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**Review Paper**

# Exploring the impact of N4-acetylcytidine modification in RNA on non-neoplastic disease: unveiling its role in pathogenesis and therapeutic opportunities

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## Abstract

RNA modifications include not only methylation modifications, such as $m^6A$, but also acetylation modifications, which constitute a complex interaction involving "writers," "readers," and "erasers" that play crucial roles in growth, genetics, and disease. N4-acetylcytidine ($ac^4C$) is an ancient and highly conserved RNA modification that plays a profound role in the pathogenesis of a wide range of diseases. This review provides insights into the functional impact of $ac^4C$ modifications in disease and introduces new perspectives for disease treatment. These studies provide important insights into the biological functions of post-transcriptional RNA modifications and their potential roles in disease mechanisms, offering new perspectives and strategies for disease treatment.

Keywords: RNA modification; N4-acetylcytidine ($ac^4C$); biological function; diseases; treatment

## Introduction

Similar to DNA and proteins, RNA undergoes chemical modifications catalyzed by specific enzymes. As early as the 1950s, it was discovered that RNA could undergo modifications on the canonical ACUG bases. To date, over 170 RNA modifications have been identified [1], with N6-methyladenosine ($m^6A$) methylation being the most prevalent and proven to play a crucial role in various advanced biological processes, such as RNA splicing, maturation, transport, and translation [2].

With the rapid development of high-throughput sequencing technologies, research on RNA modifications has become increasingly profound, and N4-acetylcytidine ($ac^4C$) acetylation

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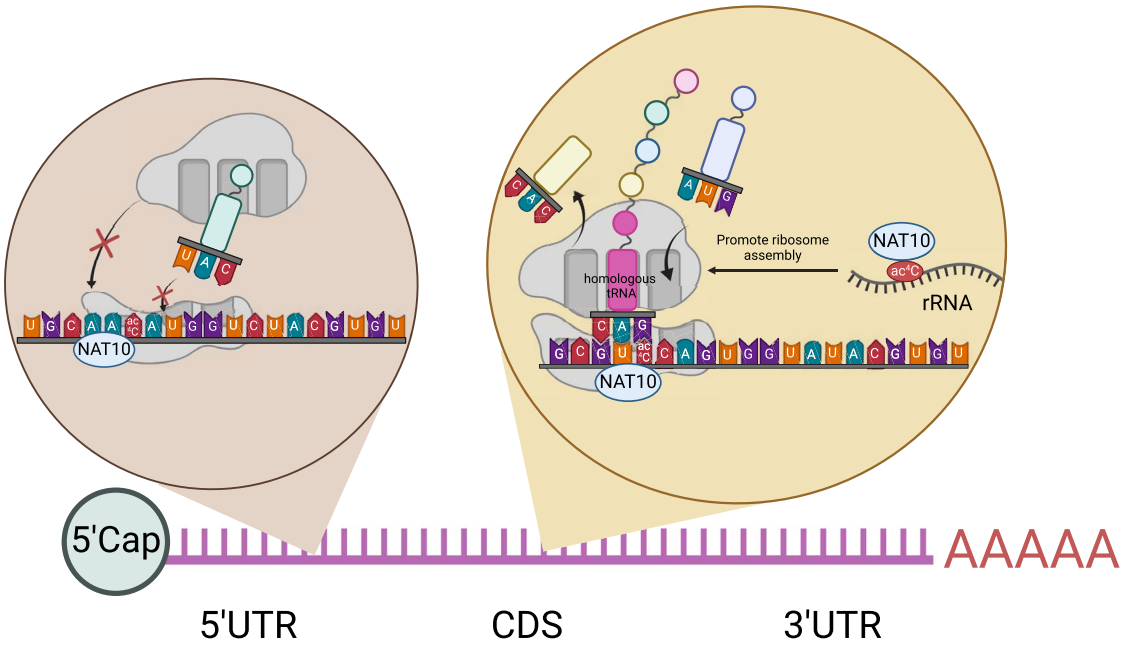


