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MicroRNAs: a new avenue to understand, investigate and treat immunoglobulin A nephropathy?

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Abstract

IgA nephropathy (IgAN) is the most common cause of primary glomerulonephritis worldwide. Up to 30% of cases develop the progressive form of the disease, eventually requiring renal replacement therapy. Diagnosis and risk stratification relies on an invasive kidney biopsy and management options are limited, with recurrence following renal transplantation being common. Thus the quest to understand the pathophysiology of IgAN has been one of great importance. MicroRNAs (miRs) are short nucleotides that suppress gene expression by hybridizing to the 3′ untranslated region of messenger RNA (mRNAs), promoting mRNA degradation or disrupting translation. First discovered in 1993, miRs have since been implicated in a number of chronic conditions, including cancer, heart disease and kidney disease. The mounting interest in the field of miRs has led to fascinating developments in the field of nephrology, ranging from their roles as biomarkers for disease to the development of miR antagonists as avenues for treatment. The translational potential for miRs in IgAN is thus well grounded and may represent a paradigm shift in current approaches to the disease. This review aims to summarize the literature with regard to miRs and their roles in IgAN.

Key words: biomarkers, epigenetic, IgA nephropathy, microRNA, therapy

Immunoglobulin A nephropathy (IgAN) is a primary kidney disease characterized by the deposition of IgA in the glomerulus. With an incidence of at least 2.5 per 100,000/year, it is the most common cause of primary glomerulonephritis worldwide [1]. The condition exacts a substantial socio-economic cost, with up to 30% of cases developing a progressive phenotype characterized by glomerular or interstitial fibrosis, eventually requiring renal replacement therapy [2]. Diagnosis and risk stratification relies on an invasive kidney biopsy [3] and management options are limited, with recurrence following renal transplantation being common [4, 5]. Thus the quest to understand the pathophysiology of IgAN has been one of the great importance.

The prevalent model for the pathogenesis of IgAN is the 'four-hit hypothesis', which postulates that the kidney is an innocent bystander of an otherwise systemic disease [6]. Fundamental to this model is an increase in poorly *O*-galactosylated IgA1 *O*-glycoforms in the serum displaying low levels of *O*-linked galactose at the IgA1 hinge [7]. A systemic IgG antibody response is directed against this altered hinge region, generating IgA–IgG antibody complexes, which are then deposited in the kidneys, triggering an inflammatory cascade eventually leading to chronic kidney disease (CKD). Indeed, a proportion of patients with increased levels of poorly *O*-galactosylated IgA1 *O*-glycoforms never develop the disease [8] and *in vitro* experiments suggest mesangial cells of patients with IgAN may be 'primed' to respond to IgG–IgA complexes [9], highlighting the necessity of multiple hits in the pathophysiology of IgAN. Furthermore, it is possible that each 'hit' may be mediated by several independent variables, such as genetic, epigenetic and environmental factors, including exposure to mucosal infections [10]. Thus there is still much to be clarified about this

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enigmatic disease, with the roles of antibodies, complement, and increasingly microRNAs (miRs), being explored.

miRs are short nucleotides that suppress gene expression by hybridizing to the 3′ untranslated region of messenger RNA (mRNA), promoting mRNA degradation or disrupting translation [11]. First discovered in 1993 [12], miRs have since been implicated in a number of chronic conditions, including cancer, heart disease and kidney disease [13–15]. The biogenesis of miRs is now well elucidated (Fig. 1A). A primary miR transcript is generated from nuclear DNA through the action of RNA polymerases. This hairpin transcript undergoes editing within the nucleus through the action of the nuclease Drosha, which cleaves the 5′ and 3′ regions of the transcript to form precursor miR (pre-miR) [16]. The pre-miR is shuttled out of the nucleus through coupling with an exportin 5–ran-GTP complex [17], which also shields the edited transcript from degradation [18]. In the cytoplasm, the pre-miR undergoes further processing through the action of the RNAse Dicer, removing the loop region of the hairpin structure to form double-stranded RNA [19]. A helicase then separates the two strands to form a passenger strand, which is degraded, and a functional guide strand. The latter is loaded onto an RNA-induced silencing complex (RISC) and the functional strand guides the RISC to potential mRNA targets [20]. The action by which the RISC silences gene expression is determined by the degree of complementarity between the functional strand's 'seed region', a six to eight nucleotide long region usually beginning at Position 2, and the target mRNA [21]. Complete complementarity results in the argonaute protein of the RISC promoting mRNA degradation, whereas partial complementarity promotes disruption of translation [21]. In this manner, miRs can control the expression of many genes, playing vital roles in the regulation of several crucial cell processes.

