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Forum

RNA modifications: disease biomarkers and signaling molecules

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RNA modifications constitute a dynamic regulatory code governing RNA structure, fate, and translation. Modified nucleosides, long dismissed as metabolic waste, are now recognized as stable biomarkers and active signaling messengers involved in metabolic and reproductive regulation. Advances in methods for detecting modified nucleosides could accelerate translation toward clinical diagnostics and monitoring.

RNA modifications are recognized as an intrinsic part and a dynamic regulatory layer of RNA, expanding RNA functionality beyond the constraints of the primary sequence. By altering charge, hydrophobicity, and structural plasticity, these marks modulate RNA folding, localization, RNA–protein interactions, stability, and translation, establishing context-dependent regulation of gene expression in health and disease. Emerging evidence shows that RNA modifications act not only within intact RNAs but also through modified nucleosides generated during RNA turnover, which can function as hormonelike signaling molecules and as stable, informative biomarkers linking RNA metabolism to organismal physiology. In this Forum, we discuss RNA modifications as biomarkers in reproductive and endocrinological disorders and also discuss downstream signaling roles of modified nucleosides; these stable chemical marks preserve cellular information beyond RNA degradation, bridging metabolism and signaling.

RNA modification code as a source of clinical diagnostic readouts

The 'RNA modification code' arises from combinatorial patterns of over 170 known modifications, orchestrated by condition-specific enzymatic machinery. Writers/erasers (e.g., enzymes) install/remove these marks, and readers (e.g., binding proteins) interpret them, creating dynamic signatures that adapt to the cellular environment, including metabolic cues. These enzymatic shifts generate disease-specific codes, detectable in biofluids, which could be harnessed for precision diagnostics (Figure 1A).

Intriguingly, the RNA modification code remains informative even when RNAs degrade into single nucleosides. These modification profiles, reflecting physiological and pathological states, retain combinatorial signatures. Pioneering applications of liquid chromatography-tandem mass spectrometry (LC–MS/MS) have showcased its power to quantify multiple RNA modifications simultaneously [1], revealing environmental and metabolic impacts. For instance, in mice exposed to a high-fat diet (HFD), LC–MS/MS profiling of sperm revealed altered transfer RNA–derived small RNAs (tsRNAs) with elevated 5-methylcytosine (m^5C) and 2-methylguanosine (m^2G), mediating intergenerational transmission of metabolic disorders such as glucose intolerance [2]. Enzymatic control by writers such as DNMT2 installs these marks, as its deletion abolished the HFD-induced changes and prevented their inheritance [3]. Recently, LC–MS/MS was used to detect RNA modifications in mouse uterine fluid and revealed changes under maternal HFD [4]. These studies established LC–MS/MS as a robust tool to decode modification signatures in mouse models, distinguishing normal from pathological states.

Critically, LC–MS/MS is clinically feasible for detecting single modified nucleosides. As early as the 1960s, methylated purines were detected in rat urine and traced to RNA turnover [5]. Intriguingly, these methylated purines increased significantly in the urine of tumor-bearing rats compared with healthy ones [5]. Recently, low-input [6] and enhanced-sensitivity [7] LC–MS/MS methods have enabled fragmented, modified nucleosides to be used as diagnostic readouts for disease detection and monitoring (Figure 1B).

Building on the foundational insights using LC–MS/MS to identify the differential RNA modification profiles in studies from mouse and human tissues [2–4,8,9], recent studies have expanded to clinical translational applications with minimally invasive approaches (e.g., blood, semen, and urine), revealing disease-specific modification profiles. In ovarian disease, blood-borne RNA modification levels track clinical indices (e.g., anti-Müllerian hormone) and support polycystic ovary syndrome (PCOS) classification. A two-RNA-modification panel (m^6A and m^7G) achieved >93% accuracy, outperforming conventional clinical approaches, based on abnormal serum androgen levels, menstrual cycle irregularities, and polycystic ovarian morphology as defined by the Rotterdam criteria [10]. In cardiopulmonary disease, a seven-RNA-modification signature in peripheral blood also distinguished pulmonary hypertension and its subtypes with approximately 90% accuracy [11]. In andrology, semen RNA modification profiles correlate with sperm motility. A 13-RNA-modification signature (seven from RNA fragments combined with six from total RNA) was strongly associated with the proportions of immotile and progressively motile sperm and may aid assessment of asthenozoospermia and teratozoospermia [12].