Figure 1. Functions of ac$^4$C modifications in RNAs. The ac$^4$C present in the mRNA CDS enhances translation rate through intramolecular hydrogen bonding and interaction with homologous tRNAs. ac$^4$C near the 5'UTP AUG strong promoter represses translation, and ac$^4$C on rRNAs promotes the assembly of ribosomes and facilitates translation.

modification is gradually being elucidated. Although studies on understanding the "writer," "reader," and "eraser" aspects of its function have not reached the same depth as m$^6$A modification [3–5], scientists have revealed the mechanism of action of ac$^4$C acetyltransferase and found that this enzyme is closely related to the occurrence and development of various diseases [6, 7]. ac$^4$C acetylation modification is mainly catalyzed by NAT10 acetyltransferase on the N4 position of cytidine in RNA and is widely present in various RNA molecules, including mRNA, tRNA, and rRNA. Among them, NAT10, as the only ac$^4$C acetyltransferase in human cells, can precisely regulate the stability and translation of RNA by catalyzing the acetylation of rRNA [8], tRNA [9], and mRNA [10], which advances our understanding of the role of RNA acetylation modification in cellular physiology and pathology.

Numerous studies have substantiated the association between ac$^4$C modification and the onset of cancer [11–14]. Additionally, the presence of ac$^4$C modification has been implicated in the physiological mechanisms of various diseases, playing a pivotal role in disease treatment. In this concise review, we provide a detailed exploration of the molecular-level regulatory functions of ac$^4$C acetylation and its roles in disease, with a prospective outlook on the involvement of NAT10 in disease therapy.

## Modulatory function of ac$^4$C RNA modification in molecular processes

The ac$^4$C modification on mRNA exerts differential effects on gene expression and translation depending on its position. Within the coding sequence (CDS), the ac$^4$C modification enhances stability through the formation of hydrogen bonds with internal N4-acetyl groups. Additionally, it interacts with homologous tRNA, thereby increasing translation rates [10, 15]. Conversely, the ac$^4$C modification present in the 5' untranslated region (5'UTR) may competitively inhibit annotated start codons, impeding protein synthesis. Particularly near strong AUG initiation codons, ac$^4$C modification in the 5'UTR may also inhibit translation [16]. Moreover, the inhibitory effect of ac$^4$C modification in the 5'UTR on translation may counteract the promoting effect observed in the CDS. Therefore, site-specificity must be considered in the development of therapeutic drugs.

In addition, ac$^4$C modification plays a crucial role in ribosome synthesis. Depletion of ribosomal RNA cytidine acetyltransferase 1 (RRA1), homologous to NAT10, leads to significant accumulation of the 23S precursor of 18S rRNA, impacting ribosome synthesis [17]. In summary, ac$^4$C acetylation on mRNA and rRNA collectively promotes gene expression (Fig. 1).

When studying ac$^4$C acetylation modification on RNA, several detection methods have been discovered. Liquid chromatography-mass spectrometry (LC-MS) and high-performance LC-MS (HPLC-MS) are among the qualitative and quantitative methods for detecting ac$^4$C modification in RNA, capable of identifying ac$^4$C modifications within microorganisms and human cells. However, due to their inability to amplify signals, these techniques have limited sensitivity, posing challenges in detecting low-abundance ac$^4$C modifications [18].

RNA modification immunoprecipitation-sequencing methods (such as RNA IP-seq) amplify signals. However, this method has a higher false positive rate and cannot accurately detect ac$^4$C modifications at the nucleotide level or quantify their abundance at presumed modification sites[18]. In 2017, Sinclair *et al.* [19] developed specific affinity reagents for analyzing the expression spectrum of ac$^4$C. Arango *et al.* [10] employed the acRIP-seq method, utilizing ac$^4$C-binding protein antibodies to enrich ac$^4$C mRNA, thereby mapping the locations of ac$^4$C in the human transcriptome.

