



microRNAs as Promising Biomarkers of Chronic Kidney Disease. The Case of miR-223

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Abstract

Pathology often results from a deregulation of the gene expression program. We now know that gene regulation is under the control of a wealth of non-coding RNAs whose existence was unknown until the turn of the 20th century. This review focuses on the control at the post-transcriptional level through small non-coding RNAs known as microRNAs (miRNAs), short single strand nucleic acids that either induce translational repression or mRNA decay through base pairing with the 3' untranslated region of their mRNA targets. Since the dawn of the new millennium, many progresses were made to understand the roles of miRNA in the nephrology field. We and others have shown that a number of miRNAs are deregulated during the course of Chronic kidney disease (CKD) and associated cardiovascular damage, eg vascular calcifications and atherosclerosis. miR-223's gene expression is enhanced *in vivo* in aortas of a murine model of CKD *in vitro* while it is decreased in the serum, and enhanced *in vitro* in vascular smooth muscle cells subjected to the uremic toxins. Recently, we have shown that miR-223 affects the gene regulation at multiple levels by using a multi-omics approach and that its expression is decreased in the serum of a large cohort of CKD patients, where it is correlated with all-cause mortality, but also cardiovascular and renal events. miR-223 could have role(s) in CKD vascular remodelling and may therefore be a useful target to prevent or treat complications of the CKD pathogenesis.

Introduction

CKD patients afflicted by later stages of chronic kidney disease (CKD) exhibit a high cardiovascular morbimortality, correlated with the appearance of cardiovascular disorders (CVD) [1]. CVD are allegedly the first killers in the world accounting for 30% of all global deaths [2]. This is particularly true in the course of CKD, and finally results in vascular calcifications. This is increasingly frequent with the ageing of the population and it is estimated that prevalence of CVD will reach 23.3 million by 2030. Most of the deaths are caused by atherosclerosis, a pathological process that will impact any artery. Due to the complexity of the described process, it is widely believed that nontraditional predictive factors will be useful for diagnosis and prognosis.

microRNAs (miRNAs) are a newly described class of molecules that are endogenous interfering RNAs. They are now new promising biomarkers for numerous pathologies. Their molecular description is 20-25 nt non-coding RNAs that regulate very precisely the gene regulatory networks by affecting both the stability and translation of mRNAs in a post-transcriptional manner [3]. Their current number is estimated at approx. 2 000, and their corresponding genes occupy approximately 3% of the genome [3,4]. They are first transcribed as a larger RNA product, pri-miRNA, a few hundred to a few thousand nucleotides long transcript. It is then cleaved in the nucleus by the RNase III Drosha into a pre-miRNA hairpin (60 to 70 nt) that will translate to the cytoplasm by way of Exportin 5. There it will be recognized and cleaved by the Dicer RNase III [5]. This results in a double stranded RNA, comprising the 5' and 3' strands that will be recognized by RNA-induced-silencing complex (RISC) that contains Argonaute 2 (Ago2), another endonuclease and be unwound to single strand RNA. Finally, the RISC complex carries the mature miRNA to its target messenger RNAs, triggering gene silencing [3].

The active strand of the miRNA, the seed sequence, (approx.7-8 nt long, located in nucleotides 2-8 of the miRNA) binds in a complementary way with target mRNAs most often in its 3 prime Untranslated Region (3' UTR). (Guo et al., 2010) Outside of the seed region, frequent mismatches are present in the binding between miRNA and mRNA targets explaining why a given miRNA can bind dozens of different messengers. Conversely, one target mRNA can be regulated by several miRNAs. This explains why nowadays it is considered that the 2 000 miRNAs known to date regulate the expression of approximately 1/3rd to 2/3rd of the human genes [6]. The binding of miRNA to its mRNA target finally results in translation inhibition, or the induction of messenger degradation. In the current paradigm, mammalian microRNAs in a vast majority act by destabilizing their target mRNAs and decreasing levels of translation [6].

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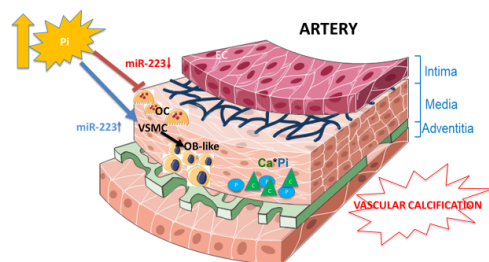


Figure 1: Summary of the central action of miR-223 on the effects of inorganic phosphate (Pi) on both vascular smooth muscle cell transdifferentiation and vessel osteoclastogenesis, triggering vascular calcification.

