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Perspective

Ribosome RNA modification in cancer: Biological functions and therapeutic targets

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The ribosome is a cellular system responsible for translating the nucleotide code of mRNA into proteins. In human cells, this complex consists of four distinct ribosomal RNAs (rRNAs): The 28S, 18S, 5.8S, and 5S rRNAs, along with 80 ribosomal proteins. Polymerase I is responsible for synthesizing the 28S, 18S, and 5.8S rRNAs, while polymerase III is responsible for synthesizing the 5S rRNA [1]. Ribosomal proteins are transcribed by polymerase II and subsequently assembled into two distinct subunits, the 60S large subunit and the 40S small subunit. The large subunit consists of three rRNA chains (5S, 5.8S, and 28S rRNA) and 47 ribosomal proteins, whereas the small subunit is composed of a single 18S rRNA chain and 33 ribosomal proteins. Over a hundred types of chemical modifications of RNA molecules have been identified over the past few decades. These modifications have been detected in both prokaryotic and eukaryotic cells and have been observed in all RNA subtypes.

To gain a more comprehensive understanding of the type and distribution of rRNA modifications, various techniques have been applied in this field, including mass spectrometry-based, quantitative PCR-based, chemical-assisted, and protein-assisted detection methods. There are 228 modification sites with 14 types of modifications determined by quantitative mass spectrometry in human 80S ribosomes: 4 in 5.8S (total length, 157 nt), 91 in 18S (1869 nt), and 133 in 28S (5064 nt) [2]. Among these modified nucleotides, methylation of 2′-OH of ribose sugar to a 2′-O-methyl-ribose and isomerizations of uridines to pseudouridines (ψ) are the most wide-ranging and extensively studied.

rRNA modifications serve crucial regulatory roles, influencing the structure, translation, and biogenesis of ribosomes. For instance, the absence of internal ψ and 2′-O-methylated sugars results in a blockade of rRNA biogenesis [3]. In addition, there is increasing evidence suggesting that rRNA modifications serve regulatory roles in human tumor cells [1]. Fig. 1 illustrates various rRNA modifications, their targets, and the associated functions in cancer. Modifications of rRNA can influence cellular functions. Furthermore, these modifications can serve as valuable biomarkers for cancer diagnosis, thereby presenting novel potential markers and targets for drug development.

rRNA modifications. In human cells, various rRNA modifications have been identified. These modifications can be classified into three types based on their positions: Methylation of the ribose sugars at the C2-position, isomerization of uridines to ψ, and base modifications. Depending on the type of chemical modification group, base modifications can also be divided into three classes: Methylation (m), acetylation (ac), and aminocarboxypropylation. The structures, distributions, regulators, and biological functions of various rRNA modifications are summarized in Table 1.

2′-O-methylation is the most abundant modification of rRNAs. In human rRNAs, it is primarily installed through a nucleolar RNA-dependent mechanism. Small nucleolar RNA (snoRNA) serves as a guide due to its sequence complementarity to the target, directing protein enzymes to the site of modification. Box C/D snoRNAs associate with ribonucleoproteins (RNPs), which include fibrillarin (FBL) methyltransferase, non-histone chromosome protein 2-like 1 (NHP2L1), NOP56 ribonucleoprotein (NOP56) and NOP58 ribonucleoprotein (NOP58), to form the C/D snoRNP complexes and catalyze the formation of 2′-O-methylation [1]. A study has demonstrated that 2′-O-methylation is present at numerous sites of rRNA [2]. In 5.8S, 2′-O-methylation has been identified at U14 and G75, while a total of 41 2′-O-methylation sites have been detected among the 91 modification sites in 18S, and an additional 67 2′-O-methylation sites have been identified in 28S.

