Abstract: **P295**

**Proteomic, functional and receptor studies of growth differentiation factor 15, GDF-15.**

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Background/Introduction: The stress induced cytokine GDF-15 is strongly associated with cardiovascular disease. GDF-15 is also increased in concentration in plasma in many other diseases and experimentally many contradicting functions have been suggested and several receptors have been identified.

Purpose: Our approach was to investigate GDF-15’s function using a proteomic array to analyse the outcome after GDF-15 stimulation in search of novel downstream activated molecules. The more precise identity of the receptor was also investigated.

Methods: The regulation of proteins in purified human monocytes after GDF-15 stimulation together with oxidative stress (H2O2) or not was analysed using the technique 2-D DIGE. Proteins that were up- or down-regulated after GDF-15 treatment were identified with mass spectrometry and three proteins from different categories of function were verified by Western blot. Two different functional assays were performed to analyse GDF-15’s effect; a transwell migration assay and a Factor Xa activity assay. For receptor studies both inhibitory chemicals and siRNA together with Western blot were used.

Results: When human monocytes were stimulated with GDF-15 under oxidative stress the expression of several cytoskeleton proteins (actin associated) but also chaperones, coagulation and glycolysis proteins were affected. The up-regulation of WD repeat 1 (WDR1, actin regulator), T complex protein 1 (TCP-1, chaperone) and protein disulfide isomerase (PDI, disulfide catalyser activating tissue factor) was confirmed by Western blot. Analysing THP-1 cells ability to migrate after stimulation with GDF-15 showed an increased effect. Analysing the coagulation activity of cells after GDF-15 treatment showed increased activity on the surface of THP-1 cells but also increased activity on released microparticles. An increased phosphorylation of Smad2 after 15 minutes of GDF-15 stimulation in human monocytes was detected, which was blocked in THP-1 cells with the inhibitors ITD 1 and A 83-01 and by siRNA treatment, indicating that TGFBR2 is involved in the binding of GDF-15. When analyzing migration after ITD 1 and A 83-01 treatment the increasing effect seen by GDF-15 is prohibited.

Conclusions: GDF-15 up-regulates the actin associated protein WDR1, the chaperone TCP-1 and the catalyser PDI. The ability of cells to migrate is increased after GDF-15 incubation proposing a way for monocytes to move to inflamed areas. Coagulation activity of cells and microparticles also increases after GDF-15 stimulation, which could be one explanation for the association between GDF-15 and thrombotic events. The GDF-15 initiated Smad2 phosphorylation transmits via TGBFR2 as shown by inhibitor studies and siRNA.