A comparative transcriptomic analysis of the regenerating neonatal mouse heart

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Heart disease is the leading cause of death in Australia, with a significant proportion of disease burden due to myocardial infarction (MI). MI results in the formation of an intractable fibrotic scar, which contributes to deterioration of heart function and, in many cases, leads to heart failure. In contrast with the adult mammalian heart, recent evidence suggests that neonatal mice are able to fully regenerate following cardiac injury and MI (Porrello *et al.*, 2011; Haubner *et al.*, 2012). To investigate this transient regenerative potential of the neonatal mouse heart, we used a GeneChip Exon 1.0 ST microarray, to determine the differential expression of protein coding and non-coding genes between infarcted mice at postnatal day 1 (MIP1; regenerative) and postnatal day 14 (MIP14; non-regenerative). Surgeries were performed as previously described, with P1 animals anaesthetized by placing on ice for 3-5 minutes prior to surgery and P14 animals anaesthetized by ventilation with 1.5-4% isofluorane (vol.:vol.) (Porrello *et al.*, 2011).

For the first time, we have identified, on a transcriptome-wide level, genes regulated during mammalian cardiac regeneration (178 genes in total). A bioinformatic pipeline, using the MetaCore^{TM} database, identified gene networks and candidate genes specifically regulated following MI at P1 (MIP1). Gene ontology analysis revealed an upregulation of cell cycle genes ($P < 0.0001$) and a downregulation of fibrosis genes ($P < 0.0001$) in MIP1 compared to MIP14. This suggests a requirement for increased cardiomyocyte proliferation and simultaneous prevention of cardiac fibrosis during regeneration. Additionally, several epicardial-enriched genes were specifically upregulated following MI at P1 but not following MI at P14 and real-time quantitative PCR (qPCR) was used to confirm these expression changes. In accordance, activation of the epicardium is known to be essential for zebrafish cardiac regeneration (Lepilina *et al.*, 2006)

Interestingly, 36 long non-coding RNAs (lncRNAs) were differentially expressed at MIP1 compared with sham-operated animals. lncRNAs are non-protein coding transcripts greater than 200 nucleotides in length. Gene set enrichment analysis based on guilt-by-association methodology was performed to implicate MI-regulated lncRNAs in possible biological processes. Several mRNA and lncRNA candidates associated with neonatal heart regeneration are currently being interrogated *in vivo* for their functional roles in the cardiac regenerative response. It is hoped that molecular players identified in this *in vivo* functional screen could begin to elucidate the signalling networks involved in cardiac regeneration.

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