Modulating bone morphogenic protein signalling in cancer cachexia

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Cancer cachexia is characterized by profound loss of lean and fat mass, resulting in whole body weakness. Debilitating frailty increases morbidity, ultimately accounting for 1 in 3 advanced cancer deaths (Fearon, 2011). Preventing muscle wasting can increase the lifespan of cachectic mice independently of tumor growth (Zhou *et al.*, 2010). The cellular mechanisms driving muscle atrophy in cachexia remain to be fully elucidated. Recent studies have demonstrated that Bone Morphogenic Protein (BMP) signalling is a key regulator of skeletal muscle mass (Sartori *et al.*, 2013, Winbanks *et al.*, 2013). We sought to investigate the role of BMP signalling in the setting of cachexia.

All *in vivo* experiments were approved by the AMREP Animal Ethics Committee in accordance with the current code of practice for the use and care of animals for scientific purposes (NHMRC). All surgical procedures were conducted under inhalation of isoflurane with post-operative analgesia. 7 week old, male, Balb/c mice were subcutaneously implanted with tumour pieces derived from a C26 colon carcinoma. Animals developed a progressive cachexia associated with a loss of lean and fat mass. Mice were administered (by intramuscular injection) adeno-associated viral vectors (AAV) encoding constructs designed to modulate BMP signalling. At endpoint, animals were anaesthetized with tribromoethanol (300mg/kg I.P injection) to facilitate terminal blood collection.

We observed diminished phosphorylation of Smad1/5/8 protein (a key BMP effector) in cachectic muscle. Disruption of BMP-Smad1/5/8 signalling displayed evidence of perturbed muscle function and was associated with increased expression of the E3 ubiquitin ligase MUSA1, previously implicated in mediating proteolysis in muscle. Expression of BMP7 by AAV was sufficient to reduce skeletal muscle atrophy in tumor baring mice.

Our data demonstrate that BMP signalling and dependent mechanisms are disrupted in the skeletal muscle of cachectic mice. Additionally, our studies provide evidence to suggest that modulation of BMP signalling may prove beneficial in the setting of cancer cachexia.

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A. Hagg and R. Sartori contributed equally to this study. M. Sandri and P. Gregorevic contributed equally to this study.