Wnt signaling pathway and sclerostin in the development of atherosclerosis and vascular calcification

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Abstract

Atherosclerosis is a complex process involving endothelial dysfunction, vascular inflammation, vascular smooth muscle cell (VSMC) proliferation, angiogenesis, and calcification. One of the pathomechanisms of atherosclerosis is the upregulation of Wnt signaling. This study aimed to summarize the current knowledge regarding the role of Wnt signaling and sclerostin in atherosclerosis, vascular calcification, aneurysms, and mortality based on the PubMed database. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendation and identified 160 papers that were included in this systematic review. The published data highlight that the upregulation of Wnt components facilitates the initiation and progression of atherosclerosis, arterial remodeling, VSMCs proliferation and phenotypic transition to the osteoblastic lineage in the arterial wall. This results in protein secretion, cell migration, calcification, fibrosis and aneurysm formation. The transformation of VSMCs into osteoblast-like cells that is observed in atherosclerosis results in sclerostin expression inhibiting the Wnt pathway. Furthermore, it was shown that sclerostin, expressed in atherosclerotic plaques, inhibits aneurysm formation in a mouse model. However, in humans, while the antisclerostin antibody romosozumab inhibits bone resorption, biochemical parameters of endothelial activation and inflammation are not affected, and the incidence of aneurysms is not increased. It was suggested that detecting sclerostin in the calcified aortic atherosclerotic plaques reflects a defense mechanism against Wnt activation and inhibition of atherosclerosis, although this has only been shown in animal models. Moreover, an increased number of vascular cells converted to osteogenic phenotypes results in increased plasma sclerostin concentrations. Therefore, plasma sclerostin derived from bone limits its importance as a global marker of vascular calcification.

Key words: atherosclerosis, aneurysm, cardiovascular disease, sclerostin, WNT-signaling

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Introduction

Cardiovascular disease (CVD) is one of the most common causes of mortality worldwide,[1] the most frequent form of which is coronary artery disease (CAD) associated with atherosclerosis, and its acute form (myocardial infarction) is responsible for most deaths. Atherosclerosis is a complex process that consists of several pathological traits, including endothelial dysfunction, vascular inflammation, vascular smooth muscle cell (VSMC) proliferation, plaque angiogenesis, and calcification.[2,3] Moreover, all these processes are associated with the Wnt signaling pathway.[2,4]

The process of arterial calcification stems from the transformation of VSMCs localized in the intima-media into osteoblast-like cells,[5,6] thereby switching functions from contractile to synthetic. The shift in VSMC phenotype is primarily related to runt-related transcription factor 2 (RUNX2) expression regulated by the Wnt pathway.[6,7] The increase in mechanical load within arteries likely releases proteins to strengthen the action on the RUNX2 factor and facilitates the action of the Wnt-enhancing calcification.[8] This process can be driven by soft tissue injury, resulting in the disruption of homeostasis and the initiation of bone matrix development, leading to ectopic calcification and mineralization of soft tissues.[9]

The main functions of Wnt signaling are the regulation of cell migration and polarity, organogenesis, fate determination, and proliferation of cells during embryonic development. Wnt signaling is also involved in the proliferation of stem cells into progenitor cells, which can subsequently differentiate into several cell types, including cardiac muscle, VSMC and endothelial cells. Therefore, the Wnt pathway is crucial during embryonic development and plays a role in the homeostasis of the adult organism. Moreover, the Wnt pathway is ubiquitous and controls many fundamental cellular processes, including osteogenesis, integrating multiple receptors, growth factors and cellular connections to transcription factors that affect gene expression.[4]

Sclerostin (SOST), an inhibitor of bone formation and calcification secreted by osteocytes,[10,11] is also a soluble inhibitor of the Wnt canonical signaling pathway. Sclerostin is involved in bone tissue homeostasis, inhibits osteogenesis and calcification, and is a modulator of bone homeostasis.[10,11] Its mechanism of action is to bind the LRP5 receptors and disrupt the canonical Wnt pathway.

Numerous studies have reported the involvement of sclerostin in the development of atherosclerosis[11,12] and its complications,[13,14] including arterial stenosis,[14] and clinical presentation in the form of ischemic heart disease,[15] cerebral ischemia[16,17] and peripheral artery disease,[18] but also more advanced complications such as vascular calcification[19–22] and aneurysm development.[22] The results of some studies suggest that sclerostin could potentially play a positive role and inhibit the progression of atherosclerosis.[23]

Moreover, it has been shown that sclerostin may be locally produced in calcified tissue and may act as a counter mechanism against enhanced calcification in arterial beds. It seems that sclerostin may constitute the intermediary between bone homeostasis and the development of vessel calcification and atherosclerosis.

Objectives

As the induction of calcification is an important element in atherosclerosis, we aimed to summarize the knowledge on the role of Wnt signaling and sclerostin in the development of atherosclerosis and vascular calcification.

Methodology

Data on the role of Wnt signaling and sclerostin in the development of atherosclerosis, arterial aneurysm and mortality presented in the article are based on published studies available in the PubMed database. Our search was based on the keywords "Wnt signaling", "sclerostin", "atherosclerosis", "vascular calcification", "aneurysm", and "cardiovascular mortality", and we initially identified 652 articles. Following a review by 2 of the authors, 160 studies were included in the article. Duplicated articles, as well as papers without full-text availability, were excluded from the review. The review included a broad range of articles, from basic molecular studies to clinical outcome investigations. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram is presented in Fig. 1.

