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Review Article

The role of CD40/CD40L signaling in vascular inflammation and aneurysm progression: a systematic review and evidence synthesis

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Abstract: Objective: This systematic review aimed to comprehensively evaluate the current evidence linking cluster of differentiation 40 and its ligand (CD40/CD40L) signaling to aneurysm development and progression across different anatomical sites. Methods: A systematic literature search was performed in PubMed, Embase, Web of Science, and Scopus databases for studies published up to July 27, 2025, according to predefined inclusion criteria. Eligible studies were those that used clinical samples, human tissues, animal models, in vitro experiments, or genetic analyses to directly investigate the role of CD40/CD40L signaling in aneurysmal disease. Data extraction was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Results: Sixteen studies met the inclusion criteria. The findings consistently demonstrated robust upregulation of CD40 and CD40L within aneurysmal walls. Circulating soluble CD40L levels were significantly elevated in patients with thoracic aortic aneurysms and ruptured intracranial aneurysms. In contrast, results in abdominal aortic aneurysm cases were variable. Genetic analyses indicated that lifelong genetically elevated CD40 expression was associated with a lower risk of aortic aneurysm. Mechanistic studies revealed that CD40/CD40L signaling promoted endothelial activation, immune cell recruitment, pro-inflammatory cytokine and chemokine release, and extracellular matrix degradation. In murine models, CD40L deficiency markedly reduced aneurysm incidence, dilation, and rupture, confirming a causal relationship. Therapeutic blockade of the CD40/CD40L axis significantly attenuated aneurysm formation in experimental models, underscoring its translational potential. Conclusion: The CD40/CD40L signaling pathway plays a pivotal role in aneurysm pathogenesis by amplifying vascular inflammation and promoting matrix remodeling. Targeting this pathway demonstrates promising efficacy in preclinical studies and warrants further investigation for potential clinical application.

Keywords: CD40/CD40L signaling, arterial aneurysm, vascular inflammation, abdominal aortic aneurysm, thoracic aortic aneurysm, intracranial aneurysm, soluble CD40L, therapeutic targets

Introduction

Arterial aneurysms are defined as the localized dilatations of the arterial wall exceeding 50% of the normal vessel diameter and represent a major cause of cardiovascular morbidity and mortality [1, 2]. The clinical significance of aneurysms is underscored not only by their propensity for progressive enlargement but also by the catastrophic consequences of rupture, which can lead to life-threatening hemorrhage or ischemic complications [3]. Although arterial aneurysms vary in anatomical distribution and etiological factors, for instance, abdominal aortic aneurysms (AAAs) typically occur in elderly individuals, thoracic aortic aneurysms (TAAs) are frequently associated with genetic syndromes and connective tissue disorders. Intracranial aneurysms (IAs) carry a substantial risk of subarachnoid hemorrhage, and a shared hallmark across these entities is the chronic, non-resolving inflammation within the vascular wall [4, 5].

This inflammatory milieu is driven by the recruitment and activation of both innate and adap-

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tive immune cells, along with the release of pro-inflammatory mediators; these factors promote the activation of proteolytic enzymes that degrade extracellular matrix (ECM) components, particularly elastin and collagen [6]. Consequently, aneurysms are increasingly regarded as immune-mediated degenerative vascular diseases characterized by persistent inflammation and matrix degradation, ultimately culminating in wall thinning and rupture [7]. Despite advances in imaging and surgical repair, no effective pharmacological therapy exists to halt aneurysm progression, underscoring the urgent need to better understand the underlying inflammatory mechanisms.

Over the past decades, cluster of differentiation 40 and its ligand (CD40/CD40L), members of the tumor necrosis factor receptor superfamily, have emerged as pivotal regulators of vascular inflammation. While initially characterized for its indispensable role in T cell-dependent B cell activation and immunoglobulin class switching, CD40/CD40L signaling is now recognized as a multifunctional pathway that contributes to the pathogenesis of various vascular diseases [8]. Among these conditions, atherosclerosis is the most extensively investigated, and provides the clearest evidence of the pathogenic role of CD40/CD40L [9]. In atherosclerosis, CD40/CD40L interactions drive endothelial activation, leukocyte recruitment and polarization, smooth muscle cell phenotypic modulation, and platelet-mediated thrombosis [10]. By engaging downstream inflammatory and proteolytic cascades, this axis amplifies immune responses and contributes to vascular remodeling and tissue destruction [11].

Over recent decades, the CD40/CD40L dyad - members of the tumor necrosis factor receptor superfamily - has been increasingly recognized as a key amplifier of vascular inflammation and immune activation. Beyond atherosclerosis, emerging evidence suggests that this signaling axis participates in multiple vascular disorders, including vasculitis, thrombosis, and aneurysm formation. These observations highlight CD40/CD40L as a central mediator linking immune activation to vascular injury.

However, despite these insights, the role of CD40/CD40L signaling in aneurysmal disease remains poorly defined and inconsistently reported. Most mechanistic understanding stems from atherosclerosis studies, whereas data directly elucidating aneurysm formation and progression are scattered across clinical, experimental, and genetic investigations. A systematic synthesis of these studies has not yet been performed.

Therefore, this systematic review and evidence synthesis aim to comprehensively synthesize evidence across human, animal, and genetic studies to elucidate the role of CD40/CD40L signaling in aneurysm biology. Specifically, the objectives of this study are: (1) to characterize the expression patterns of CD40 and CD40L across different aneurysm types and models; (2) to delineate the mechanistic contributions of this pathway to aneurysm initiation and progression; and (3) to evaluate the therapeutic potential of targeting CD40/CD40L signaling for aneurysm prevention and treatment. By consolidating multidisciplinary evidence, this review provides an innovative and translational perspective on how CD40/CD40L acts as a central amplifier of vascular inflammation and matrix degradation, highlighting its potential as a promising immunotherapeutic target in aneurysm management.

Methods

Literature search strategy

This study is a systematic review conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement.

Databases and search strategy

A comprehensive and systematic search was performed across four major electronic databases - PubMed, Embase, Web of Science, and Scopus. The search strategy was carefully developed to ensure high sensitivity and specificity for identifying studies investigating the role of CD40/CD40L signaling in aneurysmal disease. A combination of controlled vocabulary (e.g., Medical Subject Headings [MeSH] terms) and free-text keywords was employed. The Boolean search query was constructed as follows: (“CD40” OR “CD40 ligand” OR “CD40L”) AND (“aneurysm” OR “aneurysms”). The search covered all articles published up to July 27, 2025, without restrictions on study design or species. To ensure comprehensive-

ness, the reference lists of all eligible articles were manually screened to identify additional relevant studies that were not retrieved through the electronic database search.

