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REVIEW

miR-126 and miR-223 as biomarkers of vascular damage in the course of Chronic Kidney Disease

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Development of disease is often due to deregulation of gene expression. The gene program is controlled at the post-transcriptional level by the action of small non-coding RNAs known as microRNAs (miRNAs), short, single-stranded molecules that control mRNA stability or translational repression via base pairing with regions in the 3' untranslated region of their target mRNAs. Over the last decade, considerable progress has been made to elucidate the roles of miRNAs in vascular pathogenesis and develop the use of miRNAs as innovative biomarkers in diagnostics, and as groundbreaking drugs in pharmacological treatments. It has been recently shown that several miRNAs are implicated in the course of chronic kidney disease (CKD) and are associated with vessel damage, such as vascular calcifications and atherosclerosis. The inflammatory miR-223 is increased *in vitro* in vascular smooth muscle cells subjected to uremic toxins and is also increased *in vivo* in more advanced stages of CKD. The endothelial-specific miR-126 is involved in vascular remodeling in response to laminar shear stress in HUVEC cells. Finally, miR-126 levels have been found to be deregulated in murine and human serum in the course of experimental CKD and in human diabetic patients. In conclusion, these miRNAs could play a role in CKD vascular remodeling and may therefore represent useful targets to prevent or treat complications of CKD.

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Introduction

Patients with advanced stages of chronic kidney disease (CKD) exhibit higher cardiovascular morbidity and mortality, associated with the presence of cardiovascular diseases (CVD). CVD are the leading cause of mortality throughout the world. The World Health Organization has estimated that 17.3 million people died from CVD in 2008, representing 30% of all global deaths. With aging of the population, the prevalence of CVD will inevitably increase to reach 23.3 million by 2030. Most CVD are caused by atherosclerosis, a pathological process that can affect any artery in the human body[1]. The first phase of atherosclerosis, called intimal hyperplasia, starts with endothelial dysfunction and induction of smooth muscle cell migration and proliferation from the media to the intima, in turn leading to degeneration of the artery wall and local inflammation. All these phenomena are observed in the course of CKD, and finally result in vascular calcifications[2].

Due to the complexity of this process, it is generally considered that unconventional predictive factors will be increasingly useful for diagnosis. The understanding of the pathophysiological process of vascular calcifications has been substantially improved over the last decade, but further progress is still required to develop preventive and therapeutic strategies[2].

Since the turn of the millennium, microRNAs (miRNAs), a novel and abundant class of endogenous interfering RNAs, have become a new source of excitement in many fields of research in biology and medicine, as they have emerged as new promising biomarkers for numerous diseases. These small 20-24 nt non-coding RNAs fine-tune gene regulatory networks by affecting both the stability and translation of mRNAs in a post-transcriptional manner[3]. More than 1,500 miRNAs have now been described, and are listed in dedicated databases. miRNA biogenesis starts with the transcription of a larger RNA product, Pri-miRNA. This transcription is usually performed by RNA polymerase II. Pri-miRNA, a few hundred to a few thousand nucleotide long transcript, is then cleaved in the nucleus by RNase III Drosha, with the aid of protein partner, DiGeorge syndrome critical region 8 (DGCR8). The resulting pre-miRNA hairpin (approx. 60 to 70 nt) migrates from the nucleus to the cytoplasm, where it is recognized and cleaved by Dicer RNase III. The resulting double-stranded RNA, known as the miRNA/miRNA* duplex, is unwound to single strands by the action of RNA-induced-silencing *complex* (*RISC*) that contains a further endonuclease, Argonaute 2 (Ago2). Finally, the RISC complex will bring the mature miRNA to target messenger RNAs, starting gene silencing[3].

In the mature miRNA, the 7 nt long seed sequence, usually located in nucleotides 2–8 of the miRNA, base-pairs with mammalian mRNAs by complementary base-pairing, mostly to the 3 prime Untranslated Region (3'UTR). However, several studies have shown that, in some cases, miRNAs bind the coding region or 5'UTR of respective target mRNAs[4]. Due to imperfect base-pairing outside of the seed region, hundreds of target mRNAs can likely bind to a single miRNA. This clearly illustrates the role of these small RNAs in the intricate network of gene regulation. Inversely, a single mRNA can be regulated by several miRNAs. Most researchers now estimate that the 1,500 miRNAs identified to date regulate the expression of approximately 1/3 to 2/3 of all human genes[4].

