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Review

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The Ribosome-Cancer Connection: How Dysregulated Biogenesis Fuels Tumor Growth

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Abstract: Ribosomes are dynamic ribonucleoprotein complexes that function as molecular machines driving messenger RNA (mRNA) translation and sustaining cellular proteostasis. Ribosome biogenesis—a highly orchestrated, energy-expensive process involving ribosomal RNA (rRNA) transcription, processing, and assembly with ribosomal proteins—is tightly regulated by multiple checkpoints and surveillance mechanisms. Increasing evidence indicates that dysregulation of ribosome biogenesis is a hallmark of cancer, enabling tumor cells to meet heightened metabolic and proliferative demands. Beyond mutations in ribosomal proteins and rRNA processing factors, recent studies have revealed that widespread genetic and epigenetic alterations profoundly reshape ribosome biogenesis and function, contributing to oncogenic transformation and tumor heterogeneity. This resulting ribosomal heterogeneity can lead to the production of aberrant ribosomes or specialized ribosomes with substrate restrictively that selectively influence specific mRNA translation programs, thereby driving aggressive malignant phenotypes, including metastasis, and crucially contributing to the development of acquired therapeutic resistance. Moreover, defects in ribosome quality control and ribosomal stress signaling are increasingly recognized as drivers of tumor progression and poor clinical outcomes. This review summarizes the multifaceted roles of ribosome biogenesis dysfunction in cancer biology, emphasizing its regulation at the genetic, epigenetic, and post-transcriptional levels, and its contribution to drug resistance. Finally, it highlights emerging therapeutic strategies that target ribosome biogenesis or exploit ribosome-associated vulnerabilities, underscoring the potential of ribosome-focused interventions as a new frontier for innovative approaches in precision oncology.

Keywords: ribosome biogenesis; ribosomal proteins; cancer development; therapeutic target; biomarker discovery

1. Introduction

Ribosomes are complex molecular machines found in all living cells that play a central role in protein synthesis, also known as the process of translation. They are responsible for decoding messenger RNA (mRNA) sequences and linking amino acids into polypeptide chains based on the instructions encoded in the genetic material [1]. The ribosome components synthesis and assembly process, known as ribosome biogenesis, is a highly convoluted and coordinated complex multistep process that begins in the nucleolus. The assembly of mature

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ribosomes, the culmination of this process, is central to various cellular functions, including proliferation, differentiation, and maintaining homeostasis and growth [2]. In normal cells, ribosome biogenesis is tightly regulated to meet cellular demands. It involves transcription of ribosomal RNA (rRNA) genes, processing and modifying pre-rRNA, and their assembly into ribosomal subunits together with ribosomal proteins (RPs). In eukaryotes, ribosomes consist of two subunits: the small 40S subunit, composed of one 18S rRNA molecule and 33 ribosomal proteins, and the large 60S subunit, which contains 28S, 5.8S, and 5S rRNA molecules along with 47 ribosomal proteins [3–5]. Together, the 60S and 40S subunits form the mature 80S ribosome [4]. Ribosome assembly begins in the nucleolus and depends on the coordinated activity of all three RNA polymerases. The RNA polymerase I (RNA Pol I) transcribes ribosomal DNA (rDNA) into the precursor 47S rRNA. This pre-rRNA undergoes extensive cleavage and processing, facilitated by numerous processing proteins, to generate three rRNA species: 28S, 5.8S, and 18S (Figure 1) [6]. RNA polymerase II (RNA Pol II) transcribes the genes encoding ribosomal proteins and associated assembly factors, whereas RNA polymerase III (RNA Pol III) transcribes the gene for 5S rRNA, a component of the 60S subunit [7]. The assembly of mature ribosomes requires the synchronized action of all three RNA polymerases, the involvement of more than one hundred processing factors, and the integration of around 80 ribosomal proteins [8,9]. This intricate process is essential for protein synthesis and seems to be associated with various physiological and pathological conditions, including tumorigenesis and metabolic disorders [10]. In tumor cells, ribosome production is often upregulated to support rapid cell proliferation and malignant growth [11]. Dysregulated signaling pathways, metabolic reprogramming, and aberrant expression of noncoding RNAs enhance RNA Pol I transcription activity, leading to hyperactivation of ribosome biogenesis in various cancer types [12]. Over the past decade, the relationship between dysregulated ribosome biogenesis and increased cancer risk has been increasingly recognized to the extent that abnormal ribosome biogenesis is now considered a crucial hallmark of cancer cells [13]. Some pieces of evidence have demonstrated that the nucleolus, where ribosomes are assembled, appears enlarged and increases in size in cancer cells [14]. This enlargement reflects the increased demand for ribosome production to support the rapid cell proliferation typical of tumor cells. Studies have demonstrated that cancer cells enhance ribosome synthesis, driven by mechanisms such as increased GTP metabolism, which fuels nucleolar growth and ribosome production [15].

