

# **Ribosome biogenesis: a central player in cancer metastasis and therapeutic resistance**

## **Authors:**

Amr R. Elhamamsy <sup>1</sup>, Brandon J. Metge <sup>1</sup>, Heba A. Alsheikh <sup>1</sup>, Lalita A. Shevde <sup>1,2</sup>,  
Rajeev S. Samant <sup>1,2,3</sup>

## **Affiliation**

- 1 Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.
- 2 O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, USA.
- 3 Birmingham VA Medical Center, Birmingham, AL, USA.

## **Contact details for PI**

Rajeev Sharad Samant, PhD  
Professor, Department of Pathology  
The University of Alabama at Birmingham  
WTI 320E  
1824 6th Ave South  
Birmingham AL 35233  
Phone: 205-975-6262  
Email: [rsamant@uab.edu](mailto:rsamant@uab.edu)

**Number of words:** 4158

**Number of characters:** (no spaces) : 25,789

(with spaces) : 29,937

**Disclosure:** The authors declare no potential conflicts of interest.

## **Keywords:**

ribosome biogenesis; metastasis; onco-ribosome; chemotherapy resistance;  
radiotherapy resistance; ribosome-targeted therapy

**Abbreviations:**

2'-O-Me:	ribose 2'-O methylation
Ψ:	Pseudouridylation
AML:	acute myeloid leukemia
CLL:	Chronic lymphocytic leukemia
CML:	Chronic myeloid leukemia
CRC:	Colorectal cancer
CTCs:	Circulating tumor cells
CRPC:	Castrate-resistant prostate cancer
DKC1:	Dyskerin pseudouridine synthase 1
EMT:	Epithelial–mesenchymal transition
FBL:	Fibrillarin
GBM:	Glioblastoma
HGSOC:	High-grade serous ovarian cancer
HR:	Homologous recombination
IRES:	Internal ribosome entry site
MDS:	Myelodysplastic syndrome
NoRC:	Nucleolar remodeling complex
NPM1:	Nucleophosmin 1
NSCLC:	Non small cell lung carcinoma
PRC2:	Polycomb repressive complex 2
RMRP:	RNA component of mitochondrial RNA processing endoribonuclease
RNA Pol I:	RNA polymerase I
RNA Pol II:	RNA Polymerase II
RNA Pol III:	RNA Polymerase III
rDNA:	ribosomal DNA
rRNA:	ribosomal RNA
tRNA:	transfer RNA
T-ALL:	T-cell acute lymphoblastic leukemia
TOP2:	Topoisomerase II

## **Abstract:**

Ribosomes are a complex ensemble of ribosomal RNA (rRNA) and ribosomal proteins that function as mRNA translation machines. Ribosome biogenesis is a multi-step process that begins in the nucleolus and concludes in the cytoplasm. The process is tightly controlled by multiple checkpoint and surveillance pathways. Perturbations in these checkpoints and pathways can lead to hyper-activation of ribosome biogenesis. Emerging evidence suggests that cancer cells harbor a specialized class of ribosomes (onco-ribosomes) that facilitates the oncogenic translation program, modulates cellular functions, and promotes metabolic rewiring. Mutations in ribosomal proteins, rRNA processing, and ribosome assembly factors result in ribosomopathies that are associated with an increased risk of developing malignancies. Recent studies have linked mutations in ribosomal proteins and aberrant ribosomes with poor prognosis, highlighting ribosome-targeted therapy as a promising approach for treating cancer patients. Here we summarize various aspects of dysregulation of ribosome biogenesis and the impact of resultant onco-ribosomes on malignant tumor behavior, therapeutic resistance, and clinical outcome. Ribosome biogenesis is a promising therapeutic target, and understanding the important determinants of this process will allow for improved and perhaps selective therapeutic strategies to target ribosome biosynthesis.

## Introduction

Aberrant cell growth and proliferation depend on increased protein synthesis and overactive translation that requires hyperactive ribosome biogenesis. This is enabled by multiple cellular regulatory pathways that are hijacked to tune transcription and translation (1). This is consistent with the acquisition of genetic and epigenetic alterations by cancer cells and changes in regulatory layers of translation such as microRNAs, tRNA modifications, and RNA binding proteins that play significant roles during tumor progression and metastasis.

Ribosome biogenesis is a multi-step process that starts in the nucleolus and culminates in the formation of functional ribosomes in the cell. Ribosomes serve as translation machinery in the cell and are a complex assembly of ribosomal RNAs (rRNAs) and a large number of ribosomal proteins and ribosome-associated proteins. In humans, ribosomes comprise a small 40S subunit and a large 60S subunit. The small 40S subunit comprises one 18S rRNA and 33 ribosomal proteins, while the large 60S subunit contains one each of the 28S, 5.8S, and 5S rRNAs and 47 ribosomal proteins. Together, the 60S and 40S subunits constitute the 80S ribosome particle (**Figure 1**).

The nucleolus is the principal site of ribosome biogenesis and forms around nucleolar organizer regions (NORs), which contain several hundred ribosomal DNA (rDNA) gene repeats in human diploid cells. These are located on each of the five acrocentric chromosomes. RNA polymerase I (RNA Pol I) transcribes rDNA into pre-ribosomal RNA (47S pre-rRNA). A large number of processing proteins are required for the splicing and processing of pre-rRNA, resulting in the formation of three rRNA species: 28S, 5.8S, and 18S (2). RNA polymerase II (RNA Pol II) transcribes genes that encode ribosomal proteins and ribosome-associated proteins, whereas RNA polymerase III (RNA Pol III) transcribes the 5S rRNA gene. Thus, while synthesis of mature ribosomes necessitates synchronization of all three RNA polymerases, around 200 processing factors, and about 80 ribosomal proteins, transcription of 45S rRNA by RNA Pol I is considered as a rate-limiting and a key step in ribosome biogenesis. In cancer, dysregulated signaling pathways, metabolic reprogramming, and aberrant expression of non-coding RNAs

promote RNA Pol I transcription activity, resulting in ribosome biogenesis hyperactivation (3-7).

Evidence has emerged in recent decades regarding the close link between dysregulated ribosome biogenesis and tumorigenesis. For example, oncogenic c-Myc transcription factor increases protein synthesis and promotes translational capacity by modulating the expression of many genes implicated in ribosome biogenesis (8). Conversely, surveillance systems centered on tumor suppressors (e.g. TP53, PTEN, and RB1) have evolved in normal cells to oppose excessive changes in ribosome biosynthesis and halt cell growth (9). While the impacts of ribosome biogenesis on cancer metastasis, treatment resistance, and clinical outcome are not fully understood, in this review, by integrating the most current findings, we present novel insights into the relationship between ribosome biogenesis and cancer metastasis, as well as potential therapeutic approaches.

## **Dysregulation of ribosome biogenesis in cancer**

### ***Historical overview***

The first preliminary indications of the importance of ribosome biogenesis dysregulation in cancer came about with the identification of irregular numbers and shape of nucleoli in cancer cells. This feature was adopted by pathologists with AgNOR staining to mark the nucleolus and evolved to become a hallmark of malignant cells, allowing for the appreciation that nucleolar phenotypes are reflective of ribosome biogenesis (10,11). One of the first indications that defined the interplay between ribosome biogenesis and cell cycle progression was the discovery that cell proliferation could be blocked by inhibiting ribosome biogenesis (12). This study was followed by research suggesting that ribosome biogenesis might play an important role in neoplastic transformation wherein aberrations of the major tumor suppressor retinoblastoma (Rb) and p53 pathways stimulated nucleolar function and led to nucleolar enlargement (13).

One of the earliest studies by Williamson *et al.*, provided evidence that dysregulated abundance of pre-RNA transcripts correlated with poor prognosis (14). More recently, the importance of rRNA biogenesis in driving malignant phenotypes was evidenced by the observation that in contrast to normal B cells, lymphoma cells demonstrate greater

reliance upon elevated rDNA transcription, rendering them exquisitely sensitive to RNA Pol I inhibition (15). This paradigm-shifting evidence altered the perspective on RNA Pol I activity as merely a byproduct of tumorigenesis, to being a major driver. The discovery of somatic mutations in ribosomal proteins in multiple hematological and solid malignancies added to the affirmation that ribosomal changes are intimately associated with cancer. Human and animal cell models carrying these mutations show defects and abnormalities in ribosome assembly, cell proliferation, and malignant transformation. As research continues to evolve revealing the complexities of ribosome biogenesis, it has become evident that ribosome biogenesis alterations in cancer can stem from a myriad of crucial steps beginning with rDNA transcription through ribosome modifications collectively driving tumor progression and metastasis.

### ***Ribosomal heterogeneity in cancer***

Ribosomes were thought for a long time as complex machines with invariable composition. This view was challenged when Mauro and Edelman first suggested that ribosomes can have heterogeneous composition, and this heterogeneity regulates translation and modulates protein synthesis rates (16). Due to the complexity of their composition, heterogeneity in ribosomes can arise from variability in any of their components, e.g., rRNA modifications, rRNA variants, stoichiometry and paralogs of ribosomal proteins, post-translational modifications, and ribosome-associated proteins. These variabilities in ribosome composition contribute to the generation of “specialized ribosomes” or in the case of cancer, “onco-ribosomes” (17).

