Activin and BMP Signaling Mechanics in Myogenic Cells

Timothy Cho and Jonathan W. Lowery

Division of Biomedical Science, Marian University College of Osteopathic Medicine, Indianapolis, IN, USA

The Activin and BMP signaling pathways exert reciprocal effects on myogenesis and skeletal muscle regeneration after injury. Signal transduction in both pathways is mediated by ligand-induced activation of transcriptional regulators called SMAD proteins, with Activins leading to phosphorylation of SMAD2/3 and BMPs leading to phosphorylation of SMAD1/5/8. These ligands bind to their own type I receptors but can also compete for shared type II receptors (ACVR2A and/or ACVR2B). However, it is unclear how these pathways interact in skeletal muscle progenitor cells. To address this deficiency, we investigated the effects of the ligands BMP2 and Activin-A on C2C12 cells, which are an immortalized mouse myoblast cell line with myogenic potential, using a sequence of pre-treatment and co-treatment assays followed by western blot analyses to examine the activation level of their respective SMAD proteins. As expected, treatment with exogenous BMP2 or Activin-A led to phosphorylation of SMAD1/5/8 and SMAD2/3, respectively. Moreover, pre-treatment of C2C12 cells with Activin-A before BMP2 delivery resulted in an attenuated phosphorylation of SMAD1/5/8; this effect seems to be specific to Activin ligands, though, since the converse relationship was not observed with respect to phosphorylation of SMAD2/3. Similarly, attenuated phosphorylation of SMAD 1/5/8 was also observed when Activin-A and BMP2 were delivered to C2C12 cells simultaneously; this co-treatment scenario results in a lower level of SMAD1/5/8 phosphorylation indicating competition for receptor binding between the two ligands. Ongoing experiments seek to elucidate the mechanism(s) impacting the cellular responses under sequential versus simultaneous activation by Activin and BMP ligands. A better understanding of these mechanisms could lead to novel therapeutic strategies for regeneration of skeletal muscle tissue after injury.

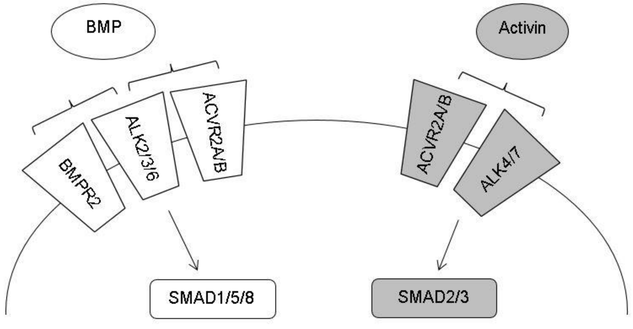


Figure 1: Signaling schematic of BMP and Activin signaling pathways. Type I receptors, ALK2/3/6 and ALK4/7 are specific to BMP and Activin binding, respectively. ACVR2A/B, a type II receptor, is shared between the two ligands. Binding of the type II receptor causes dimerization of the receptors and results in phosphorylation of respective SMAD proteins.

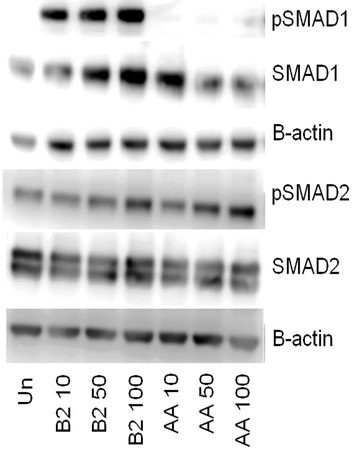
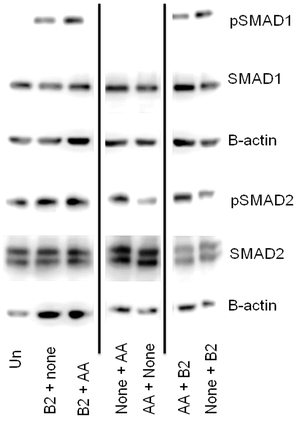


Figure 2: Single treatment assay of C2C12 cells with BMP2 and Activin-A ligands. Increasing doses of each ligand concentration (from 10 ng/ml, 50 ng/ml, and 100 ng/ml) were introduced. B-actin, total SMAD1, and total SMAD2 serve as loading controls.

Figure 3: Pre-treatment assays of C2C12 cells with BMP2 and Activin-A ligands. Max doses (100 ng/ml) of each ligand were introduced: first ligand (for 5 minutes) and second ligand (for 30 minutes). B-actin, total SMAD1, and total SMAD2 serve as loading controls.



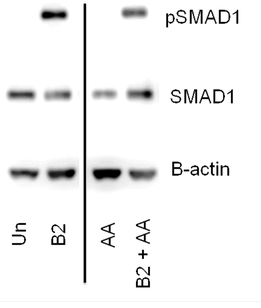


Figure 4: Co-treatment assay of C2C12 cells with BMP2 and Activin-A ligands. Max doses (100 ng/ml) of each ligand were introduced. B-actin serves as loading control.

Conclusions:

- Treatment with exogenous BMP2 or Activin-A led to phosphorylation of SMAD1/5/8 and SMAD2/3, respectively.
- Pre-treatment with Activin-A before BMP2 delivery resulted in attenuated phosphorylation of SMAD1/5/8; converse relationship not observed with respect to phosphorylation of SMAD2/3
- Co-treatment with Activin-A and BMP2 resulted in attenuated phosphorylation of SMAD1/5/8 indicating competition for receptor-binding

Future studies:

- Repeat current study to verify the indicated results
- Introduce myogenic assays to assess the functional capacity for myogenesis in C2C12 cells

For a video presentation of this poster and to join the conversation:

http://bit.ly/2nPBTHS