Ribosome Biogenesis: A Central Player in Cancer Metastasis and Therapeutic Resistance

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ABSTRACT

Ribosomes are a complex ensemble of rRNA and ribosomal proteins that function as mRNA translation machines. Ribosome biogenesis is a multistep 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 hyperactivation 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 patients with cancer. 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 multistep 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 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 1 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 (**Fig. 1**).

The nucleolus is the principal site of ribosome biogenesis and forms around nucleolar organizer regions (NOR), which contain several hundred 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 noncoding 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

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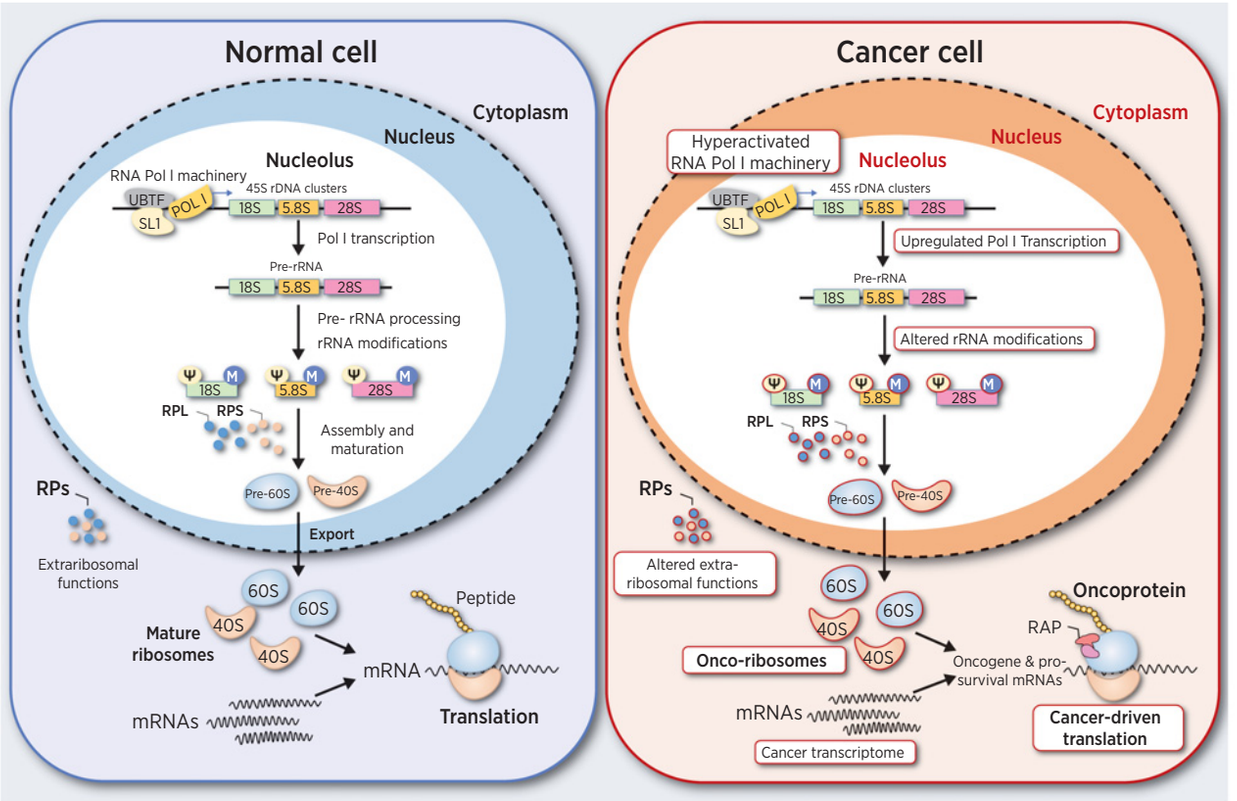

Figure 1.

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 noncanonical or abnormal rRNA modifications. Ribosomal proteins undergo posttranslational modifications. Differentially modified ribosome proteins can be incorporated in the ribosomes adding to the ribosome heterogeneity. In addition, ribosomal proteins, due to their extraribosomal 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 prosurvival genes that promote cancer progression.

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 and p53 pathways stimulated nucleolar function and led to nucleolar enlargement (13).

