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Review

Angiogenesis within atherosclerotic plaques: Mechanical regulation, molecular mechanism and clinical diagnosis

Hanxiao Chen [a,1], Chengxiu Peng [a,1], Fei Fang [a], Yuhao Li [a], Xiaran Liu [a], Ying Hu [d], Guixue Wang [b,c], Xiaoheng Liu [a,b,*], Yang Shen [a,b,**]

[a] Institute of Biomedical Engineering, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu 610041, China
[b] Jinfeng Laboratory, Chongqing 401329, China
[c] Key Laboratory of Biorheological Science and Technology of Ministry of Education, State and Local Joint Engineering Laboratory for Vascular Implants, College of Bioengineering, Chongqing University, Chongqing 400030, China
[d] Department of Obstetrics and Gynecology, Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, 610041, China

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ABSTRACT

Atherosclerosis (AS) is a disease characterized by focal cholesterol accumulation and insoluble inflammation in arterial intima, leading to the formation of an atherosclerotic plaque consisting of lipids, cells, and fibrous matrix. The presence of plaque can restrict or obstruct blood flow, resulting in arterial stenosis and local mechanical microenvironment changes including flow shear stress, vascular matrix stiffness, and plaque structural stress. Neovascularization within the atherosclerotic plaque plays a crucial role in both plaque growth and destabilization, potentially leading to plaque rupture and fatal embolism. However, the exact interactions between neovessels and plaque remain unclear. In this review, we provide a comprehensive analysis of the origin of intraplaque neovessels, the contributing factors, underlying molecular mechanisms, and associated signaling pathways. We specifically emphasize the role of mechanical factors contributing to angiogenesis in atherosclerotic plaques. Additionally, we summarize the imaging techniques and therapeutic strategies for intraplaque neovessels to enhance our understanding of this field.

Abbreviations: AS, Atherosclerosis; ECM, Extracellular matrix; VSMC, Vascular smooth muscle cells; VECs, Vascular endothelial cells; MMPs, Matrix metalloproteinases; ICG, Indocyanine green; CEUS, Contrast-enhanced ultrasonography; VCAM-1, Vascular cell adhesion molecule-1; IPH, Intraplaque-hemorrhage; MVD, Microvessel density; EPCs, Endothelial progenitor cells; VEGFR, Vascular endothelial growth factor receptor; BMP, Bone morphogenetic protein; TGF-β, Transforming growth factor-β; Ang-1, Angiopoietin 1; MMP-2, Matrix metalloproteinase 2; WSS, Wall shear stress; CFD, Computational fluid dynamics; IVUS, Intravascular ultrasound; OCT, Optical coherence tomography; FSS, Fluid shear stress; VBM, Vascular basement membrane; LSS, Low shear stress; HSS, High shear stress; NO, Nitric oxide; HSPGs, Heparan sulfate proteoglycans; uPA, Urokinase-type plasminogen activators; FAK, Focal adhesion kinase; PSS, Plaque structural stress; PSS-HI, Pss heterogeneity index; FEA, Finite element analysis; VH-IVUS, Virtual histology intravascular ultrasonography; NCLs, Non-culprit lesions; KLF2, Krüppel-like factor 2; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; Piezo1, Piezo-type mechanosensitive ion channel component 1; PLCγ, Phospholipases cγ; MAPK, Mitogen-activated protein kinase; PI3K, Phosphatidylinositol 3′-kinase; eNOS, Endothelial nitric oxide synthase; NADPH, Nicotinamide adenine dinucleotide phosphate; Foxo1, Forkhead box o1; GSK3β, Glycogen synthase kinase 3β; ALK, Activin receptor-like kinase; HO-1, Heme oxygenase-1; ROS, Reactive oxygen species; MRI, Magnetic resonance imaging; NIRS, Near-infrared spectroscopy; SMI, Superb microvascular imaging; SWE, Shear wave elastography; DCE-MRI, Dynamic contrast enhanced mri; MPI, Magnetic particle imaging; NIRAF, Near-infrared autofluorescence; FLECT, Fluorescence emission computed tomography; TMP-PF, Etramethylpyrazine and paeoniflorin combination; 3PO, 3-3-pyridinyl-1-4-pyridinyl-2-propen-1-one; SMYA, Simiao yongan.

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* Corresponding author. Institute of Biomedical Engineering, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu 610041, China.

** Corresponding author. Institute of Biomedical Engineering, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu 610041, China.

E-mail addresses: liuxiaohg@scu.edu.cn (X. Liu), shenyang@scu.edu.cn (Y. Shen).

[1] These authors contributed equally to this work.

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1. Introduction

The chronic, progressive process known as atherosclerosis (AS) is typified by the accumulation of lipids, endothelial dysfunction, infiltration of monocytes and macrophages, formation of extracellular matrix (ECM), and migration and proliferation of vascular smooth muscle cells (VSMC).[1] The disease involves the formation of plaque within the artery wall, which may lead to the narrowing of blood vessels or acute tissue ischemia. In recent years, there has been a shift in focus from solely addressing vascular stenosis to predicting plaque instability and preventing the risk of plaque rupture. Neovascularization is an emerging indicator of atherosclerotic plaque instability and adverse cardiovascular outcomes.[2]

Neovascularization is the natural development of a network of new blood vessels in tissue that are not normally contained. Angiogenesis specifically refers to the formation of new branches based on existing vessels through the proliferation and migration of vascular endothelial cells (VECs), leading to the expansion and extension of a new capillary network.[3] In atherosclerotic regions, specific local conditions such as hypoxia, inflammation, and oxidative stress[4] induce proangiogenic factors that promote the sprouting of new blood vessels from pre-existing vasa vasorum in the arterial adventitia or lumen, thus counteracting oxygen supply deficits.[5] This restoration process is known as physiological angiogenesis, which is essential for restoring normal oxygen levels and reducing inflammation, contributing to the regression of AS.[6,7] Angiogenesis is believed to be beneficial in alleviating plaque, but excessive angiogenesis with poor vessel stability and leak-prone vessel walls may ultimately increase the risk of plaque rupture.[8] The vasa vasorum angiogenesis can begin at the initiation stage and continue throughout plaque growth, while the mutual effects between plaque and neovessels are not clearly defined.[9] In advanced plaques, pathological neovascularization is susceptible to intra-plaque hemorrhage due to its structural fragility. The leakage of a mixture, consisting of erythrocytes, fibrin, and platelets within the plaque, contributes to an increase in the plaque load, leading to sudden plaque enlargement or complete vessel occlusion. This aggravates local hypoxia and inflammation within the arterial wall. The outcome may be matrix degradation, fibrous cap thinning, and plaque rupture, which can lead to serious cardiovascular events.[10]

Hypoxia and inflammation are widely recognized as the most significant factors in the progression of AS plaque. Recently, there's growing evidence that the impact of mechanical factors on AS plaque progression has received increasing attention. The mechanical microenvironment of vascular VECs includes blood flow shear stress, and matrix stiffness, which can activate mechanosensitive receptors on the cell surface and then regulate angiogenesis-related signals. New blood vessels are typically distributed at the shoulder and base of the plaque, with cellular matrix metalloproteinases (MMPs)secretion leading to degradation of the plaque's fibrous cap and subsequent alterations in mechanical properties.[5]

Therefore, we conducted a review on neovascularization in atherosclerotic plaques, describing the origins, impact factors, molecular mechanisms, and highlighting potential clinical applications such as imaging methods and therapy strategies. Noninvasive imaging methods have made significant advancements in precision, allowing for the visualization of intraplaque neovascularization and providing greater convenience for patients. Additionally, as research on neovascularization deepens, molecular imaging methods have emerged due to the discovery of multiple targets for neovascularization. Our review focuses on current and emerging therapies targeting intraplaque angiogenesis, which hold promise for the treatment of AS and the prevention of its complications. There are two main types of treatment strategies available today: inhibiting neovascularization and promoting neovascular maturation. We discuss the potential of anti-angiogenic drugs and alkaloids, as well as the challenges and limitations associated with their clinical application. Rapid progress in this area may significantly drive the development of drugs targeting intraplaque neovascularization.

2. Neovessel characteristics in atherosclerotic plaque

2.1. Two origins of plaque neovessels

The intimal vessels can originate from the vasa vasorum in the adventitia and vascular lumen as well, as first observed by Kumamoto M. et al.[11] They also discovered that neovessels originating from vasa vasorum are 28 times more likely to occur than those originating from the luminal source. While new vessels coming from the lumen were more strongly connected with hemosiderin deposits and intimal bleeding, the density of new vessels from the vasa vasorum was considerably positively correlated with the degree of chronic inflammatory cell infiltration, granulation tissue, and atherosclerotic alterations.[11] (Fig. 1A).