The Wnt pathway: general overview

The 'Wnt' name comes from the combination of the Wingless segment polarity gene name in *Drosophila* and its vertebrate homolog int-1 (integrated). This highly conserved signaling pathway is activated by membrane receptors.[24] The Wnt signaling pathway consists of at least 19 proteins and is involved in numerous biological processes, including embryonic development, organogenesis, stem cell development, cell proliferation, differentiation, migration and polarity, tissue homeostasis, and glucose and lipid metabolism.[24–27] Furthermore, Wnt signaling participates in bone formation, vascular and valvular calcification,[2,3,19,28,29] and angiogenesis.[30] In the process of angiogenesis, Wnt signaling regulates endothelial cell proliferation and survival,[31] and proliferation, migration and survival of VSMCs via the Wnt/β-catenin pathway.[2,28–30]

Alterations in Wnt signaling appear to be directly involved in the increase of cardiovascular risk. For example, in mouse models, mutations of the co-receptors of Frizzled

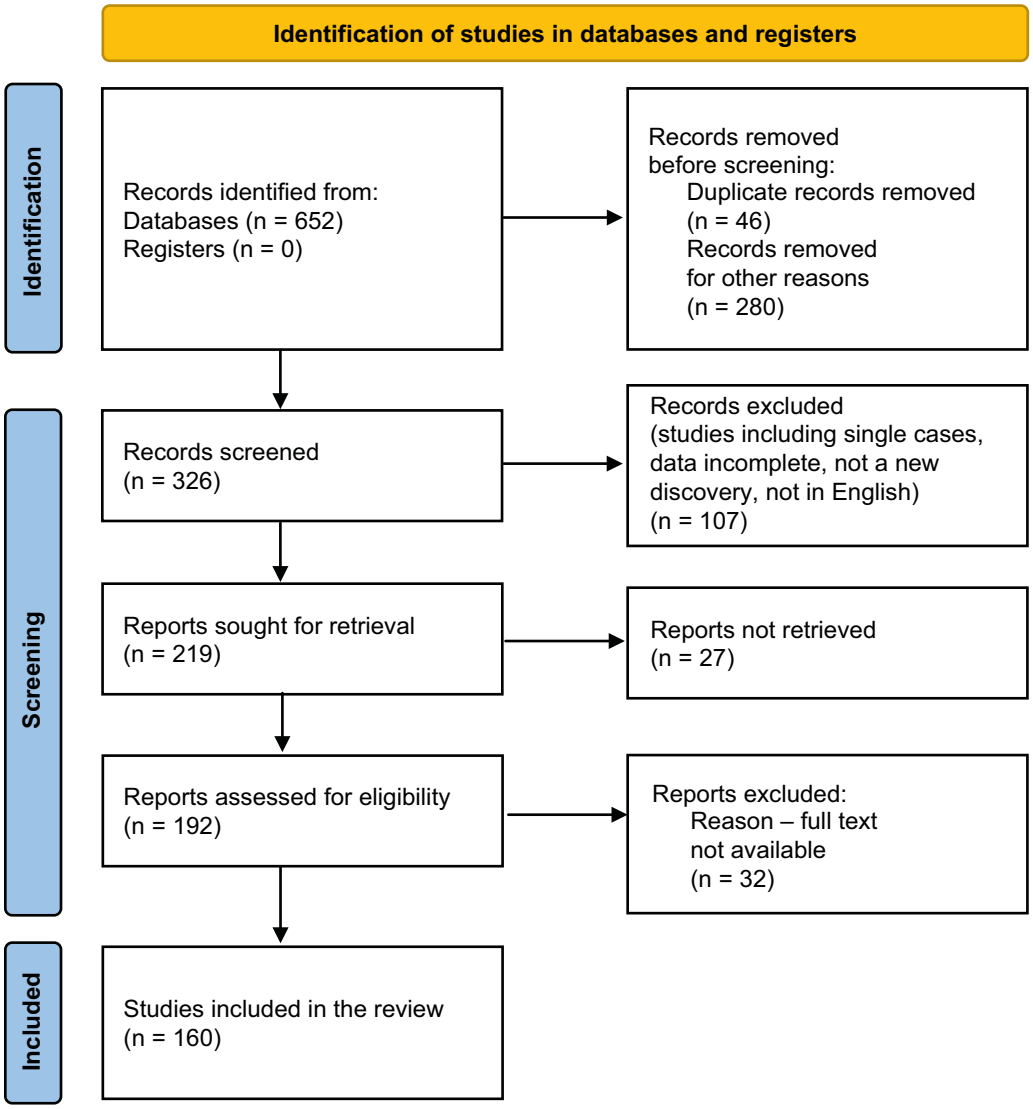

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

(Fz), a receptor in the Wnt pathway (e.g., low-density lipoprotein receptor-related protein 6 (LRP6)), are associated with an increase in morphogenesis and differentiation of adipocytes,[32] enhancement of monocyte adhesion to endothelial cells, the proliferation of VSMCs,[30] vascular calcification,[33] hypercholesterolemia and, consequently, hypertension, type 2 diabetes mellitus (T2DM) and premature CAD.[34] The current literature also shows that enhanced Wnt signaling, due to gain-of-function mutations of all elements of this signaling pathway, is associated with alterations in vascular development.[35,36]