One of the earliest studies by Williamson and colleagues 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 hematologic 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, posttranslational 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 and colleagues discovered that the number

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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 and colleagues 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 and colleagues 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 and colleagues demonstrated a novel concept that sites of rRNA hypomethylation can be used as potential drug targets (23). Metge and colleagues 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 internal ribosome entry site (IRES)–mediated translation (24). Altered expression of small nucleolar RNAs (snoRNA) accounts for further ribosome heterogeneity, impacting physiologic and pathologic cellular processes, including carcinogenesis (25, 26). rRNAs undergo extensive posttranscriptional modifications, predominantly pseudouridylation (ψ) 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 noncancerous 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 patients with breast cancer (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 pathologic 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 prosurvival genes, facilitating cancer progression (19, 32). Babaian and colleagues identified a cancer-specific single-nucleotide variation in 18S rRNA at nucleotide 1248. U in more than 45% of patients with colorectal cancer. This results in hyper-modification on 18S rRNA at the peptidyl decoding site of the ribosome. A subset of patients with colorectal cancer 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 and

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

Ribosomal protein	Expression level/status	Cancer type	Phenotype	References
Ribosomal proteins in tumorigenesis and metastasis				
RPL5	Mutated (missense)	T-ALL, melanoma, and GBM	RPL5 mutations dysregulated the HDM2/p53-mediated ribosome biogenesis checkpoint with subsequent dysregulation in ribosome biogenesis	44–46
RPL10	Mutated (missense)	T-ALL	• RPL10 R98S mutant leukemia cells showed a ribosome biogenesis defect. • RPL10 R98S mutant leukemia cells showed enhanced IRES-mediated translation and high tolerance to high oxidative stress levels	45, 47–49
RPL15	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 *in vivo*	51
RPL19	Upregulated	Prostate cancer	• Increased RPL19 expression was predictive of shorter patient survival. • Silencing RPL19 suppressed tumor growth *in vivo*.	52, 53
	Upregulated	Hepatocellular carcinoma	Overexpression of RPL19 predicted poor prognosis.	54
RPL22	Downregulated	Lung cancer (NSCLC)	Downregulation of RPL22 is associated with carcinogenesis.	55
	Deletions	T-ALL	Haploinsufficiency or monoallelic loss of RPL22 accelerated development of T-ALL.	56
RPL23	Upregulated	MDS	• Silencing RPL23 suppressed cell proliferation and increased apoptosis. • RPL23 overexpression was associated with apoptotic resistance and higher risk of MDS	57
	Upregulated	High-grade serous ovarian carcinoma	Higher RPL23 mRNA levels were associated with worse prognoses.	58
RPL26	Upregulated	Pancreatic cancer	Knockdown of RPL26 suppressed cell proliferation.	59
RPL29	Upregulated	Pancreatic cancer	Knockdown of RPL29 suppressed cell proliferation.	59
RPL34	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	• High levels of RPL34 are associated with poor prognosis for patients with osteosarcoma. • Knockdown of RPL34 inhibited cell proliferation, induced cell apoptosis.	62
	Upregulated	Oral squamous cell carcinoma	Knockdown of RPL34 inhibited cell proliferation and migration.	63
RPL41	Downregulated	Retinoblastoma	• RPL41 peptide therapy improved sensitivity to carboplatin. • RPL41 peptide therapy induced apoptosis and inhibited cell migration.	64
	Downregulated	Breast cancer	RPL41 downregulation is associated with malignant transformation.	65
RPS2	Upregulated	Prostate cancer	Knockdown of RPS2 suppressed cell proliferation and induced apoptosis in malignant prostate cells.	66
Phospho-RPS6	Upregulated	Lung cancer	High levels of phospho-RPS6 are associated with shorter metastasis-free survival.	67
RPS15	Mutated (missense)	CLL	• RPS15 mutant primary CLL cells showed altered translation efficiency and rewiring of the translational program. • Mutant RPS15 caused dysregulation of p53 pathway.	34, 68–70
RPS15A	Upregulated	Colorectal cancer	High levels of RPS15A are associated with poor prognosis.	71
RPS20	Mutated (missense)	Colorectal cancer	RPS20 mutation was associated with a defect in pre-rRNA maturation.	72, 73
RPS20	Upregulated	GBM	Higher levels of RPS20 are associated with poor prognosis.	74
RPS27L	Upregulated	Colorectal cancer	Elevated RPS27L expression in either feces or tissues is associated with better prognosis.	75
Ribosomal proteins in therapeutic resistance				
RPL3	Downregulated	Lung cancer	Overexpression of RPL3 inhibited cell migration and invasion and improved 5-FU efficacy in lung cancer cells.	88
RPL6	Upregulated	Gastric cancer	• Downregulation of RPL6 suppressed cell proliferation. • Overexpression of RPL6 promoted multidrug resistance.	89, 90
RPL13	Upregulated	Gastric cancer	• Knockdown of RPL13 suppressed cell proliferation. • Overexpression of RPL13 promoted chemoresistance.	91
RPL23	Upregulated	Gastric cancer	RPL23 overexpression promoted multidrug resistance.	92
RPL34	Upregulated	Pancreatic cancer	Knockdown of RPL34 suppressed cell proliferation, migration, and drug resistance of pancreatic cancer cells.	93
RPS6	N/A	Gastric cancer	RPS6 suppression decreased cell proliferation and tumor growth in lapatinib- and trastuzumab-resistant gastric cancer models.	94
RPS3	N/A	GBM	• In radioresistant GBM cell lines, ring finger protein 138 (RNF138) ubiquitinates RPS3 and promotes its degradation, which suppresses radiation-induced apoptosis and confers radioresistance. • Silencing of RPS3 enhanced GBM cell tolerance to irradiation *in vitro*.	95