Inclusion and exclusion criteria

Studies were selected according to predefined inclusion and exclusion criteria to ensure the relevance and scientific rigor of the evidence. Inclusion criteria: (1) Original research directly investigating the role, expression, or mechanism of CD40 and/or CD40L in aneurysm formation, progression, or pathophysiology; (2) Studies related to CD40/CD40L involving human participants, tissues, or animal models, as well as in vitro or genetic analyses; (3) Research reporting measurable outcomes such as CD40/CD40L expression levels, inflammatory markers, ECM degradation, or aneurysm-related morphological and functional changes; (4) Peer-reviewed articles published in English; (5) Studies published up to July 27, 2025. Exclusion criteria: (1) Reviews, editorials, conference abstracts, or case reports without original data; (2) Non-English publications or studies without accessible full text; (3) Research not directly related to CD40/CD40L signaling or not focused on aneurysmal disease; (4) Studies with insufficient data quality, duplicated reports, or unclear experimental methodology; (5) Articles using irrelevant models or lacking specific analysis of CD40/CD40L signaling in the context of aneurysm pathogenesis. These criteria were established to ensure the inclusion of high-quality and directly relevant evidence, thereby improving the reproducibility and transparency of this systematic review.

Data extraction

Data extraction was independently conducted by two reviewers using a standardized data collection form. For each included study, the following information was systematically recorded: first author, publication year, study design, experimental model or patient population, methodological approach, key outcome measures, and principal findings relevant to the association between CD40/CD40L signaling and aneurysm. Any discrepancies between the reviewers were resolved through discussion and consensus. When necessary, a third reviewer was consulted to reach a final decision.

PRISMA flow diagram

The overall process of study selection - including identification, screening, eligibility assessment, and final inclusion - was summarized in a flow diagram following the PRISMA guidelines (**Figure 1**). As this study is a systematic review without a quantitative meta-analysis, the PRISMA flow diagram was used to ensure transparency and reproducibility of the literature screening and selection process.

Statistical analysis

As this study is a systematic review without quantitative data pooling, no formal statistical analysis was conducted. The evidence was synthesized qualitatively according to the PRISMA 2020 guidelines. Data extraction was independently performed by two reviewers, while the overall process of data extraction and synthesis was methodologically reviewed by a specialist in biostatistics to ensure the scientific rigor and consistency of the analytical procedures.

Quality assessment

To ensure methodological consistency and reliability, all included studies were systematically assessed for quality. Two reviewers independently evaluated study quality using tools appropriate for each study design. For observational and clinical studies, the Newcastle-Ottawa Scale was applied, with studies scoring ≥6 considered high quality. For animal experiments, the Systematic Review Centre for Laboratory animal Experimentation Risk-of-Bias Tool was used to assess randomization, allocation concealment, blinding, and outcome reporting. Discrepancies between reviewers were resolved through discussion or adjudication by a third reviewer. The results of the quality assessment were used to describe the overall methodological rigor of the included studies rather than the criteria for inclusion or exclusion.

Results

Expression of CD40/CD40L in human, animal, tissue, and cellular contexts of aneurysms

Expression in human aneurysmal tissues and thrombi: Multiple lines of evidence from human studies confirmed the activation of the CD40/

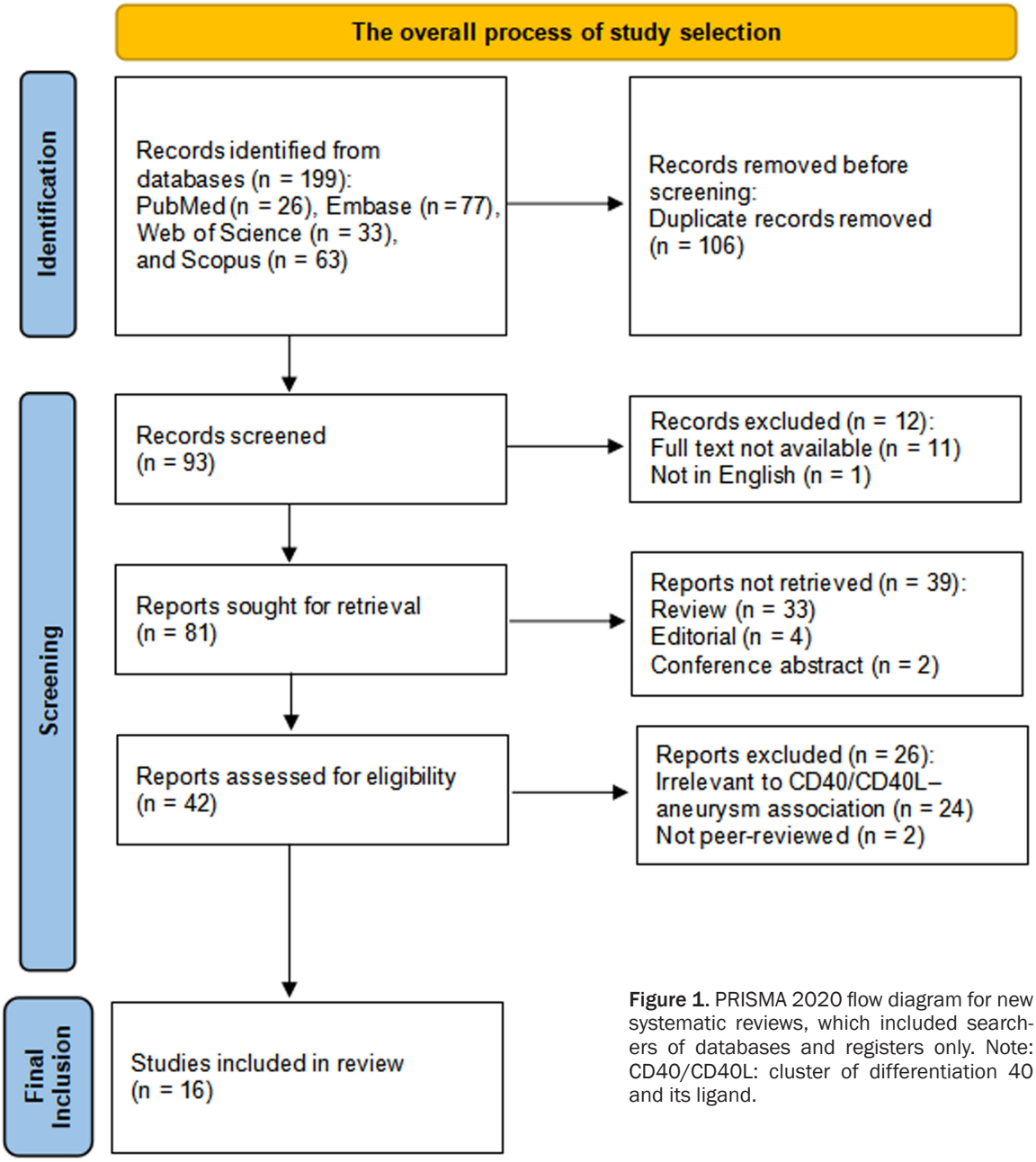

Figure 1. PRISMA 2020 flow diagram for new systematic reviews, which included searchers of databases and registers only. Note: CD40/CD40L: cluster of differentiation 40 and its ligand.

