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Review

The importance of being (slightly) modified: The role of rRNA editing on gene expression control and its connections with cancer

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ABSTRACT

In human ribosomal RNAs, over 200 residues are modified by specific, RNA-driven enzymatic complexes or stand-alone, RNA-independent enzymes. In most cases, modification sites are placed in specific positions within important functional areas of the ribosome. Some evidence indicates that the altered control in ribosomal RNA modifications may affect ribosomal function during mRNA translation. Here we provide an overview of the connections linking ribosomal RNA modifications to ribosome function, and suggest how aberrant modifications may affect the control of the expression of key cancer genes, thus contributing to tumor development. In addition, the future perspectives in this field are discussed.

Contents

1. Introduction

Increasing evidence indicates that the process of ribosome production is profoundly altered in cancer cells. The quantitative up-regulation of ribosome biogenesis – which can be reasonably considered as a consequence of the loss of the mechanisms controlling cell proliferation – is a well-known feature of cancer cells [1]. Indeed, ribosome biogenesis is under the control of the same oncogenes and tumor suppressor genes that control cell cycle progression, including c-myc, p53, pRb, and others [2]. In addition, a number of studies have reported the possibility that neoplastic transformation and progression may be supported by qualitative alterations in ribosome biogenesis, leading to the production of ribosomes whose function has been intrinsically altered [1,3].

The whole ribosome consists of 4 ribosomal RNAs (rRNAs), for a total length of almost 7000 RNA nucleotides, which are assembled with at least 82 different proteins. In addition, a significant fraction of rRNA nucleotide residues (approximately 3% of the total) are modified. Such a complex structure can be altered at many different levels, thus possibly introducing peculiar functional properties. With regard to rRNA modifications, available data indicate that their alteration affects gene expression regulation with important effects on some genes that are important for neoplastic transformation and progression [4–6]. In addition, it is also known that the expression of factors involved in rRNA modification are frequently found altered in transformed cells [6,7]. Altogether, these

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observations may suggest that the control of rRNA modification could play an important role in cancer development.

In this review we will analyze in detail the evidence linking ribosomal RNA modifications to ribosome function, and how this may affect the control of the expression of genes which play a crucial role in cancer development.

1.1. When, where, and how rRNA modifications occur

Modifications occurring in rRNA represent crucial steps in ribosome biogenesis. In recent years, many details of the modification processes have become clear, including the effectors of modifications, their timing, the cellular localization of processes, and the position on rRNAs in the three-dimensional structure of the ribosome (even though all of this information might not be available for each single modification) [8]. Still, the understanding of the function(s) of many of the modified residues remains a demanding challenge for future studies.

In human rRNAs, over 200 residues have been found to be modified: 95 uridines converted to 5-ribosyluracil (pseudouridine, ψ), 105 ribose (linked to any of the four bases) methylated on the 2′-*O*, 2 cytosines acetylated (ac^4C) and 10 bases methylated (m^7G, m^1A, $m^6{}_2A$, m^5C, m^3U, $m^1acp^3\psi$) [9]. Modified residues – which in most cases are highly conserved throughout evolution – are situated in specific positions on rRNA molecules, and are concentrated in important functional areas of the 3D ribosomal structure: the decoding site, mRNA channel, tRNA binding sites, peptidyl transferase center, and subunits' interface [8,10]. All these modifications are induced by specific enzymes or enzymatic complexes (see Box 1), which can be roughly divided into four groups: C/D Box ribonucleoproteins (RNPs) for ribose methylation; H/ACA RNPs for pseudouridylation; N-acetyltransferases (NAT) for base acetylation; and methyltransferases (MTases, stand-alone or enzymatic complexes) [9] for base methylation.

Base and sugar modifications are only one aspect of the process of preparation of rRNAs which are ready for ribosome assembly. Nonetheless, some of these modifications are considered necessary for the occurrence of ribosome biogenesis, since this extremely complex process is endowed with multiple control steps, which eventually ensure the production of competent ribosomes.

rRNAs are transcribed by two different RNA polymerases: 5S rRNA is transcribed by PolIII in the nucleoplasm, whereas 5.8S, 18S, and 28S rRNAs are transcribed in the nucleolus by PolI as a unique precursor – 47S – also harboring two external transcribed spacers (5′ and 3′ ETS) and two internal transcribed spacers (ITS1 and 2). Internal and external spacers are sequentially removed by endo- and exo-nucleolytic cleavages,

