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Role of circulating microRNA in hemodialyzed patients

Rola krążących cząsteczek mikroRNA u pacjentów hemodializowanych

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Summary

MicroRNA (miRNA) belongs to the family of non-coding RNAs, which posttranscriptionally regulate gene function. Moreover, accumulating evidence points to an essential role of miRNAs in development and monitoring of kidney disease, though the role of particular miRNAs in patients undergoing hemodialysis is still unclear. This might have consequences. It is possible that measuring a single miRNA in hemodialyzed patients may not provide adequate information about development of many pathological processes. The goal of this review is to highlight the current knowledge in the field of miRNAs, with a special emphasis on their circulation in hemodialyzed patients.

Keywords: microRNA • miRNA • hemodialysis • chronic kidney disease • acute kidney injury

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MicroRNA

MicroRNA (miRNA) belongs to the non-coding RNA family. Current estimates suggest that more than 60% of human protein-coding genes are regulated by miRNAs [9,24]. The discovery of circulating miRNA has revolutionized medicine. Currently, it is believed that the human genome encodes over 2000 miRNAs, which are abundantly expressed in almost all human cells. The single-stranded molecules have a length of 19-23 nt [3,15].

Nowadays, it is evident that mRNA is not just a simple messenger between DNA and proteins, but growing evidence supports new roles for these molecules, such as regulation of genome organization and gene expression posttranscriptionally. Since a single miRNA can bind and consequently regulate the expression of more than 100 different transcripts, it has been estimated that miRNAs may be able to regulate up to 30% of the protein-coding genes in the whole human genome. miRNAs recognize their targets based on sequence complementarity [4,8].

In humans, the binding sites for mature miRNA are located within the 3′-untranslated region (3′-UTR) of the target messenger RNA. Furthermore, each miRNA thanks to an 8 nt sequence called a "seed" may target multiple mRNAs, and each mRNA may be targeted by miRNAs regulating its transcriptional activity [5]. The mature miRNA is partially or completely complementary to the 3′-UTR of one or more mRNAs. To become effective, the mature miRNA forms a complex with proteins, termed the RNA-induced silencing complex. The miRNA incorporated into the silencing complex can bind to the target mRNA by base pairing. This base pairing subsequently causes inhibition of protein translation (full complementarity of the "seed" sequence) and/or degradation of the particular mRNAs (partial complementarity of the "seed" sequence) [8, 12, 19]. Protein levels of the target gene are consequently reduced or even totally inhibited, whereas messenger RNA levels either may or may not be decreased. In humans, miRNAs mainly inhibit protein translation of their target genes and only infrequently cause degradation or cleavage of the mRNA [3,8].

Gain and loss of function studies revealed that miRNAs play a critical role in the regulation of basically all biological cell functions such as proliferation, differentiation and apoptosis [15,20]. Moreover, the abnormalities in miRNA observed in adults have been identified in multiple disease states, including malignancies, inflammatory diseases, and cardiovascular diseases (CVD) [3,5]. Additionally to their disease-specific expression profiles, miRNAs are readily accessible for analysis from a plasma/serum or urine specimen and therefore represent a group of promising candidate molecular biomarkers that may substantially change clinical decision-making by providing further insight into specific pathophysiological mechanisms in individual patients. miRNA is suggested to be one of the main factors of the regulation of gene expression which has an impact on both physiological cardiac development and many pathological processes including cardiac arrhythmia, hypertension, heart failure, cardiac fibrosis, coronary artery disease and myocardial infarction [20].