CD40L signaling pathway within aneurysmal tissues. In AAA, Guedj et al. reported significantly elevated levels of soluble CD40L (sCD40L) in conditioned media, compared with non-aneurysmal controls, suggesting immune cells as the primary source, although precise cellular localization was not specified [12]. Subsequent investigations addressed this limitation. Kusters et al. analyzed transcriptomic data from the Stockholm AAA Biobank and demonstrated significant upregulation of both CD40 and CD40L messenger RNA in the medial layer of AAA walls, independent of thrombus presence [13]. Immunohistochemical analysis by Nagashima et al. further revealed that CD40 expression was localized to infiltrating B lymphocytes, T lymphocytes, and macrophages, but not to neutrophils or vascular smooth muscle cells [14].

In IA, bioinformatic re-analysis of the GSE540-83 dataset by Guedj et al. identified marked upregulation of CD40 and CD40L messenger RNA in ruptured tissues, with protein-protein

interaction analysis highlighting these molecules as hub genes within rupture-associated inflammatory networks [12]. Additionally, mural thrombi in AAA were identified as active sources of sCD40L. Touat et al. demonstrated a luminal-to-abluminal gradient of sCD40L within intraluminal thrombi that correlated with platelet activation markers, indicating that thrombi contributed to circulating sCD40L levels and potentially exacerbated aneurysmal wall degradation [16].

Circulating sCD40L levels and their clinical correlations: Plasma sCD40L levels were evaluated as potential biomarkers across different aneurysm subtypes. In TAA, Touat et al. observed significantly elevated sCD40L levels in patients with an aortic diameter ≥45 mm, regardless of etiology, and this elevation correlated with platelet activation [17]. Similarly, in AAA, plasma sCD40L concentrations were reported to be higher than those in controls. However, a prospective cohort study by Flondell-Sité et al. demonstrated substantial interindividual variability and no consistent correlation between sCD40L levels and aneurysm diameter, which may have been influenced by confounding factors such as antiplatelet therapy or concomitant comorbidities [18]. In patients with ruptured IA presenting as aneurysmal subarachnoid hemorrhage, Chen et al. reported that plasma sCD40L levels were markedly increased and correlated with clinical severity scores, independently predicting poor functional outcomes and mortality [19]. Flow cytometric analysis in a pediatric case of ruptured IA confirmed elevated CD40L expression on $CD4^+$ T lymphocytes in both peripheral blood and the aneurysmal wall, supporting a dual systemic and local pro-inflammatory role [20].

Studies in specific populations revealed context-dependent associations. In individuals living with human immunodeficiency virus, higher plasma sCD40L levels were paradoxically associated with a lower risk of aneurysm formation [21]. Conversely, in Kawasaki disease complicated by coronary artery aneurysms, CD40 expression on B lymphocytes showed an upward trend, although statistical significance was not achieved [22]. These observations emphasized that CD40/CD40L signaling is modulated by underlying pathophysiological and immunological conditions.

Genetic and preclinical research evidence

To investigate the causal role of the CD40/CD40L signaling pathway in aneurysm development and progression, evidence from genetic analyses, animal experiments, and methodological quality assessments was summarized. Genetic studies demonstrated that genetically elevated CD40 levels were significantly associated with a reduced risk of aortic aneurysm and dissection, suggesting a potential protective effect of long-term CD40 upregulation [23, 24]. In contrast, CD40L showed no significant genetic association, indicating that its pathogenic influence may be confined to acute inflammatory responses. Animal studies revealed that in angiotensin II (AngII)-induced $Apoe^{-/-}$ mice, CD40L expression progressively increased with disease progression; deficiency of CD40L markedly reduced aneurysm incidence, dilation, and rupture-related mortality. Immunohistochemical analyses showed that CD40L mainly originated from infiltrating immune cells, promoting the expression of inflammatory cytokines and matrix metalloproteinases (MMPs), thereby contributing to vascular wall degradation. Inhibition or deletion of CD40 signaling was associated with reduced inflammation and structural stability. Histological and bioinformatic analyses further demonstrated significant upregulation of CD40/CD40L in various aneurysm types (AAA, TAA, and IA), particularly in infiltrating T cells, B cells, and macrophages. The strongest expression was observed in ruptured IAs, while AAA thrombi exhibited a luminal-to-abluminal gradient of sCD40L concentration [20]. The overall methodological quality of the included studies was moderate to high, with most achieving acceptable rigor according to the Newcastle-Ottawa Scale and Systematic Review Centre for Laboratory animal Experimentation Risk-of-Bias Tool. Detailed information is presented in **Table 1**.

CD40/CD40L signaling in aneurysm pathogenesis: mechanistic insights

Emerging evidence from transcriptomic analyses, single-cell sequencing, and preclinical models identified the CD40/CD40L dyad as a central amplifier of vascular inflammation and ECM degradation in aneurysm pathogenesis. These mechanisms appeared to be conserved across multiple aneurysm subtypes, including

