Project Title: Roles for microRNAs in compensatory renal hypertrophy?

KHA Grant ID: KHA2018-JG

Interim Progress Report

Background

Worldwide over 2 million people are receiving end stage renal replacement therapy such as dialysis or kidney transplantation. Thus there is a critical need to develop novel therapies that prevent disease development and progression. Kidneys have a natural ability to grow and compensate in healthy individuals, however the key initiators of this response are not known. This study has helped to elucidate the mechanism driving healthy hypertrophy in order to develop targeted therapies to treat kidney disease by increasing the size and compensatory capacity of the kidneys in diseased patients. We have used gene expression array technology (messenger RNA and microRNA) following the removal of one kidney and defined novel changes in gene expression that may drive this compensatory growth. We are now pursuing evidence for the operation of these mechanisms.

Project

Our project is due for completion in December 2020. This is an interim report for the year July 2018 – July 2019. During the year of 2018, cost centre creation and cost centre availability became available in September 2018 for which we were able to start the project. At the same time, we started the process for Human Ethics approval. For reason beyond our control, the process is lengthy and has taken considerable time into 2019 to secure ethics and site specific approval for this study. While human ethics process took their course we began our laboratory studies with animals. Therefore, in the year of July 2018 to July 2019 we developed a mouse model of nephrectomy (removal of left kidney) to conduct miRNA studies post nephrectomy (Figure 1A and B). Firstly, we confirmed similar to that seen in humans, mice undergo a 27% increase in kidney weight /g of body weight. After confirmation and establishment of the model in our laboratory we began animal experiments to collect kidney tissue at various time points post nephrectomy (4h - 168h). Using these samples we assessed total RNA (Fig. 1C) and percentage miRNA content of samples at 24-72h post nephrectomy (Fig. 1D). We found that Total RNA increased within 24h post-nephrectomy, when compared to 4h samples and this seemed to be sustained until 2 weeks (168h) post nephrectomy. Furthermore, we saw significant increases in the percentage of miRNA at 24 and 72h post nephrectomy. During the year of 2018/2019, we also started our Open Array Analysis of 750 miRs by examining the 24h samples (Fig. 1E). We found that only 5 miRs were significantly upregulated, while we observed 1 miR down regulated. Of these, miR-671-5p (down regulated) and miR-363-3p (upregulated) were highly significantly different compared to sham group. Change in the expression of these miRs are currently being validated by quantitative Real-time PCR analysis. Of interest from our results so far is that miR-671-5p is known to target and down regulate the expression of FOXM1[1]. FOXM1 is a key cell cycle transcription factor involved regulating cell cycle gene expression and is interestingly required in proximal tubule proliferation during kidney injury repair[2]. Thus the down regulation of miR-671-5p after the removal of a kidney may support renal hypertrophic growth by allowing FOXM1 expression. This warrants further investigation and we are currently following this up by looking at gene expression changes by RNA-seq. In addition, the upregulation of miR-363-3p may also be an exciting lead as it is known to target p21. It has been previously demonstrated that lack of p21 in a mouse model of chronic renal failure, prevents the progression of chronic kidney disease[3]. Accordingly, we are currently assessing the expression of miRs at other time points by Open Array to identify other key miRs involved in renal hypertrophy. For the remaining time for this project, we intend to use this data to generate a panel of miRs to then examine in the blood and urine of patients undergoing a nephrectomy. We are now in the process of organising collection of human samples to examine whether these changes also occur in humans. Furthermore, we have also started to address aim 2 of this study, where we have managed to conduct preliminary high throughput cell screen optimisation experiments with the Cell Screening Facility at Flinders University using a human renal cell line HK-2. We have defined optimal cell concentrations and use of high throughput viability testing.

During the course of the year our previously written literature review examining Compensatory Renal Hypertrophy has been published, the funding has contributed to the expansion of laboratory personnel and training of two Honours Students and leveraged additional seed funding from Flinders University, College of Medicine and Public Health. This exciting preliminary data will form the basis of an NHMRC Ideas Grant Application in 2020. We are also in the process of writing a manuscript with the data obtained from the miRNA Open Array and RNA-seq Data.

