Role of Vascular Smooth Muscle Cell Phenotypic Switching and Calcification in Aortic Aneurysm Formation Involvement of Vitamin K-Dependent Processes

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Involvement of Vitamin K-Dependent Processes

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Abstract—Aortic aneurysm is a vascular disease whereby the ECM (extracellular matrix) of a blood vessel degenerates, leading to dilation and eventually vessel wall rupture. Recently, it was shown that calcification of the vessel wall is involved in both the initiation and progression of aneurysms. Changes in aortic wall structure that lead to aneurysm formation and vascular calcification are actively mediated by vascular smooth muscle cells. Vascular smooth muscle cells in a healthy vessel wall are termed contractile as they maintain vascular tone and remain quiescent. However, in pathological conditions they can dedifferentiate into a synthetic phenotype, whereby they secrete extracellular vesicles, proliferate, and migrate to repair injury. This process is called phenotypic switching and is often the first step in vascular pathology. Additionally, healthy vascular smooth muscle cells synthesize VKDPs (vitamin K-dependent proteins), which are involved in inhibition of vascular calcification. The metabolism of these proteins is known to be disrupted in vascular pathologies. In this review, we summarize the current literature on vascular smooth muscle cell phenotypic switching and vascular calcification in relation to aneurysm. Moreover, we address the role of vitamin K and VKDPs that are involved in vascular calcification and aneurysm.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2019;39:1351-1368. DOI: 10.1161/ATVBAHA.119.312787.)**

Key Words: aortic aneurysm ■ blood vessels ■ extracellular matrix ■ phenotypic switching ■ vascular calcification ■ vascular smooth muscle cell ■ vitamin K

Aortic aneurysm is a matrix degenerative disease defined by a dilated blood vessel. Aneurysms weaken the arterial vessel wall increasing risk of rupture, which results in massive, and often fatal, internal bleeding. Aortic aneurysms are the result of environmental and genetic risk factors, which lead to shear stress, inflammation, positive vascular remodeling, and ECM (extracellular matrix) degradation.[1–4] Aortic aneurysms resulted in >151 000 deaths globally in 2013 and are ranked in the top 15 causes of mortality in the United States.[5] However, there are no efficient interventions to prevent aneurysm progression.

The sequential pathophysiology of aneurysm formation is unclear, but it is believed that vascular smooth muscle cells (VSMCs) play a central role. Most VSMCs in the vessel wall display a contractile phenotype, which allows them to maintain vascular tone. However, VSMCs have the ability to differentiate into a synthetic phenotype. This process is termed phenotypic switching and is considered to be a key mechanism in arterial remodeling.[6,7] Synthetic VSMCs are characterized by decreased contractile protein expression and increased the production of elastolytic enzymes (MMPs [matrix metalloproteinases]), which degrade the ECM and facilitate migration by detaching cells from the basement membrane and ECM.[8] Additionally, synthetic VSMCs can secrete extracellular vesicles that enhance local inflammation and promote vascular calcification.[9,10] Vascular calcification, an extreme form of arterial remodeling mediated by VSMCs, is characterized by deposition of calcium phosphate crystals in the vessel wall.[11] Calcification of the medial layer of the vessel wall is associated with arterial stiffening[12] and has been observed in aneurysm.[13–16] Vascular calcification is, in part, regulated by vitamin K-dependent mineralization-inhibiting proteins, such as MGP (matrix Gla protein).

Vitamin K is a fat-soluble vitamin, whose main function is to facilitate carboxylation of VKDPs (vitamin K-dependent proteins). High dietary intake of vitamin K has been shown to be associated with reduced coronary artery calcification and all-cause mortality,[17,18] but the potential influence of vitamin K on VSMC-mediated mechanisms of aortic aneurysm formation has not been analyzed.

Received on: December 4, 2018; final version accepted on: May 14, 2019.

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Nonstandard Abbreviations and Acronyms

AAA	abdominal aortic aneurysm
BMP	bone morphogenetic protein
CHOP	C/EBP homologous protein
CNN	SM-calponin
COL3A	type III collagen
dp-ucMGP	dephospho-uncarboxylated MGP
ECM	extracellular matrix
ER	endoplasmic reticulum
Gas6	growth arrest-specific gene 6
GGCX	γ-glutamyl carboxylase
HDL	high-density lipoprotein
KO	vitamin K epoxide
LDL	low-density lipoprotein
MAPK	mitogen-activated protein kinase
MGP	matrix Gla protein
MMP	matrix metalloproteinase
MYH11	myosin heavy chain 11
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor-κB
NOX	NADPH oxidase
OPN	osteopontin
PDGF	platelet-derived growth factor
PDI	protein disulfide isomerase
ROS	reactive oxygen species
SIRT1	sirtuin 1
SMMHC	SM myosin heavy chain
STAT3	signal transducer and activator of transcription 3
TAA	thoracic aortic aneurysm
TAAD	thoracic aortic aneurysm and aortic dissection
TGF-β	transforming growth factor β
TIMP	tissue inhibitor of MMPs
TRX	thioredoxin
VKA	vitamin K antagonist
VKDPs	vitamin K-dependent proteins
VKOR	vitamin K-oxidoreductase
VKORC1	vitamin K-oxidoreductase complex subunit 1
VKORC1L1	VKORC1-like 1
VSMC	vascular smooth muscle cell
XBP1u	unspliced X box protein 1

In this review, we summarize current knowledge on mechanisms regulating VSMC phenotypic switching and calcification in the context of aortic aneurysm formation. Additionally, we explore the role of vascular vitamin K in these processes to offer insight into therapeutic implications.

Aneurysm Formation

The word aneurysm comes from the Greek word aneurysma, which means dilation. Clinically, an aneurysm is defined by a dilated blood vessel with an enlargement of 50% greater than the normal diameter.[19] The arterial wall subjected to an aneurysm is vulnerable to pressure. Increased blood pressure results in a continuing enlargement of the vessel wall, which may eventually lead to rupture and hemorrhage.

Aneurysms predominantly occur in the aorta and are clinically termed according to the location: (1) ascending aortic aneurysm, (2) aortic arch aneurysm, (3) descending thoracic aortic aneurysm (TAA), (4) thoracoabdominal aortic aneurysm, and (5) abdominal aortic aneurysm (AAA), the most common type. AAA is the 13th most commonly found cardiovascular disorder,[20] and most frequently seen in men, older than 60 years, and with one or more risk factors, including family history, high blood pressure, high cholesterol, obesity, and smoking.

Aortic aneurysms are characterized by a disrupted vessel wall structure with degraded elastic laminae and disappearance of organized VSMC layers.[21,22] For a long time, biomechanical factors were thought to be the main cause of aneurysm formation and rupture. Currently, aneurysm pathophysiology is regarded as a complex biological process involving cellular-driven remodeling of the vessel wall.[23] Active and dynamic remodeling, rather than degeneration of the vessel wall, is the cause of AAA development.[24] In support of this, AAA is characterized histologically by inflammation, oxidative stress, VSMC apoptosis, and ECM degradation.[4] Moreover, a major difference between the healthy vessel wall and the aneurysm wall is the reduced number of VSMCs.[25]

Aortic aneurysms that develop above the diaphragm in the upper aortic segment are generally termed TAAs. TAAs are predominantly a result of genetic and connective tissue disorders, such as Marfan syndrome caused by mutations in fibrillin-1, which assists proper elastic fiber formation and elastin deposition.[26] Other TAA-related diseases are Ehlers-Danlos syndrome type IV and Loeys-Dietz syndrome, caused by mutations in COL3A (type III collagen) and TGF (transforming growth factor)-β receptors (*TGF-βR1* or *TGF-βR2*) genes, respectively.[27] Moreover, TAA may develop in individuals harboring genetic predispositions without syndromic disorders, termed familial TAA and aortic dissection (TAAD).[28] Increased risk for TAAD is also found in patients with bicuspid aortic valve and mutations in VSMC contractile proteins.[29–31]

