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Review

Role of macrophages in vascular calcification: From the perspective of homeostasis

Rong Dong [a,b], Zhenjun Ji [a], Mi Wang [a], Genshan Ma [a,*]

[a] Department of Cardiology, Zhongda Hospital, School of Medicine, Southeast University, No. 87, Dingjiaqiao, Nanjing 210009, China
[b] Department of Cardiology, Yancheng No. 1 People's Hospital, No. 66 South Renmin Road, Yancheng 224000, China

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ABSTRACT

Vascular calcification (VC) is a crucial risk factor for the high morbidity and mortality associated with cardiovascular and cerebrovascular diseases. With the global population aging, the incidence of VC is escalating annually. However, due to its silent clinical process, VC often results in irreversible clinical outcomes. Inflammation is a core element in the VC process, and macrophages are the major inflammatory cells. Due to their diverse origins, microenvironments, and polarization states, macrophages exhibit significant heterogeneity, exerting strong effects on the occurrence, development, and even the regression of VC. In this review, we summarize the origin, distribution, classification, and surface markers of macrophages. Simultaneously, we explore the mechanisms by which macrophages maintain homeostasis or regulate inflammation, including the macrophage-mediated regulation of VC through the release of inflammatory factors, osteogenic genes, extracellular vesicles, and alterations in efferocytosis. Finally, we discuss research targeting inflammation and macrophages to develop novel therapeutic regimens for preventing and treating VC.

1. Introduction

Vascular calcification (VC) frequently occurs in conditions such as chronic kidney disease (CKD), diabetes, hypertension, atherosclerosis, and certain genetic disorders [1–3]. It independently predicts adverse cardiovascular outcomes and indicates aging. With global aging and the prevalence of multiple risk factors, the incidence of VC is increasing annually, posing a critical threat to public health worldwide. The silent progression of VC, the lack of early detection methods, and public unawareness complicate the issue. VC's gradual progression often leads to irreversible clinical outcomes. For example, increased aortic stiffness can lead to hypertension, aortic stenosis, and left ventricular hypertrophy [4]. Additionally, cerebral VC can cause cognitive decline [5–7], significantly decreasing patients' quality of life. Severe VC can cause ischemia and necrosis of the affected organs, resulting in stroke, acute myocardial infarction, and limb ischemia. In extreme cases, VC can be life-threatening, heavily burdening families and society Fig. 1. The current VC treatments mainly include medication and surgical interventions. Phosphate binders or calcimimetic agents are employed to control mineral disturbances in patients undergoing dialysis to prevent VC. However, the effectiveness of these treatments is uncertain, and some medications have severe side effects. Surgical treatments like endarterectomy and vascular interventions can open blocked blood vessels and significantly improve acute ischemia in tissues or organs. However, these surgeries do not alter the composition or elasticity of calcified vessels and, thus, do not address the underlying cause of VC. The lack of effective prevention and treatment methods for VC underscores the need for further research.

Traditionally, VC has been viewed as the passive deposition of calcium phosphate in the vessel wall. Recent evidence shows that VC is an actively regulated cellular process [8] and can be classified into four types based on the calcification site: intimal, medial, valvular, and calciphylaxis [9]. Intimal calcification is linked to atherosclerotic plaques and is driven by the inflammation-induced osteogenic differentiation of endothelial cells and vascular smooth muscle cells (VSMCs). Medial calcification, or Mönckeberg's calcification, seen in aging and CKD, mainly involves the osteogenic differentiation of VSMCs. In contrast, valvular calcification results from the osteogenic differentiation of valvular endothelial and interstitial cells. Calciphylaxis, common in CKD patients, involves diffuse medial calcification of the skin and subcutaneous arteries, leading to thrombosis, ischemic necrosis, non-healing wounds, and high mortality; however, its mechanisms remain unclear

* Corresponding author at: Department of Cardiology, Zhongda Hospital, School of Medicine, Southeast University, No. 87, Dingjiaqiao, Nanjing 210009, China.
E-mail address: magenshan@hotmail.com (G. Ma).

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[10]. The mechanisms underlying the broader category of VC include oxidative stress [11–13], mitochondrial stress [14], endoplasmic reticulum stress [15,16], inflammation [17–20], advanced glycation end products [21], autophagy [22], ferroptosis [23], and aging [24–26]. Understanding these mechanisms is crucial for developing preventive and therapeutic strategies for VC.

Inflammation is a key regulator of VC [17] and involves sustained and sometimes excessive innate immune responses and harmful adaptive immune responses [27,28]. Macrophage infiltration is crucial in VC development, where it plays a significant role in the initiation, progression, and regression of VC [29–31]. Advances in single-cell sequencing have identified new macrophage subtypes and contributed to research on macrophages in cardiovascular diseases. Targeting inflammation regulation, particularly macrophage functions, has become a focus of VC studies [32–34]. This review aims to provide insight into the origins, development, differentiation, phenotypes, and functional regulation of macrophages in various environments and their impact on VC. Such understanding is crucial for advancing basic research and developing macrophage-targeted therapies to prevent or treat VC.

2. Origin, Development, and classification of Macrophages

2.1. Discovery, Origin, and distribution of Macrophages

Metchnikoff and his colleagues discovered macrophages and their role in phagocytosis over 140 years ago [35], sparking extensive research into these cells. Studies have found that macrophages have functions that include engulfing pathogens to resist infection, engulfing excess cells during development, clearing necrotic tissue, and facilitating tissue repair [36]. Subsequent research revealed that macrophages, as key innate immune cells, also present antigens to T cells, initiating and regulating adaptive immunity [37]. Macrophages are also involved in intercellular communication [38] and demonstrate plasticity and heterogeneity. They can adapt their phenotype during differentiation based on the surrounding environment and produce biological effects by secreting various effector molecules [39]. In the 1960 s, van Furth [40] proposed that all macrophages derive from blood monocytes; however, this theory has been revised. Recent research showed that tissue macrophages in adult organisms, known as resident tissue macrophages (RTMs), are derived from the yolk sac (YS) and established during the embryonic stage [41]. RTMs originate from YS progenitors in the embryo and the fetal liver (FL). During embryogenesis, YS progenitors differentiate into organ-specific RTMs as the integral components of specific tissues, forming stable spatial and functional relationships with specialized tissue cells and playing crucial roles in growth, development, and homeostasis [41,42]. For example, microglia prune neuronal synapses during development, which is vital for cognitive function [43]. Osteoclasts remodel bone structure and resorb excess bone [44]. Kupffer cells phagocytize damaged red blood cells and participate in iron recycling [45], and adipose-associated macrophages regulate energy metabolism [46]. Cardiac resident macrophages assist with cardiac electrical conduction [47]. Ongoing research into RTMs in different tissues provides deeper insight into the mechanisms of maintaining homeostasis.

The two types of macrophages in the body are classified according to

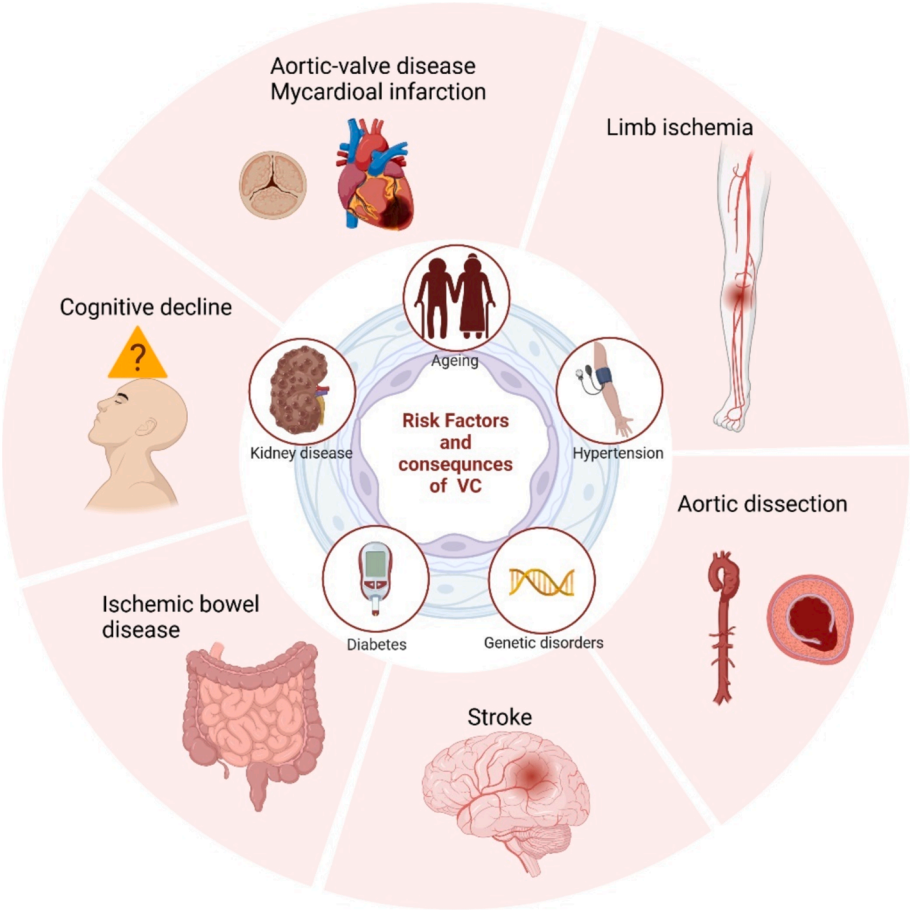

Fig. 1. Risk factors and consequences of vascular calcification (VC). Many diseases may lead to VC, such as chronic kidney disease, hypertension, diabetes, atherosclerosis, aging, and some genetic disorders. Severe VC can cause ischemia and necrosis of the affected organs, resulting in stroke, myocardial infarction, cognitive decline, ischemic bowel disease, aortic dissection, kidney atrophy, blindness and so on. Created in BioRender.com.