The Wnt pathway: mechanism of action

The Wnt proteins secreted by epithelial cells bind to the extracellular domain of the Fz surface receptor family. The Wnt ligand and the Fz receptor require LRP5/6 as co-receptors for the transduction of the signal into the cells.[24] Other ligands that can activate LRP5/6 receptors include parathormone (PTH)[37] and G-protein-coupled ligands such as isoproterenol (β-mimetic), adenosine and glucagon.[38] Furthermore, LRP5/6 are also co-receptors for a platelet-derived growth factor (PDGF) and the transforming growth factor-β (TGF-β) receptor.[39] The complex of Wnt protein/Fz receptor with LRP5/6 co-receptors transduces the signal to cytoplasmic phosphoprotein Dishevelled (Dsh/Dvl). Moreover, Wnt signaling activates 3 different pathways: canonical, planar cell polarity (PCP) and the Wnt/Ca.[24]

The canonical pathway

The canonical pathway comprises Dsh signaling to protein complexes which, in the absence of Wnt ligands, promotes the ubiquitination and finally degradation of β-catenin,[24] while the Wnt ligand and activation of the Fz-LRP5/6 receptor complex inhibits this degradation, resulting in the translocation of β-catenin from the cytoplasm into the nucleus. Finally, β-catenin interacts

with T-cell factor (TCF)/lymphoid-enhancer binding factor (LEF), which activates the transcription of Wnt-related genes that encode cyclin D1, PPAR and c-Myc, all of which are responsible for cell growth, proliferation and survival.[24]

The non-canonical pathways

The non-canonical pathways comprise the PCP pathway, which regulates the cytoskeletal organization and cell polarization,[24] and the Wnt/Ca pathway responsible for the regulation of cell movement and adhesion.[24] In these pathways, the Wnt signal is mediated through Fz receptors independent from the LRP5/6 co-receptor. The co-receptors for this pathway are likely mediated through tyrosine-protein kinase transmembrane receptor (ROR2),[40] neurotrophin-related protein 1 (NRH1),[41] receptor tyrosine kinase (Ryk),[42] and protein tyrosine kinase 7 (PTK).[43]

The transduction of the non-canonical signaling leads to the activation of cytoplasmic Dsh, which is similar to the activation of the canonical pathway, but the PCP pathway utilizes the PDZ and DEP domains of Dsh, and ultimately activates the small GTPases Rho and Rac.[44] One branch of this pathway acts through Daam 1 (Dishevelled-associated activator of morphogenesis 1), which binds to the central PDZ domain of Dsh and activates Rho GTPase through WGEF (weak-similarity GEF).[45] Active Rho GTPase can stimulate Rho-associated kinase (ROCK)[46] and myosin,[47] resulting in the modification of actin and cytoskeletal organization.

The other signaling branch depends on the C-terminal DEP domain of Dsh and stimulates Rac GTPase activity.[48] Rac triggers c-Jun N-terminal kinase (JNK)[49] in a Daam-independent manner. Both Rho and Rac GTPases can regulate transcription and alter cell organization and polarity.[50]

The 2nd arm of non-canonical Wnt signaling, the Wnt/Ca pathway, is responsible for an increase in intracellular calcium levels through trimeric G protein signaling.[51,52] Increased calcium stimulates calcium-sensitive kinases, including phospholipase C, and protein kinase C (PKC).[53] Moreover, the Wnt/Ca pathway is thought to stimulate the canonical and PCP pathways[54,55] by utilizing the PDZ and DEP domains of the Dsh protein. However, in the non-canonical pathway, the Dsh protein is localized at the cell membrane and not in the cytoplasm as in the canonical Wnt signaling pathway.[56] Finally, the Wnt/Ca pathway is essential in embryonic development, cell adhesion, tissue orientation, and organ formation.[51]

Numerous factors, such as secreted frizzled-related proteins (sFRPs)[57] and Wnt inhibitory factor-1 (WIF-1),[58] may inhibit the Wnt pathways by directly binding to Wnt and preventing its connection with the receptor. In addition, sclerostin[59] and Dickkopf (Dkk) family members[60] inhibit the transduction of the signal by binding to LRP5/6. The Wnt signaling pathways are highlighted in Fig. 2.

