microRNAs in the Diagnosis and Pathophysiology of Acute Kidney Injury and Kidney Transplantation

Kristien J Ledeganck, MD PhD[1*], Els M Gielis, MD[1*], Daniel Abramowicz, MD PhD[1,2], Peter Stenvinkel, MD PhD[4], Paul G Shiels, PhD[3], Amaryllis H Van Craenenbroeck, MD PhD[1,2]

** equally contributing*

[1] Laboratory of Experimental Medicine and Pediatrics, University of Antwerp, B-2610 Wilrijk, Belgium

[2] Department of Nephrology, Antwerp University Hospital, Wilrijkstraat 10, B-2650 Edegem, Belgium

[3] Section of Epigenetics, Institute of Cancer Sciences, University of Glasgow, Glasgow G61 1QH, UK

[4] Division of Renal Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden

Corresponding author

Amaryllis Van Craenenbroeck, MD PhD

Department of Nephrology, Antwerp University Hospital

Wilrijkstraat 10, B-2650 Edegem, Belgium

tel: ++ 32 3 821 38 72; fax: ++ 32 3 829 01 00

amaryllis.vancraenenbroeck@uantwerpen.be

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Abstract

MicroRNAs (miR) are epigenetic regulators of gene expression at the posttranscriptional level. They are involved in intercellular communication and crosstalk between different organs. As key regulators of homeostasis, their dysregulation underlies several disease conditions, including kidney disease. Moreover, their remarkable stability in plasma and urine makes them attractive biomarkers.

Beyond biomarker studies, clinical microRNA research in the nephrology field has focused the last decennia on the discovery of specific microRNA signatures and the identification of novel targets for therapy and/or prevention. Heterogeneity of conducted research is, however, striking, and there is a current need for standardization and confirmation of new findings in large prospective trials.

After discussing briefly the general concepts of microRNA, this review provides an overview of the available clinical evidence in both the pathophysiology and biomarker field for the role of microRNA in acute kidney injury and kidney transplantation.

Introduction

MicroRNAs (miRs), an evolutionary conserved class of non-coding RNAs, are negative regulators of post-transcriptional gene expression (1). Sequence-specific binding to the target mRNA results in translational inhibition or mRNA degradation. microRNA dysregulation is involved in the development and progression of numerous diseases, including cancer, cardiovascular and kidney disease (2, 3). microRNA synthesis takes place through a canonical pathway involving four key enzymes or, alternatively, via the mirtron pathway **(Figure 1)**. Genomic events or inhibition of regulatory enzymes all can lead to microRNA dysregulation in disease (5, 6).

microRNAs execute their repressive function intracellularly, but they are also released into the extracellular compartment where they can act as hormones and/or biomarkers **(Figure 1).** Apart from being released passively as a result of cell death or injury, microRNAs are actively secreted in different types of extracellular vesicles, including exosomes, microvesicles and apoptotic bodies. Circulating microRNAs form complexes with RNA binding proteins including Argonaute (AGO) 2 proteins and lipoproteins (HDL and LDL), which protects them from RNAse-dependent degradation (9). Interestingly, microRNAs are important intercellular communicators (for overview, see (7)+REF) in a paracrine or even endocrine way (for example in muscle-kidney crosstalk (8)). Following uptake, specific microRNA can exert their silencing function in the recipient cells. *In vivo* modulation (merely inhibition) of microRNA expression as a therapeutic strategy is widely explored and in chronic kidney disease (CKD), several microRNA-targeting drugs entered clinical testing based on convincing pre-clinical evidence (4, 11). In autosomal dominant polycystic kidney disease, insight in the pathogenetic role of miR-17 (12, 13) recently lead to the start of a phase I trial of an anti-miR-17 compound (known as RGLS4326). Likewise, miR-21 inhibition

in Alport syndrome shows promising results, both in an animal model and in *in vitro* studies of patients with Alport syndrome (13, 14). A phase I clinical trial with an anti-miR-21 compound (RG-012) is ongoing in patients with Alport syndrome, thereby evaluating its safety and treatment efficacy (NCT03373786). In contrast with CKD, trials targeting microRNA in the field of acute kidney injury (AKI) or kidney transplantation have not entered the clinical phase yet.

This review provides an overview of the available clinical evidence in both the pathogenic role and the diagnostic potential of microRNA in acute kidney injury and kidney transplantation. For a comprehensive review on the role of miRNA in CKD, we refer the reader to Lv *et al* (15). Table 1 and Table 2 show the current evidence for AKI and transplant-related disorders, respectively. Several caveats apply when interpreting the study results. Firstly, the considerable heterogeneity in the applied techniques (qRT-PCR, microarrays, Next Generation Sequencing) (16) and patient groups studied, makes inter-group comparisons and indepent validation difficult. Secondly, several of these studies investigated microRNA target prediction and mRNA interactions through biostatistical modelling. Experimental validation however, remains important. Thirdly, natural inter-individual variation in microRNA expression levels is not well defined, neither across the life course, between cell types nor defined in response to variation in psychosocial factors or nutrition. These are established factors that affect kidney biology (17, 18). Technological developments, principally the use of single cell sequencing technologies, could add in-depth-analysis in this area and enable a more comprehensive picture of both differing clinical epigenotypes and inter-individual variation in epigenotypes unrelated to any pathophysiology.

microRNA in the pathophysiology of acute kidney injury and transplant-related disorders

Acute kidney injury

From the limited experiments that were performed to date in human settings, microRNAs appear to act via different mechanisms. Some microRNAs repress pathways that play a protective role in renal physiology while a pro-inflammatory effect by inhibition of anti-inflammatory pathways or mitochondrial function have also been described.

A number of microRNAs appear to play a role in acute kidney injury (AKI), where the release of multiple interleukins precedes structural kidney damage (Figure 2). These include miR-101 (interleukin 2, nuclear factor kappa B (NFkB) pathway) (19), miR-494 (activating transcription factor 3 in NFkB pathway) (20), miR-16 (BCL-2) (21) and miR-107 (tumor necrosis factor (TNF)) (22). Interestingly, urinary miR-494 levels, as opposed to serum levels, were found to increase early in critically ill AKI patients compared to their counterparts without AKI and healthy controls. In turn, miR-494 inhibits the expression of the kidney protective gene ATF3, resulting in more aggravated kidney injury (20). C/EBP-β (C/ enhancer binding protein-β) upregulated miR-16, which in turn blocked one of the anti-apoptotic genes, BCL-2 after ischemia/reperfusion-induced injury (21). In septic AKI patients, increased miR-107 induced TNF-α secretion by targeting DUSP7 (dual specificity protein phosphatase 7) in endothelial cells, which may directly cause tubular injury (22). *In vitro* inhibition of this microRNA resulted in attenuated TNF secretion and prevented subsequent tubular cell injury (22). In a study by Ge *et al.* (23), 37 microRNAs were differentially expressed in the serum of sepsis-induced AKI versus sepsis non-AKI patients. Function and pathway analysis revealed that 8 of them were associated with 13 genes involved in mitochondrial oxidative stress and dysfunction response including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), sirtuin 1 (SIRT1), mammalian target of rapamycin (mTOR), oxidative stress responsive 1 (OXSR1) and NADPH oxidase 5 (NOX5) (23). Congruent with these observations, up-regulation of renal tubular miR-709 after cisplatin-induced AKI

hampers mitochondrial function and induces cell apoptosis by depressing mitochondrial transcriptional factor A expression (24).

