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5-Methylcytosine in non-communicable and communicable diseases

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ABSTRACT

5-Methylcytosine (m^5C) has attracted increasing attention as an important epitranscriptomic modification that regulates RNA metabolism and diverse cellular processes. It is dynamically controlled by writer, eraser, and reader proteins that govern the life cycle of the methylated transcript. Studies have demonstrated that aberrant m^5C enzymatic dysregulation is linked to a wide range of human diseases, ranging from non-communicable diseases like cancer to communicable diseases like viral infections. Notably, m^5C regulatory enzymes may exert context-dependent dual roles, functioning either protectively or pathogenically within and across different disease settings. However, current research efforts remain largely confined to individual disease models, and a unified understanding of m^5C functions across pathologies remains lacking. Furthermore, clinical translation targeting m^5C modification remains limited, with most findings restricted to experimental studies. This review aims to provide a comprehensive overview of current knowledge on m^5C -mediated mechanisms across both communicable and non-communicable contexts and evaluate their potential as therapeutic targets.

KEYWORDS: Epitranscriptomic; 5-Methylcytosine (m^5C); Cancer; Cardiovascular diseases; Chronic respiratory diseases; Virus infection

1. Introduction

RNA serves as a central intermediary in the genetic information flow from DNA to proteins, as described by the central dogma[1]. Precise regulation of transcription and translation is essential for cellular growth, differentiation, and homeostasis. “Epitranscriptomic”

modifications represent a newly recognized regulatory layer that fine-tunes gene expression and contributes to phenotypic diversity and environmental adaptation. To date, more than 170 distinct RNA modifications have been identified, such as N^6 -methyladenosine (m^6A), 5-methylcytosine (m^5C), N^1 -methyladenosine (m^1A), N^7 -methylguanosine (m^7G), N^4 -acetylcytidine (ac^4C) and pseudouridine (Ψ). These modifications regulate key aspects of RNA metabolism, such as stability, splicing, nuclear export, translation, localization, degradation, and interaction with RNA-binding proteins, and therefore play essential roles in both physiological and pathological processes[2]. For example, m^6A modification regulates embryonic stem cell and cancer self-renewal, and enhances cancer cell survival under DNA damage[3], while m^5C modification promotes nuclear export by recruiting reader proteins that facilitate mRNA transport from the nucleus to the cytoplasm[4].

Among these modifications, m^5C is one of the most abundant and conserved post-transcriptional modifications. Recent advances in sequencing technologies, such as RNA bisulfite sequencing (RNA-BisSeq), RNA immunoprecipitation sequencing (RIP-seq), and nanopore direct RNA sequencing, have enabled the mapping of m^5C sites across diverse RNA classes, including messenger RNA (mRNA), long non-coding RNA (lncRNA), microRNA (miRNA),

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and small nuclear RNA (snRNA)[5]. The broad distribution of m⁵C across RNA species enables it to coordinate multiple layers of gene expression and RNA regulatory networks. For example, m⁵C modification of p21 mRNA enhances its transcript stability and promotes efficient translation, thereby controlling cell cycle progression and stress responses[6]. Moreover, altered rRNA m⁵C levels in bacteria enhance the translation of stress response-decoding transcripts, promoting their survival under unfavorable conditions[7]. Therefore, the widespread regulatory functions of m⁵C make it indispensable, as its dysregulation can profoundly disrupt gene expression and cellular physiology.

Notably, m⁵C modification is reversibly and dynamically regulated by an enzymatic network of writers, readers, and erasers that deposit, recognize, and remove m⁵C marks on RNA. Disruption of this regulatory network has been strongly linked to a spectrum of human diseases, including cancer, cardiovascular disorders, and respiratory diseases. In cancer, aberrant m⁵C modification stabilizes oncogenic mRNAs and promotes tumor growth. For example, NSUN2 facilitates hepatocellular carcinoma proliferation by methylating H19 lncRNA[8]. In cardiovascular disease, m⁵C dysregulation aggravates mitochondrial dysfunction and endothelial inflammation, contributing to pathological remodeling[9,10].

Beyond non-communicable diseases, pathogens can exploit host m⁵C machinery to facilitate infection and pathogenicity. For instance, NSUN2-catalyzed m⁵C methylation of hepatitis B virus mRNA enhances viral mRNA export and protein translation while suppressing host innate immune response to evade host immunity[11]. Similarly, in the malaria parasite, m⁵C stabilizes transcripts essential for parasite development stages, thereby supporting life cycle progression and transmission[12,13].

Despite the growing recognition of m⁵C as a critical epitranscriptomic regulator, a comprehensive understanding of its disease-context-specific functions and regulatory mechanisms remains elusive. This review therefore provides a comprehensive overview of m⁵C RNA modification in non-communicable and communicable diseases, highlighting the roles of writers, erasers, and readers in regulating RNA and disease outcomes. By addressing these knowledge gaps, this review underscores the importance of studying m⁵C regulation and its potential for advancing diagnostics and therapeutic strategies.

2. m⁵C modification

m⁵C is a chemical modification resulting from methylation of the fifth carbon of cytosine residues, catalyzed by methyltransferases using S-adenosyl-methionine as the methyl donor[14]. Initially, m⁵C was identified in DNA as an epigenetic mark that controls

gene expression and maintains genome integrity[15] and was later discovered in RNA, particularly in tRNA and rRNA across multiple species[16].

The regulation of m⁵C modification in RNA is mediated by a coordinated network of regulatory enzymes comprising writers, erasers, and readers. Writers, including the NOP2/Sun RNA methyltransferase family (NSUN1-7) and DNA methyltransferase 2 (DNMT2), catalyze cytosine methylation with distinct substrate specificity[17,18]. For example, NSUN1 regulates ribosome assembly and translation efficiency, while NSUN3 supports mitochondrial translation and codon recognition[18,19]. Erasers such as ten-eleven translocation (TET1-3) and alpha-ketoglutarate-dependent dioxygenase ALKBH1 remove m⁵C modifications through oxidative reactions, converting them into 5-hydroxymethylcytidine (hm⁵C), thereby reducing the overall m⁵C levels and influencing mRNA stability and immune responses[20]. Readers recognize m⁵C-modified RNAs to mediate downstream regulatory functions. Key m⁵C readers include Aly/REF export factor (ALYREF), which facilitates mRNA nuclear export, and Y-box binding protein (YBX1), which regulates mRNA stability and translation[21,22]. More recently, MBD5 and MBD6 have also been identified as potential m⁵C readers, contributing to embryonic development, erythrocyte differentiation, iron metabolism, and behavior[23].

The interplay of writers, erasers, and readers is crucial for cellular homeostasis. At the chromatin level, NSUN2 methylates chromatin-associated RNAs (caRNAs), and the resulting m⁵C marks are recognized by MBD6 to remove repressive H2AK119ub histone marks, thereby promoting transcriptional activation. TET2 counterbalances this process by oxidizing m⁵C to 5-hydroxymethylcytosine (hm⁵C), limiting excessive activation and maintaining chromatin homeostasis[24]. At the post-transcriptional level, NSUN2-mediated m⁵C on CG-rich mRNA regions is recognized by ALYREF to recruit the Transcription-Export (TREX) complex, while NSUN2 loss impairs mRNA export and gene expression regulation[4]. In summary, coordinated m⁵C regulation is critical for proper gene expression, and dysregulation is linked to developmental defects and disease.

