Commentary

Ribosome biogenesis: An emerging druggable pathway for cancer therapeutics

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ABSTRACT

Ribosomes are nanomachines essential for protein production in all living cells. Ribosome synthesis increases in cancer cells to cope with a rise in protein synthesis and sustain unrestricted growth. This increase in ribosome biogenesis is reflected by severe morphological alterations of the nucleolus, the cell compartment where the initial steps of ribosome biogenesis take place. Ribosome biogenesis has recently emerged as an effective target in cancer therapy, and several compounds that inhibit ribosome production or function, killing preferentially cancer cells, have entered clinical trials. Recent research indicates that cells express heterogeneous populations of ribosomes and that the composition of ribosomes may play a key role in tumorigenesis, exposing novel therapeutic opportunities. Here, we review recent data demonstrating that ribosome biogenesis is a promising druggable pathway in cancer therapy, and discuss future research perspectives.

1. Introduction: Cancer cells are addicted to ribosome production

In eukaryotes, the initial steps of ribosome production take place within specialized nuclear domains called nucleoli. Nucleoli are highly dense nuclear structures that assemble around the nucleolar organizers (NORs) containing the genes coding for ribosomal RNAs (rRNAs) [1]. For many years, changes in nucleolar morphology, nucleolar size, and number of nucleoli per nucleus have been recognized as hallmarks of malignity and tumor aggressiveness [2]. The analysis of nucleolar morphology, typically after specific staining (e.g. silver nitrate-based AgNOR), has been used by pathologists to establish the tumorigenic potential of cells in patient biopsies [2–5]. However, significant technical limitations, including quantification and reproducibility, have prevented the routine implementation of such techniques by pathologists. Recently, the production of specific algorithms has addressed several of these limitations by providing tools to characterize nucleolar disruption both qualitatively (i.e. distinguishing patterns of disruption) and quantitatively (i.e. measuring severity of disruption), and by defining a precise numerical index: the "index of nucleolar disruption" or iNo score [6,7]. Hopefully, such tools will be transformed into clinical applications in order to extend the use of nucleolar morphology to

disease diagnosis and prognosis, and to integrate the nucleolus as a biomarker for improved patient stratification.