Kidney transplantation

Ischemia/reperfusion injury and delayed graft function

microRNAs are involved in the regulation of processes as angiogenesis and apoptosis through transforming growth factor beta (TGF-β), endothelin, vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) signalling (25). Up-regulation of miR-182-5p, miR-21-3p and miR146a have been reported in this context (26, 27). The overexpression of miR-146a probably represents a compensatory mechanism, since *in vitro* experiments identified the role of miR-146a as a negative regulator of inflammation in tubular cells by down-regulation of the NFkB /C-X-C motive chemokine ligand 8 (CXCL-8) pathway (27). In multivariate logistic regression analysis, the expression of miR-217 and miR-125b (both involved in cellular stress and damage responses by influencing cyclin dependent kinase inhibitor 2 (CKDN2) loci transcript expression) in pre-implantation biopsies together with donor age and type were independently associated with delayed graft function. Delayed graft function could be predicted in 84% of cases, with 92.4% specificity and 64.3% sensitivity (28). More recently (McGuinness D, Shiels P *et. al*, personal communication), delayed graft function has been identified as a manifestation of allostatic overload at a transcriptional level. A composite indicator of accumulated biological stress over the life course is defined as allostatic load, which predisposes to morbidity in case of chronic or repeated stress exposure. Organs undergoing delayed graft function exhibited a greater magnitude of change in transcriptional amplitude and elevated expression of non-coding RNAs and pseudogenes, consistent with increased allostatic load than in those showing immediate graft function. Notably, this study incorporated a validation biopsy set and

individual validation of targets transcriptionally and post-transcriptionally. Additionally, it undertook a cross-comparison with publically available data sets for kidney pathologies, used to identify significant transcriptional commonality for over 20 delayed graft function transcripts, thus providing a clear molecular signature for the burden of 'wear and tear' within the kidney and age-related physiological capability and resilience. The expression of the CDKN2 locus transcripts in this cohort related to the delayed graft function outcome and perfusion status at the transcript level. These results indicate that CDKN2A/p16^{INK4}, ARF/p14 and CDKN2B reflected the allostatic load (and biological age) of these organs pre-perfusion. Regulation of these loci by miR-125b is a notable feature.

T-cell mediated rejection

Global miR expression profiling of grafts with T-cell mediated rejection showed that miR-142-5p, miR-155 and miR-223, could each predict T-cell mediated rejection with high sensitivity and specificity (Area under the curve (AUC) 0.96-0.99) (29). Their correlation with intra-graft CD3 and CD20 mRNA levels suggests that these miRs originate from immune cells infiltrated in the graft (29). Other groups have shown similar patterns for miR-142-5p (30), miR-155 (25, 30, 31), miR-223 (30-32). In addition, miR-10b (anti-apoptotic targeting BCL211 (31)) and miR-30a-3p appeared to be down-regulated in rejecting graft tissue, while correlating with renal tubule specific mRNAs (Na^+-K^+-$2Cl^-$ cotransporter (NKCC-2) (29). In a small set of biopsies, eight miRs turned up to be upregulated and 12 miRs downregulated in T-cell mediated rejection (34), with some of them targeting pathways highly relevant in leukocyte function (for example hsa-miR-611 targeting glycosyltransferase like domain containing 1 (GTDC1)).

Vitalone *et al*. (35) identified 19 miRs that may target the differentially expressed mRNAs in T-cell mediated rejection. Validation of these miRs in an independent set of biopsies

revealed significant up-regulation of 3 miRs (Table 1) and down-regulation of 6 miRs in rejecting vs non-rejecting graft tissue. All up-regulated miRs were associated with tubulitis and interstitial inflammation, suggesting infiltrating lymphocytes as the origin of these miRs, whereas miR-204, miR-210 and miR-10b-3p negatively correlated with Banff scores. The TGF-β signalling pathway, and in particular forkhead box P3 (FOXP3) regulated transcription, is common to the regulatory action of all these miRs (35).

Oghumu *et al.* (32) have identified a panel of 25 miRs significantly different expressed in grafts from recipients with acute rejection compared to acute pyelonephritis. Interestingly, some previously reported down-regulated miRs in T-cell mediated rejection grafts including miR-23-3p (25), miR-30a-5p (29), miR-30d-5p (29), miR-30c-5p (29, 31) and miR-99b-5p (25) were significantly up-regulated in acute pyelonephritis compared to rejection biopsies (32).

Antibody-mediated rejection

Up-regulation of miR-146-5p, miR-182, miR-21-3p, miR-1228 and let-7i, involved in inflammation, chemokine and cytokine signaling, apoptosis and interleukin signaling, has been observed in grafts with antibody-mediated rejection (25). Increased miR-142-5p expression levels were observed in peripheral blood mononuclear cells and grafts from recipients with chronic antibody-mediated rejection compared to normal allografts and peripheral blood mononuclear cells from stable kidney transplant recipients. miR-142-5p overexpression was associated with down-regulation of 41 genes related to a cell-mediated immune response (36). Of note, miR-146-5p as well as miR-142-5p were also significantly up-regulated in grafts with T-cell mediated rejection (29, 30).

To unravel the molecular mechanisms underlying chronic antibody-mediated rejection, Rascio *et al.* (37) performed a combined mRNA and miR expression analysis in peripheral blood mononuclear cells from kidney recipients with chronic antibody-mediated rejection

and normal allografts. Four miRs were found to be modulators of 6 mRNAs involved in the type I interferon (IFN) signalling network. miR validation in an independent set of peripheral blood mononuclear cells revealed a significant down-regulation of miR-148b-3p, miR-29b-3p and miR-769-5p. Validation of these findings has not been forthcoming. No overlapping miR signature could be identified with the data of Danger *et al*. (36), possibly related to methodological differences and definition of the controls.

Interstitial fibrosis and tubular atrophy

Fifteen miRs have been identified as being of interest in interstitial fibrosis and tubular atrophy (IF/TA), relating to regulation of lymphocyte proliferation, B, T and natural killer (NK) cell activation/differentiation. The expression of five of these miRs has been independently validated, with miR-142-3p (38, 39) and miR-32 being up-regulated and miR-204, miR-107 and miR-211 (39) being down-regulated in grafts with IF/TA (40). The up-regulation of miR-142-5p, miR-21 (41), miR-223 and down-regulation of miR-30b, miR-30c and miR-338-3p was found by Ben-Dov *et al.* (38) and confirmed in an independent but small set of IF/TA biopsies. Bioinformatic analysis has identified SMAD 7 (an inhibitor of TGFβ mediated fibrosis) as a possible target of miR-21.

