Vascular Pharmacology 159 (2025) 107496

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Epitranscriptomics in atherosclerosis: Unraveling RNA modifications, editing and splicing and their implications in vascular disease

Victoria Stopa [a], Dimitra Dafou [c], Korina Karagianni [c], A. Yaël Nossent [d], Rosienne Farrugia [e], Yvan Devaux [a,*], Miron Sopic [a,b], on behalf of AtheroNET COST Action CA21153 (www.atheronet.eu)

[a] Cardiovascular Research Unit, Department of Precision Health, Luxembourg Institute of Health, Strassen, Luxembourg
[b] Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[c] Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece
[d] Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark
[e] Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, Msida, Malta

ARTICLE INFO

Keywords:
Epitranscriptomics
RNA splicing
RNA editing
Vascular biology
Atherosclerosis

ABSTRACT

Atherosclerosis remains a leading cause of morbidity and mortality worldwide, driven by complex molecular mechanisms involving gene regulation and post-transcriptional processes. Emerging evidence highlights the critical role of epitranscriptomics, the study of chemical modifications occurring on RNA molecules, in atherosclerosis development. Epitranscriptomics provides a new layer of regulation in vascular health, influencing cellular functions in endothelial cells, smooth muscle cells, and macrophages, thereby shedding light on the pathogenesis of atherosclerosis and presenting new opportunities for novel therapeutic targets. This review provides a comprehensive overview of the epitranscriptomic landscape, focusing on key RNA modifications such as N6-methyladenosine (m6A), 5-methylcytosine (m^5C), pseudouridine (Ψ), RNA editing mechanisms including A-to-I and C-to-U editing and RNA isoforms. The functional implications of these modifications in RNA stability, alternative splicing, and microRNA biology are discussed, with a focus on their roles in inflammatory signaling, lipid metabolism, and vascular cell adaptation within atherosclerotic plaques. We also highlight how these modifications influence the generation of RNA isoforms, potentially altering cellular phenotypes and contributing to disease progression. Despite the promise of epitranscriptomics, significant challenges remain, including the technical limitations in detecting RNA modifications in complex tissues and the need for deeper mechanistic insights into their causal roles in atherosclerotic pathogenesis. Integrating epitranscriptomics with other omics approaches, such as genomics, proteomics, and metabolomics, holds the potential to provide a more holistic understanding of the disease.

1. Introduction

Cardiovascular diseases (CVDs) remain one of the leading causes of death worldwide. Among these, conditions driven by the progression of atherosclerosis account for a significant proportion of global morbidity and mortality in both men and women [1]. Despite remarkable advances in therapeutic strategies, including the introduction of lipid-lowering agents such as PCSK9 inhibitors, which have substantially reduced LDL-C levels and mitigated the lipid-driven components of atherosclerosis, challenges persist [2]. Atherosclerosis is not merely a lipid storage disease but rather a multifaceted, progressive condition driven by a complex interplay of dysregulated lipid metabolism, endothelial dysfunction, chronic inflammation, and immune cell activation. Key immune cells involved in this process include monocytes, macrophages, dendritic cells, T cells (particularly $CD4^+$ and $CD8^+$ T cells), B cells, and innate lymphoid cells. Additionally, the disease is characterized by the dysregulation of vascular smooth muscle cells (SMCs) and extensive remodeling of the extracellular matrix (ECM) [3], both of which contribute to plaque formation, progression, and instability. The intricate crosstalk among these pathological processes underlies the

* Corresponding author at: Cardiovascular Research Unit, Department of Precision Health, Luxembourg Institute of Health, 1A-B rue Edison, L-1445 Strassen, Luxembourg.
E-mail address: yvan.devaux@lih.lu (Y. Devaux).

https://doi.org/10.1016/j.vph.2025.107496
Received 31 January 2025; Received in revised form 8 April 2025; Accepted 12 April 2025
Available online 14 April 2025

development of atherosclerotic lesions, ultimately leading to cardiovascular complications such as myocardial infarction and stroke.

The CANTOS trial, for example, demonstrated that targeting the inflammatory axis with anti-inflammatory interventions could provide significant cardiovascular benefits in patients with well-controlled LDL cholesterol [4]. However, no specific therapies are yet available to directly address endothelial dysfunction, chronic vascular inflammation, or vascular smooth muscle cell dysfunction, highlighting an unmet clinical need. Addressing these aspects is critical for developing comprehensive therapies to reduce the residual risk associated with atherosclerosis.

Epitranscriptomics has emerged as an exciting and rapidly expanding field of research that seeks to uncover how chemical modifications on RNA molecules influence cellular processes. These RNA modifications form a rich and dynamic regulatory landscape, with over 100 distinct modifications identified to date [5]. While historically, RNA modifications were primarily associated with roles in transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), recent studies have revealed their profound influence on messenger RNAs (mRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and microRNAs (miRNAs) [5]. These modifications are classified broadly into two categories: reversible and irreversible.

Reversible modifications, such as N6-methyladenosine (m6A) and 5-methylcytosine (5mC), can be dynamically added and removed during the lifespan of an RNA molecule (Table 1). This regulation is mediated by enzymes categorized as "writers" (responsible for adding modifications), "erasers" (responsible for removing them), and "readers" (which recognize and interpret these modifications to mediate downstream effects) [6]. These modifications impact critical RNA processes, including stability, translation, and splicing, thereby influencing cellular function. In contrast, irreversible modifications involve changes to the RNA nucleotide sequence itself, such as adenosine-to-inosine (A-to-I) RNA editing (Table 1). These modifications, once established, cannot be reversed and may lead to permanent alterations in RNA function.

RNA splicing, in particular, has garnered significant attention in the context of RNA modifications [7]. Differential splicing events, influenced by epitranscriptomic regulation, can generate distinct RNA isoforms, some of which may have opposing biological functions. This adds an additional layer of complexity to post-transcriptional regulation, emphasizing the therapeutic potential of targeting RNA modifications to fine-tune isoform expression and function in disease contexts.

Emerging evidence shows that RNA modifications play a significant role in atherosclerosis by regulating processes such as endothelial dysfunction, chronic inflammation, vascular smooth muscle cell dysregulation, and in the dynamic regulation of the ECM remodeling, driven in part by RNA modifications in stromal cells such as fibroblasts [8], alters ECM composition and stiffness, influencing the behavior of vascular smooth muscle cells (VSMCs) and immune cells like macrophages. This modulation of ECM properties can affect plaque stability and progression, with profound implications for vascular integrity. Recent studies have highlighted the significance of ECM remodeling in atherosclerosis pathophysiology [5,9]. This review explores recent insights into the epitranscriptomic mechanisms underlying these processes. Furthermore, we discuss their implications for RNA splicing and how these modifications could serve as potential therapeutic targets for improving the management of atherosclerosis-related diseases. By shedding light on these mechanisms, we aim to provide a framework for future research and therapeutic development in the context of vascular pharmacology.

2. Overview of Epitranscriptomic mechanisms

2.1. RNA modifications: the core components

As of today, advancements in high-throughput sequencing technologies and diverse biochemical assays have significantly expanded the field of epitranscriptomics, revealing over 170 chemical RNA modifications across various RNA species, including messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and non-coding RNA (ncRNA) [10]. Among these modifications, some are considered core components due to their distinctive properties and essential roles in cellular processes and disease mechanisms. For example, N6-

Table 1
Comprehensive overview of RNA Modifications/RNA editing and their associated enzymes: Writers, Erasers, and Readers.

		Enzymes		
		Writers	Erasers	Readers
RNA modifications	N6-methyladenosine (m6A)	Methyltransferase Like 3 (METTL3) Methyltransferase Like 14 (METTL14) Wilms Tumor 1 Associated Protein (WTAP) Vir Like m6A Methyltransferase Associated (VIRMA)	Fat Mass and Obesity Associated Protein (FTO) AlkB Homolog 5 (ALKBH5)	YTH domain family (YTHD): YTHDF1, YTHDF2, YTHDF3, YTHDC1… Insulin Like Growth Factor 2 mRNA binding proteins (IGFBP) Heterogenous Nuclear Ribonucleoproteins (HNRNPs)
	5-methylcytosine (m^5C)	NOP2/Sun RNA methyltransferase family (NSUN) (e.g., NSUN1, NSUN2, NSUN3, NSUN4) DNA methyltransferases (DNMT1, DNMT2, DNMT3)	Tet family of dioxygenases (Tet1, Tet2, Tet3) AlkB Homolog 1 (ALKBH1)	Aly/REF export factor (ALYREF)
	Pseudouridine (Ψ)	Pseudouridine synthases (PUS) H/ACA small nucleolar ribonucleoprotein (snoRNP)	-	MetRS [60]
	2′-*O*-methylation (Nm)	Fibrillarin	-	Piwi family proteins Argonaute 2 (AGO2) [61]
	N1-methyladenosine (m1A)	Nucleomethylin (NML) TRNA Methyltransferase (TRMT16B, TRMT61A complex)	Fat Mass and Obesity Associated Protein (FTO) AlkB Homolog 5 (ALKBH5) or 3 (ALHBH3)	Heat-responsive protein 12 (HRSP12)
	2'-O-ribose-methylation (2'Ome)	Fibrillarin Complex with several proteins and C/D-box snoRNAs	-	-
RNA editing	Adenosine-to-Inosine (A-to-I) Cytosine-to-uridine (C-to-U)	Adenosine deaminase acting on RNA (ADAR) family: ADAR1, ADAR2, and ADAR3. Members of the apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) family Apobec-1 complementation factor (ACF) CUG-binding protein 2 (CUGBP2) Glycine-arginine-tyrosine-rich RNA-binding protein (GRY-RBP) Heterogeneous nuclear ribonucleoprotein (hnRNP)-C1.	-	

methyladenosine (m6A), the most abundant and extensively studied modification in eukaryotic mRNA, regulates crucial processes such as mRNA stability, splicing, and translation. Similarly, modifications like 5-methylcytosine (m^5C) and pseudouridine (Ψ) have gained attention for their contributions to RNA structural stability and function. Beyond these well-characterized modifications, others, such as 2′-*O*-methylation and N1-methyladenosine (m1A), are increasingly being recognized for their potential roles [11]. Despite the growing list of known modifications, many remain poorly understood, with their functional significance and mechanisms yet to be uncovered, underscoring the complexity and vast potential of epitranscriptomics.

- N6-methyladenosine (m6A)

Among the myriad of RNA modifications identified, N6-methyladenosine (m6A), has emerged as the most prevalent and functionally versatile internal modification in eukaryotic messenger RNA and non-coding RNA. M6A perfectly exemplifies the dynamic nature of RNA modifications, functioning as a reversible chemical mark deposited by a multicomponent methyltransferase complex, commonly referred to as "writers," which includes core enzymes such as Methyltransferase Like 3 (METTL3) and Methyltransferase Like 14 (METTL14), along with auxiliary factors like Wilms Tumor 1 Associated Protein (WTAP) and Vir Like m6A Methyltransferase Associated (VIRMA) (Fig. 1A). Removal of m6A is mediated by specialized demethylases, known as "erasers," such as Fat Mass and Obesity Associated Protein (FTO) and AlkB Homolog 5 (ALKBH5) (Fig. 1A). The functional output of m6A is dictated by "reader" proteins including members of the YTH domain family (YTHD), Insulin Like Growth Factor 2 mRNA binding proteins (IGFBP) and Heterogenous Nuclear Ribonucleoproteins (HNRNPs) which recognize m6A marks and mediate downstream effects such as mRNA translation, stabilization, or degradation (Fig. 1A) [11]. Moreover, emerging evidence suggests that RNA modification enzymes can exert functions beyond their canonical roles. METTL3, primarily known as an m6A writer, has been shown to directly regulate transcription in an m6A-independent manner, potentially influencing pro-atherosclerotic gene expression [12]. The specific roles of m6A modifiers in atherosclerosis will be discussed in details below.

This modification governs key physiological processes, including RNA stability, translation, and splicing. For instance, m6A enhances mRNA degradation by recruiting specific decay factors, thereby controlling gene expression levels. In translation, it modulates the efficiency of protein synthesis, influencing cellular responses and adaptation. Furthermore, m6A plays a pivotal role in alternative splicing by interacting with splicing factors, enabling the generation of different protein isoforms critical for functional diversity [5].

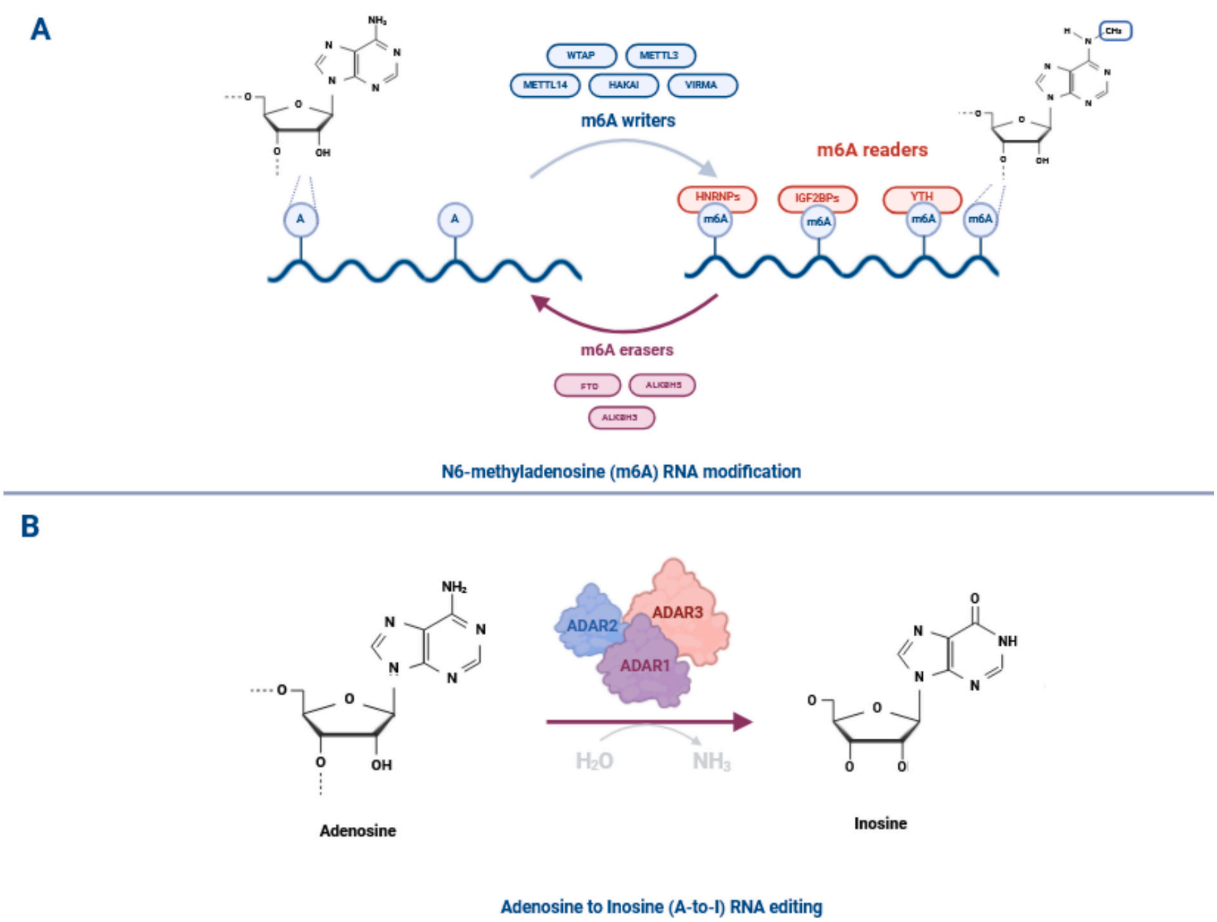

Fig. 1. Epitranscriptomic RNA modification - N6-methyladenosine (m6A) and Adenosine to Inosine (A-to-I) RNA editing. This figure illustrates two major types of RNA modifications: **A:** N6-methyladenosine (m6A) RNA modification and Adenosine-to-Inosine (A-to-I) RNA editing (bottom panel). N6-methyladenosine (m6A) RNA modification: The m6A modification involves the addition of a methyl group ($-CH_3$) to the N6 position of adenosine residues in mRNA. This process is mediated by m6A writers (methyltransferases) including METTL3, METTL14, WTAP, HAKAI, and VIRMA, which catalyze m6A deposition. The modification can be recognized by m6A readers such as YTH domain proteins, IGF2BPs, and HNRNPs, which influence RNA stability, translation, and splicing. Conversely, m6A erasers (FTO, ALKBH5, ALKBH3) are demethylases that remove the m6A mark, thereby modulating RNA function dynamically. **B:** Adenosine-to-Inosine (A-to-I) RNA editing: This process involves the enzymatic conversion of adenosine (A) to inosine (I) in RNA transcripts, catalyzed by adenosine deaminases acting on RNA (ADARs), including ADAR1, ADAR2, and ADAR3. This modification plays a critical role in transcriptome diversification, influencing codon changes, RNA stability, and splicing. Figure is created with BioRender.