Table 1. Main characteristics and quality assessment of the included studies

Author (Year)	Aneurysm Type	Study Type	Model	CD40/CD40L Expression Change	Conclusion Summary	Quality Score
Guedj et al. (2014) [12]	AAA	ex vivo	Human AAA tissues	sCD40L↑ in conditioned media from AAA tissues	AAA inflammatory microenvironment promotes immune cell recruitment and lymphoid-like structure formation, enabling CD40/CD40L signaling	7
Kusters et al. (2018) [13]	AAA	human transcriptome; animal model	human AAA wall segments; AngII-induced aneurysm model in ApoE$^{-/-}$ mice	CD40↑ and CD40L↑ in human AAA tissue (medial layer); CD40L↑ in abdominal aorta of ApoE$^{-/-}$ mice receiving AngII	CD40L promotes aneurysm formation; its deficiency-particularly in hematopoietic cells-confers protection against aneurysm and rupture, accompanied by reduced inflammation and protease activity	8
Nagashima et al. (2004) [14]	AAA	human tissue	human AAA tissues	CD40 expressed on macrophages and T/B cells, absent in neutrophils	B cells, T cells, and macrophages in AAA tissue may be regulated by the CD40/CD40L pathway	6
Wei et al. (2018) [15]	ruptured IA	bioinformatic re-analysis (microarray)	patients with ruptured IA	CD40↑ and CD40L↑ in ruptured IA vs. control superficial temporal arteries	Transcriptomic evidence supports involvement of CD40/CD40L in rupture-associated inflammation in IA	7
Touat et al. (2006) [16]	AAA	human tissue	AAA patients' aneurysmal thrombi and plasma	sCD40L↑ in thrombus luminal eluate and in plasma	Intraluminal thrombus releases sCD40L, potentially entering circulation and contributing to progression	6
Touat et al. (2008) [17]	TAAA	clinical study	TAAA patients' plasma and tissues	sCD40L↑ in TAAA with diameter >45 mm	sCD40L reflects platelet activation associated with TAAA dilatation	7
Flondell-Sité et al. (2009) [18]	AAA	prospective cohort	AAA patients	variation in plasma sCD40L between groups	No consistent relationship with AAA diameter or significant difference from controls	6
Chen et al. (2015) [19]	ruptured IA (aSAH)	prospective cohort	aSAH patients	sCD40L↑ after aSAH and, correlating with disease severity and long-term prognosis	sCD40L is elevated after aSAH and correlates with disease severity and long-term prognosis	8
Moschetti et al. (2022) [20]	pediatric ruptured IA	aneurysm wall T-cell deep phenotyping	7-years-old child with ruptured IA	CD40L↑ in CD4$^+$ T cells in peripheral blood and aneurysmal wall	This study provides the first detailed characterization of T-cell subsets in the IA wall and confirms local CD40L expression by CD4$^+$ T cells, which directly supports T cell-CD40L involvement in IA inflammation	7
Grønbæk et al. (2023) [21]	AA (TAA and AAA) in PLWH	cross-sectional analysis	COCOMO cohort	plasma sCD40L↑ associated with reduced AA risk	In HIV patients, higher plasma sCD40L was inversely associated with AA risk and linked to smaller suprarenal aortic diameter	7
Cui et al. (2022) [23]	AA and its subtypes, TAA and AAA	Mendelian randomization	European GWAS cohorts	Genetically proxied CD40↑ associated with reduced AA/TAA/AAA risk	MR evidence supports a causal association between genetically higher CD40 levels and lower risk of AA, TAA, and AAA	8
Chen et al. (2024) [24]	AAA	bidirectional Mendelian randomization and colocalization	European GWAS cohorts	Genetically predicted plasma CD40↑ associated with reduced AAA risk; colocalization evidence pointing to shared causal variants	MR evidence supports a causal association between higher genetically predicted CD40 levels and lower AAA risk	8

Note: Newcastle-Ottawa Scale: 0-3 = low; 4-5 = moderate; 6-9 = high. AA: aortic aneurysm; AAA: abdominal aortic aneurysm; CD40/CD40L: cluster of differentiation 40 and its ligand; CD40: cluster of differentiation 40; sCD40L: soluble CD40 ligand; AngII: angiotensin II; ApoE$^{-/-}$: apolipoprotein E knockout; IA: intracranial aneurysm; CD40L: CD40 ligand; CD4$^+$ T cells: CD4-positive T lymphocytes; TAA: thoracoabdominal aortic aneurysm; aSAH: aneurysmal subarachnoid hemorrhage; PLWH: people living with HIV; COCOMO: Copenhagen Comorbidity in HIV Infection Cohort.

