ABSTRACT

Aortic valve stenosis (AVS) involves the transformation of valvular interstitial cells (VIC) into an osteoblastic phenotype. Such valvular disease is mostly associated with both thickening and calcification of the valve cusps which is accompanied by inflammation and remodeling of the tissue. This process is mediated by the VIC that carry out an impressive array of functions throughout the calcification process. For this dissertation, I ***hypothesized*** that in AVS, VIC transform from a myofibroblast phenotype to osteoblast-like cells and that the canonical Wnt, TGFβ pathways and vitamin D3 interactively and collaboratively contribute to these phenomena. In order to test this hypothesis I established an *in vitro* model of calcification by culturing human primary VIC in a pro-calcification conditioned medium. Calcified cells display several molecular characteristics features of human AVS, including increased levels of alkaline phosphatase and the formation of calcium nodules. These changes increased over time and peaked at 28 days of treatment. To define possible mechanisms of AVS, I first characterized human VIC in regards to the process of calcification. I showed for the first time *in vitro*, that these VIC express bone specific markers, the characteristic of normal osteoblasts. To determine the factors involved in osteoblastic transformation in this model, I examined WNT3A and TGFβ, known to be involved in normal bone formation. Both calcified human aortic valve tissues and VIC express excess WNT3A and TGFβ1. Adding WNT3A and TGFβ1 to the VIC cultures increased the levels of cell mineralization. Further, the addition of DKK1, the WNT3A antagonist, decreased VIC calcification *in vitro*. By using various combinations of WNT3A, TGFB1 and DKK1, I made the novel observation that the suppression of DKK1 by TGFB1 allowed WNT3A to drive calcification in VIC *in vitro*. Finally, I examined the role vitamin D3 that is associated with vascular calcification in rats. Vitamin D3 can up-regulate VIC calcification *in vitro*, however its mechanism of action appears to be independent of the Wnt and TGFβ pathways. In conclusion, the canonical Wnt and TGFβ pathways function interactively through DKK1 to transform VIC to osteoblast-like cells and vitamin D3 promotes this process in an independent manner.