Ribosome heterogeneity plays a role in tumorigenesis and cancer progression (18,19). Parks *et al.*, discovered that the number of rDNA copies varied significantly within and between individual humans and also mice. Variant rRNA alleles are selectively expressed in a tissue-specific manner, and contribute to ribosome heterogeneity (20). Most malignancies have paired 5S rDNA amplification and 45S rDNA deletion, which are linked with higher proliferation rates and unregulated expression of nucleolar genes (introduced as “nucleolar activity”). Wang *et al.*, proposed that tumor suppressors and oncogenes have a variety of mutational backgrounds that are linked with recurrent alterations in copy

number of rDNA in different cancers (21). Appreciably, each of these alterations can result in ribosome heterogeneity.

Altered rRNA modifications have emerged as oncogenic drivers that can trigger tumor initiation or promote cancer progression. Several new advances contributed to solidifying the importance of altered rRNA modifications in cancer. Noteworthy among this is the report by Marcel *et al.* about the first rRNA 2'-O methylation landscape in primary human breast tumors (22). These efforts uncovered that rRNA 2'-O methylation exhibits intra- and inter-patient variability in breast tumors and is differentially associated with breast cancer subtype and tumor grade (22). Concurrent with this report, using an example of a site in the small ribosomal subunit (SSU-C1440) that is linked to diffuse large B-cell lymphoma pathogenesis, Krogh *et al.* demonstrated a novel concept that sites of rRNA hypomethylation can be used as potential drug targets (23). Metge *et al.* identified that when cancer cells are exposed to stress, e.g., hypoxia, rRNAs acquire distinct methylation patterns and create a subgroup of specialized ribosomes that are capable of performing IRES-mediated translation (24). Altered expression of small nucleolar RNAs (snoRNAs) accounts for further ribosome heterogeneity, impacting physiological and pathological cellular processes, including carcinogenesis (25,26). rRNAs undergo extensive post-transcriptional modifications, predominantly pseudouridylation ( $\psi$ ) and ribose 2'-O methylation (2'-O-Me), which are guided by snoRNAs and mediated by box H/ACA and box C/D ribonucleoprotein complexes, respectively (27). Moreover, the rRNA 2'-O-Me landscape is remarkably altered in breast cancer and varies within and across patients' tumor samples, tumor stage, and subtype (22). For example, SNORD42A, a snoRNA responsible for 2'-O-Me of the uridine116 residue in 18S rRNA, is highly expressed in individuals with acute myeloid leukemia (AML). Elevated abundance of SNORD42A is linked to AML cell proliferation and survival; deleting SNORD42A reduced cellular growth and global protein synthesis (28). In non-cancerous cells, p53 regulates the expression of fibrillarin (FBL), a central rRNA methyl transferase. Mutations or alterations in p53 remove the check on FBL, altering the 2'-O-Me marks on ribosomes, resulting in reduced translational fidelity and enhanced IRES-dependent translation. Unchecked FBL activity facilitates tumorigenesis and is associated with an unfavorable prognosis in breast cancer patients (29).

Another layer of complexity is imparted by heterogeneity of ribosomal proteins – while dysregulated ribosomal protein composition is associated with poor prognosis and worse clinical outcome, it's still somewhat enigmatic what functional relevance there is to this heterogeneity or whether ribosomal protein-dependent regulatory pathways are at work under the surface.

Heterogeneous compositions confer specialized functions to ribosomes, facilitating preferential translation of certain mRNAs in normal and pathological contexts. In cancer, mutations in ribosomal proteins have been proposed to alter the preferential translation of certain mRNAs, creating a pro-oncogenic proteome promoting cancer progression (30,31). The term “onco-ribosomes” was coined to describe a form of specialized ribosomes in cancer cells that confer preferential translation of oncogenic and pro-survival genes, facilitating cancer progression (19,32). Babaian *et al.*, identified a cancer-specific single-nucleotide variation in 18S rRNA at nucleotide 1248.U in more than 45% of colorectal cancer (CRC) patients. This results in hyper-modification on 18S rRNA at the peptidyl decoding site of the ribosome. A subset of CRC patients with hypo-modification is characterized by highly abundant ribosomal proteins that generate heterogeneous onco-ribosomes (33).

The ability of ribosomes to translate efficiently while maintaining high fidelity is critical for cell survival and proliferation. Mutations in ribosomal proteins impact the translational capacity of cells by modulating the rate and fidelity of protein synthesis. Most cell model systems engineered for mutations in ribosomal proteins show permuted translational rate and accuracy. For example, in chronic lymphocytic leukemia (CLL), mutations in the RPS15 gene result in defective ribosomes, impacting the rate and fidelity of protein synthesis (34). Thus, ribosomal heterogeneity and specialized onco-ribosomes are important players in promoting cancer progression. Further investigations are warranted to fully characterize their compositions for therapeutic purposes.

### ***Ribosomopathies and cancer***

Ribosomopathies are a group of developmental disorders caused by abnormal ribosome synthesis and dysfunctional ribosomes. Patients with ribosomopathies have a greater risk



of developing malignancy later in life (32,35,36). By modulating oncogenic signaling pathways and remodeling the translational programs in cancer cells, several studies have highlighted the close connection between mutations in ribosomal proteins and carcinogenesis (18,36-38). Diamond–Blackfan anemia is caused by mutations in the ribosomal protein genes RPS19, RPL5, RPS26, and RPL11 – these mutations disrupt the translational machinery and are also linked to an elevated risk of malignancies such as leukemia and sarcoma (39). The 5q minus syndrome which has its etiology in the loss of RPS14 coupled with deletion of the long arm of chromosome 5, is associated with a high risk of developing myelodysplastic syndrome (MDS) and AML (40). Mutations or deletions in RMRP (RNA component of mitochondrial RNA processing endoribonuclease), a pre-rRNA processing factor, cause cartilage-hair hypoplasia-anauxetic dysplasia, a ribosomopathy that is linked to an increased risk of developing non-Hodgkin’s lymphoma and basal cell carcinoma (41). Mutations in either DKC1 (Dyskerin pseudouridine synthase 1) or NPM1 (nucleophosmin 1) that impact rRNA pseudouridylation and 2-O’Me, respectively, can lead to dyskeratosis congenita, which is associated with an increased risk of MDS, leukemia, and head and neck malignancies (42,43). In **Table 1** we have presented an organized compilation of mutations in ribosomal proteins and their roles in different cancer types and clinical outcomes (34,44-75).

### ***Ribosome biogenesis in metastasis***

Metastatic colonization of cancer cells requires a precisely orchestrated series of events that allow cells to escape the primary tumor and invade at the metastatic site. One of the well-studied events during this process is epithelial to mesenchymal transition (EMT). EMT is a critical evolutionary conserved program that defines a vital process that orchestrates morphogenesis and organogenesis (76,77), and is recapitulated during cancer progression (76-79). Ribosome biogenesis is an important event for metabolically active cells, and it is logical that increased ribosome biogenesis may be essential for executing metabolic plasticity needed for the EMT program. Micalizzi *et al.*, (80) elegantly reviewed the impact of the EMT program on translation and translational regulation during

metastasis. However, the impacts of ribosome biogenesis and rRNA transcription extend far beyond the bookends of the translation process.

The link between EMT, ribosome biogenesis, and rRNA transcriptional regulation remains tenuous, though emerging reports have begun to bridge an important interplay between these processes. Initial indications of the influence of EMT on ribosome biogenesis came from Wnt5a treatment of MCF7 breast cancer cells, in which Wnt5a repressed rDNA transcription via localization of Disheveled 1 to rDNA (81). In this context, Wnt5a suppression of rDNA transcription aligned with known functions of Wnt5a in breast cancer to reduce migration and invasion. A hallmark study by Prakash *et al.*, concisely detailed an association between initiation of the EMT program concomitant with activation of rDNA transcription. Induction of EMT led to enhanced rRNA synthesis aligned with classical features of mesenchymal phenotypes, timed with the onset of the EMT program. Inhibition of rRNA synthesis shifted the EMT program and reduced metastasis (82). On the other hand, in MCF7 cells, incorporation of exogenously provided ribosomes induced EMT, accompanied by transdifferentiation in subtype marked by ER $\alpha$  suppression (83). Such shifts in tumor subtypes as a result of modulating rDNA transcription give way to the possibility that certain combination therapies may become effective in these tumors because of broad phenotypic changes incurred through impaired ribosome biogenesis.

Epigenetic regulation of rDNA transcription is an important regulatory node of ribosome biogenesis, and NoRC (nucleolar remodeling complex) maintains the silent states of rDNA clusters. Epigenetic regulation of EMT is well documented; however, recent work has linked epigenetic modulation of rDNA to increased invasion and migration. EZH2, the enzymatic catalytic subunit of polycomb repressive complex 2 (PRC2), is a major epigenetic writer that influences various aspects of tumor progression. Most recently, EZH2 was found to regulate lncRNA responsible for methylation of rDNA loci, thereby suppressing ribosome biogenesis, suggesting that the interplay between epigenetic regulation of rDNA with EMT may prime cancer cell metastasis (84).

Ribosome biogenesis is dependent on RNA Pol I transcriptional activity and incorporation of a myriad of ribosomal associated proteins that significantly impact ribosome function. A number of recent studies have demonstrated the importance of ribosomal proteins

influencing tumor progression and metastasis. Ebright *et al.*, identified a subset of ribosome gene signatures in breast cancer circulating tumor cells (CTCs) that were crucial in predicting poor clinical outcomes. RPL15 was identified as a critical driver of increased metastasis in CTCs; importantly, RPL15 overexpression promotes translation of core ribosomal proteins and drives global translation, implying the impact of ribosomal proteins on dictating metastatic potential of cancer cells (85). Single cell RNA sequencing also identified increased RPL15 and RPL27A in TNBC; moreover, RPL27A silencing diminished migration and invasion in breast cancer cells (86). Underscoring the importance of ribosomal proteins in EMT-induced ribosome biogenesis, was a recent finding reporting that La-related protein 6 (LARP6) upregulation during EMT drives localization of ribosomal proteins in migrating cells (87). LARP6 induction was found to mediate re-localization of ribosomal proteins to protrusive cell fronts, thereby enhancing ribosome biogenesis and allowing for preferential translation of mRNA subsets that exacerbate metastatic potential. This work lends further support to the importance of ribosomal associated proteins, and highlights changes in ribosomal protein content, as influenced by EMT, as an important regulatory step in transitioning cancer cells to highly migratory and invasive states.