Additionally, Yan *et al.* [20] proposed a metabolic sequencing method, FAMseq, which does not require antibodies and utilizes fluoride ions to accurately obtain the acetylation profile of the entire transcriptome RNA. There are also emerging detection tools, such as iRNA-ac$^4$C and LSA-ac$^4$C, although these

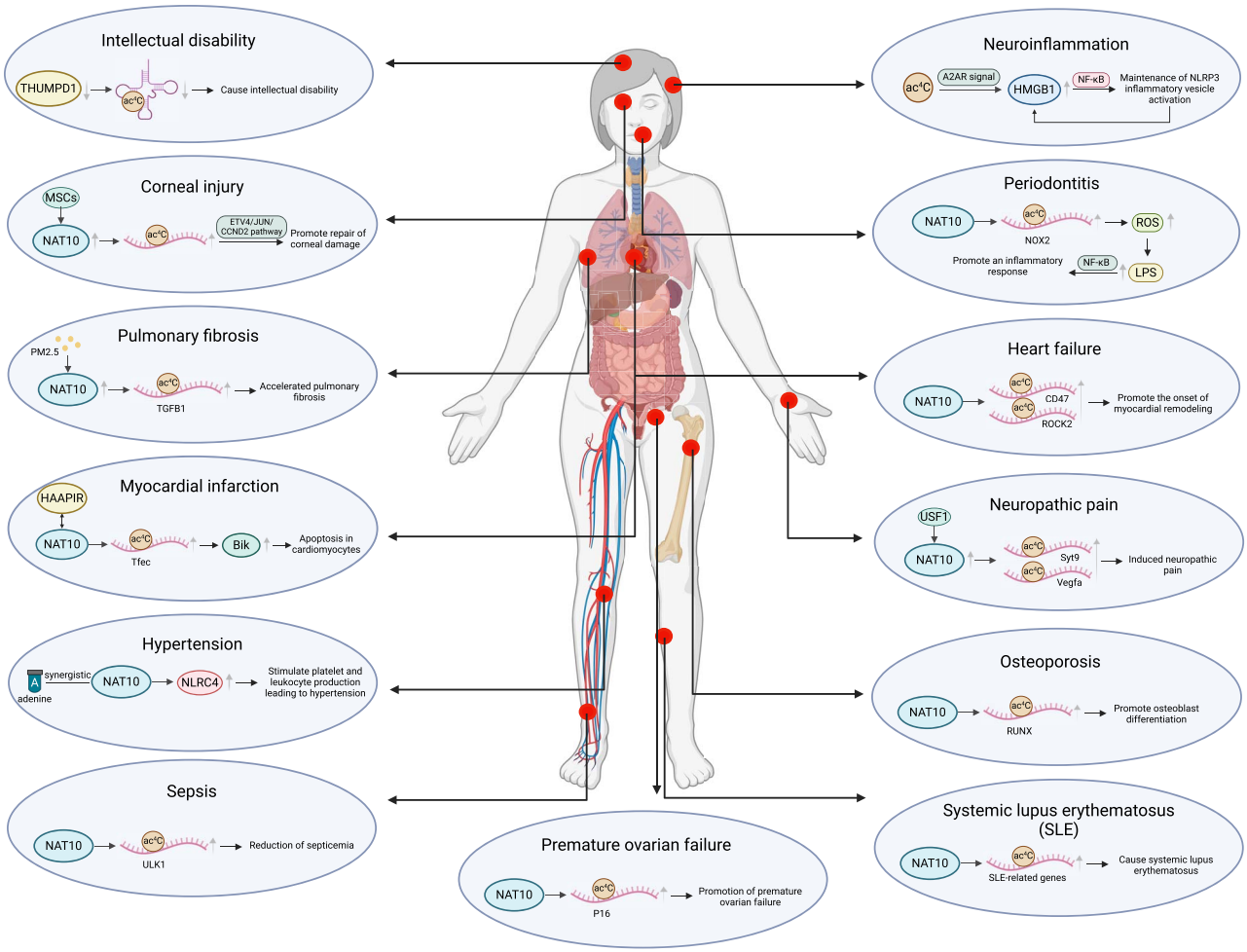


Figure 2. The role of ac$^4$C modifications in disease pathogenesis.

technologies currently cannot precisely determine the specific positions of ac$^4$C [21–23].

