Antagonism Between Bone Morphogenetic Protein and Activin Signaling Pathways in Osteoprogenitor Cells

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Antagonism between Bone Morphogenetic Protein and Activin signaling pathways in osteoprogenitor cells

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Abstract
Osteoporosis is a disease characterized by low bone mineral density due to the rate of bone resorption exceeding that of bone formation. Substantial evidence indicates the Bone Morphogenetic Protein (BMP) pathway promotes bone formation through action of the effectors SMAD1/5/8 while the Activin pathway negatively influences bone mass through action of the effectors SMAD2/3. Recent studies suggest that BMPs and Activins regulate bone mass in a see-saw-like mechanism. Here, we seek to test this hypothesis in vitro via signaling responsiveness assays using pathway specific western blots analyses in the osteogenic murine bone marrow stromal cell line Wi-20-17. We first confirmed that W-20-17 cells exhibit basal activation of SMAD1/5/8 and SMAD2/3 under serum-restricted conditions. Moreover, treatment with Follistatin, which sequesters Activin ligands in the extracellular environment, leads to an increase in BMP pathway activity. To determine the molecular mechanism allowing for this relationship, we treated Wi-20-17 cells with SMAD2/3, which is an intracellular inhibitor of Activin signaling that functions downstream of receptor engagement, and found no effect on BMP pathway activation. In contrast, treatment of Wi-20-17 cells with BMP pathway inhibitor Noggin had no effect on Activin pathway activation despite robust inhibition of BMP signaling. Collectively, our results suggest Activin-mediated repression of BMP signaling in these cells is ligand-dependent but occurs upstream of SMAD2/3 activation. Gene expression analyses indicate that W-20-17 cells express Activin A and its receptors ACVR1 and ACVR2A, and ACVR2B. Given that ACVR1 and ACVR2A also have high affinity for BMP ligands, this raises the possibility that Activin-mediated repression of BMP signaling may occur via competition for a shared pool of receptors. Overexpression studies coupled with activated activity assays are currently underway to examine this hypothesis. Collectively, our work seeks to elucidate the mechanisms that regulate antagonism of BMP and Activin signaling pathways in the osteoblast lineage to identify novel opportunities for treating low bone mass in humans.

Activin inhibition upstream of receptor engagement

Figure 3. Wi-20-17 cells were serum-restricted for twenty-four hours then treated with 250 ng/ml Follistatin for four hours. Western blots were then performed for BMP pathway activation level (phosphorylated SMAD1/5/8 compared to total SMAD1) on Activin pathway activity (BMP, phosphorylated SMAD2/3 compared to total SMAD2). These results indicate that SMAD2/3 treatment does not alleviate Activin-mediated repression of BMP signaling. * indicates p<0.05, n=4 per group.

BMP & Activin Signaling Pathways

Figure 1. The BMP and Activin signaling pathways are initiated by ligand engagement with heteromultimer complexes of type 1 and type 2 receptors in the BMP pathway ALK4/5, 1, and 6 are type 1 receptors, each of which activate the downstream effectors SMAD1, 5, and 8 in response to activation by a type 2 receptor (such as BMPR2). Similarly, Activin ligands induce the type 1 receptors ALK4 or ALK7 to activate SMAD2/3. Notably, ACVR1A and ACVR2B may serve as type 2 receptors for either. We conceptualize the effects of these pathways in the skeleton as a see-saw mechanism, with BMPs generally promoting bone mass accrual and Activin generally promoting bone mass loss.

Activin inhibition downstream of receptor engagement

Figure 4. W-20-17 cells were serum-restricted for twenty-four hours then treated with 10 mM SB431542 for four hours. Western blots were then performed for BMP pathway activation level (phosphorylated SMAD1/5/8 compared to total SMAD1) on Activin pathway activity (BMP, phosphorylated SMAD2/3 compared to total SMAD2). These results indicate SB431542 treatment does not alleviate Activin-mediated repression of BMP signaling. * indicates p<0.05, n=4 per group.

BMP inhibition upstream of receptor engagement

Figure 2. Wi-20-17 murine bone marrow stromal cells were serum-restricted for twenty-four hours then treated with 250 ng/ml Noggin for four hours. Western blots were then performed for BMP pathway activation level (phosphorylated SMAD1/5/8 compared to total SMAD1), and Activin pathway activation level (phosphorylated SMAD2/3 compared to total SMAD2), with beta-actin acting as a control. These results indicate that Noggin treatment had no effect on Activin pathway activation despite robust inhibition of BMP signaling. n=3 per group.

Current Work 1: Transduction/Overexpression

Figure 5. A. Optimization of lentiviral transduction method for Wi-20-17 cells. Fluorescent micrographs from Wi-20-17 cells transduced with GFP lentivirus (right) compared to non-transduced control (left). B. Wi-20-17 cells were treated with a lentivirus carrying cDNA encoding C-terminal V5-tagged human ACVR2A (ACVR2A-V5) or GFP (as a negative control). Cells were selected using puromycin and overexpression confirmed by western blot analysis. Western blots (left, ACVR2A, right) show confirming expression of a V5-tagged gene for ACVR2A in transduced Wi-20-17 cells compared to the beta-actin control.

Current Work 2: Inhibition of specific Activin ligands

Figure 6 (left). RT-PCR analysis of Activin subunit expression in Wi-20-17 cells after twenty-four hours of serum restriction. The presence of inhibin and activin provides the possibility that Wi-20-17 cells synthesize Activin A and B. Negative controls “RT” and “NTC” lack reverse transcriptase and template, respectively.

Working Interpretations

ACVR2A/B modulates signals for the TGF-beta superfamily of ligands, including BMP and Activin. Activin has been shown to counteract BMPs that signal through the ACVR2A/B receptors; however, Activin has not been shown to counteract BMPs that signal through BMP2.

Noggin is a BMP-specific antagonist protein, which, upon addition to the Wi-20-17 cells resulted in a decrease in phosphorylation of SMAD1/5/8 and no effect on the phosphorylation of SMAD2/3. Here we observed that BMP inhibition upstream of the receptor does not impact SMAD-2/5 phosphorylation. Such also supports the signaling competency of W-20-17 cells at the basal level, as seen in the control group.

Follistatin (FKT) is an Activin binding protein, which, upon addition to the Wi-20-17 cells resulted in an increase in the phosphorylation of SMAD1/5/8. Here we observed that BMP inhibition upstream of the receptor allows for the upregulation of BMP signaling. SB431542 is an intracellular inhibitor of Activin signaling, which upon addition to the Wi-20-17 cells resulted in a loss of SMAD2/3 phosphorylation.

Collectively, our data suggest that Activin-mediated repression of BMP signaling is ligand-dependent but occurs upstream of ACVR2A/ACVR2B expression.

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