

## Short Conceptual Overview

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# Vascular calcification: the role of microRNAs

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**Abstract:** Vascular calcification represents the deposition of calcium phosphate salts in the tunica media of the vascular wall. It occurs during aging but is accelerated and pronounced in patients with diabetes mellitus, chronic kidney disease (CKD) and established cardiovascular disease. Due to the loss of elasticity of the vessel wall, vascular calcification might result in left ventricular hypertrophy and compromise coronary perfusion. Accordingly, several studies showed that vascular calcification is associated with increased risk for cardiovascular morbidity and mortality. Accumulating data suggest that microRNAs (miRs) play an important role in vascular calcification. A variety of miRs have been implicated in the development of vascular calcification, whereas others appear to play a protective role. Accordingly, miRs might represent promising targets for the prevention of vascular calcification and its adverse cardiovascular sequelae. However, given the complexity of regulation of this process and the multitude of miRs involved, more research is needed to identify the optimal candidate miRs for targeting.

**Keywords:** cardiovascular disease; microRNAs; vascular calcification; vascular smooth muscle cells.

## Introduction

Vascular calcification represents the deposition of calcium phosphate salts in the tunica media of the vascular wall (1, 2). It occurs during aging but is accelerated and pronounced in patients with diabetes mellitus, chronic kidney disease (CKD) and established cardiovascular disease (1, 2). Due to the loss of elasticity of the vessel wall, vascular calcification might result in left ventricular

hypertrophy and compromise coronary perfusion (1, 3). Accordingly, several studies showed that vascular calcification is associated with increased risk for cardiovascular morbidity and mortality (2, 4, 5).

During vascular calcification, smooth muscle cells located at the tunica media undergo a non-reversible transformation to cells with osteoblast-like functions. These vascular smooth muscle cells (VSMCs) lose their lineage markers, including  $\alpha$ -actin and SM22a and experience an upregulation of genes normally present in osteoblasts, including runt-related transcription factor 2 (RUNX2), osteopontin, osteocalcin and alkaline phosphatase (ALP) (1, 4, 6, 7). Accumulating data suggest that microRNAs (miRs) play an important role in this transformation of VSMCs (4, 8).

miRs are non-coding small RNAs and their main role is the post-transcriptional regulation of gene expression by inducing messenger RNA (mRNA) cleavage or destabilization or by compromising mRNA translation (9, 10). miRs bind to the 3' untranslated region (UTR) of the target mRNA or to the coding region of the mRNA, leading to mRNA destabilization or inhibition of translation, respectively (11). Therefore, miRs manage genetic diversion and regulate the amount of synthesized proteins, acting as a modulator of a multitude of cellular functions (10). Accordingly, dysfunction of miRs is implicated in the pathogenesis of a wide array of diseases.

## The role of miRs in the pathogenesis of vascular calcification

miRs are more abundantly expressed within matrix cysts, allowing RNA material to be transported between cells. Disruption of miRs alters the expression of several genes that are under their control. The most important functions that are affected by miRs and contribute to vascular calcification are the contractility of the VSMCs, reaction to stress (e.g. hypoxia) and osteogenic transdifferentiation (8). A variety of miRs have been implicated in the development of vascular calcification whereas others appear to play a protective role.

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## miRs promoting vascular calcification (Table 1, Figure 1)

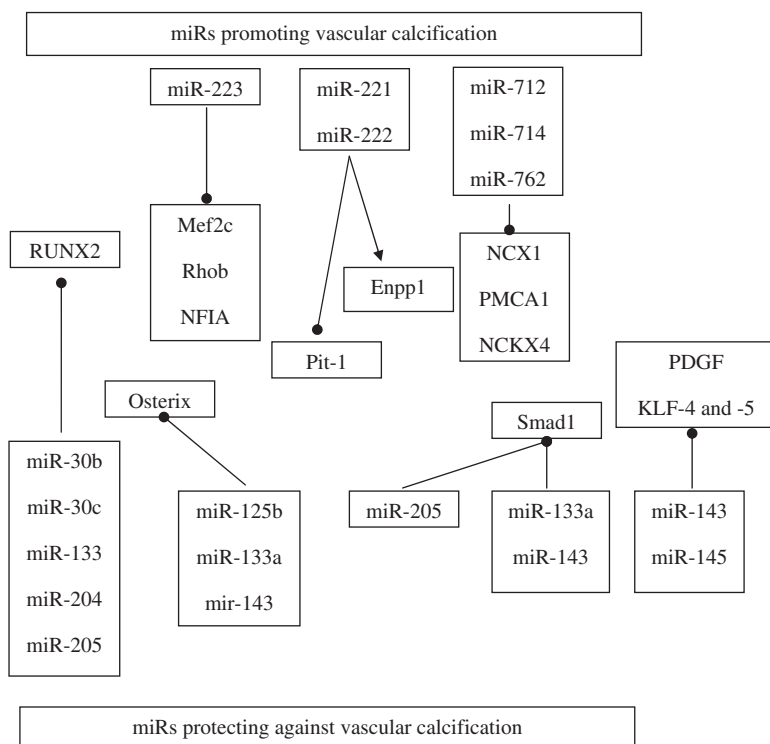
Elevated inorganic phosphate concentrations are associated with increased risk for vascular calcification and also increase the expression of miR-223, suggesting a role of the latter miR in vascular calcification (12). Physiologically, miR-223 is involved in the bone formation process.