TAAs and AAAs share common features such as dilation and rupture of the aorta, proteolysis of ECM and depletion of VSMCs.[22] While the AAA is seen as an atherothrombotic origin presenting with intraluminal thrombus, oxidative stress, and adventitial inflammation, intraluminal thrombus, and immune response are not usually observed in the TAA.[22] However, infiltration of inflammatory cells in the aortic wall of TAA patients has been documented.[32,33] Pathological observation in a mouse model suggests that noninflammatory accumulation of SMC-like cells and elastin-poor ECM, which leads to vascular remodeling, is involved in the development of TAAs.[34] Mucoid degeneration, characterized by accumulation glycosaminoglycans and observed during aging of the human aorta, is also specific to TAA.[22,35]

Vitamin K

Vitamin K is a fat-soluble vitamin. Naturally occurring vitamin K includes vitamin K1 (phylloquinone) and vitamin K2 (menaquinones). Phylloquinone is mainly found in leafy green vegetables, while menaquinones can be found in fermented

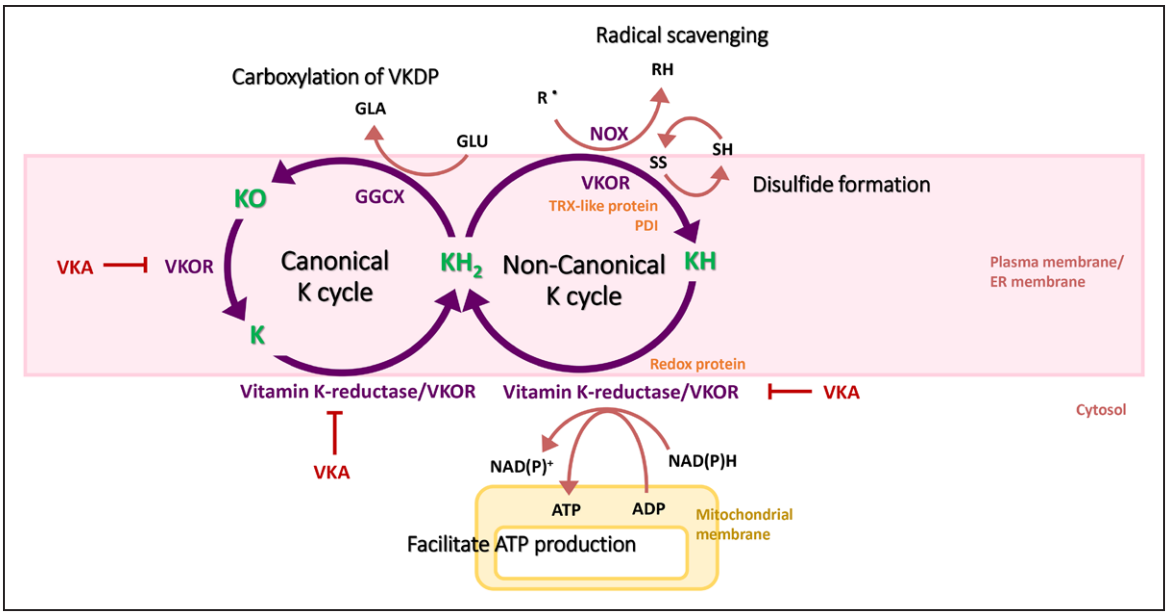

Figure 1. The canonical and noncanonical vitamin K cycle. In the canonical vitamin K cycle, vitamin K is a cofactor in the carboxylation of VKDPs (vitamin K-dependent proteins), which then become activated. The oxidation from vitamin K-hydroquinone (KH_2) to vitamin K-epoxide (KO) is driven by GGCX (γ-glutamyl carboxylase). VKOR (vitamin K-oxidoreductase) and vitamin K-reductase enable the recycling process of vitamin K through conversion of KO to K and KH_2, respectively. Imbalanced equilibrium of vitamin K forms may allow an alternative vitamin K cycle, whose reduction steps to semiquinone (KH) are also driven by VKOR. Through this noncanonical K cycle, VKOR and a redox protein (DTT or TRX [thioredoxin]) facilitate KH_2 conversion to KH, thereby exerting antioxidant properties such as preventing NADPH (nicotinamide adenine dinucleotide phosphate)-dependent lipid peroxidation and protecting the plasma membrane against reactive oxygen species (ROS) by regulating NOX (NADPH oxidase) activity. KH_2 is involved in protein disulfide-thiol interchange of NOX. Moreover, VKOR, together with PDI (protein disulfide isomerase) or TRX-like protein, contribute to disulfide formation and protein synthesis within the ER (endoplasmic reticulum) membrane. Vitamin K also improves mitochondrial oxygen consumption and is involved in ATP generation. In all cases, the presence of a VKA inhibits the recycling process of vitamin K and its subsequent functions. R* indicates free radical species; RH, organic substrates; SH, disulfide-thiol; and SS, disulfide bond.

food.[36] Menaquinones have an unsaturated aliphatic side chain with a variable number of prenyl units. The number of prenyl units indicates the respective type of menaquinone. It has been shown that menaquinone-7 is absorbed most efficiently and has the best bioavailability.[37,38] During absorption, vitamin K is taken up by enterocytes in the small intestine where it is packaged into chylomicrons, which are taken up by the liver. Vitamin K2, specifically the long chain menaquinones, is redistributed into the circulation and available for extrahepatic tissues such as the vasculature.[39,40]

Vitamin K Cycle

The main function of both vitamin K1 and K2 is acting as cofactors in carboxylation of VKDPs (Figure 1). Vitamin K serves as a cofactor for the enzyme GGCX (γ-glutamyl carboxylase), which catalyzes the conversion of glutamic acid in VKDP to γ-carboxyglutamic acid. This reaction is driven by oxidation of vitamin K-hydroquinone to vitamin K epoxide (KO). KO can be recycled by VKOR (vitamin K-oxidoreductase), which converts KO to vitamin K and back to vitamin K-hydroquinone.[41,42]

VKOR Polymorphisms Versus Aneurysm Risk

VKOR is expressed in various vascular cells and heart tissue. The first evidence of a role for vitamin K in aneurysm formation comes from a study showing increased expression of VKOR in vascular endothelial cells and ventricular aneurysm tissue of human heart.[43] Additionally, in a study of 253 cases of aortic dissection, of which 11.5% had Marfan syndrome, and 416 controls, VKORC1 (VKOR complex subunit 1) polymorphisms were shown to be associated with an almost 2-fold higher risk of aortic dissection, independent of conventional vascular risk factors.[44] In the same study, a similar observation was made in patients with stroke and coronary heart disease, suggesting VKORC1 variation as a common genetic risk factor for all vascular diseases.[44] Indeed, the role of VKORC1 was highlighted as an emerging genetic marker of TAA and acute aortic dissection in hemostasis patients.[45] Identifying the genetic polymorphism such as VKORC1 in hemostasis patients may give more specificity in identifying patients at risk of TAA than the currently used protein markers such as plasma D-dimer.[45] Another study detecting the frequency of VKORC1 polymorphisms was performed in 189 patients with an aneurysm of the ascending aorta, excluding those with genetic disease, and 188 controls with matching age, sex, body mass index, and smoking status.[46] VKORC1 polymorphism rs9923231 was significantly associated with aneurysms of the ascending aorta, suggesting that carboxylation of VKDPs might be involved in TAA formation.[46]

Role of VSMCs in Aortic Aneurysm Formation

VSMC Phenotypic Plasticity

The vessel wall of large arteries consists of different cells, including endothelial cells, fibroblasts, and VSMCs. VSMCs reside in the tunica media and comprise the majority of cells in arteries.[47] The main function of VSMCs is to regulate vascular