The exact molecular mechanisms are still subject to controversy, between an action mostly involving inhibition of translation of target mRNAs, or induction of their degradation. According to Bartel's team, in a vast majority of cases, mammalian microRNAs act by destabilizing their target mRNAs and decreasing their levels[5].

Recent data from the literature suggest that several microRNAs are involved in the pathophysiology of CKD[6-9], since they are important regulators of vascular smooth muscle cell (VSMC) and endothelial cell plasticity. This review focuses on the known roles of miR-126 and miR-223 in the endothelial cell phenotypic switch towards a deregulated state and the control of Vascular Smooth Muscle Cell (VSMC) transdifferentiation that occurs during vascular complications of CKD, illustrating that miRNAs are important modulators of vascular cell function in vascular disease.

miR-126, an important modulator of endothelial cell dysfunction

During blood vessel maturation, endothelial cells, that were motile and proliferative during vascular development, stabilize the endothelial lining, become quiescent, and generate a stable barrier between vascular tissue and blood. At that stage, endothelial cells act as sensors and respond to signals from the environment. During pathophysiological processes, such as tissue repair and the vascular pathogenesis observed in CKD, the endothelium will partially lose its cell-cell adhesion and intimal cells become mobile and proliferate. This process is called endothelial dysfunction[10]. Recent work has shown that microRNAs can modulate this phenotype switch (Figure 1A).

For example, several recent studies have shown that endothelial miRNAs play important roles during vasculogenesis and in the response to inflammation and hemodynamic stress. The pro-angiogenic miR-126 is abundant in endothelial cells, plays an important role in vascular dysfunction and modulates the expression of Vascular Cell Adhesion Molecule-1 (VCAM-1)[11-12] and chemokine Stromal cell-Derived Factor-1 (SDF-1/CXCL-12)[13-14]. MiR-126 is one of the most abundant miRNAs in the endothelial cell, where it promotes the pro-angiogenic actions of VEGF and FGF and enhances blood vessel formation[15]. Interestingly, Zhaou et al. showed that endothelial miR-126 release is reduced by atheroprotective laminar shear stress[16]. Indeed, atherosclerosis occurs in arterial regions exposed to disturbed flow. Modifications of flow conditions have been shown to regulate expression of flow-sensitive miRNAs, known as mechano-miRs in endothelial cells both *in vitro* and *in vivo*, in turn regulating endothelial dysfunction and atherosclerosis. miR-126 has been implicated as one of these mechanosensitive athero-miRNAs[17]. However, the situation is more complex than it seems, as one miR-126 can mask the effect of another. miR-126 (also referred to as miR-126-3p)

