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Vascular calcification: Role of vascular smooth muscle cells and its regulation by microRNA

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Keywords:
Calcification
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Muscle cells
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ABSTRACT

In this review, the aim is to discuss the pathological development of vascular calcification including a brief description of arterial wall structure and function, the development of atherosclerosis, highlighting normal physiological and vascular calcification with particular emphasis on their common characteristics, critically review the recent findings implicating the role of vascular smooth muscle cells in the pathogenesis of the calcification process including the role of microRNAs in regulation of these cells phenotype as a target to control cardiovascular calcification.

تكلس الأوعية الدموية: دور خلايا العضلات الملساء الوعائية وتنظيمها بواسطة microRNA

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الكلمات المفتاحية:
تعظم
تكلس
خلايا العضلات الملساء الوعائية
ميكروا ار ان اي

الملخص

في هذا البحث، الهدف هو مناقشة التطور المرضي لتكلس الأوعية الدموية بما في ذلك وصف موجز لهيكل جدار الشرايين ووظيفته، وتطور تصلب الشرايين، وإبراز التكلس الطبيعي الفسيولوجي والأوعية الدموية مع التركيز بشكل خاص على خصائصها المشتركة، ومراجعة نقدية للنتائج الأخيرة يورط دور خلايا العضلات الملساء الوعائية في التسبب في عملية التكلس بما في ذلك دور الرنا الميكروي في تنظيم النمط الظاهري لهذه الخلايا كهدف للتحكم في التكلس القلبي الوعائي.

Introduction

Ectopic calcification is the deposition of calcium phosphate in the form of hydroxyapatite crystals in tissues that do not calcify under normal physiological conditions [1] such as such as blood vessels, cardiac valves, lung, muscle and central nervous tissue. In recent years, the quantification of ectopic calcification in arteries has become the focus of attention in a number of studies to determine whether it has diagnostic and prognostic value [2], [3]. Pathological calcification in vascular tissues is found in atherosclerosis and chronic kidney disease (Mönckeberg's sclerosis) patients, where it is associated with a 20–30-fold increase in cardiovascular mortality [4]. For a long time it was thought that ectopic calcification was a degenerative process and an end stage of inflammatory reaction [5], [6]. However, there is increasing evidence that this phenomenon is the result of dysregulation of tightly controlled promoter and inhibitor mechanisms controlling mineralization [7] and is similar to the processes of bone development and bone repair [6]. Vascular calcification is categorized according to the histological deposition of crystals into two major categories; intimal calcification which is associated with atherosclerosis [1], and medial calcification which appears in association with chronic kidney disease and diabetes mellitus type II [8]. Recent studies have demonstrated the involvement of vascular smooth muscle cells in the development of vascular calcification [9]; these cells undergo transdifferentiation to osteochondrogenic precursors with the concomitant formation of calcification nodules [10]. The mechanisms of vascular calcification and its regulation remain controversial. Recently, attention has been paid to highlighting the factors affecting the calcification process and the mechanism of controlling its progression, in order to discover potential treatments and to target the critical pathways in the development of the disease as well as to limit its complications. More recently, the discovery of microRNAs and their contribution to the development of cardiovascular diseases and vascular calcification heralds the dawning of a new era in our understanding of the mechanisms of pathological processes, including cardiovascular diseases and vascular calcification.

1.1. Vascular wall structure and development of atherosclerosis.

1.1.1. Vascular wall structure

To understand the process of atherosclerosis and calcification it is worthwhile summarizing the normal structure of blood vessels. In general, the artery wall is made of three layers from inside to outside:

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1. Intima: Lines the lumen and is made of endothelium which is a single layer of endothelial cells and the sub-endothelial layer [11]. The latter comprises a basal lamina of loose fibroelastic connective tissue and occasional smooth muscle cells and an outermost part, the membrane elastic interna, rich in elastic fibers [12]. The endothelium has an important regulatory role in maintaining vascular tone and it also secretes vasodilator substances such as nitric oxide (NO) and vasoconstrictive substances like the endothelins and angiotensin II [13].
2. Media: The middle layer, which is formed of smooth muscle cells, elastic fibres and collagen fibers [14].
3. Adventitia: Supportive layer formed mainly of connective tissue and blood vessels [14] containing fibroblast and progenirator cells and the Vasa Vasorum, a microvasculature network which supplies blood to the vessel wall [15].