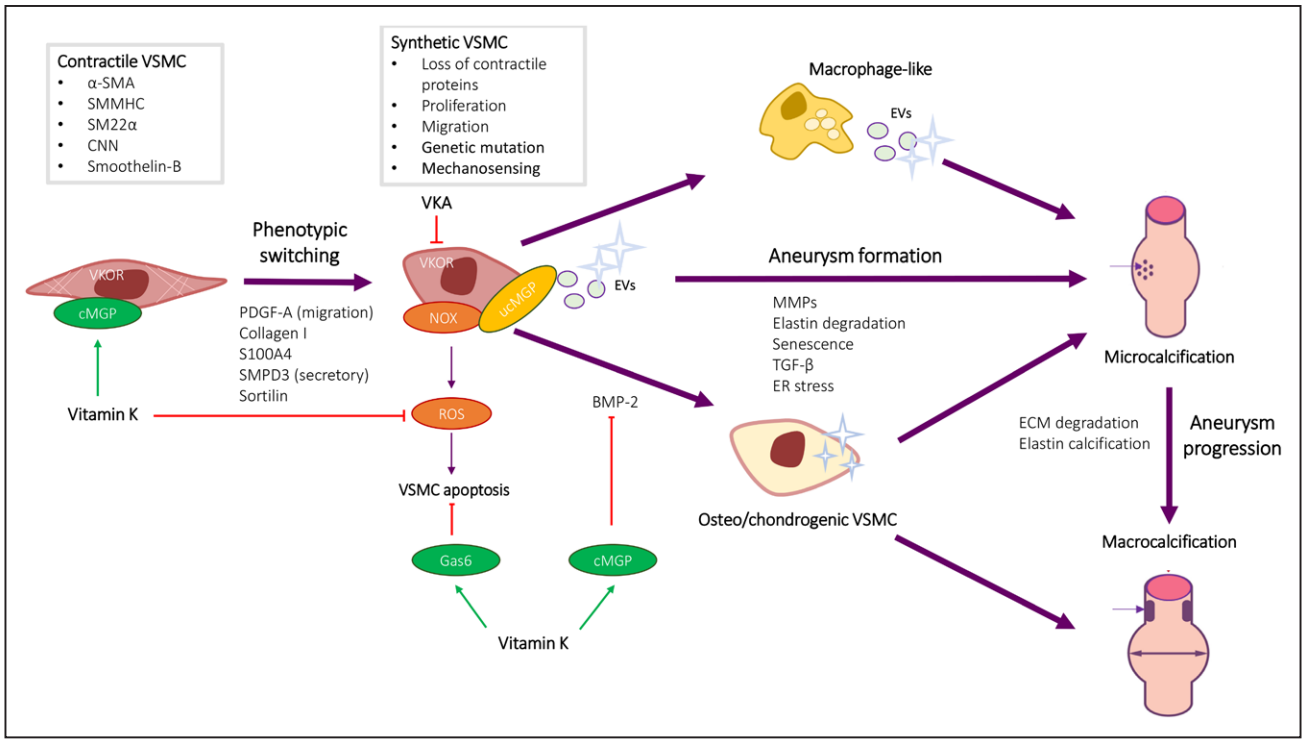

Figure 2. Vascular smooth muscle cell (VSMC) plasticity in aneurysm formation. VSMC phenotypic switching between contractile and synthetic is essential for vascular remodeling and maintaining a healthy vasculature. In the presence of stimuli (such as PDGF [platelet-derived growth factor]), a contractile VSMC can acquire a synthetic phenotype which is characterized by downregulation of contractile proteins and increase in proliferation and migration. VSMC phenotypic switching precedes aneurysm formation and differentiation of VSMCs into osteo/chondrogenic and macrophage-like phenotypes, which promote further remodeling and calcification. VSMCs fail to maintain contractility in the presence of inactive vitamin K-dependent proteins (VKDPs) and secrete calcifying vesicles. Oxidative stress drives VSMC proliferation, VSMC apoptosis, and initiates microcalcification. This, in turn, causes destructive changes in the surrounding matrix and leads to weakening of the vessel wall. Macrocalcification develops over time and further aggravates the dilation of the vessel. α-SMA indicates α-smooth muscle actin; BMP, bone morphogenetic protein; cMGP, carboxylated matrix Gla protein; CNN, SM-calponin; ECM, extracellular matrix; EVs, extracellular vesicles; Gas6, growth arrest-specific gene 6; MMP, matrix metalloproteinase; NOX, nicotinamide adenine dinucleotide phosphate oxidases; ROS, reactive oxygen species; SMMHC, SM myosin heavy chain; TGF-β, transforming growth factor β; ucMGP, uncarboxylated matrix Gla protein; VKA, vitamin K antagonist; and VKOR, vitamin K-oxidoreductase.

tone and diameter through contraction and dilatation, thereby controlling blood pressure and blood flow distribution.[48] VSMCs are not terminally differentiated, which distinguishes them from skeletal muscle cells and cardiomyocytes. Indeed, VSMCs retain remarkably high plasticity, which allows them to modulate and switch phenotype on stress signals.[7] VSMC phenotypic switching is triggered by inflammation and injury.[49] Atherosclerosis, hypertension, and postangioplasty restenosis, as well as aneurysms, are all associated with VSMC phenotypic switching.[50–52]

VSMC phenotypic modulation can be characterized by changes in morphology, protein expression, proliferation, and migration (Figure 2). In vitro, contractile VSMCs are elongated and display spindle-shape morphology, while synthetic VSMCs are rhomboidal and display cobblestone morphology.[53,54] Synthetic VSMCs express lower levels of proteins involved in contraction, such as α-SMA (α-smooth muscle actin), SMMHC (SM myosin heavy chain), SM22α (smooth muscle 22α), CNN (SM-calponin), and smoothelin-B.[55] Additionally, synthetic VSMCs are characterized by increased proliferation and migration.[55,56] VSMCs can also give rise to other cells in the vessel wall, such as osteo/chondrogenic-like and macrophage-like cells, which has been reviewed by others.[57–59]

The local environmental factors that modulate VSMC phenotype include growth factors, such as PDGF (platelet-derived growth factor)[56,60] and TGF-β,[56,61] angiotensin II,[62] nitric oxide,[56] reactive oxygen species (ROS)[63] and the components of the ECM. Moreover, other factors, such as hyaluronan and heparin, are also known to influence VSMCs.[64–66]

Phenotypic Switching of VSMCs Is Key in Aneurysm Formation

In this section, we discuss how mechanisms regulating VSMC phenotype are involved in aneurysm formation and how they could potentially be modulated by vitamin K.

Mutations in Genes Encoding Contractile Proteins and TGF-β

In line with loss of contractile function promoting aneurysm formation,[67,68] TAAs may arise from a variety of gene mutations encoding structural components of ECM, cytoskeletal/smooth muscle contraction proteins, and proteins associated with TGF-β pathway, such as TGF-β receptors and SMAD proteins.[69] For example, mutations in *MYH11* (myosin heavy chain 11) cause familial TAAD, aortic stiffness, alteration in aortic compliance, and arterial degeneration.[70] Mutations in α-SMA, a target of TGF-β-signaling, also cause TAAD,[31] as a result of impaired VSMC contractility, ability to maintain ECM,[71] differentiation, and proliferation.[69] Apart from genetic

mutations, alterations in vascular tone and interstitial pressure may induce interstitial edema, generate aortic wall stress, and initiate intraparietal dissection.[72] Pharmacological induction of VSMC contractility in a mouse model, which predisposed to aortic dissection, showed great protection against intramural delamination, an initiator of dissection.[73] These findings emphasize the importance of VSMC contractility in maintaining the structural integrity of the aorta.

