Report on the project funded by the Austrian Heart Funds:
The role of the multifunctional protein pigment-epithelium derived factor and its receptors in smooth muscle cell biology and in the development of restenosis.
PI: Johann Wojta

Introduction:

As outlined in our original proposal a possible role for PEDF in restenosis has been suggested from animal experiments. Our own results shown that PEDF and its receptors adipose triglyceride lipase/ patatin-like phospholipase containing 2 (ATGL/PLPLA2) and ribosome protein SA/laminin receptor (LR/RPSA) are present in human smooth muscle cells (SMC) isolated from aorta and its expression is downregulated in a time dependent manner after stimulation with the potent smooth muscle cell mitogen platelet derived growth factor (PDGF). As PEDF is also present in considerable quantities in adipose tissue we have expanded our project towards investigating the expression level of PEDF and its receptors in samples obtained from human adipose tissue.

We have further expanded our studies towards yet another cytokine, which has been recently shown to be involved in the development of vascular disease and restenosis, namely IL-33.

Methods:

Human adipose tissue samples

In close co-operation with Manfred Prager, who is head of the Department of Surgery at the Hospital Oberwart, we have collected samples of visceral and subcutaneous adipose tissue from morbidly obese patients with a body mass index >40 undergoing bariatric surgery. So far samples from 97 patients have been collected. Plasma samples from these patients have also been collected immediately before and after surgery and will be collected at follow-up visits 6, 12, 18 and 24 months. PEDF levels and levels of its receptors will be determined in adipose tissue samples by RT-PCR and in plasma samples by ELISA.

IL-33 in patients undergoing PCI

Blood samples were taken prospectively from 387 consecutive patients undergoing PCI. From these patients 193 had stable angina, 93 NSTEMI, and 101 STEMI, respectively. Two blood samples were taken under fasting conditions directly before PCI (at baseline) and 24 hours after PCI. IL-33 was measured with a specific enzyme-linked immunosorbent assay. Maximal lumen stenosis was measured within the stent and within the 5-mm proximal and distal edges of the stent.

Results:

In patients with decreased IL-33 (n=95), unchanged or non-detectable (n.d.) levels (n=210) or increased levels of IL-33 after PCI (n=82), the respective ISR-rate was 2.1%, 9.5% and 14.6% ($p<0.05$) (Fig. 1). IL-33 serum levels before or after PCI were not associated with ISR at follow-up ($p=0.901$ and $p= 0.790$, respectively). Accordingly, patients with ISR showed a significant increase of IL-33 upon PCI ($p<0.05$) in the entire cohort (Fig. 2A) as well as in the patients with acute coronary syndrome (ACS; Fig. 2B) or stable CAD (Fig. 2C).

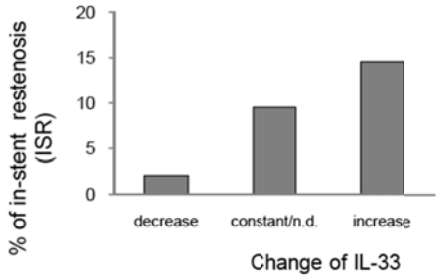

Figure 1

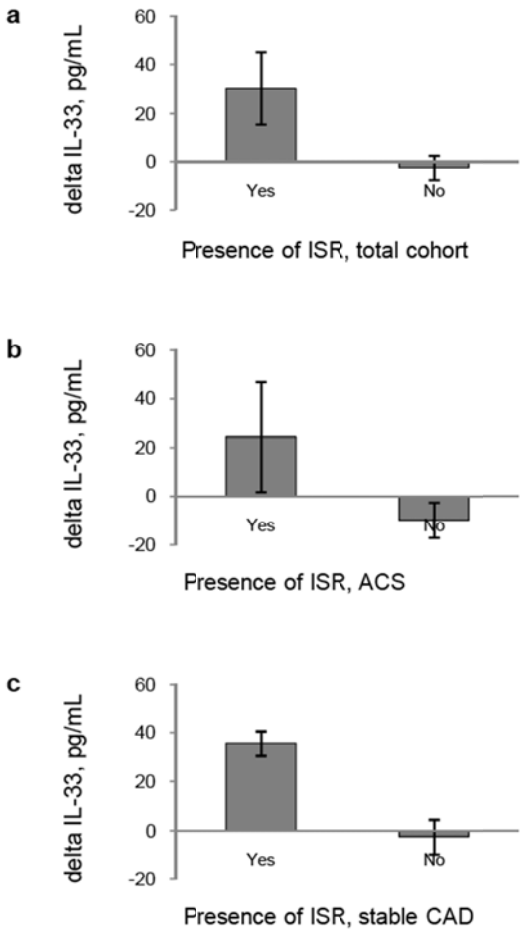

Figure 2

Conclusion:

So far we could show that a strong mitogen for smooth muscle cells, namely PDGF decreases the expression of the antiangiogenic modulator PEDF and its receptors in vascular smooth muscle cells. If operative *in vivo* this effect could impact on smooth muscle cell proliferation and plaque vascularization with PDGF not only regulating the former directly but also supporting both processes indirectly by inhibiting an antiangiogenic modulator and its receptors thereby creating a proangiogenic microenvironment in the wall of the affected vessel. Furthermore we could show that in patients with both stable and unstable coronary artery disease, a decrease of yet another newly described cytokine, namely IL-33 in serum after stent implantation is associated with a lower rate of in-stent restenosis.

Outlook:

As outlined in our original proposal, we will in the remaining part of this project continue work to elucidate the signal transduction pathways responsible for the downregulation of PEDF and its receptors by PDGF. Given the experimental evidence that PEDF and its receptors are downregulated through a growth factor for smooth muscle cells such as PDGF the planned clinical part of our study seems highly warranted. Therefore, and because PEDF has been shown to be expressed in considerable amount in human adipose tissue we will besides measuring plasma levels of PEDF in patients undergoing stent implantation also determine PEDF expression levels in adipose tissue samples and plasma levels in obese patients who are known to be at risk to develop cardiovascular disease.