Of note, similar miR expression data for miR-142-3p (29, 30, 32, 35), miR-142-5p (29, 30), miR-223 (29-31), miR-204 (29, 32, 35), miR-30c (29, 31, 32) and miR-30b (29) was found in intragraft miR profiling studies in rejecting allografts, T cell-mediated rejection in particular.

microRNA as biomarkers of kidney disease

microRNAs are highly stable in both plasma and urine, making them attractive biomarkers (10).

Acute kidney injury

AKI coincides with reduced expression levels of most, but not all, circulating miRs (42). In the plasma of AKI patients, miR-16 and miR-320 were found to be down-regulated, while miR-210 was up-regulated (43). This upregulation appeared to be a strong independent prognostic factor for 28-day survival of critically ill patients with AKI (43). Likewise, urinary levels of miR-21 and miR-155 could successfully distinguish patients with and without AKI (44). This is in keeping with other findings that urinary miR-21 appeared to be more associated with AKI prognosis and other adverse clinical outcomes than plasma miR-21 levels (45, 46). In contrast to the Du study (46), serum miR-21 was down-regulated in 2 studies that included patients who developed AKI after cardiac surgery. Serum miR-21 levels turned up to be predictive for the development of AKI when sampled prior to cardiac surgery (AUC 0.70) (48) and 6h after cardiac surgery (AUC 0.90) (49). In the latter study, also urinary miR-21 levels were predictive for AKI (49). Interestingly, ischemic preconditioning could increase endogenous miR-21 expression and further protect kidney function (50).

Urinary miR-200c and miR-423 were up-regulated in AKI patients based on microRNA array analyses (45). In a small longitudinal study, a panel of 10 miRs could be used for AKI diagnosis in Intensive Care Unit (ICU) patients with nearly 100% sensitivity and specificity and 4 of them were associated with AKI severity (47). Another set of four miRs was associated with AKI development several days before serum creatinine in cardiac surgery patients (47). miR-192 was put forth to diagnose AKI when sampled 2h after cardiac surgery, however, with a rather poor sensitivity (66.7%) and specificity (62.9%) (51). Likewise, urinary miR-30c-5p performed well also as a biomarker of AKI after cardiac surgery, and even better compared to protein-based markers such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (Kim-1) (52). In contrast-induced nephropathy, miR-30a, -c and –e appeared to be significantly higher in comparison with patients who

243 received contrast but without nephropathy, with a peak at 12h after the contrast
244 administration (53). Although all 3 miRs correlated positively with serum creatinine, they
245 only increased in 55.5% of the contrast-induced nephropathy patients while they remained
246 stable in 44.5% of the patients. The positive predictive value of these 3 microRNAs varied
247 between 91.3% and 94.9% while the negative predictive value varied between 61.3 and
248 78.2% (53). Sun *et al.* (54) confirmed the increase of miR-30a and –e and, in addition, they
249 found miR-188 to be increased in a similar population.
250
251 **Transplantation**
252 The value of microRNA as biomarkers of different graft-associated pathologies were
253 investigated either by quantification of a set of miRs known to be dysregulated in the graft
254 (27, 30, 39-41, 55-57) or involved in pathways of interest (58) or by performing a global miR
255 profiling on blood cells (36, 59), serum or plasma (33, 60) and urine (57, 61).
256
257 *Ischemic-reperfusion injury*
258 A significant up-regulation of miR-146a was observed in urine samples of recipients
259 transplanted with a deceased donor, compared to a living donor and was thus suggested as
260 a diagnostic marker for ischemia/reperfusion injury injury (27).
261
262 *T-cell mediated rejection*
263 Both senescence associated miR-223 and miR-142-3p are up-regulated in the graft and
264 peripheral blood mononuclear cells of patients with acute T-cell mediated rejection (30).
265 Increased miR-223 levels, along with increased levels of miR-10a, were also observed in the
266 serum of a small number transplant recipients during T-cell mediated rejection (55). The up-
267 regulation of serum miR-99a and miR-100 was also reported in kidney transplant recipients

with T-cell mediated rejection with serum miR-99a levels discriminating recipients with acute rejection from stable transplant recipients (AUC 0.75) and recipients with delayed graft function (AUC 0.81) (60). However, previous miR profiling studies reported decreased levels of miR-99a expression in T-cell mediated rejection kidney allografts (29, 32). Paired tissue and blood analysis should therefore be performed to determine the significance of these conflicting results.

In multivariate logistic regression analysis, a panel of 5 miRs isolated from blood cells (miR-15b, miR-16, miR103a, miR106a and miR-107) could accurately discriminate an acute vascular rejection (Banff II-III) from stable graft function (AUC 0.97). The difference between T-cell mediated vascular rejection and all other phenotypes (Borderline, Banff I and antibody-mediated rejection) was less distinct (AUC 0.82) (59).

Urinary levels of miR-10a showed to be significantly up-regulated, while miR-10b and miR-210 were down-regulated in urine samples of recipients with acute T-cell mediated rejection compared to recipients with stable graft function. Furthermore, expression levels of urinary miR-210, involved in cellular aging, related to biopsy-proven rejection severity with levels normalizing after rejection treatment. However, receiver operating characteristic (ROC) analysis revealed a rather weak specificity of 52% and sensitivity of 74% (AUC 0.70) for the distinction between acute rejection and stable graft function (61). A decreased expression of miR-210-3p was confirmed in urine pellets from transplant recipients with T-cell mediated rejection (56). In this study, higher expression levels of urine miR-155-5p – also reported as highly expressed in the graft during acute T-cell mediated rejection (25, 29-31) – were more discriminative for the diagnosis of acute rejection (AUC 0.88) and in a few cases, increased levels or miR-155-5p were observed prior to the rejection episode (56).

Antibody-mediated rejection

In peripheral blood mononuclear cells from recipients with chronic, but not acute, antibody-mediated rejection, miR-142-5p was upregulated. In ROC analysis, the discriminative capacity for chronic antibody-mediated rejection versus stable controls was rather fair with an AUC of 0.74 (36). Although the authors suggest the specificity of this miR in chronic antibody-mediated rejection, other groups also reported increased levels of this hematopoietic miR in peripheral blood mononuclear cells and grafts of recipients with an acute T-cell mediated rejection (29, 30).