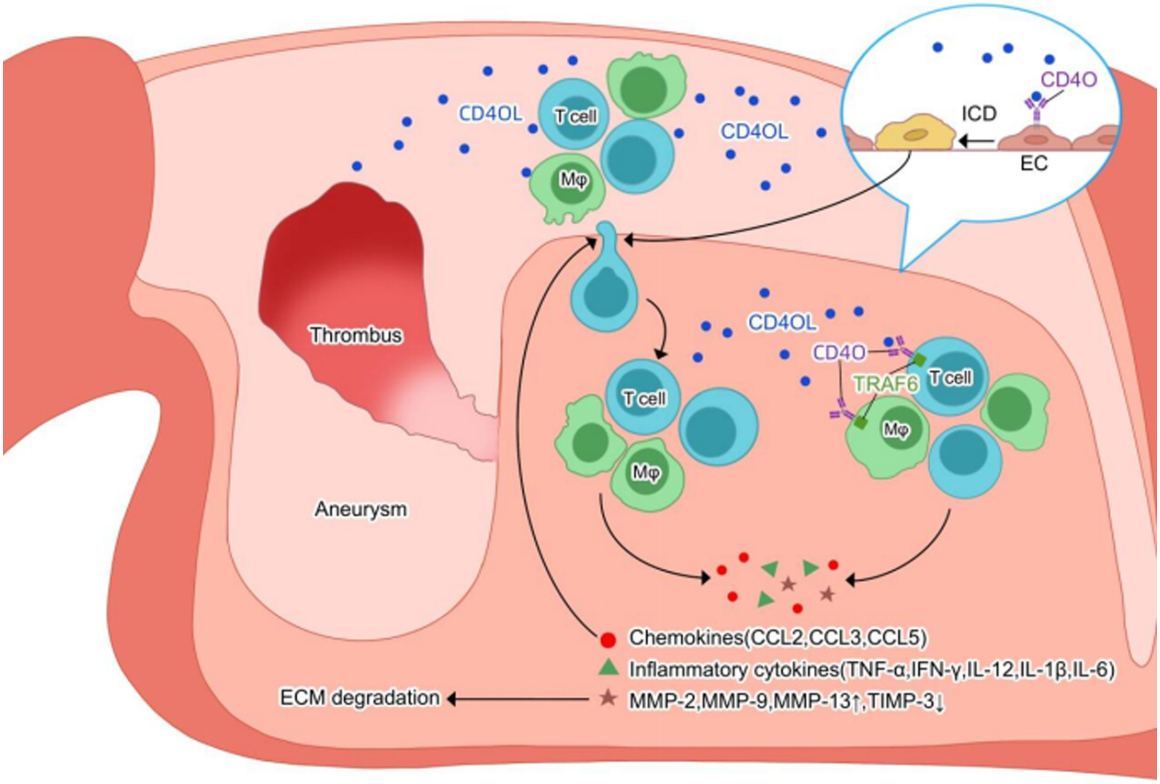

Figure 2. Proposed mechanisms by which CD40/CD40L signaling promotes aneurysm progression. Note: CD40: Cluster of Differentiation 40; CD40L: CD40 ligand; T cell: T lymphocyte; Mφ: macrophage; EC: endothelial cell; ICD: intracellular domain; TRAF6: TNF receptor-associated factor 6; ECM: extracellular matrix; CCL2: C-C motif chemokine ligand 2; CCL3: C-C motif chemokine ligand 3; CCL5: C-C motif chemokine ligand 5; TNF-α: tumor necrosis factor alpha; IFN-γ: interferon gamma; IL-12: interleukin-12; IL-1β: interleukin-1 beta; IL-6: interleukin-6; MMP-2: matrix metalloproteinase-2; MMP-9: matrix metalloproteinase-9; MMP-13: matrix metalloproteinase-13; TIMP-3: tissue inhibitor of metalloproteinases-3.

ascending thoracic aortic aneurysm (ATAA), AAA, and IA. The collective findings were synthesized into a unified mechanistic framework, as illustrated in **Figure 2**.

Role of CD40/CD40L in ATAA: Single-cell transcriptomic analysis identified the CD40/CD40L signaling pathway as one of the nine core molecular networks associated with immunogenic cell death (ICD) in ATAA. CD40L-expressing T lymphocytes interacted with CD40-expressing endothelial cells, triggering an ICD-related signaling cascade within the endothelium. This interaction induced endothelial inflammation and ICD, which in turn promoted enhanced recruitment of immune cells - particularly myeloid cells and additional T lymphocytes - thereby establishing a self-perpetuating inflammatory loop that accelerated aneurysm progression. Collectively, these findings indicated that $CD40L^+$ T lymphocytes activated the ICD program in endothelial cells via the CD40/CD40L signaling pathway, amplifying vascular inflammation and contributing to the pathogenesis of ATAA [25].

Role of CD40/CD40L in AAA: CD40L played a pivotal role in both the initiation and progression of AAA. In $Cd40l^{-/-}Apoe^{-/-}$ mice, CD40L deficiency markedly reduced the infiltration of aortic immune cells, including $CD4^+$ T lymphocytes, $Mac3^+$ macrophages, and $CD45^+$ leukocytes. In addition, this deficiency downregulated pro-inflammatory cytokines (tumor necrosis factor-α [TNF-α], interferon-γ [IFN-γ], interleukin-12 [IL-12]) as well as chemokines (C-C motif chemokine ligand 2 [CCL2], C-C motif chemokine ligand 3 [CCL3], C-X-C motif chemokine ligand 2). CD40L signaling also promoted ECM degradation by upregulating MMPs (MMP-2, MMP-9, and MMP-13) and downregulating tissue inhibitor of metalloproteinases-3 (TIMP-

3), collectively compromising the structural integrity of the aortic wall. Functionally, CD40L deficiency resulted in a significantly smaller total aortic area, a 52% reduction in vascular dilation, and improved survival due to reduced rupture risk. Bone marrow transplantation experiments confirmed that CD40L derived from hematopoietic cells - such as T lymphocytes and macrophages - was a key driver of aneurysm development. Temporal analysis revealed that CD40L influenced both early (day 7: reduced leukocyte infiltration and chemokine production) and late (day 28: decreased MMP activity and ECM degradation) stages of aneurysm progression [13].

Pharmacological inhibition of the CD40-TNF receptor-associated factor 6 (TRAF6) interaction using the small-molecule inhibitor TRAF-STOP (compound 6877002) significantly attenuated AAA formation in mice. Treated animals exhibited preserved elastic fibers, reduced MMP-2 and MMP-9 expression, and increased collagen type IV deposition. Inflammatory responses were also suppressed, as indicated by decreased TNF-α levels and altered macrophage polarization - specifically, a reduction in $CD40^+$ macrophage subsets, an upregulation of the anti-inflammatory gene Clec4a3, and a downregulation of the pro-inflammatory gene Ifit2. Consistently, ex vivo treatment of human AAA tissues with an anti-CD40L monoclonal antibody suppressed MMP-2 expression [14].

Role of CD40/CD40L in IA: Bioinformatic analyses of ruptured IA tissues revealed significant upregulation of CD40 and CD40L. Protein-protein interaction and functional enrichment analyses identified these molecules as hub genes within networks of upregulated differentially expressed genes. These networks were enriched in pathways related to “immune system development” and “primary immunodeficiency”, suggesting their involvement in immunoregulatory processes underlying IA pathogenesis [15]. Integrative network analyses combining genome-wide association study, expression quantitative trait loci, and transcriptome-wide association study data further identified CD40 as a central node within protein-protein interaction networks related to AAA, reinforcing its conserved role across different aneurysm types [26]. Histological analysis of a pediatric ruptured IA case demonstrated that infiltrating $CD4^+$ T lymphocytes within the aneurysmal wall expressed CD40L. These $CD40L^+CD4^+$ T lymphocytes functioned as multifunctional effector cells co-expressing T helper 1-type cytokines (IFN-γ, TNF, IL-2), indicating a localized pro-inflammatory phenotype that may contribute to vascular wall destruction [20].

Therapeutic targeting of CD40/CD40L in aneurysm: a summary of experimental evidence

Targeting the CD40/CD40L signaling pathway has emerged as a promising therapeutic strategy for aneurysm treatment, supported by findings from pharmacological inhibition studies, genetic deletion models, and computational drug prediction analyses.

Pharmacological inhibition of the CD40/CD40L pathway: Pharmacological agents directly targeting the CD40/CD40L signaling axis have demonstrated its significant efficacy in attenuating key pathological processes of AAA.

Trapidil and anti-CD154 antibody: Nagashima et al. showed that trapidil, an inhibitor of the CD40/CD40L pathway, significantly reduced the expression of MMP-2 at both the mRNA and protein levels in cultured human AAA tissues. This effect was specific to MMP-2, as MMP-9 expression remained unchanged [14]. The observation that an anti-CD154 (CD40L) antibody replicated this effect confirmed that the mechanism was mediated through inhibition of CD40 signaling. This study identified CD40 signaling in macrophages as a selective regulator of MMP-2 production - a key driver of ECM degradation within the AAA wall - thereby proposing trapidil as a potential therapeutic agent for AAA [16].

