Ti Particles Suppresses Osteoblast Function By Regulating Wnt/bmp Signaling Activity Through Mapk Activation

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Introduction: Biomaterials are materials intended to use in the human body to replace, augment or interact with the living tissues for improving function of the body. But, unfortunately the current biomaterials used in bone tissues tend to loosen over time. Accumulation of prosthetic implant wear in total joint arthroplasty patients is widely recognized as the major initiating event in development of periprosthetic osteolysis and aseptic loosening. Wear debris at prosthetic joint articulations either can stimulate immune cells to secrete mediators of bone resorption like TNFα and IL-1 or can contribute to osteolysis by inhibiting bone formation.

Osteoprogenitors present along with macrophages at the implant interface may play significant roles in bone regeneration and implant osteointegration. Development of osteoprogenitor cells to osteoblast are tightly regulated by bone forming signals like WNT and BMP signaling. As there is no effective protocol for preventing bone loss during periprosthetic osteolysis, the present study was undertaken to elucidate the role of osteoprogenitors in impaired bone formation as a function of their altered intracellular signaling pathways.

Methods: 1) Titanium particles were prepared by sterilization and were counted on microscope. MC3T3-E1 murine pre-osteoblast cells were treated with Ti particles (diameter 1~3 μm) with various cells to particle ratio. 2) MTT and LDH (lactate dehydrogenase) assay was carried out to evaluate the viabilities of MC3T3-E1cells to Ti particles. 3) Changes of expression of RUNX 2, Osterix, COL1A1 and Osteocalcin were analyzed using real-time RT-PCR, and transcripts levels were normalized to GAPDH levels. 4) For alkaline phosphatase activity (ALP), cell lysate was mixed with CSPD substrate and the luminescence was detected on a luminometer. 5) To detect β-catenin and BMP signaling activity, MC3T3-E1 cells were transiently transfected with β-catenin (TOPFLASH) and BRE- signaling reporter construct and luciferase activity was measured after 24 hrs of Ti treatment. 6) After stimulation with Ti particles cell lysates was collected and western blot was performed to detect activation of signaling molecules. 7) Ti particles were treated to the cells with or without specific inhibitors of JNK, ERK and NFκB signaling pathways and cell lysates was collected for western blot, ALP activity and TOPFLASH or BRE- reporter assays.

Results: 1. Ti-challenged MC3T3-E1 cells showed no significant effect on cell viability and cytotoxicity at a ratio of 1:60 (cells to particle).
2. Ti particles suppressed early (ALP activity, Runx-2, Osterix) and late (COL1A1, Osteocalcin) differentiation markers.
3. Stimulation of Ti Particles to osteoblast resulted in activation of ERK, JNK and NFkB signaling pathways as an early response.
4. Direct treatment of Ti particles to osteoblasts transfected with TOPFLASH or BRE reporter construct revealed suppressed basal BMP and WNT activity in the treated cells.
5. Inhibition of JNK and ERK signaling pathways partially restored the Ti particle induced suppression of TOPFLASH, BRE reporter and ALP activity.

Discussion: Usually long term usages of implants are associated with their failures in clinical settings. In this study, we observed adverse effect of Ti particles as wear debris on osteoblast differentiation process. Ti particle treated pre-osteoblasts showed decreased osteogenic activity including ALP activity and gene expression levels of Osteocalcin, Osterix, Osteonectin, COL1A1 and Runx2. Moreover, the basal activity of WNT and BMP pathway was suppressed by Ti particles in treated osteoblasts. As an early response to Ti particles, osteoblasts showed activation of ERK, JNK and NFκB signaling pathways. Inhibition of both ERK and JNK signaling pathways by their respective inhibitors showed partial recovery of Ti particle induced suppression of WNT, BMP and ALP activity of MC3T3 E1 cells. The inactivation of bone forming signals in osteoblasts by stimulation of ERK and JNK pathway suggests a possible direct regulatory role of these pathways in decreased bone formation found in particle-induced osteolysis. Further studies focused on intracellular communication between MAPKs and WNT or BMP signaling pathways could provide a clear insight into the signaling mechanism regulating the process of low bone formation in osteolytic condition like periprosthetic osteolysis

Significance: Further studies focused on intracellular communication between MAPKs and WNT or BMP signaling pathways could provide a clear insight into the signaling mechanism regulating the process of low bone formation in osteolytic condition like periprosthetic osteolysis

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Fig. 1. Ti particles impeded ALP activity of MC3T3 E-1 cells in dose and time dependent manner (A), and reduced expression of osteogenic genes such as Osterix, Runx2, COL1A1, and Osteocalcin (B).
**Fig. 2.** Ti particles activated ERK, JNK and NFκB signaling pathways as an early response in MC3T3 E-1 cells (A). Ti particles showed suppressed reporter activity of TOPFLASH and reduced β-catenin stability for WNT signaling. Ti particles also suppressed activity of BRE construct and phosphorylation of smad molecules (B).
Fig. 3. Combinatory inhibition of ERK and JNK signaling pathways partially restored Ti induced suppressed ALP activity in MC3T3 E-1 cells.