1.1.2. Atherosclerotic lesion pathogenesis and plaque formation

Atherosclerosis is an inflammatory disease of the arterial wall that is characterized by deposition of fatty material accompanied by the accumulation of collagen and inflammatory cells in the vascular wall in the form of fatty plaques [16]. The main pathology in atherosclerosis is a consequence of dyslipidaemia and chronic inflammation [17]. It usually starts with fatty streaks, which are considered to be the first visible stage of disease development [16]. Although the pathological processes involved in atherosclerosis are still not well understood, there is strong evidence that inflammation and oxidative stress are the major drivers of disease development and progression [18]. However, different cell types are involved in the development of lesions including endothelial cells, immune cells (primarily macrophages) and vascular smooth muscle cells (VSMC) [19]. VSMCs play an important role in atherosclerosis development. At the site of the lesion they accumulate by migration and cell proliferation where they undergo phenotypic change to a secretory phenotype and produce excess matrix components [19].
As described in fig. 1, atherosclerosis starts with endothelial cell activation at sites of predisposition (sometimes referred to as endothelial damage) due to risk factors such as hypercholesterolemia, hypertension, smoking and familial predisposition (genetic predisposition), which leads to increased endothelial permeability and promotes leukocyte and platelet adhesion. In addition, a disturbance of vasoconstriction-vasodilatation balance due to decreased nitric oxide (NO) function is evident in the early stages of atherosclerosis [11] and may also have a role in disease initiation and progression. Once low density lipoprotien (LDL) particles enter the intima they undergo modification by oxidation and methylation which, in turn, provokes an inflammatory response [14]. These oxidized phospholipids (oxPLs) are seen at high levels in the fatty streaks of experimental animals [20]. Inflammation of intima follows on from endothelial activation and the accumulation of inflammatory mediators including chemotactic factors, secreted by intimal smooth muscle cells (SMCs), leads to the recruitment of more inflammatory cells such as monocytes to the intima of the blood vessel [16]. OxPLs are up taken by VSMC and macrophages; these cells have an increased take-up of modified oxPLs compared with the native PLs, due to the down regulation of native LP-binding LDL receptors and increased binding and endocytosis of oxLPs via increased expression of scavenger receptors SR-1 and SR-2. This leads to the formation of foam cells [21], [22]. In addition, oxPLs have a role in promoting monocyte trafficking to endothelial cells [20]. Necrosis of foam cells leads to fusion of lipid content and formation of extracellular lipid deposits in the plaque core, followed by SMC migration from the medial layer of the artery – it is these cells which are responsible for the secretion of the components of fibrous cap [14]. VSMC secretion of vascular endothelial growth factor (VEGF) [23] leads to the formation of thin walled vessels in the intima and media of the artery (neo-angiogenesis), producing an increased risk of rupture and haemorrhage within the artery wall and more accumulation of fibrous tissue [16]. Complications such as atheroma, vascular stenosis and haemorrhage are the major consequences of advanced atheromatous plaque [16].

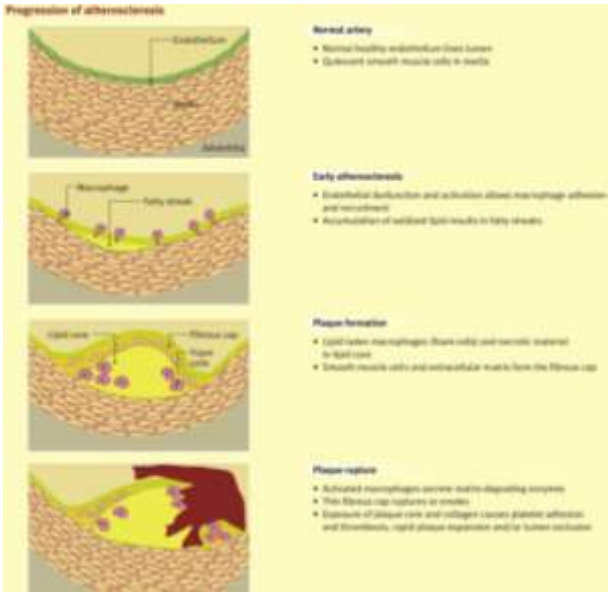

Figure 1: Progression of atherosclerosis [14].

