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Editorial Comment

EDITORIAL COMMENT

Circulating miRNAs as biomarkers of kidney disease

Alexander Hüttenhofer[1] and Gert Mayer[2]

[1]Division of Genomics and RNomics, Biocenter Innsbruck, Medical University Innsbruck, Innsbruck, Austria and [2]Department of Internal Medicine IV (Nephrology and Hypertension), Medical University Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria

Correspondence and offprint requests to: Gert Mayer; E-mail: gert.mayer@i-med.ac.at

Abstract

Interest in microRNAs (miRNAs) has dramatically increased in recent years not only because they regulate mRNA expression, and thus many physiological or pathophysiological processes, but also because they could serve as biomarkers. Next to analysis of tissue miRNA expression, measurement in body fluids such as blood or urine is attractive because miRNA in microvesicles or bound to protein is very stable. Currently it is unclear whether these circulating miRNAs are tissue and disease specific or represent more general pathologies like inflammation. In addition pre-analytical sample handling and variable analysis techniques affect the results and thus much more work needs to be done before one can draw a final conclusion about their clinical utility.

Key words: analysis technique, biomarker, circulating microRNA, kidney function, renal disease

MicroRNAs (miRNAs) are evolutionarily conserved, endogenous, non-coding RNAs. They are between 21 and 25 nucleotides in size and regulate gene expression by binding to the 3′-untranslated region of target mRNAs. Until now, ∼3000 human miRNAs have been identified and are estimated to regulate as many as 30% of all human mRNA transcripts [1]. In 2007, Valadi *et al.* [2] reported that cells also export miRNAs from the intra- to the extracellular space *in vitro* [2] and only 1 year later Chim *et al.* [3] isolated placental miRNA from maternal plasma. The exact roles of the circulating miRNAs are yet to be defined, but several lines of evidence suggest that they are not just 'waste' products disposed from cells, but rather play a significant role in intercellular communication [1]. For example, in renal disease patients, the cardiovascular adaption to physical exercise has recently been postulated to be mediated by changes in miRNA expression profiles [4], and in an animal model, structural effects of erythropoietin on the kidney are mediated by miRNAs [5].

Circulating miRNAs are also attractive biomarker candidates. In contrast to protein-free miRNAs, present in body fluids such as serum or urine, protein-bound or microvesicle-derived miRNAs (such as those in exosomes, microparticles, oncosomes or apoptotic bodies) are highly stable [6] because they are protected from nuclease degradation by these microvesicles or by binding to plasma components like high-density lipoprotein cholesterol or proteins such as Argonaute 2 or nucleophosmin [1]. At least conceptually, their expression profile in the blood or urine could mirror changes in cells involved in a particular disease process, since these cells might shed a defined population of miRNAs, for example, by secretion of miRNA-containing exosomes [7].

Oncology was the first medical discipline that investigated miRNAs for their diagnostic potential. Differences in the levels of circulating serum-derived miRNA-21, -155 and -210 were associated with the diagnosis of large B-cell lymphoma [8], miRNA-141 with prostate cancer [9] and miRNA-25 and -223 with lung cancer when compared with healthy controls [10]. Since then, numerous authors have reported on the value of miRNAs as biomarkers in areas other than oncology, but some of these reports, and the conclusions derived, require critical revision, as summarized by Witwer [11]. As demonstrated by Mitchel *et al.* [9], circulating levels of miRNA-141 are able to

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distinguish patients with prostate cancer from healthy controls. However, miRNA-141 has also been found to be elevated in plasma samples of patients with other epithelial cancers. This problem of multiple associations of one (or even a combination of several) miRNAs with different diseases has also been described for other miRNA species. Besides the fact that these findings dampen the enthusiasm that we will soon be able to routinely employ miRNA profiles as biomarkers for increasing diagnostic sensitivity and/or specificity, they also raise the question whether indeed circulating miRNAs are specific at all or merely reflect general disease states, such as inflammation.

In line with the latter assumption, Haider *et al.* [12] showed that changes in circulating miRNA-16, -155, -21 and -126 are associated with at least 10 non-neoplastic conditions [12]. Nonetheless, this does not exclude that some circulating miRNAs are specific for certain pathologies or that miRNAs might support clinical decision making, even once a disease has already been diagnosed. Unfortunately, however, results reported by one group often cannot be replicated by others. In his review, Witwer [11] analysed 32 papers dealing with breast cancer patients only. In total, 143 miRNAs were reported to be differentially regulated, 100 of them in a single publication. Of the remaining 43, discordant expression results (like up-regulation of miRNA expression in one report but down-regulation in another) were reported for 25 miRNAs. Robust concordant expression compared with controls (i.e. a >2-fold change in more than one paper), however, was reported for only 10 miRNAs. Interestingly, reproducible results were mostly reported when the same group performed the experiments, suggesting that pre-analytic factors (e.g. serum, plasma or whole blood, total or microvesicle-derived miRNAs) and the use of correct and stable reference miRNAs for quantification are crucial. Importantly, detection of miRNA levels is known to be heavily dependent on the isolation methods for miRNAs [13, 14] as well on the deep-sequencing or microarray platforms employed for expression profiling [15].