TRAF-STOP inhibitor: In a murine elastase-induced AAA model, treatment with TRAF-STOP, a small-molecule inhibitor that selectively disrupts the interaction between CD40 and the downstream adaptor TRAF6, effectively attenuated aneurysm development and progression. The treatment group exhibited a lower aneurysm incidence (66.7% vs. 87.5% in controls at day 28) and a significantly smaller increase in aortic diameter. Histological analysis revealed preserved elastic lamellae, reduced elastin degradation, and decreased inflammatory cell infiltration. Mechanistically, spatial transcrip-

tomic and RNAscope analyses demonstrated that TRAF-STOP significantly downregulated the expression of MMP-2, MMP-9, and TNF-α within the aneurysmal lesions. Furthermore, an increase in collagen type IV content in the aortic wall of treated mice suggested enhanced structural stability [21].

Robust protection with genetic deletion of CD40L: Studies employing genetic knockout models have provided compelling evidence for the central role of CD40L in aneurysm pathogenesis, underscoring its influence on inflammation, proteolysis, and clinical outcomes such as rupture [13].

Protection against formation and rupture: In an AngII infusion model using $Apoe^{-/-}$ mice, genetic deficiency of CD40L ($Cd40l^{-/-}$) markedly reduced the incidence of dissecting aneurysms from 67% in controls to 17%. This protective effect was accompanied by a 52% reduction in the total vessel area of the suprarenal aorta, indicating substantially attenuated aortic remodeling. Notably, $Cd40l^{-/-}Apoe^{-/-}$ mice were completely protected from fatal aneurysm rupture [13].

Attenuated inflammatory and proteolytic response: The protective phenotype was mechanistically associated with a profound attenuation of the inflammatory response within the aortic wall. Immunohistochemical analysis demonstrated a significant reduction in the accumulation of $CD45^+$ leukocytes, $CD4^+$ T lymphocytes, and $Mac3^+$ macrophages. This was accompanied by downregulation of proinflammatory mediators, including the chemokines CCL2 and CCL3 and the cytokine IL-12. CD40L deficiency also restored the proteolytic balance, as evidenced by markedly reduced activity of MMP-2 and MMP-9 (as assessed by gelatin zymography), decreased MMP-13 mRNA expression, and elevated transcript levels of TIMP-3 [13].

Role of hematopoietic cells: Bone marrow transplantation experiments further confirmed the essential contribution of hematopoietic cell-derived CD40L. $Apoe^{-/-}$ mice receiving bone marrow from $Cd40l^{-/-}Apoe^{-/-}$ donors exhibited a markedly reduced incidence of aneurysm formation (30% vs. 63% in controls) and were completely protected from rupture, recapitulating the phenotype observed in the global knockout model [13].

Computational docking and repurposed drug evidence: Computational approaches have identified unanticipated interactions between existing pharmacological agents and the CD40/CD40L signaling axis, offering new opportunities for therapeutic repurposing. Several of these findings have already been supported by in vivo experimental evidence.

Abciximab binding to CD40: Molecular docking simulations conducted by Sagulkoo et al. revealed that the antiplatelet agent abciximab binds strongly to CD40, exhibiting a binding energy of -198.96 kcal/mol, superior to the experimental anti-CD40 antibodies bleselumab (-158.64 kcal/mol) and dacetuzumab (-169.31 kcal/mol) [26]. The interaction was stabilized by multiple hydrogen bonds with residues K29, Q42, P43, Q45, E58, C59, G63, W71, and E98, as well as a salt bridge involving E58 [16].

In vivo validation for abciximab: These computational predictions were corroborated by experimental findings. Touat et al. demonstrated that six weeks of abciximab treatment in a rat xenograft model of AAA markedly reduced thrombus area and attenuated aneurysmal dilation [17]. Histological examination revealed decreased P-selectin expression on the luminal thrombus surface, diminished neutrophil adhesion, preserved medial elastin, and increased colonization of α-actin-positive mesenchymal cells within the thrombus, suggesting enhanced healing and structural stabilization [16].

Paclitaxel binding to CD40L: The same molecular docking analysis predicted that the chemotherapeutic agent paclitaxel exhibits strong binding affinity for CD40L, with an exceptionally low binding energy of -282.64 kcal/mol, surpassing known inhibitors such as suramin and BI08898. Key stabilizing interactions involved hydrogen bonds with residues G226, Y170, and Y172. However, the predicted high-affinity interaction and potential inhibitory effect of paclitaxel on CD40L signaling remain to be validated in experimental models of aneurysm [26].

Discussion

Aneurysms are degenerative vascular diseases driven by chronic inflammation, immune dysregulation, and ECM degradation. In recent

years, immune-regulatory signaling pathways have attracted increasing attention in aneurysm research, with the CD40/CD40L axis identified as a pivotal molecular amplifier of vascular inflammation.

To comprehensively assess its role in aneurysm development and progression, this systematic review was conducted in accordance with the PRISMA 2020 guidelines, integrating multi-level evidence from human studies, animal models, and genetic analyses. The findings consistently demonstrated upregulation of CD40/CD40L signaling across different aneurysm types, primarily localized to infiltrating T cells, B cells, and macrophages [32, 33]. Genetic or pharmacological blockade of CD40L markedly reduced aneurysm incidence, expansion, and rupture-related mortality, whereas lifelong genetically elevated CD40 expression was associated with a lower risk of aneurysm formation, suggesting a possible immunoprotective effect [34]. Collectively, these results highlight CD40/CD40L signaling as a central regulatory pathway in aneurysm pathogenesis and provide systematic evidence linking immune activation with vascular degeneration.

Current evidence indicates that the CD40/CD40L axis contributes to aneurysm progression by promoting endothelial activation, immune cell recruitment, and cytokine release [35]. This pathway primarily acts through TRAF6-dependent pro-inflammatory signaling, leading to the upregulation of cytokines (TNF-α, IFN-γ, IL-12) and chemokines (CCL2, CCL3, C-X-C motif chemokine ligand 2), along with enhanced MMP activity (MMP-2, MMP-9, MMP-13) and decreased expression of tissue inhibitor TIMP-3 [36]. These effects weaken the structural integrity of the vascular wall. Similar to findings in atherosclerosis, CD40/CD40L signaling drives a comparable inflammatory cascade; however, the morphological outcomes differ fundamentally. Atherosclerosis is characterized by inward vascular remodeling and luminal narrowing, whereas aneurysm disease manifests as outward remodeling and progressive dilation [37]. This divergence may result from differences in the affected vascular layers and cellular responses, suggesting that the same immune pathway can yield opposite structural consequences depending on the pathological context.

