Session Title: Vascular Remodeling and Engineering

Overall Session Abstract:

The vascular wall is a dynamic tissue composed of endothelial cells, smooth muscle cells, fibroblasts and extracellular matrix. Blood flow and delivery of oxygen and nutrients to tissues is an essential feature that evolutionarily allowed development of multicellular organisms. Disruption or impairment of blood flow results in ischemia and tissue damage. It is not surprising then that in response to changes in the microenvironment, "vascular remodeling" or alterations in vascular structure occur in an attempt to maintain homeostasis and provide effective delivery of oxygen, nutrients and other materials to tissues. The vasculature is sensitive to a variety of stimuli including oxygen levels, growth factors, cytokines, mechanical forces and others. Vascular remodeling is important developmentally and in response to pathological conditions and includes changes in cellular processes (growth, migration, differentiation, death, etc.) and in the vascular extracellular matrix. While substantial research has been carried out focused on vascular remodeling, many questions remain regarding the mechanisms of this process. The talks of this session will focus on mechanisms underlying remodeling of the vasculature and opportunities that exist for innovative approaches towards treatment of vascular disorders.

Mechanosensing in Bypass Graft Development

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Key words: Coronary artery bypass, histomechanical

Abstract

With high clinical success rates, the internal thoracic artery (ITA) is an excellent histomechanical candidate for coronary artery bypass grafting (CABG) compared to the radial artery (RA) or great saphenous vein (GSV). Despite this, ITAs are long vessels having distinct histomechanical properties with dramatic spatial variation. Patients undergoing multiple bypass procedures and those without patent autologous graft tissues, however, are often in need of viable graft alternatives, yet purely synthetic materials have poor patency rates and tissue engineered approaches remain underdeveloped. Whereas mechanical incompatibility underlies several vascular etiologies including ischemia, hemorrhage, inflammation, and restenosis; blood vessels are dynamic in form and function and subtle mechanical cues can be used to initiate favorable remodeling processes. These include, but are not limited to, the expression of anti-inflammatory and antithrombotic genes, quiescence of smooth muscle cells, and the synthesis and removal of extracellular matrix proteins. By leveraging the mechanical and biochemical processes involved in favorable graft remodeling the material properties of candidate graft tissues can be improved prior to implantation. This objective is achieved through extensive pre-remodeling mechanical characterization and careful examination of the dynamic macrostructure (e.g., wall thickness, radius, stress state, compliance), microstructure (e.g., protein content, cell phenotype), and gene expression profiles (e.g., Col-III, FN-1, TGFB-1, VCAM-1, TIMP-1, MMP-2, PAI-1) of blood vessels undergoing adaptation/maladaptation for 0 (acute), 7, and 21 days in dynamic ex vivo perfusion tissue culture. Then precise control over the mechanical loads that govern these remodeling processes (pressure, flow, force), through the theory of stress mediated growth-and-remodeling, are used to engineer functional and adapted tissues prior to implantation. Our approach to improving bypass grafts exploits natural biological processes inherent to these cells/tissues and provides a framework by which the properties of virtually all graft vessels could be enhanced.

Elevated Wall Tension Leads to Reduced miR-133a in the Thoracic Aorta by Exosome Release

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Key words: microRNA, thoracic aortic aneurysm, exosome

Abstract

Background: Alterations in microRNA (miR) abundance have been associated with multiple cardiovascular diseases, and while much effort has been directed toward understanding their role in modulating key cellular targets, less is known about how miR abundance is regulated within the cell. Previously, this laboratory identified several microRNAs, including miR-1, -21, -29a, -486, -720, and miR-133a, that were reduced in ascending aortic tissue from patients with thoracic aortic aneurysm (TAA). Many of these miRs, including miR-133a, displayed an inverse linear correlation with aortic diameter, such that, as diameter increased, the abundance of miR-133a was reduced. Based on the fundamentals of the Law of Laplace, we know that vessel wall tension is dependent upon the relationship between pressure and diameter (wall tension = pressure x diameter). Accordingly, during aneurysm formation wall tension increases as the aorta dilates, and this may play a role in determining miR levels in the thoracic aortic (TA) wall. Therefore, this study sought to determine a mechanism responsible for the reduction of miR-133a, and tested the hypothesis that elevated wall tension induces the loss of miR-133a in the thoracic aorta.