The alterations of nucleolar morphology observed in cancer cells directly reflect the hyper activation of ribosome production. Importantly, it appears that tumor cells are addicted to increased ribosome production (and increased translational activity), since inhibition of these processes is generally cytotoxic. It is largely assumed that cancer cells become addicted to ribosomes owing to their enhanced need for protein production to sustain their unrestricted growth. The fact that inhibitors of translation, such as the one targeting the translation initiation machinery (mTOR inhibitors, eIF4F complex inhibitors), kill cancer cells supports such a concept of addiction [8–10]. In addition, the abnormal oncogenic signaling of cancer cells prevents the efficient feedback control that coordinates the synthesis of rRNAs and ribosomal proteins (r-proteins), and eventually leads to nucleolar stress and apoptosis (see below). Increased ribosome biogenesis is a general trend in cancer cells [11–14], which is important for cell transformation and tumorigenesis, (see [15–17]), and it remains unclear why some tumor cells rely more than others on ribosome production and protein synthesis. Mechanistically, increased ribosome production in cancer cells is caused by the dysregulation of the three
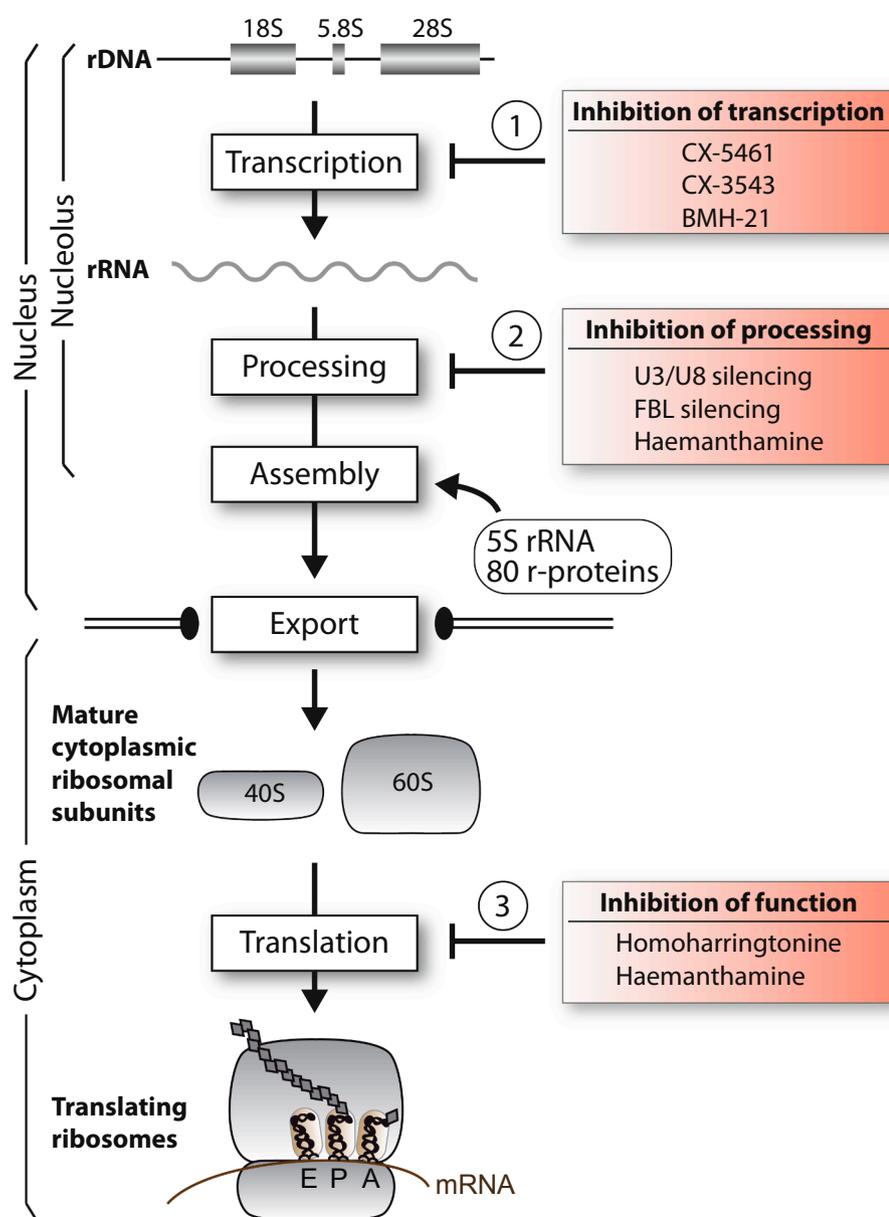
Fig. 1. Actionable steps of human ribosome biogenesis and function in anticancer therapeutics. Ribosome biogenesis starts in the nucleolus which forms around rDNA genes (up to 400 copies per cell in humans). A single pre-rRNA containing the 18S, 5.8S and 28S rRNA is synthesized by Pol I. The processing of this pre-rRNA includes numerous cleavage steps that produce the mature 5′ and 3′ ends of each rRNA [97], and the addition of > 200 chemical modifications (2′-O-methylation, pseudouridylation, base acetylation, methylation, and aminocarboxypropylation) [98]. Concomitantly, these assemble with the 5S rRNA (synthesized in the nucleoplasm by Pol III) and 80 r-proteins. These nuclear steps lead to the formation of pre-ribosomal subunits that are exported to the cytoplasm through nuclear pore complexes. In the cytoplasm, several quality control steps take place ensuring the capacity of the subunits to translate mRNAs. As detailed in the text, ribosome production and function can be pharmacologically targeted at several steps to preferentially kill cancer cells. The boxes illustrate some of the strategies and compounds that are currently being evaluated as therapeutic approaches, or that could be used in the future.

