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**P1845**

**The pro-inflammatory signaling lipid sphingosine-1-phosphate regulates gene and protein expression of both tissue factor and plasminogen activator inhibitor-1 in differentiated fat cells**

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**Background:** Generated by sphingosine kinases (SphKs) from sphingosine, the immunomodulatory lipid mediator sphingosine-1-phosphate (S1P) interlinks inflammation and coagulation as upon activation, platelets generate and release bulk amounts of S1P. Subsequently, S1P may attract immune cells to sites of arteriosclerotic lesions. Furthermore, S1P regulates the expression of protease-activated receptors (PARs) via the activation of G-protein-coupled S1P receptors (S1PR1–5) in order to enhance cellular responses to thrombin. Besides, both thrombin and the activated factor X (FXa) induce the expression of SphK1 and thereby increase the biosynthesis of S1P.
**Purpose:** This study investigates the effects of S1P on initiation and regulation of the coagulation cascade focussing on expression levels of tissue factor (TF) and the prothrombotic plasminogen activator inhibitor-1 (PAI-1). Since hyperlipidemia and obesity are typical risk factors for thrombotic events, differentiated adipocytes were used as cellular in vitro model.
**Methods:** Murine 3T3-L1 fibroblasts were differentiated with MDI (methylis-butyl-xanthine, dexamethasone, insulin) induction medium. Expression of adiponectin, PAR1–4, S1PR1–5, TF and PAI-1 was determined by RT-PCR; TF and PAI-1 protein quantified by ELISA and Western blotting.
**Results:** MDI-induced differentiation resulted in characteristic phenotypical changes and a 600-fold increase in adiponectin expression. Expression of the PARs and S1PRs was reduced in differentiated adipocytes which fitted with cellular senescence. Incubation of adipocytes with S1P (0.3 to 10 μM) resulted in a significant upregulation of the PAI-1 mRNA as well as protein expression and secretion to the culture medium implying enhanced prothrombotic signaling. This was attenuated by pharmacological inhibition of S1PR2 and -3, but not of S1PR1. In comparison, TF mRNA was downregulated by S1P whereas protein levels were markedly increased. Moreover, FXa (1 to 100 nM) induced expression of both TF and PAI-1 protein in adipocytes.
**Conclusion:** S1P regulates the expression of TF and PAI-1 in adipocytes in vitro. High S1P concentrations possibly induce prothrombotic cascades so that modulation of the S1P/S1PR axis may be a potential therapeutic strategy to prevent thrombosis in individuals at risk such as in obesity or metabolic syndrome.

## PROGENITOR CELL BIOLOGY

**P1846**

**Morphology-based identification of human induced pluripotent stem cell-derived endothelial cells by automated deep learning**

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**Background:** Endothelial cells (ECs) from human induced pluripotent stem cells (hiPSCs) are a precious source for in vitro disease modeling, tissue engineering and potential clinical application. However, guiding hiPSCs into ECs is still a challenge, and identification of fully differentiated ECs typically requires genetic manipulation in terms of lineage tracing or application of exogenous markers for immunostaining.
**Purpose:** We aimed to train a convolutional neural network to identify patterns of ECs following vascular specification of hiPSCs based on simple cellular morphological features.
**Methods:** We generated ECs from hiPSCs applying a two-steps protocol. Firstly, mesoderm was induced, and secondly, endothelial specification was prompted. Label-free phase contrast images from the end of the differentiation process were used as an input dataset. Additionally, hiPSC-derived ECs were stained with anti-CD31 antibodies, and binarized fluorescent images served as a reference to train the network. We repeated 4 independent experiments, and automatically obtained 200 images per experiment. 200 blocks of different size were randomly cropped from each image. Of 800 images in total, 640 images (i.e. 128.000 blocks) were used for training, whilst 160 images (i.e. 3.200 blocks) were used for evaluation (80:20 ratio).
**Results:** Deep learning technology could identify ECs within the target block with an accuracy and F1 score that were positively correlated to the reference block size, indicating that the surrounding area contains valuable information for the morphological identification of differentiating cells. Notably, target block size did not affect the prediction power. Moreover, a deeper neural network architecture (AlexNet vs. LeNet) improved network performance, suggesting that a more complex feature extraction by a larger network is useful for the classification of cellular morphological features (F1 score: 0,87 vs. 0,81). Finally, K-fold cross-validation confirmed generalization capability.
**Conclusion:** Morphology-based identification of hiPSC-derived ECs is possible using deep learning technology. Hence, pre-existing differentiation protocols could be optimized using a marker-free approach. However, technical improvements in terms of computational capacity need to be achieved, before applied artificial intelligence becomes a routine tool in molecular research.