- 5-methylcytosine (m^5C)

While m6A is one of the most extensively studied RNA modifications due to its broad regulatory roles, it is far from the only modification shaping the dynamic landscape of RNA biology. Other modifications, such as 5-methylcytosine (m^5C), also play crucial roles in RNA stability, processing, and functions. m^5C involve the addition of a methyl group to the cytosine base of RNA, and is regulated by a system of "writers", "readers" and erasers similar to m6A mechanisms (Table 1) [11].

Over 10 m^5C methyltransferases have been identified, including the NOP2/Sun RNA methyltransferase family (NSUN) (e.g., NSUN1, NSUN2, NSUN3, and NSUN4), as well as DNA methyltransferases (DNMT1, DNMT2, DNMT3) [13].

The removal of m^5C marks is mediated by erasers like the Tet family of dioxygenases (Tet1, Tet2, Tet3. Additionally, ALKBH1 functions as a direct RNA demethylase, specifically targeting m^5C on mRNAs and influencing their degradation, stability, or translation. Readers of m^5C, like ALYREF, bind to m^5C sites and mediate downstream regulatory functions, particularly in mRNA export. However, much remains to be uncovered regarding additional m^5C readers and their precise mechanisms. A deeper understanding of m^5C readers is essential to unravel the full complexity of this critical RNA modification.

The m^5C RNA modification is widely distributed across various types of RNA in eukaryotes and plays crucial roles in RNA synthesis, processing, translation, stability, degradation, mitochondrial function and energy metabolism…Dysregulation of m^5C has been linked to various diseases, particularly cancer, where m^5C methylation is associated with poor prognosis, tumor progression, and drug resistance [14].

- Pseudouridine (Ψ)

Another abundant RNA modification is pseudouridine (Ψ), where the ribose sugar attaches to the base through a different type of bond. This modification is catalyzed by a diverse class of enzymes called pseudouridine synthases (PUS) (Table 1) [15]. The H/ACA small nucleolar ribonucleoprotein (snoRNP) complex also plays a pivotal role in pseudouridylation, particularly in ribosomal RNA (rRNA) and small nuclear RNA (snRNA) [16]. In rRNA, this modification enhances stability, folding, and ribosomal function, while in snRNA, it ensures accurate pre-mRNA splicing.

Deficiency in pseudouridine modification, or even mutations in H/ACA snoRNP components, particularly dyskerin, are associated with diseases like dyskeratosis congenita and telomeropathies. The complex also contributes to telomere maintenance by modifying the RNA component of telomerase, underscoring its critical role in RNA biology and cellular homeostasis. Pseudouridine is broadly distributed across various RNA types, including rRNA, tRNA, mRNA, and non-coding RNA (ncRNA). It plays essential roles in tRNA function, translation, splicing, and RNA-protein interactions. Despite its prevalence and importance, the full scope of Ψ's biological significance remains poorly understood, necessitating further investigation.

- Others (e.g., 2′-*O*-methylation, N1-methyladenosine…)

Several other RNA modifications exist beyond m6A, m^5C, and pseudouridine. One example is 2′-*O*-methylation (Nm), which involves the addition of a methyl group to the 2′ hydroxyl of the ribose sugar. This modification can occur on all bases and in various types of RNA, with a predominance in rRNA and tRNA. The methyl group is transferred by specific methyltransferases, such as Fibrillarin (Table 1) [17]. Compared to other RNA modifications, Nm appears to be largely irreversible, because no erasers have been identified to date [18]. Dysregulation of Nm has been linked to various diseases, including Prader-Willi syndrome, cancers, autoimmune diseases, and Alzheimer's disease [17].

m1A, also known as N1-methyladenosine, results from the methylation of adenosine at the N1 position. It has a unique set of enzymes for its regulation but shares common similarities with m6A, such as the common erasers FTO, ALKBH members and being recognized by readers from the YTH protein family, m1A specific writers are nucleomethylin (NML), and RNA type-specific writers like TRMT16B for mitochondrial tRNA and rRNA or TRMT61A complex for mRNA and mitochondrial tRNA for instance. Beyond its role in RNA metabolism, emerging evidence suggests that m1A-modifying enzymes, particularly the TRMT6/TRMT61A complex, contribute to broader metabolic regulation, including cholesterol homeostasis and mitochondrial function. TRMT6/TRMT61A has been shown to influence cholesterol metabolism in the liver [19], an essential process linked to atherosclerosis development. Cholesterol dysregulation is a key driver of plaque formation, and alterations in tRNA modifications could impact lipid metabolism by affecting translation efficiency of key enzymes involved in sterol biosynthesis and transport. Additionally, TRMT6/TRMT61A-mediated m1A methylation in mitochondrial tRNA plays a crucial role in mitochondrial respiration and oxidative phosphorylation [20]. Given that mitochondrial dysfunction is a hallmark of vascular smooth muscle cell (VSMC) dedifferentiation and foam cell formation in atherosclerosis, dysregulation of TRMT6/TRMT61A could contribute to metabolic stress within the vasculature, further promoting disease progression [11,21]. This shared machinery highlights a dynamic interplay between these two RNA modifications in regulating RNA metabolism and function.

Overall, RNA modifications are involved in diverse biological processes, including RNA stability, translation, and splicing, and their dysregulation has been implicated in the development of numerous diseases, highlighting their importance in both health and diseases.

- 2'-O-ribose-methylation (2′Ome)

2′*O*-methylation (2'Ome) is an important modification that stabilizes RNA, supporting ribosome function and RNA splicing. It is added to ribosomal RNAs (rRNAs) and small nuclear RNAs (snRNAs) by the methyltransferase fibrillarin (Table 1), which is part of a complex with several proteins and C/D-box snoRNAs. These snoRNAs help guide the methylation process by base-pairing with target RNA sequences. [22] It has been shown that there is heterogeneity in the degree of rRNA methylation, which impacts translation of specific mRNAs, leading to altered cell fate decisions in neuronal differentiation, for example [23]. To the best of our knowledge, effects of heterogeneity of rRNA methylation on atherogenesis and cardiovascular disease has not yet been investigated.

Not all snoRNAs have known rRNA or snRNA targets, however. Such snoRNAs are usually referred to as 'orphan snoRNAs'. A cluster of 41 orphan snoRNAs transcribed from the 14q32 DLK1-DIO3 locus was shown to play a role in cardiovascular disease [24] and heart failure risk expression of these snoRNAs is increased in coronary bypass grafts that failed due to atherosclerosis, and their plasma levels are associated with platelet function in patient with peripheral artery disease. In mRNAs, the 14q32 snoRNA SNORD113–6, was shown to guide methylation of both mRNAs and tRNAs influencing arterial fibroblast cell-cell and cell-matrix interactions, fibroblast-induced matrix contraction and fibroblast migration [9]. The same snoRNA also guides methylation of $tRNA^{Leu}$(-TAA), protecting it from site-specific degradation under ischemia and oxidative stress.

Earlier studies also indicate a potential role for canonical C/D box snoRNAs in atherosclerosis, albeit through non-canonical mechanisms. For example, snoRNA U60, was shown to regulate intracellular cholesterol trafficking, independently of its canonical function, which is to guide methylation of the 28S rRNA [25]. Cholesterol trafficking is an essential process for cellular homeostasis and maintenance, but a potential link to atherosclerosis is easily made. Similarly, the canonical C/D box snoRNAs U32a, U33 and U35a protect from lipotoxic and oxidative stress, again independent of their canonical functions [26].

Although evidence for a direct role of 2'Ome in atherogenesis and progression of atherosclerotic lesions is lacking, the above-mentioned studies do provide a foundation for further investigations of C/D box snoRNA and 2'Ome, both canonical and noncanonical, in atherosclerosis.

2.2. RNA editing

RNA editing is a co/post-transcriptional modification that introduces specific changes to RNA sequences, significantly diversifying the transcriptome and affecting regulation of gene expression [27]. Among various types of RNA editing, adenosine-to-inosine (A-to-I) and cytosine-to-uracil (C-to-U) conversions are the most studied in mammals (Table 1) [28–30]. Depending on the type of RNA being edited and the specific positions within the transcript that are modified, RNA editing can impact RNA stability, localization, and the efficiency of transcript processing and turnover. Emerging evidence underscores the critical role of RNA editing as a regulator of gene expression, functioning both autonomously and in synergy with RNA interference pathways. In coding regions, editing can introduce non-synonymous changes, leading to protein recoding or premature stop codons. These modifications diversify the proteome and influence key cellular functions, underscoring RNA editing's role as a dynamic regulator of transcript and protein diversity [31–37].

Adenosine-to-inosine (A-to-I) RNA editing, mediated by the adenosine deaminase acting on RNA (ADAR) family of enzymes, represents the most prevalent form of editing in vertebrates (Fig. 1B) [34]. ADAR enzymes, including ADAR1, ADAR2, and ADAR3, are key players in this process. ADAR1 exists in two primary isoforms: a 150 kDa isoform induced by interferons and a constitutively expressed 110 kDa isoform. The 150 kDa isoform shuttles between the nucleus and cytoplasm, where it binds endogenous double-stranded RNAs (dsRNAs) to prevent their recognition by melanoma differentiation-associated gene 5 (MDA5) and subsequent activation of the mitochondrial antiviral signaling (MAVS)-dependent type I interferon response. In contrast, the 110 kDa isoform primarily localizes to the nucleus and facilitates nuclear RNA editing. Both isoforms play critical roles in modulating immune responses and maintaining cellular homeostasis [38–41]. ADAR1 and ADAR2 catalyze the site-specific deamination of adenosine-to-inosine in dsRNA substrates, selectively targeting repeat-rich regions such as *Alu* elements and long interspersed nuclear elements (LINEs). The density of editing sites is particularly high in introns and untranslated regions (UTRs), especially the 3'UTRs of protein-coding genes [34,42,43]. ADAR3, which is primarily expressed in the brain, has no proven catalytic activity [44], but appears to act as a regulatory modulator of RNA editing, potentially inhibiting ADAR1 and ADAR2 activity on certain substrates, thereby contributing to the fine-tuning of the editing landscape [45].

Cytosine-to-uridine (C-to-U) RNA editing, catalyzed by members of the apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) family, is a highly specific enzymatic process that modifies a single cytidine within RNA molecules in single-stranded RNA regions [46,47]. A prominent example is the editing of apolipoprotein B (APOB) mRNA, producing distinct protein isoforms essential for lipid metabolism. ApoB-48, produced in the intestine, is essential for chylomicron assembly and dietary fat absorption, while apoB-100, synthesized in the liver, facilitates the formation of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), key players in cholesterol transport. The editing of apoB mRNA introduces a premature stop codon, truncating apoB-48 to 48 % of the full-length apoB-100, thereby altering its interaction with receptors and enabling distinct physiological functions [48]. Unlike A-to-I editing, which primarily occurs in dsRNA regions, C-to-U editing depends on auxiliary proteins and cofactors to achieve site specificity. Apobec-1 works in conjunction with apobec-1 complementation factor (ACF), which recognizes RNA substrates. Additional proteins such as CUG-binding protein 2 (CUGBP2), glycine-arginine-tyrosine-rich RNA-binding protein (GRY-RBP), and heterogeneous nuclear ribonucleoprotein (hnRNP)-C1 contribute to the regulation of editing activity [49].

RNA editing significantly impacts immune responses and endothelial cell integrity, both of which are central to atherosclerosis pathogenesis [50]. In immune cells, adenosine-to-inosine (A-to-I) RNA editing, mediated by ADAR enzymes, influences the expression and stability of transcripts encoding cytokines, chemokines, and immune receptors. This regulatory mechanism plays a critical role in modulating inflammatory signaling pathways and maintaining immune homeostasis, which are essential for the recruitment and activation of inflammatory cells at sites of vascular injury [51]. The enzyme ADAR1 is central to this regulation, as it suppresses the recognition of endogenous double-stranded RNA (dsRNA) by innate immune receptors such as melanoma differentiation-associated protein 5 (MDA5). This suppression may occur through ADAR1's catalytic activity of RNA editing, which prevents the accumulation of unedited dsRNA [52–54], or through its ability to bind dsRNA and physically interact with receptors like RIG-I, thereby modulating their activation [55]. When RNA editing is impaired, unedited dsRNA accumulates, triggering aberrant activation of type I interferon (IFN) responses and interferon-stimulated genes (ISGs), thereby exacerbating inflammation [35,52–54,56,57]. Additionally, in endothelial cells, RNA editing modulates the stability of transcripts that govern cell adhesion, migration, and barrier function [58].

Advances in RNA sequencing technologies and analysis strategies have enabled precise detection and analysis of RNA editing, unraveling its complexity and therapeutic potential. High-throughput methods such as single-cell and long-read sequencing now capture cell-specific editing events with unprecedented resolution. These advancements have revealed RNA editing's dual role in gene regulation and disease pathology [59].