Methods and Results: Elevated tension (1.5g; 150 mmHg) applied to murine TA *ex vivo* using a tissue myograph, and resulted in reduced miR-133a tissue abundance compared to TA held at normo-tension (0.7g; 70 mmHg). Similarly, when tension was applied to isolated primary smooth muscle cell (SMC) and fibroblast cell lines, miR-133a levels were reduced in response to biaxial stretch of isolated murine TA fibroblasts, while SMCs were not affected. Mechanisms contributing to the loss of miR-133a abundance were further investigated in TA fibroblasts. Biaxial stretch did not reduce pri-miR-133a transcription, and had no effect on the expression/abundance of three microRNA-specific exoribonucleases. Remarkably, biaxial stretch increased exosome secretion. Moreover, exosomes isolated from TA fibroblasts contained more miR-133a. Inhibition of exosome secretion using the Neutral Sphingomyelinase-2 inhibitor, GW4869, prevented the biaxial stretch-induced reduction of miR-133a. Subsequently, two mouse models of hypertension were utilized to determine the effect of *in vivo* elevated wall tension on miR-133a abundance in the TA: 1) subcutaneous implantation of mini-osmotic pumps delivering angiotensin-II over a 4-week period (AngII, 1.44mg/kg/day) in wild-type mice, and 2) spontaneously hypertensive mice (BPH/2) that are angiotensin-independent. Interestingly, the abundance of miR-133a was decreased in TA tissue and increased in the plasma in both models of hypertension, as compared to a normotensive controls. Lastly, miR-133a was measured in plasma collected from hypertensive

human subjects, and compared to normotensive patients. Interestingly, miR-133a abundance was elevated in the plasma of the hypertensive patients.

Development of chemically-defined hydrogels for therapeutic angiogenesis

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Key words: Chemically-defined hydrogels, therapeutic angiogenesis

Abstract

Therapeutic angiogenesis holds remarkable promise to treat ischemic diseases and develop viable tissue engineering strategies. As extracellular matrix (ECM) proteins play a central role in physiological angiogenesis, significant efforts have been devoted to developing hydrogels that mimic the provisional ECM to promote angiogenesis. Numerous angiogenic factors (e.g. VEGF and bFGF) have been incorporated into hydrogels to achieve controlled, local delivery. In parallel, cell adhesive peptides (e.g. RGDS, RGDSP) have been conjugated onto hydrogels to engage integrins expressed on endothelial cells (EC) and facilitate cellular infiltration of host vasculature. Furthermore, recent studies explored the development of bi-functional hydrogels that can simultaneously bind to $\alpha_v\beta_3$ integrins and VEGFR2 receptors to leverage their positive crosstalk to improve endothelial cell morphogenesis. In this talk, I will discuss our research efforts to prepare chemically-defined hydrogels for therapeutic angiogenesis.

The Role of Endothelial Extracellular Vesicles in the Pathogenesis of Port Wine Stain Blood Vessels

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Key words: Vascular malformations, endothelial cells, extracellular vesicles, Port wine stain

Abstract

Background - Port wine stain (PWS) is a congenital vascular malformation of human skin which is characterized as differentiation-impaired venule-like vasculatures. The pathogenesis of PWS remains incompletely understood. We hypothesized that PWS endothelial cells release extracellular vesicles (EVs) which contain crucial molecular signatures causing vascular phenotypes of PWS.

Methods - We isolated and quantified the $CD31^+$ serum EVs from PWS patients and normal control subjects. The molecular phenotypes of PWS $CD31^+$ serum EVs were characterized and compared to normal controls. Functional analysis of $CD31^+$ EVs on human dermal microvascular endothelial cells (hDMVECs) was performed. The angiogenic potency of PWS $CD31^+$ serum EVs was evaluated in the nude mouse model.

Results - There was a significant increase in quantity of serum $CD31^+$ EVs in PWS patients as compared to normal subjects. PWS $CD31^+$ EVs contained EphB1, EphrinB2 and ADAM30. EphB1/EphrinB2/ADAM30 signaling in PWS $CD31^+$ EVs could activate c-Src, which subsequently promote the proliferation of endothelial cells. Furthermore, intradermal implantation of EphB1/EphrinB2/ADAM30 EVs from PWS patient serum into nude mouse resulted in an enhanced angiogenesis *in vivo*.

Conclusions - EphB1/EphrinB2/ADAM30 signaling in PWS $CD31^+$ EVs played essential roles in development of PWS vascular phenotypes *via* a direct activation of c-Src pathway.