SA1

Dynamics of shear stress-induced remodelling

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Chronic changes in wall shear stress lead to vascular remodelling, characterized by increased vascular wall diameter and thickness, to restore wall shear stress values to baseline. Release of nitric oxide (NO) from endothelial cells exposed to excessive shear is a fundamental step in the remodelling process, and potentially triggers a cascade of events, including growth factor induction and matrix metalloproteinase (MMP) activation, that together contribute to restructuralisation of the vessel wall. MMPs, which are secreted as inactive zymogens (pro-MMPs), are rapidly cleaved and activated in in vivo models of chronic increased blood flow, and remain active until shear stress is normalized. Enhanced production of NO in high flow conditions, along with generation of reactive oxygen species through NADPH oxidase, combine to form peroxynitrite, which is important for MMP cleavage in the early phase of arterial remodelling. However, the later phase of this process implicates not only the activation of MMPs but also their ongoing synthesis. In this respect, we have uncovered a role for NF-kappaB as a key factor regulating the expression of MMP-9 and thus participating to the remodelling of vessels.

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SA2

Stem cell differentiation into vascular cells induced by mechanical stress

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It was established that stem cells could repair lost endothelial cells and participate in the formation of neointimal lesions, because stem cells can differentiate into a variety of cells to replace dead cells or to repair damaged tissues. In this process, stem cells homing to the surface of injured vessels have to differentiate into vascular cells to exert their repairing functions (Xu Q. Circ Res. 2008;102:1011). Obviously, microenvironment where stem cells are attached play a crucial role in cell differentiation, although the spectrum of the initiators/stimulators responsible for such a differentiation remain to be clarified. It is well known that atherosclerotic lesions in the arteries are localized in some areas where blood flow is disturbed resulting in endothelial dysfunction/death in the presence of hyperlipidemia. Since recent findings suggest the potential role of stem cells in endothelial regeneration, it can be hypothesized that mechanical stress induced by blood flow can influence the differentiation process of stem cells (Xu Q. Nature Clin. Pract. Cardiovasc. Med. 2006;3:94). Support this hypothesis is recent findings that shear stress can induce differentiation of stem cells towards endothelial cell phenotype (Zeng et al. J Cell Biol. 2006; 174:1059), while stretch stress leads to differentiate into smooth muscle cells. It indicates that "good" blood flow (laminar shear stress) promotes endothelial differentiation from stem cells that tethering the surface of the vessel wall. How the stem cells sense and transduce the extracellular physical stimuli into intracellular biochemical signals is a crucial issue for understanding the mechanisms of stem cell differentiation. Collecting data derived from our and other laboratories showed that many kinds of molecules in the cells such as receptors, G proteins, cell cytoskeleton, kinases and transcriptional factors could serve as mechanoceptors directly or indirectly in response to mechanical stimulation implying that the activation of mechanoreceptors existing on the surface of stem cells is a crucial event. The sensed signals can be further sorted and/or modulated by processing of the molecules both on the cell surface and by the network of intracellular signalling pathways resulting in a sophisticated and dynamic set of cues that enable stem cell responses. The new findings indicate that signal pathway VEGF-Akt-HDAC-p53/p21 is crucial for stem cell differentiation into endothelial cells. The present presentation will summarize the data on shear stress-induced stem cell differentiation and the impact of such a differentiation on the pathogenesis of vascular diseases.

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SA3

Stretch-dependent growth and differentiation in vascular smooth muscle

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Vascular adaptation to pressure and flow involves an intricate interplay of the endothelium, the smooth muscle layer and the extracellular matrix. Shear stress on the endothelium causes vasodilatation, which in turn increases tension in the vascular wall and causes stretch-induced growth (outward remodelling). Reduced endothelial function causes instead increased vascular tone, which leads to inward remodelling of the vascular wall and an increased wall-to-lumen ratio. Vascular smooth muscle cells exposed to physiological levels of stretch grow in a maintained contractile phenotype, in contrast to the

tion by altered glycocalyx properties, for example, in atherogenesis, should be considered.