**P1847**

**Localization of vascular progenitor cells in atherosclerosis-prone areas**

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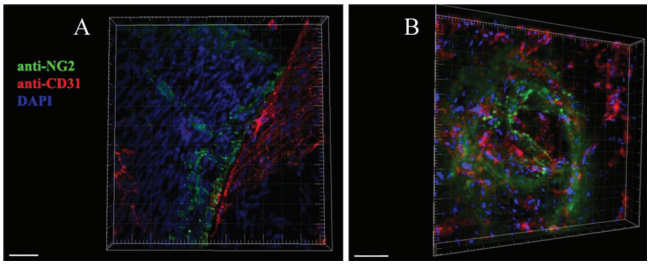
**Introduction:** It is well known fact that atherosclerosis usually develops in typical sites - curvatures and bifurcations of arteries. Atherogenic factors act systemically, so there must be a special mechanism of local atherogenesis induction. Shear stress plays a great role in this process, however, there are relatively straight sections of vessels in humans in which atherosclerosis frequently occurs. We suppose that in typical sites of atherogenesis some cells affecting early stages of this process can be located. The most likely candidates for this role are pericytes - progenitor cells that form clusters under the endothelium, and have the ability to accumulate lipids. Markers of these cells include NG2 (neural glial antigen 2), PDGFRβ (beta-type platelet-derived growth factor receptor), CD146 and nestin. Population of pericytes is heterogenous, so they can be either nestin-positive or nestin-negative, also it may be difficult to distinguish pericytes from immature smooth muscle cells. Recently we demonstrated that there is a small population among progenitor cells that constantly reacts to angeotensin II - a powerful atherogenic factor.
**Purpose:** To study vascular progenitor cells' distribution in the intima of large vessels and its response to angiotensin II.
**Methods:** C57BL/6 mice and transgenic mice carrying the eGFP gene under the nestin promoter were used. After euthanasia aortas were isolated from the heart to the renal arteries, and then were dissected longitudinally for whole-mount immunohistochemical staining or were frozen for sectioning. Antibodies to NG2, PDGFRbeta, eGFP and CD31 were used for staining. The visualization was accomplished by a confocal microscope. NG2 and PDGFRbeta double positive cells were isolated by cell sorting. The response to angiotensin II was assessed by using Ca2+ imaging in individual cells.
**Results:** We demonstrated that nestin-positive cells are localized in the adventitia of the aorta but they are absent in the subendothelial layer. We discovered that NG2-positive cells are located in bifurcations of the aorta and its major branches (Fig. A). Double staining showed that NG2-positive cells are also PDGFRβ-positive. These cells respond to an angiotensin II application in vitro. In whole-mount stained aortas we detected a concentric arrangement of NG2-positive cells around the ostia of small vessels (Fig. B) and on the lesser curvature of the aortic arch.




Localization of NG2-positive cells

**Conclusions:** In the subendothelial layer of the aorta and large arteries there is a population of nestin-negative, NG2 and PDGFRβ positive progenitor cells located in atherosclerosis-prone regions, including curvatures and branching sites. These cells respond to angiotensin II and may promote early stages of atherogenesis.
**Funding Acknowledgements:** The reported study was funded by RFBR according to the research project No. 18-015-00372

**P1848**

**Primary cilium-autophagy-cell cycle axis defects impair cardiac progenitor specification in hypoplastic left heart syndrome**

