Therapeutic methods for physiological cardiac hypertrophy: The role of MicroRNAs

G. Mazzarino, S. Lamon and G.D. Wadley, School of Exercise and Nutrition Sciences, Faculty of Health , Deakin University, 221 Burwood Hwy, Burwood, VIC 3125, Australia.

Cardiovascular disease (CVD) is the leading cause of death in Australia and worldwide. Exercise protects against CVD by inducing morphological changes to the heart, by way of cardiomyocyte hypertrophy and cardiomyocyte proliferation, which result in sustained or enhanced cardiac function (McMullen and Jennings, 2007). However, the mechanisms by which this occurs are unclear. As such, this study investigates the increasing evidence that cardiac regeneration is influenced by complex molecular mechanisms, largely controlled by microRNAs (miRNAs) (Porrello, 2013). MiRNAs are small, non-coding RNAs that regulate gene expression post transcriptionally, by manipulating their target mRNAs and altering cellular pathways. *In vitro* and *in vivo* studies have demonstrated the regenerative capacity of cardiac cells after manipulating specific miRNAs in the heart (Carè *et al*., 2007, Liu *et al*., 2015, Ramasamy *et al*., 2015, Eulalio *et al*., 2012). Furthermore, various miRNA species are physiologically upregulated in the heart following exercise training (Ramasamy *et al*., 2015). However, there are still many cardiac miRNA species that have not yet been identified, validated and evaluated *in vitro* and *in vivo*.

Based on these findings, the aim of this study was to perform a miRNA Array and bioinformatics analysis on an established rat exercise training model of cardiac hypertrophy, and to identify the miRNA species that are differentially regulated in cardiomyocyte proliferation and growth in this model.

5-week old male Wistar Kyoto rats underwent treadmill running for 5 days per week for 4 weeks. Rats were subsequently killed at 9 weeks of age with an intraperitoneal injection of ilium xylazil (30 mg kg $^{-1}$) and ketamine (225 mg kg $^{-1}$) (Wadley *et al*., 2016). Total RNA was extracted from the left ventricle of hearts with Trizol and DNase on-column digestion. Subsequent quantitative miRNA analyses were performed using Taqman Array Rodent MicroRNA A+B Cards (v3.0) and 7900HT Real-Time PCR. A bioinformatics analysis was conducted to determine miRNA-mRNA interactions for all miRNA species with an expression level that was significantly different between groups (P<0.05). Stringency was set at ‘highly predicted’ and ‘experimentally validated’. The experimental mRNA targets were identified by miRecord and literature search. In response to exercise training, our research team observed a significant increase in left ventricle mass and left ventricle/ body mass ratio (P<0.05) compared to sedentary rats, and a ~40% increase in cardiomyocyte number (P<0.05). Furthermore, our miRNA Array identified 23 miRNA species that were differentially regulated (P<0.05) in the heart following exercise training, compared to sedentary rats. Of these, *miR-10a, miR-150 and miR-210* were upregulated 1.45 fold, 1.32 fold and 1.33 fold, respectively (P<0.05). Furthermore, our bioinformatics analysis identified gene targets *BCL-6* and *MapkIp1*, *c-Myb* and *Vegfa*, and *Nptx-1* and *Efna-3* as mRNA targets for *miR-10a, miR-150 and miR-210*, respectively.

Taken together, these data suggest that *miR-10a, miR-150 and miR-210* may regulate exercise-induced cardiac hypertrophy and cardiomyocyte proliferation. Our future research aims to manipulate these miRNA species in cardiomyocytes in vitro, and to then identify a clinically relevant miRNA delivery method for cardiac-specific miRNAs *in vivo*, which may lead to prolonged stimulation of cardiomyocyte proliferation. Such research may provide a strong rationale for the development of pharmacological therapies that aim to repair damaged cardiac tissue and reduce the burden of CVD.

Carè A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Høydal M, Autore C, Russo MA, Dorn GW 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G. (2007). *Nature Medicine* **13**, 613-618.

Eulalio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, Giacca M. (2012). *Nature*, 492, 376-381.

Liu X, Xiao J, Zhu H, Wei X, Platt C, Damilano F, Xiao C, Bezzerides V, Boström P, Che L, Zhang C, Spiegelman BM, Rosenzweig A. (2015). *Cell Metabolism* **21**, 584-595.

McMullen JR, Jennings GL. (2007). *Clinical & Experimental Pharmacology & Physiology* **34**, 255-262.

Porrello ER. (2013). *Clinical Science* **125**, 151-166.

Ramasamy S, Velmurugan G, Shanmugha Rajan K, Ramprasath T, Kalpana K. (2015). *PLoS ONE* 10, 1-12.

Wadley GD, Laker RC, McConell GK, Wlodek ME. (2016). *Physiol Rep* **4**: pii: e12720. doi: 10.14814/phy2.12720.