MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

7TH MUI-START SYMPOSIUM

PROGRAM

CENTER OF CHEMISTRY & BIOMEDICINE, INNRAIN 80

PROGRAM

14:45 H Welcome Address of Prof. Christine Bandtlow (Vice Rector of Research and International Relations)

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Assoz. Prof. Priv.-Doz. Dr.med.univ. Isabel Heidegger PhD (Department of Urology) "Robo 4 - the double-edged sword in prostate cancer: impact on cancer cell aggressiveness and tumor vasculature"	P 01
Katalin Andrea Csanaky MD PhD (Division of Neuroanatomy) "Light-induced subcellular FGFR1 activation induces neuronal differentiation"	P 02
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Poster presentations	**Abstract**
Dr.med.univ. Thomas Resch (Department of Visceral, Transplant and Thoracic Surgery) "Toll-like receptor (TLR)-3 – a promising novel target for the prevention of ischemia-reperfusion injury in solid organ transplantation"	P 07
Coffee and discussion	

ABSTRACTS

P 01: Robo 4 - the double-edged sword in prostate cancer: impact on cancer cell aggressiveness and tumor vasculature

Andreas Pircher[1], Georg Schäfer[2], Andrea Eigentler[3], Renate Pichler[2], Martin Puhr[3], Eberhard Steiner[3], Wolfgang Horninger[3], Eberhard Gunsilius[1], Helmut Klocker[3] and Isabel Heidegger[3]

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Background: The magic roundabout receptor 4 (Robo 4) is a tumor endothelial marker expressed in the vascular network of various tumor entities. However, the role of Robo 4 in prostate cancer (PCa), the second common cause of cancer death among men in –developed countries, has not been described yet. Thus, the present study investigates for the first time the impact of Robo 4 in PCa both in the clinical setting and *in vitro*.

Methods and Results: Immunohistochemical analyses of benign and malignant prostate tissue samples of 95 PCa patients, who underwent radical prostatectomy (RPE), revealed a significant elevated expression of Robo 4 as well as its ligand Slit 2 protein in cancerous tissue compared to benign. Moreover, increased Robo 4 expression was associated with higher Gleason score and pT stage. In advanced stage we observed a hypothesis-generating trend that high Robo 4 and Slit 2 expression is associated with delayed development of tumor recurrence compared to patients with low Robo 4 and Slit 2 expression, respectively.

In contrast to so far described exclusive expression of Robo 4 in the tumor vascular network, our analyses showed that in PCa Robo 4 is not only expressed in the tumor stroma but also in cancer epithelial cells. This finding was also confirmed *in vitro* as PC3 PCa cells express Robo 4 on mRNA as well as protein level. Overexpression of Robo 4 in PC3 as well as in Robo 4 negative DU145 and LNCaP PCa cells was associated with a significant decrease in cell-proliferation and cell-viability.

Conclusion: In summary we observed that Robo 4 plays a considerable role in PCa development as it is expressed in cancer epithelial cells as well as in the surrounding tumor stroma. Moreover, higher histological tumor grade was associated with increased Robo 4 expression; controversially patients with high Robo 4 tend to exert lower biochemical recurrence possibly reflecting a protective role of Robo 4.

P 02: Light-induced subcellular FGFR1 activation induces neuronal differentiation

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Background: Fibroblast growth factor receptor 1 (FGFR1) activates signaling pathways involved in neuronal differentiation. FGFR signaling is dynamically regulated in space and time, therefore FGFR1 represents a useful candidate for optogenetic stimulation of subcellularly targeted FGFR kinase.

Methods: Chimeric murine opto-FGFR1 consists of the catalytic domain of FGFR1 tyrosine kinase and an algal light-oxygen-voltage-sensing (LOV) domain. The latter dimerizes upon blue light stimulation, and the phosphorylated tyrosine kinase then activates downstream signaling cascades. Four opto-FGFRs were produced to target different subcellular compartments, such as plasma membrane, endosomes, cytosol and nucleus. Constructs were tested in HEK293 cells and PC12 cells were used for analysis of neurite outgrowth.

