Dickkopf-2 (Dkk2) is Required for Osteoblast Terminal Differentiation on Titanium Microstructured Surfaces

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Statement of Purpose: Physical-chemical properties such as micron and sub-micron scale topographies, and surface energy, decrease osteoblast proliferation and increase osteoblast differentiation (osteocalcin, OCN) and local factor production, including TGF-β1, osteoprotegerin (OPG), and VEGF (1). Moreover, cells cultured on these surfaces respond to exogenous regulatory factors in a manner comparable to more differentiated osteoblasts.

While it is clear that cells grown on substrates with rough microtopographies are more differentiated, the surface-dependent factors that contribute to the expression of the differentiated osteoblast phenotype are not well understood. Diverse studies have shown that initial activation of the Wnt/β-catenin pathway induces osteoblast differentiation and up-regulates osteoblast-specific genes; however, down-regulation of the Wnt canonical pathway is needed in order to complete osteoblast terminal differentiation and produce bone-like mineral (2,3). Wnt activity is tightly regulated by several kinds of secreted antagonists, including Dickkopf-1 (Dkk1) and Dickkopf-2 (Dkk2). Expression of Dkk proteins upstream of the Wnt canonical pathway inhibits osteoblast formation. Lately, it has been shown that Dkk2 can act as an agonist or antagonist of the Wnt canonical pathway and the Dkk2 knockout model exhibits an osteopenic phenotype (3). In this study, we hypothesized that surface micro-topography and hydrophilicity modulate Wnt/Dkk2 signaling and Dkk2 is necessary for surface-dependent osteoblast differentiation.

Methods: MG63 cells were grown for 6 days on tissue culture polystyrene (TCPS, plastic) and titanium microstructured surfaces (PT [Ra<0.2μm], SLA [Ra=4μm], modSLA [hydrophilic-SLA]). Changes in mRNA levels were measured by an RNA-microarray and confirmed by real time PCR. Surface effects on Dkk2 protein were measured by ELISA. MG63 cells were transduced with lentivirus particles containing the Dkk2-siRNA template and selected with 0.025 μg/mL of puromycin. MG63 cells and selected Dkk2-silenced (siDkk2) cells were cultured on the test surfaces +/- exogenous Dkk1 and Dkk2 protein. Total cell number, alkaline phosphatase specific activity (ALP), OCN, OPG, TGF-β1, VEGF and Dkk2 protein levels were analyzed.

Results: Rough surfaces (SLA and modSLA) increased the expression (RNA microarray and real time PCR) (data not shown) and Dkk2 levels (ELISA) (Fig 1a). Dkk2 was reduced in siDkk2 cells by 70% in comparison with control cells based on RT-PCR and Western blot using anti-human/anti-mouse Dkk2-antibodies. Validated siDkk2 knockdown cells exhibited lower levels of Dkk2 protein in the conditioned media of cells grown on all surfaces based on an ELISA assay using anti-human antibodies with mouse recombinant Dkk2 protein as the standard. The silenced cells produced less Dkk2 protein and this reduction was surface dependent. These cells also produced less OCN (Fig 1b), OPG, TGF-β1, VEGF and alkaline phosphatase activity than MG63 cells on all surfaces tested, with the greatest effect on the rougher surfaces, particularly on modSLA (not shown). Similar results were observed when MG63 cells were treated with anti-Dkk2 antibodies (Fig 1c). Treatment with exogenous Dkk2 but not Dkk1 partially rescued the differentiated osteoblast phenotype in siDkk2 cells (data not shown).

Conclusions: These results indicate a major role for Dkk2 in late-stage osteoblastic differentiation on microstructured and hydrophilic surfaces, and suggest the importance of Wnt/beta-catenin-signaling and Dkk2 during implant osteointegration in vivo. Increased Dkk2 production on rougher surfaces inhibits Wnt stimulation of the β-catenin pathway, switching cells from proliferation to differentiation.

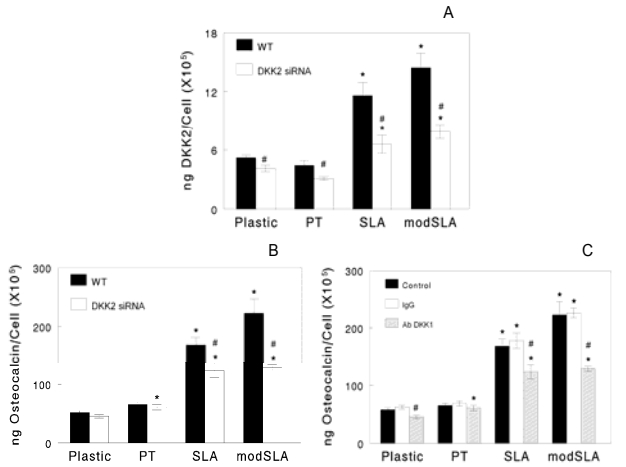

Figure 1. Dkk2 (A) and osteocalcin (B) levels on MG63 and siDkk2 cells cultured on different surfaces. Dkk2 antibody inhibition affected osteocalcin levels on MG63 cells on all surfaces (C).

References:

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