In human studies, the relationship between circulating sCD40L and aneurysm severity remains inconsistent. Plasma sCD40L levels are elevated in patients with TAA and ruptured IA, but findings in AAA vary substantially. Prospective cohort data indicate that sCD40L concentrations are influenced by antiplatelet or statin therapy, comorbidities, and the presence of intraluminal thrombus, which may lead to intermittent release of sCD40L and fluctuations in plasma levels. These observations suggest that circulating sCD40L likely reflects transient local inflammatory activity rather than a stable systemic biomarker [23].

Genetic studies provide further insight into the dual role of the CD40/CD40L axis. Mendelian randomization analyses by Elgueta et al. revealed that lifelong genetically elevated CD40 levels are causally associated with reduced risk of aortic aneurysm and dissection, whereas CD40L showed no significant genetic association [24]. This apparent discrepancy aligns with the distinction between acute pro-inflammatory activation and long-term immune homeostasis. CD40 signaling can be mediated through distinct TRAF adaptor molecules, with TRAF6 driving pro-inflammatory responses and TRAF2/3 contributing to immune regulation and tolerance [25]. Hence, sustained genetic upregulation of CD40 may preferentially activate TRAF2/3-mediated immunomodulatory signaling, providing long-term vascular protection. This “context-dependent duality” is further supported by observations from the Copenhagen Comorbidity in HIV-infection cohort, where elevated circulating sCD40L in individuals living with human immunodeficiency virus paradoxically correlated with a lower risk of aneurysm. Such findings imply that in states of chronic immune activation, CD40/CD40L signaling may undergo adaptive reprogramming, shifting from a pro-inflammatory to a regulatory role [26].

The influence of the CD40/CD40L axis extends beyond the aorta. In patients with coronary artery ectasia, plasma sCD40L levels were significantly elevated and correlated with disease severity [27]. Histopathological analyses revealed medial degeneration, inflammatory infiltrates, and matrix breakdown - features paralleling those of aneurysmal disease [28]. This suggests that CD40/CD40L-mediated inflam-

mation may represent a shared pathogenic mechanism among diverse vascular dilation syndromes [29].

From a translational perspective, inhibition of CD40/CD40L signaling demonstrates promising therapeutic potential in preclinical models. Approaches such as anti-CD40L monoclonal antibodies, CD40-TRAF6 interaction inhibitors (e.g., TRAF-STOP), and drug repurposing strategies (e.g., trapidil, abciximab, paclitaxel) consistently reduced vascular inflammation and preserved wall integrity. Although encouraging, clinical translation requires caution. Early-generation anti-CD40L antibodies were associated with thrombotic complications in other disease settings, underscoring the importance of developing cell-specific or signaling-selective strategies to balance efficacy and safety [30, 31].

This review has several limitations. Most human studies are cross-sectional with small sample sizes, limiting causal inference and temporal analysis. Animal models largely rely on acute induction methods such as AngII infusion or elastase perfusion, which may not fully mimic the chronic progression of human disease. Methodological heterogeneity across studies could also contribute to variable outcomes, and publication bias toward positive results may overestimate effect sizes. Future research should focus on longitudinal human studies, mechanistic differentiation of CD40 versus CD40L signaling in specific cell types, and validation of targeted interventions in models that better recapitulate the chronic nature of aneurysm development.

In conclusion, the CD40/CD40L pathway represents a critical molecular link between immune inflammation and vascular remodeling. Acute CD40L-mediated activation plays a clearly pathogenic role in aneurysm formation, whereas homeostatic CD40 signaling may confer long-term protection. Therapeutic modulation of this pathway holds significant promise but requires further mechanistic elucidation and clinical validation to ensure both efficacy and safety.

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Disclosure of conflict of interest

None.

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References

[1] Albini A, Di Paola L, Mei G, Baci D, Fusco N, Corso G and Noonan D. Inflammation and cancer cell survival: TRAF2 as a key player. Cell Death Dis 2025; 16: 292.

[2] Antoniades C, Bakogiannis C, Tousoulis D, Antonopoulos AS and Stefanadis C. The CD40/CD40 ligand system: linking inflammation with atherothrombosis. J Am Coll Cardiol 2022; 54: 669-677.

[3] Askarizadeh F, Karav S, Jamialahmadi T and Sahebkar A. Impact of statin therapy on CD40: CD40L signaling: mechanistic insights and therapeutic opportunities. Pharmacological Reports 2025; 77: 43-71.

[4] Wang Y, Zhang L, Jiang W, Kuang Q and Qin X. Molecular mechanisms underlying the co-pathogenesis of abdominal aortic aneurysm and rheumatoid arthritis: evidence based on bioinformatics analysis and clinical data. BMC Medical Genomics 2025; 18: 155.

[5] Wang X, Cheng H, Feng M, Jiang B, Ren C, Chen Q, Zhi X and Li Y. Causality of genetically proxied immunophenotypes on cardiovascular diseases: a Mendelian randomization study. Front Immunol 2024; 15: 1344773.

[6] Vermersch P, Wagner D, Mars LT, Noelle R and Giovannoni G. Inhibiting CD40 ligand in multiple sclerosis: a review of emerging therapeutic potential. Curr Treat Options Neurol 2025; 27: 7.

[7] Tulamo R, Frösen J, Hernesniemi J and Niemelä M. Inflammatory changes in the aneurysm wall: a review. J Neurointerv Surg 2018; 10: i58-i67.

[8] Touat Z, Ollivier V, Dai J, Huisse MG, Bezeaud A, Sebbag U, Palombi T, Rossignol P, Meilhac O and Guillin MC. Renewal of mural thrombus releases plasma markers and is involved in aortic abdominal aneurysm evolution. Am J Pathol 2022; 168: 1022-1030.

