

## MICRORNAS IN THE DEVELOPING OVINE KIDNEY

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### INTRODUCTION

The sudden loss of kidney function is known as acute kidney injury (AKI), and is referred to as pediatric AKI or pAKI in the neonate, infant, and child. Characterized clinically by a decrease in kidney filtration that can usually be reversed, as well as the inability of the kidney to appropriately regulate fluid and electrolyte homeostasis [1,2], pAKI can lead to serious complications in kidney function later in life, known as chronic kidney disease (CKD). To date, underlying factors involved in the etiology of pAKI are not known.

MicroRNAs (miRNAs) are a class of functional single stranded non-coding, small ribonucleic acid molecules [3]. These post-transcriptional regulators modify the expression of genes by acting on the mRNA that is transcribed from a gene (or multiple mRNAs), and thus, affect the translation of encoded protein [3,4]. miRNAs have been implicated in the etiology of AKI in adult animals, but their role in pAKI is not known. This preliminary research aims to determine potential regulatory genes targeted by miRNAs in the developing ovine kidney, and to explore whether any of these genes are altered in an experimental model of pAKI.

### METHODS

Animals used were *Ovis aries* (sheep, ~15 to 20 days postnatal, sacrificed). Kidney tissues were harvested and snap frozen. Two tissues were evaluated: AKI [Treated with lipopolysaccharide (0.03 mg/kg) and Indomethacin (1 mg/kg)] and Control (Untreated).

miRNA was extracted from both tissues using the mirVana miRNA Isolation Kit. Samples were sequenced in the ACHRI Genomes Facility. Using MicroCosm Targets genes/miRNAs were evaluated and plotted using Microsoft Excel. After generation of all genes, those with  $\geq 1.5$  fold change (AKI vs. Control) were selected for analyses.

### RESULTS

A total of 103 miRNAs were present in Control and AKI tissue, in which the number of down-regulated genes (n=74) far exceeded the number up-regulated (n=27). A greater number of down-regulated genes exhibited  $\geq 1.5$ -fold,  $\geq 2.0$ -

fold and  $\geq 3.0$  fold changes as compared to up-regulated genes. Oar-miR-380-3p\_st (GGUGGAUAUCCUUCUAUGUUU, fold-change = -2.1521) was found to be down-regulating the expression of cyclo-oxygenase (COX1, COX7C) and nitric oxide (NOX1) genes.

**Table 1.** MicroRNAs measured in ovine renal tissue. The number of genes down-regulated, up-regulated, and unchanged are shown as fold-change in AKI as compared to Control tissue.

Fold change	Down-regulated	Up-regulated	No change
$\geq \pm 3.0$	4	1	0
$\geq \pm 2.0$	9	2	0
$\geq \pm 1.5$	34	5	0
0	0	0	2

### SUMMARY AND CONCLUSIONS

There were notable changes in miRNAs in AKI compared to Control tissue. Substantially fewer up-regulated genes than down-regulated genes, including COX1, COX7C, and NOX1 genes were observed (Table 1). Target genes within the COX and NOX pathways which are known to modulate kidney haemodynamics and function may reveal their altered roles in AKI.

Given the evolutionary conservation of miRNA regulatory circuits [4,5], a subset of candidate miRNAs in ovine tissue could be identified and validated for application to pAKI in future studies. Through the successful application of miRNA analyses to establish regulatory genes in ovine kidney tissue, we may further our understanding of the causes and consequences of pAKI, the deleterious effects of pAKI on kidney function, and the progression from pAKI to CKD.

### REFERENCES

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