2.3. The role of RNA modifications and RNA editing in alternative splicing

Introns in precursor mRNAs are removed by RNA splicing to form mature mRNA. This process may occur via either constitutive splicing or alternative splicing mechanisms. Whilst in constitutive splicing all exons are retained and introns removed, in alternative splicing different combinations of exons can be retained or skipped resulting in diverse mRNA isoforms for the same gene. Alternative RNA isoforms have been shown to be tissue specific [62], to vary between developmental stages [63] and between healthy and disease states [64]. Alternative RNA splicing can be broadly subdivided into five subtypes: (i) Exon skipping where one or more exons and their adjacent introns are skipped, (ii) intron retention where a part or all of an intron sequence is retained within the mature transcript, (iii) selection between mutually exclusive exons where either one or another exon are retained but never both together, (iv) the use of alternative 5′ or 3′ splice site recognition sequences to give longer or shorter versions of the same exon and (v) back splicing which results in circularization of the RNA (circRNA) [65].

Epigenetic changes, at both histone [66] and RNA [67] level have been shown to influence alternative splicing contributing to different layers of RNA regulation. Histone modifications alter chromatin structure and regulate splicing by influencing the rate of transcription [66,68] or by altering spliceosome recruitment [69]. On the other hand, RNA modifications which are often added to the mRNA as it is being transcribed can directly change availability of splice site recognition sequences.

Pre-mRNA molecules are not linear molecules but take up secondary structures by intramolecular base pairing. This may sequester the sequence elements needed for binding to the spliceosome or to RNA Binding Proteins (RBP) by including such sequences within the inaccessible double stranded regions of the folded mRNA molecule. In such instances, when the secondary pre-mRNA molecule is more stable than the intermolecular bonding of the RNA-spliceosome or RNA-RBP complex, the binding sites within the double stranded portion are rendered unavailable, favouring splicing of alternative RNA isoforms [70]. RNA is subject to many chemical modifications which regulate mRNA splicing and function(reviewed in [67]) through modifications of RNA-RNA and RNA-protein interactions.

N6-methyladenosine (m6A) is among the commonest mRNA internal modifications [71,72] and the most studied. The methylation of Adenosine (A) residues alters the energetic requirements for the formation of hydrogen bonds between A and Uracil (U) in double stranded RNA conformations due to steric hindrance caused by the additional methyl group. Thus, m6A destabilizes double stranded regions of RNA [73] making their sequences more accessible to RBPs and therefore splicing [74]. Additionally m6A modifications near splice junctions have been associated with faster splicing kinetics, [75] whilst intronic m6A modifications appear to slow down the rate of splicing.

Notwithstanding various studies that have attempted to map m6A modifications through knockdown of the enzymes that write these modifications, [75,76] data is severely lacking for modified nucleotides within introns which contain most splicing regulatory elements. Data is also often limited to highly expressed genes leaving much to be discovered in the field of splicing regulation by mRNA modifications [67].

Pseudouridine (Ψ), N5 methlycytidine (m^5C), and N1 methyadenosine (m1A), modifications and their role in splicing are far less studied. The RNA-modifying enzymes that write these modifications also modify tRNA. Thus the typical experimental set-up that knocks down the enzymes that make these RNA modifications also abolishes tRNA modifications changing tRNA stability and function [77]. Unfortunately changes to the tRNA pool will invariably affect splicing through changes in translation and availability of splicing factors. Thus, new methods need to be explored to enable in depth studies of these modifications on only RNA splicing. One such proposed method uses short-term chemical inhibitors, rather than gene knockdowns or conditional knockouts, to study the effects of blocking RNA-modifying enzymes on splicing [78]. Alternative methods, not dependent on the availability of suitable chemical inhibitors, utilize ligand induced-degrons which trigger the ubiquitin-proteasome pathway to degrade enzymes fused with a degron tag [79,80].

However, Ψ, an isomer of uridine, has been shown to limit the flexibility of single stranded RNA molecules [81] and to stabilize double stranded RNA [82]. Thus the incorporation of Ψ, particularly within polypyrimidine tracts located immediately upstream of the 3′ ends of introns, decreases splicing efficiency due to reduced binding of the spliceosome U2 auxiliary factor at the splice acceptor site [83]. It has also been suggested that intronic mRNA modifications, specifically m^5C sites, mark improperly spliced RNA and sequester these molecules in the nucleus [84] where they are degraded without being exported to the cytoplasm, or degraded immediately upon export. It is not yet clear at which point in the splicing process these intronic m^5C modifications are introduced into the mRNA and how they affect the fate of the mRNA molecule.

Finally, and more directly, Adenosine Deaminases Acting on RNA (ADARs) convert Adenosine (A) residues to Inosine (I) in double stranded RNA molecules [85]. Inosine is recognized structurally as Guanosine (G), thus the conversion of AA→AI within the pre-mRNA molecules generates novel 3′ splice sites. Similarly, a change of AU → IU creates a novel 5′ splice site and both create new transcript isoforms. The converse, the destruction of a canonical splice site recognition sequence, is also possible through the conversion of AG → IG [43,86,87].

3. RNA modifications and RNA editing in cellular adaptation and pathological processes in vascular tissue

3.1. Roles of RNA Modifications in atherosclerosis

3.1.1. The role of m6A modifiers in atherosclerosis

RNA modifications are under constant, active regulation in response to signals and stressors which include factors relevant to vascular disease such as ischemia, exposure to cytokines and oxidized LDL (ox-LDL) [5]. This dynamic regulation is best understood for m6A for which writers, erasers and readers have been identified and studied. Transcriptome wide m6A modifications have been identified in both human and mouse cells. m6A modifications are found most often close to stop codons and within large internal exons. The methylation marks are highly conserved and most of them are stable in response to stimuli. However, a subset has been shown to change in response to stimuli [88]. In the context of vascular tissue, changes in m6A patterns influence gene expression, angiogenesis, inflammation and atherosclerotic plaque development.

The m6A writers N6-methyladenosine methyltransferase (METTL3) and Methyltransferase-like 14 (METTL14) have been shown to influence mRNA stability and through this a number of physiological and pathological processes within the vasculature. Increased levels of METTL3 have been reported with increasing fibrosis in cardiac fibroblasts in in vitro models when cultured fibroblasts are exposed to TGF-β1 as well as in vivo in MI mouse models [89]. Higher levels of m6A methylation have also been observed in human cardiomyopathy [90] and METTL3-dependent m6A modifications have been shown to regulate atherogenic gene expression within vascular tissue and trigger atherosclerosis in response to disturbed blood flow and oscillatory stress at vessel branch points [91]. Inflammation induced by ox-LDL significantly increases m6A modifications in macrophages (RAW 264.7 cell line) and HUVECs [92]. Knockdown of METTL3 inhibits m6A modification and dampens the inflammatory response. METTL3 downregulation also delays progression of atherosclerosis in an atherosclerosis mouse model and prevents angiogenesis in developing mouse embryos by regulating JAK2 expression via the m6A reader IGF2BP1 [93]. Similarly, knockdown of METTL14 decreases m6A content in ox-LDL exposed HUVECs and resulted in a decrease in plaque size in $APOE^{-/-}$ mice on a high fat diet [92]. Recent research has elucidated a significant connection between oscillatory shear stress and the expression of METTL3 in endothelial cells, providing deeper insights into the mechanisms underlying atherosclerosis development [91]. Oscillatory shear stress, characterized by its disturbed flow patterns, is a critical factor that induces endothelial dysfunction, thereby contributing to the initiation and progression of atherosclerotic lesions. Under these conditions, there is an upregulation of METTL3 leading to increased m6A modifications which results in the stabilization and upregulation of NLRP1 transcripts and the degradation and downregulation of KLF4 transcripts, both of which are pivotal in promoting atherogenic responses. Importantly, silencing METTL3 has been shown to mitigate these effects, underscoring its central role in the pathogenesis of atherosclerosis. .

In addition to METTL3, METTL14 is also recognized as important driver of atherosclerotic process. Reducing Mettl14 expression in macrophages has been shown to encourage their shift towards the anti-inflammatory M2 phenotype, while also decreasing foam cell formation and migration capabilities [94]. This effect is associated with a reduction in Myd88 and IL-6 levels, as Mettl14 influences Myd88 mRNA stability through m6A modifications. Additionally, Myd88 modulates IL-6 transcription by altering nuclear p65 distribution, rather than through direct m6A modification of IL-6. In animal models, knocking out the Mettl14 gene led to a significant decrease in macrophage-driven inflammation and atherosclerotic plaque development. These findings suggest that Mettl14 plays a crucial role in macrophage-mediated inflammation in atherosclerosis via the NF-κB/IL-6 signaling pathway, highlighting its potential as a therapeutic target for treating the disease.

The m6A eraser ALKB homolog 5 (ALKBH5) is expressed in cardiac microvascular endothelial cells and becomes significantly upregulated under states of hypoxia. When ALKBH5 is upregulated, due to hypoxia, global m6A marks decrease and the expression of WNT5A is dysregulated. This causes a decrease in endothelial cell proliferation and migration. Knockdown of the eraser in hypoxic cells reverses the phenotype with preservation of m6A marks and the promotion of angiogenesis [95]. Recent research has identified the m6A demethylase FTO as a significant mediator of statin effects in human endothelial cells

[96]. Atorvastatin treatment has been observed to reduce FTO protein expression in ECs. Silencing the FTO in endothelial cells enhances the expression of Kruppel-like factor 2 (KLF2) and endothelial nitric oxide synthase (eNOS), while reducing tumor necrosis factor-alpha (TNFα)-induced expression of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) [96]. This leads to decreased monocyte adhesion to endothelial cells. Conversely, overexpressing FTO increases VCAM-1 and ICAM-1 levels and decreases KLF2 and eNOS expression, counteracting atorvastatin's beneficial effects on KLF2 and eNOS induction. Mechanistically, FTO interacts with KLF2 and eNOS transcripts, modulating their expression in an m6A-dependent manner. Following FTO silencing, increased m6A modifications in the 3′ untranslated regions of KLF2 and eNOS transcripts enhance their stability through YTHDF3 binding, leading to elevated protein expression of KLF2 and eNOS.

3.1.2. m6A Modification in atherosclerosis

Among the emerging molecular mechanisms implicated in atherosclerosis progression, m6A RNA modifications have garnered significant attention. Recent studies comparing carotid artery tissues from healthy individuals and those with atherosclerosis highlight the altered expression of m6A methylases, demethylases, and binding proteins in atherosclerotic samples [97]. Gene Ontology (GO) and KEGG pathway analyses revealed these m6A regulators' involvement in cholesterol metabolism, the PPAR signaling pathway, smooth muscle cell proliferation, the MAPK cascade, and inflammatory responses [98]. These findings underscore the potential of m6A as a critical regulator of atherosclerosis, linking it to key cellular processes and offering promising therapeutic targets.

Endothelial dysfunction is one of the earliest events in atherosclerosis development, disrupting vascular homeostasis and enabling monocyte adhesion and infiltration. The regulation of endothelial function by m6A modifications is exemplified by METTL14, an m6A methyltransferase whose expression is induced by TNF-α. METTL14 enhances FOXO1 mRNA translation through YTHDF1 recognition, driving the expression of adhesion molecules and promoting endothelial-monocyte interactions [99].

MicroRNAs also contribute to endothelial dysfunction, and their regulation via m6A modifications further highlights the epitranscriptomic landscape's complexity. METTL3 facilitates the maturation of microRNAs such as miR-18a-5p and let-7e-5p, which enhance endothelial function and angiogenesis [100]. Similar mechanisms are observed in atherosclerotic contexts, where METTL3 and METTL14 regulate the biogenesis of microRNAs like miR-19a and miR-375-3p, impacting cell proliferation, vascular integrity, and plaque stability [101].

Vascular smooth muscle cells (VSMCs) play a dual role in plaque stability, switching between contractile and synthetic phenotypes in response to pathological stimuli. m6A modifications orchestrate this phenotypic switch. METTL3-driven biogenesis of miR-375-3p promotes VSMC proliferation and plaque vulnerability by suppressing PDK1, a key regulator of cell survival and metabolism [102]. Additionally, m6A-modified UCHL5 mRNA enhances its stability and expression, activating the NLRP3 inflammasome and driving inflammation in VSMCs [103]. These findings reveal how m6A-mediated regulatory mechanisms contribute to the dynamic interplay between VSMCs and the inflammatory environment in atherosclerosis. VSMCs also play a critical role in the neointima formation. Recent studies have shown that METTL3 is upregulated in the neointima following vascular injury, both in animal models and human patients [104]. The deficiency of METTL3 in VSMCs significantly attenuates neointima formation, supporting the hypothesis that METTL3-mediated m6A RNA modification promotes VSMC proliferation and migration. Beyond their established roles in VSMC phenotypic modulation and inflammatory responses, RNA modifications such as m6A may also regulate transcription and translation efficiency of key genes in vascular pathology. For instance, METTL3-mediated m6A methylation of SGK1 mRNA promotes its stability and transcription, leading to increased VSMC proliferation and migration, processes that contribute to neointima formation and vascular remodeling [104]. Additionally, Dong et al. [105] demonstrated that METTL3 facilitates VSMC phenotypic switching via m6A-dependent regulation of MRTFA translation through YTHDF3, thereby promoting atherosclerotic progression. These studies show the complex interplay between m6A methylation and VSMC plasticity.

The immune system is deeply intertwined with atherosclerosis, with macrophages playing a central role in plaque formation and progression. The METTL3-STAT1 axis, for instance, stabilizes STAT1 mRNA through m6A modifications, driving M1 macrophage polarization and amplifying pro-inflammatory signaling [106]. Conversely, METTL14 dampens inflammatory responses in endothelial cells, inhibiting atherosclerotic plaque formation [99]. These contrasting roles reflect the nuanced regulatory capacity of m6A in inflammatory pathways.

Programmed cell death mechanisms are crucial in atherosclerosis, contributing to plaque formation and instability. Apoptosis, or programmed cell death, is modulated by m6A modifications. METTL3 hypermethylates NPC1L1 mRNA, promoting endothelial apoptosis and accelerating atherosclerotic plaque development [107]. In contrast, the m6A eraser FTO reduces m6A levels on ULK1, stabilizing the protein and promoting autophagy—a protective mechanism in cells under stress [108]. METTL3-mediated autophagy regulation in VSMCs further underscores the dual role of m6A in maintaining vascular homeostasis [109].

Pyroptosis, an inflammatory form of cell death, is similarly influenced by m6A modifications. m6A-modified lncRNA MALAT1 promotes macrophage pyroptosis by degrading USP8 mRNA, exacerbating inflammation [110]. Interestingly, external factors such as exercise mitigate these effects by modulating m6A-dependent pathways, suggesting lifestyle interventions could influence epitranscriptomic regulation. Additionally, H19 was identified as the primary target lncRNA molecule of METTL3-mediated m6A modification in the pathogenesis of atherosclerosis. METTL3-mediated m6A modification regulates H19 expression, thereby aggravating atherosclerosis by activating pyroptosis, further highlighting the critical role of m6A modifications in inflammatory processes associated with cardiovascular diseases [111].

