

O9: Biorheology and Biotechnology-2

O9-1 Proteomic analysis of ApoE^{-/-} mice with disturbed flow model

Li Tianhan, Wang Guixue

Key Laboratory for Biorheological Science and Technology of Ministry of Education, State and local Joint Engineering Laboratory for Vascular Implants, Bioengineering College of Chongqing University, Chongqing 400030, China

The formation of atherosclerotic plaque is easy to induce human cardiovascular disease and the disturbed flow can accelerate the process. However the mechanism of atherogenesis in the disturbed flow region is still not clear. We used apoE^{-/-} mice to establish the disturbed flow model as previously reported, which was verified through a small animal ultrasound instrument. We extracted total protein of left carotid artery at 48 h later after surgery, then made proteomics analysis. Totally 168 significant differential expressed proteins were found between the left carotid of disturbed flow group and shame group, including 18 down-regulated and 150 up-regulated proteins. Further gene ontology analysis showed that these proteins were related with single-organism process, macromolecule metabolic process, cellular component organization or biogenesis, response to stimulus, localization and so on. KEGG pathway results indicated that they were enriched in complement and coagulation cascades, hematopoietic cell lineage, phagosome, fat digestion and absorption, phagocytosis, cell adhesion molecules signal pathways, such like Itgb2, CD9, CD36, VACM, which were consistent with the previous reports and closely related to atherogenesis. We also found some proteins that are less concerned in atherogenesis in disturbed flow regions. The function and mechanism of these proteins need to be studied. Through the construction of disturbed flow model and proteomics results, we get the differential proteins involved in biological process classification and signal pathway, which provides the foundation for further understanding mechanism of disturbed flow influencing the progression of atherosclerosis.

[Supported by the NSFC (11572064, 11332003, 31701275), the NKTR&DP (2016YFC110230 5)]

O9-2 Effects of suspension state on the biological behavior of breast cancer cells

Yonggang Lv, Xiaomei Zhang, Ying Zhang, Ya Wang

Chongqing University, China

Mechanical microenvironment can strongly affect the metastatic efficiency of circulating tumor cells. However, the effect of suspension state on their biological behavior and its mechanism are still unclear. The objective of this study was to investigate the effect of suspension state on the metastasis, extravasation and drug resistance of breast tumor cells. MDA-MB-231 cells were suspension cultured in complete medium, while the adherent cells were used as control. Our study demonstrated that (1) The suspension state significantly increased the metastatic potential of breast cancer cells, but slightly suppressed their tumor growth. The cytoskeleton state and activation of Ca²⁺/CaN/NFAT are responsible for the up-regulation of cyclooxygenase-2 (COX-2), which plays an important role in suspension-promoted metastasis. (2) The increasing expression of integrin β 1 induced by suspension culture promoted the adhesion and transendothelial migration of MDA-MB-231 cells, but had no significant influence on their spreading. (3) Suspension state plays a vital role in promoting

methotrexate (MTX) resistance of MDA-MB-231 cells by inducing adenosine triphosphate binding cassette subfamily C member 3 (ABCC3) overexpression. These findings highlight the important role of suspension state for tumor cells in tumor metastasis. This work was supported by the National Natural Science Foundation of China (11672051).

O9-3 Preliminary study of endothelial cell tight junction protein in response to different mechanical stimuli

Yazhou Wang, Desha Luo, Tieying Yin, Guixue Wang

Key Laboratory for Biorheological Science and Technology of Ministry of Education, State and Local Joint Engineering Laboratory for Vascular Implants, Bioengineering College of Chongqing University, China