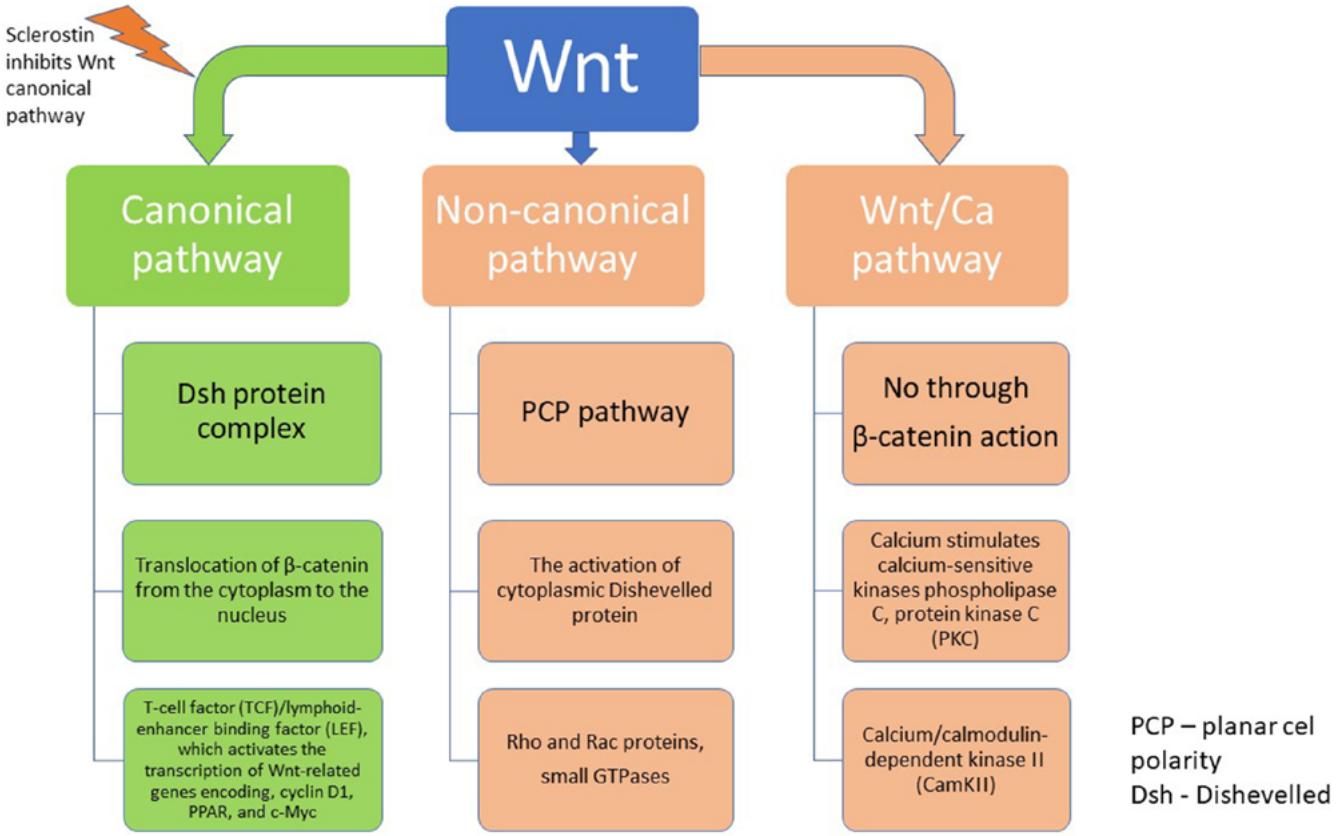

Fig. 2. Wnt signaling

Wnt signaling and bone formation

Wnt signaling participates in bone formation by increasing the transformation of mesenchymal stem cells (MSCs) to osteoblasts while inhibiting osteoclast differentiation.[61,62]

Bone cells, including osteoblasts, osteocytes, chondrocytes and bone marrow cells, produce many Wnt ligands.[63] In the mouse, these are secreted from osteoblasts in an autocrine manner and participate in their mineralization and maturation. Moreover, Wnt16 induces osteoprotegerin expression in osteoblasts via the Wnt-β-catenin pathway[64] and inhibits osteoclasts formation independent from osteoprotegerin (OPG) action.[65] Furthermore, the Wnt5a ligand involved in the non-canonical signaling pathway is responsible for osteoblast lineage formation from mesenchymal precursors and can inhibit adipocyte differentiation.[66] Even though the receptor for Wnt5a is the tyrosine kinase orphan receptor 2 (Ror2), its action results in enhancement in LRP5/6, which activates β-catenin and enhances the expression of OPG, and promotes osteoblast differentiation.[66]

Wnt3a, a Wnt ligand in the canonical signaling pathway, inhibits calcitriol-induced, but not Rankl-induced osteoclast formation induced by OPG expression in osteoblasts.[67] Moreover, Wnt16 secreted from osteoblasts inhibits its human and mouse osteoclast formation by disrupting Rankl in a Wnt-independent manner.[64] This is achieved through inhibiting Rankl-induced activation of NF-kB and calcitriol-induced mice osteoclast formation.[68] Wnt4 is also expressed in osteoblasts and inhibits osteoclasts formation independently from the Wnt pathway by enhancing OPG expression.[69]

Wnt5a enhances LRP5/6 expression in osteoblasts and simultaneously promotes Wnt10b and activates the Wnt/β-catenin pathway to induce osteoclasts formation. The interplay between Wnt5a and Wnt16 may also regulate osteoclastogenesis and osteolysis, and it is known that Wnt5a mediates osteoclast formation by binding to and stimulating Ror-2 receptors.[70] Conditions such as arthritis with a high level of Wnt5a may reverse the inhibitory effect of Wnt16 on osteoclast formation.[66,70]

In summary, secreted Wnt signaling ligands regulate osteoblast and osteoclast differentiation, and their interplay defines the balance between bone formation and bone resorption. The surrounding environmental conditions determine the induction of different Wnt ligands and the regulation of bone homeostasis.

Wnt signaling and atherosclerosis

All aspects of Wnt signaling are closely associated with the initiation and progression of atherosclerosis.[26,30] The upregulation of Wnt signaling (increased expression of the components of Wnt signaling, including WNT5a, WNT5b and WNT11) was detected in human aortic calcified atherosclerotic lesions and related aneurysms.[23,71] Furthermore, shear stress appears to be the primary mechanism that triggers the upregulation of Wnt signaling.[72] In addition, upregulated Wnt signaling affects endothelial cell proliferation and survival, enhances monocyte adhesion and transendothelial migration,[3,73] and results in dysregulation of proliferation and apoptosis of VSMCs.[28] Wnt signaling participates in bone formation by increasing the transformation of MSCs to osteoblasts and inhibiting differentiation to osteoclasts.[61,62] Therefore, inappropriate activation of the Wnt signaling pathway may play a role in osteoblastic transition into the arterial wall[74] and vascular calcification.[30] It appears that the link between atherosclerosis and bone loss is mediated through the canonical Wnt signaling pathway.[11]