1.2. Intimal and medial calcification

Vascular calcification is categorized into two main categories; intimal and medial [24]. Intimal calcification is associated with atherosclerosis and seems to be a complication or advanced stage of atherosclerotic plaque with a characteristic, patchy distribution throughout the vessel wall lesion [17]. The risk factors involved in intimal calcification are similar to those implicated in atherosclerosis [25]. However, the effect of vascular calcification on plaque stability is a controversial issue. Abedin et al., (2004) [5] has suggested that as the plaque calcification coalesces from small to larger deposits, the risk of rupture decreases, thus the risk of rupture is biphasic and depends on the interference area (contact area between plaque and vessel). In Mönckeberg's disease, medial calcification of muscular arteries is associated with chronic kidney disease, diabetes, osteoporosis and old age [26]. Medial calcification appears as a circumferential band in the medial layer of the artery [27], [28]. However, this term does not have a clear definition; it is used to describe both an accelerated atherosclerosis and general vascular calcification in CKD [29]. Whether the internal elastic lamina (IEL) is involved or not remains controversial. [26] examined the medial calcification in human arteries and found that IEL was involved in all cases, although the twelve cases studied represent a preliminary finding. Complications of medial calcification include vascular stiffness [30] and high systolic pressure with subsequent left ventricular hypertrophy [31]. Risk factors for medial calcification include impaired renal function and elevated levels of calcium (Ca^{2+}), inorganic phosphate (Pi) and long term warfarin treatment [32]. However, oxidative stress and inflammation are also major risk factors in both types of calcification [33], [34]. The pathogenesis of both forms of calcification is not well understood: medial arterial calcification is suggested to undergo an ossification mechanism similar to intra-membranous ossification [7]. In contrast, calcified lesions in chronic renal failure (CRF) animal models showed expression of chondrogenic proteins and chondrocytes [35]. In addition, examination of medial calcification in human arteries revealed evidence of active endochondral ossification (the process of bone development) in the media of these arteries [35], [36]. Intimal calcification is believed to mimic endochondral calcification in which bone formation occurs from a cartilage anlage [3]. Further studies are required to fully elucidate the relationship between medial and intimal calcification.

1.3. Physiological calcification and similarities between bone formation and vascular calcification.

The two distinct types of bone formation are intramembranous ossification, which involves the mineralization of collagen I based matrix mainly by the inner periosteal osteogenic layer [37], and endochondral ossification which is the ossification of a cartilaginous template and is organized by chondrocyte [38]. Studies suggest that

vascular calcification is an active process that mimics the bone development [39], [40]. There is emerging evidence of the expression of bone matrix proteins in vascular smooth muscle cell populations known as calcified vascular cells (CVC) [5], [1]. In addition, vascular mineralization starts with the formation of apoptotic bodies or membrane vesicles (MV) within the cells [39], [41] resembling the vesicular structures that normally appear in bone and cartilage [42]. In bone, these vesicles then become nucleation centres for hydroxyapatite [Ca10(PO4)6(OH)2] deposition. [39]. It has be shown that binding of MV to glycosaminoglycan (GAG) molecules is a feature of competent MV mineralization [42]. Similarly, in VSMCs, MVs have the capacity for Ca2+ and Pi nucleation [43], [10]. In bone, this process starts in response to retinoic acid exposure [10] and is controlled by calcium signalling that mediates the vesicle mineralization [39]. In VSMC the secretion of mineralization competent vesicles could be achieved in the presence of high calcium and phosphate alone [10]. In addition, similarities in the structure between both membrane vesicles in VSMC calcification and bone formation vesicles have been demonstrated [44]. Moreover, atherosclerosis ossification lesions often demonstrate organization of cell types including haemopoietic cells, fat tissue and multinucleated cells with positive osteoclast markers, which is reminiscent of bone marrow architecture [3]. Osteoclast like cells were seen at the edge of calcified lesions in vessels which were consistent with the remodelling process in bone [45]. The histological morphology of vascular calcification is shown in fig. 2.

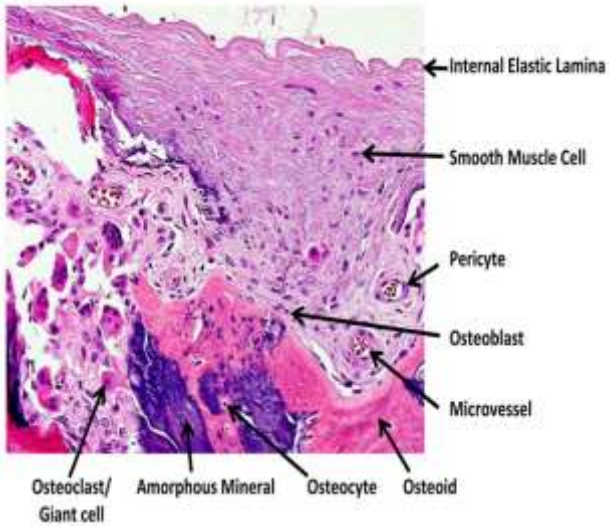

Figure 2: The histological morphology of vascular calcification [36].