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SA11

Endothelial phenotype plasticity in unstable flow regions of the cardiovascular system: differential microRNA expression

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Arterial endothelial phenotype heterogeneity significantly influences athero-suceptibility and athero-protection in vivo. Differential transcript profiling of endothelium in susceptible arterial regions of normal adult swine displays a balance of pro-pathological and protective transcript profiles when compared with adjacent regions that rarely, if ever, develop atherosclerosis. The endothelial phenotype in vivo and in vitro is highly sensitive to the local blood flow characteristics via mechanotransduction and transport mechanisms. Athero-susceptible locations map to regions of hemodynamic (and biomechanical) spatio-temporal complexity where transient vortices within flow separation zones promote flow reversal, oscillatory shear stresses, low flow velocities and low mean shear stresses, steep spatial shear stress gradients, and occasional turbulence (chaotic flow). Differential phenotypes are detectable in endothelium in vivo at the mRNA, protein, post-translational, and functional levels. We now demonstrate that differential microRNA expression that targets specific gene and protein expression is part of the regulation of endothelial phenotype.

Regulation of mRNA stability and translation occurs by highly conserved small non-coding microRNAs (miRNAs). Microarrays identified 3 miRNA families (let-7, miR10, miR26) as upregulated in endothelium from an atheroprotected region of thoracic aorta relative to a nearby atherosusceptible region (aortic arch). By qRT-PCR, expression levels of miR10a and 10b were 4.9 and 20.7-fold higher respectively at protected (n=8) vs susceptible (n=10) regions; in contrast miR7d and miR26b were elevated <2-fold. The copy number of miR10a was greater than that of miR10b and its preferential expression in endothelium in situ was detected by immunofluorescence. 854 putative targets of miR10a/b were organized into interactive pathways using IngenuityTM. Sequences of 138 of the most interactive genes were entered into the Sfold program that assesses target secondary structure as an important predictor of miRNA-target hybridization sensitivity. Among miR10a/b targets showing high total hybridization energy were Flt-1 (VEGFR1), Hox-D10 and VEGFA. Endothelial expressions of these genes were suppressed in protected vs susceptible regions in a reciprocal relationship with miR10a/b. Cultured endothelial cells overexpressing miR10 suppressed Flt-1 gene expression. The data show miRs to be flow responsive and suggest miR10a/b to be important regulators of endothelial gene expression in atheroprotection/susceptibility.

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SA12

Effects of shear flow on selectin expression in endothelial cells co-cultured with smooth muscle cells

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ABSTRACT

In vitro co-culture of endothelial cells (ECs) with smooth muscle cells (SMCs) induced rapid and sustained increases in EC expression of E-selectin. By using inhibitors, dominant-negative mutants, and siRNA, we found that activations of JNK and p38 are critical for the co-culture-induced E-selectin expression. Gel shifting and chromatin immunoprecipitation assays showed that SMC-co-culture increased the NF-κB-promoter binding activity in ECs. Inhibition of NF-κB activation blocked the co-culture-induced E-selectin promoter activity. Protein arrays and neutralizing antibodies showed that IL-1β and IL-6 produced by EC/SMC co-cultures contribute to the co-culture-induction of EC signaling and E-selectin expression. Pre-shearing of ECs inhibited the co-culture-induced EC signaling and E-selectin expression. These findings serve to elucidate the molecular mechanisms underlying the SMC-induction of EC E-selectin expression and the shear stress-protection against this SMC-induction.

INTRODUCTION

The aim was to elucidate the mechanisms that regulate the SMC-induced E-selectin expression in ECs and its inhibition by shear stress. This article reviews several publications in our labs [1-5].

MATERIALS AND METHODS

Cell culture. ECs were isolated from fresh human umbilical cords. SMCs were obtained from Clonetics (Palo Alto, CA).

Preshearing of ECs. ECs were seeded onto the outer side of the membrane (10-μm-thick, 0.4-μm pores, pre-coated with fibronectin) of a transwell. After incubation for 24 h, the membrane with ECs was incorporated into a flow chamber on the underside of the transwell for shear stress applications at a high (HSS, 12 dyn/cm2) or low level (LSS, 0.5 dyn/cm2) for 4 or 24 h.

