

# How Ribosomes Translate Cancer



Sergey O. Sulima<sup>1</sup>, Isabel J.F. Hofman<sup>1</sup>, Kim De Keersmaecker<sup>1</sup>, and Jonathan D. Dinman<sup>2</sup>

## ABSTRACT

A wealth of novel findings, including congenital ribosomal mutations in ribosomopathies and somatic ribosomal mutations in various cancers, have significantly increased our understanding of the relevance of ribosomes in oncogenesis. Here, we explore the growing list of mechanisms by which the ribosome is involved in carcinogenesis—from the hijacking of ribosomes by oncogenic factors and dysregulated translational control, to the effects of mutations in ribosomal components on cellular metabolism. Of clinical importance, the recent success of RNA polymerase inhibitors highlights the dependence on “onco-ribosomes” as an Achilles’ heel of cancer cells and a promising target for further therapeutic intervention.

**Significance:** The recent discovery of somatic mutations in ribosomal proteins in several cancers has strengthened the link between ribosome defects and cancer progression, while also raising the question of which cellular mechanisms such defects exploit. Here, we discuss the emerging molecular mechanisms by which ribosomes support oncogenesis, and how this understanding is driving the design of novel therapeutic strategies. *Cancer Discov*; 7(10); 1069–87. ©2017 AACR.

## INTRODUCTION

Does the ribosome translate cancer? This provocative question was first posed in a comprehensive review shortly after the turn of the century, at a time when evidence supporting an oncogenic role of ribosomes was beginning to emerge (1). The ensuing years have seen remarkable progress in this field, and the central question has shifted from “does” to “how does” the ribosome influence cancer progression?

The ribosome is posited to have originated in the prebiotic world where it evolved to bridge the realms of RNAs and proteins (2). It was first detected approximately 3.5 billion years later as a “small particle” by Albert Claude in the early 1940s (3). In the 1950s, these particles became experimentally linked to protein synthesis, ushering in a golden age of translation that lasted through the invention of molecular biology in the mid-1970s. These years firmly established the integral role of the more descriptively retitled “ribosome” in the central

dogma of biology. The first links between the ribosome and cancer were established early in the 21st century, shortly after congenital disorders of ribosome dysfunction—aptly named “ribosomopathies”—were first described. The first such reports involved recurrent mutations in the ribosomal protein (RP) S19 (RPS19) in patients with the rare bone marrow failure syndrome Diamond–Blackfan anemia (DBA; ref. 4). Subsequently, more mutations in RPS19 and other ribosomal proteins were described in additional ribosomopathies, including Shwachman–Diamond syndrome (SDS), X-linked dyskeratosis congenita (DC), cartilage hair hypoplasia, and Treacher Collins syndrome (5). Paradoxically, many patients with these syndromes progress from an initial phase characterized by cellular hypoproliferative disorders (e.g., anemia) toward elevated risk for cellular hyperproliferative diseases (cancer) later in life, providing the first indications that ribosomal defects might play a role in cancer progression (6).

Additional evidence has strengthened the link between ribosomes and oncogenesis. In the early 2000s, it was established that ribosomal protein genes act as haploinsufficient tumor suppressors in zebrafish (7). More recently, recurrent somatic ribosomal protein mutations were discovered in 10% to 35% of multiple tumor types, including T-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia, multiple myeloma, glioblastoma, breast cancer, and melanoma (8–13). It is also becoming clear that oncogenic factors can extrinsically influence the function of wild-type ribosomes. Despite the general concept of the ribosome as a “molecular machine,” which perhaps stereotypes its outdated view as a static entity, the ribosome is now emerging as dynamic and malleable (14), and recent evidence discussed below points to various ways of modulating and fine-tuning its function in response to specific conditions. Known oncogenic and tumor suppressor proteins can exploit these mechanisms, which

<sup>1</sup>Department of Oncology, KU Leuven, University of Leuven, LKI, Leuven Cancer Institute, Leuven, Belgium. <sup>2</sup>Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland.

S.O. Sulima and I.J.F. Hofman contributed equally to this article.

Current address for I.J.F. Hofman: Center for Hematology and Regenerative Medicine (HERM), Department of Medicine (MedH), Karolinska Institutet, Stockholm, Sweden.

**Corresponding Authors:** Kim De Keersmaecker, KU Leuven, University of Leuven, Department of Oncology, LKI, Leuven Cancer Institute, Campus Gasthuisberg O&N1, Box 603, Herestraat 49, 3000 Leuven, Belgium. E-mail: kim.dekeersmaecker@kuleuven.be; and Jonathan D. Dinman, University of Maryland, Department of Cell Biology and Molecular Genetics, 4062 Campus Drive, College Park, MD 20742. E-mail: dinman@umd.edu

doi: 10.1158/2159-8290.CD-17-0550

©2017 American Association for Cancer Research.

normally regulate the ribosomal “machine” to drive novel protein expression profiles beneficial to cancerous cells.