As the second most abundant modification of rRNAs, ψ is also called the fifth nucleotide. In mRNA and transfer RNA (tRNA), ψ can be facilitated by pseudouridine synthases without snoRNA, whereas the installation of ψ in rRNA requires snoRNA. Unlike 2′-O-methylation, box H/ACA snoRNAs, instead of box C/D snoRNAs, direct the uridine isomerase dyskerin pseudouridine synthase 1 (DKC1) to install ψ. ψ has been identified at U60 and U61 in 5.8S, with 43 ψ sites detected in 18S and 61 ψ sites identified in 28S [2].

In addition to 2′-O-methylation and ψ, there are other less abundant modifications of rRNA. A nucleolar factor, nucleomethylin (NML), has been demonstrated to be required for N-1-methyladenosine (m^1A) modification at A1309 and A1322 in 28S [3]. N-6-methyladenosine (m^6A) is found to be present in 28S (A4220) and 18S (A1832). In 28S, the m^6A modification is catalyzed

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by zinc finger CCHC-type containing 4 (ZCCHC4), while in 18S, it is installed by methyltransferase like 5 (METTL5), cooperating with the tRNA methyltransferase activator subunit 11–2 (TRMT112) [1,4]. 5-methylcytosine (m^5C) in 28S is methylated by NOP2/Sun RNA methyltransferase 5 (NSUN5) at the C3782 site and by NOP2 nucleolar protein (NSUN1) at the C4413 and C4447 sites [1]. N-7-methylguanosine (m^7G) modification has been revealed at the G1639 site in 18S, and this is mediated by Williams-Beuren syndrome chromosomal region 22 protein (WBSCR22; also known as BUD23 rRNA methyltransferase and ribosome maturation factor) [3]. Furthermore, 3-methyluridine has been identified in 28S, N6, N6-dimethyladenosine, and 1-methyl-3-(3-amino-3-carboxypropyl) pseudouridine have been identified in 18S, and N4-acetylcytidine (ac^4C), catalyzed by the enzyme N-acetyltransferase 10 (NAT10) under the guidance of box C/D snoRNAs, has been demonstrated to be present at the C137 and C1842 sites in 18S [1,2].

Functions of rRNA modifications. Changes in post-transcriptional modifications of rRNAs can influence rRNA structure, ribosome biogenesis, and the translation process, including translation initiation and translational fidelity. A deficiency in translational accuracy may result in nonsense suppressions or amino acid misincorporations. It has been demonstrated that 2′-O-methylation contributes to the stabilization of the nucleotide conformation and restricts the rotational freedom of 3′-phosphate, potentially influencing the conformation and flexibility of the RNA strand [5]. Apart from its effect on rRNA structure, 2′-O-methylation also serves an important role in the translation process. Deletion of a box C/D containing snoRNA, SNORD42A, results in reduced 2′-O-methylation at uridine 116 of 18S rRNA, which is associated with a specific decrease in the translation of ribosomal proteins [6]. Another study has revealed that a reduced level of ψ inhibits tRNA binding to rRNA [3]. This biochemical impairment in ribosome activity manifests as decreased translational fidelity and internal ribosome entry site (IRES)-dependent translation initiation.

Methylation of nitrogenous bases has a profound impact on their physical and chemical properties. This modification promotes base stacking by increasing hydrophobicity and polarizability. Furthermore, it exerts its influence on the structure of rRNA by enhancing steric hindrance, blocking the conventional Watson-Crick hydrogen bonding and fostering noncanonical Hoogsteen base pairing. For instance, the addition of a methyl group at the N1 position of adenosine can block the normal Watson-Crick base pairing of A:U, resulting in an unstable mismatch with other nucleosides through the formation of Hoogsteen base pairs [7]. This may help rRNAs attain and maintain specific conformations essential for their corresponding function, both with respect to their structure and their enzymatic activity. In addition to their capacity to impact rRNA structures, base modifications can also regulate the biological processes of ribosomes. Notably, the knockdown of NML reduces methylation levels around A1309 and disrupts interactions between ribosomal subunits, providing further evidence for the role of NML in the formation of the 60S ribosomal subunit [3]. On the other hand, m^6A modification of rRNA enhances the translation process, and m^5C modification exerts an influence on rRNA structure, while m^7G and ac^4C modifications serve crucial roles in ribosome biogenesis [8].

