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Kinetics of Phosphate Induced Calcification and Osteoblast Differentiation in Rat Aortic Valve Cells

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Objectives: Calcific aortic stenosis is a common disease in renal failure patients. The mechanisms leading to this disorder remain unclear. We have previously demonstrated osteoblast-like cell transformation in an in vivo rat model of uremia. In the present study, we used an in vitro system to dissect the mechanisms playing a role in aortic valve interstitial cells (AVICs) calcification in the uremic model.

Methods and results: We identified phosphate as the most efficient inducer of AVICs calcification. This was evident by analyses of mineralization and expression of osteoblast related genes. The mineralization was accompanied by decreased cell viability. Consistently, increased apoptosis was observed by TUNEL staining and activated caspase-3 expression. Comparisons of the kinetics of mineralization (evident at 3 days), osteoblastic gene induction (evident within hours) and apoptotic markers (evident at 6 days) indicate that apoptosis cannot be the trigger of the calcification. The effect of phosphate was abolished by inhibition of its uptake.

Conclusions: Our findings suggest that phosphate is a major determinant in AVICs calcification, furthermore, the induction of osteochondrogenic genes started as early as 2 hours post phosphate treatment, preceding the apoptosis markers and the mineralized nodules. **Key words:** Aortic valve, calcification, phosphate, apoptosis, renal failure.

A Role for MITF in Cardiac Progenitor Cell Proliferation and Differentiation

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Background: There is growing evidence that cardiac progenitor cells (CPCs) exist in the adult heart, but mechanisms of CPC differentiation and proliferation are poorly defined. We recently grew, defined and differentiated CPC's from left atrial appendages of adult mice.

Microphthalmia transcription factor (MITF) is critical in differentiation and proliferation of several cell types, including neural crest and mast cells. We recently showed that MITF is expressed in the adult heart, and has a prominent role in cardiac hypertrophy. Here we explored the potential role of MITF in CPC proliferation and differentiation.

Methods: Cells were grown from atrial appendages of adult wild-type and MITF mutated mice. Cardiomyogenic differentiation was induced by dexamethasone. CPC presence and differentiation capability was investigated by immunostaining with CPC and adult cardiomyocyte markers. MITF transcript was detected using PCR.

Results: CPC's expressed MITF-a instead of the adult MITF-h isoform. Ki-67 staining showed that there was a reduced number of proliferating cells from the MITF mutated mice when compared to wild-type mice. Both wild-type and MITF mutated cells expressed the specific CPC markers Nkx2.5 and Gata-4. Three weeks after dexamethasone treatment, normal cells expressed organized sarcomeric proteins (myosin heavy chain (MHC) and actinin) and atrial natriuretic factor (ANF). In contrast, the expression of MHC and sarcomeric actinin in MITF mutated cells was undetectable, although expression of ANF and Nkx2.5 was not affected.

Conclusion: Our preliminary results demonstrate that MITF is expressed in CPCs, and suggest a novel role for MITF in both CPC proliferation and differentiation.