3. Role of m⁵C in non-communicable diseases

Non-communicable diseases represent one of the biggest global health challenges, accounting for over 43 million deaths annually. These chronic conditions, which develop progressively over extended latency periods, include cardiovascular diseases (19 million deaths), cancers (9.3 million deaths), chronic respiratory diseases (4.1 million deaths) and diabetes (2.0 million deaths) annually[25]. To explore this regulatory layer, this section will discuss the

mechanisms of m⁵C modification across major non-communicable diseases, including cancer, cardiovascular diseases, chronic respiratory diseases (CRDs), type-2 diabetes mellitus (T2DM) and autoimmune diseases.

3.1. Cancer

Among non-communicable diseases, cancer represents the most extensively studied context of m⁵C dysregulation. Dysregulated

Table 1. Functional roles, mechanisms, and outcomes of m⁵C regulators in cancer progression.

Cancer types	Functional role	Mechanisms	Outcomes/Impacts	Ref
Leukemia	Oncogenic	NSUN1 and NSUN2 bind BRD4 and upregulate RNA Pol II to activate m ⁵ C-enriched chromatin and form drug-resistant transcription complexes	Confers resistance to venetoclax	[26]
	Oncogenic	NSUN2 upregulates PHGDH and SHMT2 in the serine/glycine biosynthesis pathway	Promotes colony formation and inhibits ferroptosis to support cell survival	[27]
	Tumor suppressor	TET2 suppresses TSPAN13 expression <i>via</i> mRNA m ⁵ C demethylation	Reduces LSC homing to the bone marrow niche and suppresses LSC self-renewal and proliferation	[28]
HCC	Oncogenic	NSUN2 upregulates lncRNA H19 to activate oncogenic G3BP1 signaling	Promotes HCC proliferation, migration, and invasion	[8]
	Oncogenic	NSUN2 upregulates PKM2 to promote HCC glycolysis	Supports tumor growth and migration	[29]
	Oncogenic	NSUN2 upregulates GRB2/AATF/RNF115 to activate Ras/p-ERK signaling	Confers resistance to sorafenib	[30]
	Oncogenic	NSUN5 upregulates SMAD3 expression	Promotes EMT-mediated HCC invasion/metastasis	[31]
	Tumor suppressor	NSUN7 maintains CCDC9B expression to prevent excessive IVNS1ABP activity that stabilizes MYC	Helps restrain MYC-associated tumor-promoting signaling in liver cancer	[32]
	Tumor suppressor	NSUN2 upregulates XPD	Inhibits HCC proliferation/migration/invasion	[33]
Lung cancer	Oncogenic	NSUN2 upregulates QSOX1 <i>via</i> m ⁵ C modification; YBX1 stabilizes to promote QSOX1 translation in EGFR-mutant NSCLC	Confers gefitinib resistance	[34]
	Oncogenic	NSUN4 and YBX1 upregulate and stabilize DHCR24 expression	Promotes cancer cell proliferation	[35]
	Tumor suppressor	NSUN6 upregulates NM23-H1 to suppress EMT markers	Inhibits lung cancer cell proliferation, migration, and EMT	[36]
CRC	Oncogenic	NSUN2 methylates MALAT1 and stabilizes SLC7A11 to enhance GSH synthesis and resist ferroptosis	Inhibits ferroptosis and promotes apoptosis resistance	[37]
	Oncogenic	NSUN4 stabilizes NXPH4 and activates HIF1A signaling	Enhances angiogenesis and hypoxic tumor survival	[38]
	Oncogenic	NSUN5 upregulates Rb-CDKs signal transduction pathway, which causes G ₁ /S cell cycle progression	Promotes tumor cell proliferation by regulating the cell cycle	[39]
	Oncogenic	ALYREF recruits ELAVL1 to enhance nuclear export of RPS6KB2 and RPTOR, activating mTOR/S6K	Promotes CRC growth and migration	[22]
	Oncogenic	ALYREF recruits NSUN2 to m ⁵ C-methylate CASC19 lncRNA, stabilizing the HNRNPC/SCD complex	Promotes cell proliferation, metastasis, and stemness; reduces apoptosis and ferroptosis	[40]
Gastric cancer	Oncogenic	NSUN2 downregulates <i>CDKN1C</i> (p57 ^{Kip2}) mRNA and promotes cyclin-CDK activity	Promotes gastric cancer development	[41]

BRD4: bromodomain-containing protein 4; RNA Pol II: RNA polymerase II; PHGDH: phosphoglycerate dehydrogenase; SHMT2: serine hydroxymethyltransferase 2; TSPAN13: tetraspanin 13; LSC: leukemia stem cell; HCC: hepatocellular carcinoma; G3BP1: GTPase-activating protein SH3 domain-binding protein 1; PKM2: pyruvate kinase M2; GRB2: growth factor receptor-bound protein 2; AATF: apoptosis antagonizing transcription factor; RNF115: ring finger protein 115; p-ERK: phosphorylated extracellular signal-regulated kinase; NSCLC: non-small cell lung cancer; DHCR24: 24-dehydrocholesterol reductase; NM23-H1: NME1/NM23-H1 nucleoside diphosphate kinase; EMT: epithelial-mesenchymal transition; CRC: colorectal cancer; MALAT1: metastasis-associated lung adenocarcinoma transcript 1; SLC7A11: solute carrier family 7 member 11; GSH: glutathione; NXPH4: neurexophilin 4; HIF1A: hypoxia-inducible factor 1 alpha; ELAVL1: ELAV-like RNA-binding protein 1; RPS6KB2: ribosomal protein S6 kinase B2; RPTOR: regulatory-associated protein of mTOR; mTOR/S6K: mechanistic target of rapamycin/S6 kinase; CASC19: cancer susceptibility candidate 19; HNRNPC: heterogeneous nuclear ribonucleoprotein C; SCD: stearyl-CoA desaturase; CDKN1C (p57^{Kip2}): cyclin-dependent kinase inhibitor 1C.

expression or activity of m⁵C regulatory enzymes plays a critical role in tumor progression, influencing processes ranging from tumor initiation and metabolic reprogramming to immune evasion and therapeutic resistance. Rather than modifying a single pathway, these enzymes exert diverse and context-dependent functions by modifying distinct RNA substrates and interacting with different molecular partners across cancer types (Table 1).

NSUN2 is one of the most well-characterized oncogenic m⁵C methyltransferases that promotes tumor growth by enhancing the stability and translation of proliferation-associated transcripts across multiple malignancies. In hepatocellular carcinoma, NSUN2 methylates lncRNA H19 and cyclin-dependent kinase 1 (CDK1) mRNA to maintain high proliferation activity[8], while in bladder cancer, it methylates oncogenic transcripts such as RAB-like 6 (RABL6) and thymidine kinase 1 (TK1) to enhance their splicing, stability, and translation[42]. Similar oncogenic roles of NSUN2 have also been reported in gastric, breast, and ovarian cancers[41,43,44]. Other NSUN family members such as NSUN5 also exhibit pro-tumorigenic effects. For instance, NSUN5 overexpression correlates with advanced stage and poor prognosis in colorectal cancer, while its depletion suppresses proliferation and induces cell cycle arrest[39]. Likewise, NSUN5 downregulation in glioma impairs 28S rRNA methylation and protein synthesis, activating stress responses that limit tumor growth and improve treatment outcomes[45]. Despite these oncogenic roles, certain NSUN enzymes exhibit tumor-suppressive roles. NSUN6 suppresses tumor growth in both pancreatic and lung cancers by maintaining proper tRNA methylation and inhibiting proliferation, migration, and epithelial-mesenchymal transition[36,46].