Release of microRNA into blood

It is known that miRNA expression is usually specific to the tissue, which is why it may be used as a biomarker of various human diseases. Moreover, it can be detected not only intracellularly but also in body fluids (plasma or serum, saliva, urine, etc.) (Figure 1)[20,21]. On the one hand, it is unknown how and why disease causes changes in the levels of specific circulating miRNAs. It is possible that cell lysis or an increase in the number of exosomes shed from diseased cells contributes to increased levels of certain circulating miRNAs. However, critical check-points of the cell cycle such as robust cell proliferation, secretion and apoptosis also induced by the immunological system may be responsible for changes of miRNA yield in the circulation. One mechanism of miRNA secretion was further investigated in COS7 and HEK293 cell culture by Kosaka et al. [14], who demonstrated that miRNAs are released through a ceramide-dependent secretory machinery. Ceramide, a bioactive sphingolipid whose biosynthesis is tightly controlled by neutral sphingomyelinase 2, triggers the secretion of exosomes. Blockade of neutral sphingomyelinase 2 by either a chemical inhibitor or by small interfering RNAs reduced secretion of miRNAs, whereas overexpression of neutral sphingomyelinase 2 increased the amount of extracellular miRNAs [6,14]. Another mechanism explaining the presence of miRNAs in the blood is the process of apoptosis or necrosis (Figure 1). The end-products of this programmed cell death process are fragmented cell remnants and apoptotic bodies, including RNA transcripts and short DNA fragments. In contrast to apoptosis, necrosis is a random process. The cell swells rather than shrinks and releases long DNA strands. RNA transcripts released by apoptotic and necrotic cells form complexes with specific RNA-binding proteins. In the blood, miRNAs can also circulate in various secreted extracellular vesicles, such as apoptotic bodies and exosomes (Figure 1). Within the endosomal system, exosomes are built by inward budding of the limiting cell membrane of the multivesicular body, a late endosomal compartment. The fusion of the multivesicular body with the plasma membrane leads to the active secretion of exosomes into the blood circulation. These circulating vesicles contain specific miRNAs and are thought to be involved in cell-to-cell communication. This would suggest that miRNAs are selectively targeted for secretion in 1 cell and taken up by a distant, target cell, possibly to regulate gene expression.

Chronic kidney disease

Chronic kidney disease (CKD) is a known cardiovascular risk factor, and most patients with CKD die of cardiovascular disease before reaching the need for dialysis [10]. Once on dialysis, CVD accounts for 30% of hospitalizations and 50% of the mortality [13]. CKD is associated with a high prevalence of hypertension and diabetes, explaining some of this increased risk. Cardiovascular risk cannot be completely elucidated by traditional risk factors. A few specific miRNAs have been implicated in the functional regulation of endothelial cells as well as inflammatory responses and peripheral angiogenic signaling. Thus, differentially expressed miRNAs are responsible for non-traditional risk factors of CKD. In particular, miR-126 and miR-155 have been shown to be involved in vascular dysfunction and inflammation. Moreover, miR-126 can facilitate the vascular endothelial growth factor signaling pathway and modify vascular inflammation by suppressing leukocyte adhesion to endothelial cells (ECs). MiR-155 regulates the expression of adhesion molecules in inflammatory ECs as well as the inflammatory response in ECs mediated by angiotensin α. Therefore, miR-126 and miR-155 might be responsible for endothelial activation and increased CVD risk in ESRD patients. This has raised the possibility that miRNAs may be probed in the circulation and can serve as novel diagnostic markers.