Ferroptosis, a form of cell death driven by iron-dependent oxidative stress, also intersects with m6A regulation. METTL3-mediated m6A modifications on GPX4 destabilize this critical antioxidant protein, promoting ferroptosis and plaque progression [112]. More recently, the role of m6A modification gained increasing attention in atherosclerosis with new discoveries pointing to m6A-associated ferroptosis-related genes [113] including NOX4, CDO1 and SLC3A2 which are involved in the disease, m6A modified and correlated with immune cell activity, suggesting their potential role in ferroptosis-related pathways. These mechanisms demonstrate how m6A modifications regulate diverse cell death pathways, each contributing to the multifaceted nature of atherosclerosis.

Ischemia and hypoxia are key drivers of atherosclerosis-related complications. m6A modifications emerge as significant regulators of these processes (Woudenberg et al. (BJP, in press). In intracranial atherosclerosis, METTL4-mediated methylation of miR-494-3p destabilizes plaques by suppressing tight junction protein ZO1, impairing endothelial barrier integrity. In ischemic myocardial tissues, METTL3 expression and m6A levels are elevated, suppressing autophagic flux and increasing apoptosis under hypoxic conditions. These findings highlight the centrality of m6A in cellular responses to ischemic stress, with implications for both vascular and cardiac health.

Recent studies have highlighted clonal hematopoiesis of indeterminate potential (CHIP) as an emerging risk factor for cardiovascular diseases, including atherosclerosis. CHIP is characterized by the clonal expansion of hematopoietic stem cells harboring somatic mutations, with TET2 mutations being the most common. These mutations are thought to contribute to a chronic inflammatory state, which plays a significant role in the pathogenesis of atherosclerosis [114]. RNA modifications, such as m6A, in hematopoietic cells may influence the inflammatory pathways that are central to atherosclerosis development. For example, TET2-mutant clones have been shown to promote the activation and polarization of pro-inflammatory macrophages [115] which might contribute to plaque formation and instability. Additionally, RNA modifications in these cells may regulate the expression of inflammatory cytokines and adhesion molecules, further enhancing the inflammatory microenvironment in atherosclerotic plaques. This mechanism opens the door for targeting the underlying RNA modifications in hematopoietic cells, such as those occurring in TET2-mutant clones, and be a novel therapeutic strategy to mitigate inflammation and plaque progression in atherosclerosis.

Among the emerging strategies targeting m6A, recent studies have focused on METTL3 inhibitors, such as STM2457, which have shown potential in preclinical models for mitigating vascular inflammation [116]. By inhibiting METTL3, these compounds can modulate the expression of key inflammatory mediators, providing a novel approach to counteract the inflammatory processes driving atherosclerosis. The development of specific and potent inhibitors or activators targeting m6A regulators and non-coding RNAs (ncRNAs) is essential. These tools could play a key role in validating the functional importance of the identified targets and may eventually be translated into therapeutic agents.

3.1.3. m^5C, Pseudouridine and m1A in atherosclerosis

Other types of RNA modification also found their link with the development and progression of atherosclerotic plaques. Different studies highlighted the potential link between m^5C -related molecules and atherosclerosis. Vascular inflammation is a critical step in the development of atherosclerosis. The Intercellular Adhesion Molecule 1, ICAM-1, a cell surface protein expressed primarily on endothelial cells, which are the inner lining of blood vessels. During inflammatory conditions, such as atherosclerosis, the expression of ICAM-1 is upregulated in response to pro-inflammatory signals like cytokines and oxidative stress. In 2016, it has been shown that the NOP2/Sun domain family, member 2 (NSUN2) deposes m^5C methylation on ICAM-1 mRNA, thus upregulating expression of ICAM1 and increasing leukocyte adhesion to the endothelium [117]. Although the role of other RNA modifications in the pathophysiology of atherosclerosis remains largely unexplored, their study could represent a valuable avenue for advancing the understanding of disease mechanisms and identifying novel therapeutic targets.

Other forms of RNA methylation such as m1A also participate in vascular remodeling processes. In aortic aneurysm data sets, proteins of the m1A writing machinery were found to be dysregulated when compared to healthy aortic tissue [118]. Similarly in an acute stroke mouse model, m1A levels in brain tissue were decreased over time following an ischemic event, most likely due to increased levels of the m1A eraser ALKBH3. The 5′ m7G methyl cap and 2'Ome marks were also reduced [119]. Global RNA m^5C marks as well as m^5C -related methyltransferases, such as NSUN2, NSUN5 and the export adapter Aly/REF, were also increased in aortic aneurysm tissue when compared to healthy aorta [120] suggesting a potential role for m^5C in the formation of aneurysms.

3.2. Roles of A-to-I RNA modifications in atherosclerosis

Over the past decade, the understanding of A-to-I RNA editing's role in health and disease has expanded significantly. However, its contribution to cardiovascular diseases, particularly atherosclerosis, remains a relatively nascent area of research. Atherosclerosis, a chronic inflammatory condition characterized by the accumulation of lipid-laden plaques within arterial walls, underpins many cardiovascular events, including myocardial infraction and acute ischemic stroke. Emerging evidence suggests that A-to-I RNA editing intersects with key atherogenic processes, such as lipid metabolism, inflammatory signaling and endothelial function, potentially influencing plaque development and stability. By elucidating these connections, we seek to highlight the therapeutic potential of targeting RNA editing pathways in the prevention and treatment of vascular diseases. As this field evolves, it offers exciting prospects for developing precision medicine approaches tailored to the epitranscriptomic landscape of atherosclerosis.

3.2.1. Impact on inflammatory signaling and lipid metabolism in atherosclerotic plaques

Inflammation has increasingly been recognized as a pivotal factor in the development of atherosclerosis and its complications [121,122]. Despite extensive research employing diverse methodologies [121,122] [120,121] [120,121] [120,121] [12,13] [119,120] the primary drivers of inflammatory signaling in these conditions remain unclear. RNA editing has emerged as a contributor to the inflammatory cascade, with ADAR1 p150 expression being notably regulated by interferon signaling [56,57,123–125].

Evidence from Mendelian interferonopathies highlights the broader implications of ADAR1 in vascular health. Rare genetic variants causing loss of function in ADAR1 or gain of function in MDA5 are linked to conditions such as Aicardi-Goutières Syndrome and Singleton-Merton Syndrome, both characterized by severe vascular calcification and inflammation [53,126]. These findings underscore the critical role of RNA editing and dsRNA sensing in vascular disease and highlight potential parallels in atherosclerosis.

Deregulated ADAR1 activity, exacerbates chronic inflammation in vascular tissues by promoting aberrant RNA editing events that upregulate key pro-inflammatory mediators. Notably, increased editing at the conserved Insulin-like growth factor binding protein 7 (IGFBP7) gene site and *Alu* elements correlates with heightened inflammation and disease progression. RNA editing promotes vascular inflammation through pathways such as IFN, IL-1, and IL-6 signaling, contributing to monocyte recruitment and plaque formation [127].

Another significant example that uncovers a link between RNA editing and inflammatory signaling in atherosclerotic plaques is the editing of NEAT1, a long non-coding RNA (lncRNA) involved in endothelial inflammation. NEAT1 expression, elevated in CAD and atherosclerotic plaques, is induced by TNF-α, promoting vascular inflammation through key pro-inflammatory markers. ADAR1-mediated A-to-I RNA editing enhances NEAT1 stability by modulating AUF1 binding to inosine-rich regions. Silencing ADAR1 or AUF1 reduces NEAT1 levels and attenuates TNF-α-induced inflammation, suggesting a regulatory axis where ADAR1-driven RNA editing influences NEAT1-mediated inflammatory signaling in atherosclerosis, independent of traditional cardiovascular risk factors [128].

A link between RNA editing and inflammatory signaling in atherosclerosis is highlighted through ADAR1-mediated inosine modifications and ENDOV function. Elevated ADAR1 and inosine levels in plaques enhance inflammatory responses, as ENDOV cleaves inosine-containing RNA, promoting CCL2-mediated monocyte recruitment. EndoV deficiency in mice reduces plaque size, lipid content, and inflammation, linked to attenuated CCL2 signaling and increased CD47 expression, a key inflammation regulator [129].

In addition to inflammatory signaling, RNA editing can have a potential connection to lipid metabolism in atherosclerotic plaques. Editing quantitative trait loci (edQTLs) have been identified as genetic determinants of plasma lipid profiles. In disease-relevant tissues, risk variants colocalize with RNA editing QTLs (edQTLs), reducing dsRNA editing and contributing to inflammation-driven diseases like dyslipidemia and atherosclerosis. These dsRNAs, including Alu elements and cis-natural antisense transcripts (cis-NATs), act as potent immunogenic substrates for MDA5 [123]. Additionally, editing of transcripts encoding key lipid transporters has been associated with altered cholesterol efflux and foam cell formation—processes integral to atherogenesis [130].

These findings underscore RNA editing as a central mechanism linking immune dysregulation to atherosclerotic disease, providing a novel target for therapeutic intervention.

- Influence on Endothelial and Smooth Muscle Cell Function

Endothelial cells (ECs) and smooth muscle cells (SMCs) play pivotal roles in the pathogenesis of atherosclerosis, contributing to both the progression and stabilization of plaques [131–133].

Smooth muscle cells (SMCs) can adapt to their environment by shifting from a contractile state to a proliferative one when exposed to signals from vascular endothelial or inflammatory cells. This transformation, while contributing to plaque growth in atherosclerosis, also plays a stabilizing role in advanced plaques by reinforcing the fibrous cap and reducing rupture risk [134,135]. Evidence suggests that ADAR1-mediated RNA editing may influence this phenotypic switch. During platelet-derived growth factor (PDGF)-BB-induced transition, intronic RNA editing of SMC markers such as SMC myosin heavy chain (MYH11) and smooth muscle α-actin (ACTA2) leads to their pre-mRNA accumulation, accompanied by decreased mature mRNA and protein levels. This reduction, potentially caused by alternative splicing, aligns with hallmark features of the phenotypic switch [136]. Although these findings suggest a regulatory role for RNA editing in SMC biology, further in vitro and in vivo studies are needed to elucidate the underlying mechanisms, including the involvement of alternative splicing variants.

Furthermore, RNA editing on the pre-mRNA of Filamin A by ADAR2 has proven to be a crucial mechanism for maintaining smooth muscle cell function. This process introduces a Q-to-R codon change at the end of exon 42, which is necessary for regulating key contraction pathways, including phospholipase C (PLC) and Rho-associated protein kinase (ROCK) signaling. Lack of editing in Filamin A pre-mRNA produces a Filamin A isoform that only encodes a glutamine (Q) residue. This alteration disrupts the localization of p190RhoGAP, impairs PLC and ROCK activity, increases MLC phosphorylation, and leads to aortic hypercontractility. These effects contribute to thickening of the smooth muscle layer and increased perivascular collagen deposition, ultimately resulting in persistently high diastolic blood pressure and left ventricular hypertrophy in mice [137]. This A-to-I editing event also affects endothelial cell function and the endothelial response to VEGFA. Filamin A editing in endothelial cells leads to reduced endothelial cell migration and reduced angiogenesis, both in tumor models, as well as in a mouse model for peripheral artery disease [138]. Intraplaque angiogenesis can lead to plaque destabilization, thereby aggravating atherosclerotic disease [139], but angiogenesis is also a crucial process in blood flow recovery after acute cardiovascular disease.

- Potential Contributions of A-to-I Editing to RNA Stability and Translation Efficiency in Atherosclerotic Lesions

Adenosine-to-inosine (A-to-I) RNA editing can also impact RNA stability and translation efficiency, two crucial factors in regulating gene expression within atherosclerotic plaques. Stellos et al. [58] uncovered a novel mechanism involving ADAR1-mediated clustered RNA editing within the 3' UTR of the cathepsin S (CTSS) mRNA, a gene with known pro-inflammatory role in vascular function and atherosclerosis [140–142]. The edited regions were located within inverted Alu repeats in the CTSS 3' UTR, forming double-stranded RNAs essential for A-to-I editing [143,144]. These editing-induced structural changes, create loops in the RNA structure that facilitate the binding of Human Antigen R (HuR), a single-stranded RNA-binding protein [58]. HuR binding stabilizes the CTSS mRNA, enhancing its expression at both the mRNA and protein levels, with functional consequences for vascular endothelial cells [145,146]. Interestingly, hypoxia and inflammation were identified as key regulators of ADAR1-mediated editing of CTSS, encompassing various stages of cardiovascular disease. In more detail, editing of CTSS is increased under hypoxic or pro-inflammatory conditions in vitro, and in individuals with coronary or carotid atherosclerotic disease [58]. Another example of the role of A-to-I RNA editing in enhancing RNA stability is highlighted by NEAT1, a long non-coding RNA involved in endothelial inflammation. As described in detail earlier, ADAR1-mediated RNA editing stabilizes NEAT1 by increasing AUF1 binding to inosine-rich regions, a mechanism crucial in promoting pro-inflammatory signaling in atherosclerotic plaques [128].

- The Role of RNA Modifications and Editing in MicroRNA Biology and Their Implications for Atherosclerosis

A-to-I RNA editing also intersects with microRNA (miRNA) biology, which plays a central role in post-transcriptional gene regulation. miRNAs regulate the expression of target genes by binding to complementary sequences in mRNA molecules, leading to their degradation or translational repression. RNA editing can influence miRNA biology through both direct and indirect mechanisms. Directly, A-to-I editing of miRNA precursor molecules can affect their maturation, stability, and target specificity, altering the repertoire of genes they regulate. Indirectly, RNA editing can modify the binding sites for miRNAs within the 3' UTRs of mRNA targets, affecting miRNA accessibility and the regulation of those transcripts [147].

RhoA and cell division control protein 42 homolog (Cdc42) are key members of the Rho GTPase family, which are widely expressed and play critical roles in cellular processes. The activity of RhoA is regulated by Rho GTPase-activating protein 26 (ARHGAP26), which converts the active GTP-bound form of RhoA to its inactive GDP-bound state, influencing various Rho-related pathways, including those implicated in cardiovascular diseases [148,149]. Interestingly, the 3' UTR of ARHGAP26 transcript undergoes significant A-to-I RNA editing by ADAR1, which disrupts the binding sites for miR-30b-3p and miR-573. This mechanism enhances the ability of ARHGAP26 to negatively regulate RhoA and Cdc42, a critical cell cycle regulator. In the absence of ADAR1-mediated A-to-I RNA editing, this regulatory mechanism is compromised, leading to increased RhoA-GTP activity and a loss of proper RhoA control [150].

