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Recent advance in treatment of atherosclerosis: Key targets and plaque-positioned delivery strategies

Li Li[1], Sainan Liu[1], Jianying Tan[1], Lai Wei[1], Dimeng Wu[2], Shuai Gao[2], Yajun Weng[1] and Junying Chen[1]

Abstract

Atherosclerosis, a chronic inflammatory disease of vascular wall, is a progressive pathophysiological process with lipids oxidation/depositing initiation and innate/adaptive immune responses. The coordination of multi systems covering oxidative stress, dysfunctional endothelium, diseased lipid uptake, cell apoptosis, thrombotic and pro-inflammatory responding as well as switched SMCs contributes to plaque growth. In this circumstance, inevitably, targeting these processes is considered to be effective for treating atherosclerosis. Arriving, retention and working of payload candidates mediated by targets in lesion direct ultimate therapeutic outcomes. Accumulating a series of scientific studies and clinical practice in the past decades, lesion homing delivery strategies including stent/balloon/nanoparticle-based transportation worked as the potent promotor to ensure a therapeutic effect. The objective of this review is to achieve a very brief summary about the effective therapeutic methods cooperating specifical targets and positioning-delivery strategies in atherosclerosis for better outcomes.

Keywords

Atherosclerosis, targets, site-homing delivery, stent, balloon, nanoparticle

Introduction

Cardiovascular diseases (CVDs) are increasing prevalence in the worldwide with an estimated 17.9 million deaths representing 32% of all world's deaths in 2019 (data from World Health Organization), and atherosclerosis is the manifest contributor to CVDs, marked by these major clinical entities including ischemic heart disease (IHD), ischemic stroke, and peripheral arterial disease (PAD). According to the Global Burden of Disease, of the total deaths, 16.2% and 11.6% were due to IHD and stroke respectively. Under this circumstance, vast researches have got started to resolve atherosclerotic conditions.

Atherosclerosis, chronic inflammation of the vessel wall, is a progressive pathophysiological process, characterized by lipids depositing initiation and innate/adaptive immune responses. Responding to disturbed flow, phenotypic transformation of endothelial cells from rest phenotype into proatherogenic phenotype, which commonly is described as the starting point of atherosclerosis and introduces excessive oxidative system activation, prothrombotic effects, foam cells formation, inflammatory releasing and sensitized SMCs, as shown in Figure 1. Extensive studies have proved that targeting these pro-atherosclerotic processes can effectively postpone the growth of plaque and even make it regression. In the following description, therapeutic targets in the progression of atherosclerosis will be introduced.

After initiating atherogenesis by disturbed flow, most arterial wall cells (covering ECs, SMCs, and macrophages expressing NOX) participate in producing oxidants, like ROS. Lipoproteins highly accumulate in tunica intima in two main pathways, including increased permeability for

[1]Key Laboratory of Advanced Technology of Materials, Ministry of Education, Southwest Jiaotong University, Chengdu, PR China
[2]Chengdu Daxan Innovative Medical Tech. Co., Ltd., Chengdu, PR China

Corresponding author:
Junying Chen, Key Laboratory of Advanced Technology of Materials, Ministry of Education, Southwest Jiaotong University, No. 111, 1st North Section, 2nd Ring Road, Chengdu, Sichuan 610031, PR China.
Email: chenjy@263.net

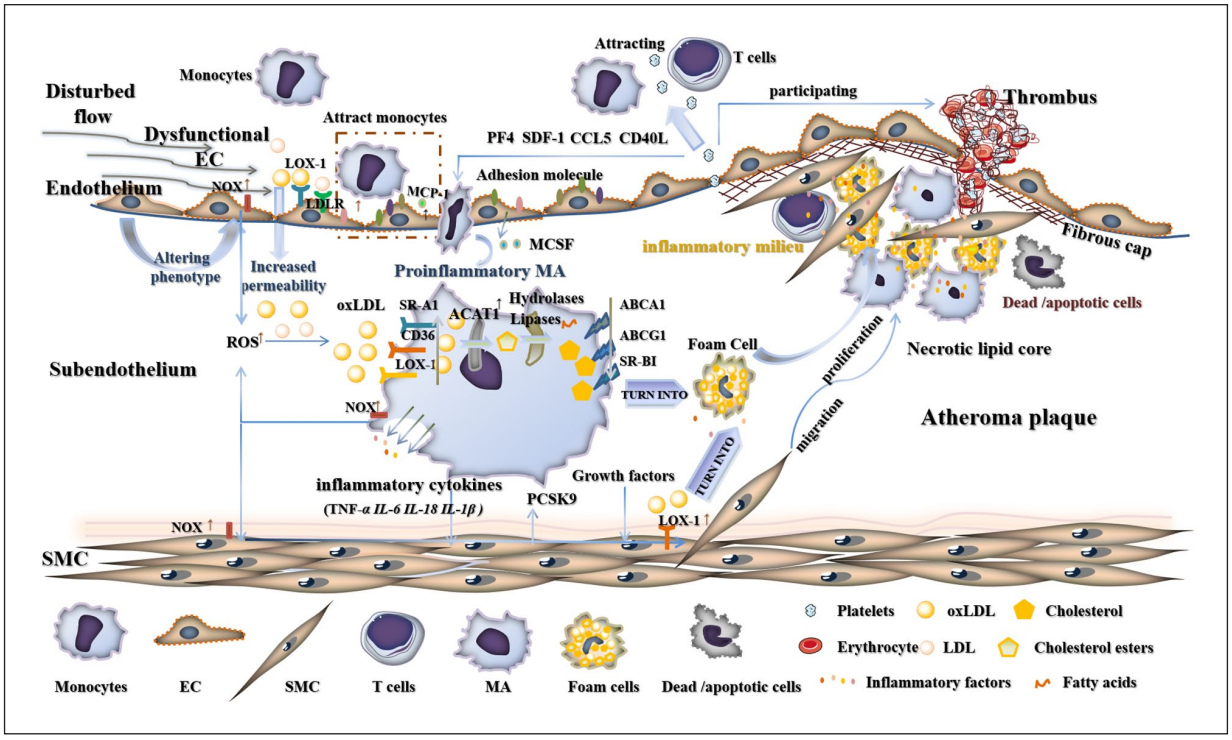

Figure 1. The main progresses of atheromatous plaques.

lipoproteins and rising lipoprotein affinity receptors,[1] such as LDL receptors for LDL uptake and LOX-1 corresponding to the passing of oxLDL. Meanwhile, dysfunctional endothelium up-regulates adhesion molecules (P-selectin, ICAM-1, and ICAM-1) and chemokines MCP-1(CCL2),[2] CCL5 and CX3CL1 for highly attracting circulating monocytes into the vascular media.[3] Besides MCP-1, oxidized LDL itself is a direct chemoattractant for monocytes.[4] Subsequently, infiltrating monocytes differentiate into inflammatory macrophages (M1 macrophages) and MCSF released by injured EC drives this transformation.[4] M1 macrophages display increased scavenge receptors (SR-A1, CD36, and LOX-1) on cytomembrane for taking in oxLDL, unregulated enzymes in the cell (ACAT1 turning oxLDL into cholesterol esters, hydrolase, and lipase for disassemble cholesterol esters to fatty acids and free cholesterol), downregulated inversus cholesterol transporter (ABCA1, ABCG, and SR-BI), which makes a dent in cholesterol efflux, enhancement of lipids afflux and accumulation of oxLDL, cholesterol and cholesterol esters, giving rise to foam cells formation. Furthermore, proinflammatory macrophages and foam cells secrete signaling molecules including inflammatory factors, ROS as well as growth factors (PDGF), stimulating SMCs migration and proliferation, accelerating the growth of plaque in arterial walls. SMCs imbibe oxLDL by elevated LOX-1 on the surface and change into lipid-laden foam cells.[5] Platelets answering the imperfect endothelium and succedent thrombosis are associated with the all stages of atherosclerotic process. Thrombosis-promoting molecules exposed from ECM in damaged endothelial system interplay with platelets, and trigger them. Accordingly, activated platelets generate active signals covering CD40L[6] and CCL5, CXCL4(PF-4), CXCL12(SDF-1) and CXCL8 (IL-8), etc. to prompt atheroma development by exacerbating inflammatory reaction.[7] Importantly, attracting other platelets and immune/inflammation cells (monocytes, macrophages, and B cells) aggravates inflammatory processes and thrombi by expressing signaling molecules CD40L.[6,8] And frequently, CD40L also triggers endothelial inflammation via CD40 on ECs.[8]

Generally, atherosclerotic disease has a blocked vessel lumen. As known, stenosis can be divided into several grades, namely less than 50%, 50%, 70%, and exceeding 70%.[9] In angiography-guided therapies, the patients with more than 70% diameter luminal narrowing would accept interventional treatment. While, vessel lumen with 50%–70% narrowing would be treated with intervention or only medical therapy, which decided by operator's assessment.[10] According to the collection of scientific studies and clinical practices/trials, systemic administrations evoked by nanoparticles and interventional strategies induced by stent and balloon have positive impacts on treating atherosclerotic plaque. Worked as vehicles for drugs, nanoparticles encapsulating therapeutic agents with improved blood half-life selectively get to lesional sites and release agents for treating, and tethering of locating moieties to particles can give this system better

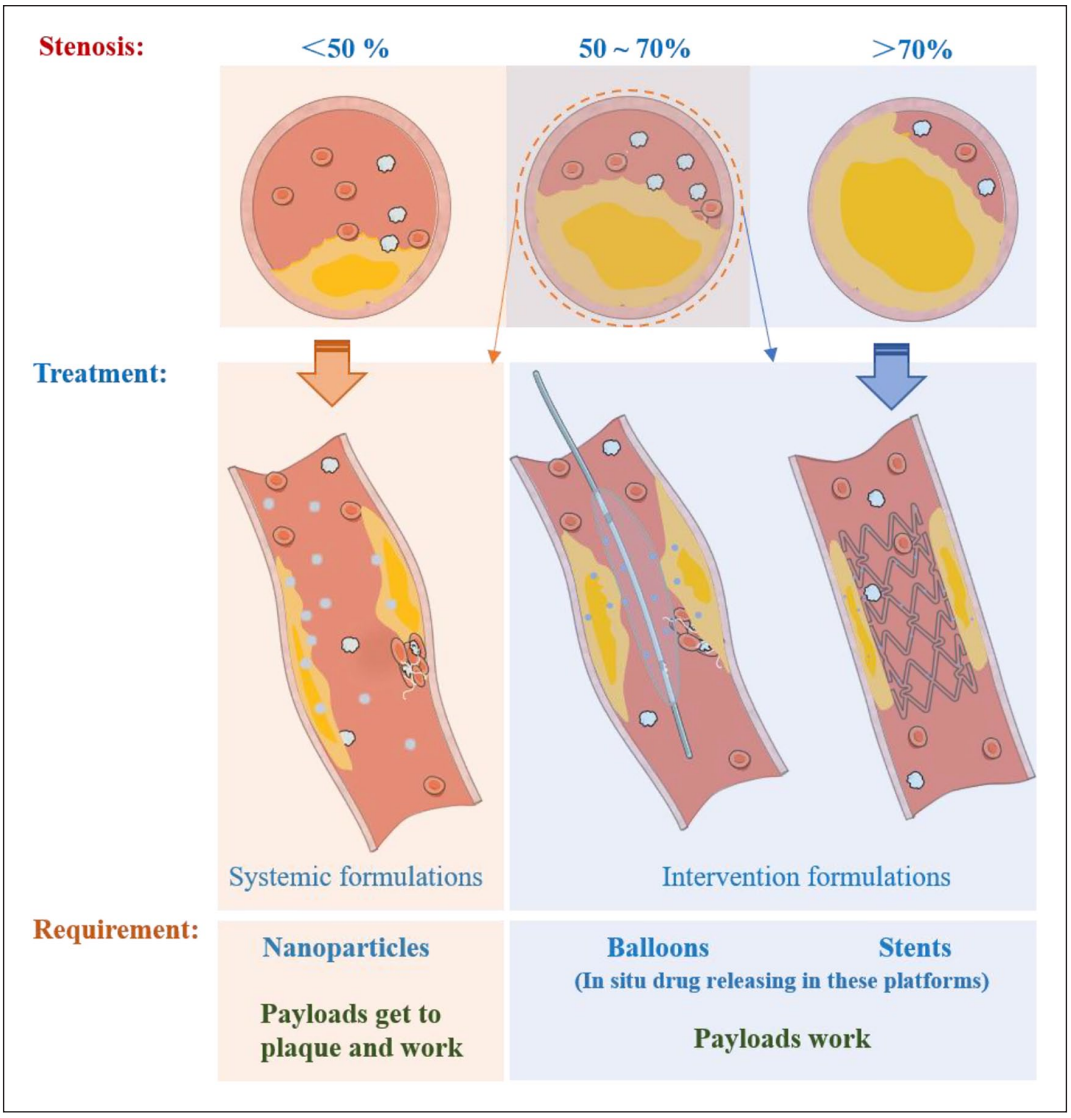

Figure 2. The stenosis severity-guided delivery strategies.

performance of active targeting.[11] Stent opens blocked blood vessel and maintains its luminal structure, but results in restenosis. Seeing that balloon also makes blood vessel unobstructed, balloon, especially drug eluting balloon, is mainly applied to solve in-stent restenosis and small artery disease with de nove lesion.[12,13] The safety and effectiveness of balloon angioplasty and stenting procedures have been approved beyond doubt, although there are postoperative complications and limitations, seen in the later parts. Noteworthily, some efforts to ameliorate these minus factors, primarily adding payloads, proved to be of avail. In this review, nanoparticles, stents, and balloons were described as site-specific drug delivery platforms, shown as Figure 2.

Taken together both pathological circumstance and positioning-delivery strategies, the objective of this review is to achieve a very brief summary about the tried and true therapeutic methods connecting specifical targets and positioning-delivery strategies in atherosclerosis, mainly aiming at treatment targets in program of inflammation, lipid metabolism, coagulation, apoptosis as well as lesion-populating cells, and local-fixed transportation formulations including stent, balloon, and nanoparticles.

Focusing on several pivotal cells

Inflamed endothelial cells

Considering activated endothelium in atheromatous plaque, available active targets may be centered on expressed adhesion molecules, receptors as well as impaired function, such as P selectins, ICAM 1, VCAM 1, αVβ3 integrin, and LOX-1 major receptors for LDL uptake by ECs,[14] biosynthesis of NO, MCP-1 secretion. Overexpressed P and E

selectins, ICAM 1, VCAM 1 induced by inflammatory stimuli, mediating leukocytes adhering to lesional region, are the hallmark of atherosclerosis. Sterilizing these adhesive molecules plays a positive role in prevention of plaque progression. Sager et al.[15] proposed that small interfering RNA (siRNA) targeting P and E selectins, ICAM1, ICAM2, and VCAM1 would combat leukocyte recruitment into plaque and lesional inflammation. Alicaforsen, an antisense phosphorothioate oligonucleotide, another antagonist of ICAM-1, selectively inactivates ICAM-1 mRNA coinciding with lessened membrane-bound protein ICAM-1.[16] Meanwhile, active atherosclerotic plaques excessively expressed P-selectin embodying bioactivity of plaques and getting prominent during thrombus formation.[17] Powerful blocking-up in P-selectin actions inhibited the inflammatory and thrombotic events, which incidentally decreased neointimal hyperplasia after balloon injury.[18] Seeing that high affinity and specificity of fucoidan for P selectins exceeding PSGL-1, stronger fucoidan-P selectins interaction blocking P selectins activities may abolish selectin-dependent recruitment of leukocytes.[19,20] The main ligands of P-selectin on sensitized ECs and platelets incorporate innate PSGL-1 on the membrane of leukocytes and other recognition effector Sialyl Lewis X, synthetic Sialyl Lewis × mimics, inclacumab, sulfated oligosaccharides as well as polysaccharides (such as fucoidan, heparin and dextran sulfate), reacting with P-selectin.[19,20] Beyond anticoagulation, heparin is also seen as a direct modulator of adhesion mediated by P-selectin. Sevuparin, a heparin-derived polysaccharide, keeps potent anti-P-selectin activities (anti-adhesive feature) without the anticoagulation properties.[21] CX3CL1/CX3CR1 process also exerts a possible therapeutic target, since CX3CL1 on inflamed ECs mediates monocytes recruitment onto the pathological vessel wall via CX3CR1 of monocytes and this interaction of CX3CL1 and its receptor also stimulates SMC migration, platelet activation and neo-angiogenesis.[22] Disruption of CX3CL1/CX3CR1 interaction with CX3CL1-Fc prevented monocyte-endothelial cell reacting and reduced atherosclerosis formation.[23] More than targeted therapies, growing proof validated that adhesion molecules could serve as location targets. Peptide ligands decoration for binding to ICAM-1, such as fibrinogen-derived peptide (NNQKIVNLKEKVAQLEA) and the sequence VHPKQHR, yielded a specific and high-affinity system directing to inflamed endothelial surface in atherosclerotic lesions.[24,25] For orientating atheroma, superfluous VCAM-1 can straight tether ligand-modified objects to lesional site, and these ligands included specific antibody or some peptides, like anti-VCAM-1 antibody, anti-VCAM-1 nanobody, and VHSPNKK.[26–28] The ligand with VHSPNKK sequence also blocked leukocyte-endothelium interactions.[28] Activated or growing cells under pathological conditions (such as differentiated macrophages and angiogenic endothelial cells) reveals high density αVβ3 integrin, but the counterpart is minimal in quiescent cells of normal vascular tissues,[29] and RGD or RGD derivatives could achieve ligation to αVβ3-positive cells in atherosclerosis.[30] Beyond expressing adhesion molecules for capturing monocytes, TGF-β stimulation through TGFβR1/2 in endothelium drives inflammatory phenotype of EC, which enhances atherogenesis.[31] Inhibition of endothelial TGF-β-TGFβRs signaling might be effective in arresting progression of building plaque by reducing vascular inflammation.[32]