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**Background:** Hypoplastic left heart syndrome (HLHS) is characterized by underdevelopment of the left ventricle–aorta complex. Induced pluripotent stem cells (iPSCs) offer a unique opportunity to investigate early human heart development. Many signaling pathways important for early cardiac specification are bundled in

primary cilia, whose expression at the cell surface is closely connected to cell cycle patterning. The regulation of signaling at the base of the primary cilium, in response to cellular fate changes or stress, is thought to be closely linked to the activation of autophagy, that is an active process during embryonic development in multiple organs.
**Purpose:** To decipher the early developmental steps resulting in the cardiac phenotype of HLHS patients, we investigated in iPSC-derived cardiovascular progenitors (CVPCs), generated from three HLHS patient-derived samples, the role of the primary cilium-autophagy-cell cycle axis during cardiovascular specification.
**Methods:** During the course of CVPCs differentiation we measured from healthy and HLHS-derived CVPCs: expression levels of key cardiac developmental genes, proliferation and apoptosis rates, cell cycle phase distribution, primary cilium expression and autophagy activation.
**Results:** Expression levels of key cardiac developmental markers were altered during the course of CVPCs differentiation. HLHS-derived CVPCs showed a unique pattern of proliferation and cell-cycle-phases distribution at the time of early specification, without a significant difference in terms of apoptosis rates. All HLHS-derived CVPCs showed abnormalities in primary cilium and autophagy. Remarkably, inhibition of autophagy in healthy control lines recapitulated the defects observed in the HLHS-derived CVPCs.
**Conclusion:** Collectively, these findings indicate a key role of the primary cilium-autophagy-cell cycle axis in CVPC specification and HLHS pathology.
**Funding Acknowledgements:** This work was supported by grants from the European Research Council and the German Research Foundation.

## P1849
## Prevention of tumorigenesis in human pluripotent stem cell-derived cardiomyocytes by immunological cytotoxicity against oncofetal antigen

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**Background and objective:** Recently, transplantation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) was evaluated to treat patients with severe heart failure. However, to ensure safety of the transplanted hiPSC-CMs, complete removal of contaminating undifferentiated cells is essential. Additionally, the management strategy of patients with iPSC-derived tumors developing after transplantation of hiPSC-CMs has not been established. To address these issues, we applied anti-cancer immunotherapy with peptide vaccination to prevent hiPSC-derived tumorigenesis for the safeguard of hiPSC-CM transplantation.
**Methods and results:** To confirm immunological elimination of contaminating undifferentiated cells in hiPSC-CMs, we explored carcinoembryonic antigens in hiPSCs. Glypican-3 (GPC3), known as a oncofetal antigen, was identified as a new pluripotent state-specific immunogenic antigen. In the course of cardiac differentiation from hiPSCs, expression level of GPC3 was significantly decreased and almost undetectable in terminally differentiated hiPSC-CMs. Undifferentiated hiPSCs were rejected by cytotoxic T cell (CTL) clones that were sensitized by using HLA-class I-restricted GPC3 peptides; however, hiPSC-CMs were not rejected. Furthermore, GPC3-reactive CTLs removed undifferentiated cells from hiPSC-derivatives selectively in vitro and inhibited teratoma formation in vivo (fig.1–3). These results indicated that GPC3-reactive CTLs was attractive application for preventing hiPSC-derived tumorigenesis caused by contaminating undifferentiated cells in hiPSC-CMs.

Figure 1

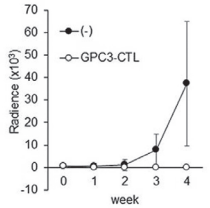


Figure 2

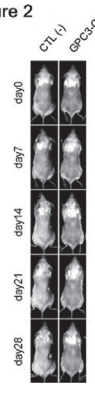


Figure 3

| samples | Teratoma formation |
|---|---|
| CTL (-) | 5/6 |
| | 83% |
| GPC3-CTL | 0/6 |
| | 0% |

**Conclusion:** Our results demonstrated that oncofetal antigen GPC3 worked as a pluripotent state-specific immunogenic antigen for the first time. Additionally, GPC3 was an effective target for purification of hiPSC-CMs, and thus future applicability of immunotherapy against GPC3 would be a perfect strategy to eliminate remained undifferentiated cells in human body to prevent tumor formation. Anti-hiPSCs immunotherapy will be useful approach as a safety net of regenerative therapy involving hiPSC-derivatives.
**Funding Acknowledgements:** Research Project for Practical Application of Regenerative Medicine from Japan Agency for Medical Research and Development (AMED)