## The role of ac$^4$C acetylation modification in diseases

In recent years, ac$^4$C acetylation modification has been implicated in the pathogenesis of various diseases. In neuroinflammation, the nucleoside metabolite ac$^4$C induces the expression of alarmin high mobility group box-1 (HMGB1 protein), activating nuclear factor $\kappa$B (NF-$\kappa$B). This, in turn, upregulates NOD-like receptor thermal protein domain associated protein 3 (NLRP3) gene expression, maintaining the activation of NLRP3 inflammasomes and activating the immune function of microglial cells [24]. In Alzheimer's disease (AD), ac$^4$C modification on lncRNA is also speculated to be associated with AD [25], although the specific regulatory mechanisms require further in-depth investigation. Additionally, the co-increase of the nucleoside metabolite ac$^4$C and adenine may be associated with hypertension [26]. This is hypothesized to be due to the activation of inflammatory cells and factors by the binding of ac$^4$C and adenine, stimulating platelet and leukocyte generation, leading to elevated blood pressure. In sepsis, inhibition of NAT10 leads to restricted expression of UNC-52-like kinase 1 (ULK1), activating the STING-IRF3 signal and ultimately causing an increase in NLRP3 inflammasomes, leading to neutrophil apoptosis and septicemia [27].Furthermore, in systemic lupus erythematosus (SLE), NAT10, by mediating ac$^4$C acetylation of certain SLE-related gene mRNAs, enhances their stability, ultimately activating immune cells and inflammatory responses [28].

These studies provide diverse perspectives for understanding the intrinsic mechanisms of diseases and offer new insights for overcoming them. Subsequently, we summarize the latest research on ac$^4$C acetylation modification in the pathogenesis of various diseases, such as osteoporosis and periodontal diseases, to highlight its closely associated characteristics with diseases and explore its broad prospects as a potential therapeutic target (Fig. 2, Table 1).

### Osteoporosis

Osteoporosis, a common condition among middle-aged and elderly individuals, is notably exacerbated in patients who have undergone oophorectomy due to reduced estrogen secretion, which accelerates bone resorption and increases the likelihood of developing osteoporosis [29]. Previous studies have confirmed a close association between the occurrence of osteoporosis and epigenetic modifications [30]. NAT10 has been identified as a facilitator of acetylation on Runt-related transcription factor 2 (RUNX2) mRNA, thereby increasing its expression by extending its half-life. This promotion of RUNX2 acetylation by NAT10 stimulates the differentiation of bone marrow mesenchymal

Table 1. The role of $ac^4C$ acetylation modification in diseases

| Disease | Enzyme | Target | Description | Ref |
|---|---|---|---|---|
| Chronic neurogenic diseases | NAT10 | A2AR | $ac^4C$ activates BV2 microglia *via* A2AR signaling and maintains NLRP3 inflammatory vesicles | [24] |
| Hypertension | NAT10 | N.A. | $ac^4C$ and adenine together promote NLRC4 expression, activating platelets and leukocytes to cause hypertension | [26] |
| Sepsis | NAT10 | ULK1 | $ac^4C$ modification of ULK1 mRNA attenuates sepsis by increasing ULK1 transcription | [27] |
| Osteoporosis | NAT10 | RUNX2 | $ac^4C$ modification of RUNX2 mRNA increases RUNX2 expression to promote osteoblast differentiation | [31] |
| Periodontitis | NAT10 | NOX2 | $ac^4C$ modification of NOX2 mRNA in macrophages enhances NOX2 expression to cause inflammatory responses | [33] |
| Myocardial infarction | NAT10 | Tfec | HAAPIR promotes Tfec gene expression, while Tfec promotes Bik accumulation, leading to cardiomyocyte death. | [38] |
| Heart failure | NAT10 | CD47/ROCK2 | Upregulation of CD47 and ROCK2 mRNA $ac^4C$ modification promotes myocardial remodeling | [39] |
| Pulmonary fibrosis | NAT10 | TGFB1 | $ac^4C$ can accelerate lung fibrosis by increasing TGFB1 mRNA stability | [41] |
| Corneal injury | NAT10 | mRNAs | MSCs enhance mRNA $ac^4C$ acetylation and activate the ETV4/JUN/CCND2 signaling axis to promote corneal injury recovery | [43] |
| Neuropathic pain | NAT10 | Syt9 | Activation of USF1 by nerve injury upregulates $ac^4C$ in Syt9 mRNA to increase SYT9 protein to trigger neuropathic pain | [6] |
| Neuropathic pain | NAT10 | Vegfa | HNRNPK binding to Vegfa mRNA increases $ac^4C$ modification and upregulates VEGFA-induced neuropathic pain | [45] |
| Premature ovarian failure | NAT10 | P16 | $ac^4C$ acetylation increases P16 mRNA stability and promotes premature ovarian failure | [7] |
| Intellectual disability | THUMPD1 | small RNAs/tRNA-SerCGA | Aberrant THUMPD1 expression leads to mental retardation syndrome caused by deletion of the $ac^4C$ modification in small RNAs and individually purified tRNAs-Ser-CGA | [50] |
| Systemic lupus erythematosus | NAT10 | $CD4^+$T cell mRNAs | Significant differences in mRNA $ac^4C$ modification abundance of certain SLE-related genes in CD4+ T cells from SLE patients | [28] |