**Table 1:** Major microRNAs (miRs) involved in the pathogenesis of vascular calcification.

microRNAs promoting vascular calcification	microRNAs protecting against vascular calcification
miR-221	miR-30b
miR-222	miR-30c
miR-223	miR-125b
miR-712	miR-133a
miR-714	miR-143
miR-762	miR-145
	miR-155
	miR-204
	miR-205

However, during vascular calcification, the ability of this miR to move from inflammatory cells of atherosclerotic lesions to VSMCs might represent a contributing factor to the phenotypic transition of VSMCs (13). Indeed, miR-223 overexpression increases VSMC proliferation and migration (13). It is also associated with reduced production of the cytoskeleton protein alpha-actin, resulting in altered morphology of the VSMCs (13). The key targets of miR-223 are myocyte enhancer factor 2c (Mef2c), a protein playing a crucial role in VSMC phenotypic transition, and Rhob, a protein involved in the contractility of VSMCs and in their response to stressful stimuli (e.g. hypoxia) (13). Both Mef2c and Rhob are expressed in lower levels when miR-223 is upregulated in VSMCs (12). Another target of miR-223 is nuclear factor IA (NFIA), an inhibitor of VSMC calcification (12, 14). The upregulation of miR-223 induces the degradation of NFIA (12, 14). Interestingly, a recent study reported a downregulation of serum miR-223 levels in patients with CKD stages 4 and 5, which was restored after kidney transplantation (15).

miR-221 and miR-222 are another pair of miRs that promotes vascular calcification. In particular, they exert a synergistic effect on altering the VSMC phenotype towards



**Figure 1:** Molecular pathways mediating the effects of microRNAs (miRs) on vascular calcification.

RUNX2, Runt-related transcription factor 2; Mef2c, myocyte enhancer factor 2c; NFIA, nuclear factor IA; Pit-1, type III sodium-dependent Pi cotransporter-1; Enpp1, ectonucleotide phosphodiesterase; NCX1, sodium/calcium exchange member 1; PMCA1, plasma membrane calcium pump isoform 1; NCKX4, sodium/potassium/calcium exchange member 4; PDGF, platelet-derived growth factor; KLF, Krüppel-like factor.

→ Stimulation; —●— Inhibition.

an osteoblast-like one (16). Their role appears to be more prominent during the early stages of VSMC transformation as their concentrations decrease as vascular calcification progresses (16). Culture of VSMCs under calcification-promoting conditions suppresses the expression of miR-221 and miR-222 (16). miR-221 and miR-222 upregulate the expression of ectonucleotide phosphodiesterase (Enpp1), which generates the mineralization inhibitor pyrophosphate (16). In addition, miR-221 and miR-222 suppress the expression of type III sodium-dependent Pi cotransporter-1 (Pit-1), which promotes vascular calcification (16).

miR-712, miR-714 and miR-762 also induce vascular calcification. These miRs directly target several calcium transporter channels including sodium/calcium exchange member 1 (NCX1), plasma membrane calcium pump isoform 1 (PMCA1) and sodium/potassium/calcium exchange member 4 (NCKX4) and thus lead to elevated intracellular calcium levels. Inhibition of miR-762, miR-714 and miR-712 was shown to substantially delay the calcification process (17).

### miRs protecting against vascular calcification (Table 1, Figure 1)

miR-30b and miR-30c are downregulated in VSMCs from calcified coronary arteries. These miRs target RUNX2, a protein that plays a significant role in the transdifferentiation of VSMCs to osteoblasts (7, 18). RUNX2 regulates osteocalcin, receptor activator of nuclear factor  $\kappa$ -B ligand (RANKL) and osteopontin, which in turn modulate bone matrix formation (4). miR-30b and miR-30c bind to a site at the 3' UTR region of RUNX2 resulting in its downregulation and thus leading to inhibition of ALP activity and reduced secretion of osteopontin and osteocalcin. In addition, these two miRs are subject to the control of bone morphogenetic protein-2 (BMP-2), which promotes vascular calcification by increasing intracellular levels of inorganic phosphate and by stimulating the expression of genes encoding osteoblast-phenotype-related proteins in VSMCs (19, 20). Indeed, treatment of VSMCs with BMP-2 decreases the expression of miR-30b and miR-30c and increases RUNX2 levels (21).