their origin. One type originates from the YS and FL, while the other is derived from monocytes produced by hematopoietic stem cells (HSC) in the bone marrow. These two types of macrophages coexist in most organs. Transplant studies showed that RTMs can proliferate independently of the peripheral mononuclear system. Even years after transplantation, donor-derived macrophages can still be detected in organs such as the heart and lungs [48,49]. However, embryonically derived RTMs decrease with age due to their limited replication capacity. Their niches are often filled by RTMs derived from peripheral monocytes. Some organs, such as the brain and aorta, rely on embryonic RTMs for maintenance rather than replenishment by monocyte-derived RTMs [49,50]. Compared to mononuclear cells, which participate in immune defenses during adulthood, RTMs have a longer lifespan. Except for a few organs, such as placenta, embryo and brain [51,52], RTMs are vital to the development and homeostasis of most organs [53]. RTMs primarily maintain homeostasis, contribute to tissue growth and development, and repair damaged tissues. In contrast, mononuclear cells clear external pathogens and necrotic tissues, exhibiting strong phagocytic activity and antigen presentation for immune coordination [50].

2.2. Macrophage heterogeneity

Macrophage heterogeneity refers to the differences in macrophage morphology, biochemistry, phenotype, and function. In 2000, Mills et al.[54] proposed the M1/M2 macrophage polarization model, noting that macrophages from C57BL/6J and BALB/c mice metabolize arginine through opposing pathways, which influence their inflammatory responses. The M1/M2 macrophage polarization model categorizes macrophages into classical M1 macrophages and alternative M2 macrophages and is most frequently used in disease research [54,55]. M1 macrophages emerge in inflammatory environments dominated by Toll-like receptor (TLR) and interferon signaling, typically induced by Th1 cytokines and bacterial wall components. The key regulatory factors comprise interferon-γ (IFN-γ) and lipopolysaccharide (LPS). M1 macrophages participate in immune responses against bacteria and intracellular pathogens, generating pro-inflammatory factors like interleukin (IL)-6, IL-12, and tumor necrosis factor (TNF). M2 macrophages are activated by Th2 cytokines in environments dominated by TH2 responses and are controlled by factors such as IL-4, IL-10, and IL-13. They are involved in immune responses to helminths, asthma, and allergies, and primarily secret anti-inflammatory cytokines to facilitate tissue repair and remodeling [55,56]. M2 macrophages can be further categorized into M2a, M2b, M2c, and M2d subtypes based on the triggering stimuli [57]. M2a is activated by IL-4 and IL-13, M2b by immune complexes and TLR agonists, M2c by IL-10 and transforming growth factor (TGF)-β, and M2d by M1 activation through adenosine A2A receptor (A2AR) modulation, which indicates that M1 and M2 macrophages can undergo phenotype conversion [56]. An example of these processes is that macrophages in infected tissues initially polarize to the M1 phenotype to combat pathogens and later switch to the M2 phenotype for tissue repair. This adaptive response to the surrounding microenvironment demonstrates the high plasticity of macrophages, forming the basis for the theory of disease prevention and treatment by regulating macrophages. However, technological limitations have hindered the identification of macrophage phenotypes between M1 and M2. The current model is based on *in vitro* studies of primary macrophages [54], which oversimplify the complex *in vivo* environment and macrophage plasticity. Nevertheless, the M1/M2 polarization model has provided a foundational understanding of immune responses [54]. Recent technological advancements, such as fluorescence-activated cell sorting, cytometry by time-of-flight, and single-cell RNA sequencing (scRNA-seq), have significantly enhanced our understanding of macrophages [58,59]. In particular, scRNA-seq has become a crucial tool in biomedical research.

3. Perivascular Macrophages

3.1. Discovery, Origin, and development of perivascular Macrophages

In 1927, Kubie discovered elongated cells in the perivascular spaces of the central nervous system capable of phagocytizing trypan blue injected into the subarachnoid space [60]. Further research revealed that these cells not only clear metabolic waste but also present antigens. Consequently, they were identified as perivascular microglia. Gene expression and other studies have confirmed these cells as a type of RTMs, and they are now referred to as perivascular macrophages (PVMs) [61,62]. Unlike most organs, vascular tissues are widespread throughout the body and closely linked with the development, growth, metabolism, damage, and repair processes of the affected organs. PVMs represent a unique subset of RTMs highly associated with vascular tissues. Research on PVMs, which were the first discovered non-parenchymal brain macrophage subset, has expanded from neuroscience and neurology to tissue and organ health and disease, reflecting a growing trend toward multidisciplinary integration [63]. Research on RTMs in different tissues has provided deeper insight into PVMs [30,64–67]. During normal vascular development, certain RTMs closely interact with vascular formation, regulating one another and maintaining tissue homeostasis and repair during pathological processes [68]. PVMs can be distinguished from other macrophage subsets by their proximity to blood vessels and certain surface markers [69]. Most steady-state tissues contain two distinct RTM subsets located in the interstitial spaces of the organ, particularly in the vascular lumen adjacent to endothelial cells. Some of these cells also interact with neuronal fibers along the blood vessels. Thus, these cells are collectively referred to as PVMs due to their unique anatomical location in close contact with the vascular system [70].

Various fate mapping methods have shown that, under steady-state conditions, these cells originate from two sources: (1) $CX3CR1^{+}$ precursor cells derived from YS endothelial cells, known as *erythro*-myeloid progenitors (EMPs) and (2) fetal monocytes that colonize arterial tissues immediately after birth [71]. Zhao et al.[70] identified two distinct PVM populations: TIM4 PVMs, characterized by high levels of T-cell immunoglobulin and mucin domain-containing protein 4 (TIM4), lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), folate-binding protein-2 receptor (FOLR2), and CD206, and low or no major histocompatibility complex II (MHCII). These PVMs possess a long lifespan and appear in multiple organs during embryogenesis, originating from EMPs. The other population, MHCII PVMs, is characterized by high levels of MHCII and CD206, low to moderate levels of LYVE1 and FOLR2, and low or no TIM4. These cells originate from HSC-derived monocytes, with their lifespans varying across organs. Some studies [64,66] have reported a third subpopulation, CCR2 PVMs Fig. 2. Table 1. However, the origin and classification of this subset are controversial [70]. Similar to RTMs, PVMs are named according to their residence location, such as brain PVMs. In the gut or serous cavities, EMP-derived RTMs are gradually replaced by bone marrow-derived circulating monocytes shortly after birth [72,73], whereas cardiac RTMs are progressively replenished by peripheral monocytes [74,75]. Aortic PVMs, a unique type of RTM, are closely related to vascular physiological and pathological processes, although their maintenance differs from most RTMs. In adults, PVMs primarily consist of EMP-derived macrophages, which maintain their longevity independently of bone marrow hematopoiesis. These macrophages depend on CX3CR1-CX3CL1 interactions and local proliferation rather than relying on peripheral monocyte differentiation [76,77]. The number of EMP-derived RTMs peaks in adulthood and decline with age, while the proportion of HSC-derived RTMs increases during puberty and remains stable thereafter [71]. The decline in EMP-derived RTMs with age leads to changes in PVM composition, reflecting a shift in vascular homeostasis. During aging, the immune system gradually becomes dysregulated, leading to age-related chronic low-grade inflammation, known as inflammaging. The relationship between inflammaging and macrophages is still being explored,