Interstitial fibrosis and tubular atrophy

Lower miR-211 and miR-204 expression levels and an up-regulation of miR-142-3p were found in urine pellets of recipients with a biopsy proven IF/TA compared to recipients with a normal histology and graft function (40). miR levels in the urine appeared to be correlated with miR expression levels in the graft (40). These findings were confirmed in a cohort of recipients with established IF/TA (57). A significant down-regulation of miR-200b, miR-375, miR-193b and up-regulation of miR-423-5p and miR-345 has been observed in these two miR discovery datasets. A larger prospective validation study revealed a significant down-regulation of miR-200b in urine pellets of recipients with established IF/TA one year after transplantation compared to recipients without IF/TA. No correlation was found between the expression of miR-200b and proteinuria (57). A significant down-regulation of miR-200b was also reported in plasma samples of recipients with IF/TA compared to stable kidney transplant recipients (58). Higher expression levels of miR-21 were measured in serum from recipients with a biopsy proven IF/TA thereby showing a gradually increase with IF/TA severity. ROC analysis for the diagnosis of severe IF/TA (grade III) revealed an AUC of 0.89. Furthermore, no correlations were found between miR-21 levels and the presence of other acute or chronic Banff lesions in this study, although study groups were small (41).

Discussion and conclusion

Insight in the pathophysiological role of microRNA in chronic kidney disease is growing, and miR targeting therapies are being introduced in the clinic. In acute kidney disease and transplantation, on the contrary, the role of microRNAs in the pathophysiological processes are still in the exploratory phase and thus need several confirmation and validation steps before they can be seen as a therapeutic target. From the few papers that have been published to date in acute kidney injury, microRNAs is a strong regulator of the NFκB pathway. This pathway has long been considered as a major target in –inflammatory diseases because of its role in proinflammatory cytokine production, cell survival and leucocyte recruitment. Lately, it became, however, clear that the NFκB pathway also plays a role in the protection processes against inflammation thanks to its anti-apoptitic functions. This dual mechanism hampered the development of anti-NFκB pathway drugs. In the transplantation field, more insights in the pathophysiology of transplant related processes as well as diagnostic biomarkers for diagnosis are eagerly awaited. A biomarker is 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic responses, or pharmacological responses to a therapeutic intervention' (ref). As their remarkable stability in body fluids make them attractive biomarkers, several microRNAs are put forward as biomarkers for the diagnosis of kidney diseases and transplant related pathologies. Clinically useful biomarkers should have a high sensitivity and specificity, a high negative and positive predictive value and a diagnostic AUC nearing 1.0. Currently, these latter characteristics remain under the desired results, which hamper the clinical implementation of microRNAs as diagnostic or prognostic markers of disease. After transplantation, a combined panel of five miRs, however, was able to discriminate T-cell mediated vascular rejection form stable graft function with an AUC of

0.97 (ref). Most probably, combining the right microRNAs to a diagnostic panel will be the future. Nowadays, most studies remain in the exploratory phase and there is an urgent need for larger clinical prospective trials to validate the results and thoroughly investigate their diagnostic and prospective potential. Next, a standardized method for sampling and analysis is highly recommended to improve between-group comparison in external validation set-ups.

Search terms

The following databases were used: Pubmed and Web of Science. No limits were applied on publication date and last data base search was performed on May 25th 2018. The following Mesh terms were used: 'microRNA or microRNA or miR AND acute kidney injury'; microRNA or microRNA or miR AND renal function'; 'microRNA or microRNA or miR AND acute renal impairment' 'MicroRNAs AND Kidney Transplantation'. Only papers on human research were withheld for this review.

Disclosure

The authors have nothing to declare

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Table 1: microRNAs in acute kidney injury

microRNA AND PATHOPHYSIOLOGY							
Phenotype	**Study (author)**	**Study population**	**Sample**	**miR**	**Internal validation (independent cohort)**	**Overlap with other studies**	
						Upregulation	**Downregulation**
AKI	Lan YF *et al.* (2012)(1)	Critical patients with AKI (n=16) Critical patients without AKI (n=10) Healthy controls (n=14)	Serum and urine samples	↑ urinary miR-494 in AKI patients	-	-	-
AKI	Wang S *et al.*(2017)(2)	Septic AKI (n=15) Non-septic AKI (n=15) Septic non-AKI (n=15) Healthy volunteers (n=15)	Circulating endothelial cells	↑ miR-107 in septic AKI patients	-	-	-
AKI	Chen *et al* (2016)(3)	Critical patients with AKI (n=11) Critical patients without AKI (n=7) Healthy volunteers (n=4)	Serum and urine samples	↑ urinary let-7d, life-26-3p, miR-16, miR-451, miR-486-5p, miR-518*, miR-720 ↓ 21 miRs	miR-16 was further validated in animal studies	-	-
AKI	Kang *et al.* (4)	Children after cardiac surgery: Control group (n=249): - AKI (n=115) - Non-AKI (n=134) RIPC group (n=200)	Plasma and urine	↑ miR-21 after RIPC	-	-	-

		- AKI (n=38) - Non-AKI (n=162)					
AKI	Guo *et al.* (5)	AKI patients with cisplatin (n=21)	Kidney biopsy	↑ miR-709	-	-	-
AKI	Liu *et al.* (6)	AKI patients (n=4)	peripheral blood mononuclear cells	Overexpression of miR-101 led to reduced c-Rel and IL-2 expression			
AKI	Ge *et al.* (7)	**Discovery cohort:** Septic AKI (n=6) Septic non-AKI (n=6) Controls (n=3) **Validation cohort:** Septic AKI (n=35) Septic non-AKI (n=30)	Serum	40 miRs were differentially expressed between AKI and non-AKI patients	↑ miR-4270, miR-4321, miR-3165 ↓ miR-142-5p, miR-22-3p, miR-191-5p, miR-23a-3p, miR-4456	-	-
microRNA AS BIOMARKERS							
Phenotype	**Study (author)**	**Study population**	**Sample**	**miR**	**Internal validation (independent cohort)**	**Overlap with other studies**	
						Upregulation	**Downregulation**
AKI	Lorenzen (2011) (8)	**Discovery cohort:** Critically ill patients with AKI (n=5) Healthy controls (n=5) **Validation cohort:** AKI (n=77) Healthy controls (n=30) AMI (n=18)	Plasma	13 miRs were different between AKI patients and healthy controls	↓ miR-16 and miR-320 in AKI ↑ miR-210 in AKI	-	-
Ischemic or	Saikumar (2012) (9)	Critically ill patients with	Urine	↑ miR-21 in AKI	-	↑ miR-21 (10, 11)	-

septic AKI		elevated Scr and elevated levels of urinary KIM-1 (n=22) Healthy volunteers 'n=25)		↓ miR-155 in AKI			
Severe AKI	Du (2013) (10)	Stage 1 or 2 AKI defined by AKIN after cardiac surgery (n=80) Non-AKI group (n=40)	Urine and plasma	↑ miR-21 in AKI in both urine and plasma samples	-	↑ miR-21 (9, 11)	-
AKI	Ramachandran (2013) (11)	**Discovery cohort:** ICU patients with AKI (n=6) Healthy volunteers (n=6) **Validation cohort:** Healthy volunteers (n=74) ICU patients without kidney disease (n=23) ICU patients with AKI (n=71) Kidney Tx patients with tubular injury (n=27)	Urine	378 microRNAs were selected for validation with qPCR in the validation cohort	↑ miR-21, miR-200c, miR-423 in AKI patients ↓ miR-4640 in AKI patients	↑ miR-21 (9, 10)	-
AKI	Aguado-Fraile (2015) (12)	**Discovery cohort:** ICU patients (n=4) Healthy volunteers (n=10) **Validation cohort:** ICU patients (n=35) Cardiac surgery patients (n=41) Healthy volunteers (n=20)	Serum	10 miRs were selected (more than 2-folds change)	↓ miR-101-3p, miR-127-3p, miR-210-3p, miR126-3p, miR-26b-5p, miR-29a-3p, miR-146a-5p, miR-27a-3p, miR-93-3p, miR-10a-5p in AKI in ICU patients ↓ miR-127-3p, miR-26b-5p, miR-146a-5p, miR-93-3p in patients after	-	-