[9] Touat Z, Lepage L, Ollivier V, Nataf P, Hvass U, Labreuche J, Jandrot-Perrus M, Michel JB and Jondeau G. Dilation-dependent activation

of platelets and prothrombin in human thoracic ascending aortic aneurysm. Arterioscler Thromb Vasc Biol 2023; 28: 940-946.
[10] Tian S, Wang Y, Wan J, Yang M and Fu Z. Costimulators CD40-CD40L, a potential immunetherapy target for atherosclerosis: a review. Medicine 2024; 103: e37718.
[11] Texakalidis P, Sweid A, Mouchtouris N, Peterson EC, Sioka C, Rangel-Castilla L, Reavey-Cantwell J and Jabbour P. Aneurysm formation, growth, and rupture: the biology and physics of cerebral aneurysms. World Neurosurg 2019; 130: 277-284.
[12] Guedj K, Khallou-Laschet J, Clement M, Morvan M, Delbosc S, Gaston AT, Andreata F, Castier Y, Deschildre C, Michel JB, Caligiuri G and Nicoletti A. Inflammatory micro-environmental cues of human atherothrombotic arteries confer to vascular smooth muscle cells the capacity to trigger lymphoid neogenesis. PLoS One 2014; 9: e116295.
[13] Kusters PJH, Seijkens TTP, Beckers L, Lievens D, Winkels H, de Waard V, Duijvestijn A, Lindquist Liljeqvist M, Roy J, Daugherty A, Newby A, Gerdes N and Lutgens E. CD40L deficiency protects against aneurysm formation. Arterioscler Thromb Vasc Biol 2018; 38: 1076-1085.
[14] Nagashima H, Aoka Y, Sakomura Y, Uto K, Sakuta A, Aomi S, Kurosawa H, Hagiwara N, Kawana M and Kasanuki H. Matrix metalloproteinase 2 is suppressed by trapidil, a CD40-CD40 ligand pathway inhibitor, in human abdominal aortic aneurysm wall. J Vasc Surg 2004; 39: 447-453.
[15] Wei L, Wang Q, Zhang Y, Yang C, Guan H, Chen Y and Sun Z. Identification of key genes, transcription factors and microRNAs involved in intracranial aneurysm. Mol Med Rep 2018; 17: 891-897.
[16] Touat Z, Ollivier V, Dai J, Huisse MG, Bezeaud A, Sebbag U, Palombi T, Rossignol P, Meilhac O, Guillin MC and Michel JB. Renewal of mural thrombus releases plasma markers and is involved in aortic abdominal aneurysm evolution. Am J Pathol 2006; 168: 1022-1030.
[17] Touat Z, Lepage L, Ollivier V, Nataf P, Hvass U, Labreuche J, Jandrot-Perrus M, Michel JB and Jondeau G. Dilation-dependent activation of platelets and prothrombin in human thoracic ascending aortic aneurysm. Arterioscler Thromb Vasc Biol 2008; 28: 940-946.
[18] Flondell-Sité D, Lindblad B, Kölbel T and Gottsäter A. Cytokines and systemic biomarkers are related to the size of abdominal aortic aneurysms. Cytokine 2009; 46: 211-215.
[19] Chen XD, Sun J, Lu C, Ba HJ, Chen MH, Lin JH and Cai JY. The prognostic value of plasma soluble CD40 ligand levels following aneurysmal subarachnoid hemorrhage. Thromb Res 2015; 136: 24-29.
[20] Moschetti G, Vasco C, Clemente F, Galeota E, Carbonara M, Pluderi M, Locatelli M, Stocchetti N, Abrignani S, Zanier ER, Ortolano F, Zoerle T and Geginat J. Deep phenotyping of T-Cells derived from the aneurysm wall in a pediatric case of subarachnoid hemorrhage. Front Immunol 2022; 13: 866558.
[21] Grønbæk SK, Høgh J, Knudsen AD, Pham MHC, Sigvardsen PE, Fuchs A, Kühl JT, Køber L, Gerstoft J, Benfield T, Ostrowski SR, Kofoed KF and Nielsen SD. Aortic aneurysms and markers of platelet activation, hemostasis, and endothelial disruption in people living with HIV. Front Immunol 2023; 14: 1115894.
[22] Patra PK, Jindal AK, Rikhi R, Kaur A, Srivastava P, Suri D, Rawat A, Pilania R and Singh S. CD40 gene polymorphism and its expression in children with Kawasaki disease from North India: a preliminary case-control study and meta-analysis. Front Pediatr 2023; 11: 1252024.
[23] Cui X, Xuan T, Chen S and Guo X. Causal associations between CD40/CD40L and aortic diseases: a mendelian randomization study. Front Genet 2022; 13: 998525.
[24] Chen Z, Chen T, Lin R and Zhang Y. Circulating inflammatory proteins and abdominal aortic aneurysm: a two-sample Mendelian randomization and colocalization analyses. Cytokine 2024; 182: 156700.
[25] Tian Z, Zhang P, Li X and Jiang D. Analysis of immunogenic cell death in ascending thoracic aortic aneurysms based on single-cell sequencing data. Front Immunol 2023; 14: 1087978.
[26] Sagulkoo P, Chuntakaruk H, Suratanee A, Plaimas K and Teerakulkittipong N. Integrative bioinformatics frameworks for abdominal aortic aneurysm using GWAS meta-analysis, biological network construction, and structural modeling. Sci Rep 2025; 15: 22331.
[27] Obeagu EI and Obeagu GU. Platelet-driven modulation of HIV: unraveling interactions and implications. J Home Page 2024; 12.
[28] Obare LM, Temu T, Mallal SA and Wanjalla CN. Inflammation in HIV and its impact on atherosclerotic cardiovascular disease. Circ Res 2024; 134: 1515-1545.
[29] Norman P and Powell J. Site specificity of aneurysmal disease. Circulation 2023; 121: 560-568.
[30] Flondell-Sité D, Lindblad B, Kölbel T and Gottsäter A. Cytokines and systemic biomarkers are related to the size of abdominal aortic aneurysms. Cytokine 2023; 46: 211-215.
[31] Elgueta R, Benson MJ, De Vries VC, Wasiuk A, Guo Y and Noelle RJ. Molecular mechanism

and function of CD40/CD40L engagement in the immune system. Immunol Rev 2022; 229: 152-172.
[32] Chen Y, Yang M, Zhang M, Wang H, Zheng Y, Sun R and Li X. Single-cell transcriptome reveals potential mechanisms for coronary artery lesions in kawasaki disease. Arterioscler Thromb Vasc Biol 2024; 44: 866-882.
[33] Chen XD, Sun J, Lu C, Ba HJ, Chen MH, Lin JH and Cai JY. The prognostic value of plasma soluble CD40 ligand levels following aneurysmal subarachnoid hemorrhage. Thromb Res 2022; 136: 24-29.
[34] Chatzigeorgiou A, Lyberi M, Chatzilymperis G, Nezos A and Kamper E. CD40/CD40L signaling and its implication in health and disease. Biofactors 2022; 35: 474-483.
[35] Chalouhi N, Hoh BL and Hasan D. Review of cerebral aneurysm formation, growth, and rupture. Stroke 2023; 44: 3613-3622.
[36] Cai D and Chen SY. Elastin in the pathogenesis of abdominal aortic aneurysm. Cells 2025; 14: 1597.
[37] Bossone E and Eagle KA. Epidemiology and management of aortic disease: aortic aneurysms and acute aortic syndromes. Nat Rev Cardiol 2021; 18: 331-348.