Compared to vasa vasorum, there are significantly fewer studies on luminal neovessels. In a study using Indocyanine Green (ICG) video angiography, researchers conducted a dynamic assessment of neovascularization and presented evidence that luminal neovessels are more closely linked to inflammation and intraplaque bleeding.[12] Recent findings revealed that symptomatic plaques had a greater contrast effect from the luminal side than from the adventitial side using contrast-enhanced ultrasonography (CEUS) and histology. Furthermore, a stronger correlation exists between symptomatic plaque and intraplaque neovascularization originating from the luminal side instead of the adventitia.[13] The actual images of intraplaque angiogenesis, obtained by hematoxylin-eosin (HE) staining, ultrasound, and fluorescence staining, are depicted in Fig. 1B.

The vasa vasorum stretching into the intima tends to be immature, possibly due to decreased cellular connectivity and increased permeability, leading to easier leakage of intravascular materials into the plaques[14,15] and the presence of leaked lipoproteins in the plaques.[9] The earliest oxLDL deposition in AS is found deep in the tunica intima, not in the inner intima, contradicting the hypothesis that oxLDL particles enter the outer intima straight through the wounded artery endothelium. Thus, the “outside-in theory” postulated that oxLDL and monocytes might reach the outer intima directly via the vasa vasorum vasculature.[16] Additionally, a fully coupled mechano-chemo-biological model has been introduced for the first time to support the “outside-in theory” of AS.[17] Despite the very limited perfusion of plaque neovessels, the role of recruiting macrophages and leukocytes has been demonstrated, and early studies have found that expression of monocyte adhesion factor (VCAM-1) is greatly increased in neovascular endothelial cells[18] which does favor of monocytes adhering and penetrating and exacerbates the process of AS. The new blood vessels are most commonly distributed in the shoulder of the plaque, and the collagen and fibrin matrix is heavily degraded due to high expression of MMPs,[19] causing a series of mechanical properties changes along its route, dovetailing with the plaque rupture region of high incidence.[20]

2.2. Intraplaque-hemorrhage (IPH)

Sprouting and expanding Immature plaque vasa vasorum are extremely delicate, porous, and susceptible to bleeding (Fig. 2A). Neovessel injury and the worsening of IPH may be caused by MMPs and inflammatory cytokines that are generated by mast cells and activated macrophages.[24] An established mathematical modeling system demonstrated IPH area can be reduced by decreasing the microvessel density (MVD) and the permeability of the neovasculature.[25] The close correlation between IPH and acute plaque rupture has been widely acknowledged, although the underlying mechanisms have not been fully elucidated. Plaque hemorrhages in the coronary arteries are thought to be more common in patients who die from rupture than in those who have stable lesions. Erythrocyte membrane accumulation within an atherosclerotic plaque may be a strong atherogenic stimulus in addition to interfering with the advancement of IPH—the free cholesterol deposition, macrophage infiltration, and necrotic core growth.[26]

Intraplaque bleeding has a significant impact on rupture, but it is not always the sole cause. It has been found that the number of neovessels in plaques returned to normal physiological values once significant calcification occurred, and glycoprotein A and iron cores could be detected in post-calcified plaques, suggesting that IPH had occurred in the past. These findings suggest that intraplaque hemorrhage does not always lead to IPH or cause plaque rupture or instability.[27,28] Additionally, a recent study found that there was no clear affiliation between plaque microvasculature and IPH several weeks after a cerebrovascular incident, implying that other variables may contribute to its development.[29]

3. Process of atherosclerotic angiogenesis

Pathological angiogenesis takes place in many diseases, such as tumors and diabetic retinopathy.[30] The mechanisms of neovascularization have been described in detail focusing on several different models of angiogenesis and branching, sprouting, vasculogenesis and intussusception.[31] Vasculogenesis mostly arises from circulating endothelial progenitor cells (EPCs), in contrast, sprouting is a more prevalent mechanism for forming new blood vessels from existing ones in AS.[32]

3.1. The initial stages of angiogenesis

Angiogenesis begins with vasodilation and enhanced local vascular permeability, which allows plasma proteins to extravasate and deposit in a temporary matrix layer, as well as the remodeling of pre-existing interstitial matrix by proteases.[31] These processes facilitate cell migration, followed by basement membrane degradation, pericellular detachment, and loosening of intercellular junctions to support the emergence of selected tip cells (as shown in Fig. 2A).

3.2. Tip cell competition and spouting

Tip cells migrate and give rise to the top buds, which are predominantly enriched in vascular endothelial growth factor receptor (VEGFR), and the gradient of VEGF concentration determines the migration direction and location.[25] Compared to cells maintained in a quiescent state, called phalanx cells, tip cells tend to migrate while stalk cells tend to propagate.[33] VEGFA signaling via binding to VEGFR2 is essential for physiological vascular development.[34] VEGF/VEGFR2 enhances the expression of DLL4 (NOTCH ligand) in endothelial cells as it enables a

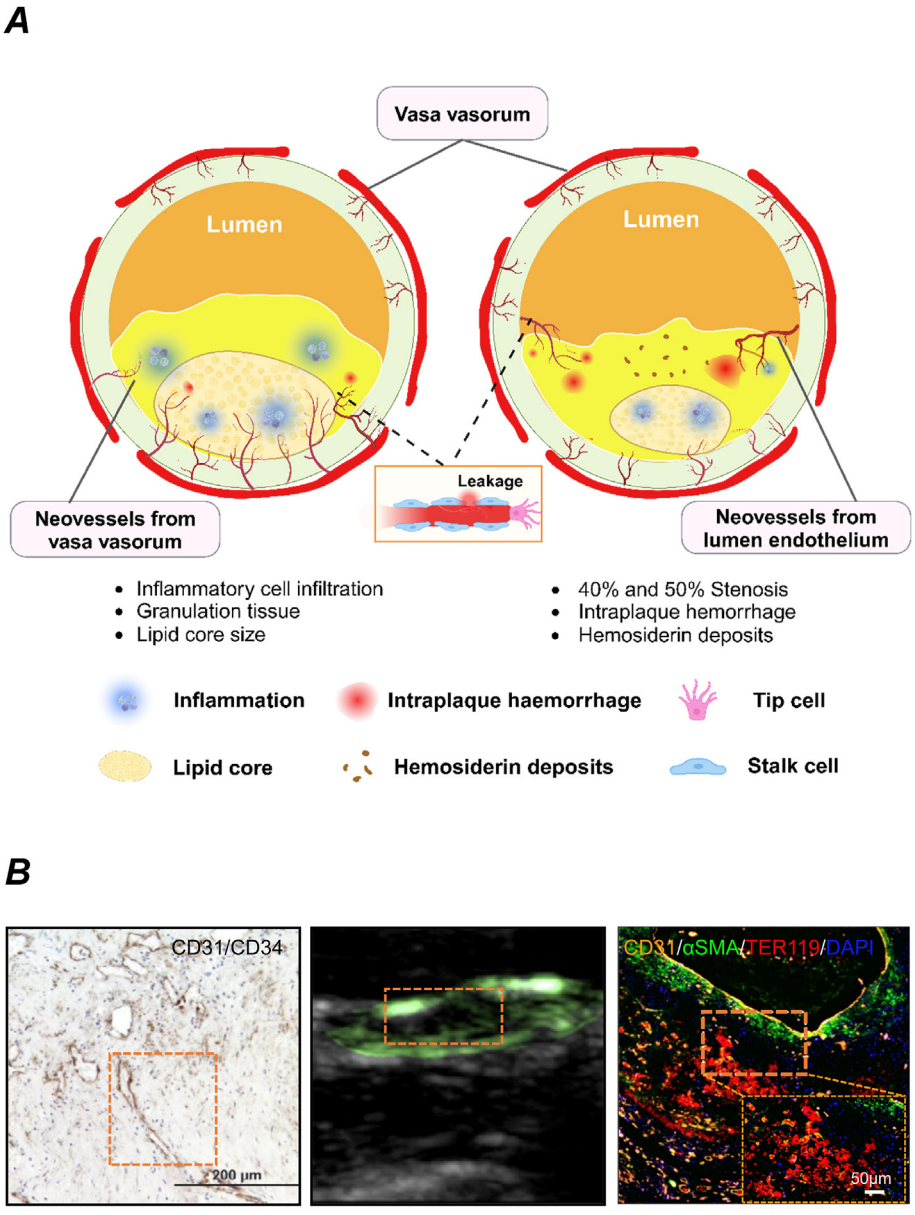

Fig. 1. Neovessels in atherosclerosic plaque. (A) Neovascularization that protrudes into the plaque may originate from the vasa vasorum in the adventitia or the luminal endothelium of the artery. Vasa vasorum-derived neovessels showed a significantly positively correlated with chronic inflammatory cell infiltration, and granulation tissue, while luminal neovessels are more closely associated with inflammation and intraplaque hemorrhage. The presence of luminal vessels is most frequent when the severity of stenosis reaches 40 %–50 %.[11] Created with Biorender.com. (B) HE staining, ultrasound imaging and immunofluorescence staining of intraplaque angiogenesis.[21–23] The dotted areas indicate the presence of neovascularization within the plaque. The figures are reprinted with permission.