Regulating phenotype-switched SMCs

In atherosclerosis, SMC proliferation is held responsible for plaque growth. And, in the past decades, the phenotypic heterogeneity theory of SMCs, also called as phenotypic switching from a contractile/quiescent phenotype supporting the arterial structure toward a synthetic one with increased migration, replication, and protein synthesis activities, has been expounded partly. Conventionally, this process is believed to have a bearing on atherosclerosis. Dapperly, VSMC plasticity also contains the transformed phenotypes of resembling foam cells, macrophage-like cells, and MSC-like cells.[33]

Theoretically, reversing or inhibiting those pro-transformation programs (as seen in Figure 3) would hold back phenotypic changes and put switched SMCs into reverse. In one example, Vengrenyuk and his team[34] validated that maintaining the producing of myocardin could conduce to contractile phenotypic SMCs after cholesterol loading. As a key enhancer in regulating phenotypic transition of SMCs, KLF4 bears on atherosclerotic plaque pathogenesis. Knocking down KLF4 in SMCs specifically cuts down lesion size, while heightens plaque stability.[35] Furthermore, alternative targets miR-143 and miR-145 accelerate myocardin expression for keeping SMCs in a contractile state and also affect a network of transcription factors relating to Klf4, myocardin, and Elk-1, which generate repressed proliferation of SMCs.[34,36,37] And, the inhibiting effect of miR-145 for SMC modulating is also due to regulating the L-type calcium channel expression partly.[38] Intriguingly, normal SMCs with a quiescent, differentiated state express calcium ion channels. In parallel with cell proliferation, L-type calcium channel (LTCC) will die away and the recurring of LTCCα1C (a LTCC subunit) is followed by reappearance of contractile phenotype markers in an earlier investigation.[39] In this study, the authors stated that activated RhoA, ERK1/2, and p38 MAPK pathways inspired inhibited LTCCα1C production under PDGF stimulation and fluvastatin upregulated LTCCα1C expression via inactivating those pathways for retaining a more differentiated VSMC phenotype. Indeed, it had been corroborated that increased miR-133 could coax modulated SMCs back to quiescence for regulating VSMC growth via suppressing the expression of transcription factor Sp-1.[40] Furthermore, KLF5 also takes an important part in regulating SMC phenotype and inhibition of this factor (e.g. by

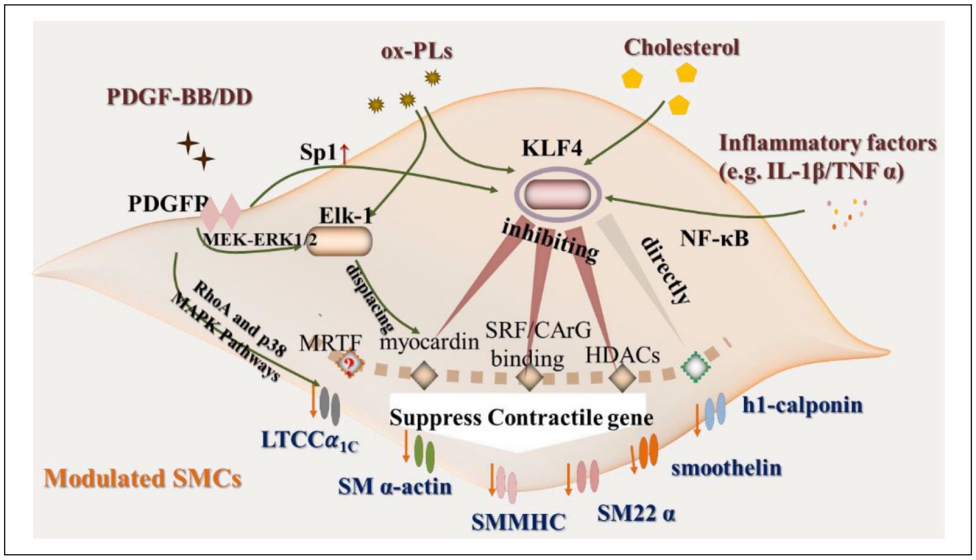

Figure 3. The main external stimulus-mediated phenotypic modulation of SMCs.

synthetic retinoid Am80) could achieve suppressed smooth muscle phenotypic modulation.[41] Dramatically, STAT3 (activator of transcription 3) protein also contributes to SMC phenotypic switch by interacting with myocardin.[42] Naturally, PERK-STAT3-MRTFA signaling axis could serve as a target.[43] A PERK inhibitor GSK2606414 obstructed STAT3 while triggering SRF by dampening down PERK activity in smooth muscle cells, which suppressed SMC's phenotypic change.[43] In another case, crocin effectively prevented VSMCs proliferation and phenotypic switch induced by PDGF-BB through STAT3 pathway.[44] In SMC phenotype switching, abrogating of glycolytic enzyme PKM2 (pyruvate kinase muscle 2) obtained inhibiting effects of SMC proliferation, migration, phenotypic switching and neointimal hyperplasia, accompanied with decreased ERK (extracellular signal-regulated kinase), mTOR (mammalian target of rapamycin), and STAT3 signaling.[45] To recapitulate briefly, any signal molecule for promoted activation of contractile differentiation and inhibited synthetic/dedifferentiated pathway, including SRF, myocardin and myocardin related transcription factor (MRTFs), or SRF/CArG-box complex and KLF4, might work as a mediator in modulated SMCs.[46]

Diverse circumstance stimuli (e.g. platelet-derived growth factor-BB/DD, oxidized phospholipids, cholesterol, and inflammatory factors-TNFα and IL-1β) could evoke transition of SMCs phenotype toward synthetic type with loss of contractile markers involving SMα-actin, SM22 α, SMMHC, and others, mainly via KLF4 and MEK-ERK1/2-Elk-1 pathway.[34,47–51] For details, in MEK-ERK1/2-Elk-1 pathway, phosphorylated Elk-1 replaced myocardin from SRF, which reduced differentiation marker genes.[48] And, the mechanisms of KLF4 mediated SMC phenotypic modulation have been described as several types, including KLF4 direct integrating to SMC marker gene promoters; blocking SRF/CArG-box binding; lessoning myocardin production and HDACs recruiting.[49]

Apoptotic cells and efferocytosis of MAs in atherosclerosis

Regulating apoptosis procedures of plaque-residing cells. Rupture-prone plaque is typically actuated by cell death (may be a marker of plaque instability), fundamentally macrophages (in necrotic core) and smooth muscle cells (in fibrous cap) apoptosis germinating necrotic core change in size and fibrous cap thinning respectively.[52] Apoptosis accompanies the whole process of atherosclerosis. As a momentous feature of atherosclerosis, decided by cell type and plaque stage, apoptosis of cell exerts profitable and deleterious effects. Importantly, foam cell apoptosis contributes to the formation of the acellular lipid core and endothelial apoptosis is directly seen at post-stenotic area with low shear stress.[53] EC apoptosis is a contributor of initiating plaque development, involving in EC dysfunction, increased endothelium permeability, thrombosis and instable plaque.[54] Depending on cell types, the stimulus for apoptosis (different dead pathway) as well as cell at different pathological stages, apoptosis regulation needs to be considered in detail. The brief apoptotic pathways of several pivotal cells are shown in Figure 4. In the early stages of atherosclerosis, increased macrophage apoptosis diminished lesion cellularity and decreased lesion progression,[55] but in advanced plaque, decreased macrophage apoptosis reduced necrotic core formation and lesion size, promoted plaque stability.[56] Generally, induction of VSMC apoptosis could be beneficial for lessening cellular accumulation and following stenosis. However, after vessel injury

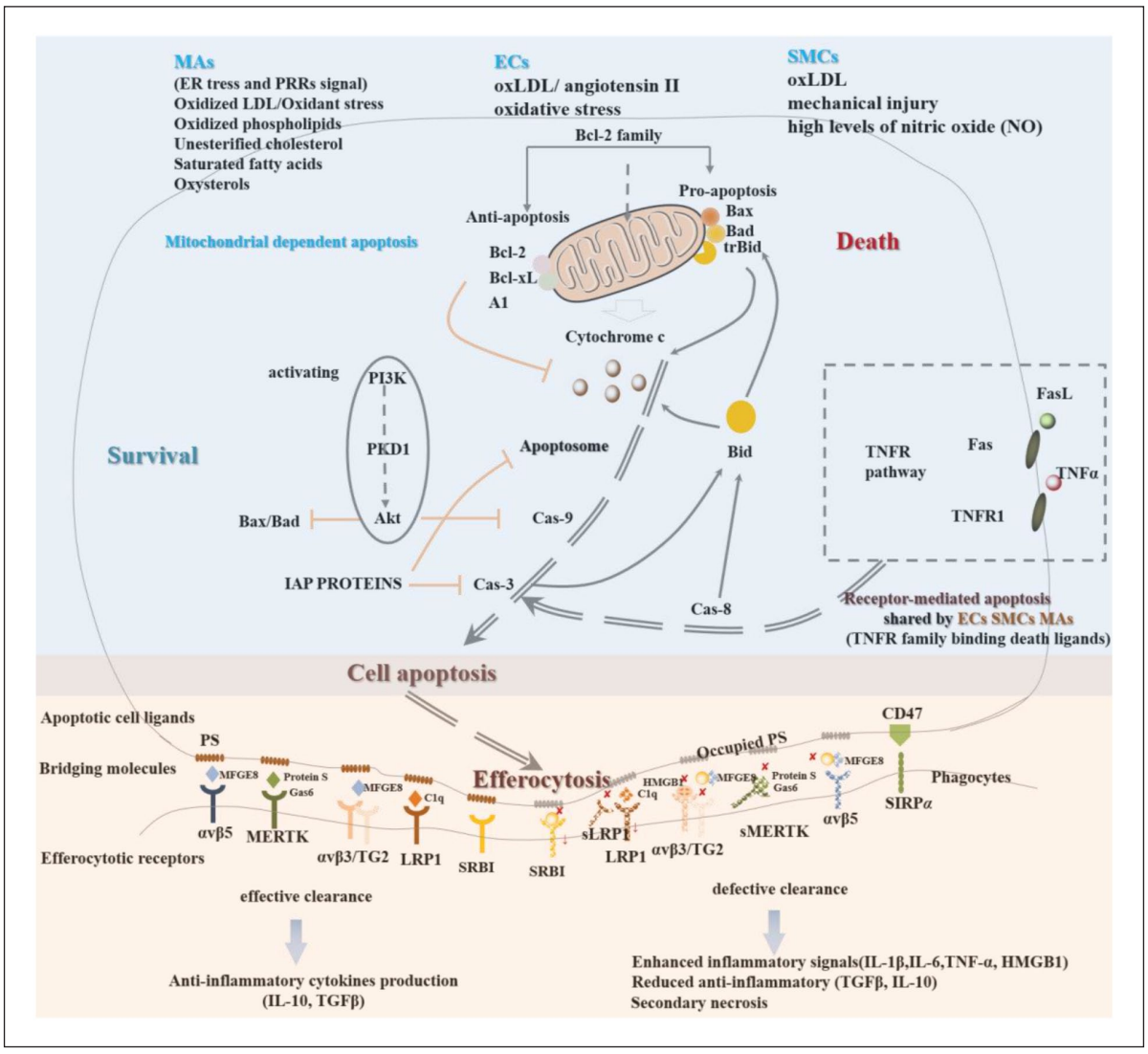

Figure 4. Apoptosis and efferocytosis of vascular wall cells (endothelial cells, smooth muscle cells and macrophages) in atherosclerosis.

including after angioplasty and stenting, protection against apoptosis aids to reduce neointima formation.[57] In the whole, conceived therapeutic strategies may selectively facilitate apoptosis of macrophages and SMCs in early lesional vessel and preclude death of macrophages at late stage, SMC after injury and EC in all expanding of plaque.

Centering on MicroRNAs (miRNAs) has found that miRNAs affect apoptosis of vascular cells conducing to the pathogenesis of atherosclerosis. Impediment of EC apoptosis has attracted for designing novel means against atherosclerosis, and the following all may be potential therapeutic targets for atherosclerosis: miR-210 upregulation repressed PDK1 favoring endothelial apoptosis[58]; miR-26a with anti-apoptotic effect downregulating TRPC3 or TRPC6 overexpression alleviating the development of atherosclerosis[59,60]; MicroRNA-122 promoting endothelial cell apoptosis by targeted XIAP inhibition[61]; MiR-365 potentiating ox-LDL-induced ECs apoptosis paralleling damaged Bcl-2 expression[62]; MiR-429-mediated down regulation of Bcl-2 giving impetus to atherosclerosis-associated endothelial cell apoptosis[63]; MicroRNA-142-3p also monitoring endothelial cell apoptosis.[64] Absorbed in regulatory roles of micro RNA in apoptosis, Chen et al.[65] proposed that inciting expression of miR-26a by tanshinol could attenuate the endothelial cells apoptosis for endothelial protection and dwindling formation of atherosclerosis. Additionally, Liang et al.[66] unfolded that direct inhibition of p38 via MiR-124 overexpression restrained macrophage apoptosis accompanied by climbing anti-inflammatory cytokines and dropping pro-inflammatory factors. Pointing toward another example, Tian et al.[67] also drew a conclusion that inhibiting Fas/FasL pathway by D4F (apolipoprotein A-I mimetic peptide) prevented macrophages from ox-LDL-induced apoptosis. For breaking TNFα-TNFR-1

signal transmission, Cho's group[68] subdued TNFR-1 expression by trafficking small interfering RNA to win anti-apoptotic effects of EC with higher ratio of anti-apoptotic factor (Bcl-xL) to pro-apoptotic factor (Bax) as well as apparently stronger HUVEC proliferation and capillary formation caused by angiogenic factors (KDR/Flk-1 and eNOS). Another critical mitogen-activated protein kinase (MAPK) signaling pathway provides pro-apoptotic signals through JNK or p38 activation or pro-survival signals through ERK1/2, ERK5 activation.[69–71] ERK5 engaging in PKB/Akt survival pathway protects cell from apoptosis.[72] Raising ERK5 in macrophages (such as by statins) upregulated macrophage efferocytosis halting plaque formation.[73] All in all, given regulating apoptosis, besides suppression of death signal (by anti-oxidants[74]) and signal transmission, elevating Bcl-2/Bax ratio for decreased cytochrome c excretion, apoptosome formation, sensitizing PI3K-Akt signals with diminished Bax/Bad and caspase 3, or raising IAPs expression also have been regard as beneficial adjustments.

Apoptosis mainly embraces mitochondria dependent apoptosis (Bcl protein family, anti-apoptosis Bcl-2/Bcl-XL/A1, and pro-apoptosis protein Bax/Bad/trBid) and receptor-mediated apoptosis. TNF receptors family (TNFR1, Fas) binding death ligands (TNFα, Fas ligand) sensitize caspase 8 and caspase 3 sequentially, and produced caspase 3 is the onset of apoptosis and sensitive caspase 8 turns Bid into trBid localizing to mitochondria for enhancing mitochondrial dependent apoptosis. Once mitochondria responds to death signals, pro-apoptosis members (Bax/Bad/trBid) are conducive to mitochondrial cytochrome c release, forming apoptosome with APAF-1 and pro-caspase 9 to activate caspase 9 and caspase 3 in order. Bcl-2/Bcl-XL/A1, IAPs (inhibitors of apoptosis proteins) including XIAP (X-chromosome linked IAP), cIAP1, cIAP2, NAIP (neuronal apoptosis inhibitor protein), active PI3K/Akt signal, and some survival factors help cell live. Apoptotic cells display prophagocytic signals, especially phosphatidylserine (PS), for clearance of phagocytes. Adequate efferocytosis is imperative to defend against atherosclerosis, producing inflammation-counteracted TGFβ and IL-10. But, impaired efferocytosis (increased cell death, sufficient numbers of phagocytes, and damaged engulfment mechanisms) in atheroma, including overloaded macrophages forming foam cells, oxidized LDL and phospholipids insensitizing phagocytic receptors (SR-BI) or bridging molecules (MFGI8), hampered devouring molecules expression (SR-BI or LRP1), HMGB1 binding αvβ3 and PS, shedding of MERTK and LRP1 as well as TNFα/TNFR1 pathway evoking CD47 (a do not eat me signaling, repulsing phagocytes) boosting, causes defective clearance and conversion of apoptotic into necrotic cells, emerging proatherogenic factors (IL-1β, IL-6, TNFα, HMGB1) and secondary necrosis[55,71,75–77].

