SUPPLEMENT

Materials & Methods

Surface Plasmon Resonance (Biacore) Analysis of Sclerostin-BMP Interactions

Recombinant BMPs were hydrated (100µg/ml) in PBS (pH 7.3) with 1mg/ml RIA grade BSA and 1mg/ml carboxyl-methyl dextran. The running buffer for Biacore analysis was HBS-EP CMD (10mM HEPES, 150mM NaCl, 3mM EDTA, 0.005% polysorbate 20, and 1mg/ml carboxyl-methyl dextran). For kinetic analysis, Biacore CM5 sensor chips were made with 200 and 400 response units (RU) of purified human sclerostin-FLAG using NHS/EDC chemistry, as recommended by the manufacturer (Biacore, Piscataway, NJ). BMPs were diluted in running buffer and injected over the sensor chip using the Biacore 3000 instrumentation. The data was processed using the Bia-evaluation software by first correcting for background binding in a non-functionalized flow cell and analyzing the resulting binding curves for on/off rates.

SOST Expression in Human Osteoclasts

Human osteoclast precursor cells and human osteoclasts were purchased from Cambrex Bioscience. Human osteoclasts were identified as large, multi-nucleated cells that stained histochemically for tartrate-resistant acid phosphatase (TRAP, SIGMA). RNA was prepared from these cells (Invitrogen) and analysed by RT-PCR for SOST and TRAP (ACP5) expression. As a control, RNA was isolated from hMSC cells grown in osteogenic differentiating media to yield osteoblasts. Primer sets crossed intron/exon boundaries to eliminate amplification of genomic DNA or generation of larger size amplicons. All PCR products were verified by sequencing.

PCR Primers:

Human SOST (product size 186nt): Sense: 5’-CCGGAGCTGGAGAACAACAAG-3’, Antisense: 5’-GCACCTGGCCGGGAGCACACC-3’

Human DAD (626nt): Sense: 5’-GCAGTTATGTCGGCGTCGGTA-3’, Antisense: 5’-GTGGCATGGAGTTCTTTAATTTGGA-3’

Human ACP5 (350nt): Sense: 5’-TCGCTCGGACTGTGCAGATC-3’, Antisense: 5’-GCTGCTGGCTGAGGAAGTCA-3’

Effect of Sclerostin in hMSC

To determine the effect of human sclerostin on collagen production, hMSC cells (Cambrex Bioscience, Walkersville, Maryland) cultured in osteogenic media (MSCGM with 100nM dexamethasone, 50µg/ml ascorbic acid, and 10mM β-glycerophosphate (βGP)) and increasing concentrations of sclerostin-FLAG or control protein (purified
proteins from Sf9 conditioned media). Media were collected 8 to 10 days later and analysed for Type I collagen production (Prolagen C ELISA, Quidel, Mountainview, CA).

**Results**

The kinetic parameters of the BMP-sclerostin interaction were characterized using surface plasmon resonance (Biacore). Human sclerostin was coupled to a CM5 sensor chip. Purified recombinant BMP-2, BMP-4, BMP-5, BMP-6, and BMP-7 were injected at various concentrations. The resulting binding curves were used to determine the on and off rates. Under the conditions used in the study, BMPs-2, 4, 5, 6 and 7 exhibited similar binding kinetics and affinities ($K_D = 0.9$ to 3.4nM, see Table 1).

<table>
<thead>
<tr>
<th>BMP</th>
<th>$ka$ (1/s)</th>
<th>$kd$ (1/Ms)</th>
<th>$KD$ (M)</th>
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</thead>
<tbody>
<tr>
<td>BMP-6</td>
<td>6.36E+05</td>
<td>6.12E-04</td>
<td>9.63E-10</td>
</tr>
<tr>
<td>BMP-5</td>
<td>5.37E+05</td>
<td>1.47E-03</td>
<td>3.25E-09</td>
</tr>
<tr>
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<td>BMP-2</td>
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<td>1.71E-03</td>
<td>1.00E-09</td>
</tr>
<tr>
<td>BMP-7</td>
<td>8.65E+05</td>
<td>1.56E-03</td>
<td>1.80E-09</td>
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</tbody>
</table>

**Table 1** – Kinetics of BMP-binding to human sclerostin. Sclerostin exhibited similar binding kinetics for all BMPs tested.

To determine whether osteoclasts expressed $SOST$, we used RT-PCR to look for the expression of the gene in RNA isolated from human osteoclasts and osteoclastic precursors. As can be seen in Figure 2, neither human osteoclasts nor their precursors expressed $SOST$ in contrast to the findings with hMSC cells grown in osteogenic media.

We examined the effect of partially purified preparations of human sclerostin on the production of Type I collagen in hMSC cells grown in osteogenic media. In similar studies, we showed that sclerostin significantly decreased ALP activity and mineralization under these conditions. In Figure 3, we show that sclerostin also significantly reduced the production of Type I collagen in a dose-dependent manner (ANOVA, $p < 0.001$).

**Supplement Figure Legends**

**Figure 1** – BMP-4 competes with BMP-6 binding by Biacore. No significant binding of BMP-4 on a BMP-6 presaturated chip (a bulk shift is seen).
Figure 2 – (A) Human osteoclasts were multi-nucleated cells that stained positive for TRAP. (B) Human osteoclast precursor cells and human osteoclasts did not express SOST (right panel). In contrast, hMSC grown in differentiating media to yield osteoblasts, expressed SOST.

Figure 3 – Sclerostin, but not a control protein preparation, decreased production of Type I collagen in a dose-dependent manner in cultures of hMSC grown in osteogenic media (ANOVA, p < 0.001). Collagen production expressed as mean±SD, % vehicle-treated cells (vehicle = 154 ± 24 ng/ml).