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Table 1. Dysregulations in ribosomal proteins are associated with tumor progression and therapeutic resistance. (Cont'd)

Ribosomal protein	Expression level/status	Cancer type	Phenotype	References
Phospho-RPS3	N/A	Lung cancer (NSCLC)	In radioresistant NSCLC cells, RPS3 phosphorylation plays a key role in radiation resistance and initiating a prosurvival transcriptional program.	96
RPS11	Upregulated	GBM	• Knockdown of RPS11 impaired apoptosis and led to resistance to etoposide and doxorubicin. • Higher levels of RPS11 are associated with poor prognosis.	74, 97
RPS13	Upregulated	Gastric cancer	RPS13 promotes cell proliferation and multidrug resistance.	92, 98
RPS27A	Upregulated	CML	• Patients with CML-accelerated or blast phase have higher levels of RPS27A compared with chronic phase patients. • Knockdown of RPS27A improved therapeutic efficacy of tyrosine kinase inhibitor Imatinib.	99
RPS27L	N/A		Rps27l deficiency sensitized Trp53 +/− mice to irradiation by inhibiting cell proliferation, impairing DNA damage response, and inducing apoptosis.	100
RPLP1	N/A	HNSCC	• Silencing of RPLP1 promoted apoptosis and decreased radioresistance *in vitro*. • Invasive HNSCC showed higher expression levels of RPLP1.	101

Abbreviations: CML, chronic myeloid leukemia; GBM, glioblastoma; HNSCC, head and neck squamous cell carcinoma; N/A, information on clinical expression is not available; NSCLC, non–small cell lung carcinoma; T-ALL, T-cell acute lymphoblastic leukemia.

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colleagues (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 and colleagues, 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 transdifferention in subtype marked by ERα 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 long noncoding RNA (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 and colleagues identified a subset of ribosome gene signatures in breast cancer circulating tumor cells (CTC) 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 triple-negative breast cancer; 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 anticancer 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 radioresistance and chemoresistance 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 radioresistance; its knockdown reduced cell proliferation, suppressed apoptosis, and increased the radiosensitivity 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 because 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 upstream binding transcription factor (UBTF) stabilization, and thus reducing the recruitment of RNA Pol I to the rDNA promoter (113). In preclinical 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 hematologic 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 homologous recombination (HR)-proficient castration-resistant prostate cancer (CRPC) to the PARP inhibitor talazoparib, synergistically inhibiting tumor growth in a preclinical 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 holocomplex, resulting in p53 activation (123). While not yet tested in clinical trials, in various preclinical studies BMH21 has shown promising therapeutic efficacy towards different hematologic and solid tumors (124–127). A second generation RNA Pol I inhibitor molecule, PMR-116, demonstrates greater efficacy and improved chemical properties compared with CX-5461. Unlike CX-5461, PMR-116 induces phosphorylation and accumulation of p53 without nonspecifically activating CHK2 (128). Altogether, because 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/radioresistance of tumor cells. Therefore, it would be useful to investigate the utility of genetic screening of ribosomal proteins in treatment-naïve patients with cancer. The influence of the tumor microenvironment, intratumoral heterogeneity, and ribosome heterogeneity are all new, unexplored fields. Stressors like intratumoral 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

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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). In addition, Abraham and colleagues, 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 (sincRNA) 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 noncanonical roles of RNA polymerases and novel potential regulators of ribosome biogenesis in physiologic and pathologic contexts. In addition, several lncRNA species, e.g., promoter-associated RNAs (pRNA), pyrimidine-rich noncoding 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, because ribosome biogenesis is critical for cell survival, various players in this process may potentially have functional redundancy. In addition, 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.

Authors' Disclosures

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