Beyond intrinsic tumor cell regulation, m⁵C methylation plays a critical role in shaping the tumor microenvironment, a complex ecosystem that governs tumor growth and therapy response[47]. NSUN2 is enriched in macrophages and proliferative T cells, suggesting a role in influencing inflammatory signaling, while NSUN6 is preferentially expressed in regulatory T cells (Tregs), a key mediator of antitumor immune suppression[48]. Functional studies have shown that loss of NSUN6 in colon cancer disrupts methylation balance, leading to degradation of immune-regulatory transcripts and activation of oncogenic pathways such as MAPK/p38 and NF-κB. These molecular changes result in reduced immune cell infiltration and the establishment of an immunosuppressive microenvironment, which is associated with poor prognosis[49]. Besides, m⁵C methylation remodels the tumor microenvironment through metabolic reprogramming. For example, NSUN2 with YBX1 stabilizes enolase 1 (ENO1) mRNA (a glycolytic enzyme), enhancing glycolysis and lactic acid production (Warburg effect), thereby promoting tumor growth and creating an acidic microenvironment that facilitates invasion and metastasis[50]. m⁵C enzymes can also influence innate immune signaling, specifically

via the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway. NSUN2 and NSUN5 exhibit opposing regulatory effects on this pathway, with NSUN2 stabilizing three prime repair exonuclease 2 to suppress cGAS-STING-interferon signaling, enabling tumor cells to evade immune recognition[51], whereas NSUN5 activates cGAS-STING signaling *via* glutathione peroxidase 4 methylation, enhancing interferon production and promoting anti-tumor immune responses[52]. The contrasting roles of NSUN2 and NSUN5 suggest that m⁵C regulators may act in a coordinated manner to fine-tune immune responses within the tumor microenvironment, though this hypothesis requires further experimental validation.

Resistance to apoptosis is a hallmark of cancer and a major contributor to chemotherapy failure. m⁵C methylation has emerged as an important regulator of treatment response through its effects on DNA repair mechanisms, apoptosis and autophagy, cancer stemness, and non-coding RNA regulation[53]. In ovarian cancer, YBX1 stabilizes chromodomain helicase DNA-binding protein 3 mRNA *via* its interaction with poly(A) binding protein cytoplasmic 1, enhancing homologous recombination repair and conferring resistance to platinum-based therapy[21]. This phenomenon is also observed in anaplastic thyroid cancer, where NSUN2-ALYREF-mediated upregulation of serine/arginine-rich splicing factor 6 promotes alternative splicing of UDP-N-acetylglucosamine pyrophosphorylase 1 toward the AGX2 isoform. This promotes N-linked glycosylation and stabilizes ABC transporters, thereby enhancing chemotherapeutic drug efflux and ultimately driving resistance[54]. Conversely, in osteosarcoma, DNMT1 represses the NSUN2 promoter, reducing m⁵C modification and destabilizing anti-apoptotic mRNAs, thereby enhancing chemotherapy-induced apoptosis and highlighting a potential DNMT1-NSUN2 therapeutic axis[55]. Overall, these findings underscore that m⁵C regulatory enzymes play multifaceted roles in cancer biology. Their dual functions in promoting or suppressing tumor progression highlight both the complexity of epitranscriptomic regulation and the potential of m⁵C-targeted strategies for therapeutic intervention.

3.2. Cardiovascular diseases

Cardiovascular diseases remain the leading cause of death worldwide, accounting for approximately 20 million deaths annually, with increasing incidence among young populations[56]. Major cardiovascular diseases include ischemic heart disease, cardiomyopathy, hypertensive heart disease, arrhythmia, and inflammatory conditions such as myocarditis. The diverse functional roles, regulatory mechanisms, and clinical outcomes associated with these m⁵C regulators in cardiovascular pathologies are summarized in Table 2.

Mitochondrial homeostasis plays a central role in maintaining

cardiac function by regulating adenosine triphosphate production, calcium signaling, and redox balance. Dysregulation of mitochondrial biogenesis and degradation triggers reactive oxygen species overproduction, leading to cardiomyocyte death and adverse tissue remodeling[66]. In this context, RNA methyltransferases such as NSUN3 and NSUN4 are critical regulators of mitochondrial function. Both proteins localize predominantly to the mitochondrial matrix, where they facilitate mitochondrial translation and oxidative phosphorylation protein synthesis. NSUN3 methylates mitochondrially encoded methionine transfer RNA (mt-tRNA^{Met}) for 5-formylcytosine (f⁵C) formation, a modification essential for accurate mitochondrial protein synthesis[9]. This role is supported by findings from cardiac-specific NSUN3 knockout mice, which exhibit enlarged mitochondria with fragmented cristae, enhanced cardiac contractility, and age-associated mild heart enlargement[57]. Similarly, NSUN4 is required for mitochondrial ribosome maturation, and its deficiency similarly impairs oxidative phosphorylation protein synthesis, reduces ATP production, and leads to cardiomyopathy[67].

Beyond mitochondrial regulation, m⁵C enzymes exert protective effects in regulating cardiovascular stress responses and inflammation through mRNA stabilization. Under acute injury conditions, NSUN2 exerts protective effects through the enhancement of nuclear factor erythroid 2-related factor 2 expression

in doxorubicin-induced myocardial injury, thereby activating antioxidant pathways and mitigating reactive oxygen species-mediated damage and apoptosis[59]. In sepsis-induced myocardial injury, NSUN2 suppresses excessive ferroptosis by downregulating nuclear receptor coactivator 4 expression, leading to reduced lactate dehydrogenase leakage and inflammation[60]. Furthermore, other m⁵C writers like DNMT2 play a protective role during cardiac stress by stabilizing inhibitory RNAs such as Rn7sk, thereby preventing positive transcription elongation factor b activation and suppressing transcription of hypertrophic genes including α -myosin heavy chain (*Myh6*)/ β -myosin heavy chain (*Myh7*)[58].

Conversely, aberrant m⁵C modification frequently drives chronic inflammation. In homocysteine-induced atherosclerosis, NSUN2 exacerbates vascular inflammation by methylating intercellular adhesion molecule 1 (*ICAM-1*) and *IL-17A* mRNAs, thereby enhancing endothelial leukocyte adhesion, T-cell activation, and monocyte recruitment. This process establishes a positive feedback loop, whereby recruited monocytes further elevate homocysteine levels, sustaining NSUN2 activation and chronic inflammation[63]. Other than NSUN2, the demethylase TET3 contributes to disease progression in acute coronary syndrome by removing m⁵C modifications, which suppresses colon cancer associated transcript 2 expression and disrupts its interaction with RNA-binding protein

Table 2. Functional roles, mechanisms, and outcomes of m⁵C regulators in cardiovascular diseases.