Intriguingly, miRs were recently found to exist in stable forms outside of the cell [22]. These miRs are durable even in relatively harsh conditions, including extremes of temperatures and pH, and are resistant to immediate degradation from

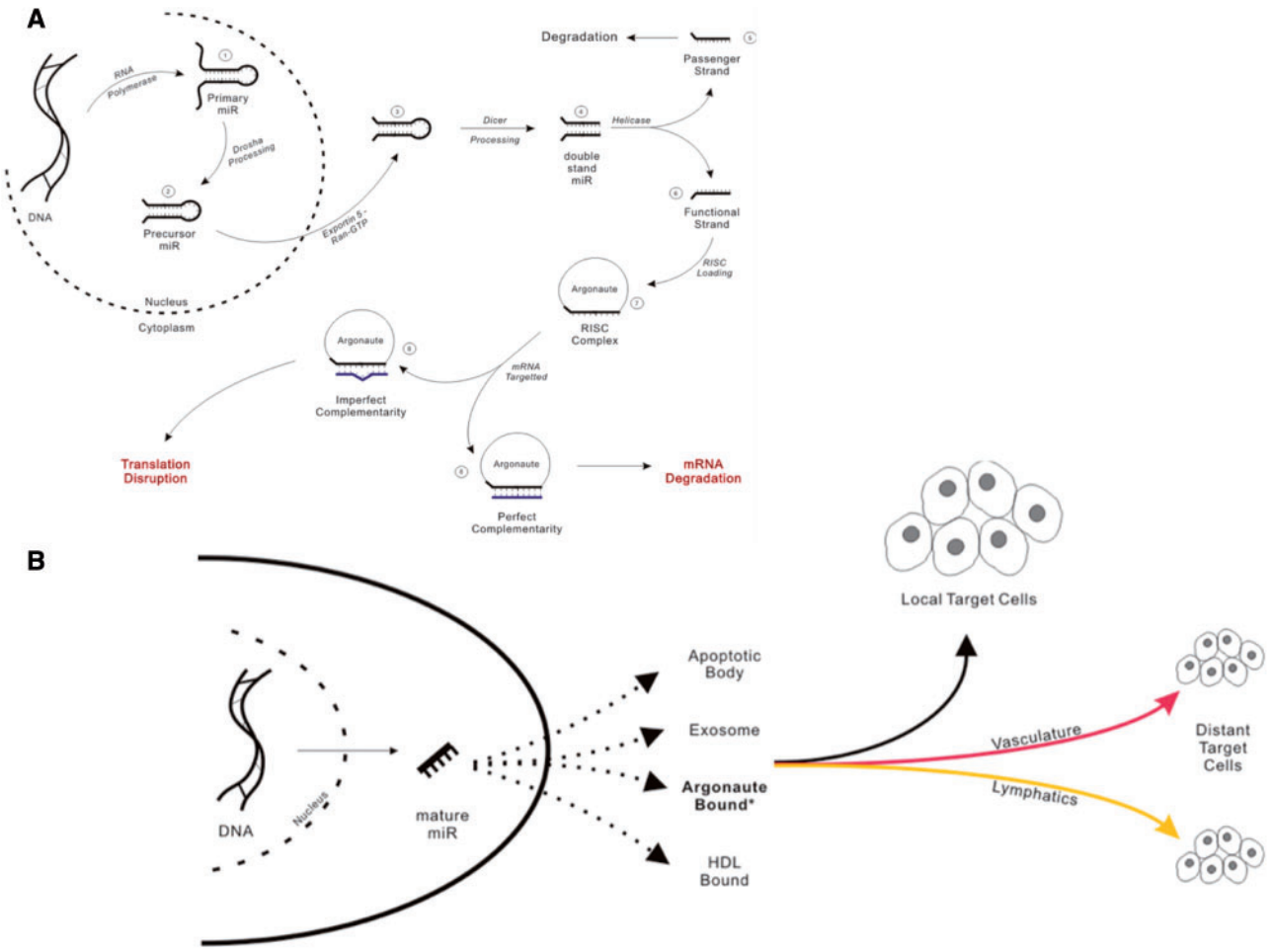

Fig. 1. **(A)** The biogenesis of miRs. (1) The biogenesis of miRs begins in the nucleus, with the production of a hairpin loop transcript known as a primary miR (pri-miR), under the action of RNA polymerase. (2) The pri-miR undergoes post-transcriptional editing by the action of Drosha, an RNAse, supported by the action of DGCR8. This process cleaves the pri-miR at its 5′ and 3′ regions to produce a precursor miR (pre-miR). (3) The pre-miR is exported out of the cell in complex with exportin 5–Ran-GTP, which also prevents degradation of the edited transcript. (4) The pre-miR then undergoes further editing under the action of Dicer, which cleaves the loop structure to produce a double-stranded RNA. (5) The strands are separated by helicases to produce a passenger strand that is left exposed in the cytoplasm and is thus swiftly degraded. (6) The remaining functional strand is the final mature miR. (7) The mature miR is then complexed with argonaute proteins to form the RISC. This complex moves on to degrade mRNA or disrupt translation, guided by the complementarity of the mature miR within the complex. (**B**) Extracelluar miRs. Extracellular miRs can be found within apoptotic bodies (released during cell apoptosis) or exosomes or in complex with argonaute proteins or high-density lipoproteins (HDLs). Of the extracellular miRs, 95–99% are argonaute bound, making this the predominant miR complex in biological fluids. Extracellular miRs can thus travel to target cells either locally within the tissue space or to distant target cells via the vasculature or lymphatic system. Here, they are able to silence gene expression following uptake into target cells. The mechanisms of secretion and uptake of extracellular miRs are yet to be clarified, although several hypotheses exist.

