BMP signaling in skeletal muscle and bone

Shi, S.T.

Summary

In the studies described in this thesis we aimed to investigate BMP signaling in skeletal muscle cells and osteoblast differentiation. The presented research work can be divided into three different sections. In the first section, we investigated the role of BMP signaling in endothelial to osteoblast transdifferentaition as related to a bone disease *i.e.* Fibrodysplasia Ossificans Progressiva (FOP). The occurrence of FOP has been linked to the mutations in BMP type I receptor ALK2, The mutation endows constitutive activity to this receptor, and thereby result in elevated BMP signaling in FOP patients. Our key findings for this part are:

a. BMP signaling induces osteoblast differentiation in mouse embryonic endothelial cells (MEECs).
b. The presence of TGFβ signaling sensitizes MEECs to BMP-induced osteoblast differentiation.
c. *Snail* is induced in MEECs by TGFβ, and enhances BMP-induced osteoblast differentiation in MEECs.

The second part mainly focused on the effect of BMPs on myoblast differentiation and their involvement in the muscle degenerative disease Duchenne Muscular Dystrophy (DMD). Our key findings in this part are:

a. Canonical Wnt signaling induces *Id3* expression.
b. Wnt3a induced *Id3* expression does not depend on BMP activation.
c. *Id3* is an effector of canonical Wnt activity on myoblast

proliferation and osteoblast differentiation in C2C12 myoblast cells.

d. BMP signaling represses myoblast differentiation. BMP-Smad activity is repressed during myoblast differentiation into myotubes. The expression of BMP antagonist Noggin increases during differentiation.
e. The BMP inhibitors, LDN-193189, Dorsomorphin, and Noggin, promote myoblast differentiation into myotubes in both C_2C_{12} and human primary myoblasts.
f. Overexpression of Noggin improves muscle histology as characterized by decreased necrotic area in the limb muscle and enhanced expression of muscle regeneration markers.

From the above results, we may hypothesize that repression of BMP signaling might be beneficial to counteract progression of diseases discussed in the above sections. Therefore the main research focus in the last section is on the characterization of the effects of different BMP inhibitors. We have investigated the interplay between Noggin and BMPs. In addition, we have developed an antisense oligonucleotide (AON) to decrease BMP receptor activity. Our key findings in this part are:

a. Both BMP6 and BMP7 induce Noggin expression. On the contrary, Noggin only represses BMP7 activity, but has only modest effect on BMP6 activity.
b. A crucial amino acid was identified in BMP6 which endows BMP6 resistance to Noggin inhibition. It is possible to make BMP2 and BMP7 resistant to Noggin inhibition by amino

acid substitution,

c. To be able to use exon skipping technology as an alternative and potentially more specific way of inhibiting BMP activity. We have developed an AON that specifically targets ALK2 and decreases ALK2 expression in a diverse set of cells. The ALK2 AON successfully reduces BMP-induced osteoblast differentiation.