An impressive body of evidence has accumulated in recent years supporting the notion that a wide spectrum of both congenital and somatically acquired ribosomal defects, as well as modulation of ribosomal activity by oncogenic factors, contribute to a cancer phenotype. Many excellent animal models have been generated to study ribosome-defective cancers. Although it is not the purpose of this review to specifically describe these models in detail, they are listed

and referenced in Tables 1 and 2. Additionally, a novel ribosomal protein nomenclature has recently been proposed in an attempt to conform to ribosome structures from all three domains of life (15). Although we refer to the traditional nomenclature of ribosomal genes throughout this review, they are cross-referenced with the proposed universal ribosomal protein names in Table 1 and Fig. 1. In this review, we summarize and comment on the exponential accumulation of discoveries in the field in the past several years and address the next big questions: What are the molecular and cellular

**Table 1. RPs involved in cancer and cancer-predisposing disease**

RPs involved in disease for which animal models are available					
Old name	New name	Implicated disease	Model (organism: modification)	Phenotype	Reference
<b>RPS14</b>	<b>uS11</b>	5q- MDS	Zebrafish: MO against <i>Rps14</i>	Severe anemia Morphologic abnormalities	(188)
			Zebrafish: CRISPR/Cas inactivating <i>Rps14</i>	No phenotype in heterozygous mutants Embryonically lethal Decreased hemoglobin Morphologic abnormalities	(167)
			Mouse: deletion of chromosomal region including <i>Rps14</i>	Anemia	(70)
			Mouse: conditional inactivation of <i>Rps14</i>	Anemia	(189)
<b>RPS19</b>	<b>eS19</b>	DBA	Zebrafish: MO against <i>Rps19</i>	Impaired erythropoiesis Morphologic abnormalities	(190, 191)
			Mouse: <i>Rps19</i> KO	KO lethal before implantation No phenotype in heterozygous mutant	(192)
			Mouse: inducible <i>Rps19</i> <sup>R26W</sup> transgenic	Mild anemia Growth retardation	(193)
			Mouse: inducible shRNA against <i>Rps19</i>	Severe anemia Bone marrow failure	(194)
			Mouse: spontaneous heterozygous hypomorphic mutation in <i>Rps19</i>	Mild anemia	(195)
<b>RPS24</b>	<b>eS24</b>	DBA	Zebrafish: MO against <i>Rps24</i>	Morphologic abnormalities Hematopoietic defects	(196)
			Mouse: deletion of exons 2–3 of <i>Rps24</i>	Embryonically lethal No phenotype in heterozygous mutant Development of sarcoma in advanced age	(197)
<b>RPS29</b>	<b>uS14</b>	DBA	Zebrafish: insertional <i>Rps29</i> mutant	Defective erythropoiesis	(87)
			Zebrafish: insertional <i>Rps29</i> mutant	Homozygous embryonically lethal Defective erythropoiesis Apoptosis in head region	(198)
<b>RPS27</b>	<b>eS27</b>	DBA, melanoma	Zebrafish: MO against <i>Rps27</i>	Morphologic abnormalities Defective erythropoiesis	(199)
<b>RPL5</b>	<b>uL18</b>	DBA, multiple cancer types	Zebrafish: MO against <i>Rpl5</i>	Morphologic abnormalities Anemia	(196)
			Mouse: deletion of exons 1–8 of <i>Rpl5</i>	Embryonically lethal No phenotype in heterozygous mutant Development of sarcoma in advanced age	(197)