miR-133a is mostly present in skeletal and cardiac muscle cells but has also been identified in VSMCs. It appears to protect against vascular calcification. More specifically, miR-133a is downregulated in VSMCs that have transitioned to osteoblast-like cells (18). miR-133a binds to the 3' UTR area of RUNX2, leading to reduced production of osteocalcin and ALP and ultimately protecting the VSMCs from transforming into osteoblast-like cells (18).

miR-125b also protects against the osteogenic transdifferentiation of VSMCs in its early stages. Indeed, miR-125b levels decrease during the progression of vascular calcification. In addition, suppression of miR-125b promotes osteogenic transdifferentiation and matrix mineralization and increases ALP activity. Osteoblast transcription factor SP7 (osterix) appears to represent the principal target of miR-125b and mediates its protective effects against calcification (22).

miR-135a was also shown to inhibit the calcification process of VSMCs *in vitro*. Reduced miR-135a levels cause unrestrained matrix mineralization and increases in calcium concentrations and in ALP and osteocalcin activity. These effects appear to be mediated through the Krüppel-like factor-4 (KLF-4)/signal transducer and activator of transcription 3 (STAT3) pathway (23). However, others reported that miR-135a induces vascular calcification by affecting the activity of calcium transporter channels NCX1, PMCA1 and NCKX4, leading to increased intracellular calcium levels (17).

miR-204 has been identified as another inhibitor of vascular calcification both *in vitro* and *in vivo*. miR-204 targets RUNX2 and decreases the levels of this osteogenic factor by binding to its 3' UTR region. This results in decreased ALP activity and osteocalcin production, which protect against calcification (24). miR-205 also prevents calcification not only by targeting RUNX2 but also by modulating the activity of Smad1, which affects osteoblastic activity and bone development (25). A recent study reported a downregulation of miR-204 in the kidneys of patients with CKD, which correlated with the severity of impairment of renal function (26).

miR-145 and miR-155 play an important role in maintaining the contractility of VSMCs by stimulating the activity of myocardin. However, decreased levels of these miRs are also associated with the phenotypic transition of VSMCs to osteoblast-like cells and thus contribute to vascular calcification (27).

miR-133a and miR-143 are also downregulated in cells undergoing inorganic phosphate-induced calcification. These miRs target osterix and Smad1 and these mediators are excessively secreted during calcification. Interestingly, Mg was shown to prevent the downregulation of miR-133a and miR-143, suggesting that it might protect against vascular calcification (28). It has also been reported that elevated inorganic phosphate concentrations reduce the levels of both miR-143 and miR-145. The reduced bioavailability of these miRs results in increased expression of platelet-derived growth factor (PDGF) and KLF-4 and -5, which are associated with the propagation of vascular calcification (12, 14).

Another pair of miRs that appears to play an important role in vascular calcification is miR-29a and miR-29b, which regulate the activity of both osteoblasts and osteoclasts (29). Disruption of the fine-tuning of these miRs is not only associated with vascular calcification but also with arterial stiffening. The main target of miR-29 is a disintegrin and metalloproteinase with thrombospondin motifs-7 (ADAMTS-7). ADAMTS-7 is downregulated by miR-29 and this results in reduced activity of BMP-2 and the decay of cartilage oligomeric matrix protein (COMP), which is a glycoprotein involved in maintaining VSMC contractility. The end result is protection against vascular calcification and preservation of arterial elasticity (29). However, others reported that miR-29 promotes vascular calcification through the downregulation of elastin production, where it appears to play an important role. Decreased production of elastin is in turn associated with the transition of VSMCs to an osteogenic phenotype and with augmented calcium deposition in the vascular wall (30).

## Conclusions

Accumulating data suggest that miRs play an important role in the pathogenesis of vascular calcification. Emerging evidence suggests that complex networks of multiple miRs are responsible for the development of vascular calcification, primarily by targeting the osteogenic master regulator RUNX2 (31, 32) (Figure 1). Accordingly, miRs might be useful as biomarkers of early vascular calcification, allowing timely intervention to prevent progression of this process (32). In addition, miRs might represent promising targets for the prevention of vascular calcification and its adverse cardiovascular sequelae. Blocking of vascular-calcification-promoting miRs with antisense-based miR inhibitors or enhancing the activity of miRs than protecting against vascular calcification using nano-carrier-based approaches or vessel-specific vectors might represent future therapeutic options (33). However, given the complexity of regulation of this process and the multitude of miRs involved, more research is needed to identify optimal candidate miRs for targeting.

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