1.4. Calcification regulation

As a biological process, calcification is controlled by stimulatory and inhibitory factors and a disturbed balance between these factors can induce calcification [46].

1.4.1. Calcification inducers:

• Calcium and phosphate: calcium is considered to be the main stimulator of VSMC to produce MV [47], the presence of high phosphate is essential to the calcification process [10], as shown in in vitro studies [48].

• Alkaline phosphatise (ALP): is an ectoenzyme that drives bone formation by hydrolysing inorganic pyrophosphate (PPi) to produce inorganic phosphate (Pi) which is involved in MV formation [49]. In vitro induction of tissue nonspecific alkaline phosphatase in human VCSMs stimulated the development of calcification, while its suppression inhibited calcification [50]. This could be targeted for future therapeutic purposes.

• Bone morphogenic proteins (BMPs): BMP2 and 4 are members of the transforming growth factor–beta (TGF-beta) super family [41] that are involved in vascular calcification [51]. BMP2 has been identified in atherosclerotic lesions [46]. In vitro studies of the effect of BMP2 on VSMC shows that it can only induce calcification in high phosphate medium while no effect was seen in cultures with normal phosphate levels, the calcification was inhibited in BMP2 knockdown animal models [52].

• Receptor activator nucleation ligand (RANKL): RANKL is a tumour necrosis factor that is expressed by osteoblasts - binding of its receptor results in osteoclast activation [53]. RANKL can induce VSMC calcification by increasing the expression of BMP4 [54].

1.4.2. Calcification inhibitors:

• Pyrophosphate: is a potent inhibitor of calcification that can directly inhibit hydroxyapatite formation [51]. An in vitro study of calcification in normal artery showed that pyrophosphate is an essential inhibitor of calcification even with high levels of calcium and phosphate [55].

• Matrix Gla protein (MGP): this is a vitamin K-dependent inhibitor that is normally expressed in SM and cartilage and inhibits calcification by binding to BMP2 [56]. Decreased expression of MGP was seen in calcified arteries [1].

• Fetuin A: a circulatory inhibitor that inhibits ectopic calcification at various stages of disease development. Its deficiency is associated with intimal calcification [57].

• Osteopontin (OPN): is a non-collagenous adhesive protein that is produced by macrophages in atherosclerotic plaques [58]. It inhibits the development of calcification in human VSMCs [59]. Although its deficiency does not cause calcification; it can enhance it in MGP-/- mice [32].

• Osteoprotegrin (OPG): is an inhibitory glycoprotein that belongs to tumour necrosis factor super family and has been found to inhibit calcification by targeting the Msx2 gene in VSMC [60] as well as regulating the procalcific effects of RANKL [61].

1.5. Mechanism of vascular calcification

Vascular calcification is found in close association with vascular pathology and is considered to be a prognostic indicator for the severity of cardiovascular conditions and kidney diseases [2]. The mechanisms of vascular calcification are still not well understood due to its multi-factorial nature. However, there are different theories about the origin of differentiated cells in vascular calcification, including VSMCs [9], resident pericytes [7], multipotent mesenchymal progenitor cells [9] and endothelial cells [62], [47] have reviewed the calcification process and have suggested that it is the result of inactivation of calcification inhibitory proteins such as Fetuin A and MGP [39], coupled with the formation of calcified matrix and the phenotypic changes identified in VSMC [47] (fig. 3).

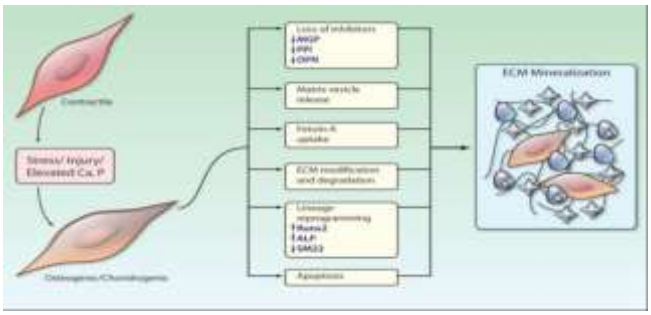

Figure 3: Mechanism of vascular calcification [47].

The molecular basis of vascular calcification depends mainly on the activation of BMPs which induce downstream signalling molecules via the induction of Msx2 and Runx2/cbfa1 to stimulate osteochondrogenic differentiation [63]. However, in cases of osteogenic differentiation, Mxs2 stimulates osteogenesis via Runx2-independent activation of osterixs (Osxs) which are transcriptional regulators of mineralization and differentiation of osteoblasts [64], while in chondrogenic calcification Runx2 is the major driver [65]. The expression of chondrocyte differentiation factor SOX9 and the absences of Wnt, osterix and low Msx favours a chondrocyte pathway (fig. 4) [9].