more efficient response to the NOTCH receptor neighboring stalk cells,[35] with cells exhibiting faster or higher DLL4 expression having greater potential to differentiate into tip cells (Fig. 2). Activation of Cdc42 gene by VEGF triggers the formation of filopodia of the tip cell, which migrates and elongates in response to VEGF concentration. The binding of highly expressed NOTCH on stalk cells with ligand DLL4 leads to the down-regulation of VEGFR-2, thus reducing the responsiveness of stalk cells to VEGF in order to maintain their phenotype and prevent excessive sprouting.[36] When two tip cells converge, they merge to form a vascular lumen (Fig. 2B). The regulation of new sprouting during vascular expansion depends on the coordination of NOTCH, VEGF, and bone morphogenetic protein (BMP) signaling.[37]

3.3. Lumen formation

The vasculature is further stabilized by forming basement membrane and recruiting mural cells (VSMCs and pericytes), which are regulated by the signaling pathways of transforming growth factor-β (TGF-β), angiopoietin 1 (Ang-1)/Tie-2, and platelet-derived growth factor (PDGF)/PDGF receptor-β (Fig. 2BV).[5] The secretion of PDGF-BB by microvascular endothelial cells that sprout and migrate is thought to drive nearby pericytes into synchronized expansion and chemotaxis.[38,39] Secreted by pericytes, tissue inhibitor of metalloproteinase-3 inhibits the activities of MMP-1 and MMP-10, thereby maintaining both newly formed and established vascular systems.[40] Ang-1 is produced by pericytes and VSMCs, activating endothelial Tie-2 to enhance interactions between VECs and pericytes. Meanwhile, VEGF promotes the production of Ang-2, which competes with Ang-1 for binding to Tie-2, resulting in detachment of pericytes from vessels. In stalk cells, low expression of Ang-2 and high expression of Tie-2 favor Ang-1/Tie-2 signaling, promoting vascular remodeling and stabilization.[41,42] According to Simone P. et al., plaques with high microvessel density have a local balance between Ang-1 and Ang-2 that favors Ang-2. Additionally, there is an association between plaque Ang-2 levels and matrix metalloproteinase 2 (MMP-2) activity, which explains the instability of intra-plaque neovessels.[43]

Early-stage VEGF production in AS is mostly produced by smooth muscle, which initiates the pathological response in the early stage of AS and even earlier. It is important to note that this response is not the cause of AS, but rather a response to early lipid deposition.[44] Furthermore, there is no significant correlation between the region expressing the most abundant growth factor and neointima neovascular.[45] These findings lend support to the concept that the presence of endothelial growth factor is required but not sufficient for angiogenesis in atherosclerotic lesions.[46]

4. Mechanical microenvironment contributing to angiogenesis

As previously mentioned, intraplaque neovessels can originate either vasa vasorum or the lumen. However, due to their growth in different microenvironments, they are influenced by distinct factors to varying extents. In this context, we primarily focus on mechanical factors and propose that vasa vasorum stretching from adventitia to the bottom of the plaque is likely regulated by basal stiffness and plaque structural stress. Conversely, endothelial cells germinated from the lumen are more significantly impacted by shear stress. Apart from mechanical regulators, we also review the recent advancements in biochemical factors such as hypoxia, inflammation, and metabolism (Fig. 3).

4.1. Shear stress

VECs directly experience a force (wall shear stress, WSS) resulting from the frictional drag of blood on their surface.[47] Atherogenesis preferentially occurs in the outer walls of vascular bifurcations and point locations where blood flow recirculates and stagnates. Shear stress at these sites has been measured or modeled to be lower than the average physiological shear of the arteries and is usually oscillating.[48] But with endothelial thickening and plaque formation, lumen stenosis is caused, and local shear stress changes.[47] The area near the plaque was divided into three parts: upstream, center, and downstream, and it was found that the WSS values and flow velocity were highest in the center and lowest in the downstream; the pressure values were lowest in the center and

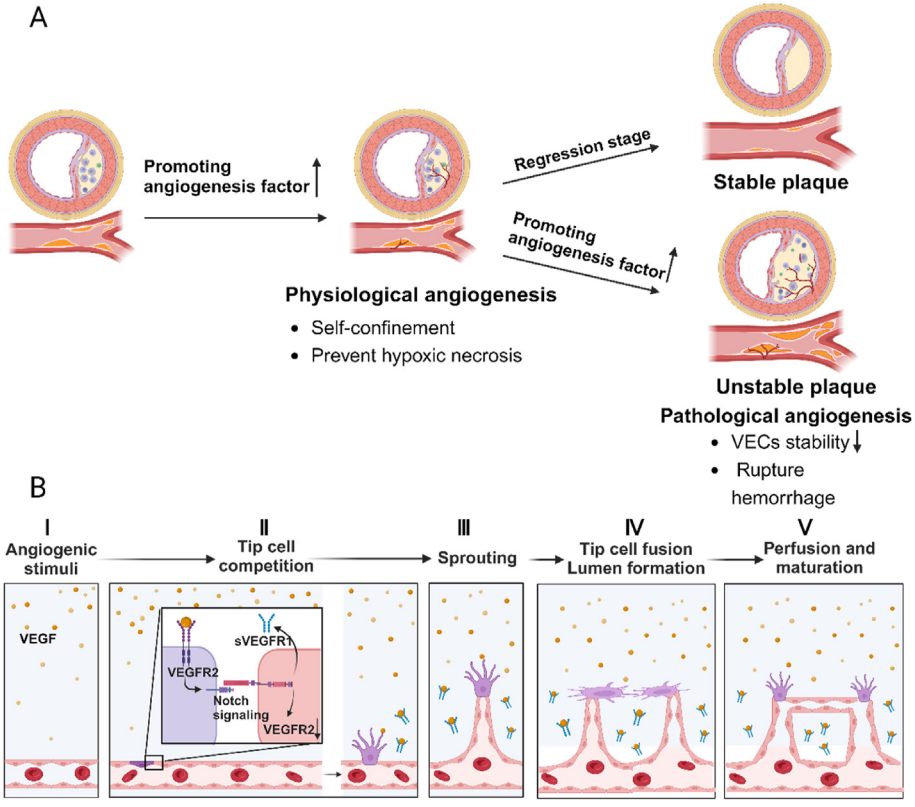

Fig. 2. Stepwise mechanism of angiogenesis within AS plaque. (A) Distinct outcomes of plaques with angiogenesis. When physiological angiogenesis progresses to unlimited, pathological angiogenesis's characteristics of easy leakage and rupture will transform plaques into vulnerable plaques. (B) The initial stages of angiogenesis involve degradation of the basement membrane, pericyte detachment and endothelial loose cell junctions. (I) under stimulation of VEGF, (II) tip cell that is mostly rich in VEGFR and highly expressed DLL4 is selected and (III) initiates sprouting in response to guidance signals. DLL4 binds to NOTCH on stalk cells and stalk cells proliferate to support the stretch of tip cells. (IV) when the two tip cells meet, the bud merges, forming a vascular cavity. (V) Pro-maturation signals regulate mural cell recruitment and vascular maturation. Created with Biorender.com.

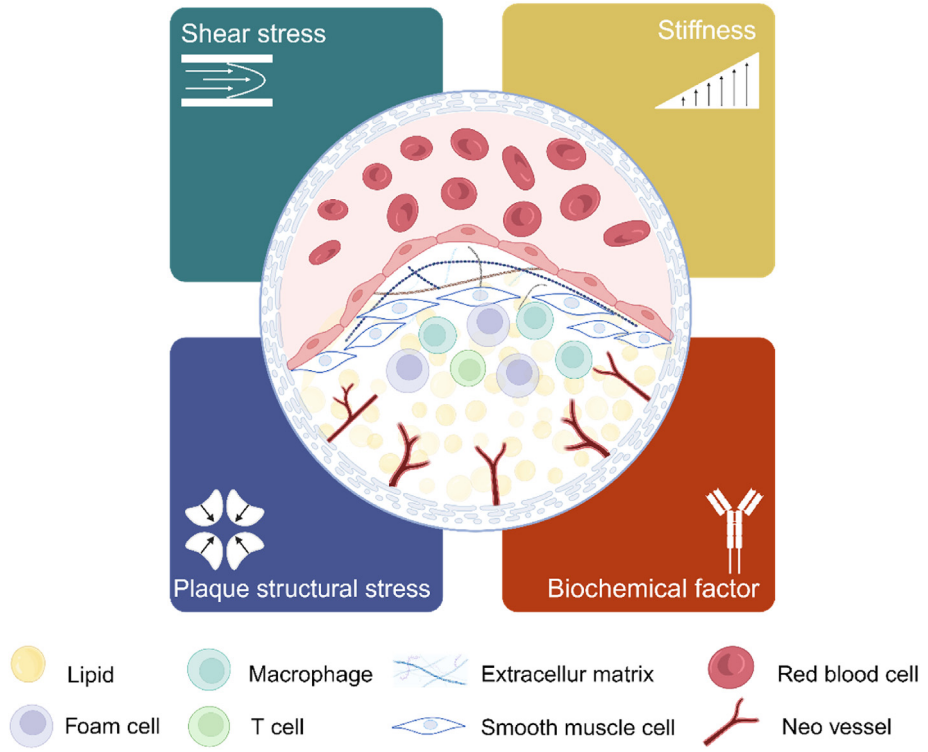

Fig. 3. Mechanical traits of plaque angiogenesis. Based on the advancements in the understanding of biomechanics in plaque angiogenesis, we propose that the mechanical microenvironment can be categorized into three major groups: fluid shear stress, matrix stiffness, and plaque structural stress. The beginning and progression of angiogenesis within atherosclerotic plaques are significantly influenced by these factors. Created with Biorender.com.