TGF-β is another factor implicated in regulating VSMC contractility.[26] Stimulation of VSMCs with TGF-β1 increases α-SMA, SMMHC, and CNN mRNA expression[61] and reduces proliferation.[74] However, since reduced proliferation results in fewer VSMCs in the vessel wall, leading to AAA formation,[75] the exact effect of TGF-β1 signaling on VSMCs in aneurysm formation is likely dependent on the context and other mechanisms at play. Increased TGF-β expression is observed in human AAA and TAA tissue[75,76] and in TAAs related to Marfan syndrome.[26,77] Increased TGF-β activity has been shown to play a role in the pathology of TAAD in Marfan syndrome.[78,79] In line with that, a loss-of-function mutation in *TGF-β2* was sufficient to cause aortic root dilation in a mouse model of Marfan syndrome.[80] Paradoxically, these mice showed an upregulation of TGF-β signaling and TGF-β1 expression.[80] Milewicz et al[68] suggested that overactivity of TGF-β may be a secondary response to tissue injury in thoracic aortic diseases, rather than the primary cause. Indeed, aortic dilation and medial elastin damage in young Marfan mice developed in the absence of elevated VSMC TGF-β signaling.[81] Furthermore, Loeys-Dietz syndrome mice (TGF-β receptor knockin; *TGF-βR2*$^{G357W/+}$) showed increased TGF-β signaling only in later stages of TAA development.[82] Conditional disruption of VSMC *TGF-βR2* in postnatal mice impaired VSMC contractile apparatus induced a proliferative response and rapidly result in TAAD.[83] Moreover, TGF-β neutralization in these mice exacerbated aortic disease, suggesting that basal TGF-β is required to maintain structural integrity of VSMCs.[83] These studies highlight the fact that TGF-β signaling contributes to aneurysm formation regardless of the presence of the fibrillin-1 mutation. Indeed, a growing body of evidence suggests emerging concepts such as mechanosensing and vascular tone regulation may better explain how aneurysms are formed in the context of TAAD.[84]

Interestingly, TGF-β neutralization was shown to enhance angiotensin II–induced aortic rupture and aneurysm in mice, both at the thoracic and abdominal regions.[85] In a similar mouse model, TGF-β was shown to protect against inflammatory aortic aneurysm progression and complications.[34] Whereas VSMC-extrinsic TGF-β signaling protects against AAA, VSMC-intrinsic TGF-β signaling protects the thoracic aorta in the angiotensin II–induced mice.[86] A recent study revealed that TGF-β1 suppressed a broad array of proinflammatory genes in cultured human VSMCs, partially through the STAT3 (signal transducer and activator of transcription 3) and NF-κB (nuclear factor-κB) pathway.[87] In parallel, TGF-β1 potently induced the expression of VSMC contractile genes.[87] Observation from this study suggests that the 2 events are independent, and suppression of the proinflammatory genes is not the consequence of the induced contractile VSMC phenotype.[87]

The interaction of vitamin K and TGF-β has not been studied in the context of aneurysm and VSMC biology. A study in osteoblast-like cells derived from osteosarcoma showed that vitamin K promoted TGF-β mRNA expression.[88] However, a study in cancer cells showed no alteration in the expression of TGF-β1.[89] Conversely, TGF-β was found to induce expression of VKDPs, such as MGP, in embryonic lung cell culture and Gas6 (growth arrest-specific gene 6) protein in VSMCs.[90,91]

Although mutational defects may not be easily amended, vitamin K may alleviate their downstream effects, pathologies, and vessel integrity in aortic aneurysm, such as elastin degradation, VSMC apoptosis, oxidative stress, and calcification, which we discuss in the following sections.

Elastin Degradation

Phenotypic switching of VSMCs underlies the destructive changes that lead to aneurysm formation in animal models of TAA.[67,92] Investigating genetic diseases, in this case Marfan syndrome, provided a better understanding of this process.[6] The defective elastin lamellae impair VSMC attachment, which leads to switching of VSMCs towards a synthetic, proelastolytic phenotype.[6,92] The proelastolytic phenotype of VSMCs isolated from human AAA tissue is characterized by increased production of MMPs.[93] MMPs are a family of endopeptidases that exert proteolytic activity towards elastin and collagen. Increased MMPs, in particular, MMP-2 and MMP-9, degrade the ECM, thereby weakening the vascular wall and leading to AAA formation. TAA and AAA were found in ApoE mice with TIMP (tissue inhibitor of MMPs)-1 deficiency.[94] Moreover, VSMC migration is inhibited by inhibition of MMPs.[95] End-stage AAA usually features a destruction of elastin which is compensated by an increased synthesis of collagen.[96,97]

Although the effect of vitamin K on elastin degradation has not been studied in the context of aneurysm, a study in cancer cells showed that vitamin K2 inhibits production of MMPs by suppressing NF-κB and MAPK (mitogen-activated protein kinase) activity.[98] Vitamin K2 (45 mg/d) was shown to reduce serum MMP-3 in rheumatoid arthritis patients in a cross-sectional (n=158) and a longitudinal study (n=52).[99] However, vitamin K1 (10 mg/d) does not altered serum MMP-3 of rheumatoid arthritis patients as revealed by a randomized control trial study (n=64).[100]

Apoptosis of VSMCs

AAAs have a reduced number of VSMCs,[25] leading to limited capacity to produce connective tissue and to repair elastin breaks. The reduced number of VSMCs is likely the consequence of apoptosis, because apoptotic VSMCs have been observed in the medial layer of AAAs.[101] VSMC apoptosis can result from proteolytic degradation of the ECM[102] and also from oxidative stress.[103] Other factors that may contribute to a proapoptotic milieu include inflammatory mediators, proliferative triggers, such as PDGF and cell stretch, hypoxia, and DNA damage.[102] Also, mechanical stress can contribute by enhancing endoplasmic reticulum (ER) stress-induced apoptosis.[104] In addition, VSMC senescence can ultimately progress into apoptosis.[102] Apoptosis is accompanied by the

generation of apoptotic bodies, which, if not cleared by phagocytosis, can stimulate calcification.[105] Calcium deposits, in turn, can aggravate inflammation and mechanical stress[106,107] indicating the fueling of an amplification loop spiraling down towards a mechanically compromised vessel wall.

Vitamin K may be associated with VSMC apoptosis through activation of Gas6, which is a VKDP containing γ-carboxyglutamic acid.[108] Gas6 inhibits VSMC apoptosis by binding to Axl, a tyrosine kinase receptor, thereby activating Akt.[109] Vitamin K2 was shown to inhibit rat VSMC calcification in culture through restoration of the Gas6/Axl/Akt antiapoptotic pathway.[110] Downregulation of the Gas6-Axl interaction is associated with inorganic phosphate (Pi)-induced human VSMC apoptosis and addition of human recombinant Gas6 inhibits Pi-induced apoptosis and calcification.[111] Additionally, Axl was found to be upregulated in cultured rat VSMCs after vascular injury and may mediate migration and proliferation of VSMCs.[112] Circulating Gas6 and soluble Axl in plasma has been measured in healthy controls (n=141) and patients with large (n=123) or small (n=122) AAA.[113] Gas6 concentration was found to positively correlate with AAA size, and the correlation was stronger with Gas6/Axl ratio.[113] While the authors of this study suggest that the higher Gas6 concentration in plasma may reflect the higher *Gas6* gene expression and Gas6/Axl plasma ratio might be a useful marker for AAA,[113] the mechanisms of action were not demonstrated. All of these studies point to the possibility that vitamin K could protect from aneurysm via Gas6/Axl and inhibition of apoptosis.

ER Stress

Recently, aortic aneurysm formation has been shown to correlate with increased ER stress.[114] In line with this, transcription factor XBP1u (unspliced X box protein 1), which is expressed in the absence of ER stress, has been shown to maintain VSMC contractile phenotype. XBP1u deficiency causes VSMC dedifferentiation, enhances proinflammatory and proteolytic VSMC phenotype, thus aggravating TAA and AAA in vivo.[115] ER stress also promotes TAAD formation through CHOP (C/EBP homologous protein), an effector of the ER stress-induced unfolded protein response which regulates ER stress-induced apoptosis.[116,117] CHOP deletion prevents VSMC apoptosis and TAAD development, without affecting VSMC proliferation.[117] Moreover, ER stress inhibition was able to attenuate AAA in angiotensin II–induced ApoE$^{-/-}$ mice.[118]

The effect of vitamin K on ER stress has not been studied in the context of aneurysm. However, it is known that VKOR is associated with the ER disulphide forming pathway,[119,120] and ER transcription factors, such as XBP1, may affect the expression of *VKORC1* gene.[121]