					CS		
AKI	Zou (2017) (13)	AKI after cardiac surgery (n=27) Non-AKI after cardiac surgery (n=44)	Urine	↑ miR-30c-5p, miR-192-5p, miR-378a-3p	-	↑ miR-30c (14) ↑ miR-192(15)	-
Contrast-induced AKI	Gutiérrez-Escolano (2015) (14)	contrast-induced nephropathy patients (n=92) Non-contrast-induced nephropathy patients (n=92)	Plasma	↑ miR-30a, -c and -e	-	↑ miR-30a (16) ↑ miR-30c (13) ↑ miR-30e (16)	-
Contrast-induced AKI	Sun *et al.* (2016) (16)	Patients with AKI after elective coronary angiography or percutaneous coronary intervention (n=71)	Plasma	↑ miR-188, miR-30a and -e	-	↑ miR-30a (14) ↑ miR-30e (14)	-
AKI	Arvin *et al.* (2017)(17)	Stage 2-3 AKI after cardiac surgery (n=18) Stage 0-1 AKI after cardiac surgery (n=97)	Serum and urine	↓ urinary and serum miR-21	-	-	↓serum miR-21(18)
AKI	Gaede *et al.* (2016)(18)	AKI after cardiac surgery (n=14) Non-AKI after cardiac surgery (n=14)	Serum	↓ serum miR-21	-	-	↓serum miR-21(17)
AKI	Zhang *et al.* (2017) (15)	AKI after cardiac surgery (n=35) Non-AKI after cardiac surgery (n=35)	Plasma	↑ miR-192	-	↑ miR-192(13)	

Abbreviations: n: number; AKI: acute kidney injury; AMI: acute myocardial infarction; Scr: serum creatinine; KIM-1: kidney injury molecule-1 ; AKIN: acute kidney injury Network; ICU: intensive care unit; miR: microRNA, RIPC: remote ischemic preconditioning, qPCR: quantitative polymerase chain reaction.

Table 2: microRNA in kidney transplantation

microRNA AND PATHOPHYSIOLOGY							
Phenotype	**Study (author)**	**Study population**	**Sample**	**miR**	**Internal validation**	**Overlap with other studies**	
						Upregulation	**Downregulation**
ATN/delayed graft function	Wilflingseder *et al.*, 2013 (19)	ATN (n=14) normal PBX (n=10)	Biopsy	↑ 7 miRs	-	↑ miR-21-3p (20) ↑ miR-182-5p (20)	-
ATN/delayed graft function	Wilflingseder *et al.*, 2014 (20)	ATN + T0BX (n=8) normal PBX + T0BX (n=10)	Biopsy	↑ 29 miRs	-	↑ miR-21-3p (19) ↑ miR-182-5p (19)	-
ATN/delayed graft function	Amrouche *et al.*, 2016 (21)	ATN (n=19) Normal PBX (n=15)	Biopsy	↑ miR-146a	-	-	-
ATN/delayed graft function	McGuinness *et al.*, 2016 (22)	**Discovery cohort:** T0BX good performing allografts within 2 years post-Tx (n=5) T0BX poor performing allografts within 2 years post-Tx (n=5) **Validation cohort:** T0BX delayed graft function (n=27) T0BX no delayed graft function (n= 67) **Model validation cohort:** T0BX delayed graft function (n=10)	Biopsy	11 differentially expressed miRs (fold changes not reported)	↓ miR-125b ↓ miR-217	-	-

		T0BX no delayed graft function (n=14)					
Acute T-cell mediated rejection (Banff I)	Sui *et al.*, 2008 (23)	T-cell mediated rejection (n=3) resected tissue RCC (n=3)	Biopsy	↑ 8 miRs ↓ 12 miRs	-	↑ miR-125a (24) ↑ miR-602 (24) ↑ miR-628 (24) ↑ miR-658 (24)	↓ miR-17-3p (24) ↓ miR-330 (24) ↓ miR-483 (24) ↓ miR-611 (24) ↓ miR-663 (24)
Acute T-cell mediated rejection (Banff I)	Anglicheau *et al.*, 2009 (25)	**Discovery cohort:** T-cell mediated rejection (n=3) normal PBX (n=4) **Validation cohort:** T-cell mediated rejection (n=9) normal PBX (n=17)	Biopsy	↑ 10 miRs ↓ 43 miRs	↑ miR-142-5p ↑ miR-155 ↑ miR-223 ↓ miR-10b ↓ miR-30a-3p	↑ miR-142-3p (26-28) ↑ miR-155 (19, 24, 28) ↑ miR-223 (24, 27, 28) ↑ miR-342-3p (26, 27) ↑ miR-142-5p (28) ↑ miR-21 (24) ↑ miR-146a (24) ↑ miR-650 (24)	↓ miR-30c (24, 27) ↓ miR-125a (19, 27) ↓ miR-204 (26, 27) ↓ miR-30a-5p (27) ↓ miR-30d-5p (27) ↓ miR-32 (24) ↓ miR-125b-5p (27) ↓ miR-193b (19) ↓ miR-99a-5p (27) ↓ miR-100-5p (27) ↓ miR-126-3p (27) ↓ miR-130a-3p (27) ↓ miR-10b (24) ↓ miR-30a-3p (24) ↓ miR-27b (19)
Acute T-cell mediated rejection (Banff I-II)	Wilflingseder *et al.*, 2013 (19)	T-cell mediated rejection (n=30) normal PBX (n=10)	Biopsy	↑ 4 miRs ↓ 18 miRs	-	↑ miR-155 (24, 25, 28) ↑ miR-150-5p (27)	↓ miR-125a (25, 27) ↓ miR-27b (25) ↓ miR-193b (25) ↓ miR-181a (26) ↓ miR-23b-3p (27) ↓ miR-99b-5p (27)
Acute rejection	Oghumu *et al.*, 2014 (27)	AR (heterogeneous) (n=5) normal T0BX (n=4)	Biopsy	↑ 13 miRs ↓ 16 miRs	-	↑ miR-142-3p (25, 26, 28) ↑ miR-223-3p (24, 25, 28) ↑ miR-342-3p (25, 26)	↓ miR-30c-5p (24, 25) ↓ miR-125a-5p (19, 25) ↓ miR-204-5p (25, 26) ↓ miR-23b-3p