Enhancing effective efferocytosis. Efferocytosis, referring to scavenging/engulfment of apoptotic cells by phagocytic cells like macrophages, activates anti-inflammatory, and proresolving signaling pathways that are crucial for the resolution of inflammation and effective efferocytosis of apoptotic can suppress inflammation and growth of necrotic core.[78] Efficient efferocytosis by macrophages takes a pivotal part in limiting the progression of atherosclerotic plaque. Three prerequisites depicted in Figure 4, apoptotic cell ligands (mainly PS), efferocytotic receptors (MERTK, LRP1, TG2, SRBI and integrin αvβ5, αvβ3), and bridging molecules (MFGE8, Protein S, Gas6, and complement C1q), collectively determine the clearance of apoptotic cell in vascular lesion.[55,71,75] Increased cell death, sufficient number of phagocytes and blocked digestion pathway induced by oxLDL competition inhibition, shielded PS, occupied linker molecules, decreased efferocytotic receptors, along with augmented CD 47-rejection phagocytosis via discerning SIRPα engender impaired efferocytosis[79] in atherosclerosis, playing a major role in extending lesion. Strengthening efficient efferocytosis is a promising and pivotal way to limit the progression and vulnerability of atherosclerotic plaque. During atherogenesis, progressively upregulated CD47 co-localizes to necrotic core, and special blocking-up anti-phagocytic CD47 signaling could restore eliminating of diseased and apoptotic SMCs or macrophages to prevent atherosclerosis.[80,81] Targeting CD47-SIRPα axis by miR-378a that depletes SIRPα level could hoist the phagocytic activity of oxLDL-stimulated macrophages.[82] SHP-1, a downstream effector molecule of CD47-SIRPα signaling, suppressed phagocytic function, and PEG-functionalized single-walled carbon nanotubes (SWNTs) with monocyte/macrophage-selectivity loaded by SHP-1 inhibitors (NSC-87877) accumulated within lesional macrophages and renewed lesional phagocytosis, linked to lowered the plaque burden and inflammatory gene levels.[83] TPI-1 (tyrosine phosphatase inhibitor 1, toward SHP-1 inhibition)-carrying SWNTs also could reactivate macrophage efferocytosis.[84] Huang et al.[85] also gave a description that increased SHP-1 expression during carotid atherosclerotic plaque progression possessed a position by macrophage polarization-mediated efferocytosis and deleting SHP-1 function could shift macrophages toward an anti-inflammatory phenotype preferring to promote efferocytosis. Attentionally, CD47 linking ligand TSP1 reinforces ROS release and abates eNOS activation and NO production. Consequently, inhibiting CD47 may ameliorate atheroma, as a consequence of elevated NO levels and decreased oxidative stress.[86] Another instrumental thing may be an increase of "eat me" signals of dying cells and PS functions as a therapeutic target to heighten phagocytosis of apoptotic cells. Schutters et al.[87] employed RGD-introduced annexin A5 interacting with αvβ3/5 on the phagocytes to target expressed phosphatidylserine (PS) for enhanced engulfment of apoptotic cells and IL-10 secretion.

Aiming at critical pathological proceeding

Targeting of lipid metabolism process

Targets in forward direction. The phagocytosis of oxidized low density lipoprotein is implicated in endothelial dysfunction, inflammation, formation of foam cells, migration and proliferation of smooth muscle cells, platelets activation, cell apoptosis, and atherosclerotic plaque instability.[88] Oxidized low density lipoprotein transits through endothelium in connect with LOX-1, and under the pathologic pathway of atherogenesis, undue presence of scavenger receptors on induced-atherogenesis cells (mainly LOX-1, CD36, SR-A1 on macrophages and LOX-1 on SMCs) is a significant cause of foam cell production. Resting platelets constitutively express CD36, mediating OxLDL binding to platelets, and activated platelets exhibit LOX-1.[89] Thus, scavenger receptors appear to be an available target of curing and arriving in plaque sites.

LOX-1, a cell-surface receptor for oxidized LDL (Ox-LDL), is dramatically associated with atherosclerosis. Its expression of human carotid arteries in advanced atherosclerotic plaques has been observed in intimal smooth muscle cells, as well as macrophages, endothelial cells, and active platelets.[90] LOX-1 is also colocalized with the apoptotic cells highly in lesional site.[91] Ishino et al.[92] deemed LOX-1 was expressed in the macrophage-rich lipid core area. And they also took advantage of anti-LOX-1 antibody to direct LOX-1 receptor for imaging of vulnerable plaque,[93] indicating the effectiveness of targeting LOX-1 for plaque homing. Analogously, targeting LOX-1 can realize controlled delivery of therapeutic agents into arterial plaques. Saito et al.[94] found that LOX1-targeted liposome loading fasudil notably prevented intimal hypertrophy and matrix metalloproteinase-9 expression. And anti-LOX1 antibody bound liposomes reached carotid artery lesions with effect.

As a therapeutic target, given that remarkable increase of LOX-1 in the neointima after balloon injury has been reported, the loss-function of LOX-1 has obvious inhibitory effects on intimal hyperplasia, oxidative stress, leukocyte infiltration by anti-LOX-1 antibody administration.[95] Gene silencer also has been recognized as a feasible means to LOX-1 deficiency, and PIP (pyrrole-imidazole polyamide) is an optional molecule for inhibiting the expression of LOX-1, monocyte chemoattractant protein-1, intercellular adhesion molecule-1, matrix metalloproteinase-9, and thickening neointimal.[96] Kaimin et al.[97] designed PIP targeting LOX-1 eluting stents and unmasked that the stent could dwindle the area of neointima and in-stent restenosis (ISR) without impairing re-endothelialization. Fan et al.[98] revealed that casein kinase 2-interacting protein-1 (CKIP-1) minified LOX-1 transcription on account of interplaying with proteasome activator REGγ for promoted degradation of transcriptional factor Oct-1. CKIP-1 harbors a protective role during foam cell formation and atherosclerosis.[99] Attractively, use of scavenger receptor inhibitors (for example, CD36 inhibitors AP5055/AP5258[100] and micellar nanolipoblockers (NLBs) functionalized with anionic carboxylate group for SR-A[101]) or molecules competing with oxLDLs for binding to SRs (such as long-chain fatty acids[102]) can prevent scavenger receptors-dependent oxLDL uptake, which is a promising avenue of the treatment and prevention of atherosclerotic development. Considering Ox-LDL metabolic process, well-directedly suppressed ACAT1 expression or promoted transfer-out proteins for cutting down cholesterol gathering in macrophages could limit further foam cell formation. Liraglutide could down-regulate ACAT1 with suppressed macrophage foam cell formation, which interdicts the development of atherosclerotic plaque.[103]

Modulating reversing procedure. Possible mechanisms of rapid regression of atheroma plaque relate to efflux of cholesterol from phagocyte, emigration of foam cells out of plaque, influx of healthy phagocytes for remove necrotic debris, and other components of the plaque.[104,105] This part would focus on transfer-out of cholesterol. Backward cholesterol transport occupies an important position against atherosclerosis development, and cholesterol out from foam cells has been identified as one powerful therapeutic strategy for meliorating lesion. ABCA 1 is the key reverse cholesterol transporter mediating cholesterol exporting from cells. Chen et al.[106] reported that carvedilol could boost cholesterol out and ABCA1 expression for halting atherosclerosis possibly through NF-κB. A great deal of targets can pose as a primer mover for contributory ABCA1 expression.

Enabled liver X receptor (LXRs, LXRα and LXRβ forms, ligand-activated transcription factors), upon ligands binding, augment target genes expression involved in reverse cholesterol transport (considerable ABCA1 and ABCG1 prompting cholesterol excretion) and mitigate proinflammatory gene expression (TNFα, IL-1β, and CCL2.[107,108] Some endogenous (non-enzymatically generated oxysterols-weak or no agonistic activity, and other cholesterol derivatives-24(S)-hydroxycholesterol, FF-MAS, desmosterol) and exogenous (T0901317, GW3965) agonists are widely accepted as LXR ligands.[107,108] LXRs signaling can be seen as a potent signal for cholesterol efflux from foam cells, some reports had elaborated this property. Quercetin (one of flavonoids) motivates increasing cholesterol efflux from foam cells derived from ox-LDL-induced macrophages through PPARγ-LXRα (more ABCA1 expression) pathway.[109] PPARγ (peroxisome proliferator-activated receptor-γ) motivating significantly enhances foam/macrophages to expel cholesterol through the expression of ABCA1 and LXRα, and functional ABCA1 expression is necessary for PPARγ-induced cholesterol efflux from macrophages. Ren et al.[110] uncovered that mangiferin promoted cholesterol efflux of acetylated LDL-loaded macrophage via elevated ABCA1 and ABCG1

mediated by the activated PPARγ-LXRα pathway and ameliorated atherogenesis (decreased plaque size). Libby and Plutzky[111] summarized that the activation of PPARγ also minified biomarkers of inflammation (TNF-α, IL-1β), inflammatory associated adhesion molecules, oxLDL-internalized receptors (LOX-1, scavenger receptor A) as well as MMPs expression, which suppressed inflammation and atherosclerosis. Zimmer et al.[112] also authenticated CD (cyclodextrin)-mediated LXR agonism exhibited the anti-atherosclerotic and anti-inflammatory effects with augmented removal of cholesterol and regression of plaque. For permitting cholesterol out from foam cells or macrophages and amending atherosclerosis, Han et al.[113] availed Urolithin A (UA) to promote cholesterol efflux from formed foam cells and attenuate cholesterol accumulation via modulating related microRNA-33a (decreased miR-33a but increased expression ABCA1 and ABCG1) and ERK/AMPK/SREBP1ignaling pathways. More specifically, miR-33a strongly repressed the levels of ABCA1 to dampen cellular cholesterol efflux, inhibition of endogenous miR-33a downwards adjusted ABCA1 expression.[114] Additionally, inhibiting macrophage miR-34a to up-modulate ABCA1 and ABCG1 gives impetus to cholesterol efflux or reverse transport, which gooses atherosclerosis regression. miR-34a inhibition also alters M1 into M2 macrophage polarization via liver X receptor, together with shrinking inflammation (reduced TNF-α, IL-6, and MCP-1).[115] PCSK9 downregulates ABCA1 gene and protein expression for weak cholesterol efflux and PCSK9 could also serve as a powerful therapeutic target.[116] As a regulator of LDLR (LDL receptor) and LDL-C (LDL cholesterol), the peptide-based anti-PCSK9 vaccines could obtain long-term therapeutic effect against atherosclerosis.[117]

Inflammation pathway

Classical targets: NLRP3/IL-1β pathway and downstream mediators. Atherosclerosis development is not merely caused by accumulating lipid within the arterial wall, also a chronic inflammatory disease responsible for vascular injury. As a consequence, targeting inflammation itself has stimulated novel approaches to reduce cardiovascular events and risks induced by atherosclerotic walls. IL-1β is a primary form of circulating IL-1 that induces various secondary inflammatory cytokines (including IL-6, TNFα) synthetizing when answering vascular injury and is manufactured in the setting of NLRP3 inflammasome activation.[118] The activation of the NLRP3 inflammasome (more likely driven by cholesterol crystal) drives the initiation and progression of atherosclerosis fundamentally by predominant IL-1β effect originating from macrophages.[119] NLRP3 inflammasome activation is discovered mainly in macrophages/monocytes and foam cells, sporadically in SMCs, ECs, and T cells in plaque site. And NLRP3, ASC and caspase 1 as well as IL-1β and IL-18 increase in arterial wall.[120] A growing elaboration sharpens that, owing to PRRs signaling, ox-LDL, ROS and cholesterol/calcium phosphate crystals phagocytosis (proatherogenic mediators), NLRP3 pathway gets activated, which enables the next IL-1β and IL-18 release, causing vascular inflammation in the progression of atherosclerosis,[121–123] as shown in Figure 5. IL-1β acts on cells (abundant SMCs, ECs, and macrophages) in the diseased vessel system, then alters cell functions including inflammatory transform releasing IL-6 and TNF-α, autocrine factors, and self-active state, resulting in endothelial dysfunction, infiltrative monocytes, MAs inflamed state and SMC proliferation (a catastrophic road of atherogenesis).[124–126] Taking these together, pointing at NLRP3 inflammasome, IL-1β and downstream inflammatory mediators (IL-6, TNF-α) may obtain positive outcome for impeding atherosclerotic extension. In these regards, there are three directions for working: smothering of IL-1β emerging by inhibiting active NLRP3 inflammasome; incapacitating IL-1β through blocking IL-1 receptors binding or nullifying IL-1β itself; making generative secondary inflammatory factors (IL-6, TNF-α) ineffective for abrogating cells inflammatory response.

Combating NLRP3 inflammasome activation by thioredoxin-1 significantly mitigates ROS-stimulating NLRP3 generation, IL-1β secretion and gets atherosclerosis stunted, unfolding atheroprotective functions.[127] Some other tactics of indirect or direct depressing NLRP3 inflammasome have been demonstrated to block inflammasome activation for diminished IL-1β with selectivity and advantage, embodying small-molecule inhibitors (MCC950,[128] β-hydroxybutyrate, arglabin) and microRNA(microRNA-223, suppressing NLRP3 protein expression),[129] and more inhibitors also had been summarized by Zahid and their colleagues.[130] The new therapeutic strategies inhibiting NLRP3 inflammasome activation are burgeoning. Peng's group[131] unveiled 13-methylberberine opposed NLRP3 inflammasome activation for inhibited cell injury induced by H_2O_2. Melatonin inhibits NLRP3 inflammasome and pyroptosis with diminished caspase1, IL-1βand IL 18 production and suppressive NF-κB activation.[132] Pyroptosis (inflammatory form of cell death), observed in monocytes, macrophages, dendritic cells, VSMCs, vascular endothelial cells, is dependent on caspase-1 and triggered by activated inflammasomes.[133] As a pyroptosis promoter, active enzyme caspase-1 also plays a forceful part in aiming inflammation. Caspase-1 retardant, VX-765, remarkably reduces VSMCs pyroptosis and IL-1β processing in OxLDL circumstance and impedes the growth of atherosclerosis.[134] As a pivotal intermediator in inflammatory responses, with regulating a myriad of pro-inflammatory genes, the transcription factor NF-κB also partakes inflammasome regulation.[135] Growing evidence indicates that interfering with NF-κB signaling could mediate vascular inflammation, and some nuclear factor kappa B inhibitors have been investigated in different cell models, like resveratrol for endothelial

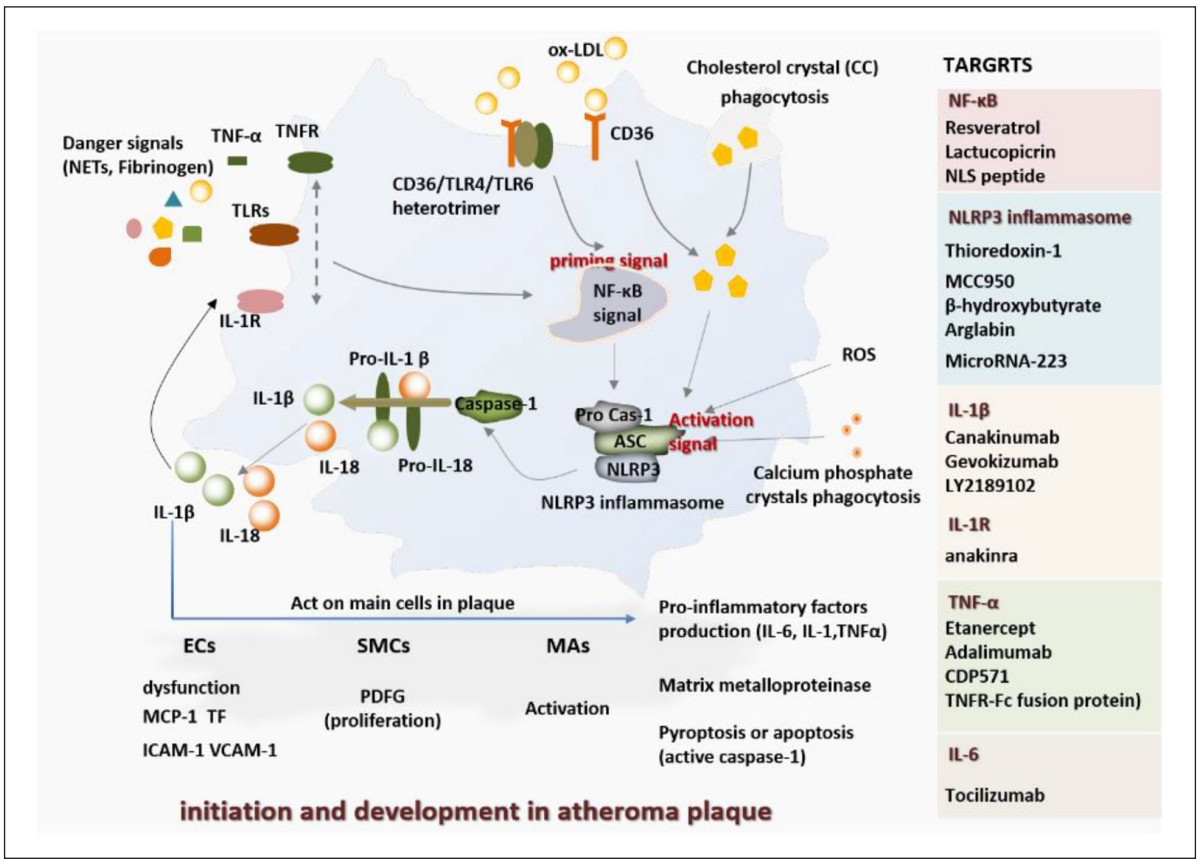

Figure 5. The key pro-atherogenic role of NLRP3 inflammasome and IL-1β in initiation and development in atheroma plaque.

cells, lactucopicrin for macrophages and NLS (NF-kB nuclear localization sequence) peptide for SMCs and MAs.[136–139]

For taking aim at primary and secondary inflammatory products, antagonists or therapeutic agents have got groundbreaking findings for anti-inflammation or atheroprotection by directly targeting TNF-α with etanercept, adalimumab, TNF-specific antibody CDP571, TNF receptor–Fc fusion protein, IL-1β with canakinumab, gevokizumab, LY2189102, IL-6 with tocilizumab, IL-1R with anakinra[126,140–142] or indirectly inhibiting the production of these factors, like silencing tumor necrosis factor alpha converting enzyme (TACE, cleaving precursor of TNF-α) expression.[143] IRAK4, recognized as a result of danger signal acting on TLR or IL-1 receptors, is an essential signal transducer downstream of TLR and IL-1 receptors.[144] IRAK4 of macrophages as a target, is directly reacted by FC-99 (benzenediamine derivate), and, as a result, this treatment attenuates proinflammatory mediators (TNF-a, IL-6, MCP-1) production.[145] Extensive inhibitors of IRAK4 have been reviewed and used, such as N-Acyl-2-aminobenzimidazole inhibitors, a diarylamide and an unrelated imidazo[1,2-a] pyridine series of IRAK4 inhibitors and quinazoline based inhibitors.[144,146,147] However, targeting any inflammatory mediators cannot completely block all the inflammatory pathways in atherosclerosis.

