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.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

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

proliferative growth seen in vascular lesions and plaque formation. Organ culture of intact blood vessels is a useful technique to study molecular mechanisms of stretch-induced growth with maintained cell-cell and cell-matrix interactions. This technique has been applied to pressurized large and small arteries as well as to veins, revealing basically similar molecular mechanisms but with functional effects dependent on tissue organization and physiological role of the vessel. A convenient model is the rat or mouse portal vein, which has a dominantly longitudinal muscle layer that rapidly hypertrophies in vivo in response to increased portal pressure. Organ culture of portal veins under longitudinal stretch reproduces this growth response (1), and analysis of signal mechanisms shows that stretch causes synthesis of smooth muscle-specific contractile and cytoskeletal proteins, such as SM 22α, calponin, desmin and α-actin, by a sequence of events including biphasic (minutes and hours) phosphorylation of focal adhesion kinase (FAK), early (minutes) phosphorylation of the proliferation-related signal ERK1/2, and late (hours) Rho activation, cofilin phosphorylation and actin polymerisation (2,3). Synthesis of smooth muscle-specific proteins is regulated by the transcription factor serum response factor, in concert with cofactors such as myocardin and myocardin-related transcription factors, which are dependent on the state of actin polymerisation (reviewed in ref. 4). The actin filament stabilising agent jasplakinolide causes increased synthesis of smooth muscle proteins in the mouse portal vein, and also activates the ERK1/2 pathway, whereas effects of stretch on FAK phosphorylation or contractility are abolished (3). This suggests that actin filament dynamics are crucial for vascular remodelling responses. Cholesterol-rich membrane caveolae are important for integrating signal mechanisms in the plasma membrane, and studies in caveolin-1 deficient mice suggest that caveolae are needed for endothelium-dependent relaxation in response to flow, involving Akt phosphorylation and NO production. In contrast, stretch-induced responses in mouse portal vein do not involve Akt phosphorylation and are unaffected in caveolin-1 deficient mice (5). The signal mechanisms regulating contraction and growth responses seem to be integrated partly via the intracellular Ca^{2+} concentration, and evidence suggests that voltage-dependent Ca^{2+} influx via L-type membrane channels elicits smooth muscle differentiation via a Rho kinase dependent mechanism (6). In mouse portal vein, stretch-induced effects on cofilin phosphorylation, regulating actin polymerisation, are attenuated by L-type channel inhibition, correlating with decreased synthesis of smooth muscle marker proteins, while inhibition of Ca^{2+} influx via store-operated channels causes a global decrease in protein synthesis but does not inhibit stretch-induced cofilin phosphorylation or synthesis of smooth muscle-specific proteins. Thus Ca^{2+} exerts a dual influence on the regulation of protein synthesis in vascular smooth muscle. Since phenotype modulation of smooth muscle cells is associated with loss of voltage-dependent channels and gain of store-operated channels, the contractile and synthetic cellular phenotypes may be specifically susceptible to interventions targeting the different modes of Ca^{2+} entry.

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SA4

Bio-mechanical activation and notch signaling - how vascular cells respond to stress !

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Over the last couple of years it has become increasingly clear that the Notch signaling pathway plays a pivotal role in the development and homeostasis of the cardiovascular system. The rapid increase in the number of publications that focus on the role of Notch signaling in regulating vascular cell function both in vitro and in vivo reflects the degree of interest in understanding the role of this pathway in vascular homeostasis. An emerging paradigm suggests that developmental gene regulatory networks are often recapitulated in the context of phenotypic modulation, vascular remodeling and repair in adult vascular disease. Notch receptor-ligand interactions, in conjunction with vascular endothelial growth factor (VEGF) and components of the Hedgehog (Hh) signaling pathway have all been implicated in vascular morphogenesis and modeling of the embryonic vasculature. The presentation will focus on the specific role of a Hh/VEGF/Ang axis in controlling vascular smooth muscle cell (SMC) growth (proliferation and apoptosis) through regulation of Notch signaling [1-5]. Using dynamic in vitro cultures of SMC under flow and pressure and ligated murine carotid arteries in vivo to mimic vascular injury, the components of these pathways in dictating the vascular SMC response to bio-mechanical injury will be addressed. Collectively, data will provide an insight into the coordinate regulation of Notch by sonic hedgehog (shh) and VEGF-A in adult SMC and thus may represent a future novel therapeutic target for intervention in vascular proliferative disorders.