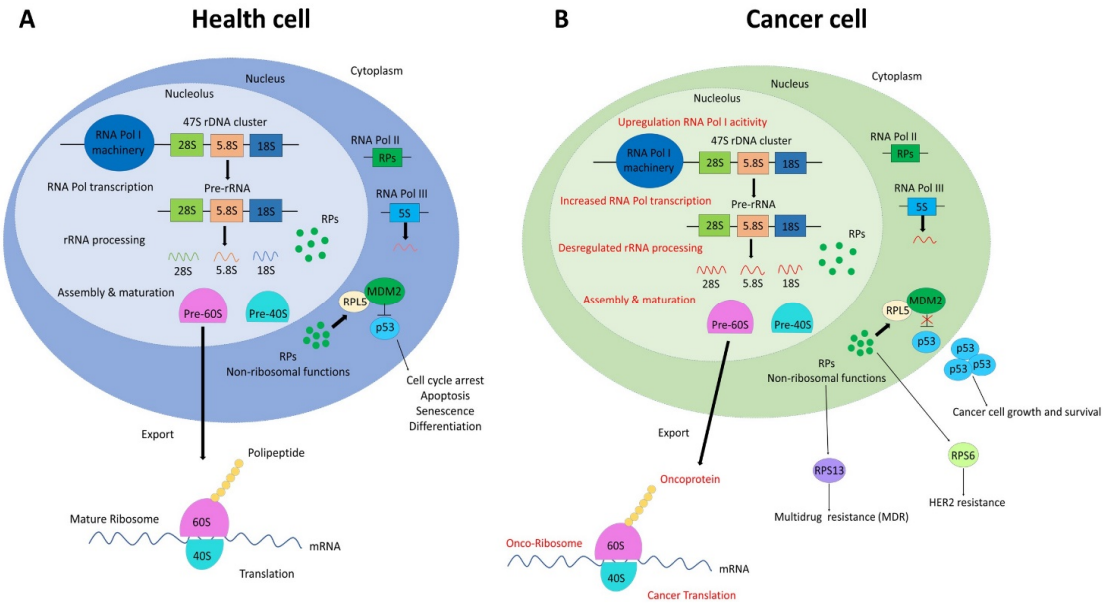


Figure 1. Ribosome diversity plays a key role in the development and progression of cancer. (**A**). In healthy cells, the production of ribosomes begins in the nucleolus, where multiple copies of ribosomal DNA (rDNA) are located. These rDNA regions are transcribed by RNA Pol I, generating a precursor RNA that is processed into the mature rRNA components: 28S, 5.8S, and 18S. These rRNAs are then assembled with ribosomal proteins (RPs) to form the pre-60S and pre-40S ribosomal subunits. After further maturation, these subunits are exported to the cytoplasm to engage in protein synthesis. (**B**). In cancer cells, rapid and uncontrolled growth demands elevated levels of protein production. To meet this need, RNA Pol I activity is often increased, resulting in enhanced rRNA production. Furthermore, cancer cells frequently display atypical or abnormal rRNA modifications. Ribosomal proteins, beyond their structural roles, also have additional functions that can promote tumor progression and resistance to drug therapy. These changes contribute to the formation of specialized ribosomes that drive specific abnormal translation, favoring the synthesis of oncogenic and survival-related proteins, thereby fueling cancer progression.

Nucleolar enlargement is now recognized as a marker of tumor progression due to its role in simultaneously promoting nucleotide, protein, and ribosome biogenesis, while also inhibiting cell cycle arrest and apoptosis, ultimately leading to a more aggressive cancer phenotype [16]. Moreover, emerging studies have demonstrated that tumor suppressors, oncogenes, and proto-oncogenes can influence the malignant progression of cancer by altering ribosome biogenesis. For example, the oncogenic transcription factor c-Myc increases protein synthesis and promotes transcriptional capacity by modulating many genes involved in ribosome biogenesis [17]. Conversely, a surveillance system centered on tumor suppressors has evolved in normal cells to counteract excessive changes in ribosome biosynthesis and halt uncontrolled cell growth [18]. Consequently, increasing attention has been given to unraveling the correlation between ribosome activity and tumor development, including molecular regulatory mechanisms and cancer treatment strategies. In this review, we discuss the impact of ribosome biogenesis on cancer metastasis, treatment resistance, and clinical outcomes, integrating the most up-to-date findings to provide novel insights into targeting ribosomes as a potential therapeutic target.

2. Ribosomopathies and Cancer

Congenital defects affecting ribosomal components and ribosome biogenesis cause a class of diseases collectively known as ribosomopathies. Among the most studied and well-known ribosomopathies are Diamond-Blackfan anemia (DBA, MIM #105650–#620072), Shwachman-Diamond syndrome (SDS, MIM #260400), X-linked dyskeratosis congenita (DKCX, MIM #305000), cartilage-hair hypoplasia (CHH, MIM #250250), and Treacher Collins syndrome (TCS, MIM #154500). Some of the named syndromes represent “disease families” with up to 21 different forms like SDSs; also some of them are characterized by larger DNA deletions e.g., 5q syndrome; and some genes are not RPs but rather genes involved in ribosome biogenesis e.g DKC1 and RMRP. The wide number of affected genes yields a highly variable spectrum of symptoms. This variability is further influenced by the incomplete penetrance of certain mutations [19]. Early symptoms, often manifested in infancy, typically include impaired cell proliferation, leading to anemia or developmental defects. However, some ribosomopathies—specifically DBA (MIM #105650, MIM #620072), DKCX, 5q- syndrome (MIM #153550), SDS, and CHH—paradoxically exhibit an increased susceptibility to [20]. Myelodysplastic syndromes, non-Hodgkin lymphoma, and acute myeloid leukemia are highly prevalent in 5q syndrome, CHH, and SDS, respectively [21]. Additionally, colon carcinoma and osteosarcoma are associated with DBA, while DKCX is frequently linked to squamous cell carcinoma (of both mucosal and skin tissues) and acute myeloid leukemia [22–24]. Although the precise mechanisms underlying the Dameshek’s riddle that triggers cells from hypoproliferation to hyperproliferation remain unclear, it is plausible that mutations in RPs create a stressful cellular environment that fosters clonal selection, ultimately leading to carcinogenesis. Somatic deletions of ribosomal protein genes, predominantly heterozygous cancer (~95%), have been identified in approximately 43% of a cancer cohort comprising 10,744 tumor samples and cell lines. These deletions often co-occur with p53 mutations due to the regulatory connection between p53 and ribosomal protein mutations [25]. Some researchers have termed this phenomenon “somatic ribosomopathies”. However, whether ribosomal protein mutations or deletions are a cause or consequence of cancer progression remains an open question, requiring further investigation. Nonetheless, ribosome biogenesis presents a promising therapeutic target and a potential diagnostic marker.

3. Ribosomal Protein Mutations and Ribosomal Biogenesis Dysregulation in Cancer Progression

The intricate link between ribosomes and cancer extends far beyond their fundamental role in protein synthesis. Mutations in genes encoding RPs or factors involved in ribosome assembly are strongly associated with various diseases, including cancer. Beyond their structural functions, RPs possess extra-ribosomal roles crucial for DNA repair, replication, and apoptosis, and their dysregulation can profoundly impact cellular physiology. Several studies indicate that RPs possess extra-ribosomal functions, playing a crucial role in DNA repair, replication, division, apoptosis and chemoresistance [26]. Consequently, these mutations and the resulting dysfunctions can significantly disrupt cellular processes, profoundly impact cellular physiology and contribute to a wide range of cancer diseases [11]. For instance, several RPs are over-expressed in numerous human tumors, including colon, prostate, liver, pancreatic, gastric, lung and breast cancers (Table 1) [27]. Among these, ribosomal protein S19 (RPS19/eS19), a component of the 40S ribosomal subunit, plays a crucial role in ribosome biogenesis and protein synthesis [28]. RPS19/eS19 deficiency disrupts the maturation of the 40S subunit and has been implicated in several cancers due to its involvement in the regulation of cell growth, proliferation, and apoptosis [29]. Arthurs and co-workers found that RPS19/eS19 expression levels were upregulated in several cancer tissues collected from 82 patients compared to normal tissues [30]. Markiewski and colleagues observed overexpression of RPS19/eS19 and RPL39/eL39 in breast cancer. Specifically, RPS19/eS19 induces the production of immunosuppressive