Diseases/Conditions	Functional role	Mechanisms	Outcomes/Impacts	Ref
Cardiac hypertrophy	Pathological	NSUN2 upregulates LARP1 stability <i>via</i> m ⁵ C-YBX1 to elevate GATA4 expression	Promotes hypertrophic growth	[10]
	Protective	NSUN3 stabilizes mt-tRNA ^{Met} , increasing mitochondrial translation and OXPHOS	Maintains cardiac homeostasis	[57]
	Protective	DNMT2 stabilizes Rn7sk-P-TEFb complex and reduces <i>Myh6/Myh7</i> gene expression	Prevents cardiomyocyte hypertrophy	[58]
Doxorubicin-induced myocardial injury	Protective	NSUN2 upregulates Nrf2 expression and activates antioxidant response	Reduces ROS-mediated cardiomyocyte injury and apoptosis	[59]
Sepsis-induced myocardial injury	Protective	NSUN2 downregulates NCOA4	Reduces ferroptosis and inflammation	[60]
Post-MI remodeling	Protective	ALYREF upregulates Col1a2/Col3a1/Eln proteins and promotes fibroblast proliferation	Promotes necessary scar formation that prevents cardiac rupture	[61]
Atherosclerosis	Protective	ALYREF facilitates m ⁵ C-dependent CSD1 stabilization	Reduces endothelial apoptosis, inflammation, lipid dysfunction, and ferroptosis	[62]
	Pathological	NSUN2 upregulates ICAM-1 and IL-17A	Promotes leukocyte adhesion to endothelium, inflammation, and arteriosclerosis	[63]
	Pathological	NSUN3 increases M0/M1 macrophages, enhances proinflammatory cytokines, and reduces anti-inflammatory cytokines	Promotes plaque inflammation	[64]
Acute coronary syndrome	Pathological	TET3 downregulates CCAT2, reduces FUS binding and elevates TRIM14 expression	Promotes endothelial damage	[65]

LARP1: La-related protein 1; YBX1: Y-box binding protein 1; GATA4: GATA binding protein 4; mt-tRNA^{Met}: mitochondrial transfer RNA methionine; OXPHOS: oxidative phosphorylation; Rn7sk: RNA, 7SK small nuclear; P-TEFb: positive transcription elongation factor b; Myh6: myosin heavy chain 6; Myh7: myosin heavy chain 7; NCOA4: nuclear receptor coactivator 4; Nrf2: nuclear factor erythroid 2-related factor 2; ROS: reactive oxygen species; CSD1: CDGSH iron sulfur domain 1; ICAM-1: intercellular adhesion molecule 1; IL-17A: interleukin 17A; Col1a2: collagen type I alpha 2 chain; Col3a1: collagen type III alpha 1 chain; Eln: elastin; MI: myocardial infarction; TET3: ten-eleven translocation methylcytosine dioxygenase 3; CCAT2: colon cancer-associated transcript 2; FUS: fused in sarcoma; TRIM14: tripartite motif-containing 14.

FUS. This leads to upregulation of tripartite motif containing 14, thereby worsening coronary microvascular endothelial cell damage in acute coronary syndrome[65].

Persistent inflammation further drives pathological cardiac remodeling, leading to fibrosis and hypertrophy that compromise cardiac function. During myocardial infarction-induced cardiac fibrosis, NSUN2 and ALYREF act cooperatively to promote fibroblast activation and extracellular matrix deposition. NSUN2 stabilizes *HuR* mRNA, thereby upregulating fibrosis-associated markers (e.g., TGF- β , CTGF, and α -SMA) and promoting fibroblast proliferation and myofibroblast differentiation[68]. Meanwhile, ALYREF binds to m⁵C-modified transcripts of extracellular matrix components such as collagen and elastin, thereby promoting fibroblast proliferation and the synthesis of these matrix proteins[61]. NSUN2 and YBX1 promote maladaptive cardiac hypertrophy, where NSUN2-mediated m⁵C modification of La-related protein 1 (*LARPI*) mRNA enhances its stability *via* interaction with YBX1, which in turn protects the pro-hypertrophic transcription factor GATA binding protein 4 from degradation. Additionally, YBX1 boosts global protein synthesis through the mechanistic target of rapamycin complex 1 activation under stress conditions, collectively driving hypertrophic signaling[10,69].

3.3. CRDs

CRDs comprise a group of long-term conditions that affect the

lungs and airways, leading to persistent breathing difficulties. Major CRDs include chronic obstructive pulmonary disease (COPD), asthma, pneumoconiosis, interstitial lung disease, and pulmonary sarcoidosis. CRDs represent the third leading cause of death worldwide, accounting for approximately 400 000 deaths annually[70].

Recent studies reveal a role of m⁵C in regulating airway inflammation and structural remodeling, as summarized in Table 3. In asthma, NSUN2 has been identified as a pro-inflammatory regulator by stabilizing heterogeneous nuclear ribonucleoprotein K (*HNRNPK*) mRNA. This promotes platelet-derived growth factor-BB-induced changes in airway smooth muscle cells, such as proliferation, migration, invasion, and inflammatory cytokine production, key processes of airway thickening and chronic inflammation. While knockdown of NSUN2 suppressed these pathogenic effects, overexpression of HNRNPK restored them, establishing a direct mechanistic link between m⁵C modification and asthma pathogenesis[71].

Besides, m⁵C has also been implicated in environmentally induced pulmonary fibrosis. Exposure to fine particulate matter (PM_{2.5}) induces NSUN2 expression in lung epithelial cells, where it cooperates with the m⁵C reader ALYREF to enhance nuclear export and translation of chitinase-3-like protein 1 (*CHI3L1*) mRNA. Elevated CHI3L1 subsequently activates pro-fibrotic AKT/ERK signaling, driving epithelial-mesenchymal transition and fibrotic remodeling[81]. Consistent with this mechanism,

Table 3. Functional roles, mechanisms, and outcomes of m⁵C regulators in respiratory diseases, diabetic complications, and autoimmune diseases.

Diseases	Functional role	Mechanisms	Outcomes/Impacts	Ref
Asthma	Pathological	NSUN2 upregulates HNRNPK stability, enhancing PDGF-BB-induced ASMC pathological remodeling	Exacerbates airway remodeling and chronic inflammation	[71]
Pulmonary fibrosis	Pathological	NSUN2 upregulates CHI3L1 expression, leading to activation of the pro-fibrotic AKT/ERK signaling pathway	Promotes epithelial-mesenchymal transition and fibrotic remodeling	[72]
Type 2 diabetes mellitus	Pathological	NSUN2 upregulates ACSL6 to reduce glucose tolerance, insulin sensitivity, and hepatic lipid metabolism	Promotes hepatic steatosis, dyslipidemia, and hyperglycemia	[73]
Diabetic nephropathy	Pathological	NSUN2 upregulates SOCS1, enhancing macrophage infiltration and inflammatory responses	Exacerbates renal injury	[74]
	Protective	TET2 oxidizes Bcas3 m ⁵ C to promote mitophagy	Protects glomerular cells and attenuates renal damage	[75]
Diabetic retinopathy	Pathological	NSUN2 upregulates MUC1 and enhances microvascular endothelial cell proliferation, migration, and angiogenesis	Promotes retinal neovascularization and vision loss	[76]
Systemic lupus erythematosus	Protective	NSUN2 maintains CD4 ⁺ T cells m ⁵ C levels to suppress hyperactive proinflammatory pathways	Prevents tissue damage, systemic flares, and autoantibody production	[77]
	Pathological	NSUN4 promotes CD74 expression to cause CD8 ⁺ T-cell dysfunction	Promotes autoimmune responses and renal damage	[78]
Rheumatoid arthritis	Pathological	NSUN2 promotes FLS activation <i>via</i> Wnt/ β -catenin pathway	Promotes synovial inflammation and joint destruction	[79]
	Pathological	NSUN2 enhances CCL2 expression and drives M1 macrophage polarization	Promotes inflammatory responses and accelerates arthritis progression	[80]

HNRNPK: heterogeneous nuclear ribonucleoprotein K; PDGF-BB: platelet-derived growth factor-BB; ASMC: airway smooth muscle cell; CHI3L1: chitinase-3-like protein 1; ALYREF: Aly/REF export factor; AKT: protein kinase B; ERK: extracellular signal-regulated kinase; EMT: epithelial-mesenchymal transition; ACSL6: acyl-CoA synthetase long-chain family member 6; MUC1: mucin 1; SOCS1: suppressor of cytokine signaling 1; BCAS3: breast carcinoma amplified sequence 3; CD74: cluster of differentiation 74; FLS: fibroblast-like synoviocytes; CCL2: C-C motif chemokine ligand 2.

m⁵C epitranscriptome profiling reveals that NSUN2-mediated m⁵C modification also upregulates additional fibrosis-associated transcripts, including adiponectin (*ADIPOQ*), complement component 1q (*CIQ*), and matrix metalloproteinase 9 (*MMP9*), highlighting a broader pro-fibrotic RNA regulatory network[72].