Results: Membrane-opto-FGFR1 (mem-opto-FGFR1) localized predominantly to the surface membrane but was also detected in endosomes. Cyto-opto-FGFR1 (cyto-opto-FGFR1) diffused freely in the cytoplasm, while nuclear-opto-FGFR1 (nucl-opto-FGFR1) was found exclusively in the nucleus. The FYVE-containing endosomal-opto-FGFR1 by immunoelectron microscopy revealed unspecific localization in the nucleus, cytoplasm and plasma membrane. Western blot results showed that blue light stimulation following cyto-opto-FGFR1 transfection induced a significant increase in pERK levels, and an even greater increase was seen in mem-opto-FGFR1 transfected cells. The pAKT/tAKT ratio did not change, while an increase of pPLCɣ1/tPLCɣ1 ratio was shown after light stimulation of mem-opto-FGFR1 transfected cells. PC12 cells expressing mem-opto-FGFR1 exhibited significantly longer neurites after blue light stimulation than after ligand (NGF or FGF) treatment, and significantly more neurites extended from the cell bodies. The cyto- and nucl-opto-FGFR1 did not show any measurable effect in this experimental setting.

Conclusion: FGFR1 dependent neurite outgrowth can be controlled and manipulated optogenetically, which allows us to study subcellular receptor activation with spatial and temporal precision.

P 03: Iron metabolism is a conserved regulator of whole body cholesterol homeostasis

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This abstract will not be published because it contains highly sensitive data. If you are interested in the results, visit the poster or directly contact the author.

P 04: The role of miRNA 6240 during neonatal cardiac regeneration

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In 2011 Porrello et al. reported a short postnatal window of complete cardiac regeneration following apical resection in the neonatal mouse. Stimulated by their work we independently established a neonatal mouse model of left anterior descending artery (LAD) ligation and proved excellent recovery of murine neonatal hearts after clinical relevant myocardial infarction (MI). The underlying pathways are still poorly defined. Thus, we carried out a comprehensive analysis of the coding and non-coding transcriptome in the normal developing mouse heart and the post-MI mouse heart. These data revealed a previously unreported transition in microRNA expression in the developing heart between postnatal day three (P3) and P5 that associates specifically with cessation of cardiomyocyte cell division. To test our conclusions that these data define the major coding and non-coding transcriptional pathways for normal cardiac development and post-MI repair, we selected exemplars of miRNAs that were implicated as regulators of cardiomyocyte proliferation. Of the successfully tested miRNAs we now selected miR-6240 for further *in vitro* and *in vivo* analysis.

miR-6240 is a recently discovered miRNA that was previously found in a transcriptome analysis of murine cardiac hypertrophy. To date, no functional data for miR-6240 are available. However, *in silico* target prediction for miR-6240 and our preliminary data suggest a pivotal role of miR-6240 in cell proliferation and possibly hypoxia signaling.

We now aim to understand the role of miR-6240 during murine cardiac development and neonatal cardiac regeneration. In order to test our hypothesis that miR-6240 is involved in the regulation of cardiomyocyte proliferation we established overexpression and decoy constructs for miR-6240.

So far we confirmed the time dependent regulation of miR-6240 in the neonatal heart and found that there is no physiological difference in heart function and morphology by cardiomyocyte specific miR-6240 overexpression. Hence, following establishment of the genetic tools we aim to test more cardiac in vivo conditions, to decipher the role of miR-6240 during neonatal cardiac regeneration.

P 05: Ongoing comprehensive multi-omic profiling of tumor endothelial cells of prostate tissue

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Background: Current anti-angiogenic therapies for cancer therapy destroy tumor vessels by blocking VEGF, with the ultimate goal to starve cancer cells. Anti-VEGF therapy has been approved for various cancers, including metastatic colorectal cancer (CRC) and non-small cell lung cancer (NSCLC). However, VEGF-targeted therapy poses major problems and showed no effect in prostate cancer (PCA) claiming for alternative anti-angiogenic strategies, based on fundamentally different mechanisms. Therefore, the aim of the present translational study is to isolate tumor endothelial cells (TECs) and normal endothelial cells (NECs) from PCA patients undergoing a radical prostatectomy as well as to perform multi-omic profiling (including targeted metabolomics, transcriptomics) to identify new targets for anti-angiogenic therapies.

Methods: We isolated NECs and TECs from 50 radical prostatectomy specimens. After successful enrichment of NECs/TECs by culturing and CD31 magnetic bead purification we confirmed endothelial cell phenotype by immune-fluorescence and FACS analysis. Next we analyzed cell proliferation using 3H-thymidine incorporation assay. Furthermore we collected NECs and TECs for unbiased RNA sequencing. Sample collection for targeted metabolomics is ongoing and will be performed in the near future.