It is generally accepted that the damage of the structure and function of endothelial cells (EC) is the first step in the development of atherosclerosis (AS) and plays an important role in the development of AS. Subendothelial lipoprotein deposition is one of the foundations of AS plaque formation, indicating that the permeability of EC has changed. It has been found that paracellular pathway opening is the main reason for increased vascular permeability. Among them, tight junctions mainly determine the strength of intercellular junctions and play an integral role in maintaining endothelial barrier. Among the physiological stimuli that impact on the endothelium, mechanical or hemodynamic forces associated with blood flow are of central importance. These include cyclic circumferential strain, caused by a transmural force acting perpendicularly to the vessel wall, and shear stress, the frictional force of blood dragging against cells. As such, one can hypothesize a dynamic regulatory association between endothelial permeability and hemodynamic stimuli. To study the effect of different mechanical models on the tight junctions of human umbilical vein endothelial cells (HUVECs), HUVECs were subjected to flow Shear stress, static pressure and Cyclic Strain. To detect tight junction protein changes, including Claudin-5, Occludin, ZO-1 and Tricellulin. The results showed that the action of flow shear stress and cyclic Strain, the morphology of the cells changed from fusiform to polygonal shape. After withdrawal of mechanical stimulation, the cells tended to recover from the initial morphology. Fluorescence quantitative PCR results showed that the tight junction protein response was different under different mechanical models.

O9-4 PI3K-nos2b Signaling is Crucial for Simulated Microgravity-mediated angiogenesis in Zebrafish CVP Network

Daoxi Lei, Guixue Wang

Bioengineering College of Chongqing University, China

Microgravity was reported to regulate angiogenesis and vascular remodeling. However, the mechanism that how the microgravity affects the vascular development remains unclear. Our study aims to evaluate the effects of microgravity on vascular development by the caudal vein plexus (CVP) network of zebrafish and to delineate the roles of PI3K-nos2b signaling in this process.

Using a ground-based simulation microgravity bioreactor, we found that the simulated microgravity (SM) could significantly promote the angiogenesis in CVP of zebrafish larvae. Then we also found that injection with nos2b-Morpholino or treatment with PI3K inhibitors

LY294002 could partially rescue the CVP network abnormality caused by SM, indicating that nos2b was involved in SM mediated CVP development. Furthermore, overexpression of nos2b could partly rescue LY294002-caused CVP network failure. Taken together, our results indicates that SM can affect zebrafish CVP angiogenesis by promoting PI3K-nos2b signaling. [Supported by the NSFC (11572064, 31771599), the NKTR&DPC (2016YFC1102305, 2016YFC1101101), the FRFCU (CDJXY230002, CDJZRPY0021)]

O9-5 Ferric iron, lipopolysaccharide and lipoteichoic acids can induce anomalous fibrin amyloid formation: an assessment with novel amytracker™ stains and thioflavin T

Martin Page^a, Douglas Kell^b, Etheresia Pretorius^a

^a*Stellenbosch University, South Africa*

^b*University of Manchester, United Kingdom*

Most chronic diseases include an inflammatory component. This inflammatory component is closely linked to a procoagulant phenotype of some kind, appending thrombotic conditions as comorbidities of inflammatory diseases. A potential trigger of this state may be highly inflammatory bacterial wall components; and these components may have a prominent role in hypercoagulability. In recent work, we discovered that the presence of a tiny amount of lipopolysaccharide (LPS) from Gram-negative bacteria caused fibrinogen clotting to lead to the formation of an amyloid form of fibrin. Here, we show that the broadly equivalent lipoteichoic acids (LTAs) from two species of Gram-positive bacteria, as well as ferric iron, have similarly potent effects. The ability of these inflammagens to divert fibrin formation to an amyloid form was confirmed with the fluorescent markers thioflavin T, as well as the novel amyloid-selective AmyTracker dyes, were the products were added to human plasma, clotted via thrombin and detected by confocal microscopy. We show that all three bacterial wall inflammagens and iron give very large fluorescence enhancements. The staining patterns differ significantly as a function of both the amyloidogens and the dyes used to assess them, indicating the altered nature of the clots formed. These results highlight the amyloid-forming potential of fibrin(ogen) in the presence of various inflammagens. Thus, the data provide further evidence for an important role of bacterial cell wall products and also increased iron, in the various coagulopathies that are observable in inflammatory diseases. Finally, these assays may have an application in diagnostics and these findings may offer novel therapeutic targets in the treatment of anomalous clot formation.