nucleases [23]. There is a growing body of evidence to suggest that extracellular miRs are selectively secreted [24] and owe their stability to complexes with high-density lipoproteins, argonaute proteins or their containment within exomes (Fig. 1B) [25]. *In vitro* experiments have demonstrated that extracellular miRs are capable of being taken up by 'target' cells, where they are then able to silence gene expression and thus influence the phenotype [26]. This has raised the possibility of these nucleotides playing a role in cell-to-cell communication, acting as endocrine or paracrine agents.

The increasing interest in miRs has led to a number of developments in the field of nephrology, ranging from their roles as biomarkers for disease [27] to the development of miR antagonists as avenues for treatment [28]. The translational potential for miRs in IgAN is thus well grounded and may represent a paradigm shift in current approaches to the disease. This review aims to summarize the literature with regard to miRs and their role in IgAN. We begin by exploring the broad differential expression of miRs in IgAN and provide an overview of associations between individual miRs and clinical correlates, exploring their potential roles in diagnosis and monitoring. Finally, the pathophysiological roles of miRs in IgAN are explored and their potentials as targets for therapy are discussed.

miRs are differentially expressed in the blood, urine and renal tissue in IgAN

Demonstrating differential expression of miRs in IgAN compared with other types of kidney disease and in healthy subjects is fundamental to establishing a precedent for further investigation of miRs in IgAN. Several small studies have been conducted using microarray analysis to measure miR expression, with real-time quantitative polymerase chain reaction (qPCR) to validate differentially expressed miRs. While microarray analysis has a role in profiling the differential expression of miRs, its main disadvantage is that novel miRs are inevitably omitted from detection. This problem is overcome by next-generation sequencing (NGS). However, the technique is expensive and time consuming; and as a consequence, there are limited data from NGS in IgAN.

The kidney

Dai *et al.* [29] demonstrated, by microarray analysis, reduced expression of 31 miRs and elevated levels of 34 miRs in renal biopsies of IgAN patients compared with healthy subjects [29]. However, no statistical analysis was performed to demonstrate significance. Tan *et al.* [30] used NGS to profile the renal tissue of six IgAN patients and compared this with healthy subjects, and identifying 84 miRs that were differentially expressed. Additionally, seven novel miRs were detected in the IgAN cohort. Twenty of the differentially expressed miRs were common to other microarray studies in IgAN (Table 1). However, the cohort studied was small, no disease controls were included and the results were not validated in a separate cohort. Consistent with alteration of miR expression in the kidney in IgAN, human mesangial cells (HMCs) exposed to salivary secretory IgA from IgAN patients differentially expressed 56 miRs [31].

Peripheral blood mononuclear cells (PBMCs)

A single study has shown differential expression of 37 miRs in PBMCs in IgAN [32].

Urine

Wang *et al.* [33] used microarray analysis to investigate miR expression in the urinary sediment of IgAN patients and compared these to that seen in disease controls, which included membranous nephropathy (MN) and minimal change disease (MCD) [33]. While no miR was significantly dysregulated compared with all three controls, subgroup analysis by histological severity of IgAN (Lee's grade [34]) revealed four were dysregulated in the Grade I/II group, six were dysregulated in the Grade III group (with the down-regulation of miR-3613-3p and 4668-5p being common to both) and none were dysregulated in the Grade IV/V group compared with all controls. These findings suggest that miR profiles in IgAN may be dynamic, varying with the time course and severity of the disease. A similar study reported by Konta *et al.* [35] that included diabetic nephropathy (DN) and crescentic glomerulonephritis as disease controls also found no single miR to be differentially expressed, although subgroup analysis was not performed.

Current evidence would suggest that there is generally a good correlation between urinary and renal miR expression [35]. However, in IgAN this is not always the case, with miRs-150-5p and 223-3p abundantly expressed in urinary sediment [33] but down-regulated in renal tissue [29]. One possible explanation in IgAN is that urinary miRs may be arriving as passengers in erythrocytes [36]. Duan *et al.* [36] found 112 miRs to be differentially expressed in the urinary sediment of IgAN patients by microarray analysis and validated the three highest expressed miRs (25-3p, 144-3p and 486-5p) in a cohort of IgAN patients against disease controls (MN, MCD, focal segmental glomerular sclerosis, Henoch–Schönlein nephritis, renal amyloidosis) and healthy controls. Curiously, expression of these miRs was greatest in urinary sediment containing erythrocytes compared to the urinary sediment without (erythrocytes were removed using immunomagnetic seperation). Furthermore, expression was highest within urinary erythrocytes compared with other urinary cell types. This 'loading' of miRs appeared to be occurring within the kidney, as no difference in expression was identified between the blood erythrocytes of IgAN patients and healthy controls [36].

