Post-translational modifications in atherosclerosis: Roles, mechanisms and therapeutic potential (Review)

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Abstract. Atherosclerosis (AS) is the principal pathological basis of cardiovascular disease and develops through the coordinated progression of endothelial dysfunction, lipid accumulation, macrophage foam-cell formation, vascular smooth muscle cell remodeling, chronic inflammation and plaque destabilization. Protein post-translational modifications (PTMs) provide a reversible regulatory layer through which metabolic, oxidative, inflammatory and mechanical cues are translated into changes in protein activity, stability, localization and molecular interactions. In this review, the roles of major PTMs in AS were summarized, including lysine modifications, cysteine modifications, phosphorylation, glycosylation, nitration and ADP-ribosylation. Rather than treating these modifications as isolated events, their integration into disease-related regulatory networks is emphasized. In particular, lysine lactylation is discussed as a metabolic-epigenetic mechanism linking lactate accumulation to endothelial dysfunction, macrophage polarization, vascular smooth muscle cell senescence and vascular calcification. PTM crosstalk is also highlighted, including SUMOylation-ubiquitination and ubiquitination-phosphorylation interactions, as a mechanism that coordinates inflammasome activation, oxidative stress and vascular cell phenotypic switching. Finally, the therapeutic potential of targeting PTM-related enzymes and pathways, such as histone deacetylases, poly(ADP-ribose) polymerases, kinases and palmitoylation regulators was evaluated, while addressing key challenges including context-dependent PTM effects, limited site-specific validation, insufficient human plaque evidence and the translational gap between experimental findings and clinical application. This review provides an integrated framework for understanding PTM-mediated regulation in AS and for developing more precise PTM-based therapeutic strategies.

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Contents

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of death globally (1,2). Atherosclerosis (AS), a pathological process characterized by dysregulated lipid metabolism within the arterial wall and chronic inflammatory responses, constitutes the most common and critical pathological basis of CVD (3). Its progression involves intricate interactions among multiple cellular and molecular mechanisms (Fig. 1). Typically, AS is initiated by endothelial injury induced by haemodynamic abnormalities, oxidative stress or metabolic disorders (4,5). Following endothelial injury, low-density lipoprotein (LDL) permeates the endothelium into the intima, undergoes oxidation to form oxidised LDL (ox-LDL) and is subsequently phagocytosed by macrophages. Macrophages then transform into lipid-laden foam cells, contributing to the formation of lipid cores (6,7). These foam cells release inflammatory cytokines to amplify inflammation, whilst macrophages further escalate the inflammatory cascade by secreting additional cytokines during ox-LDL phagocytosis (8). Concurrently, abnormal proliferation, migration and phenotypic transformation of vascular smooth muscle cells (VSMCs) contribute to fibrous cap formation (9,10). This fibrous cap encapsulates the lipid core, forming a mature atherosclerotic plaque. Plaque stability determines disease progression, as thinning of the fibrous cap destabilizes plaques and increases the risk of rupture (11). Plaque rupture triggers thrombosis, leading to acute cardiovascular events

such as myocardial infarction and stroke (12). Furthermore, oxidative stress accelerates lipid peroxidation and inflammation by stimulating the production of reactive oxygen species (ROS) (13). Vascular calcification, a late-stage feature of AS, exacerbates arterial stiffening and luminal narrowing via calcium deposition (14). Therefore, elucidating the molecular regulatory mechanisms underlying these cellular events is essential for understanding AS progression and identifying new therapeutic opportunities.

Although lipid-lowering, antithrombotic and anti-inflammatory therapies have substantially improved the prevention and treatment of AS-related cardiovascular events, residual cardiovascular risk remains common. This limitation partly reflects the fact that AS progression is not driven by a single upstream pathway, but by intertwined metabolic stress, oxidative injury, inflammatory activation, vascular cell-state transitions and plaque microenvironmental remodelling. Therefore, mechanisms that integrate these signals at the protein-function level may provide additional therapeutic opportunities beyond conventional risk-factor control. PTMs are particularly relevant in this context because they rapidly and reversibly regulate protein activity, localization, stability and interactions in response to pathological stimuli. Targeting PTM writers, erasers, readers or specific modified substrates may therefore enable more precise intervention in AS, although substrate specificity, cell-type dependence and long-term safety remain major translational challenges.

Against this background, post-translational modifications (PTMs) serve as key molecular regulatory mechanisms throughout AS progression. PTMs dynamically regulate protein activity, localization, stability and molecular interactions by enzymatically adding or removing specific chemical groups on defined amino acid residues (15,16). In recent years, PTMs have garnered significant attention for their role in AS as key mechanisms regulating cellular signaling and pathophysiological processes, including fine-tuned control over endothelial function (17), inflammatory responses (18), lipid metabolism (19) and cell fate determination (20). Common modification types include lysine residue modifications, such as the emerging lactylation, alongside SUMOylation [a modification in which small ubiquitin-like modifier (SUMO) proteins are covalently attached to specific lysine residues on target proteins], ubiquitination, acetylation and methylation; cysteine residue modifications, including S-palmitoylation, S-nitrosylation and S-glutathionylation; classical serine/threonine/tyrosine modifications termed phosphorylation; and other modifications including glycosylation, nitration and ADP-ribosylation. Notably, these PTMs do not operate in isolation but interact through competitive, synergistic and causal crosstalk to collectively shape protein function and cellular fate. Understanding how this network integrates environmental signals, coordinates cellular responses and ultimately determines plaque fate is crucial for elucidating AS pathogenesis and developing novel therapeutic strategies. Accordingly, this paper systematically reviews recent advances in studies on these PTMs in AS, focusing on the mechanisms of various modifications at key pathological stages. It also summarizes current PTM-targeted drug development strategies, aiming to provide theoretical foundations and innovative insights for the precise treatment of AS.

2. Role of key PTMs in AS pathogenesis

PTMs serve as vital intracellular regulatory signals. By adding specific chemical groups (such as phosphate, acetyl and methyl groups) to or removing them from designated amino acid residues, PTMs alter protein structure, activity and function, thereby participating in numerous physiological and pathological processes (16,21). Different PTMs possess distinct modification mechanisms and target specific sites, as detailed in Table I. Building upon this foundation, the following section systematically elaborates on the specific roles and underlying mechanisms of these PTMs during AS progression and explores their potential as therapeutic intervention targets. Accordingly, for each major mechanism discussed below, distinct evidence derived from ox-LDL- or agonist-stimulated cell systems, $ApoE^{-/-}$ or $LDLR^{-/-}$ atherosclerosis models, other non-AS vascular or metabolic disease models, and human plaque tissue or clinical cohorts is discussed. Human tissue associations are described separately from clinical cohort evidence and are not interpreted as establishing mechanistic causality.

Lysine modification in the pathogenesis of AS

Lactylation. Lysine lactylation (Kla), first identified by the Zhao Yingming group at the University of Chicago in 2019 (22), has rapidly emerged as a novel PTM, garnering significant attention in studies of metabolic regulation and disease pathogenesis. Notably, this epigenetic modification uniquely links cellular metabolism to chromatin remodelling through distinct biochemical pathways (23). Catalysed by lactyl transferases, the modification process attaches lactate molecules to lysine residues on proteins, thereby altering protein function or chromatin architecture to regulate gene expression and cellular activity (24,25). Lysine lactylation is an emerging post-translational modification that provides a mechanistic link between cellular metabolism and epigenetic regulation. Although its role in AS has not yet been fully elucidated, current evidence indicates that lactylation participates in AS pathogenesis by modulating gene transcription, cellular reprogramming and disease-related functional responses through multiple regulatory pathways. These include cellular reprogramming and the induction of irreversible cellular damage (Fig. 2).

One of the most fundamental roles of lactylation is its direct remodelling of chromatin through histone modification, thereby regulating the transcriptional activity of specific genes. This mechanism is particularly prominent under AS pathological conditions. In ox-LDL-stimulated endothelial cells, high-fat diet (HFD)-fed apolipoprotein (Apo)$E^{-/-}$ mice and arteries from patients with atherosclerosis, Dong *et al* (26) reported that ox-LDL enhanced glycolysis, increased intracellular lactate accumulation and promoted histone H3 lysine 18 lactylation (H3K18la). In that study, anti-silencing function 1A histone chaperone-dependent recruitment of p300 increased H3K18la enrichment at the snail family transcriptional repressor 1 (SNAI1) promoter and activated the endothelial-to-mesenchymal transition (EndMT) programme; the human vascular observations support disease relevance but do not independently establish site-specific causality (26-28). The macrophage solute carrier family 16, member 3

Table I. Modified amino acid residues affected by PTMs, modification mechanisms and year of discovery.

Modified amino acid residues	PTM	Modification mechanism	Year (Refs.)
Lys	Lactylation	Lys + Lactyl-CoA → Lactyl-Lys	2019 (22)
	SUMOylation	Lys + SUMO + ATP → ADP + SUMO-Lys	1996 (46)
	Ubiquitination	Lys + Ub + ATP → ADP + Ub-Lys	1977 (64)
	Acetylation	Lys + Ac-CoA → CoA + Ac-Lys	1964 (71)
	Methylation	SAM → SAH + CH_3-Lys/Arg	1959 (88)
Cys	S-Palmitoylation	Palmitoyl-CoA + Cys → CoA + S-palmitoyl-Cys	1979 (109)
	S-Nitrosylation	Cys + NO → SNO	1992 (110)
	S-Glutathionylation	Cys + GSH → Cys-GSH	1983 (111)
Ser/Thr/Tyr	Phosphorylation	Ser/Thr/Tyr + ATP → ADP + PO_3-Ser/Thr/Tyr	1906 (136)
Asn/Ser/Thr	Glycosylation	Asn-X-Ser/Thr + UDP-GlcNAc + UDP-Man + UDP-Glc → Asn-X-Ser/Thr-$GlcNAc_nMan_mGlc_k$ + UDP Ser/Thr + UDP-GalNAc → Ser/Thr-GalNAc + UDP	1981 (157)
Tyr/Trp	Nitration	Tyr + $ONOO^-$ → Tyr-NO_2	1992 (164)
Arg/Glu	ADP-Ribosylation	Arg/Glu + NAD^+ → Arg/Glu-ADP-Ribose + nicotinamide	1963 (170)

Ac, acetyl; Ac-CoA, acetyl coenzyme A; ADP, adenosine diphosphate; Arg, arginine; Asn, asparagine; ATP, adenosine triphosphate; CoA, coenzyme A; Cys, cysteine; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; Glu, glutamate; GSH, reduced glutathione; Lys, lysine; Man, mannose; NAD^+, oxidized nicotinamide adenine dinucleotide; NO, nitric oxide; $ONOO^-$, peroxynitrite; PTM, post-translational modification; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; Ser, serine; SNO, S-nitrosothiol; SUMO, small ubiquitin-like modifier; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Ub, ubiquitin; UDP, uridine diphosphate; UDP-GalNAc, uridine diphosphate N-acetylgalactosamine; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; UDP-Man, uridine diphosphate mannose.

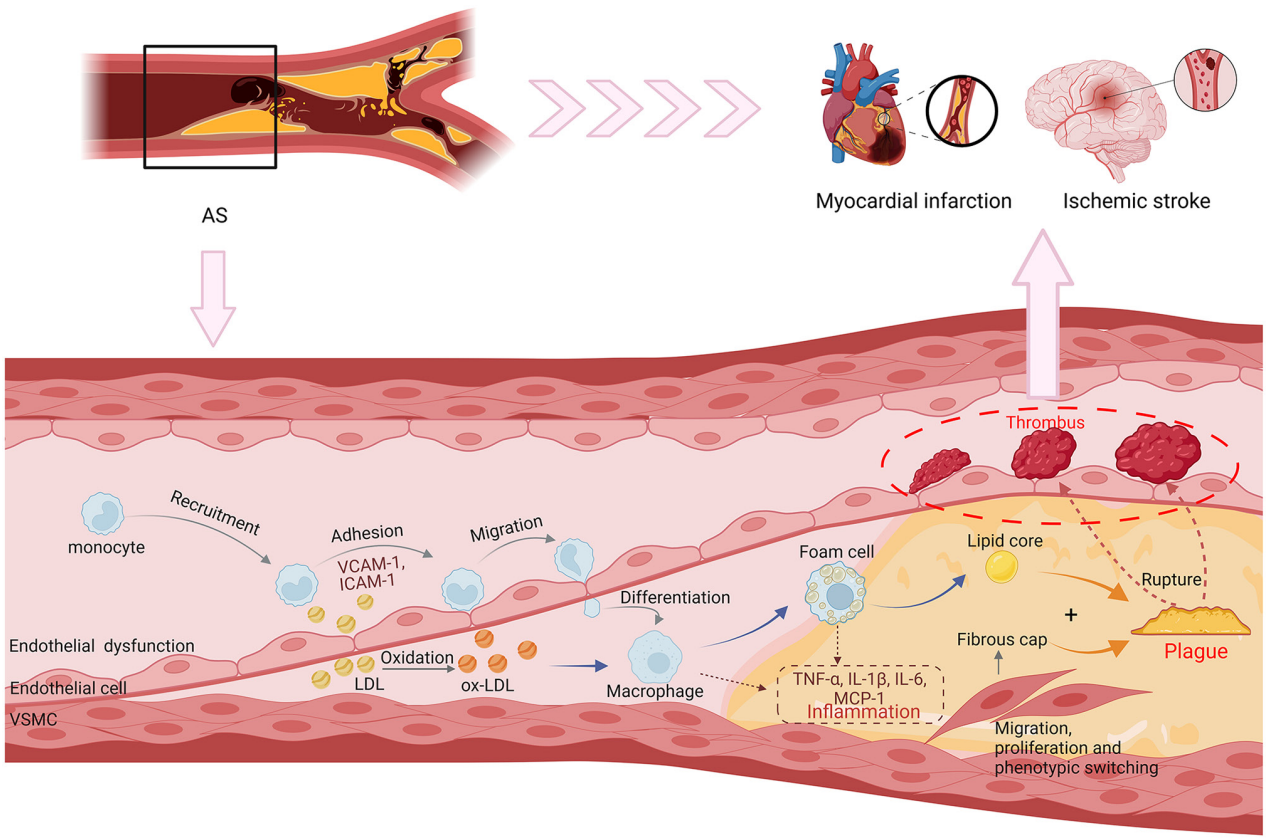

Figure 1. The process of AS: Monocyte recruitment, adhesion, migration and differentiation; LDL penetrates the endothelium and is oxidized under the influence of ROS. The resulting ox-LDL is taken up by macrophages, which become foam cells and form a lipid core; VSMC migration and proliferation; AS plaque formation and plaque rupture result in the formation of a thrombus, which ultimately causes myocardial infarction or ischaemic stroke. AS, atherosclerosis; ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1β; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein 1; ox-LDL, oxidized LDL; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; VSMC, vascular smooth muscle cell.