Atherosclerosis development is associated with the proliferation and migration of VSMCs and endothelial dysfunction.[75] The canonical Wnt/β-catenin signaling pathway results in the upregulation of proliferation genes, such as *cyclin D*1 responsible for VSMCs proliferation.[74] Some of the *WNT* family genes encoding proteins such as *WNT1*[74,76] and *WNT5a*[77] in VSMCs and macrophages from atherosclerotic plaques have been identified as the initiators of VSMCs proliferation and release of pro-inflammatory cytokines. Additionally, overexpression of Wnt inhibitors like sFRPs has been shown to constrain VSMC proliferation.[76] In contrast, Wnt3a exerts an anti-inflammatory effect by modulating NFκB-related gene expression in a mouse model.[78] Moreover, an increased DKK-1 level promotes pro-inflammatory cytokine release,[79] and the Wnt co-receptor LRP5 is responsible for enhancing lipid uptake, transforming macrophages into foam cells, and macrophage migration through enhanced regulation of Wnt-related proteins such as osteopontin (OPN), bone morphogenetic protein 2 (BMP2), cyclin D1, c-jun, lymphoid enhancer factor 1 (LEF1), and β-catenin.[80]

The Wnt pathway regulates the expression of OPG and OPN associated with extracellular matrix mineralization.[81] Osteopontin has pro-inflammatory properties[82] and activates the proteolytic activity of metalloproteinases,[83] while OPG expressed in endothelial cells and VSMCs[84] plays a role in the pathogenesis of atherosclerosis and the progression of aortic aneurysms.[85]

Wnt signaling is also involved in the process of fibrogenesis through TGF-β activation.[86] Pathological activation of the canonical Wnt pathway has been detected in pulmonary,[87] dermal,[88] renal,[89] and myocardial infarction-related fibrosis,[90] and in muscles of mice from a model of musculoskeletal dystrophy.[91] The DKK proteins are thought to play a significant role in inhibiting the Wnt canonical pathway by either binding to the LRP5/6 receptor and its co-receptor Kremen-1/2, internalizing the receptor and facilitating its degradation, or by disrupting the interaction between WNT and the LRP5/6 and Fz co-receptor complex.[92] In cultured human fibroblasts, TGF-β signaling

led to lower DKK-1 expression, which in turn activated the Wnt pathway. Both lower expression of DKK-1 and the use of DKK-1-neutralizing antibodies resulted in aggravation of fibrosis, whereas overexpression of DKK-1 prevented the initiation of fibrosis in the skin obtained from patients with systemic sclerosis.[91] Overexpression of Wnt proteins in fibroblasts has been detected in enhanced generalized dermal fibrosis mouse models.[91] This evidence demonstrates that the interrelation of the TGF-β pathway and Wnt signaling plays a pivotal role in the pathogenesis of fibrosis.[91]

During atherosclerosis, fibrosis is present in the wall of the artery and heart valves. WNT5b and WNT11 proteins were detected in aortic valvular interstitial cells with extensive fibrosis, underscoring the role of the canonical Wnt pathway in the development and exacerbation of atherosclerosis in humans.[71]

Wnt signaling and vascular calcification

The involvement of Wnt signaling in physiological bone turnover may be the herald of calcification, as the process of calcifying smooth muscle cells resembles the process of osteogenesis. Vascular calcification is one of the most common locations of ectopic soft tissue calcification and represents the congregation of hydroxyapatite preferentially in the tunica media[9] during diabetes and chronic kidney disease (CKD), and contributes to the development of hypertension and cardiovascular complications.[93] The primary pathological process is that of the transition of mesenchymal VSMCs into a single-lineage osteogenic cell type.[94] In the presence of calcified arterial plaques, a loss of elasticity increases the constant strain exerted on arteries resulting in VSMC proliferation and differentiation.[6] In transformed VSMCs, osteogenic genes have been found, albeit their mRNA expression is significantly lower than in osteoblasts.[95] Moreover, high plasma levels of calcium and phosphate initiate the process of calcium deposition in arteries by changing the phenotype of VSMCs and increasing the expression of osteogenic proteins.[96,97] The main regulatory mechanism involved in the process of calcification and plaque formation is the canonical Wnt signaling pathway.[98] The target gene of the Wnt cascade is the transcription factor RUNX2 responsible for the phenotypic change of VSMC,[7] osteoblast differentiation and initiation of calcification.[24]

The arterial Wnt signaling pathway is induced by hypercalcemia and hyperphosphatemia, RUNX2, BMP-2 and -4, and stress or injury, which results in the upregulation of Wnt-related genes.[99] The Wnt signaling pathway induces vascular calcification by promoting the expression of genes responsible for VSMC differentiation like *RUNX2* (osteogenic differentiation),[100] *VCAN* (cell proliferation and migration due to vessel injury),[101] *OPG* to inhibit osteoclast formation,[102] and *RANKL* (responsible for the recruitment of osteoblast-like precursors).[100]

Wnt3a has been shown to activate β-catenin and RUNX2 expression, thereby increasing arterial calcium deposition and osteocalcin expression resulting in the promotion of VSMC calcification[103] as well as migration by increased adherence to type 1 collagen fibrils.[104] Moreover, Wnt7b plays a role in the development of neo-vasculature via the Wnt signaling pathway,[105] while Wnt16 has been implicated in changing the phenotype of VSMCs from contractile to osteogenic lineage.[106]

Sclerostin: mechanism of action

Sclerostin, the product of the *SOST* gene[107] and secreted by osteocytes,[10] acts mainly in an autocrine and paracrine manner. The physiological role of sclerostin is the inhibition of bone formation and calcification,[11] and it has been suggested that the serum concentration of sclerostin reflects the pool of mature osteocytes.[107] Its expression was also detected in many other tissues, including the heart, lungs and cancers.[108] The mechanisms of sclerostin action are summarized in Table 1.

