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**Cellular mechanisms of aortic valve calcification in primary human valvular interstitial cell culture**

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**Background:** Aortic stenosis (AS) is the most common acquired valvular heart disease. Calcification of the aortic valve (AV) cusps is the main pathogenetic mechanism of AS formation, however triggering and progression mechanisms of it are not fully understood. Aortic valve interstitial cells (AVIC) are one of the main cell populations responsible for the AV structure and homeostasis.

The aim of the study was to characterize the ability of AVIC to osteogenic differentiation using the model of the primary culture of AVIC of patients with aortic stenosis and assess the expression of genes involved in osteogenesis (RUNX2, BMP2, SPRY1, SOX9, CTNNB1, POSTN, OPG and OPN).

**Materials and methods:** The study was carried out on primary human AVIC culture obtained by enzymatic dissociation of experimental valve specimens from patients with AS (n=15) and the control specimens from the orthotopic heart transplantation recipients (n=10). To assess the osteogenic potential of AVIC, the routine stem cell osteodifferentiation protocol based on the culture medium with addition of inductors (10 mM ß-glycerophosphate, 0.1 μM dexamethasone, and 50 μg/ml ascorbic acid) was used. Calcium deposits were demonstrated by Alizarin Red staining. Analysis of the expression of osteogenic differentiation genes, such as RUNX2 (runtrelated transcription factor 2), BMP2 (bone morphogenetic protein 2), SPRY1 (Sprouty RTK signaling antagonist 1), SOX-9 (SRY-box9), CTNNB1 (ß-catenin1), POSTN (periostin), OPG (osteoprotegerin), and OPN (osteopontin), was performed by real-time PCR.

**Results:** The inductors of osteogenic differentiation provoked greater mineralization of AVIC cultures derived from the patients with AS than that observed in control group (p=0.0003). The expression of RUNX2 and SPRY1 in non-differentiated cells was reduced compared with control cells in patients with bicuspid AV (p = 0.02). After 21 days of the osteogenic induction, the expression of RUNX2 and SPRY1 increased in all three groups. At the same time, the expression of SPRY1 was lower in the group with bicuspid AV compared with tricuspid AV (p = 0.007). The expression level of BMP2 did not differ between groups in unstimulated AVIC, however, it increased after osteogenic differentiation in the group of patients with tricuspid AV (p = 0.017). OPN expression was higher in cells from tricuspid AV, while OPG expression was reduced in patients with both bicuspid and tricuspid AV (p <0.01), No differences were found between the groups for the remaining genes (POSTIN, CTNNB1, SOX9) before and after stimulation with an osteogenic medium.

**Conclusions:** The osteogenic potential of AVIC is increased in patients with aortic stenosis. The gene expression profile of osteogenic differentiation differs in patients with a bicuspid and tricuspid aortic valve. Damage of protective mechanisms may be a potential mechanism for accelerated valve calcification mostly in patients with tricuspid aortic valve.