**Table 1. RPs involved in cancer and cancer-predisposing disease (Continued)**

RPs involved in disease for which animal models are available					
Old name	New name	Implicated disease	Model (organism: modification)	Phenotype	Reference
<b>RPL11</b>	<b>uL5</b>	DBA, T-ALL	Mouse: deletion of exons 3–4 of <i>Rpl11</i>		(200)
			Cre constitutively and ubiquitously expressed	Heterozygous embryonically lethal	
			KO Cre ubiquitous inducible	KO in adult mice lethal due to bone marrow failure	
			HTZ Cre ubiquitous inducible	Anemia Prone to radiation-induced lymphomas	
			Zebrafish: mutant identified in shRNA screen	Homozygous embryonically lethal Morphologic abnormalities Defective erythropoiesis	(7, 201)
			Zebrafish: MO against <i>Rpl11</i>	Morphologic abnormalities Defective erythropoiesis	(98)
<b>RPS20</b>	<b>uS10</b>	Colorectal cancer	Mouse: spontaneous heterozygous mutation in <i>Rps20</i>	Mild anemia	(195)
<b>RPL10</b>	<b>uL16</b>	T-ALL	Zebrafish: MO against <i>Rpl10</i>	Morphologic abnormalities	(202)
<b>RPL22</b>	<b>eL22</b>	T-ALL	Mouse: gene trap in <i>Rpl22</i> between exons 3–4	Anemia Specific block in $\alpha\beta$ T-cell development Heterozygous accelerates lymphoma Homozygous limits spread of lymphoma	(9, 102)
			Zebrafish: MO against <i>Rpl22</i>	T-cell development block	(203)
RPs involved in disease for which no models are available yet					
<b>RPS17</b>	<b>eS17</b>	DBA			
<b>RPL35A</b>	<b>eL33</b>	DBA			
<b>RPS7</b>	<b>eS7</b>	DBA			
<b>RPL26</b>	<b>uL24</b>	DBA			
<b>RPS10</b>	<b>eS10</b>	DBA			
<b>RPS26</b>	<b>eS26</b>	DBA			
<b>RPL15</b>	<b>eL15</b>	DBA			
<b>RPS28</b>	<b>eS28</b>	DBA			
<b>RPL31</b>	<b>eL31</b>	DBA			
<b>RPS15</b>	<b>uS19</b>	CLL			
<b>RPSA</b>	<b>uS2</b>	Gastric cancer			
<b>RPL23A</b>	<b>uL23</b>	Endometroid cancer			

NOTE: Table shows all RPs that have been implicated in ribosomopathies (blue), in cancer (red), or in both (purple).

Abbreviations: CLL, chronic lymphocytic leukemia; HTZ, heterozygous; KO, knockout; MO, morpholino; T-ALL, T-cell acute lymphoblastic leukemia.

mechanisms by which ribosomes can promote oncogenesis, and how can this knowledge be therapeutically exploited?

## THE RIBOSOME AND DYSREGULATION OF TRANSLATIONAL CONTROL

The ribosome converts genetic information into proteins with great speed and accuracy: Elongation of the polypeptide

chain by one amino acid occurs in approximately 60 milliseconds with an error rate of  $10^{-3}$ – $10^{-4}$ /codon (16). In eukaryotes, the small (40S) and large (60S) subunits combine to form the active 80S ribosome. In humans, the small subunit consists of a single ribosomal RNA (rRNA) chain and 33 ribosomal proteins (RPS) whereas the large subunit entails three rRNA chains and 47 ribosomal proteins (RPL; 17). Initially, ribosomal proteins were considered to be the central players in

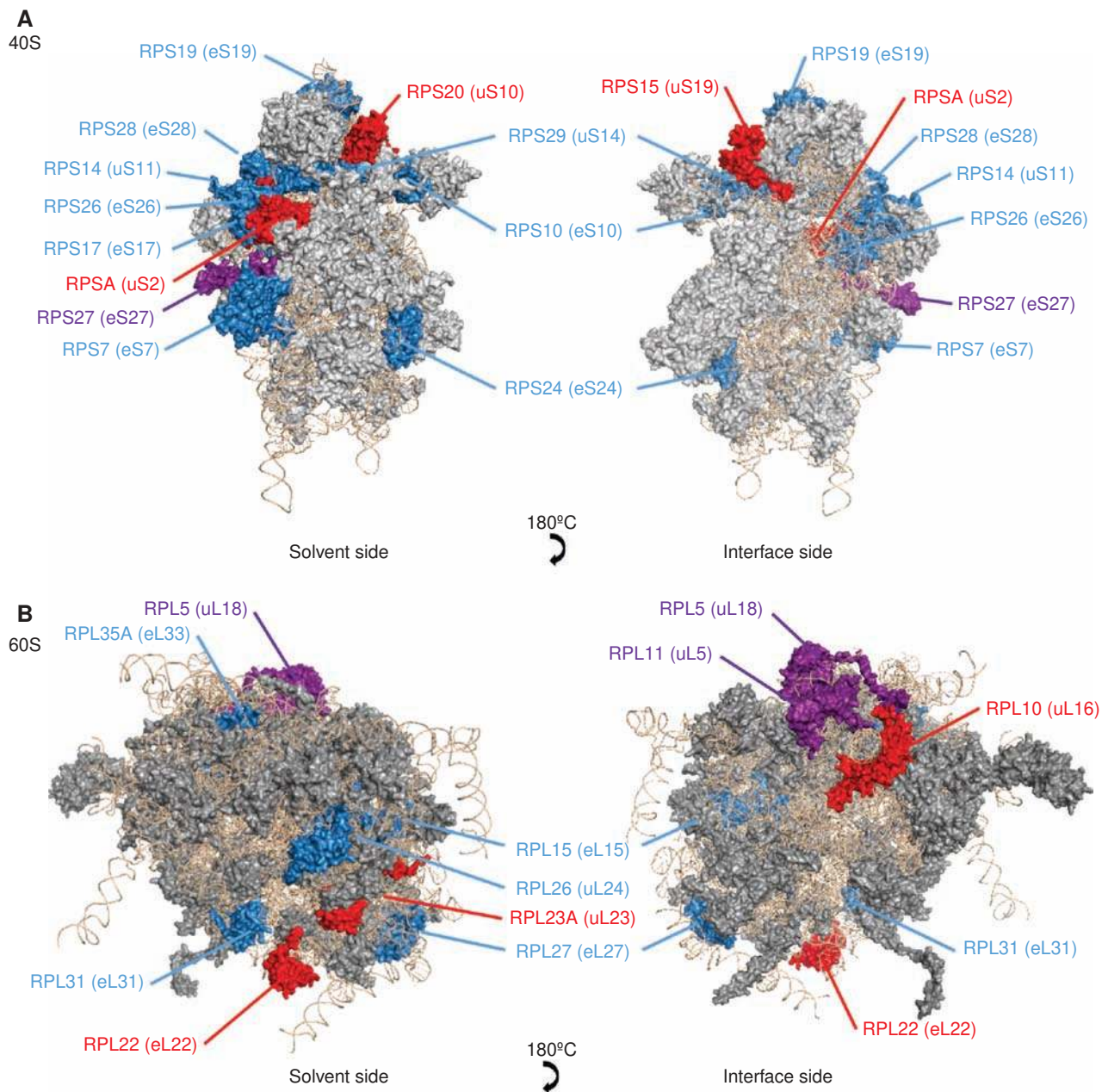
**Table 2. Ribosome biogenesis factors involved in cancer-predisposing disease**