In addition, transcriptomic studies further support the clinical relevance of m⁵C regulators as potential diagnostic and prognostic biomarkers in COPD and idiopathic pulmonary fibrosis, linking them to key pathways involved in disease progression. In COPD, m⁵C regulators (NSUN2, NSUN3, DNMT2, NOP2, TRMT112, YBX1, and ALKBH1) are increased during disease progression, driving NF-κB-mediated immune dysregulation and Th1/Th2 imbalance, thereby contributing to disease exacerbation—a finding validated in patient samples and smoke-exposure models[82]. In idiopathic pulmonary fibrosis, m⁵C is strongly linked to immune cell infiltration, cytokine signaling, and receptor activation, suggesting that m⁵C regulators contribute to disease progression by modulating immune-fibrotic signaling networks rather than individual genes[83].

3.4. Type 2 diabetes mellitus (T2DM)

T2DM is the most common form of diabetes, accounting for about 90%-95% of cases worldwide. It is characterized by chronic hyperglycemia due to insulin resistance and progressive beta-cell dysfunction[84]. Emerging epitranscriptomic studies have implicated m⁵C modification as a regulatory layer influencing metabolic homeostasis in T2DM. Transcriptomic profiling of peripheral blood from T2DM patients has identified 13 dysregulated m⁵C regulators, many of which are involved in glucose and lipid metabolism pathways[85]. Although specific mechanisms remain largely unknown, these findings suggest m⁵C as a promising therapeutic target requiring further validation.

Functional studies have further provided mechanistic evidence implicating NSUN2 as a key pro-diabetic m⁵C writer. NSUN2 upregulation has been reported in T2DM patients and high-fat diet-induced mouse models, whereas its silencing improved glucose tolerance, insulin sensitivity, and hepatic lipid metabolism by reducing m⁵C methylation of acyl-coA synthetase long-chain family member 6 (*ACSL6*) mRNA and destabilizing its transcript[73].

Beyond systemic metabolism, dysregulated m⁵C signaling also contributes to tissue-specific diabetic complications. In diabetic nephropathy, NSUN2 exacerbates renal injury by enhancing macrophage infiltration and inflammatory responses *via* m⁵C-dependent stabilization of suppressor of cytokine signaling 1 (*SOC1*) mRNA[74], whereas TET2 plays a protective role by limiting m⁵C accumulation on breast carcinoma amplified sequence 3 (*BCAS3*) mRNA, thereby preserving mitophagy (selective clearance of damaged mitochondria) in glomerular cells and attenuating renal

damage[75]. Similarly, in diabetic retinopathy, NSUN2 stabilizes m⁵C-modified mucin-1 (*MUC1*) transcripts and facilitates their ALYREF-mediated nuclear export, driving pathological endothelial proliferation and angiogenesis[76]. Collectively, these findings demonstrate the importance of balanced m⁵C writer-eraser activity in maintaining tissue homeostasis and highlight m⁵C regulators as context-dependent therapeutic targets in T2DM.

3.5. Autoimmune diseases

Autoimmune disease arises from immunological dysregulation, in which the immune system fails to differentiate self from non-self and mistakenly attacks its own healthy cells and tissues. This manifests in autoimmune conditions, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis, and type 1 diabetes. Notably, the global incidence and prevalence of autoimmune diseases have increased following the 2019 COVID-19 pandemic, with accelerated disease burdens like psoriasis and type 1 diabetes[86]. Among autoimmune diseases, this section will focus on RA and SLE as representative models.

Notably, NSUN2 is involved in the pathogenesis of both SLE and RA, but through different mechanistic pathways. In SLE, m⁵C modification modulates immune cell function and autoimmune pathogenesis. For instance, NSUN2 expression is reduced in CD4⁺ T cells from SLE patients alongside increased m⁵C sites specifically on cytokine signaling transcripts[87]. This selective hypermethylation likely promotes T-cell hyperactivity characteristic of SLE, where dysfunctional CD4⁺ T cells produce excess IL-17, infiltrate tissues, and drive autoantibody production[77]. Hence, these findings suggest NSUN2 downregulation may contribute to the pro-inflammatory T-cell phenotype. In parallel, NSUN4 has also been implicated in SLE pathogenesis, particularly in regulating CD8⁺ T-cell function. NSUN4-mediated m⁵C modification promotes CD74 expression, disrupts mitochondrial pathways, and contributes to CD8⁺ T-cell dysfunction. This observation was further supported by the functional studies showing that nanoparticle-mediated siRNA knockdown of NSUN4 in SLE mouse models significantly reduced autoimmune responses and renal damage, providing direct evidence for the pathogenic role of NSUN4 in SLE[78].

In contrast to SLE, NSUN2 is consistently upregulated in RA, where it promotes disease progression by modulating key inflammatory and signaling pathways. Mechanistically, NSUN2 contributes to fibroblast-like synoviocyte dysregulation by activating the Wnt/β-catenin signaling pathway, resulting in increased production of matrix-degrading enzymes (*e.g.*, MMP3), extracellular matrix components such as fibronectin, and multiple pro-inflammatory interleukins. Consistently, NSUN2 expression is significantly upregulated in both RA patients and collagen-

induced arthritis rat models compared to healthy controls[79]. In addition, NSUN2 facilitates M1 macrophage polarization *via* m⁵C modification of *CCL2* transcripts, thereby amplifying inflammatory responses and accelerating arthritis progression, while NSUN2 knockdown alleviates these effects, underscoring its potential as a therapeutic target in RA[80].

4. Role of m⁵C in communicable diseases

Communicable diseases, also known as infectious diseases, are caused by pathogenic agents, such as viruses, bacteria, parasites and fungi, which are transmitted through direct human contact or *via* vector-mediated routes. Major infectious diseases such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), tuberculosis, malaria, influenza, and COVID-19 are leading contributors to global morbidity and mortality. This section will discuss the mechanisms of m⁵C modifications across major communicable diseases, including viral infections, parasitic infections, and bacterial infections, which are summarized in Table 4.

4.1. Virus infections

m⁵C modification has emerged as a critical regulator in virus-host interactions. Its presence across diverse DNA and RNA viruses suggests a conserved and functionally important role in shaping viral infection outcomes[99]. Notably, m⁵C can exert both pro-viral and antiviral effects depending on the context. While m⁵C modification may enhance viral RNA stability and translation to support replication, it can also regulate host antiviral gene expression and immune signaling pathways. This dual functionality highlights the complex role of m⁵C in fine-tuning virus-host dynamics.

One major mechanism through which m⁵C promotes viral replication is by directly enhancing viral RNA stability, translation efficiency, and nuclear export. Host m⁵C methyltransferases, particularly NSUN2, frequently install m⁵C marks directly onto viral transcripts to act as a physical shield against RNA degradation, thereby facilitating viral replication and assembly. For instance, NSUN2-mediated m⁵C modification of HIV-1 viral RNA enhances translation efficiency, particularly of the *gag* transcript, which is essential for virion assembly[89]. Similar

Table 4. Functional roles, mechanisms, and outcomes of m⁵C regulators in communicable diseases (viral, parasitic, and bacterial infections).