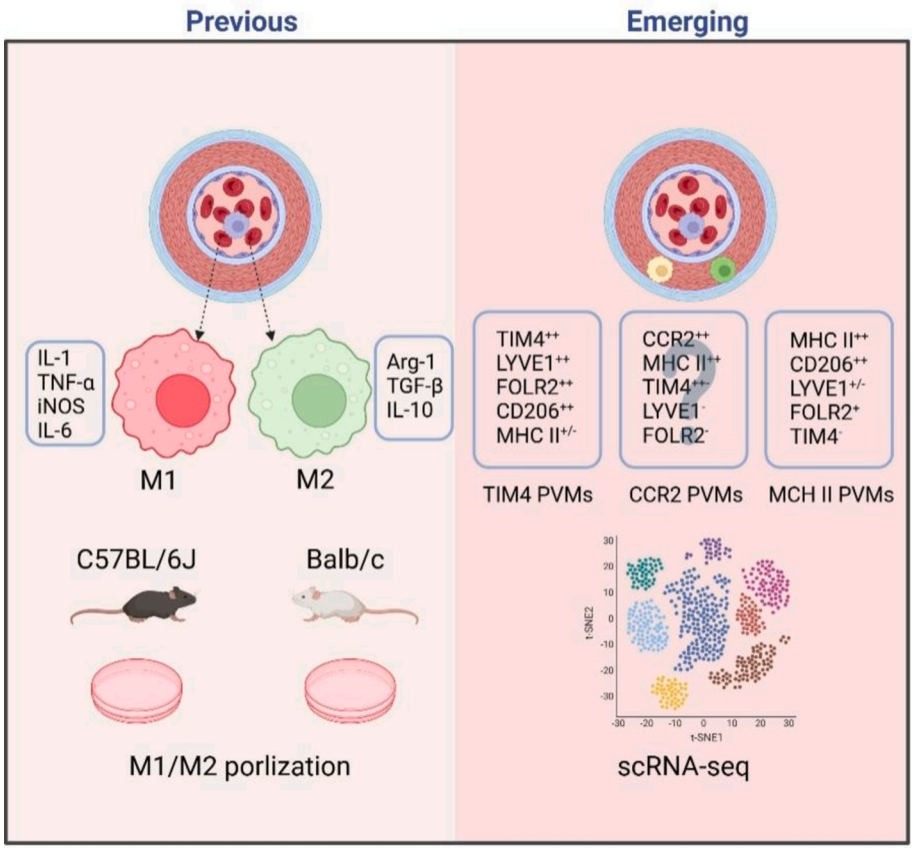

Fig. 2. Updated understanding of macrophage subpopulations. Macrophages are divided into two populations: M1 macrophages and M2 macrophages. This model was proposed based on the observation that macrophages from C57BL/6J and BALB/c mice metabolize arginine through opposing pathways and influence inflammatory responses. However, emerging technologies have revealed the high heterogeneity of macrophage development, phenotype, and function. In particular, the emergence of single-cell RNA sequencing (scRNA-seq) technology has changed the traditional binary cognition of macrophages and provided new ideas for macrophage-targeted treatment. Created in BioRender. Dong, R. (2024) https://BioRender.com/t80f160.

Table 1
Subpopulations of PVMs in homeostasis.

Clusters	Origin	Phenotype	Lifecycle
TIM4 PVMs	EMPs	TIM4^{hi}, LYVE1^{hi}, FOLR2^{hi}, CD206^{hi}, MHCII$^{lo/-}$	In situ proliferation
MHCII PVMs	HSC-derived monocytes	MHCII^{hi}, CD206^{hi}, LYVE1$^{lo/+}$, FOLR2$^{lo/+}$, TIM4 $^{lo/-}$	In situ proliferation and peripheral monocyte replenishment
CCR2 PVM	HSC-derived monocytes?	CCR2^{hi}, MHCII^{hi}, TIM4$^{hi/-}$, LYVE1^{-}, FOLR2^{-}	Monocyte replenishment

TIM4, T-cell immunoglobulin and mucin domain-containing protein 4; LYVE1, lymphatic vessel endothelial receptor 1; FOLR2, folate-binding protein-2 receptor; MCH II, major histocompatibility complex II; HSC, hematopoietic stem cell; EMP, *erythro*-myeloid progenitor; PVM, perivascular macrophage; CCR2, C–C chemokine motif receptor 2.

particularly how disrupted homeostasis and chronic inflammaging might be related. Investigating the mechanisms of selective apoptosis of EMP-derived RTMs may provide potential strategies for delaying aging.

3.2. Pvms and angiogenesis

Early research has indicated that macrophages regulate angiogenesis through paracrine mechanisms [78]. Numerous studies have demonstrated that macrophages secrete growth factors and enzymes that promote angiogenesis by remodeling the extracellular matrix and inducing endothelial cell migration [79]. *In vitro* experiments demonstrated that macrophages secrete various pro-angiogenic factors, including vascular endothelial growth factor A (VEGFA), TNF, TGF-β1, and fibroblast growth factor 2 [80]. Among these, VEGFA is the most potent angiogenic factor, capable of inducing the formation of endothelial tip cells [81,82]. However, *in vivo* studies showed that, although VEGF is involved in macrophage-mediated vascular fusion, it does not originate from macrophages. This suggests that macrophages do not promote angiogenesis through a VEGF-dependent mechanism [83]. Additionally, research on tumors has shown that VEGF ablation in inflammatory cells can enhance vascular normalization and maturation [84]. The discovery of RTMs and research on tissue embryology have provided further evidence of macrophages' role in promoting vascular development and showed that macrophages derived from EMPs are involved in all stages of vascular network development [85]. During early embryonic vascular development, YS endothelial cells differentiate into EMPs, which mature into macrophages. These PVMs support vascular development by directly interacting with endothelial tip cells. This model, in which PVMs act as cellular partners in vascular development, has been validated in various tissues, including the brain, retina, and muscle [83,86]. Similar to the pruning of synapses by brain microglia during development, these embryonic RTMs guide growing blood vessels in connecting with each other and subsequently phagocytizing excess vascular tissue. The depletion of this macrophage type can lead to widespread vascular distribution, impaired vessel anastomosis, and disrupted vascular remodeling [87] Fig. 3. Macrophages also promote vascular development and growth by secreting soluble mediators [83,87]. Research has shown that these embryonic macrophages are similar to M2-type tumor-associated macrophages [83], expressing

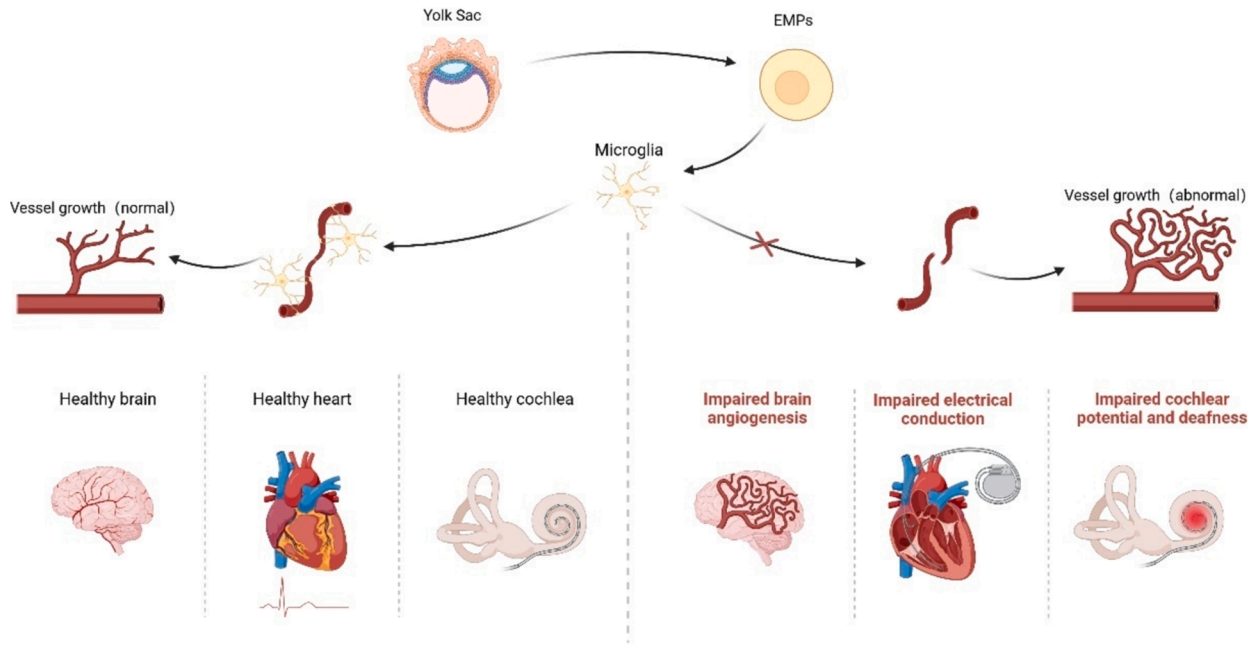

Fig. 3. The origin of perivascular macrophages (PVMs) and angiogenesis. During embryonic development, PVMs mainly originate from *erythro*-myeloid progenitors derived from yolk sac endothelial cells. They can later differentiate into microglia and are distributed along blood vessels in fetal organs. These macrophages guide the developing vascular network in connecting with each other and eliminating excess tissue. The depletion of these PVMs can lead to diffuse vascular distribution, impaired vessel anastomosis, and disrupted vascular remodeling, resulting in abnormal vascular development. Created in BioRender. Dong, R. (2023) BioRender.com/r58u626.

angiopoietin receptor TIE2 [88] and neuropilin 1 (NRP1). Recently, Wang et al.[89] conducted single-cell transcriptomic sequencing of 19 human embryonic tissue samples from weeks 4 to 26 of pregnancy and identified a pro-angiogenic macrophage population. Spatial and differentiation trajectory analyses suggested that this cell population might be YS-derived microglia distributed around blood vessels in fetal organs.

3.3. Pvms in homeostasis maintenance and tissue repair

As a subtype of RTMs, PVMs are crucial for maintaining body homeostasis. Early studies demonstrated that depleting macrophages using clodronate liposomes or antibodies increased capillary permeability. However, M2-like macrophages could counteract this effect, indicating that PVMs can repair tissues and play an important role in regulating vascular permeability [90]. Vascular permeability is vital for tissue growth, development, and homeostasis, as it helps maintain the metabolic balance and spatial structure of organs [91–93]. Research has shown that PVMs in the cochlea regulate the permeability of the blood-labyrinth barrier and maintain the internal environment, which is crucial for cochlear potential formation and normal hearing [92–94]. Morphological and functional changes in PVMs in noise-induced and age-related sensorineural hearing loss lead to macrophage activation, replacing normal homeostatic balance with excessive inflammation. Persistent immune activation damages the cochlear immune microenvironment, ultimately resulting in hearing loss and even deafness [95]. Similar observations have been made in other tissues; brain PVMs regulate cerebrospinal fluid flow through arterial motion [96], and cardiac PVMs are associated with cardiac electrical conduction [97].

