Serum miRNAs as potential biomarkers for assessing skeletal muscle regeneration

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Skeletal muscles can be injured by myriad insults that compromise their functional capacity. Regenerative processes are often slow and incomplete, and so developing novel therapeutic strategies to enhance muscle regeneration represents an important research area. One limitation for the field is that in order to accurately determine the regenerative status of muscles, the current approach is to take a muscle biopsy, which is invasive, can be painful, may act as a potential source of infection and, in patients suffering from myopathies, causes damage to already-compromised muscles. microRNAs (miRNAs) are small non-protein coding RNAs that act as regulators of gene expression by binding to the 3' untranslated regions (3'-UTR) of messenger RNA (mRNA) and either repressing transcription of or degrading the mRNA (Baggish *et al.*, 2011; Taylor *et al.*, 2012). It has recently been discovered that miRNAs are stably secreted into the bloodstream, and are detectable in both serum and plasma (Mitchell *et al.*, 2008). Serum miRNAs have been proposed as circulating biomarkers for a number of diseases, and recent studies have also found that miRNA expression in serum is altered following exercise (Baggish *et al.*, 2011; Uhlemann *et al.*, 2012) or in muscle pathologies such as Duchenne muscular dystrophy (Cacchiarelli *et al.*, 2011). The aim of this study was to examine miRNA expression in blood serum during muscle regeneration after myotoxic injury, in order to assess its potential as a biomarker for assessing muscle repair.

Male C57BL/6 mice (8-9 weeks, n = 24) were used in these experiments. Mice were anaesthetised with 4% isoflurane at 2 L/min such that they were unresponsive to tail or toe pinch, and anaesthesia was maintained with 1-2% isoflurane at 0.5 L/min. The tibialis anterior (TA) muscle of the right hindlimb was injected with cardiotoxin (50 µl of 10 µM solution, *i.m.*) to cause complete muscle fibre degeneration, and mice were injected with Carprofen (5 mg/kg, *i.p.*) as an analgesic. Mice were allowed to recover for 7, 14 or 21 days, after which they were anaesthetised deeply (60 mg/kg, sodium pentobarbital, *i.p.*) and blood samples were obtained by cardiac puncture. After blood samples were obtained mice were killed by cardiac excision while still anaesthetised and muscles were excised and stored at -80°C for further analysis. miRNA was isolated from serum as previously described (Taylor *et al.*, 2012) and transcribed to cDNA using a miScript® II RT kit (Qiagen). miRNA levels were quantified using a miScript SYBR® Green PCR Kit with mScript Primer Assays (Qiagen).

We found that a number of miRNAs (including miRNA-206, miRNA-133a, miRNA-212 & let-7f) were detectable in serum samples from mice during recovery from myotoxic injury. Furthermore, there were significant changes in serum miRNA expression during regeneration. These data suggest that analysis of a cohort of 'signature' serum miRNAs may have potential as a relatively fast and less invasive alternative to muscle biopsies for examining the regenerative status of skeletal muscles, allowing easier assessment of the effectiveness of treatments in patients recovering from muscle injury or suffering from muscle disease.

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