Sclerostin is a soluble inhibitor of the canonical Wnt signaling pathway and therefore regulates the proliferation and differentiation of osteoblasts and bone formation.[117] It antagonizes BMP signaling, thus stimulating osteoblast and osteocyte apoptosis.[118,119] The autocrine action of sclerostin also involves stimulating RANKL expression in osteocytes, thus supporting osteoclast activity and bone resorption.[120] In addition, the paracrine action of sclerostin on osteoblasts and osteoclasts by the LRP5 receptor inhibits bone formation.[121]

Sclerostin signaling is modulated by numerous factors, including calcitriol, which facilitates its action by modulating the expression of LRP5/6, the sclerostin receptor. In addition, as shown in mice models, calcitriol enhances the expression of Dkk-1 and secretion of frizzled-related protein 2 (Sfrp2), which are antagonists of the Wnt signaling pathway.[122] Other factors modulating the action of sclerostin are PTH,[123] tumor necrosis factor alpha (TNF-α)[110] and glucocorticoids.[111]

Thus, the physiological role of sclerostin in the regulation of bone mineralization is the inhibition of the canonical Wnt/β-catenin pathway via LRP5/6 binding[109] It also enhances the degradation of β-catenin, resulting in the inhibition of osteoblast differentiation and proliferation.

As mentioned above, numerous studies have shown the essential role of the Wnt signaling pathway in vascular development and remodeling.[30] An anti-calcification effect related to the inhibition of the Wnt pathway was demonstrated in carotid plaques and calcified aortas.[112] Thus, the presence of sclerostin in human arteries is not unexpected.[124] Some studies have also reported sclerostin and DKK-1 expression in calcified human aortas and carotid plaques.[124]

Table 1. Mechanisms of sclerostin action

Models	Genes mutation	Effects or results
In vitro human, mice osteoblast[11,109]	–	sclerostin and Dickkopf family bind to LRP5/6 receptors and suppress osteogenesis
A human with atherosclerosis and heart valves calcifications[21]	–	sclerostin has been identified in vascular smooth muscle cells and aortic valves
A human with chronic kidney disease[22]	–	sclerostin is produced locally in calcified arteries
In vitro mice, cell osteoblasts culture incubated with TNF-α[110]	–	decreased sclerostin levels
Mice osteocytes culture incubated with glucocorticoids[111]	–	increased sclerostin levels
In vitro and ex vivo mice VSMC arterial cells with atherosclerosis and calcifications[112]	*Enpp1*$^{-/-}$ mouse	sclerostin expression identified in mature osteocyte – VSMC of aortic tissue
A human with sclerosteosis and VBD[113]	gene chromosome 17q12-q21 of sclerostin	loss of sclerostin function in bones
Mice limb bud[114]	***SOST*** gain of function mutations	loss of Wnt pathway in limbs
A human with bone overgrowth[115]	*LRP4* genes: mutations – R1170W, W1186S	loss of function of LRP4 – sclerostin receptor in bone
Postmenopausal women treated with calcitriol[116]	–	enhanced serum sclerostin levels

VSMC – vascular smooth muscle cells; VDB – Van Buchem's disease; TNF-α – tumor necrosis factor alpha.

Sclerostin expression in VSMCs likely reflects their transition to osteoblast-like cells.[112] This hypothesis is supported by a positive correlation between serum sclerostin concentration and the severity of aortic calcification.[21,125] In addition, it was shown that β-catenin activity is crucial in initiating VSMC proliferation and neointima formation, processes essential in arterial physiology. Reactive oxygen species (ROS) are among the factors that can enhance β-catenin activity.[74] Sclerostin and DKK-1 inhibit the β-catenin-dependent Wnt signaling pathway, and therefore a high sclerostin level may indicate a defensive mechanism against enhanced Wnt pathway stimulation by ROS.[33] The process of vascular calcification resembles that of bone morphogenesis.[126] Wnt signaling mediates the differentiation of progenitor and VSMCs into an osteo/chondro phenotype.[127] This was seen in cultured rat VSMCs, in which Dkk-1 acts as a potent inhibitor of the canonical Wnt signaling pathway reducing the expression of Runx2, an essential transcription factor for osteogenic differentiation.[103] In human knee chondrocytes, the incubation with sclerostin resulted in a decrease of RUNX-2 mRNA.[128] Therefore, both sclerostin and Dkk-1 proteins may neutralize the process of vascular calcification and modify arterial stiffness and arteriosclerotic plaque stability.[129]

Sclerostin and atherosclerosis

Interestingly, a higher sclerostin concentration was found in the media compared to the intima of atherosclerotic plaques of patients undergoing carotid endarterectomy, and a similar finding was demonstrated for VSMCs when compared to infiltrating macrophages.[130] Sclerostin was also found in the aorta of patients undergoing aortic valve surgery and was upregulated in calcifying VSMCs and calcified valvular plaques.[21] Serum sclerostin levels have been associated with the presence of thoracic aortic calcification (TAC), the severity of calcification, and sclerostin expression in the vessel wall.[131] Numerous studies have shown associations between sclerostin levels and aortic or carotid plaques and vascular calcifications in patients with T2DM and CVD and in postmenopausal women.[12,132,133] In addition, sclerostin levels were higher in elderly patients with peripheral arterial disease (PAD) than in patients with a normal value of the ankle-brachial index (ABI), and higher sclerostin levels were shown to be an independent predictor of PAD.[18] Therefore, it seems that sclerostin may be considered a surrogate marker of vascular calcification, and may even be a surrogate of vascular disturbances in patients with CKD.[134] Previous literature also suggests that increased sclerostin levels in VSMCs may protect against excessive vascular calcification in dialysis patients.[133] However, this mechanism has limited efficacy.