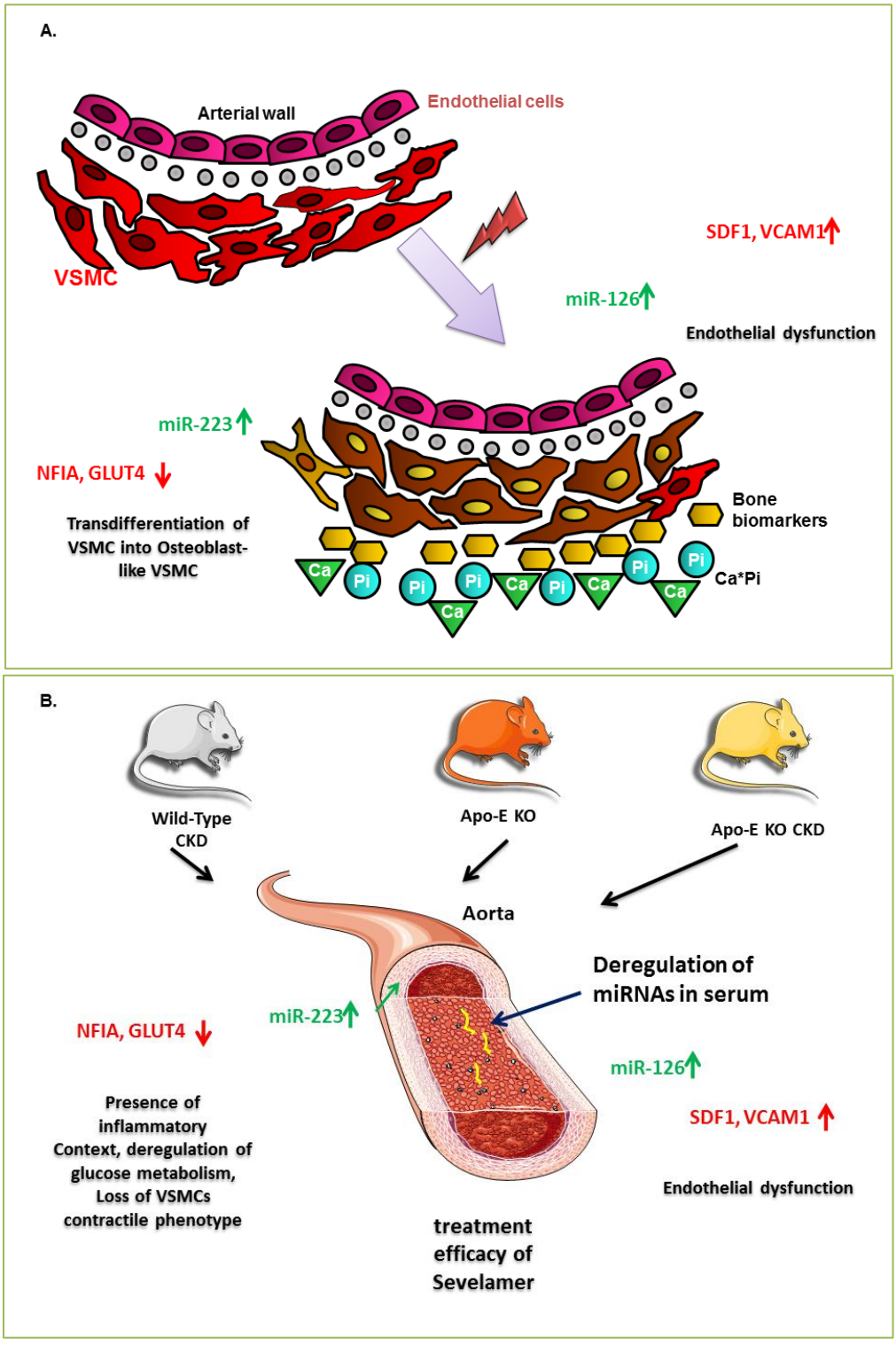

Figure 1. miR-126 and mir-223 are biomarkers of Chronic Kidney Disease (CKD), atherosclerosis and vascular calcification. Synoptic representation of the relevance of miR-126 and miR-223, and specific mRNA targets in (A) a pictorial representation of the cell types present in normal and calcified aorta and (B) murine models of Chronic Kidney Disease (CKD), atherosclerosis and vascular calcification. In an inflammatory context in CKD, atherosclerosis and vascular calcification, contractile vascular smooth muscle cells (VSMCs) transdifferentiate into a synthetic phenotype, reflecting a pathogenic context, and endothelial cells become dysfunctional.

and miR-126* (also known as miR-126-5p) are highly expressed in the endothelium and have been shown to be differentially regulated by flow. miRNA* is generally considered to be degraded and therefore does not have any biological relevance. However, Schober surprisingly claimed that miR126* (-5p) is more highly expressed than miR-126 in the endothelium and is the predominant atheroprotective regulator[18]. In this review, miR-126-3p is referred to as miR-126.