The cytokine interleukin-1β (IL-1β) rooting in macrophages is a major driver in pathogenesis of atherosclerosis. Priming and activating signals trigger active NLRP3 inflammasome drawing forth IL-1β and IL-18. ROS, disturbed flow, phagocytic calcium phosphate crystals, and cholesterol crystal (CC) or CC from untaken oxLDL position themselves as enable signals of NLRP3 inflammasome. And danger signals recognizing receptors (IL-1βwith IL-1R, NETs/fibrinogen/ CC with TLRs, oxLDL-dependent CD36/TLR4/TLR6 heterotrimer, as well as TNFαengaged TNFR) sever as priming signal to trigger NLRP3 inflammasome activation via NF-κB pathway. Activated NLRP3 inflammasome elicits capase-1 responsible for cleaving pro-IL-1 β and pro-IL-18 for IL-18 and IL-1β generation. For one thing, IL-1β ligating to IL-1R as a priming signal irritates added IL-1β production. For another, IL-1β acts on cells in plaques (including ECs, SMCs, MAs) via IL-1 receptor family, contributing to cells sensitization for enhanced pro-inflammatory factors production (IL-6, IL-1, and TNFα). Graver ECs dysfunction, increased vascular permeability and anabatic expression of MCP-1, TF, and adhesion molecules cause pro-coagulation effects, leukocyte infiltration. Autocrine factor PDGF of SMCs works on SMC proliferation. Of note, these cells biosynthesize ascending matrix metalloproteinase, MMPs

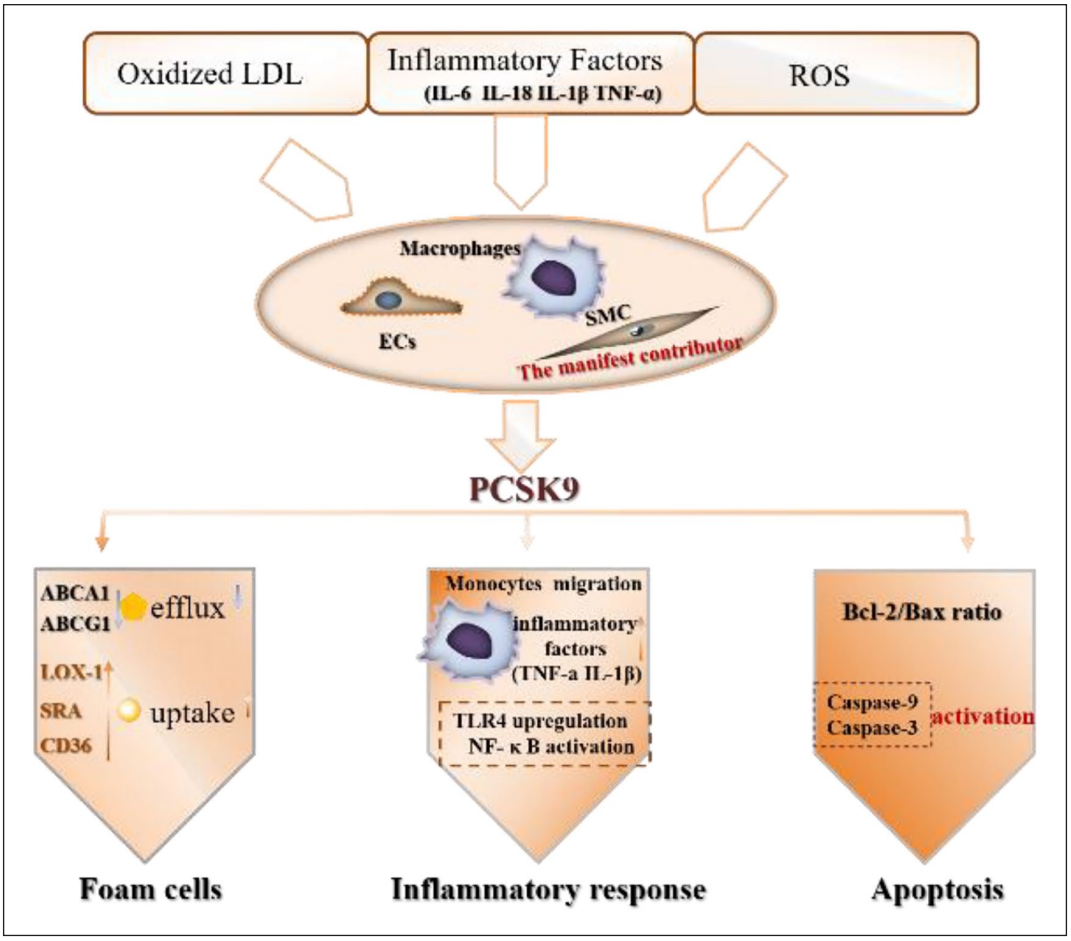

Figure 6. The effect of PCSK9 on atherosclerotic progression.[116,160,161] Covered by inflammatory conditions, oxLDL and ROS at the plaque site, response cells (ECs, SMCs, and MAs) synthesize PCSK9, elevating SR (LOX-1, SRA, CD36) expression for more oxLDL intake, lowering ABCA1 and ABCG1 with decrease cholesterol outflow. Under this modulation, lipid-laden cells emerge as foam cells. Concomitantly, PCSK9 upregulates adhesion molecules VCAM-1 expression (more monocytes trafficking), and provokes macrophages to liberate inflammatory molecules (TNFα and IL-1β) based on TLR4/NF-κB pathway activation.

2/9 of ECs for erosion, MMP 3 and MMPs 2/9 of SMCs for remodeling and migration respectively, and MMP 1/8/13 of macrophages for plaque rupture (collagenase).[119,148,149] For the enzyme caspase-1 (a promoter of apoptosis) mentioned earlier, it can trigger pyroptosis (the inflammatory cell death), precipitating the development of atherosclerosis.[124]

Some momentous regulators for inflammation

PCSK9. PCSK9 (the proprotein convertase subtilisin/kexin type 9), an enzyme, possibly wields its pro-atherogenic power through inherent pro-inflammatory effects, beyond regulation of cholesterol homeostasis.[150,151] Uplifted PCSK9 secreted by ECs, SMCs as well as macrophages in plaque regions is provoked by immoderate oxLDL gather, inflammatory milieu,[152] a mass of ROS generation. Synthetic PCSK9 has pleiotropic effects on atherogenesis, covering modulation of inflammatory, engulfing oxLDL, apoptosis/autophagy, and cholesterol efflux, specifically seen in Figure 6. More importantly, PCSK9 directly increases inflammation in atherosclerotic lesion.[153] The deficiency of PCSK9 could reduce atherosclerosis markedly.[154] Targeted treatments against PCSK9 overtly lessens inflammation, endothelial dysfunction, and plaque size.[154–156] As an emerging target for treating atherosclerosis, the therapeutic capacity of PCSK9 has been confirmed. Monoclonal antibodies, gene silencing, and mimetic peptide (inspired by EGF-A binding domain of the LDLR, which interacts with PCSK9) are several methods of restraining PCSK9 for exhibiting anti-atherosclerosis effects.[156–158] Such as, Tang et al. found that, in terms of PCSK9 quantity, PCSK9 was more clearly observed in the atherosclerotic plaques than normal aortic tissues. Gene interference, specifically blocking PCSK9 expression, weakened inflammatory factors secretion (TNF-α, IL-1β) and plaque area.[155] What's more, other researchers also exposed inhibition of PCSK9 could inhibit HUVEC apoptosis induced by ox-LDL via Bcl/Bax–caspase9–caspase3 pathway.[159]

Protease-activated receptors (PARs). Veritably, inflammation and coagulation systems converge at injury and plaque tissue, which can be delineated as proinflammatory factors bring about active coagulation pathway, in return, coagulation considerably contributes to inflammation.[162] Tissue factor (TF, on the membrane or in the plasma) is deemed as a cross-talked linker between inflammation and coagulation system, and in response to inflammatory mediators (TNFα, IL-1β), ROS, LPS or other injurious stimuli, heightened TF expression is witnessed.[163,164] TF activates FVII, forming TF/FVIIa complex which signals to generate FXa and thrombin, which fix to PARs for inflammation through PAR-2 and PAR-1, respectively.[164] Thrombin interacts with PAR-1, -3, and -4 by the great affinity (PAR-1 is the major receptor of thrombin), FXa is usually sensitive to PAR-1, -2, and -4 (PAR-2 is the most prime for FXa and not activatable by thrombin, may function as a FVIIa/FXa complex receptor). Veritably, PAR1 and PAR2 appeared at MA, EC, SMC and monocytes.[165–170] Thrombin and FXa activate a variety of cell types including MA, SMC, and EC for actuating cellular inflammatory response, which works in arterial injury and in neointima of human atherosclerotic lesions. Based on the precondition, PAR1 and PAR2 are the charming targets. Hara et al.[171] found that FXa-PAR-2 signaling activates macrophages and promotes vascular inflammation, increasing atherosclerosis involving MCP-1, IL-6 and TNF-α production, activation of NF-κB (upregulated inflammation through NF-κB pathway). It is trust worthy that PAR-2 is a underlying remedial target. Contributing PAR route on endothelial cells causes vWF, TF, adhesion molecules expression, and P-selectin relocation onto the cell surface. Vorapaxar treatment for competitive binding to PAR-1 (inhibiting thrombin-induced PAR-1 activation) realized lower coagulation activation, inflammatory response and endothelial activation, containing descending level of TNF-α, IL-6, vWF, and soluble E-selectin.[172]

GLP-1R/GLP-1 signal. At present, the localization of GLP-1R (glucagon-like peptide-1 receptor) in blood vessels is on vascular smooth muscle, ECs, platelets, and monocytes/macrophages. And native GLP-1 (glucagon-like peptide-1, thought as anti-atherogenic and anti-inflammatory actions) or GLP-1 analogs (liraglutide, semaglutide, exendin-4) as GLP-1R agonists heighten GLP-1R expression for modifying cells or tissue function. Overall, GLP-1R signaling in multiple pathways impacts abnormal vascular tissue possibly relating to enhanced plaque stability, endothelial function and subdued smooth muscle proliferation, platelet aggregation, oxidative stress, and inflammation with attenuating atherosclerotic development.[173–175] Some investigators considered that GLP-1Rs deadened plaque tissue pullulation and affected atherosclerosis through an anti-inflammatory mechanism.[176,177] GLP-1R activation in endothelial cells blunted eNOS uncoupling and vascular inflammation, prevented vascular oxidative stress, and elevated NO bioavailability.[175] Furthermore, GLP-1 and its analogs prevented the development of aortic atherosclerotic lesions by reducing the monocyte/macrophage infiltration and macrophage foam cell formation.[103] Shrinkage of GLP-1 inactivation and degradation has shown as anti-inflammatory effects. Dipeptidyl peptidase IV (DPP-IV), CD26, a cell-surface, and secreted peptidase with increased dipeptidyl peptidase-4 activities in atherosclerosis, is in charge of degrading GLP-1.[178–182] Dipeptidyl peptidase-IV (DPP-IV) inhibition has been widely appreciated as a possible therapeutic target for atherosclerosis, and its anti-atherogenesis could be explained by increased GLP-1 biological activity for direct vascular protective effects with better EC function, impaired monocytes/macrophage inflammation.[183,184] Matsubara et al.[184] concluded that desfluoro-sitagliptin (DFS), a DPP-IV inhibitor, augmented GLP-1 activity to reduce the releasing of proinflammatory mediators and ROS in macrophages, as well as attenuate EC dysfunction, exhibiting antiatherogenic effects.

The coagulation system

The rupture of an atherosclerotic plaque primarily triggers arterial thrombosis formation. As plaque ruptures, exposed collagen and von Willebrand factors(vWF), acting as specific platelet cell-surface receptors, capture circulating platelets to the site via bonding integrin α2β1, GPVI, and GPIb on platelets, respectively. And GPIbα mediates platelets to adhere to injured and inflamed endothelium. After adhering, major platelet integrin αIIbβ3 and GPIbα binds other platelets, then leading to rapid growth of the thrombus. As a more important role, high concentration of tissue factor exiting in lesions generated by atherogenic cells, referring to active platelets, monocytes/macrophages, inflamed ECs, SMCs, and foam cells, notably initiates the extrinsic coagulation pathway. Ultimately, combining faulty platelets, stable thrombus rich in fibrin, platelets and red cells take shape, which further enlarges ongoing plaque size. More concerned, stent implantation for treating narrowing vessel tissue would trigger clotting formation, encompassing acute (0–24 h), subacute(24 h to 30 days), late (30 days to 1 year), and very late (beyond 1 year-likely associated with hypersensitivity reaction, excessive fibrin deposition, or neoatherosclerosis) stent thrombosis, sharing similar mechanisms with thrombus formation.[185,186] As a consequence, concentrating on thrombosis formation and platelets is a charming way to AS (atherosclerosis) targeting, therapy, and suppressing plaques growing.