cytokines and promotes tumor growth, effects that can be impaired by the blockage of RPS19/eS19 [31]. Furthermore, RPS19/eS19 overexpression was also observed in human colon cancer compared to normal tissues, indicating its high malignant potential in colon carcinoma [32]. Similarly, RPS19/eS19 mRNA expression is increased in primary colon carcinoma tissues, and high expression levels correlate with increased malignancy and progression in colon carcinoma [33]. Moreover, some studies have shown that alterations in the protein RPS7/eS7, which is involved in rRNA maturation and the assembly of the 40S ribosomal subunit, can affect protein synthesis and the cellular stress response, thereby contributing to tumorigenesis in different types of cancer [34–36]. Zhou et al. showed that RPS7/eS7 is significantly upregulated in hepatocellular carcinoma (HCC) tumor tissues and is associated with poor prognosis. Overexpression of RPS7/eS7 promoted tumor cell adhesion, migration, and invasion, whereas its depletion reduced these capacities. Mechanistically, RPS7/eS7 stabilizes the mRNA of LOXL2, an enzyme involved in extracellular matrix formation, thereby activating the ITGB1/FAK/SRC signaling pathway, which facilitates metastasis [35]. Another crucial RP is the ribosomal protein RPL5/uL18 that is involved in 60S ribosomal subunit biogenesis by binding to the 5S rRNA. Along with RPL11/uL5 and 5S RNA, RPL5/uL18 forms the 5S ribonucleoproteins (5S RNP) complex, which is involved in assembling the large ribosomal subunit. When the nucleolus is under stress, impaired ribosome biogenesis causes this complex to accumulate in the nucleoplasm. There, it binds to and inhibits MDM2, preventing p53 degradation and promoting p53 stabilization (Figure 1A, B) [37–39]. This step is crucial for both the structural integrity and function of the ribosome, and this interaction stabilizes p53, leading to cell cycle arrest or apoptosis in response to ribosomal stress. RPL5/uL18 loss or mutation can disrupt ribosome biogenesis and protein synthesis, leading to cellular stress and genomic instability (Figure 1B). Several studies demonstrate that downregulation or mutation in the RPL5/uL18 gene impairs the p53 pathway, allowing unchecked cell proliferation and tumor progression [40,41]. Oršolić et al. identified novel mechanisms by which RPL5/uL18 mutations contribute to tumorigenesis in various human cancer types [42]. De Keersmaecker and colleagues demonstrated that 2% of patients with acute lymphoblastic leukemia carry heterozygous inactivating RPL5/uL18 mutations [43]. Moreover, RPL5/uL18 deletion or mutation has been reported in 11% of glioblastomas, 28% of melanomas, 34% of breast cancers, and over 20% of multiple myeloma tumors [12,44]. Further studies have shown the crucial role of RPL5/uL18 in counteracting the oncogenic effects of c-Myc by mediating ribosomal stress, thereby suppressing Myc-driven cancers [45]. Meanwhile, reduced expression of RPL5/uL18 in breast cancer is linked to poor prognosis [46]. Research by Fancello et al. also demonstrated that silencing RPL5/uL18 in breast cancer cell lines accelerates progression through the G2/M phase of the cell cycle and increases tumor growth in xenograft mouse models, supporting the potential tumor-suppressive function of RPL5/uL18 in breast cancer [47]. Ribosomal protein L10 (RPL10/uL16), a component of the 60S ribosomal subunit, has also garnered attention for its involvement in cancer, extending beyond its canonical role in protein synthesis [48]. RPL10/uL16 dysregulation or mutation has been implicated in tumorigenesis, contributing to the progression of various cancers. The R98S mutation in the RPL10/uL16 gene is a prominent cancer-related mutation, notably prevalent in T-cell acute lymphoblastic leukemia (T-ALL). In T-ALL, this mutation alters ribosome function and reduces translational fidelity, creating favorable conditions for tumorigenesis [49]. Furthermore, RPL10/uL16 mutation in T-ALL cells affect proliferation by increasing the reactive oxygen species (ROS) levels and BCL-2 expression, highlighting the involvement of mutated RPs in tumorigenesis [49]. RPL10/uL16 overexpression has been observed in some solid tumors, potentially contributing to enhanced ribosomal activity and protein synthesis, thereby driving rapid tumor growth. Shi et al. reported upregulation of RPL10/uL16 mRNA and protein levels in epithelial ovarian cancer, with higher expression in malignant tumors compared to benign tumors [50]. Karan et al. demonstrated that the RPL10/uL16 overexpression correlates with tumor progression in human prostate cancer cells [51]. Moreover, RPL10/uL16 is deregulated in pancreatic cancer, as shown by Shi et al., who demonstrated that dimethylaminoparthenolide (DMAPT) treatment reduces RPL10/uL16 expression in pancreatic cancer cell lines. Mechanistically, DMAPT directly binds to RPL10/uL16, downregulating the expression of p65 and IKKγ, key components of the NF-κB signaling pathway, thereby inhibiting cell proliferation [52]. Mutations in the RPL22/eL22 protein, a component of the 60S ribosomal subunit, have also been frequently observed in T-ALL, gastric cancer, and endometrial cancer [53–55]. In particular, point mutations in RPL22/eL22 occur at high frequency in cancer characterized by microsatellite instability-high (MSI-H) [56,57]. Rao and colleagues reported that RPL22/eL22 is deleted or mutated in approximately 36% of colorectal cancers (CRC), and its inactivation triggers the upregulation of its paralog RPL22L1. Overexpression of RPL22L1 is correlated with poor survival in CRC patients and contributes to enhanced proliferation and tumor progression [58]. Beyond mutations in individual RPs, the overall dysregulation of ribosome biogenesis is considered a significant driver of cancer progression and neoplastic transformation. Experimental evidence demonstrates a connection between altered ribosome production rates and the development of neoplasms, indicating that this is a fundamental driver of tumorigenesis. Guetget et al. demonstrated that

depletion of TTF-1-interacting protein-5 (TIP5), a component of the nucleolar remodeling complex (NORC) essential for maintaining rDNA genes in a silent state within heterochromatin organization, including centric and pericentric repeats, stimulated rRNA transcription. This upregulation of rDNA transcription induced cell transformation in embryonic mouse fibroblast cell lines [59]. Rossetti and colleagues, through RNA interference knockdown experiments, demonstrated that loss of the MTG16a protein, a known ribosomal gene repressor, induced morphological and molecular changes contributing to the initial steps of breast tumorigenesis [60]. Furthermore, ribosome biogenesis deregulation has been implicated in metastatic colonization, where cancer cells escape the primary tumor, invade other organs and establish metastatic sites through a precisely orchestrated series of events. These events characterize the epithelial-to-mesenchymal transition (EMT) process, a pivotal mechanism in both morphogenesis and organogenesis [8,61]. Although the link between EMT, ribosome biogenesis, and rRNA transcription remains unclear, emerging reports are clarifying this connection. For example, Brighenti et al. showed that interleukin-6, a major inflammatory cytokine, stimulated rRNA transcription, downregulated p53 expression, induced EMT and increased the invasiveness of human colon mucosal epithelial cells [62]. Moreover, Dass and colleagues demonstrated the involvement of Wnt5a signaling in repressing rRNA synthesis by stimulating the nucleolar accumulation of Dishevelled 1 (DVL1), a downstream effector of the Wnt5a pathway. Mechanistically, Wnt5a activation induced nucleolar accumulation of DVL1, directly impairing rRNA synthesis, and suggesting a tumor-suppressive effect in breast cancer cell lines [63]. Prakash et al. reported a strong association between EMT initiation and rDNA transcription activation. They showed EMT induction in breast cancer models correlated with tumor metastasis through ribosome biogenesis upregulation during G1/S arrest. This process was accompanied by the release of the repressive nucleolar chromatin remodeling complex (NORC) from rDNA, along with the recruitment of the EMT-driving transcription factor Snai1 (Snail1), RNA Pol I, and the Upstream Binding Factor (UBF) [64].