References

1. Tan, X., Fu, Y., Chen, L., Lee, W., Lai, Y., Rezaei, K., Tabbara, S., Latham, P., Teal, C.B., Man, Y.G. and Siegel, R.S., 2016. miR-671-5p inhibits epithelial-to-mesenchymal transition by downregulating FOXM1 expression in breast cancer. *Oncotarget*, *7*(1), p.293.
2. Chang-Panesso, M., Kadyrov, F.F., Lalli, M., Wu, H., Ikeda, S., Kefaloyianni, E., Abdelmageed, M.M., Herrlich, A., Kobayashi, A. and Humphreys, B.D., 2019. FOXM1 drives proximal tubule proliferation during repair from acute ischemic kidney injury. *The Journal of clinical investigation*, *129*(12).
3. Megyesi, J., Price, P.M., Tamayo, E. and Safirstein, R.L., 1999. The lack of a functional p21WAF1/CIP1 gene ameliorates progression to chronic renal failure. *Proceedings of the National Academy of Sciences*, *96*(19), pp.10830-10835.

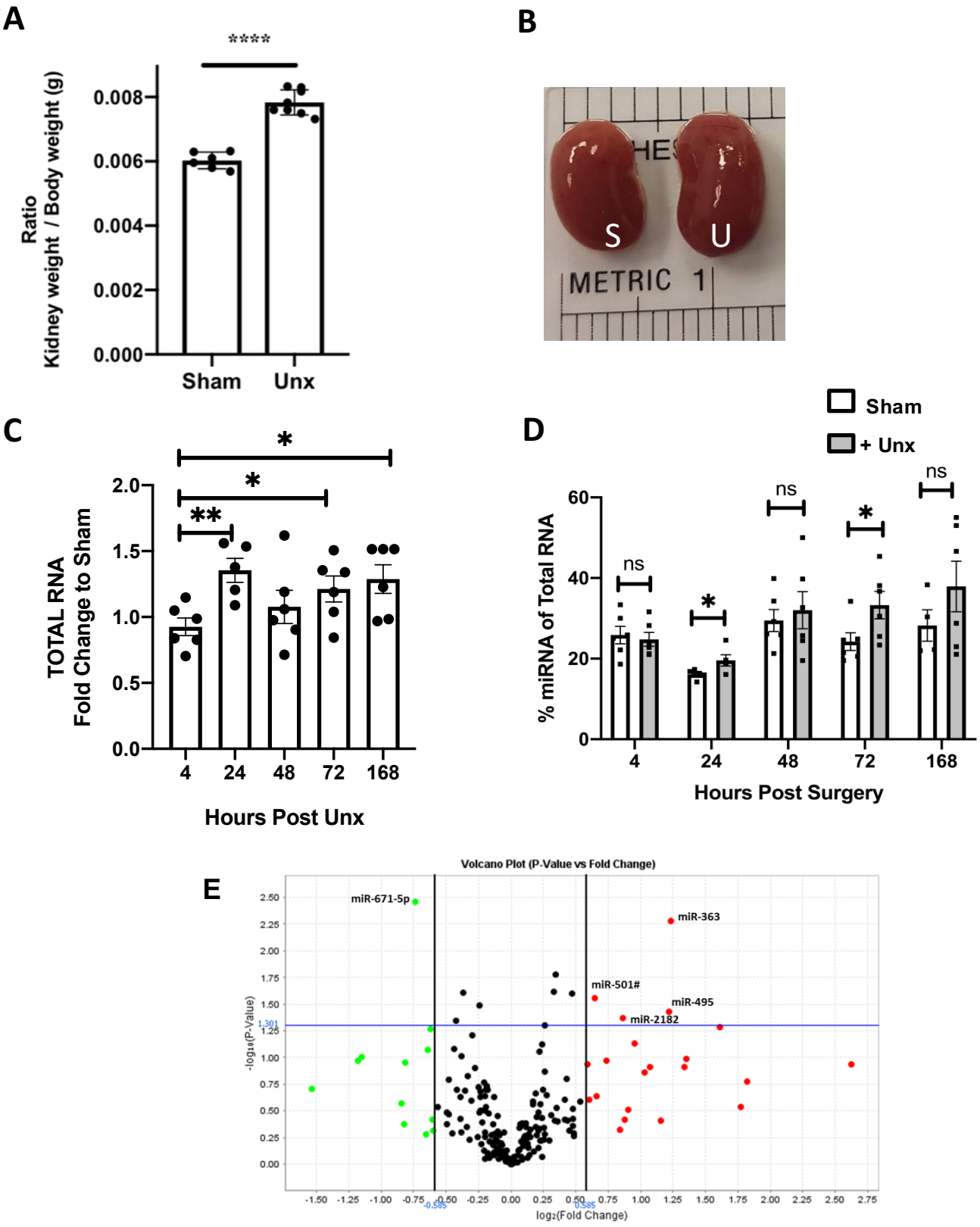

Figure 1 : ***Unilateral nephrectomy (Unx) causes renal hypertrophy in remanent kidney of C57BL6 mice resulting in changes to miRNA expression profile.*** Mice underwent a unilateral nephrectomy, where the left kidney was removed or underwent a sham operation. **A)** After 2 week the remanent kidney (right kidney) was collected and kidney to body weight ratio calculated (n=6-8). **B)** Representative image of right remanent kidney, **S** = sham operation and **U** = nephrectomy. Total RNA **(C)** and miRNA **(D)** was quantified from remanent kidney from mice 4h to 168h (2 weeks) post nephrectomy (Unx) or sham operation (4-6 mice per group). **E)** Total RNA from kidney tissue 24h post nephrectomy was analysed in a high throughput Open Array platform examining