RNA polymerases (Pol) by molecular mechanisms involving cancer-promoting proteins, including major oncogenic and tumor suppressive pathways, like c-Myc, mTOR, p53, pRb and PTEN (reviewed in details in [14,18]).

Ribosome biogenesis is a complex process involving the synthesis, modification, assembly, and transport of eighty-four core ribosomal components: four rRNAs and eighty r-proteins (Fig. 1) [19]. It is highly energy-consuming, involves all three RNA Pol, and most cell compartments as it is initiated in the nucleolus, progresses to the nucleoplasm, and ends in the cytoplasm [20]. Three of the four mature rRNAs are produced from a single long polycistronic precursor synthesized by Pol I requiring extensive processing (RNA cleavage), the fourth rRNA, namely the 5S, is produced by Pol III [19], while r-proteins are encoded in mRNAs synthesized by Pol II. All of these components work synergistically to ensure their faithful assembly into precursor (pre-) ribosomal subunits. This requires the participation of over 200 assembly factors. Each of these steps is closely regulated and intimately interconnected, to such a level that inhibiting one may severely impact another [21]. Finally, important quality control is exerted all along the pathway to ensure that only properly assembled ribosomes are produced [22].

Ribosomes were traditionally considered to be "monolithic blocks", and it was believed that cells exclusively produce a single type of ribosome. It was recently shown that cells produce heterogeneous populations of ribosomes, the composition of which may differ, producing functional consequences on translation [23]. For instance, ribosomes may differ because their rRNAs are differentially modified (rRNAs contain over 200 modifications including the abundant 2′-O-methylation, and pseudouridylation, as well as a dozen other base methylations, see below), or because an r-protein is absent or replaced by tissue-specific isoforms [24,25]. This, in turn, may impact the selection of mRNAs that ribosomes translate, thus influencing either normal processes, such as cell differentiation or embryogenesis, or disease etiology, such as cancer initiation and progression [26]. In this review, we will refer to "ribosome variants" when we discuss compositionally different ribosomes, to "specialized ribosomes", when a specific function in the translation of specific mRNA transcripts has been attributed to these different ribosomes, and to "cancer ribosomes" or "oncoribosomes", when specialized ribosomes have been associated with tumorigenesis. Despite the current efforts to systematically identify ribosome assembly factors in human cells [27–31], the fine molecular mechanisms and the kinetics of processing, assembly, nuclear export

and quality control remain largely unexplored in humans compared to budding yeast (the historical reference model) [19,32].

Until recently, targeting ribosomes to develop anticancer therapeutic strategies was not seriously envisaged for several reasons. Firstly, because human ribosome biogenesis is a highly complex process, the coordinated regulation of which is not fully deciphered. Secondly, because it involves the contribution of hundreds of cellular factors, many of which remain to be identified. Thirdly, and most importantly, because inhibiting ribosome biogenesis or function was expected to indistinctively kill healthy and diseased cells. Surprisingly, however, cancer cells are more sensitive to treatments that inhibit ribosome production.

In this review, we summarize recent evidence supporting the notion that targeting ribosome biogenesis and/or function in cancer cells represents a promising strategy to develop specific powerful anticancer drugs. So far, such strategies have led to the development of several molecules that interfere with distinct steps of ribosome production and function, and some of these molecules have entered clinical trials (Fig. 1).