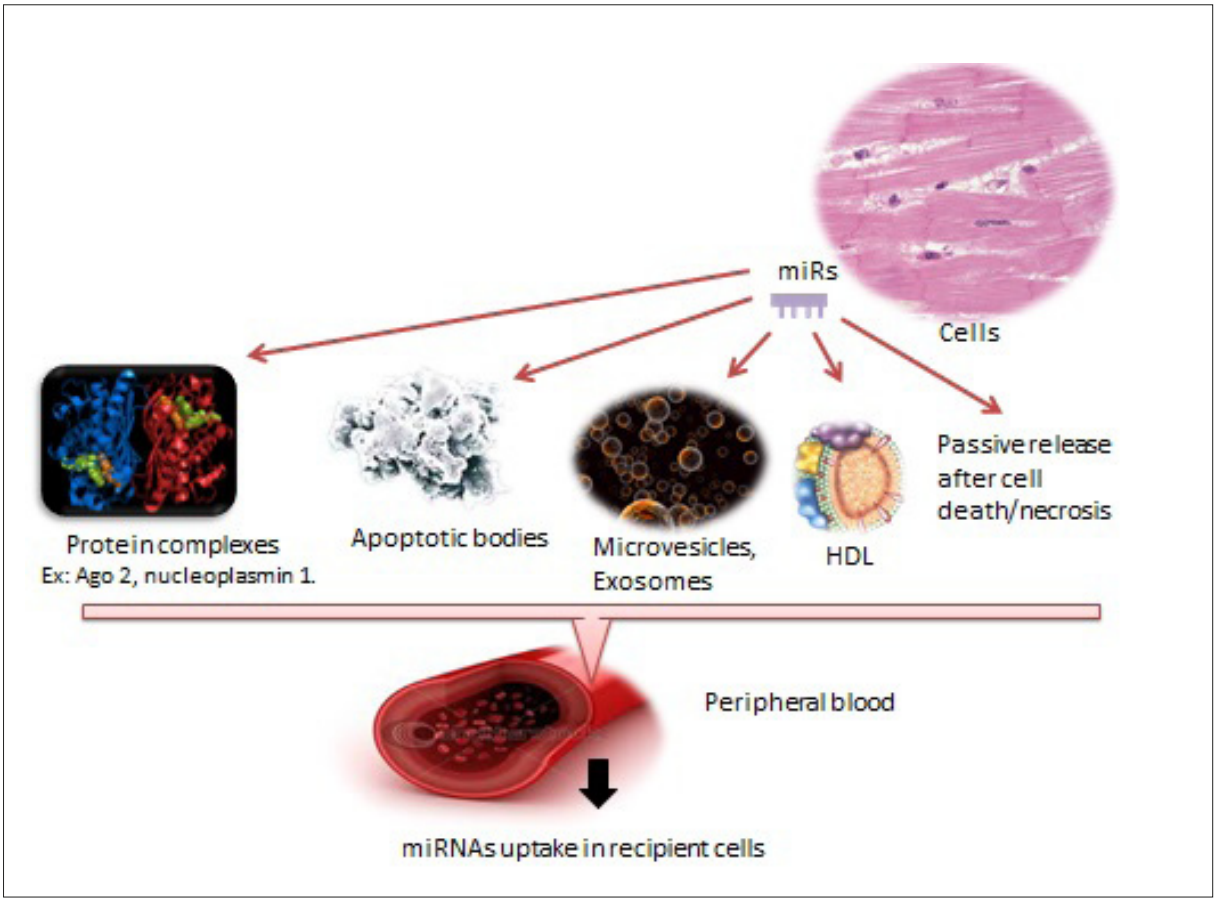

Fig. 1. The various mechanisms of miRNA release from cells in the peripheral blood circulation and their uptake in recipient cells. Ago2 Argonaute 2; High Density Lipoprotein (HDL) [18]

Circulating microRNA among patients with acute kidney injury undergoing dialysis as well as chronic kidney disease in pre-dialysis and hemodialyzed patients

One of the major challenges in hemodialysis patients is the identification of reliable biomarkers that can be measured routinely in easily accessible samples, such as serum/plasma. Circulating miRNAs are stably detectable in serum/plasma and other body fluids. Recent studies revealed that miRNAs are detectable in extracellular human body fluids such as blood or urine in a rather stable form [21]. There are several possibilities of stabilizing circulating miRNAs in the blood; two main transporter systems have been identified. miRNAs are transported in microvesicles and/or exosomes, apoptotic bodies and other microparticles (Figure 1) [11]. In addition, miRNAs are transported by non-vesicle associated protein and lipoprotein complexes [25]. Turchinovich et al. [22] reported that the majority of circulating miRNAs are bound to a molecular family called Argonaute proteins. Especially Ago 2, a 97 kDa member of the RNA-induced silencing complex (RISC), is the most frequent miRNA carrier (Figure 1)[2]. Furthermore, Gupta et al. [11] suggest that other transport forms such as exosomes may play only a minor role and more than 97% of the miRNAs are not exosome-associated. The role of urinary miRNAs as biomarkers of kidney disease is under active research [21]. There are several advantages of quantifying miRNAs in urine as biomarkers. First, the measurement of cytokines (IL-1, – 6, – 8, – 10) and growth factors in urine would seem a logical means to assess the severity of kidney damage and provide prognostic information. Also, measurement of urinary cytokine protein may not be clinically relevant because most of the cytokines exert paracrine effects and are not excreted in the urine, and the amount of cytokine protein found in urine may not correlate with the local concentration in the tubulointerstitium [21]. Little is known about circulating microRNA and the association with pre-dialysis among patients with chronic kidney disease and following their transition to dialysis as well as in patients with acute kidney injury undergoing dialysis.

