Cathepsins are upregulated in vascular calcification

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Statement of Purpose: Cathepsins are lysosomal enzymes known to degrade various extracellular matrix components. Cathepsin S is upregulated in atherosclerosis, balloon angioplasty and aneurysms and it has been shown to play an important role in atherosclerotic plaque destabilization[12]. Cathepsin K is one of the most potent collagenase and exhibits increased expression in atherosclerotic arteries[3]. Cathepsin L is another potent elastase that is found in high amounts in aneurysmal and atherosclerotic arteries. Serum levels of cathepsin L are also significantly higher in patients with coronary artery stenosis [4]. Our aim was to study the expression of these cathepsins in an *in vitro* and *in vivo* model of vascular calcification.

Methods: In vitro vascular smooth muscle cell calcification model: Vascular smooth muscle cell undergo calcification in presences of elastin and transforming growth factor (TGF-b)[5]. Briefly, elastin degradation products in the form of elastin peptides (Elastin products #CB 573) and recombinant TGFb-1 (PeproTech) together cause osteogenic responses in rat aortic smooth muscle cells. Primary rat aortic smooth muscle cells were cultured in DMEM with 10% fetal bovine serum and treated with 100ug/ml elastin peptides and 10ng/ml recombinant TGFb-1. At the end of 7 days, RNA was extracted using Qiagen RNeasy tissue kit, quantified with Aglient Bioanalyzer 2100 RNA 6000 kit. Quantity and quality of the extracted RNA was measured using Agilent Bioanalyzer 2100 RNA 6000 kit. Reverse transcription was conducted with 1ug RNA using Retroscript kit, Ambion. Real-time PCR was conducted with SYBR green PCR kit (Qiagen) using primers from SABiosciences specific for rat cathepsin S, K & L. GAPDH was used as the housekeeping gene and delta-delta Ct method utilized for PCR data analysis (n=3).

In vivo elastin calcification model: Purified elastin was prepared from porcine aortic segments using the Partridge method. 30-40mg of purified elastin was implanted subdermally in juvenile male rats. At the end of 7 days, the elastin along with surrounding capsule was explanted. RNA was extracted using the Qiagen RNeasy fibrous tissue kit. Reverse transcription and real-time PCR were conducted as described above. B2MG was used as the housekeeping gene and delta-delta Ct method utilized for PCR data analysis (n=3).

Results: When SMCs were treated with elastin fragments, 3-fold upregulation of cathepsin S and K was seen with slight increase in Cat-L. When elastin was subdermally implanted, it was calcified significantly and capsule surrounding elastin showed significantly higher Cat S and K expression. Protein data also corroborated gene data (not shown). Cathepsin L expression levels was not significantly higher than the control in the subdermal model.

Conclusions: Degradation of extracellular matrix proteins especially elastin has been associated with vascular calcification. In this study, the increased expression of cathepsins S and K indicate the involvement of these collagenases/elastases in the SMCs and elastin calcification. Future studies will include inhibition of these cathepsins using inhibitors or siRNA. Inhibition studies can help us to further confirm their role in these cathepsins in vascular calcification.

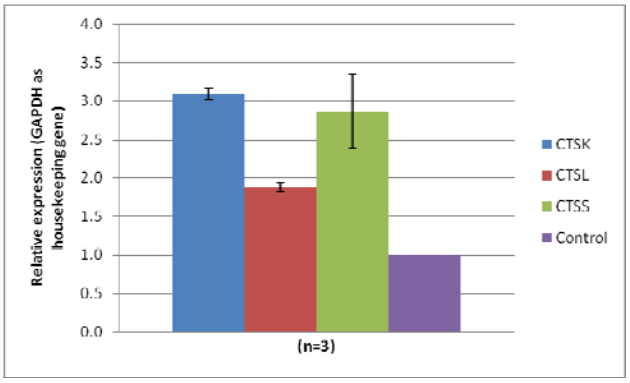

Figure 1. Cathepsin expression in an *in vitro* model of SMC calcification

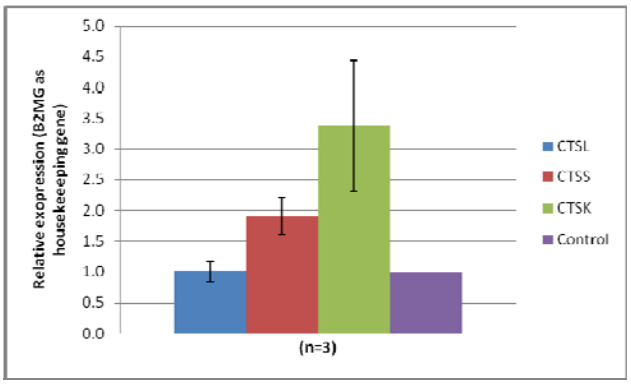

Figure 2. Cathepsin expression surrounding implanted elastin in a rat subdermal elastincalcification model

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