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Wnt/β-catenin activation attenuates osteogenic differentiation and inflammation in female aortic valve interstitial cells

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Calcific aortic valve disease (CAVD) is characterized by progressive fibro-calcific remodeling of the aortic valve, driven in part by osteogenic differentiation of human aortic valve interstitial cells (HAVICs). Clinically, men tend to exhibit greater calcification burden, while women display more fibrosis, yet the molecular mechanisms underlying these sex-specific differences remain unclear. We analyzed clinical, transcriptomic, and proteomic data from patients with aortic stenosis to dissect sex-specific mechanisms of calcification. Calcification severity and activity were assessed using Agatston scores, ^{18}F-NaF PET/CT, and echocardiography. HAVICs were isolated from male and female patients and subjected to osteogenic differentiation *in vitro*. Bulk RNA sequencing and LC-MS/MS proteomics were performed. Gene set enrichment and differential expression analyses identified sex-specific regulatory pathways. Clinically, men had significantly higher aortic valve calcium scores and ^{18}F-NaF uptake, whereas in women, ^{18}F-NaF uptake correlated with OPG/sRANKL ratios. *In vitro*, female HAVICs showed higher osteogenic calcification than male cells. Both sexes shared enrichment in canonical osteogenic pathways (Wnt, PI3K-Akt, ECM-receptor interaction), but female HAVICs also upregulated angiogenic and hormone response pathways. DKK1 – a Wnt pathway inhibitor – was significantly upregulated in female cells, suggesting suppression of this pathway during differentiation. To explore the role of Wnt signaling, β-catenin was overexpressed in HAVICs from female donors. Activation of Wnt/β-catenin in female HAVICs reduced calcification (Alizarin Red), osteogenic marker expression, and caspase-1 activity. This study reveals sex-specific molecular programs in HAVIC calcification, with Wnt inhibition potentially shaping the female phenotype, and highlights Wnt/β-catenin as a target for sex-specific CAVD therapies.

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