Regulation of neutrophil gelatinase-associated lipocalin in aortic valve stenosis

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Background: Aortic valve (AV) stenosis is the commonest form of adult valvular heart disease (VHD) and affects 4.5% of the general population aged over 60 years. Owing to multifactorial and complex molecular events, the valve interstitial cell (VIC) undergoes myofibroblast and osteoblast differentiation. Neutrophil gelatinase-associated lipocalin (NGAL) is a pleiotropic glycoprotein belonging to the lipocalin family and it is expressed in a wide range of tissues and cell types. It is deregulated in several diseases with both detrimental and beneficial effects. NGAL mainly signals towards 24p3R.

Purpose: We aimed to confirm the expression of NGAL in human AV stenosis and its association with inflammation, oxidative stress, fibrosis/remodeling and calcification, and its regulation in calcifying VICs.

Methods: Surgical AV leftovers were harvested from patients undergoing elective surgical valve replacement with no kidney disease. Serum samples were collected within the 24h before the surgery. AV were histologically assessed by hematoxylin-eosin, Movat, Alizarin Red and Alcian blue/Sirius Red staining and immunohistochemistry. Western blotting, ELISA and zymography were used in molecular biology studies. VICs were challenged for 2, 4 and 8 days with hyperphosphate (2.6mM, HP) media ± rhNGAL for in vitro validation studies.

Results: NGAL was quantified in AVs and serum samples from 126 donors (57.4% men). Circulating NGAL was associated with circulating inflammation (Tumor Necrosis Factor-α, Interleukin (IL)-6 and ICAM-1) and oxidative stress (Myeloperoxidase (MPO) and 8OHdG) markers. Likewise, tissue NGAL was correlated with inflammation (IL-6, RANTES and Galectin-3), oxidative stress (MPO, Endothelial Nitric Oxide Synthase, Malondialdehyde (MDA) and Carboxy Methyl Lysine (CML)) and fibrosis (Collagen type I). Osteoblast markers, metalloproteinase (MMP)-9 expression or its activity were not associated with NGAL. NGAL was greater expressed in men than women (217.70±23.41 vs. 119.5±11.31, p=0.0098). In vitro, intracellular NGAL and 24p3R were strongly down-regulated in calcifying men-derived VICs (n=6) whereas secretion of NGAL was enhanced from day 4 (0.55±0.15, p=0.0283; 0.32±0.09 $p<0.0001$; 8.00±2.32, p=0.0053 fold changes, respectively). This may reflect reduced 24p3R-dependent signalling in osteoblast-like VICs. Such effects were overall negated in women-derived VICs (n=3). RhNGAL endowed calcifying VICs with increased necrosis (52KDa-cPARP1), apoptosis (cCaspase 3) and oxidative stress (CML, MDA, nitrotyrosine and pNF-kB) at day 8.

Conclusions: NGAL is associated with inflammation, oxidative stress and fibrosis in AV stenosis, and promotes pro-apoptotic and necrotic phenotypes in vitro. Our results suggest that NGAL signaling may drive sex-dependent differences clinically relevant to the VHD pathogenesis. The challenge is now to understand how NGAL signals in men/women-derived VICs.