

## **The Role of Pit-1 in Phosphate Induced Rat Aortic Valve Interstitial Cells Calcification**

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### **Background:**

Cardiovascular disease is the most frequent cause of death in patients with end-stage kidney disease (ESKD). Despite the high prevalence of valvular calcification in ESKD, the pathogenesis of the disorder is still obscure. In this study, we used an in vitro system to dissect the mechanisms of renal failure associated aortic valve calcification (AVC) and to evaluate the role of specific mediators relevant to renal failure associated calcification.

### **Methods:**

Aortic valve Interstitial cells (ICs) were isolated from valve leaflets of Sprague–Dawley rats. In order to evaluate the specific mediator of calcification, ICs were incubated with different mediators- Phosphate 3.5 mM or PTH 50ng/ml or adenine 10 $\mu$ M.

Mineralization of the cells was identified after 7 day qualitatively by von-Kossa staining and quantitatively by measuring calcium levels using cresolphthalein method. Osteoblast related proteins: osteocalcin, osteopontin and Runx-2 were evaluated after phosphate treatment using immunostaining, real time PCR and western blot analysis. To further specify the role of phosphate in AVC, The cells were pretreated either with foscarnet a phosphate transporter inhibitor or by silencing the pit-1 (phosphate receptor) gene.

### **Results:**

We identified phosphate as the most efficient inducer of AVICs calcification; this was evident by analyses of positive von-Kossa staining, increased in Ca quantification and upregulation of osteoblast related genes (osteopontin, osteocalcin and Runx-2). Foscarnet treatment abolished the phosphate effect, while silencing pit-1 decreased Ca quantification and staining compared to the control but it didn't affect Runx-2 and osteocalcin expression.

### **Conclusions:**

Our findings suggest that phosphate is a major determinant in AVICs calcification. Our studies suggest that several cellular pathways in addition to osteoblast transformation pathway are involved in the pathogenesis of phosphate related aortic valve calcification.