						↑ miR-150-5p (19)	(19) ↓ miR-30a-5p (25) ↓ miR-30d-5p (25) ↓ miR-99b-5p (19) ↓ miR-99a-5p (25) ↓ miR-100-5p (25) ↓ miR-125b-5p (25) ↓ miR-126-3p (25) ↓ miR-130a-3p (25)
Acute rejection	Liu *et al*., 2015 (24)	AR (n.o.s) (n=15) normal TxBX (n=15)	Biopsy	75 differentially expressed miRs (fold changes not reported)	-	↑ miR-155 (19, 25, 28) ↑ miR-223 (25, 27, 28) ↑ miR-21 (25) ↑ miR-125a (23) ↑ miR-146a (25) ↑ miR-602 (23) ↑ miR-628 (23) ↑ miR-629 (23) ↑ miR-650 (25)	↓ miR-30c (25, 27) ↓ miR-10b (25) ↓ miR-17-3p (23) ↓ miR-30a-3p (25) ↓ miR-32 (25) ↓ miR-330 (23) ↓ miR-483 (23) ↓ miR-611 (23) ↓ miR-663 (23)
Acute T-cell mediated rejection	Bijkerk *et al*., 2017 (29)	**Discovery cohort:** stable Tx (clinical) (n=4) T-cell mediated rejection (n=6) **Validation cohort:** stable Tx (clinical) (n=13) T-cell mediated rejection (n=13)	**Plasma**	not all differentially expressed miRs reported	↑ miR-17 ↑ miR-140-3p ↑ miR-130b ↑ miR-122 ↑ miR-192 ↓ miR-135a ↓ miR-199a-3p ↓ miR-15a		
Acute T-cell mediated rejection	Vitalone *et al.*, 2015 (26)	T-cell mediated rejection (n=29) normal TxBX (n=68)	Biopsy	↑ 3 miRs miR-142-3p miR-342-3p miR-25 ↓ 6 miRs	-	↑ miR-142-3p (25, 27, 28) ↑ miR-342-3p (25, 27)	↓ miR-204 (25, 27) ↓ miR 181a (19)

				miR-181a miR-192 miR-204 miR-215 miR-10b-3p miR-615-3p			
Acute T-cell mediated rejection (Banff I)	Soltaninejad *et al.*, 2015 (28)	T-cell mediated rejection (n=17) normal TxBX (n=18)	Biopsy	↑ 4 miR miR-142-5p miR-155 miR-142-3p miR-223	-	↑ miR-155 (19, 24, 25) ↑ miR-142-3p (25-27) ↑ miR-223 (24, 25, 27) ↑ miR-142-5p (25)	-
Acute antibody-mediated rejection	Wilflingseder *et al.*, 2013 (19)	morphologic antibody-mediated rejection (n=11) normal PBX (n=10)	Biopsy	↑ 6 miRs	-	-	-
Chronic antibody-mediated rejection	Danger *et al*., 2013 (30)	chronic antibody-mediated rejection (n=18) stable Tx (clinical) (n=30) AR (heterogeneous) (n=9) chronic antibody-mediated rejection (n=21) normal TxBx (n=18)	peripheral blood mononuclear cell Biopsy	not all differentially expressed miRs reported ↑ miR-142-5p	↑ miR-142-5p -	- -	- -
Chronic antibody-mediated rejection	Rascio *et al.,* 2015 (31)	**Discovery cohort:** chronic ABRM (n=5) normal PBX (n=5) **Validation cohort:**	peripheral blood mononuclear cell	↓ 16 miRs	↓ miR-148b-3p ↓ miR-769-5p ↓ miR-29b-3p	-	-

		chronic antibody-mediated rejection (n=5) normal PBX (n=5)					
Acute pyelonephritis	Oghumu *et al.*, 2014 (27)	APN (n=11) AR (heterogeneous) (n=5)	Biopsy	↑ 24 miRs ↓ 1 miR	-	-	-
IF/TA	Scian *et al.*, 2011 (32)	**Discovery cohort:** IF/TA (n=13) normal PBX (n=5) **Validation cohort:** IF/TA (n=19) normal PBX (n=8)	Biopsy	56 differentially expressed miRs (fold changes not reported)	↑ miR-142-3p ↑ miR-32 ↓ miR-204 ↓ miR-107 ↓ miR-211	↑ miR-142-3p (33, 34)	↓ miR-211 (34)
IF/TA	Ben-Dov *et al.*, 2012 (33)	**Discovery cohort:** n=4 IF/TA n=4 normal PBX **Validation cohort:** n=10 IF/TA n=8 normal PBX	Biopsy	↑ 28 miRs ↓ 7miRs	↑ miR-142-3p ↑ miR-142-5p ↑ miR-21-5p ↑ miR-21-3p ↑ miR-223 ↓ miR-30b ↓miR-30c ↓ miR-338-3p	↑ miR-142-3p (32, 34) ↑ miR-21-5p (35) ↑ miR-142-5p (34)	-
IF/TA	Glowacki *et al.*, 2013 (35)	severe graft fibrosis (explant) (n=11) non-pathologic parenchyma of urologic cancer (kidney/urinary tract) (n=12)	Biopsy	↑ miR-21	-	↑ miR-21 (33)	-
IF/TA	Soltaninejad *et al.*, 2015 (34)	IF/TA (n=16) normal TxBX (n=17)	Biopsy	↑ miR-142-3p ↑ miR-142-5p ↓ miR-211	-	↑ miR-142-3p (32, 33) ↑ miR-142-5p (33)	-
microRNAs AS BIOMARKERS							

Phenotype	Study (author)	Study population	Sample	miR	Internal validation	Overlap with other studies	
						Upregulation	Downregulation
Ischemia/reperfusion injury	Amrouche *et al.*, 2016 (21)	LD (n=16) DD (n=35)	**Urine pellet**	↑ miR-146a	-	-	-
Acute T-cell mediated rejection (Borderline, Banff I-II)	Lorenzen *et al.*, 2011 (36)	**Discovery cohort:** T-cell mediated rejection (n=5) normal PBX (n=5) **Validation cohort:** T-cell mediated rejection (n=68) normal PBX (n=20) UTI (n=13)	**Total urine**	↑ 5 miRs ↓ 16 miRs	↑ miR-10a ↓ miR-210 ↓ miR-10b	-	-
Acute T-cell mediated rejection (Banff I-II)	Betts *et al*., 2014 (37)	T-cell mediated rejection (n=8) pre-T-cell mediated rejection (n=3) post-T-cell mediated rejection (n=6) 1 year-T-cell mediated rejection (n=6) healthy controls (n=4)	**Serum**	↑ miR-223 ↑ miR-10a	-	-	-
Acute rejection	Tao et al., 2015 (38)	**Discovery cohort:** AR (n.o.s) (n= 4) stable Tx (clinical) (n= 4) **Validation cohort:** AR (n.o.s) (n=12) delayed graft function (n=15) stable Tx (clinical) (n=11)	**Serum**	↑ 6 miRs	↑ miR-99a ↑ miR-100	-	-
T-cell mediated rejection	Millán *et al*., 2017 (39)	T-cell mediated rejection (n=8)	**Urine pellet**	↑ miR-155 ↑ miR-142-3p	-	-	-