Table 1. Ribosomal protein alterations and cancer development.

Ribosomal Proteins	Cancers	References
RPS19/eS19	Prostate, breast, CRC	[29–33]
RPS7/eS7	Lung, HCC, Prostate	[34–36]
RPL5/uL18	T-ALL, AML, glioblastoma, melanoma, multiple myeloma, breast	[37,40–47]
RPL11/uL5	T-ALL, melanoma, gastric	[37–39]
RPL10/uL16	T-ALL, ovarian, prostate, pancreatic	[48–52]
RPL22/eL22	T-ALL, gastric, endometrial, CRC	[53–57]
RPL22L1	CRC	[58]

4. Regulation of Ribosome Biogenesis by Genetic and Epigenetic Mechanisms

Oncogenesis and tumor progression are driven by the activation of oncogenes and/or the inactivation of tumor suppressor genes. Recent data link the activity of certain oncogenes and tumor suppressors to the precise regulation of ribosome processing [65,66]. Some studies have demonstrated the critical role of the proto-oncogene c-Myc in stimulating RNA Pol II activity, responsible for RP-encoding mRNA synthesis, as well as RNA Pol I and III activities, essential for rRNA synthesis [67–69]. Other studies have shown that c-Myc stimulates RNA Pol I activity by promoting SL1 recruitment to gene promoters, increases ribosomal protein synthesis by enhancing RNA Pol II transcription, and stimulates RNA Pol III transcription by activating TFIIIB [17,70,71]. Additional data from Arabi et al. revealed c-Myc nuclear function, demonstrating that c-Myc and Max interaction is crucial for rDNA transcription in response to mitogenic signals [72]. Consequently, c-Myc-driven enhancement of ribosome biogenesis involves both stimulating rDNA transcription and upregulating RP gene expression. Barna and co-workers demonstrated that c-Myc overexpression increases RP expression levels, playing a crucial role in lymphoma progression, accelerating cell cycle progression, and contributing to genomic instability [73]. Thus, uncontrolled Myc activity, observed in many cancers, intensifies ribosome biogenesis and promotes aberrant translation, sustaining tumor progression [70,74]. Mechanistically, Mayer and co-workers demonstrated that mitogenic growth factors activate the mammalian target of rapamycin (mTOR) pathway, which induces Pol I transcription by stimulating UBF and TIF-IA, and RNA Pol III transcription by promoting TFIIIB and TFIIIC association with 5S rRNA genes [75]. Collectively, these data indicate that ribosome biogenesis activation correlates with factors stimulating cell proliferation. Conversely, evidence suggests that ribosome biogenesis also controls cell cycle progression. Indeed, the ribosome biogenesis rate modulates p53 expression through the interaction of RPs and MDM2 [76]. Studies have shown that p53 negatively regulates ribosome biogenesis by inhibiting RNA Pol I transcription through its interaction with the selectivity factor SL1 and suppressing Pol III transcription via TFIIIB binding, thereby downregulating ribosome biogenesis [18,77]. Treré et al. demonstrated

that pRB and p53 expression are linked to ribosome biogenesis rate. This suggests that in tumors with altered pRB and p53 expression, rRNA synthesis upregulation may consistently support cancer cell growth, leading to uncontrolled cell cycle progression (Figure 1B) [78]. Moreover, Belin and colleagues showed that the aggressive breast cancer cell phenotype is associated with strong activation of ribosome biogenesis, triggered by rRNA processing pathways and methylation pattern alterations, impaired translational initiation via CAP- or IRES-dependent mechanisms, and significant perturbation in translational fidelity [79]. Although these genetic mechanisms are established drivers of tumor progression, recent evidence emphasizes that epigenetic regulation of rDNA transcription has emerged as a key determinant of cancer progression and invasiveness. Epigenetic mechanisms —including DNA methylation, histone modifications, and the action of noncoding RNAs—govern the dynamic chromatin state of rDNA repeats, thereby controlling ribosome biogenesis, a process that is frequently dysregulated in cancer. Chu and colleagues found that EZH2, the enzymatic component of Polycomb Repressive Complex 2 (PRC2), promoted ribosome synthesis by targeting and silencing the long noncoding RNA (lncRNA) PHACTR2-AS1, which is responsible for rDNA loci methylation. The authors showed that PHACTR2-AS1 depletion led to hyperactivation of ribosome synthesis and rDNA instability, thereby promoting breast cancer growth and metastasis [80]. Additionally, Galbiati and collaborators investigated the role of JmjC domain-containing histone demethylase 1B (JHDM1B), a negative regulator of gene transcription, in the epigenetic control of rDNA transcription. Knockdown experiments of JHDM1B revealed a significant stimulation of ribosome synthesis, conferring a more aggressive phenotype to mammary gland cancer cells [81]. Data obtained by Chen et al. revealed that elevated levels of nucleolar protein 9 (NOL9), a 5′ polynucleotide kinase crucial for pre-rRNA cleavage and 28S rRNA maturation, are linked to poor prognosis in HCC. The authors found that NOL9 knockdown hindered cell proliferation and promoted apoptosis in vitro experiments, while NOL9 depletion reduced tumor growth in *in vivo* HCC models. Mechanistically, NOL9 expression is regulated by DNA methylation and the transcription factor ZNF384, driving HCC proliferation through a β-catenin-dependent pathway [82].

5. Ribosome Biogenesis in Cancer Drug Resistance

Drug treatment often fails as tumors develop resistance to conventional therapies like chemotherapy and radiotherapy, leading to poor clinical outcomes. In this context, ribosome biogenesis plays a significant role in drug resistance by enhancing ribosome production and protein synthesis, enabling cancer cells to adapt to and circumvent therapeutic interventions. Recent findings by Yang and colleagues identified a novel radioresistance mechanism in non-small cell lung cancer (NSCLC). The phosphorylation of ribosomal protein S3 (RPS3/uS3), a component of the 40S ribosomal subunit, and TRAF2, an anti-apoptotic protein, induced the dissociation of the RPS3/uS3-TRAF2 complex. This dissociation activated NF-κB, leading to the upregulation of pro-survival genes in NSCLC models [83]. Furthermore, Russo and colleagues demonstrated the crucial role of RPL3/uL3 in overcoming drug resistance to 5-Fluorouracil in human lung adenocarcinoma models. The authors reported that RPL3/uL3 expression, induced by 5-fluorouracil, negatively regulated NF-κB activation by preventing nuclear translocation. This significantly increased apoptosis and inhibited migration and invasion in the Calu-6 cell line [84]. Wu and co-workers further revealed the potential role of RPL6/eL6 in multidrug-resistant gastric cancer cell models. The authors reported that patients with RPL6/eL6 -positive expression had shorter survival times than those with RPL6/eL6 -negative expression. Mechanistically, loss- and gain-of-function experiments showed that RPL6/eL6 downregulation reduced colony formation, suppressed proliferation and cell cycle progression in vitro, and inhibited tumor growth in vivo [85]. Moreover, data obtained by Zhang and colleagues proved the critical role of RPL6/eL6 in lung cancer cell lines. Their findings showed that knockdown of RPL6/eL6 inhibited cell proliferation and migration, while inducing cell apoptosis by suppressing the AKT signaling pathway in H1299 and H1975 cells [86]. Using loss- and gain-of-function experiments, Kobayashi et al. reported that the knockdown of RPL13/eL13 expression led to a significant reduction in the growth of various gastrointestinal cancer cells, accompanied by pronounced G1 and G2/M cell cycle arrest. Interestingly, RPL13/eL13 silencing increased cellular sensitivity to DNA-damaging agents, suggesting an inverse correlation between RPL13/eL13 expression and chemosensitivity [87]. On the other hand, Shi et al. investigated the roles of RPS13/uS15 and RPL23/uL14 in multidrug resistance in gastric cancer cell lines and showed that overexpression of RPS13/uS15 and RPL23/uL14 enhanced the resistance of SGC7901 cells to vincristine, Adriamycin, and 5-fluorouracil by suppressing drug-induced apoptosis [88]. Furthermore, Song et al. explored the role of human regulator of ribosome synthesis 1 (RRS1) in cisplatin-resistance in breast cancer cells. The data demonstrated that RRS1 silencing increased sensitivity to cisplatin in MCF-7/DDP cell lines, leading to inhibited cancer cell proliferation by disrupting cell cycle progression and enhancing apoptosis. Mechanistically, RRS1 interacted with and stabilized astrocyte-