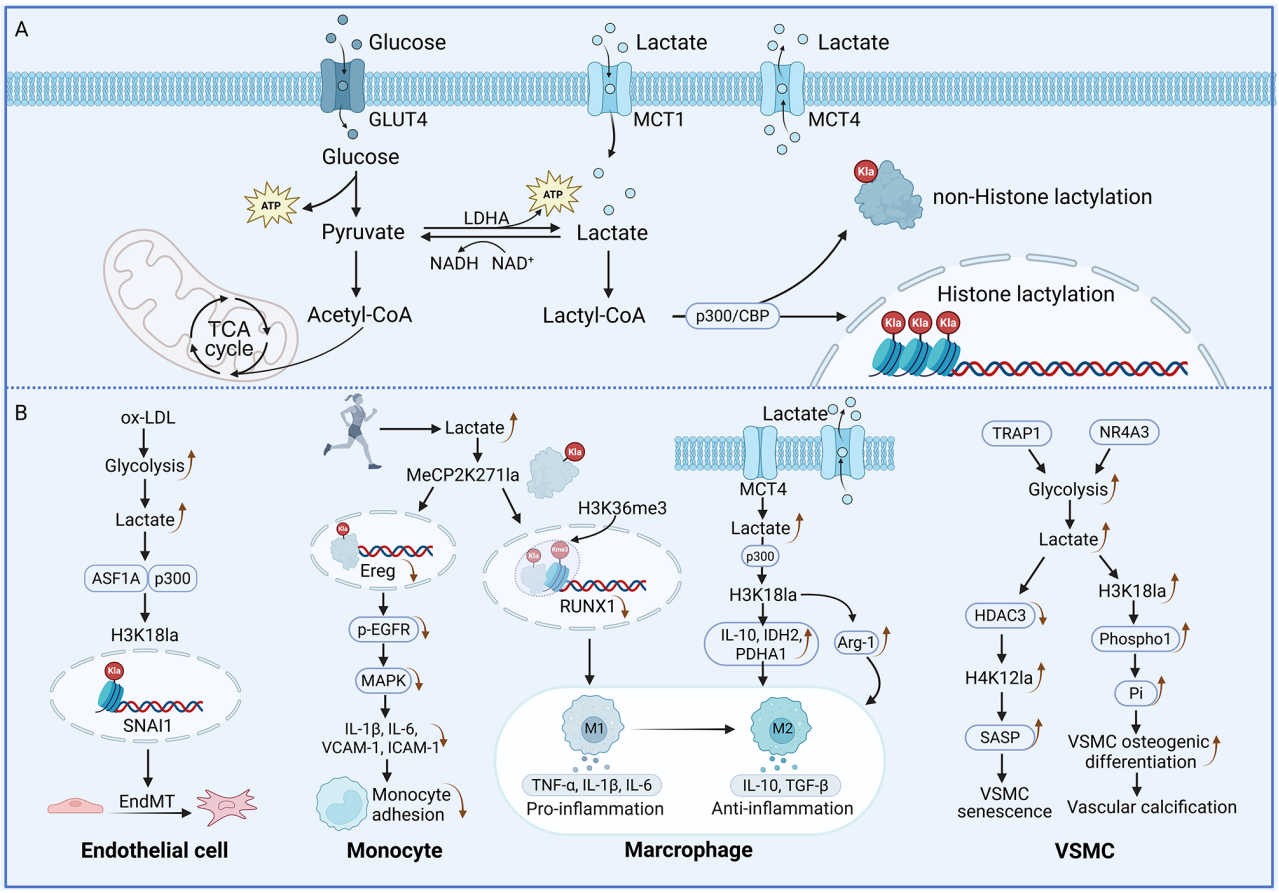

Figure 2. Mechanisms of lactylation and its roles in AS. (A) Mechanisms of intracellular lactate metabolism and lactylation. Lactate is transported into the cytoplasm via MCT or produced via glycolysis. In the cytoplasm, lactate catabolism occurs via two pathways. In one pathway, lactate is oxidized to pyruvate, which then enters the mitochondria and is metabolized via the TCA cycle. In the other metabolic pathway, lactate is converted into lactyl coenzyme A, which is involved in the lactylation of histones and non-histone proteins. (B) The accumulation of lactic acid drives lactylation modifications, which exert multifaceted effects during AS. Lactylation regulates the expression of the EndMT transcription factor SNAI1, thereby promoting monocyte adhesion via the Ereg/MAPK pathway. Simultaneously, it modulates Arg1 expression through the MeCP2/H3K36me3/RUNX1 complex and H3K18la modification, influencing macrophage polarization. Furthermore, H4K12la modification governs the expression of SASP factors, contributing to VSMC senescence. Additionally, H3K18la modification upregulates Phospho1 expression, exacerbating vascular calcification. Arg-1, arginase 1; ASF1A, anti-silencing function 1A histone chaperone; CBP, CREB-binding protein; CoA, coenzyme A; EGFR-P, phosphorylated epidermal growth factor receptor; EndMT, endothelial-to-mesenchymal transition; EREG, epiregulin; GLUT4, glucose transporter 4; ICAM-1, intercellular adhesion molecule 1; IDH2, isocitrate dehydrogenase 2; IL-1β, interleukin-1β; Kla, lysine lactylation; LDHA, lactate dehydrogenase A; MAPK, mitogen-activated protein kinase; MCT, monocarboxylate transporter; MeCP2, methyl-CpG-binding protein 2; NR4A3, nuclear receptor subfamily 4 group A member 3; ox-LDL, oxidized low-density lipoprotein; p300, E1A-binding protein p300; PDHA1, pyruvate dehydrogenase E1 subunit α1; RUNX1, runt-related transcription factor 1; SASP, senescence-associated secretory phenotype; SNAI1, snail family transcriptional repressor 1; TCA, tricarboxylic acid; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α; TRAP1, TNF receptor-associated protein 1; VCAM-1, vascular cell adhesion molecule 1; VSMC, vascular smooth muscle cell.

[SLC16A3, also known as monocarboxylic acid transporter 4 (MCT4)]-H3K18la pathway is supported by macrophage manipulation experiments and ApoE$^{-/-}$ mouse atherosclerosis: MCT4 inhibition or deficiency increased lactate-dependent H3K18la, upregulated IL-10 and pyruvate dehydrogenase E1 subunit alpha 1, promoted M1-to-M2 polarization and reduced lesion burden, whereas direct validation in human plaques remains unavailable (29). By contrast, the nuclear receptor subfamily 4 group A member 3 (NR4A3)-H3K18la axis is supported by VSMC calcification cultures, experimental medial-arterial-calcification models and calcified vascular specimens rather than by conventional ApoE$^{-/-}$ or LDL receptor (LDLR)$^{-/-}$ plaque models; it should therefore be interpreted as a vascular-calcification mechanism with potential relevance to advanced AS (30). Collectively, these findings demonstrate that histone lactylation serves as a dynamic epigenetic marker that directly regulates gene transcription.

Beyond direct transcriptional regulation, lactylation also modifies non-histone proteins to modulate key signaling molecules, thereby reprogramming cellular functional states. This process exhibits a distinctive duality in the inflammatory regulation of AS. In ApoE$^{-/-}$ mouse exercise models together with endothelial mechanistic assays, Wang *et al* (31) found that exercise-induced lactylation of methyl-CpG binding protein 2 (MeCP2) at the K271 site in endothelial cells enhances its interaction with histone H3K36me3, leading to downregulation of epiregulin expression. This in turn inhibits EGFR phosphorylation and the downstream mitogen-activated protein kinase (MAPK) signaling pathway, reduces the expression of adhesion molecules and pro-inflammatory factors, and suppresses monocyte adhesion, a critical early event in AS (31,32). A related but cell-type-distinct mechanism has been described in macrophages: In exercise-treated ApoE$^{-/-}$ mice and macrophage assays, increased lactate promoted

MeCP2 K271 lactylation, suppressed the transcription factor RUNX family transcription factor 1 (RUNX1), favored anti-inflammatory M2 polarization and reduced plaque lipid deposition (33). In MCT4-deficient macrophage models, lactate accumulation similarly upregulated H3K18la through a p300-dependent pathway, boosted IL-10 expression and promoted anti-inflammatory M2 macrophage polarization, with protective effects in experimental AS (29). Nevertheless, lactylation exerts bidirectional effects in macrophages. Other agonist- or stress-stimulated cell models have demonstrated that lactylation can also promote the expression of pro-inflammatory cytokines under specific conditions (34). Such divergent outcomes may stem from differences in lactate concentration, disease stage, cell type and substrate availability: Moderate lactylation may initiate adaptive anti-inflammatory programs, whereas sustained high-level lactylation can trigger severe pathological alterations. Seemingly contradictory findings regarding lactylation in AS therefore reflect strong cell-type specificity and microenvironment sensitivity rather than a single uniform pro- or anti-atherosclerotic role.

Whether involving histone or non-histone lactylation, these regulatory processes remain reversible, but sustained signals may contribute to persistent VSMC dysfunction. The TNF receptor associated protein 1 (TRAP1)-histone deacetylase (HDAC)3-H4K12la axis is supported by VSMC senescence assays, VSMC-specific Trap1 deletion in ApoE$^{-/-}$ mice and expression analyses in human atherosclerotic aortic tissue: TRAP1 enhances aerobic glycolysis and lactate production, reduces HDAC3 abundance and increases H4K12la at senescence-associated secretory phenotype gene promoters (35,36). The human data are tissue-level associations rather than residue-mutant causal evidence. In parallel, the NR4A3-H3K18la mechanism was established mainly in induced vascular-calcification systems rather than longitudinal human AS cohorts (30). Accordingly, these pathways may contribute to cap weakening or calcification, but they should not yet be described as proven drivers of irreversible plaque damage in humans (37).

In summary, lactylation participates in AS progression through a multilayered regulatory framework (38). Histone lactylation directly reshapes chromatin accessibility and transcriptional programs, whereas non-histone lactylation modulates key signaling cascades and cellular functional states. Together, these mechanisms link metabolic stress to endothelial dysfunction, macrophage polarization, VSMC senescence, osteogenic differentiation and plaque instability. When lactylation-mediated adaptive responses fail to restore cellular homeostasis, sustained lactate accumulation and persistent lactylation signals may further promote persistent pathological outcomes, including cellular senescence and vascular calcification. These findings suggest that lactylation may represent a promising regulatory node for AS intervention, particularly through modulation of lactate metabolism, inhibition of lactylation-related enzymes or blockade of pathogenic lactylation events that promote terminal cell-state transitions.

However, the therapeutic translation of lactylation-targeted strategies remains limited by several unresolved issues. First, the specificity of current detection methods remains a major concern, as pan-lactylation antibodies and site-specific antibodies such as anti-H3K18la may cross-react with other acyl modifications (39,40). Second, most studies do not distinguish among different lactylation-related isoforms, including L-lactylation, D-lactylation and N-ε-(carboxyethyl)lysine, which may lead to inaccurate conclusions regarding the metabolic origin and biological function of lactylation (41). Third, many AS-related studies primarily demonstrate correlations between lactate accumulation and lactylation levels, whereas robust *in vivo* causal evidence remains insufficient (42,43). In addition, although p300 is widely regarded as a major lactyltransferase, emerging evidence suggests that other acetyltransferases, such as lysine acetyltransferase 7 (KAT7, also known as HBO1) and histone acetyltransferase 1, may also catalyze lactylation with distinct substrate preferences (44,45). Therefore, future studies should combine high-resolution mass spectrometry, isoform-specific detection strategies and cell-type-specific genetic models to define the writer enzymes, substrate sites and downstream effector pathways of lactylation in AS. Such efforts will be essential for determining whether lactylation can be reliably translated into a precise therapeutic target for AS.

SUMOylation. SUMOylation is a dynamic and reversible post-translational modification that has been increasingly linked to cardiovascular diseases (46), particularly AS. It plays a pivotal role throughout multiple stages of AS progression by regulating endothelial function, inflammatory responses and vascular smooth muscle cell phenotypic switching (Fig. 3A). This reversible process is tightly modulated by the sentrin/SUMO-specific protease (SENP) family (47). Similar to lactylation, the regulatory effects of SUMOylation in AS cannot be explained by a single mechanism; instead, it influences disease progression via multiple pathways: Acting as a molecular switch in response to environmental cues, stabilizing target proteins and modulating diverse cellular functions.

One of the most distinctive regulatory mechanisms of SUMOylation is its role as a haemodynamic sensor, exerting opposing biological effects under different shear stress conditions to determine the activation state of endothelial cells. This mechanism converts mechanical signals into molecular events, thereby regulating the initiation sites of atherosclerotic lesions. Flow-chamber endothelial studies and disturbed-flow mouse models indicate that turbulent flow activates p90RSK-mediated phosphorylation of SENP2 at Thr368, inhibits SENP2 de-SUMOylation activity and increases SUMOylation of ERK5 and p53, thereby promoting vascular cell adhesion molecule 1/intercellular adhesion molecule (ICAM)-1 expression, monocyte infiltration and endothelial inflammation (48). Laminar-flow endothelial models show that checkpoint kinase-dependent phosphorylation of SENP2 at Ser344 enhances de-SUMOylation and supports endothelial barrier integrity (48). A transplant-arteriosclerosis model, rather than a conventional lipid-driven AS model, further implicates SENP1-mediated de-SUMOylation of GATA binding protein 2 in endothelial activation (49). Protein inhibitor of activated signal transducer and activator of transcription (STAT) (PIAS)1-mediated endothelial nitric oxide synthase (eNOS) SUMOylation is supported by endothelial genetic and vascular-homeostasis experiments, without direct human plaque validation (47,50). PIAS3-mediated vimentin SUMOylation has stronger AS-specific support from VSMC

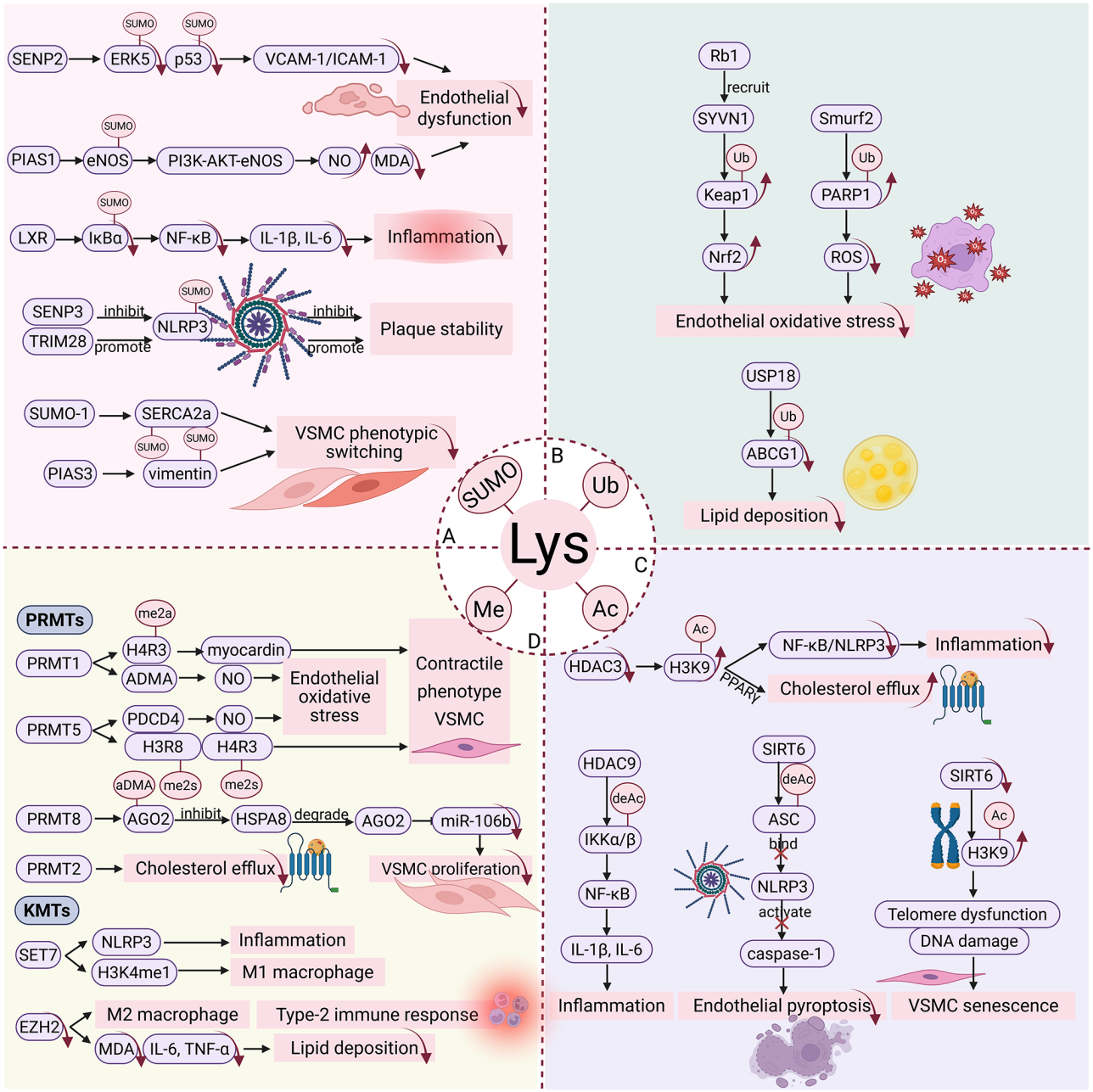

Figure 3. Roles of other PTMs on lysine residues in atherosclerosis. (A) SUMOylation affects endothelial dysfunction (SENP2-p53/ERK5-eNOS, PI3K-AKT-eNOS), inflammation (IκBα-NF-κB), plaque stability (NLRP3) and VSMC phenotypic switching (SUMO1-SERCA2A, PIAS3-vimentin). (B) Ubiquitination affects endothelial oxidative stress (SYVN1-Keap1-Nrf2/Smurf2-PARP1-ROS) and lipid deposition (USP18-ABCG1). (C) Acetylation. HDAC3 affects inflammation and cholesterol efflux by influencing H3K9, HDAC9 affects inflammation by influencing IKKα/β, SIRT6 limits excessive H3K9 acetylation at telomeric regions and thereby restrains VSMC senescence. (D) Methylation. PRMTs cause cholesterol efflux, endothelial oxidative stress, VSMC proliferation and phenotypic switching through histones and non-histones. The SET7 and EZH2 of KMTs affect inflammation, the polarization state of macrophages, immune response and lipid deposition. ABCG1, ATP binding cassette transporter; ADMA, asymmetric dimethylarginine; aDMA, asymmetric dimethylarginine modification AGO2, Argonaute RISC catalytic subunit 2; Akt, protein kinase B; ASC, apoptosis-associated speck-like protein; eNOS, endothelial nitric oxide synthase; ERK5, extracellular signal-regulating kinase 5; EZH2, enhancer of zeste homolog 2; IKK, inhibitory κB kinase; IL-1β, interleukin-1β; IL-6, interleukin-6; IκBα, inhibitor of nuclear factor-κB α; Keap1, kelch-like ECH-associated protein 1; KMTs, lysine methyltransferases; LXR, liver X receptor; Lys, lysine residue; MDA, malondialdehyde; NF-κB, nuclear factor κB; NLRP3, NLR family pyrin domain containing 3; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; p53, tumor protein p53; PARP1, poly(ADP-ribose) polymerase-1; PDCD4, programmed cell death 4; PI3K, phosphatidylinositol 3-kinase; PIAS1, protein inhibitor of activated STAT1; PPARγ, peroxisome proliferator-activated receptor γ; PRMTs, protein arginine methyltransferases; PTMs, post-translational modifications; Rb1, ginsenoside Rb1; ROS, reactive oxygen species; SENP2, SUMO-specific protease 2; SERCA2A, sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a; SET7, SET domain-containing lysine methyltransferase 7; SIRT6, sirtuin 6; Smurf2, SMAD-specific E3 ubiquitin protein ligase 2; SUMO, small ubiquitin-like modifier; SUMO-1, small ubiquitin-like modifier 1; SYVN1, synoviolin 1; TNF-α, tumor necrosis factor-α; TRIM28, tripartite motif containing 28; USP, ubiquitin-specific protease; VCAM-1, vascular cell adhesion molecule 1; VSMC, vascular smooth muscle cell.

assays and ApoE$^{-/-}$/Pias3-deficient mouse experiments, in which it promotes ubiquitin-dependent vimentin degradation and limits VSMC phenotypic switching (51). The SUMO-pathway inhibitor 2-D08 was used as an *in vitro* mechanistic probe

in this study and should not be presented as having demonstrated anti-AS efficacy in animals. SUMOylation of serum response factor has instead been described in vascular-remodelling models (52). Sarco/endoplasmic reticulum (ER) Ca^{2+}-ATPase 2a (SERCA2a) SUMOylation is supported by diabetic vascular-injury/atherosclerosis and VSMC experiments, but its site-specific status in human plaques and clinical cohorts remains elusive (9).