highest in the upstream.[49] The maximum stress is mostly found in the necrotic core during the initial growth phase of the plaque and progressively shifts toward the left shoulder of the plaque as the plaque grows, increasing the instability of the plaque and the risk of plaque detachment, according to a fluid-solid bidirectional coupling model.[50]

However, opinions differ on WSS predicting plaque progression, with the prevailing view being that low shear stress promotes plaque formation and high shear stress promote plaque rupture.[51,52] Previous studies have largely assessed the impact of shear stress by employing *in vivo* models, *in vivo* imaging, and computational fluid dynamics (CFD). However, the criteria used to classify low, medium, and high shear stress vary among different vessel types. Additionally, the diversity of imaging techniques, such as intravascular ultrasound (IVUS), optical coherence tomography (OCT), and multimodal imaging, contributes to the ongoing uncertainty regarding how shear stress can predict plaque progression.[53] Exploring the stability of fluid shear stress (FSS) in plaque remains a significant challenge. Angiogenesis is an indispensable factor in plaque progression, and endothelial cells will inevitably react to mechanical stimuli. Therefore, our research concentrates on understanding how shear stress influences plaque angiogenesis and further affects plaque advancement from the following four aspects.

Basement membrane degradation The vascular basement membrane (VBM) is composed of collagen type IV, collagen type I, laminin, vitronectin, and several proteoglycans. MMPs perform as a pillar to degrade VBM.[54] Both low shear stress (LSS) and high shear stress (HSS) can up-regulate the expression of MMPs in VEC, potentially promoting angiogenesis through ECM degradation and contributing to the transformation of plaques into high-risk types by degrading collagen and elastic fibers.[55,56] Nevertheless, VECs subjected to HSS (10 and 30 dyn/cm^2) had higher type IV collagen mRNA levels than VECs exposed to a static environment or LSS (3 dyn/cm^2).[57] Reduced collagen concentration and significant fibrous cap thinning are encouraged in areas with persistent LSS by the combination of decreased collagen production and increased MMP-mediated collagen degradation.[58] Recently, laminins have appeared as new contributors in mouse and human myoendothelial junctions and shear stress communication, which can be a new target for angiogenesis mechanotransduction research.[59]

Sprouting Peter et al. proposed that endothelial cells within the initial capillary sprout do not experience WSS, while endothelial stalk cells are located in LSS regions, with peak shear stress (50 dyn/cm^2 μm^{-1}) occurring at the entrance of the sprout.[60] This phenomenon is applicable to venous vasculature similar to capillaries, but luminal neovessels exposed to both arterial and vessel blood flow are more susceptible to shear stress. *In vitro* studies have identified a shear threshold of ~10 dyn/cm^2 as a stimulus to produce sprouting, which is lower than the physiological shear experienced in nonsprouting vessels *in vivo*.[61] Besides, it was observed that with the increase in shear stress levels, the endothelial cell budding area, the number of junction points, the average bud length, and the longest bud length all decreased significantly.[62] However, debates arise regarding whether WSS levels between 3 and 15 dyn/cm^2 can attenuate or enhance sprouting, possibly due to the difficulty in determining an absolute value for high or low shear stress in vitro.[63] *In vivo* studies have also correlated sprouting with both low and high levels of WSS.[64,65]

Proliferation and Migration In the initial stage of AS, LSS stimulates angiogenesis by inducing VEC migration and proliferation, eventually resulting in atherosclerotic stenosis and thickening of the intima.[66] In contrast, HSS may increase cell migration while suppressing apoptosis and VEC proliferation[67] in a co-culture system with VSMCs.[68] Correspondingly, nitric oxide(NO) and VEGF up-regulated by HSS mediate VEC angiogenesis,[69] resulting in a higher density of neovessels at the upstream compared to the low-shear area.[56] Laminar shear stress has been shown to enhance the migration of microvascular endothelial cells and OSS (0.5 ± 4 dyn/cm^2) inhibits angiogenesis by downregulating migration and invasion enhancer 1 in our recent study.[70] OSS promotes EC proliferation, while laminar shear stress acts the opposite.[71]

In conclusion, the effects of mechanics on neovascularization are extensively debated and are linked to the intricate mechanical

environment surrounding plaques and the lack of model support. Heparan sulfate proteoglycans (HSPGs), the predominant proteoglycans on the surface of VECs,[72] have recently been reported to serve as mechanical sensors of shear stress, influencing the physiology of predominant VECs, including NO production, cell migration, proliferation, tissue, permeability, etc.[63,73] Furthermore, it has been discovered that high shear stress tends to hinder the initiation of neovascularization and stabilize the vasculature, which is mediated by HSPGs.[62]

4.2. Stiffness

The disturbed flow in the bifurcated or curved part of the artery destroyed the neatly arranged structure of VECs, resulting in increased permeability, and thus induced the formation of plaques. With the development of AS, the aorta stiffness undergoes dynamic changes, initially presenting as lower than normal aorta due to early lipid deposition[74,75] and eventually reaching or exceeding 100 kPa due to the formation of vascular calcification.[76] Clinical data indicates that the hardness of atherosclerotic plaques varies from lipidized (1 kPa) to fibrous plaques (35.5–54 kPa) to calcified (80–300 kPa).[77] Previous studies on how the mechanical microenvironment regulates angiogenesis in AS plaques have mostly focused on the shear stress created by blood flow. However, new research has shown that ECM matrix rigidity has a direct influence on angiogenesis. In addition to signaling, ECM influences VEC behavior during angiogenesis. Furthermore, once blood vessels begin to form, specialized VECs known as tip cells initiate the germinating process.

Plenty of studies have shown that matrix stiffness can affect cell proliferation, diffusion, migration, and differentiation, as well as regulate matrix gene expression and protein interactions.[75] Modulating ECM stiffness at different stages of angiogenesis requires particular chemical and mechanical cues.[78] Within the cell, mechanotransduction allows mechanical signals to be translated into biochemical signals. A variety of ECM proteins or binding sites influence VEC adhesion, diffusion, migration, and proliferation in different ways.[79] High matrix stiffness can both loosen intercellular connections and stimulate the proliferation, migration, and infiltration of VECs to promote VEC germination.[80] Moderately increased ECM stiffness can enhance cell migration, as cells can respond to a harder matrix by increasing contractile force and traction. However, excessive ECM rigidity may create a mechanical barrier that inhibits cell migration. ECM proteins[81] and crosslinking enzymes[82] are made by VECs and have a direct impact on the stiffness and structure. The MMPs' activity rises in tandem with the stiffness.[83] Angiogenesis is frequently influenced by protease systems, especially urokinase-type plasminogen activators (uPA) and MMPs by degrading the basement membrane and infiltrating surrounding tissue.[84] A crucial function of uPA and its receptor uPAR in the fibrinolytic system is to activate pro-MMPs into active MMPs, which in turn controls the composition of extracellular matrix (ECM). Because they are expressed in the filamentous foot of these migratory cells, uPAR and MMPs have a direct relationship to tip cells. Selecting between tip cells and stalk cells is a crucial phase in angiogenesis, which comes after the start of cell migration.

By acting on VECs through biochemical, chemotactic, and mechanical signals, ECM can influence the differentiation of tip/stalk cells and the development of blood vessels. The ECM releases chemotactic signals, and variations in matrix density and elasticity generate mechanical forces.[85] An increase in matrix stiffness enhances active Rac1 levels by promoting focal adhesion of Focal Adhesion Kinase (FAK) and p-PXN, leading to increased cytoskeletal tissue and cell stiffness. This activation triggers YAP, facilitating its nuclear entry to up-regulate the expression of target genes and ultimately promote the formation of tip cells.[86] The selection of tip cells and stalk cells depends on lateral inhibition of the Notch1-Dll4 signaling pathway. Dll4 expression is triggered by biochemical signals in response to alterations in the extracellular matrix, governing stalk cell behavior.[87] Additionally, ECM stiffness also activates the classical mechanically-sensitive transcription coactivators YAP and TAZ in endothelial cells.[88] YAP/TAZ regulates stalk cell proliferation and governs endothelial sprouting and bud structure through mechanical stimuli such as matrix stiffness and cell stretching.[89] Increased matrix stiffness enhances cell–cell contact,[90] which contributes to stabilizing neovessels in the final stage of angiogenesis. The interaction between endothelial cells and recruited pericytes is vital for supporting and maintaining newly formed blood vessels.[91] By inhibiting certain MMPs, the degradation of matrix structures can be effectively prevented, thereby hindering the infiltration of VECs into the ECM.

