Elucidating the antagonistic relationship between Bone Morphogenetic Protein and Activin signaling pathways in osteoprogenitor cells

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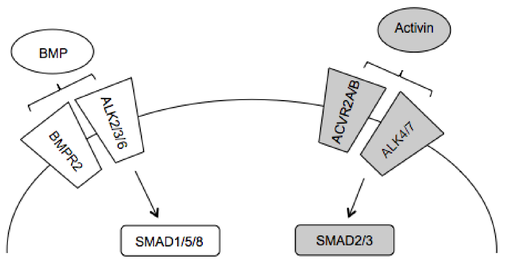
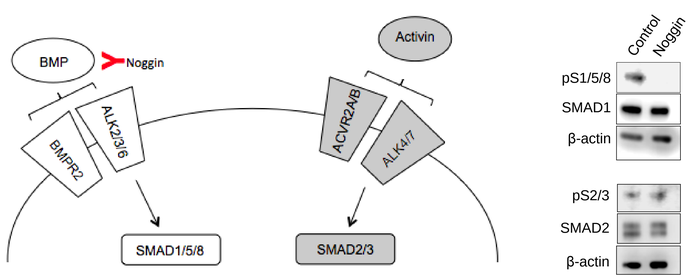
Abstract

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 from our lab suggest that BMP and Activin ligands regulate bone mass in a see-saw-like mechanism via competition for a shared pool of receptors, *i.e.* receptor-level competition. In the present study we seek to test this hypothesis in vitro via signaling responsiveness assays using pathway-specific western blot analyses in the osteogenic cell line W-20-17. We first confirmed that W-20-17 cells respond to exogenous stimulation by BMP2 and Activin-A. Then, we administered recombinant versions of naturally-occurring extracellular ligand traps for BMP2 or Activin ligands (Noggin and Follistatin, respectively) to examine basal antagonism between these pathways. This revealed that, under basal conditions, SMAD1/5/8 activation is repressed by Activin signaling; interestingly, the converse relationship was not observed. To determine the molecular mechanism allowing for this relationship, we treated W-20-17 cells with SB431542, which is an intracellular inhibitor of Activin signaling that functions downstream of receptor engagement, and found no effect on SMAD1/5/8 activation. Collectively, our results suggest Activin-mediated repression of BMP signaling is ligand-dependent but occurs upstream of SMAD2/3 activation.

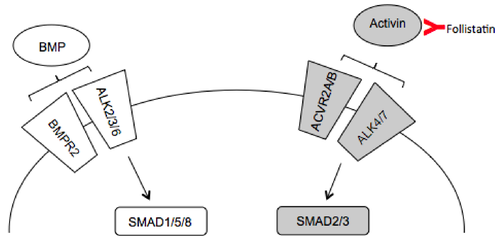
BMP & Activin Signaling Pathways

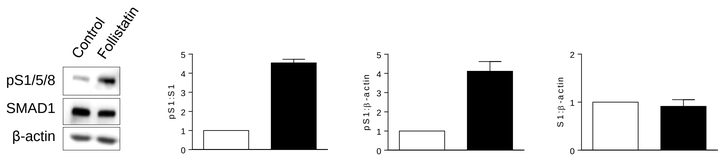


BMP inhibition upstream of receptor engagement

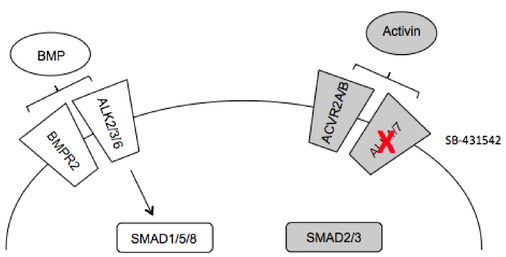


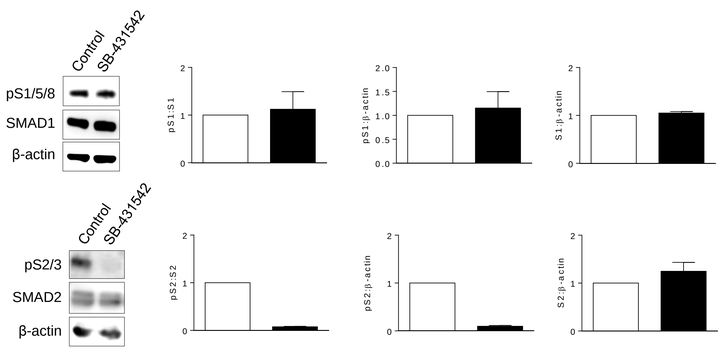
Activin inhibition upstream of receptor engagement





Activin inhibition downstream of receptor engagement





Working interpretations

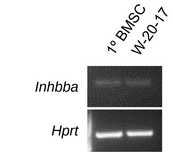
ACVR2A/B modulates signals for the TGF-beta superfamily of ligands, including BMP and Activin. Activin has been shown to counteract BMPs that signal through the ACVR2A/B receptors, however, Activin has not been shown to counteract BMPs that signal through BMPR2.

Noggin is a BMP-specific antagonist protein, which upon addition to the W-20-17 cells resulted in a decrease in phosphorylation of SMAD 1/5/8 and no effect on the phosphorylation of SMAD 2/3. Here we observed that BMP inhibition upstream of the receptor does not impact SMAD 2/3 phosphorylation. Such also supports the signaling competency of W-20-17 cells at the basal level, as seen in the control group.

Follistatin (FST) is an Activin binding protein, which upon addition to the W-20-17 cells resulted in an increase in the phosphorylation of SMAD 1/5/8. Here we observed that Activin inhibition upstream of the receptor allows for the upregulation of BMP signaling. SB-431542 is an intracellular inhibitor of Activin signaling, which upon addition to the W-20-17 cells resulted in a loss of SMAD 2/3 phosphorylation.

Collectively, our data suggest that Activin mediated repression of BMP signaling is *ligand dependent but occurs upstream of effector activation.*

Future direction: Inhibition of specific Activin subunits



Hypothesis
Sequestering individual Activin subunits will mimic the effect of Follistatin on W-20-17 cells.

Methods
Identify which Activin subunits are endogenously expressed by W-20-17 cells. Then, deliver neutralizing antibodies against these subunits and compare to pan-Activin inhibition by Follistatin.

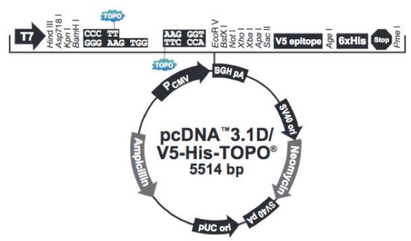
Predicted Results
We predict that delivery of anti-Activin-A antibody will mimic the effect of Follistatin in W-2-17 cells and lead to increased phosphorylation of SMAD1/5/8.

Future direction: ACVR2B overexpression

Hypothesis
Increasing the expression level of ACVR2B (which is shared by BMP and Activin ligands) will alleviate Activin-mediated inhibition of BMP signal transduction.

Methods
cDNA encoding *hACVR2B* will delivered to W-20-17 cells. The expression vector contains an C-terminal V5 epitope tag and overexpression of hACVR2B will be confirmed by western blot. Subsequently, control and hACVR2B-overexpressing W-20-17 cells will be treated with Follistatin and .

Predicted Results
We predict that overexpression of hACVR2B will cause Follistatin treatment to no longer increase phosphorylation of SMAD1/5/8 in W20 cells.



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