Another example of the interplay between A-to-I editing and miRNA regulation can be found in the arylhydrocarbon receptor (AhR), known to contribute to atherosclerosis development [151]. More specifically, ADAR1-mediated editing in the 3' UTR of *AhR* mRNA creates a recognition motif for miR-378. The enhanced interaction between miR-378 and the edited *AhR* mRNA leads to the repression of both *AhR* mRNA and protein expression [152].

A direct interaction between RNA editing and miRNA is evident in the role of ADAR2-mediated editing in cardiac health, as evident by Altaf et al. [153]. ADAR2 editing affects both coding and non-coding RNAs, including miRNAs, which play a pivotal role in heart development and function. In ADAR2-deficient mouse hearts, small RNA sequencing revealed a significant reduction in miR-29b levels. miR-29b is known to inhibit cardiac fibrosis through the TGF-β/Smad3 pathway, suggesting that the loss of ADAR2 editing disrupts miR-29b regulatory functions, potentially promoting fibrosis [153]. In addition to miR-29b, other miRNAs, including the let-7 family exhibited significant downregulation in ADAR2-deficient mouse hearts [153]. Dysregulated expression of let-7 miRNAs has been associated with various cardiovascular diseases, including fibrosis, hypertrophy, myocardial infarction, and atherosclerosis [154–156].

A different study identified increased RNA editing in the primary transcript of miR-487b-3p in mouse gastrocnemius muscle lysates, as well as in primary human arterial fibroblasts, following ischemia [157]. The critical editing event was found to occur within the seed region of the mature miRNA, which plays a crucial role in determining mRNA target specificity [157,158]. As a result, the alteration in the seed region changes the miRNA binding preferences, leading to the de-repression of several proangiogenic genes that are no longer complementary to the edited seed sequence of miR-487b-3p. Using both in vitro and in vivo assays, this study revealed that angiogenesis and arterial sprouting were driven specifically by the edited variant of miR-487b-3 [158]. A few years later, the same authors demonstrated that A-to-I RNA editing occurs in 35 miRNAs associated with vascular activity [159]. Notably, under ischemic conditions, levels of specific primary miRNAs from vascular fibroblasts and endothelial cells, along with their editing rates, showed a significant increase. In some mature miRNAs, editing was detected within the seed region, altering their target specificity and enhancing angiogenesis in both in vitro and ex vivo settings. Editing of vasoactive microRNAs depended on both ADAR1 and/or ADAR2 in a microRNA-specific manner. Furthermore, silencing ADAR1 or ADAR2 resulted in a substantial reduction of expression of most vasoactive miRNAs. These findings highlight ADAR's pivotal role in vascular responses and its potential as a therapeutic target in atherosclerosis.

In conclusion, miRNAs, which have been widely proposed as potential biomarkers and effectors of cardiovascular diseases (CVD) [160,161], are increasingly recognized for their susceptibility to RNA editing events in the context of atherosclerosis and cardiovascular ischemia. These findings not only expand our understanding of RNA editing as a crucial layer of gene regulation but also highlight the potential of edited miRNAs as novel biomarkers or therapeutic targets for atherosclerosis and related cardiovascular diseases. Further exploration of these interactions could pave the way for advanced diagnostic and treatment strategies in CVD management.

- <u>Potential Contributions of A-to-I Editing in circular RNAs Biology and Their Implications for Atherosclerosis</u>

Emerging research highlights the significant roles of circRNAs in the development and progression of cardiovascular diseases [162]. Notable examples include circANRIL, which exerts atheroprotective effects in endothelial cells, circDLGAP4, which is associated with beneficial outcomes in ischemic stroke, and circTLK1, which is linked to adverse effects in the same condition [163–165]. Increased RNA editing in intronic regions has been shown to reduce circRNA levels [166], potentially contributing to structural heart diseases such as heart failure and dilated cardiomyopathy, as well as to atherosclerosis [162,167,168].

A recent study highlights the critical role of A-to-I RNA editing in regulating the formation of circular RNAs (circRNAs) in the human heart, particularly under conditions of heart failure. A key finding is the marked reduction in RNA editing, particularly within intronic Alu elements of protein-coding genes, in failing hearts. This decrease in editing was attributed to reduced levels of ADAR2 and was associated with an increase in circRNA levels, with circAKAP13 being one of the most significantly upregulated circRNAs. Functional experiments further revealed that overexpression of circAKAP13 in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) impaired sarcomere regularity, indicating its potential pathological role in heart failure. Restoration of ADAR2 reversed these effects, decreasing circRNA levels and restoring RNA editing activity [33].

4. Challenges and future perspectives

Epitranscriptomic research is rapidly advancing, revealing tremendous potential for unraveling pathophysiological processes and developing novel therapeutic interventions. However, to fully harness its capabilities, significant challenges must be addressed at both technical and conceptual levels.

Recent advances in sequencing technology have enabled high-throughput discovery of epitranscriptomic signatures across the transcriptome. RNA modifications like N6-methyladenosine (m6A) were identified using immunoprecipitation-based approaches, such as meRIP-seq, which rely on antibodies to enrich modified RNA fragments for sequencing. While these methods facilitated the initial discovery of m6A modifications within DRACH motifs [169], they have limitations in resolution and specificity. The advent of Oxford Nanopore Technologies (ONT) and its direct RNA sequencing approach represents a significant leap forward [170,171]. This technology provides direct information on RNA modifications through changes in electrical current during sequencing, complemented by bioinformatics pipelines. Additionally, the integration of adaptive sampling in ONT sequencing allows for the real-time enrichment of low-abundance RNAs, such as non-coding RNAs. This feature enhances the detection of rare transcripts by selectively focusing sequencing efforts on these target sequences [172]. This capability is especially useful for profiling plaque heterogeneity, where low-abundance RNA species can provide critical insights into the molecular dynamics of atherosclerosis. While currently limited to identifying m6A and 5-methylcytosine (5mC), rapid advancements in computational methods are expected to expand its capabilities to include other modifications like inosine (A-to-I editing) and pseudouridine. Despite these breakthroughs, several technical limitations persist. Direct RNA sequencing requires substantial amounts of starting material, which restricts its use for low-input samples. Additionally, the current protocols are dependent on polyadenylated RNAs, thereby excluding non-poly(A)-tailed RNAs, such as certain long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), from analysis. Furthermore, existing ONT protocols are not yet capable of studying small RNA species, such as miRNAs, leaving their modification landscapes largely unexplored. Additionally, the validation of specific RNA modification sites, remains challenging due to technical constraints. Techniques like reverse transcription-based qPCR, which leverage the reduced retrotranscription efficiency of enzymes like BstI at methylated sites, offer a promising and cost-effective alternative for residue-specific m6A quantification [173]. BstI polymerase struggles to retrotranscribe RNA at m6A-modified residues due to the conformational hindrance caused by the methyl group, resulting in diminished efficiency at these sites. However, the sensitivity of the method to detect subtle differences in methylation levels and their applicability to diverse RNA contexts require further optimization.

To address these challenges, single-cell RNA sequencing (scRNA-seq) offers a powerful approach for resolving cellular heterogeneity. Unlike bulk sequencing, which averages gene expression across populations, scRNA-seq enables the analysis of individual cells, allowing for the identification of rare or distinct subpopulations. This is particularly important in complex tissues like atherosclerotic plaques, where different VSMC subpopulations and macrophage subsets exhibit unique roles in disease progression [174]. By providing insights into cell-type-specific epitranscriptomic signatures, single-cell sequencing can uncover how RNA modifications contribute to cellular behavior and the overall pathology of atherosclerosis, potentially revealing new therapeutic targets.

Beyond technological challenges, understanding the biological relevance of specific RNA modifications remains limited. Most studies focus on global changes in m6A levels using knockout or overexpression of key enzymes, such as METTL3 or FTO. While these approaches provide valuable insights, they often overlook the nuanced effects of individual RNA modification sites. Investigating the functional impact of single modifications is crucial, particularly as observed changes in m6A levels in biological systems are often subtle (10–30 %). Determining whether such differences exert significant regulatory effects and how to study them remains an open question. One promising strategy involves introducing target RNAs with and without specific modifications into cells to measure phenotypic outcomes. For instance, modified and unmodified miRNAs have been transfected to assess their functional differences, yielding intriguing insights [175]. Another approach leverages CRISPR technology, which can be coupled with RNA modification enzymes. Using guide RNAs to direct catalytically inactive CRISPR-associated proteins, such as dCas13b, to specific RNA sites enables precise targeting of RNA modification enzymes [176]. Writers or erasers, can then be recruited to edit specific RNA transcripts at defined locations. This approach allows researchers to study the effects of individual RNA modifications on cellular processes without affecting global RNA modification levels.

Epitranscriptomics is beginning to transition from bench research to clinical applications, offering exciting therapeutic possibilities. For instance, RNA editing has emerged as a promising strategy for treating genetic disorders. One notable example is the application of adenosine-to-inosine (A-to-I) editing to correct mutations responsible for diseases like Alpha-1 Antitrypsin Deficiency (AATD) [177]. This genetic disorder, caused by the E342K mutation in the *SERPINA1* gene, leads to misfolded alpha-1 antitrypsin (AAT) protein and associated lung and liver pathology. Targeted oligonucleotide platforms, such as Wave Life Sciences' WVE-006 and Korro Bio's KRRO-110™ [178], recruit endogenous ADAR enzymes to specifically edit the mutated RNA, restoring functional AAT protein production and addressing the root cause of the disease. These pioneering efforts highlight the therapeutic potential of RNA editing and epitranscriptomic modulation. Extending these technologies to treat atherosclerosis-related diseases could open new frontiers in cardiovascular medicine by targeting RNA modifications that influence endothelial dysfunction, inflammation, and smooth muscle cell behavior.

To fully realize the potential of epitranscriptomics in transforming therapeutic strategies for atherosclerosis and other diseases, several key advancements are needed. First, the development of more sensitive and versatile technologies is crucial. This includes improving methods for low-input and non-poly(A)-dependent RNA sequencing, as well as establishing protocols capable of analyzing small RNAs and other challenging RNA classes. Second, there is a need to deepen our understanding of site-specific modification effects. This entails shifting the focus from global RNA modifications to single-site analyses, supported by functional assays and innovative tools like CRISPR-based RNA editing. Additionally, integrating epitranscriptomics with other omics approaches—such as genomics, transcriptomics, proteomics, and metabolomics—can provide a comprehensive view of regulatory networks in both health and disease. Finally, translating these insights into therapeutic applications, including RNA-targeting drugs for atherosclerosis and other complex conditions, represents a promising frontier for the field.

5. Conclusion

The role of epitranscriptomic marks in physiology and disease development, including atherosclerosis, has only recently been recognized. Changes in RNA methylation status, RNA editing or alternative splicing associated with disease development, remain to be fully characterized. The discovery of new molecular tools to assess or reproduce RNA modifications in experimental conditions has allowed a greater knowledge of the functional role of these modifications in the maintenance and dysregulation of vascular homeostasis. With the excitement of the research community around the RNA World consecutive to mRNA-based vaccines against COVID-19, strengthened by three recent Nobel Prizes dedicated to non-coding RNAs, RNA modifications and RNA editing, the RNA field is in a booming phase and may become the next generation of molecular targets for "theranostic" approaches [179]. Combined with other omics data in a multiomics manner, epitranscriptomics may help advance personalized healthcare to reduce the burden of atherosclerotic cardiovascular disease.

CRediT authorship contribution statement

Victoria Stopa: Writing – original draft, Visualization. **Dimitra Dafou:** Writing – review & editing, Writing – original draft. **Korina Karagianni:** Writing – original draft. **A. Yaël Nossent:** Writing – review & editing, Writing – original draft. **Rosienne Farrugia:** Writing – review & editing, Writing – original draft. **Yvan Devaux:** Writing – review & editing, Writing – original draft, Conceptualization. **Miron Sopic:** Writing – review & editing, Writing – original draft, Conceptualization.

Funding

V.S. is funded by the Heart Foundation Daniel Wagner of Luxembourg (YMCA project). K.K. is funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the 4th Call for H.F.R.I. PhD Fellowships (Fellowship no. 10829). A.Y.N. is funded by the Novo Nordisk Foundation in Denmark (NNF Young Investigator Award 2023 NNF23OC0079200). R.F is funded by HORIZON-WIDERA-2022-TALENTS-01: BioGeMT (Project 101086768) and HORIZON EIC 2022 PATHFINDER CHALLENGE CARDIOGENOMICS: TargetMI (Project 101114924).Y.D. is funded by the National Research Fund (grant INTER/NCN/23/17880314/RVH), the Innovative Medicines Initiative (H2020, IMI; grant number: 821508), the Ministry of Higher Education and Research, and the Heart Foundation-Daniel Wagner of Luxembourg. M.S. is funded by the Horizon Europe (HORIZON-MSCA-2021-PF-MAACS 101064175), the Ministry of Science, Technological Development and Innovation, Republic of Serbia through a Grant Agreement with the University of Belgrade-Faculty of Pharmacy No: 451-03-47/2024-01/200161.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yvan Devaux reports financial support was provided by European Cooperation in Science and Technology. Victoria Stopa reports financial support was provided by Heart Foundation Daniel Wagner of Luxembourg. Yvan Devaux reports financial support was provided by National Research Fund. Yvan Devaux reports financial support was provided by Innovative Health Initiative. Yvan Devaux reports financial support was provided by Ministry of Higher Education and Research. Miron Sopic reports financial support was provided by Horizon Europe. Miron Sopic reports financial support was provided by Ministry of Science, Technological Development and Innovation. Ann-Yael Nossent reports financial support was provided by Novo Nordisk Foundation. Yvan Devaux reports a relationship with Firalis that includes: board membership. Yvan Devaux has patent licensed to Firalis. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This article is based upon work from COST Action AtheroNET (CA21153) funded by COST (European Cooperation in Science and Technology).

Data availability

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

References

[1] C.W. Tsao, A.W. Aday, Z.I. Almarzooq, C.A.M. Anderson, P. Arora, C.L. Avery, et al., Heart disease and stroke Statistics-2023 update: a report from the American Heart Association, Circulation 147 (8) (2023) e93–e621.
[2] J.L. Katzmann, C.J. Packard, M.J. Chapman, I. Katzmann, U. Laufs, Targeting RNA with antisense oligonucleotides and small interfering RNA: JACC state-of-the-art review, J. Am. Coll. Cardiol. 76 (5) (2020) 563–579.
[3] A. Di Nubila, G. Dilella, R. Simone, S.S. Barbieri, Vascular extracellular matrix in atherosclerosis, Int. J. Mol. Sci. 25 (22) (2024).
[4] P.M. Ridker, B.M. Everett, T. Thuren, J.G. MacFadyen, W.H. Chang, C. Ballantyne, et al., Antiinflammatory therapy with Canakinumab for atherosclerotic disease, N. Engl. J. Med. 377 (12) (2017) 1119–1131.
[5] A.Y. Nossent, The epitranscriptome: RNA modifications in vascular remodelling, Atherosclerosis 374 (2023) 24–33.
[6] D. Benak, F. Kolar, L. Zhang, Y. Devaux, M. Hlavackova, RNA modification m(6) am: the role in cardiac biology, Epigenetics 18 (1) (2023) 2218771.

