WS15.1 Development of a human model of airway epithelial cells invalidated for *CFTR*

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Objectives: The sequence of events occurring at the early onset of pulmonary infection and inflammation is poorly understood. Primary cultures of well-differentiated CF human airway epithelial cells (HAECs) are valuable but difficult to generate and heterogeneous. Our objective is to genetically modify non-CF HAECs by disrupting the *CFTR* gene and create a CF-like 3D model of the airway epithelium.
Methods: shRNA sequences targeting the *CFTR* mRNA (shCFTR) were screened and the three best silencing sequences were cloned as single and triple constructs to produce lentiviral (LV) vectors co-expressing the green fluorescent protein. Their efficiency was assessed by transduction of Calu-3 airway epithelial cells. GFP positive cells sorted by FACS were cultured on filters for polarization to measure transepithelial currents in Ussing chambers and *CFTR* protein levels by Western blot. Preliminary results showed a partial knock-down of the *CFTR* protein coupled with variations in the transepithelial currents. Primary HAECs were also transduced with LV vectors expressing GFP only or a shRNA sequence targeting the gap junction protein Connexin26. Immunofluorescence analyses showed a good knock-down efficiency of Cx26 while GFP remained expressed in cells grown at the air-liquid interface for at least 30 days.
Conclusion: These results validate our strategy to knock-down *CFTR* sequences in primary HAECs. Experiments are ongoing to identify more efficient shCFTR sequences. Ultimately, we expect to investigate the phenotypic consequences of CFTR knock-down/knock-out compared to the parental cells, therefore eliminating the variability of CF specimen.

WS15.2 Derivation of normal and cystic fibrosis human induced pluripotent stem cells (iPSCs) from airway epithelium

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The repopulation of diseased airway epithelium with gene-corrected, patient-specific cells derived from iPSCs and expressing functional CFTR may represent a future therapeutic option for Cystic Fibrosis (CF). However, recent studies indicate that the full differentiation potential of iPSCs may be restricted to lineages related to the donor cell.
We hypothesized that for efficient airway epithelium differentiation it may be advisable to derive iPSCs from airway epithelial cells.
We therefore aimed to derive iPSCs from primary human bronchial airway epithelial cells, obtained from normal and CF donors, and assess their differentiation potential into airway epithelium.
Primary human airway epithelial cells, normal and CF (ΔF508/ΔF508), were transfected with expression vectors encoding L-Myc, Klf4, Oct3/4 and Sox2. iPSC, normal and CF, were then subjected to an in vitro differentiation protocol to generate proximal airway progenitors and lung multipotent progenitors.
Airway-derived human iPSCs, normal and CF, expressed alkaline phosphatase and pluripotency markers NANOG, OCT-4, SSEA-1, SSEA-3, SSEA-4, TRA-1–60, TRA-1–81. Morphology of airway-derived iPSC, normal and CF, was consistent with typical morphology of human iPSCs.
We were then able to differentiate both normal and CF iPSC into double-positive TTF-1/SOX2 proximal airway progenitor cells and into TTF-1/SOX9 lung multipotent progenitors.
We report for the first time the derivation of human iPSC from primary airway epithelial cells, normal and CF ΔF508/ΔF508.
Airway-derived iPSCs may have higher differentiation potential towards lung epithelium and may be the iPSCs of choice for airway epithelium correction in CF.

WS15.3 Human amniotic mesenchymal stem cells modify the function and cytokine production of F508del airway epithelial cells upon coculture

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Background and Objectives: In cystic fibrosis (CF), there is a lack/dysfunction of CF Transmembrane Conductance Regulator (CFTR) and of epithelial sodium channel (ENaC) at the level of the respiratory epithelium. We evaluated human amniotic mesenchymal stem cells (hAMSCs) obtained from end term placentae for their ability to modify defective function of CFTR and EnaC in in vitro cell model.
Methods: hAMSCs and CF bronchial epithelial cells (CFBE41o-) were cocultured on Transwell filters seeded at different ratios. Real-time PCR was used to study transcript levels of CFTR and ENaC. Expression of the CFTR protein was investigated by western blot analysis. Chloride efflux was studied by fluorimetry. ENaC activity was assayed by apical fluid reabsorption. Cytokine secretion was studied in conditioned media.
Results: hAMSCs expressed at low levels CFTR mRNA, and gamma but not alpha and beta subunits of ENaC. Cocultures of hAMSCs with CFBE cells demonstrated that the mature band of CFTR appeared. Fluorimetric measure of ion chloride efflux allowed to detect an increased function of the CFTR channel in cocultures as compared with CFBE cells and hAMSCs alone. Amiloride-dependent fluid reabsorption decreased when CFBE cells were cocultured with hAMSCs respect to CFBE cells alone. After three days of coculture IL-1beta, IL-6, IL-8 and TNF-alpha showed an increase depending on the hAMSC-CFBE ratio; this increase was not observed after six day of coculture.
Conclusions: hAMSCs are capable to induce CFTR maturation and to correct the CF phenotype (chloride efflux and fluid absorption). Finally, the transient increase in cytokine levels should be further investigated in its significance.

WS15.4 Purinergic signalling regulates pancreatic epithelial transport and pancreatic stellate cells

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Objectives: Pancreas is one of the major organs affected by cystic fibrosis. Purinergic receptors may provide novel strategies for regulation of epithelial and stromal cell functions and the aim of our studies is to clarify the role of purinergic signalling in coordination of the whole gland function in health and disease.
Methods: We have used rodent pancreas and human pancreatic cell lines and applied various molecular/cell/physiological techniques including Ussing chamber recordings and confocal microscopy.
Conclusion: Pancreatic ducts produce bicarbonate-rich fluid in response to secretin that stimulates CFTR Cl^- channels. P2 receptors, e.g. P2Y2, stimulate in addition Ca^{2+} regulated Cl^- channels, most likely TMEM16A/ANO1 channels that we find expressed in duct epithelia. In addition, P2Y receptors stimulate Ca^{2+}-activated K^+ channels (i.e. $K_{Ca}3.1$), which increases the driving force for anion-dependent transport and significantly amplify secretion. ATP is released from pancreatic ducts in response to a number of patho-/physiological stimuli, but the main provider may be pancreatic acini. Acini accumulate ATP in zymogen granules by means of vesicular nucleotide transporter (VNUT). Overstimulation or damage to acini releases ATP and digestive enzymes to interstitium facing various stromal cells, such as pancreatic stellate cells (PSC). PSC express the P2X7 receptor, which may have a double role. In low ATP concentrations it supports cell proliferation, while at high ATP concentrations cells die. Taken together, purinergic signalling may play significant role in epithelial secretion and development of fibrosis in pancreatic diseases, such as cystic fibrosis.