The increased sclerostin concentrations observed during the course of atherosclerosis in a clinical setting seem to be ineffective in exerting protective anti-calcification effects in damaged vessels. Moreover, clinical studies show sex-related differences in sclerostin concentrations, which are higher in men, and in the frequency and course of CVD. However, even higher serum sclerostin concentrations in men do not prevent the occurrence and progression of atherosclerosis, suggesting that the levels of circulating sclerostin are not effective in inhibiting the pathological process in vessels.[135]

Sclerostin is independently positively associated with increased carotid intima-media thickness (CIMT) and with the risk of carotid plaque presence and aortic calcification.[12] However, Gaudio et al. showed higher sclerostin and DKK-1 concentrations in postmenopausal women with

T2DM than in healthy controls and a negative correlation with CIMT only in the T2DM group.[11] Thus, sclerostin concentration was an independent predictor of CIMT in T2DM patients. In patients with T2DM, sclerostin was likely higher due to the presence of atherosclerotic lesions and the presence of cells derived from an osteogenic lineage inside the arterial wall, which may be the source of circulating sclerostin.[136] Therefore, higher sclerostin levels in patients with CVD may reflect the advanced progression of atherosclerosis and plaque calcification.

Sclerostin and vascular calcification

Recently, it was shown that *SOST* knockout mice or the administration of anti-sclerostin antibodies resulted in enhanced bone formation and mineralization.[137,138] However, the data describing the role of sclerostin as an important risk factor for vascular calcification raise doubt.[139] It has been found that induction of renal failure in *SOST* knockout mice resulted in the development of vascular calcification.[140] However, while low levels of sclerostin increased bone formation,[141,142] this process did not prevent increased vascular mineralization.[138] In addition, in DBA/2J mice that are more susceptible to the development of ectopic calcifications without renal failure,[143] treatment with anti-sclerostin antibodies and a diet that included warfarin resulted in the development of aortic and renal arteries calcifications.[138] Thus, these results suggest that sclerostin prevents vessel calcification in the aorta, kidney and cardiac arteries. This hypothesis seems to be supported by observations that expression of sclerostin mRNA and protein occurs in calcified vessels in both mice and humans,[144] and plasma sclerostin levels are inversely associated with mortality among patients with CKD.[145] It seems that locally produced sclerostin in the calcified tissues may act as a counter mechanism against further ectopic calcification. The mechanism may be similar to bones in that sclerostin binds to LRP5 receptors and inhibits the Wnt pathway in VSMCs. It seems that sclerostin may also act by indirect stimulation of FGF-23,[146] resulting in urinary phosphate excretion, which lowers the plasma phosphate level.

Sclerostin and aneurysms

Under physiological conditions, VSMCs produce collagen and elastin, which are responsible for the strength and elasticity of arteries and the aorta. However, during atherosclerosis, the phenotype of VSMCs is modified, and they start producing matrix metalloproteinases (MMPs) that are involved in the degradation of the extracellular matrix, which in turn contributes to the development of aneurysms.[112]

A study by Kirshna et al. reported the downregulation of sclerostin and activation of the Wnt/β-catenin pathway in abdominal aortic aneurysms (AAA)[23] in both mouse and human aortas. Upregulation of Wnt target genes was also detected in that arterial intima and media during the aging processes.[147] The development of an aneurysm may stem from epigenetic changes in several genes, including excessive methylation of one of the CpG islands in the *SOST* promoter and subsequent inhibition of gene activity by up to 75%, as shown in human osteocytes.[148,149]

Physiologically, collagen and elastin fibers maintain arterial width and elasticity. During the development of an aneurysm, fragmentation of collagen and elastin fibers occurs, resulting in decreased arterial wall strength.[150] Results of studies performed on mouse fibroblasts indicate that by inhibiting the Wnt pathway, sclerostin enhances the expression of genes encoding extracellular matrix proteins responsible for maintaining the aorta structure.[151]

It is known that Wnt signaling controls the expression of OPG and OPN. Osteopontin activates proteolytic pathways and MMP-9 activity,[81] and is engaged in the promotion of inflammation.[80] In a mouse model, low OPN levels limited the development of AAA,[152] and it is interesting to note that OPG promotes the MMP-2 and MMP-9 release and activity from monocytes and VSMCs,[153,154] leading to instability of the arterial wall. Furthermore, OPG concentration correlated positively with AAA progression[148] and was positively associated with aortic diameter, MMP-2 and MMP-9 activity, cathepsin activity, and the number of lymphocytes inside the wall of aortic aneurysms, all being well-established parameters of AAA pathogenesis and severity.[83,85] Moreover, OPG deficiency protected against aortic angiotensin II-induced aneurysm development and rupture in mice.[155]

In a study performed in a mouse model, results showed that sclerostin overexpression or administration inhibited angiotensin II-induced aneurysm formation in the thoracic and abdominal aorta and the development of atherosclerosis.[23] In line with this finding, inhibition of the Wnt pathway by sclerostin protected against the AAA development by downregulation of pro-aneurysmal genes in mice.[23] Potentially, the inhibition of Wnt signaling may decrease the expression of OPN, OPG and MMP-9, and thus attenuate aortic wall inflammation and extracellular matrix degradation.[23]