Overall, growing evidence has strongly identified an important link between the EMT program and ribosome biogenesis, which culminates in enhanced migration, invasion, and ultimately metastasis. Collectively, current studies demonstrate not only the importance of rRNA transcriptional regulation, but also highlight epigenetic modifications and ribosome associated proteins as important factors that allow cancer cells to manipulate cellular programs such as EMT, thereby promoting metastatic potential. Ultimately, therapies aimed at targeting ribosome biogenesis induced during EMT may be a viable approach for a subset of patients. Clearly, more in depth studies are needed to unravel the complexities linking EMT and ribosome biogenesis.

### **Ribosome biogenesis in therapeutic resistance**

Despite anti-cancer treatments, cancer cells have the capacity to survive and become resistant to chemotherapy and radiation, resulting in a poor clinical outcome. Several

studies have demonstrated that proteins involved in ribosome biogenesis mediate radio-resistance and chemo-resistance in cancer models. Highlighting the importance of ribosome biogenesis in therapy resistance, **Table 1** details a number of ribosomal proteins that modulate therapeutic resistance in various cancers (74,88-101). In addition to ribosomal proteins, rRNA processing, rRNA modifying, and assembly proteins involved in ribosome biogenesis may have important roles in therapeutic resistance. Inactivation of the 60S subunit assembly factor, Bop1, provides cancer cells with a survival advantage to resist high dose chemotherapy (102). Conversely, nucleolin, a protein essential for ribosome synthesis and RNA processing, improves glioma stem cell sensitivity to temozolomide, partially by DNA repair regulation (103). The rRNA modifying protein NOP2/Sun RNA Methyltransferase 5 (NSUN5) is a candidate RNA methyltransferase for 5-methylcytosine on 28S rRNA at position C3782. Its loss spurs an adaptive translational program that enables tumor cell survival in conditions of stress but paradoxically is associated with a favorable clinical outcome (104). Similarly, rRNA and rDNA processing proteins influence radiation resistance in different cancer models. As an example, rRNA processing protein NOB1 is involved in radio-resistance; its knockdown reduced cell proliferation, suppressed apoptosis, and increased the radio-sensitivity in *in vitro* and *in vivo* models of papillary thyroid carcinoma (105). As such, apart from traditional roles in translation, a complex ensemble of ribosomal proteins and associated ribosome biogenesis factors collectively mediate cancer cell therapeutic response.

### Targeting Ribosomes

For decades, the nucleolus and its related pathways have been shown to exert control over several cellular functions that contribute to tumorigenesis and cancer progression. Thus, RNA Pol I and ribosome biogenesis were thought-provoking targets for cancer therapeutics. The ability to provide therapeutic selectivity for cancer cells and minimize the side effects of cancer therapeutics has been the optimum goal in targeting cancer generally and ribosome biogenesis specifically. Several cancer chemotherapeutic agents such as chemotherapeutic reagents like cisplatin, oxaliplatin, doxorubicin, and mitoxantrone were found to inhibit rRNA transcription and processing (106-110).

Oxaliplatin and phenanthriplatin were demonstrated to induce ribosome biogenesis stress and impact pre-rRNA formation without inducing DNA damage, unlike cisplatin. Further investigations determined that oxaliplatin and phenanthriplatin induced cytotoxicity through RPL11, and silencing RPL11 led to resistance to these drugs (107).

Inhibiting RNA Pol I transcription triggers nucleolar stress and results in translocation of ribosomal proteins from the nucleolus to the nucleoplasm, where proteins like RPL5 and RPL11 bind to MDM2, triggering its dissociation and therefore stimulation of p53 (111). Thus, by sustaining high levels of RNA Pol I transcription, cancer cells maintain nucleolar integrity and keep p53 under check (111). Therefore, the concept of inhibiting RNA Pol I for cancer therapeutics attracted investigators to design specific inhibitors to target RNA Pol I, with the expectation that normal cells would be spared since they are much less dependent on RNA Pol I transcription activity than cancer cells.

CX-5461 was the first selective and orally available inhibitor of RNA Pol I transcription (112,113). CX-5461 acts by perturbing the SL1-rDNA complex, compromising UBTF stabilization, and thus reducing the recruitment of RNA Pol I to the rDNA promoter (113). In pre-clinical models of melanoma and pancreatic cancer CX-5461 showed significant antitumor activity and induced potent cytotoxicity in cancer cells regardless of their P53 mutation status. CX-5461 was also found to work against hematological malignancies, for example, in a MYC-induced lymphoma mouse model (11). Inhibition of ATM/ATR in combination with CX-5461 showed improved therapeutic benefit in treating tumors that lack P53 (114,115). CX-5461 demonstrated clinical efficacy in AML and multiple myeloma (116-118) and showed a promising therapeutic effect in ovarian carcinomas by P53-independent initiation of DNA damage (119,120). CX-5461 also sensitized HR-proficient castrate-resistant prostate cancer (CRPC) to the PARP inhibitor talazoparib, synergistically inhibiting tumor growth in a pre-clinical CRPC PDX model (121).

BMH-21 is another potent small molecule RNA Pol I inhibitor that was discovered by a chemical compound library screen for p53 pathway activation in a human cancer cell line by Laiho and colleagues (122). BMH-21 inhibits RNA Pol I transcription by proteasome-dependent degradation of RPA194, the large catalytic protein subunit of RNA Pol I holo-complex, resulting in p53 activation (123). While not yet tested in clinical trials, in

various pre-clinical studies BMH21 has shown promising therapeutic efficacy towards different hematological and solid tumors (124-127). A second generation RNA Pol I inhibitor molecule, PMR-116, demonstrates greater efficacy and improved chemical properties compared to CX-5461. Unlike CX-5461, PMR-116 induces phosphorylation and accumulation of p53 without non-specifically activating CHK2 (128). Altogether, since ribosome biogenesis is a central process that contributes to cell survival and stress adaptive response, several investigations have explored the prospects of targeting ribosome biogenesis to interfere with the evolution of resistance to radiotherapy and chemotherapy.

Independent of RNA Pol I, the perinucleolar compartment (PNC) has emerged as a promising niche to screen for targeting ribosome biogenesis inhibitors (129). Remarkably PNC is a phenotypic marker that reflects metastatic capability (130). High PNC prevalence in primary tumors is associated with poor patient outcomes, including overall survival of patients with breast, colorectal, and ovarian cancer (130-132). Using PNC reduction as a surrogate marker, multiple high-content screens were performed and those yielded several lead compounds (129,133). One such well described, promising compound is Metarrestin that inhibits Pol I transcription, induces nucleolar segregation, reduces nucleolar volume, and reduces metastasis in an experimental model of prostate cancer (134). Overall, ribosome biogenesis inhibition by RNA Pol I inhibition or disruption of PNC presents a novel therapeutic avenue to overcome the chemotherapeutic resistance in multiple tumor types.

### **Perspective: Opportunities and Challenges**

Aberrations in ribosomal proteins are documented in multiple ribosomopathies and cancers. This is suggestive of a potential for monitoring ribosomal proteins in prognosticating chemo/radio-resistance of tumor cells. Therefore, it would be useful to investigate the utility of genetic screening of ribosomal proteins in treatment-naïve cancer patients. The influence of the tumor microenvironment, intra-tumoral heterogeneity, and ribosome heterogeneity are all new, unexplored fields. Stressors like intra-tumoral hypoxia and acidosis can create tumor regions with differential activation of ribosome

biogenesis, which may confer irregular therapeutic responsiveness and a selective pressure for cancer evolution toward more aggressive and treatment-resistant phenotypes. Furthermore, identifying translational changes linked to metastasis may lead to new therapy targets, particularly at critical points in the metastatic cascade where there is a higher dependence on translating a new set of mRNAs (135,136).

Nucleolar integrity and rRNA transcription are maintained by RNA Pol II-mediated transcription of Alu elements (alu RNAs). Since alu RNAs are localized in the nucleoli and interact with NPM1 and nucleolin, rRNA biogenesis and nucleolar architecture are disrupted when expression of alu RNAs is altered (137). Additionally, Abraham *et al.*, uncovered that nucleolar RNA Pol II plays an essential role in promoting rRNA and ribosome biogenesis by binding to rDNA-flanking regions and forming R-loops. As a consequence, RNA Pol I mediated transcription of sense intergenic noncoding RNAs (sincRNAs) is inhibited preserving the nucleolar architecture and maintaining rRNA transcription (138). While it is speculated that increased level of sincRNAs may lead to aberrant nucleolar morphology seen in cancer, these findings open the doors to further investigations into the non-canonical roles of RNA polymerases and novel potential regulators of ribosome biogenesis in physiological and pathological contexts. In addition, several long non-coding RNA species, e.g., promoter-associated RNAs (pRNAs), pyrimidine-rich non-coding transcript (PNCTR), promoter and pre-rRNA antisense (PAPAS), regulate RNA Pol I transcription and rRNA biogenesis, yet their relevance in cancer is understudied (139-141)

Another unexplored area is the impact of malignant cell transformation on the liquid/liquid phase separation of nucleoli (nucleolar dynamics). It was shown that the NORs can undergo prominent changes in response to chemical or environmental stress. However, to date no comprehensive studies have been conducted to establish if NORs are altered in cancer cells and whether these changes are cancer-type specific. Besides, little is known about the impact of aneuploidy in cancer cells on the nucleolar dynamics – specifically if aneuploidy alters rRNA and ribosome biogenesis, ultimately impacting cellular response to RNA Pol I inhibitors.