Angiogenesis relies largely on biochemical and mechanical cues. Biochemical stimuli influence the blood vessel microenvironment, conversely convert mechanical signals and impact cell behaviors.[78] Understanding the effects of ECM stiffness on angiogenesis within plaques could lead to potential treatments for AS by preventing or reversing stromal sclerosis.

4.3. Plaque structural stress

Recent work suggests that plaque structural stress (PSS) is crucial to the evolution and crack of AS plaques.[20] PSS refers to stress within AS plaques due to the dilation and stretching of blood vessels under arterial pressure, and it is affected by several factors such as plaque size, composition, and lumen geometry.[92] Acute coronary syndrome, severe adverse cardiovascular events, and plaque rupture are substantially correlated with high PSS, according to the majority of current studies that primarily focus on this relationship.[20,93] The increase in PSS heterogeneity index (PSS–HI) also contributes to the progression and vulnerability of plaque.[94] Li et al. performed stress analysis on carotid plaques using finite element analysis (FEA) and high-resolution MR imaging and found that symptomatic plaques had greater mean maximal stresses than asymptomatic ones.[95]

Furthermore, Tang et al. found that plaque inflammation was linked to increased stress in the shoulder area and the thin fibrous cap.[96] Similarly, elevated PSS may be related to intra-plaque neovascularization, which can induce neovascular rupture and IPH.[97] When neovascularization or IPH was present, the maximum main stress increased, suggesting that neovascularization within the plaque may diminish stability by increasing internal tension.[98] The interaction between plaque structural stress and angiogenesis is intricate. On the one hand, the stress within the plaque may regulate the process of angiogenesis by affecting inflammatory response and cell activity. On the other hand, neovessels may alter the mechanical environment of the plaque and affect its distribution of structural stress. In conclusion, further urgent study is required to learn about the effect of PSS on angiogenesis within plaques, which may emerge as a key research frontier.

It is proposed that biomechanical stress is intimately related to inflammation, both of which serve as indications of plaque vulnerability in atherosclerosis. Thus, biomechanical engineering can be applied to anticipate plaque physiology *in vivo*.[96] Given the low predictive value of anatomy-based plaque risk assessment alone, biomechanical modeling may be used in conjunction with plaque imaging to stratify coronary non-culprit lesions.[99] The combination of FSS and PSS, along with virtual histology intravascular ultrasonography (VH-IVUS) and biomechanical analysis, may further improve plaque risk stratification.[94,100] In a prospective natural history investigation, a new study using comprehensive combinations of mechanistically synergistic FSS and PSS, as well as baseline anatomical characteristics, discovered relationships with future significant adverse cardiovascular events from non-culprit lesions (NCLs).[101]

4.4. Hypoxia, inflammation and metabolism

The pathophysiological factors in the plaque, hypoxia, inflammation, and metabolism are also regulated by mechanical factors. The classical hypoxic factor HIF-1 can be upregulated by LSS and OSS,[102] which in turn promotes angiogenesis. HIF-1α enhances inflammation and EC

proliferation by activating glycolysis, facilitating the occurrence of lesions at the site of atherosclerotic translocation.[103] Previous research has identified Krüppel-like factor 2 (KLF2) as a gene that is substantially activated by laminar flow but repressed by disturbed flow. In contrast to HIF-1α, KLF2 has been found to suppress inflammatory signals.[104] 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3 (PFKFB3), an important rate-limiting enzyme of glycolysis, is regulated by HIF-1 and KLF. During vascular sprouting, tip cells have a higher rate of glycolysis, so PFKFB3 plays an important role in sprouting angiogenesis by regulating endothelial tip-cell competition like VEGF.[105] Laminar shear stress activates KLF2, which inhibits PFKFB3-mediated glycolysis in ECs, reducing angiogenesis and vessel sprouting.[106] Most of studies on the HIF-1-PFKFB3 pathway have been conducted in diabetes models,[107] and it can be speculated that LSS and OSS activate HIF-1 to encourage PFKFB3-mediated glycolysis.

Piezo-Type Mechanosensitive Ion Channel Component 1 (Piezo1) is also a mechanoreceptor in VECs, and the deletion of Piezo1 in mice impairs shear stress-mediated angiogenesis, germination angiogenesis, and vascular lumen formation. In addition, EC Piezo1 can sense blood flow disturbance and is associated with inflammatory signaling. Matrix stiffness activated Piezo1, reducing HIF-1αubiquitination and subsequently enhancing the expression of downstream pro-angiogenic factors to provoke hepatocellular carcinoma angiogenesis.[108]

5. Mechanobiologic mechanisms of angiogenesis

The endothelial cells comprising the body's vascular system exhibit a diverse array of mechanotransductive behaviors and responses to biomechanical stimuli, working together to regulate overall blood vessel structure and function. Here, we introduce various molecular signals related to angiogenesis in plaque, all of which are capable of responding to mechanical or biological regulatory factors (Fig. 4). Most of these signals have been well established, while some of them are newly discovered molecules. Understanding these signal events will enhance our comprehension of the underlying mechanobiology mechanism that governs the mechanical regulation of angiogenesis.

5.1. VEGF/VEGFR

VEGF is the principal angiogenic growth factor that modulates angiogenesis through VEGFRs. It has been discovered that physical factors such as shear stress, stiffness, and plaque structural stress are significant effectors and drivers of angiogenesis that rely on VEGFR-2 phosphorylation.[109] The binding of VEGF to VEGFR-2 leads to the activation of several pathways, including PLCγ (phospholipases Cγ) and PKC-Raf kinase-MEK-MAPK (mitogen-activated protein kinase) pathway, signal-regulated kinases (Src) activated phosphatidylinositol 3′-kinase (PI3K)-Akt pathway and p38 MAPK pathway. Ras/MAPK signaling initiates DNA synthesis to promote VEC proliferation,[110–112] while PI3K/AKT signaling is provoked to increase VEC survival.[113] The p38 MAPK signaling is activated to further trigger actin reorganization and ultimately promote VEC migration.[114] Interestingly, endothelial nitric oxide synthase (eNOS) can be activated by both the AKT pathway and PLCγ/PKC pathway, leading to increased cell permeability.[115] Mechanical stresses may have an impact on VEGFR-2 phosphorylation, according to recent study, and mechanobiological agents like ERK/MAPK, c-Src, Rho/ROCK, and YAP/TAZ have been linked to VEGFR-2 activation.[116] Wang et al. found that increasing matrix stiffness dramatically elevated VEGFR2 expression in HUVECs. The integrin αVβ5/Akt/Sp1 pathway played a role in this stiffness-mediated effects.[117] Recently, Kang et al. revealed that oscillatory flow triggers H_2O_2-mediated VEGFR2 oxidation in endothelial cells via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-4 induction. Conversely, laminar flow enhances endothelial NO synthase expression, leading to VEGFR2 S-nitrosylation at Cys1206, neutralizing oxidative inactivation.[118]

5.2. NOTCH/DLL4

Activation of Notch is sensitive to mechanical cues directly and indirectly, controlling cell proliferation, differentiation, and apoptosis, while also inhibiting angiogenesis and promoting neovascular maturation.[119] During the process of angiogenesis, DLL4 induces high NOTCH expression in stalk cells, leading to receptor cleavage by γ-secretase in the near-membrane structural domains to release NICD. This NICD is then