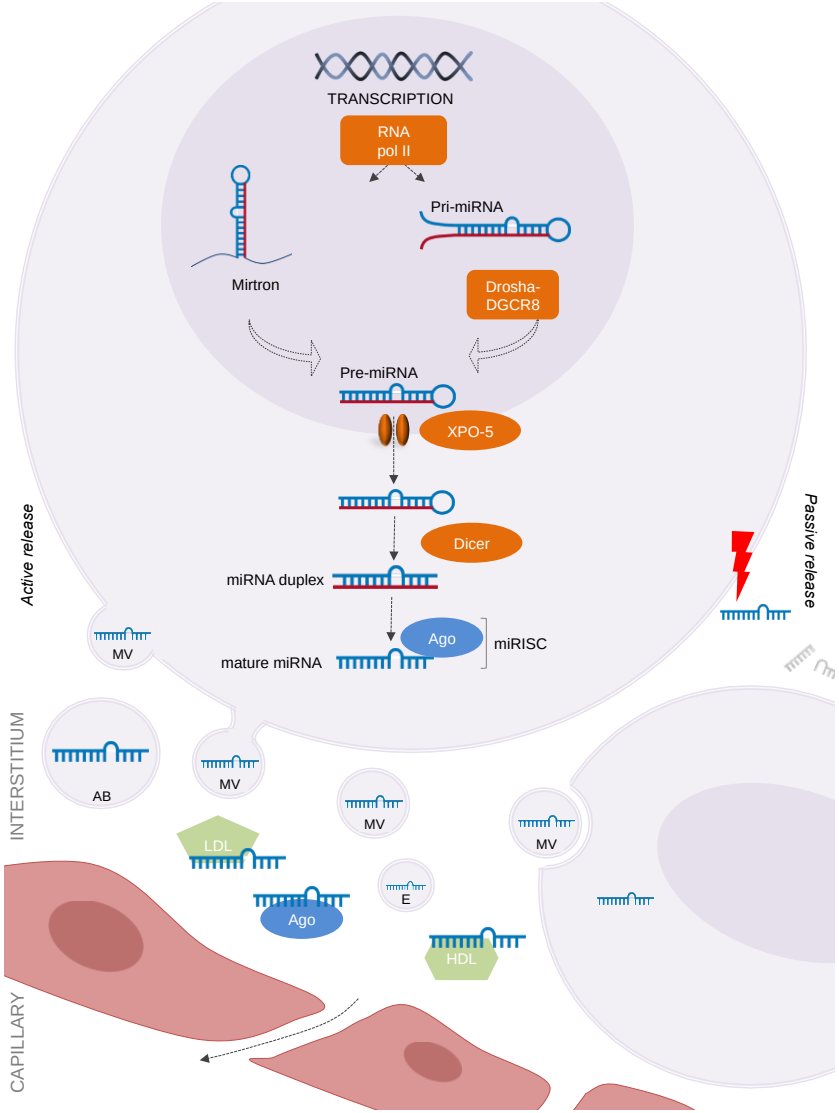
		no T-cell mediated rejection (n=71)		↓ miR-210-3p			
Acute T-cell mediated vascular rejection (Banff II-III)	Matz *et al.*, 2016 (40)	**Discovery cohort:** stable Tx (clinical) (n = 4) T-cell mediated vascular rejection (n = 4) **Validation cohort:** T-cell mediated vascular rejection (Banff II-III) (n=24) stable Tx (clinical) (n=40) UTI (n=11) Borderline (n=17) Banff IA-IB (n=15) antibody-mediated rejection (n=15) Mixed T-cell mediated rejection-antibody-mediated rejection (n=6) IF/TA (n=33)	**Blood cells**	29 miRs differentially expressed miRs (fold changes not reported)	↓ miR-15b ↓ miR-16 ↓ miR-106a ↓ miR-103a ↓ miR-107 ↓ miR-15a	-	-
Chronic antibody-mediated rejection	Danger *et al.*, 2013 (30)	**Discovery cohort:** chronic antibody-mediated rejection (n= 9) stable Tx (clinical) (n=10) **Validation cohort:** chronic antibody-mediated rejection (n=18) stable Tx (clinical) (n=30)	**peripheral blood mononuclear cell**	not all differentially expressed miRs reported	↑ miR-142-5p	-	-

		AR (heterogeneous) (n=9)					
IF/TA	Scian *et al.*, 2011 (32)	IF/TA (n=7) normal PBX (n=7) **Prospective validation cohort:** n=36 kidney Tx recipients (108 samples)	**Urine pellet**	↑ miR-142-3p ↓ miR-211 ↓ miR-204	-	↑ miR-142-3p (41)	↓ miR-211 (41) ↓ miR-204 (41)
IF/TA	Maluf *et al.*, 2014 (41)	**1st Discovery cohort:** IF/TA (n= 10) normal PBX (n= 12) **Validation cohort:** IF/TA (n=7) normal PBX (n=10) **2nd discovery cohort** (3 months post Tx with IF/TA 24 months) (n=10) (3 months post Tx without IF/TA 24 months) (n=10) **Prospective validation cohort:** n=66 kidney Tx recipients (132 samples) 3-6 months 18-24 months	**Urine pellet**	**1st Discovery cohort:** ↑ 10 miRs ↓ 12 miRs **2nd discovery cohort** 48 miRs differentially expressed miRs (fold changes not reported)	↑ miR-142-3p ↓ miR-125b ↓ miR-203 ↓ miR-204 ↓ miR-211 **Prospective validation cohort** ↑ miR-200 ↑ miR-140-3p ↓ miR-99a ↓ miR-200b	↑ miR-142-3p (32)	↓ miR-204 (32) ↓ miR-211 (32)
IF/TA	Glowacki *et al.*, 2013 (35)	IF/TA grade I (n=12) IF/TA grade II (n=7) IF/TA grade III (n=10)	**Serum**	↑ miR-21	-	-	-

		no IF/TA (n=13)					
IF/TA	Zununi *et al*., 2017 (42)	stable Tx (clinical) (n=27) IF/TA (n=26) (grade I: n=16, grade III: n=10)	**Plasma**	↑ miR-150 ↑ miR 423-3p ↓ miR-192 (only IF/TA grade III) ↓ miR-200b	-	-	-

Abbreviations: n: number; Scr: serum creatinine; Tx: transplantation; CKD: chronic kidney disease; SLE: systemic lupus erythematosus; DD: deceased donor; LD: living donor; AR: acute rejection; n.o.s.: not otherwise specified; IF/TA: interstitial fibrosis/tubular atrophy; UTI: urinary tract infection; Tx: transplantation; TxBX: transplant biopsy; PBX: protocol biopsy; miR: microRNA; qPCR: quantitative polymerase chain reaction.