Another core function of SUMOylation is regulation of target-protein stability, particularly within inflammasome signaling. Tripartite motif containing 28-mediated SUMO1/2/3 conjugation, mitochondrial E3 ubiquitin ligase 1-mediated SUMO2/3 modification and SENP3-dependent removal of SUMO1 at NLR family pyrin domain containing 3 (NLRP3) Lys204 have been defined mainly in macrophage or inflammasome-focused cell systems (53-55). These experiments support isoform- and site-dependent regulation of NLRP3 activity, but the individual mechanisms have not been directly validated by residue-specific perturbation in $ApoE^{-/-}$ or $LDLR^{-/-}$ plaques or in human atherosclerotic tissue. Consequently, the apparently opposing effects of SUMO1 and SUMO2/3 should be interpreted as context-dependent cellular mechanisms rather than as a fully resolved *in vivo* AS model (56). Liver X receptor (LXR)-related endothelial and inflammatory-cell studies also suggest that SUMO-related regulation of inhibitor of NF-κB (IκB)α can restrain NF-κB signaling, although AS-specific *in vivo* and human plaque validation remains limited (57,58).

SUMOylation also influences plaque-related cell states through VSMC calcium handling and macrophage lysosomal function. In diabetic VSMC and vascular-disease models, high glucose and palmitate reduce SUMO1 and SERCA2a SUMOylation, whereas SUMO1 restoration improves calcium homeostasis and limits pathological proliferation and migration (9). In macrophages, the disease relevance of transcription factor EB (TFEB) SUMOylation is supported by ox-LDL-stimulated cell experiments and TFEB-KR (a SUMOylation-deficient TFEB K316R mutant) expression in $LDLR^{-/-}$ mice, in which reduced TFEB SUMOylation enhanced lysosomal activity and cholesterol efflux and limited foam-cell formation (59,60). Direct confirmation of the responsible TFEB site and modification occupancy in human plaques is still lacking.

These three mechanisms collectively form the network foundation for SUMOylation-mediated regulation of AS. As a molecular switch in environmental responses, SUMOylation converts haemodynamic signals into adaptive changes in endothelial function; modulates the stability of target proteins to finely control inflammatory thresholds; and maintains VSMC differentiation while regulating macrophage lipid metabolism. These mechanisms exhibit spatiotemporal synergy: During the early stage of AS, the switching function of SUMOylation determines the initiation sites of lesions; during disease progression, its steady-state regulation controls inflammatory amplification; and during plaque formation, its effects influence plaque cellular composition and stability.

However, pan-SUMO antibodies widely used in current SUMOylation research cannot distinguish SUMO1 from SUMO2/3 and are prone to interference from non-specific binding (61). In addition, SUMOylation undergoes rapid turnover, which may lead to signal loss during standard experimental procedures. Several NLRP3- and enzyme-specific mechanisms remain confined to cell systems, whereas others have been tested only in short-term or non-classical vascular animal models. Because AS is chronic and progressive, these mechanisms require confirmation in long-term disease models (62). Future studies should characterize the spatiotemporal landscape of SUMOylation in chronic AS and develop isoform-selective probes to define E3 ligase/SENP-substrate pairs across disease stages (63).

Ubiquitination. Protein ubiquitination is mediated by a multi-step enzymatic cascade involving E1 activating enzymes, E2 conjugating enzymes and E3 ligases (64). Through the selective attachment of ubiquitin to substrate proteins, this modification regulates protein stability, signaling activity and cellular stress responses. In cardiovascular diseases, particularly AS, ubiquitination participates in multiple pathological processes by modulating oxidative stress, antioxidant defense, inflammatory signaling and cholesterol metabolism (Fig. 3B) (65).

The most classical function of ubiquitination is targeted substrate degradation through the proteasome. In oxidative-stress-stimulated endothelial cells, the E3 ligase SMAD specific E3 ubiquitin protein ligase 2 interacts with poly(ADP-ribose) polymerase (PARP)1 and promotes its ubiquitination-dependent degradation, thereby reducing ROS production and endothelial apoptosis (66). This mechanism is currently supported only by stimulated endothelial cell experiments and has not been validated in $ApoE^{-/-}$ or $LDLR^{-/-}$ mice or in human atherosclerotic plaques.

Ubiquitination can also enhance endogenous antioxidant defense by removing inhibitory regulators of protective signaling pathways. The Nrf2/kelch like ECH associated protein 1 (Keap1) axis provides a representative example. Keap1 is a negative regulator of Nrf2; its ubiquitination-mediated proteasomal degradation releases Nrf2, promotes Nrf2 nuclear translocation and induces the transcription of antioxidant genes (67). In high glucose/ox-LDL-stimulated endothelial cells and streptozotocin (STZ)-induced $ApoE^{-/-}$ mice fed an HFD, ginsenoside Rb1 directly binds to Keap1 and promotes synoviolin 1-dependent Keap1 ubiquitination and proteasomal degradation, with K108, K323 and K551 identified as key ubiquitination-related lysine residues. This process activates the Nrf2/PPARG coactivator-1α antioxidant pathway. In parallel, Rb1 reduces p47^phox phosphorylation and membrane translocation, thereby suppressing of the NADPH oxidase 2 (NOX2; cytochrome b-245 β chain) complex assembly and ROS production. Thus, Rb1 alleviates endothelial oxidative injury by coordinating Keap1 ubiquitination with p47^phox phosphorylation, highlighting functional crosstalk between ubiquitination and phosphorylation in AS-related oxidative stress (68).

Beyond E3-ligase-mediated ubiquitination, the balance between ubiquitination and deubiquitination constitutes another regulatory layer in AS. Ubiquitin specific peptidase 18 (USP18) expression is increased in human coronary atherosclerotic plaques and macrophage experiments indicate that USP18 interacts with ATP binding cassette subfamily G member 1 (ABCG1) and reduces its ubiquitination, thereby stabilizing ABCG1 and promoting cholesterol efflux. In $ApoE^{-/-}$ mice, USP18 knockdown aggravates lesion formation and decreases

plaque ABCG1 expression (69). Thus, this mechanism has cell, ApoE$^{-/-}$ mouse and human plaque-expression support; however, the human evidence remains associative and does not establish that USP18-dependent ABCG1 deubiquitination is causally required in patients. ABC transporters, including ABCA1 and ABCG1, are essential for macrophage cholesterol homeostasis, and their dysfunction promotes cholesterol accumulation and inflammatory signaling (69,70). Therefore, ubiquitin-dependent regulation of ABCG1 stability provides a rapid and reversible mechanism by which macrophages adapt to intracellular cholesterol burden and inflammatory stress during AS progression. Collectively, ubiquitination contributes to AS regulation through multiple interconnected mechanisms. As a protein degradation signal, it mediates the timely clearance of damaged or pro-oxidative proteins; as a regulator of antioxidant signaling, it relieves transcriptional repression of Nrf2-dependent protective pathways; and through crosstalk with deubiquitination, it fine-tunes the stability of cholesterol transporters such as ABCG1. These mechanisms indicate that ubiquitination is not merely a general protein degradation process, but a dynamic regulatory system that integrates oxidative stress, inflammatory signaling and lipid metabolic homeostasis. However, most current evidence remains centered on individual ubiquitination events, whereas the temporal hierarchy among E3 ligases, deubiquitinating enzymes, oxidative stress and cholesterol overload during AS progression remains insufficiently defined.

Acetylation. Protein acetylation is a core epigenetic modification that profoundly governs pathophysiological processes including gene expression, inflammatory responses and oxidative stress (71). These biological events are dynamically regulated through alterations in histone and non-histone acetylation levels (72). Accumulating evidence indicates that disrupted acetylation homeostasis is closely correlated with atherosclerotic progression (73), highlighting the critical involvement of histone deacetylases (HDACs) (Fig. 3C). Based on structural and functional heterogeneity, HDACs are classified into five subfamilies: Class I (HDAC1/2/3/8), Class IIa (HDAC4/5/7/9), Class IIb (HDAC6/10), Class III [the sirtuin (SIRT) family] and Class IV (HDAC11) (74). These subtypes form a multilayered regulatory network during AS pathogenesis, specifically modulating key pathological processes such as endothelial injury, foam cell formation and plaque stability.

Class I HDACs (HDAC1/2/3/8) are extensively involved in inflammatory and metabolic regulation, among which HDAC3 plays a particularly prominent role in diabetes-associated AS (75). Human plaque studies indicate that HDAC3 expression is elevated in plaque macrophages from patients with diabetes and correlates with circulating lipid measures, whereas macrophage/endothelial experiments and mouse AS models provide mechanistic support for effects on inflammation, endothelial dysfunction and lesion formation (76). Pro-inflammatory cytokines increase endothelial HDAC3 and promote EndMT in cell models, and the HDAC3 inhibitor RGFP966 suppresses EndMT and improves plaque features in ApoE$^{-/-}$ mice (77,78). Sodium butyrate provides a less selective example: Dietary treatment reduced atherosclerotic inflammation in HFD-fed ApoE$^{-/-}$ mice and was linked to macrophage polarization through a free fatty acid receptor 2/HDAC-microRNAs (miRNAs) axis (79). Related non-AS macrophage and nutritional studies also illustrate its broader effects on NF-κB/NLRP3 signaling, histone acetylation and receptor-mediated pathways (80,81). Because butyrate activates G-protein-coupled receptors and affects multiple HDAC isoforms and metabolic pathways, this evidence should not be interpreted as selective validation of HDAC3 or of a single acetylated substrate.

As a class IIa histone deacetylase, HDAC9 has comparatively strong translational support. HDAC9 is increased in human carotid and aortic plaques and represses macrophage cholesterol-efflux regulators ABCA1 and ABCG1 (82). Mechanistic cellular studies, ApoE$^{-/-}$ mouse experiments and *ex vivo* monocytes from patients with established atherosclerosis indicate that HDAC9 binds component of inhibitor of NF-κB kinase complex (IKK)α/β, promotes their deacetylation and increases IKK-NF-κB signaling; class IIa inhibition with TMP195 reduces plaque inflammation and improves stability in the mouse model (83). Indole-3-carboxaldehyde also alters HDAC9-related macrophage cholesterol handling and polarization in experimental systems (84). TMP195 nevertheless inhibits several class IIa HDACs, and its broad macrophage-reprogramming effects have also been demonstrated in nonvascular tumour models (83,85); these observations do not constitute clinical evidence of HDAC9-selective efficacy in AS.

Class III HDACs (the SIRT family) maintain plaque stability via multiple cellular regulatory mechanisms. As a core member of this subfamily, SIRT6 confers protection against AS by modulating endothelial cell survival and death. Both HFD-fed ApoE$^{-/-}$ mice and ox-LDL-stimulated endothelial cells exhibit significant downregulation of SIRT6 expression (86). Conversely, SIRT6 overexpression reduces atherosclerotic plaque size, ameliorates dyslipidemia and suppresses the secretion of pro-inflammatory cytokines such as IL-1β and IL-18. Mechanistically, SIRT6 deacetylates the apoptosis-associated speck-like protein, thereby blocking its interaction with the NLRP3 inflammasome and inhibiting caspase-1 activation. This cascade reduces endothelial cell injury and prevents plaque destabilization. These findings demonstrate that SIRT6 regulates endothelial cell fate through deacetylation-dependent modulation of cell death signaling. Furthermore, SIRT6 exerts analogous protective effects in VSMCs. It preserves VSMC homeostasis by restricting excessive H3K9 acetylation at telomeric regions, thereby alleviating cellular senescence (87). VSMC-specific SIRT6 overexpression thickens the fibrous cap and shrinks the necrotic core; by contrast, catalytically inactive SIRT6 mutants exacerbate plaque instability, validating the therapeutic potential of SIRT6 deacetylase activity against AS. Collectively, these data confirm that SIRT6 maintains plaque stability by sustaining VSMC functional homeostasis, with its deacetylase activity directly linked to the progression of atherosclerotic lesions.

Protein acetylation and HDACs regulate inflammatory transcription, lipid handling, endothelial injury and VSMC homeostasis through mechanisms that differ by isoform and cell type. Class I and IIa HDAC pathways have cell and atherosclerosis-prone mouse support, and selected HDAC3/HDAC9 observations have also been made in human plaques or patient-derived monocytes. SIRT6-related protection is supported by ox-LDL-stimulated cells and ApoE$^{-/-}$ or

cell-specific mouse experiments (86,87). Nevertheless, human data are predominantly expression or *ex vivo* associations, and no individual acetylation site has yet been validated as a causal therapeutic target in a clinical AS cohort. Accordingly, statements that a specific HDAC class predominantly controls a defined disease stage should be regarded as a conceptual synthesis rather than an established temporal hierarchy.

Methylation. Protein methylation is a dynamic PTM coordinated by methyltransferases and demethylases, playing a pivotal role in vascular pathophysiology, particularly in the progression of AS (88). This process involves the transfer of methyl groups from S-adenosylmethionine to lysine or arginine residues on target proteins (89). Methyltransferases are classified into two major families: Protein arginine methyltransferases (PRMTs) and lysine methyltransferases. Both families drive atherosclerotic development through distinct yet interconnected molecular mechanisms (Fig. 3D).

The most classical function of methyltransferases is the direct regulation of chromatin state and gene transcription through histone methylation. PRMT1 supports smooth-muscle contractile gene expression through H4R3 asymmetric dimethylation, but this conclusion is derived mainly from VSMC and non-AS aortic-disease models, including inducible Prmt1 ablation causing contractile dysfunction and aortic dissection (90). VSMC mechanistic studies indicate that PRMT5-mediated H3R8/H4R3 symmetric dimethylation suppresses contractile transcription and promotes pathological remodeling (20). SET domain containing 7, histone lysine methyltransferase (SET7)/9 can also methylate non-histone substrates, although those broader functions are not themselves AS-specific evidence (91). SET7/9 has stronger AS-specific support from its increased expression in human carotid plaques and ApoE$^{-/-}$ mouse arteries and its effects on NADPH-oxidase and NLRP3-related inflammatory transcription (92). EZH2 is elevated in advanced human plaques, particularly in T-cell nuclei, and CD4+ T-cell-specific Ezh2 deletion in ApoE$^{-/-}$ mice promotes anti-inflammatory immune remodelling and limits AS (93-95). The human observations for SET7/9 and EZH2 establish disease association, whereas causal evidence is provided primarily by the mouse and cellular experiments.

Beyond histone modifications, PRMT1 also participates in endothelial dysfunction through non-histone methylation-related mechanisms. In a lipopolysaccharide-induced endothelial injury model, inflammatory stimulation increased PRMT1 expression, global arginine methylation and intracellular asymmetric dimethylarginine (ADMA) levels, while reducing SIRT1 expression, NAD$^+$ availability and nitric oxide production. Combined treatment with cholecalciferol and metformin reversed these changes, suggesting that modulation of the SIRT1-PRMT1-ADMA axis may help restore NAD$^+$/NO bioavailability and alleviate endothelial senescence and dysfunction (96). However, this conclusion is mainly based on an agonist-stimulated endothelial injury model rather than direct AS plaque validation. Furthermore, PRMT1 methylates the class II transactivator, promoting its degradation and inhibiting IFN-γ-induced major histocompatibility complex II transcriptional activation in macrophage-related immune models (97). PRMT5 has also been implicated in ox-LDL-induced endothelial dysfunction through programmed cell death 4-related mechanisms, but this evidence remains mainly cell-model based (98). These findings reveal that non-histone methylation governs cellular responses to pathological stimuli by regulating the stability or interactions of key functional proteins, further expanding the regulatory role of protein methylation in AS pathogenesis.

Furthermore, methylation directly shapes the cellular and immune microenvironment during AS progression by modulating cell proliferation, polarization and immune cell function. Experimental neointimal and adipose-related models indicate that PRMT8 mediates asymmetric dimethylation of argonaute RISC catalytic component 2 (AGO2), enhances AGO2- heat shock protein family A (Hsp70) member 8 binding and triggers AGO2 degradation through chaperone-mediated autophagy, thereby suppressing miR-17 family maturation, VSMC proliferation and neointimal formation (99). This mechanism directly links methylation to the miRNA processing pathway, revealing a unique post-transcriptional regulatory mode for controlling cell proliferation. SET7/9 sustains pro-inflammatory M1 macrophage polarization by elevating H3K4 monomethylation (H3K4me1), and its AS relevance is supported by human carotid lesion and ApoE$^{-/-}$ mouse data (92). EZH2 within CD4$^+$ T cells reshapes systemic immune responses, which is supported by human plaque correlation data and gene knockout experiments in ApoE$^{-/-}$ mice: Inhibition of EZH2 or T-cell-specific deletion of the Ezh2 gene promotes anti-inflammatory macrophage polarization, favors type 2 T-helper cell (Th2) immune deviation and enriches protective immune populations [type 2 invariant natural killer T cells (iNKT2), Th2 cells, memory T cells and anti-inflammatory macrophages], thereby restricting AS progression (95). Experiments in ApoE-deficient mice demonstrated that USP7 knockout reduces EZH2 levels, downregulates pro-inflammatory cytokines (IL-6, TNF-α) and oxidative stress markers (malondialdehyde), alleviates lipid deposition and ultimately attenuates plaque formation (100). By contrast, PRMT2 regulation of macrophage lipid metabolism remains cell-model based: PRMT2 overexpression inhibits ox-LDL-induced foam cell formation in RAW264.7 macrophages by promoting ABCA1-mediated cholesterol efflux (101). This finding broadens the regulatory spectrum of methylation from inflammatory control to macrophage cholesterol homeostasis.