On the other hand, it is unknown whether circulating levels of miRNAs are affected in patients undergoing hemodialysis. Recent studies suggest that circulating miRNAs in general might have biosignaling functions and are transported by microvesicles and/or proteins [16]. Therefore it is hypothesized that a dialysis procedure could remove miRNAs at least partially from the blood, which leads to altered circulating levels of these small ribonucleotides in the circulation. This might have

biological consequences. Emilian et al. [7] observed that circulating miR-499 levels were decreased after hemodialysis. Therefore, these observations mitigate the potential of miR-499 as a marker of myocardial injury in patients with ESRD (end-stage renal disease). It should be noted that this study was limited by the small size of the group of patients with ESRD. Thus, the authors could not investigate the association between miRNA levels and various grades of renal dysfunction. Interestingly, evidence indicates that in pre-dialysis patients with CKD the levels of circulating miRNAs are reduced, suggesting altered miRNA kinetics depending on renal function. This is consistent with the study of Neal et al. [19], who demonstrated that miRNA levels similarly correlated inversely with decreasing glomerular filtration rate (GFR), and also found decreasing miR-155 with decreasing eGFR in patients with mild to severe CKD and end-stage renal disease receiving hemodialysis treatment. This result was in agreement with Wang et al. [23], who found in the study a significant reduction in miRNA126 in ESRD patients. Moreover, Neal et al. [18] hypothesized that this was due to degradation by circulating RNase and performed an ex vivo study on exosomes from colon cancer epithelium, demonstrating increased degradation when incubated with plasma from CKD patients compared to control plasma [17]. It worth pointing out that dialysis membranes do not usually allow passage of larger molecules (30-40 kDa). However, the dialysis procedure itself might influence the amount of circulating miRNAs. Martino et al. [16] analyzed the effect of hemodialysis on circulating miRNA levels in blood and collected spent dialysate in order to investigate whether the procedure of hemodialysis removes miRNAs in patients with acute kidney injury requiring dialysis. The salient finding in this research is that miRNAs, although only small in size, are not removed by various dialyzer membranes, even those designed to remove medium-sized molecules. Also, the authors [16] did not observe any effect of the dialysis procedure on circulating miRNA-21 and – 210 levels comparing pre-dialyzer and post-dialyzer blood samples. This leads to the conclusion that dialysis therapy does not deplete the patient's blood of possibly biologically active miRNAs in the circulation of the patients with acute kidney injury requiring dialysis.

Referring to research in acute kidney injury conducted by Martino et al. [16], it should be noted that the use of renal replacement therapy by hemodialysis did not differ in the same procedure in patients with acute kidney injury and those undergoing hemodialysis with ESRD. However, it is a specific group of patients who represented a whole series of metabolic disorders, which may have reflected differences in the expression profile of circulating biomarkers, including those which are new miRNA.

In another study [1], the expression levels of miR-155 and miR-126 were significantly reduced in ESRD patients. However, they did not significantly differ between pre--HD and post-HD, being slightly higher pre-HD [1]. Data suggested that miR-126 and miR-155 might be useful predictive tools in ESRD. Therefore, the reduction of circulating miR-126 and miR-155 might be accompanied by a series of clinical symptoms. Alternatively, the miRNA levels in CKD may have been so low due to kidney disease that this overshadowed any pathological processes. It is also possible that measuring a single miRNA may not provide adequate prediction. Recent studies clearly demonstrate that multiple miRNAs work in concert to regulate key physiologic functions [5].

Conclusion

In summary, miRNAs are readily accessible for analysis from plasma or urine specimens and other bodily fluids. Circulating miRNAs may represent a potential noninvasive molecular biomarker for various pathological conditions. In addition, miRNA is relatively stable and resistant to degradation, making it an ideal substance to be tested for large scale clinical use or research with archive specimens. Moreover, the detection of circulating miRNAs can provide further insight into specific pathophysiological mechanisms in hemodialysis patients. It is unknown whether circulating levels of microRNAs are affected in patients undergoing hemodialysis. This might have consequences. It is possible that measuring a single miRNA in hemodialyzed patients may not provide adequate information about development of many pathological processes, because it is a specific group of patients who represent a whole series of metabolic disorders, which may have reflected differences in the expression profile of circulating biomarkers, including miRNA. Consequently the results should be validated in large clinical populations. The identification of miRNA seems to represent only the tip of an iceberg and the prediction of an individual miRNA. A deeper understanding of the involvement of miRNAs in hemodialyzed patients is needed before these regulatory pathways can be explored as therapeutic approaches for CKD patients.

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