Figure 1. microRNA biogenesis and function



microRNA coding regions in the human genome are found either intergenic or in the introns of annotated genes. microRNA synthesis starts in the nucleus where most of the miRs are transcribed by RNA polymerase II into primary miR transcripts (pri-miR) of several kilobases that contain local stem-loop structures. The first step of miR maturation is cleavage at the stem of the hairpin structure by a microprocessor complex consisting of Drosha (an RNase III protein) together with its cofactor DiGeorge Syndrome Critical Region 8 (DGCR8) which releases a small hairpin structure of 70 nucleotides that is termed a precursor miR (pre-miR). Following nuclear processing, pre-miRs are exported to the cytoplasm by exportin 5 (XPO-5) where they are cleaved near the terminal loop by another RNase enzyme called Dicer thereby releasing a ~22 nucleotide miR duplex. This duplex is loaded onto an Argonaute (AGO) protein to generate the RNA-induced silencing complex (RISC). One strand (guide strand) remains in the AGO protein as a biologically active miR

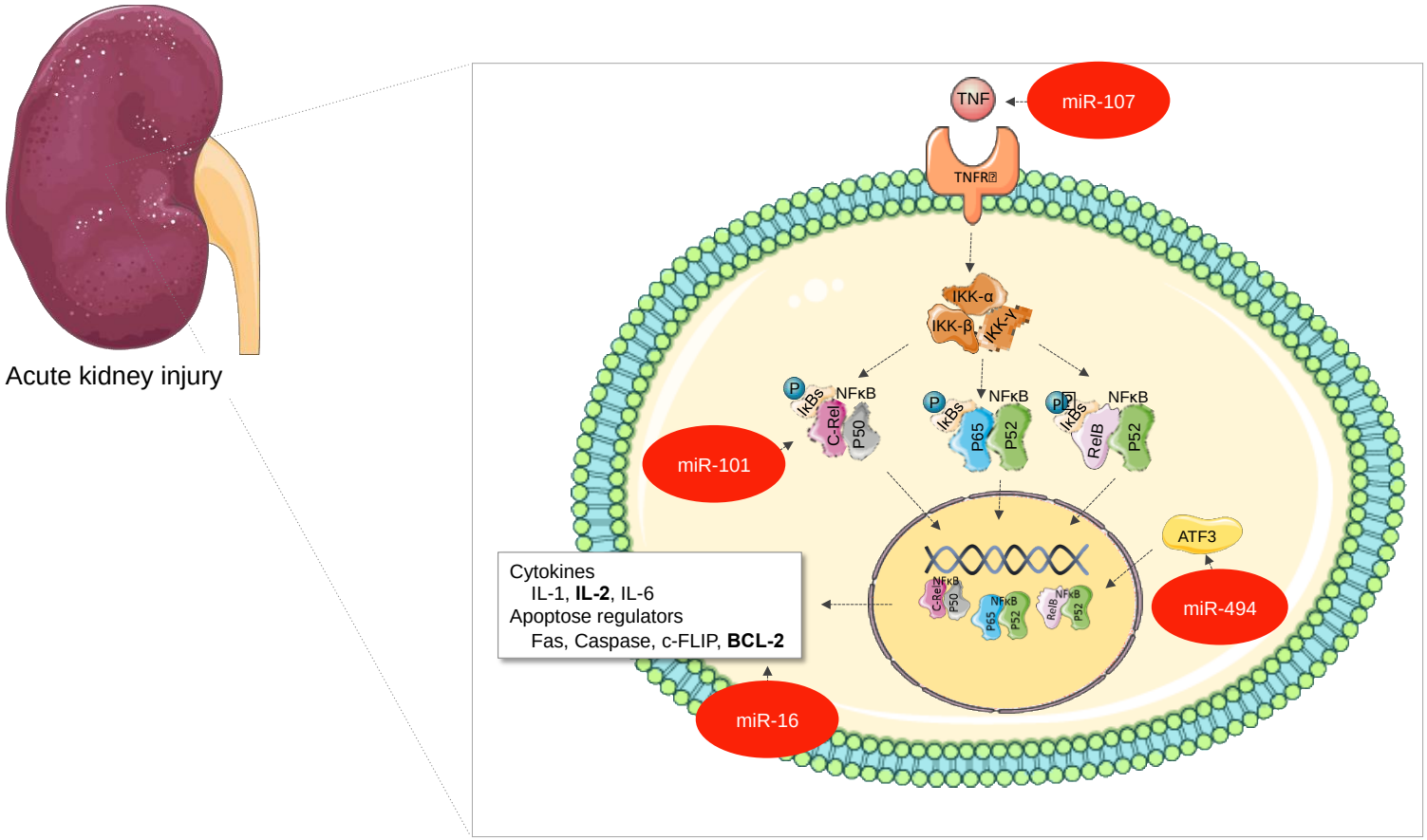
whereas the other stand (passenger strand, known as miR*) is degraded. The mature miR as part of the effector RISC binds to the 3' UTR region of the mRNA and mediate mRNA degradation, destabilization or translational inhibition.

Apart from this canonical pathway, there is an alternative 'mirtron' pathway, independent from Drosha and DGCR8. Mirtrons are miRs that originate from spliced-out introns and that are created when small RNAs bind to the termini of small intronic hairpins (6). Pre-microRNA hairpins with 3' overhangs are so formed and can mature into 22 nucleotides structures, which look and function as normal miRs.

microRNAs exert their repressive function intracellularly, but are also released into the extracellular compartment, with this initiating their role as important intercellular communicators as they are taken up by recipient cells. microRNA can be released passively following cell death or injury, or can be actively secreted in different types of extracellular vesicles, including exosomes, microvesicles and apoptotic bodies. Circulating microRNAs form complexes with RNA binding proteins including Argonaute 2 proteins and lipoproteins (HDL and LDL), which protects them from RNAse-dependent degradation.

Abbrevations: RNA pol II: RNA polymerase 2; DGCR8: DiGeorge Syndrome Critical Region 8; XPO-5: exportin-5; miRISC: microRNA-induced silencing complex; AB: apoptotic body; MV: microvesicle; E: exosome; Ago: Argonaute protein; LDL: low density lipoprotein; HDL: high density lipoprotein

Figure 2. microRNA involved in the NFkB pathway in AKI



miR-107, miR-101, miR-16 and miR-494 and their targets in the NFκB pathway in the pathophysiology of acute kidney disease.

Abbrevations: TNF: tumor necrosis factor; TNFR: TNF receptor; NFκB: nuclear factor κB; IKK: I κB kinase; c-Rel, p50, p52, p65 and RelB : NF-κB transcription factor family members ; ATF3: activating transcription factor 3; IL: interleukin; Fas: first apoptosis signal; c-FLIP: cellular FLICE-like inhibitory protein ; BCL-2: B-cell lymphoma-2