2. Targeting ribosome biogenesis for therapeutic intervention

2.1. Targeting ribosome biogenesis at the level of rRNA synthesis

2.1.1. Rationale and proof-of-concept

The rationale for targeting ribosome biogenesis in cancer cells is based on the general observation that cancer cells produce more ribosomes and are thus likely to be more vulnerable to a reduction in ribosome production than healthy cells [13]. Historically, such a strategy was first attempted by using inhibitors of Pol I such as actinomycin D [33]. However, the limited specificity of actinomycin D, and its important toxicity towards cells, prevented its further development. Nonetheless, the effect on ribosome biogenesis of thirty-six drugs belonging to different chemical categories and routinely used in chemotherapeutic regimens was systematically investigated revealing that half of them displayed strong inhibitory effects on ribosome biogenesis by interfering specifically with either rRNA synthesis or pre-rRNA processing [34]. Quite unexpectedly, inhibition of ribosome biogenesis appears to represent the primary mode of action of some of these well-established anticancer compounds, as it was recently reported in the myc-driven Eμ-Myc Cdkn2aArf−/− lymphoma model for several platinum derivatives including oxaliplatin [35]. These data prompted the idea that targeting ribosome production may be a promising approach to develop innovative anticancer drugs.

Proof-of-concept that targeted therapies which selectively inhibit ribosomal subunit biogenesis are efficient at killing cancer cells was made independently by Australian and American teams. They characterized several compounds, namely: CX-5461, CX-3543, and BMH-21 [36–39]), which selectively inhibit the function of Pol I (Fig. 1 – Box 1). CX derivatives and BMH-21 act *via* distinct mechanisms. CX derivatives are G-quadruplex (G4) DNA motif binders while BMH-21 binds to GC-rich DNA sequences [36,37,39,40]. Both G4 and GC-rich motifs are abundant in rDNA. CX derivatives inhibit the function of Pol I by preventing the binding of nucleolin and UBF (Upstream Binding Factor) [37,38], both of which are important for RNA synthesis, to Pol I promotors, while BMH-21 triggers the proteasomal-dependent degradation of the large catalytic subunit of Pol I [39]. Despite being DNA-binding molecules, these compounds were initially shown to not induce DNA damage in cultured cells, a feature that support their selectivity toward Pol I activity [36,37,39,40]. However, a recent study on DNA repair deficient cells raised the possibility that DNA damage might also contribute to their toxicity, at least in cells with particular genetic background ([41] and see below). In the original studies, the compounds were tested *in vitro* on a large panel of cancer cell lines representative of different types of cancers (NCI60 panel or an extended panel). For each of the three compounds, there was no association between the cancer of

origin of the cell lines and the observed IC_{50} values, indicating that they do not have an organ-specific activity [37–39]. Yet, the IC_{50} values ranged from 90- to more than a 100-folds among cell lines for CX-5461 and BMH-21, respectively. This shows that these compounds have a selective toxicity toward cells with particular molecular profiles, which remain to be characterized. Once characterized, these molecular profiles might lead to identification of tumor subtypes that would benefit from treatment with these compounds. To date, CX-5461 has been the most extensively studied, and its anticancer activity has been reported in multiple cancer backgrounds, including: leukemia, lymphomas and myelomas [36,42–44], prostate [45], osteosarcoma [46], ovary [47] and neuroblastoma [48], illustrating the potentially broad applicability of such compounds. In addition to interfering with the function of Pol I, CX derivatives also cause severe replication and DNA repair issues since they stabilize G4 DNA motifs which form naturally within guanine-rich DNA sequences and consist of a four-stranded helical structure [41]. The tumor suppressor BRCA2 is important for replication fork stabilization and for DNA damage repair, including for repairing G-quadruplexes. Treatment with CX-3543 or with CX-5461 eventually kills any cell, irrespective of its origin, but cancer cells, in particular those that have lost their ability to repair DNA damage, are particularly sensitive to treatment with both CX derivatives [41]. This is typically the case for BRCA1/2-deficient cancer cells, and for polyclonal patient-derived xenografts (PDX), including tumors resistant to PARP inhibitors (poly ADP ribose polymerase) and/or to platinum salts [41]. The deleterious effect of CX-5461 on BRCA2-deficient cells was reported in two colorectal cell lines, one ovarian cell line, in breast tumor cells, and in bone osteosarcoma cells, vouching for the general applicability of treatments with G4 stabilizers in DNA-repair deficient background, while not calling the effect on Pol I activity into question [41].