elevated gene-1 (AEG-1) by preventing its ubiquitination and subsequent proteasomal degradation, which in turn promoted drug efflux by upregulating multidrug resistance gene 1 (MDR1) [89]. In a hallmark study, Gambardella and colleagues established that RPS6/eS6 and NRF2 activation was associated with anti-HER2 drug resistance in HER2-amplified gastric cancer cell lines. Using loss- and gain-of-function experiments, the authors demonstrated that RPS6/eS6 inhibition reduced NRF2 expression and restored sensitivity to anti-HER2 therapy, leading to decreased cell proliferation and tumor growth in HER2-amplified gastric cancer, both in vitro and *in vivo* [90]. Moreover, Guo et al. by loss- and gain-of-function experiments of RPS13/uS15 in gastric cancer, demonstrated that downregulation of RPS13/uS15 decreased cell proliferation, induced cell cycle arrest in the G1 phase, reduced colony-forming ability in vitro, and inhibited tumor growth *in vivo* [91]. Taken together, these data indicate the critical role of ribosomal proteins in drug resistance across various types of tumors.

6. Ribosome-Targeting Therapeutic Approaches

Ribosome biogenesis plays a crucial role in cancer growth, proliferation, and progression, making it an attractive target for cancer therapy. Targeting ribosome biogenesis offers several advantages, particularly the enhanced selectivity of therapies due to the elevated rate of this process in tumor cells compared to normal cells. Inhibiting ribosome biogenesis also holds promise for preventing cancer recurrence, progression, and metastasis. As highlighted earlier, emerging evidence underscores the importance of increased ribosome biogenesis in tumor growth and drug resistance to treatments. Cancer cells, with their higher rate of rRNA synthesis, are also more vulnerable than normal cells to disruptions in rRNA production. Consequently, there is growing interest in developing novel therapeutic strategies that aim to inhibit rRNA transcription in cancer, as summarized in Table 2. Over the years, various cancer therapeutic agents used in clinical practice, including cisplatin, doxorubicin, and oxaliplatin, have been utilized to target and inhibit rRNA transcription, processing, and assembly [8,92]. Cisplatin and other platinum analogues primarily induce cell death through the DNA damage response. While both cisplatin and oxaliplatin are DNA-damaging platinum analogues, oxaliplatin and DNA intercalators such as actinomycin D induce nuclear stress and ribosome biogenesis stress, especially at low concentrations [93–95]. This stress leads to cell death by repressing RNA Pol I activity through degradation of the RPA194 subunit [96]. Cortés and colleagues demonstrated that low concentrations of Actinomycin D selectively inhibited RNA pol I-dependent rRNA transcription, leading to reduced cell viability and induction of apoptosis in neuroblastoma (NBL) cell lines, as well as tumor regression in xenograft models [97]. Moreover, findings obtained by Taylor and co-workers showed that actinomycin-D treatment significantly inhibited tumor growth in a panel of 12 patient-derived glioma stemlike cell lines [98]. Gionfriddo et al. demonstrated that treatment with low-dose actinomycin D elicited a more robust stress response in acute myeloid leukemia (AML) cells harboring nucleophosmin-1 (NPM1) mutations compared to wild-type cells, suggesting that the NPM1-mutated AML models may be more sensitive to nuclear stress [99]. Furthermore, in a single-center, single-arm, open-label, phase II pilot study the safety and efficacy of dactinomycin were evaluated in NPM1-mutated AML patients with hematological relapses or refractory disease. These results demonstrate that single-agent actinomycin D 15 μg/kg/day for 5 consecutive days may induce a complete response in relapsed/refractory NPM1-mutated AML [99]. However, promising small molecules, including CX-5461, CX-3543, and BMH-21, have been identified as selective inhibitors of cancer cell growth and proliferation. CX-5461 was the first selective, orally available inhibitor of RNA Pol I transcription initiation, targeting the SL1-rDNA complex and disrupting the stabilization of the upstream binding transcription factor (UBTF) [100,101].

CX-5461's mechanism of action appears to extend beyond the inhibition of RNA Pol I-mediated transcription. Evidence suggests that it can also act as a topoisomerase II poison and induce DNA damage through the stabilisation of G-quadruplex (G4) DNA structures. This suggests that its antitumor activity involves both nucleolar and non-nucleolar mechanisms. Therefore, the biological effects of CX-5461 should not be exclusively attributed to the inhibition of ribosome biogenesis. The relative contribution of these distinct mechanisms may depend on tumour type and molecular context.

In vivo, CX-5461 induced p53-mediated cellular senescence and autophagy in various solid tumor models [102]. This multifaceted mechanism of action is consistent with the way in which CX-5461 has been shown to induce ATM/ATR-dependent DNA damage responses. It has also been shown to induce G2 cell-cycle arrest. In addition, it has been shown to alter rDNA chromatin organization. Furthermore, it has been shown to induce G4-mediated genomic instability [103–106]. These findings emphasise the importance of distinguishing its effects on ribosome biogenesis from its other DNA-damage-related activities when evaluating its anti-tumor efficacy.

Table 2. Summary of therapeutic approaches targeting the ribosome.