Collectively, methyltransferases regulate AS-related transcription, vascular-cell phenotypes and immune responses through both histone and non-histone methylation. However, evidence maturity varies considerably, ranging from human plaque and ApoE$^{-/-}$ mouse support for SET7/9 and EZH2 to predominantly cell-based evidence for several PRMT pathways.

Emerging lysine acylations with potential relevance to AS: Succinylation, crotonylation and malonylation. In addition to the lysine modifications discussed above, emerging lysine acylations, including succinylation, crotonylation and malonylation, may also have potential relevance to AS, although direct evidence remains limited. These modifications are metabolically sensitive because their acyl donors, such as succinyl-CoA, crotonyl-CoA and malonyl-CoA, are closely connected to intermediary metabolism (102,103). Studies in related cardiovascular and metabolic contexts have shown that lysine succinylation is regulated by SIRT5 and can affect mitochondrial metabolism, fatty acid oxidation, ATP production

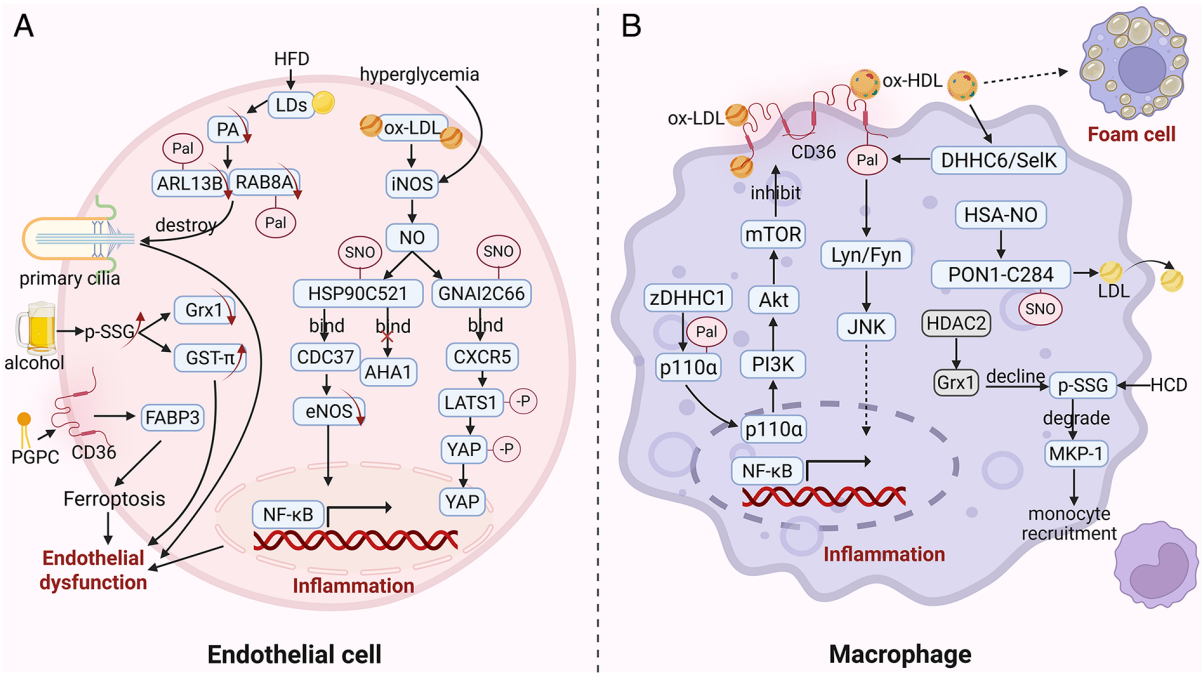

Figure 4. The roles of PTMs on cysteine residues - S-palmitoylation, S-nitrosylation and S-glutathionylation - in AS. (A) In endothelial cells, loss of ciliary-protein palmitoylation promotes ciliary dysfunction, whereas PGPC-induced FABP3 upregulation promotes lipid peroxidation and ferroptosis. S-nitrosylation mediated by iNOS induces inflammation via the NF-κB pathway, while increased glutathionylation levels lead to endothelial dysfunction. (B) In macrophages, palmitoylation regulates CD36 expression through the PI3K-Akt-mTOR and JNK signaling pathways, affecting macrophage phagocytosis of oxHDL and foam cell formation. Nitrosylation of PON1 leads to LDL penetration into macrophages and increased glutathionylation levels promote monocyte recruitment. AHA1, activator of Hsp90 ATPase activity 1; ARL13B, ADP-ribosylation factor-like GTPase 13B; CDC37, cell division cycle 37; CD36, cluster of differentiation 36; CXCR5, C-X-C motif chemokine receptor 5; DHHC6, zinc finger DHHC-type palmitoyltransferase 6; eNOS, endothelial nitric oxide synthase; FABP3, fatty acid-binding protein 3; GNAI2, G protein subunit αi2; Grx1, glutaredoxin 1; GST-π, glutathione-S-transferase π; HCD, high-calorie diet; HFD, high-fat diet; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; LATS1, large tumor suppressor kinase 1; Lyn/Fyn, Src-family tyrosine kinases Lyn and Fyn; MKP-1, MAPK phosphatase 1; mTOR, mechanistic target of rapamycin; ox-HDL, oxidized high-density lipoprotein; mTOR, mammalian target of rapamycin; PA, palmitic acid; PGPC, 1-palmitoyl-2-glutamyl-sn-glycero-3-phosphocholine; PON1, paraoxonase 1; p-SSG, protein glutathionylation; RAB8A, RAB8A small GTPase; SelK, selenoprotein K; SNO, S-nitrosylation; YAP, Yes-associated protein; ZDHHC1, zinc finger DHHC-type palmitoyltransferase 1.

and cardiac function (104,105). Lysine crotonylation has been reported in VSMC phenotypic remodeling, where crotonylome changes may interact with ubiquitination and glycolytic remodeling (106). Lysine malonylation, also regulated by SIRT5, has been linked to glycolytic flux, mitochondrial metabolism and fatty acid oxidation (107,108).

However, whether these acylations directly regulate AS initiation, plaque progression or plaque instability remains largely unresolved. Based on their known links to mitochondrial metabolism, inflammatory signaling and vascular cell remodeling, it can be hypothesized that succinylation, crotonylation and malonylation may influence endothelial dysfunction, macrophage lipid handling or VSMC phenotypic switching during AS. Nevertheless, these possibilities should currently be regarded as hypothesis-generating rather than established mechanisms. Future studies should combine high-resolution mass spectrometry-based acylome profiling, enrichment of low-abundance acylated peptides, isotope tracing of acyl-CoA donors, site-specific functional validation and cell-type-specific AS models to determine whether these emerging acylations are functionally involved in AS rather than merely reflecting metabolic disturbance.

Cysteine modifications in AS: Impact on key cellular events. Modifications of cysteine residues in PTMs primarily involve the covalent attachment of chemical groups via thiol or thioester bonds, thereby regulating protein function, structure, stability and molecular interactions. Accumulating evidence indicates that cysteine residues undergo diverse PTMs, including palmitoylation (109), nitrosylation (110) and glutathionylation (111), as well as a broader range of oxidative and sulfur-related modifications (112). These modifications exert critical regulatory roles in the pathophysiology of AS, particularly within vascular endothelial cells and macrophages (113). Importantly, cysteine-based PTMs should not be interpreted as uniformly pro-atherogenic or atheroprotective modifications. Their biological consequences are highly context-dependent and are shaped by the modified substrate, the functional role of the targeted cysteine residue, the cellular or extracellular compartment in which the modification occurs, the local redox and nitric oxide donor environment, substrate abundance and the stage of AS progression. Therefore, the same PTM may produce opposite effects in different biological settings.

Cysteine modifications in endothelial dysfunction. In vascular endothelial cells (Fig. 4A), HFD-triggered lipid droplet accumulation consumes cytoplasmic palmitate via esterification. In male mouse atherosclerosis models supported by endothelial mechanistic experiments, such palmitate depletion suppresses palmitoylation of ciliary proteins, including ARF like GTPase 13B (ARL13B) and RAB8A, member RAS

oncogene family (RAB8A), inducing ciliary loss and endothelial dysfunction (114). Independent ApoE$^{-/-}$ studies showing that endothelial primary-cilium loss accelerates atherosclerosis support the broader importance of cilia, but do not specifically validate the ARL13B/RAB8A palmitoylation axis (114,115). Direct confirmation of this site-specific mechanism in human plaques remains unavailable.

Oxidized lipid metabolites further link lipid stress to endothelial dysfunction. 1-palmitoyl-2-glutamoyl-sn-glycero-3-phosphocholine (PGPC), an oxidized phospholipid, is internalized by endothelial cells via the cluster of differentiation 36 (CD36) receptor (116). In oxidized lipid-stimulated endothelial cell models, PGPC upregulates fatty acid-binding protein 3 (FABP3), drives iron-dependent lipid peroxidation and glutathione depletion and ultimately triggers endothelial ferroptosis (117). These changes disrupt the mitochondrial membrane potential, increase ROS and impair endothelium-dependent vasodilation, but the PGPC-FABP3 ferroptosis mechanism is currently supported mainly by endothelial functional assays rather than direct human plaque-level validation.

Beyond palmitoylation and its associated oxidized lipid metabolites, specific S-nitrosylation events have emerged as critical regulators of endothelial dysfunction and atherosclerotic progression. Endothelial functional assays and relevant studies in HFD-fed ApoE$^{-/-}$ mice with endothelial-specific expression of Cys521-mutant Hsp90 confirm that S-nitrosylation of the heat shock protein Hsp90 at Cys521 acts as a conformational switch: It disrupts the interaction between Hsp90 and its activator Hsp90 ATPase activity 1, while enhancing Hsp90 binding to CDC37 (118). This molecular rearrangement suppresses eNOS activity and activates NF-κB signaling, thereby exacerbating endothelial oxidative stress, upregulating pro-inflammatory cytokines, promoting monocyte adhesion and ultimately accelerating atherosclerotic plaque formation (119). This pathway has cell and animal support, but direct evidence that the same site-specific Hsp90 S-nitrosylation event operates in human plaques remains limited. Similarly, S-nitrosylation at Cys66 of guanine nucleotide-binding protein G(i) subunit alpha-2 (GNAI2) promotes coupling of the C-X-C motif chemokine receptor (CXCR)5 receptor. The GNAI2-Cys66 pathway has evidence across all three major experimental levels. High glucose plus ox-LDL increased GNAI2 S-nitrosylation in human umbilical vein endothelial cells and human aortic endothelial cells; increased aortic S-nitrosated (SNO)-GNAI2 was detected in STZ/HFD-treated LDLR$^{-/-}$ mice, in which endothelial expression of the non-nitrosylatable GNAI2-C66A mutant reduced lesion formation; and elevated SNO-GNAI2 was also observed in coronary artery samples from patients with diabetes and coronary artery disease (120,121). Notably, pharmacological targeting of these pathways confers promising therapeutic potential. For example, melatonin exerts endothelial protective effects by inhibiting inducible (i)NOS expression and the subsequent S-nitrosylation of downstream substrates (122).

S-glutathionylation further accelerates atherosclerotic progression by impairing the activity of endothelial functional proteins such as eNOS. Specifically, S-glutathionylation of eNOS induces enzymatic uncoupling, redirecting its catalytic activity from nitric oxide production toward superoxide generation; this shift exacerbates vascular oxidative stress and inflammatory responses (123). Chronic binge-alcohol exposure in ApoE$^{-/-}$ mice also increased aortic protein S-glutathionylation, reduced glutaredoxin 1 activity and altered glutathione-S-transferase π activity (124). These molecular alterations promote endothelial dysfunction and oxidative vascular injury, thereby contributing to AS progression.

Cysteine modifications in macrophage activation. During macrophage foam cell formation (Fig. 4B), oxidized high-density lipoprotein (oxHDL) activates the Asp-His-His-Cys (DHHC6) acyltransferase 6/selenoprotein K (SelK) complex, thereby enhancing CD36 palmitoylation. This modification promotes CD36 incorporation into plasma membrane lipid rafts, strengthens its interaction with caveolin-1 and facilitates CD36 membrane targeting. These events subsequently activate Lyn/Fyn kinases and downstream c-Jun N-terminal kinase (JNK) signaling, thereby increasing oxHDL uptake and driving macrophage foam cell formation (125). The DHHC6/SelK-CD36 mechanism is currently supported mainly by macrophage cellular and biochemical experiments, without direct validation in ApoE$^{-/-}$ or LDLR$^{-/-}$ plaques or in human plaque tissue. The palmitoyltransferase zDHHC1 also modulates macrophage function via the PI3K pathway. Accumulating evidence (126) demonstrates that zDHHC1 knockout reduces palmitoylation of the p110α subunit, promotes its nuclear translocation and suppresses activation of the PI3K-Akt-mTOR signaling axis. These alterations downregulate CD36 expression and oxLDL internalization, effectively restraining foam cell generation. Notably, therapeutic potential extends beyond palmitoyltransferases to the regulation of depalmitoylases (127). Targeted modulation of acylprotein thioesterase 2 (APT2) provides novel intervention opportunities: Sulforaphane (SFN) binds specifically to the C56 site of APT2 (128). This interaction inhibits APT2 palmitoylation and membrane localization, thereby potentially disrupting the depalmitoylation of downstream substrates such as Scribble. Although the effects of SFN on canonical APT2 substrates (e.g., H-Ras) require further validation, its ability to modulate APT2-mediated palmitoylation highlights a promising metabolic strategy against atherosclerotic progression.

S-nitrosylation also confers atheroprotective effects through distinct mechanistic pathways. Biochemical and macrophage-based experiments indicate that trans-nitrosylation of paraoxonase 1 (PON1) at Cys284 enhances its enzymatic and antioxidant activities (129). Human serum albumin-released nitric oxide, acting as a trans-nitrosylating donor, modifies PON1 at the Cys284 residue via S-S trans-nitrosylation (130). This PTM markedly enhances PON1 hydrolase activity, including its lactonase and esterase functions, and strengthens its antioxidant capacity. Compared with native PON1, S-nitrosylated PON1 more effectively suppresses Cu^{2+}-induced LDL oxidation and shows increased penetration into macrophage cells (131). Together with the pro-atherogenic effect of Hsp90 S-nitrosylation in endothelial cells, this example highlights that the net effect of S-nitrosylation is determined by substrate identity, affected cellular process and biological context rather than by the modification itself.

Furthermore, HCD animal models show that oxidative stress triggered by a HCD further enhances the S-glutathionylation

Table II. Representative kinase-mediated phosphorylation modules involved in AS pathogenesis.

Kinase module	Representative phosphorylation-related axis	Main AS-related process	Overall effect
MAPK family	p38 MAPK-IL-6; ERK1/2-STAT1 Ser727	Inflammation, VSMC proliferation, macrophage lipid accumulation	Mainly pro-atherogenic
AMPK	AMPK-ULK1 Ser317/Ser777; AMPK-mTORC1	Autophagy, lipid handling, metabolic homeostasis	Mainly protective
ROCK/PERK	ROCK-MLCP-TβR1/Smad2C; PERK-eIF2α/Drp1 Ser616	LDL retention, ER stress, endothelial apoptosis, mitochondrial injury	Mainly pro-atherogenic
JAK2/Src	JAK2-STAT2/STAT3; Src Tyr416; Src-STAT3 Tyr705	VSMC phenotypic switching, macrophage inflammation, endothelial activation	Mainly pro-atherogenic
IKKε	IKKε-STAT1-NLRP3	Low-shear-stress-induced endothelial pyroptosis	Pro-inflammatory/pro-atherogenic

AMPK, AMP-activated protein kinase; AS, atherosclerosis; Drp1, dynamin-related protein 1; eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinases 1 and 2; IKKε, inhibitor of nuclear factor-κB kinase subunit epsilon; IL-6, interleukin-6; JAK2, Janus kinase 2; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; MLCP, myosin light-chain phosphatase; mTORC1, mechanistic target of rapamycin complex 1; NLRP3, NLR family pyrin domain containing 3; PERK, protein kinase R-like endoplasmic reticulum kinase; ROCK, Rho-associated coiled-coil-containing protein kinase; Smad2C, C-terminally phosphorylated Smad2; Src, proto-oncogene tyrosine-protein kinase Src; STAT, signal transducer and activator of transcription; TβR1, transforming growth factor-β receptor type 1; ULK1, Unc-51-like autophagy-activating kinase 1; VSMC, vascular smooth muscle cell.

of key macrophage proteins, leading to the inactivation and degradation of critical regulatory molecules such as MAPK phosphatase 1 (MKP-1) (132). Notably, macrophage-specific overexpression of glutaredoxin (Grx) effectively reverses excessive HCD-driven protein S-glutathionylation, restores endogenous MKP-1 activity and attenuates monocyte recruitment as well as macrophage infiltration within atherosclerotic plaques - thereby halting atherosclerotic progression (133,134). Myeloid HDAC2 inhibition has also been shown in HCD/experimental AS settings to upregulate Grx1, normalize monocyte function, limit macrophage migration into plaques and reduce atherosclerotic progression (135). These S-glutathionylation mechanisms have relatively strong animal-model support, but human plaque confirmation remains limited.

Collectively, these findings highlight the strong substrate dependence of cysteine-based PTMs. The same modification may exert opposing effects on different proteins, as illustrated by the pro-atherogenic effect of Hsp90 S-nitrosylation and the anti-atherogenic effect of PON1 S-nitrosylation. Therefore, therapeutic interventions should target specific proteins and modification sites rather than globally modulating cysteine PTMs.