We have previously observed endothelial dysfunction in our murine model of chronic kidney disease[19], which is an indicator of subtle disturbances of endothelial function and which may potentially play an important role at this disease stage (Figure 1B). *In vivo*, we examined changes of miRNA expression in aortas of CKD and/or atherosclerotic mice and showed that miR-126 expression was markedly increased and expression of protein targets VCAM-1 and SDF-1 was altered during the course of CKD and/or atherosclerosis[8]. Even more marked changes were observed in mice developing both CKD and atherosclerosis, known to display vascular calcifications[20]. Finally, Sevelamer, a drug commonly used in CKD, partially corrected these changes in miR expression, suggesting a direct link between the observed miRNA alterations and uremic vascular toxicity.

Mitchell et al.[21] have demonstrated the presence of circulating miRNAs in human plasma. Circulating miRNAs are transported by micro vesicles, conferring the required stability and protection against RNase activity[22] or in complexes with the chaperon Argonaute 2 and/or lipoproteins. Several studies have demonstrated a correlation between plasma miRNA and diseases such as cancer[21], highlighting their roles as potential noninvasive biomarkers[23]. Stephanie Dimmeler's team was the first to show that miRNA levels are altered in the serum of patients with coronary artery disease (CAD) compared to healthy counterparts. In cardiovascular disease studies, various endogenous circulating miRNAs (eg miR-454, U6 or miR-17-5p) were considered to normalize the levels of circulating miRNAs, but most people add a known amount of spiked-in exogenous non-human miRNA (eg synthetic *Caenhorabditis elegans* miR-39), to avoid further experimental bias[24].

Plasma levels of miR-126 had been shown to be decreased in type 2 diabetes[25]. We were the first to study miR-126 expression in damaged vessels due to CKD. Our results established that miR-126 is decreased during the later stages of CKD in all pathological conditions. At the later stages of uremia, Apo-E KO mutation induced a decrease of serum miR-126 levels. On the other hand, CKD at this later stage of uremia triggered a decrease of miR-126. Interestingly, several miRNAs, including miR-126, have been shown to be secreted by endothelial cells, more precisely in blood micro-vesicles, which are predicted to regulate cellular differentiation[22, 25].

It has been shown that administration of miR-126 rescued EC proliferation at predilection sites of limited atherosclerosis[13]. Systemic treatment of mice with microparticles after electric denudation of the endothelium accelerated re-endothelialization *in vivo*. Other experiments have revealed that micro particles released from apoptotic endothelial cells are integrated by neighbouring cells to promote EC migration and proliferation, two critical steps in endothelial repair[26]. miR-126 was the predominant miRNA in these microparticles. Microparticles derived from glucose-treated ECs contained fewer miR-126 and showed reduced endothelial repair capacity. This observation is particularly interesting, as the same authors reported that miR-126 is decreased in circulating microparticles from patients with stable coronary artery disease with and without diabetes mellitus[26].

Cantaluppi et al.[27] also showed that microvesicles derived from endothelial progenitor cells are able to protect kidney cells (per tubular capillaries and tubular cells) in a rat model of acute kidney injury. Here again, miR-126 was found to be the main miRNA expressed in micro vesicles, in tandem with miR-296. The combined results of these studies strongly support the hypothesis that miR-126 promotes endothelial regeneration in vessels, but is also able to protect kidney tissue, and that increasing the production of this miRNA would be a potential therapeutic approach to limit the development of atherosclerosis and damage due to CKD and diabetes.

miR-223 expression in smooth muscle and endothelial cells: a marker of inflammation?

Unlike other muscle cells (skeletal and cardiac), VSMCs have an important plasticity enabling them to undergo a switch from a "synthetic" to a "contractile" phenotype, in response to physiological and pathological environmental signals. Growth factors, such as PDGF, or vascular damage induce VSMC dedifferentiation and consequently their transdifferentiation into migratory and proliferative ("synthetic") cells. This phenotypic change is instrumental for angiogenesis and vascular repair, and is associated with significant changes in the VSMC gene expression pattern (ie an increase in the expression of VSMC-specific genes, such as calponin-1 (CNN), smooth muscle β-actin (SMβA) and SM22 β-actin (SM22). Two independent teams have highlighted the important role of miRNAs in VSMC development, differentiation, and pathophysiology after knocking out the

miRNA processing enzyme Dicer in murine VSMCs, which induced profound vascular abnormalities, resulting in embryonic lethality. Among miRNAs found to be expressed in the vasculature, miR-143 and miR-145 are the miRNAs most highly expressed in smooth muscle cells, and their down-regulation is directly associated with a phenotypic switch from contractile, i.e. fully differentiated, to synthetic, i.e. proliferative, VSMCs[28].