Drug	Therapeutic Intervention	Targets	Tumour types in Preclinical Models	Tumour Types in Clinical Trials	Phase of Clinical Trials	References
Actinomycin D	DNA intercalators/ Pol I preferential inhibition at low dose	At low doses preferentially inhibit RNA Pol I → nucleolar stress	NBL, AML, glioblastoma	AML	Phase II	[97–99]
CX-5461	First generation of RNA Pol I inhibitor	Block rDNA transcription by RNA Pol I → nucleolar stress → G-quadruplex stabilisation/DNA damage	Pancreatic, breast, ovarian, prostate, CRC, melanoma, pediatric NBL, hematologic malignancies	HR-deficient (BRCA/PALP2) tumours, breast, ovarian, pancreatic, hematologic malignancies	Phase I Phase II	[100–117]
CX-3543	First generation of RNA Pol I inhibitor	Fluoroquinolones-derivatives → breaking down nucleolin/G-quadruplex (G4) complexes to rDNA	Lung, CRC, breast, pancreatic, and acute promyelocytic leukaemia	Advanced solid tumours and lymphomas	Phase I	[118–121]
BMH-21	First generation of RNA Pol I inhibitor	Block rDNA transcription → binding to GC-rich rDNA, preventing the formation of Pol I pre-initiation complex	Prostate, breast, CRC, ovarian, pediatric NBL	N/A	N/A	[122–131]
PMR-116	Second generation of RNA Pol I inhibitor	Block rRNA transcription	HCC, prostate, CRC, breast, lymphoma, AML	Advanced solid tumours and lymphomas	Phase I	[132–136]

Furthermore, when combined with other drugs, such as ATM/ATR pathway inhibitors, CX-5461 has been shown to selectively and synergistically promote cancer cell death in lymphoma, advanced prostate cancer and high-grade serous ovarian carcinoma [107–109]. Moreover, preclinical studies have demonstrated that CX-5461 significantly reduced tumor mutation burden in mouse models of c-Myc-driven hematological cancers [110]. A remarkable study demonstrated that ovarian cancer cells, which had developed resistance to carboplatin and paclitaxel treatments, became sensitive to CX-5461 treatment, thereby enhancing the therapeutic potential of this drug [111]. Yu et al. also demonstrated that CX-5461 treatment exhibited anti-proliferative effects and promoted the redifferentiation of thyroid cancer by inhibiting TERT activation [112]. Interestingly, Chung et al. demonstrated that CX-5461, when combined with anti-PD-1 therapy, exerted synergistic growth-inhibitory effects in models of colorectal and breast cancers, thereby supporting its potential role in cancer immunotherapy [113]. To date, four clinical trials involving CX-5461 have been registered, of which two have been completed and two are currently recruiting. The first phase I clinical trial of CX-5461 (ANZCTR12613001061729) was conducted in patients with advanced hematological malignancies, demonstrating promising anti-tumor activity with an acceptable safety profile in 16 patients, although phototoxicity remains an important consideration for treatment management. This study's primary endpoints focused on safety and tolerability, setting an MMTD of 170 mg/m^2 and conducting a DLT assessment. Secondary endpoints included antitumor activity, pharmacokinetics, and pharmacodynamics of RNA-FISH. Exploratory endpoints explored response-associated biomarkers, such as TP53 and ATM mutations, MYC expression, and homologous recombination deficiency [114]. The second multicenter phase I clinical trial of CX-5461 (NCT02719977), conducted by the Canadian Cancer Trials Group, evaluated the CX-5461 treatment in 40 patients with advanced solid cancers harboring BRCA1/2, PALB2, or other homologous recombination deficiency (HRD) mutations. This study demonstrated that CX-5461 was well-tolerated, with a recommended phase II dose of 475 mg/m2 on days 1, 8, and 15 every 4 weeks, and dose-limiting phototoxicity [115]. Notably, these findings suggested that the antitumor activity of CX-5461 is primarily observed in patients with HR-defective cancers. Based on these results, CX-5461 is currently being studied in an open-label, multi-center, phase 1b study (NCT04890613) designed to determine a tolerable dose in patients with selected solid tumors harboring BRCA1/2, PALB2, or other HRD mutations [116]. Preliminary data obtained by the expansion phase of the study indicate that the drug exerted durable disease control is tolerated by heavily pretreated HRD mutated patients with manageable photosensitivity [117]. Meanwhile, another clinical trial (NCT06606990) has recently begun recruiting participants, with the primary objective of evaluating the safety, side effects and optimal dose of CX-5461 in patients with metastatic solid tumors, both with and without HR-deficiency genetic mutations, who have progressed after at least one previous treatment. These trials provided clinical proof-of-concept to further investigate the use of CX-5461 in multiple tumors with particular attention to those with defects in DNA repair. Currently, CX-5461 is being evaluated in phase II clinical trials and has received fast-track designation from the Food and Drug Administration (FDA) for the treatment of tumors with mutations in BRCA1/2 and PALB2 [118]. Other studies have shown that CX-3543, a small molecule, selectively disrupts nucleolin/rDNA G-quadruplex complexes

in the nucleolus, inhibiting rRNA transcription and leading to p53 stabilization and cell apoptosis [119,120]. Furthermore, data from Yao and colleagues demonstrated that CX-3543 treatment reduced the expression of c-Myc and colon cancer-associated transcript-1 (CCAT1) in colon cancer cell lines by promoting apoptosis and inhibiting cell proliferation [121]. A phase I open-label, multi-center, dose-escalation study of CX-3543 (NCT00955786) in patients with advanced solid tumors and lymphomas has been completed, showing that the drug was well-tolerated up to 360 mg/m^2 and exhibited a favorable pharmacokinetic profile [137]. Another class of small compounds, known as BMH compounds, also appears to inhibit RNA Pol I transcription by causing disassembly of the Pol I complex at the rDNA promoter through binding to GC-rich DNA [122]. Among these, BMH-21 inhibits RNA Pol I transcription, induces proteasome-dependent degradation of the catalytic subunit RPA194, and exhibits strong cytotoxicity, ultimately leading to p53 activation [123,124]. The molecular target and mechanisms of action of BMH-21 are distinct from those of CX-5461; however, this compound also effectively inhibits rRNA synthesis. Jacobs and colleagues demonstrated that BMH-21 inhibited the Pol I transcription initiation, promoter escape and elongation process in in vitro and *in vivo* models [125]. Moreover, Peltonen and colleagues showed antitumor activity of BMH-21 in prostate tissues isolated from patients and *in vivo* models [126]. Low et al. observed inhibited tumor growth in enzalutamide-resistant prostate cancer models, accompanied by a significant decrease in 45S rRNA synthesis [127]. Jouines and colleagues demonstrated that treatment with BMH-21 in pediatric NBL models suppressed cell proliferation and induced ribosomal stress, leading to activation of apoptosis and the p21 pathway [128]. Interestingly, BMH-21 reduced cell proliferation and induced cell cycle arrest in triple-negative breast cancer (TNBC) in *in vitro* models and inhibited tumor growth in human TNBC xenografts [129]. In ovarian cancer models, Fu and colleagues demonstrated that BMH-21 treatment induced nuclear stress in SKOV3 cell lines, inhibited cell viability, and promoted apoptosis [130]. Furthermore, Nakano and co-workers demonstrated that BMH-21 treatment killed tumor cells and reduced the tumor size and number of tumor nodules in CRC organoid cultures of patient-derived cancer cells [131]. In a landmark study, Metge et al. demonstrated that inhibition of RNA biogenesis by BMH-21 reprogrammed pro-tumor macrophages into tumor-suppressive macrophages, promoted an inflammatory tumor microenvironment and, in combination with radiotherapy, markedly suppressed tumor progression in breast cancer models [132]. In addition to the advancement of RNA Pol I inhibitors, Pimera Inc. has developed a second-generation inhibitor, PMR-116, which demonstrates improved chemical properties and greater efficacy compared to CX-5461. Preclinical data demonstrated the anti-tumor effects of PMR-116 in various solid and hematological malignancy models, both in vitro and *in vivo*. Specifically, PMR-116 significantly improved survival and reduced tumor burden in several preclinical models, including a transgenic model of acute myeloid leukemia, xenograft models of colorectal and breast cancer, and patient-derived xenografts of prostate tumors [133,134]. Interestingly, the data obtained by Ferreira et al. demonstrated that PMR-116 is a promising new broad-spectrum anti-cancer drug for advanced solid tumors driven by Myc, which are highly refractory to standard therapies. Based on these data, the authors launched a phase I clinical trial of PMR-116 in patients with advanced solid malignancies (CTRN12620001146987) [135]. Another open-label, phase I study (ACTRN12620001146987) was conducted to assess the safety and tolerability of PMR-116 in patients with advanced solid tumors [136,138]. Overall, ribosome biogenesis inhibition by RNA Pol I inhibitors represents a novel therapeutic approach to reduce tumor progression and overcome drug resistance in multiple tumor types.