rRNA modifications modulate cancer progression. There has been increasing evidence demonstrating that alterations in the levels of rRNA modifications can regulate cancer cell biological processes. Therefore, enzymes and snoRNAs that are responsible for modifying rRNAs serve essential roles in the modulation of cancer progression. As aforementioned, under the guidance of snoRNAs, FBL methyltransferase catalyzes ribose residues and affects every type of nucleotide to form 2′-O-methylation by associating with box C/D snoRNAs [1]. High levels of FBL have been demonstrated to accompany modifications in rRNA methylation patterns, impair translational fidelity, and increase IRES-dependent translation initiation of key cancer genes (insulin like growth factor 1 receptor, Myc,

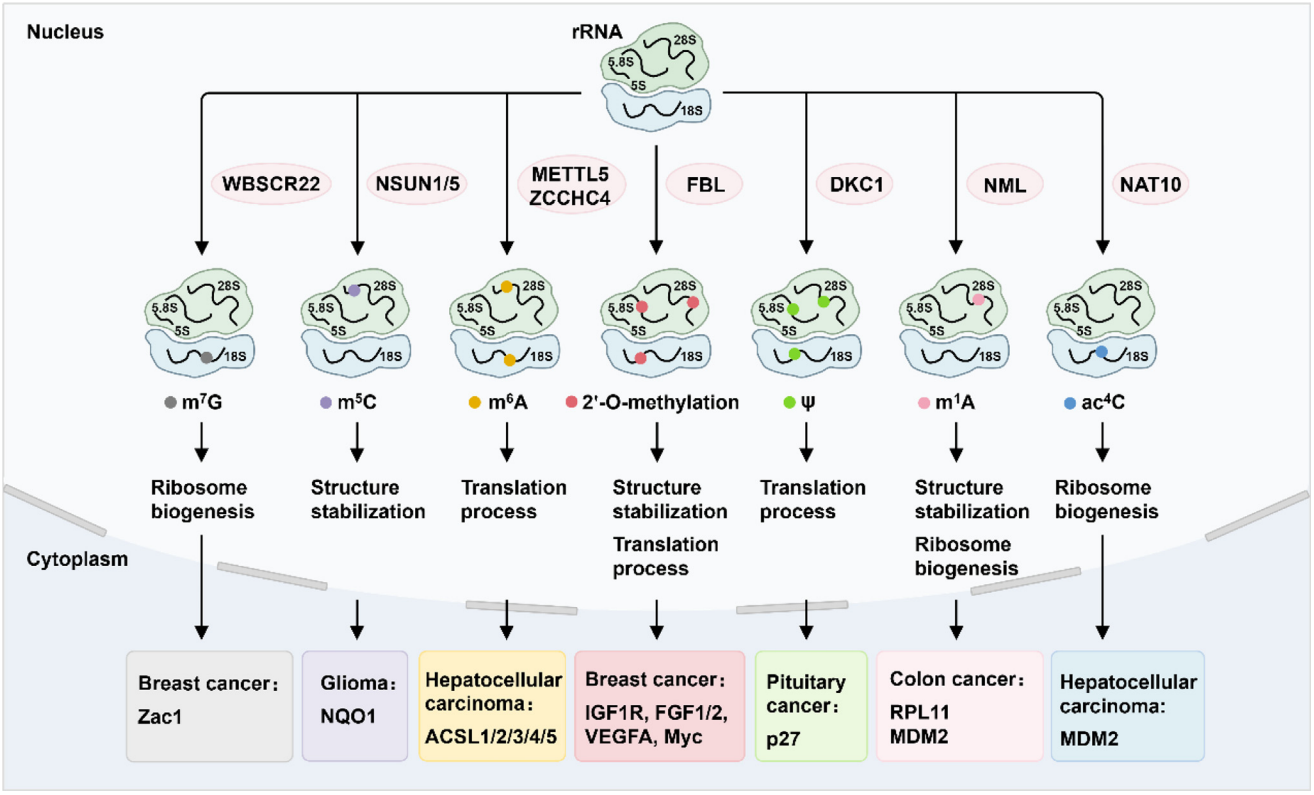
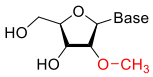
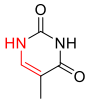
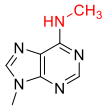
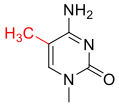
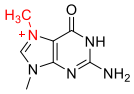
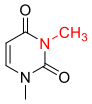
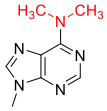
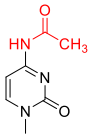
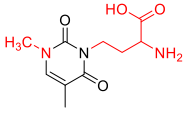