Advances in rRNA epitranscriptomics in the context of tumor growth and metastasis present several promising prospects. These rRNA epitranscriptomic modifications may potentially be important diagnostic markers or may help patient stratification. The molecular machinery responsible for these marks bears promise as novel drug targets. Specifically, differential snoRNA expression and differential rRNA 2'-O methylation in various cancers offer new opportunities for cancer prognostics and therapeutics. These will become a reality following controlled studies involving significantly large cohorts of patients. However, since ribosome biogenesis is critical for cell survival, various players in this process may potentially have functional redundancy. Additionally, diverse tumor cell populations due to intratumoral variability contributes to complexity of ribosomal heterogeneity. Thus, it is challenging to define a unique event to target ribosome biogenesis of all the cells in a tumor. However, as a combination treatment, ribosome biogenesis inhibition may offer a logical way to make tumor cells vulnerable to classical cytotoxic chemotherapy. As such, it is evident that additional investigations are necessary to mechanistically understand the significance of ribosome biogenesis in the context of tumor formation, progression, metastasis, and therapeutic resistance.



## **Acknowledgements**

The work is supported in part by I01 BX003374 (VA), CA194048 (NCI/NIH), and O'Neal Invests Award to R. S. Samant; Department of Defense (W81XWH-18-1-0036 and W81XWH-10-1-0755) and O'Neal Invests Award to L. A. Shevde.

## **Author Contributions**

R. S. S. and L. A. S. conceptualized the review. All authors were involved in writing and editing the manuscript. All authors have read and approved the manuscript.

**Table 1. Dysregulations in ribosomal proteins are associated with tumor progression and therapeutic resistance**

Ribosomal Protein	Expression level/status	Cancer type	Phenotype	Ref.
<b>Ribosomal proteins in tumorigenesis and metastasis</b>				
<i>RPL5</i>	Mutated (missense)	T-cell acute lymphoblastic leukemia (T-ALL), melanoma, and glioblastoma (GBM)	RPL5 mutations dysregulated the HDM2/p53-mediated ribosome biogenesis checkpoint with subsequent dysregulation in ribosome biogenesis	(44-46)
<i>RPL10</i>	Mutated (missense)	T-ALL	<ul style="list-style-type: none"> <li>• RPL10 R98S mutant leukemia cells showed a ribosome biogenesis defect.</li> <li>• RPL10 R98S mutant leukemia cells showed enhanced IRES-mediated translation and high tolerance to high oxidative stress levels</li> </ul>	(45,47-49)
<i>RPL15</i>	Upregulated	Colon cancer	Silencing of RPL15 inhibited cell proliferation and induced apoptosis	(50)
	Upregulated	Gastric cancer	Knockdown of RPL13 inhibited cell proliferation, migration, and tumor growth <i>in vivo</i>	(51)
<i>RPL19</i>	Upregulated	Prostate cancer	<ul style="list-style-type: none"> <li>• Increased RPL19 expression was predictive of shorter patient survival.</li> <li>• Silencing RPL19 suppressed tumor growth <i>in vivo</i>.</li> </ul>	(52,53)
	Upregulated	Hepatocellular carcinoma	Overexpression of RPL19 predicted poor prognosis.	(54)
<i>RPL22</i>	Downregulated	Lung cancer (Non small cell lung carcinoma; NSCLC)	Downregulation of RPL22 is associated with carcinogenesis.	(55)
	Deletions	T-ALL	Haploinsufficiency or monoallelic loss of RPL22 accelerated development of T-ALL	(56)

<i>RPL23</i>	Upregulated	Myelodysplastic syndrome (MDS)	<ul style="list-style-type: none"> <li>• Silencing RPL23 suppressed cell proliferation and increased apoptosis.</li> <li>• RPL23 overexpression was associated with apoptotic resistance and higher-risk of MDS</li> </ul>	(57)
	Upregulated	High-grade serous ovarian carcinoma	Higher RPL23 mRNA levels were associated with worse prognoses.	(58)
<i>RPL26</i>	Upregulated	Pancreatic cancer	Knockdown of RPL26 suppressed cell proliferation	(59)
<i>RPL29</i>	Upregulated	Pancreatic cancer	Knockdown of RPL29 suppressed cell proliferation	(59)
<i>RPL34</i>	Upregulated	Glioma	Knockdown of RPL34 suppressed proliferation and migration of glioma cells	(60)
	Upregulated	NSCLC	Knockdown of RPL34 suppressed cell proliferation and enhanced apoptosis in NSCLC cell lines.	(61)
	Upregulated	Osteosarcoma	<ul style="list-style-type: none"> <li>• High levels of RPL34 are associated with poor prognosis for osteosarcoma patients.</li> <li>• Knockdown of RPL34 inhibited cell proliferation, induced cell apoptosis.</li> </ul>	(62)
	Upregulated	Oral squamous cell carcinoma	Knockdown of RPL34 inhibited cell proliferation and migration	(63)
<i>RPL41</i>	Downregulated	Retinoblastoma	<ul style="list-style-type: none"> <li>• RPL41 peptide therapy improved sensitivity to carboplatin.</li> <li>• RPL41 peptide therapy induced apoptosis and inhibited cell migration.</li> </ul>	(64)
	Downregulated	Breast cancer	RPL41 down-regulation is associated with malignant transformation.	(65)
<i>RPS2</i>	Upregulated	Prostate cancer	Knockdown of RPS2 suppressed cell proliferation	(66)

			and induced apoptosis in malignant prostate cells.	
<i>Phospho-RPS6</i>	Upregulated	Lung cancer	High levels of Phospho-RPS6 are associated with shorter metastasis-free survival.	(67)
<i>RPS15</i>	Mutated (missense)	Chronic Lymphocytic Leukemia	<ul style="list-style-type: none"> <li>• RPS15 mutant primary CLL cells showed altered translation efficiency and rewiring of the translational program.</li> <li>• Mutant RPS15 caused dysregulation of p53 pathway.</li> </ul>	(34,68-70)
<i>RPS15A</i>	Upregulated	Colorectal cancer (CRC)	High levels of RPS15A are associated with poor prognosis.	(71)
<i>RPS20</i>	Mutated (missense)	CRC	RPS20 mutation was associated with a defect in pre-rRNA maturation.	(72,73)
<i>RPS20</i>	Upregulated	GBM	Higher levels of RPS20 are associated with poor prognosis.	(74)
<i>RPS27L</i>	Upregulated	Colorectal cancer	Elevated RPS27L expression in either feces or tissues is associated with better prognosis	(75)
<b>Ribosomal proteins in therapeutic resistance</b>				
<i>RPL3</i>	Downregulated	Lung cancer	Overexpression of RPL3 inhibited cell migration and invasion and improved 5-FU efficacy in lung cancer cells.	(88)
<i>RPL6</i>	Upregulated	Gastric cancer	<ul style="list-style-type: none"> <li>• Down-regulation of RPL6 suppressed cell proliferation</li> <li>• Overexpression of RPL6 promoted multidrug resistance</li> </ul>	(89,90)
<i>RPL13</i>	Upregulated	Gastric cancer	<ul style="list-style-type: none"> <li>• Knockdown of RPL13 suppressed cell proliferation</li> <li>• Overexpression of RPL13 promoted chemoresistance</li> </ul>	(91)

<i>RPL23</i>	Upregulated	Gastric cancer	RPL23 overexpression promoted multidrug resistance	(92)
<i>RPL34</i>	Upregulated	Pancreatic cancer	Knockdown of RPL34 suppressed cell proliferation, migration, and drug-resistance of pancreatic cancer cells.	(93)
<i>RPS6</i>	N/A	Gastric cancer	RPS6 suppression decreased cell proliferation and tumor growth in lapatinib-and trastuzumab-resistant gastric cancer models	(94)
<i>RPS3</i>	N/A	GBM	<ul style="list-style-type: none"> <li>• In radioresistant GBM cell lines, Ring Finger Protein 138 (RNF138) ubiquitinates RPS3 and promotes its degradation, which suppresses radiation-induced apoptosis and confers radio-resistance.</li> <li>• Silencing of RPS3 enhanced GBM cell tolerance to irradiation in vitro.</li> </ul>	(95)
<i>Phospho-RPS3</i>	N/A	Lung cancer (NSCLC)	In radioresistant NSCLC cells, RPS3 phosphorylation plays a key role in radiation resistance and initiating a pro-survival transcriptional program.	(96)
<i>RPS11</i>	Upregulated	GBM	<ul style="list-style-type: none"> <li>• Knockdown of RPS11 impaired apoptosis and led to resistance to etoposide and doxorubicin.</li> <li>• Higher levels of RPS11 are associated with poor prognosis.</li> </ul>	(74,97)
<i>RPS13</i>	Upregulated	Gastric cancer	RPS13 promotes cell proliferation and multidrug resistance.	(92,98)
<i>RPS27A</i>	Upregulated	Chronic myeloid leukemia (CML)	<ul style="list-style-type: none"> <li>• Patients with CML-accelerated or blast phase</li> </ul>	(99)