An important consequence of drug-induced inhibition of ribosome biogenesis is the activation of a regulatory loop known as nucleolar stress [49]. The nucleolar stress response is a p53-dependent anti-tumoral pathway activated following a dysfunction in ribosome biogenesis [50]. Briefly, in normal cells, the antitumor protein p53 is maintained at a low level as it is constitutively targeted to the proteasome for degradation by Hdm2-mediated ubiquitination. Upon stress inhibiting ribosome biogenesis, free unassembled ribosomal components accumulate. This is notably the case of the uL5-uL18-5S rRNA trimeric complex which titrates Hdm2, sequestering it away from p53 [51,52]. The consequence of Hdm2 titration is the stabilization of p53 and the activation of a cell death program leading to the killing of the stressed cell. [Note that on the basis of structural conservation between the three kingdoms of life, a coherent nomenclature has recently been proposed for r-proteins [53]. In this unified nomenclature, RPL11 and RPL5 have been renamed uL5 and uL18, respectively]. It is quite remarkable that among the eighty ribosomal proteins, uL5 and uL18 are the most important for maintaining the structure of the nucleolus [6]. In addition, the cytotoxic activity of these compounds is not limited to p53-dependent apoptosis, since other cell death modalities were reported including necrosis, senescence and autophagy, thus broadening the potential application of inhibitors of ribosome biogenesis for killing cancer cells [36–39]. However, the fine molecular mechanisms underlying these effects have as yet not been elucidated. Furthermore, the deleterious effects of CX-5461 have been observed in cells and polyclonal PDX models that lack p53 [41].

The rationale for establishing ribosome biogenesis as a druggable pathway in cancer therapeutics further resides in the observation that CX-derivatives and BMH-21 drugs seem to preferentially kill cancer cells, leaving "normal" untransformed cells less affected. For example, the CX-5461 compound displays an IC_{50} of around 5 μM and 3 nM in non-transformed cells (e.g. BJ-hTert fibroblasts) and cancer cells (e.g. EOL-1 leukemia cells), respectively, while retaining the same Pol I inhibitory activity [38]. Drug selectivity is also supported by animal studies, in which these compounds displayed efficient antitumor activity without impacting the weight of mice over the 5-week time

course of the treatment [36,39,40]. In these studies, growth inhibition was observed using xenografts of pancreatic cancer MIA PaCa-2 cells (CX-3543 and CX-5461), melanoma A375 cells (CX-5461 and BMH-21), and colorectal cancer HCT116 cells (BMH-21) [36–40]. Evaluation of CX-5461 in advanced phase I clinical trial for patients with BRCA1/2 deficient tumors is ongoing in Canada (NCT02719977). CX-3543 has been assessed in solid and neuroendocrine tumors, and lymphomas as phase I/II studies in the US, and no other trial is currently active (https://clinicaltrials.gov). In conclusion, independent works have demonstrated that the pharmacological inhibition of ribosome biogenesis is an efficient means of preferentially killing cancer cells. This was a turning point in the demonstration that the nucleolus is a bona fide target for anti-cancer intervention [54,55].

2.1.2. Limitations

Although targeting ribosome production by inhibiting the function of Pol I appears to be appealing, several limitations have been identified that will have to be overcome to fulfill the requirements of further clinical developments. Firstly, not all cell lines are sensitive to Pol I inhibitors (see NCI60 response [37–39]), and this is irrespective of their p53 status. Therefore, it is crucial to better understand the pathways modulating the sensitivity of cells to these inhibitors. Secondly, the tested compounds only have a limited selectivity towards rRNA genes. Indeed, both CX-derivatives and BMH-21 are DNA binding compounds that are able to directly interact with any DNA sequence that is GC-rich or contains G4, which means that they can bind elsewhere in the genome than onto rDNA. As such, these compounds are not specific to Pol I, they preferentially affect Pol I because rDNA genes are enriched in GC- and G4-motifs. Thirdly, there are reported cases of acquired resistance to CX-5461, indicating that the anticancer activity of the drug can somehow be bypassed.