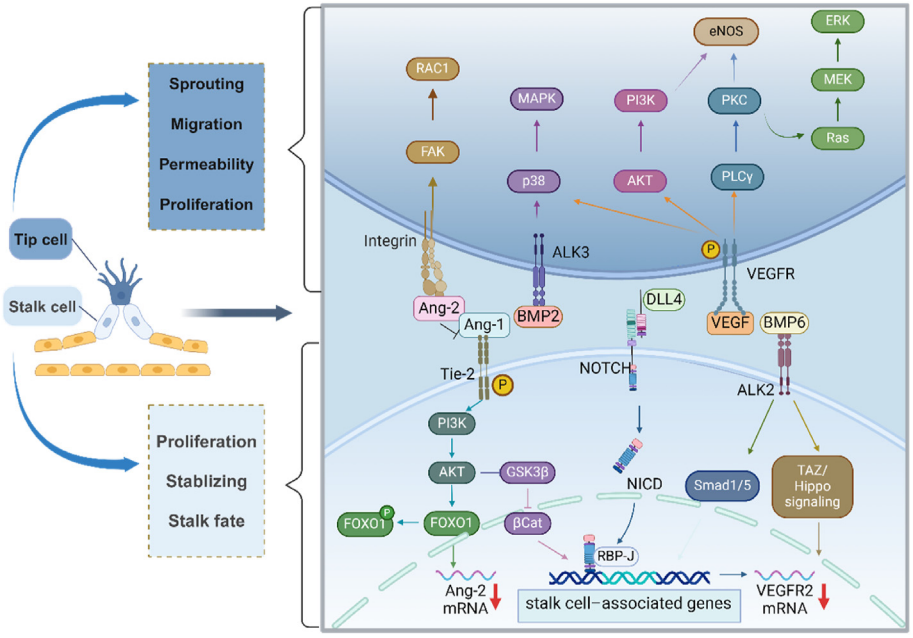

Fig. 4. Signaling pathways of intraplaque neovascularization. The tip cell interacts with stalk cells to facilitate vessel formation in a dynamic balance. We mainly discuss VEGF/VEGFR, NOTCH, Ang/Tie-2, BMP/ALK signaling. The downstream pathways include RAC/FAK, MAPK, PI3K/AKT, PLCγ/PKC pathway, which can regulate sprouting, migration, permeability and proliferation of tip cells. Correspondingly, stalk cells appear to exhibit proliferation, stable vascular network and maintain stalk fate, which avoids continuous sprouting and plays an assistant role. Interestingly, Ang-1 stimulates Tie-2 and therefore inhibits Ang-2 mRNA level, and Ang-2 acts as an antagonist inversely. DLL4 secreted by tip cells binds to NOTCH on stalk cells and decreases VEGFR mRNA level, reducing their possibility of becoming tip cells. BMPs downstream activation also presents discrepancies for tip and stalk cells to regulate their different behaviors. Created with Biorender.com.

translocated into the nucleus, where it activates target gene expression with CSL/RBPJ/CBF1.[120] A major downstream effector gene is the hairy/enhancer of split related with the YRPW motif (HEY) family of transcription factors 1 (HEY1), which has been found to inhibit VEGFR2 at the transcriptional level.[121] Recently, researchers found that SUMOylation promotes the formation of HEY1 homodimers, thereby maintaining the DNA-binding ability of HEY1 by recognizing E box promoter elements. This indicates that SUMOylation maintains HEY1 as an inhibitory transcription factor TF that regulates multiple angiogenic genes.[122] Tip cells with higher levels of VEGFR signaling also exhibit elevated levels of DLL4, which targets NOTCH receptors on surrounding stalk cells, leading to a reduction in pERK and a less pronounced up-regulation of p21. Consequently, the stalk cells demonstrate increased proliferative compared to tip cells with high levels of pERK and p21.[123] The ligand Jagged 1 binds to Notch receptors on pericytes, promoting their maturation and adhesion to the endothelial basement membrane.[124]

Notch is a mechanoresponsive receptor, and proteomic and *in vivo* experiments have shown that the activation of NOTCH1 is promoted by the high shear stress characteristic of arterial flow.[125] In addition, due to its crucial role in endothelial quiescence and the acquisition and maintenance of arterial identity, NOTCH1 expression was upregulated under low oscillatory shear stress in models in modeling using cuff tubes to manipulate carotid blood flow in mice, indicating a strong correlation between NOTCH1 and the progression of atherosclerosis.[126]

5.3. Ang/Tie-2

The angiopoietin (Ang) regulatory system comprises two closely related ligand proteins Ang-1 and Ang-2, which respectively act on the Tie-2 membrane surface receptor of VECs. The cascade of signaling pathway initiated by Ang is also subject to FSS. The formation of Ang-1/Tie-2 complexes at cell–cell junctions inhibits nuclear localization of forkhead box O1 (Foxo1) transcription factor via PI3K/Akt-dependent phosphorylation, leading to the translocation of Foxo1 to the cytoplasm and subsequent inhibition of the transcription of its target genes include Ang-2.[127] It is noteworthy that under shear stress (6 dyn/cm^2, 24 h), the PI3K/Akt/FOXO1 pathway is responsive to mechanical stimuli, as indicated by decreased expression of Foxo-1 and its target gene, Ang-2.[128] Additionally, it has been investigated that OSS activated Ang-2 expression via Wnt signaling.[129] Furthermore, research has shown that Through the inhibition of glycogen synthase kinase 3β (GSK3β) mediated by the PI3K/AKT pathway, Ang-1 activates β-catenin, which in turn leads to enhanced expression of Dll4 induced by the Notch signal. This is achieved through complex formation with NICD/RBP-J on the Dll4 intron3 enhancer, strengthening the Dll4/Notch signal cascade that ultimately leads to vascular stabilization.[130]

Ang-1 is known to preferentially facilitate vascular stabilization and maturation while Ang-2 has the opposite effect.[131] The expression levels of Tie-2 are determined by the type of VEC (tip or stalk). In stalk cells, there is low expression of Ang-2 and high expression of Tie-2, favoring Ang-1/Tie-2 signaling for to vascular remodeling and stabilisation.[132] As Tie-2 levels are low in angiogenic tip cells, Ang-2 binds to integrins directly to initiate signaling that promotes endothelial tip cell migration and vessel sprouting through activation of FAK.[133] Increased Ang-2 levels during hyperglycemia or hypoxia activate Tie-2, leading to pericyte detachment from the basement membrane and migration.[134] In addition to having a high microvessel content, atherosclerotic plaques have been shown to have an imbalance in the ratio of Ang-1 to Ang-2 levels that is biased towards Ang-2.[43] Furthermore, hemorrhagic plaques showed an Ang-1/Ang-2 ratio favoring Ang-2, suggesting an underlying involvement for the angiotensin system in microvessel immaturity.[135] Recombinant variants such as COMP-Ang-1 have been reported for use as novel treatment in neovascularization-related diseases due to their stabilizing effects on neovessels.[131]

5.4. BMP/ALK

The TGF-β superfamily is activin receptor-like kinase (ALK), and BMP is a member of the TGF-β family.[136] BMP receptors can be modulated by hypoxia and mechanic stimuli, particularly shear stress. There is compelling evidence that BMP-4 expression is transcriptionally downregulated in a variety of endothelial cell types across multiple species by atheroprotective laminar shear stress, while BMP-2 transcript levels remain unaffected.[137] Turbulent shear stress promotes VEC proliferation and induces SMAD1/5/8 phosphorylation(Zhou et al., 2013[138]), whereas VEC quiescence is facilitated by laminar shear stress via BMP9-ALK1/ENG-SMAD axis.[139]

BMP2 and BMP6 have been identified as being expressed in endothelial cells, both proangiogenic and promoting increased vascular density through the induction of new branch formation.[140] It has been determined that their respective receptors, ALK3 is required for efficient tip cell formation, while ALK2 represses sprout formation.[141] Andreas B et al. proposed a model suggesting ALK2-dependent activation of SMAD1/5 signaling enhances stalk cell development while simultaneously repressing tip cell competence, and ALK3-dependent activation of p38 signaling enhances tip cell competence and cell migration.[142] BMP2 activates the expression of genes associated with tip cells, such as DLL4 and KDR through p38 signaling, whereas BMP6 promotes phosphorylation of SMAD1/5 and p38 in VECs, resulting in the up-regulation of genes associated with stalk cells, including ID1, ID2, HES1, and FLT1.[142] Meanwhile, VEGFR2 and DLL4 mRNA transcription is regulated by BMP2 and BMP6, which contributes to tip cell formation. BMP receptors have the ability to initiate PI3K and MAPK signaling pathways in addition to Smad signaling cascades.[143] BMP2 synergistic affects tip germination behavior with VEGF, while the role of BMP in up or down-regulating VEGF remains controversial. Additionally, BMP6 downregulates the expression of VEGFR2 by the Hippo pathway and also decreases DLL4.[144]

5.5. Bach1

Bach1, a well-known transcriptional repressor of the heme oxygenase-1 (HO-1) gene, is highly expressed in human VECs, and has been recently identified as a transcription factor related to angiogenesis. Increased BACH1 expression has been observed in patients with cerebrovascular symptoms and in mice with hyperlipidemia-induced atherosclerosis. In the thoracic aorta, BACH1 is mainly located in the cytoplasm, while in the internal curvature of the aortic arch with disordered blood flow, BACH1 is located at the nucleus.[145] Bach1 deficiency was found to be linked to a significant increase in capillary density, arteriole density and VEGF expression,[146] indicating that BACH1 probably promotes atherosclerosis and inhibits neovascularization. Deletion of endothelial BACH1 led to decreased levels of TNF-α and IL-1β in the plasma of atherosclerotic mice and reduced macrophage accumulation within atherosclerotic plaques.[145]