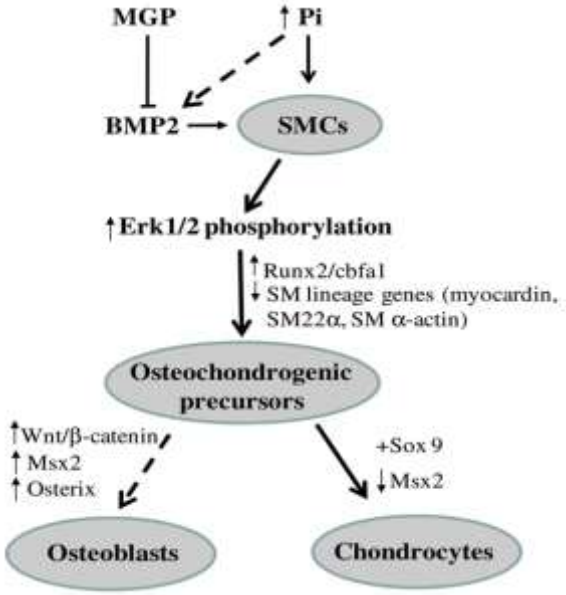

Figure 4: Osteogenic and chondrogenic differentiation of VSMCs [1].

Bostrom et al., (2011) have reviewed the role of BMPs in vascular calcification [38]. BMPs induce an effect by binding to their receptors which activates the phosphorylation of transcription factors known as "mothers against decapentaplegia" homolog (Smads). Activated Smads complex together with the common mediator (co)-Smad4, followed by translocation to the nucleus resulting in the modulation of gene expression in concert with Runx2 and other calcification stimulatory factors (fig. 5). Additionally, BMPs can induce calcification via a Wnt/β-catenin paracrine pathway [66].

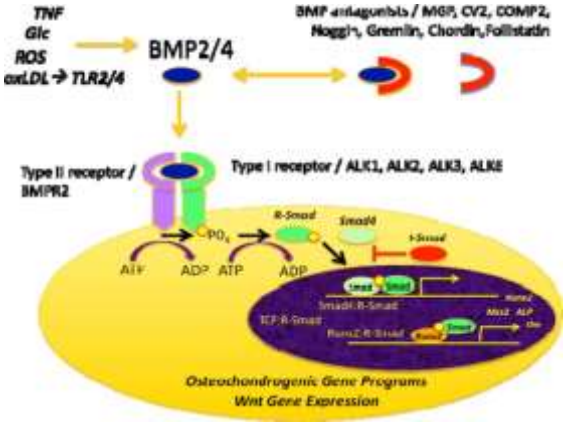

Figure 5: BMP induced activation of Smads [38].

1.6. Vascular smooth muscle cells in vascular calcification

Vascular smooth muscle cells are the most abundant cell type in the arterial wall [1] as well as in atherosclerotic plaque [20]. In recent studies it has been shown that the majority of differentiated cells in vascular calcification are VSMCs [9]. They have a characteristic of phenotypic plasticity in response to diseases [67], which makes these cells of great importance in understanding the pathogenesis of vascular diseases. Normal contractile smooth muscle cells express SMC marker genes such as SM-alpha actin, SM-22 alpha and myocardin [24]. As they differentiate they lose expression of these proteins and acquire the expression of bone protein markers such as osteopontin and alkaline phosphatase, with subsequent secretion of proteins necessary to start the calcification process [24]. In calcifying lesions, they lose these markers and acquire osteochondrogenic markers [24]. However, many studies have shown that VSMC have the ability to differentiate to other cell types when exposed to specific stimuli [1] such as inflammatory mediators [17], hyperphosphatemia, oxidized phospholipids (oxPLs) [20] and oxidative stress [19].