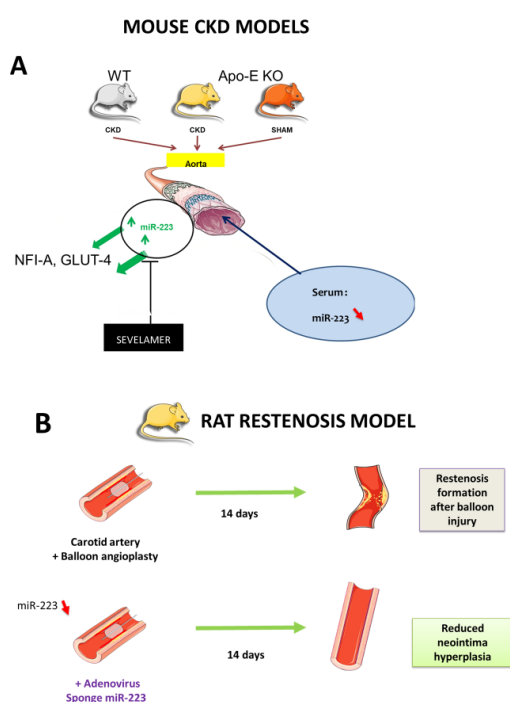


Figure 2: Animal models showing the effects of miR-223 in vivo on cardiovascular events linked to vascular calcification: (A) Murine model of chronic kidney disease (CKD) and atherosclerosis (ApoE KO). Mice with both CKD and ApoE KO display vascular calcifications. (B) Rat restenosis model. Sponge treatment decreases miR-223 levels and diminishes restenosis in carotid.

It has been shown since 2008 that circulating miRNAs are present in the human blood [7,8] in which they are carried by microvesicles, that confer the stability and the protection needed against RNase activity [9] or complexed with chaperon protein argonaute 2. Various papers in the cancer field suggested a link between several miRNA's seric levels with tissue amounts [7], emphasizing their roles as potential non-invasive biomarkers. A pioneer paper from Dimmeler's team has shown that miRNA levels are significantly different in the serum of patients with coronary artery diseases (CAD) compared to healthy counterparts [10]. A spike-in of a known amount of exogenous synthetic *Caenorhabditis elegans* miR-39 miRNA is increasingly common as no further experimental bias is usually added as reference [8].

CKD is characterized by a slow and progressive loss of kidney

function leading to vessel and bone damage. microRNAs were suggested to be involved in the pathophysiology of CKD by us and other teams [11-13] with relevance to the control of vascular smooth muscle cell (VSMC trans-differentiation that occurs during the vascular complications of CKD), suggesting that miRNAs are important modulators of cell vessel function in CKD-related cardiovascular disease [14] (Figure 1).

Our team was the first to show that levels of miR-223 are altered in damaged vessels and plasma of a murine model of CKD [4,15]. In aorta, miR-223 levels were twice higher in CKD mice whereas they were more 3-times higher in mice that had endured both CKD surgery and ApoE KO, that had vascular calcification [15]. On the other hand, our results established that miR-223 seric levels decreased during the course of CKD at the latter stages in all pathological conditions. Finally, administration of the calcium-free phosphate binder sevelamer carbonate partially corrects the miRNA deregulations in aortas, suggesting a possible direct link between the observed miRNA alterations and the vascular damage caused by CKD [14]. miR-223 also has a role in osteoclast differentiation, with an impact on the vascular calcification and osteoporosis process [16] (Figure 2).

miR-223 is highly expressed in recently isolated endothelial cells, but its levels diminish very quickly during routine cell subculture [17]. Vascular endothelial cell growth factor and basic fibroblast growth factor further decreased its expression. We also recently found miR-223 to be expressed in freshly isolated endothelial cells from the brain microvasculature [4]. It is thus reasonable to consider that in vessels, miR-223 is present in both aortic cells from the media and endothelial cells from the intima. It is now widely recognized that chronic low-grade inflammation plays a key role in the initiation and propagation of CKD. Thus, the strong increase in the inflammatory miR-223 expression during the course of CKD and atherosclerosis in our experimental models is striking, and it will be interesting to find out if this increase is beneficial (but not enough to alleviate symptoms in the latter stages of CKD) or detrimental. Our experimental results on a rat model of restenosis seem to indicate that miR-223 increase is detrimental and that an inhibitory strategy using a sponge titrating miR-223 decreases restenosis [18].