The researchers further identified BACH1 as a mechanosensor of s stress and discovered that the BACH1-YAP transcriptional network is essential to vascular inflammation and atherogenesis. Li Jiang et al. also confirmed that inhibition of Bach1 expression increased VEGF level and promoted VEC migration, with high levels of Wnt/β-catenin downstream target genes (such as IL-8 and VEGF).[146] They proposed that Bach1 negatively impacts Wnt/β-catenin signaling and confirmed that its disruption in the interaction between β-catenin and TCF4.[146,147] As a result, targeting Bach1 could be a promising therapeutic option for individuals with peripheral artery disease by reducing oxidative stress and potentially improving angiogenesis.[148]

5.6. SIRT6

SIRT6, a member of the Sirtuins family, is involved in metabolism, antiaging, DNA repair, and other physiological and pathological processes.[149] Several studies have confirmed SIRT6 as a protective factor for

AS.[150] However, with a co-location of HIF-1α, SIRT6 is overexpressed in unstable carotid plaques. *In vitro* experiments confirmed that under both normoxia and hypoxia, SIRT6 increases the expression of HIF-1α at the protein level, thereby enhancing the migration, proliferation and tube formation ability of VECs. Additionally, SIRT6 also induces reactive oxygen species (ROS) accumulation by inhibiting catalase which directly impairs vascular endothelial function leading to endothelial barrier dysfunction. On the one hand, SIRT6 stimulates angiogenesis in both normoxia and hypoxia. In contrast, SIRT6 causes apoptosis and mitochondrial malfunction in response to oxidative stress. As a result, newly formed blood vessels often exhibit inadequate endothelial function, leading to a higher risk of intrahemorrhage or rupture of the plaque.[151] Additionally, previous studies have revealed that SIRT6 blocks vascular stiffness, which warrants further investigation in the context of intraplaque neovascularization.[152]

6. Clinical imaging diagnosis

Previous studies have shown that mechanical factors such as shear stress, PSS, and matrix stiffness can affect angiogenesis and consequently plaque stability. Current imaging instruments not only have the capability to identify plaque components but also to assess shear stress to evaluate its stability. For instance, magnetic resonance imaging (MRI) is able to visualize specific elements within the plaque, including the lipid-rich necrotic core, fibrous tissue, calcification, and IPH.[153] FSS affects the size, composition, and progression of AS plaques which can be evaluated by ultrasound or 4D-MRI.[154]

Invasive imaging A prospective study demonstrated that increased plaque vascular density and IPH were associated with a deteriorating cardiovascular prognosis.[155] Therefore, there is an urgent need for accurate observation of neovascularization using imaging techniques. Plaque imaging techniques can be categorized as either invasive or non-invasive. Invasive imaging uses specially developed high-resolution catheters to detect the distinctive features of these lesions, such as OCT, IVUS, and near-infrared spectroscopy (NIRS) for combined detection of vulnerable plaques in non-obstructive lesions.[156] While these invasive imaging techniques hold significant research value, they are not suitable for screening high-risk patients for atherosclerosis. Non-invasive imaging, on the other hand, has lesser spatial resolution than invasive techniques, but it can detect pathological features of susceptible plaques based on lesion shape. Molecular imaging can detect lesions based on the increased expression of specific markers, although it has relatively low spatial resolution (3–5 mm), it is effective in identifying vulnerable plaques with enhanced marker expression. Overall, this discussion primarily focuses on advanced non-invasive imaging techniques and molecular imaging methods for the diagnosis of AS plaque.

Ultrasound imaging Advancements in ultrasound technology have enable visualization of not only blood flow but also the vessel walls, including atherosclerotic plaques. Color Doppler ultrasound can show the formation of new blood vessels within the plaque,[157] and Vector Doppler imaging can detect WSS in the narrowed part of the artery.[158] CEUS examination enables objective observation of atherosclerotic plaque neovascularization and identifies active inflammation, which is a characteristic of susceptible plaque.[159] Superb microvascular imaging (SMI) is a novel Doppler technique that can effectively evaluate the formation of new blood vessels in carotid atherosclerotic plaques.[160] A novel ultrasound technique called shear wave elastography (SWE) is useful in discriminating between stable and unstable atherosclerotic plaques in the carotid arteries by measuring tissue elasticity.[161]

Non-invasive imaging In addition to ultrasound imaging, there are alternative imaging techniques available for evaluating AS plaques. Dynamic contrast enhanced MRI(DCE-MRI) is a novel functional imaging technique that assesses the physiologic properties of lesions and tissues based on the microvascular system, which can be used to quantify plaque microvasculature.[162] Magnetic Particle imaging (MPI) can identify atherosclerotic plaques associated with IPH by imaging the degradation products of superparamagnetic hemoglobin (hemosiderin, ferritin).[163] Near-infrared Autofluorescence (NIRAF) is another technique used for imaging using autofluorescence in the near-infrared spectral region. Fluorescence emission computed Tomography (FLECT) can monitor IPH *in vivo*, establishing a preclinical technology to assess and monitor plaque instability.[164] However, it is important to note that recent research suggests NIRAF signal in unstable plaques may be multifactorial and further exploration into other potential factors leading to NIRAF in unstable plaques is needed.[165]

Molecular imaging Compared with the non-invasive imaging, molecular imaging has the ability to detect vulnerable plaques using specific markers, despite having the relatively low spatial resolution. For example, researchers have developed a ^{99m}Tc-MAG$_3$-bevacizumab probe that binds strongly to all VEGF-A subtypes, allowing for non-invasive diagnosis and assessment of plaque neovascularization through *in vivo* micro SPECT/CT imaging.[166] Additionally, Bevacizumab-800CW is a near-infrared fluorescent tracer that targets VEGF-A and can be used to visualize its distribution in atherosclerotic plaque.[167] Furthermore, radiolabeled RGD tracers have been utilized for imaging angiogenesis in atherosclerosis due to their high affinity for integrin αVβ3 expressed on activated endothelial cells. Many radiolabeled RGD tracers have been used for imaging angiogenesis in atherosclerosis.[168,169] $MnFe_2O_4$ represent an important class of magnetic ternary compound nanoparticles. The PFP-HMME@PLGA/$MnFe_2O_4$-RAM nanoplatform actively targets the mitochondria of VECs and increases the nanoparticle accumulation in plaque neovascular, offering a novel strategy for early clinical diagnosis and real-time evaluation of atherosclerotic plaque neovascularization.[170]

New imaging techniques enable us to acquire detailed information on plaque inflammation and neovascularization, facilitating the detection of atherosclerotic plaque components and evaluation of plaque stability in combination with shear force, plaque structural stress and other factors. The imaging techniques of neovascularization in plaque were summarized in Fig. 5. The ultimate goal for future work would be early identification of atherosclerotic disease prior to the onset of clinical symptoms.

7. Drug therapy

Inhibiting angiogenesis within atherosclerotic plaques is essential for plaque stabilization, with numerous therapeutic strategies primarily targeting VEGF to mitigate intraplaque neoangiogenesis. However, recent studies indicate that this approach may also hinder compensatory angiogenesis in ischemic tissue, leading to certain limitations. The lack of mural cells in the neovasculature can result in high permeability, potentially causing plaque rupture and bleeding. Therefore, promoting the maturation of neovascularization within atherosclerotic plaques can enhance their stability. In summary, drug treatment strategies for plaque angiogenesis will be discussed from two perspectives: (I) inhibition of plaque angiogenesis, (II) promotion of new blood vessel maturation (Table 1).

Increasing the concentration of VEGF-A can significantly promote angiogenesis within the plaque. Based on the current findings, VEGF-A shows promises as a target for inhibiting angiogenesis within plaques. By blocking VEGF receptor signaling, axitinib, a strong and selective inhibitor of VEGFR tyrosine kinases 1, 2, and 3, can reduce angiogenesis and plaque instability.[171] Through VEGF-related signaling pathways, the modified recombinant human endostatin known as Endostar demonstrates its anti-angiogenic properties.[172] In an experimental porcine model of early AS, it prevents the neovascularization of plaque vasa vasorum and the progression of AS.[173] Tetramethylpyrazine and paeoniflorin combination (TMP-PF) have the functions of antioxidation, anti-inflammatory, and regulation of angiogenesis. Recent studies have shown that TMP-PF inhibits VEGF expression through up-regulation of miR-126, thereby reducing the binding of VEGF and VEGFR2 and blocking this signaling pathway to inhibit angiogenesis.[174] Melatonin relies on the regulation of angiogenesis to carry out its functions. Ding et al. discovered that giving melatonin for nine weeks might dramatically