NAT10, N-acetyltransferase 10; $ac^4C$, N4-acetylcytidine; tRNA, transfer RNA; mRNA, messenger RNA; A2AR, adenosine A2A receptor; NLRP3, NOD-like receptor thermal protein domain associated protein 3; NLRC4, NOD-like receptor family CARD-containing 4 protein; ULK1, UNC-51-like kinase 1; RUNX2, runt-related transcription factor 2; NOX2, the NADPH oxidase 2; Tfec, transcription factor EC; HAAPIR, heart-apoptosis-associated piRNA; Bik, BCL2-interacting killer; CD47, integrin-associated protein, IAP; ROCK2, rho-associated coiled-coil containing protein kinase 2; TGFB1, transforming growth factor beta 1; MSCs, marrow-derived mesenchymal stem cells; ETV4, ets translocation variant 4; CCND2, cyclin D2; Syt9, synaptotagmin 9; USF1, upstream transcription factor 1; Vegfa, vascular endothelial growth factor A; HNRNPK, heterogeneous nuclear ribonucleoprotein K, hnRNPK; P16, cyclin-dependent kinase inhibitor, MTS; THUMPD1, THUMP domain containing 1; SLE, systemic lupus erythematosus; N.A., not available.

stem cells (BMSCs) into osteoblasts [31]. Notably, overexpression of NAT10 in mice subjected to bilateral oophorectomy has been found to reverse bone loss, mitigating the detrimental effects on the organism [31]. Consequently, NAT10 emerges as a potential direction for the prevention and treatment of osteoporosis.

## Periodontal diseases

Periodontitis, a chronic inflammation caused by bacterial invasion of periodontal tissues within dental plaque, stands as a leading cause of adult tooth mobility and loss [32]. In the context of periodontitis, NAT10 mediates acetylation on the mRNA of NADPH oxidase 2 (NOX2) within macrophages, enhancing the expression of the NOX2 gene. This process promotes the generation of reactive oxygen species (ROS), subsequently accelerating the lipopolysaccharide (LPS)-induced NF-$\kappa$B signaling pathway, culminating in heightened inflammatory responses. Deletion of NAT10 in mice results in reduced levels of IL-6 and TNF-$\alpha$, while NAT10 overexpression increases the content of inflammatory cytokines [33]. Thus, NAT10 inhibitors have the potential to alleviate inflammatory reactions caused by macrophages during the pathological progression of periodontitis, offering a promising avenue for effective therapeutic interventions.

Furthermore, recent research has unveiled that NAT10, through $ac^4C$ acetylation, regulates the vascular endothelial growth factor A (VEGFA)-mediated PI3K/AKT signaling pathway, enhancing osteogenic development in human periodontal ligament stem cells [34]. During osteogenic differentiation, NAT10 overexpression results in elevated expression of osteogenic markers, increased alkaline phosphatase (ALP) activity, and enhanced osteogenic capability. These findings hold promise for applications in periodontal tissue engineering based on human periodontal ligament stem cells, providing assistance in the treatment of periodontal diseases [34].

