S16: Special Symposium to Celebrate the Centennial of Distinguished Professor Yuan-Cheng B. Fung-1

### S16-1 Morphogenesis and mechanobiology of airway smooth muscle cells on 3D tubular micropatterns as mechanism of bronchial airway development

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During early development of the lung, airway smooth muscle cells (ASMCs) emerge and form bundles at the terminal airways, and eventually organize as helical structures that surround the airway walls with certain axial orientations. However, it remains unclear how such ASM structures form on the tubular wall. Here we cultured ASMCs on tubular micropatterns fabricated with PDMS coated with collagen, and subsequently assessed in real time the ASMCs' adhesion, migration and self assembly into stable morphology together with cell stiffness and cytoskeleton remodelling. The tubular micropattern had a diameter ranging from 50 to 150 µm. ASMCs were inoculated on the micropattern for up to 72h while being imaged by live cell imaging system, fluorescence microscopy, and probed with optical magnetic twisitng cytometry (OMTC) and atomic force microscopy (AFM), respectively. We found that ASMCs cultured on the tubular micropatterns seemed to be agile to adjust their shape and orientation to probe and cope with the uneven microenvironment. Ultimately the cells oriented on the curved surface with the cell's long axis in angle conducive to their survival. The cells also change their stiffness. most likely through active remodeling of the cytoskeleton to correlate the changing tubular curvature. Interestingly, the cells rarely oriented to be parallel to the axial direction of the tube, demonstrating that plane surface was not optimal for cell growth based on mechanical equilibrium and energy minimization. In conclusion, our results show that 3D tubular curvature is indeed a factor to affect the structural arrangement and mechanical properties of ASMCs (at least in vitro) that may contribute to the morphogenesis and functionalization of the bronchial airways during lung development.

# S16-2 Glycosylation is a strong molecular determinant of MUC5AC rheology in airway mucus at both single protein and bulk solution levels

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Airway mucus is an important physical barrier that traps exogenous hazardous matters and excludes them out of body via cilia beating and coughing. This defense function largely relies on the appropriate rheological properties of the airway mucus. As airway mucus contains

abundant proteins known as mucins (primarily the subtype MUC5AC), it has been shown that airway mucus rheology is closely related to the glycosylation of MUC5AC, but the mechanism remains unclear. Therefore, we modified MUC5AC's O-glycosylation to various extent by using O-glycosidase. Subsequently, we characterized the rheological behavior of the O-glycosylation modified MUC5AC at either molecular level using atomic force microscopy (AFM) or bulk volume level using advanced rotational rheometry (Kinexus II, Malvern). We demonstrate that at molecular level, O-glycosylation had a strong effect on the mechanical properties of MUC5AC. At the bulk volume level, the MUC5AC solution in all cases exhibited a gel-like rheological behavior that fitted well to classical Burgers model with a typical shear-thickening at shear rate  $\leq 0.02$  s<sup>-1</sup> and nonlinearly shear-thinning afterwards. However, both the storage and loss moduli of the MUC5AC solution were highly dependent on the level of O-glycosylation. We also found that the network structure of MUC5AC polymer filaments was determined at least in part by the extent of O-glycosylation. Taken together, this study provides rheological characteristics of MUC5AC with variable Oglycosylation from micro- to macro scale, which may help fully understand the mucus physiology and associated disease pathology and the development of drug delivery systems as well as ideas and methods for treating chronic respiratory diseases such as asthma or COPD with MUC5AC as a target.

## S16-3 Dynamics of neutrophil transmigration mediated by beta-2 integrin via P- and E-selectins

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Leukocyte transendothelial migration is a key step in their recruitment to the sites of inflammation. However, the synergic regulation of endothelium-expressed selectins on leukocyte transmigration remains unclear. Here, an in vitro model was developed to investigate the dynamic contributions of P- and E-selectin to PMN transmigration under static condition. PMN transmigration is significantly increased on LPS stimulation, which is higher on 4 h LPS-treated HUVECs than on 12 h LPS-treated HUVECs. Blocking and competitive tests indicate that P-selectin engages PSGL-1 to activate b<sub>2</sub>-integrin and initiate PMN transmigration within the first 15 minutes, while E-selectin engages CD44 to influence PMN transmigration after 15 minutes. All these P- and E-selectin-induced b<sub>2</sub>-integrin activation is likely transduced via Syk signaling pathway. And complicated complementary and competitive mechanisms are involved in the interactions of P-/E-selectin and their ligands to promote PMN transmigration.

## S16-4 Membrane structural protein analysis and mechanical property analysis of rat erythroblasts in different developmental stages

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Both in the evolution of vertebrates and the mammalian process of erythropoiesis, erythrocytes undergo critical events including cell volume reduction, changing from nucleated to enucleated, critical membrane protein assembly and enhancement in deformability, viscoelasticity and oxygencarrying capacity. In this study, these significant changes were studied to elucidate the relationship between the variation of mechanical properties and the molecular events during erythropoiesis. Using phasecontrast microscopy, different color fluorescence labeling proteins were observed in situ. Using electrophoresis and Western Blot analysis, expression levels of  $\alpha$  and  $\beta$  spectrin, band 3 and actin were compared in erythroblasts at different developmental stages. Extracted erythroblasts were loaded onto an AFM to determine their Young's Modulus, and a micropipette aspiration system was used to determine their viscoelasticity. Increased deformability and viscoelasticity were gradually established during the erythroblast development and mainly in late stages. Changes in mechanical properties were closely related to the membrane protein molecular events. Thus, the present studies offer a novel approach to examining the relationship between biomechanics, molecular biology and developmental biology. This work was supported by grants from the National Natural Science Foundation of China (No. 1010200620150034), Chinese National 863 High Tech Research and development program (No. 1010103920110136).

## S16-5 Influence of different rhythms sound wave to serotonin concentration in rats hippocampus

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Background: In our study, we focused on the infuence of music to serotonin concentration variation in hippocampus. rhythm was another parameter needed. We hope our research could answer what kind of music is able to improve mind situation of different people.

Method: (1) Heart rates of SD rats were detected under anaesthesia and normal situation, calculated averages, set rhythms (300beats/min, 350beats/min, 400beats/min); (2) Made sound waves according to setting rhythms with Finale 2011 software; (3) Rats were grouped randomly. Some were received different sound waves under anaesthesia or normal situation, some were received nothing as control. (4) Left and right hippocampus were isolated from brains into tubes filled with 0.9% NaCl solution, weighed. Then ultrasonicated tissues and

centrifigured. Supernatant was used to serotonin ELISA.

Conclusion: (1) Right and left hippocampus have different responds to same sound wave; (2) Under anaesthesia situation, right hippocampus from group received 300 beats/min sound wave secreted the most serotonin concentration, 0.202ng/(ml\*mg); (3) Under normal situation, right hippocampus from group received 400 beats/min sound wave secreted the most serotonin concentration, 0.128ng/(ml\*mg); (4) Nearer to the current heart rate under certain situation the sound wave rhythm received was, the more serotonin secreted.