

## SA32

**Matrix-induced gene expression and growth factor release in endothelial cells under shear stress**

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Flow induced shear stress belongs to mechanical forces which play a significant role in controlling the vasculature. Acutely shear stress may be understood as the control variable for local diameter regulation by autacoids like nitric oxide (NO). However, if flow changes are persistent shear stress may be elevated chronically and adaptive remodeling processes of the vessel wall are induced in a long term range. Those adaptations like changes in wall thickness and new vessel formation are dependent on fine tuned signals composed mainly by changes in gene expression, release of growth factors, and degradation of extra cellular matrix. All those processes have been shown to be directly induced by shear stress. The exact mechanism translating a mechanical force into an cellular biochemical signal, however, is still only partly understood and it is hypothesized that cell-matrix interactions may be involved.

To study the direct effect of cellular matrix binding on shear stress dependent eNOS-expression endothelial cells (EC) grown on different matrix components were subjected to shear stress in vitro.

Only when they are seeded on a laminin containing matrix they responded to shear stress with a 2fold increase of the eNOS mRNA expression. This eNOS-mRNA increase could be prevented by an inhibition of cellular laminin binding with the peptide YIGSR. This peptide represents a sub-sequence of the  $\beta$  1 chain of laminin which is bound by a laminin receptor (LBP) of 67kDa. The shear stress dependent alterations of eNOS expression are due to an increased transcription no increase was found during incubation with actinomycin D. Similar to the eNOS mRNA western blots against eNOS protein showed elevated values only when the cells were grown on laminin. As for the mRNA the elevation of eNOS-protein could be prevented by inhibition of LBP with YIGSR. Western blots showed that the expression of the LBP itself appeared to be up-regulated by shear stress indicating a feedback loop mechanism.

Beside these non-integrin matrix receptor-effects we could show that EC exposed to elevated shear stress release bFGF a cytokine stored in large amounts within the cells. We could further show that this release is not due to unspecific cell damage but rather be part of a regulated pathway involving the integrin  $\alpha$ v $\beta$ 3 and indicating again a participation of cell matrix interactions in sensing shear stress. Basic FGF regulates EC proliferation/migration and angiogenesis and therefore may participate in vascular remodeling. Due to its lack of a signal sequence it is evident that bFGF is not secreted via the classical vesicular pathway of the golgi. It was shown earlier that small heat shock protein, hsp27, and bFGF are co-expressed in EC and that enhanced expression of hsp27 facilitates release of bFGF. Based on our hypothesis that shear stress induced bFGF release is controlled by cell-matrix interaction we investigated whether matrix modulation by proteases contribute to the signaling cascade in bFGF release and studied the role of hsp27 in this process. In conditioned media of EC subjected to shear stress a 3-fold enhanced elastase activity was found together with a 10-fold higher bFGF release. This

bFGF release was significantly reduced by protease inhibition. Moreover, static cells treated with elastase demonstrated a similarly increased bFGF release. As shown earlier for shear stress this elastase induced bFGF release could be prevented by inhibition of integrin  $\alpha$ v $\beta$ 3. Furthermore, like in shear stress hsp27 was phosphorylated upon elastase treatment which could be prevented by both, inhibition of integrin  $\alpha$ v $\beta$ 3 and p38 MAP Kinase. Finally, co-precipitation experiments indicated a close physical interaction of bFGF with phosphorylated hsp27. These results indicate that the mechanism of shear stress induced bFGF release is critically dependent on matrix modulation via proteases (elastase) which might subsequently alter specific cell-matrix interactions via certain integrins (here  $\alpha$ v $\beta$ 3).

We further investigated whether matrix fragments generated by elastase would have specific own effects on EC and might contribute to vascular remodeling. EC subjected to shear stress exhibited enhanced elastase activity in conditioned media. This went along with an increased occurrence of the laminin fragment E8 within the matrix. EC seeded on purified E8 resulted in a reduced proliferation and exhibited a 2-fold higher apoptosis rate as well as an increased O<sub>2</sub>- production. Aortic ring sprouting was significantly inhibited by E8. This inhibition was revoked by application of radical scavengers.

These results indicate that during shear stress the matrix is altered proteolytically, a mechanism which might be part of a feed back loop to limit shear stress induced proliferation and differentiation of EC in adaptive vascular remodeling to increased flow.

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*Where applicable, the experiments described here conform with Physiological Society ethical requirements.*

## SA33

**Integrins in the vascular system: function and signaling pathways**

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Integrins are  $\alpha\beta$ heterodimeric cell surface adhesion receptors that mediate the physical interaction with the extracellular matrix and initiate signaling events necessary for cell adhesion, migration and positioning, and induce signaling event. The main signaling pathways activated by integrins include: MAPK, PI-3K-PKB/Akt, Rho family GTPases and Nuclear Factor-kB (NF-kB). Endothelial cell integrins are critical mediators and regulators of angiogenesis and vascular homeostasis. Extracellular integrin antagonists (i.e. small peptides or antibodies) suppress angiogenesis in many experimental models. In our laboratory we are exploring alternative approaches of suppressing angiogenesis by targeting integrin cytoplasmic domain and integrin-dependent intracellular signaling events.

