Bone Morphogenetic Protein (BMP) signalling is a positive regulator of skeletal muscle mass

C.E. Winbanks,[1] J.L. Chen,[1,2,6] H. Qian,[1] Y. Liu,[1] B.C. Bernardo,[1] C. Beyer,[1] K.I. Watt,[1] R.E. Thomson,[1] T. Connor,[3] B.J. Turner,[4] J.R. McMullen,[1] L. Larsson,[5] S.L. McGee,[3] C.A. Harrison[2,6] and P. Gregorevic,[1,6]
[1]Division of Cell Signaling and Metabolism, Baker IDI Heart and Diabetes Institute, Melbourne, VIC 3004, Australia, [2]Prince Henry's Institute of Medical Research, Clayton, VIC 3168, Australia, [3]School of Medicine, Deakin University, Geelong, VIC 3217, Australia, [4]Florey Institute of Neuroscience & Mental Health, The University of Melbourne, VIC 3010, Australia, [5]Department of Neuroscience, Uppsala University Hospital, 75185 Uppsala, Sweden and [6]Department of Biochemistry and Molecular Biology, Monash University, VIC 3800, Australia.

The Transforming Growth Factor–β (TGF-β) signalling network regulates skeletal muscle development and adaptation. Myostatin, activin and other TGF-β ligands stimulate intracellular Smad2/3 signalling to repress muscle growth, and promote catabolism (McPherron *et al.*, 1997; Zhou *et al.*, 2010). However, a role in muscle for the parallel signalling pathway comprising bone morphogenetic proteins (BMP), BMP receptors and the regulatory Smad proteins 1, 5 and 8 has not been defined. Phosphorylation of Smad1/5/8 by BMP-stimulated receptors promotes complex formation with Smad4 and nuclear retention where, in cooperation with transcriptional co-regulators, they govern gene expression in a cell and context-dependent manner (Massagué *et al.*, 2005). As a means to test the role of this pathway in skeletal muscle growth and wasting, recombinant adeno-associated viral (rAAV) vectors were administered to male C57Bl/6 mice. Mice anaesthetised using isoflurane were administered a single intramuscular injection of either rAAV:BMP6, rAAV:ALK3 or rAAV:Smad6 into the tibialis anterior muscle. For denervation experiments, mice were subjected to isoflurane anaesthesia, and a 1 mm portion of the peroneal nerve that supplies the tibialis anterior muscle was excised prior to injection with the listed rAAV vectors.

We show for the first time that BMP signalling positively regulates muscle mass. Phosphorylation of Smad1/5 *via* the expression of BMP7 or ALK3 in murine muscles stimulated myofibre hypertrophy *via* increased protein anabolism, but hypertrophy was prevented *via* Smad1/5 inhibition. Blockade of mTOR-dependent anabolic signalling prevented BMP-mediated hypertrophy despite increased Smad1/5 phosphorylation, indicating that BMP-mediated muscle hypertrophy involves recruitment of mTOR-dependent processes. Our data demonstrate that Smad1/5 phosphorylation was elevated in conditions of wasting associated with disruption of the neuromuscular junction (NMJ) and that the BMP axis is stimulated in muscles following disruption of the NMJ as a mechanism to minimise muscle atrophy. Accordingly, administration of Smad6 as a means to specifically block the phosphorylation of Smad1/5 in denervated muscles exacerbated muscle atrophy, whereas increased phosphorylation of Smad1/5 was protective. Moreover, our data demonstrate that BMP signalling regulates the HDAC4-myogenin axis, an established promoter of skeletal muscle wasting in denervation induced wasting. Combined, our studies demonstrate a new role for BMP signalling as a positive regulator of skeletal muscle mass (Moresi *et al.*, 2010). Interventions that stimulate BMP-Smad1/5 signalling may have potential to ameliorate the pathology of muscle wasting in neuromuscular disorders and other conditions associated with disruption of the NMJ.

McPherron AC, Lawler AM & Lee SJ.(1997) *Nature* **387**, 83-90.
Massagué J, Seoane J & Wotton D.(2005) **19**, 2783-810.
Moresi V, Williams AH, Meadows E, Flynn JM, Potthoff MJ, McAnally J, Shelton JM, Backs J, Klein WH, Richardson JA, Bassel-Duby R & Olson EN.(2010) *Cell* **143**, 35-45.
Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q, Rosenfeld R, Chen Q, Boone T, Simonet WS, Lacey DL, Goldberg AL & Han HQ.(2010) *Cell* **142**, 531-43.