We have reported that miR-223 plays a significant role in vascular smooth muscle cell calcification induced by inorganic phosphate (Pi), both *in vitro*[9] and *in vivo*[8] (Figure 1A and B). Increased serum phosphate level has been recognized as a major risk factor in kidney disease and downstream vascular complications. Our data demonstrated the direct molecular and cellular effects of Pi on vascular smooth muscle cells, showing major impacts on calcification, proliferation and migration properties by modulating the expression of associated miRNAs and phenotypic marker genes. In our calcification models, we confirmed the previously described important roles of miR-143 and miR-145 on other normal and pathological cardiovascular events[9]. We also reported, for the first time, a similar role for miR-223, since this miRNA (first reported as an oncomiR, a marker of muscle damage and a key factor in osteoclast differentiation[7]) is expressed in VSMCs and is significantly upregulated in Pi-treated cells. Moreover, over-expressing miR-223 in VSMCs increased proliferation and markedly enhanced VSMC migration[9]. Our results therefore suggested that miR-223 is a potential biomarker of vascular calcification.

In support of these *in vitro* findings, we also observed upregulation of miR-223 in aorta samples collected from experimental murine models of CKD and atherosclerosis, which display vascular calcification[8]. Moreover, the vascular expression of miR-223 is altered in these pathological conditions. Importantly, the levels of expression of miR-223 and its specific protein targets vary according to the stage of CKD. Finally, administration of the calcium-free phosphate binder, sevelamer carbonate, partially corrects miRNA deregulations, suggesting a possible direct link between the observed miRNA alterations and the vascular damage caused by CKD[8].

One of our study's most important findings was the up-regulation of miR-223 in aortas from both WT-CKD and Apo-E CKD mice with surgically induced CKD. Lu et al.[29] have already reported that miR-223 overexpression triggers the cellular uptake of glucose in cardiomyocytes by modulating GLUT-4 expression. We recently reported that miR-223 is expressed in VSMC and that this upregulation is a potential marker of VSMC damage[9]. Our present results further suggest that this is also true *in vivo*. miR-223 was initially considered to be not significantly expressed in endothelial cells *in vitro*[25] (and our unpublished results). However, another team has recently shown that miR-223 is actually highly expressed in freshly isolated endothelial cells, but that miR-223 expression decreases very rapidly during routine cell subculture[30]. Interestingly, various transcription factors, such as vascular endothelial cell growth factor and basic fibroblast growth factor, further decreased miR-223 expression. We also recently found miR-223 to be expressed in freshly isolated endothelial cells from the brain microvasculature[31]. It is therefore reasonable to postulate that 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. Consequently, the marked increase in inflammatory miR-223 expression during the course of CKD and atherosclerosis in our experimental models is striking, and it would be interesting to determine whether this increase is beneficial (although insufficient to alleviate symptoms in the later stages of CKD) or detrimental.

The essential question is how miR-223 can be transferred to vascular cells? Tabet et al.[32] suggested that high-density lipoproteins (HDL) deliver functional microRNAs (miRNA) and 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 in which it is not transcribed.

Microvesicles such as exosomes from urine have also been demonstrated to contain miRNA[33]. The miRNA content in exosomes has been proposed to reflect the underlying pathophysiology of certain kidney diseases that obviously represent another new reservoir for biomarker discovery, but exosomes are also able to shuttle their cargo between kidney cells and change the recipient cell's proteome and function, partly by means of miR-126[27].

Altogether, 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, even further increasing this complexity.