[7] Z.M. Zhu, F.C. Huo, J. Zhang, H.J. Shan, D.S. Pei, Crosstalk between m6A modification and alternative splicing during cancer progression, Clin. Transl. Med. 13 (10) (2023) e1460.
[8] M. Ilieva, S. Uchida, Epitranscriptomics in fibroblasts and fibrosis, Am. J. Phys. Cell Phys. 322 (6) (2022) C1110–C1116.
[9] E. van Ingen, D.A.L. van den Homberg, M.L. van der Bent, H. Mei, N. Papac-Milicevic, V. Kremer, et al., C/D box snoRNA SNORD113-6/AF357425 plays a dual role in integrin signalling and arterial fibroblast function via pre-mRNA processing and 2'O-ribose methylation, Hum. Mol. Genet. 31 (7) (2022) 1051–1066.
[10] S. Delaunay, M. Helm, M. Frye, RNA modifications in physiology and disease: towards clinical applications, Nat. Rev. Genet. 25 (2) (2024) 104–122.
[11] C. Wang, X. Hou, Q. Guan, H. Zhou, L. Zhou, L. Liu, et al., RNA modification in cardiovascular disease: implications for therapeutic interventions, Signal Transduct. Target. Ther. 8 (1) (2023) 412.
[12] I. Barbieri, K. Tzelepis, L. Pandolfini, J. Shi, G. Millan-Zambrano, S.C. Robson, et al., Promoter-bound METTL3 maintains myeloid leukaemia by m(6)A-dependent translation control, Nature 552 (7683) (2017) 126–131.
[13] X. Yang, Y. Yang, B.F. Sun, Y.S. Chen, J.W. Xu, W.Y. Lai, et al., 5-methylcytosine promotes mRNA export - NSUN2 as the methyltransferase and ALYREF as an m(5) C reader, Cell Res. 27 (5) (2017) 606–625.
[14] Q. Zhang, F. Liu, W. Chen, H. Miao, H. Liang, Z. Liao, et al., The role of RNA m(5) C modification in cancer metastasis, Int. J. Biol. Sci. 17 (13) (2021) 3369–3380.
[15] S.J. Hermon, A. Sennikova, S. Becker, Quantitative detection of pseudouridine in RNA by mass spectrometry, Sci. Rep. 14 (1) (2024) 27564.
[16] M. Khanna, H. Wu, C. Johansson, M. Caizergues-Ferrer, J. Feigon, Structural study of the H/ACA snoRNP components Nop10p and the 3′ hairpin of U65 snoRNA, RNA 12 (1) (2006) 40–52.
[17] D.G. Dimitrova, L. Teysset, C. Carre, RNA 2'-O-methylation (nm) modification in human diseases, Genes (Basel) 10 (2) (2019).
[18] K.I. Zhou, C.V. Pecot, C.L. Holley, 2'-O-methylation (nm) in RNA: progress, challenges, and future directions, RNA 30 (5) (2024) 570–582.
[19] Y. Wang, J. Wang, X. Li, X. Xiong, J. Wang, Z. Zhou, et al., N(1)-methyladenosine methylation in tRNA drives liver tumourigenesis by regulating cholesterol metabolism, Nat. Commun. 12 (1) (2021) 6314.
[20] M. Safra, A. Sas-Chen, R. Nir, R. Winkler, A. Nachshon, D. Bar-Yaacov, et al., The m1A landscape on cytosolic and mitochondrial mRNA at single-base resolution, Nature 551 (7679) (2017) 251–255.
[21] L. Cui, R. Ma, J. Cai, C. Guo, Z. Chen, L. Yao, et al., RNA modifications: importance in immune cell biology and related diseases, Signal Transduct. Target. Ther. 7 (1) (2022) 334.
[22] T. Kiss, Small nucleolar RNA-guided post-transcriptional modification of cellular RNAs, EMBO J. 20 (14) (2001) 3617–3622.
[23] S.J. Hafner, M.D. Jansson, K. Altinel, K.L. Andersen, Z. Abay-Norgaard, P. Menard, et al., Ribosomal RNA 2'-O-methylation dynamics impact cell fate decisions, Dev. Cell 58 (17) (2023) 1593–609 e9.
[24] K.E.J. Hakansson, E.A.C. Goossens, S. Trompet, E. van Ingen, M.R. de Vries, R. van der Kwast, et al., Genetic associations and regulation of expression indicate an independent role for 14q32 snoRNAs in human cardiovascular disease, Cardiovasc. Res. 115 (10) (2019) 1519–1532.
[25] K.A. Brandis, S. Gale, S. Jinn, S.J. Langmade, N. Dudley-Rucker, H. Jiang, et al., Box C/D small nucleolar RNA (snoRNA) U60 regulates intracellular cholesterol trafficking, J. Biol. Chem. 288 (50) (2013) 35703–35713.
[26] C.I. Michel, C.L. Holley, B.S. Scruggs, R. Sidhu, R.T. Brookheart, L.L. Listenberger, et al., Small nucleolar RNAs U32a, U33, and U35a are critical mediators of metabolic stress, Cell Metab. 14 (1) (2011) 33–44.
[27] S. Farajollahi, S. Maas, Molecular diversity through RNA editing: a balancing act, Trends Genet. 26 (5) (2010) 221–230.
[28] R.W. Wagner, J.E. Smith, B.S. Cooperman, K. Nishikura, A double-stranded RNA unwinding activity introduces structural alterations by means of adenosine to inosine conversions in mammalian cells and Xenopus eggs, Proc. Natl. Acad. Sci. USA 86 (8) (1989) 2647–2651.
[29] L.M. Powell, S.C. Wallis, R.J. Pease, Y.H. Edwards, T.J. Knott, J. Scott, A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine, Cell 50 (6) (1987) 831–840.
[30] S.H. Chen, G. Habib, C.Y. Yang, Z.W. Gu, B.R. Lee, S.A. Weng, et al., Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon, Science 238 (4825) (1987) 363–366.
[31] K. Licht, M.F. Jantsch, Rapid and dynamic transcriptome regulation by RNA editing and RNA modifications, J. Cell Biol. 213 (1) (2016) 15–22.
[32] O. Solomon, A. Di Segni, K. Cesarkas, H.T. Porath, V. Marcu-Malina, O. Mizrahi, et al., RNA editing by ADAR1 leads to context-dependent transcriptome-wide changes in RNA secondary structure, Nat. Commun. 8 (1) (2017) 1440.
[33] K.E. Kokot, J.M. Kneuer, D. John, S. Rebs, M.N. Mobius-Winkler, S. Erbe, et al., Reduction of A-to-I RNA editing in the failing human heart regulates formation of circular RNAs, Basic Res. Cardiol. 117 (1) (2022) 32.
[34] K. Nishikura, A-to-I editing of coding and non-coding RNAs by ADARs, Nat. Rev. Mol. Cell Biol. 17 (2) (2016) 83–96.
[35] H. Chung, J.J.A. Calis, X. Wu, T. Sun, Y. Yu, S.L. Sarbanes, et al., Human ADAR1 prevents endogenous RNA from triggering translational shutdown, Cell 172 (4) (2018) 811–24 e14.
[36] J. Chen, H.F. Liu, L.B. Qiao, F.B. Wang, L. Wang, Y. Lin, et al., Global RNA editing identification and characterization during human pluripotent-to-cardiomyocyte differentiation, Mol. Ther. Nucleic Acids. 26 (2021) 879–891.
[37] K. Karagianni, S. Pettas, G. Christoforidou, E. Kanata, N. Bekas, K. Xanthopoulos, et al., A systematic review of common and brain-disease-specific RNA editing alterations providing novel insights into neurological and neurodegenerative disease manifestations, Biomolecules 12 (3) (2022).
[38] C.X. George, C.E. Samuel, Human RNA-specific adenosine deaminase ADAR1 transcripts possess alternative exon 1 structures that initiate from different promoters, one constitutively active and the other interferon inducible, Proc. Natl. Acad. Sci. USA 96 (8) (1999) 4621–4626.
[39] X. Fang, L. Zhang, ZBP1 contributes to interferonopathies caused by impaired ADAR1 function, Mol Biomed. 3 (1) (2022) 39.
[40] R. de Reuver, S. Verdonck, E. Dierick, J. Nemegeer, E. Hessmann, S. Ahmad, et al., ADAR1 prevents autoinflammation by suppressing spontaneous ZBP1 activation, Nature 607 (7920) (2022) 784–789.
[41] C.V. Riella, M. McNulty, G.T. Ribas, C.F. Tattersfield, C. Perez-Gill, F. Eichinger, et al., ADAR regulates APOL1 via A-to-I RNA editing by inhibition of MDA5 activation in a paradoxical biological circuit, Proc. Natl. Acad. Sci. USA 119 (44) (2022) e2210150119.
[42] C.R. Walkley, J.B. Li, Rewriting the transcriptome: adenosine-to-inosine RNA editing by ADARs, Genome Biol. 18 (1) (2017) 205.
[43] K. Karagianni, D. Dafou, K. Xanthopoulos, T. Sklaviadis, E. Kanata, RNA editing regulates glutamatergic synapses in the frontal cortex of a molecular subtype of amyotrophic lateral sclerosis, Mol. Med. 30 (1) (2024) 101.
[44] C.X. Chen, D.S. Cho, Q. Wang, F. Lai, K.C. Carter, K. Nishikura, A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains, RNA 6 (5) (2000) 755–767.
[45] R. Raghava Kurup, E.K. Oakes, A.C. Manning, P. Mukherjee, P. Vadlamani, H. A. Hundley, RNA binding by ADAR3 inhibits adenosine-to-inosine editing and promotes expression of immune response protein MAVS, J. Biol. Chem. 298 (9) (2022) 102267.
[46] Z. Chen, T.L. Eggerman, A.P. Patterson, ApoB mRNA editing is mediated by a coordinated modulation of multiple apoB mRNA editing enzyme components, Am. J. Physiol. Gastrointest. Liver Physiol. 292 (1) (2007) G53–G65.
[47] M.P. Sowden, N. Ballatori, K.L. Jensen, L.H. Reed, H.C. Smith, The editosome for cytidine to uridine mRNA editing has a native complexity of 27S: identification of intracellular domains containing active and inactive editing factors, J. Cell Sci. 115 (Pt 5) (2002) 1027–1039.
[48] N.O. Davidson, G.S. Shelness, APOLIPOPROTEIN B: mRNA editing, lipoprotein assembly, and presecretory degradation, Annu. Rev. Nutr. 20 (2000) 169–193.
[49] N. Fossat, K. Tourle, T. Radziewic, K. Barratt, D. Liebhold, J.B. Studdert, et al., C to U RNA editing mediated by APOBEC1 requires RNA-binding protein RBM47, EMBO Rep. 15 (8) (2014) 903–910.
[50] J. Yuan, L. Xu, H.J. Bao, J.L. Wang, Y. Zhao, S. Chen, Biological roles of A-to-I editing: implications in innate immunity, cell death, and cancer immunotherapy, J. Exp. Clin. Cancer Res. 42 (1) (2023) 149.
[51] J.H. Yang, X. Luo, Y. Nie, Y. Su, Q. Zhao, K. Kabir, et al., Widespread inosine-containing mRNA in lymphocytes regulated by ADAR1 in response to inflammation, Immunology 109 (1) (2003) 15–23.
[52] P. Vitali, A.D. Scadden, Double-stranded RNAs containing multiple IU pairs are sufficient to suppress interferon induction and apoptosis, Nat. Struct. Mol. Biol. 17 (9) (2010) 1043–1050.
[53] G.I. Rice, P.R. Kasher, G.M. Forte, N.M. Mannion, S.M. Greenwood, M. Szynkiewicz, et al., Mutations in ADAR1 cause Aicardi-Goutieres syndrome associated with a type I interferon signature, Nat. Genet. 44 (11) (2012) 1243–1248.
[54] G.I. Rice, Duany Y. Del Toro, E.M. Jenkinson, G.M. Forte, B.H. Anderson, G. Ariaudo, et al., Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling, Nat. Genet. 46 (5) (2014) 503–509.
[55] S. Yang, P. Deng, Z. Zhu, J. Zhu, G. Wang, L. Zhang, et al., Adenosine deaminase acting on RNA 1 limits RIG-I RNA detection and suppresses IFN production responding to viral and endogenous RNAs, J. Immunol. 193 (7) (2014) 3436–3445.
[56] N.M. Mannion, S.M. Greenwood, R. Young, S. Cox, J. Brindle, D. Read, et al., The RNA-editing enzyme ADAR1 controls innate immune responses to RNA, Cell Rep. 9 (4) (2014) 1482–1494.
[57] B.J. Liddicoat, R. Piskol, A.M. Chalk, G. Ramaswami, M. Higuchi, J.C. Hartner, et al., RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself, Science 349 (6252) (2015) 1115–1120.
[58] K. Stellos, A. Gatsiou, K. Stamatelopoulos, L. Perisic Matic, D. John, F.F. Lunella, et al., Adenosine-to-inosine RNA editing controls cathepsin S expression in atherosclerosis by enabling HuR-mediated post-transcriptional regulation, Nat. Med. 22 (10) (2016) 1140–1150.
[59] K. Karagianni, A. Bibi, A. Made, S. Acharya, M. Parkkonen, T. Barbalata, et al., Recommendations for detection, validation, and evaluation of RNA editing events in cardiovascular and neurological/neurodegenerative diseases, Mol. Ther. Nucleic Acids. 35 (1) (2024) 102085.
[60] O. Levi, Y.S. Arava, Pseudouridine-mediated translation control of mRNA by methionine aminoacyl tRNA synthetase, Nucleic Acids Res. 49 (1) (2021) 432–443.
[61] Y. Tian, D.K. Simanshu, J.B. Ma, D.J. Patel, Structural basis for piRNA 2'-O-methylated 3′-end recognition by Piwi PAZ (Piwi/Argonaute/Zwille) domains, Proc. Natl. Acad. Sci. USA 108 (3) (2011) 903–910.
[62] E.T. Wang, R. Sandberg, S. Luo, I. Khrebtukova, L. Zhang, C. Mayr, et al., Alternative isoform regulation in human tissue transcriptomes, Nature 456 (7221) (2008) 470–476.
[63] Y. Barash, J.A. Calarco, W. Gao, Q. Pan, X. Wang, O. Shai, et al., Deciphering the splicing code, Nature 465 (7294) (2010) 53–59.

