NF-κB Decoy Oligodeoxynucleotide Enhanced Osteogenesis in Mesenchymal Stem Cells Exposed to Polyethylene Particle

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Introduction: Total joint replacement is a highly successful surgical procedure for end-stage arthritis. However, the high revision rate after surgery remains a major concern. Biomaterial-induced tissue responses may lead to chronic inflammation and local bone destruction (periprosthetic osteolysis). Modulation of the key transcription factor NF-κB in immune cells could potentially mitigate the osteolytic process. The potential effects of NF-κB targeting therapy on other important cells must be considered, especially mesenchymal stem cells (MSCs) and osteoprogenitors that are important to bone remodeling and are involved in particle induced osteolysis.

Methods: Mouse bone marrow MSCs were harvested from male C57BL-6J at 6-8 weeks of age. Human MSCs were purchased from Lonza (healthy male donors, aged 20-40). Decoy NF-κB oligodeoxynucleotide (ODN) was used to modulate NF-κB activity. Ultra-high molecular weight polyethylene (UHMWPE) particles (0.48±0.10μm) were obtained from joint stimulation test samples provided by Dr. Tim Wright from the Hospital of Special Surgery. The MSCs treated with various combinations of UHMWPE particles, decoy ODN (0.5μM), lipopolysaccharide (100 ng/ml) and appropriate controls were analyzed for their cell viability, apoptosis, and osteogenic differentiation ability. 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: The numbers of viable cells were reduced to 42.9±3.4% (without LPS) and 38.5±4.8% (with 1μg/ml LPS) when cells were exposed to UHMWPE particles compared to the untreated controls; this reduction was reversed by decoy ODN treatment. MSCs exposed to UHMWPE particles caused 11.0% (without LPS) or 10.1% (with 1μg/ml LPS) of cell apoptosis. Transforming growth factor β1 (TGFβ1) secretion was increased by NF-κB decoy ODN treatment in MSCs (24.79±4.07pg/ml) compared to the untreated controls (11.96±1.77pg/ml). The induction persisted when the cells were exposed to UHMWPE particles but at significantly higher levels (decoy ODN: 64.66±3.79pg/ml; UHMWPE only: 110.39±1.77pg/ml). Osteoprotegerin secretion was increased by NF-κB decoy ODN treatment in MSCs (4302.96±681.6pg/ml) compared to the untreated controls (2685.36±309.23pg/ml), but it did not reach statistical significance. MSCs exposed to UHMWPE particles had significantly lower OPG expression (124.26±2.03pg/ml), while NF-κB ODN treatment enhanced the expression level (543.77±58.36pg/ml). Mechanistic studies using a TGFβ1 receptor kinase inhibitor showed that decoy ODN up-regulated osteoprotegerin expression through a TGF-β1 dependent pathway. In an osteogenesis assay, alkaline phosphatase (ALP) activity by MSCs (day 14) was reduced from 95.97±4.09IU/L to 9.13±3.35IU/L when
the cells were exposed to UHMWPE particles. Decoy ODN treatment enhanced the ALP activity to 34.08±9.24IU/L in UHMWPE particle-exposed cultures. Runx2 and osteopontin RNA expression at day 14 was reduced by either decoy ODN alone or particle exposure. Osteopontin but not Runx2 expression was increased by decoy ODN in the presence of particles. Bone mineralization (Alizarin red stain) of MSCs at day 21 was reduced 58.7±2.0% by particle exposure, and decoy ODN reversed the reduction. Decoy ODN also increased bone mineralization in MSCs with no particles by 32.6±5.5%. Similar phenotypes of cell viability and osteogenic differentiation in the presence of particles and decoy ODN were also confirmed using human MSCs.

**Discussion:** Our results suggest that suppression of NF-κB activity by decoy ODN may protect MSCs from the adverse effects of wear particle exposure via 3 distinct pathways including 1) suppressing the immune response by induction of TGF-β1 expression, 2) enhancing osteogenesis signaling and cell viability, and 3) suppressing osteoclastogenesis by enhancing osteoprotegerin expression. The strategic use of NF-κB ODN could potentially mitigate wear particle-induced peri-prosthetic osteolysis.

**Significance:** Modulation of wear particle induced inflammation by NF-κB decoy ODN had no adverse effects on MSCs, and may potentially further mitigate peri-prosthetic osteolysis by protecting MSC viability and osteogenic ability.

**Figure 1** NF-κB decoy ODN treatment increased mouse MSC viable cell amounts in response to UHMWPE particles. The viable cell amounts were quantified by lactate dehydrogenase activity assay. UNT: untreated control; S-ODN: scrambled ODN. *p< .05, ***p<.005.
Figure 2 NF-κB decoy ODN increased osteogenic ability in mouse MSCs exposed to UHMWPE. Bone mineralization of the cells was fixed and assayed by Alizarin red stain. The cells were de-stained and quantified by reading at O.D. 562 nm. UNT: untreated control; S-ODN: scrambled ODN. ** p < .01, *** p < .005.