Co-culture of ECs and SMCs. After EC preshearing, the inner side of the membrane was seeded with SMCs under static condition, thus forming an EC/SMC co-culture system. Controls had no cells or ECs instead of SMCs on the inner side. To study the effect of distance of EC/SMC separation, ECs seeded on the

membrane were separated by 1 mm from the SMCs plated on an outer chamber (EC/M/SMC).

RESULTS AND DISCUSSION

Pre-exposure of ECs to HSS, but not LSS, for 24 h inhibits SMC-induced E-selectin expression in ECs. EC/SMC co-culture induced an increase in E-selectin mRNA expression in ECs within 1 h. Separation of ECs from SMCs by 1 mm retarded the E-selectin expression. Pre-shearing of ECs at HSS for 24 h inhibited the co-culture-induced E-selectin expression; this was not seen with LSS. Thus, SMCs induced EC expression of E-selectin via a paracrine effect that can be inhibited by HSS.

SMC-induced EC expression of E-selectin and its inhibition by shear stress are mediated by the JNK and p38 pathways. The phosphorylation of ERK, JNK, p38, and Akt in ECs showed transient increases after co-culture with SMCs. The co-culture-induced E-selectin expression was inhibited by inhibitors for only JNK and p38. Pre-shearing at HSS, but not LSS, for 24 h inhibited the co-culture-induced JNK and p38 phosphorylation. JNK- or p38-specific siRNA caused significant inhibition of the co-culture-induced E-selectin expression. The increase in E-selectin-Luc promoter activity in ECs by SMC-co-culture was prevented by pre-shearing at HSS, but not LSS. Thus, the SMC-induction of EC expression of E-selectin is mediated by JNK and p38 and blocked by HSS.

SMC-induced EC expression of E-selectin and its inhibition by shear stress are dependent on NF-κB. Inhibition of NF-κB abolished the co-culture-induced E-selectin promoter activity. Co-culture with SMCs increased the NF-κB-DNA binding activity in EC nucleus, which was inhibited by HSS (but not LSS). Thus, the co-culture induced E-selectin expression is mediated by NF-κB, and this effect is inhibited by HSS.

IL-1β and IL-6 produced by EC/SMC are the major factors contributing to the SMC-induced signaling and E-selectin expression in ECs. Using a human cytokine array system, we identified IL-1β and IL-6 as the proteins released from EC/SMC at significantly higher levels than EC/EC (>4-fold). Neutralizing antibodies against IL-6 and/or IL-1β inhibited the co-culture-induced increases in E-selectin mRNA, JNK and p38 phosphorylation, and NF-κB-DNA binding activity.

IRAK and gp130 are involved in regulatory effects of SMC-co-culture and shear stress on EC E-selectin expression. The SMC-induced E-selectin expression in ECs was suppressed by siRNAs against gp130 (IL-6 receptor) and IRAK (complex with the IL-1β receptor upon its stimulation). The co-culture-induced phosphorylations of gp130 and IRAK were inhibited by pre-shearing at HSS (but not LSS) for 24 h.

These results indicate that the SMC-induction of E-selectin in ECs involves the paracrine action of IL6 and IL-1β on their receptors to activate the JNK, p38 and NF-κB, and that this effect can be inhibited by high shear stress.

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SA13

Shear stress, inflammation and atherosclerosis

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Atherosclerosis is the disease with the highest mortality in the western world. Despite its large socio-economical impact, the underlying mechanisms are only partially known. It has been accepted for decades that atherosclerosis is a lipid driven disease, despite the fact that risk factors related to lipid metabolism only partially explain atherogenesis. Furthermore, new therapies specially focussed upon lipid metabolism only partially reduce plaque size. Recently two concepts – inflammation and blood flow/shear stress – have undergone a renaissance and gained a lot of interest as complementary explanations for plaque formation and these concepts will be the topic of the present manuscript.