Kinase-mediated (Ser/Thr/Tyr) phosphorylation in the pathogenesis of AS. Protein phosphorylation modulates protein function and subcellular localization through kinase-catalyzed transfer of the ATP γ-phosphate group to specific amino acid residues of substrate proteins, thereby governing core cellular signaling and pathological processes (136). In AS, serine/threonine (Ser/Thr) and tyrosine (Tyr) phosphorylation participate in plaque initiation, progression and destabilization by regulating endothelial inflammation, macrophage lipid handling, VSMC proliferation and phenotypic switching, oxidative stress, autophagy, ferroptosis and thrombosis-related signaling (137). Rather than acting through isolated linear pathways, these kinases form interconnected signaling modules. Therefore, this section discusses representative kinase-mediated phosphorylation events according to their predominant functional contexts, including MAPK-dependent inflammatory and stress responses, AMP-activated protein kinase (AMPK)-mediated metabolic homeostasis, Rho-associated coiled-coil kinase (ROCK)/protein kinase R-like ER kinase (PERK)/protein kinase C (PKC)δ-related cytoskeletal remodeling and cellular stress responses, non-receptor tyrosine kinase signaling mediated by Janus kinase 2 (JAK2) and SRC proto-oncogene, non-receptor tyrosine kinase (Src), and additional kinase-linked pathways involved in endothelial pyroptosis and atherothrombosis (Table II).

First, the MAPK family serves as core regulators of cellular stress and inflammatory responses. As key serine/threonine (Ser/Thr) kinases, MAPKs govern fundamental signal transduction cascades, with major subfamilies including p38α MAPK, ERK and JNK. These kinases are activated by multiple pathological stimuli, such as oxidative stress, proinflammatory cytokines and altered shear stress. In AS, the MAPK axis profoundly modulates inflammation, cell proliferation, apoptosis and lipid metabolism throughout disease progression (21). In atherosclerotic rat models, aerobic exercise alleviates dyslipidemia and vascular calcification by elevating aortic 5-methoxytryptophan levels and suppressing the p38 MAPK/IL-6 pathway (138). Under hyperglycemic VSMC conditions, enhanced ERK1/2 phosphorylation upregulates TGF-β and MMP9 expression and promotes VSMC proliferation, indicating a mainly cell-model-based mechanism relevant to diabetic vascular remodeling (139). In contrast, the ERK1/STAT1 Ser727 axis has stronger

in vivo support: In LDLR-deficient mice fed an HFD, ERK1 deficiency and STAT1 S727A modification reduced plaque lipid content and modulated plaque inflammatory responses, while accompanying macrophage experiments helped define the effects on migration, proliferation, phagocytosis and oxLDL handling (140,141). Toll-like receptor (TLR)4 is an important regulator of VSMC-derived foam cell formation. In ox-LDL-stimulated VSMCs, 1,25(OH)2D3 reduces lipid uptake and enhances cholesterol efflux by downregulating TLR4, CD36 and scavenger receptor class A while upregulating ABCA1, ABCG1 and LXR-α; therefore, the proposed vitamin D-JNK-TLR4 pathway should be interpreted primarily as a VSMC foam-cell mechanism unless further human plaque validation is available (142).

AMPK serves as a core regulator of metabolic homeostasis and exerts comprehensive protective effects against atherosclerotic pathogenesis. As a highly conserved serine/threonine (Ser/Thr) kinase, AMPK functions as a central energy sensor governing cellular metabolism, with well-documented anti-atherosclerotic and vasoprotective properties. Mechanistically, AMPK mitigates oxidative stress (143), suppresses proinflammatory cascades (144), and inhibits aberrant proliferation of VSMCs (145). Macrophage and vascular-cell models suggest that AMPK reduces intracellular lipid accumulation by initiating autophagic flux through phosphorylation of unc-51 like autophagy activating kinase 1 at Ser317/Ser777 (146), whereas experimental AS models suggest that AMPK/mTOR-dependent autophagy contributes to plaque stabilization (147). In macrophage ferroptosis and lipid-handling models, Fer-1 enhances cholesterol efflux by upregulating scavenger receptor class B member 1 through AMPK activation and alleviates lipid deposition and iron overload (148). Thus, AMPK signaling is well supported in experimental AS models, although direct human plaque validation of specific AMPK substrate phosphorylation events remains limited.

Beyond the MAPK and AMPK families, multiple kinases contribute to AS progression by regulating cytoskeletal dynamics, ER stress (ERS) and cell migration. These include ROCK, PERK, IKK and Akt, as well as PKC isoforms. Among them, PKCδ exerts a unique regulatory role in vascular injury repair.

ROCK drives AS progression by phosphorylating and inhibiting myosin light chain phosphatase, thereby potentiating endothelin-1-mediated transforming growth factor β receptor 1 activation (149,150). This signaling cascade ultimately promotes Smad2C phosphorylation, chondroitin 4-sulfotransferase 1 upregulation and LDL retention in the vascular wall, directly exacerbating atherosclerotic plaque formation. As a core regulatory kinase of ERS, PERK induces endothelial cell apoptosis by phosphorylating its downstream target eukaryotic initiation factor 2α (151). Additionally, PERK activates the calcium/calmodulin dependent protein kinase IIβ/dynamin-related protein 1 (Drp1) signaling pathway, driving mitochondrial hyperfragmentation and increasing ROS production by enhancing phosphorylation of Drp1 at Ser616 (p-Drp1 Ser616). In vascular injury models, PKCδ promotes the expression of CXCR2 ligands in VSMCs, modulates chemokine secretion, supports endothelial repair after arterial injury and reduces thrombosis-related complications (152). Direct human plaque evidence for ROCK-, PERK- or PKCδ-specific phosphorylation events remains limited.

Non-receptor tyrosine kinases play a pivotal role in regulating vascular cell function and the inflammatory microenvironment by phosphorylating STAT family transcription factors. The non-receptor tyrosine kinase JAK2 phosphorylates STAT3, driving the transition of VSMCs from a contractile to a synthetic phenotype and accelerating vascular remodeling (153). In homocysteine-stimulated macrophage models and $ApoE^{-/-}$ mouse atherosclerosis, FABP4 activates RAP1A, member of RAS oncogene family-dependent JAK2/STAT2 signaling, promotes STAT2 nuclear translocation, and increases IL-1β, IL-6 and TNF-α secretion, giving this JAK2/STAT2 pathway both macrophage mechanistic and *in vivo* support (154). Src signaling has also been validated beyond cell culture: Autophosphorylation of Src at Tyr416 mediates membrane translocation and amplifies homocysteine-induced macrophage inflammation, while endothelial-specific Src knockout in a carotid ligation mouse model markedly attenuated plaque formation. c-Src also phosphorylates STAT3 at Tyr705, promoting nuclear translocation and endothelial inflammatory responses (17). These data support Src as an experimentally validated *in vivo* target, although direct human plaque validation of the specific Src-STAT phosphorylation events remains limited.

Furthermore, the non-canonical IKK family member IKKε mediates low shear stress-induced endothelial pyroptosis through the STAT1/NLRP3 axis, providing mechanistic insight into how disturbed flow links endothelial inflammation to AS progression (155). In addition to inflammatory regulation, kinase signaling also participates in atherothrombotic processes. Junctional cadherin 5 associated (JCAD) has comparatively stronger translational support: Human vascular/thrombotic association data and experimental pathway validation indicate that JCAD promotes arterial thrombosis by enhancing endothelial expression of procoagulant and anti-fibrinolytic factors, including tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1). Mechanistically, JCAD appears to restrain PI3K/Akt signaling under this context, as JCAD silencing increases PI3K/Akt activation and thereby downregulates TF and PAI-1 expression. The causal involvement of PI3K/Akt is supported by the observation that wortmannin, a PI3K/Akt pathway inhibitor, abolishes the inhibitory effects of JCAD silencing on TF and PAI-1. Thus, the JCAD-PI3K/Akt axis links endothelial kinase signaling to coagulation and fibrinolytic balance, providing a potential mechanism by which JCAD contributes to atherothrombosis (156).

Protein phosphorylation exerts fine-tuned regulation across multiple dimensions of AS through four major kinase categories: Stress and inflammatory response kinases, metabolic and homeostasis regulatory kinases, cytoskeletal remodelling kinases and tyrosine kinase signal-initiating kinases. These kinases not only execute specific functions independently but also intersect through intricate signaling networks, collectively determining vascular cell fate and plaque stability.

Other PTMs in the pathogenesis of AS. Beyond the primary PTMs outlined above, glycosylation/glycation, tyrosine nitration and ADP-ribosylation contribute to vascular pathology

through distinct mechanisms and with markedly different evidence maturity. Their effects should not be treated as uniformly pro-atherogenic: Certain pathways have been tested in ApoE$^{-/-}$ mice or human plaque tissue, whereas others remain limited to stimulated vascular cells or non-AS disease models.

Protein glycosylation in AS involves enzymatically regulated N-glycosylation and non-enzymatic glycation; canonical N-glycosylation commonly occurs at Asn-X-Ser/Thr motifs (157). Hypoglycosylated ICAM-1 has been identified in mouse and human models of endothelial dysfunction and displays enhanced monocyte adhesion; its enrichment in lesion-associated regions provides tissue-level association rather than direct proof that a defined glycan causes plaque progression (158). CD36 contributes substantially to ox-LDL uptake in mouse and human macrophages (159). Neuraminidase 1 (NEU1) was identified as a membrane interaction partner of CD36, and cell-based experiments indicate that NEU1-mediated desialylation increases CD36-dependent ox-LDL uptake (160). However, the NEU1-CD36 glycan mechanism has not been directly validated by site-specific manipulation in ApoE$^{-/-}$ or LDLR$^{-/-}$ plaques or human tissue.

At the non-enzymatic glycation level, circulating and tissue advanced glycation end products are increased in diabetes, but biomarker elevation alone does not establish a causal AS mechanism. The RAGE-NF-κB-Caveolin-1 pathway has been supported by endothelial experiments and ApoE$^{-/-}$ mice, where it increases LDL transcytosis (161). By contrast, AGE-induced NADPH-oxidase-derived oxidative stress and foam-cell formation are supported mainly by macrophage assays (162), and AGE-RAGE-PI3K/AKT-dependent proliferation and migration were demonstrated in cultured human aortic smooth-muscle cells (163). The use of human cells should not be equated with evidence from human plaques or clinical cohorts.

Nitration is a PTM of proteins mediated by reactive nitrogen species, particularly the nitration of tyrosine residues induced by peroxynitrite ($ONOO^-$), which alters protein structure and function (164). Evidence from stimulated vascular cell and *ex vivo* vascular models indicates that angiotensin II activates the angiotensin II type 1 receptor (AT1 receptor)-iNOS pathway, increases NO and superoxide production, and promotes $ONOO^-$ formation and protein tyrosine nitration, thereby impairing NO-dependent endothelial function (165). However, this mechanism has not been directly validated as a site-specific causal pathway in ApoE$^{-/-}$ or LDLR$^{-/-}$ AS models.

Myeloperoxidase (MPO) further contributes to oxidative and nitrative stress by generating reactive oxidants, promoting LDL oxidation and reducing vascular NO bioavailability. These processes facilitate endothelial inflammation, macrophage activation and foam-cell formation, although the contribution of individual MPO-dependent nitration events to plaque progression remains incompletely defined (166). Human tissue studies provide associative evidence: Increased 3-nitrotyrosine has been detected in advanced carotid and coronary atherosclerotic lesions, with higher levels reported in unstable than in stable carotid plaques, and site-specific nitration of ApoA-I has been identified in human coronary tissue (167,168). Nevertheless, these human observations do not establish that a specific nitration site causally drives AS progression.

ADP-ribosylation regulates DNA-damage responses, transcription, chromatin organization and cell fate through the covalent attachment of mono-ADP-ribose (MAR) or PAR to diverse amino acid residues, including serine, glutamate, aspartate and lysine, in an enzyme- and context-dependent manner (169,170). Its vascular effects should not be regarded as uniformly pro-atherogenic, because PARP family members differ in catalytic activity, substrates and biological functions. Nevertheless, sustained PARP1-mediated PARylation has generally been associated with vascular oxidative stress, inflammation and cellular injury (171). Transient PARP1 activation facilitates the recruitment of DNA-repair factors following oxidative DNA damage, whereas persistent activation consumes intracellular NAD^+ and may impair NAD^+-dependent metabolic and stress-response pathways, thereby promoting vascular-cell dysfunction or death (172,173). More direct mechanistic evidence indicates that PARP1 also regulates VSMC phenotypic switching. Increased PARP1 activity has been detected in VSMCs within human coronary atherosclerotic plaques and in experimental vascular-injury models. In VSMC and non-AS vascular-injury experiments, PARP1-mediated PARylation of myocardin and serum response factor impaired myocardin-SRF-dependent contractile gene transcription, while PARylation-dependent disruption of the myocardin-c-Jun interaction promoted VSMC proliferation and migration (174). However, this substrate-specific mechanism has not yet been directly validated in ApoE$^{-/-}$ or LDLR$^{-/-}$ atherosclerosis models. Evidence from other vascular disease models further supports a relationship between excessive PARylation and VSMC loss. In patient-derived VSMCs and LmnaG609G/G609G mice modelling Hutchinson-Gilford progeria syndrome, trifluridine reduced PARP1 activity and restored NAD^+ levels, while the trifluridine/tipiracil combination TAS-102 alleviated aortic VSMC loss and structural abnormalities (175). Because this evidence was obtained from a premature vascular-ageing model rather than a conventional ApoE$^{-/-}$ or LDLR$^{-/-}$ model, its relevance to common AS requires further validation. PARP inhibition has also shown anti-inflammatory effects in AS-related cell models. In ox-LDL-stimulated THP-1 monocytes and related monocyte-endothelial adhesion and foam-cell assays, olaparib reduced mitochondrial ROS production, NF-κB and NLRP3 inflammasome activation, monocyte adhesion and foam-cell formation (176,177). These findings remain limited to *in vitro* experiments and have not been validated in ApoE$^{-/-}$ or LDLR$^{-/-}$ mice or in human plaques. Compared with PARP1-mediated PARylation, the role of MARylation in AS remains less established. In cytokine-stimulated macrophages, PARP14-mediated MARylation of STAT1 at Glu657 and Glu705 suppressed STAT1 Tyr701 phosphorylation and pro-inflammatory gene expression, and PARP14 deficiency aggravated arterial lesion formation in non-classical mouse models of acute and chronic vascular disease (178). Nevertheless, direct validation of this pathway in AS-prone mice and human plaques is lacking. Overall, although PARP1 activity and PAR accumulation have been observed in human AS tissue, site-specific causal evidence for ADP-ribosylation substrates in naturally progressing human AS remains limited.

Taken together, these modifications occupy different positions on the translational spectrum. Glycosylation/glycation

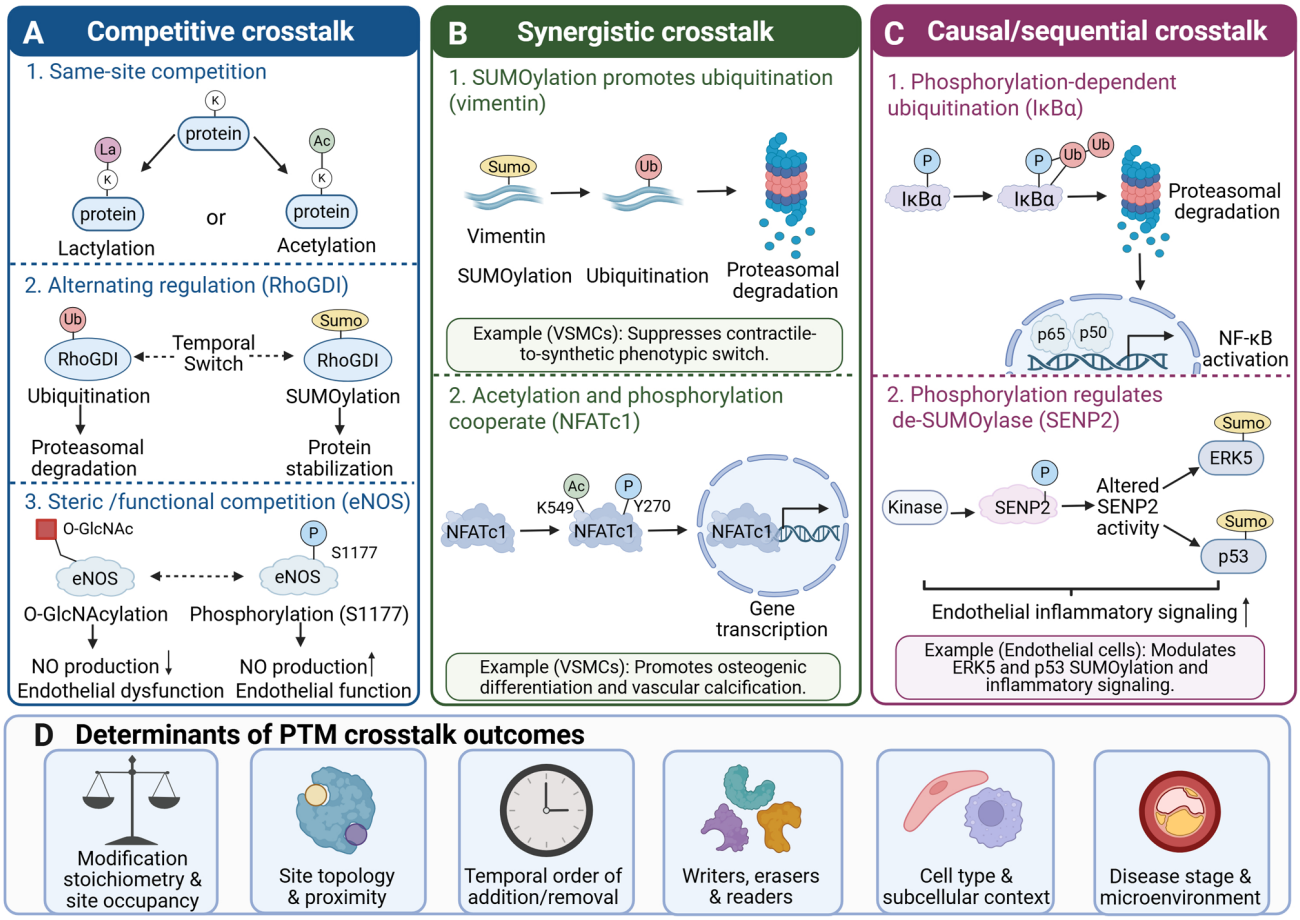

Figure 5. Context-dependent post-translational modification crosstalk in atherosclerosis. (A) Competitive crosstalk. Lactylation and acetylation may compete for the same lysine residue. Temporally alternating ubiquitination and SUMOylation regulate RhoGDI stability in VSMCs, whereas reciprocal O-GlcNAcylation and phosphorylation of eNOS influence endothelial nitric oxide production. (B) Synergistic crosstalk. SUMOylation promotes vimentin ubiquitination and degradation, while NFATc1 acetylation and phosphorylation cooperate to enhance nuclear localization, VSMC osteogenic differentiation and vascular calcification. (C) Causal or sequential crosstalk. IκBα phosphorylation triggers its ubiquitination and degradation, leading to NF-κB activation. Phosphorylation of SENP2 alters its de-SUMOylase activity and downstream ERK5 and p53 SUMOylation. (D) PTM crosstalk outcomes are determined by modification stoichiometry, site topology, temporal order, PTM-regulatory enzymes, cell type and disease stage. eNOS, endothelial nitric oxide synthase; ERK5, extracellular signal-regulated kinase 5; IκBα, inhibitor of nuclear factor κBα; NFATc1, nuclear factor of activated T cells 1; NF-κB, nuclear factor κB; NO, nitric oxide; O-GlcNAc, O-linked β-N-acetylglucosamine; p50, NF-κB p50 subunit; p53, tumor protein p53; p65, NF-κB p65 subunit; PTM, post-translational modification; RhoGDI, Rho GDP-dissociation inhibitor; SENP2, SUMO-specific protease 2; SUMO, small ubiquitin-like modifier; VSMC, vascular smooth muscle cell.

includes cell, ApoE$^{-/-}$ and human tissue/clinical-association evidence, but many glycan-specific mechanisms remain unresolved. Protein nitration has substantial human plaque association, whereas causal validation of individual nitrated sites is scarce. ADP-ribosylation is supported mainly by cell and non-AS vascular models, with selected human plaque observations but little site-specific human causality. Proposed interactions among AGE-RAGE signaling, oxidative stress, nitration and PARP activation remain mechanistically plausible but should be treated as hypothesis-generating until matched PTM measurements and perturbation experiments are performed in the same AS model.