Platelets. Platelets occupy an important position in developing thrombus and plaques, looking like Figure 7. And targeting it for preventing thrombosis mainly covers specifically inhibiting the receptors themselves, inactivating the promoters of platelets and hindering these promoters to

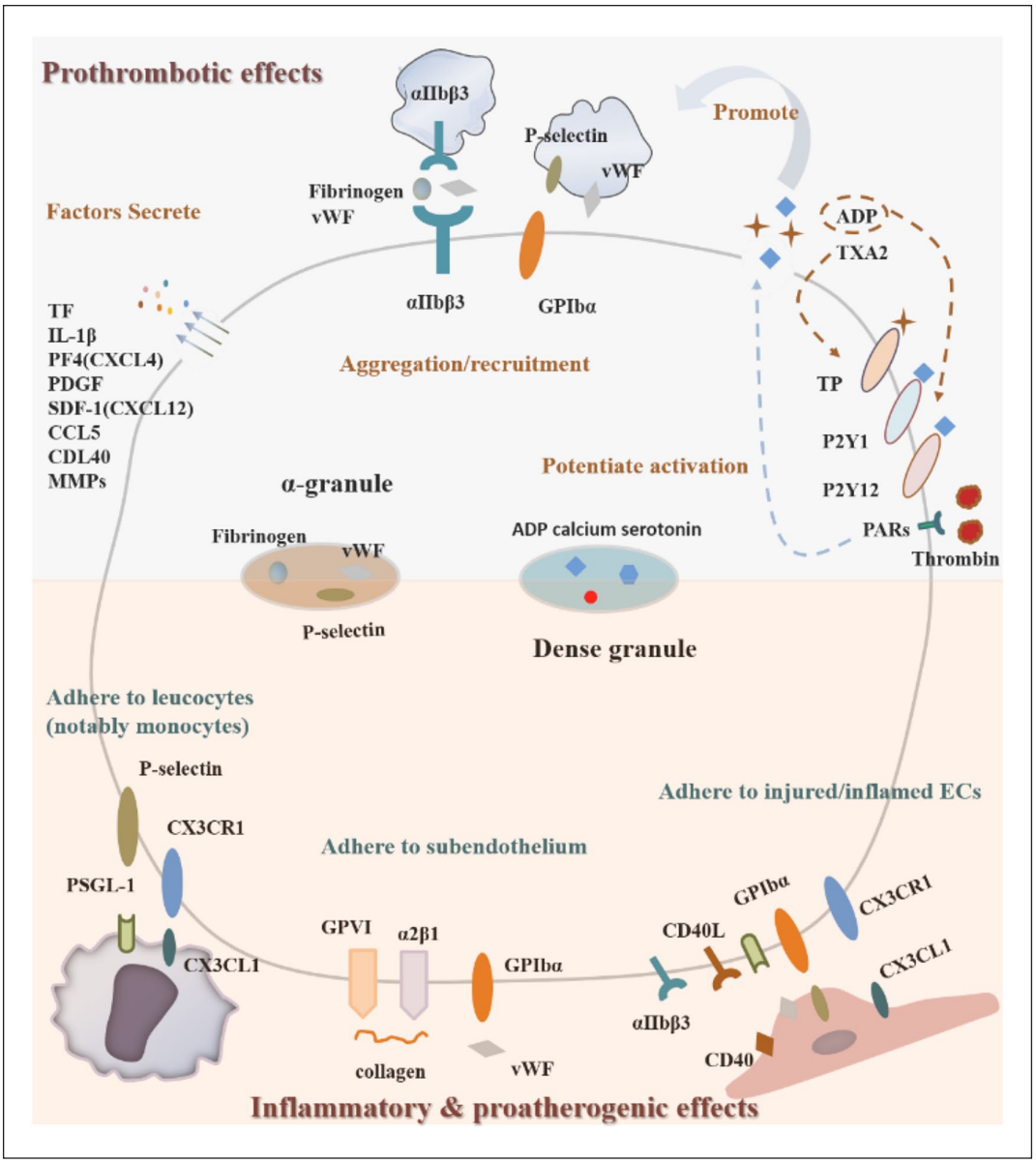

Figure 7. The major portion of platelets in growing atherosclerotic plaques and prothrombotic events. There are abundant membrane-anchored receptors for adhering to injured/inflamed endothelial layer (GPIbα for P-selectin and vWF, PSGL-1 binding to P-selectin, CD40L-CD40 interaction, CX3CR1 for CX3CL1), ruptured lesion (GPVIα and α2β1 for collagen, GPIb for vWF), monocytes/macrophages (P-selectin for PSGL-1, CX3CR1 for CX3CL1), and active platelets (GPIbα and αIIbβ3 grasp additional platelets). PAR1/PAR4 coupling thrombin and P2Y1/P2Y12 binding ADP quietly potentiate platelet activation. Thrombin binding PAR promotes TXA2 and ADP producing, in addition to this, more ADP generating has a lot to do with TXA2-bound TP receptors.[187]

yield. Hypothetically, blocking-up of platelets adhesion to bare collagen (via GPVI, α2β1), vWF (via GPIb) after plaque fracture and injured/inflamed endothelium (such as via GPIbα binding to P-selectin and vWF) could decrease vascular occlusion with effect. Currently, anti-GPVI treatment could be a powerful strategy to specifically passivate the collagen-GPVI pathway in platelets, reviewed by Nieswandt et al.[188] Meanwhile, other payloads have been employed as efficacious antithrombotic agents, containing assorted receptors inhibitors: glycoprotein IIb/IIIa inhibitors-abciximab, eptifibatide, tirofiban, and lamifiban[189]; ADP receptor antagonists-Y2P1 inhibitor A2P5P and P2Y12 inhibitors (AR-C69931MX, clopidogrel, ticagrelor, and prasugrel[190,191]); incapacitating thromboxane A2 receptor by thromboxane receptor antagonists, such as ifetroban, domitroban, variprost, and others, more details seen in Kontogiorgis's review.[192] Peptide (SP-14 with sequence SHIHGDYSSPSGAP) is also used to inhibit the binding of

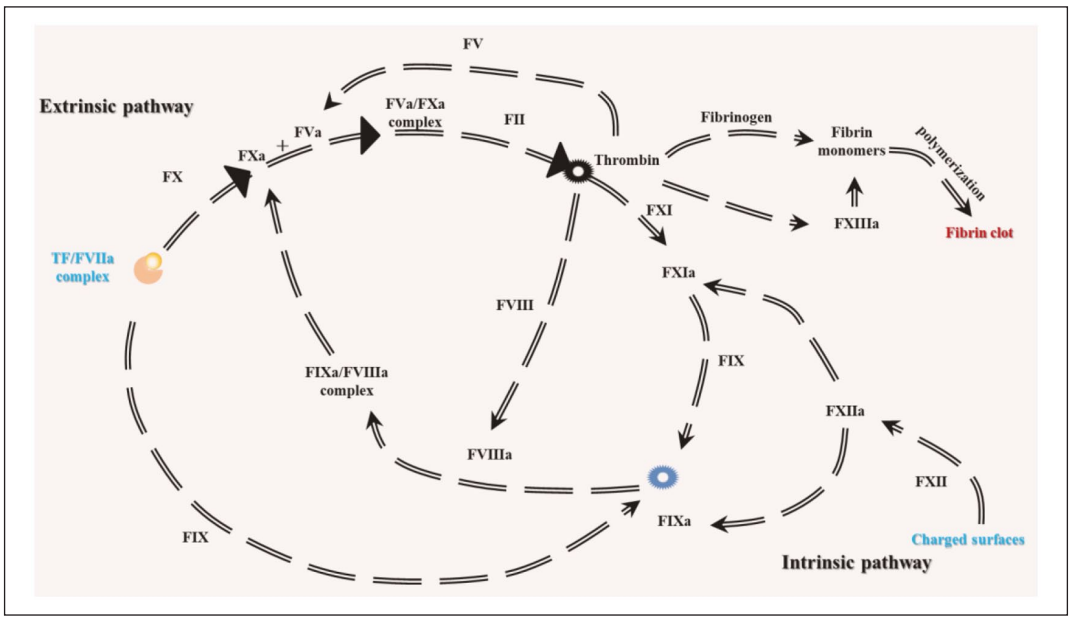

Figure 8. The extrinsic and intrinsic clotting pathway.[226] Ample TF combining coagulation factor FVIIa transforms FIX into FIXa, and FX into FXa, is closely linked to extrinsic and intrinsic coagulation pathway, finally meeting at bio-synthesizing thrombin and stable fibrin clot formation imbued with platelets and erythrocytes. More than TF-FVIIa pathway, FXII interacts with negatively charged surface or molecules, followed by forming FIXa and FXIa resulting from activated FXII (FXIIa), which means intrinsic pathway starts.

TXA2 to TP receptor for reducing platelet aggregation.[193] More than blocking receptors, extinguishing platelet autocrine factors (ADP, TXA2) production is also a potent strategy for opposing thrombogenesis, and in some cases, TXA2 biosynthesis could be blocked powerfully by aspirin targeting COX-1[194] or thromboxane synthase inhibitors(e.g. dazoxiben, dazmagrel, pirmagrel, isbogrel, and ozagrel).[192] TXA2 was produced via cyclooxygenase (COX)-thromboxane synthase pathway.[192] Later, bifunctional regulators with TP receptors blocking and thromboxane synthase inhibition were also exploited, including ribogrel, terbogrel, picotamide and BM-351/573, etc.[192] Not merely incurring platelet aggregation and activation, TXA2 may lead to plaque evolution and thrombus in human atherosclerosis, and infiltrated monocytes and macrophages in lesion make TXA2, which constitutes a significant source of TXA2.[195] Worthily mentioning, synergistic effect of blocking TP receptor and selectively suppression of TXA2 may have a positive impact on reducing plaque tissue.[196] Thrombin is a central part of forming fibrinous thrombus and platelet acting, and PAR-1 is the main thrombin receptor on platelets. PAR-1 antagonists have properties of antiplatelet and antiarterial thrombosis, and vorapaxar is a novel antiplatelet agent that selectively inhibits the cellular actions of thrombin through antagonism of PAR-1,[197] some other PAR-1 antagonists had been summarized by Chackalamannil.[165] A newly-found receptor, GLP-1R, may serve as an antiplatelet-target. For instance, GLP-1 and other GLP-1 receptor agonists (liraglutide, exenatide) treatment could hinder platelets aggregation and thrombosis induced by collagen and thrombin very likely through triggering of GLP-1R signaling for increased cAMP level.[198,199] Once platelets are in active state, PS flips to the outer side of the plasma membrane, exerting pro-coagulation activity with initiation and propagation of coagulation via formed TF/FVIIa complex and assembly of intrinsic tenase (FVIIIa/FIXa), prothrombinase (FVa/FXa) on PS-exposed platelets.[200] Based on this feature, outside PS owns both targeting properties and therapeutic capacities for platelets-containing thrombi. Competitive ligation of PS-binding ligands (such as lactadherin and annexin V) to PS inhibits platelet prothrombinase and factor Xase activity, effectively restrains FXa, and thrombin generation, which leads to delayed thrombosis formation.[201] According to the avidity of PS and annexin V, annexin V acted as a guiding molecules for selective targeting of platelet-containing thrombi.[202] Annexin V fusing curative stuffs, such as Kunitz protease inhibitor (KPI),[203] hirudin,[204] are shown to have thrombosis inhibitory activity with specifical PS affinity. Jing et al.[202] constructed disintegrin protein echistatin-annexin V system to obtain an antithrombotic effect via competitively binding to αIIbβ3 integrin resulting in reduced fibrinogen linking and platelet aggregation, and to PS molecules for dwindling prothrombinase complex and thrombin formation.

Coagulation factor as targets. Figure 8 represents the extrinsic and intrinsic clotting pathways. Antithrombotic modulations come in all shapes and sizes, mainly centering on neutralizing thrombogenic molecules through various proteins, antibodies, peptides, aptamers, oligonucleotide, and

other small molecules. Notably, targeting pro-coagulation factors gets attractive, including TF, thrombin, FXIIa, FXII, FXa, FXIa, kallikrein, and so on. Designedly making these coagulation factors invalid has been confirmed potently antithrombotic capacities. Concretely, targets and their corresponding inhibitors are showed, mainly delineated as: thrombin is a prime target, directly/indirectly inhibited by antithrombin activators(heparin), warfarin, hirudin and its derivatives (lepirudin, desirudin, bivalirudin), dabigatran, argatroban,[205] thrombin-specific aptamers (HD1 and NU172),[206] thrombalexin (TLN),[207] and avathrin[208]; aptamers (RNA_{11F7t} and $RNA_{BA}4$), rivaroxaban, apixaban, edoxaban, ACH-11, are effective for FXa blocking.[206,209,210] The factor Xa can excite platelets by PAR-1 pathway, so tempering FXa with rivaroxaban would be antiplatelet effect more than reduced atherothrombotic events through PAR-1 derived platelet activation.[211] Sterilizing intrinsic factors FXIIa, FXII, and FXIa are also prevention of thrombosis formation by: RNA aptamer R4cXII-1 targeting FXIIa and FXII[206]; Infestin-4 with FXIIa inhibitory effects[212]; BF9,[213] Boophilin,[214] and DEF (an mAb to FXIa)[215] for inhibiting FXIa as well as Ir-CPI (Ixodes ricinus contact phase inhibitor) binding both FXIIa and FXIa.[216] For preventing undesirable thrombotic events, some natural anticoagulants have also been investigated, including antithrombin, activated protein C (APC), tissue factor pathway inhibitor (TFPI), and protein S. In some examples, protein S, a regulator in intrinsic coagulation pathway, subdues FIXa and Xase complex (FIXa–FVIIIa) through tethering to FIXa heparin-binding exosite, thereby in keeping with lower FX activation.[217] And, protein S also activates protein C for suppressing FV into FVa and FVIII into FVIIIa.[206] TFPI, found in endothelial cells and platelets, plays an anticoagulant part in early stages of clotting cascade and regulates tissue factor (TF)-induced coagulation via inactivating FXa and binding to FVIIa of TF/factor VIIa complex for inhibiting further FX activation.[218] As a cell-signaling receptor, TF also keeps a direct proinflammatory role for macrophages/SMC and promotes migratory and mutagenic effects of SMCs, so that TFPI has protective effects against MA/SMC inflammation and SMC migration and proliferation in addition to abating thrombus formation.[219–221]

Coagulation holds a vital position in the onset of atherosclerosis, on good grounds, depriving effects of coagulation factors, beyond preventing sequential thrombotic events and abolishing subsequent occlusive arterial thrombus formation, may also be remarkably effective in impeding atherosclerotic progression or postoperative restenosis. Nationally, targeting coagulation factor Xa or thrombin has been elucidated as a promising treatment for holding back plaque starting and propagation or injury-induced neointima formation, and promoting lesion stability and plaque regression.[210,222,223] Posthuma et al.[210] corroborated and extended that, except for inhibiting newly-formed plaque and increased stability of brittle plaque, FXa inhibition by rivaroxaban also facilitated regression of pre-existing atherosclerotic lesion with reduced macrophages, enhanced collagen deposition, diminished necrotic core, lower expression of PARs, thrombin, FXa, and MMPs. Some other researchers had also made it clear that direct thrombin inhibition by anticoagulant dabigatran improved endothelial function, reduced atherosclerotic lesion size, collagen content, and oxidative stress (ROS production),[224] retarded the initiation and progression of lesion and macrophage accumulation in Apolipoprotein E-deficient mice.[225]

Important ROS generation system

Reactive oxygen species (ROS) participates in the modulation of cell functions and biological processes for promoted atherosclerotic progression, shown as oxidative modification of lipoproteins, inflammatory response, EC dysfunction and SMC proliferation and phenotypic switching, promoted cell death, etc.[227] Scavenging of generated ROS and arresting of its emergence have been considered potential for counteracting atherosclerosis. ROS-scavenging polysaccharide β-cyclodextrin (TPCD) nanoparticles with Tempol (a free radical scavenger) and phenylboronic acid pinacol ester (PBAP, for eliminating hydrogen peroxide effectively) evidently have inhibitory effects for atherosclerosis development by elimination of overproduced ROS, which led to diminished ROS-induced inflammation and apoptosis in macrophages, and inhibited foam cell formation.[228] And, NADPH oxidases (NOX) and renin-angiotensin systems are important sources of ROS in the cardiovascular system, shown in Figure 9. NADPH oxidases (NOX) system exists in nearly all plaque cell types including EC, SMC, MA, monocytes, and platelets.[229,230] NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase) may serve as a significant target. Neutralizing agents targeting NADPH oxidase, such as antibody, peptide or siRNA have been demonstrated to attenuate neointimal formation after arterial injury.[231] Nanoparticles loaded by siRNA targeting NOX2 were transferred into balloon injured artery in an atherosclerotic rat model, which would prevent neointimal area and lumen loss.[231] An available Nox inhibitor VAS2870 could inhibit ROS liberation induced by PDGF in SMCs and abolish PDGF-guided SMC migration effectively.[232] The importance of Ang II in ROS formation in renin-angiotensin system had been stressed over recent years. Blocking Ang II-induced ROS generation involves two parts: abolishing of Ang II biosynthesis (Ang II biosynthesis means that angiotensinogen is cleaved by renin to form angiotensin I, and subsequently generated angiotensin I is converted into Ang II under angiotensin-converting enzyme (ACE)[233]); blocking the interaction between Ang II and AT1R. For combating oxidative stress, the

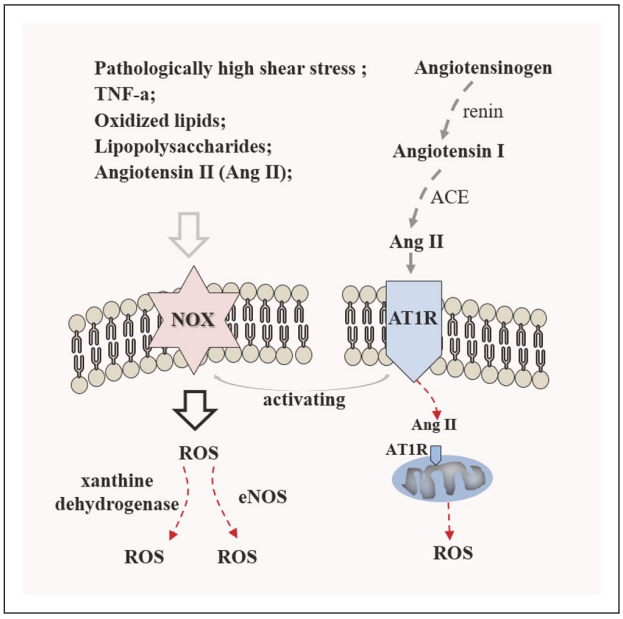

Figure 9. The NOX-derived ROS pathway and important renin-angiotensin system. The mediators of NOX activity and expression are involved in excessive proatherogenic factors, such as pathological shear stress, TNFα, PDGF, oxLDL, and important angiotensin II (Ang II).[237] By renin-angiotensin pathway, generated Ang II linking to angiotensin type I receptor (AT1R) activates membrane-bound NADPH oxidase in ECs, SMCs, and macrophages,[227] and it also traffics to AT1R on outer mitochondria membranes, inducing mitochondria-derived ROS.[236] Importantly, NOX-derived ROS aggravates ROS overproduction by acting on eNOS and xanthine dehydrogenase.[230]

synergism of ACE inhibitor and AT1 receptor blocker had been observed.[234]

More than inducing ROS generation for inflammatory expression, synthetic SMC, EC dysfunction as well as cell apoptosis, Ang II-AT1R interaction guides apoptosis by Fas pathway, synthetic response of SMC by JAK2-STAT or JAK2-ERK1/2 pathway,[227] and stimulates angiogenic action for enhanced plaque neovessel formation,[235] which all resulted in aggravated atherosclerosis. Wu et al.[235] considered that renin inhibitor aliskiren, suppressing angiotensin II (Ang II) biosynthesis, reduced the atherosclerotic plaque area and plaque neovessel density, even to the extent of decreasing the vascular inflammatory action. An absorbing point in Ang II is that activation of Ang II is involved in all phases of atherosclerosis, presented as stimulating TF expression, resultant endothelial dysfunction, promoting to form foam cells via regulating LOX-1 expression (SMCs) and contributory oxLDL absorption, inducing apoptosis of ECs and SMCs.[236] Thus, blocking-up in Ang II-AT1AR (Angiotensin II Type 1A Receptor) could be attainable tactics for disposing of atherosclerosis through antagonizing AT1AR or lessoning Ang II.

