Changes in BMP and TGF-β1 Signaling in MC3T3-E1 Cells Challenged with Polymethylmethacrylate Particles

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Introduction: Implant loosening may result from the biological effects of prosthetic wear debris on osteo-progenitor cells. However, the molecular mechanisms of particle-induced inhibition of osteogenic differentiation have not been elucidated. Two members of the transforming growth factor-beta (TGF-β) superfamily are of interest because of their involvement in osteoblast differentiation: the bone morphogenetic proteins (BMPs) and TGF-β1. TGF-β1 is a member of the TGF-β superfamily that is also involved in the induction of osteoblast differentiation. In this study, we demonstrate that MC3T3-E1 cells subjected to polymethylmetha-crylate(PMMA) particle challenge demonstrate changes in the expression of genes in the BMP and TGF-β1 signaling pathways with concurrent inhibition of osteoblast differentiation markers.

Methods: ***Cell cultures*** **-** MC3T3-E1 subclone 14 preosteoblast cells were grown to confluency in ascorbic acid-free α-MEM. To induce osteogenesis, cells were grown in osteogenic α-MEM with 10% FBS, 50 μg/mL ascorbic acid, and 10mM β-glycerophosphate. ***Alkaline Phosphatase -*** Cells were lysed and vortexed. Alkaline phosphatase activity of was assayed using Quantichrome Alkaline Phosphatase Assay Kit. ***Von Kossa Staining -*** Cells were fixed with formaldehyde solution and rinsed with dH2O. Mineralized nodules were visualized by incubating the cells in 5% silver nitrate solution under UV light. NIH Image 1.62f was used to measure the area of von Kossa staining. ***RT-PCR -*** Cells were lysed with Trizol Reagent and RNA was purified with RNeasy Kit. RNA was converted to cDNA using High Capacity cDNA Reverse Transcription Kit. RNA expression levels were quantified with Osteogenesis PCR Array.

Results: MC3T3-E1 cells were grown for 4 days or 8 days with or without 0.30% v/v PMMA particle challenge. Particle challenge significantly downregulated expression of TGF-β1 1.80-fold on day 4, and by day 8 downregulation was not seen(Fig. 1). Neither the expression of TGF-β1's receptors nor its downstream effector Smad2 were significantly affected by particle challenge(Fig. 1). The expression of Runx2, which is a downstream effector of both TGF-β1 and the osteogenic BMPs, was significantly downregulated 2.05-fold on day 4 of particle challenge and no longer downregulated by day 8(Fig. 1). The difference in Runx2 expression between days 4 and 8 was significant (Fig. 1).

Expression of BMP2 was not significantly affected by day 4; by day 8, expression was downregulated significantly by 5.15-fold (Fig. 2). Contrastingly, BMP4 demonstrated a significant 1.73-fold downregulation on day 4 and was not significantly affected on day 8 (Fig. 2). BMP1, an important regulator of the osteogenic BMPs 2 and 4, was significantly downregulated 4.25-fold by day 4(Fig. 2). The expression of two negative regulators of osteogenesis, BMP3 and Sost, were shown to have similar patterns of expression with particle challenge. Both showed significant upgregulation on day 4 and then significant downregulation by day 8(Fig. 2).

Particle challenge also caused significantly reduced alkaline phosphatase activity on days 1, 4, and 8 of particle challenge and also significantly reduced mineralization in a dose-dependent manner(Fig. 3).

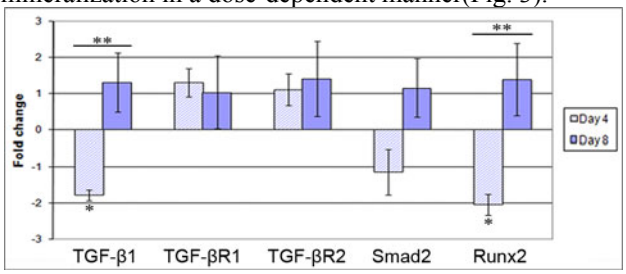

Figure 1

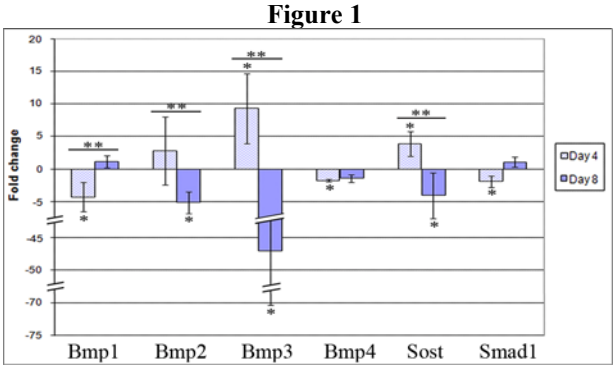

Figure 2

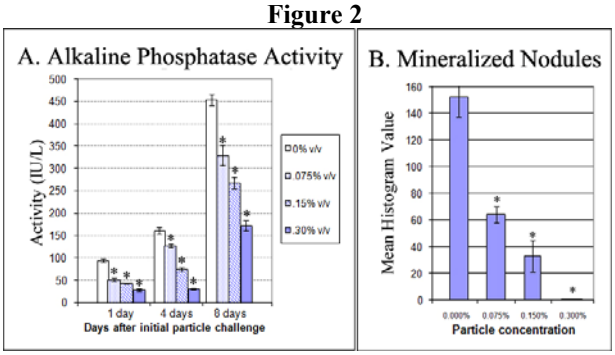

Figure 3

Discussion: This study has demonstrated that challenging differentiating osteoblasts with PMMA particles causes changes in the TGF-β1and BMP signaling pathways. TGF-β1 expression is significantly downregulated by day 4 of particle challenge while its receptors and downstream effector Smad2 are not significantly affected. The most striking change with the BMP signaling pathway is the dynamic change in expression of BMP3 and SOST, which are negative regulators of osteoblast differentiation; respective expression of each gene is upregulated on day 4 and then downregulated on day 8. Further studies manipulating these signaling pathways to restore them to a differentiating state free of particles could lead to therapies to counteract the effects of wear particles.

Acknowledgements: This study was funded by Stanford SOM Medical Scholars Research Fund and the Ellenburg Chair in Orthopaedic Surgery.