## P1850
## Changes in coexpression of pericytes and endogenous cardiac progenitor cells from heart development to disease state

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**Introduction:** Focussing on the potential role of cardiovascular cell therapy, we investigated the spatial relationship between pericytes (cells with cardiac repair capabilities that ensheath blood vessels) and endogenous cardiac progenitors within stem cells' niches. We explored possible changes in their co-localisation in developing human hearts from foetal to adult stage and following ischaemia.
**Methods:** Foetal and adult human heart specimens, obtained under ethical consent (University of Edinburgh ethics committee), were used for immunohistochemistry, cell isolation, culture and differentiation. Multi-lineage differentiation in culture, by single and double staining was completed for CD 146+ foetal pericytes and c-kit+ cells. Endothelial markers (CD31) gene expression was quantified by qPCR.
**Results:** c-kit+ cells frequency and coexpression with pericytes decrease with heart development, already evident by gestation week 19th. Pericytes and c-kit+ cells express the early cardiac transcription factors Nkx2.5 and Islet 1. Only c-kit+ cells express the stemness marker SSEA3 (24%), known to progressively decrease with cell differentiation. Endothelial differentiation assessment shows that cardiac pericytes and c-kit+ cells do not form CD31+ networks. This finding correlates with absence of staining for CD31 marker in both cultured cells' types. The cardiac marker $\alpha$-actin was present in both cell populations. In healthy adult heart, pericyte markers CD146 localise within the vasculature. Following ischaemia this pericyte marker becomes also evident outside the vasculature.In healthy adult atrium, c-kit expression is low and coexpression with other markers inconspicuous. Ischaemia leads to increased c-kit expression, particularly in blood vessels $<$50um diameter. Furthermore, following ischaemia c-kit, endothelium and pericyte markers co-localise within the same atrial cells. Blood vessels $>50\mu$m diameter showed mostly only staining for endothelial (vWF) and pericyte (CD146) markers, with no co-expression of c-kit marker identified. Staining patterns within the ischaemic regions of the right and left atrial appendages revealed low levels of colocalisation between vWF and CD146. Acute ischaemia of the left ventricle affected the detection of cardiac stem cells markers in the area of injury, due to myocardium disruption.
**Conclusion:** Foetal heart pericytes and c-kit+ cells express early cardiac transcription factors and show trans-differentiation potential, which decreases in healthy adult hearts. The preservation and activity of cardiac stem cells niches within the atrium vasculature appears re-activated in post-ischaemic hearts. Better understanding of cardiac c-kit+ and pericyte cells during-human embryonic development and during ischaemia may identify alternative novel therapeutic strategy against coronary artery disease.
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## P1851
## Catecholamine-dependent cAMP signaling in a patient-specific induced pluripotent stem cell takotsubo-model

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**Aims:** Takotsubo syndrome (TTS) is characterized by acute transient left ventricular dysfunction in the absence of obstructive coronary lesions. The pathogenic mechanism leading to TTS is still unknown. However, an adrenergic overstimulation and a genetic predisposition were shown to play an important role in TTS. The aim of the study was to use an in vitro induced pluripotent stem cell (iPSC)-TTS model to prove the hypothesis of an altered cAMP signaling under catecholamine stress.
**Methods and results:** Functional TTS-iPSC-cardiomyocytes (CMs) were generated, treated with catecholamines to mimic a TTS-phenotype and analyzed regarding cAMP signaling and cardiac function.
In our previous work we found an increased susceptibility and sensitivity to catecholamines under stress conditions in iPSC-CMs of TTS patients compared to control. This was among others shown by an increase of catecholamine-induced cytoplasmic cAMP as well as altered electrical activity in TTS iPSC-CMs compared to control. In our new study, we aimed to analyse the cardiac stunning phenotype of TTS patients. Therefore, the conduction velocity of TTS iPSC-CMs was investigated and resulted in a significant decrease compared to control. Furthermore the reduced receptor desensitization in catecholamine-treated TTS-iPSC-CMs is underlined by significantly increased $\beta$1-adrenergic receptor (AR) mRNA