All of these observational cross-sectional studies demonstrate a differential expression of miRs in IgAN and highlight the variation in miR profiles with tissue type, disease severity and ethnicity. Although no single miR was differentially expressed across all of these studies, five dysregulated miRs (134, 185, 233, 615p and let-7a) were common to renal tissue and either PMBCs or cultured HMCs in IgAN (Table 1).

miR expression in blood, urine and renal tissue correlates with histological and clinical parameters in IgAN

Diagnosis and risk stratification of IgAN currently relies on kidney biopsy and histopathological assessment, which is invasive, time consuming and expensive. miRs can be remarkably stable [37] and are easily obtained from blood and urine, providing readily available sources in the clinical setting for diagnosis and monitoring [27]. As such, clinical and histopathological correlations between miR expression in renal tissue, urine and plasma have been investigated. The miRs selected for these studies have been based on earlier studies reporting differential expression using microarray or NGS or because of their prominent roles in inflammation and fibrosis in settings outside of IgAN.

Table 1. miRs differentially expressed in at least two different studies in IgA N

Tissue control used	Renal tissue[a], healthy	Renal tissue[b], healthy	PMBCs[c], healthy	SIgA HRMCs[d], healthy	Urinary sediment[e], healthy + disease	Urinary sediment[f], healthy + disease
15b	↑	↓				
17		↓	↑			
23a	↑	↓				
30a-5p	↑	↓				
30d	↓	↓				
98		↑	↑			
99a	↓	↓				
128		↓	↑			
133a	↓	↑				
133b	↓	↑				
134	↑		↑			
148b		↓	↑			
150	↓	↑			↑	↑
185	↑		↑			
195		↓	↑			
199b-3p		↓	↑			
221		↓	↑			
223	↓			↓	↑	
374b		↓	↑			
486	↓	↑				↑
502-3p		↓	↑			
572				↑	↑	
615	↓			↓		
625	↓			↑		
628	↑	↓				
3613-3p				↓	↓	
let-7a	↑	↓	↑			
let-7c		↓	↑			
let-7d	↓		↑			

miRs differentially regulated in at least two microarray/RNA sequencing studies. ↑, increased expression compared with controls (≥2.0 fold); ↓, reduced expression compared with controls (≤0.5 fold).

[a]Dai *et al.* [29]; microarray analysis of IgAN renal tissue compared with healthy controls.

[b]Tan *et al.* [30]; RNA sequencing of IgAN renal tissues compared with healthy controls.

[c]Serino *et al.* [32]; microarray analysis of IgAN PMBCs compared with healthy controls.

[d]Liang *et al.* [31]); microarray analysis of HRMCs stimulated with secretory IgA from patients compared to stimulation with secretory IgA from healthy controls.

[e]Wang *et al.* [33]; microarray analysis of IgAN urinary sediment compared with healthy and disease controls.

[f]Duan *et al.* [36]; microarray analysis of IgAN urinary sediment compared with healthy and disease controls.

Correlation of renal miR expression with renal histology and clinical outcome

Four studies have explored 12 intrarenal miRs and their associations with clinical features, histological findings and mRNA markers of fibrosis [38–41]. miRs 21-5p, 155, 199a-5p, 205 and 214-3p correlated with the extent of interstitial fibrosis, miRs 21-5p and 214-3p were associated with an increased risk of renal failure {hazard ratio [HR] 4.08 [95% confidence interval (CI) 1.32–12.55] and HR 3.81 (95% CI 1.06–13.74), respectively} and miR-192 was associated with an increased rate of estimated glomerular filtration rate decline. Of note, levels of miR-141 and -200c expression in the kidney correlated with vimentin and E-cadherin mRNA, respectively, both of which are markers of epithelial-to-mesenchymal transition (EMT), which is often observed in CKD (Table 2).

Although many of these miRs were also noted to be differentially regulated using NGS [30], it is noteworthy that none were found to be specific for IgAN when studies included disease controls. Dysregulation of these miRs is thus likely to be as a result of generic renal fibrosis pathways common to CKD; indeed, miR-21-5p alone has been linked to renal fibrosis secondary to many pathologies, including DN [42].

Correlation of urinary miR expression with renal histology and clinical outcome

Four studies from a group in Hong Kong have investigated urinary miR profiles in IgAN and their clinical correlates. miRs 93 and 429 were associated with glomerulosclerosis [43, 44], while miRs 217 and 377 were associated with tubulointerstitial scarring [45]. miRs-200b inversely correlated with the rate of GFR decline [44], and various correlations with markers for EMT (SMAD3, vimentin and ZEB2) and cytokines [interleukin 1β (IL-1β), Interleukin 6 (IL6) and tumour necrosis factor α (TNF-α)] were reported (Table 2). Most of these studies, like those investigating renal miR expression, are limited by the lack of disease controls. It is therefore not possible to determine if the changes in miR expression are specific for IgAN or simply generic to CKD. The study by Szeto *et al.* [45] included diabetic and hypertensive nephropathy as disease controls, but no glomerulonephritis controls, and found miR-17 to be overexpressed in IgAN (although healthy controls were not included), but found no specific correlation between any of the eight other miRs investigated and clinical correlates.