Using genetic fate mapping in a MGP-/- mouse model of arterial calcification, SMCs were labelled with (SMC)-Cre recombinase and Cre reporter Rosa26-LacZ (R26R-LacZ) 22 during embryonic development. SMCs were identified to be the main cell that underwent transdifferentiation to osteoprogenitor precursor cells [1]. Cultured human VSMCs have also been shown to transdifferentiate into osteochondrogenic precursors and start to form calcified nodules [68]. In an in vitro study of the effect of extracellular calcium (Ca2+) and phosphate (Pi) on VSMCs, it has been shown that high Pi levels, even with moderate Ca2+ levels, can induce membrane vesicles release and subsequent calcification [10]. In addition, loss of VSMC calcium sensation receptor was associated with increased membrane vesicles in in vitro studies of human and bovine VSMCs [69]. There are different pathways that promote the phenotypic switching of VSMC depending on the stimulating factor. For example, oxidative stress is an essential contributor to vascular diseases by the formation of reactive oxygen species (ROS), especially hydrogen peroxide H2O2 [19]. Byon et al., (2008) found that in vitro exposure of VSMC to H2O2 induced their transdifferentiation and calcification and this was associated with up regulation of bone markers and down regulation of SMC markers (fig. 6) [19]. These effects were found to be induced through increasing the expression of Runx2 transcription factor rather than the induction of VSMCs apoptosis [19]. These findings were consistent with another study in regard to the calcification induction effect of oxidative stress [70].

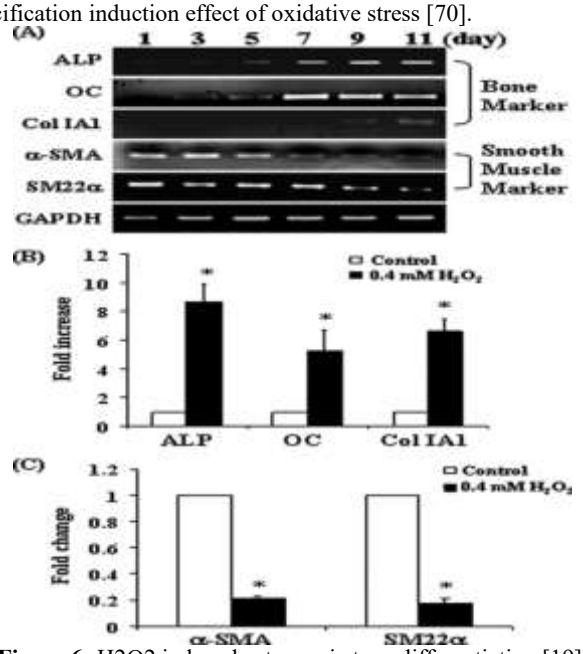

Figure 6: H2O2 induced osteogenic transdifferentiation [19].

Another crucial factor influencing VSMCs transdifferentiation is oxidized phospholipids such as 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), which have been implicated in the pathogenesis of atherosclerosis [34]. In addition, they were demonstrated to induce phenotypic switching in vivo and in vitro [20]. It has been shown that SMC marker genes such as SM alpha-actin and SM myosin were suppressed after being cultured for 24 hours in high oxPLs while the expression of bone protein markers MCP-1 and MCP-2 simultaneously increased. These effects were completely blocked by targeting Kruppel-like transcription factor 4 (KLF4) [20]. In a preliminary study of vascular calcification in end stage renal disease patient (ESRD), immunohistochemical examination of radial artery showed increased expression of inflammatory factors in correlation with increased expression of BMP-2 and collagen type 1 (fig. 7) in comparison to healthy controls. These effects were mediated by disrupting the low density lipoprotein receptor pathway [17]. Although the study data were limited by the low number of both patients and controls, in addition to variation of their health conditions, they indicate roles for BMP2 and oxLDL in medial artery calcification.

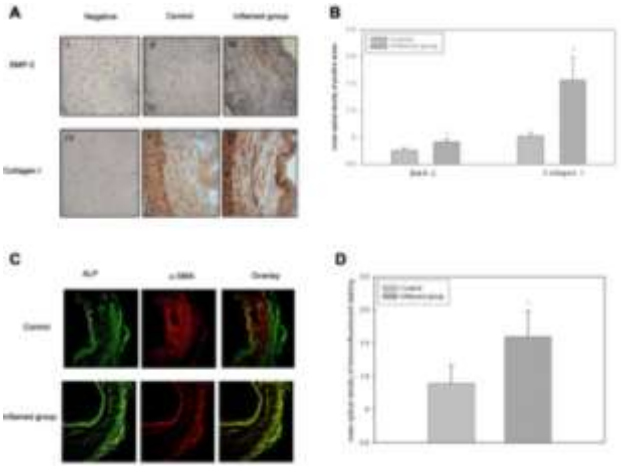

Figure 7: Immunohistochemical staining shows increased the expression of BMP and collagen I in inflammed radial arteries of ESRD patients [17].

