In vitro studies to understand gender differences in calcific aortic valve disease: crosstalk between JAK-STAT and TLR pathways

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Introduction
Calcific aortic valve disease (CAVD) has become an important social and economic burden. Inflammation has been pointed out as a key event in CAVD pathogenesis, in which the role of the immune modulator type I interferon (IFN) remains unknown. On the other hand, as male sex is a risk factor for CAVD, possible gender differences may have gone unnoticed in studies performed mostly with cells explanted from male patients.

Purpose
To elucidate the role of type I IFN on inflammation and calcification of human aortic valve interstitial cells (AVIC) isolated from male and female patients.

Methods
Control and stenotic AVICs were exposed to IFN-a and lipopolysaccharide (LPS) alone or combined. Western Blot and ELISA were used to analyze pro-inflammatory/pro-osteogenic molecules and signaling pathways. To test whether stimuli promotes osteogenic differentiation of AVIC, osteoblast markers and a-smooth-muscle actin were analyzed by qPCR and immunofluorescence, respectively. Alizarin red staining and calcium deposits quantification were performed to evaluate in vitro calcification.

Results
Our data showed that IFN-a and LPS cooperated to activate signal transducer and activator of transcription (STAT)-1 and nuclear factor (NF)-κB. The combination of stimuli also triggered the secretion of pro-inflammatory and pro-osteogenic molecules with a reported role in AVIC calcification. Consistent with this, IFN-a alone and combined with LPS promoted a-smooth muscle actin downregulation, thus suggesting cell differentiation. Further analysis indicated an IFN-a mediated early upregulation of osteoblast markers in male but not in female AVIC, pointing to potential differences in calcification. In vitro calcification assays revealed that male AVIC were more prone to calcification, as suggested by higher calcium deposition. Strikingly, IFN-induced calcification was totally abrogated by using the JAK inhibitor tofacitinib. qPCR revealed the tendency to lower levels of IFN-a receptor subunit-1 transcripts in female AVIC. In addition, signaling pathways analysis showed a synergistic phosphorylation of protein-kinase-B (Akt) only in female AVIC, a kinase that may be involved in calcification differences.

Conclusions
IFN-a and LPS crosstalk promotes higher inflammatory and osteogenic responses in male AVIC, which correlates with the higher rate of metaplasia reported in male patients. Our data point to JAK-STAT pathways as potential therapeutic targets for CAVD.