MicroRNAs in Acute Kidney Injury

Timothy F. Jones^{a, b} Soliana Bekele^{a, b} Michael J. O'Dwyer^{a–c}
John R. Prowle^{a, b}

^aWilliam Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; ^bAdult Critical Care Unit, Royal London Hospital, Barts Health NHS Trust, London, UK;

^cDepartment of Anaesthesia, St. Vincent's University Hospital, Dublin, Ireland

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Abstract

Background: It is increasingly recognised that improved diagnosis, prognosis and treatment of acute kidney injury (AKI) requires an understanding of distinct underlying cellular and molecular mechanisms (endotypes) that may distinguish overtly similar clinical AKI presentations. One important avenue of research is the post-transcriptional regulation of gene expression in response to kidney injury mediated by microRNAs. **Summary:** This mini-review summarises the use of microRNAs as diagnostic and prognostic biomarkers in AKI. The contribution of microRNAs to the pathophysiology of AKI will be highlighted along with the potential for therapeutic applications. **Key Messages:** While there is great potential for a better understanding of AKI, microRNAs form a complex regulatory network. Understanding the role and significance of microRNAs in the context of AKI and critical illness is a major endeavour in translational medicine, requiring the integration of clinical and experimental data.

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Introduction

Acute kidney injury (AKI) is a common complication of critical illness and is strongly associated with increased risk of death in hospital. In those who survive, AKI is a major risk factor for the development of subsequent chronic kidney disease, and its associated health and socioeconomic burdens. However, the AKI syndrome, as defined by contemporary consensus criteria, represents a collection of overlapping conditions, initiated by a variety of renal insults, so it remains unclear to what extent there are differing AKI endotypes (distinct underlying functional or pathobiological mechanisms) as opposed to a common set of pathophysiological mechanisms [1]. Over recent years, consideration of differences in the underlying molecular signatures of tumours with superficially

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similar clinical and histopathological manifestations has revolutionised the diagnosis, risk assessment and therapeutic strategies in oncology, and such endotypic classification of clinical diseases or syndromes is now being extended to nonmalignant conditions, such as asthma, as part of the drive to deliver precision medicine. Consequently, the characterisation of the cellular and molecular differences between differing paradigms of AKI is crucial to determine to what extent there are distinct endotypes of AKI and to eventually enable individualised therapeutic approaches [1]. To this end, several recent publications have sought to distinguish AKI endotypes by examining the patterns of transcriptional gene expression in differing AKI settings [2]. However, beyond the level of gene transcription, post-transcriptional regulation of messenger RNA (mRNA) translation also regulates gene expression by regulating the production of the final protein product encoded by the gene, thus playing a key role in determining endotypes and phenotypes in disease states. MicroRNAs are important post-transcriptional regulators [3], which are now being extensively studied in the characterisation of a variety of disease processes, including AKI.

MicroRNA

MicroRNAs are short, non-protein coding RNA molecules between 19 and 25 nucleotides in length, which regulate gene expression at the post-transcriptional level through mRNA signal repression [3]. In excess of 2,500 microRNAs have now been identified in humans [4]. Typically microRNAs bind to mRNA at the 3' untranslated region in a sequence specific fashion and, once bound, silencing is achieved through a combination of direct inhibition of ribosomal translation and acceleration of mRNA degradation [3]. Each microRNA has a "seed sequence" spanning nucleotide positions 2–7 which determines mRNA target specificity. The majority of human protein-encoding mRNA transcripts possess microRNA binding sites and individual mRNAs may contain multiple binding sites for different microRNAs, while due to the relative short length of the seed sequence, each microRNA may target as many as 100 individual mRNAs. The large number of microRNAs and potential mRNA targets and the promiscuous binding of the seed sequences thus imply a complex regulatory network of gene expression. MicroRNAs have been shown to be involved in a wide range of biological processes including cell cycle control, apoptosis, stem cell differentiation, haematopoiesis, hypoxia re-

sponses, metabolism, aging, immunity, viral replication and oncogenesis. Importantly, as microRNAs have highly tissue-specific expression and distinct temporal expression patterns during embryogenesis, they play a key role in the differentiation and maintenance of tissue identity. MicroRNAs are also released from cells and may be present in body fluid within exosomes, micro-vesicles or bound to plasma proteins, making them both potential mediators of intercellular communication and measurable biomarkers of tissue responses to injury and disease. MicroRNAs are readily detected in body fluids [5] and are stable to freeze-thaw cycles; thus, individual microRNAs can be easily measured by quantitative PCR, while next-generation sequencing techniques can be used to investigate the entire human microRNA repertoire within a given sample. Finally, microRNA levels can be manipulated *in vivo* by delivery of either microRNA mimics (to increase activity) or antisense analogues (to bind to and inhibit the action of the endogenous microRNA), providing avenues for experimental investigation and potential for clinical intervention.