## Heart diseases

Myocardial infarction (MI) is a prevalent cardiac disease with a higher incidence in the elderly population [35, 36]. Piwi-interacting

RNAs (piRNAs) are abundantly expressed in the heart, and their levels significantly increase under stressful conditions [37]. HAAPIR, a piRNA associated with cardiac apoptosis, targets NAT10 to mediate acetylation of transcription factor EC (Tfec), thereby increasing its gene expression. Concurrently, Tfec activates the pro-apoptotic factor BCL2-interacting killer (Bik), leading to Bik accumulation and subsequent cardiomyocyte death following MI [38]. Inhibition of NAT10 could potentially offer a therapeutic window for patients with MI.

Furthermore, heart failure patients commonly exhibit characteristic cardiac remodeling. NAT10 catalyzes acetylation of integrin-associated protein (CD47) and rho-associated coiled-coil containing protein kinase 2 (ROCK2) mRNA, enhancing the expression of proteins necessary for cardiac remodeling. In contrast, remodelin, a NAT10 inhibitor, can suppress cardiac fibrosis, providing effective prevention against cardiac functional impairment [39]. Thus, NAT10 emerges as a crucial target for the treatment of myocardial remodeling.

## Pulmonary fibrosis

Long-term exposure to high concentrations of PM2.5 increases the risk of pulmonary fibrosis in the human respiratory system [40]. Experimental evidence reveals that $ac^4C$ modification and NAT10 levels increase in lung epithelial cells exposed to PM2.5, with the upregulation of NAT10 positively correlated with exposure duration and concentration. Further investigations confirm that NAT10 accelerates PM2.5-induced pulmonary fibrosis through $ac^4C$-dependent stabilization of transforming growth factor beta 1 (TGFB1) mRNA. As an upstream driver, TGFB1 expedites the epithelial-mesenchymal transition (EMT) and fibrotic processes in lung epithelial cells. Simultaneously, inhibiting NAT10 significantly protects against PM2.5-induced EMT and fibrosis [41]. The experimental findings suggest that NAT10 could be a potential target for blocking PM2.5-induced pulmonary epithelial transformation and fibrosis.

## Corneal injury

Corneal injury represents the most common type of trauma affecting the eyes, involving various factors, such as infection, allergic reactions, and more. Mesenchymal stem cells (MSCs) with potent differentiation and repair capabilities are found in various tissues [42]. Research suggests that MSCs enhance mRNA $ac^4C$ acetylation by stimulating the expression of NAT10, subsequently activating the ETV4/JUN/CCND2 signaling axis and increasing its stability. This ultimately promotes the repair of corneal injuries [43]. Therefore, $ac^4C$ acetylation modification emerges as a crucial therapeutic avenue for treating corneal injuries.

## Neuropathic pain

Neuropathic pain (NPP), resulting from neurological injuries caused by trauma or diseases, has been identified as a complex condition that significantly impacts individuals [44]. Recent studies indicate that NAT10 plays a role in the formation of NPP through multiple pathways, presenting itself as a promising new target for treating neuropathic pain. In damaged dorsal root ganglia, the activation of upstream transcription factor 1 (USF1) promotes NAT10 expression, upregulating $ac^4C$ in synaptotagmin 9 (Syt9) mRNA and increasing the $ac^4C$ site and SYT9 protein levels. Inducing these physiological processes in the absence of injury can lead to the occurrence of neuropathic pain-like behaviors. Therefore, USF1-regulated NAT10 targets Syt9 and $ac^4C$ in peripheral nociceptive neurons, modulating neuropathic pain [6].