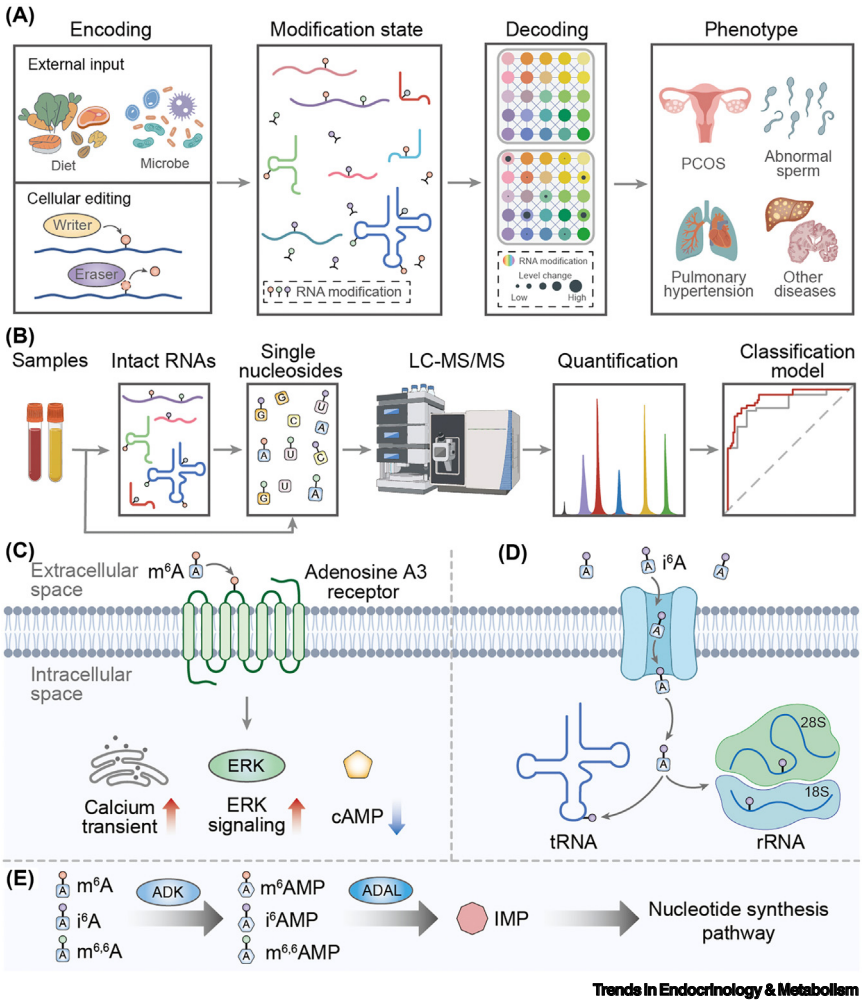

Figure 1. RNA modifications act as detectable biomarkers and signaling molecules. (A) Schematic diagram illustrating the establishment and biological interpretation of 'RNA modification code'. External input (e.g., diet and microbes) or cellular editing processes (e.g., altered expression or activity of RNA modification writers and erasers) shape a coordinated landscape of RNA modification types and abundance, referred to as the RNA modification code. Dysregulation of this code may contribute to disease pathogenesis, including PCOS, abnormal sperm, pulmonary hypertension, and other diseases. (B) LC–MS/MS enables simultaneous quantification of multiple RNA modifications; intact RNAs isolated from clinical samples are enzymatically digested into individual nucleosides, which are then simultaneously detected and quantified by LC–MS/MS. Integration of these quantitative data with classification models enables decoding of the RNA modification code, serving as a powerful tool for precise disease detection and biomarker discovery. (C) Extracellular m^6A binds to the adenosine A3 receptor, inducing calcium transients and ERK signaling activation while reducing cAMP levels. (D) Extracellular i^6A is taken up by cells and incorporated into tRNAs and rRNAs. (E) Cells employ a clearance pathway to detoxify excess modified nucleosides. Figure was created with BioRender (https://biorender.com/). ADAL: adenosine deaminase-like enzymes; ADK: adenosine kinase; IMP: inosine monophosphate; LC–MS/MS: liquid chromatography-tandem mass spectrometry; PCOS: polycystic ovary syndrome.

Such panels capture snapshots of the RNA modification code, where combinatorial shifts, driven by enzymatic dysregulation, reflect underlying pathophysiology. For instance, PCOS-associated m^6A/m^7G changes may stem from ovarian tissues, offering causal insights beyond symptom-based classification. These modification signatures might arise from dynamic enzymatic orchestration in affected tissues and create combinatorial codes that vary by condition, which lead to detectable modification changes in biofluids.

The stability and chemical diversity of modified nucleosides confer analytical robustness compared to labile RNA or protein biomarkers. Their detection in accessible fluids offers a minimally invasive window into metabolic and reproductive health. However, clinical translation requires standardized reference ranges, normalization for renal function and dietary variability, and longitudinal studies linking dynamic modification changes to treatment outcomes.

Modified nucleosides: from waste to signaling messengers

Interestingly, beyond their utility as biomarkers, individual modified nucleosides can act as active signaling molecules that elicit downstream pathways and shape systemic regulation.

RNA catabolism releases both unmodified and modified nucleosides. Unmodified nucleosides can be recycled through the nucleotide salvage pathway, whereas many modified nucleosides are historically regarded as terminal catabolites that are excreted and measurable in extracellular fluids as metabolic waste. Recent mass spectrometry studies show that modified nucleosides comprise nearly 50% of all nucleosides in human plasma and rodent serum, and over 90% in mouse and human urine, exceeding unmodified nucleosides [13]. Despite variation driven by diet, renal function, microbial contributions, and the analytical platform, the recurrent enrichment of extracellular modified nucleoside signals suggests active physiological roles rather than inert waste.