2.2. Targeting ribosome biogenesis at other levels than RNA synthesis

rRNA synthesis is only one of several steps of ribosome biogenesis that represents a druggable target, another being pre-rRNA processing. Processing depends on dozens of *trans*-acting factors which can either be proteinaceous or ribonucleic in nature. In addition to the actual catalytic activities, directly responsible for RNA processing (i.e. the endo- and exoribonucleolytic activities), many factors contribute to the proper folding of pre-rRNAs to enable them to acquire a cleavage-competent conformation. This is notably the case of the C/D box small nucleolar RNAs (snoRNAs) U3 and U8 which interact with the pre-rRNA through the formation of Watson-Crick base-pairing interactions. U3 and U8 are essential for early and late pre-rRNA processing reactions leading to the formation of small and large ribosomal subunits, respectively [56]. Using a mouse xenograft model, it was recently shown that aggressive cancer cells in which the expression of U3 or U8 was silenced lost their tumorigenic potential once implanted into the flanks of nude animals [56]. When tumors formed, these were not only smaller but also more heterogeneous in composition as a consequence of healthy tissue repopulating the microenvironment. Increased tumor heterogeneity, as established by positive emission tomography that relies on the use of a metabolic tracer, is considered to be a positive clinical parameter owing to the presence of healthy tissues [56]. Consistently, knockdown of fibrillarin, one of the essential proteins of the U3/U8 C/D box complex, resulted in the reduced growth of MCF7 xenografts [57].

Other steps of ribosome biogenesis that may also be targeted are ribosome assembly and chemical modification of its components. There are only a few known pharmacological inhibitors of ribosome assembly. The best characterized being diazaborin, which inhibits the formation of the large ribosomal subunit at a late stage in budding yeast [58]. The search is on for similar active compounds on human cells. Finally, the chemical modifications of rRNAs also constitute a crucial step of ribosome biogenesis that can be targeted to design anticancer drugs since

they lead to the production of cancer ribosomes (see below).

3. Targeting the function of ribosomes

Owing to the central role of ribosomes in modulating gene expression, innovative therapeutics inhibiting their function could also be pertinent. Indeed, this would represent a novel means of reducing the translational capacity of cells, which plays a primary role in tumorigenesis (reviewed in [11]). The control of translation has also been proposed as a novel target to circumvent resistance to targeted therapies, for example by inhibiting the eIF4F translation initiation complex in the context of anti-BRAF and anti-MEK resistance [9]. However, similarly to inhibiting ribosomal subunit biogenesis, a major challenge remains to be addressed, namely the level of specificity of such an inhibition. Indeed, (i) Would it target a particular transcript or a subset of transcripts? (ii) Would it preferentially target diseased than healthy cells? Despite these concerns, this is a rapidly developing field of research, and several molecules are already in use to treat human diseases, including neurodevelopmental syndromes and cancer [59]. Indeed, homoharringtonine, a natural plant alkaloid extracted from the Japanese plum-yew tree Cephalotaxus harringtonii, is an FDA-approved drug in the treatment of chronic myeloid leukemia traditionally used in Chinese medicine (Fig. 1 – Box 3), which functions by inhibiting translation. Homoharringtonine is also known as omocetaxine mepesuccinate or Synribo®.

Alternatively, molecules that simultaneously target ribosome production (by blocking synthesis of components, or their assembly) and function (by blocking an active site on the ribosome) (Fig. 1 – Boxes 2 and 3) could be developed. One such case is haemanthamine, a natural alkaloid extracted from Daffodil bulbs, the anticancer properties of which have been used in folk medicine for centuries and have only recently been deciphered [60]. Indeed, it was demonstrated that this molecule targets both ribosome function, by sterically blocking translation, and ribosome production, by inhibiting RNA processing specifically; this latter property providing the added advantage of a potent activation of the nucleolar stress response leading to strong p53 stabilization [60].