Another study revealed that nerve injury increases NAT10 expression in dorsal horn neurons and the interaction between NAT10 and vascular endothelial growth factor A (Vegfa) mRNA. In spared nerve injuny (SNI), heterogeneous nuclear ribonucleoprotein K (HNRNPK) binding to Vegfa mRNA facilitated NAT10 recruitment. Elevated NAT10 expression, through increased $ac^4C$ modification, enhanced the translation efficiency of Vegfa mRNA, resulting in upregulation of VEGFA. Upregulated VEGFA enhances central sensitization and neuropathic pain induced by SNI or AAV-hSyn-NAT10. Blocking this cascade reaction may provide a novel therapeutic approach for patients with neuropathic pain [45].

## Premature ovarian failure

Premature ovarian insufficiency (POI), a dysfunctional ovarian condition occurring in females under the age of 40, although not highly prevalent, has profound and far-reaching effects on affected individuals [46, 47]. Granulosa cells play a crucial role in the growth and development of follicles, promoting this process through the secretion of hormones [48]. Cyclin-dependent kinase inhibitor P16 is associated with cellular senescence, and interferes with the cellular proliferation process by inhibiting the expression of Cyclin D1 (CCND1). In a mouse model of premature ovarian insufficiency, NAT10 has been identified to promote acetylation of P16 mRNA, thereby inhibiting the proliferation and differentiation of ovarian granulosa cells by increasing their stability, ultimately contributing to the onset of premature ovarian insufficiency [7]. Therefore, NAT10 exhibits the potential for therapeutic intervention in the context of premature ovarian insufficiency.

## Intellectual disability

THUMPD1, a tRNA-binding protein found on human serine and leucine tRNAs, plays a crucial role in the acetylation process [49]. Biallelic mutations in the THUMPD1 gene are associated with intellectual disability syndrome, characterized by developmental delays, behavioral abnormalities, impaired hearing and vision, and facial dysmorphisms. Experimental evidence confirms that targeted THUMPD1 knockout results in the loss of $ac^4C$ modification in small RNAs and tRNAs. In summary, dysregulated THUMPD1 expression leads to tRNA acetylation deficiency, impairing protein function, and ultimately giving rise to a syndromic form of intellectual disability [50].

## Viral Diseases

Following infection with influenza A virus, the $ac^4C$ modification of DAZAP1 in the host cells is significantly enriched in the 5'UTR. Meanwhile, decreased expression of NAT10 in infected host cells can inhibit viral growth [51]. These findings suggest that by decreasing the expression of host cell NAT10, viral replication and pathogenicity can be effectively reduced, resulting in an antiviral effect [51].

In HIV-1 (human immunodeficiency virus type 1), the expression level of NAT10 is abnormally high. Studies have found that inhibiting NAT10 expression not only reduces the $ac^4C$ modification of viral RNA and the expression of HIV-1 genes simultaneously, but also inhibits HIV-1 replication without affecting normal cellular physiological functions. This result highlights the importance of $ac^4C$ modification as a potential target for antiviral drug development. Additionally, mutations in the $ac^4C$ site on the HIV-1 env gene are part of the viral messenger RNA 3'UTR encoding gag, leading to a significant decrease in gag protein expression and accelerating virus spread [52]. HIV-1 Tat is a virus-encoded transactivator that activates viral transcription. NAT10, as one of the host proteins associated with HIV-1 Tat, can help

HIV-1 latency in Jurkat cells by inhibiting Tat-mediated HIV-1 transcription [53].

On the other hand, studies have shown that $ac^4C$ modification can enhance the translation efficiency and stability of viral RNA, thereby enhancing virus replication and pathogenicity [51]. For example, in the 5'UTR of enterovirus 71 mRNA, $ac^4C$ modification enhances the recruitment of PCBP2 to the internal ribosome entry site (IRES) selectively, promoting the translation of viral RNA and enhancing virus infectivity. These findings provide new insights into viral biology and important biomarkers and targets for the development of antiviral strategies [54].

