Serum miRNAs are unsuitable for use as biomarkers for assessing skeletal muscle regeneration in a commonly used mouse model of myotoxic injury

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Skeletal muscles can be injured by myriad insults that compromise their functional capacity, and developing novel therapeutic strategies to enhance muscle regeneration represents an important research area. One limitation for the field is that in order to make a definitive determination of the regenerative state of muscle post-injury, either a muscle biopsy (in the case of human and larger laboratory animals) or post-mortem removal of the muscle (in the case of mouse and rat models of injury) is required for biochemical and histological analysis. This poses a problem for rodent models in particular as it prevents monitoring of muscle regeneration over time in the same animal, while also using large numbers of experimental animals. It is therefore of great interest, both from a technical and an ethical point of view, to develop more accurate non-terminal methods of assessing muscle regeneration in rodent models. microRNAs (miRNAs) have appeal as biomarkers as they are relatively stable when released into the bloodstream and are detectable in both serum and plasma (Mitchell *et al.*, 2008). While there is a growing body of evidence that miRNAs are viable biomarkers for use in cases of whole-body muscle pathology and damage to large muscle groups in humans, the ability to detect muscle damage and/or regeneration in a smaller experimental model of muscle injury (*i.e.* myotoxic damage to a single muscle in mice) has not been investigated. The aim of this study was to examine miRNA expression in blood serum following an experimentally induced myotoxic injury in mice, and to test the hypothesis that serum miRNAs could be used as biomarkers to assess muscle regeneration in this experimental model.

Male C57BL/6 mice (8-9 weeks, *n*=36) were used in these experiments. Mice were anaesthetized 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 (50µl of 10µM solution, *i.m.*) to cause complete muscle fibre degeneration, and mice were injected with Carprofen (5mg/kg, *i.p.*) as an analgesic. Mice were allowed to recover for 2, 5, 7, 14 or 21 days, after which they were anaesthetized 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 anaesthetized and muscles were excised and stored at -80°C. Total RNA (including miRNA) was isolated from serum as previously described (Taylor *et al.*, 2012) and miRNA was isolated from muscle samples using using a miRNeasy® RNA extraction kit (Qiagen, Doncaster, VIC, Australia), according to manufacturer's instructions. miRNA was transcribed to cDNA using a miScript® II RT kit (Qiagen) and miRNA expression was quantified using a miScript SYBR® Green PCR Kit with mScript Primer Assays (Qiagen).

We found that, while expression of a number of miRNAs was significantly altered in regenerating TA muscle, there was little change in expression of circulating serum miRNAs, nor was there significant correlation between serum expression and muscle miRNA expression. The inability to detect changes in circulating miRNAs is probably due to the relatively small size of the injured muscle, and indicate that circulating miRNAs are not a suitable measurement of muscle regeneration in this experimental model.

Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB & Tewari M. (2008) *Proc Natl Acad Sci USA* **105**, 10513-10518.

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