			<p>have higher levels of RPS27A compared to chronic phase patients.</p> <ul style="list-style-type: none"> <li>• Knockdown of RPS27A improved therapeutic efficacy of tyrosine kinase inhibitor Imatinib.</li> </ul>	
<i>RPS27L</i>	N/A		<p>Rps27l deficiency sensitized Trp53 +/- mice to irradiation by inhibiting cell proliferation, impairing DNA damage response, and inducing apoptosis.</p>	(100)
<i>RPLP1</i>	N/A	<p>Head and neck squamous cell carcinoma (HNSCC)</p>	<ul style="list-style-type: none"> <li>• Silencing of RPLP1 promoted apoptosis and decreased radio-resistance <i>in vitro</i>.</li> <li>• Invasive HNSCC showed higher expression levels of RPLP1.</li> </ul>	(101)

N/A: Information on clinical expression is not available

## References

1. Uniacke J, Perera JK, Lachance G, Francisco CB, Lee S. Cancer cells exploit eIF4E2-directed synthesis of hypoxia response proteins to drive tumor progression. *Cancer Res* **2014**;74:1379-89
2. Pelletier J, Thomas G, Volarevic S. Ribosome biogenesis in cancer: new players and therapeutic avenues. *Nat Rev Cancer* **2018**;18:51-63
3. Drygin D, Rice WG, Grummt I. The RNA polymerase I transcription machinery: an emerging target for the treatment of cancer. *Annu Rev Pharmacol Toxicol* **2010**;50:131-56
4. Ferreira R, Schneekloth JS, Jr., Panov KI, Hannan KM, Hannan RD. Targeting the RNA Polymerase I Transcription for Cancer Therapy Comes of Age. *Cells* **2020**;9
5. Schneider DA. RNA polymerase I activity is regulated at multiple steps in the transcription cycle: recent insights into factors that influence transcription elongation. *Gene* **2012**;493:176-84
6. McCool MA, Bryant CJ, Baserga SJ. MicroRNAs and long non-coding RNAs as novel regulators of ribosome biogenesis. *Biochem Soc Trans* **2020**;48:595-612
7. Donati G, Montanaro L, Derenzini M. Ribosome biogenesis and control of cell proliferation: p53 is not alone. *Cancer Res* **2012**;72:1602-7
8. van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat Rev Cancer* **2010**;10:301-9
9. Zhai W, Comai L. Repression of RNA polymerase I transcription by the tumor suppressor p53. *Mol Cell Biol* **2000**;20:5930-8
10. Derenzini M, Farabegoli F, Trere D. Relationship between interphase AgNOR distribution and nucleolar size in cancer cells. *Histochem J* **1992**;24:951-6
11. Derenzini M, Trere D, Pession A, Montanaro L, Sirri V, Ochs RL. Nucleolar function and size in cancer cells. *Am J Pathol* **1998**;152:1291-7
12. Volarevic S, Stewart MJ, Ledermann B, Zilberman F, Terracciano L, Montini E, *et al.* Proliferation, but not growth, blocked by conditional deletion of 40S ribosomal protein S6. *Science* **2000**;288:2045-7
13. Derenzini M, Montanaro L, Trere D. What the nucleolus says to a tumour pathologist. *Histopathology* **2009**;54:753-62
14. Williamson D, Lu YJ, Fang C, Pritchard-Jones K, Shipley J. Nascent pre-rRNA overexpression correlates with an adverse prognosis in alveolar rhabdomyosarcoma. *Genes Chromosomes Cancer* **2006**;45:839-45
15. Bywater MJ, Poortinga G, Sanij E, Hein N, Peck A, Cullinane C, *et al.* Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. *Cancer Cell* **2012**;22:51-65
16. Mauro VP, Edelman GM. The ribosome filter hypothesis. *Proc Natl Acad Sci U S A* **2002**;99:12031-6
17. Li D, Wang J. Ribosome heterogeneity in stem cells and development. *J Cell Biol* **2020**;219
18. Sulima SO, Hofman IJF, De Keersmaecker K, Dinman JD. How Ribosomes Translate Cancer. *Cancer Discov* **2017**;7:1069-87
19. Bastide A, David A. The ribosome, (slow) beating heart of cancer (stem) cell. *Oncogenesis* **2018**;7:34

20. Parks MM, Kurylo CM, Dass RA, Bojmar L, Lyden D, Vincent CT, *et al.* Variant ribosomal RNA alleles are conserved and exhibit tissue-specific expression. *Sci Adv* **2018**;4:eaa0665
21. Wang M, Lemos B. Ribosomal DNA copy number amplification and loss in human cancers is linked to tumor genetic context, nucleolus activity, and proliferation. *PLoS Genet* **2017**;13:e1006994
22. Marcel V, Kielbassa J, Marchand V, Natchiar KS, Paraqindes H, Nguyen Van Long F, *et al.* Ribosomal RNA 2'O-methylation as a novel layer of inter-tumour heterogeneity in breast cancer. *NAR Cancer* **2020**;2:zca036
23. Krogh N, Asmar F, Come C, Munch-Petersen HF, Gronbaek K, Nielsen H. Profiling of ribose methylations in ribosomal RNA from diffuse large B-cell lymphoma patients for evaluation of ribosomes as drug targets. *NAR Cancer* **2020**;2:zca035
24. Metge BJ, Kammerud SC, Pruitt HC, Shevde LA, Samant RS. Hypoxia re-programs 2'-O-Me modifications on ribosomal RNA. *iScience* **2021**;24:102010
25. Lafontaine DL. Noncoding RNAs in eukaryotic ribosome biogenesis and function. *Nat Struct Mol Biol* **2015**;22:11-9
26. Gay DM, Lund AH, Jansson MD. Translational control through ribosome heterogeneity and functional specialization. *Trends Biochem Sci* **2021**
27. Sloan KE, Warda AS, Sharma S, Entian KD, Lafontaine DLJ, Bohnsack MT. Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. *RNA Biol* **2017**;14:1138-52
28. Pauli C, Liu Y, Rohde C, Cui C, Fijalkowska D, Gerloff D, *et al.* Site-specific methylation of 18S ribosomal RNA by SNORD42A is required for acute myeloid leukemia cell proliferation. *Blood* **2020**;135:2059-70
29. Marcel V, Ghayad SE, Belin S, Therizols G, Morel AP, Solano-Gonzalez E, *et al.* p53 acts as a safeguard of translational control by regulating fibrillarin and rRNA methylation in cancer. *Cancer Cell* **2013**;24:318-30
30. Guimaraes JC, Zavolan M. Patterns of ribosomal protein expression specify normal and malignant human cells. *Genome Biol* **2016**;17:236
31. Genuth NR, Barna M. The Discovery of Ribosome Heterogeneity and Its Implications for Gene Regulation and Organismal Life. *Mol Cell* **2018**;71:364-74
32. Kampen KR, Sulima SO, Vereecke S, De Keersmaecker K. Hallmarks of ribosomopathies. *Nucleic Acids Res* **2020**;48:1013-28
33. Babaian A, Rothe K, Girodat D, Minia I, Djondovic S, Milek M, *et al.* Loss of m(1)acp(3)Psi Ribosomal RNA Modification Is a Major Feature of Cancer. *Cell Rep* **2020**;31:107611
34. Bretones G, Alvarez MG, Arango JR, Rodriguez D, Nadeu F, Prado MA, *et al.* Altered patterns of global protein synthesis and translational fidelity in RPS15-mutated chronic lymphocytic leukemia. *Blood* **2018**;132:2375-88
35. Aspesi A, Ellis SR. Rare ribosomopathies: insights into mechanisms of cancer. *Nat Rev Cancer* **2019**;19:228-38
36. Sulima SO, Kampen KR, De Keersmaecker K. Cancer Biogenesis in Ribosomopathies. *Cells* **2019**;8
37. Farley KI, Baserga SJ. Probing the mechanisms underlying human diseases in making ribosomes. *Biochem Soc Trans* **2016**;44:1035-44