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SA5

Mechanotransduction and the glycocalyx

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The surface of endothelial cells (ECs) is decorated with a wide variety of membrane-bound macromolecules that constitute the glycocalyx (GCX). As the most apical structure on the EC, the GCX senses the force (shear stress) of flowing blood and transmits it via the cytoskeleton throughout the cell to sites where transduction of force to biochemical response (mechanotransduction) may occur. In this presentation the structure of the GCX and many of the experiments that demonstrate its role in mechanotransduction and vascular remodeling will be reviewed. Experiments with enzymes that degrade specific glycosaminoglycan components have been used to show that the GCX mediates the shear-induced production of nitric oxide, a central process in cardiovascular control, while the same enzyme treatments do not affect shear-induced production of prostacyclin, another hallmark of EC mechanotransduction. These experiments reinforce the concept of distributed sites of mechanotransduction in EC. The characteristic remodeling of the EC cytoskeleton and intercellular junctions in response to shear stress are dependent on the GCX as well, and the experiments that support the role of the GCX in these processes will be reviewed as well.

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SA6

Modulation of inflammatory responses of endothelial cells by changes in local shear stress

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Stable local haemodynamic microenvironments may determine the phenotype of endothelial cells (EC) in different regions of the circulation, but acute changes in flow might also modulate functional responses. We aim to understand how different levels or patterns of shear stress applied to endothelial cells regulate inflammatory responses, and in particular, leukocyte recruitment. For this purpose, we developed models in which human EC of various types (HUVEC from umbilical veins; HUAEC from umbilical arteries; HCAEC from coronary arteries) were cultured in glass capillaries coated with desired substrates. These constructs were conditioned by different levels of shear stress for different periods, or exposed to abrupt changes in shear. Conditioning could be combined with treatment with cytokines such as tumour necrosis factor-α (TNF) and interleukin-1β (IL-1), and adhesion and migration of flowing neutrophils analysed as an 'inflammatory' readout.

Initial studies showed that conditioning of HUVEC for 24h at increasing shear stress acted to powerfully suppress responses to TNF, but not IL-1, judged by neutrophil recruitment (Sheikh et al., 2003; 2005). However, in subsequent studies, responses to both cytokines were suppressed by shear conditioning for HUAEC and HCAEC. Studies in which culture medium constituents, such as basic fibroblast growth factor, were swapped, indicated that this difference between the endothelial cells arose from culture conditions rather than from an in vivo imprinted phenotype. The fact that the original 'static' cultures of each cell type showed similar abilities to support adhesion and migration of neutrophils also indicated that the phenotypes of EC were plastic and could be re-set by conditioning in vitro. Taking this further, we analysed expression of selected genes in HUVEC immediately after digestion from veins, after standard culture in vitro and then after shear conditioning. Changes were induced by initial culture, which were reversed in part at least by the return to a shear environment. Thus it seems that endothelial phenotype is highly pliable, with enviromental factors, such as shear stress and growth factors, modifying responses in an interlinked but reversible manner.

We thus investigated whether the less responsive state induced in vitro by shear stress would change when flow was ceased. This might be relevant to ischaemic conditions in vivo (for instance linked to thrombo-embolism, surgical interventions or organ transplantation), where an inflammatory response typically follows reperfusion. We found that response of EC to TNF only increased slowly over 24-48h after cessation of flow, and that if a very low level of shear stress was retained, then the response remained suppressed (Matharu et al.,2008). In all of the above, functional