Despite these reservations, circulating miRNAs are an interesting area of research not only in oncology, but also in diabetes [16], cardiovascular medicine [17, 18] and nephrology, where they have important roles during kidney development, homeostasis and disease. Our group performed quantitative polymerase chain reaction miRNA and microarray mRNA expression profiling on renal biopsy sections in discovery and validation cohorts [19] to differentiate stable and progressive cases of chronic kidney disease (CKD). Subsequently, differential expression data of miRNAs were inversely correlated with predicted mRNA targets, which were further characterized by Kyoto Encyclopedia of Genes and Genomes pathway analysis. In the discovery cohort, we observed that miRNA-30d, -140-3p, -532-3p, -194, -190, -204 and -206 were down-regulated in progressive cases of CKD and inversely correlated with 29 up-regulated target mRNAs. These were involved in inflammatory response, cell–cell interaction, apoptosis and intracellular signalling. In the validation cohort, we were able to confirm decreased expression of miRNA-206 and -532-3p and an inverse correlation of these miRNAs with the expression of 9 of 12 predicted mRNA target genes.

In this issue of *Clinical Kidney Journal*, Brigant *et al.* [20] report their results about circulating miRNA levels in controls and patients with various stages of chronic kidney disease (CKD stages 3–5, haemodialysis and after renal transplantation). Based on initial experimental data, they focused on five miRNA species. The strength of the study is that the authors used a 'spike' in miRNA as an internal control to avoid a bias introduced by an unreliable reference gene; in addition, they correlated their miRNA findings with established serum markers of uraemic toxicity such as indoxyl sulphate, p-cresyl sulphate or serum parathyroid hormone and phosphate levels. In stage 3–5 CKD patients, expression of all five miRNA levels was found to be increased in serum. In contrast, expression of these miRNA levels was decreased after transplantation when compared with control subjects; in haemodialysis patients, some were up-regulated, while others were down-regulated. A potential concern regarding the study is the fact that comorbidities, medication used and even some demographic features such as age, which also affect miRNA expression profiles, were not perfectly matched between groups. Clinical data on renal clearance of miRNAs are sparse, but in case of acute myocardial infarction it has been suggested that a change in glomerular filtration rate affects serum levels of miRNAs [21]. This is in contrast to a paper by Neal *et al.* [22], who reported that total miRNA levels and levels of five specific miRNAs (-16, -21, 155, -210 and -638) all were reduced in patients with advanced renal failure when compared with controls [22]. Circulating miRNA-499 (a marker for myocardial injury) levels are reduced during haemodialysis [23], in contrast to the serum concentrations of miRNA-21 and -210 [24]. In nephrotic syndrome and focal segmental glomerulosclerosis, a specific profile of miRNAs was described by several authors [25, 26]. The same is reported for diabetic nephropathy [27], systemic vasculitis [28] and lupus nephritis [29]. Circulating miRNA-21 might serve as a biomarker for kidney fibrosis [30] and Sui *et al.* [31] and Bijkerk *et al.* [32] reported the use of miRNAs in renal transplantation. Finally, Lorenzen [33] summarized the miRNA expression profile changes in renal ischaemia-reperfusion injury.

As far as the pathogenesis of the altered expression profiles of the miRNAs investigated by Brigant *et al.* [20] is concerned, several options besides differences in renal clearance are possible. An important question in this context, which was not answered by the authors in their study, is whether circulating miRNAs indeed reflect changes in the tissue or cells they are predicted to be derived from (in the case of the study by Brigant *et al.*, the injured kidney or renal cells) or reflect a more general pathology. In this respect, most circulating miRNAs have been shown to originate from blood or endothelial cells [20]. Thus it would be of utmost importance to investigate the origin of serum-derived miRNAs described in this or other studies dealing with circulating miRNAs. If differentially expressed serum miRNAs are derived from kidney-related exosomes, these exosomes should exhibit hallmarks of surface proteins, which are also found in kidney cells (such as renal cells). The rationale behind this assumption is that exosomes are produced from cells by fusion of multivesicular bodies with the cellular membrane and hence will contain host cell–derived protein surface markers.

Exosomes have been reported to be preferentially secreted from cells under stress conditions [6]. Therefore, one might also speculate that the increased abundance of miRNAs observed by Brigant *et al.* might be an indication of progressive endothelial stress (not only within the kidney) and subsequent increase in the abundance of exosomes, coinciding with more advanced renal failure. Following renal transplantation, which will subsequently reverse cellular stress, the abundance of serum-derived miRNAs also reverses, which is compatible with this assumption. Alternatively, it is also possible that the differences in miRNA abundance reflect inflammation in general since the levels in subjects after transplantation, under immunosuppressive therapy, are below those observed in healthy controls.

Despite these open questions, the findings by Brigant *et al.* [20] might serve as a starting point for additional efforts to clarify the role of circulating miRNAs and microvesicles as prognostic or predictive biomarkers in renal disease subjects.

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Conflict of interest statement

None declared.

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