3. Interactions and crosstalk of PTMs in AS pathogenesis

Protein PTM crosstalk refers to the functional interaction between two or more modifications that jointly regulate protein conformation, activity, stability, subcellular localization, molecular interactions and turnover. Such interactions may occur on the same amino acid residue, between neighboring residues within a short peptide region, between spatially separated sites connected by conformational changes or through sequential modification cascades involving different proteins and modifying enzymes (179). In AS, PTM crosstalk provides a molecular mechanism through which metabolic stress, disturbed blood flow, oxidative stress, inflammatory stimulation and cell-state transitions can be integrated into coordinated pathological responses.

In the present framework, PTM crosstalk is discussed at three interconnected levels: Competitive, synergistic and causal crosstalk (Fig. 5). However, these categories should not be interpreted as completely mutually exclusive. Competitive and synergistic crosstalk primarily describe the direction of the functional interaction, whereas causal crosstalk describes the dependency and temporal order between modifications. Accordingly, a phosphorylation event that induces substrate ubiquitination is both causal, because phosphorylation is required for ubiquitination, and synergistic, because the two modifications cooperate to promote degradation. The same PTM pair may also shift between competitive and synergistic

relationships depending on the substrate, modified residue, modifying enzyme, cell type and disease stage.

To avoid overinterpreting co-occurring modifications as direct crosstalk, at least four levels of experimental evidence should be distinguished. Level I evidence is the co-detection of two PTMs in the same tissue, cell population, protein, peptide or intact proteoform. Level II evidence is a regulatory association in which manipulation of a writer, eraser, reader, metabolic pathway or upstream stimulus alters both PTMs. Level III evidence requires substrate- and site-resolved demonstration that changing the first modification alters the second modification and the corresponding molecular function. Level IV evidence additionally demonstrates that the site-specific crosstalk contributes to AS-related phenotypes *in vivo*. This evidence hierarchy is particularly important because large-scale proteomic datasets are powerful for identifying candidate crosstalk networks but generally do not, by themselves, establish causal relationships.

Conceptual framework and evidence hierarchy of PTM crosstalk. Recent mass spectrometry-based approaches have expanded PTM crosstalk research from individual substrates to proteome-wide networks. An enrichment-free workflow combining liquid chromatography with high-field asymmetric waveform ion mobility spectrometry substantially increased the identification of peptides containing multiple modifications. Searching simultaneously for phosphorylation, acetylation and methylation identified ~6-fold more candidate PTM crosstalk sites than a conventional workflow, providing a publicly available resource for discovering modification combinations that may occur on the same peptide (180). Because multiple PTMs are detected within the same peptide sequence, this approach provides stronger evidence for local coexistence than separately identifying modified peptides from the same protein. Nevertheless, coexistence still does not demonstrate that one PTM functionally regulates another.

Site-resolved protein turnover profiling (SPOT) further linked PTM states to protein dynamics. By combining stable-isotope labeling with phosphopeptide, acetyl-peptide and ubiquitin-remnant enrichment, SPOT quantified >120,000 peptidoforms, including >33,000 phosphorylated, acetylated or ubiquitinated peptides derived from >9,000 proteins (181). Modified and unmodified forms of the same protein frequently displayed different apparent turnover behavior. Importantly, the direction of the association was site-dependent: Different modified forms of the same protein could show accelerated, delayed or unchanged turnover. This result argues against assigning a universal functional consequence to an entire PTM class. For example, ubiquitination is not invariably equivalent to rapid degradation and acetylation is not invariably equivalent to protein stabilization.

Phosphoproteomic analysis using DeltaSILAC (delta determination of turnover rate for modified proteins by stable isotope labeling with amino acids in cell culture) similarly demonstrated that individual phosphorylation sites may be associated with either accelerated or delayed protein turnover (182). However, subsequent protein-peptide turnover profiling showed that differences between modified and unmodified peptide-labeling kinetics do not necessarily indicate that the PTM directly alters protein degradation. Because modified and unmodified proteoforms interconvert during the lifetime of a protein, such differences can instead reveal whether a PTM is added early or late during protein maturation and whether it is subsequently removed (183). Therefore, turnover-based datasets should be considered resources for prioritizing candidate sequential crosstalk rather than definitive evidence that a specific PTM controls protein stability.

Direct multi-PTM profiling in human AS plaques remains limited. Nevertheless, available disease-focused datasets provide an important basis for constructing AS-related PTM networks. Phosphoproteomic analysis of VSMCs showed that P2RY12 signaling regulated the PI3K-AKT-mTOR-autophagy pathway and promoted VSMC-derived foam-cell formation in advanced AS (184). Phosphoproteomic analysis of cells deficient in the cytoplasmic protein tyrosine kinase FES (FES proto-oncogene, tyrosine kinase) identified migration-associated phosphorylation networks, while genetic and *in vivo* analyses supported an atheroprotective function of FES (185). These datasets do not directly demonstrate crosstalk between phosphorylation and a second PTM, but they identify disease-relevant phosphoproteins that can be prioritized for matched ubiquitinomic, acetylomic, SUMOylomic or glycoproteomic analysis.

Human plaque total-proteome resources are equally important for interpreting PTM changes. A proteomic atlas comprising 219 carotid plaque samples from 120 patients identified distinct regional protein signatures associated with plaque inflammation, calcification, SMC content and extracellular matrix organization (186). Such total-proteome datasets can be integrated with PTM-enrichment datasets to determine whether an apparent change in a modified peptide reflects altered PTM occupancy or merely altered abundance of the corresponding protein. They can also help identify cell-composition confounding, because an apparent increase in a macrophage-associated PTM may result from greater macrophage abundance rather than increased modification within each macrophage.

These datasets collectively establish a practical discovery pipeline for AS PTM crosstalk: Disease-relevant proteins are first identified by plaque proteomics or cell-specific phosphoproteomics; candidate modification combinations are then prioritized using multi-PTM datasets; and the interactions are subsequently tested using site-specific perturbation, temporal analysis and *in vivo* disease models. Thus, proteomics should be viewed as a hypothesis-generating platform that transforms isolated PTM observations into testable modification networks.

Three mechanistic modes of PTM crosstalk in AS

Competitive crosstalk. Competitive crosstalk occurs when the formation or function of one PTM limits another PTM. At least three mechanistically distinct forms of competition should be distinguished. First, site-exclusive competition occurs when two modifications target the same amino acid residue and are therefore chemically mutually exclusive. Second, steric or conformational competition occurs when modifications at neighboring or structurally coupled residues alter the accessibility, recognition or functional effect of another modification. Third, resource competition occurs when multiple PTMs share a limited writer, eraser, reader, cofactor or metabolic donor. Only the first mechanism constitutes strict same-residue

competition; the other two represent indirect but biologically relevant forms of competition.

The proposed relationship between lysine lactylation and acetylation illustrates these distinctions. Both modifications can occur on lysine residues, and hypoxic and inflammatory regions within atherosclerotic plaques exhibit metabolic conditions that may favor glycolysis and lactate accumulation. Increased lactate availability can promote lysine lactylation, whereas acetyl-CoA availability contributes to protein acetylation (187-189). Consequently, lactylation and acetylation have the potential to compete when they target the same lysine. However, reciprocal changes in global lactylation and acetylation are not sufficient to demonstrate direct mutual exclusion at an individual residue.

The relative abundance of lactylation and acetylation should also not be interpreted simply as a direct quantitative indicator of pyruvate partitioning between lactate production and mitochondrial oxidation. It instead integrates several variables, including lactate and acetyl-CoA availability, formation of activated acyl donors, writer and eraser activities, substrate accessibility, protein turnover and the abundance of the underlying protein. Therefore, metabolic flux can bias the modification equilibrium without being its sole determinant.

The enzymatic basis of lysine lactylation has also become increasingly complex. p300 was initially implicated as a histone lactylation writer in the original description of histone lactylation (22). More recent studies identified alanyl-tRNA synthetase as a lactate sensor and lactyltransferase and HBO1/KAT7 as a lysine lactyltransferase in specific biological contexts (44,190). In parallel, class I histone deacetylases HDAC1-3 were shown to possess delactylase activity (43). Because some of these enzymes also regulate acetylation, lactylation-acetylation crosstalk may involve not only same-site competition but also competition for multifunctional enzymes. Nevertheless, the substrate range and relative contribution of each writer or eraser are cell- and context-dependent, and their roles in endothelial cells, macrophages and VSMCs in AS remain to be defined.

Competition between ubiquitination and SUMOylation represents a more established lysine-centered mechanism. Rho-specific guanine nucleotide dissociation inhibitor (RhoGDI), a regulator of Rho GTPase signaling, participates in Ang II-induced phenotypic modulation of VSMCs (191,192). During the early and late phases of Ang II stimulation, enhanced ubiquitination is associated with proteasome-dependent RhoGDI degradation. By contrast, SUMOylation predominates during an intermediate time window and limits ubiquitin-chain formation, thereby maintaining RhoGDI stability and promoting VSMC proliferation (193). This regulation is dependent on AT1-receptor signaling but displays marked temporal specificity.

The RhoGDI example demonstrates that competitive crosstalk is not a static molecular property. The dominant PTM state depends on the relative kinetics of SUMO and ubiquitin conjugation, the abundance and localization of their respective enzymes, the accessibility of substrate lysines and the duration of upstream stimulation. It also indicates that a single endpoint measurement may obscure sequential changes in the modification equilibrium.

Reciprocal O-GlcNAcylation-phosphorylation provides an additional vascular example. In endothelial cells exposed to hyperglycemia, increased O-GlcNAc modification of eNOS was accompanied by decreased activating phosphorylation at Ser1177 and reduced eNOS activity. Similar changes were observed in arteries from diabetic animals (194). Because eNOS-derived nitric oxide restrains leukocyte adhesion, platelet activation, vascular permeability and VSMC proliferation, this reciprocal PTM regulation provides a mechanistic connection between metabolic stress and endothelial dysfunction.

Nevertheless, reciprocal O-GlcNAcylation and phosphorylation should not automatically be interpreted as direct competition at the same residue unless both sites are structurally resolved. Competition may also arise because O-GlcNAcylation alters local conformation, inhibits kinase access, recruits different binding proteins or changes upstream AKT signaling. Thus, this example illustrates both the strength and the limitation of modification-specific immunochemical evidence: It demonstrates a functionally important reciprocal relationship but does not necessarily define the complete molecular configuration of the modified proteoform.

Collectively, competitive crosstalk allows vascular cells to choose between alternative protein states. Same-site competition produces mutually exclusive proteoforms; structural competition changes the accessibility or consequence of neighboring sites; and resource competition links PTM patterns to metabolic and enzymatic capacity. These mechanisms enable metabolic and inflammatory signals to be integrated at the level of a single substrate.

Synergistic crosstalk. Synergistic crosstalk occurs when two PTMs cooperate to generate a functional output that is greater, more persistent, or more specific than that produced by either modification alone. One PTM may expose a second site through conformational remodeling, create a docking site for another writer or reader, promote substrate localization to a particular cellular compartment or stabilize an interaction complex required for the second modification. Synergy may therefore occur without the two PTMs occupying adjacent residues.

The cooperation between SUMOylation and ubiquitination in the regulation of vimentin provides an instructive example. PIAS3, acting as a SUMO E3 ligase, enhances vimentin SUMOylation and is associated with increased vimentin ubiquitination and degradation. This process suppresses the transition of VSMCs from a contractile to a synthetic phenotype (51). In contrast to RhoGDI, in which SUMOylation protects the substrate from ubiquitination, vimentin SUMOylation facilitates ubiquitin-dependent degradation.

The opposite outcomes of SUMOylation-ubiquitination crosstalk on RhoGDI and vimentin reveal an important principle: Functional direction cannot be predicted solely from the identities of the two PTMs. It depends on whether the PTMs occupy the same or different lysines, the distance and three-dimensional relationship between modified sites, the type of SUMO chain, the recruitment of SUMO-targeted ubiquitin ligases and the temporal sequence of modification. Accordingly, the substrate-centered proteoform, rather than the PTM pair in isolation, should be considered the fundamental unit of crosstalk.

Acetylation-phosphorylation cooperation also contributes to VSMC dysfunction. In diabetic vascular calcification, carboxymethyl-lysine suppresses SIRT3 and promotes nuclear factor of activated T cells 1 (NFATc1) acetylation at K549, while focal adhesion kinase-related signaling regulates NFATc1 phosphorylation at Y270 (195). These modifications jointly enhance NFATc1 nuclear accumulation and transcriptional activity, thereby promoting osteogenic differentiation of VSMCs and vascular calcification. Because the two modifications occur on different residues, their synergy is likely mediated through changes in conformation, nuclear trafficking, transcriptional-complex assembly or resistance to nuclear export rather than direct competition.

Synergistic crosstalk may introduces threshold behavior into pathological signaling. A single modification may be insufficient to produce a phenotype, whereas the combined presence of two PTMs shifts a sufficiently large fraction of the substrate into an active, stable, nuclear or degradation-prone state (15,179,195,196). This may explain why modest changes in two PTMs can produce a substantial functional effect even when neither change is individually dominant.

Synergy can also occur at the pathway level rather than on a single protein molecule. For example, phosphorylation may activate a transcription factor while acetylation stabilizes its transcriptional coactivator (195). Although the two PTMs occur on different proteins, their effects converge on the same pathological output. Such pathway-level crosstalk is biologically relevant but should be distinguished from direct intramolecular crosstalk. The former can be supported by integrated pathway perturbation, whereas the latter requires evidence that both modifications occur on the same substrate or proteoform (180,197).

Causal and sequential crosstalk. Causal crosstalk occurs when one modification is mechanistically required for the formation, removal, recognition or functional consequence of another. It is therefore defined by dependency and temporal order rather than simply by whether the final effects are antagonistic or synergistic. Causal crosstalk may involve a substrate modification that creates a recognition motif, modification of a writer or eraser that alters its enzymatic activity, or modification-dependent changes in substrate localization and accessibility (181-183).

Phosphorylation-directed ubiquitination is a prototypical causal mechanism. In the NF-κB pathway, phosphorylation of IκBα creates a phosphodegron that is recognized by the ubiquitination machinery, resulting in polyubiquitination and proteasomal degradation of IκBα (57,198). The loss of IκBα releases NF-κB and permits its nuclear translocation and inflammatory transcriptional activity. Phosphorylation is therefore not merely associated with IκBα ubiquitination but provides the recognition signal required for the subsequent event.

This sequential mechanism increases signaling specificity by ensuring that degradation occurs only after sufficient kinase activation. However, the process should not be described as universally irreversible. IκBα is resynthesized as part of NF-κB negative feedback, and the duration of inflammatory signaling is determined by the balance between degradation, transcriptional induction and protein resynthesis (199). Therefore, PTM cascades may convert transient signals into sustained responses without necessarily producing permanent molecular states.

Modification of PTM-regulating enzymes represents another form of causal crosstalk. The phosphorylation state of SENP2 regulates its de-SUMOylase activity and consequently alters the SUMOylation levels of substrates including ERK5 and p53, thereby influencing endothelial inflammatory responses under disturbed flow (48). In this case, phosphorylation does not directly compete with substrate SUMOylation. Instead, phosphorylation acts at the enzyme level and propagates its effect to multiple downstream SUMOylated proteins. Such enzyme-centered crosstalk can amplify an upstream signal but also complicates causal attribution because manipulating SENP2 may simultaneously affect numerous substrates.