Table 2. miR expression in IgAN with clinical correlates

Study	Tissue	miR	eGFR correlation	Proteinuria correlation	Histopathology associations	Renal failure risk	Other mRNA correlations
Hennino *et al.* [40]	Kidney	21-5p			Interstitial/glomerular fibrosis	HR 4.08 (95% CI 1.32–12.55)	
		199a-5p					
		214-3p				HR 3.81 (95% CI 1.06–13.74)	
Wang *et al.* [38]	Kidney	200c		Positive			Correlated with E-cadherin
		205		Positive	Tubular interstitial fibrosis		
		192	Correlated with rate of decline		Glomerular fibrosis		
		141					Inverse correlation with vimentin
Wang *et al.* [39]	Kidney	146a	Inverse	Positive			
		155	Inverse	Positive	Tubular interstitial fibrosis		
Bao *et al.* [41]	Kidney	21			Interstitial/glomerular fibrosis		
Wang *et al.* [39]	Urinary sediment	146a		Positive			Inverse correlation with urinary IL-1β, IL-6 and TNF-α. Positive correlation with RANTES
		155		Positive			Inverse correlation with urinary IL-1β and TNF-α. Positive correlation with RANTES and FOXP3
Szeto *et al.* [45]	Urinary sediment	15	Positive	Inverse			
		17	Positive				
		192	Positive	Inverse			
		216a		Inverse			
		217	Positive		Tubular interstitial fibrosis (inverse correlation)		
		377			Tubular interstitial fibrosis		
Wang *et al.* [43]	Urinary sediment	21	Positive				Positive correlation with SMAD3
		29b	Positive	Positive			Positive correlation with SMAD3
		29c		Positive			Positive correlation with SMAD3
		93			Glomerular fibrosis		Positive correlation with SMAD3
Wang *et al.* [44]	Urinary sediment	200a		Positive			Inverse correlation with vimentin
		200b	Correlated with rate of decline	Positive			Inverse correlation with vimentin, ZEB2
		429	Positive	Positive	Glomerular fibrosis (inverse correlation)		Inverse correlation with vimentin
Hu *et al.* [46]	Bcells	374b		Positive	Correlates with MEST score		
Bao *et al.* [47]	GECs	233		Positive	Correlation with glomerular endothelial proliferation		

GECs, Glomerular Endothelial Cells; MEST, mesangial hypercellularity, endocapillary hypercellularity, segmental glomerulosclerosis, tubular atrophy/interstitial fibrosis; RANTES, regulated on activation, normal T cell expressed and secreted.

Correlation of blood miR expression with renal histology and clinical outcome

Hu *et al.* [46] and Bao *et al.* [47] reported that expression levels of miRs 374b and 233 in B-lymphocytes and circulating endothelial cells, respectively, correlated with histological findings in IgAN (Table 2). Serino *et al.* [32] noted that miRs let-7b and 148 were up-regulated in PMBCs using microarray, and in a retrospective multicentre study, it was demonstrated that the two miRs combined could discriminate between IgAN patients, healthy subjects and disease controls (MCD, MN, FSGS). The combined miR biomarkers also held their diagnostic validity across two ethnic cohorts (Caucasians and East Asians) [48].

Although these data represent an exciting first step towards a non-invasive diagnostic test for IgAN, it is yet to be validated prospectively by a separate research group or in further cohorts of patients. Such steps are crucial, particularly as IgAN miR profiles can be variable even within the same ethnic group at different stages of development. Indeed, two groups from China demonstrated that the presence of a polymorphism in pre-miR-146a was associated with IgAN susceptibility in paediatric patients of the Chinese Han population but not in adults of the same ethnicity [49, 50].

miRs have demonstrable roles in the pathophysiology of IgAN

The role of miRs in the pathogenesis of IgAN is an area of growing interest, fuelled by evidence of miR involvement in a spectrum of other chronic diseases, including cancer and heart failure [13, 14], and in the development of miR-based treatment strategies such as miR-21 antagonism for the treatment of Alport's disease.

PBMCs: miRs 148b, 374b and let-7b regulate IgA1 *O*-glycosylation

The extent of *O*-galactosylation of IgA1 is widely accepted as being an important contributor to the development of IgAN. IgA1 hinge region *O*-glycosylation involves the initial addition of *N*-acetylgalactosamine (GalNAc) to threonine or serine residues, catalysed by *N*-acetylgalactosaminyltransferase 2 (GALNT2) [51]. GalNAc is subsequently *O*-galactosylated by core 1 β1,3-galactosyltransferase (C1GALT1) acting with a chaperone, Cosmc [52, 53]. Three studies have investigated the roles of miRs 148b, 374b and let-7b in regulating glycosylation of IgA1 in PBMCs.