Stress increases extracellular m^6A-modified adenosine in culture medium, serum, and interstitial fluids. This pool derives predominantly from intracellular RNAs, such as

rRNAs, via endoribonucleolytic turnover, independent of changes in m^6A writer/eraser expression or generic decay factors [13]. Interestingly, extracellular m^6A acts as an endogenous human A3 adenosine receptor (hA3R) agonist, with nearly tenfold higher apparent potency than adenosine, activating cellular Extracellular signal-regulated kinase 1/2 (ERK1/2) signaling and calcium transients while decreasing cAMP levels in allergic/inflammatory contexts, suggesting that extracellular modified nucleosides can function as signaling molecules [13] (Figure 1C).

Beyond acting at cell surface receptors, some extracellular modified nucleosides can be taken up by cells and fed back into RNA synthesis. For example, exogenous N6-isopentenyladenosine (i^6A) added to culture medium can be transported into multiple cell lines and is cotranscriptionally incorporated into 18S/28S rRNA in a Pol I-dependent manner [14]. Notably, i^6A is also detected in tRNA fractions, yet ms^2i^6A—the product of tRNA isopentenyltransferase 1 (TRIT1) (i^6A-modifying enzyme)—does not increase, and i^6A incorporation persists in TRIT1-deficient cells, indicating a TRIT1-independent uptake/integration route [14]. The erroneous incorporation of i^6A into rRNAs and tRNAs can disrupt translation through the impairment of their cellular functions, ultimately resulting in cell death (Figure 1D).

To counterbalance this ligand activity and the potential cytotoxicity of extracellular modified nucleosides, cells engage a conserved clearance pathway. Adenosine kinase (ADK) first phosphorylates m^6A, i^6A, and other modified adenosines (e.g., N6,N6-dimethyladenosine, $m^{6,6}A$), after which adenosine deaminase-like enzymes (ADAL family) deaminate the phosphorylated species to inosine monophosphate, which then re-enters cellular metabolism [15] (Figure 1E). This ADK/ADAL axis is conserved across species and likely safeguards cells from supraphysiologic exposure to modified adenosines. Disrupting this clearance pathway impairs the handling of modified adenosines and perturbs lipid metabolism [15].

These discoveries open a new frontier for endocrine research. Given the known roles of adenosine signaling in both metabolic and reproductive tissues, it is tempting to speculate that extracellular modified nucleosides may act within endocrine tissues. For example, does extracellular m^6A released from stressed adipocytes act on A3Rs in nearby preadipocytes or macrophages to modulate insulin sensitivity or local inflammation? In the ovary, could modified nucleosides in the follicular fluid act as paracrine signals, influencing granulosa cell function or oocyte maturation? Exploring these modification–receptor axes within endocrine organs is a critical next step to unite the biomarker and signaling fields.

The capacity for extracellular modified nucleosides to be reincorporated into recipient cell RNAs [14] suggests a novel mechanism of intercellular communication, moving beyond simple receptor agonism. This posits that one cell type (e.g., stressed hepatocyte) could 'donate' modification building blocks, directly influencing the epitranscriptomic landscape and subsequent gene expression programs of another cell type (e.g., pancreatic beta cells). Extending this to reproductive contexts, in the ovary, modified nucleosides in follicular fluid may engage adenosine receptors on granulosa cells to regulate steroidogenesis and oocyte maturation, potentially exacerbating PCOS. In the testis, similar paracrine signaling could modulate spermatogenesis, intersecting with insulin pathways to link metabolic disorders with male infertility. This 'epitranscriptomic seeding' could be a powerful way for the maternal metabolic environment to directly shape the oocyte's RNA modification profile, with potential implications for intergenerational inheritance. Determining the full extent of this pathway, which other modifications are transported, and by what mechanisms should be a key priority.

Together, these findings blur classical boundaries between metabolism, RNA biology, and endocrinology, suggesting that modified nucleosides may represent a previously unrecognized layer of hormonal communication—chemically encoded, dynamically regulated, and evolutionarily conserved. This paradigm shift repositions RNA turnover from a simple end point to an active, regulated process of intercellular signaling.

Concluding remarks and future perspectives

Increasing evidence supports the idea that RNA modifications not only regulate RNA fate and translation within cells but also function as extracellular messengers that transmit cellular information and can be harnessed as minimally invasive readouts of physiological states for diagnostics. As quantitative diagnostics often reflect upstream enzymatic dysregulation, RNA modification signatures offer a potentially transformative, chemically grounded readout. Looking ahead, systematic mapping of extracellular modification and receptor networks could uncover a previously unappreciated 'epitranscriptomic endocrine system', with open questions for the field. Coupled with machine learning–based integration of LC–MS/MS and single-cell modification profiles, these efforts may redefine how metabolic information is encoded, transmitted, and therapeutically targeted across organ systems, enabling precision monitoring of endocrine health.

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Declaration of interests

The authors declare no competing interests.

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