In contrast to small blood vessels like capillaries, large vessels such as the aorta have a well-developed tunica media consisting of vascular smooth muscle and elastic fibers. Maintaining aortic vascular tension is essential for stabilizing blood pressure. Atherosclerotic aortas often show increased pulse pressure, which is a sign of worsening cardiovascular health and is associated with higher risks of chronic diseases such as cardiovascular disease and chronic kidney disease [98]. Age-related changes, such as extracellular matrix protein cross-linking and increased matrix deposition, contribute to atherosclerosis. Aging affects the quantity and/or structure of elastin and collagen in arterial walls, which can impact arterial mechanical function [99]. PVMs are involved in maintaining appropriate aortic tension. The selective depletion of aortic PVMs with drugs results in atherosclerosis, vessel dilation, and increased collagen deposition [100]. EMP-derived macrophages are characterized by the high expression of genes such as LYVE-1, Stab1, growth arrest specific 6, and Cd163 [71]. Lim et al.[100] used phenotype analysis, transcriptional analysis, and the targeted deletion of Csf1r to show that LYVE-1^+ PVMs in mouse and human vascular tissues bind to smooth muscle cells (SMCs) via hyaluronan binding, releasing matrix metalloproteinase 9 (MMP-9) and reducing collagen expression in SMCs. This process decreases collagen deposition in arterial tissues, which is a key factor in regulating arterial tension. LYVE-1^+ PVMs exhibit independent self-renewal and function differently from monocyte-derived macrophages. Ulndreaj et al.[101] found that exogenously recruited macrophages lacking LYVE-1 caused aortic dilation and aneurysms in an angiotensin II mouse model. These findings suggest that LYVE-1 is a crucial marker for resident macrophages in cardiovascular tissues and that LYVE-1^+ PVMs play a significant role in maintaining vascular homeostasis. However, scRNA-seq results have provided contrasting conclusions. Fabienne et al.[102] reported that LYVE-1^+ resident-like macrophages in human atherosclerotic plaques promote VSMC transdifferentiation into osteoblasts via CCL24 secretion, potentially contributing to VC. These studies suggest that differences in macrophage origins contribute to macrophage heterogeneity.

Peripheral monocyte infiltration into tissues is essential for restoring homeostasis during inflammation. In addition to decreases in EMP-derived PVMs, increased peripheral monocyte infiltration is a significant factor in the inability to maintain arterial or organ homeostasis. Florentin et al.[91] found that inflammatory macrophages around lung blood vessels are key factors leading to increased right ventricular systolic pressure and lung remodeling. Wang et al.[103] identified the chemokine receptor CXCR6 as important in vascular aging and remodeling by increasing macrophage recruitment, mediating pressure overload-induced atherosclerosis, and potentially leading to aortic

dissection or aneurysm.

Given the high plasticity of macrophages, researchers must consider more complex scenarios: 1) Does the pre-restoration environment affect macrophage phenotype and function afterward? 2) After peripheral monocyte infiltration subsides, is the so-called homeostasis consistent with the pre-event state? Reductions in LYVE-1^{+} embryonic RTMs during aging initiate atherosclerosis. The subsequent matrix deposition and increased matrix stiffness can affect macrophage polarization [104]. Stiff matrices can lead to macrophage polarization toward a pro-inflammatory phenotype [105,106], exacerbating atherosclerosis and creating a vicious cycle. These findings explain the accelerated development of atherosclerosis in seniors. Decreases in embryonic PVMs, inflammatory invasion, and increased arterial matrix stiffness significantly disrupt vascular homeostasis, leading to accelerated aortic stiffness and possibly arterial calcification, aneurysms, dissection, or rupture, which can impair organ function and lead to irreversible clinical outcomes or death.

4. The dual effects of Macrophages in VC

This section will review the current research findings on macrophage-secreted inflammatory factors, osteogenic genes, extracellular vesicles, macrophage subtypes, and macrophage efferocytosis dysfunction in the VC process. It will also discuss the dual effects of macrophages in promoting and inhibiting VC and address drugs and methods targeting macrophages for VC treatment. The aim is to provide references for future research on targeting inflammation or macrophage therapy for VC.

4.1. Advancement of Knowledge related to VC

VC is commonly associated with CKD, diabetes, atherosclerosis, hypertension, aging, and certain genetic disorders. As an independent risk factor for cardiovascular disease, VC has become a growing concern in the context of global population aging [107–110]. The traditional theory that calcium phosphate passively deposits on the vascular wall to form VC was challenged at the end of the 20th century. Demer proposed the concept that VC originates from highly osteogenic vascular tissues [111]. Increasing evidence supports that VC is a highly regulated active process, similar to bone formation. As discussed, macrophages are key participates in the development of VC [90,91]. They can either promote VC by releasing inflammatory factors and extracellular vesicles or inhibit it by releasing anti-inflammatory factors, differentiating into osteoclast-like cells, or internalizing, encapsulating, and absorbing calcium deposits. However, the origin of macrophages in vascular lesions remains to be explored. The recruitment of circulating monocytes to the vascular wall, where they differentiate into macrophages and infiltrate the wall, is a crucial step in the development of vascular diseases such as atherosclerosis, aortic aneurysm, and VC. Experimental results suggest that targeting macrophage recruitment can alleviate vascular inflammation and pathology. However, changes in PVMs when vascular homeostasis is challenged have not been reported. Some studies suggest that macrophages in vascular lesions may originate from local proliferation rather than the recruitment of circulating monocytes [112]. Given the updates in macrophage origin and VC concepts, as well as the high plasticity of macrophages, further research into the relationship between macrophages of different origins and VC may provide new insight into the prevention and treatment of VC.

4.2. Regulation of VC by Macrophage-secreted cytokines

Traditional research on the relationship between macrophages and VC has focused on the cytokines secreted by macrophages. Early studies have found that macrophages, especially in the inflammatory environment, can secrete a variety of inflammatory factors, such as the IL-1 superfamily, IL-6, and TNF-α, which have a strong pro-calcification effect [113–116]. These cytokines not only promote the osteogenic differentiation of VSMCs but also stimulate the production of reactive oxygen species (ROS) and osteogenic proteins [114]. For example, IL-6 promotes Runt-related transcription factor 2 (Runx2) expression through the STAT3/JMJD2B pathway, regulating the differentiation of VSMCs into osteoblast-like cells, which ultimately leads to VC [117,118]. The androgen receptor (AR) can directly bind to the androgen response element sequence in the IL-6 gene promoter, accelerating IL-6 transcription and expression. This accelerates VSMC calcification and osteogenic differentiation in CKD. Silencing the AR in monocyte-derived macrophages significantly reduced IL-6 expression and inhibited phosphate-induced VSMC calcification [119], which may partly explain the higher prevalence of VC in men. Song et al.[120] found that M1 macrophages promoted VC by secreting TNF-α, which increased the expression of CA1 and CA2 in VSMCs. Inflammation disrupts the existing homeostatic balance, with inflammatory factors like TNF-α promoting VC formation. Hydroxyapatite particles in calcified blood vessels have pro-inflammatory effects, recruiting macrophages [121] and stimulating them to produce more TNF-α, creating a vicious cycle that continues to promote VC [122]. TNF-α can also induce Rac activation, increasing the synthesis of inflammatory cytokines like IL-6 and IL-8 [123]. Targeting TNF-α can effectively suppress VC [124]. Among the inflammatory factors, IL-1β is particularly significant as it is a key inflammatory factor in inflammatory VC [125]. Plasma levels of IL-1β are positively correlated with high coronary calcium load, and elevated plasma IL-18 levels are highly associated with cardiovascular mortality in patients with high coronary calcium load [126]. IL-1β promotes VC in three ways [127]: by inducing endothelial-to-mesenchymal transition, inhibiting the mobilization and infiltration of bipotent mesodermal progenitor cells, and activating non-specific alkaline phosphatase, which exacerbates VC. Thus, regulating macrophage secretions of IL-1β can help inhibit VC. Aortic calcifications can be inhibited by IL-1β monoclonal antibody in LDLR-deficient mice [128]. These pro-inflammatory cytokines were shown to favour osteochondrogenic transition of vascular cells and the release of matrix vesicles and apoptotic bodies able to act as Ca/P nanocrystal nucleation points [129]. Racs are a group of small GTPases in inflammatory cells involved in key signaling pathways, affecting the expression of growth factors and cytokines. Rac1 and Rac2 have a competitive relationship. Macrophage Rac1 activates nuclear factor-kappaB (NF-κB) via NADPH oxidases, leading to the production of inflammatory cytokines like IL-1β and TNF-α, as well as ROS [130]. Rac2 prevents progressive VC by inhibiting Rac1-dependent IL-1β expression in macrophages. Statins increase active Rac1 in macrophages by disrupting the Rac1-RhoGDI complex, leading to increased NF-κB activation, elevated IL-1β mRNA, and the enhanced secretion of Rac1-dependent IL-1β protein, thus promoting VC [131]. Conversely, the loss of Rac2 in macrophages results in increased Rac1 activity, which promotes NF-κB signaling and reactive oxygen production via NADPH oxidase, leading to IL-1β maturation and VC mediation [126]. Canakinumab, an anti-human IL-1β monoclonal antibody, neutralizes IL-1β signaling, thereby suppressing inflammation in autoimmune diseases. The CANTOS trial confirmed the critical role of inflammation in atherosclerosis and marked the beginning of a new era of research on the secondary prevention of cardiovascular diseases targeting inflammation [132]. Theoretically, antagonistic therapy targeting IL-1β might have anti-VC effects, and future long-term follow-up studies should monitor VC development. In contrast, research on statin-related VC could explore methods to promote Rac1 binding with RhoGDI to reduce Rac1 activity and, consequently, VC occurrence.