Glycosylation-dependent regulation of CD36 stability represents a plausible but less completely established causal pathway. NEU1-mediated desialylation reduces CD36 glycosylation and enhances macrophage uptake of oxidized LDL (160). Changes in glycosylation may alter CD36 conformation, membrane residence, endocytosis, exposure of cytoplasmic lysines or accessibility to ubiquitin ligases. However, direct causal crosstalk would require demonstration that manipulation of a defined CD36 glycosylation site changes its ubiquitination independently of total CD36 expression, trafficking and endocytosis. Until such evidence is available, this relationship should be presented as a candidate glycosylation-ubiquitination pathway rather than an established sequential cascade.

Temporal resolution is essential for identifying causal PTM interactions. A modification that precedes another may represent an upstream trigger, but both PTMs may alternatively be induced in parallel by a shared stimulus. Time-resolved perturbation, pulse-chase labeling, kinase or ligase inhibition and analysis of modification-deficient mutants are required to distinguish sequential dependency from coincident regulation (200). Furthermore, the effect of inhibiting the first PTM on the second PTM should be normalized to total substrate abundance, because loss of the protein itself may produce an apparent reduction in all associated modifications (201).

Context-dependent determinants of PTM crosstalk outcomes

PTM stoichiometry as a determinant of crosstalk direction and biological effect. PTM stoichiometry, also described as site occupancy, is a central but frequently overlooked determinant of crosstalk. For a single lysine that can exist in mutually exclusive unmodified, acetylated, lactylated, ubiquitinated or SUMOylated states, an increase in one state necessarily limits the fraction available for the others. The functional importance of this competition depends not only on the fold change in PTM abundance but also on the absolute fraction of substrate molecules carrying each modification.

Proteomic measurement of 6,829 acetylation sites in human cells found that most acetylation sites occurred at very low stoichiometry, with a median occupancy of ~0.02%, whereas higher-occupancy sites were enriched in nuclear proteins and transcriptional regulators (196). This finding demonstrates why PTM fold changes cannot be interpreted independently of occupancy. A 10-fold increase from 0.001 to 0.01% occupancy may affect only a small fraction of the total protein pool,

whereas a 20% change at a highly occupied regulatory site may alter a substantial proportion of substrate molecules.

Low global occupancy does not necessarily imply biological irrelevance. A PTM may be restricted to a small but functionally critical subcellular compartment, protein complex, plaque region or cell population. A low-abundance modified proteoform may also exert a disproportionate functional effect, for example by initiating a signaling cascade or nucleating a multiprotein complex (15). Therefore, occupancy should be interpreted together with localization, effect size per modified molecule, substrate abundance and the amplification properties of the downstream pathway.

This issue is particularly relevant to AS plaques, in which bulk tissue measurements average signals across heterogeneous cell populations. A large change in a macrophage-specific PTM may appear quantitatively small after dilution by extracellular matrix and other cell types (202,203). Conversely, an apparent increase in PTM abundance may reflect expansion of the cell population expressing the modified protein. Matched measurement of the modified peptide, corresponding unmodified peptide, total protein and cell-type abundance is therefore required.

Stoichiometry also helps explain why the same PTM pair can produce different effects on different substrates (196). If SUMOylation occupies a major fraction of a ubiquitination-prone lysine on RhoGDI, it can effectively protect the substrate from degradation (191). If vimentin SUMOylation occurs at a separate site that recruits a ubiquitin ligase, even relatively low SUMO occupancy may facilitate degradation (51). Thus, site occupancy interacts with site topology and reader recruitment to determine whether SUMOylation-ubiquitination crosstalk is competitive or synergistic.

Cell type, disease stage and spatial context of PTM crosstalk in AS. PTM crosstalk should be mapped onto the cellular and temporal architecture of AS. In endothelial cells, disturbed flow, hyperglycemia and oxidative stress can alter phosphorylation, SUMOylation, ubiquitination and O-GlcNAcylation networks that regulate nitric oxide production, inflammatory activation and barrier integrity. The reciprocal O-GlcNAcylation-phosphorylation of eNOS and phosphorylation-dependent regulation of SENP2 are representative examples (49,50,194).

In macrophages, metabolic reprogramming, lipid loading, hypoxia and inflammatory activation can change the availability of acyl donors and the activities of PTM enzymes (22,25,29,43). These changes make macrophages a likely context for lactylation-acetylation crosstalk and glycosylation-dependent regulation of lipid receptors such as CD36 (160,187-189). Nevertheless, direct site-resolved lactylation-acetylation competition in plaque macrophages remains to be demonstrated. In VSMCs, PTM crosstalk regulates protein stability, migration, proliferation, foam-cell formation, osteogenic differentiation and phenotypic switching (35). RhoGDI SUMOylation-ubiquitination, vimentin SUMOylation-ubiquitination, NFATc1 acetylation-phosphorylation and P2RY12-associated phosphorylation networks represent distinct mechanisms through which PTMs influence VSMC fate (51,184,191,195).

Disease stage may alter both the direction and consequence of crosstalk. During early endothelial activation (17,48), phosphorylation-dependent signaling may dominate rapid responses. During chronic plaque inflammation and hypoxia, metabolic PTMs may become more prominent (29,33,36). In advanced lesions, PTM regulation of VSMC survival, extracellular matrix production, calcification, macrophage lipid handling and cell death may influence plaque stability (11,35,186). Therefore, the same PTM pair should not be assumed to exert identical effects in early lesions, advanced stable plaques and rupture-prone plaques.

Challenges and strategies for defining PTM crosstalk in vivo. A major obstacle is the cellular and spatial heterogeneity of atherosclerotic plaques. Endothelial cells, macrophages, lymphocytes, multiple VSMC-derived states, fibroblast-like cells and extracellular material coexist within the same lesion. The fibrous cap, shoulder region, necrotic core, calcified regions and adjacent arterial tissue also differ substantially in molecular composition (6,7,202,203). Bulk-tissue PTM changes may therefore reflect altered cell composition or plaque architecture rather than altered PTM occupancy in a specific cell type. This limitation can be addressed by combining PTM proteomics with cell sorting, lineage tracing, laser-capture microdissection, spatially resolved sampling and single-cell or spatial transcriptomic information. Parallel measurement of cell-type markers and total protein abundance can further determine whether a PTM change results from cell enrichment or intracellular regulation (180,186).

A second challenge is the low and dynamic stoichiometry of many PTMs (196). Enrichment-based proteomics improves detection but introduces differences in antibody affinity, peptide recovery, ionization efficiency and sequence coverage. A modified peptide may therefore change even when the underlying occupancy does not. Matched total-proteome analysis, quantification of the corresponding unmodified peptide, isotope-labeled internal standards and targeted parallel reaction monitoring can improve interpretation (204). Where technically possible, absolute or fractional occupancy should be measured rather than relying only on relative PTM intensity.

A third challenge is the loss of intact proteoform information in conventional bottom-up proteomics. Proteolytic digestion makes it difficult to determine whether modifications detected on separate peptides coexist on the same protein molecule. This is particularly problematic for long-distance crosstalk mediated by protein conformation. Middle-down, top-down and native top-down mass spectrometry can preserve more information about intact combinations of PTMs and protein complexes (197). However, these technologies currently have lower throughput and sensitivity for complex plaque samples than conventional bottom-up proteomics.

A fourth challenge is temporal resolution. Human endarterectomy samples provide only a cross-sectional endpoint, making it difficult to establish whether one modification precedes another. Time-resolved cell experiments, pulse-labeling approaches, inducible animal models and sampling across defined disease stages can help reconstruct PTM order. Because pulse-labeling kinetics can reflect both protein turnover and proteoform interconversion, these data should be interpreted with explicit kinetic models (181-183).

A fifth challenge is the pleiotropy of PTM enzymes. Writers, erasers and readers usually regulate multiple substrates, and their inhibition may alter metabolism, transcription or signaling independently of the proposed crosstalk event. Similarly, substitution of a modified lysine, serine, threonine or tyrosine may change local charge, folding or molecular interactions independently of preventing the PTM (15,21,179). Robust causal analysis therefore requires complementary approaches, including endogenous site-specific genome editing, modification-deficient and appropriate control mutants, rescue experiments, direct measurement of both PTMs on the same substrate and restoration of the downstream phenotype.

Finally, conventional animal models do not completely reproduce human plaque rupture, thrombosis and long-term lesion evolution (205). Candidate crosstalk mechanisms identified in cultured cells or mouse aortas should therefore be validated in defined cell populations and regions of human plaques whenever possible. Conversely, associations observed in human plaques require mechanistic testing in experimentally tractable models.

Therapeutic and conceptual implications. The competitive, synergistic and causal modes of PTM crosstalk collectively form a context-dependent regulatory network rather than three isolated pathways (57,179). Competitive crosstalk integrates metabolic and enzymatic constraints by allocating substrates among alternative proteoforms. Synergistic crosstalk produces cooperative or threshold-dependent functional outputs. Causal crosstalk transmits information through ordered modification cascades and enzyme-centered networks.

This framework suggests that globally inhibiting an entire PTM class is unlikely to provide sufficient specificity. A writer or eraser may exert protective effects on one substrate while promoting pathology through another (21). More selective approaches may include targeting substrate-enzyme interfaces, PTM-reader interactions, disease-enriched proteoforms or specific temporal windows of modification. Such strategies remain conceptual until substrate selectivity, delivery and systemic effects are established *in vivo* (206).

Overall, PTM crosstalk in AS is determined by at least six variables: Residue identity and spatial topology, modification stoichiometry, order of PTM addition and removal, writer/eraser/reader availability, cell type and subcellular localization and disease stage. Future studies integrating matched total proteomics and multiple PTM-omics with spatial sampling, temporal perturbation and intact-proteoform analysis will be essential for converting candidate PTM correlations into causal AS networks. This transition from cataloging individual modifications to defining dynamic proteoform networks may provide a more precise framework for understanding and eventually modulating AS pathogenesis.

4. Therapeutic targeting of PTMs in AS

The regulatory network formed by protein PTMs provides multiple potential targets for the treatment of AS. Current strategies include modulation of PTM-modifying enzymes, protection of modified substrates and regulation of upstream metabolic or redox pathways. However, most reported agents remain at the *in vitro* or animal stage, and several clinically available drugs influence PTMs only indirectly and were approved for indications other than AS. To facilitate translational evaluation, Table III classifies each agent according to its AS-specific evidence stage, regulatory status, selectivity and major safety limitations. To date, no drug has been approved specifically for AS through a defined PTM-targeting mechanism.

Targeting PTM-modifying enzymes. Lactylation links glycolytic metabolism to transcriptional regulation in AS. Endothelial p300-mediated H3K18 lactylation and SLC22A6-associated H3K9 lactylation promote endothelial dysfunction and lesion development, whereas narciclasine reduces endothelial H3K18 lactylation and early atherogenesis (26,207,208). By contrast, macrophage MCT4 inhibition increases H3K18 lactylation at reparative genes and attenuates AS (29). These opposing effects indicate that the therapeutic consequences of lactylation depend on the modified locus and vascular cell type. Because current interventions primarily alter glycolysis, lactate transport or p300 activity rather than an individual lactylated residue, cell-targeted modulation is likely to be more feasible than systemic suppression of lactate metabolism. All of these lactylation-directed interventions remain preclinical; human vascular observations do not establish therapeutic target engagement or clinical efficacy.

SUMOylation-directed interventions further illustrate the substrate dependence of PTM regulation. The SUMO E2 inhibitor 2-D08 has been used as an *in vitro* mechanistic probe of PIAS3-dependent vimentin regulation and VSMC phenotypic switching, but has not demonstrated efficacy in an AS animal model (51). Conversely, *Polygonatum odoratum*-derived flavones enhanced SUMO-activating enzyme subunit 1 expression and hepatocyte nuclear factor 1β SUMOylation, reduced sortilin-mediated lipid accumulation and attenuated plaque formation in LDLR-deficient mice (209). The opposing directions of these findings suggest that indiscriminate activation or inhibition of the SUMO pathway is unlikely to produce consistent benefit.

Ubiquitination-directed strategies have focused mainly on E3 ligases. The F-box protein 3 inhibitor BC-1215 reduced inflammatory signaling in cellular and experimental AS systems (210), whereas the MDM2 inhibitor JNJ-165 reduced retinoid X receptor β ubiquitination, mitochondrial dysfunction and vascular inflammation in experimental AS (211). Neither agent has human plaque target-engagement or clinical outcome evidence. Because MDM2 also regulates p53 and other DNA-damage and survival pathways, its vascular effects must be distinguished from the broad consequences of sustained systemic inhibition.

Acetylation-directed interventions have primarily targeted HDACs and sirtuins. The pan-HDAC inhibitor trichostatin A (TSA) produced context-dependent effects: HDAC inhibition reduced foam-cell formation in one $ApoE^{-/-}$ study (212), whereas TSA increased macrophage CD36, ox-LDL uptake and lesions in $LDLR^{-/-}$ mice (213). Toxicities observed with broad HDAC inhibitors in oncology restrict their suitability for chronic AS prevention (214). MC1568 restored acetylation-related endothelial responses under nitric-oxide stress *in vitro* (215), while TMP195 reduced plaque inflammation in $ApoE^{-/-}$ mice and suppressed inflammatory activation in patient-derived

Table III. Evidence stage and translational assessment of potential PTM-directed agents and strategies for AS.

PTM/pathway	Agent or strategy	PTM-related action	AS-specific evidence	Development status	Key selectivity or safety limitation	Translational assessment
Lactylation	Endothelial ASF1A-p300 pathway inhibition (26)	Reduces H3K18 lactylation and EndMT	Ox-LDL-stimulated endothelial cells; ApoE $^{/-}$ mice; human atherosclerotic arteries	Target only	p300 is not lactylation-specific; no selective pathway inhibitor	Multilevel mechanistic support; low direct tractability
	Narciclasine (208)	Suppresses glycolysis-H3K18la-NF-κB signaling	HUVECs; ApoE$^{-/-}$ mice	Experimental compound	Pleiotropic and antiproliferative effects	Preclinical proof-of-concept
	Endothelial SLC22A6 pathway inhibition (207)	Reduces H3K9 lactylation	Endothelial cell assays; ApoE$^{-/-}$ mice	Preclinical target strategy	Broad transporter and metabolic functions	Requires endothelial targeting and safety validation
	Macrophage MCT4 inhibition (29)	Enhances reparative H3K18 lactylation	Macrophage assays; HFD-fed ApoE$^{-/-}$ mice	Experimental strategy	Opposing cell-type-specific effects	Context-dependent target strategy
SUMOylation	2-D08 (51)	Inhibits UBC9-dependent SUMO conjugation	VSMCs *in vitro*; PIAS3-vimentin pathway, no 2-D08 efficacy, validated in ApoE$^{-/-}$ mice	Research compound	Global suppression of numerous SUMO substrates	Mechanistic probe only; no *in vivo* drug efficacy
	Polygonatum odoratum flavones (209)	Increases SAE1 expression and HNF1β SUMOylation	Cell assays; LDLR$^{-/-}$ mice	Preclinical natural-product mixture	Undefined active constituent and pharmacokinetics	Early animal proof-of-concept
Ubiquitination	BC-1215 (210)	Inhibits FBXO3-dependent inflammatory signaling	Ox-LDL-stimulated THP-1 macrophages; human carotid plaque and genetic association; no AS animal efficacy	Research compound	Multiple FBXO3-dependent substrates; no *in vivo* inhibitor target engagement	Human target association, but inhibitor evidence remains *in vitro*
	JNJ-165 (211)	Inhibits MDM2-mediated RXRβ ubiquitination	Ox-LDL-stimulated endothelial cells; HFD-fed LDLR$^{-/-}$ mice	Preclinical compound	Also affects p53 and other MDM2 substrates	Animal proof-of-concept; chronic safety unresolved
Acetylation	Trichostatin A (212,213)	Pan-HDAC inhibition	ApoE$^{-/-}$ and LDLR$^{-/-}$ mice; opposing effects across studies	Research pan-HDAC inhibitor	Broad HDAC blockade and systemic toxicity	Context-dependent efficacy; low chronic-AS feasibility
	MC1568 (215)	Class IIa HDAC inhibition	Nitric-oxide-stressed HUVECs *in vitro*	Research compound	Limited isoform and cell-type selectivity	*In vitro* mechanistic evidence only
	TMP195 (83)	Class IIa HDAC inhibition	ApoE$^{-/-}$ mice; *ex vivo* monocytes from patients with established AS	Preclinical compound	Class-selective, but not isoform- or substrate-selective	Preclinical proof-of-concept with human *ex vivo* support

Table III. Continued.