Serino *et al.* [32, 54] identified up-regulation of miRs 148 and let-7b in PMBCs of Caucasian IgAN patients and *in silica* analysis revealed C1GALT1 and GALNT2 to be targets for miRs 148b and let-7b, respectively. Indeed, both these enzymes were expressed less in PMBCs of IgAN patients compared with healthy subjects and levels negatively correlated with their respective associated miRs. Subsequent transfection experiments with miR mimics and inhibitors demonstrated miRs 148b and let-7b significantly suppressed expression of C1GALT1 and GALNT2, both at an mRNA and protein level. The group also found a positive correlation between miR-148b and the serum levels of poorly *O*-galactosylated IgA1 *O*-glycoforms. In parallel, Hu *et al.* [46] showed that miR-347b was overexpressed in B-lymphocytes of Chinese IgAN patients, and following transfection with miR-347b there was suppression of Cosmc and a proliferation pathway regulator [phosphatase and tensin homolog (PTEN)] at both an mRNA and a protein level, leading to greater proliferation of B-cells and higher serum levels of poorly *O*-galactosylated IgA1 *O*-glycoforms.

These studies demonstrate the regulatory potential of miRs in IgA1 *O*-glycosylation, a fundamental component in the development of IgAN (Fig. 2). Although each study restricted their patient cohorts to a single ethnic group, Serino *et al.* [48] subsequently demonstrated dysregulation of miRs 148b and let-7b in an East Asian cohort as well as in Caucasians, providing some evidence that these miRs may have a universal role in the pathophysiology of IgAN.

The kidney: dysregulated miRs lead to activation of local inflammatory pathways in IgAN

There is also evidence that specific miR networks may be important in the development of inflammatory and profibrotic changes in the glomeruli and tubulointerstitium in IgAN.

There is convincing evidence that secretory IgA (SIgA) is an important component of the pathogenic fraction of circulating IgA in IgAN [55]. Liang *et al.* [31, 56] examined the effect of exposing HMCs to purified SIgA and showed that SIgA from IgAN patients resulted in release of several pro-inflammatory cytokines from HMC, including IL-6, IL-8 and IL-1β, compared with SIgA from healthy subjects. Subsequent *in silica* analysis predicted IL-6, IL-8 and IL-1β to be targets for miRs 16, 100-3p and 877-3p, respectively. Of note, these three miRs were down-regulated in IgAN on both NGS and microarray compared with healthy subjects [30, 31]. This down-regulation was validated and transfection experiments with miR mimics confirmed miRs 16, 100-3p and 877-3p to be negative regulators of IL-6, IL-8 and IL-1β, respectively. Of note, the three miRs had no effect on their respective cytokines at an mRNA level, suggesting the miRs disrupt translation instead of triggering mRNA degradation [31, 56].

Xing *et al.* [57] conducted a similar set of experiments using a human embryonic kidney cell line (HEK293), focusing on miR-29b-3p, which had been documented to be down-regulated in previous NGS experiments [30]. Transfection with miR-29b-3p down-regulated CDK6, a p65 kinase, at a protein level, and inhibiting miR-29b-3p increased TNF-α-induced p65 phosphorylation, leading to an increase in TNF-α-induced IL-8 expression [57].

There have been two laser capture microdissection studies (LCMS) using renal biopsies to investigate the effects of miRs within specific compartments of the nephron [41, 47]. In one study, Bao *et al.* [46] sought to clarify the roles of miRs in glomeruli displaying endothelial proliferation/hypercellularity (the E1 lesion of the Oxford Classification), a histological lesion associated with an increased risk of progression to fibrosis and possibly responsiveness to immunosuppression in IgAN. Bao *et al.* [47] found that miR-233 was down-regulated in glomeruli with hypercellularity compared with those without. Subsequently, through an extended series of experiments, IgA from these patients was shown to trigger IL-6 release in cultured HMC, resulting in a reduction of miR-233 levels in cultured glomerular endothelial cells (GEnCs). This reduction caused GEnC proliferation, monocyte–endothelial adhesion and the expression of intracellular adhesion molecule 1 (responsible for the migration of inflammatory cells) through the upregulation of importin α5 and importin α4, causing increased p65 and STAT3 activity, respectively [47]. All of these changes are consistent with down-regulation of miR-233, playing some role in the development of endocapillary hypercellularity in susceptible patients with IgAN.