Oxidative Stress

Oxidative stress is a well-established factor promoting the development of aortic aneurysm, and it has been implicated in regulating features of VSMC phenotype. Both VSMCs and infiltrating inflammatory cells can contribute to the increase in ROS in the vessel wall.[122] Several sources of ROS exist (NOX [NADPH (nicotinamide adenine dinucleotide phosphate) oxidases]), lipoxygenases, cyclo-oxygenase, and nitric oxide synthase; however, NOX enzymes have been predominantly implicated in AAA pathology thus far.[123] NOX enzymes form a family involved in the production of superoxide anion, and NOX1, NOX2, NOX4, and NOX5 are expressed in human VSMCs.[124,125] Genetic deletion of p47^{phox}, a cytosolic subunit that associates with NOX1-4, reduced the incidence of AAA in an angiotensin II–induced mouse model of aneurysm.[126] Interestingly, systemic NOX2 deficiency reduced ROS in AAA lesion, however, it exacerbated angiotensin II–induced AAAs in mice by increasing vascular inflammation.[127] This was because of disruption of macrophage function, the main source of increased NOX2 expression in the AAAs.[127] NOX4-mediated oxidative stress has been shown to be implicated in VSMC apoptosis,[124] while increased NOX2 and NOX5 activity have been shown to induce VSMC proliferation.[128–130] Moreover, NOX2 and NOX5 are most prominently expressed NOX isoforms in the aorta of AAA patients.[131] NOX1-mediated ROS generation has been shown to decrease contractile protein expression in cerebral aneurysms.[132]

More generally, scavenging ROS attenuated the formation of AAA in mice and protected against aortic aneurysm development.[133] In another mouse model of AAA, aneurysm formation and rupture were decreased by vitamin E, a well-known antioxidant.[134] Flow loading and systemic antioxidant therapy lowered oxidative stress and early aortic dilation in a rat model of AAA.[135] Antioxidant treatment not only reduced macrophage infiltration into the abdominal aorta but also delayed AAA formation and reduced aortic rupture.[134] Additionally, in human subjects, increased circulating level of TRX (thioredoxin), a protein released from cells in response to oxidative stress, and release of TRX in the luminal part of AAA patients positively correlated with AAA size and expansion.[136] Correspondingly, proteomic analysis showed that TRX protein in the human AAA wall sample is negatively correlates with the growth rate, which is in line with hypothesis that upregulation of TRX in defense against ROS may slow down the aneurysm expansion rate.[137] A similar dependency of TAA on increased ROS has been observed, as in vitro and ex vivo studies on human TAA tissue revealed that oxidative stress mediates VSMC phenotypic switching towards synthetic phenotype through connective tissue growth factor.[52] These results were further validated in a mouse model of TAA, which showed similar pathology as observed in human tissue.[52] Taken together, these studies suggest that decreasing oxidative stress attenuates aneurysm formation; however, the underlying mechanisms have not been studied in detail.

Recent findings suggest a role for vitamin K as an antioxidant. Human VKORC1L1 (VKORC1-like 1), a paralogue enzyme of VKORC1 found in the ER, was found to regulate vitamin K-dependent intracellular antioxidant function in the cell membrane.[138] Both vitamin K1 and K2 were shown to mediate a VKORC1L1-dependent increase in cell viability. Genome-wide expression studies showed that VKORC1L1 is expressed throughout many tissues and may serves as a potential target for the regulation of intracellular redox homeostasis.[138]

Additionally, Mukai et al[139] showed that vitamin K-hydroquinone is a potent free radical scavenger. Kinetic studies showed vitamin K-hydroquinone has 10- to 100-fold higher antioxidant activity than other radical scavengers such

as α-tocopherol and ubiquinone. In another study, menadione (a synthetic form of vitamin K without a side chain) and vitamin K1 and K2 inhibited NADPH-dependent microsomal lipid peroxidation in the presence of dithiothreitol, a non-physiological redox protein which drives VKOR.[140] This antioxidant effect was abolished when a vitamin K antagonist (VKA), namely warfarin, was added into the reaction. Vitamin K1 and K2 were shown to protect against oxidative stress in primary oligodendrocytes and immature cortical neurons.[141] Additionally, vitamin K1 and K2 prevented oxidative stress-induced cell death by inhibiting activation of 12-lipoxygenase in primary oligodendrocytes.[142] This vitamin K action was independent of the GGCX, though facilitated by the VKOR redox cycle.

Vitamin K has also been shown to directly modulate NOX activity. Bridge et al[143] have shown that reduced phylloquinone ($K1H_2$) serves as a natural electron donor for NOX in the soybean plant and might protect the plasma membrane against ROS. In addition, $K1H_2$ is also involved in protein disulfide-thiol interchange of NOX.[143] Electron transfer via hydroquinone was proposed as a mechanism sensing the redox changes across the plasma membrane that occur during aging and senescence.[143–145] Additionally, the VKOR recycling requires redox partners such as PDI (protein disulfide isomerase) and TRX-like protein to deliver reducing equivalence.[146] PDI is known to regulate the activity of NOX in various cell types and is essential for redox-mediated VSMC migration via PDGF, which is associated with NOX activation.[147] Intracellular PDI regulates expression and activity of the NOX, which contributes to ROS generation.[148] Further to that, VKOR significantly contributes to disulfide formation and redox homeostasis within the ER.[120] Reduction of VKOR activity by warfarin, in combination with other disulfide bond formation proteins, resulted in cell death.[120]

All these studies show that oxidative stress is an important factor promoting both AAA and TAA formation, and that vitamin K, as an antioxidant, has great potential in this field. However, as the antioxidant mechanisms of vitamin K have not been analyzed in VSMCs in the context of aneurysm, this warrants further study.

Senescence of VSMCs

Many hallmarks of cellular senescence have been reported in aneurysms and aneurysm-derived VSMCs. Stress signals, such as uremic toxins and ROS, induce a cellular senescence response, which is characterized by a quiescent state and secretion of proinflammatory cytokines.[149] Senescent VSMC releases factors which drive differentiation of local VSMCs to become osteo/chondrogenic.[150] An in vitro study by Liao et al[151] indicated that VSMCs derived from human AAA exhibited a distinct phenotype resembling accelerated replicative senescence. These VSMCs displayed distortion in morphology and limited proliferation capacity in comparison to VSMCs derived from the inferior mesenteric artery. Additionally, a genome-wide association study revealed a common variant, rs10757278, found in human AAA.[152] Rs1075728 is adjacent to *CDKN2A/CDKN2B* (genes encode the cell cycle regulators), which is expressed in senescent VSMCs.[59,153,154]

Another senescence mechanism is related to lamin A. Persistent DNA damage and accumulation of prelamin A, the precursor to the component of nuclear membrane lamin A, is a key mediator that links premature senescence and accelerated vascular calcification.[155] Increased expression of lamin A was observed in the region of high wall stress and not in the nonaneurysmal vascular beds of AAA patients.[156] Furthermore, a physiological pulsatile stretch of VSMC in vitro caused a time-dependent elevation of prelamin A and lamin A expression.[156] Lamin A is also a direct activator of SIRT1 (sirtuin 1).[157] SIRT1 is a nicotinamide adenine dinucleotide–dependent protein deacetylase, highly expressed in the vasculature.[158] SIRT1 is known for its protective function in vascular aging and is involved in variety of cellular processes including the inhibition of apoptosis.[159,160] Inhibition of SIRT1 in VSMCs results in DNA damage, early senescence, and apoptosis.[160] In addition, reduction of SIRT1 in animal models induces medial degeneration, AAA formation, and aortic dissection providing a potential molecular basis for aneurysm formation in patients.[160] Concurrently, human AAA wall showed significantly lower SIRT1 immunoreactivity in comparison to nonaneurysmal aortic sections.[161] In addition, overexpression of VSMC-specific SIRT1 was able to suppress AAA formation in an animal model.[161] VSMCs cultured from end-stage human AAA expressed elevated levels of differentiation marker (microRNA 145), lower expression of SIRT1, and exhibited higher level of DNA damage compared with nonaneurysmal human saphenous vein.[162] These features did not correlate with patients' chronological age.[162]

Telomeres, which are DNA repeats at the end of chromosomes, have a protective role against age-associated diseases.[163] Telomere attrition (shortening) is one of the hallmarks of cellular aging and one of the causes of cellular senescence.[164] Shortening of telomeres has been documented in biopsies of AAA of aortic tissue.[165]