7. Biomarkers for Clinical Translation

The clinical translation of RNA Pol I inhibitors necessitates robust biomarkers that reflect both target engagement and therapeutic responsiveness. In this context, assessing ribosomal and nucleolar alterations becomes essential for developing diagnostic tools and guiding therapeutic strategies. These changes, whether quantitative or qualitative, offer valuable insights into the molecular consequences of Pol I inhibition and its impact on ribosome function. Assessing these alterations is crucial for the development of novel diagnostic tools and therapeutic strategies. The changes under investigation can be broadly classified as quantitative or qualitative, with clinical applications spanning diagnostics, prognostics, and therapeutic targeting. Quantitative changes include alterations in the abundance of ribosomes and the expression of their components. In contrast, qualitative changes involve mutations or differential expression of specific RPs or rRNA, as well as post-transcriptional modifications of rRNA. Such alterations reprogram ribosome function, biasing translation toward subsets of mRNAs that promote cancer survival, proliferation, and differentiation. Measuring these changes requires an integrated toolkit of molecular and imaging approaches, including RNA sequencing (RNA-seq), quantitative PCR (qPCR), western blotting (WB), immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH) [139]. At the functional level, inhibition of rRNA transcription can be monitored by quantifying pre-rRNA species via qPCR or

in situ hybridization (ISH) in preclinical and early clinical settings [140]. More advanced readouts include 5-ethynyl uridine (EU) incorporation coupled with click chemistry, enabling imaging or flow cytometric detection of nascent rRNA synthesis. Interestingly, Berry and colleagues demonstrated that treatment of several human cell lines with CX-5461 resulted in an 89–92% reduction in nucleolar EU incorporation, quantified by fluorescence imaging [141]. Concurrently, nucleolar disruption, a hallmark of RNA Pol I inhibition, can be assessed by immunofluorescence or IHC against nucleolar proteins such as fibrillarin (FBL), NPM1, nucleolin (NCL), or UBF (upstream binding factor), providing morpho-functional readouts in patient biopsy samples [142–144]. Pol I inhibition perturbs nucleolar phase behavior and structure, disrupting NPM1–rRNA interactions and inducing nucleolar fragmentation or stress caps—observable features that correlate with transcriptional blockade, given that rRNA transcription is integral to nucleolar phase separation. In parallel, stabilization of G-quadruplex (G4) structures can be evaluated: in vitro by biophysical assays, and in tissue samples using structure-specific antibodies such as BG4, which have demonstrated robust IHC staining in patient-derived tumor sections, including gastric and liver cancers [145]. Importantly, genomic alterations that modulate sensitivity to nucleolar stress or rRNA biogenesis inhibition can be identified by next-generation sequencing (NGS), FISH, or IHC [146,147]. These include BRCA1/2 and PALB2 mutations (homologous recombination deficiency), c-Myc amplification or overexpression, and additional markers such as Ki-67 proliferation index or p53 stabilization—both reflecting functional consequences of nucleolar stress [148]. Furthermore, emerging evidence highlights the role of epitranscriptomic rRNA modifications (e.g., pseudo uridylation, 2′-O-methylation), which can be quantified by specialized sequencing approaches such as RiboMeth-seq or Ψ-seq, as potential biomarkers of ribosome heterogeneity in cancer [149–154]. Finally, ribosome profiling (Ribo-seq) provides a high-resolution functional readout, capturing the subset of mRNAs actively engaged by ribosomes under altered ribosome states [155,156]. Together, multi-layered approaches—integrating molecular, imaging, and functional tools—enable a comprehensive, multidimensional characterization of ribosome biogenesis and nucleolar stress. To translate this knowledge into useful tools, we need to develop and test biomarker-based approaches that can capture various aspects of ribosomal biogenesis and compare these with individual markers or simpler panels. This complex process cannot be fully characterised by a single biomarker, and a multidimensional panel is also risky. So, an integrated approach combining genomic alterations, rRNA activity, ribosomal composition, rRNA modifications, nucleolar morphology, and functional translation parameters is best. This added value must be demonstrated through validation studies comparing its performance with single biomarkers or more basic panels.

In developing these composite panels, it is critical to distinguish between prognostic, predictive, and pharmacodynamic biomarkers. Assessing these parameters longitudinally—before, during, and after treatment—allows clinicians to track therapeutic efficacy in real time and detect emerging drug resistance early. To achieve this non-invasively, liquid biopsy approaches can complement traditional tissue-based analyses, while single-cell and spatial transcriptomics offer unprecedented resolution to identify specific tumor sub-populations with distinct ribosomal dependencies (Figure 2). Ultimately, these combined strategies enhance the translational potential of ribosome-based therapies, paving the way for personalized medicine. Nevertheless, before entering routine clinical practice, these candidate signatures must undergo rigorous prospective validation. This process requires establishing high reproducibility, standardizing assays, defining clinically relevant cut-off values, and ensuring compatibility with standard clinical specimens such as FFPE tissue and peripheral blood. Overcoming these logistical and analytical hurdles to create feasible companion diagnostics—integrating baseline tumor profiles with evolving resistance markers—will be pivotal to guiding patient stratification, identifying candidates for RNA Pol I inhibition, and successfully embedding ribosome-targeted therapies into precision oncology.