MicroRNAs in AKI

As crucial determinants of cellular phenotypes, microRNAs have been extensively studied in a number of clinical and experimental settings as biomarkers of disease states and as ways of differentiating the mechanisms underlying heterogeneous clinical conditions. Due to their involvement in cellular differentiation and regulation of cell cycle, they have been most widely studied in malignancy, as well as in a range of chronic diseases. AKI with its diverse aetiology, complex pathogenesis and uncertain outcomes represents an acute disease process where microRNA profiles could provide valuable insights into underlying mechanism. The importance of microRNAs as a class of regulator in the pathogenesis of AKI was first demonstrated in a transgenic mouse model possessing a renal proximal tubular-specific, genomic deletion of an enzyme (Dicer) that is required to produce microRNAs [6]. This model exhibited a global down-regulation of microRNAs in the renal cortex, and the proximal tubular Dicer knockout mice showed resistance to AKI after ischaemia-reperfusion, with significantly better renal function, less tissue damage, less tubular apoptosis and better survival than in wild-type mice [6]. These findings therefore suggest a net pathogenic role for microRNAs expression in ischaemic AKI. Overall, more than 50 individual microRNAs have now been described as having differen-

tial expression in AKI [7]. In the context of experimental and clinical AKI, many of these microRNA have been associated with molecular mechanisms that promote inflammation, apoptosis and fibrosis; conversely, other AKI-associated microRNAs may act protectively by exerting anti-inflammatory, anti-apoptotic, anti-fibrotic and pro-angiogenic effects. Thus, there are several important considerations when interpreting microRNA patterns in AKI. First, for individual microRNAs, it may be unknown whether the response is a protective physiological response to injury, a bystander to the disease process or a mediator of a pathophysiology, implying that the clinical implications of any differential microRNA expression must be understood in the context of the molecular pathways regulated. Second, type of sample from which microRNA is derived may be crucial, in clinical studies, plasma or urine has been most often studied; however, it is unclear if these are a suitable surrogates for tissue expression in the kidney that currently can be examined only in animal models or in renal biopsies. Finally, given the complexity of microRNA regulation, it may be that a signature of expression of a number of microRNAs may be more important than any individual one.

The difficulty in interpreting diverse microRNA-AKI associations is illustrated in studies performed in human clinical AKI intensive care unit settings (Table 1). While a large number of publications have been generated in a short time, these have been generally small in size and have often compared microRNAs in plasma, serum or urine in critically ill AKI patients compared to healthy control individuals, limiting AKI-specific inferences. In this evolving field, the most convincing mechanistic evidence is derived from a combination of human studies, to demonstrate clinical relevance, and animal models to explore the mechanism and the effect of intervention. For instance, Lan et al. [8] demonstrated that increased urinary miR-494 was associated with AKI and that this rise preceded changes in serum creatinine and urea nitrogen; as there were no differences in serum concentrations of miR-494, renal or urinary tract origin was suggested. The same investigators then used a murine model of ischaemia-reperfusion injury to demonstrate an up-regulation of miR-494 after ischaemia-reperfusion injury, which inhibited the expression of activating transcription factor 3 (ATF3 – a stress-response, reno-protective protein). Finally, the delivery of antisense-miR-494 to mice prior to ischaemia ameliorated renal injury (as measured by serum creatinine), reduced proinflammatory cytokines and caused suppression of caspase-3 apoptotic ac-

tivity, thus establishing a firm rationale for a pathophysiological role of miR-494 in this AKI model [8].

Despite interesting individual results, the current literature remains characterised by the diversity of microRNA responses described and the lack of consistency between studies (Table 1). This may reflect a diversity of AKI endotypes, or it may be a reflection of the limited power of small studies in the context of a complex regulatory network. Even miR-21, which is the most consistently reported association with AKI, is difficult to interpret. While miR-21 has been shown to be raised in response to AKI, lower baseline levels were associated with the risk of AKI prior to cardiac surgery [9]. These findings may reflect a pleiotropy of miR-21 targets, so that miR-21 has the potential not only to protect against injury by inhibiting apoptosis and inflammation but also to amplify injury responses and promote fibrosis in differing contexts.

The role of individual microRNAs may be clearer in better defined paradigms of AKI. For instance, in the setting of cisplatin-induced AKI, an elegant set of recent experiments has shown induction of miR-375 expression in cisplatin-treated renal tubular cells, both in-vivo and in-vitro [10]. In these models, the inhibition of miR-375 decreased cisplatin-induced apoptosis, suggesting that miR-375 is a cell-damaging or pro-apoptotic agent after cisplatin exposure. Important pathogenic pathways known to be involved in acute renal tubular injury including p53 expression (indirectly) and nuclear factor- κ B activation (directly) were shown to promote miR-375 expression after cisplatin exposure. Induced miR-375 then in turn inhibited hepatocyte nuclear factor-1 β protein expression, reducing its endogenous cytoprotective activity [10]. It is this kind of in-depth mechanistic description that is required to improve our understanding of human clinical microRNA data and its relevance.