In addition to secreting pro-inflammatory cytokines that promote VC, macrophages can also secrete anti-inflammatory cytokines to inhibit VC formation. IL-10 is the most studied anti-inflammatory cytokine. It inhibits the production of pro-inflammatory factors in blood leukocytes by activating the STAT3 pathway [133]. IL-10 also acts as an antagonist of inflammatory factors such as IL-1β, IL-6, and TNF-α [134], exhibiting anti-inflammatory and anti-atherosclerotic effects. Clinical studies

suggest that plasma IL-10 levels are negatively correlated with vulnerable plaques and at-risk patients [135]. Additionally, studies of VC in patients undergoing peritoneal dialysis confirmed the anti-VC effect of IL-10 [136].

4.3. Regulation of VC by Macrophage-secreted osteogenic genes

Macrophages release various osteogenic genes, including bone morphogenetic protein 2 (Bmp2) [137], Runx2, osteopontin (OPN), and tissue nonspecific alkaline phosphatase. Bmp2 is a multifunctional growth factor within the TGF-β superfamily. It initiates BMP signaling by binding to type I or type II BMP receptors (transmembrane serine/threonine kinases) and forming a receptor-ligand complex. Bmp2 is crucial for bone regeneration and repair, serving as a key regulator of *in situ* bone formation [138,139]. As a potent osteogenic factor, Bmp2 acts upstream to induce the expression of muscle segment homeobox 2 (Msx2) and Runx2 in VSMCs. Msx2 is a transcription factor that promotes osteogenic gene expression [139]. Runx2 is essential for osteoblast differentiation and chondrocyte maturation [140] and also promotes the osteogenic transformation and calcification of VSMCs, thereby inducing VC [8,141–143]. Inactivating or knocking out Runx2 was reported to prevent VSMC osteogenic transformation and calcification [8,144], indicating that targeting macrophage-secreted osteogenic genes could help prevent or treat VC. Transient receptor potential canonical 3 (TRPC3) is a non-selective Ca^{2+} channel. An *in vitro* study by Dube et al.[145] found that macrophages exhibited significant constitutive autocrine/paracrine Bmp2 osteogenic signaling. Further *in vivo* studies showed that the absence of TRPC3 channels in macrophages reduced macrophage apoptosis and impaired the expression of osteogenic regulators Bmp2 and Runx2, leading to decreased VC formation in advanced atherosclerotic plaques. Among the osteogenic genes secreted by macrophages, OPN plays dual roles. In bone tissue, OPN is secreted by both osteoblasts and osteoclasts. Osteoclasts secrete OPN to inhibit hydroxyapatite formation, which suppresses bone formation and links OPN to bone destruction [146]. However, studies of cardiovascular diseases showed that OPN is highly expressed at sites of atherosclerotic plaques, especially where macrophages and foam cells are present. This suggests that OPN promotes plaque formation and further contributes to VC [147]. Other research indicates that macrophages near calcified plaques secrete large amounts of OPN to inhibit plaque calcification, showing an anti-VC effect [148]. Macrophage-secreted OPN not only functions similarly to scavenger receptors but also facilitates the phagocytosis of hydroxyapatite-coated microspheres by macrophages [149], which may be one of the mechanisms of OPN's anti-VC effects. Most studies suggest that OPN inhibits VC by blocking hydroxyapatite crystal formation and modulating acidification [148]. Ge et al.[150] found that both macrophage OPN mRNA levels and serum OPN levels were increased in hypertensive patients with VC. OPN inhibited the differentiation of these patients' macrophages into osteoclasts and reduced the expression of macrophage-derived inflammatory factors, thereby counteracting macrophage-mediated VC. However, other studies indicated that elevated serum OPN levels were associated with an increased risk of vascular disease [151,152]. Therefore, the effects of strategies aimed at regulating VC solely by adjusting macrophage-derived OPN may be limited. Research should focus on understanding discrepancies in the OPN-VC relationship, including whether differences in the rate and amount of OPN released by macrophages under various pathological conditions contribute to VC progression and whether the concentration and duration of OPN in tissues correlate with disease phenotypes.

4.4. Regulation of VC by Macrophage-secreted extracellular vesicles

Extracellular vesicles (EVs) are membrane-bound structures released by cells that transport nucleic acids, proteins, and various metabolites to target cells, facilitating intercellular communication. EV-mediated intercellular signaling has been demonstrated in various disease models and is also crucial in the development of VC. Ectopic calcification often originates from EVs [153]. During VC progression, different cell types, including VSMCs, valve interstitial cells, and macrophages, produce EVs, which accumulate between collagen fibers. Macrophage-derived EVs are distinctive because they specifically express annexin V on their surface [154].

Early research showed that early mineralization sites bind phosphatidylserine (PS) with Ca^{2+} to form complexes in bone tissue that promote bone mineralization. Similarly, PS in necrotic cell debris has a high affinity for Ca^{2+} in ectopic calcification, which may contribute to calcification at necrotic tissue sites. Annexin V binds PS and forms complexes on EV surfaces. Macrophage-derived EVs can form complexes with annexin V, PS, and S100A9, which facilitates VC and serves as a nucleation site in the VC process [155]. As in early bone mineralization, complexes containing Ca^{2+} in EVs can become nucleation sites for calcification, leading to microcalcifications or larger calcifications.

In addition to surface complexes, EVs can carry cargo, such as nucleic acids, proteins, and various metabolites, and influence VC development. For example, Li et al.[34] found that exosomes derived from high-phosphate-stimulated macrophages (Mexo-P) inhibited the expression of let-7b-5p in VSMCs, leading to the upregulation of transforming growth factor beta receptor 1 and enhanced SMAD3/RUNX2 signaling, which promotes VC. This study explains the mechanism of CKD-related VC from the perspective of macrophage-secreted EVs. M1 macrophage-derived EVs can coordinate intercellular communication by delivering non-coding RNAs, such as tRNA-derived small RNAs (tsRNAs). Xia et al. [156] discovered that M1 macrophage-derived EVs carrying tsRNA-5006c regulated mitochondrial autophagy, promoting the calcification of aortic interstitial cells. Li et al.[157] found that M1 macrophages release EVs containing miR-214, which downregulates TWIST1 in valve endothelial cells, thus promoting heart valve calcification. In diabetes, the S100A9-RAGE axis accelerates macrophage EV secretion, promoting VC formation [158]. LPS-treated macrophages secrete EVs containing various cytokines, *cis*-aconitate decarboxylase, plasminogen activator inhibitor-1, serum amyloid A-3 protein, and other proteins, which contribute to inflammation, oxidative stress, and VC. These EVs promoted VC by inducing pro-inflammatory and pro-oxidative responses under calcification conditions [159]. Research on macrophage-released EVs during the VC process represents significant progress in identifying effective treatment targets for VC, particularly using miRNA-based therapies Fig. 4.

4.5. The relationship between Macrophage subtypes and VC

As an inflammatory disease, VC involves the differentiation of macrophages into various subtypes, each playing an active role in the process. The M1/M2 macrophage polarization model was discussed previously. M1 macrophages, characterized by their pro-inflammatory phenotype, produce cytokines such as IL-1, IL-1β, IL-6, and TNF-α, which promote VC. Toshiaki et al.[33] found the uptake of indoxyl sulfate by the organic anion transporter polypeptide OATP2B1 in macrophages in a uremia model, triggering the DII4-notch signaling pathway and activating pro-inflammatory macrophages. This finding provides an inflammatory perspective on the mechanism of VC in CKD. Li et al.[157] showed that M1 macrophages released microvesicles containing microRNA-214, which downregulated TWIST1 in valve interstitial cells, thereby promoting aortic valve calcification. In contrast, M2 macrophages exhibit an anti-inflammatory phenotype. They release anti-inflammatory factors and prostaglandin E2 or engulf apoptotic cells, reducing potential VC nucleation sites [160]. Farias-Itao et al.[161] found that M2 macrophages in perivascular fat were associated with reduced plaque calcification. Villa-Bellosta et al.[162] reported that M2 macrophages increased the accumulation of extracellular ATP and pyrophosphate, inhibiting calcium-phosphate deposition and slowing vascular intimal calcification.