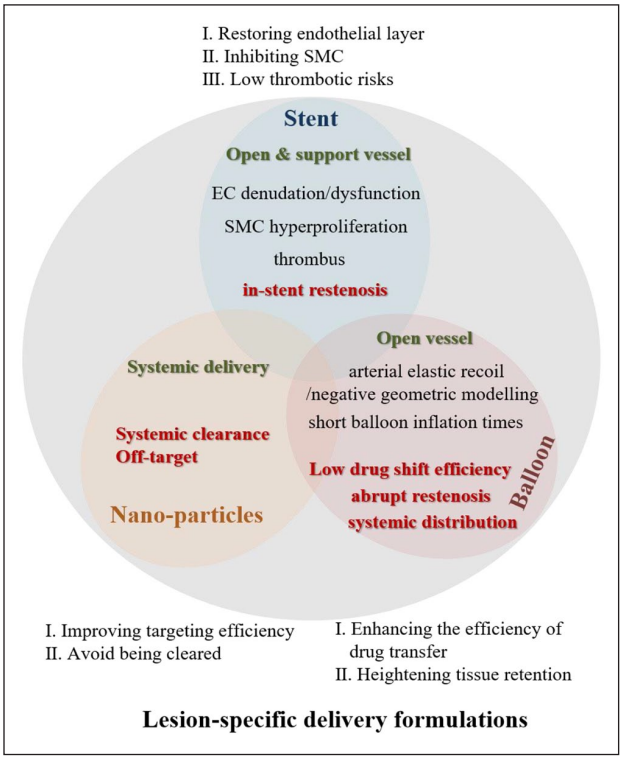

Figure 10. The schematic representation of the main lesion-specific delivery formulations.

Strategies of lesion-localizing delivery for treating atherosclerosis

Site-specific treatments of atherosclerotic plaque, relating to inhibition of restenosis and thrombus formation or modifying plaque with less prone to rupture/grow and promoting plaque regression, demand therapeutic agents to collect to the target lesion and into the vessel wall and cells. The section mainly states the tactics of effective loading enriched in atherosclerotic lesion, like Figure 10, including balloon catheter-driven local delivery, stent-based, and nanoparticle models.

Systematic delivery mode by nanoparticles

Bio-recognition based on natural ligand-receptor interaction. Biomarkers including changed cells (proinflammatory MA, inflamed EC, and switched SMC), biological factors and ECM components (collagen, vWF, fibrin) as well as deposited clots in plaque have been applied for the location of nanoparticles with therapeutic benefit in atherosclerosis. Bio-recognition based on natural ligand-receptor interaction may work as a potent formulation and resident cells in lesion contribute to this procedure. Currently, all kinds of ligands for targeting treatments embrace antibodies, peptides, aptamers including RNA- or DNA-based ligands. Growing evidence demonstrates that scavenger receptors highly express in atherosclerosis and

targeting these molecules with affinitive ligands (like decadeoxyguanine[238] and DNA oligonucleotides[239] for SRA and anti-LOX1 antibody for LOX-1) could make therapeutic drugs get to lesion site.[94] Fasudil, a rho-kinase inhibitor, is capsulized into liposomes embellished with anti-LOX1 antibody and successfully reaches the arterial plaque with inhibited intimal hypertrophy.[94] Based on leukocytes binding to endothelial ICAM-1 by LFA-1 (lymphocyte function-associated antigen-1) integrin, inflamed leukocyte-mimetic nanoparticles with LFA-1 I domain preferentially reach the site of inflammation.[240] Antibodies[241] and peptides targeting ICAM-1 (cyclo(1,12)PenITDGEATDSGC peptide[242]) and VCAM-1 (VHPKQHR[243] and ^{18}F-4V peptide[244]) also have emerged. More than targeting to inflamed endothelium, a specific sequence with VHSPNKK motif also blocks the interaction between leukocyte and endothelial layer.[28] Furthermore, targeting the underlying basement membrane had become another important point, such as KLWVLPK peptide binding to collagen.[245] Nanoparticles coupling with KLWVLPK peptide for paclitaxel delivery exhibits greater vascular retention in vivo.[245] And this particles system also transports IL-10 to atherosclerotic plaques for deactivating macrophages and T cells and resolving acute inflammation.[246] Modery's group[247] had designed RGD and EWVDV peptides-modified liposomes to target active αIIbβ3 and P-selectin respectively, and manifested these particles had higher selectivity as well as retention to activated platelets. The CREKA peptide can mediate nanogels loaded by recombinant hirudin to get to fibrous clots, winning anticoagulant therapy by binding to fibrin and fibronectin.[248] Based on high affinity of CREKA for fibrin as well as abundance of fibrin and H_2O_2 in thrombi, Kang et al.[249] developed a fibrin-targeted nano-platform composed of tirofiban (a glycoprotein IIb/IIIa receptor inhibitor), FBAP for H_2O_2-scavenging, BAP with H_2O_2-response, and proved its antioxidant and antithrombotic activity. p32(gClqR), also named as p33, p32, C1qBP, HABP-1, is biosynthesized on the surface, in cells and as a secreted protein. Sharply inflammatory region and atherosclerotic plaque, exist in violently activated cells including active platelet (or collagen-induced aggregation), inflamed endothelial cells as well as inflammatory answering cells (macrophages/monocytes), foam cells and smooth muscle cells, highly expressing p32, in return, p32 activation irritates these cells to be active.[250,251] Sufficient grounds have been available as the affinity interaction of LyP-1 (a 9 residues peptide, CGNKRTRGC) and p32. LyP-1-carrying nanoparticles exposed enhanced affinity to macrophage in vitro and converged at carotid lesions in vivo.[252] LyP-1-coated nanoparticles could penetrate the plaque to advantage, substantially accumulate in the plaque interior not at the surface of the plaque.[253] LyP-1 liposomes loaded by GW3965 also locate to atherosclerotic plaques and cut down the number of macrophages.[254]

Biomimetic targeting strategies

Membrane cloaking derived from platelet and macrophage. Platelets participate in inflammatory response and clot formation in atheroma through reacting with activated endothelial layer, subendothelial layer (collagen and vWF), or leukocyte.[7,255] Considering this intrinsic affinity of platelet to plaques, mimicking of platelet morphology, size, flexibility, and surface biology has been followed with interest.[256] In one example, Anselmo's group took nanoparticles and PAH/BSA as template and flexible shell separately to imitate the morphology and mechanical flexibility of platelets, followed by bearing the collagen-binding peptide (CBP; $[GPO]_7$), vWF binding peptide (VBP, TRYLRIHPQSQVHQI) and integrin GPIIb-IIIa linked FMP peptide (GRGDS, linear fibrinogen-mimetic peptide) for limited biological functions, and validated that this mimetic particles coupled to activated natural platelets and injured endothelial sites.[256] As a novel interfacing approach, the application of cell membrane has many natural advantages based on its multiple biological properties and functions, such as immunosuppression and selective recognition.[257] On treatment and checking, platelet membrane cloaking maintains inherent platelet properties with immune evasion (originating from CD47), suppressed complement system activation (due to CD55, CD59), localizing to clotting wall of atherosclerosis or angioplasty-induced denuded/injured vessels (on account of GPIb-IX-V, GPIa-IIa, and GPVI).[258,259] Homing efficacy of platelet membrane coating to plaque may give the credit to their membrane proteins, such as GPIb linking to vWF, GPVI and integrin α2β1 (GPIa/IIa) tying to collagen, GPIIb/IIIa, and GPVI fixing to fibrin.[260] At present, except for detection of the condition,[261] growing evidence confirmed that platelet membrane modification significantly elevates therapeutic effects for atheromatous plaque, ascribing to more effective homing capacity to the focus.[262] Encapsulation rapamycin with platelet membrane-coated nanoparticles effectively homes to plaques in atherosclerotic mice modes, and evidently weakens the progression of atherosclerosis.[262] In another example, GSK2606414 (PERK inhibitor)-loaded nanoclusters were coated by platelet membrane, which thwarted phenotypic modulation of SMC and EC dysfunction, mitigated restenosis, and thrombosis in the rat model of carotid artery balloon angioplasty.[43] Absorbingly, platelet membrane fused by other counterpart of selective cell would carry more perfect properties combining various function of both source cells.[263]

Termed macrophage naturally homing to the inflammatory plaque, such as macrophage membrane with high expression of α4 integrin reacting with the vascular cell adhesion molecule-1 (VCAM-1),[264] macrophage membrane embellishment has been applied to target and solve atherosclerosis.[265] Given that atherosclerosis is characterized by inflammation and ROS overproduction, Gao et

al.[266] prepared macrophage membrane coated ROS-responsive nanoparticles (NPs) for improved therapeutic efficacy in atherosclerosis with reduced inflammation and plaque burden, owing to specific targeting of inflammatory site as well as tied and sequestered multiped pro-inflammatory substances by membrane antigens (TNFR2, CD36, and CCR2).

Lipoprotein modalities (mimicking HDL). Lipoproteins, innate plasma particles transporting lipid, are commonly classified as five groups according to their density and size: chylomicrons, high-density (HDL), low-density (LDL), intermediate-density (IDL), and very low-density lipoprotein (VLDL).[267] HDL exerts to remove excess cholesterol from cells (e.g. foam cells/macrophages in atherosclerotic plaques), reduce inflammation, and improve endothelial function. HDL has certain key features, including: nano-diameter with 7- to 13-nm range; a hydrophobic core of predominately cholesteryl esters covered by phospholipids monolayer with embedded apolipoproteins, especially apolipoprotein (apo) A-I[268]; bio-functions involving cholesterol carriage, anti-inflammatory effects, antimicrobial activity, anticoagulation, and specifically targeting several cell surface receptors (such as scavenger receptor B1, ATP-binding cassette A1, and ABCG1 for removing the excess cholesterol from cells).[269–272] Considering HDL's nature with inherent plaque affinity, forming "HDL-like" nanoparticles by simulating its structure and elements is now contributing to targeting treatments.

Rather than achieving the characteristics of HDL itself, recombined HDL with or without decoration is used for a novel potent transit system for agents directly entering into plaque. HDL is naturally marked by binding to and interacting with macrophage cells and hepatocytes during reverse cholesterol transportation, which has an important protective effect in atherosclerosis by reverse efflux of cholesterol from plaque macrophages.[273] In Cormode's study, inorganic nanocrystal replacing the hydrophobic core of HDL incorporated phospholipid to come into being micelles. Whereafter, for producing HDL-like nanoparticles, apo A-I, the primary protein constituent of HDL and providing targeting abilities, had been used as a working component. Their research results validated that these artificially analogous particles to natural HDL possessed the capacity of being specifically ingested by macrophages with high affinity.[274] As a proof-of-principle study, these nanocrystal core HDL had lodged in the atherosclerotic wall in mice pathological model. In addition, the targeting abilities of imitated HDL particles to inflammatory artery had been verified in a wide of investigation, and these particles could be used as a carrier for therapeutic drugs or imaging agents. Marrache and Dhar[275] developed the biodegradable synthetic HDL mimic containing hydrophobic core, and a phospholipid bilayer coat decorated with triphenylphosphonium (TPP) and apolipoprotein (apo) A-I mimetic 4F peptide as a vulnerable plaques targeting agent to lodge in atherosclerotic areas, markedly in mitochondria. Lameijer et al.[276] used HDL biomimetic particles to successfully transport small molecule 68770028 (a CD40-TRAF6 inhibitor) to plaque, gaining immunotherapy with impaired monocyte migration and recruitment and reduced plaque macrophage content. 4F peptide-modified nanosystem also was applied to successfully home GW3965 to plaque, which reduced plaque burden without increased liver toxicity.[277] Artificial HDL particles have limited cholesterol efflux ability, tailoring artificial HDL properties by HA recognizing overexpressing CD44 receptors in injured endothelial, would exhibit anti-atherogenic effects with a greater cholesterol efflux capacity and better targeting efficiency.[278] Luthi et al.[279] unveiled that orienting size and surface composition of high-density lipoprotein (HDL) biomimics altered the biomimic-mediated the cholesterol binding capacities and efflux cholesterol from macrophage cells.

Apoptotic mimicry. "Eat me" signals of apoptotic cell are composed of phosphatidylserine (PS), intercellular adhesion molecule-3 (ICAM-3), carbohydrates, and calreticulin, selectively recognized by phagocytes via phagocytic receptors.[280] On account of phagocytic ligand-induced effective recognition and engulfment, apoptotic bionics equipped with "eat me" signal molecules could target to the inflammatory macrophages in atherosclerosis and PS equipment has been widely concerned.[281] Naturally occurring membrane molecular alterations is ubiquitous in the process of apoptosis. Significantly, phosphatidylserine (PS) abandons membrane asymmetry and flips to the surface of apoptotic cells, and externalized PS is the best representative "eat me" signal for macrophages recognition to remove dead cells.[282] Conversely, PS can pass for a "tethering" ligand for macrophages. Zhao et al.[283] prepared PS-modified microbubble and proved its targeting capability to activated macrophages. Wu et al.[284] designed the biomimic liposome embellished with cRGDfK and PS to delivery pioglitazone (PIO, a PPARγ agonist) into atherosclerotic macrophages, which kept macrophages weakened the release of IL-1β and TNFα, and strengthened the secretion of anti-inflammatory substances (such as 1L-4 and IL-10) with attenuated progression of atherosclerosis. The cRGDfK modification facilitated liposomes to fasten onto activated ECs in plaque and penetrate into plaque, followed that intra-plaque particles were intendedly discerned and absorbed by macrophages owing to PS signal. Sometimes, PS also articulating other phagocytic ligand, such as oxidized cholesterol ester derivative cholesterol-9-carboxynonanoate, co-mediated inflammatory macrophages targeting.[285] Significantly, Hosseini et al.[286] gave proof that phosphatidylserine liposomes (PSLs) simulating apoptotic cells attenuated atherosclerosis by targeting B1a cell activation. Another hot spot, the growing evidence has

been certificated that specifically targeting PS could also act as delivery system for imaging and treating agents, and several frequently-used PS affine ligands have been used in this policy, including annexin V,[87] lactadherin or its PS-binding C domains-fused protein,[287]and some PS-recognizing peptides PSP1[288] and LIKKPF.[289]

Interventional device-based delivery approaches to lesion

Stent as coping style of local delivery. As an intervention program of occlusive atherosclerotic arteries, stent opens vessel via balloon expansion and maintains its original tubular shape for remodeling blood flow. From the earliest bare metal stents (BMS) to current drug-eluting stents (DES) and bioabsorbable stents (BRS), the development of stents has been in line with clinical outcomes.[290] After stenting, vascular injury response (endothelial disruption, overgrowth of SMCs) often favors re-narrowing of treated arteries. Surmounting in-stent restenosis is a long-term goal in the field of stent technology. Compared with permanent one, novel bioabsorbable stents degrade naturally with vascular healing, which requires the stent degradation rate to match the mechanical properties for supporting artery and velocity of mending. Considering this, for BRS, regulating degradation rate should be paid close attention. In this part, eternal stent would be uniquely reviewed. For stent-guided local transport, coatings on stents clearly occupy an important position at performance. From the earliest bare metal stent to the current coated stent, investigators have indicated that coated technologies endued stent more exceptional service properties, such as gold-coated NIR stents.[291] In efforts to coat metal stents, drug-eluting designs, bionic tactics with virous functional factors have been available.