Senescence has been shown to accompany not only AAAs but also TAAs. VSMCs derived from bicuspid and tricuspid aortic valve-associated aneurysms of TAA patients displayed reduced proliferation and migration capacity. In addition, telomere length analyses showed that these VSMCs had significantly shorter telomeres compared with VSMCs derived from healthy tissue.[166] Aschacher et al[167] showed that telomere shortening was associated with reduced activity of telomerase, which in turn is involved in VSMC proliferation. It was concluded that VSMCs in TAAs undergo premature senescence. The cause-consequence relationship of VSMC senescence and TAA formation and the role of telomere shortening remains to be unraveled.[167]

Mitochondrial dysfunction is another hallmark of cellular senescence, and it has been implicated in vascular aging and cardiovascular diseases.[168] Mitochondrial respiratory chain dysfunction causes reduction of ATP synthesis and increased ROS generation.[168,169] The bioenergetic profiling of proliferating human VSMCs revealed that they rely on mitochondrial oxidative phosphorylation and have a high respiratory reserve capacity at rest, while aging cells have lower resting oxidative phosphorylation and reduced reserve capacity.[170] Bioinformatic analyzes suggest the importance of mitochondria and oxidative

phosphorylation in AAA.[171] Indeed, mitochondrial dysfunction was observed in synthetic VSMCs of human AAA tissue and of AAA mouse model.[172] A recent study demonstrated that VSMC mitochondrial respiration is reduced in TAAs of fibulin-4 mutant mice; this was coupled with increased ROS and dysregulated expression of genes involved in energy metabolism.[173] The reduction of mitochondrial respiration in VSMC was also observed in VSMCs of Loeys-Dietz syndrome mice and human fibroblasts of Marfan and Loeys-Dietz syndrome patients, suggesting that altered mitochondrial respiration may contribute to TAA formation.[173]

However, the direct effects of vitamin K on VSMC senescence have not been studied, with one exception. Vitamin K2 has been reported to be involved in ATP generation. A study in Drosophila mitochondria revealed that vitamin K2 acts as an electron carrier that can rescue deficiency of *PINK1* (PTEN-induced putative kinase 1),[174] a mitochondrial regulator of autophagy that determines VSMC fate.[175,176] In this study, vitamin K2 was shown to rescue mitochondrial dysfunction, improve mitochondrial oxygen consumption, and facilitate the production of ATP.[174]

Taken together, the studies of noncanonical roles of vitamin K at the background of our current understanding of aneurysm biology suggest that vitamin K antioxidative property seems to be the most promising effects on the pathogenesis of aneurysms (Figure 1). In the following parts, we discuss how the canonical function of vitamin K might play a role in the mechanisms of aneurysm formation through inhibition of VSMC phenotypic switching towards osteo/chondrogenic and VSMC calcification.

VSMC Calcification in Aneurysm Formation

Calcification, the process of deposition of calcium phosphate crystals of the medial layer of the vessel wall, is associated with arterial stiffening and causes isolated systolic hypertension.[177] VSMCs are key players in vascular calcification.[178] Phenotypic switching of contractile VSMCs into osteo/chondrogenic VSMCs is accompanied by expression of bone-specific proteins that regulate ECM mineralization (Figure 2).[179,180] BMP-2 (bone morphogenetic protein 2), the main regulator of osteogenesis, binds the BMP receptor and activates SMAD signaling, which leads to expression and increased activity of osteo/chondrogenic transcription factors such as RUNX2 (Runt-related transcription factor 2), Osterix, and SOX9.[59,181] These transcription factors control expression of mineralization regulators, such as alkaline phosphatase, osteocalcin, OPN (osteopontin), osteoprotegerin, and bone sialoprotein.[59] In response to a variety of stress signals, VSMCs can cause and enhance calcification via several mechanisms, including increased apoptosis,[105] extracellular vesicle release,[182] and loss of natural calcification inhibitors, such as MGP.[183]

Calcification of bovine VSMCs suppressed the expression of elastic fiber protein, tropoelastin (elastin monomer), and fibrillin-1.[184] Conversely, the addition of recombinant tropoelastin was able to inhibit VSMC calcification.[185] Study in bovine VSMC culture shows that Vitamin K2 inhibits calcium deposition in a dose-dependent manner, and the addition of vitamin K to bisphosphonate treatment enhances the expression of tropoelastin.[186] Moreover, Vitamin K2 and bisphosphate inhibit bovine VSMC differentiation to osteo/chondrogenic phenotype.[186] Vitamin K alone increased MGP and decrease OPN expression of inorganic Pi-induced bovine VSMC calcification in culture.[186]

Apart from VSMCs, macrophages are now recognized as important contributors to the progression of calcification and proinflammatory macrophages release microcalcification-inducing extracellular vesicles.[187,188] Cholesterol loading converts VSMCs into a macrophage-like foam cells.[189,190] Interestingly, recent findings show that macrophage-like human VSMCs, which were induced by enzyme-modified nonoxidized LDL (low-density lipoprotein), have a genetic profile associated with calcification including upregulation of BMP and downregulation of MGP and had a higher propensity to calcify.[191] Taken together, these studies suggest that differentiation of VSMCs in to macrophage-like cells may contribute to vascular calcification (Figure 2).

Calcification of Aneurysms

Vascular calcification has been shown to be increased in symptomatic AAA and contribute to rupture risk in AAA, as it induces changes in mechanical properties of the aneurysm (Figure 2).[192,193] However, when categorized based on the size as microcalcification (<50 µm) and macrocalcification (>50 µm),[194] calcification has been suggested to promote different outcomes with respect to aneurysms.

Microcalcification, which precedes macrocalcification, is unequivocally considered detrimental and is present during a biologically active fast-dilating aneurysm.[13] Microcalcification colocalized with elastin degradation in the aorta of Marfan patients.[15] In Marfan mice, microcalcification was abundant in the ascending aorta and strongly correlated with the aortic root diameter.[15] In the same study, macrocalcification was found to be prominent in the aortic root of Marfan mice and correlated with aortic dilation to a lesser extent.[15] The data on macrocalcification are more complex. Recently, a prospective case-control and observational cohort study of 72 patients with asymptomatic AAA revealed that macrocalcification was not associated with aneurysm expansion or AAA events,[13] and it was suggested that macrocalcification of the aneurysm segment might stabilize the degenerated vascular wall.[13] However, research is conflicting and recently published data on the role of calcium scoring in aneurysmal aortic disease revealed macrocalcification of TAAs and AAAs was associated with significantly higher overall and cardiovascular mortality.[16] This was a retrospective, observational, single-center study performed on 319 patients (TAA=123 and AAA=196), who underwent computed tomography. Aortic aneurysm calcification scores were derived from computed tomography, and multivariate regression analysis was performed after correcting for potential confounders (age and blood pressure).[16] Although the study reflects clinical practice, renal function, a known cause for vascular calcification, was not analyzed.[16]

While macrocalcification may be less detrimental to the aneurysmal vessel wall than microcalcification, it does start as microcalcification. Therefore, detection of microcalcification could be useful to measure biological activity in the aneurysmal wall, thus identifying risk for AAA events.[195] Recently, it was shown that active mineralization in AAA, represented

