rescued by over-expression of HDAC6, demonstrating that this enzyme mediates some of the effects that Erg exerts on the cytoskeleton. To study cytoskeletal dynamics we performed live-cell imaging of Erg-deficient EC by transfecting cells with actin-RFP and recording images by time-lapse microscopy. Control cells exhibited a characteristic asymmetrical shape with lamellae orientated towards the direction of migration; Erg-deficient cells exhibited an angular shape with cortical actin and markedly reduced lamellipodia. Quantification using kymographs showed that Erg inhibition results in loss of lamellipodia formation. In vivo, inhibition of Erg expression in angiogenic EC resulted in decreased HDAC6 expression with increased tubulin acetylation. In conclusion, these results indicate that Erg is required for endothelial cell migration, regulating HDAC6 expression and tubulin acetylation, and for the dynamic movement of actin-rich lamellipodia. These pathways are essential for cell migration and angiogenesis and point to a novel crucial function of this transcription factor in endothelial biology.

doi:10.1016/j.vph.2011.08.120

P.1.11
Impaired angiogenesis: A prediction for digital ulcers in Raynaud Phenomenon

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Introduction: Structural changes in the microcirculation of the fingers with Raynaud Phenomenon (RP), diagnosed by capillaroscopy, lead to stenotic lesions and occlusions with impairment of blood flow with consequent digital ischemia and ulcers. Hypoxia stimulates angiogenesis in physiological conditions. The aims of this study were to identify biomarkers of angiogenesis and to analyze the impact of pro-angiogenic and angiostatic factors in regenerating the affected microcirculation in RP patients.

Materials and methods: We reviewed a cohort of 140 RP patients attending our Raynaud's Clinic. In an attempt to define impaired angiogenesis as a risk of a patient with RP to develop digital ulcers we compared capillaroscopy patterns with angiogenesis biomarkers and the clinical outcome, namely digital ulcers. Vascular endothelial growth factor (VEGF) as pro-angiogenic biomarker and endostatin and endoglin as angiostatic biomarkers were measured in our cohort.*Results* Four groups of patients were compared: primary RP, secondary RP with and without digital ulcers and healthy controls. Data analysis showed that patients with RF and ANA>1/1280 anti-centromere mottled pattern, Sscl 70 positive, VS increased, C-reactive protein>30, pitting of the digital pads of the fingers, calcinosis lesions and changes of digital capillaroscopy stages (early/active or active/late) have a high risk of developing digital ulcers. VEGF was increased in primary RP and secondary RP patients without digital ulcers. In patients with digital ulcers endoglin and endostatin were significantly higher when compared with the other groups. Capillaroscopy patterns showed attempts of angiogenesis in secondary RP patients. Avascular areas were more frequent in patients with digital ulcers.

Conclusions: In our cohort we identified patients at risk of developing DUs as having increased angiostatic biomarkers and a lower level of pro-angiogenic biomarker VEGF. We might conclude that impaired angiogenesis is a consequence of increased inhibiting factors in patients with digital ulcers rather than an absence of angiogenesis stimulation. These data may help us in understanding the pathogenesis and natural history of RP and DUs and defining a correct preventive target therapy in patients without active ulcer but at risk of developing ulcers.

doi:10.1016/j.vph.2011.08.121

SESSION 3
Smooth muscle cells and cell death

P.3.1
The protease MT4-MMP is essential for maintenance of vascular smooth muscle cell phenotype and vessel homeostasis in vivo