In conclusion, we are now at a stage where miRNA expression can be studied in human CKD populations, before and after the vascular calcification stage, in order to develop miRNAs as new biomarkers, useful for diagnosis and treatment evaluation, but also to develop miRNAs as innovative targets for groundbreaking treatments (Figure 2).

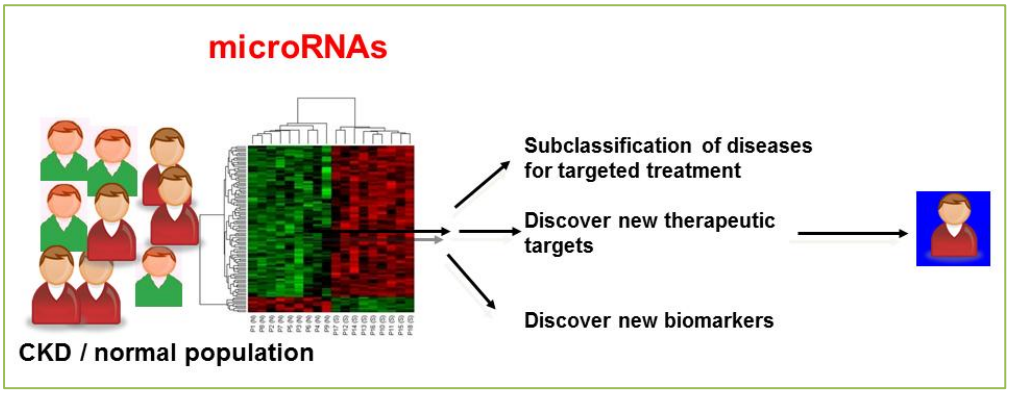

Figure 2. Principle of the study of a disease by the use of miRNAs. Normal and CKD patient populations are compared in terms of expression of a miRNA signature.

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References

1. Madonna R, De Caterina R. Potential roles of vessel wall heparan sulfate proteoglycans in atherosclerosis. Vascul Pharmacol 2014; 60:49-51.
2. Massy ZA, Drueke TB. Vascular calcification, Curr Opin Nephrol Hypertens 2013; 22:405-412.
3. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136:215-233.
4. Vijayarathna S, Oon CE, Jothy SL, Chen Y, Kanwar JR, Sasidharan S. MicroRNA pathways: an emerging role in identification of therapeutic strategies. Curr Gene Ther 2014; 14:112-120.
5. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 2010; 466:835-840.
6. Chen NX, Kiattisunthorn K, O'Neill KD, Chen X, Moorthi RN, Gattone VH, et al., Decreased microRNA is involved in the vascular remodeling abnormalities in chronic kidney disease (CKD). PLoS One; 2013 8:e64558.
7. Taibi F, Metzinger-Le Meuth V, Massy ZA, Metzinger L. miR-223: An inflammatory oncomiR enters the cardiovascular field. Biochim Biophys Acta 2014; 1842:1001-1009.
8. Taibi F, Metzinger-Le Meuth V, M'Baya-Moutoula E, Djelouat EM, Louvet L, Bugnicourt JM, et al. Possible involvement of microRNAs in vascular damage in experimental chronic kidney disease. Biochim Biophys Acta 2014; 1842:88-98.
9. Rangrez AY, M'Baya-Moutoula E, Metzinger-Le Meuth V, Henaut L, Djelouat MS, Benchitrit J, et al. Inorganic phosphate accelerates the migration of vascular smooth muscle cells: evidence for the involvement of miR-223. PLoS One 2012; 7:e47807.
10. Pushpakumar SB, Kundu S, Sen U. Endothelial dysfunction: The link between homocysteine and hydrogen sulfide. Curr Med Chem 2014; epub ahead of print.
11. Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci USA 2008; 105:1516-1521.
12. Asgeirsdottir SA, van Solingen C, Kurniati NF, Zwiers PJ, Heeringa P, van Meurs M, et al., MicroRNA-126 contributes to renal microvascular heterogeneity of VCAM-1 protein expression in acute inflammation. Am J Physiol Renal Physiol 2012; 302:F1630-1639.
13. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci Signal 2009; 2:ra81.
14. van Solingen C, de Boer HC, Bijkerk R, Monge M, van Oeveren-Rietdijk AM, Seghers L, et al. MicroRNA-126 modulates endothelial SDF-1 expression and mobilization of Sca-1(+)/Lin(-) progenitor cells in ischaemia. Cardiovasc Res 2011; 92:449-455.
15. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis, Dev Cell 2008; 15:261-271.
16. Zhou J, Li YS, Nguyen P, Wang KC, Weiss A, Kuo YC, et al. Regulation of vascular smooth muscle cell turnover by endothelial cell-secreted microRNA-126: role of shear stress, Circ Res 2013; 113:40-51.
17. Kumar S, Kim CW, Simmons RD, Jo H. Role of Flow-Sensitive microRNAs in Endothelial Dysfunction and Atherosclerosis: Mechanosensitive Athero-miRs. Arterioscler Thromb Vasc Biol 2014; epub ahead of print.
18. Schober A, Nazari-Jahantigh M, Wei Y, Bidzhekov K, Gremse F, Grommes J, et al. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat Med 2014; 20:368-376.
19. Chillon JM, Mozar A, Six I, Maizel J, Bugnicourt JM, Kamel S, et al. Pathophysiological mechanisms and consequences of cardiovascular calcifications: role of uremic toxicity. Ann Pharm Fr 2009; 67:234-240.
20. Massy ZA, Mentaverri R, Mozar A, Brazier M, Kamel S. The pathophysiology of vascular calcification: are osteoclast-like cells the missing link? Diabetes Metab 2008; 34 Suppl 1:S16-20.
21. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 2008; 105:10513-10518.

22. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L et al. Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 2008; 3:e3694.

23. Menendez P, Villarejo P, Padilla D, Menendez JM, Montes JA. Diagnostic and prognostic significance of serum microRNAs in colorectal cancer. J Surg Oncol 2013; 107:217-220.

24. 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; 9:e89237.

25. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, et al. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 2010; 107:810-817.

26. Jansen F, Yang X, Hoelscher M, Cattelan A, Schmitz T, Proebsting S, et al. Endothelial microparticle-mediated transfer of MicroRNA-126 promotes vascular endothelial cell repair via SPRED1 and is abrogated in glucose-damaged endothelial microparticles. Circulation 2013; 128:2026-2038.

27. Cantaluppi V, Gatti S, Medica D, Figliolini F, Bruno S, Deregibus MC, et al., Microvesicles derived from endothelial progenitor cells protect the kidney from ischemia-reperfusion injury by microRNA-dependent reprogramming of resident renal cells. Kidney Int 2012; 82:412-427.

28. Albinsson S, Suarez Y, Skoura A, Offermanns O, Miano JM, Sessa WC. MicroRNAs are necessary for vascular smooth muscle growth, differentiation, and function. Arterioscler Thromb Vasc Biol 2010; 30:1118-1126.

29. Abedin M, Tintut Y, Demer LL. Vascular calcification: mechanisms and clinical ramifications. Arterioscler Thromb Vasc Biol 2004; 24:1161-1170.

30. Shi L, Fisslthaler B, Zippel N, Fromel T, Hu J, Elgheznawy A, et al. MicroRNA-223 antagonizes angiogenesis by targeting beta1 integrin and preventing growth factor signaling in endothelial cells. Circ Res 2013; 113:1320-1330.

31. Metzinger-Le Meuth V, Andrianome S, Chillon JM, Bengrine A, Massy ZA , Metzinger L. microRNAs are dysregulated in the cerebral microvasculature of CKD mice. Front Biosci (Elite Ed) 2014; 6:80-88.

32. Tabet F, Vickers KC, Cuesta Torres LF, Wiese CB, Shoucri BM, Lambert G et al. HDL-transferred microRNA-223 regulates ICAM-1 expression in endothelial cells. Nat Commun 2014; 5:3292.

33. Dear JW, Street JM, Bailey MA. Urinary exosomes: a reservoir for biomarker discovery and potential mediators of intrarenal signalling. Proteomics 2013; 13:1572-1580.