Combination therapy is routinely used in oncology and could enhance ribosome inhibition approaches. A direct effect of combining drugs is that the molecules are administered at lower concentrations thereby reducing their level of toxicity. The first attempts at combining Pol I inhibitor treatments with other drugs *in vitro* have revealed promising results, for example by combining CX-5461 with the mTOR inhibitor Everolimus on Myc-driven lymphoma xenograft [61], or by combining CX-5461 and the pan-PIM-kinase inhibitor CX-6258 on prostate cancer PDX [45]. Furthermore, combining molecules that target ribosome biogenesis and/or function with other anticancer treatments could prevent resistance and prolong the effects of individual drugs. For instance, as discussed above, inhibition of ribosome biogenesis results in an increase in the intracellular pool of free ribosomal proteins, which plays a major role in promoting apoptosis. Considering that free ribosomal proteins have a short half-life [62], drugs that target the proteasomes could be used to stabilize them. Finally, reducing the ribosome pool of cancer cells may weaken their metabolism and might render them more sensitive to stresses induced by other drugs.

4. Targeting "cancer ribosomes": Myth or reality?

Today we are facing a paradigm shift with the demonstration that cells do not produce ribosomes with a unique composition, but instead produce a variety of ribosomes that differ in their protein and RNA composition [24,63]. Ribosome composition was shown to vary in r-proteins or rRNAs according to the status of the cell, environmental conditions, developmental stages, or physiological and pathological conditions [64–73]. Interestingly, ground-breaking studies have

demonstrated changes in rRNA composition in cancer cells supporting the existence of cancer-associated ribosome variants, exhibiting altered rRNA 2′-O-methylation [69,70]. In addition, mutations of the DKC1 gene encoding the pseudouridine synthase dyskerin were identified in dyskeratosis congenita patients, which are characterized by an increased cancer susceptibility. These patients display an altered rRNA pseudouridine content [72,74]. Hence, these findings substantiate the notion of cancer ribosomes (see definition in the Section 1). Continuous technological advances in mapping rRNA modifications and characterizing the ribosomal proteome will be instrumental in identifying cancer-specific ribosomal signatures and in reinforcing the cancer ribosome hypothesis.

Among the different types of RNA modifications, 2′-O-methylation (2′-O-Me) is the most abundant on eukaryotic rRNA with 55 and 106 sites mapped in yeast and in human rRNA, respectively [71,73,75–77]. 2′-O-Me patterns of such cancer ribosomes were shown to be different from those of their healthy counterparts, i.e. the level of 2′-O-Me at some of the sites was different [70]. Importantly, 2′-O-Me provides an unanticipated "plasticity" to rRNA and ribosomes, since changes in the 2′-O-Me pattern is a molecular means to affect ribosome composition, which in turn contributes to modulating ribosomal activity [69–71,78–81]. For instance, modulation of 2′-O-Me may provide functional specificity to cancer ribosomes, favoring the translation of mRNAs with key roles in tumorigenesis, cell survival and resistance to chemotherapeutic treatments [70,71]. Interestingly, modifications in 2′-O-Me are associated with alterations of internal ribosome entry site (IRES)-dependent translation initiation, which is preferentially activated under stress (e.g. hypoxia or nutrient deprivation). The mRNA of many major genes implicated in tumorigenesis contain an IRES-element, including oncogenes (c-Myc), tumor suppressors (p53), growth factors and their receptors (IGF-1R, VEGF, FGF) and apoptosis modulators (XIAP, Bcl-xl) [11,82]. The alteration of 2′-O-Me levels was associated with increased IRES-dependent translation initiation of c-Myc, FGF, VEGF and IGF-1R [70]. How such regulatory mechanisms contribute to tumorigenesis *in vivo* remains to be explored. Interestingly, a similar role is attributed to pseudouridylation, which, remarkably, affects a different set of IRES-elements compared to 2′-O-Me, and further supports the notion that chemical modifications of rRNA plays a central role in modulating the translational capacity of ribosomes, notably during tumorigenesis [74,83,84].