PTM/pathway	Agent or strategy	PTM-related action	AS-specific evidence	Development status	Key selectivity or safety limitation	Translational assessment
Methylation	EZH2 inhibition or T-cell-specific Ezh2 deletion (95)	Reduces H3K27me3-dependent inflammatory remodeling	Human plaque association; ApoE$^{-/-}$ mice	Target-validation strategy	Broad transcriptional effects; genetic deletion is not drug-equivalent	Strong target support; pharmacological translation unresolved
S-Palmitoylation	2-BP (125,216)	Broadly inhibits protein S-palmitoylation	Primarily *in vitro* macrophage and biochemical assays	Research probe	Nonselective effects on DHHC, non-DHHC and lipid-metabolic proteins	Low translational feasibility
	Sulforaphane (128)	Modulates APT2 palmitoylation and localization	APT2 biochemical/cell mechanism; no AS animal validation	Pleiotropic natural compound	NRF2 activation and multiple additional pathways	Indirect, unvalidated PTM mechanism in AS
S-Nitrosylation	Melatonin (121)	Reduces GNAI2-Cys66 S-nitrosylation	HG/ox-LDL-stimulated endothelial cells; diabetic LDLR$^{-/-}$ mice; human coronary tissue association	Clinically available outside AS	Pleiotropic redox, mitochondrial and circadian effects	Mechanism-linked animal proof-of-concept; human evidence is associative
S-Glutathionylation	Liraglutide (123,219,220)	Reduces eNOS-S glutathionylation	PTM mechanism in non-AS vascular mice; separate efficacy in ApoE$^{-/-}$ mice; clinical CV outcomes	Approved for metabolic indications	Multiple metabolic and vascular mechanisms	Clinical CV benefit established, but PTM causality unproven
Phosphorylation	Rapamycin/sirolimus (221)	Inhibits mTOR-dependent phosphorylation and promotes autophagy	ApoE$^{-/-}$ mice	Approved immunosuppressant; local coronary delivery established	Immunosuppression, dyslipidemia and impaired healing	Local delivery may be feasible; chronic systemic use is limited
	Tofacitinib (19,222)	Inhibits JAK signaling and alters macrophage cholesterol handling	Macrophages *in vitro*; no AS animal target engagement	Approved for inflammatory diseases	Serious infection, mortality, malignancy, MACE and thrombosis risks	Poor systemic AS candidate
Glycosylation	DANA and related sialidase inhibitors (223)	Inhibit NEU1/NEU3-dependent LDL desialylation and uptake	Human cultured macrophages; ApoE$^{-/-}$ and LDLR$^{-/-}$ mice	Research inhibitors	Limited neuraminidase isoform selectivity	Animal proof-of-concept for early lesion prevention

Table III. Continued.

PTM/pathway	Agent or strategy	PTM-related action	AS-specific evidence	Development status	Key selectivity or safety limitation	Translational assessment
Nitration	Ebselen (225)	Reduces upstream oxidative and nitrative stress	Diabetic ApoE/GPx1 double-knockout mice	Investigational redox compound	Broad thiol and redox effects; not site-	Indirect upstream preclinical strategy
specific	M-hydroxy ebselen (224)	Reduces oxidative and nitrative stress	Diabetic ApoE/GPx1 double-knockout mice	Experimental derivative	Broad redox activity; not site-specific	Indirect upstream preclinical strategy
	GKT137831/setanaxib (226)	Inhibits NOX1/4 upstream of peroxynitrite formation	Diabetic ApoE$^{-/-}$ mice	Clinical-stage investigational compound outside AS	Not nitration-specific; may affect physiological NOX signaling	Repurposing potential; AS trials and target engagement required
ADP-Ribosylation	Olaparib (176)	Inhibits PARP activity	Ox-LDL-stimulated THP-1, adhesion and foam-cell assays; no AS animal or human plaque validation	Approved for cancer	DNA-repair and hematological toxicity	*In vitro* evidence only; poor fit for chronic AS use
	TAS-102 (175)	Reduces PARP1 activity and restores NAD^+ homeostasis	Patient-derived VSMCs; LmnaG609G/G609G progeria mice; non-AS model	Approved anticancer drug	Antimetabolite and cytotoxic effects	Supportive vascular-aging evidence only

Evidence refers to the PTM-related anti-atherosclerotic mechanism, not the regulatory status of the agent. ApoE$^{-/-}$ and LDLR$^{-/-}$ models are specified where available; other vascular models are identified separately. Human cultured cells, *ex vivo* monocytes and tissue associations do not constitute clinical efficacy evidence. Approval for another indication does not constitute clinical-stage AS evidence. None of the listed agents is approved as a PTM-directed treatment for AS. 2-BP, 2-bromopalmitate; ApoE, apolipoprotein E; APT2, acyl-protein thioesterase 2; AS, atherosclerosis; ASF1A, anti-silencing function 1A histone chaperone; CV, cardiovascular; DANA, 2-deoxy-2,3-dehydro-N-acetylneuraminic acid; DHHC, Asp-His-His-Cys; EndMT, endothelial-to-mesenchymal transition; eNOS, endothelial nitric oxide synthase; EZH2, enhancer of zeste homolog 2; FBXO3, F-box protein 3; GNAI2, G protein subunit alpha i2; GPx1, glutathione peroxidase 1; HFD, high-fat diet; HNF1β, hepatocyte nuclear factor 1β; HUVEC, human umbilical vein endothelial cell; JAK, Janus kinase; LDLR, low-density lipoprotein receptor; MACE, major adverse cardiovascular events; MCT4, monocarboxylate transporter 4; MDM2, mouse double minute 2 homolog; mTOR, mechanistic target of rapamycin; NEU, neuraminidase; NOX, NADPH oxidase; NRF2, nuclear factor erythroid 2-related factor 2; p300, E1A-binding protein p300; PARP, poly(ADP-ribose) polymerase; PIAS3, protein inhibitor of activated STAT3; PTM, post-translational modification; RXRβ, retinoid X receptor β; SAE1, SUMO-activating enzyme subunit 1; THP-1, human monocytic leukaemia cell line; UBC9, SUMO-conjugating enzyme; VSMC, vascular smooth muscle cell.

monocytes (83). These *ex vivo* human data do not represent a clinical trial. Resveratrol influences SIRT1-associated deacetylation but remains a pleiotropic, low-bioavailability compound rather than a selective SIRT1 therapy (74).

Among methylation-directed interventions, pharmacological EZH2 inhibition and T-cell-specific Ezh2 deletion reduced inflammatory remodeling and lesion development in ApoE$^{-/-}$ models (95). Although compounds such as GSK126 are biochemically selective for EZH2, they alter broad transcriptional programmes across immune and vascular cells; lesion reduction therefore does not establish a single H3K27me3 site as the therapeutic mechanism, and human plaque target-engagement or clinical efficacy has not been shown.

Available S-palmitoylation modulators remain predominantly mechanistic tools. The commonly used inhibitor 2-bromopalmitate alters CD36 S-palmitoylation and modified-lipoprotein uptake but lacks sufficient selectivity to be considered a therapeutic candidate (125,216). Sulforaphane can interact with APT2 (128), although its vascular effects also involve NRF2-dependent and other antioxidant pathways. Disulfiram inhibits gasdermin D Cys192 palmitoylation (217), but the principal cardiovascular evidence was obtained in acute myocardial infarction rather than AS.

S-nitrosylation can be influenced by reducing nitric-oxide production or nitrosative stress, but available interventions generally act upstream of the modified substrate. In diabetes-accelerated AS, melatonin reduced GNAI2-Cys66 S-nitrosylation and suppressed CXCR5-Hippo-Yes-associated protein signaling in cells and LDLR$^{-/-}$ mice (121). Melatonin also modulates endothelial NLRP3-related pyroptotic pathways in separate cell models (218), underscoring its pleiotropy. Endothelial expression of non-nitrosylatable Hsp90-Cys521 reduced lesions in ApoE$^{-/-}$ mice (119), but no pharmacological strategy has selectively protected this residue in humans.

S-glutathionylation represents another redox-sensitive mechanism. Liraglutide reduces eNOS S-glutathionylation and improves nitric-oxide bioavailability in vascular experimental systems, and it reduces lesion progression in ApoE$^{-/-}$ mice (123,219). Cardiovascular outcome benefit has been demonstrated in patients with type 2 diabetes (220), but neither that trial nor the animal study established eNOS deglutathionylation as the causal clinical mechanism. Liraglutide should therefore be described as an approved metabolic drug with a PTM-related vascular mechanism, not as a validated PTM-directed AS therapy.

Phosphorylation-directed interventions mainly target kinase pathways. Oral rapamycin attenuated plaque progression in ApoE$^{-/-}$ mice (221), but its effects reflect broad mTOR inhibition rather than validation of a single substrate-phosphorylation event; systemic exposure may cause immunosuppression, dyslipidaemia and impaired wound healing. Tofacitinib alters macrophage cholesterol handling through JAK inhibition *in vitro* (19), but cardiovascular and malignancy risks in susceptible patients make it more useful as mechanistic evidence than as an AS-repurposing candidate (222).

Among glycosylation-related strategies, inhibition of pathological desialylation has received particular attention. The sialidase inhibitor DANA reduced NEU1-dependent CD36 desialylation and macrophage ox-LDL uptake, while inhibition of the NEU1/NEU3 pathway limited LDL desialylation and early lesion formation in AS models (223). Because parent DANA has limited neuraminidase isoform selectivity, the development of isoform-restricted derivatives will be important for determining whether this mechanism is therapeutically tractable.

Protein nitration is less amenable to direct pharmacological intervention because it is generated mainly by reactive nitrogen species rather than by a single substrate-selective enzyme. Ebselen, M-hydroxy ebselen and the NOX inhibitor GKT137831 reduced nitrative stress and lesion formation in diabetic AS models (224-226), but acted through broad redox regulation rather than selective prevention of a defined nitrated residue. Protein nitration may therefore currently be more useful as an indicator of pathological redox stress than as an independently druggable PTM.

Finally, PARP1-dependent ADP-ribosylation is therapeutically tractable but incompletely validated in AS. Olaparib reduced NF-κB/NLRP3 activity, monocyte adhesion and foam-cell formation only *in vitro* (176). TAS-102 improved VSMC survival and aortic pathology in a progeria model (175), not in ApoE$^{-/-}$ or LDLR$^{-/-}$ AS. Geniposide reduced lesions and altered PARP1/PI3K/AKT-associated lipophagy in an experimental AS model (227), but site-specific PARylation target engagement was not demonstrated. Prolonged PARP inhibition may also compromise DNA repair and haematopoietic homeostasis.

Collectively, these studies demonstrate that PTM pathways can be manipulated at the levels of modifying enzymes, upstream metabolic or redox signals, and individual modified substrates. However, the therapeutic meaning of such manipulation varies substantially according to the substrate, modification site, vascular cell population and disease context. These differences necessitate a cross-cutting assessment of target selectivity, mechanistic attribution, safety and translational evidence, as discussed below.

Drug development potential and barriers to clinical translation across PTMs. PTM-directed therapies can theoretically intervene at several levels, including catalytic enzymes, enzyme-substrate recognition, individual modification sites and upstream metabolic or redox pathways. Enzyme-regulated PTMs, such as ubiquitination, acetylation, methylation, phosphorylation and ADP-ribosylation, are particularly attractive because their writers and erasers often contain pharmacologically accessible catalytic or regulatory domains. Nevertheless, the tractability of a PTM enzyme does not by itself establish the therapeutic selectivity of its inhibition (15,21).

A central challenge is the distinction between enzyme selectivity and substrate selectivity. Most PTM-modifying enzymes act on numerous proteins; consequently, even a compound with high biochemical selectivity for one enzyme may alter multiple physiological substrates across different tissues. This problem is exemplified by HDAC and PARP inhibitors, which can suppress vascular inflammatory pathways while simultaneously disturbing lipid handling, DNA repair or haematopoietic homeostasis (74,175,212). Evaluation

of PTM-directed drugs must therefore extend beyond conventional enzyme selectivity to include substrate-, site-, cell- and tissue-level effects.

The consequences of a PTM are also highly context dependent. The same modification may be pathogenic in one vascular cell population but protective in another, as illustrated by the opposing effects of endothelial and macrophage lactylation (26,29,31). Disease stage may introduce an additional layer of complexity: Suppression of inflammatory signaling during early lesion development may not produce the same outcome in advanced plaques, where VSMC survival, extracellular-matrix production and fibrous-cap stability become critical (35,186). Global activation or inhibition of an entire PTM pathway may therefore eliminate beneficial responses together with pathogenic ones.

Mechanistic attribution represents a further limitation. Several clinically available or experimentally effective compounds - including resveratrol (74), melatonin (121,218), sulforaphane (128) and liraglutide (123,219) - affect multiple metabolic, inflammatory and redox pathways. A change in global PTM abundance after treatment does not establish that regulation of the reported PTM is responsible for the therapeutic phenotype. Stronger evidence requires demonstration of the relevant substrate and residue, confirmation that the compound changes this modification in the disease-relevant cell population, and genetic or pharmacological rescue showing that the site is necessary for the observed benefit.

Translation is additionally constrained by the chronic nature of AS. Many PTM-modulating agents were originally developed for cancer, transplantation or severe inflammatory disorders, in which a relatively narrow therapeutic window may be acceptable. In patients with stable or subclinical AS, prolonged immunosuppression, impaired wound healing, haematological toxicity, metabolic disturbance or increased cardiovascular risk may outweigh a moderate reduction in plaque burden (214,222). Current cellular and ApoE- or LDLR-deficient mouse models also incompletely reproduce human plaque ageing, rupture, thrombosis, comorbidities and polypharmacy, further limiting direct extrapolation (205).

These limitations suggest an important hierarchy of therapeutic strategies. Conventional catalytic-site inhibitors are often more straightforward to develop, but they usually affect most substrates processed by the targeted enzyme. By contrast, disruption of a disease-relevant enzyme-substrate interaction could, in principle, prevent modification of a pathogenic substrate while preserving other physiological functions of the same enzyme. Interface-directed small molecules, peptides, molecular glues or engineered biologics may therefore provide greater substrate discrimination than broad catalytic inhibition (228). This approach should not, however, be regarded as universally superior, because many enzyme-substrate interfaces are shallow, dynamic or transient and may be difficult to target intracellularly. Structural definition of the interaction, direct target-engagement assays and cell-specific delivery will be required to establish its practical advantage.

Future development should consequently integrate interface-directed or allosteric modulation with plaque-, cell- or organelle-targeted delivery. Site-specific PTM proteomics, chemoproteomic target-engagement methods, single-cell profiling and spatial analysis could be used to determine whether a candidate drug reaches the relevant plaque cell population and changes the intended substrate residue (202,203). These approaches may also identify patient subgroups in which a particular PTM pathway is dominant and provide pharmacodynamic biomarkers for early clinical studies.

Overall, the field remains weighted toward demonstration of animal efficacy but comparatively weak in molecular specificity. Many studies report changes in lesion area, inflammatory markers or global PTM abundance without confirming site-specific target engagement in the relevant plaque cell population. This phenotype-centred approach makes it difficult to distinguish direct PTM-mediated efficacy from secondary effects of altered metabolism, inflammation or redox balance. Future research should therefore move from general phenotypic observation toward mechanism-anchored, site-resolved validation by combining PTM proteomics with single-cell and spatial analyses, residue-mutant rescue experiments and direct pharmacodynamic assessment (181,197). To date, no PTM-directed agent has demonstrated AS-specific clinical efficacy through a defined modification mechanism.

5. Conclusions and future prospects

AS is not driven by a single pathological stimulus, but emerges from the long-term interaction of lipid metabolic disorder, oxidative stress, inflammation, haemodynamic disturbance, vascular cell remodeling and plaque microenvironmental changes. Within this complex setting, PTMs provide a rapid and reversible regulatory layer that connects extracellular and metabolic cues to protein function. The evidence summarized in this review indicates that PTMs are not merely downstream biochemical markers of AS, but participate in shaping endothelial dysfunction, macrophage lipid handling, inflammatory activation, VSMC phenotypic switching, vascular calcification and plaque instability (6,137).

A major insight from recent studies is that the biological meaning of a PTM depends strongly on context. The same modification may lead to different, or even opposite, outcomes depending on the modified substrate, residue site, cell type, subcellular localization, disease stage and local metabolic or redox environment. Lysine lactylation provides a representative example. By linking lactate accumulation to histone and non-histone protein regulation, lactylation may influence endothelial activation, macrophage polarization, VSMC senescence and osteogenic differentiation. However, its net effect cannot be simply defined as protective or detrimental, because adaptive metabolic reprogramming and pathological cell-state transition may occur under different conditions. Similar context dependence is also evident in cysteine modifications, SUMOylation, ubiquitination and phosphorylation, emphasizing that PTM-based interpretation requires substrate- and cell-specific resolution (26,29,30,33,36).

Another important conclusion is that PTMs rarely function as isolated events. Instead, they form interconnected regulatory networks through competitive, synergistic and sequential crosstalk. For example, SUMOylation and ubiquitination may jointly regulate substrate stability, phosphorylation can create docking or degradation signals for subsequent modifications, and metabolic acylations may compete for lysine residues or share common regulatory enzymes (51,57,191,195). Such

crosstalk provides a mechanism by which metabolic stress, inflammatory signaling, oxidative injury and mechanical forces are integrated into coordinated vascular responses. This network view helps explain why modulation of a single PTM enzyme may produce divergent effects across substrates, vascular cell types and disease stages. From a therapeutic perspective, PTM-related enzymes and pathways offer promising but still incompletely validated opportunities for AS intervention.

Pharmacological modulation of HDACs, PARPs, kinases, methyltransferases, palmitoylation-related enzymes and other PTM regulators has shown experimental anti-atherosclerotic potential in cellular or animal models (21,74). Nevertheless, most current strategies remain limited by insufficient substrate specificity, incomplete target-engagement validation, potential systemic effects and limited evidence from human plaques. Therefore, global inhibition or activation of an entire PTM class is unlikely to be an ideal long-term strategy for a chronic disease such as AS. More precise approaches may require targeting disease-relevant enzyme-substrate interactions, specific modified residues, pathological proteoforms or defined vascular cell populations (186,213,228).

Future studies should move beyond cataloging changes in global PTM abundance and focus on determining which PTM events are causal, cell-type specific and therapeutically actionable in AS. Site-resolved PTM proteomics, single-cell and spatial omics, isotope tracing, residue-mutant rescue experiments, cell-specific animal models and validation in human plaque specimens will be essential for this transition (186). In particular, emerging modifications such as succinylation, crotonylation, malonylation and MARylation should be investigated with caution, as their relevance to AS remains largely hypothesis-generating rather than established. Integrating these approaches may help convert PTM research from descriptive association into mechanism-based intervention, ultimately providing a more precise framework for understanding and targeting AS progression (229).

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XX was involved in the literature search, visualization and writing-original draft. FM revised and polished the manuscript. WY designed the framework of this article and revised the manuscript. Data authentication is not applicable. All authors have read and agreed to the published version of the manuscript.

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Competing interests

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