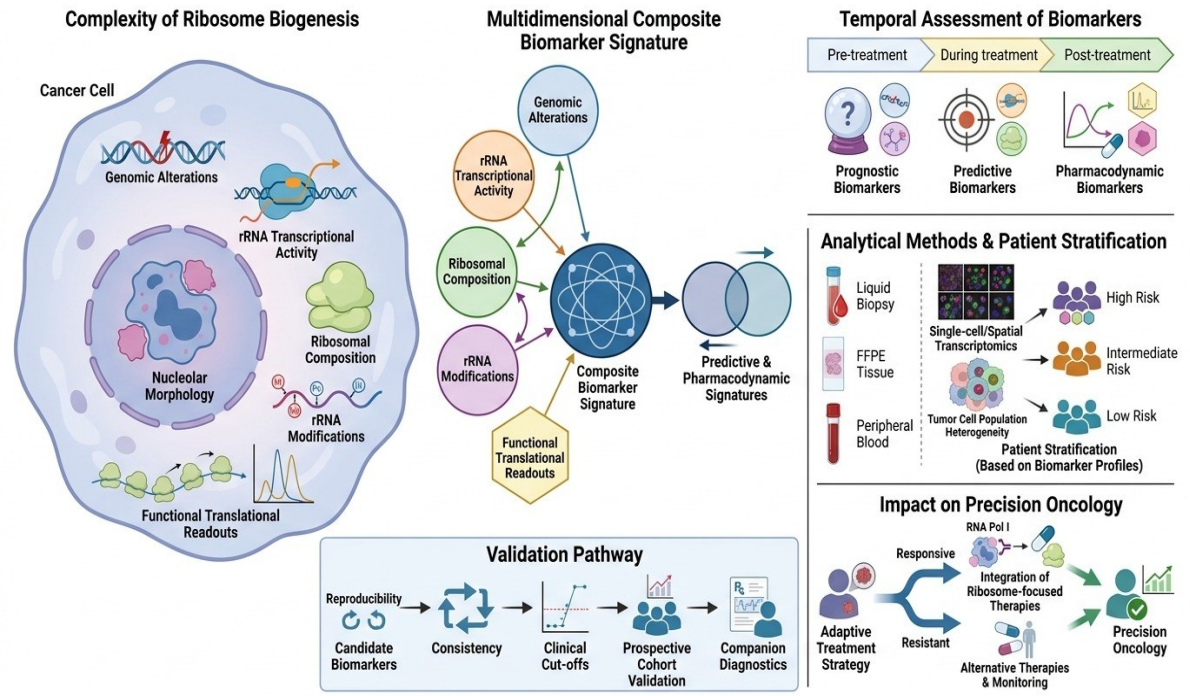


Figure 2. Proposed research framework for developing and validating an integrated, multidimensional biomarker signature of ribosome biogenesis in cancer. The framework shows how prognostic, predictive and pharmacodynamic biomarkers can be integrated across treatment periods. These categories reflect their clinical function rather than fixed sampling times. Predictive biomarkers may be assessed before treatment to identify patients who will benefit from a specific intervention. Pharmacodynamic biomarkers are typically monitored during treatment to check engagement with the biological target and treatment-induced changes. The clinical uses, assays, biological specimens, evidence and validation steps of each candidate marker should be explicitly defined. The framework differentiates the biological contributions of individual markers, including to ribosome biogenesis, tumor biology, treatment sensitivity and therapeutic response. It does not assume all markers provide equivalent or interchangeable information. This proposed research framework requires validation before implementation.

8. Conclusions and Prospects

Ribosome biogenesis remains a crucial area of cancer research due to its fundamental role in sustaining the hyperactive protein synthesis required for tumor growth and progression. While significant advances have been made in understanding ribosomal dysregulation in cancer, several gaps remain regarding the molecular intricacies of rRNA synthesis, ribosome assembly, and their broader implications for oncogenesis. Furthermore, ribosome dysregulation contributes to cancer metastasis through the production of non-canonical ribosomes, also called onco-ribosomes, interactions with oncogenic signaling pathways, and alterations in nucleolar proteins. As a result, the RNA Pol I transcription rate increased in various cancers, highlighting its pivotal role in ribosome activity and establishing it as a hallmark of human malignancy. Some studies have specifically highlighted the use of novel small-molecule inhibitors to address RNA Pol I hyperactivation and ribosomes [157,158]. The development of drugs targeting ribosome biogenesis is an active area of research, showing great potential as a novel approach in cancer therapy. Currently, available inhibitors primarily focus on the Pol I machinery. However, it has recently been proposed that drugs such as CX-5461 exert cytotoxic effects through alternative mechanisms, including stabilizing G-quadruplex DNA structures and acting as a topoisomerase II poison [105,159–161]. Additionally, these drugs may exert part of their cytotoxic effects through non-nucleolar mechanisms, which require further investigation. Investigating the downstream processes of rRNA synthesis, including its processing and ribosome assembly, may offer valuable insights and robust strategies for inhibiting cancer cell proliferation. Interestingly, recent studies have revealed that ribosomes exhibit unexpected heterogeneity, forming diverse subpopulations that can selectively modulate translational programs based on their cellular context [162]. This heterogeneity, arising from variations in RP composition and the presence of rRNA modifications, enables ribosomes to differentially translate specific subsets of transcripts involved in processes such as cell cycle regulation and development. Since most rRNA processing and modifications simultaneously occur with transcription, the factors involved are likely to exhibit precise spatial and temporal coordination with RNA Pol I elongation. This suggests that changes in RNA Pol I transcription could alter rRNA modification patterns. However, whether the observed increase in Pol I activity

directly drives these changes or if additional coordinating mechanisms are involved remains unclear. Moreover, factors such as c-Myc, which are known to enhance rRNA transcription, also stimulate the transcription of snoRNAs and the rRNA methyltransferase fibrillarin, thereby influencing rRNA modifications [163].

In conclusion, this review shows the importance of understanding ribosome biogenesis in cancer. This is important not only as a basic biological process but also as a way to find ways to treat patients and check for signs of disease. This study suggests that RNA Pol I hyperactivation is a promising therapeutic target, supported by preclinical evidence and initial clinical demonstration of target engagement in patients. This shows how they can be used to check for signs of disease, how to treat patients and how to find new ways to check for disease. Some treatments are already being used in people, and some are being tested. But we do not know about all the other ways to treat the disease yet. This includes problems with how ribosomes are made, how they are made and how they are used. We also need to check how good these treatments are. Future research should focus on identifying the molecular determinants of therapeutic response and establishing robust translational frameworks integrating mechanistic, pharmacodynamic and clinical evidence. A more precise understanding of ribosome biogenesis dependencies and their associated biomarkers may help to develop more effective therapeutic strategies.

Author Contributions

V.B.: conceptualization, writing-original draft, review and editing; M.C. and A.A.: writing and review; E.S. and V.C.: methodology; A.B.: conceptualization, supervision and writing. All authors have read and agreed to the published version of the manuscript.

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All data supporting the findings of this study are available from the corresponding author upon reasonable request. All relevant data are included in the manuscript. No publicly accessible repository was used for data deposition.

Conflicts of Interest

The authors declare no competing interests.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors occasionally used AI as a language support tool to assist with translation and improve linguistic accuracy. AI tools were not used to generate data, results, scientific interpretations, or conclusions. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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