Intramyocardial delivery of miR-29a improves cardiac function and prevents pathological remodeling following myocardial infarction

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MicroRNAs are endogenous, small, noncoding RNA molecules that regulate the expression of protein-coding genes. They have been reported to impact on numerous biological processes and diseases including cardiovascular physiology and diseases (Hudson & Porrello, 2013). Following myocardial infarction (MI), members of the miR-29 family have been shown to be down-regulated in the region of the heart adjacent to the infarct border and repress the expression of extracellular matrix proteins associated with fibrosis (van Rooij *et al.*, 2008). It has been demonstrated that inhibition of miR-29 *in vivo* induces collagen mRNA expression, and also that over-expression of miR-29 in cultured cardiac fibroblasts reduces collagen expression *in vitro* (van Rooij *et al.*, 2008). Together these studies indicate that miR-29 could be an anti-fibrotic therapy for heart disease, however, the therapeutic potential of miR-29 in the setting of acute myocardial infarction remains unclear as systemic delivery of microRNA mimics has proven challenging. In order to verify whether miR-29 can improve cardiac function and exert anti-fibrotic effects *in vivo*, we developed an intramyocardial injection protocol for microRNA mimic delivery to the infarct border zone following permanent occlusion of the left anterior descending (LAD) coronary artery. Briefly, 8-week-old CD-1 male mice were anaesthetized with a combination of ketamine (50mg/kg, i.p.) and metetomidine (10mg/kg i.p.). Following anaesthesia, mice were intubated and ventilated (Harvard Mini-Vent, Type 845, USA) prior to thoracotomy. An incision was made between the 3rd and 4th intercostal space and the LAD was permanently ligated. Synthetic microRNA mimics (QIAGEN) for either miR-29a or a scrambled control sequence were injected into the infarct border zone at a low dose (0.1mg/kg) using a Hamilton syringe (30 gauge). Mice were euthanized by CO_2 asphyxiation followed by cervical dislocation and infarcted cardiac tissue samples were separated from non-infarcted regions for further analysis. All protocols were approved by the UQ Animal Ethics Committee. Quantitative PCR (qPCR) analysis showed that our intra-cardiac delivery strategy leads to miR-29a up-regulation specifically in the infarct border zone at day 3 post-MI. Additionally, the miR-29 was functional as it down-regulated its target genes, collagen 1 (Col1a1) and collagen 3 (Col3a1).. We next assessed whether these anti-fibrotic affects could improve cardiac function following MI. Cardiac function was assessed by echocardiography in mice injected with miR-29a or the scrambled sequence control mimic at baseline and at 3, 7, 14 and 21 days post-MI. Several cardiac functional parameters including ejection fraction, fractional shortening and left ventricular internal diameter were significantly improved in the miR-29a treatment group. Furthermore, histological sectioning and staining with Masson's trichrome demonstrated a marked reduction in infarct size at day 21 post-MI. Moreover, injection with miR-29a prevented the development of cardiac hypertrophy and associated increases in cell size of cardiomyocytes following MI. Interestingly, isolation of different cell types from adult hearts at Day 3 following intramyocardial delivery of microRNA mimics revealed that miR-29a was specifically increased in cardiomyocytes. The present findings suggest that the anti-fibrotic and cardioprotective effects of miR-29a following myocardial infarction may be mediated through an as yet unidentified mechanism involving cardiomyocytes in the infarct border zone.

Hudson JE, Porrello ER. (2013). The non-coding road towards cardiac regeneration. *J Cardiovasc Transl Res* **6**, 909-923.

van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN. (2008). Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci USA* **105**, 13027-13032.