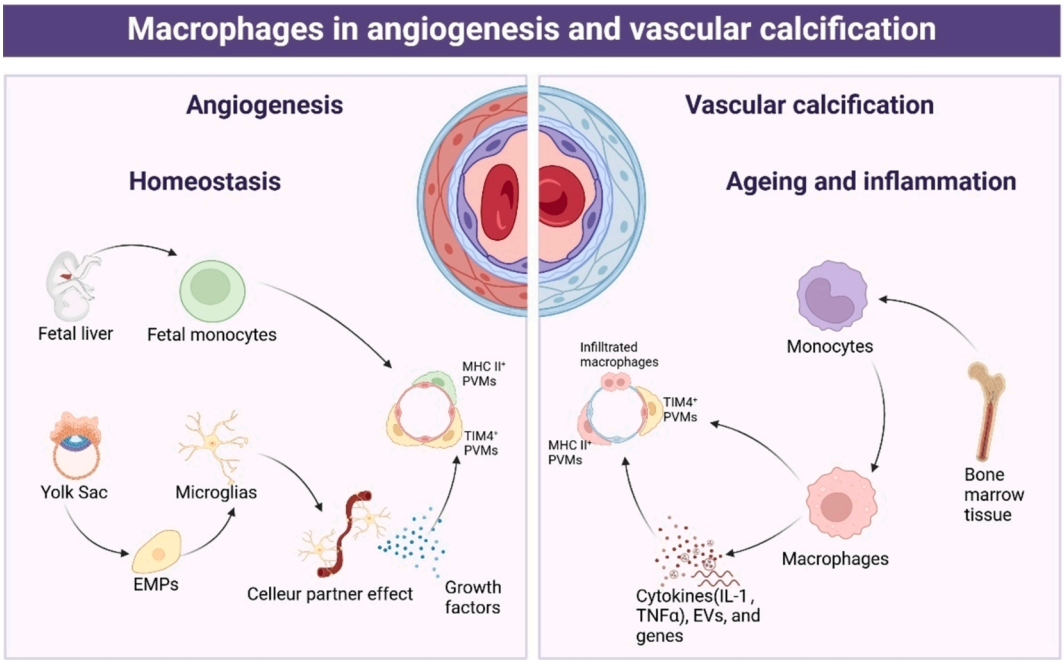

Fig. 4. Macrophages in angiogenesis and vascular calcification. In the normal development process, yolk sac endothelial cells, namely *erythro*-myeloid progenitors (EMPs), first differentiate into microglia, participate in angiogenesis during development, and finally differentiate into TIM4 perivascular macrophages (PVMs). These become the dominant component in maintaining vascular homeostasis. In an aging or inflammatory environment, monocytes derived from bone marrow hematopoietic tissue infiltrate and polarize into pro-inflammatory macrophages, which promote vascular calcification by releasing various inflammatory factors and osteogenic genes. At the same time, the empty niche where inflammation leads to PVM death can be occupied by infiltrating inflammatory macrophages, which becomes the basis of vascular homeostasis imbalance. Created in BioRender. Dong, R. (2024) https://BioRender.com/h66k594.

However, macrophages have high plasticity and heterogeneity, and the M1/M2 polarization model cannot fully explain their role in VC. With rapid advancements in sequencing technologies, more macrophage subtypes associated with cardiovascular diseases are being discovered or defined [163]. Notably, Boyle et al.[164] identified a macrophage subtype known as Mhem. These macrophages, derived from blood monocytes and activated by phagocytosing hemoglobin, express CD163 and induce cholesterol efflux core genes such as LXR-α and ABCA1, preventing foam cell formation [165]. Boyle et al.[166] also identified another macrophage subtype, HA-mac, similar to Mhem macrophages, with anti-atherosclerotic and anti-inflammatory functions. Like M2 macrophages in atherosclerotic plaque regression, both macrophage subtypes express CD163, suggesting they may have vascular-protective roles. New research indicated that CD163 macrophages enhance hyaluronan synthase through NF-κB induction, thereby inhibiting VC [166], which explains why vulnerable high-risk plaques often do not exhibit widespread calcification. Conversely, some studies showed that after phagocytosing oxidized hemoglobin (ferrylHb), CD163-expressing macrophages polarize into a pro-inflammatory phenotype, promoting atherosclerosis and secreting IL-1β and TNF-α, which facilitates VC [167]. These findings highlight the need to consider the high plasticity and heterogeneity of macrophages in research. Conclusions based solely on a single molecular target may be incomplete.

Emerging technologies, such as scRNA-seq and flow cytometry, have enabled successful studies of complex macrophage phenotypes and functions in calcification-related lesions. Previous research on the development of PVMs showed that under healthy conditions, YS-derived aortic resident macrophages are predominant and express CX3CR1 and LYVE-1. These cells, similar to M2 macrophages, express anti-inflammatory markers and play a crucial role in maintaining aortic matrix components and arterial tension homeostasis. The specific depletion of LYVE-1 macrophages led to increased aortic collagen deposition and stiffness [100]. Kim et al.[168] used scRNA-seq to identify three LYVE-1-expressing macrophage subtypes in mouse atherosclerotic plaques. Gene enrichment analysis revealed two subtypes associated with endocytosis pathways and one with inflammatory gene pathways. Another scRNA-seq study [102] in ApoE$^{-/-}$ mice with hypercholesterolemia found that LYVE-1^{+} resident-like macrophages also expressed CCL24, an eosinophil chemokine. CCL24 increased VSMC osteoblastic/chondrocytic markers, alkaline phosphatase activity, and Runx2 expression [169,170]. This macrophage subtype is present in vulnerable human atherosclerotic plaques, and its proliferation leads to elevated plasma CCL24 levels, promoting VC in human atherosclerosis. This suggests that LYVE^{+}CCL24^{+} macrophages have an osteogenic effect on VSMCs [102]. Research on the two anti-inflammatory macrophage subtypes showing pro-inflammatory and calcification-promoting effects has highlighted the need for a deeper understanding of macrophage polarization [102,168]. The diverse functional states of macrophages reflect their high plasticity and heterogeneity, responding to different environmental signals rather than being defined by a single cell surface marker. Contradictory findings also highlight the potential gaps in current research on macrophages and VC: 1. *In vitro* studies may introduce biases in macrophage selection, often using bone marrow-derived monocytes while neglecting YS-derived RTMs in VC. 2. Inflammatory processes vary and cannot follow the same paradigm for macrophage activation and subsequent biological processes. Thus, *in vitro* results may not fully represent *in vivo* VC development. 3. Given macrophages' high plasticity, research often focuses on biochemical signals in the environment and rarely considers physical structural changes, especially in VC, which affects tissue stiffness, morphology, and structure and involves hemodynamic changes. Such complex pathological processes are challenging in *in vitro* models, and standardized *in vivo* models combined with advanced sequencing technologies may be required to compare early and late-stage macrophages in VC to address these research gaps.

4.6. The relationship between Altered efferocytosis and VC

Efferocytosis is the process by which professional or non-professional

phagocytes clear apoptotic cells, which is crucial for maintaining tissue homeostasis. Defects in efferocytosis may lead to chronic inflammatory diseases [171]. The efferocytosis process can be divided into three main stages: recognition, ingestion, and digestion [172]. Abnormalities at any of these stages can lead to impaired efferocytosis function and the development of disease. For example, in atherosclerosis, lipid and lipoprotein accumulation beneath the endothelium leads to macrophage infiltration. Initially, macrophages have normal phagocytic function. However, as lipid accumulation increases, both resident and infiltrating macrophages engulf large amounts of lipids, forming foam cells. This results in a defect in macrophage phagocytic function and leads to macrophage apoptosis, with apoptotic bodies becoming nucleation sites for VC [173]. The formation of a necrotic core in atherosclerotic plaques provides a structural basis for VC. Studies have shown that apoptotic macrophages (foam cells) are present in microcalcified tissues, which lead to a decrease in the ability of subsequently infiltrating macrophages to clear these apoptotic cells and ultimately cause the necrotic core to enlarge [174]. Additionally, phospholipid fragments from apoptotic cells provide nucleation sites for calcification [175]. Inflammaging causes various tissue damage and contributes to several age-related diseases [176,177]. Defects in efferocytosis function are one mechanism of aging, and aging also leads to efferocytosis dysfunction. The mechanisms by which aging affects macrophage efferocytosis function include changes in “find-me” signals and increased monocyte migration, which alter the migration of phagocytes to apoptotic cells during the recognition stage; immune cell aging, which affects the macrophage microenvironment, leading to changes in macrophage phagocytic activity and abnormalities in the ingestion stage; and alterations in the phagocytic process due to changes in dying cells during aging [172]. Research on VC has also confirmed these mechanisms. Fang et al.[178] found that aging triggers the recruitment of monocytes and macrophages through the senescence-associated secretory phenotype. The pro-inflammatory microenvironment not only affects immune cell function but also impacts the differentiation of myeloid immune cells. The clearance of senescent cells is impaired in aged macrophages, and this effect is exacerbated by the pro-inflammatory microenvironment, promoting VSMC calcification. Their other research revealed that LPS could induce macrophage aging, with aged macrophages expressing high levels of interferon-induced transmembrane protein 3, which worsens vascular smooth muscle calcification and aging [179]. Galectin-3 is a multifunctional carbohydrate-binding protein that regulates cell growth, proliferation, and apoptosis by coordinating interactions between cells and the ECM [180]. It is associated with inflammation and is considered a key promoter of atherosclerosis [181,182]. Studies have shown that galectin-3 is related to aging [183–185], and high plasma levels of galectin-3 are associated with frailty in elderly people [186]. However, a deficiency in galectin-3 can reduce the ability of macrophages to phagocytose apoptotic cells [187,188]. The upregulation of galectin-3 in macrophages was shown to promote the migration of VSMC-derived extracellular vesicles to the intima and induce the calcification of the diabetic vascular intima, mediating ectopic calcification [189]. Elevated plasma galectin-3 levels in elderly individuals may lead to changes in macrophage phagocytic function. Thus, further investigation is required to determine whether the high incidence of VC in elderly patients is related to phagocytic dysfunction caused by increased galectin-3 levels.