Drug-eluting designs. As a drug carrier platform, stent systems are expected to achieve high local drug concentration with low systemic toxicity and long actuation duration. Therapeutic agents and their loading systems are two important research directions. A large number of clinical drugs or ones under ongoing trails had been reviewed, involving in anti-inflammatory, antiproliferative, and immunosuppressive drugs, including sirolimus and its analogs (e.g. everolimus, zotarolimus, biolimus A9), paclitaxel, dexamethasone, batimastat, 17-beta estradiol, actinomycin D, or tacrolimus.[292,293] Antiplatelet drugs also have been being under investigation. Ticagrelor, a P2Y12 receptor inhibitor, is coated onto stent. More than affecting platelet, the ticagrelor eluting stents preserves endothelial recovery and favors suppressive smooth muscle proliferation.[294] The drug release profile largely determines the overall performance of stents in a variety of evaluation models.[291] Polymers, synthetic or biological polymers, acting as versatile basal layers demanding to provide good biocompatibility (blood or tissue), generally are utilized to incorporate pharmacologic agents for mediating drug release behavior, covering poly(ethylene-co-vinylacetate), poly(n-butylmethacrylate), poly(styrene-b-isobutylene-b-styrene) fluoropolymer, phosphorylcholine, poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA).[295] Additionally, biological molecules, hyaluronan (HA) and chitosan (CH) polyelectrolyte multilayers[296] or phosphorylcholine,[297] also may be potential candidate polymers for local drug delivery. Adequate coating techniques are essential to acquire excellent drug loading and releasing. Dipping, spray, electrospunning nanofiber[298] as well as hydrogel[299,300] coating have been developed. Nevertheless, increasing researchers held the view that polymer coating could answer for SMC proliferation, endothelial incompetence, proinflammatory responses, and thrombotic events.[301] In this regard, polymer-free drug-coated stents have been seen. Considering drug-binding matrix without polymer, some stent developers had exploited nitrogen-doped titanium oxide (N-TiO2) coatings for drug elution, and found that this polymer-free everolimus (EVL)-eluting stent prevented the platelet adhesion and restenosis, compared with bare metal stent.[302] Some novel smooth muscle-sensitive drugs are essential, as drugs against SMC proliferation often inhibit EC proliferation and migration without distinction, which leads to a detrimental effect on successful vascular repair. CTP synthase1 inhibitors and PERK inhibitors exhibit cell-specific inhibitory effects on VSMC not ECs.[43,303] Tang et al.[304] demonstrated that cyclopentenyl cytosine (CPEC), a CTP synthase inhibitor, promoted contractile SMC showing pro-angiogenic property, and lessened neointima formation after injury, while accelerating re-endothelialization. Of note, CPEC-induced SMCs energized proliferative and migratory effects of EC via a pro-angiogenic paracrine effect.

Enhancing or restoring the normal function of cells populating lesion. The health and continuous endothelium occupy an important position in regulating local hemostasis, thrombogenesis as well as VSMC proliferation, particularly at the injured locus post-stenting. Meddling endothelial cell selectively and specifically for promoting ECs repopulation has been presumed to be a rational choice to boost vascular repair and cripple neointima formation after stent placement.[305] Generally, coating stent with biological factors, such as proteins (antibodies, ECM proteins), cytokines and chemokines, gas signal molecule (nitric oxide, NO), or adapters (DNA, RNA, and peptides) for joining stent surface with the endothelial wall, has been utilized to make an increment in attachment, migration, vitality, and function of endothelial cells. As the native microenvironment provided by ECM, multifarious ECM-derived proteins, active peptide sequence, or short fragment of protein have been surfaced onto stent for improving endothelial cell, covering fibronectin (FN)[306] or FN+ phosphatidylcholine,[307]

fibrinogen,[308] laminin,[309] collagen,[310] vitronectin,[311] and their derived peptide: fibronectin fragments RGD, REDV, GRGDSP, and PHSRN; YIGSR, PDSGR, LRE, IKLLI, and IKVAV enriched in laminin; and DGEA and CAG from collagen type I[312] and collagen IV[313] respectively. Furthermore, other types of peptides also have emerged. SVVYGLR, synthetic peptide with binding integrins such as α4β1 and α9β1 and mimicking VEGF, enhances EC adhesion, migration, and maintains ECs proliferation.[314,315] Immobilizing P8RI (a soluble synthetic peptide, like a CD31 agonist) onto stent could favor vascular endothelium repair, prevent in-stent stenosis and thrombosis.[316] Other than those enhancements, biological factors can provide reinforced cues for positive effects in inducing ECs repopulating and function. Chemotactic/growth factors including CXCL1,[317] SDF-1, VEGF,[318] chemokine (CC motif) ligand 2 (CCL2) and insulin-like growth factor-1 (IGF-1),[319] NGF,[320] hepatocyte growth factor (HGF),[321] sphingosine 1-phosphate (S1P),[322] erythropoietin or recombinant erythropoietin[323] have been applied to thrust complete endothelial covering via augmenting attachment, proliferation, migration, or nitrogen monoxide (NO) production. Noteworthily, NO releasing from ECs naturally plays an important role in anti-thrombosis and anti-restenosis through inhibiting excessive proliferation of smooth muscle cells, propagating wall endothelium, and deterring adhesion/aggregation of platelets. Justifiably, covering materials with NO-producing layers has caught hold of attention of researchers. At present, NO transportation platform mainly includes NO-releasing materials (involving NO donors like NONOates, SNAP and RSNOs, and NO-releasing peptide like the sequence GTAGLIGQ linked with polylysine KKKKK[324]) and NO-generating strategies (covering loading of catalyzer selenocystamine, CuII and NOS gene, eNOS and iNOS).[325–327] Fixation of antibody can work as another potent candidate, as antibody fragments scFv, single chain fragment variable binding to VEGFR2 mediating VEGFR2-positive ECs to adhere and reproduce on the stent surface.[328] Meanwhile, burgeoning gene therapies also serve as the encouragement of migratory and proliferative effects of vascular endothelial cells, such as ZNF580 gene,[310] DNA encoding for human VEGF[329] and CD39-encoding mRNA.[330]

Responding to mechanical injury (e.g. stenting, balloon injury) and pathological stimulus (e.g. growth factor PDGF, inflammatory cytokines TNFα), activated SMCs evinces augmented proliferative and migratory activities associating with their de-differentiation, which induced the narrowing of vascular lumen or neointima formation in stent.[331] For SMCs in abnormal state, some efforts have been done. In some cases, as inhibiting SMC activity potently and stimulating EC proliferation modestly, perlecan, and a perlecan-inducing compound (e.g. RUS3108, inducing perlecan synthesis in SMC) may be used for stents to prevent ISR (in-stent restenosis).[332] And, through regulating SMC phenotypic modulation, diminishing in restenosis after stenting is possible. In light of synthetic smooth muscle phenotype featured with active RhoA expression, Huang et al.[333] built RhoA inhibitor-eluting stent with rhosin loading, and subsequently demonstrated that this RhoA inhibitor down-regulated YAP (Yes-associated protein) expression in SMC phenotypic modulation, which attenuated neointimal formation. Furtherly, selective Sp-1 inhibition with Mithramycin A also abrogates partly restenosis via disturbing YAP-mediated SMC phenotypic modulation.[334] And, sorafenib couples to myocardin by competing with YAP for increased SRF-myocardin interaction, which modulates SMC phenotypic switching and attenuates in-stent restenosis.[335] Blocking signal transduction of PDGF-PDGFR pathway works as another candidate. PDGF receptor (PDGFR) tyrosine kinase inhibitor, Sunitinib, targets SMC proliferation, necrosis of SMCs and migration, mediating reduced neointimal formation after stenting.[336] Stent-based delivery of D-65495, a bis(1H-2-indolyl)methanone, another PDGF-receptor inhibitor, could cause diminished neointima formation.[337] Through jamming the G0/G1 cell cycle and PDGF receptor β-Akt pathway, statins prevent SMCs from the reception of PDGF-BB signal for inhibited the pathological proliferation and migration of SMCs. Rosuvastatin inhibits the smooth-muscle-cell phenotypic modulation in PDGF-BB-induced synthetic SMC model and stents covered by heparin and rosuvastatin can reduce the incidence of stenosis and late thrombosis.[338]

As answering environmental stimuli (i.e. ox-LDL), macrophages in plaque could convert their polarized forms to M1 macrophage, which would release pro-inflammatory factors and reactive oxygen or nitrogen species. Inevitably, a corresponding M2 macrophage also have been deemed as immunosuppression and eliminating inflammation of lesion.[339] Given phenotypic tunability of macrophages, a novel formulation transforming inflammatory state of plaque macrophages to anti-inflammatory M2 macrophages might be used to treat atherosclerosis. To name a few, Zhao et al.[340] constructed nanospindles with TiO2 and Ti4Ni2O on NiTi alloy, which manipulated macrophages to produce a favorable immune microenvironment with TGF-β, BMP-2, and VEGF for enhancing the functionality of the ECs. Similarly, Xu and co-workers[341] considered that induced M2 macrophage polarization could secrete VEGF for promoted endothelialization through ERK1/2 and PI3K/AKT pathways.

Capturing new cells or guiding neighboring ECs to injury site. Trapping circulating cells (covering ECs, EPCs, and SMCs) from blood flow is an alternative option of winning self-endothelialization. Endothelial progenitor cells (EPCs) are generally defined as a group of cells featuring differentiation into mature endothelial cells,[342] which have great application potential to expedite reendothelialization

and limit intimal thickening in stent usage. Coupling of the stuff possessing excellent ligand accessibility to receptors on EPCs to stent has succeeded in self-endothelialized implants, including antibodies and peptides. In one example, linked CGRGDS peptide onto materials could harvest ECFCs and HBOECs under shear stress, which has the ability to grow into successive worked endothelial layer in vivo.[343] Recently, antibody-immobilized stent has been designed to catch EPCs (particularly late stage of EPCs) efficiently and specifically. Park et al.[344] unwrapped that stents integrating anti-CD146 antibody could capture more late-EPCs as well as mesenchymal stem cells (MSCs) in vitro. More biomarkers of EPCs have emerged in this research field, touching upon CD34,[345] CD133,[346] vascular endothelial-cadherin (VE-cadherin),[347] CD146,[344] endoglin,[348] vascular endothelial growth factor type 2 receptor (VEGFR2),[328] CD144, and CD309.[349] Fixation of antibody for bounding these biomarkers onto materials could potently attract these cells to pathological sites. Antibody fragments scFv, single chain fragment variable binding to VEGFR2 can be utilized to guide attachment and multiplying of VEGFR2-positive cells covering circulating ECs and EPCs.[328] What's more, specific peptide ligands also have been defined, involving integrin-combined peptide ligand, such as αvβ3-integrin-binding peptide cyclic Arg-Gly-Asp peptide[350] and LXW7 (an octamer disulfide cyclic peptide (cGRGDdvc)).[351] In the example, LXW7 potently and specifically immobilized to αvβ3-integrin of EPC/EC, but presented weaker binding to platelets and no binding to THP-1 monocytes. A 12-mer peptide, termed as TPS (TPSLEQRTVYAK), an EPC-specific ligand, also unfolds high affinity and specific binding ability for human endothelial progenitor cells (EPCs).[352] WKYMVm (Trp-Lys-Tyr-Met-Val-D-Met), a hexapeptide, according to Li's group,[353] possessed a fixed characteristics showing up remarkable EPC-induced recruitment for vasculogenesis guided by activating the formyl peptide receptor. Newly, HGGVRLY, hemocompatible peptide-1 (HCP-1), α4β1 integrin ligand, a recently defined stem cell homing peptide, could combine with EPCs and boost them spreading, which confirmed by Hsu et al.[354] Going further, HGGVRLY also fastened a high proportion of BMSC (bone marrow stromal cells) having VCAM-1. In other investigations, HCP-1-immobilized surface was very productive in gaining mitigated thrombus by lowing platelet adhesion and enhanced endothelial cell adhesion.[355,356] Another α4β1 integrin ligand, REDV displaying peptide sequences Arg-Glu-Asp-Val, has been recognized as optionally and effectively catching endothelial cells (ECs) in fluid shear stress.[357] Markedly, for mesenchymal stem cells, another promoter cell, incremental evidence had argued that MSCs possessed inhibitory potential to restenosis via boosting re-endothelialization[358]and anti-inflammatory effects of SMC promoted by paracrine factors from MSCs.[359] Additionally, DNA aptamers[360] also could act as potent cell-bonded molecules, especially for harvesting self-seeding of EPCs onto vascular stent. More than promoted migration and proliferation for ECs, ZNF580 or VEGF gene also served as an attractor of EPCs in circulating blood.[310] Another type of candidate, introduction of growth (NGF, nerve growth factor)[320]/chemotactic CXCL1 (CXCL1 binding his receptor CXCR2)[317] factors on stent had been investigated in homing circulating EPCs.

Balloon catheter-based systems. Balloon catheter-driven local delivery comes to the foreground for lesional trafficking in recent years. Based on existing circumstances, compared with old plain balloon, apart from opening artery and improving blood flow, coated balloons could carrier a sufficient dose of an effective payload, for example paclitaxel, to the lesional tissue. Upon the balloons inflated, the drugs, adhering onto balloon membrane or hidden underneath the folds wrapped around the shaft, are pushed into the wall of arterial blood vessel and surrounding tissue.[361] The loss and transfer to the wall can be balanced by designing physical surface features or coating formulation of the balloons. For coating balloon, some elements would be appropriately added, such as medication for treatment, an inactive excipient with aiding in drug transfer. More often, balloon surface would be also designed, such as micro-porous. Furthermore, designing balloon catheter itself has emerged as a novel approach of local liquid delivery to achieve uniform agents into vessel wall, such as occlusion perfusion catheter. Occlusion perfusion catheter (OPC), a catheter containing three balloons, comprises two compliant occlusion balloons defining the treatment chamber at both ends and a center space occupying balloon (treatment chamber) through which the therapeutic agent would be infused. Perfusion catheter opens a direct manner of delivering payloads into medial artery for handling restenosis.[362] Bunch et al. exploited the occlusion perfusion catheter as an actual drug delivery catheter to locally deliver liquid paclitaxel to the stenotic lesions and assessed the feasibility, safety, and efficacy of this tactic for disposing of restenosis. Ultimately, they demonstrated that liquid paclitaxel treatment using occlusion perfusion catheter could be safely and effectively prevent restenosis.[363] In this part, therapeutic ingredients coated balloons will be introduced mainly.

Effective payloads. Lesion-homing delivery via active principle (like antiproliferative drug, gene, antithrombotic agents as well as cells) laden balloon may occupy the crude preponderance with leaving no foreign bodies in the blood vessel in management of in-stent restenosis and de novo lesion in small-vessel. For remedying atherosclerotic arterial disease, drug-coating balloon has emerged as an available alternative. The balloons carrying medicine (such as cytotoxic agent paclitaxel and sirolimus) coat the inner side of atherosclerotic vessels, which arrests or puts

off adverse complications and inhibits pathological pathway of atherosclerosis and neointimal hyperplasia.[364–366] Paclitaxel, a highly lipophilic antineoplastic drug showing sustained inhibition of the cell proliferation, produces a rapid inhibitory effects after the exposure of cells or tissues to paclitaxel for just a few seconds to a few minutes.[367] Based on paclitaxel eluting, drug-coated balloons have recently displayed enhanced efficacy in treating recurrence of stenosis.[368,369] During treatment of coronary in-stent restenosis, early studies suggested that paclitaxel-coated balloon catheter with 3 μg/mm^2 of balloon surface area apparently weakened the secondary occurrence of stenosis, and second stent implantation and sustained drug release at the site of injury may not be necessary for the inhibition of restenosis.[370] Meanwhile, Bonaventura et al. generalized that, differing from drug-eluting stents, handling with drug-coated balloon carrying paclitaxel received lower late lumen loss and similar restenosis and revascularization rates to DES for treating small-vessel de novo lesions.[371] Chowdhury[372] represented that diminished inflammation and plaque burden in nonobstructive lesions had been observed in paclitaxel drug-coated balloon treatment and proved that paclitaxel drug-coated balloon with suppressed capacity for atherosclerotic proceedings could safely and potentially serve as regional anti-atherosclerosis therapy. Another candidate sirolimus is also a coping style of reducing intimal growth. In one investigation, various sirolimus coating (crystalline coating and amorphous one) had been produced, and their inhibitory capability of neointimal proliferation had been confirmed, although they showed significantly unlike residence time in the coronary artery wall.[373] Of particularly note is that characteristics of drug molecules themselves embodying lipophilicity, molecular size and surface charge, would affect drug penetration and cell uptake into specified target, which had been narrated by Tesfamariam in detail.[374]

For local administration with angioplasty balloon catheter, inhibition of thrombosis is also of great concern. Back in 1994, Nunes et al.[375] transported a synthetic antithrombin to the arterial wall for suppressive platelet-dependent thrombosis via a hydrogel-coated angioplasty balloon catheter, concurrently without altered bleeding parameters. Other antithrombotic drugs (heparin, argatroban) had been sent to injured arteries via porous balloon catheter to prevent thrombosis or partial intimal hyperplasia without obstructing system coagulability.[376,377] Furthermore, over the past decades, balloon catheter-based systems have been described as useful mode of therapeutical gene shipping for treating cardiovascular conditions, incorporating micro-needle, double-balloon, channel balloon, and porous balloon catheters, while, for efficiently, selectively and rapidly mediating gene transfer into given cell types of artery wall, appropriate system should be opted.[378,379] For the efficient delivery and long-term expression of therapeutic genes targeting the medial SMCs, Pankajakshan et al. applied irrigating balloon catheter to transfer gene (SM22α promoter) wrapped in a AAV2/9 vector into intended site after inflating balloon catheter with minute pores to injure the coronary artery SMCs. In the light of their exploration, object gene was successfully expressed in the middle layer with lasting for at least 2 months.[380] On these foundations, suppress intimal hyperplasia via balloon catheter-based gene delivery is a promising concept. More than the foregoing payloads, endothelial cells also had been conveyed to balloon-dilated rabbit arteries through a local delivery catheter (dispatch), and adhered onto scathed arteries with modest cellular retention.[381]