Fig. 1. rRNA modifications and their functions in cancer. FGF1/2: fibroblast growth factor 1/2; IGF1R: insulin like growth factor 1 receptor; Myc: MYC proto-oncogene, bHLH transcription factor; p27: p27/Kip1 tumor suppressor; VEGFA: vascular endothelial growth factor A.

Table 1
Chemical structures of rRNA modifications and their functions in rRNA.

Modification	Structure	Distribution	Modifier	Cofactor	Biological function
2′-O-methylation		5.8S, 18S, 28S	FBL	box C/D snoRNAs NHP2L1 NOP56, NOP58	Structure stabilization Translation process
Pseudouridine		5.8S, 18S, 28S	DKC1	box H/ACA snoRNAs	Translation process
N-1-methyladenosine		28S	NML	unknown	Structure stabilization Ribosome biogenesis
N-6-methyladenosine		18S, 28S	18S: METTL5 28S: ZCCHC4	TRMT112 unknown	Translation process
5-methylcytosine		28S	NSUN1/5	unknown	Structure stabilization
N-7-methylguanosine		18S	WBSCR22	unknown	Ribosome biogenesis
3-methyluridine		28S	unknown	unknown	unknown
N6, N6-methyladenosine		18S	unknown	unknown	unknown
N-4-acetylcytidine		18S	NAT10	box C/D snoRNAs	Ribosome biogenesis
1-methyl-3-(3-amino-3-carboxypropyl) pseudouridine		18S	unknown	unknown	unknown

fibroblast growth factor 1/2, and vascular endothelial growth factor A), which contributes to tumorigenesis and is associated with poor survival in breast cancer [1]. Furthermore, the loss of function of the tumor suppressor DKC1 disrupts the regulation of p27 translation, serving a role in the development of pituitary cancer [1]. These findings demonstrate that 2′-O-methylation and ψ modifiers serve crucial oncogenic roles by upregulating these specific rRNA modifications. This observation not only emphasizes the significance of these modifications in cancer-related processes but also highlights their potential as therapeutic targets.

rRNA m^6A modification-related enzymes are also able to modulate cancer. In hepatocellular carcinoma (HCC), the depletion of METTL5-mediated 18S rRNA m^6A modification results in impaired 80S ribosome assembly and a decline in the translation of acyl-CoA synthetase long-chain (ACSL) family genes (ACSL1/2/3/4/5) involved in fatty acid metabolism, thereby promoting cancer progression [9]. Additionally, ZCCHC4 knockout eliminates the m^6A4220 modification in 28S rRNA, reduces global translation, inhibits cell proliferation, and markedly decreases tumor size in HCC [1]. In addition to rRNA m^6A modifiers, NML serves a role in large ribosomal subunit formation through m^1A modification in 28S rRNA, thereby increasing the ribosomal protein L11 (RPL11)-murine double minute 2 (MDM2) interaction and promoting cell proliferation in colon cancer through the p53 signaling pathway [3]. Other rRNA modifiers have been associated with various types of human cancer, although the underlying mechanisms require further investigation. For instance, the loss of NSUN5 in glioma cells activates the selective synthesis of specific stress-related target NAD(P)H quinone dehydrogenase 1 (NQO1), and metastasis-related methyltransferase 1 (MERM1)/WBSCR22 enhances tumor cell survival in the vasculature by regulating PLAG1 like zinc finger 1 (Zac1)/p53-dependent apoptosis in breast cancer, while NAT10 increases mutant p53 levels by counteracting MDM2 action in HCC cells [1,10]. Taken together, increasing evidence highlights the important roles of these modifiers in regulating rRNA modifications across various aspects of cancer cell biology, including tumorigenesis, cell proliferation, and metabolism.