Expression of chimeric isolated  $\beta$ 1 or  $\beta$ 3 integrin subunit cytoplasmic domains, causes rapid disruption of focal adhesions and actin stress fibers, dissolution of VE-cadherin/ $\beta$  catenin local-

ization at cell-cell contacts, cell rounding and detachment. In vitro, HUVEC expressing isolated  $\beta 1$  integrin subunit cytoplasmic domain detached in the absence of detectable cleavage of caspase-8, -3, and PARP, suppression of FAK, ERK, PKB and Ik-B phosphorylation, and significant increased in cell death up to 24 hours after construct expression. In vivo expression of isolated  $\beta$  integrin in endothelial cells of quiescent vessels caused acute endothelial cell rounding and detachment followed by an increase in DNA-fragmentation in the detached cells. These results demonstrate that cell detachment induced by expressed isolated  $\beta 1$  cytoplasmic domain preceded cell death and revealed an essential role of integrins for the adhesion and survival of quiescent arterial endothelial cells in vivo.

Inhibition of endothelial cell COX-2 by non steroidal anti-inflammatory drugs suppress  $\alpha V\beta 3$ -dependent Rac activation, endothelial cell spreading, migration in vitro and FGF-2-induced angiogenesis in vivo. The COX-2 metabolite PGE2 accelerated  $\alpha V\beta 3$ -mediated HUVEC adhesion and promoted Rac activation and cell spreading.  $\alpha V\beta 3$ -mediated adhesion induced a transient COX-2-dependent rise in cAMP levels, while the cell permeable cAMP analogue 8-brcAMP accelerated adhesion, promoted Rac activation and cell spreading. We have recently shown that integrin-mediated cell adhesion and soluble integrin ligands contribute to maintaining COX-2 steady-state levels in endothelial cells by the combined prevention of lysosomal-dependent degradation and the stimulation of mRNA synthesis involving multiple signaling pathways.

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### SA34

#### Expression and Function of Laminins in the Embryonic and Mature Vasculature

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Basement membranes of vascular endothelium, like those of other tissues, are composed of laminins, type IV collagens, heparan sulphate proteoglycans and nidogens. However, isoforms of all four classes of molecules exist, which combine to form structurally and functionally distinct basement membranes. The endothelial cell basement membranes have been shown to be unique with respect to their laminin isoform composition. Laminins are a family of glycoprotein heterotrimers composed of an  $\alpha$ ,  $\beta$  and  $\gamma$  chain. To date 5  $\alpha$ , 4  $\beta$  and 3  $\gamma$  laminin chains have been identified that can combine to form 15 different isoforms. The laminin  $\alpha$  chains are considered to be the functionally important portion of the heterotrimers, as they exhibit tissue-specific distribution patterns and contain the major cell interaction sites. Vascular endothelium expresses only two laminin isoforms and their expression

varies depending on the developmental stage, vessel type and the activation state of the endothelium. Laminin 8 (composed of laminin  $\alpha 4$ ,  $\beta 1$ ,  $\gamma 1$  chains) is expressed by all endothelial cells regardless of their stage of development and its expression is strongly upregulated by cytokines and growth factors that play a role in inflammatory events. Laminin 10 (composed of laminin  $\alpha 5$ ,  $\beta 1$ ,  $\gamma 1$  chains) is detectable primarily in basement membranes of capillaries and venules commencing 3-4 weeks after birth. In contrast to laminin 8, endothelial cell expression of laminin 10 is upregulated only by strong proinflammatory signals and, in addition, angiostatic agents such as progesterone. Current data suggest that laminin 10 contributes to the barrier function of endothelium, in particular to the extravasation of inflammatory cells. The data argue for a dynamic endothelial cell extracellular matrix that presents different molecular information depending on the type of endothelium and/or physiological situation.

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### SA35

#### INTEGRINS AND ANGIOGENESIS

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Members of the integrin family play central roles in regulating not only cell adhesion and migration but also cell differentiation and signalling. Aberrant integrin expression and signalling have been observed in various pathological conditions such as tumour growth and metastasis. Changes in integrin expression patterns have also been reported in some physiological processes such as wound healing. Elucidating the precise roles of these molecules in vivo is essential for our understanding of these biological processes and diseases, and for developing strategies for inhibiting or modulating adhesive function for therapeutic applications. To this end, using integrin-deficient mice, we have focused our efforts on the functions of integrins in pathological angiogenesis and wound healing.

Our work has shed significant light on the roles of  $\alpha v\beta 3$ -integrin in angiogenesis, and the roles of both  $\alpha 3\beta 1$  and  $\alpha v\beta 3$  in wound healing. Inhibition of  $\alpha v\beta 3$ -integrin function has been reported to suppress neovascularisation and tumour growth, suggesting that this integrin is a critical modulator of angiogenesis. Our work showed that, surprisingly, mice lacking  $\beta 3$ -integrins not only support tumourigenesis, but even show enhanced tumour

growth. Moreover, the tumours in these integrin-deficient mice display enhanced angiogenesis, suggesting strongly that b3-integrins are not essential for neovascularisation. We have demonstrated that avb3 integrin plays a key role in the control of angiogenesis by its regulation of the pro-angiogenic receptor, Flk-1.

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