

miR-141 Mediates Acute Kidney Injury Recovery

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Background

Acute kidney injury (AKI) is a global clinical problem that places a significant financial burden on the NHS. AKI is characterised by a sudden decline in renal function and mortality as high as 60%. Current AKI biomarkers have limited ability to classify disease and stratify rapidly progressing patients, and the underlying pathological mechanisms are poorly understood. In this study we hypothesised that alterations in urinary microRNA (miRNA) profiles could predict AKI recovery/nonrecovery after 90 days and that injury-specific changes would signify miRNA mediators of AKI pathology. To test this hypothesis we compared urinary miRNAs from recovered and nonrecovered AKI patients with unaffected individuals to identify biomarkers, then manipulated selected miRNA expression in injury models to investigate mechanisms.

Methods

Biomarker Study: Taqman Low Density Array analysis profiled 377 miRNAs in pooled urine samples from recovered (n = 6) and nonrecovered (n = 5) stage III AKI patients. Selected candidate biomarker miRNAs were then analysed by RT-qPCR in our complete patient cohort (n = 30) and controls (n = 10). **Cell Model:** Renal proximal tubular epithelial cells (PTECs) were incubated with 1 mM H₂O₂ for 24 h to induce tubular injury via oxidative stress, and expression of candidate miRNAs was manipulated using miRNA mimics. **miRNA Target Selection:** Computer algorithms predicted messenger (m)RNA targets for candidate miRNAs, and target mRNA expression was knocked down using siRNAs. **Reporter Assay:** Cells were transfected with control Renilla reporter vector plus either our luciferase reporter construct p-miR-Report-PTPRG (protein tyrosine phosphatase receptor type G) containing the PTPRG 3'-untranslated region (UTR) or empty luciferase vector, followed by miRNA mimics or controls. **Animal Model:** Our unilateral ischemia reperfusion injury (IRI) Lewis rat model was used in which the left kidney was clamped for 45 min, animals were then sacrificed 48 h later.

Results

Biomarker Study: Comparison of urinary miRNAs from AKI patients with controls detected significant injury-specific increases in miR-21, miR-126, miR-141 and corresponding decreases in miR-192 and miR-204; miR-141 best predicted nonrecovery. **AKI models:** Expression of miR-141 increased under oxidative stress conditions in vitro and unilateral IRI in vivo. RNA-sequencing confirmed miR-141 upregulation in tubular injury and implicated other miRNAs in AKI pathology. Forced miR-141 expression in the presence of H₂O₂ led to increased PTEC death and decreased cell viability. Analysis in silico identified 9 mRNA targets with 2 or more miR-141 3'-UTR binding sites, expression analysis of these mRNAs in PTECs highlighted PTPRG for further study. Luciferase analysis confirmed PTPRG was a direct miR-141 target, PTPRG siRNA knockdown under oxidative stress increased PTEC death and decreased cell viability.

Conclusion

We have identified association of increased miR-21, miR-126, miR-141 and decreased miR-192 and miR-204 detection with AKI. Forced miR-141 expression in our novel in vitro model caused increased PTEC death and reduced cell viability in tubular injury. PTPRG was shown to be a direct target of miR-141 and siRNA knockdown increased PTEC death and reduced cell viability, identifying PTPRG as a potential target for AKI therapy.