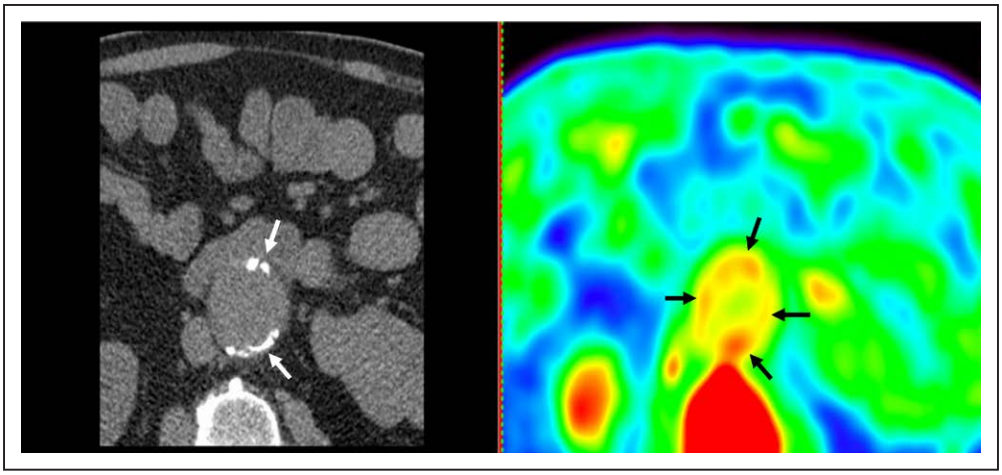

Figure 3. Example of an aneurysm with areas of ^{18}F-NaF uptake (positron emission tomography [PET]-computed tomography [CT], **right**) that do not correlate with calcification visible on CT (**left**). On the CT scan, calcification is visible at the anterior and posterior wall of the aorta (white arrows); however, there is almost circumferential ^{18}F-NaF uptake (black arrows). This shows that there are areas where the PET-CT tracers detect active mineralization, where none is detected by CT scan. The signal intensity of ^{18}F-NaF uptake visualized by PET-CT requires a careful quantitative analysis, which includes calculation of tissue-to-background ratios using background blood pool activity in the right atrium. Moreover, manual adjustment of regions of interest is important when analyzing ^{18}F-NaF uptake in structures adjacent to the bone to ensure that any signal attributed to bone is discarded.

by a marked increase in ^{18}F-NaF uptake on positron emission tomography-computed tomography (see Figure 3 as a visual example), correlates with presence and progression of AAA.[13] ^{18}F-NaF specifically detects active calcification and puts forward microcalcification as a promising risk factor amendable for intervention in AAA.[13,196]

Vitamin K-Dependent Calcification Inhibitors

For many decades, it was thought that calcification of the vasculature was a passive process. The discovery of inhibitors actively preventing vascular calcification revealed that this process is highly regulated by cells and proteins. Of specific interest are VKDPs, whose activity can be modulated by vitamin K or VKAs.[42,197] VKDPs can be found in hepatic and extrahepatic tissues (summarized in Table). In all cases, VKDPs only exhibit their function when being carboxylated with the aid of its unequivocal cofactor vitamin K.

VSMCs synthesize several VKDP calcification inhibitors (Table). One of the most potent inhibitors for vascular calcification known in is MGP.[183] MGP regulates VSMC phenotypic switching through BMP signaling which transdifferentiates VSMC into osteo/chondrogenic phenotype.[42,55,208] This transdifferentiation, together with loss of VSMC markers; α-SMA and SM22α, was found in MGP-deficient mice.[205,222] MGP also acts locally to inhibit ECM mineralization in calcified vascular lesions.[209–211] MGP maintains contractility of VSMCs by binding to BMP-2 and thus inhibiting osteo/chondrogenic differentiation.[208,223,224] MGP also prevents calcification when it is loaded into extracellular vesicles that are secreted during the process of vascular remodeling.[212–214] Moreover, intrasection analysis of human coronary arteries (n=12) showed that MGP is associated with microcalcifications in early atherosclerotic lesions,[225] which is associated with increased plaque vulnerability.[107,226,227] A patient-based proteomic analysis revealed that MGP is increased in calcified AAA (n=6) and TAA (n=6) tissue sample in comparison to the adjacent normal aorta tissues.[228] It was suggested that upregulation of the calcification inhibitor might be a feedback mechanism to prevent further calcium deposit.[228] However, circulating MGP has not been studied in the context of AAA and TAA.

Phenotypic Switching of VSMCs in Aneurysm Promotes Calcification

VSMC phenotypic switching, as shown by loss of contractility markers and increases in MMPs, precedes AAA in mice.[51] Accordingly, synthetic VSMCs and upregulation of OPN was shown in the dilated region of human TAA.[52]

In the context of aneurysm formation, additional specific mechanisms promoting calcification are at play. MMP-mediated elastin degradation and misfolding of elastin were shown to be associated with calcium deposition on elastin fibers and calcification.[210,229] Additionally, elastic fiber degradation might cause detachment of VSMCs thereby altering their phenotype.[92] Elastin peptides, which are generated though elastin breaks, upregulate ALP and downregulate MGP expression of human VSMCs in vitro.[15] Absence of the calcification inhibitory protein MGP also promotes phenotypic switching of VSMCs.[209] Hence, VSMC phenotypic switching increases both cellular and ECM stiffness.[26] Elastin-derived peptides and TGF-β1 were shown to promote osteo/chondrogenic differentiation of VSMCs, which may lead to calcification.[230] Indeed, VSMCs of Marfan mice showed enhanced ALP activity and upregulation of osteogenic gene profile including ALP, bone γ-carboxyglutamic acid-containing protein, and RUNX2, in comparison to wild-type mice.[15]

It was proposed that osteo/chondrogenic differentiation of VSMCs is an attempt to prevent vessel wall degeneration by increased ECM production.[230] Differentiation of macrophages into osteoclast-like cells is also observed in the development of AAAs in both humans and mice.[231,232] It was shown that pharmacological inhibition of the activity of these osteoclasts (cells involved in resorbing calcification) using bisphosphonate was able to prevent AAA in a $CaCl_2$-induced

Table. Hepatic and Extrahepatic VKDPs

Vitamin K-Dependent Proteins	Function	Reference
Hepatic		
Coagulation factor VII, IX, and X	Procoagulant. Aid in the coagulation cascade.	Danziger[198]
Prothrombin (coagulation factor II)	Inhibits vascular calcification through binding of phosphatidylserine on EVs, thus activates coagulation. Loading of EVs with prothrombin reduces both their procalcific and procoagulant properties.	Kapustin et al[199]
Activated coagulation factor (FIIa, FVIIa, and FXa)	Activates cellular protease-activated receptors, thereby inducing cellular processes, such as inflammation, apoptosis, migration, fibrosis, and calcification.	Borissoff et al[200] and Schurgers and Spronk[201]
Protein C	Coagulation inhibitor. Assembles anticoagulant complex on cell surface.	Danziger,[198] Matsuzaka et al,[202] and Esmon et al[203]
Protein S	Coagulation inhibitor. Cofactor for activated protein C.	Danziger[198] and Matsuzaka et al[202]
Protein Z	Coagulation inhibitor. Cofactor for the inactivation of FXa.	Danziger[198] and Han et al[204]
Extrahepatic		
Osteocalcin	Bone tissue-specific protein, which is also expressed in osteo/chondrogenic VSMCs.	Willems et al,[42] Steitz et al,[205] Neve et al,[206] and Iyemere et al[207]
	Regulates mineral deposition.	
Matrix Gla protein	Regulates VSMC phenotypic switching through BMP signaling.	Willems et al,[42] Rensen et al,[55] Malhotra et al,[208] Luo et al,[209] Murshed et al,[210] Schurgers et al,[211] Kapustin et al,[212] Schurgers et al,[213] and Wallin et al[214]
	Locally, MGP inhibits ECM mineralization in vascular lesions.	
	Prevents calcification when loaded into EVs that are secreted during the process of vascular remodeling.	
Gla-rich protein (also called Ucma)	Inhibits vascular calcification through direct binding at calcification sites, thus inhibiting crystal formation/maturation, and via loading into EVs and calcifying protein particles. Binds and inhibits BMP-2.	Viegas et al, [215–217] and Willems et al[42,218]
Growth arrest-specific gene 6	Involved in vascular homeostasis.	Clauser et al,[90] Nakano et al,[108,219] Melaragno et al,[109] Son et al,[111] Fridell et al,[220] and Laurance et al[221]
	In VSMCs, Gas6 inhibits apoptosis, induces migration, and promotes cell survival.	

