Phosphorylating apatite-specific peptide inhibits osteoblast mineralization

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Purpose: Immobilization of multifunctional peptides (containing material- and cell-specific domains) on biomimetically mineralized implants can be used to enhance cell attachment and differentiation, to accelerate bone-implant integration. For this purpose, we used a combinatorial phage display approach to identify peptides with high and specific affinity to biomimetic apatite and discovered the sequence VTKHLNQISQSY (VTK) [1]. To study the effect of post-translational modifications, we phosphorylated the serine residues of VTK (pVTK), and found a 10-fold increase in binding to biomimetic apatite [2]. Adding pVTK to MC3T3 pre-osteoblast cultures also caused a dose-dependent inhibition of mineralization. Understanding the mechanism involved in this inhibition can create new peptide-based drugs to treat pathological calcification. This study investigated the mechanism by which pVTK inhibits mineralization, specifically exploring the role of the mineral influencing proteins tissue non-specific alkaline phosphatase (TNAP) and ectonucleotide pyrophosphatase phosphodiesterase1 (Enpp1).

Methods: MC3T3 cells were seeded at 10,000 cells/cm^2 and cultured for up to 12 days in growth medium, osteogenic medium only (growth medium supplemented with β-glycerophosphate and ascorbic acid) or osteogenic medium supplemented with 300uM pVTK peptide. Matrix deposition and mineralization were measured using Picrosirius Red staining and von Kossa staining respectively. Enzyme activity of Enpp1 and TNAP were measured colorimetrically from cell lysates (normalized to DNA content). qRT-PCR was used to assess gene expression of Enpp1 and TNAP.

Results and discussion: Treatment of MC3T3 cultures with 300uM pVTK peptide caused inhibition of mineralization compared to non-treated controls (Fig. 1A). Mineral inhibition is not due to insufficient matrix deposition (Fig. 1B); rather treatment with peptide results in significantly higher amounts of collagen.

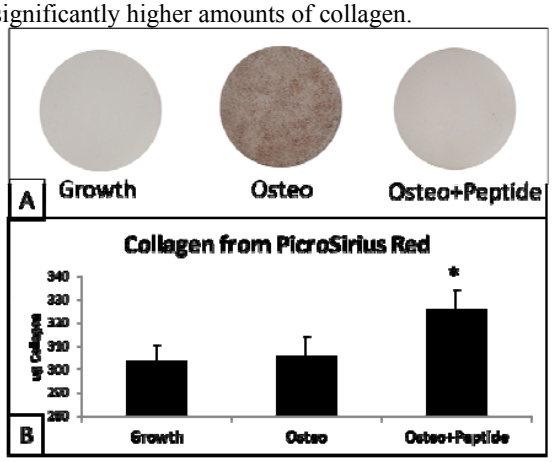

Fig. 1: (A) von Kossa staining shows inhibition of mineralization with peptide treatment (B) Quantification of destained Picrosirius; * indicates statistical significance compared to all other groups (P<0.05)

The enzymes TNAP and Enpp1 regulate osteoblast mineralization by influencing the ratios of inorganic phosphate (Pi) and pyrophosphate (PPi). Enpp1 generates extracellular PPi, inhibiting mineralization, while TNAP cleaves inhibitory PPi to generate Pi and promote mineralization (Fig. 2). To study the effect of pVTK on these molecules, Enpp1 and TNAP protein and gene expression were measured after peptide treatment. TNAP protein levels are not affected, while gene expression was significantly higher with peptide treatment. Enpp1 was significantly inhibited with 12 days of peptide treatment, at both protein and gene levels (Fig. 3).

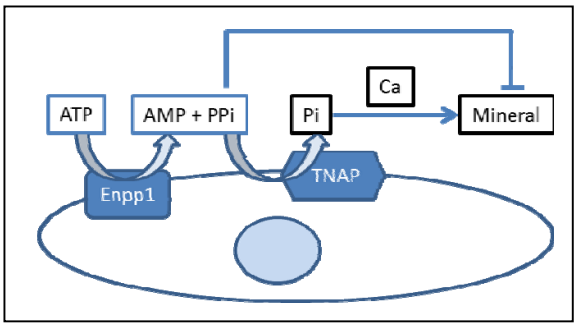

Fig. 2: Schematic showing roles of TNAP and Enpp1 in promotion and inhibition of mineralization

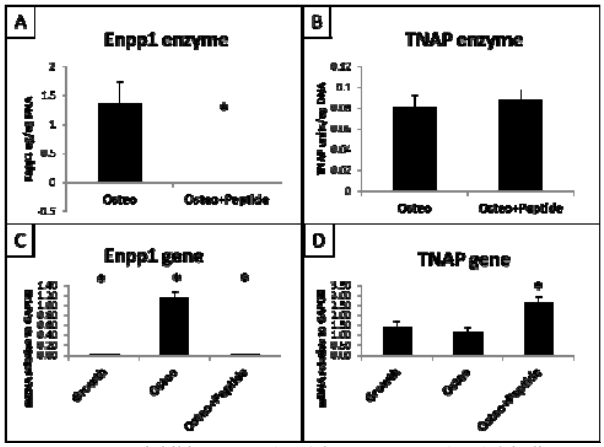

Fig. 3: pVTK inhibits Enpp1 (A,C) but not TNAP (B,D) * indicates statistical significance compared to all other groups in each graph (P<0.05)

Conclusions: pVTK treatment inhibited MC3T3 mineralization without affecting the quantity of collagen matrix deposition. Inhibition appears to occur via disrupting the mineralization-influencing enzyme Enpp1. Further studies are required to understand the complete cellular mechanisms of inhibition. Based on its mineral-inhibiting properties, the pVTK peptide can potentially be used in biomaterial delivery systems to treat pathological calcification of blood vessels and cardiac valves or heterotopic ossification of bone.

References: [1] Segvich SJ. Biomat. 2009;30 1287-1298, [2] Addison WN. Biomat. 2010: 31 9422-9430