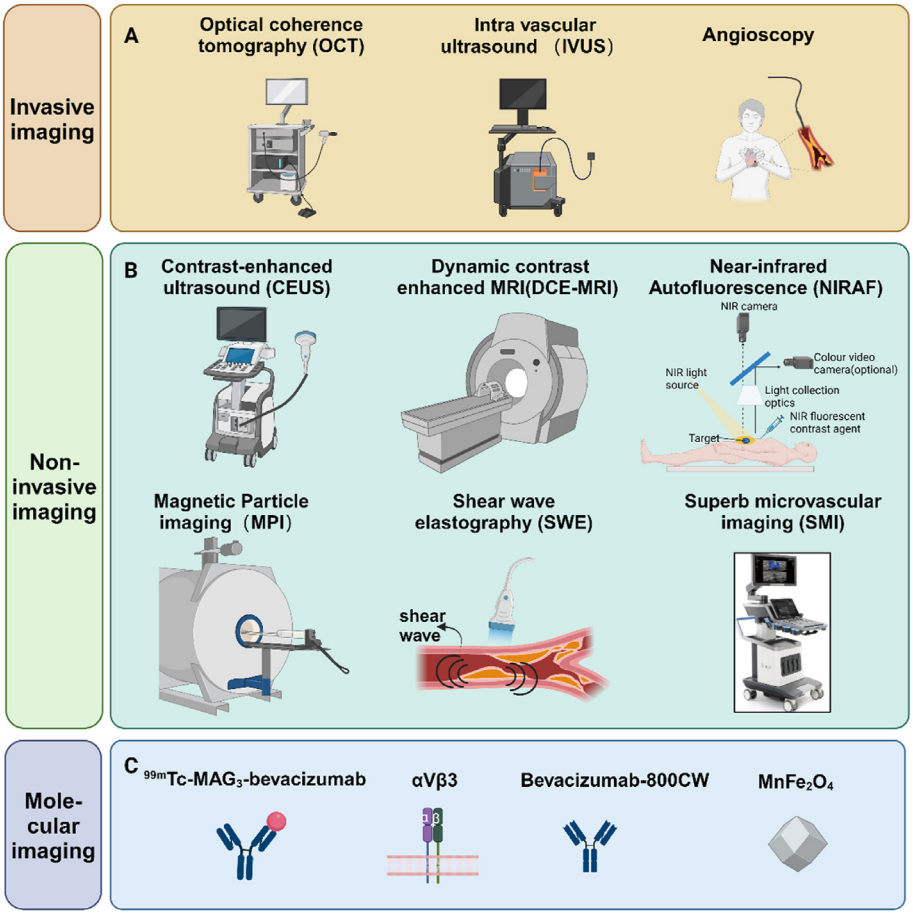

Fig. 5. The imaging technique of neovascularization in plaque. (A) Invasive imaging detects the specific characteristics of these lesions using specially constructed high-resolution catheters. Including OCT, IVUS, and angioscopy.[156] (B) Non-invasive imaging has low spatial resolution and can identify pathological features of vulnerable plaques, mainly including CEUS, DCE-MRI, NIRAF, SMI, MPI, and SWE.[159–164] (C) Molecular imaging identifies lesions by increasing the expression of specific markers. This paper mainly introduces ^{99m}Tc-MAG$_3$--bevacizumab, Integrin $\alpha_V\beta_3$, Bevacizumab-800CW, and $MnFe_2O_4$.[166–170] Created with Biorender.com.

Table 1
Compounds studied to treat Intra-plaque angiogenesis.

	Medicine	Mechanism of action	Reference
Inhibit angiogenesis in plaque	Axitinib	VEGFR1,2,3-inhibitor	171,182, 183
	Endostar	Blocks VEGF-induced tyrosine phosphorylation of VEGFR-2	173,184
	TMP-PF	Up-regulation of miR-126, reducing the binding of VEGF and VEGFR2	174,185
	Melatonin	miR-424–5p/VEGFA pathway PPARγ-RhoA-ROCK pathway	176,186
Inhibiting glycolysis flux	3PO	Glycolysis inhibitor	178,187
	PFK15	Reduced glucose uptake by ECs	179,188
Promote VV maturation	Si-Miao-Yong-An(SMYA)	Dll4/Notch1/Hey1/VEGF pathway	180,189, 127
	FGF-2&PDGF-BB	Enhanced the VEGFR-2 degradation	181,190

reduce the risk of vulnerable plaque rupture in a rupture-prone vulnerable carotid plaque model, therefore lowering the chance of IPH.[175] Recent studies have revealed that melatonin can supress the migration of VECs via the PPARγ-RhoA-ROCK pathway, thereby restraining the development of plaque and IPH formation.[176]

Blocking VEGF has been the primary approach to reduce neovascularization, but its limited efficacy and adverse effects have led to a need for a different strategy. Targeting ECs metabolism, given their reliance on glycolysis for energy, may offer a promising new avenue for inhibiting neovascularization.[177] 3-(3-Pyridinyl)-1-(4-pyridinyl)-2-propen-1-one(3PO) and PFKFB3 inhibitors have shown potential in inhibiting intraplaque neovascularization and pathological angiogenesis by downregulating VECs adhesion molecules[178] and reducing glucose uptake.[179] Combining inhibition of VECs glycolytic activity with growth factor receptor blocking holds promise as an anti-angiogenic therapy.

Improper utilization of antiangiogenic therapy may lead to hypoxia, apoptosis of VECs, and loss of endothelial vascular intimal integrity. Therefore, a noval therapeutic approach involves enhancing pericyte coverage and maturity to encourage new blood vessels to maturate. Simiao Yongan (SMYA), a traditional Chinese medicine consisting of honeysuckle, scrophulariae, angelica and licorice, has been shown to increase pericytes recruitment and density of mature vasa vasorum in aortic plaques, contributing to plaque stabilization.[180] SMYA is capable of regulating the expression of key mRNA in Dll4/Notch1/Hey1/VEGF signaling pathway, affecting the recruitment of peripheral cells around vasa vasorum and ultimately stabilizing vulnerable plaques. The early-stage upregulation of VEGF as the upstream Dll4 target promotes Notch1 binding with DII4 to facilitate vasa vasorum maturation; meanwhile, late-stage vulnerable plaque stability is achieved by inhibiting VEGF expression within the plaque through this mechanism. Additionally, the combination of FGF-2 and PDGF-BB enhances VEGFR-2 degradation while increasing neovessel pericyte coverage to promote function and maturation of plaque neovessels.[181]

8. Conclusion

This paper provides a detailed overview of intraplaque angiogenesis from the perspective of neovessel origin, tip/stalk cell interaction mechanisms, molecular pathways, mechanical regulation, and diagnostic and therapeutic approaches. Since the concept of intraplaque angiogenesis was proposed, VV has been the focus of research, and we also focus

on luminal neovascularization which gradually attracted people's attention. Neovascularization of different origins is associated with different chemicals within the plaque. Furthermore, intraplaque neovascularization from the luminal side has a stronger correlation with symptomatic plaque than neovascularization from the adventitia. However, current research is still insufficient to support all of the points involved. Most of the neovascularization is located in the shoulder of the plaque, and the mechanical environment is complex. In vitro organoid models, co-culture models, microfluidic chips, arterial ring models, etc. have been developed to simulate the growth of plaques, and IVUS-VH with a 3D reconstruction method also are developed to explore the mechanical factors within plaques. We mainly focus on the effects of flow shear force, matrix hardness, and stress in plaques on angiogenesis, and interestingly, stress can also be used as a marker to assess plaque vulnerability as well as neovascularization.

Imaging of atherosclerotic plaques is essential for early diagnosis of the disease, development of treatment plans, and evaluation of treatment effects. In addition to introducing the traditional imaging technologies (OCT, IVUS, CEUS, DCE-MRI, etc.), this paper also lists some novel imaging technologies in recent years, such as NIRAF, SMI, MPI, and SWE. With the continuous progress of imaging technology, In the future, we hope to enable earlier disease detection, more personalized treatment strategies, and more effective long-term disease management. Meanwhile, imaging technology advancement has significantly enhanced our comprehension of the pathological mechanism of this complex disease, and provided more accurate diagnostic and treatment tools for the clinic, combined with artificial intelligence and machine learning algorithms, these technologies will further increase the precision of diagnosis and the success rate of treatment, and bring better prognosis for patients. The clinical application of these drugs is promising, but it is also accompanied by rigorous evaluation of safety, efficacy, and long-term effects. Future research needs to focus on optimizing the dosing of these drugs, how they are administered, and how they can be combined with other treatments to maximize therapeutic effectiveness. In addition, an in-depth exploration of the molecular mechanisms of angiogenesis and individual differences in clinical applications will help to develop more personalized treatment options. We look forward to these studies leading to more effective and safe treatment options for patients with atherosclerosis shortly.

CRediT authorship contribution statement

Hanxiao Chen: Writing – original draft. **Chengxiu Peng:** Writing – original draft. **Fei Fang:** Writing – review & editing. **Yuhao Li:** Writing – original draft. **Xiaran Liu:** Writing – original draft, Conceptualization. **Ying Hu:** Investigation. **Guixue Wang:** Writing – review & editing. **Xiaoheng Liu:** Writing – review & editing. **Yang Shen:** Writing – review & editing, Writing – original draft.

Ethical approval

This study does not contain any studies with human or animal subjects performed by any of the authors.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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