38. Kang J, Brajanovski N, Chan KT, Xuan J, Pearson RB, Sanij E. Ribosomal proteins and human diseases: molecular mechanisms and targeted therapy. *Signal Transduct Target Ther* **2021**;6:323
39. Horos R, Ijspeert H, Pospisilova D, Sendtner R, Andrieu-Soler C, Taskesen E, *et al.* Ribosomal deficiencies in Diamond-Blackfan anemia impair translation of transcripts essential for differentiation of murine and human erythroblasts. *Blood* **2012**;119:262-72
40. Lee JH, List A, Sallman DA. Molecular pathogenesis of myelodysplastic syndromes with deletion 5q. *Eur J Haematol* **2019**;102:203-9
41. Makitie O, Vakkilainen S. Cartilage-Hair Hypoplasia - Anauxetic Dysplasia Spectrum Disorders. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, *et al.*, editors. *GeneReviews*((R)). Seattle (WA)1993.
42. AISabbagh MM. Dyskeratosis congenita: a literature review. *J Dtsch Dermatol Ges* **2020**;18:943-67
43. Nachmani D, Bothmer AH, Grisendi S, Mele A, Bothmer D, Lee JD, *et al.* Germline NPM1 mutations lead to altered rRNA 2'-O-methylation and cause dyskeratosis congenita. *Nat Genet* **2019**;51:1518-29
44. Orsolich I, Bursac S, Jurada D, Drmic Hofman I, Dembic Z, Bartek J, *et al.* Cancer-associated mutations in the ribosomal protein L5 gene dysregulate the HDM2/p53-mediated ribosome biogenesis checkpoint. *Oncogene* **2020**;39:3443-57
45. De Keersmaecker K, Atak ZK, Li N, Vicente C, Patchett S, Girardi T, *et al.* Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. *Nat Genet* **2013**;45:186-90
46. Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, *et al.* Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* **2014**;505:495-501
47. Kampen KR, Sulima SO, Verbelen B, Girardi T, Vereecke S, Rinaldi G, *et al.* The ribosomal RPL10 R98S mutation drives IRES-dependent BCL-2 translation in T-ALL. *Leukemia* **2019**;33:319-32
48. Girardi T, Vereecke S, Sulima SO, Khan Y, Fancello L, Briggs JW, *et al.* The T-cell leukemia-associated ribosomal RPL10 R98S mutation enhances JAK-STAT signaling. *Leukemia* **2018**;32:809-19
49. Abdelfattah N, Rajamanickam S, Panneerdoss S, Timilsina S, Yadav P, Onyeagucha BC, *et al.* MiR-584-5p potentiates vincristine and radiation response by inducing spindle defects and DNA damage in medulloblastoma. *Nat Commun* **2018**;9:4541
50. Dong Z, Jiang H, Liang S, Wang Y, Jiang W, Zhu C. Ribosomal Protein L15 is involved in Colon Carcinogenesis. *Int J Med Sci* **2019**;16:1132-41
51. Wang H, Zhao LN, Li KZ, Ling R, Li XJ, Wang L. Overexpression of ribosomal protein L15 is associated with cell proliferation in gastric cancer. *BMC Cancer* **2006**;6:91
52. Bee A, Ke Y, Forootan S, Lin K, Beesley C, Forrest SE, *et al.* Ribosomal protein l19 is a prognostic marker for human prostate cancer. *Clin Cancer Res* **2006**;12:2061-5
53. Bee A, Brewer D, Beesley C, Dodson A, Forootan S, Dickinson T, *et al.* siRNA knockdown of ribosomal protein gene RPL19 abrogates the aggressive phenotype of human prostate cancer. *PLoS One* **2011**;6:e22672
54. Rao B, Li J, Ren T, Yang J, Zhang G, Liu L, *et al.* RPL19 Is a Prognostic Biomarker and Promotes Tumor Progression in Hepatocellular Carcinoma. *Front Cell Dev Biol* **2021**;9:686547

55. Yang M, Sun H, Wang H, Zhang S, Yu X, Zhang L. Down-regulation of ribosomal protein L22 in non-small cell lung cancer. *Med Oncol* **2013**;30:646
56. Rao S, Lee SY, Gutierrez A, Perrigoue J, Thapa RJ, Tu Z, *et al.* Inactivation of ribosomal protein L22 promotes transformation by induction of the stemness factor, Lin28B. *Blood* **2012**;120:3764-73
57. Qi Y, Li X, Chang C, Xu F, He Q, Zhao Y, *et al.* Ribosomal protein L23 negatively regulates cellular apoptosis via the RPL23/Miz-1/c-Myc circuit in higher-risk myelodysplastic syndrome. *Sci Rep* **2017**;7:2323
58. Kang H, Choi MC, Kim S, Jeong JY, Kwon AY, Kim TH, *et al.* USP19 and RPL23 as Candidate Prognostic Markers for Advanced-Stage High-Grade Serous Ovarian Carcinoma. *Cancers (Basel)* **2021**;13
59. Li C, Ge M, Yin Y, Luo M, Chen D. Silencing expression of ribosomal protein L26 and L29 by RNA interfering inhibits proliferation of human pancreatic cancer PANC-1 cells. *Mol Cell Biochem* **2012**;370:127-39
60. Ji P, Wang L, Liu J, Mao P, Li R, Jiang H, *et al.* Knockdown of RPL34 inhibits the proliferation and migration of glioma cells through the inactivation of JAK/STAT3 signaling pathway. *J Cell Biochem* **2019**;120:3259-67
61. Yang S, Cui J, Yang Y, Liu Z, Yan H, Tang C, *et al.* Over-expressed RPL34 promotes malignant proliferation of non-small cell lung cancer cells. *Gene* **2016**;576:421-8
62. Luo S, Zhao J, Fowdur M, Wang K, Jiang T, He M. Highly expressed ribosomal protein L34 indicates poor prognosis in osteosarcoma and its knockdown suppresses osteosarcoma proliferation probably through translational control. *Sci Rep* **2016**;6:37690
63. Dai J, Wei W. Influence of the RPL34 gene on the growth and metastasis of oral squamous cell carcinoma cells. *Arch Oral Biol* **2017**;83:40-6
64. Geng W, Ren J, Shi H, Qin F, Xu X, Xiao S, *et al.* RPL41 sensitizes retinoblastoma cells to chemotherapeutic drugs via ATF4 degradation. *J Cell Physiol* **2021**;236:2214-25
65. Wang S, Huang J, He J, Wang A, Xu S, Huang SF, *et al.* RPL41, a small ribosomal peptide deregulated in tumors, is essential for mitosis and centrosome integrity. *Neoplasia* **2010**;12:284-93
66. Wang M, Hu Y, Stearns ME. RPS2: a novel therapeutic target in prostate cancer. *J Exp Clin Cancer Res* **2009**;28:6
67. McDonald JM, Pelloski CE, Ledoux A, Sun M, Raso G, Komaki R, *et al.* Elevated phospho-S6 expression is associated with metastasis in adenocarcinoma of the lung. *Clin Cancer Res* **2008**;14:7832-7
68. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, *et al.* Mutations driving CLL and their evolution in progression and relapse. *Nature* **2015**;526:525-30
69. Ljungstrom V, Cortese D, Young E, Pandzic T, Mansouri L, Plevova K, *et al.* Whole-exome sequencing in relapsing chronic lymphocytic leukemia: clinical impact of recurrent RPS15 mutations. *Blood* **2016**;127:1007-16
70. Ntoufa S, Gerousi M, Laidou S, Psomopoulos F, Tsiolas G, Moysiadis T, *et al.* RPS15 mutations rewire RNA translation in chronic lymphocytic leukemia. *Blood Adv* **2021**;5:2788-92
71. Chen J, Wei Y, Feng Q, Ren L, He G, Chang W, *et al.* Ribosomal protein S15A promotes malignant transformation and predicts poor outcome in colorectal cancer through misregulation of p53 signaling pathway. *Int J Oncol* **2016**;48:1628-38
72. Nieminen TT, O'Donohue MF, Wu Y, Lohi H, Scherer SW, Paterson AD, *et al.* Germline mutation of RPS20, encoding a ribosomal protein, causes predisposition to hereditary

- nonpolyposis colorectal carcinoma without DNA mismatch repair deficiency. *Gastroenterology* **2014**;147:595-8 e5
73. Broderick P, Dobbins SE, Chubb D, Kinnersley B, Dunlop MG, Tomlinson I, *et al.* Validation of Recently Proposed Colorectal Cancer Susceptibility Gene Variants in an Analysis of Families and Patients-a Systematic Review. *Gastroenterology* **2017**;152:75-7 e4
  74. Yong WH, Shabihkhani M, Telesca D, Yang S, Tso JL, Menjivar JC, *et al.* Ribosomal Proteins RPS11 and RPS20, Two Stress-Response Markers of Glioblastoma Stem Cells, Are Novel Predictors of Poor Prognosis in Glioblastoma Patients. *PLoS One* **2015**;10:e0141334
  75. Huang CJ, Yang SH, Lee CL, Cheng YC, Tai SY, Chien CC. Ribosomal protein S27-like in colorectal cancer: a candidate for predicting prognoses. *PLoS One* **2013**;8:e67043
  76. Aiello NM, Kang Y. Context-dependent EMT programs in cancer metastasis. *J Exp Med* **2019**;216:1016-26
  77. Ye X, Weinberg RA. Epithelial-Mesenchymal Plasticity: A Central Regulator of Cancer Progression. *Trends Cell Biol* **2015**;25:675-86
  78. Bakir B, Chiarella AM, Pitarresi JR, Rustgi AK. EMT, MET, Plasticity, and Tumor Metastasis. *Trends Cell Biol* **2020**;30:764-76
  79. Lambert AW, Weinberg RA. Linking EMT programmes to normal and neoplastic epithelial stem cells. *Nat Rev Cancer* **2021**;21:325-38
  80. Micalizzi DS, Ebright RY, Haber DA, Maheswaran S. Translational Regulation of Cancer Metastasis. *Cancer Res* **2021**;81:517-24
  81. Dass RA, Sarshad AA, Carson BB, Feenstra JM, Kaur A, Obrdlik A, *et al.* Wnt5a Signals through DVL1 to Repress Ribosomal DNA Transcription by RNA Polymerase I. *PLoS Genet* **2016**;12:e1006217
  82. Prakash V, Carson BB, Feenstra JM, Dass RA, Sekyrova P, Hoshino A, *et al.* Ribosome biogenesis during cell cycle arrest fuels EMT in development and disease. *Nat Commun* **2019**;10:2110
  83. Kudo M, Anam MB, Istiaq A, Ahmad SAI, Ito N, Ohta K. Ribosome Incorporation Induces EMT-like Phenomenon with Cell Cycle Arrest in Human Breast Cancer Cell. *Cells Tissues Organs* **2021**:1-10
  84. Chu W, Zhang X, Qi L, Fu Y, Wang P, Zhao W, *et al.* The EZH2-PHACTR2-AS1-Ribosome Axis induces Genomic Instability and Promotes Growth and Metastasis in Breast Cancer. *Cancer Res* **2020**;80:2737-50
  85. Ebright RY, Lee S, Wittner BS, Niederhoffer KL, Nicholson BT, Bardia A, *et al.* Deregulation of ribosomal protein expression and translation promotes breast cancer metastasis. *Science* **2020**;367:1468-73
  86. Zhao W, Li X, Nian W, Wang J, Wang X, Sun L, *et al.* Ribosome Proteins Represented by RPL27A Mark the Development and Metastasis of Triple-Negative Breast Cancer in Mouse and Human. *Front Cell Dev Biol* **2021**;9:716730
  87. Dermit M, Dodel M, Lee FCY, Azman MS, Schwenzer H, Jones JL, *et al.* Subcellular mRNA Localization Regulates Ribosome Biogenesis in Migrating Cells. *Dev Cell* **2020**;55:298-313 e10
  88. Russo A, Saide A, Cagliani R, Cantile M, Botti G, Russo G. rpL3 promotes the apoptosis of p53 mutated lung cancer cells by down-regulating CBS and NFkappaB upon 5-FU treatment. *Sci Rep* **2016**;6:38369

