Wnt-BMP Cross-Talk Regulates Osteoblastic Differentiation of Human MSCs on Microstructured Surfaces

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Statement of Purpose: Previously we have reported a surface modification that induces osteoblastic differentiation without addition of exogenous molecules or factors using topographical cues on biomaterials. These modifications use micro/nanostructures to regulate important molecules associated with osteoblastic differentiation including alkaline phosphatase specific activity and osteocalcin, and important growth factors for osteoblastic differentiation. Bone formation is a well-orchestrated process that requires the activation and regulation of numerous molecules including bone morphogenetic proteins (BMPs) and Wnts. BMP and Wnt signaling have been shown to be crucial for osteoblastic differentiation and bone induction. Bone formation around implants is less understood and the mechanism of progenitor cell differentiation on these biomaterials is not well explored. It has been reported that BMP and Wnt molecules increase after exposure to specific topographical cues. However, it is not clear if there is a possible cross-talk between Wnt and BMP molecules. Here we examine whether Wnt signaling is involved BMP signaling and if BMP signaling is involved in Wnt signaling in MSC differentiation on microstructured surfaces.

Methods: Human MSCs were cultured in Mesenchymal Basal Growth Media (Lonza) on tissue culture polystyrene (TCPS) or titanium surfaces with different topographies. Smooth titanium (PT) Ra=0.4μm; rough titanium (SLA) Ra=3.4μm; or high-energy rough titanium (modSLA) were used as substrates. Three studies were performed: (1) Time course study after 2, 4, or 6 days of culture. After incubation, gene expression was assessed for Wnt and BMP molecules; (2) Effect of WNT5A knockdown (shWNT5A) or BMP2 knockdown (shBMP2) on MSCs ± exogenous Wnt5a or BMP2 on osteoblast differentiation (alkaline phosphatase activity [ALP], osteocalcin [OCN]) and soluble factors (osteoprotegerin [OPG], VEGF, FGF2) in conditioned media; (3) MSCs treated with Wnt5a or BMP2 and levels of BMP2 and Wnt5a measured in conditioned media. Data are mean±SEM, n=6 cultures/variable (ANOVA, post-hoc Bonferroni's Student's t-test).

Results: Surface roughness increased WNT5A and BMP2 expression at day 4 and 6, while WNT3A and CTNNB expression decreased on rough surfaces at the same time points. Increased WNT5A and BMP2 expression on rough surfaces correlated with increased ITGB1 on the same substrates. WNT5A-silencing and BMP2-silencing abolished markers of osteoblastic differentiation such as alkaline phosphatase activity and osteocalcin levels on rough surfaces. Additionally, silencing WNT5A decreased levels of angiogenic factors VEGF and FGF2 in all surfaces tested. BMP2 silencing decreased levels of VEGF and FGF2 only on rough surfaces. Exogenous Wnt5a treatment of shWNT5A cells rescued all parameters tested, yielding similar levels to control MSCs (Fig. 1A). Addition of BMP2 to shWNT5A cells rescued ALP, OCN (Fig. 1B), BMP2, and partially rescued BMP4 and FGF2 levels to WT control group. Addition of BMP2 to shWNT5A failed to rescue OPG levels. However, addition of exogenous BMP2 to shWNT5A cells decreased dramatically VEGF levels in all surfaces. Exogenous BMP2 to shBMP2 cells rescued ALP, OCN, OPG, BMP4, and FGF2 levels, but failed to rescue VEGF levels. Addition of Wnt5a to shBMP2 cells failed to rescue ALP, OCN, OPG, VEGF, and FGF2 levels, partially rescued BMP4 levels.

BMP2 treatment had no effect on WNT5A or WNT3A expression in MSCs. MSCs treated with Wnt5a increased expression of BMP2 and decreased expression of WNT3A.

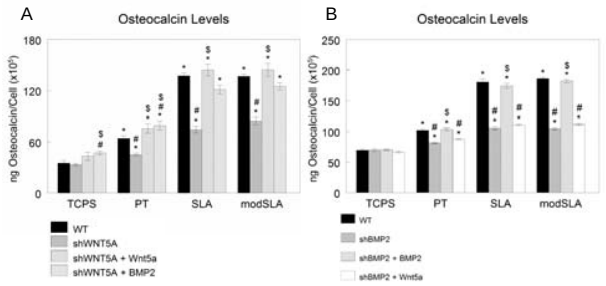

Figure 1. Effect of Wnt5a or BMP2 treatment on osteocalcin production in silenced WNT5A-MSCs (shWNT5A, A) or silenced BMP2-MSCs (shBMP2, B). *p<0.05, vs. TCPS; #p<0.05, vs. WT cells; $p<0.05, vs. silenced cells.

Conclusions: Our data show that BMP and Wnt molecules are regulated by surface micro/nanostructure. WNT5A and BMP2 silenced cells failed to differentiate on microstructured surfaces. Exogenous BMP2 rescued osteoblastic markers in WNT5A silenced cells but exogenous Wnt5a failed to rescue BMP2 silenced cells. Additionally, Wnt5a treatment increased BMP2 expression while BMP2 had no effect on WNT5A expression. Taken together, our results suggest that micro/nanostructure modulates expression of WNT5A and this signaling pathway is needed for BMP2 expression and subsequent osteoblastic differentiation.