Another pathway was found to mediate VSMC transdifferentiation in response to high phosphate levels is the Erk1/2 signalling pathway [1]. This pathway is involved in the PDGF-induced inhibition of SMC gene expression [85]. This effect was attributed to the phosphorylation of Elk transcription factor and its binding to surface response factor (SRF) which interferes with myocardin and SMC gene expression [85]. An additional factor that was identified to affect the transdifferentiation of VSMC in vascular calcification is DNA methylation [2]. Using 5-aza-deoxycytidine (5-aza-dc) which is a DNA methyl-transferase inhibitor on human VSMCs culture, it was found that 5-aza-dc influenced the calcification of human VSMCs by inducing the expression of ALP as an essential factor for its action, as well as increasing the expression of sodium-dependent phosphate cotransporter (Pit-1), which is essential for Pi induced vascular calcification [86]. Interestingly, this study also found an up-regulation of SMC genes [2] which does not correlate with induction of calcification, and further evaluation is required to identify the mechanisms implicated in this controversy. In another study, Wen et al., (2013) has demonstrated the involvement of the Nalp-3 inflammatory pathway in induction of VSMC calcification via triggering of pro-inflammatory mediators such as cytokines, thus inducing cell apoptosis [71]. A recent study by Lei et al., (2014) of the role of elastin fibers in vascular calcification suggests that elastin degradation by metalloproteinases results in exposure of calcium binding sites in elastin with subsequent mineral deposition and VSMC differentiation [24]. It was found that rat VSMCs switched to an osteochondroblast phenotype when cultured on calcified elastin and hydroxyapatite crystals. These changes were reversed after the removal of calcifying medium. A longer term procedure might retard this reversal and further in vivo studies are suggested to determine whether elastin induced calcification precedes the phenotypic changes. In vitro study of the effect of vitamin D on VSMCs showed an increase in the mineralization associated with the expression of bone markers and loss of myogenic markers; this effect was accompanied by increasing expression of Runx2 [72]. However, the role of vitamin D in vascular diseases is complex and dependent on the dose [73].

1.7. Micro RNA targeting in regulation of VSMC function

MicroRNAs are small non-coding RNAs that control gene expression via targeting messenger RNA (mRNA) [74]. They can negatively control gene expression by complementary annealing with the 3' untranslated regions (UTRs) of mRNA [75]. One miRNA is able to target large numbers of genes and regulate many biological processes [76]. Recent studies have shown the involvement of miRNAs in various diseases such as cancer and inflammatory disorders [64]. Many miRNAs have been recognized to be involved in the regulation of function and phenotype of VSMCs by targeting the transcriptional factors regulating differentiation [40]. As reviewed by [87] (fig. 8), miRNAs are involved in the regulation of VSMCs either by promoting or inhibiting the expression of the contractile cell phenotype. CARG box promoters of contractile genes bind to SRF in order to activate contractile gene transcription in the presence of myocardin and myocardin related factors (MRFs).

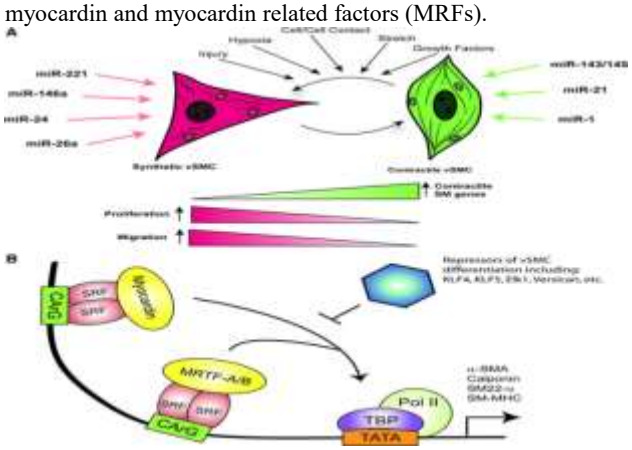

Figure 8: MicroRNA regulation of VSMCs [87].

