RapKO) mice.

Results:
HC-RicKO mice suffer from early onset, progressive, and profound hearing loss with impaired outer HC function. HC loss occurs only secondary to hearing loss in HC-RicKO mice. Instead, HC-RicKO mice show abnormal stereocilia. Synapse numbers are also significantly reduced in HC-RicKO mice, with a reduced synaptic actin cytoskeleton and disrupted organization of synaptic proteins. In contrast to auditory function, vestibular function is unaffected in HC-RicKO mice. Results of HC-RapKO mice will also be presented at the congress.

Conclusions:
mTORC2 regulates auditory HC structure and function and is essential for hearing. These results provide novel insights on mTOR signaling in inner ear sensory HCs.

Evolution of hypoxia and hypoxia-inducible factor asparaginyl hydroxylase (FIH) regulation in chronic kidney disease

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Introduction:
The roles of hypoxia inducible factor (HIF) during chronic kidney disease (CKD) are much debated. Interventional studies with HIF-α activation in rodents yielded contradictory results. The pathway is regulated by prolyl and asparaginyl hydroxylases; little is known about the effect of the asparaginyl hydroxylase Factor Inhibiting HIF inhibiting (FIH) in CKD.

Material and Methods:
We used a model of proteinuric CKD and a model of obstructive nephropathy. In these models, we assessed hypoxia with pimonidazole and vascularization with 3D micro-CT imaging. We analyzed a database of 217 CKD biopsies and we collected 15 CKD biopsies from various severity degrees to assess FIH expression. Finally, we modulated FIH activity *in vitro* and *in vivo* using a pharmacologic approach, to assess its relevance in CKD.

Results:
In our model of proteinuric CKD, we show that early CKD stages are not characterized by hypoxia or HIF activation. At late CKD stages, some areas of hypoxia are observed, but these are not colocalizing with fibrosis. In mice and in humans, we observed a downregulation of the HIF pathway, together with an increased FIH expression in CKD, according to its severity. Modulating FIH in vitro affects cellular metabolism, as described previously. *In vivo*, pharmacologic FIH inhibition increases the glomerular filtration rate of control and CKD animals and is associated with a reduced development of fibrosis.

Discussion/Conclusions:
The causative role of hypoxia and HIF activation in CKD progression is questioned. A pharmacological approach of FIH downregulation seem promising in proteinuric kidney disease.

Molecular Characterization of BK Polyomavirus Replication in Allogeneic Hematopoietic Cell Transplantation Patients

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Introduction:
High-level BK polyomavirus (BKPyV) replication in allogeneic hematopoietic cell transplantation (HCT) predicts failing immune control and BKPyV-associated hemorrhagic cystitis. However, there is limited understanding of the underlying molecular features and pathophysiology. In this study, we provide the first systematic molecular characterization of BKPyV replication and BKPyV-associated hemorrhagic cystitis in HCT patients.

Material and Methods:
To identify molecular markers of BKPyV replication and disease, we scrutinized longitudinal urine and plasma pairs from 20 HCT patients. This through assessing: BKPyV DNA-load using quantitative nucleic acid testing (QNAT), DNase-I treatment prior to QNAT and next-generation sequencing (NGS). Additionally, we tested cell-mediated immunity by measuring CD8 T-cell INF-y response to stimulation with immunodominant epitopes.

Results:
Use of larger QNAT amplicons led to under-quantification and false-negatives results ($P < .001$). DNase-I reduced urine and plasma BKPyV-loads by >90% ($P < .001$), indicating non-encapsidated BKPyV genomes. DNase-resistant urine BKPyV-loads remained infectious in cell culture. BKPyV genome fragmentation of ≤250 bp impaired NGS coverage of genetic variation using 1000-bp and 5000-bp amplicons. Conversely, 250-bp amplicons captured viral minority variants. We identified genotype-specific and genotype-independent changes in capsid Vp1 or T-antigen predicted to escape from antibody neutralization or cytotoxic CD8 T-cells, respectively. Genotype-specific changes in immunodominant 9mers were associated with reduced or absent CD8 T-cell responses.