rRNA modifications as diagnostic biomarkers and therapeutic targets. The identification of prognostic and predictive markers in the early stages of cancer is imperative. Ribosome biogenesis might be an innovative source of biomarkers that remains to be evaluated. To date, research has demonstrated that both rRNA modifications and their modifiers can serve as valuable biomarkers for cancer diagnosis and prognosis prediction. For instance, in breast cancer, the absence of the FBL protein has been linked to an unfavorable prognosis in patients [11]. In clear cell renal cell carcinoma (ccRCC), the upregulation of DKC1 is associated with unfavorable clinicopathological characteristics, which suggests that DKC1

may serve as an independent prognostic indicator for patients with ccRCC [12]. Despite recent progress, further validation is necessary to assess the feasibility of using rRNA modifications as tumor biomarkers in clinical practice. Furthermore, it remains unclear whether rRNA modifications are linked to specific cancer stages or have the potential to be used to monitor the effects of therapies.

It is widely acknowledged that using inhibitors to regulate epigenomic modifications is a promising choice for cancer treatment. Given that these rRNA modification-related enzymes also serve oncogenic roles in cancer cells, developing inhibitors for them offers a direct approach to cancer treatment. Some of these enzymes already have available inhibitors. For instance, the DKC1 inhibitor pyrazofurin can markedly decrease the total RNA ψ content, which culminates in the suppression of the growth of colorectal cancer (CRC) cells and the formation of human CRC organoids [13]. Remodelin, an inhibitor of NAT10, has been illustrated to be able to suppress melanogenesis and melanoma growth and attenuate doxorubicin resistance in breast cancer [14]. However, due to the absence of clinical trials, it remains uncertain whether these inhibitors can be employed in the treatment of cancer.

Apart from small molecule inhibitors, modifications can also be reversed by targeted editing methods. Remarkable progress has been made in this field in recent years. For example, targeted specific demethylation of pyruvate dehydrogenase kinase 4 (PDK4) m^6A by the $dm^6ACRISPR$ system can markedly decrease the expression levels of PDK4 and glycolysis of cancer cells, thus inhibiting tumor growth and progression [15]. Compared with inhibitors, this technology allows us to change the modification status of a specific RNA at a specific site. However, this system seems difficult to function in matured rRNA because most modifications are buried within the ribosome, while only a few are located on the ribosome surface. Therefore, whether changes of modifications can occur after significant folding of rRNA and the maturation of the ribosome remains a question.

Ribosomes are the cellular machines responsible for protein synthesis and serve a crucial role in cell function. Increasing research has revealed that rRNA modifications serve pivotal roles in influencing rRNA structure, ribosome biogenesis, and translation processes, thereby exerting a significant impact on cell biology. It has been demonstrated that rRNA modifications are involved in human cancer. Consequently, enzymes and their cofactors responsible for these rRNA modifications have emerged as potential targets for both cancer diagnosis and therapy. By further investigating the mechanisms underlying rRNA modifications, novel opportunities to develop innovative cancer treatments will be identified.

Conflict of interest

The authors declare that they have no conflict of interest.

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