Results: NECs and TECs could be successfully isolated with an estimated success rate of 80%. Phonotypical investigations showed high CD31 positivity reflecting EC phenotype. Furthermore NECs and TECs differed from morphological aspects (cell size, cell nuclei and junctions formation). In addition TECs are hyper-proliferative compared to NECs reflecting are hyper-motile states. Preliminary RNA-Seq analyses reveal that TECs and NECs differ in cholesterol metabolism.

Conclusion and outlook: Here we show for the first time that human NECs and TECs can be isolated and cultured from fresh prostate tissue. First analysis of NECs and TECs show morphological and functional differences. Preliminary RNA-Seq analysis revealed difference in cholesterol metabolism between TEC and NEC, currently untargeted cholesterol metabolism analysis are ongoing.

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P 06: Paving thE way towaRds tailored-made medicine. The uSe of 3-dimensiONAL-prInting models to simulate an individualIZEd transcatheter aortic valve implantation (TAVI). The PERSONALIZE-TAVI Project

Fabian Plank[1],

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Background: Aortic stenosis is the most common valve disease in the industrial world and is associated with very high mortality rates if left untreated. In elderly and high-risk patients, transcatheter aortic valve has been established as an excellent treatment with comparative results as surgical conventional surgery. Nonetheless, this procedure relies solely on precise preprocedural planning and can therefore be further enhanced using 3-D models to simulate implantation and prevent complications.

Methods: We will examine 35 patients (incl. 5 pilot patients) prospectively which will undergo a preprocedural and postprocedural cardiac CT exam.

The CT scans are performed during routine CT-evaluation for TAVI eligibility and procedural planning. Based on CT reconstructions, 3-dimensional replicas of the aortic root and adjacent structure will be constructed for planning and subsequently implanting the transcatheter valve. Part of this process will involve the production of standardized prosthesis models to simulate individual size selection, landing zone, expansion level, anatomic details and potential procedural adjustments.

An additional post-procedural CT will be performed to assess implantation success and further evaluate computational fluid dynamics for flow patterns and shear stress.

First results: This first-in-hospital approach of 3 dimensional planning of aortic valve implantation was planned to guide proceduralists through individual anatomical differences and reduce complications. An initial approach using various software to overcome the conjunction between raw computed tomography data and 3-D printer compatible data has delayed the scheduled timeline. However, a new cooperation with Materialise (Leuven, Belgium) has connected the missing data link and set us at the verge of our first pilot prints. Additionally, we could present our project´s blueprint at the Inaugural Symposion of the Austrian Platform for Personalized Medicine enhance our cooperation with the health and life science university (UMIT) and subsequent flow dynamic measurements.

Next steps: With the new software solution, we are eager to advance to the first 3-D printed models and further procedure guidance. Further adjacent fluid dynamic analysis will be initialized.

P 07: Toll-like receptor (TLR)-3 – a promising novel target for the prevention of ischemia-reperfusion injury in solid organ transplantation

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Background: Toll-like receptor (TLR)-3 represents a pattern recognition receptor as part of the innate immune system. Recently it has been proposed as a possible candidate molecule for the modulation of cardiac ischemia reperfusion (IRI) in vitro.

Methods: In order to investigate the detailed effects of TLR3 on cardiac IRI in vivo, syngeneic heart transplantation was performed in either C57BL/6 wild type (WT) or TLR3 knockout (TLR3-/-) mice following 9 h of cold ischemia.

Results: TLR3 knockout significantly diminished IRI-related injury 48 h after reperfusion as demonstrated by a cumulative histological damage score (TLR3-/-: 5.8±0.8 vs. WT: 8.8±0.3; p=0.006). In particular, epicardial and myocardial damage was alleviated ($p<0.05$, respectively). Furthermore, the presence of infiltrating lymphocytes significantly decreased (p=0.0009). This was accompanied by reduced intragraft (CCL3, CCL4) and splenic mRNA expression of pro-inflammatory cytokines (TNFα, CCL4; all $p<0.05$). Whereas elevated levels of anti-inflammatory factors (TGFβ) were registered, those indicating hypoxia (HIF1α) significantly declined ($p<0.05$, respectively). Importantly, in contrast to the depletion of TLR3 expression in TLR3-/- recipient grafts and spleens ($p<0.01$ respectively), other toll-like receptors (TLR2, TLR4) remained unaffected, indicating that the observed protective effects were solely due to TLR3 exclusion.

Conclusion: This study outlines for first time the detrimental influence of TLR3 on IRI after cardiac transplantation. Our data indicate that TLR3 represents a possible novel target for future pharmacologic therapies in solid organ transplantation.

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