Discussion/Conclusions:
Our results provide new insights for patient evaluation and risk assessment. They highlight the need for standardization of diagnostic tests and the contribution of both viral adaptation and host factors to BKPyV replicative success and disease potential. Finally, they provide the basis for future prospective evaluation.

Bioengineering of vascularized insulin-secreting constructs for type 1 diabetes

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Introduction:
Disruption of islet extracellular matrix (ECM), inflammation and delayed vascularization lead to a significant islet loss following intraportal islet transplantation. Human amniotic membrane (HAM) is known for its rich ECM and anti-inflammatory properties. Blood outgrowth endothelial cells (BOECs) constitute an ideal source of autologous endothelial cells. This project aims to assess whether incorporation of pre-vascularized insulin-secreting organoids (PVO) into a pre-vascularized HAM-derived hydrogel will support their engraftment and function.

Material and Methods:
PVOs were generated on microwell platforms by combining different ratios of insulin-secreting cells, human amniotic epithelial cells (hAECs) and BOECs. PVOs were encapsulated within BOEC-containing HAM-hydrogel (constructs) and cultured in a vasculogenic media. Cell distribution and insulin-secreting cells function was assessed *in vitro*. To evaluate *in vivo* function, the best performing PVOs were transplanted in the epidydimal fat pad of diabetic NSG mice. Following, insulin-secreting constructs composed of PVOs and BOECs-vascularized HAM-hydrogel were transplanted under the skin of diabetic NSG mice.

Results:
Generated PVOs displayed a good function *in vitro*. The best performing PVO ratio was composed of 50% insulin-secreting cells, 25% hAECs and 25% BOECs. PVOs transplantation in epidydimal fat pad significantly improved engraftment, leading to reversal of diabetes in 6 out 7 mice, which stayed within normoglycemic range for 3 months. Furthermore, the engineered constructs supported PVO function and the development of an integrated vascular-like network.

Conclusions:
Our findings suggest that insulin-secreting constructs composed of PVOs and BOECs-vascularized HAM-derived hydrogel could be a promising strategy of β cell replacement therapies in alternative sites other than the liver.

Investigating the role of nonsense-mediated mRNA decay in hepatocellular carcinoma

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Introduction:
Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism that degrades transcripts containing premature termination codons arising from transcription or splicing errors. In cancer, NMD eliminates most frameshifted transcripts encoding neoantigens, thereby impeding potential anti-tumor immune responses. Conversely, NMD negatively regulates mRNAs of several stress factors, such as ATF4 and ATF5. Consequently, NMD inhibition leads to the upregulation of the integrated stress response, making cancer cells more resistant. How these pro vs. anticancer roles of NMD play out *in vivo* remains unclear.

Material and Methods:
We use a novel genetic mouse model to inactivate NMD (*Smg6*$^{mut/mut}$) in the context of hepatocellular carcinoma (HCC), to evaluate the outcome of NMD inactivation on HCC development and progression. Additionally, we utilize a genetic HCC mouse model that relies on overactivation of the mTOR pathway through *Tsc1* and *Pten* double knockout (Liver double knock-out: *L-dKO*), which triggers HCC.

Results:
L-dKO Smg6$^{mut/mut}$ mice exhibit significant hepatomegaly (Liver/body weight: 19%). In contrast, *L-dKO Smg6*$^{wt/wt}$ mice display characteristics of NAFLD along with mild hepatomegaly (Liver/body weight: 12%). Moreover, *L-dKO Smg6*$^{mut/mut}$ livers exhibit a proliferative phenotype, validated by the significantly increased expression of PCNA. Our immunofluorescence analyses reveal a high degree of immune cell infiltration in the *L-dKO Smg6*$^{mut/mut}$ livers.

Discussion/Conclusion:
Our analyses show that *Smg6* mutation in addition to *L-dKO* induces a strong inflammatory response, causing immune cell infiltration to the liver, leading to a massive hepatomegaly. We expect our findings to provide important insights into the potential of NMD inhibition as a therapeutic option for HCC.

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