angiogenesis is a physiologically process, while in most adult tissues angiogenesis is initiated only during tissue-repair or in pathological conditions. Pericellular proteolysis plays an important role in angiogenesis being required for endothelial cell migration, invasion and tube formation. The aim of this study was to clarify which proteases are involved in physiological and pathological angiogenesis, represented by in vitro capillary tube formation by respectively human endometrial (hEMVEC) and foreskin MVEC (hFMVEC) in a 3D matrix.

Urokinase (u-PA)-inhibiting antibodies and the metalloproteinase inhibitor Batimastat (BB94) both reduced VEGF-A and TNF alpha induced tube formation by hMVEC in a fibrin matrix. The presence of collagen in the matrix increased the relative contribution of matrix metalloproteinases (MMPs) to tube formation. hMVEC expressed various MMPs mRNAs and proteins as detected by RT-PCR, western blotting, zymography. MMP-2, MT1-, MT3- and MT4-MMP mRNAs were abundantly expressed by both cell types under basal as well as VEGF-A- and TNF alpha-stimulated conditions. MMP-1 was only expressed in hFMVEC under TNF alpha-stimulated conditions, while the expression in hEMVEC was present under all conditions. MT3- and MT4-MMP mRNA expressions were significantly higher in hEMVEC than in hFMVEC. MMP-9 and the other MT-MMP mRNAs were hardly detectable. Immunohistochemistry confirmed the presence of MT3-MMP in endothelial cells of endometrial tissue. Overexpression of TIMP-1 or TIMP-3 by adenoviral transduction of hEMVEC reduced tube formation to the same extent, while only TIMP-3 was able to inhibit tube formation by hFMVEC.

These data indicate that the angiogenic process in hFMVEC, which closely reflects the inflammation/repair-associated angiogenesis, depends largely on MT1-MMP. In contrast, the inhibition of capillary-like tube formation by hEMVEC by both TIMP-1 and TIMP-3 suggests that other MMPs than MT1–MMP, in particular MT3-MMP, play a dominant role in the physiological process of endometrial angiogenesis.

P435

OSCILLATORY SHEAR STRESS (OS) UPREGULATES CATHEPSIN EXPRESSION WHILE INHIBITING CYSTATIN C EXPRESSION IN ENDOTHELIAL CELLS (EC) - IMPLICATION IN ATHEROSCLEROSIS. Manu Platt, Randy Ankeny, Won kyong Shin, Michelle Sykes, Hanjoong Jo. *Georgia Tech / Emory University, Atlanta, GA.*

Cathepsins are a family of cysteine proteases, which can break down elastin and collagen components in vascular wall, and their activities are known to be controlled by the specific inhibitor cystatin C. Unstable shear conditions such as OS are pro-atherogenic, while laminar shear (LS) is atheroprotective. Here, we examined the hypothesis that OS and LS differentially regulate expression of cathepsins and cystatin C in EC. Initially, our DNA microarray study showed that exposure of EC to OS for 1 day decreased cystatin C by 2.5-fold but increased cathepsin K by 2.6-fold above the LS control at the mRNA level. Exposure of EC to OS decreased cystatin C protein level by 67% of LS control in the conditioned media as determined by Western blot and ELISA. Conversely, OS increased cathepsin K protein level by 2-fold above LS control in the conditioned media. We also found that OS increased protein levels of two additional potent elastases, cathepsins S and L, by 3- and 5-fold, respectively, above the LS control in the conditioned media. These results clearly demonstrate that the pro-atherogenic OS stimulates expression of cathepsins, while preventing expression of their inhibitor cystatin C in EC. The shift in balance toward a more elastolytic and collagenolytic environment induced by OS may lead to degradation of internal elastic lamina and plaque development.

This work was supported by funding from the NIH and NASA (HJ).

P436

UROKINASE (UPA) INDUCES CAPILLARY-LIKE TUBE FORMATION VIA NF-KAPPAB DEPENDENT CELL SURVIVAL AND CDC42 INDUCED CYTOSKELETAL REARRANGEMENT. Gerald W. Prager, Yuri Koshelnick, Katharina Leithner, Johannes Breuss, Bernd R. Binder. *Department of Vascular Biology and Thrombosis Research, University of Vienna.*

For angiogenesis the generation of a proteolytic machinery is indispensable with the urokinase/plasmin system as essential components. Urokinase does not only contribute to angiogenesis via its proteolytic properties, but also effectuates itself proangiogenic signalling. We have demonstrated that VEGF induced pro-uPA activation and it has been shown that endothelial cell migration, differentiation and proliferation are synergistically induced by urokinase independent of its proteolytic activity. However, signalling pathways involved in the uPA-mediated angiogenic responses are not fully understood.

When we compared the most important angiogenic growth factor vascular endothelial growth factor (VEGF) with urokinase in respect to capillary like tube formation, we observed an pivotal anti-apoptotic effect of both stimuli. This effect on cell survival was dependent on the type of matrix used as well as on integrin-matrix interaction itself as we could inhibit the anti-apoptotic activities of uPA or VEGF by functional blocking anti-integrin beta 3 antibodies. While VEGF led to a PI3-kinase dependent phosphorylation of Akt, which resulted in CDC42 activation, which itself phosphorylated PAK leading to IKK-1 phosphorylation, uPA activated CDC42 and its downstream effectors in a PI3-kinase/Akt independent manner. This indicates that the anti-apoptotic properties of uPA are not Akt, but NF-kappaB mediated. Indeed, when we used adenovirus overexpressing I-kappaB to block the NF-kappaB pathway, uPA but also VEGF were ineffective to support cell survival. Therefore, we can conclude that NF-kappaB dependent survival pathways are induced by VEGF and uPA. Capillary-like tube formation was also dependent on the CDC42 pathway, because overexpression of a dominant negative form of CDC42 blocked capillary-like tube formation even when apoptosis was prevented by overexpression of the X-linked inhibitor of apoptosis protein (XIAP), an NF-kappaB-dependent member of the IAP gene family, which mediates anti-apoptotic effect upon NF-kappaB stimulation.

From these data we conclude that both VEGF and uPA mediate CDC42-dependent anti-apoptosis and capillary-like tube formation, but in contrast to VEGF the uPA dependent activation of this pathway is PI3-kinase independent.

P437

PLASMINOGEN ACTIVATOR INHIBITOR-1 IS REGULATED BY GLYCOPROTEIN 130 LIGANDS IN HUMAN ADIPOSE TISSUE. Gersina Rega, Christoph Kaun, Thomas W. Weiss, Svitlana Demyanets, Manfred Frey, Gerald Maurer, Kurt Huber, Johann Wojta. *Department of Internal Medicine II, University of Vienna, Austria, Department of Surgery, University of Vienna, Austria, 3rd Department of Medicine, Wilhelminenspital, Vienna, Austria.*

Background: Adipose tissue is a prominent source of plasminogen activator inhibitor-1 (PAI-1), the primary physiological inhibitor of plasminogen activation. Elevated levels of PAI-1 are found in obese subjects and are associated with an increased risk of thromboembolic events. On the other hand a correlation between levels of the glycoprotein 130 (gp130) ligand interleukin-6 (IL-6) and obesity has been described. We could show recently that gp130 ligands upregulate PAI-1 in cardiac myocytes (Macfelda and Weiss et al., J Mol Cell Cardiol. 2002 Dec;34(12):1681-91). Here we investigate whether the gp130 ligands oncostatin M (OSM), IL-6, leukemia inhibitory factor (LIF) and cardiotrophin-1 (CT-1) regulate PAI-1 expression in human adipose tissue.