The role of Fn14 in mouse skeletal muscle recovery post Notexin injury: Effects on myogenic regulatory factors, catabolic markers, and structural proteins

A. Pascoe,[1] A.J. Johnston,[1] L. Jenkinson,[1] C. van der Poel[2] and R.M. Murphy,[1] [1]La Trobe Institute for Molecular Sciences, La Trobe University, VIC 3086, Australia and [2]School of Human Biosciences, La Trobe University, VIC 3086, Australia.

Muscle wasting is a devastating comorbidity associated with an array of chronic and acute conditions including, but not limited to, diabetes, immobilisation, cancer, ageing, and muscular dystrophies (Jackman and Kandarian, 2004). Maintenance of muscle mass and function is essential for improving health outcomes and quality of life in these individuals.

TNFα-like weak inducer of apoptosis (TWEAK) is an emerging cytokine regulator of muscle homeostasis. TWEAK has been shown to regulate muscle growth, repair, and remodelling *via* interaction with its receptor, fibroblast growth factor-inducible 14 (Fn14) (Bhatnagar and Kumar, 2012).

The current study investigates the effects of two anti-Fn14 antibody variants on the degeneration and recovery of mouse *tibialis anterior* (TA) skeletal muscle following a snake venom (Notexin) induced injury. Notexin solution (40 µl, 10 µg/ml) was injected intramuscularly in the right TA and 40 µl saline injected in the left TA under isoflurane anaesthesia (2-5% flow rate, recovery within 5 minutes). Buprenorphine (0.05mg/kg) was injected subcutaneously as analgesic immediately post-injury, and during recovery as prescribed by the supervising veterinarian.

Mice received 20 mg/kg intraperitoneal injections of either anti-Fn14 A, anti-Fn14 B, or no antibody treatment, 3-4 hours and 7 days post-injury. Mice were culled by CO_2 asphyxiation at days 3, 7, and 14 post injury and both TA muscles collected (n=4 for each treatment and time point).

Tissue architecture was assessed with H+E staining and key myogenic regulatory factors (myogenin and MyoD), catabolic markers (calpain-1 and HSP27), and structural proteins (actin, desmin, and myosin) were measured with semi-quantitative western blotting to establish progression of muscle recovery. Recovery of actin, desmin, and myogenin to baseline were all significantly delayed in antibody B treated mice with pathological scores persistent at 14 days post-injury.

While preliminary results indicate that Fn14 may contribute to mouse skeletal muscle regeneration following Notexin injury, further work is needed to clarify the *in vivo* mechanistic actions of the antibody treatments employed and direct future studies.

Bhatnagar S, Kumar A. (2012). The TWEAK-Fn14 system: breaking the silence of cytokine-induced skeletal muscle wasting. *Curr Mol Med* **12**, 3-13.

Jackman RW, Kandarian SC. (2004). The molecular basis of skeletal muscle atrophy. *Am J Physiol Cell Physiol* **287**, C834-43.