89. Wu Q, Gou Y, Wang Q, Jin H, Cui L, Zhang Y, *et al.* Downregulation of RPL6 by siRNA inhibits proliferation and cell cycle progression of human gastric cancer cell lines. *PLoS One* **2011**;6:e26401
90. Du J, Shi Y, Pan Y, Jin X, Liu C, Liu N, *et al.* Regulation of multidrug resistance by ribosomal protein l6 in gastric cancer cells. *Cancer Biol Ther* **2005**;4:242-7
91. Kobayashi T, Sasaki Y, Oshima Y, Yamamoto H, Mita H, Suzuki H, *et al.* Activation of the ribosomal protein L13 gene in human gastrointestinal cancer. *Int J Mol Med* **2006**;18:161-70
92. Shi Y, Zhai H, Wang X, Han Z, Liu C, Lan M, *et al.* Ribosomal proteins S13 and L23 promote multidrug resistance in gastric cancer cells by suppressing drug-induced apoptosis. *Exp Cell Res* **2004**;296:337-46
93. Wei F, Ding L, Wei Z, Zhang Y, Li Y, Qinghua L, *et al.* Ribosomal protein L34 promotes the proliferation, invasion and metastasis of pancreatic cancer cells. *Oncotarget* **2016**;7:85259-72
94. Gambardella V, Gimeno-Valiente F, Tarazona N, Martinez-Ciarpaglini C, Roda D, Fleitas T, *et al.* NRF2 through RPS6 Activation Is Related to Anti-HER2 Drug Resistance in HER2-Amplified Gastric Cancer. *Clin Cancer Res* **2019**;25:1639-49
95. Kim W, Youn H, Lee S, Kim E, Kim D, Sub Lee J, *et al.* RNF138-mediated ubiquitination of rpS3 is required for resistance of glioblastoma cells to radiation-induced apoptosis. *Exp Mol Med* **2018**;50:e434
96. Yang HJ, Youn H, Seong KM, Jin YW, Kim J, Youn B. Phosphorylation of ribosomal protein S3 and antiapoptotic TRAF2 protein mediates radioresistance in non-small cell lung cancer cells. *J Biol Chem* **2013**;288:2965-75
97. Awah CU, Chen L, Bansal M, Mahajan A, Winter J, Lad M, *et al.* Ribosomal protein S11 influences glioma response to TOP2 poisons. *Oncogene* **2020**;39:5068-81
98. Guo X, Shi Y, Gou Y, Li J, Han S, Zhang Y, *et al.* Human ribosomal protein S13 promotes gastric cancer growth through down-regulating p27(Kip1). *J Cell Mol Med* **2011**;15:296-306
99. Wang H, Xie B, Kong Y, Tao Y, Yang G, Gao M, *et al.* Overexpression of RPS27a contributes to enhanced chemoresistance of CML cells to imatinib by the transactivated STAT3. *Oncotarget* **2016**;7:18638-50
100. Zhao Y, Tan M, Liu X, Xiong X, Sun Y. Inactivation of ribosomal protein S27-like confers radiosensitivity via the Mdm2-p53 and Mdm2-MRN-ATM axes. *Cell Death Dis* **2018**;9:145
101. Williams PD, Owens CR, Dziegielewska J, Moskaluk CA, Read PW, Lerner JM, *et al.* Cyclophilin B expression is associated with in vitro radioresistance and clinical outcome after radiotherapy. *Neoplasia* **2011**;13:1122-31
102. Sapio RT, Nezdyur AN, Krevetski M, Anikin L, Manna VJ, Minkovsky N, *et al.* Inhibition of post-transcriptional steps in ribosome biogenesis confers cytoprotection against chemotherapeutic agents in a p53-dependent manner. *Sci Rep* **2017**;7:9041
103. Ko CY, Lin CH, Chuang JY, Chang WC, Hsu TI. MDM2 Degrades Deacetylated Nucleolin Through Ubiquitination to Promote Glioma Stem-Like Cell Enrichment for Chemotherapeutic Resistance. *Mol Neurobiol* **2018**;55:3211-23
104. Janin M, Ortiz-Barahona V, de Moura MC, Martinez-Cardus A, Llinas-Arias P, Soler M, *et al.* Epigenetic loss of RNA-methyltransferase NSUN5 in glioma targets ribosomes to drive a stress adaptive translational program. *Acta Neuropathol* **2019**;138:1053-74

105. Meng W, Wang PS, Liu J, Xue S, Wang GM, Meng XY, *et al.* Adenovirus-mediated siRNA targeting NOB1 inhibits tumor growth and enhances radiosensitivity of human papillary thyroid carcinoma in vitro and in vivo. *Oncol Rep* **2014**;32:2411-20
106. Burger K, Muhl B, Harasim T, Rohrmoser M, Malamoussi A, Orban M, *et al.* Chemotherapeutic drugs inhibit ribosome biogenesis at various levels. *J Biol Chem* **2010**;285:12416-25
107. Bruno PM, Liu Y, Park GY, Murai J, Koch CE, Eisen TJ, *et al.* A subset of platinum-containing chemotherapeutic agents kills cells by inducing ribosome biogenesis stress. *Nat Med* **2017**;23:461-71
108. Gilder AS, Do PM, Carrero ZI, Cosman AM, Broome HJ, Velma V, *et al.* Coilin participates in the suppression of RNA polymerase I in response to cisplatin-induced DNA damage. *Mol Biol Cell* **2011**;22:1070-9
109. Likovsky Z, Peterka M, Peterkova R. Drug-induced changes of rRNA biosynthesis--a marker of toxic damage to embryonal cell population. *Funct Dev Morphol* **1993**;3:3-9
110. Kacerovska H, Likovsky Z, Smetana K. Nucleolar silver stained granules in rat Yoshida sarcoma cells after RNA synthesis inhibition. *Neoplasma* **1981**;28:513-6
111. Deisenroth C, Zhang Y. Ribosome biogenesis surveillance: probing the ribosomal protein-Mdm2-p53 pathway. *Oncogene* **2010**;29:4253-60
112. Haddach M, Schwaebe MK, Michaux J, Nagasawa J, O'Brien SE, Whitten JP, *et al.* Discovery of CX-5461, the First Direct and Selective Inhibitor of RNA Polymerase I, for Cancer Therapeutics. *ACS Med Chem Lett* **2012**;3:602-6
113. Drygin D, Lin A, Bliesath J, Ho CB, O'Brien SE, Proffitt C, *et al.* Targeting RNA polymerase I with an oral small molecule CX-5461 inhibits ribosomal RNA synthesis and solid tumor growth. *Cancer Res* **2011**;71:1418-30
114. Quin J, Chan KT, Devlin JR, Cameron DP, Diesch J, Cullinane C, *et al.* Inhibition of RNA polymerase I transcription initiation by CX-5461 activates non-canonical ATM/ATR signaling. *Oncotarget* **2016**;7:49800-18
115. Negi SS, Brown P. rRNA synthesis inhibitor, CX-5461, activates ATM/ATR pathway in acute lymphoblastic leukemia, arrests cells in G2 phase and induces apoptosis. *Oncotarget* **2015**;6:18094-104
116. Hein N, Cameron DP, Hannan KM, Nguyen NN, Fong CY, Sornkom J, *et al.* Inhibition of Pol I transcription treats murine and human AML by targeting the leukemia-initiating cell population. *Blood* **2017**;129:2882-95
117. Maclachlan KH, Cuddihy A, Hein N, Cullinane C, Harrison SJ, Hannan R, *et al.* Novel Combination Therapies with the RNA Polymerase I Inhibitor CX-5461 Significantly Improve Efficacy in Multiple Myeloma. *Blood* **2017**;130
118. Lee HC, Wang H, Baladandayuthapani V, Lin H, He J, Jones RJ, *et al.* RNA Polymerase I Inhibition with CX-5461 as a Novel Therapeutic Strategy to Target MYC in Multiple Myeloma. *Brit J Haematol* **2017**;177:80-94
119. Cornelison R, Dobbin ZC, Katre AA, Jeong DH, Zhang Y, Chen D, *et al.* Targeting RNA-Polymerase I in Both Chemosensitive and Chemoresistant Populations in Epithelial Ovarian Cancer. *Clin Cancer Res* **2017**;23:6529-40
120. Sanij E, Hannan KM, Xuan J, Yan S, Ahern JE, Triglos AS, *et al.* CX-5461 activates the DNA damage response and demonstrates therapeutic efficacy in high-grade serous ovarian cancer. *Nat Commun* **2020**;11:2641