The question is how miR-223 can be transferred to vessel cells? Tabet et al. [19] suggest that high-density lipoproteins (HDL) deliver miR-223 deliver functional microRNAs (miRNA). They showed that HDL delivers miR-223 to endothelial cells in order to suppress expression of intercellular adhesion molecule 1 (ICAM-1). This would be the first example of an extracellular miRNA regulating gene expression in cells where it is not transcribed.

Also, microvesicles such as exosomes from urine have been demonstrated to contain miRNA [9]. The miRNA content in exosomes has been proposed to reflect the underlying pathophysiology of certain kidney diseases that represent of course another new reservoir for biomarker discovery but exosomes have also the capacity to shuttle their cargo between kidney cells and change the recipient cell's proteome and function [20].

miR-223 impacts various levels of gene regulation in an osteoclast cell line

We used a multi-omics approach combining microarray transcriptomics, SELDI-TOF and MALDI-TOF proteomics and NMR-based metabolomics to study the impact of over-expression and inhibition of miR-223, a pleiotropic regulator of metabolic-related disease, in the RAW monocyte-macrophage cell line [21]. We analyzed the levels of proteins, mRNAs, and metabolites to identify genes involved in miR-223 regulation, candidate disease biomarkers and potential therapeutic targets. We observed that both up- and down-regulation of miR-223 induced profound changes in the mRNA, protein and metabolite profiles of the RAW monocyte-macrophage cells. Among the deregulated genes, we found that 52 proteins were significantly altered when comparing scramble, pre- and anti-miR223 treatment. Using dedicated databases, 16 out of

these genes were predicted to have binding sites for miR-223. Among these, transcription factors CARM1, Ube2g2, Cactin and Ndufa4 were confirmed to be miR-223 targets by stability of mRNAs, bone remodeling and immune response. Transcriptomics evidenced a change in 120 genes, among which 30 genes encoding long noncoding RNAs. Genes were linked to histone acetylation, but also to bone remodeling and RNA regulation. The most important discriminant metabolites found in the metabolomics study were found to be hydrophilic amino acids, carboxylic acids linked to metabolism and pyrimidine nucleotides.

miR-223 as predictor of CKD progression

As CKD is associated with disorders of mineral and bone metabolism and is closely linked to elevated cardiovascular risk, levels of some miRNAs could be associated with CKD progression. However, their association with clinical outcomes remains poorly understood. To develop new biomarkers in order to evaluate the appearance and course of vascular calcifications which are a hallmark and an aggravating factor of the latter stages of CKD and atherosclerosis, we studied the expression of two circulating miRNAs, miR-223 and miR-116, in a CKD cohort, and to evaluate the miRNAs' link with cardiovascular and renal events and all-cause mortality [22].

We used RT-qPCR to measure serum levels of miR-223 and miR-126 in a cohort of 628 patients (CKD stage 1 to 5 patients or on renal replacement therapy – CKD 5D –, and healthy controls) in Ghent University Hospital (Belgium). The CKD patients were followed over 6 years. The all-cause mortality, and cardiovascular and renal events were collected.

The serum levels of miR-223 were significantly lower in the CKD3B, CKD4, CKD5 and CKD5D groups than in the healthy controls. Compared with controls, serum levels of miR-126 were significantly lower in the CKD2, CKD3A, CKD3B, CKD 4, CKD 5 and CKD 5D groups. When taking into account all events, patients having below-median levels of miR-223 suffered the lower survival rate. We observed the same results for miR-126. The associations between miRNA levels and overall mortality were however not significant after adjustment for baseline eGFR. In summary, CKD is associated with a decrease in circulating miR-223 and miR-126 levels in humans with impaired kidney function. Neither miR-223 nor miR-126 were a prognostic marker of all-cause mortality, cardiovascular events or renal events. The mechanism that underlies the low expression of circulating miRNAs in CKD has yet to be identified.

Together, all the results discussed here provide new clues concerning the status of miRNA regulation during the course of CKD. MiRNAs are expressed in a cell- and tissue-specific manner, and have reshaped our view, increasing this complexity even further.

To conclude, we are now at a stage where miRNAs expression can be studied in human CKD populations, before and after the vascular calcification stage, in order to develop them as new biomarkers, useful for diagnosis and treatment evaluation, but also to detect innovative targets for future therapeutic strategies.