[64] M.M. van den Hoogenhof, Y.M. Pinto, E.E. Creemers, RNA splicing: regulation and dysregulation in the heart, Circ. Res. 118 (3) (2016) 454–468.
[65] A. Beqqali, Alternative splicing in cardiomyopathy, Biophys. Rev. 10 (4) (2018) 1061–1071.
[66] S.J. Brown, P. Stoilov, Y. Xing, Chromatin and epigenetic regulation of pre-mRNA processing, Hum. Mol. Genet. 21 (R1) (2012) R90–R96.
[67] W.V. Gilbert, S. Nachtergaele, mRNA regulation by RNA modifications, Annu. Rev. Biochem. 92 (2023) 175–198.
[68] C. Hodges, L. Bintu, L. Lubkowska, M. Kashlev, C. Bustamante, Nucleosomal fluctuations govern the transcription dynamics of RNA polymerase II, Science 325 (5940) (2009) 626–628.
[69] R.J. Sims 3rd, S. Millhouse, C.F. Chen, B.A. Lewis, H. Erdjument-Bromage, P. Tempst, et al., Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing, Mol. Cell 28 (4) (2007) 665–676.
[70] M.B. Warf, J.A. Berglund, Role of RNA structure in regulating pre-mRNA splicing, Trends Biochem. Sci. 35 (3) (2010) 169–178.
[71] N. Liu, T. Pan, N6-methyladenosine-encoded epitranscriptomics, Nat. Struct. Mol. Biol. 23 (2) (2016) 98–102.
[72] N. Liu, Q. Dai, G. Zheng, C. He, M. Parisien, T. Pan, N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions, Nature 518 (7540) (2015) 560–564.
[73] C. Roost, S.R. Lynch, P.J. Batista, K. Qu, H.Y. Chang, E.T. Kool, Structure and thermodynamics of N6-methyladenosine in RNA: a spring-loaded base modification, J. Am. Chem. Soc. 137 (5) (2015) 2107–2115.
[74] N. Liu, K.I. Zhou, M. Parisien, Q. Dai, L. Diatchenko, T. Pan, N6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein, Nucleic Acids Res. 45 (10) (2017) 6051–6063.
[75] A. Louloupi, E. Ntini, T. Conrad, U.A.V. Orom, Transient N-6-Methyladenosine transcriptome sequencing reveals a regulatory role of m6A in splicing efficiency, Cell Rep. 23 (12) (2018) 3429–3437.
[76] S. Ke, A. Pandya-Jones, Y. Saito, J.J. Fak, C.B. Vagbo, S. Geula, et al., M(6)a mRNA modifications are deposited in nascent pre-mRNA and are not required for splicing but do specify cytoplasmic turnover, Genes Dev. 31 (10) (2017) 990–1006.
[77] E.M. Phizicky, A.K. Hopper, tRNA biology charges to the front, Genes Dev. 24 (17) (2010) 1832–1860.
[78] E. Yankova, W. Blackaby, M. Albertella, J. Rak, E. De Braekeleer, G. Tsagkogeorga, et al., Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia, Nature 593 (7860) (2021) 597–601.
[79] M.T. Kanemaki, Ligand-induced degrons for studying nuclear functions, Curr. Opin. Cell Biol. 74 (2022) 29–36.
[80] G. Wei, M. Almeida, G. Pintacuda, H. Coker, J.S. Bowness, J. Ule, et al., Acute depletion of METTL3 implicates N (6)-methyladenosine in alternative intron/exon inclusion in the nascent transcriptome, Genome Res. 31 (8) (2021) 1395–1408.
[81] D.R. Davis, Stabilization of RNA stacking by pseudouridine, Nucleic Acids Res. 23 (24) (1995) 5020–5026.
[82] E. Kierzek, M. Malgowska, J. Lisowiec, D.H. Turner, Z. Gdaniec, R. Kierzek, The contribution of pseudouridine to stabilities and structure of RNAs, Nucleic Acids Res. 42 (5) (2014) 3492–3501.
[83] C. Chen, X. Zhao, R. Kierzek, Y.T. Yu, A flexible RNA backbone within the polypyrimidine tract is required for U2AF65 binding and pre-mRNA splicing in vivo, Mol. Cell. Biol. 30 (17) (2010) 4108–4119.
[84] T. Amort, D. Rieder, A. Wille, D. Khokhlova-Cubberley, C. Riml, L. Trixl, et al., Distinct 5-methylcytosine profiles in poly(a) RNA from mouse embryonic stem cells and brain, Genome Biol. 18 (1) (2017) 1.
[85] L.P. Keegan, A. Gallo, M.A. O'Connell, The many roles of an RNA editor, Nat. Rev. Genet. 2 (11) (2001) 869–878.
[86] O. Solomon, S. Oren, M. Safran, N. Deshet-Unger, P. Akiva, J. Jacob-Hirsch, et al., Global regulation of alternative splicing by adenosine deaminase acting on RNA (ADAR), RNA 19 (5) (2013) 591–604.
[87] S.M. Rueter, T.R. Dawson, R.B. Emeson, Regulation of alternative splicing by RNA editing, Nature 399 (6731) (1999) 75–80.
[88] D. Dominissini, S. Moshitch-Moshkovitz, S. Schwartz, M. Salmon-Divon, L. Ungar, S. Osenberg, et al., Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq, Nature 485 (7397) (2012) 201–206.
[89] T. Li, Y. Zhuang, W. Yang, Y. Xie, W. Shang, S. Su, et al., Silencing of METTL3 attenuates cardiac fibrosis induced by myocardial infarction via inhibiting the activation of cardiac fibroblasts, FASEB J. 35 (2) (2021) e21162.
[90] V. Kmietczyk, E. Riechert, L. Kalinski, E. Boileau, E. Malovrh, B. Malone, et al., M (6)A-mRNA methylation regulates cardiac gene expression and cellular growth, Life Sci Alliance. 2 (2) (2019).
[91] C.S. Chien, J.Y. Li, Y. Chien, M.L. Wang, A.A. Yarmishyn, P.H. Tsai, et al., METTL3-dependent N(6)-methyladenosine RNA modification mediates the atherogenic inflammatory cascades in vascular endothelium, Proc. Natl. Acad. Sci. USA 118 (7) (2021).
[92] Y. Liu, G. Luo, Q. Tang, Y. Song, D. Liu, H. Wang, et al., Methyltransferase-like 14 silencing relieves the development of atherosclerosis via m(6)a modification of p65 mRNA, Bioengineered 13 (5) (2022) 11832–11843.
[93] G. Dong, J. Yu, G. Shan, L. Su, N. Yu, S. Yang, N6-Methyladenosine methyltransferase METTL3 promotes angiogenesis and atherosclerosis by upregulating the JAK2/STAT3 pathway via m6A reader IGF2BP1, Front. Cell Dev. Biol. 9 (2021) 731810.
[94] Y. Zheng, Y. Li, X. Ran, D. Wang, X. Zheng, M. Zhang, et al., Mettl14 mediates the inflammatory response of macrophages in atherosclerosis through the NF-kappaB/IL-6 signaling pathway, Cell. Mol. Life Sci. 79 (6) (2022) 311.
[95] Y. Zhao, J. Hu, X. Sun, K. Yang, L. Yang, L. Kong, et al., Loss of m6A demethylase ALKBH5 promotes post-ischemic angiogenesis via post-transcriptional stabilization of WNT5A, Clin. Transl. Med. 11 (5) (2021) e402.
[96] W. Mo, Z. Chen, X. Zhang, G. Dai, D. Ma, J. Pan, et al., N6-Methyladenosine demethylase FTO (fat mass and obesity-associated protein) as a novel mediator of statin effects in human endothelial cells, Arterioscler. Thromb. Vasc. Biol. 42 (5) (2022) 644–658.
[97] A. Quiles-Jimenez, I. Gregersen, Mittelstedt Leal, M. de Sousa, A. Abbas, X. Y. Kong, I. Alseth, et al., N6-methyladenosine in RNA of atherosclerotic plaques: an epitranscriptomic signature of human carotid atherosclerosis, Biochem. Biophys. Res. Commun. 533 (4) (2020) 631–637.
[98] W. Zhao, Y. Xu, J. Zhu, C. Zhang, W. Zhou, S. Wang, M6A plays a potential role in carotid atherosclerosis by modulating immune cell modification and regulating aging-related genes, Sci. Rep. 14 (1) (2024) 60.
[99] D. Jian, Y. Wang, L. Jian, H. Tang, L. Rao, K. Chen, et al., METTL14 aggravates endothelial inflammation and atherosclerosis by increasing FOXO1 N6-methyladeosine modifications, Theranostics 10 (20) (2020) 8939–8956.
[100] A. Chamorro-Jorganes, W.K. Sweaad, R. Katare, M. Besnier, M. Anwar, N. Beazley-Long, et al., METTL3 regulates angiogenesis by modulating let-7e-5p and miRNA-18a-5p expression in endothelial cells, Arterioscler. Thromb. Vasc. Biol. 41 (6) (2021) e325–e337.
[101] B.Y. Zhang, L. Han, Y.F. Tang, G.X. Zhang, X.L. Fan, J.J. Zhang, et al., METTL14 regulates M6A methylation-modified primary miR-19a to promote cardiovascular endothelial cell proliferation and invasion, Eur. Rev. Med. Pharmacol. Sci. 24 (12) (2020) 7015–7023.
[102] J. Chen, K. Lai, X. Yong, H. Yin, Z. Chen, H. Wang, et al., Silencing METTL3 stabilizes atherosclerotic plaques by regulating the phenotypic transformation of vascular smooth muscle cells via the miR-375-3p/PDK1 Axis, Cardiovasc. Drugs Ther. 37 (3) (2023) 471–486.
[103] X. Yang, C. Wang, G. Zhu, Z. Guo, L. Fan, METTL14/YTHDF1 axis-modified UCHL5 aggravates atherosclerosis by activating the NLRP3 inflammasome, Exp. Cell Res. 427 (2) (2023) 113587.
[104] J Huang, Q Feng, Z Dong, Z Li, Y Liu, R Xu, Z Liu, Q Ding, X Yang, F Yu, Y Jia, METTL3 Aggravates Intimal Hyperplasia by Facilitating the m6A-YTHDC1-dependent SGK1 Gene Transcription, bioRxiv (2025 Jan 9), 2025-01.
[105] Z Dong, Y Jin, Y Shen, J Huang, J Tan, Q Feng, Z Gong, S Zhu, H Chen, F Yu, W Li, METTL3-catalyzed m6A methylation facilitates the contribution of vascular smooth muscle cells to atherosclerosis, Cardiovasc. Res. (2025 Feb 20) cvaf029.
[106] Y. Liu, Z. Liu, H. Tang, Y. Shen, Z. Gong, N. Xie, et al., The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 facilitates M1 macrophage polarization through the methylation of STAT1 mRNA, Am. J. Phys. Cell Phys. 317 (4) (2019) C762–C775.
[107] G. Zhang, X. Li, X. Huang, m6A-related bioinformatics analysis and functional characterization reveals that METTL3-mediated NPC1L1 mRNA hypermethylation facilitates progression of atherosclerosis via inactivation of the MAPK pathway, Inflamm. Res. 72 (3) (2023) 429–442.
[108] S. Jin, X. Zhang, Y. Miao, P. Liang, K. Zhu, Y. She, et al., M(6)a RNA modification controls autophagy through upregulating ULK1 protein abundance, Cell Res. 28 (9) (2018) 955–957.
[109] Z.M. Fang, S.M. Zhang, H. Luo, D.S. Jiang, B. Huo, X. Zhong, et al., Methyltransferase-like 3 suppresses phenotypic switching of vascular smooth muscle cells by activating autophagosome formation, Cell Prolif. 56 (4) (2023) e13386.
[110] B. Shu, Y.X. Zhou, H. Li, R.Z. Zhang, C. He, X. Yang, The METTL3/MALAT1/PTBP1/USP8/TAK1 axis promotes pyroptosis and M1 polarization of macrophages and contributes to liver fibrosis, Cell Death Dis. 7 (1) (2021) 368.
[111] F. Tang, L.H. Tian, X.H. Zhu, S. Yang, H. Zeng, Y.Y. Yang, METTL3-mediated m6A modification enhances lncRNA H19 stability to promote endothelial cell inflammation and pyroptosis to aggravate atherosclerosis, FASEB J. 38 (20) (2024) e70090.
[112] H. Zhang, J. Liu, Y. Zhou, M. Qu, Y. Wang, K. Guo, et al., Neutrophil extracellular traps mediate m(6)a modification and regulates sepsis-associated acute lung injury by activating ferroptosis in alveolar epithelial cells, Int. J. Biol. Sci. 18 (8) (2022) 3337–3357.
[113] M. Jiang, W. Zhao, L. Wu, G. Zhu, Screening of m6A-associated ferroptosis-related genes in atherosclerosis based on WGCNA, Front Cardiovasc Med. 11 (2024) 1469805.
[114] M.A. Sikking, S.L.V.M. Stroeks, O.J. Waring, M.T.H.M. Henkens, N.P. Riksen, A. Hoischen, et al., Clonal hematopoiesis of indeterminate potential from a heart failure specialist's point of view, J. Am. Heart Assoc. 12 (15) (2023) e030603.
[115] E. Pronier, A. Imanci, D. Selimoglu-Buet, B. Badaoui, R. Itzykson, T. Roger, et al., Macrophage migration inhibitory factor is overproduced through EGR1 in TET2 (low) resting monocytes, Commun. Biol. 5 (1) (2022) 110.
[116] D. Zhang, Z. Gou, Y. Qu, X. Su, Mechanistic insights into vascular biology via methyltransferase-like 3-driven N(6)-adenosine methylation of RNA, Front. Cell Dev. Biol. 12 (2024) 1482753.
[117] Y. Luo, J. Feng, Q. Xu, W. Wang, X. Wang, NSun2 deficiency protects endothelium from inflammation via mRNA methylation of ICAM-1, Circ. Res. 118 (6) (2016) 944–956.
[118] Y. Wu, D. Jiang, H. Zhang, F. Yin, P. Guo, X. Zhang, et al., N1-Methyladenosine (m1A) regulation associated with the pathogenesis of abdominal aortic aneurysm through YTHDF3 modulating macrophage polarization, Front Cardiovasc Med. 9 (2022) 883155.