121. Lawrence MG, Porter LH, Choo N, Pook D, Grummet JP, Pezaro CJ, *et al.* CX-5461 Sensitizes DNA Damage Repair–proficient Castrate-resistant Prostate Cancer to PARP Inhibition. *Molecular Cancer Therapeutics* **2021**
122. Peltonen K, Colis L, Liu H, Jaamaa S, Moore HM, Enback J, *et al.* Identification of novel p53 pathway activating small-molecule compounds reveals unexpected similarities with known therapeutic agents. *PLoS One* **2010**;5:e12996
123. Peltonen K, Colis L, Liu H, Trivedi R, Moubarek MS, Moore HM, *et al.* A targeting modality for destruction of RNA polymerase I that possesses anticancer activity. *Cancer cell* **2014**;25:77-90
124. Low JY, Sirajuddin P, Moubarek M, Agarwal S, Rege A, Guner G, *et al.* Effective targeting of RNA polymerase I in treatment-resistant prostate cancer. *Prostate* **2019**;79:1837-51
125. Guner G, Sirajuddin P, Zheng Q, Bai B, Brodie A, Liu H, *et al.* Novel Assay to Detect RNA Polymerase I Activity In Vivo. *Mol Cancer Res* **2017**;15:577-84
126. Fu X, Xu L, Qi L, Tian H, Yi D, Yu Y, *et al.* BMH-21 inhibits viability and induces apoptosis by p53-dependent nucleolar stress responses in SKOV3 ovarian cancer cells. *Oncol Rep* **2017**;38:859-65
127. Kammerud SC, Metge BJ, Elhamamsy AR, Weeks SE, Alsheikh HA, Mattheyses AL, *et al.* Novel role of the dietary flavonoid fisetin in suppressing rRNA biogenesis. *Lab Invest* **2021**;101:1439-48
128. Dauban L, Cerezo E, Henras A, Gadad O. Meeting report from the first European OddPols meeting: Toulouse 2018. *Gene* **2019**;702:215-9
129. Frankowski K, Patnaik S, Schoenen F, Huang S, Norton J, Wang C, *et al.* Discovery and Development of Small Molecules That Reduce PNC Prevalence. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD)2010.
130. Slusarczyk A, Kamath R, Wang C, Anchel D, Pollock C, Lewandowska MA, *et al.* Structure and function of the perinucleolar compartment in cancer cells. *Cold Spring Harb Symp Quant Biol* **2010**;75:599-605
131. Kamath RV, Thor AD, Wang C, Edgerton SM, Slusarczyk A, Leary DJ, *et al.* Perinucleolar compartment prevalence has an independent prognostic value for breast cancer. *Cancer Res* **2005**;65:246-53
132. Norton JT, Pollock CB, Wang C, Schink JC, Kim JJ, Huang S. Perinucleolar compartment prevalence is a phenotypic pancancer marker of malignancy. *Cancer* **2008**;113:861-9
133. Norton JT, Titus SA, Dexter D, Austin CP, Zheng W, Huang S. Automated high-content screening for compounds that disassemble the perinucleolar compartment. *J Biomol Screen* **2009**;14:1045-53
134. Frankowski KJ, Wang C, Patnaik S, Schoenen FJ, Southall N, Li D, *et al.* Metarrestin, a perinucleolar compartment inhibitor, effectively suppresses metastasis. *Sci Transl Med* **2018**;10
135. Grzmil M, Hemmings BA. Translation regulation as a therapeutic target in cancer. *Cancer Res* **2012**;72:3891-900
136. Silvera D, Formenti SC, Schneider RJ. Translational control in cancer. *Nat Rev Cancer* **2010**;10:254-66
137. Caudron-Herger M, Pankert T, Seiler J, Nemeth A, Voit R, Grummt I, *et al.* Alu element-containing RNAs maintain nucleolar structure and function. *EMBO J* **2015**;34:2758-74

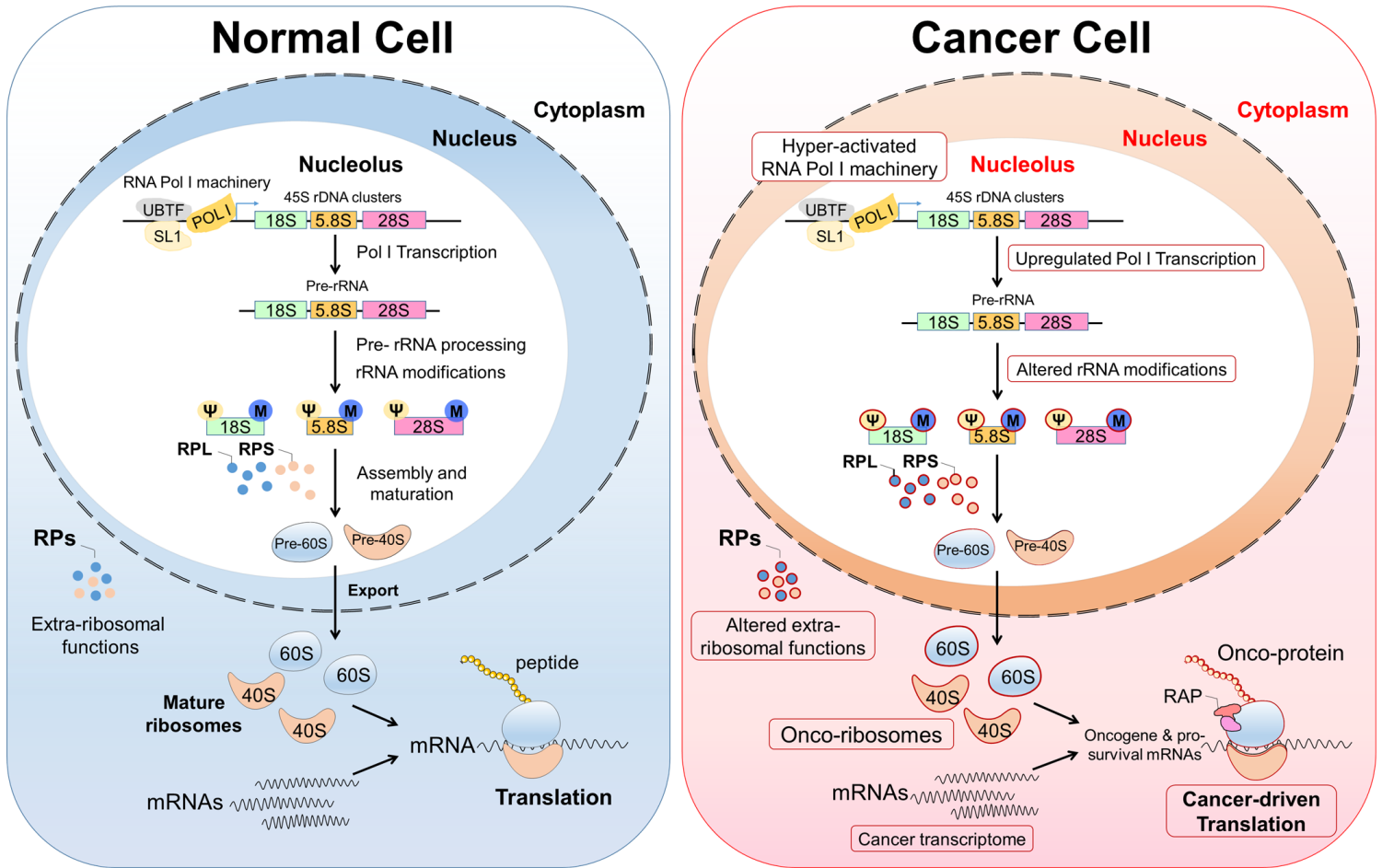
138. Abraham KJ, Khosraviani N, Chan JNY, Gorthi A, Samman A, Zhao DY, *et al.* Nucleolar RNA polymerase II drives ribosome biogenesis. *Nature* **2020**;585:298-302
139. Hao Q, Prasanth KV. Regulatory roles of nucleolus organizer region-derived long non-coding RNAs. *Mamm Genome* **2021**
140. Shiao YH, Lupascu ST, Gu YD, Kasprzak W, Hwang CJ, Fields JR, *et al.* An intergenic non-coding rRNA correlated with expression of the rRNA and frequency of an rRNA single nucleotide polymorphism in lung cancer cells. *PLoS One* **2009**;4:e7505
141. Yan Q, Zhu C, Guang S, Feng X. The Functions of Non-coding RNAs in rRNA Regulation. *Front Genet* **2019**;10:290

## Figure legend

### **Figure 1. Ribosome heterogeneity plays a role in tumorigenesis and cancer progression.**

Ribosome biogenesis begins in the nucleolus where repeats of rDNA reside. RNA Pol I transcription factors, such as UBTF and SL1 bind to active clusters of rDNAs to initiate RNA Pol I transcription and pre-rRNA biosynthesis. Then, pre-rRNA passes through a series of processing and rRNA modifications. Processed rRNA species are combined with ribosomal proteins to generate pre-60S and pre-40s subunits, which are matured and transported to the cytoplasm to participate in protein synthesis. Owing to their uncontrollable proliferation and high demand for ribosomes, cancer cells have upregulated activity of RNA Pol I leading to increased rRNA biogenesis. In cancer, there are also non-canonical or abnormal rRNA modifications. Ribosomal proteins undergo post-translational modifications. Differentially modified ribosome proteins can be incorporated in the ribosomes adding to the ribosome heterogeneity. Additionally, ribosomal proteins, due to their extra-ribosomal functions, can contribute to chemo/radioresistance and cancer progression. It is hypothesized that all these alterations and modifications create “onco-ribosomes”, which carry out an aberrant translational program and direct the preferential translation of oncogenes and pro-survival genes that promote cancer progression.





**Figure 1.** Ribosome heterogeneity plays a role in tumorigenesis and cancer progression