Diseases	Functional role	Mechanisms	Outcomes/Impacts	Ref
HIV	Antiviral	NSUN1 competes with Tat for TAR binding and suppresses transcription	Restricts replication and promotes latency	[88]
	Proviral	NSUN2 methylates HIV-1 RNA to increase Gag expression	Promotes virus replication and assembly	[89]
HBV	Proviral	NSUN2 upregulates HBV RNA stability	Promotes virus replication	[90]
HCV	Proviral	YBX1 enhances HCV RNA stability	Promotes viral replication and facilitates virion assembly and budding	[91]
MLV	Proviral	ALYREF facilitates the nuclear export and expression of viral RNA	Enhances virus replication	[92]
SARS-CoV-2	Antiviral	NSUN2 downregulates viral transcripts	Reduces replication and virulence	[93]
	Proviral	TET2-mediated cellular hm ⁵ C enhances viral nucleocapsid stability	Promotes replication	[94]
Influenza	Proviral	NSUN2 modulates IAV vRNP transcriptional activity and genomic packaging rather than mRNA stability, thereby promoting proper assembly of infectious viral particles	Promotes infectious viral particle production and replication	[95]
Malaria	Pathological	PfNSUN1 regulates the late intraerythrocytic developmental cycle and supports schizont maturation	Enhances parasite proliferation and asexual-stage development	[96]
	Pathological	Pf/PyNSUN2 maintains global m ⁵ C levels and regulates gametocyte development	Promotes gametocyte production and parasite transmission	[12]
	Pathological	PfNSUN3 stabilizes m ⁵ C-modified <i>var</i> transcripts and regulates genes involved in parasite development	Supports early asexual-stage progression and facilitates immune evasion	[13]
Leishmaniasis	Pathological	NSUN2 maintains m ⁵ C-dependent stability of mRNAs involved in stage transitions and stress adaptation	Supports metacyclogenesis, promastigote-to-amastigote differentiation, and parasite stress adaptation	[97]
<i>Helicobacter pylori</i> infection	Pathological	m ⁵ C DNA methylation at GCGC motifs regulates metabolism, natural competence, adherence, and <i>crdRS</i> transcription	Supports copper efflux, metal resistance, and persistent gastric colonization	[98]

HIV: human immunodeficiency virus; Tat: trans-activator of transcription; TAR: trans-activation response element; Gag: group-specific antigen; HBV: hepatitis B virus; HCV: hepatitis C virus; YBX1: Y-box binding protein 1; MLV: murine leukemia virus; ALYREF: Aly/REF export factor; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; IAV: influenza A virus; vRNP: viral ribonucleoprotein; PfNSUN1: *Plasmodium falciparum* NSUN1; Pf/PyNSUN2: *Plasmodium falciparum*/*Plasmodium yoelii* NSUN2; PfNSUN3: *Plasmodium falciparum* NSUN3; GCGC: recognition motif for SC01731-mediated DNA methylation; *crdRS*: copper-responsive two-component regulatory system.

proviral effects are observed in hepatitis B virus, Influenza A virus, and Nipah virus, where NSUN2 modifies viral transcripts to boost replication[11,95,100]. Furthermore, viral RNA modification often occurs through coordinated actions of multiple enzymes. In HIV-1 infection, DNMT2 acts cooperatively with NSUN2 to regulate m⁵C modifications, with DNMT2 re-localizing to stress granules to methylate viral RNA and enhance its post-transcriptional stability throughout the viral replication cycle[101].

Following methylation on viral transcripts, host m⁵C reader proteins such as YBX1 and ALYREF recognize these modifications and execute proviral functions. For instance, YBX1 binds to an m⁵C site at position C7525 within the NS5A-encoding region of the HCV genome, significantly enhances HCV RNA stability, promotes viral replication, and facilitates virion assembly and budding[91]. Similarly, ALYREF binds to m⁵C modifications on murine leukemia virus transcripts, acting as a transport adaptor that facilitates the nuclear export and subsequent expression of the viral RNA[92]. Beyond exploiting reader proteins, viruses can also hijack m⁵C erasers that dynamically remodel the epitranscriptomic landscape by oxidizing m⁵C to generate hm⁵C. During severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, endogenous TET2 expression and TET2-mediated m⁵C hydroxylation are significantly upregulated alongside NSUN2, leading to increased cellular hm⁵C that enhances viral nucleocapsid stability, elevates protein levels, and promotes replication[94].

m⁵C modification is not limited to mammalian systems but is also present in mosquito vectors, where it modulates viral replication within the vector and impacts arboviral transmission. In mosquito tissues, infection with arboviruses such as Dengue, Chikungunya, and Sindbis viruses induces upregulation of the host methyltransferase DNMT2, which methylates viral genomes to enhance viral replication in the mosquito vector[102]. Notably, current biocontrol strategies employing *Wolbachia* bacteria suppress arboviral transmission by downregulating host DNMT2 expression in mosquito vectors. This reduction in m⁵C deposition on viral RNA limits replication in the mosquitoes, thereby contributing to pathogen blocking in mosquitoes[103].

In contrast to their proviral roles, the host can also utilize m⁵C regulators as viral restriction factors. For example, host NSUN1 acts to restrict HIV-1 replication and promote viral latency by suppressing viral transcription. Mechanistically, NSUN1 directly competes with the viral Tat protein for binding to the TAR RNA stem-loop structure at the HIV-1 5'-LTR. By displacing Tat, NSUN1 prevents the recruitment of positive transcription elongation factor b (P-TEFb; CDK9/CCNT1) and the subsequent RNA polymerase II (RNA Pol II)-driven transcriptional elongation, which ultimately reduces viral protein production and restricts replication[88]. Interestingly, NSUN2 can exhibit context-dependent effects on viral RNAs, including promoting their degradation. In Epstein-Barr

Virus (EBV) infection, NSUN2 installs m⁵C modifications onto EBV-encoded small RNA 1 (EBER1), which is further cleaved by RNase angiogenin, thereby reducing its levels to prevent viral RNA overload in infected cells and limiting the immune-suppressing effects during latency[104]. Likewise, NSUN2 knockout in Caco-2 cells infected with SARS-CoV-2 reduces m⁵C modification of viral RNA and consequently enhances viral replication, suggesting that NSUN2-mediated methylation is a host restriction mechanism that limits viral replication[93].

Viruses can modulate host innate immune responses to favor their own replication. Studies have demonstrated that NSUN2 negatively regulates type I interferon (IFN-I) responses by inhibiting the retinoic acid-inducible gene I (RIG-I) pathway and promoting the degradation of interferon regulatory factor 3 (IRF3). In particular, NSUN2 suppresses type I IFN signaling by depositing m⁵C modifications on Pol III-transcribed ncRNAs (RPPH1, 7SL, 7SK), thereby limiting their expression, which effectively suppresses the RIG-I-mediated immune signaling pathway[105]. On the other hand, NSUN2 negatively regulates type I IFN by catalyzing m⁵C methylation on *IRF3* mRNA, which accelerates *IRF3* mRNA degradation and reduces IRF3 protein levels, thereby limiting interferon-beta/interferon-stimulated gene production[106]. In response, host cells can dynamically counter this viral-mimicking suppression by downregulating endogenous NSUN2 levels during infection. This reduction in NSUN2 effectively boosts the host's antiviral IFN-I responses to facilitate more efficient viral clearance. This effect has been observed in infections with Sendai virus, vesicular stomatitis virus, herpes simplex virus type 1, Zika virus, and SARS-CoV-2[106].