In humans, 2′-O-Me of rRNA is carried out by the methyl transferase fibrillarin associated with the RNA-binding protein 15.5 kDa and the core proteins NOP56 and NOP58 [85,86]. Methylation at each site is guided by snoRNAs from the C/D box snoRNA family, which carry a complementary sequence to the target rRNA [87]. As the mechanisms of partial rRNA modification remain largely unknown, it is interesting that progressively reducing the concentration of fibrillarin in cells only impacts a subset of the 106 2′-O-methylated nucleotides, and that these mostly correspond to positions that are naturally hypomodified [71,73]. This suggests that hypo-modification is regulated by limiting the amounts of specific functional snoRNPs [71,73,86]. Moreover, of these 106 2′-O-Me sites, several are sensitive to the presence of p53 in cells [70,73], which may indicate that modulation of 2′-O-Me levels contributes to regulating cell proliferation or to other aspects of the tumor suppressive functions promoted by p53. In addition, fibrillarin controls the amount of ribosome production through various molecular mechanisms, including rDNA histone methylation, pre-rRNA processing, and pre-rRNA modification [88], and its expression is controlled by p53 [70,73]. Therefore, targeting such a master regulator of ribosome biogenesis could also be a promising approach to interfere with the production of ribosomes. However, to our knowledge no inhibitors of fibrillarin have so far been described.

Lastly, developing drugs directly targeting specific ribosome variants, such as cancer ribosomes, can seriously be envisaged in view of the mode of action of several antibiotics that bind to highly specific ribosomal structures [89]. In particular, recent structural analysis of prokaryotic and eukaryotic ribosomes demonstrated that selectivity of ligand [Note: By "ligand", we refer to any molecule that can bind to the ribosome, i.e. tRNA, r-protein, translational factor, or small molecule inhibitors, including antibiotics and compounds active towards eukaryotic ribosomes] binding is often provided by subtle differences in the structure of the ribosome [60,90–95]. For instance, the anticancer compound haemanthamine, presented above, binds exclusively to eukaryotic ribosomes, since its binding site is slightly different in bacterial compared to human ribosomes, and is consequently only active in human cells [60]. Interestingly, resistance to antibiotics often relies on chemical modifications of the ribosomes [90,96], illustrating that changes as faint as a single chemical modification are sufficient to prevent the binding of small molecule inhibitors. This provides the proof-of-concept that it may be possible to develop antibiotic-like molecules to target the ribosomes that accumulate in cancer cells providing they exhibit structural features that distinguish them, even slightly, from those of healthy cells.

5. Conclusions and future directions

In this commentary, we presented evidence that ribosome biogenesis and function have emerged as novel cellular processes that can be efficiently targeted to treat cancer. This description included strategies that are currently under evaluation in clinical trials (RNA synthesis inhibition), as well as those that we deem should be further investigated in the future (targeting ribosome variants with small molecule inhibitors). However, we are but at the dawn of anticancer ribosome therapeutics, and many essential questions remain, the most important being why cancer cells are more sensitive than healthy ones to drugs that inhibit ribosome production and/or function. We speculate that the propensity of cancer cells to rely on more abundant ribosome production and increased protein synthesis to sustain their unrestricted cell growth renders them more vulnerable to cellular alterations of ribosome levels. Alternatively, cancer cells may be rewired by compositionally and functionally different ribosomes, offering a theory based on the preponderance of the "quality" of the ribosomes produced, rather that their "quantity", in tumorigenesis.

So far, inhibition of ribosome biogenesis has only been tested in a relatively limited number of cancer models, and should thus be evaluated more broadly, in particular in cancers with higher therapeutic demands (e.g. advanced pancreatic or colorectal cancer, triple negative breast cancer, and melanoma) or in those displaying stronger proliferative traits.

Conflict of interest

None.

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