In a separate study, Bao *et al.* [41] used LCMS to investigate the role of miR-21, an miR associated with a number of CKDs, in

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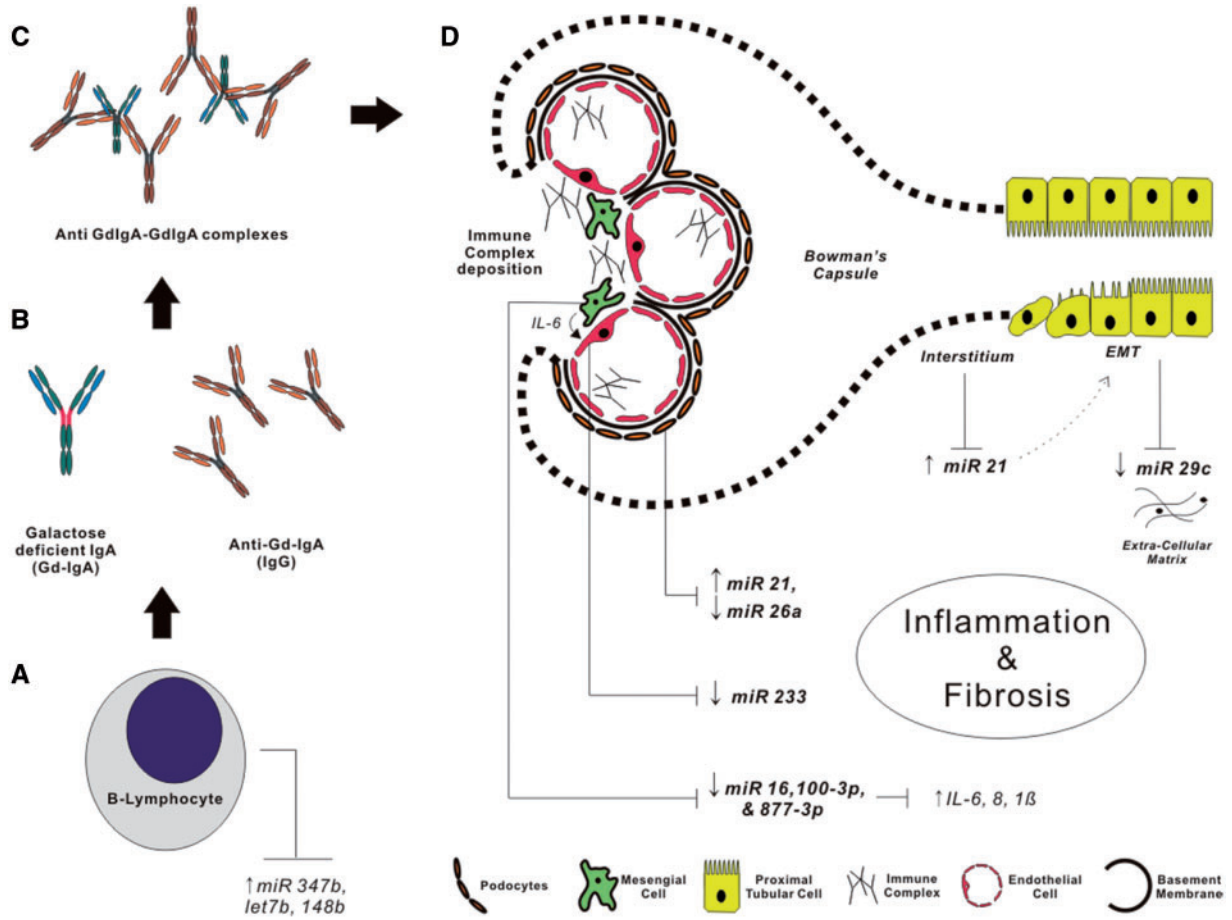

Fig. 2. MicroRNAs and the four-hit hypothesis. (**A**) IgA1 is *O*-galactosylated in B cells under the action of C1GALT, GALNT2 and the chaperone protein Cosmc. miRs 148b, let-7b and 347b suppress these proteins, respectively, and thus their increased expression generates poorly *O*-galactosylated IgA1 *O*-glycoforms. (**B**) Hit 1 and Hit 2. The first hit of the four-hit hypothesis is the generation of poorly *O*-galactosylated IgA1, the second is the generation of anti-IgA1 hinge IgG antibodies. (**C**) Hit 3. As poorly *O*-galactosylated IgA1 is targeted by anti-IgA1, IgA1–IgG immune complexes are formed in circulation. (**D**) Hit 4. Deposition of IgA–IgG immune complexes in the kidneys results in an inflammatory process leading to glomerulonephritis, fibrosis and eventually CKD. miRs in mesangial cells: miRs 16, 100-3p and 877-3p are down-regulated in mesangial cells stimulated by secretory IgA taken from IgAN patients [31, 56]. These miRs suppress IL-6, IL-8 and IL-1β, respectively, resulting in overactivity of these cytokines in IgAN. miRs in endothelial cells: IL-6 produced by mesangial cells suppresses miR-233 in GEnCs, increasing p65 and STAT3 activity, promoting local inflammatory effects [47]. miRs in podocytes: miR-26a expression is low in the glomeruli of IgAN patients. Its expression positively correlates with markers of healthy podocyte function [60]. miRs in proximal tubular cells: miR-29c, a known suppressor of extracellular matrix proteins, is reduced in expression in IgAN [58, 59]. miRs of the interstitium: miR-21 increases in IgAN, promoting EMT [41].