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The homeostasis of the arterial vessel wall is crucial for proper function of the cardiovascular system and alteration of the vascular structure is the underlying cause of common diseases such as atherosclerosis, aneurysms, and hypertension. This homeostasis mainly depends on vascular smooth muscle cells (VSMC) in the media layer and the extracellular matrix that these cells produce and organize. For good performance of the vasculature, VSMCs need to be kept at the contractile phenotype since dedifferentiation to a synthetic phenotype can favor the development and progression of vascular disease. Therefore, it is of special interest to identify the molecular mechanisms responsible for VSMC quiescence and proper matrix assembly. In this context, we have investigated the possible function in the vessel wall of the membrane type 4-matrix metalloproteinase (MT4-MMP, *Mmp17*) that is abundantly expressed in VSMCs. First, we found that the structure and ultrastructure of conductance (aorta) and resistance (mesenteric artery) vessels were altered in MT4-MMP-deficient mice. In particular, media layer was thicker and contained an increased number of VSMCs. Moreover, electron microscopy revealed important morphological changes of MT4-MMP null VSMCs that were larger, rounder and aberrant and were surrounded by matrix deposits and aggregates. These features reminded of activated VSMCs and suggested that MT4-MMP might be required for maintenance of the normal VSMC phenotype. Accordingly, the response of MT4-MMP null VSMCs to injury in the carotid ligation model was enhanced with a significant increase in neointima formation and an abnormal outward remodeling. Finally, since the vessel phenotype was observed in both conductance and resistance arteries we analyzed the possible hemodynamic impact; notably, a significant decrease in the diastolic pressure in vivo and an increase in vessel distensibility ex vivo were observed in the absence of MT4-MMP. We are currently investigating the mechanisms by which MT4-MMP is regulating VSMC phenotype and matrix organization in the vessel wall. In sum, we have identified the protease MT4-MMP as a novel gate-keeper of vessel homeostasis in vivo.

doi:10.1016/j.vph.2011.08.122

P.3.2
Adenosine A_{2B} receptor agonism inhibits vascular smooth muscle cell proliferation and intimal hyperplasia in ApoE deficient mice

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The A_{2B} adenosine receptor ($A_{2B}R$) is highly expressed in macrophages and vascular smooth muscle cells (vSMC) and has been established as an important regulator of inflammation and vascular adhesion. Recently, it has been demonstrated that $A_{2B}R$ deficiency enhances neointimal lesion formation after injury. Therefore, we hypothesize that $A_{2B}R$ agonism may protect against injury-induced intimal hyperplasia.

To test this hypothesis, nine week old female ApoE deficient mice were fed a Western-type diet for 1 week, after which the left common carotid artery was denuded using a guide wire. Mice ($n = 10$ per group) were treated daily with either vehicle control or the $A_{2B}R$ agonist BAY 60-6583 (50 μg/mouse), leading to peak plasma concentrations of approximately 1 μg/mL at 2 hours after injection. After 18 days, mice were sacrificed and lesions analyzed.

Interestingly, lumen stenosis as defined by the neointima/lumen ratio was inhibited by treatment with the $A_{2B}R$ agonist from 2.1 ± 0.3 in the controls to 1.3 ± 0.2 ($P<0.05$) in treated mice. Total vessel area remained unaffected (controls: $140 \pm 8 * 10^3$ μm^2 versus BAY 60-6583: $131 \pm 8 * 10^3$ μm^2, P = NS), demonstrating absence of outward remodeling. Collagen content was increased from $10.7 \pm 10.9\%$ in the control animals to $17.0 \pm 2.0\%$ in the BAY 60-6583 treated mice ($P<0.05$), while macrophage content was unchanged.

To determine whether $A_{2B}R$ agonism affects vSMC proliferation, cultured murine vSMCs were stimulated with BAY 60-6583 for 24 hours, after which [^{3}H]Thymidine incorporation was measured. We observed a dose-dependent reduction in vSMC proliferation from $240 \pm 11 * 10^3$ dpm to $111 \pm 13 * 10^3$ dpm at 1 μg/mL BAY 60-6583 ($P<0.001$) and even up to $5 \pm 2 * 10^3$ dpm at a concentration of 10 μg/mL. Within this concentration range BAY 60-6583 did not induce cell death. Furthermore, collagen production by cultured vSMCs as determined using a picrosirius red staining was increased by 20% and 50% at increasing BAY 60-6583 concentrations ($P<0.05$).