the expression of 750 miRNA. Data was normalised to endogenous controls: snoRNA202, snoRNA135 and U87 using the Expression Suite software (v1.0, Thermofisher). **D)** Volcano plot of normalised data, showing P-Value vs fold change to Sham operation controls, where fold change boundaries were set to +/-1.5 and significance level to 0.05 (blue line). *P<0.05, **p<0.01 ****p<0.0001 and NS= no statistical significance based on an unpaired, two-tailed students T-Test. Error bars, +/- SEM.

NOTE: If 2006 has funds brought forward this project commenced under the previous finance system and therefore the project totals will not be correct.

Flinders Uni
40 Inc/Exp for Life by Project
Current Month July 2020

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	2018	*2019*	*2020*	*Total Project Actuals*	*Commitment*
Cost Centre: 523 (Hypertension/Cardiology)					
Project: 42443 (18/20 Kidney Health Australia - J Gleadle)					
Source: 062 (Contracts)					
Old Cost Centre/ Project:					
Funds brought forward					
9298 - Funds Brought Forward	**0**	**25,000**	**4,531**	0	0
Income					
0333 - Foundations Research Grant	25,000	25,000	0	50,000	0
TOTAL INCOME & CARRY FORWARD	25,000	50,000	4,531	50,000	0
Salaries					
Total Salaries	0	0	0	0	0
Non Salaries					
3218 - Facility Charges	0	540	0	540	0
3219 - Laboratory Items	0	18,509	1,150	19,659	0
3220 - Chemicals	0	11,134	0	11,134	0
3221 - Sequencing & Services	0	15,287	0	15,287	0
Total Non Salaries	0	45,469	1,150	46,619	0
TOTAL EXPENDITURE	0	45,469	1,150	46,619	0
BALANCE	25,000	4,531	3,381	3,381	0
COMMITMENTS				0	
BALANCE INCL COMMITMENTS				3,381.02	

Project total correct