References

- Massy Z, Drüeke T. Vascular calcification. *Curr Opin.Nephrol Hypertens*. 2013 July;22(4):405–412.
- Fan J, Salameh H. Impact of Chronic Kidney Disease on Risk for Vascular Events. *Curr Vasc Pharmacol*. 2016;14(5):409-414.
- Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009 Jan;136(2):215-233.
- Metzinger-Le Meuth V, Andrianome S, Chillon JM, Bengrine A, Massy ZA, et al. microRNAs are dysregulated in the cerebral microvasculature of CKD mice. *Front Biosci (Elite Ed)*. 2014 Jan ;1;6:80-88.
- Stavast CJ, Erkeland SJ. The Non-Canonical Aspects of MicroRNAs: Many Roads to Gene Regulation. *Cells*. 2019 Nov;8(11):1465.
- Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature*. 2010 Aug;466(7308):835-840.
- Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*. 2008 Jul;105:10513-10518.
- Roberts TC, Coenen-Stass AM, Wood MJ. Assessment of RT-qPCR normalization strategies for accurate quantification of extracellular microRNAs in murine serum. *PLoS One*. 2014 Feb;9(2):e89237.
- Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, et al. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One*. 2008;3(11):e3694.
- De Rosa R, De Rosa S, Leistner D, Boeckel JN, Keller T, et al. Transcoronary Concentration Gradient of microRNA-133a and Outcome in Patients With Coronary Artery Disease. *Am J Cardiol*. 2017 Jul;120(1):15-24.
- Chen NX, Kiattisunthorn K, O'Neill KD, Chen X, Moorthi RN, et al. Decreased microRNA is involved in the vascular remodeling abnormalities in chronic kidney disease (CKD). *PLoS One*. 2013 May;8(5):e64558.
- Metzinger-Le Meuth V, Fournier O, Charnaux N, Massy ZA, Metzinger L. The expanding roles of microRNAs in kidney pathophysiology. *Nephrol Dial Transplant*. 2019 Jan;34(1):7-15.
- Rangrez AY, M'Baya-Moutoula E, Metzinger-Le Meuth V, Hénaut L, Djelouat MS, et al. Inorganic phosphate accelerates the migration of vascular smooth muscle cells: evidence for the involvement of miR-223. *PLoS ONE*. 2012 Oct;7(10):e47807.
- Metzinger-Le Meuth V, Burtsey S, Maitrias P, Massy ZA, Metzinger L. microRNAs in the pathophysiology of CKD-MBD: Biomarkers and innovative drugs. *Biochim Biophys Acta Mol Basis Dis*. 2017 ;1863 :337-345.
- Taïbi F, Metzinger-Le Meuth V, M'Baya-Moutoula E, Djelouat Ms, Louvet L, et al. Possible involvement of microRNAs in vascular damage in experimental chronic kidney disease. *Biochim Biophys Acta*. 2014 Jan;1842(1):88-98.
- M'baya-Moutoula E, Louvet L, Molinié R, Guerrera IC, Cerutti C, et al. A multi-omics analysis of the regulatory changes induced by miR-223 in a monocyte/macrophage cell line. *Biochim Biophys Acta Mol Basis Dis*. 2018 Aug;1864(8):2664-2678.
- Shi L, Fisslthaler B, Zippel N, Frömel T, Hu J, et al. MicroRNA-223 antagonizes angiogenesis by targeting $\beta 1$ integrin and preventing growth factor signaling in endothelial cells. 2013 Sep;113:1320-1330.
- M'baya-Moutoula E, Marchand A, Six I, Bahrar N, Celic T, et al. Inhibition of miR-223 expression using a sponge strategy decreases restenosis in rat injured carotids. *Curr Vasc Pharmacol*. 2019 Jul.
- Tabet F, Vickers KC, Cuesta Torres LF, Wiese CB, Shoucri BM, et al. HDL-transferred microRNA-223 regulates ICAM-1 expression in endothelial cells. *Nat Commun*. 2014 Feb;5:3292.
- Lv LL, Cao YH, Ni HF, Xu M, Liu D, et al. MicroRNA-29c in urinary exosome/microvesicle as a biomarker of renal fibrosis. *Am J Physiol Renal Physiol*. 2013 Oct;305(8):F1220-1227.
- M'Baya-Moutoula E, Louvet L, Metzinger-Le Meuth V, Massy ZA, Metzinger L. High inorganic phosphate concentration inhibits osteoclastogenesis by modulating miR-223. *Biochim Biophys Acta*. 2015 Oct;1852(10 Pt A):2202-12.
- Fournier O, Schepers E, Metzinger-Le Meuth V, Glorieux G, Liabeuf S, et al. Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. *Sci Rep*. 2019 Mar ;9 :4477.