## Discussion

In the 1970s, $ac^4C$ acetylation modification was first discovered on tRNA-ser and tRNA-leu in eukaryotic organisms and subsequently detected in rRNA and mRNA [8, 55]. As an acetylation modification identified on mRNA, $ac^4C$ exhibits robust biological functions. In this review, we delve into the role of $ac^4C$ modification in RNA post-transcriptional regulation, emphasizing its significance in the mechanisms underlying various diseases, particularly neurological and cardiac disorders. The ubiquity and specificity of $ac^4C$ modification reveal its crucial role as a biological regulatory mechanism, especially in the context of diseases. The presence of $ac^4C$ modification on different RNA types, such as mRNA and rRNA, underscores its importance in biology, while its specificity in specific disease contexts emphasizes the importance of considering the type and location of modifications when studying the relationship between RNA modification and diseases.

Regarding therapeutic potential, NAT10, identified as the sole $ac^4C$ modification catalyzing enzyme found in the human body [8], plays a pivotal role in the development of various diseases. Therefore, the development of inhibitors or activators targeting NAT10, particularly in the treatment of heart diseases and neuropathic pain, represents a promising strategy. For instance, in MI, NAT10 mediates the acetylation of Tfec and activates the expression of the pro-apoptotic factor Bik [38]. Furthermore, remodelin, which inhibits NAT10 expression, can reduce cardiac remodeling and treat premature aging syndrome. Simultaneously, in neuropathic pain, NAT10 is involved in NPP formation through various pathways, such as its interaction with USF1 and VEGFA mRNA. Targeting NAT10 can modulate key molecules like Syt9 and VEGFA, providing new strategies for treatment.

Meanwhile, research on the relationship between $ac^4C$ modification on RNA and diseases is still limited. First, the specific regulatory mechanisms of $ac^4C$ modification remain unclear. Currently, only one "writer" enzyme, NAT10, has been identified in humans, with no other enzymes or "reader" and "eraser" functions similar to NAT10 found yet. Second, the detailed involvement of $ac^4C$ modification in the pathogenesis of many diseases has not been fully elucidated. Additionally, the presence of $ac^4C$ modification is not the sole pathogenic factor in disease occurrence. Therefore, the significance of $ac^4C$ modification in the development of diseases requires further evaluation.

A deeper understanding of the specific mechanisms of $ac^4C$ modification in different diseases, particularly how it affects RNA stability, translation efficiency, or interaction with specific proteins, will help reveal the subtle regulatory mechanisms of RNA modification in disease occurrence. This insight offers new perspectives for future treatments.

In summary, our research further confirms the significant role of RNA modification, especially $ac^4C$ modification, in the occurrence and development of diseases. These findings not only provide new approaches for the diagnosis and treatment of diseases but also hold crucial implications for understanding the molecular mechanisms of diseases. By thoroughly investigating this modification, we can offer new strategies and methods for disease treatment.

**Key Points**

- Complex interplay of post-transcriptional RNA modifications: describes the intricate process of post-transcriptional RNA modifications involving "writers," "readers," and "erasers," playing crucial roles in growth, heredity, and disease.
- Significance of N4-acetylcytidine ($ac^4C$): emphasizes the profound effects of $ac^4C$ as an ancient and highly conserved RNA modification in the pathogenesis of various diseases.
- In-depth exploration of ac4C modification in disease: delves into the functional implications of ac4C modification in disease, revealing its critical role in the pathogenesis of different conditions.
- Introduction of novel perspectives on disease treatment: highlights the introduction of fresh perspectives and strategies for disease treatment based on the study of ac4C modification, providing innovative approaches for addressing diseases.
- Revelation of biological functions of post-transcriptional RNA modifications: indicates that these investigations not only offer critical insights into the biological functions of post-transcriptional RNA modifications but also unveil their potential roles in disease mechanisms.
- In summary, the text underscores the importance of post-transcriptional RNA modifications, particularly the ac4C modification in disease, and introduces new perspectives and strategies for disease therapy, shedding light on the biological functions of these modifications.

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## Author contributions

Y.H. and J.B. were responsible for literature selection and retrieval. K.Y.W. and T.T.N. drafted the manuscript. W.H.O.Y. and Y.J.X. compiled and summarized the table and figures. Z.J.H. and X.H.Z. reviewed and revised the manuscript. L.L. funded our project. All authors approved the final draft.

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## Data availability

The authors verify that the data underpinning the study's conclusions can be found both in the main text and the supplementary resources provided.

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