Modifying of the balloon surface. Considering more effective local drug delivery for facilitated drug retention on balloon and shift into tissue while weakened drug loss into blood, modifying balloon surface is considered to be applicable and potential, including adding excipients (iopromide, dimethyl sulfate, dextrane, urea, polyethylene glycol, and so on) or carriers/coatings (hydrogel, nanoparticle, and polyethylene oxide coating) and microstructure (perforating, arrays).[365,382] The immobilization of ionic monomers (acrylic acid and 2(dimethylamino) ethyl methacrylate) onto polyethylene balloon catheters meets an instantaneous local high concentration of argatroban (an available anti-thrombin agent) within dilated artery, though encountering a decrease after restoring blood flow.[383] Given surface design could manifest discrepant kinetic characteristics for a certain kind, different in rate of release, binding capacity to treated sites, systemic drug concentration, tissue penetration depth, and retention capacity. Obviously, adding controlled release coating, such as hydrogel or others, could obtain better pharmacokinetics. In one example, Dick et al. employed a porous balloon catheter and a gel-coated one as model of local drug delivery system and compared the differences of drug behavior between them. Evidently, it could be concluded that, compared to the hydrogel-coated balloon, the porous one obtained more payloads within treated segment at the cost of higher system concentration, which rendered low transport efficiency.[384] Tomaru et al.[376] used a novel porous balloon catheter consisting of dumbbell-shaped balloon with 12 small holes as the delivery system and demonstrated that this new porous balloon catheter created less mechanical vascular trauma comparing with the conventional ones. Microstructure of surface determines the drug transfer efficiency and retention efficiency in treated vessel. Developing microneedle array as drug supporter also enables to eliminate low drug delivery efficiency of standard DEB, which implies the superior therapeutic efficacy for atherosclerosis. And microneedle drug eluting balloon is proven to be suitable solution for intraluminal delivery led by microneedles embedding tissue and going deep into the tunica media of the vascular tissue.[385] Still further, Tzafriri and his partners[386] devised various micro-

features (amorphous, flaky, and microneedle) on balloon surface to move paclitaxel, observed that microneedle system reaped larger coverage, higher tissue concentration than amorphous/flaky surface. Based on nanomotors with the property of penetrating into target cell, Huang et al.[387] developed a novel balloon-coating with a paclitaxel packaged into MJAMS/aV (platelet membrane coated Janus mesoporous silica nanomotor modified with anti-VCAM-1 antibody) for the long-term anti-proliferative effects of paclitaxel. Specifically, nanomotor could penetrate into the atherosclerotic plaque deeply, which promoted by near-infrared (NIR) light, wrapping of nanomotor with platelet membrane minimized drug loss before reaching the plaque and anti-VCAM-1 antibody linked to the excessive VCAM-1of the plaque site. Concurrently, solvents also pose as a controller of healing potion transfer. Take for example, when methanol, water, or acetone were used as solvent for paclitaxel-contained balloon, high drug loss into blood entailed low drug transfer rates to the applied site. But used ethyl acetate-solutions, smaller drug loss and more un-transferred PTX onto balloon had been observed.[388]

Comprehensive analysis of these delivery strategies

As a systematic transportation strategy, more than therapeutic agents, the transport mode on the basis of nanoparticles encounters their challenges: how to colocalize and how to work. Its final therapeutic effects are derived from drug molecule, efficiency of targeted transport as well as degree of plaque damage caused by particle fixation. In this regard, the specificity of molecules (including therapeutic molecules and targeting moieties) embellished onto/into nanoparticles is required for positioning, retention and cure. More than payloads, the shape and size of particles may induce the discrepancies in targeting ability and distribution. Zhang et al.[389] elaborated that the order of targeting capacity is platelet (the highest), cylinder, blade, sphere and brick (the lowest), and enlarged size mediated the increased targeting efficiency. Moreover, the level of plaque injury is closely related to the changes in hydrodynamic characteristics caused by particles. Virtually, the onset, growth and rupture of atherosclerosis are very sensitive to fluid dynamics. Hemodynamics plays a central part in the development of atherosclerosis, and shear stress, the friction force exerted by blood flow on the blood vessel wall, is a key driving force.[390] According to the principle of hydrodynamics in atherogenesis, the regions of predisposition for plaque forming possess some clear attributes: curvature, branching, bifurcation, tapering, or external attachment (like plaque) inducing disturbed flow.[391] In these susceptible zones, weaker shear force acts on endothelial cells, comparing with protected zones.[392] And, in these areas of vascular stenosis, the cap of the plaque presents the maximum wall shear stress (WSS) and the regions with low WSS exist at the rear of plaques like shoulder.[393] High shear stress tends to make plaque rupture, while the sites with low shear stress are more likely to obtain lipid deposition. Additionally, aggravated stenosis is parallel to the rising WSS.[393] Given these conditions, the effect of transport vehicle induced hemodynamic changes on plaque progression should be considered. And seeing the principle of fluid dynamic in stenosed vascular lumen, nanoparticles themselves can be used as drug agents for regulating the hemodynamics of stenotic artery toward atheroprotective flow environment, like minimizing the stresses on the wall and resistance to blood flow.[394] Injected nanoparticles with different size, shape, and dose (volume fraction) have distinct effects upon mechanical forces in plaque, which induces plaque injury of different severity. Generally, nanoparticles with platelet shape show the highest WSS, meaning the most serious damage to plaque, compared with cylinder-, brick-, and sphere-shaped nanoparticles.[395] Enlarged dose and particle size would trigger appearance of high shear stress, which may lead to the rupture of plaque.[389,395] Together, more than optimum presentation of therapeutic or targeted molecules, it is necessary to optimize the particle body design.

As a major interventional formulation, the long-term effectiveness of stent implantation in the treatment of atherosclerosis is approved. But its adverse consequences have also attracted attention. Fast recovery of blood flow and in situ supply of therapeutic molecules provide favorable factors. Thrombotic and inflammatory properties of the material itself accelerate negative effects. And acute injury (endothelium denudation/dysfunction) caused by stent expansion was once dubbed the important cause in the formation of stenosis. For dispelling ultimate unfavorable performance, multifarious drugs are loaded onto the surface of stent for restoring endothelial layer, SMC-selective prevention, or lowering the thrombosis/inflammation of materials. Ulteriorly, in addition to drug dependent wall response, it has been deliberated that the local hemodynamic forces induced by rigid protruding and malapposed struts after stent graft are a significant inducement of in-stent restenosis and thrombosis. Insufficient stent design (strut thickness and shape) and deployment (i.e. underexpansion inducing larger distance of the struts from the vessel wall) may impede healing response post-stent.[396] Concretely, in stented segments, the zones between the struts have a strong disposition to growing neointima considering low WSS in these regions while high WSS on the surface of struts linked to platelet activation. And, the large struts engender heavy protruding and hindrance for blood flow while less embedding into vascular lumen, accompanied by increased shear rate. Regions with high shear rate reside in the top and bottom of malapposed struts, forming a thrombotic surface. As a consequence of incomplete stent expansion, residual stenosis further deteriorates the mechanical environment of dilated

artery (i.e. decreasing WSS, more disturbed), thus accelerates the stent failure with restenosis, stent thrombosis.[397] And, it is obvious that severe residual stenosis heralds more disturbance. Compared with non-streamlined struts (rectangular), struts with appropriate streamlining (circular/ovoid) could abate the magnitude of shear rate at the top of strut and the area of recirculation regions around the struts.[398] Overall, the final clinical outcome of stent implantation is the comprehensive result of drugs, stent design and implantation technology.

As another alternative mode of drug transportation based on interventional device, more than opening blocked blood vessels quickly, balloon transport also has advantages in uniform drug distribution on arterial wall, immediate drug release, a shorter duration of antiplatelet therapy, and no long-term residue of foreign material. Now, balloon dilatation is widely used to deal with in-stent restenosis implicated in EDS and bare metal stent, neo-plaques at small vessels and bifurcated vessels.[12] But, the expansion of balloon causes vascular injury of the host artery, featured by endothelial dissection and damage, which expedites ensuing acute platelet deposition, SMC hyperplasia, and restenosis. The acute and subsequent results of balloon angioplasty are the most susceptible to inflation pressure, balloon size (balloon to vessel ratio), and time (inflating time). High inflation pressure obtains high acute lumen acquisition, but results in severe vascular trauma with significantly higher incidence of dissection, thrombus and restenosis. And, unrelated expansion pressure, excessive balloon size leads to large vascular wall trauma and restenosis.[399] As reported Li et al.,[400] excessively rapid balloon dilatation also works as a considerable cause of arterial dissection (referring to a tear along the inner wall of an artery). Commonly, it is recommended that inflated time of 30–60 s at nominal pressure (about 7–8 atm) and matched balloon-to-vessel ratio (about 0.8–1.0) are appropriate to avert vascular dissection.[12] But, for drug delivery, expansion time should be considered more carefully. Notably, due to elastic recoil of blood vessels after deflating balloon or insufficient dilatation, the existence of residual stenosis limits the therapeutic effect of balloon. Based on hemodynamic analysis, less loss of blood vessel gain helps to maintain a normal hydrodynamic environment, as large residual stenosis implies large vessel wall shear stress, increasing the risk of restenosis.[401] Significantly, selecting the optimal balloon size and expansion pressure, small residual stenosis could be obtained. Optimizing balloon design is also a way to achieve small residual stenosis. By the combination of incision (cutting the lumen of the thickened vascular wall and possibly breaking the elastic force) and dilatation of the plaque, cutting balloon angioplasty minimizes arterial wall trauma and decreases residual stenosis for lessened neoproliferative response, and subsequent restenosis.[402,403] For inhibiting the response of excessive SMC proliferation and eventual restenosis induced by trauma, the selected drug is loaded on the balloon surface. Low drug transport efficiency to target tissue and the use of nonspecific drugs are still the main limitations. In order to enhance drug transport efficiency, coating technologies (like hydrogel), balloon surface design as well as the employment of drug carriers have been carefully recommended. Ultimately, integrating these factors, the optimization of therapeutic agent selection, balloon design (surface and construct), and surgical procedure improves the targeting delivery of treatment drugs into plaque and lessens the trauma of target artery for accelerated positive vascular repair response.

Conclusions and perspectives

Inevitably, the pathological progression of atherosclerosis is the ramification of cross- and cascade-response of multipathways, covering macrophage inflammatory phenotype and damaged phagocytosis, inflamed endothelium, phenotype-modulated SMC, abnormal lipid metabolism, production of reactive oxygen species as well as deposited thrombus, etc. Based on this circumstance, it is a legitimate basis for treating atherosclerotic plaque to eliminate/inhibit pathogenic factors and revive native cell functions. Focusing on how the therapeutic agents reach the lesion, stent-, balloon-, or nanoparticles-based delivery modes are under processive investigation. Naturally, since the idea delivery strategies require maximal bioavailability of target tissue/cells in plaque for therapeutic molecules to restore functions of health vascular, delivery modes mediated by stent, balloon, or nanoparticles counter their drawbacks. Post-stenting restenosis induced by vascular injury response (e.g. endothelial discontinuity and growth of SMC), late thrombus and nonspecific therapeutic drugs (like antiproliferative agents) limits stent-based treatment. Additionally, coagulation and inflammation induced by stent itself should be solved, like ticagrelor[294] for resolving coagulation and recombinant CD47[404] for reduced inflammatory cell attachment. For this formulation guided by balloon,[374,385] mostly relying on EC-toxic drugs, arterial elastic recoil, and negative geometric modeling may cause an abrupt restenosis and short balloon inflation times (30–60 s) would be not conducive to drug shift efficiency from balloon surface to the luminal lesion. Moreover, cytotoxic levels associated with high drug concentrations locally and systemic distribution also should be considered. Therefore, stent/balloon-free strategies meet the clinical needs. The handicaps of systemic transportation induced by nanoparticles are concentrated on how to arrive in plaque tissue/cells successively rather than being cleared. And this systemic trafficking scheme is required to achieve lessened plaque progression, potentiated regression and stability.

From the perspective of therapeutic molecules, the effects should be directionality and uniqueness with annihilating harmful factors while protecting or

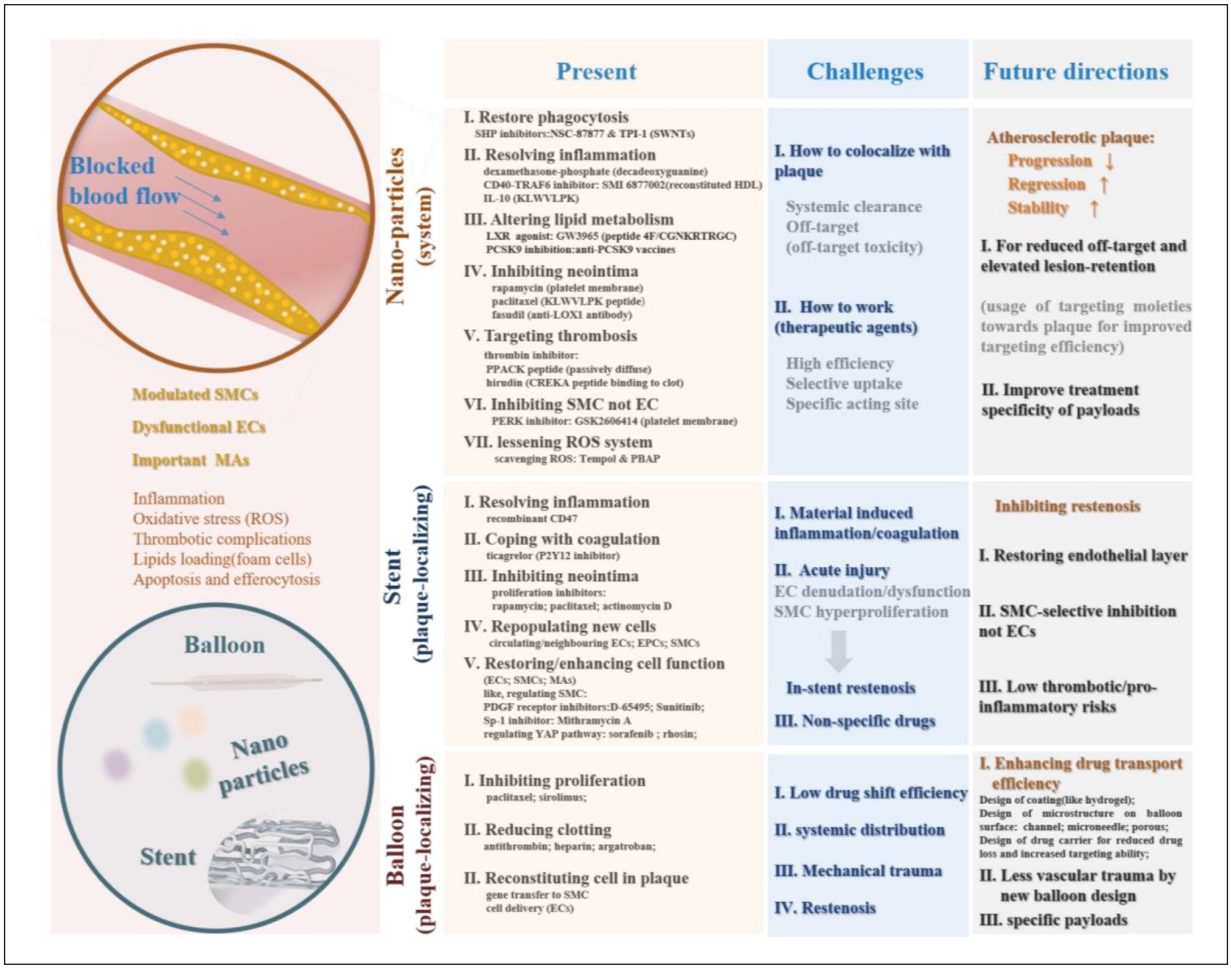

Figure 11. The promising treatment of atherosclerosis in development and the trends.

promoting favorable events one or more. In the case of RGD presenting in fibronectin, fibrinogen, von Willebrand factor, vitronectin, and thrombospondin,[189] this peptide captures platelets via binding to αIIbβ3 (GPIIb/IIIa), αvβ3, and α5β3, while blocks fibrinogen binding to platelets guided by competitive effect for αIIbβ3. RGD on surface would capture platelets. More importantly, integrins αvβ3 and α5β3 are also expressed on endothelial cells.[405] So, RGD can link ECs and platelets. Therefore, selected payloads should be specific for treated targets. General drug-eluting stents have impeded neointimal smooth muscle cell hyperplasia, but aggravating dysfunction of endothelial cell, which lead to late thrombogenic events and post-angioplasty restenosis. For supporting stent applications, new specific agents should alleviate disease phenotypes of SMCs but facilitate health one of ECs and nonthrombogenic effects. The novel PERK inhibition (like GSK2606414) mitigated phenotypic switching of smooth muscle cells by activating SRF and inhibiting STAT3, while rescued endothelial cells from impaired growth and releasing of prothrombogenic tissue factor.[43]

Taken together, given reliving more normal functions of narrowing vessels, election of appropriate drugs and local delivery modes is required for: treating inflammation and restoring effective efferocytosis; targeting thrombi; preventing monocyte recruiting and subsequent macrophage accumulation; altering lipid metabolism; holding back SMC phenotypic modulation, reducing ROS production, and protecting endothelium. And, based on different shift strategies, the future directions should be considered separately as depiction of Figure 11.

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ORCID iD

Li Li https://orcid.org/0000-0001-8834-9837

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