The role of inflammation became apparent from a series of mouse studies where systematically parts of the immune system were knocked down, before the induction of atherosclerosis. These studies identified inflammation as an independent mechanism attributing to plaque formation, and based upon these results and further studies atherosclerosis is considered a lipid driven inflammatory disease. The effect of blood flow in atherosclerosis is based upon the observation that plaques are not evenly distributed over the arterial system. These predilection sites are at or near side branches, i.e. where blood flow is non-uniform, or at the lesser curvature of bends, i.e. where blood velocity is relatively low. The effect of blood flow on the vessel wall is through shear stress which alters the physiology of endothelial cells. Shear stress (τ N/m2 or Pascal (Pa)) arises from the friction between two virtual layers in a fluid, and is induced by the difference in movement of the two layers (dv/dr s-1; in case of a cylindrical tube) and the "roughness" (or viscosity Pa.s) between these layers ($\tau = dv/dr * \eta$). Shear stress also arises at the interplay between blood and the endothelial layer, where it induces a shearing deformation of the endothelial cells. This shearing deformation affects the phenotype of the endothelial cells and thereby the inflammatory component and plaque progression/composition.

This paper describes the interaction between shear stress and inflammation. We will first describe recent findings on the sensing mechanism of shear stress by the endothelium. Subse-

quently, pro-inflammatory pathways modulated by shear stress in endothelial cells, followed by the effect of shear stress on plaque progression and plaque composition. At the end we will discuss new findings related to longitudinal plaque heterogeneity.

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SA14

Engineering vascular grafts

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Coronary artery and peripheral vascular disease are associated with significant morbidity and mortality. In these patients, surgical intervention including small diameter bypass grafting with autologous veins or arteries is a common treatment. However, many patients lack suitable autologous vessels, either because these vessels are diseased themselves or because of previous surgery, and in these cases, synthetic grafts are often used. Unfortunately, many of these grafts fail because of the low number of endothelial cells and the proportion of the endothelialised surface remaining after exposure to flow, which results in acute thrombosis and subsequent occlusion of the vessel.
At the University of Manchester, we are developing small calibre vascular grafts for coronary or peripheral bypass and vascular access grafts for haemodialysis. These grafts are based on electrostatically spun polyurethane and polycaprolactone with controlled porosity and biodegradability and are coated with specific vascular matrix molecules to regulate cell adhesion, migration, and growth factor bioavailability. This talk will focus on the approaches we are using to improve both the initial adhesion of endothelial cells to the graft surface and the retention of these cells to this surface following restoration of flow. Our studies have revealed significant new insights into the biology of endothelial cell attachment to surfaces coated with specific vascular matrix molecules, with important implications for the design of the next generation of vascular grafts.

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SA15

Waveform analysis and microcirculatory function

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Risk factors for cardiovascular disease mediate their effects by altering the structure and function of wall and endothelial components of arterial blood vessels. Pathological change in the microcirculation plays a pivotal role in promoting end-organ dysfunction that not only predisposes to further organ damage but also increases the risk for future macrovascular events. The microcirculation is recognised as the site where the earliest manifestations of cardiovascular disease occur that may play a pivotal role in driving the atherosclerotic process in conduit vessels(1).
Ultrasound and the Doppler effect have been long used to measure blood velocity and its temporal and spatial variation within the vascular tree in order to diagnose and monitor vascular disease. Changes in morphology of the linear flow velocity spectral envelope is not representative of any single vessel but is determined by changes in the properties and total cross-sectional area of downstream vascular networks(2). Quantitative analysis of Doppler-time velocity waveforms that reflect measures of flow pulsatility (eg resistive index, pulsatility index) can mirror changes in downstream vascular resistance and may predict future adverse clinical outcomes(3). In a series of studies in different patient groups we have shown these derived indices often provide misleading information in relation to the haemodynamic actions of drug interventions and are not sensitive in detecting early microvascular dysfunction in different vascular beds in humans(4,5). Novel algorithms that enable quantitative analysis of the Doppler velocity spectral envelope over the duration of the cardiac cycle provides more sensitive information in relation to the haemodynamic action of drugs and identification of early microvascular abnormalities in humans. Data will be presented showing the superiority of this approach in identifying early microvascular abnormalities in waveforms obtained from different arterial territories in different disease states associated with increased cardiovascular risk.
Techniques capable of detecting microvascular damage and monitoring response to therapeutic interventions, especially in vulnerable target organs of interest, may improve risk stratification and could represent a valuable surrogate for future cardiovascular outcome.

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