Ca^{2+} is a particularly interesting factor in the phagocytic process. On the one hand, Ca^{2+} plays a crucial role in macrophage migration, survival, and foam cell formation and is essential for efferocytosis. In phagocytic macrophages, Ca^{2+} induces actin polymerization, which facilitates the formation of the phagocytic cup structures necessary for phagocytosis. Additionally, Ca^{2+} enhances the secretion of anti-inflammatory factors by phagocytic macrophages, suggesting that Ca^{2+} can improve macrophage phagocytic function and reduce nucleation sites for VC. On the other hand, dysfunctional Ca^{2+} homeostasis is a key factor in VC formation. Elevated Ca^{2+} concentrations promote atherosclerotic plaque calcification and lead to VC [190]. Regulating Ca^{2+} homeostasis in tissues could be a potential method to promote phagocytosis and inhibit VC.

4.7. Research on VC treatment targeting Macrophage regulation

As previously mentioned, macrophages can influence VC through various mechanisms. They can release inflammatory factors, osteogenic genes, and EVs or polarize into different phenotypes to either promote or inhibit inflammation. Macrophage dysfunction or death can also contribute to VC. The multiple ways in which macrophages affect VC suggest that strategies to target macrophages could be effective in combating VC.

Research on dietary supplements indicates that several supplements can reduce VC formation by modulating macrophages. Vitamin D is essential for bone health and development. Adequate levels of vitamin D not only prevent osteoporosis and fractures [191] but also help prevent VC [192]. Vitamin D deficiency leads to macrophage recruitment and inflammation, worsening VC, whereas sufficient vitamin D supplementation improves macrophage infiltration and local inflammation, thus preventing VC [193]. Vitamin K2 supplementation also shows promise in preventing VC in the general population [194,195]. Additionally, vitamin K deficiency is a risk factor for coronary calcification in patients with human immunodeficiency virus infections [196]. Studies have demonstrated vitamin K’s anti-inflammatory effects. For example, Ohsaki Y et al.[197] showed that vitamin K could inhibit LPS-induced inflammation in macrophage-like THP-1 cells. Further research revealed that menaquinone-4 (a form of vitamin K2) reduces IL-6 expression by decreasing the activation of NFκB and inhibiting the phosphorylation of IKKα/β in LPS-treated THP-1 and RAW264.7 cells. Pan et al.[198] found that the pre-treatment of human monocyte-derived macrophages with menaquinone-7 suppressed TNFα, IL-1α, and IL-1β gene expression and protein production upon activation with TLR agonists, showing a dose-dependent downregulation. Vitamin K may protect blood vessels through its anti-inflammatory effects, thus inhibiting VC. Astaxanthin (AST), a potent carotenoid antioxidant, has anti-inflammatory properties. Wu et al.[199] discovered that AST alleviates LPS-induced oxidative stress by inhibiting NF-κB activation and reducing inflammation. AST can also bind to IL-6, inhibiting the positive inflammation feedback loop and significantly reducing ROS in LPS-stimulated macrophages. Curcumin, a dietary polyphenol, is an antioxidant that suppresses macrophage inflammation, induces polarization toward the M2 phenotype, and enhances antigen capture and phagocytosis by macrophages [200,201].

As discussed, macrophages play a role in VC through various mechanisms, and targeting these processes can help suppress or reverse VC. Research on macrophage regulation in VC showed significant clinical potential, but clinical translation is still weak. This gap is partly due to differences between *in vitro* and *in vivo* studies, which often focus on disease mechanisms without considering the impact of regulatory factors or drug side effects. Additionally, the limited variables in experiments mean that only some mechanisms are studied and the complex needs of clinical work may not be fully addressed. The integration of medicine and modern engineering technology, known as “medical-engineering integration,” represents a deep fusion of cutting-edge science, including the combination of material science and medical research. This interdisciplinary approach could bridge the gap between disease mechanisms and clinical applications, including studies on macrophage regulation in VC.

Intravenous sodium thiosulfate (STS) can attenuate the progression of VC and arterial stiffness in patients on hemodialysis but carries risks of gastrointestinal symptoms, increased anion gap acidosis, and reduced bone density [202,203]. Inspired by natural grapefruit-derived EVs, Feng et al.[203] fabricated a biomimetic nanocarrier comprising EVs loaded with STS and further modified with hydroxyapatite crystal binding peptide (ESTP) for the VC-targeted delivery of STS. The ESTP

nanodrug significantly prevented VC by driving M2 macrophage polarization, reducing inflammation, and suppressing the bone-vascular axis by effects such as inhibiting the osteogenic phenotype *trans*-differentiation of VSMCs and enhancing bone quality. Additionally, the ESTP nanodrug did not induce hemolysis or damage other organs.

Nanocrystals are crystalline particles smaller than 1 μm that improve the delivery of poorly soluble drugs [204]. Nanocrystals combine the advantages of crystalline materials and colloidal nanoparticles, offering significant benefits over traditional drug formulations in terms of delivery, absorption, and bioavailability [205]. Curcumin, as mentioned earlier, is poorly soluble, limiting its bioavailability. Lizoňová et al. [206] developed a stable curcumin nanocrystal by combining curcumin with phospholipids rich in polyethylene glycol, which remained colloidally stable in culture medium with low macrophage clearance and no toxicity to healthy cells. Phospholipid-stabilized nanocrystals allow for the conversion of poorly soluble compounds into highly efficient "solution-like" drug delivery systems, enhancing curcumin's bioavailability and pharmacological effects on macrophage regulation and inflammation, thus suppressing VC.

Poly(aspartic acid) (PASP) is a strong calcium-chelating agent, which may be effective in inhibiting VC; however, its direct administration may lead to side effects [207]. Adelnia et al.[207] employed polysuccinimide, a precursor of PASP, to prepare targeted polysuccinimide-based nanoparticles (PSI-NPs) that not only acted as a prodrug but also functioned as a carrier of additional therapeutics to provide powerful synergistic vascular anti-calcification effects. Chemically modified PSI-NPs can serve as effective nanocarriers for the loading of hydrophobic drugs, and possess anti-calcification and anti-ROS activities. Although highly effective in preventing calcium deposition, PSI-NPs could not prevent the osteogenic *trans*-differentiation of VSMCs. The presence of curcumin addressed this problem. It not only further reduced ROS levels in macrophages but also prevented the osteogenic differentiation of VSMCs *in vitro*. The inclusion of curcumin and PSI-NPs combined the therapeutic effects of both. Cur-loaded NPs significantly reduced calcium deposition in the aorta without adversely affecting bone integrity or noticeable side effects/toxicity, according to organ histological and serum biochemical analyses.

IFNγ signaling plays a complex role in atherogenesis [208,209]. The IFNγ stimulation of macrophages permits the *in vitro* exploration of pro-inflammatory mechanisms and the development of novel immune therapies [210]. Decano et al.[211] characterized two clusters of macrophages in atherosclerosis and combined their cellular data with a cell-signature drug library to identify a novel compound BI-2536, which targets a subset of macrophages in atherosclerosis. *In vitro* and *in vivo* experiments demonstrated that the drug could shift the balance of macrophage subtypes, regulate inflammation, and reduce atherosclerosis and calcification. Their approach is a precision medicine strategy to identify new drugs that target atherosclerosis or VC.

Research on traditional Chinese medicine has shown that active components can regulate macrophages to inhibit VC. *Ganoderma lucidum* spore powder (GLSP), derived from the traditional herb Ganoderma, demonstrates anti-atherosclerotic and anti-VC effects [32]. Further