On the other hand, Krupple like factor-mediated inhibition of these mechanisms causes suppression of contractile gene expression [87]. In an in vitro study of miR-204 role in vascular calcification, there was clear evidence that miR204 is involved in the calcification process as a negative regulator, where its expression was decreased in mouse VSMCs during calcification [76]. Using luciferase reporter assays, it was shown that this action is mediated via the down regulation of Runx2 which has been identified as a main target for miR204. Inhibition of miR204 induced the expression of Runx2 protein, while miR204 mimics decreased both its expression and osteoblastic differentiation [76]. The same finding was found in an in vivo experiment using agomir-204-chemically modified oligonucleotides on mouse models [76]; however, the in vivo effect of miR-204 on normal calcification in bone was not definitely identified because of the short time for treatment used in the study. In addition, members of the miR221/222 cluster have been demonstrated in vascular wall [77] and their expression was found to be up-regulated in intimal lesions [78]. In cultured VSMCs they were verified by qRT-PCR [77] and their expression was considerably higher than that found with microarray analysis [78]. This difference is thought to be due to differences in the age of the experimental rats; this finding was supported by recent evidence that miRNA expression alters with age [79]. Knock down of both miR-221 and miR-222 was associated with decrease of VSMC growth both in vivo and in vitro [80], [77]. Using gain of function and loss of function approaches, the tumour suppressor mRNA p27 (kip1) and p57 (kip2) have been demonstrated to have binding sites for miR-221 and miR-222, suggesting them to be the target genes of miR-222 and miR-221 regulation. However, other targets still need to be identified as the proliferation promoting effect of miR221 and miR-222 was not totally blocked in p27(kip1) and p57(kip2) deficient VSMs cells [80], [77]. In addition to VSMCs, up regulation of miR-221and miR-222 expression was found in endothelial cells in coronary artery disease [79]. Further studies are required to elucidate the role of miR-221 and miR-222 in these cells in relation to vascular proliferation.

Another study by Chen et al., (2013) found that the expression of miR-125b, miR-145 and miR-155 are low in the blood of CKD patients in comparison to healthy patients (fig. 9) and the same findings were found using animal models [74].

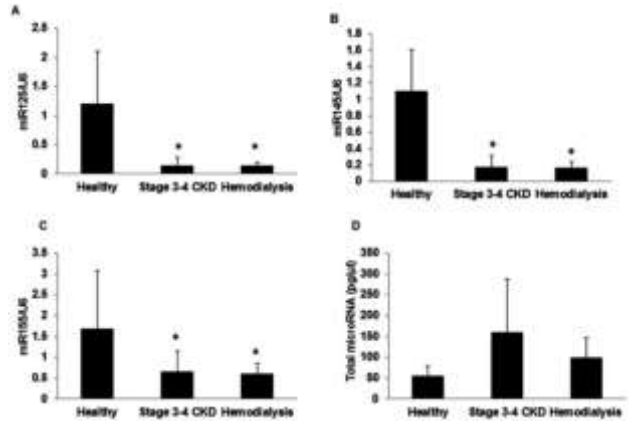

Figure 9: Reduced miRNAs expression in chronic kidney deseases [74]

MicroRNA-145 is the most abundant miRNA in vascular wall [78] and has been shown to be greatly down regulated in neointimal lesions [81]. MiR-145 expression changes have been shown to be closely associated with VSMCs marker gene expression changes in platelet derived growth factor (PDGF)-induced vascular calcification in vivo and in vitro. Computational analysis revealed that its effect is mediated via targeting the KLF-5 transcription factor [81]. In addition, miR-145 has been found to influence VSMC normal differentiation [82]. Both miR-143 and miR-145 are highly expressed miRNAs in smooth muscle cells that regulate cardiovascular function and VSMC differentiation through serum response protein (SRP). Loss of these miRNAs inhibits VSMC phenotypic differentiation [75]. Additionally, Goettsch et al., (2011) have shown that miR-125b was down regulated in ApoE-/- mouse models, as well as in neointimal lesions [78]. It was demonstrated that miR-125b inhibits BMP4 induced mesenchymal cell transdifferentiation into osteoblasts while its knockdown increases alkaline phosphatase expression [40]. Moreover, miR-155 was demonstrated to control VSMCs phenotype by repressing angiotensin type 1 receptor (ATR1) [74] and its down regulation in arteries of CKD animal models was associated with aortic calcification and end organ vascular abnormalities [83]. Another in vivo study has shown that miR-155 is reduced in association with low glomerular filtration rate [84], which suggests that miR-155 has an essential role in renal and vascular diseases. MicroRNA-31 is another miRNA that has been shown to control phenotypic modulation of VSMCs [85]. Using gain of function and loss of function approaches, miR-31 mimics were found to decrease the expression of SMC genes and miR-31 inhibitor increased them. These effects were found to be mediated by targeting cellular response of E/A stimulating genes (CREG) [85]. However, this study needs further confirmation by in vivo animal studies as well as human tissue experiments.

Conclusion

To conclude, vascular calcification appears to be an important predictor for cardiovascular and renal disease progression. Whether the development of calcification is a complication of disease or a protective mechanism to prevent pathological expansion is an important point for future studies. Further understanding of the development of calcification and the differences between medial and intimal calcification mechanisms is required. The similarities between vascular and normal bone calcification are useful in developing our understanding of the complex nature of calcification pathology, and further understanding of the role of osteoclasts in the progression of calcification could be an important link between the different theories of disease mechanism.

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