

NF- κ B Decoy Oligo-deoxynucleotide Decreases Polyethylene Particle-induced Suppression of Osteogenesis in Bone Marrow Derived Mesenchymal Stem Cells

Lin, Tzu-hua¹, Yao, Zhenyu¹, Barcay, Katherine R.¹, Waters, Heather¹, Sato, Taishi¹, Yang, Fan^{1,2}, Goodman, Stuart B.^{1,2}

¹. Department of Orthopaedic Surgery, Stanford University, Stanford, CA, USA. ². Department of Bioengineering, Stanford University, Stanford, CA, USA.

Statement of Purpose: Chronic inflammation induced by wear particles from the bearing surfaces of total joint replacements (TJRs) is crucial for the development of periprosthetic osteolysis. Modulation of the key transcription factor NF- κ B in macrophages could potentially mitigate so called “particle disease”. Using a novel murine particle infusion model, we previously showed that continuous delivery of UHMWPE particles attracts osteoprogenitor cells, which could potentially reverse particle-induced peri-prosthetic osteolysis. However, the potential adverse effects of modulating the NF- κ B signaling pathway on osteoprogenitors and mesenchymal stem cells (MSCs) remain unknown. The purpose of this study is to define the effects of NF- κ B decoy oligo-deoxynucleotide (ODN), which interferes with NF- κ B transcription, on osteoprogenitor cells.

Methods: Mouse bone marrow MSCs were harvested from male C57BL-6J, 6-8 weeks of age. The characteristics of the isolated MSCs were confirmed by flow cytometry analysis of surface markers, and cells at passage 4-7 were used. Human MSCs from males, aged 20-40 years were purchased from Lonza. Decoy NF- κ B ODN was used to modulate NF- κ B activity. UHMWPE particles (0.48 \pm 0.10 μ m) were obtained from joint stimulation test samples provided by Dr. Tim Wright at the Hospital of Special Surgery. MSC cultures were treated with various combinations of UHMWPE particles, lipopolysaccharide (LPS, 0.1 μ g/ml), decoy and scrambled ODN (0.5 μ M), and appropriate controls, and analyzed for cell characteristics, viability, and cytokine (including TGF- β 1) expression. Cell differentiation and osteogenesis were determined by measuring alkaline phosphatase activity, osteocalcin expression, and Von Kossa staining. The experiments were performed at least in triplicate, and repeated twice independently. Statistical analysis was performed using Graph-Pad Prism. The animal protocol was approved by the Stanford University Animal Care Committee.

Results: UHMWPE particles, LPS, and decoy ODN alone had no significant effects on MSC viability after 48 hrs of treatment. The combination of particles plus LPS reduced MSC cell viability to 36.6% (p<0.005); the reduction was to 58.7% (p<0.05) when combined with decoy NF- κ B ODN treatment. In human MSCs, UHMWPE particles alone reduced cell viability to 20.8%, whereas the viability with decoy NF- κ B ODN treated cells was 83.2%. TGF- β 1 protein expression by MSCs was enhanced by exposure to particles (7.3 fold, p<0.05) for 24 hrs. Decoy NF- κ B ODN increased TGF- β 1 expression in MSCs 2.1 fold (p<0.005). Importantly,

mouse MSCs exposed to UHMWPE particles significantly reduced the osteogenesis differentiation ability, including alkaline phosphatase, osteocalcin, and cell mineralization (Von Kossa staining) (Fig.1). Decoy ODN increased mineralization of murine MSCs exposed to UHMWPE particles. Furthermore, decoy ODN increased osteoprotegrin (OPG) expression (an antagonist of RANKL) in mouse MSCs exposed to UHMWPE particles.

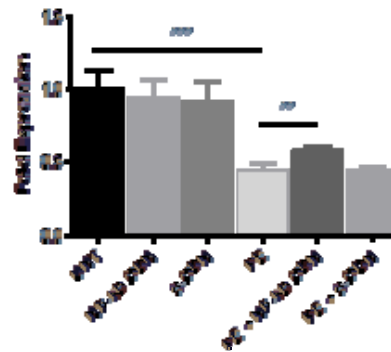


Fig.1 NF- κ B decoy ODN mitigates the suppression of osteogenic differentiation by murine MSCs exposed to UHMWPE particles. Primary mouse MSCs were exposed to particles and cultured in osteogenic differentiation medium for 21 days. Cellular mineralization was evaluated by Von Kossa staining and quantified by ImageJ software.

** p < 0.01; *** p < 0.005. PE=UHMWPE particles

UNT=untreated; S-ODN=scrambled ODN

Discussion: If NF- κ B inhibitors are to be used clinically to limit chronic inflammation and periprosthetic osteolysis, their efficacy and safety must be established. The efficacy of NF- κ B decoy ODN on wear particle induced inflammation has previously been demonstrated using macrophage cell culture experiments. Modulation of wear particle induced inflammation by NF- κ B decoy ODN may further mitigate peri-prosthetic osteolysis by enhancing MSC viability and facilitating the production of OPG. TGF- β can increase OPG expression, while NF- κ B signaling can negatively regulate the TGF- β pathway. Modulation of wear particle induced peri-prosthetic osteolysis by NF- κ B decoy ODN could potentially mitigate the inflammatory reaction to wear particles and facilitate the viability and osteogenic ability of MSCs.

Acknowledgement: This work was supported by NIH grants 2R01AR055650, 1R01AR063717 and the Ellenburg Chair in Surgery at Stanford University.