These VKDPs have a high affinity for calcium ions because of their negatively charged Gla residues and are involved in inhibiting ectopic calcification. BMP indicates bone morphogenetic protein; ECM, extracellular matrix; EVs, extracellular vesicles; Gas6, growth arrest-specific gene 6; MGP, matrix Gla protein; VKDPs, vitamin K-dependent proteins; and VSMC, vascular smooth muscle cell.

mouse model of the aneurysm.[231] Furthermore, bisphosphonate attenuated AAA in angiotensin II–induced mouse model of aneurysm through reduction of vascular inflammation.[233] Conversely, inhibiting osteoclast activity with bisphosphonate failed to prevent aneurysm progression in an animal model of severe Marfan syndrome.[234] The contradictory outcome may lie on the different basic pathophysiology between TAA and AAA.[233]

VKAs Promote and Vitamin K Rescues Calcification

The importance of vitamin K status in vascular calcification is supported by studies of VKAs,[235] which counteract the function of vitamin K by blocking VKOR and subsequently inhibiting vitamin K recycling.[236] VKAs, such as warfarin, are widely used as anticoagulants to prevent thromboembolic complications in cardiovascular diseases.[237]

In a study in mice, VKA-induced calcification was associated with increased apoptosis and reduced cellularity in the medial vascular layer in mice.[238] In rats, VKA treatment was shown to rapidly calcify the elastic lamellae of major arteries and aortic heart valves.[239] This was similar to what has been observed in MGP-deficient mice, suggesting that VKAs induce calcification by inhibiting MGP functionality.[209] It must be noted that a daily high dose of VKA in combination with high-dose vitamin K1 was used to prevent bleeding and cause arterial calcification of rats (15 mg warfarin/100 g of body weight administered twice daily, while typical maintenance dose of VKA for human ranges between 2 and 10 mg/d).[239] This dosage is required to maintain a constant level of VKA in plasma without oscillation,[239] because of rapid clearance of warfarin from plasma in rats and considering the half-life of warfarin.[240,241] However, the authors showed no significant differences in blood chemistry between the VKA-treated and the control groups. Additionally, although observations in animal models are based on much higher doses of VKAs than human dosage, it appears that the international normalized ratio in these animals falls within the same target international normalized ratio range for patients.[242]

Ample evidence exists that patients using VKAs have higher levels of vascular calcification than VKA nonusers.[243,244] More than a decade ago, it was reported that VKAs are associated with increased valvular calcification in patients.[243] Since then, this has been verified in many subsequent studies. A large population-based cohort of 15 010 individuals enrolled in the Gutenberg health study, of which 278 patients received VKA treatment, demonstrated an increased arterial stiffness in

VKA-treated patients.[245] Interestingly, enhanced inflammation was also observed in VKA users in this study. However, the cross-sectional design of this study does not allow interpretation of cause and effect, and only a limited number of patients received direct-acting anticoagulants for oral anticoagulation therapy for comparison. Another study analyzed atherosclerotic plaques of patients with nonvalvular atrial fibrillation and showed higher coronary calcium score and significantly higher amount of spotty calcification (calcification of <3 mm) in VKA users compared with a matching demographics (age, sex, body mass index, family history, etc) control group (n=101 per group).[246] It must be noted that despite the propensity score matching, this study ignored the difference between VKA-treated patients and control with regards to some parameters, including HDL (high-density lipoprotein) and stroke risk score in patients with atrial fibrillation ($CHADS_2VASC_2$), which might contribute to spotty calcification. Serial coronary intravascular ultrasound examinations in a post hoc analysis of 8 prospective randomized clinical trials showed a significant progression of coronary artery calcification, independent of changes in atheroma volume in VKA users compared with the propensity matching control group (n=164 per group).[247] In a different study, VKA users had significantly more coronary artery calcification than VKA nonusers with matching gender and Framingham risk score (n=133 per group).[242] A small cross-sectional study on long-term VKA treatment showed an association of VKA users with extracoronary calcification.[248] Recently, small cross-sectional studies conducted in atrial fibrillation patients pointed towards increased prevalence of vascular calcification as compared to nonvitamin K antagonist oral anticoagulants or no treatment.[249] Data on vascular calcification from subgroup analyses of large controlled randomized clinical trials comparing warfarin with direct oral anticoagulants for various clinical indications might further clarify the role of vitamin K in aortic calcification or aneurysm formation. Such results would provide a valuable piece of the puzzle, given the limitations of smaller clinical studies of warfarin and of animal studies that used several log-fold excess of warfarin compared with that used clinically in humans.

In experimental animals, VKA-induced medial calcification was rescued by cotreatment with a high dose of vitamin K2.[238] Additionally, regression of arterial calcification was shown in an animal model with high vitamin K diet.[250] In an interventional randomized control trial of 53 long-term end-stage renal disease patients, who had a vitamin K deficiency, supplementation of vitamin K2 significantly decreased the levels of inactive MGP (dp-ucMGP [dephospho-uncarboxylated MGP]) in a dose- and time-dependent manner.[251] This indicates that circulating dp-ucMGP reflects bioavailability of vitamin K in the vasculature.[213,251,252] Indeed, low vitamin K status or intake is linked to vascular calcification in human subjects.[253,254] A study of 115 patients with suspected coronary artery disease showed reduction of carboxylated MGP correlates with coronary artery calcification progression.[255] Recent results from a prospective interventional study of 72 patients with aortic stenosis showed that vitamin K1 supplementation over a 12-month period significantly decelerated the progression of aortic valve calcification.[252] Hence, the level of dp-ucMGP can be used as a marker for vascular vitamin K deficiency. As vascular calcification is suggested to increase risk of aneurysm rupture, treatment with vitamin K could hold promise to decrease this risk.

VKAs in Aneurysm

The effect of VKAs on AAA progression has not been fully explored yet. They have been linked to continued AAA expansion of >5 mm in diameter and increased risk of persistent blood flow in aneurysm sac (endoleak) after endovascular aneurysm repair.[256,257] A few clinical cases have been reported in which the use of anticoagulants induced spontaneous rupture of bronchial artery aneurysms in patients who took heparin and VKAs and did not have an apparent aortic pathology.[258] Additionally, VKAs promote many effects similar to what has been observed in aneurysm pathology. In a mouse model of atherosclerosis, high-dose VKAs induce a vulnerable atherosclerotic plaque phenotype with elastin breaks.[242] High-dose warfarin treatment in rats results in activation of MMP-9, elastin degradation, vascular elastocalcinosis, and stiffness.[259] In addition, impaired vitamin K status in patients with end-stage renal disease is linked to VSMC phenotypic switching towards senescence-associated secretory phenotype, thus promoting premature vascular aging.[260] Taken together, VKAs affect VSMC properties, weaken the vascular media and might thus increase the susceptibility of aneurysms to progress and rupture.

Conclusions

In summary, in this review, we presented what is known about the role of VSMC phenotypic switching in the pathophysiology of aneurysm formation. NOX enzymes in VSMCs are a major source of increased oxidative stress in aneurysms. Oxidative stress induces VSMC phenotypic switching, which results in ECM degradation and weakening of the arterial wall. VSMC phenotypic changes also lead to calcification, which has been recently proposed as a risk factor for aneurysm progression and rupture, as it results in segmental aortic stiffening generating distally increased aortic wall stress. Vitamin K and VKOR are involved in the regulation of NOX and ROS generation. Vitamin K is known to activate MGP by which it prevents vascular calcification. Moreover, vitamin K has the ability to scavenge free radicals, reduce oxidative stress, and decrease vascular calcification. Therefore, it is tempting to postulate that vitamin K deficiency plays a role in aneurysm formation. Vitamin K supplementation holds the potential to lower the risk of aortic aneurysms and improve cardiovascular outcome.

More studies are needed in patients to examine the effects of vitamin K supplementation on aneurysm progression and risk of rupture. Moreover, shedding light on the effect that vitamin K has on hallmarks of VSMC phenotype (such as proliferation, migration, and contractile protein expression) and the associated mechanisms (oxidative stress and ER stress) would help to fully understand the role of vitamin K in aneurysm formation.

Sources of Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675111.

Disclosures

Dr Schurgers is an unpaid consultant to or involved in clinical trials for Nattopharma ASA and Immunodiagnosticsystems (IDS).

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Highlights

- Vascular smooth muscle cell phenotypic switching and extracellular vesicle release contribute to microcalcification driven aortic aneurysm formation.
- Calcification is involved in both abdominal aortic aneurysm and thoracic aortic aneurysm formation. Early detection of microcalcification may help to hold aortic aneurysm progression.
- Vitamin K-dependent processes are involved in the inhibition of calcification, and vitamin K might be a potential treatment option for an aortic aneurysm.