In conclusion, our data show that activation of the adenosine A_{2B} receptor protects against vascular injury, while it also enhances plaque stability as indicated by increased collagen content. These outcomes thus point to A_{2B} receptor agonism as a new therapeutic approach in the prevention of restenosis.

doi:10.1016/j.vph.2011.08.123

P.3.3
PAR-2 depletion protects against the development of pulmonary hypertension

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Background: A hallmark of the vascular remodeling process underlying pulmonary artery hypertension (PAH) is the aberrant proliferation and migration of pulmonary artery smooth muscle cells (PASMC). Accumulating evidence suggests that mast cell mediators play a role in the pathogenesis of PAH. Therefore, in the present study we investigated the importance of mast cell tryptase and protease-activated receptor (PAR)-2 in the development of PAH.

Methods and results: Our results revealed strong increase in PAR-2 and tryptase expression in idiopathic (I)PAH patients, mice exposed to hypoxia, and rats treated with monocrotaline (MCT). Elevated tryptase levels were also detected in plasma samples from IPAH patients. Hypoxia and PDGF-BB upregulated PAR-2 expression in PASMC. This effect was reversed by PDGF-BB neutralizing antibodies or the PDGF-BB receptor antagonist Imatinib. Attenuation of PAR-2 expression was also observed in the lungs of mice exposed to hypoxia and rats challenged with MCT in response to Imatinib treatment. Stimulation of PASMC with tryptase resulted in ERK1/2 activation, increased cell proliferation and migration as well as in enhanced synthesis of fibronectin and MMP-2. Depletion of PAR-2 attenuated the PASMC response to tryptase, demonstrating PAR-2 dependent signaling. Furthermore, PAR-2−/− mice were protected against hypoxia induced pulmonary hypertension.

Conclusions: Our study identified a novel role of PAR-2 in vascular remodeling in the lung. Interference with this pathway may offer novel therapeutic options for the treatment of PAH.

doi:10.1016/j.vph.2011.08.124

SESSION 4
Reactive oxygen species and vascular dysfunction

P.4.1
Dual effect of homocysteine on rat neutrophils

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Effect of homocysteine (HC) on functional activity of intact and activated rat neutrophils was studied. Intact neutrophils isolated from blood of healthy animals and neutrophils activated *in vivo* by administration of zymozan 4 h before cell preparation were compared. HC effect on zymozan-induced respiratory burst *in vitro* measured by chemiluminescence procedure, intensity of degranulation process, intracellular cAMP content and participation of adenosine and NMDA receptors in neutrophil activation were tested.

HC has been found to suppress the levels of reactive oxygen species (ROS) produced by myeloperoxidase in intact neutrophils. Moreover, HC (10–100 mkM) inhibits purified human myeloperoxidase. This effect is supposed to be caused by homocysteinylation of the enzyme. HC can also perform as a trap for hypochlorite anion similar to carnosine or taurine in the same concentration range. Milk xanthine oxidase (0.5–10 mkM) is also inhibited by HC. These data suggest an antioxidative property of HC. Opposite effect of HC has been described when *in vivo* activated neutrophils were used in the experiments: high concentrations of HC (corresponded to toxic levels of HC in blood of human beings under neurodegenerative and cardiac diseases) strongly activated ROS production. Similar stimulation of ROS production by activated neutrophils was shown in the presence of NMDA. Moreover, effect of both HC and NMDA has been prevented by antagonist of NMDA receptors, MK-801. Activation of neutrophils is accompanied by *de novo* mRNA expression of NR1 subunit of NMDA receptors and internalization of NR1 and NR2B subunits on neutrophil membranes.

Additionally, we have demonstrated that incubation of activated neutrophils with HC results in stimulation of degranulation process and increase of intracellular cAMP levels, the latter being suppressed by ZM 241385, antagonist of A2a adenosine receptors (Ki = 0.125 nM).