IgAN. The group found miR-21 expression was significantly elevated in both the glomeruli and tubulointerstitium in IgAN compared with healthy subjects. *In vitro*, HMC exposed to IgA from IgAN secreted higher levels of TGF-β1 and TNF-α compared with those stimulated with IgA from healthy subjects. Addition of these cytokines to podocytes and a proximal tubule epithelial cell line (HK2 cells) significantly increased levels of miR-21, which was absent when neutralizing antibodies were introduced. Anti-miR-21 reduced the expression of EMT markers and PTEN activation [41], suggesting a downstream role of miR-21 in IgAN fibrosis.

In contrast to the IgAN-specific effect of miRs 148b, 374b and let-7b in regulating *O*-glycosylation of IgA1 in PBMCs, it is likely that the data generated from miR expression in renal tissue are likely to represent pathways common to all fibrosing renal diseases. What these data do highlight, however, is the complex interaction between miRs and inflammatory cytokines in IgAN, with multiple miRs regulating a single cytokine (as with IL-8) and single miRs potentially regulating a multitude of inflammatory pathways (as with miR-233).

Animal studies: can they help us delineate the role for miRs in IgAN?

While there remains controversy regarding the applicability of current animal models of IgAN to human disease, acceptable animal models of CKD do exist. As the glomerular and tubulointerstitial fibrosis seen in IgAN is likely to share many common pathways with other types of CKDs, these models may provide useful avenues to investigate the pathophysiological role of miRs in IgAN.

Fang *et al.* [58] demonstrated that miR-29c, an miR known to supress extracellular matrix proteins [57], was down-regulated in a rat model of tubulo-interstitial fibrosis and in human kidneys with progressive IgAN. Having previously demonstrated a protective effect of hypoxia inducible factor (HIF) on fibrosing renal disease, the group postulated an interaction between HIF and miR-29c. The group used an established model of CKD in rats (5/6 nephrectomy) and exposed the rats to L-mimosine, an inducer of HIF, after which the renal tissue and blood samples of the rats were compared with control animals. The group

found that miR-29c expression decreased following nephrectomy and increased in response to L-mimosine. This increase correlated with HIF levels and inversely correlated with interstitial fibrosis. This was also demonstrated *in vitro* by stimulating cultured HK2 cells to release HIF, which positively correlated with an increase in miR-29c. Furthermore, this effect was reduced when small interfering RNAs (siRNAs) were introduced to block the effect of HIFa, demonstrating a causal relationship between HIF and miR-29c. The group thus postulated a similar mechanism may be occurring in humans, suggesting that progressive IgAN may be associated with HIFa induced down-regulation of miR-29c.

Ichii *et al.* [60] demonstrated that miR-26a was present at higher levels in the glomeruli of healthy renal tissue compared with the interstitium, and this reverses in IgAN. Using a mouse model of autoimmune glomerulonephritis, the group verified that glomerular miR-26a was significantly reduced compared with control mice and that miR-26a levels positively correlated with the mRNA levels of podacalyxin and synaptopodin. The group subsequently used miR silencing experiments in cultured mouse podocytes to demonstrate that inhibiting miR-26a down-regulated the expression of several structural podocyte proteins. These changes were also observed in a murine lupus nephritis model, suggesting changes in glomerular levels of miR-26a may not be specific to IgAN [60].

Can miRs be targets for treatment in IgAN?

There is a growing body of evidence to support miR-targeted therapy as a novel approach to treating a range of diseases, including Alport syndrome [28], hepatitis C infection and various cancers [61]. While it is unlikely that targeting a single miR will be effective for the treatment of IgAN, it is possible that a multi-antago-miR approach may be efficacious to target different elements of the disease. For instance, blocking the actions of miRs let-7b, 374b and 148b could conceivably blunt the 'first hit' of IgAN by reducing circulating poorly O-galactosylated IgA1 O-glycoform levels, while antagonizing miR-21 may be a suitable target to reduce downstream fibrosis developing as a consequence of glomerular injury and the development of proteinuria. Antagonizing miRs is, however, not without consequences, as a single miR has the potential to regulate hundreds of transcripts. miR-specific therapy must therefore be developed with caution, with the aim of minimizing 'off-target' effects that may arise from the unintended suppression of transcripts unrelated to IgAN.

Conclusion

It is clear from the literature that while we have a tantalizing early insight into the role of miRs in IgAN, it is still too early for concrete conclusions. We need properly validated studies, spanning different ethnic groups and encompassing diverse populations before we can reliably comment on the role of particular miRs in IgAN. The role of miRs in regulating IgA1 O-glycosylation is currently the most convincing data we have; however, this is likely to change in the coming years as large studies report on the value of serum and urine miRs and miR expression in the kidney in IgAN and other forms of kidney disease.

Conflict of interest statement

None declared.

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