Next Steps

The relative ease of microRNA measurement and their potential importance make them a tempting target for clinical investigators to study in ill-defined conditions such as AKI. However, understanding the complex results of these analyses is likely to be challenging. The current literature focusses on a small proportion of microRNAs and further studies may have a confirmation biased toward the inclusion of known associations; unbiased analyses using next-generation sequencing to examine the entire micro RNA profile may thus be preferred to explore unknown associations. Such broader

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examination of microRNA profiles in differing causes of AKI (i.e., septic, ischaemic, nephrotoxic) could determine the underlying endotypes distinguishing these conditions. However, for any microRNA associations, it will be necessary to clarify the upstream regulators of microRNA expression, the downstream effectors mechanisms targeted by microRNA induction and whether microRNA expression is associated with protective or pathological effects. Finally, while microRNAs may be implicated in kidney injury, they are far from being disease specific; for example, miR-21 has also been associated with allergic inflammatory disease and breast cancer. Thus, individual microRNA expression patterns are unlikely to be clinically definitive in isolation; however, combined signals from multiple microRNAs could be more disease-specific.

Conclusions

Diverse changes in microRNA profiles have been observed after AKI. MicroRNAs are abundant and stable in plasma and urine, are often elevated before conven-

tional markers of renal dysfunction. MicroRNA mimics or inhibitors could afford a therapeutic role in renal protection. However, observational study populations often display heterogeneous AKI aetiology, where microRNA responses might be expected to vary, while individual microRNAs have a diverse range of targets that are likely to vary between pathological states. Furthermore, while there are biologically plausible mechanisms and suggestive animal data, no direct causation has been demonstrated in in-vivo human AKI studies. Finally, the use of microRNAs as biomarkers may be confounded by non-AKI pathological states, which perturb the “microRNA-ome.” In conclusion, we have begun to explore this important area of gene regulation, and advancing our scientific and clinical understanding of the role of microRNAs in AKI pathophysiology is likely to require close integration of laboratory-based and bedside studies.

Disclosure Statement

There are no conflicts of interest to disclose.

References

- 1 Kellum JA, Prowle JR: Paradigms of acute kidney injury in the intensive care setting. *Nat Rev Nephrol* 2018;14:217–230.
- 2 Xu K, Rosenstiel P, Paragas N, Hinze C, Gao X, Huai Shen T, et al: Unique transcriptional programs identify subtypes of AKI. *J Am Soc Nephrol* 2017;28:1729–1740.
- 3 Jonas S, Izaurralde E: Towards a molecular understanding of microRNA-mediated gene silencing. *Nat Rev Genet* 2015;16:421–433.
- 4 Treiber T, Treiber N, Plessmann U, Harlander S, Daiss JL, Eichner N, et al: A compendium of RNA-binding proteins that regulate microRNA biogenesis. *Mol Cell* 2017;66:270–284.e13.
- 5 Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al: The microRNA spectrum in 12 body fluids. *Clin Chem* 2010;56:1733–1741.
- 6 Wei Q, Bhatt K, He HZ, Mi QS, Haase VH, Dong Z: Targeted deletion of Dicer from proximal tubules protects against renal ischemia-reperfusion injury. *J Am Soc Nephrol* 2010;21:756–761.
- 7 Fan PC, Chen CC, Chen YC, Chang YS, Chu PH: MicroRNAs in acute kidney injury. *Hum Genomics* 2016;10:29.
- 8 Lan YF, Chen HH, Lai PF, Cheng CF, Huang YT, Lee YC, et al: MicroRNA-494 reduces ATF3 expression and promotes AKI. *J Am Soc Nephrol* 2012;23:2012–2023.
- 9 Gaede L, Liebetrau C, Blumenstein J, Troidl C, Dörr O, Kim WK, et al: Plasma microRNA-21 for the early prediction of acute kidney injury in patients undergoing major cardiac surgery. *Nephrol Dial Transplant* 2016;31:760–766.
- 10 Hao J, Lou Q, Wei Q, Mei S, Li L, Wu G, et al: MicroRNA-375 is induced in cisplatin nephrotoxicity to repress hepatocyte nuclear factor 1-β. *J Biol Chem* 2017;292:4571–4582.
- 11 Lorenzen JM, Kielstein JT, Hafer C, Gupta SK, Kümpers P, Faulhaber-Walter R, et al: Circulating miR-210 predicts survival in critically ill patients with acute kidney injury. *Clin J Am Soc Nephrol* 2011;6:1540–1546.
- 12 Saikumar J, Hoffmann D, Kim TM, Gonzalez VR, Zhang Q, Goering PL, et al: Expression, circulation, and excretion profile of microRNA-21, -155, and -18a following acute kidney injury. *Toxicol Sci* 2012;129:256–267.
- 13 Ramachandran K, Saikumar J, Bijol V, Koyner JL, Qian J, Betensky RA, et al: Human miRNome profiling identifies microRNAs differentially present in the urine after kidney injury. *Clin Chem* 2013;59:1742–1752.
- 14 Du J, Cao X, Zou L, Chen Y, Guo J, Chen Z, et al: MicroRNA-21 and risk of severe acute kidney injury and poor outcomes after adult cardiac surgery. *PLoS One* 2013;8:e63390.
- 15 Aguado-Fraile E, Ramos E, Conde E, Rodríguez M, Martín-Gómez L, Lietor A, et al: A pilot study identifying a set of microRNAs as precise diagnostic biomarkers of acute kidney injury. *PLoS One* 2015;10:e0127175.