In summary, the biological outcome of NSUN2-mediated m⁵C regulation is dictated by the dynamic interplay between host and viral control mechanisms. Viral dominance is associated with stabilization of NSUN2, leading to suppression of IFN signaling and enhanced viral RNA stability and translation. In contrast, host-dominant responses are characterized by downregulation of NSUN2, which relieves IFN suppression, promotes antiviral signaling, and facilitates the degradation of viral transcripts.

4.2. Parasitic infections

Parasitic infections represent a global health crisis, with intestinal parasites alone impacting approximately 3.5 billion people and causing 450 million illnesses annually[107]. Despite their diverse life cycles and host environments, parasite pathogenicity commonly relies on coordinated and complex gene expression programs required for host adaptation and immune evasion. In this context, m⁵C modification has also been linked to these regulatory processes, with most current studies focusing primarily on malaria parasites.

Malaria is caused by *Plasmodium* species and transmitted through

the bite of infected *Anopheles* mosquitoes. Recent studies have revealed that *Plasmodium* parasites encode homologs of human NSUN proteins, including NSUN1, NSUN2 and NSUN3, referred to as Pf/PyNSUN1-3. These enzymes regulate multiple stages of the parasite life cycle, encompassing asexual growth, sexual differentiation, transmission, and immune evasion. For instance, PfNSUN1 functions primarily during the late intraerythrocytic developmental cycle, where it supports schizont maturation and efficient parasite proliferation[96]. Comparable findings have been reported for Pf/PyNSUN2, where it was shown to regulate sexual-stage development. Knockout of Pf/PyNSUN2 reduces global m⁵C levels and significantly impairs gametocyte production in both *Plasmodium falciparum* and *Plasmodium yoelii*[12]. Gametocyte formation is essential for transmission to mosquitoes rather than direct host cell invasion; thus NSUN2 represents a potential transmission-blocking target. PfNSUN3 regulates early asexual-stage progression, particularly during the ring stage, through downregulating gene expression involved in cytoskeletal organization, motility, adhesion, and DNA replication. Other than developmental regulation, PfNSUN3 also contributes to parasite virulence and immune evasion. For instance, *Plasmodium falciparum* evades detection by switching *var* gene expression to display variant PfEMP1 surface proteins. This is evidenced by knockdown of *PfNSUN3*, which disrupts *var* gene transcriptional regulation, leading to loss of PfEMP1 expression and impaired immune concealment. Collectively, these findings demonstrate that PfNSUN enzymes coordinate diverse processes throughout the intraerythrocytic life cycle[13]. However, m⁵C readers and erasers in *Plasmodium* have not yet been identified, representing an important gap for future research.

Aside from *Plasmodium*, similar roles for m⁵C modification have been observed in other protozoan parasites. In *Leishmania*, the causative agent of cutaneous leishmaniasis, NSUN2 knockout disrupts the parasite cycle (metacyclogenesis) and alters promastigote-to-axenic amastigote differentiation under stress conditions. These defects are linked to impaired mRNA stability of stage-specific surface proteins and pathways involved in protein folding and nucleotide metabolism, underscoring the role of m⁵C in parasite differentiation and stress adaptation[97]. In *Toxoplasma gondii*, m⁵C modifications have been identified on mRNAs encoding proteins involved in replication, invasion, metabolism, and virulence, suggesting a regulatory role in parasite pathogenicity. Notably, hypermethylation has been observed in virulence-associated transcripts, including those encoding dense granule proteins (GRAs), rhoptry proteins (ROPs) and micronemal proteins (MICs)[13]. GRA12 exemplifies a key regulatory mechanism. Deletion of *GRA12* in IFN- γ -activated macrophages results in collapse of the parasitophorous vacuole and increased host cell necrosis, while the hypermethylation of *GRA* transcripts suggests that m⁵C modification may act as a critical stabilizer, maintaining

sufficient GRA12 expression to facilitate parasite survival and virulence[13]. In conclusion, m⁵C modification functions as a key regulatory mechanism in parasites, supporting their adaptation to host environments and the coordination of complex life cycle transitions. Moreover, it also stabilizes virulence-associated transcripts and facilitates immune evasion, thereby contributing to parasite persistence and overall pathogenic potential.

4.3. Bacterial infections

Bacteria exhibit m⁵C modifications on both DNA and RNA, where these epigenetic marks serve as critical regulators of environmental adaptation. For example, DNA m⁵C methylation has been well characterized in *Streptomyces coelicolor*, where it regulates bacterial growth and secondary metabolism. In particular, the m⁵C DNA methyltransferase SCO1731 is essential for the normal life cycle of *Streptomyces coelicolor*, including spore germination, mycelial development, and the production of antibiotics such as undecylprodigiosin and actinorhodin[108]. Although *Streptomyces coelicolor* is a non-pathogenic model organism, insights from its study may extend to pathogenic relatives. These findings suggest that m⁵C methylation could contribute to virulence-associated processes in species such as *Streptomyces somaliensis*, though direct evidence remains limited. In *Helicobacter pylori*, a major cause of gastric disease, m⁵C methylation at specific DNA motifs (e.g., GCGC sequences) regulates multiple cellular processes, including metabolism, natural competence, and adherence to gastric epithelial cells[98]. Importantly, m⁵C-dependent regulation ensures proper transcription of copper-responsive (*crdRS*) two-component regulatory system, which is required for copper efflux, metal toxicity resistance, and persistent colonization within the hostile gastric environment[98].

m⁵C modifications are also present in bacterial RNA, occurring predominantly at rRNA and tRNA sites. One of the earliest identified bacterial m⁵C methyltransferases is RsmB in *Escherichia coli*, which catalyzes methylation at C967 in 16S rRNA, stabilizing the decoding center and ensuring translational fidelity[109]. Another example of bacterial methyltransferases is RsmB and RsmF, which are responsible for m⁵C modifications in 16S rRNA of *Thermus thermophilus*[110]. Dynamic remodeling of these modifications has been implicated in bacterial stress adaptation. For instance, disruption of *rsmF*, which mediates m⁵C1407 modification, impairs the growth of *Escherichia coli* under oxidative stress conditions, particularly during the lag phase of adaptation[7]. Collectively, these findings suggest that m⁵C modifications support bacterial stress responses by preserving translational efficiency and enabling rapid cellular adaptation under adverse conditions. However, the direct role of m⁵C-mediated regulation in bacterial pathogenicity during infection remains largely unexplored.