Sclerostin and mortality

An investigation by Zeng et al. found a U-shaped association between sclerostin levels and vascular calcification and mortality.[156] Even though atherosclerosis progresses with aging, it was shown that sclerostin concentrations did not predict the occurrence of cardiovascular events during a 15-year observational period in a population-based prospective study, whereas DKK-1 level was such a predictor.[157] Moreover, some data has shown that DKK-1

Table 2. Association of sclerostin expression with cardiac and vascular pathologies

Models	Material	Results
Human – postmenopausal type 2 diabetic women with atherosclerosis[12]	serum	Serum sclerostin level positively correlates with plaque volume and vascular calcifications.
Humans over 65 years[18]	serum	Serum sclerostin levels were higher in patients with PAD than in patients with normal ABI.
Human – Afro-Caribbean men[19]	serum	Serum sclerostin levels positively correlate with coronary and aortic calcifications.
Human with atherosclerosis[21]	calcified and atherosclerotic aorta wall	Upregulated expression of sclerostin was detected in VSMCs.
Human with atherosclerosis[21]	serum	Serum sclerostin level positively correlates with aortic calcification.
Human with atherosclerosis[124]	aorta wall, atherosclerotic plaques	Sclerostin expressions were detected in the heart, calcified aorta and atherosclerotic plaques.
Human with atherosclerosis[130]	atherosclerotic plaques and aortic calcifications	Sclerostin expressions were detected in aortic calcifications and plaques. Higher levels of sclerostin in the media than in the intima.

VSMC – vascular smooth muscle cells; PAD – peripheral arterial disease; ABI – ankle-to-brachial index.

is released mainly from endothelial cells[79] and can activate platelets,[158] causes endothelial cell apoptosis and enhances the expression of molecules including pentraxin-3 and plasminogen activator inhibitor type 1, which contributes to inflammation and inhibits fibrinolysis.[159]

The effects of inhibition of sclerostin in the vasculature

Locally enhanced sclerostin production can potentially inhibit vascular calcification at the site in the arterial wall, although at the same time may exert a negative effect on the bones by increasing bone resorption and inhibiting bone formation after being released into the circulation.[120] Administration of the sclerostin inhibitor romosozumab, an anti-sclerostin antibody used to treat osteoporosis, resulted in a decrease in bone resorption and an increase in bone formation.[160] The results of the ARCH study involving postmenopausal women with osteoporosis revealed a higher frequency of severe cardiovascular adverse events in the group treated with romosozumab than in patients treated with alendronate (2.5% compared to 1.9%).[161] The most common cardiovascular events were myocardial infarctions and stroke. However, the results of another large study, FRAME, did not find an increase in cardiovascular risk between romosozumab and placebo groups.[162] Several nonclinical studies have also been performed to elucidate the potential biological mechanisms mediating the increase in adverse cardiovascular events. It has been shown that romosozumab did not induce vasoconstriction in isolated human coronary artery cultures,[163] and did not have any impact on cardiovascular or respiratory function in monkeys.[164] Moreover, it did not initiate or exacerbate the process of arterial calcification in the absence of atherosclerosis in rats, even during lifetime exposure to this drug.[165] In mouse models of atherosclerosis, administration of anti-sclerostin antibodies did not result in changes to plaque volume or mineralization, and histopathological examination of the aortas did not reveal increased hemorrhages, thrombosis or necrosis in a high-fat diet model of atherosclerosis due to treatment with romosozumab.[163] Therapeutic anti-sclerostin antibodies did not increase the incidence of aneurysms[23] and did not change biochemical parameters, platelet and endothelial activation or markers of inflammation in mouse models of the aortic aneurysm.[23]

Thus, the studies in animal models have not shown a significant effect of anti-sclerostin antibodies on the cardiovascular system. Furthermore, the data did not show evidence of the detrimental effects of sclerostin inhibition on the development of inflammation or exacerbation of atherosclerosis. The summary of findings concerning sclerostin levels in different clinical conditions is presented in Table 2.

Summary

Emerging data suggest there are similarities between bone homeostasis and vascular pathologies.[166] Bone constitutes the buffering capacity for calcium and phosphorus, although the conditions of hypercalcemia and hyperphosphatemia result in stimulation of the arterial Wnt pathway. This Wnt pathway enhancement results in the initiation of transdifferentiation of VSMCs into a phenotype that secrets proteins, migrates, and induces mineralization and atherosclerosis. The Wnt pathway also stimulates the release and activity of other signaling regulators and growth factors, exacerbating RUNX2 expression and resulting in vascular calcification.

Conclusions

Sclerostin and DKK-1 detection in the calcified aorta in carotid plaques supports the hypothesis that upregulation of Wnt pathway inhibitors may be a defensive mechanism

to restrain atherosclerosis. However, these methods have so far only been demonstrated under specific laboratory conditions and in animal models. It is also suggested that serum sclerostin concentrations mirror the advancement of arterial remodeling and vessel wall calcification, and it may represent the increased number of vascular cells transformed into osteogenic phenotypes. Indeed, higher serum sclerostin concentrations are observed in patients with atherosclerosis and vessel calcification when compared to healthy subjects. However, the value of serum sclerostin levels as a marker of advancement of global vascular calcification is lowered by the fact that they reflect 2 pools of sclerostin; one released by VSMCs due to their pathogenic transition to the osteogenic-like phenotype in arterial walls and a second that is derived physiologically from the bones.

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