Box 1

C/D Box RNPs

C/D Box RNPs are complexes formed by a C/D Box small nucleolar RNA (snoRNA) and four conserved proteins, three of which with structural functions (Nop56, Nop58, and 15.5 KDa), plus one with catalytic activity (Fibrillarin, FBL) (Fig. 1). snoRNAs may either be expressed from their own promoters by RNA polymerase II or III or, more often, be spliced-out introns of genes encoding functional mRNAs which are involved in ribosome synthesis or translation. C/D box snoRNAs range in length between 60 and 200 nucleotides; their secondary structure is characterized by a stem-bulge-stem shape, containing the box C (RUGAUGA, where R stands for purine) close to the 5′ terminus, and the box D (CUGA) near to the 3′ end. Similar boxes (C′ and D′) are located close to the apex of the stem. In the folded snoRNA, following the base pairing of the 5′ and 3′ termini, the C/D and C′/D′ boxes become close to one another, in a kink-turn called K-loop. D and D′ guides are 10–21 nt guide sequences, constituting part of the stem, which are complementary to the target RNA to be modified. The first event necessary for ribose methylation to occur is 15.5 kDa snoRNP protein binding to the kink-turn formed by the C and D boxes; after this, Nop56, Nop58 and fibrillarin bind to complete the RNP, and 2′-O-Methylation occurs on the target RNA, which is base-paired to the guide RNA 5 nt upstream of the D or D′ box.

H/ACA RNPs

H/ACA RNPs are complexes formed by one H/ACA snoRNA and four conserved proteins: NOP10, GAR1 and NHP2 with structural function, and dyskerin (DKC1), with pseudouridylase activity (Fig. 1). The length of H/ACA snoRNA ranges between 120 and 250 nucleotides; their secondary structure is characterized by a double hairpin, separated by a hinge region containing the H box (ANANNA, where N stands for any nucleotide), and an ACA box, 3 nucleotides before the 3′ terminus. The H box appears to be important for the correct assembly of the RNP complex, while the ACA box probably has a stabilizing activity. Each of the two hairpin structures harbors a pseudouridylation pocket, whose sequence confers the target specificity by Whatson/Crick base paring with the target sequence and, therefore, may be used to predict the pseudouridylation site (a web-based database of snoRNAs has been created and is available at https://www-snorna.biotoul.fr).

NOP10 and GAR1, as well as dyskerin, bind directly to the H/ACA snoRNA, thus locking the substrate RNA in place. After the initial binding, a number of structural changes ensure a precise and stable positioning of the target nucleotide for modification at the dyskerin catalytic site. GAR1 does not act directly either on the substrate or on the guide RNA anchoring; it is probably involved in the final release of the target RNA from the complex. For a broader review of the topic the reader is referred to Ge and Yu [89] and McMahon et al. [41].

Base methyltransferases (MTases)

Enzymes for rRNA base methylation have been best characterized in yeast, and some of them have been found to be conserved in humans. The catalytic domains of most of the rRNA base MTases belong to the class of Rossmann-fold enzymes (except for Emg1, which belongs to the SPOUT class); all of them use S-adenosyl-l-methionine (SAM) as a methyl donor [90], and they act either alone or in concert with a co-activator, with stabilizing functions (see Sharma and Lafontaine [8]). In contrast to what happens for C/D box RNPs and H/ACA RNPs, where a single enzyme can modify almost one hundred different residues (since target specificity is provided by the snoRNA guide), for base methylation each enzyme is target-specific, or at most can modify two different residues. This is the case for DIMT1L, with the MTase taking care of A_{1850} and A_{1851} dimethylation in 18S rRNA [19]. Among other MTases identified in humans, TRM112 acts in concert with WBSCR22 (MERM1) to methylate G_{1639} in 18 s rRNA [19,20]; nucleomethylin (NML) methylates A_{1322} on 28S rRNA [21]; p120 nucleolar antigen, belonging to the Nop2/NSUN/NOL1 proteins family, methylates C_{4447} on 28S rRNA [56], and NSUN5 methylates C_{3782} on 28S rRNA [55].

Base acetyltransferases

The only N-acetyltransferase (NAT) described so far as acting on human rRNA is NAT10, which catalyzes the transfer of one acetyl group from acetyl-CoA to N^4 of cytidine [22,23]. NAT10 is an enzyme that has no exclusive activity on RNA, since it was originally found to be a histone and microtubule modifier [23]. It is active on C residues in position 1837 and 1842 of 18 s rRNA; interestingly, it seems to work as a stand-alone enzyme for the modification of C_{1837} [22], while it has been described as cooperating with the C/D box snoRNA U13 for the modification of C_{1842} [23].