[119] A.K. Chokkalla, K. Pajdzik, X. Dou, Q. Dai, S.L. Mehta, V. Arruri, et al., Dysregulation of the Epitranscriptomic mark m(1)a in ischemic stroke, Transl. Stroke Res. 14 (6) (2023) 806–810.
[120] Y. He, H. Zhang, F. Yin, P. Guo, S. Wang, Y. Wu, et al., Novel insights into the role of 5-Methylcytosine RNA methylation in human abdominal aortic aneurysm, Front. Biosci. (Landmark Ed). 26 (11) (2021) 1147–1165.
[121] H. Winkels, E. Ehinger, M. Vassallo, K. Buscher, H.Q. Dinh, K. Kobiyama, et al., Atlas of the immune cell repertoire in mouse atherosclerosis defined by single-cell RNA-sequencing and mass cytometry, Circ. Res. 122 (12) (2018) 1675–1688.
[122] C. Cochain, E. Vafadarnejad, P. Arampatzi, J. Pelisek, H. Winkels, K. Ley, et al., Single-cell RNA-Seq reveals the transcriptional landscape and heterogeneity of aortic macrophages in murine atherosclerosis, Circ. Res. 122 (12) (2018) 1661–1674.
[123] Q. Li, M.J. Gloudemans, J.M. Geisinger, B. Fan, F. Aguet, T. Sun, et al., RNA editing underlies genetic risk of common inflammatory diseases, Nature 608 (7923) (2022) 569–577.
[124] C.E. Samuel, Adenosine deaminase acting on RNA (ADAR1), a suppressor of double-stranded RNA-triggered innate immune responses, J. Biol. Chem. 294 (5) (2019) 1710–1720.
[125] J.C. Hartner, C.R. Walkley, J. Lu, S.H. Orkin, ADAR1 is essential for the maintenance of hematopoiesis and suppression of interferon signaling, Nat. Immunol. 10 (1) (2009) 109–115.
[126] X. Guo, S. Liu, Y. Sheng, M. Zenati, T. Billiar, A. Herbert, et al., ADAR1 Zalpha domain P195A mutation activates the MDA5-dependent RNA-sensing signaling pathway in brain without decreasing overall RNA editing, Cell Rep. 42 (7) (2023) 112733.
[127] T.D. Mann, E. Kopel, E. Eisenberg, E.Y. Levanon, Increased A-to-I RNA editing in atherosclerosis and cardiomyopathies, PLoS Comput. Biol. 19 (4) (2023) e1010923.
[128] N.I. Vlachogiannis, M. Sachse, G. Georgiopoulos, E. Zormpas, D. Bampatsias, D. Delialis, et al., Adenosine-to-inosine Alu RNA editing controls the stability of the pro-inflammatory long noncoding RNA NEAT1 in atherosclerotic cardiovascular disease, J. Mol. Cell. Cardiol. 160 (2021) 111–120.
[129] X.Y. Kong, C. Huse, K. Yang, J. Ogaard, N. Berges, E.S. Vik, et al., Endonuclease V regulates atherosclerosis through C-C motif chemokine ligand 2-mediated monocyte infiltration, J. Am. Heart Assoc. 10 (14) (2021) e020656.
[130] O. Franzen, R. Ermel, K. Sukhavasi, R. Jain, A. Jain, C. Betsholtz, et al., Global analysis of A-to-I RNA editing reveals association with common disease variants, PeerJ 6 (2018) e4466.
[131] A.C. Doran, N. Meller, C.A. McNamara, Role of smooth muscle cells in the initiation and early progression of atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 28 (5) (2008) 812–819.
[132] M. Li, M. Qian, K. Kyler, J. Xu, Endothelial-vascular smooth muscle cells interactions in atherosclerosis, Front Cardiovasc Med. 5 (2018) 151.
[133] M.E. Pepin, R.M. Gupta, The role of endothelial cells in atherosclerosis: insights from genetic association studies, Am. J. Pathol. 194 (4) (2024) 499–509.
[134] P. Libby, P.M. Ridker, G.K. Hansson, Progress and challenges in translating the biology of atherosclerosis, Nature 473 (7347) (2011) 317–325.
[135] P. Libby, P.M. Ridker, A. Maseri, Inflammation and atherosclerosis, Circulation 105 (9) (2002) 1135–1143.
[136] J. Fei, X.B. Cui, J.N. Wang, K. Dong, S.Y. Chen, ADAR1-mediated RNA editing, a novel mechanism controlling phenotypic modulation of vascular smooth muscle cells, Circ. Res. 119 (3) (2016) 463–469.
[137] M. Jain, T.D. Mann, M. Stulic, S.P. Rao, A. Kirsch, D. Pullirsch, et al., RNA editing of Filamin a pre-mRNA regulates vascular contraction and diastolic blood pressure, EMBO J. 37 (19) (2018).
[138] M. Jain, G. Manjaly, K. Maly, M.R. de Vries, M. Janisiw, L. Konig, et al., Filamin a pre-mRNA editing modulates vascularization and tumor growth, Mol. Ther. Nucleic Acids. 30 (2022) 522–534.
[139] L. Parma, F. Baganha, P.H.A. Quax, M.R. de Vries, Plaque angiogenesis and intraplaque hemorrhage in atherosclerosis, Eur. J. Pharmacol. 816 (2017) 107–115.
[140] G.P. Shi, G.K. Sukhova, M. Kuzuya, Q. Ye, J. Du, Y. Zhang, et al., Deficiency of the cysteine protease cathepsin S impairs microvessel growth, Circ. Res. 92 (5) (2003) 493–500.
[141] H. Wu, Q. Du, Q. Dai, J. Ge, X. Cheng, Cysteine protease Cathepsins in atherosclerotic cardiovascular diseases, J. Atheroscler. Thromb. 25 (2) (2018) 111–123.
[142] A. Gatsiou, N. Vlachogiannis, F.F. Lunella, M. Sachse, K. Stellos, Adenosine-to-inosine RNA editing in health and disease, Antioxid. Redox Signal. 29 (9) (2018) 846–863.
[143] G. Ramaswami, W. Lin, R. Piskol, M.H. Tan, C. Davis, J.B. Li, Accurate identification of human Alu and non-Alu RNA editing sites, Nat. Methods 9 (6) (2012) 579–581.
[144] D.D. Kim, T.T. Kim, T. Walsh, Y. Kobayashi, T.C. Matise, S. Buyske, et al., Widespread RNA editing of embedded alu elements in the human transcriptome, Genome Res. 14 (9) (2004) 1719–1725.
[145] G.K. Sukhova, Y. Zhang, J.H. Pan, Y. Wada, T. Yamamoto, M. Naito, et al., Deficiency of cathepsin S reduces atherosclerosis in LDL receptor-deficient mice, J. Clin. Invest. 111 (6) (2003) 897–906.
[146] J. Reiser, B. Adair, T. Reinheckel, Specialized roles for cysteine cathepsins in health and disease, J. Clin. Invest. 120 (10) (2010) 3421–3431.
[147] M. Correia de Sousa, M. Gjorgjieva, D. Dolicka, C. Sobolewski, M. Foti, Deciphering miRNAs' action through miRNA editing, Int. J. Mol. Sci. 20 (24) (2019).
[148] A. Cai, L. Li, Y. Zhou, Pathophysiological effects of RhoA and rho-associated kinase on cardiovascular system, J. Hypertens. 34 (1) (2016) 3–10.
[149] K.P. Nunes, R.C. Webb, New insights into RhoA/rho-kinase signaling: a key regulator of vascular contraction, Small GTPases. 12 (5–6) (2021) 458–469.
[150] Q. Wang, H. Hui, Z. Guo, W. Zhang, Y. Hu, T. He, et al., ADAR1 regulates ARHGAP26 gene expression through RNA editing by disrupting miR-30b-3p and miR-573 binding, RNA 19 (11) (2013) 1525–1536.
[151] D. Wu, N. Nishimura, V. Kuo, O. Fiehn, S. Shahbaz, L. Van Winkle, et al., Activation of aryl hydrocarbon receptor induces vascular inflammation and promotes atherosclerosis in apolipoprotein E−/− mice, Arterioscler. Thromb. Vasc. Biol. 31 (6) (2011) 1260–1267.
[152] M. Nakano, T. Fukami, S. Gotoh, M. Takamiya, Y. Aoki, M. Nakajima, RNA editing modulates human hepatic aryl hydrocarbon receptor expression by creating MicroRNA recognition sequence, J. Biol. Chem. 291 (2) (2016) 894–903.
[153] F. Altaf, C. Vesely, A.M. Sheikh, R. Munir, S.T.A. Shah, A. Tariq, Modulation of ADAR mRNA expression in patients with congenital heart defects, PLoS One 14 (4) (2019) e0200968.
[154] E. Brennan, B. Wang, A. McClelland, M. Mohan, M. Marai, O. Beuscart, et al., Protective effect of let-7 miRNA family in regulating inflammation in diabetes-associated atherosclerosis, Diabetes 66 (8) (2017) 2266–2277.
[155] M. Liu, G. Tao, Q. Liu, K. Liu, X. Yang, MicroRNA let-7g alleviates atherosclerosis via the targeting of LOX-1 in vitro and in vivo, Int. J. Mol. Med. 40 (1) (2017) 57–64.
[156] L. Zhu, Q. Li, D. Qi, F. Niu, Q. Li, H. Yang, et al., Atherosclerosis-associated endothelial cell apoptosis by miRNA let7-b-mediated downregulation of HAS-2, J. Cell. Biochem. 121 (8–9) (2020) 3961–3972.
[157] R. van der Kwast, E. van Ingen, L. Parma, H.A.B. Peters, P.H.A. Quax, A. Y. Nossent, Adenosine-to-inosine editing of MicroRNA-487b alters target gene selection after ischemia and promotes neovascularization, Circ. Res. 122 (3) (2018) 444–456.
[158] Y. Kawahara, B. Zinshteyn, P. Sethupathy, H. Iizasa, A.G. Hatzigeorgiou, K. Nishikura, Redirection of silencing targets by adenosine-to-inosine editing of miRNAs, Science 315 (5815) (2007) 1137–1140.
[159] R. van der Kwast, L. Parma, M.L. van der Bent, E. van Ingen, F. Baganha, H.A. B. Peters, et al., Adenosine-to-inosine editing of vasoactive MicroRNAs alters their Targetome and function in ischemia, Mol. Ther. Nucleic Acids. 21 (2020) 932–953.
[160] A. Churov, V. Summerhill, A. Grechko, V. Orekhova, A. Orekhov, MicroRNAs as potential biomarkers in atherosclerosis, Int. J. Mol. Sci. 20 (22) (2019).
[161] M.M.J. Mens, A. Heshmatollah, L. Fani, M.A. Ikram, M.K. Ikram, M. Ghanbari, Circulatory MicroRNAs as potential biomarkers for stroke risk: the Rotterdam study, Stroke 52 (3) (2021) 945–953.
[162] Y. Devaux, E.E. Creemers, R.A. Boon, S. Werfel, T. Thum, S. Engelhardt, et al., Circular RNAs in heart failure, Eur. J. Heart Fail. 19 (6) (2017) 701–709.
[163] C.L. Song, J.P. Wang, X. Xue, N. Liu, X.H. Zhang, Z. Zhao, et al., Effect of circular ANRIL on the inflammatory response of vascular endothelial cells in a rat model of coronary atherosclerosis, Cell. Physiol. Biochem. 42 (3) (2017) 1202–1212.
[164] Y. Bai, Y. Zhang, B. Han, L. Yang, X. Chen, R. Huang, et al., Circular RNA DLGAP4 ameliorates ischemic stroke outcomes by targeting miR-143 to regulate endothelial-mesenchymal transition associated with blood-brain barrier integrity, J. Neurosci. 38 (1) (2018) 32–50.
[165] F. Wu, B. Han, S. Wu, L. Yang, S. Leng, M. Li, et al., Circular RNA TLK1 aggravates neuronal injury and neurological deficits after ischemic stroke via miR-335-3p/TIPARP, J. Neurosci. 39 (37) (2019) 7369–7393.
[166] A. Ivanov, S. Memczak, E. Wyler, F. Torti, H.T. Porath, M.R. Orejuela, et al., Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals, Cell Rep. 10 (2) (2015) 170–177.
[167] J.N. Boeckel, N. Jae, A.W. Heumuller, W. Chen, R.A. Boon, K. Stellos, et al., Identification and characterization of hypoxia-regulated endothelial circular RNA, Circ. Res. 117 (10) (2015) 884–890.
[168] M. Vausort, A. Salgado-Somoza, L. Zhang, P. Leszek, M. Scholz, A. Teren, et al., Myocardial infarction-associated circular RNA predicting left ventricular dysfunction, J. Am. Coll. Cardiol. 68 (11) (2016) 1247–1248.
[169] W.K. Sweaad, F.M. Stefanizzi, A. Chamorro-Jorganes, Y. Devaux, C. Emanueli, CA EU-CCA, Relevance of N6-methyladenosine regulators for transcriptome: implications for development and the cardiovascular system, J. Mol. Cell. Cardiol. 160 (2021) 56–70.
[170] A. Leger, P.P. Amaral, L. Pandolfini, C. Capitanchik, F. Capraro, V. Miano, et al., RNA modifications detection by comparative Nanopore direct RNA sequencing, Nat. Commun. 12 (1) (2021) 7198.
[171] M. Jain, R. Abu-Shumays, H.E. Olsen, M. Akeson, Advances in nanopore direct RNA sequencing, Nat. Methods 19 (10) (2022) 1160–1164.
[172] S. Martin, D. Heavens, Y. Lan, S. Horsfield, M.D. Clark, R.M. Leggett, Nanopore adaptive sampling: a tool for enrichment of low abundance species in metagenomic samples, Genome Biol. 23 (1) (2022) 11.
[173] A. Castellanos-Rubio, I. Santin, A. Olazagoitia-Garmendia, I. Romero-Garmendia, A. Jauregi-Miguel, M. Legarda, et al., A novel RT-QPCR-based assay for the relative quantification of residue specific m6A RNA methylation, Sci. Rep. 9 (1) (2019) 4220.
[174] J. Vallejo, C. Cochain, A. Zernecke, K. Ley, Heterogeneity of immune cells in human atherosclerosis revealed by scRNA-Seq, Cardiovasc. Res. 117 (13) (2021) 2537–2543.
[175] T. Woudenberg, M.L. van der Bent, V. Kremer, I.S.E. Waas, M. Daemen, R. A. Boon, et al., Site-specific m6A-miR-494-3p, not unmethylated miR-494-3p, compromises blood brain barrier by targeting tight junction protein 1 in intracranial atherosclerosis, Br. J. Pharmacol. (2024) 1–15.

[176] H. Shi, Y. Xu, N. Tian, M. Yang, F.S. Liang, Inducible and reversible RNA N(6)-methyladenosine editing, Nat. Commun. 13 (1) (2022) 1958.

[177] D.M. Erion, L.Y. Liu, C.R. Brown, S. Rennard, H. Farah, Editing approaches to treat Alpha-1 antitrypsin deficiency, Chest 167 (2) (2025 Feb) 444–452.

[178] D. Erion SG, K. Su, D. De Silva, M. Patel, J. Flum, D. Jenness, M. Popovici-Muller, D. Ulkoski, A. Wantz, W. Fedyk, H. Kenney, T. Bradshaw, S. Hu, M. Maciejewski, A. Saha, M. Shadid, M. Pink, C. Brown, L. Liu, V. Krishnamurthy, S. Colletti, KRRO-110, An RNA Editing Oligonucleotide Encapsulated in a Lipid Nanoparticle (LNP) Delivered to Liver Cells for the Treatment of Alpha-1 Antitrypsin Deficiency (AATD), Am. J. Respir. Crit. Care Med. 209 (2024) A3797.

[179] M. Sopic, V. Stopa, Y. Devaux, Leveraging epitranscriptomics for cardiovascular disease theranostics, Eur. Heart J. 45 (13) (2024) 1098–1100.