5. Potential m⁵C therapeutic intervention

m⁵C modification has gained significant prominence, particularly as a high-precision diagnostic and prognostic indicator that often outperforms standard biomarkers (Table 5). Unlike conventional protein markers, dysregulated m⁵C signatures in patient biofluids offer a non-invasive approach, facilitating accessible and early-stage screening for both metabolic and malignant pathologies. In cardiovascular disease, machine learning-integrated transcriptomics has been used to refine m⁵C diagnostic models. For instance, four m⁵C regulatory markers—DNMT3B, NOP2, NSUN6, and DNMT1—have been identified as constituting a good diagnostic model, which shows superior diagnostic accuracy (AUC = 0.869) for heart failure[47]. Parallel to these findings in oncology, m⁵C levels in peripheral blood immune cells (predominantly monocytes) distinguish colorectal cancer patients from healthy controls with high sensitivity (AUC = 0.888-0.909), and this signature significantly outperforms the standard carcinoembryonic antigen marker. This is supported by upregulated NSUN5/YBX1 expression in blood samples of colorectal cancer patients[111]. Crucially, m⁵C also exhibits dynamic clinical correlations that reflect disease progression and therapeutic response. In non-small cell lung cancer, leukocyte m⁵C levels progressively decrease from NSCLC stage I to IV, particularly in metastatic cases, but significantly increase post-chemoradiotherapy[112]. This demonstrates that m⁵C serves as both a diagnostic tool and a real-time therapeutic response indicator. Despite these promising observations, m⁵C diagnostic markers remain preclinical due to systemic challenges such as limited cohort studies and lack of large-scale prospective validation. Hence, advancing detection technologies to achieve the high sensitivity and specificity required for precise m⁵C mapping will lay the essential foundation for transitioning this research into validated clinical diagnostics and targeted therapeutic interventions.

Furthermore, identifying m⁵C regulatory enzymes as druggable targets offers therapeutic potential for recalibrating gene expression across diverse pathological contexts, extending beyond their role as mechanistic regulators. To date, the established clinical trajectory of m⁶A modulation provides a robust blueprint for this shift. Notably, the m⁶A RNA methyltransferase METTL3 inhibitor STC-15 (NCT05584111) has demonstrated favorable safety and preliminary

efficacy in Phase 1 clinical trials involving patients with advanced solid tumors ($n = 33-40$). Adverse events were predominantly low-grade (grade 1-2), with only a single dose-limiting toxicity observed, indicating good tolerability. In addition, treatment also induced approximately a 63% reduction in blood m⁶A levels within 24 hours, together with regulation of interferon-associated transcripts[113]. Building on the clinical success of m⁶A modulation, the field of m⁵C research has recently pivoted toward targeted therapeutic intervention. Discovery of MY-1B as a covalent NSUN2 inhibitor has emerged as a significant breakthrough. MY-1B exerted potent anti-leukemic effects by disrupting the m⁵C-YBX1 signaling axis. Mechanistically, it blocks the methylation of the ferroptosis suppressor protein 1 (*FSP1*) mRNA 3'UTR, thereby destabilizing this oncogenic ferroptosis suppressor and synergizing with standard chemotherapy to trigger cancer cell death[114]. Moreover, MY-1B has shown promise in treating viral infections, specifically suppressing NiV replication in both *in vitro* and animal models by destabilizing essential viral matrix (M) transcripts and halting virion assembly[100]. Together, these findings establish MY-1B not only as a potent anti-tumor agent but also as a versatile tool for antiviral intervention, underscoring the broad therapeutic potential of targeting the NSUN2-mediated m⁵C landscape. Complementing these findings, the selective NSUN3 inhibitor B19 has demonstrated similar therapeutic potential. B19 disrupts mitochondrial oxidative phosphorylation, inducing energy stress that activates the AMPK pathway and subsequently suppresses STAT3 signaling[115]. Although NSUN3 inhibition shows significant promise in treating colorectal cancer, its vital role in cardiac function requires tissue-selective delivery to avoid potential cardiotoxicity.

Beyond traditional drug discovery, the field is simultaneously advancing through AI-driven virtual screening and innovative gene-editing technologies. Computational screening identified non-covalent NSUN2 inhibitors (ZINC-1000507789) targeting the SAM cofactor pocket with high affinity. These are predicted to reduce m⁵C levels by reversibly blocking methyltransferase activity, avoiding the permanent modifications associated with covalent binders like MY-1B[115]. Complementing virtual screening, an AI-driven platform *de novo* designed NSUN1/2 inhibitors that overcome venetoclax resistance in acute myeloid leukemia and lung cancer cells. These inhibitors act by disrupting active chromatin structure complexes

Table 5. Potential of m⁵C regulatory machinery as clinical biomarkers for non-communicable diseases.

Diseases	Potential markers	Sample type	Diagnostic value (AUC)	Clinical outcomes	Ref
CRC	Peripheral blood immune cell m ⁵ C levels	Blood	0.888-0.909	Tumor biomarker	[111]
NSCLC	Leukocyte m ⁵ C levels	Blood	0.912	Therapeutic response marker	[112]
Heart failure	DNMT3B/NOP2/NSUN6/DNMT1	Left ventricular tissue	0.869	Diagnosis & classification	[47]
Type 2 diabetes	ZBTB4 (DNA 5mC reader)	Skeletal muscle tissue	0.655	Diabetes diagnosis	[85]

DNMT3B: DNA methyltransferase 3B; NOP2: nucleolar protein 2; NSUN6: NOP2/Sun RNA methyltransferase family member 6; DNMT1: DNA methyltransferase 1; ZBTB4: zinc finger and BTB domain containing 4; AUC: area under the curve.

that drive drug resistance, and their efficacy has been validated *in vivo* using syngeneic mouse models of acute myeloid leukemia[26]. Besides, development of the RNA-targeted CRISPR-mediated m⁵C modification system has improved the precision of epitranscriptomic manipulation. One such approach is the dCasRx-based system, which uses a catalytically inactive Cas13 protein (dCasRx) to recruit RNA-modifying enzymes to specific target transcripts. This system enables precise and reversible manipulation of m⁵C modifications on specific mRNAs by recruiting methyltransferases or demethylases. For example, dCasRx-NSUN6 has been shown to increase m⁵C modification on ribosomal protein SA mRNA (transcript for proliferation), resulting in significantly increased protein levels and accelerated wound healing[116]. This demonstrated that RCMS serves as a powerful, reversible model for exploring m⁵C function, offering therapeutic potential that extends far beyond proliferation control.

Overall, the m⁵C epitranscriptome has transitioned from a fundamental biological concept into a potent clinical reality, encompassing high-precision biomarkers, small-molecule inhibitors, and RCMS gene-editing platforms. Although current translational efforts remain predominantly cancer-focused, emerging successes in metabolic, viral, and parasitic disease models highlight its broad therapeutic potential across both non-communicable and communicable diseases. Increasing structural and mechanistic insights into m⁵C regulatory machinery enable the design of precision drugs and RNA-targeted editing tools, positioning m⁵C-based therapeutics as a cornerstone of future precision medicine.

6. Conclusion

Discovery of m⁵C as a dynamic epitranscriptomic mark has revolutionized the understanding of post-transcriptional gene regulation in human disease. This review has synthesized evidence across diverse pathologies, revealing that m⁵C regulators act as context-dependent modulators whose dysregulation is frequently hijacked in both communicable and non-communicable diseases. While the transition from laboratory observation to clinical application remains ongoing, the emergence of site-specific intervention tools marks a critical turning point. However, the context-dependency of these regulators introduces a significant therapeutic paradox. For example, while NSUN2 predominantly drives pathological processes in cancer and viral infections, it also exerts vital protective functions in SLE and certain host antiviral responses. Broad systemic inhibition could inadvertently trigger severe, off-target pathological or immunological consequences. Therefore, the field must resolve this mechanistic duality and prioritize the development of highly targeted, tissue-specific delivery systems before these interventions can be safely transitioned to

clinical application. Ultimately, m⁵C epitranscriptomic landscape is poised to serve as a foundation for next-generation precision medicine, bridging high-precision diagnostics with targeted therapies across diverse human pathologies.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Authors' contributions

SYK and KLC conducted a comprehensive literature search and contributed to manuscript preparation. KLC, NZ, and SA contributed to manuscript editing, reviewing, and refining the content. All the authors have reviewed and approved the final manuscript.

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