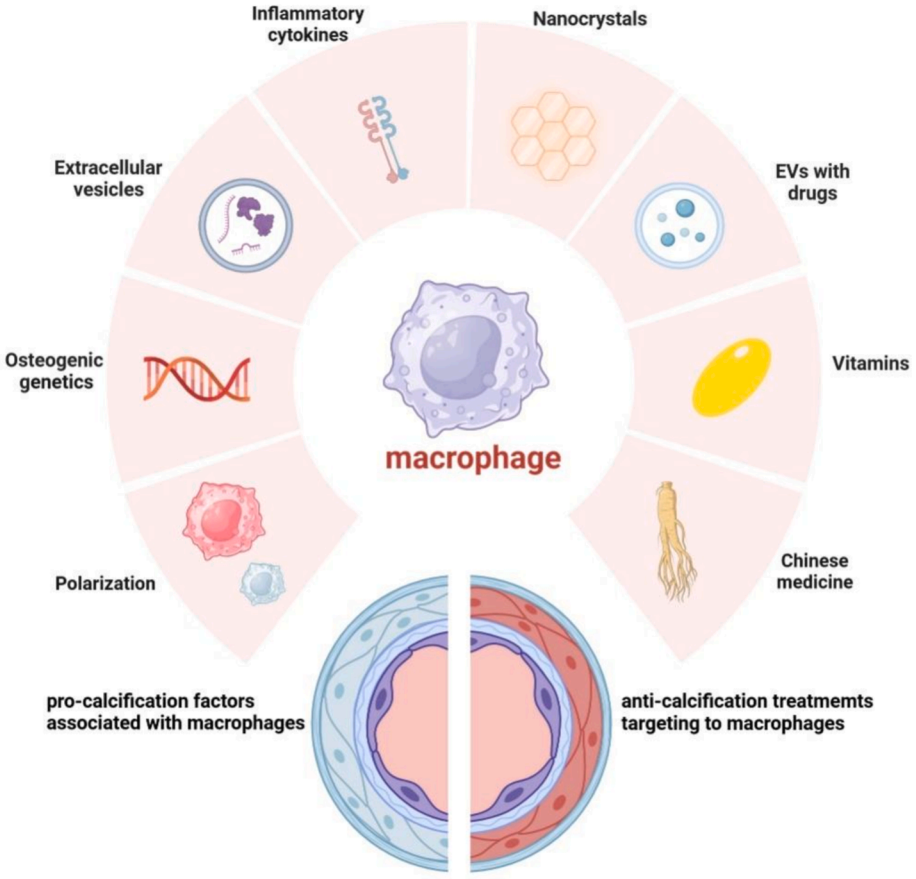

Fig. 5. Pro-calcification factors and anti-calcification treatments. In a pro-inflammatory microenvironment, macrophages promote the occurrence of vascular calcification by releasing pro-inflammatory factors, osteogenic genes, and exosomes in various ways. Research on the mechanism of macrophages and vascular calcification provides clues for the prevention and treatment of vascular calcification. Targeted nanocrystals and exosomes carrying drugs can achieve the precise regulation of macrophages and achieve the goal of preventing and treating vascular calcification. At the same time, dietary supplements and traditional Chinese medicine research also provide a theoretical basis for the prevention of vascular calcification. Created in BioRender. Dong, R. (2024) https://BioRender.com/l27a910.

research revealed that GLSP and its triterpenes promoted ABCA1/G1-mediated cholesterol efflux and suppressed macrophage inflammation, inactivating VSMC-mediated osteogenesis and reducing atherosclerosis and aortic calcification. Ginseng saponins also have anti-inflammatory and anti-atherosclerotic effects. Zhang et al.[212] used ginseng saponin Rb1 in C57BL/6 and ApoE-/- mouse models of atherosclerosis and found that it increased macrophage polarization to the M2 phenotype. Further studies showed that Rb1 increased IL-4 and IL-13 secretion and continuously activated STAT6 phosphorylation, promoting M2 polarization in macrophages Figs. 5 and 6.

5. Summary and Prospect

Recent research on VC and inflammation, particularly the relationship between VC and macrophages, has provided a solid scientific basis for preventing and treating related diseases. The discovery of RTMs has deepened our understanding of maintaining homeostasis. Understanding VC development from a homeostatic balance perspective highlights the importance of controlling inflammation and provides a basis for research on tissue repair and regeneration after injury. Single-cell technologies, such as scRNA-seq and high-resolution sequencing, have advanced the study of cellular heterogeneity, while spatial transcriptomics has revealed cellular spatial distribution, complementing the limitations of scRNA-seq. However, research on spatial transcriptomics in PVMs is still limited, and further exploration is needed. Investigating special PVM subgroups in the homeostatic process may enable reverse vascular aging and the regeneration of damaged tissues. An in-depth exploration of traditional Chinese medicine provides convincing evidence for regulating macrophages to prevent VC, while the emerging interdisciplinary integration of material science and medicine addresses the limitations of traditional drug formulations by improving drug bioavailability and reducing adverse reactions, offering new benefits to patients.

Research on the macrophage regulation of VC identifies many potential targets for modulation. However, the lack of clinical treatment options for VC indicates insufficient clinical translation. Increased investments in translational medicine research will help bridge this gap. Given the slow progression of VC and its lengthy pathological process, multiple cross-sectional studies in different age groups may offer a more comprehensive view of macrophage regulation. The high plasticity and heterogeneity of macrophages complicate the disease process, necessitating further in-depth studies. Finally, while new drug formulations or delivery systems show great potential in experiments, issues such as EV stability and the biological and intracellular fate of drug nanocrystals indicate that safety and practicality require further validation.

CRediT authorship contribution statement

Rong Dong: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Zhenjun Ji:** Methodology, Investigation. **Mi Wang:** Methodology, Investigation. **Genshan Ma:** Supervision, Project administration, Methodology,

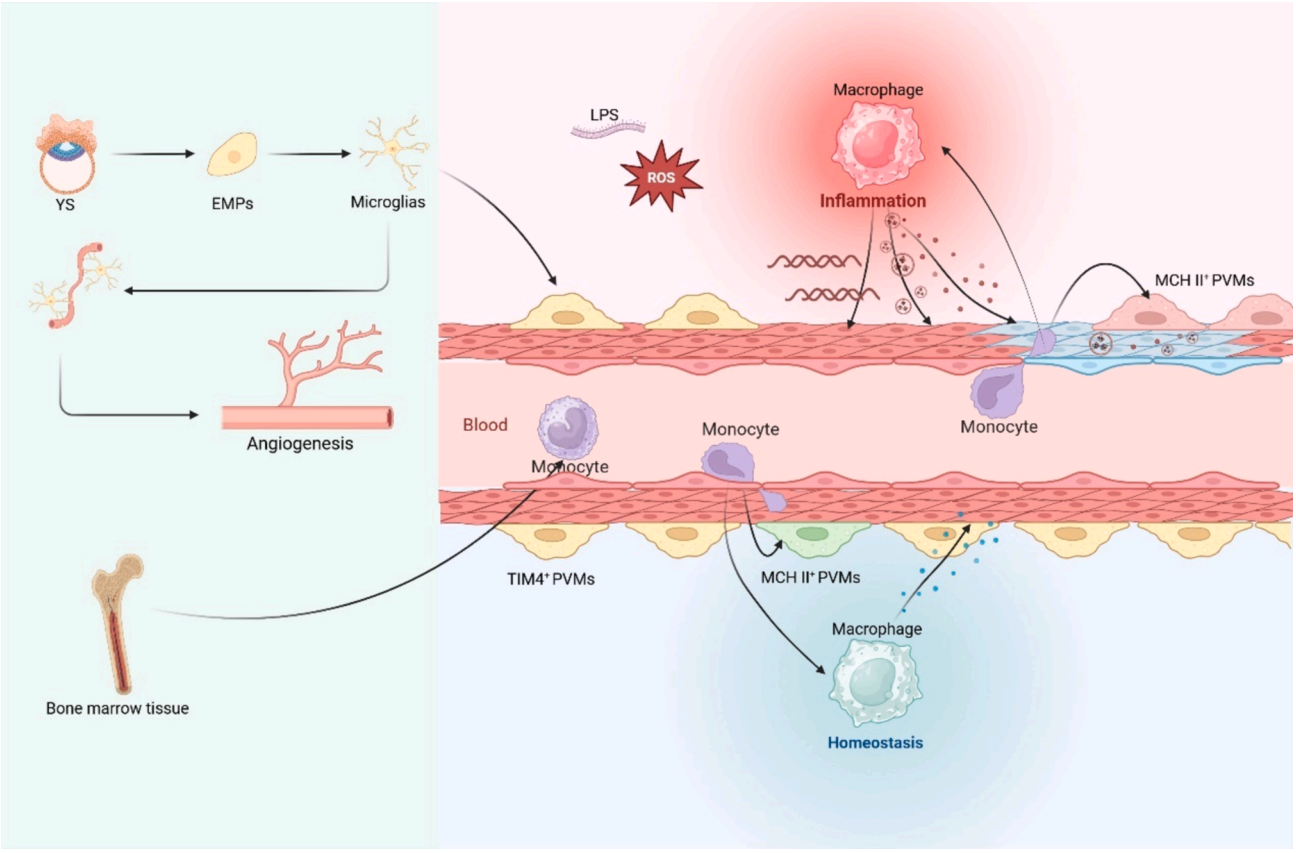

Fig. 6. Overview of the role and function of macrophages in vascular physiology and calcification. Before birth, macrophages originate from the yolk sac or fetal liver. In particular, the erythroid progenitor cells derived from the yolk sac differentiate into microglia, which assist in angiogenesis and vascular anastomosis in a "cell partner" manner and drive into perivascular macrophages (PVMs), performing a homeostatic maintenance function. Under a homeostatic condition, macrophages and perivascular macrophages secrete growth factors to maintain vascular health. During the aging process, the selective apoptosis of a small number of PVMs may be supplemented by peripheral monocytes. In a pro-inflammatory state, peripheral monocytes derived from bone marrow hematopoietic tissue massively infiltrate and differentiate into macrophages. The secretion of inflammatory mediators can lead to vascular calcification. Meanwhile, inflammation causes the cell death of perivascular macrophages, and the macrophages that differentiate in the inflammatory environment replenish the empty epitopes, providing a basis for the gradual imbalance of vascular homeostasis and the development of vascular calcification. Created in BioRender.com.

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Declaration of Competing Interest

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Data availability

No data was used for the research described in the article.

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