Gene	Disease	Model (organism: modification)	Phenotype	Reference
<b>DKC1</b>	DC	Mouse: <i>Pot1b</i> del and reduced telomerase activity	Hyperpigmentation Bone marrow failure at 4-5 months	(204)
		Mouse: <i>Dkc1</i> <sup>Δ15</sup> truncating mutation	Impaired proliferation Increased DNA damage response independent of telomere length	(205)
		Mouse: <i>Dkc1</i> <sup>m</sup> hypomorphic mutation	DC clinical phenotypes Impaired ribosomal RNA pseudouridylation before onset of symptoms Telomere defects only later, might exacerbate DC	(206)
<b>SBDS</b>	SDS	Zebrafish: MO KD of <i>Sbds</i>	Pancreatic hypoplasia Neutropenia Skeletal defects	(207)
		Zebrafish: CRISPR/Cas inactivation of <i>Sbds</i>	Pancreatic hypoplasia Neutropenia Morphologic defects Growth retardation	(208)
		Mouse: transplant of <i>Sbds</i> deficient fetal liver cells	Neutropenia Hypocellular bone marrow Myeloid differentiation block	(209)
		Mouse: homozygous and heterozygous loss of <i>Sbds</i>	Embryonically lethal	(210)
		Mouse: <i>Sbds</i> disruption in pancreas	Pancreatic hypoplasia Growth retardation	(211)

ribosome function, whereas rRNA was relegated to a minor, scaffolding role. As our understanding of the ribosome progressed and its activity as a ribozyme was established, these perceived roles were completely reversed. However, ribosomal proteins are now reappreciated as more than just structural glue: Over half are essential, and some are even required for catalytic activity (18). Moreover, the ribosomal protein:RNA mass ratio increases along with organismal complexity: from ~1:2 in bacteria to ~1:1 in higher eukaryotes (19), providing evidence for the importance of these proteins for specialized ribosomal function. The presence of additional ribosomal proteins and rRNA expansion elements in human ribosomes is also likely indicative of the contribution of each to ribosomal structure and function. Indeed, the large surface area of the 4.3 MDa human ribosome provides opportunities for interactions with a myriad of protein and/or RNA binding *trans*-acting factors and for modulation of ribosomal activity.

As with other cellular polymerization reactions, translation can be divided into three distinct steps: initiation, elongation, and termination (Fig. 2). Cap-dependent initiation is widely considered to be the rate-limiting step of translation and is thus a principal regulatory target through numerous inputs and *trans*-acting factors (for a comprehensive review, see ref. 20). Cancer cells can exploit this key regulatory nexus for their oncogenic programs. Amplification of genes encoding translation initiation factors (EIF), as well as aberrations in oncogenic factors such as mTOR, c-MYC, and RAS that upregulate the function of ribosomes by increasing rates of ribosome production and initiation, has been extensively

described in a variety of human cancers (21, 22). Aberrations in translation initiation factors outside of the canonical cap-dependent initiation machinery are also relevant. For example, EIF6 is a regulator of 80S formation that can promote tumor growth and is overexpressed in many cancers (23). Moreover, the recent emergence of EIF2A-driven non-canonical translation initiation in 5' untranslated regions (UTR), as well as the use of a CUG codon rather than the conventional AUG initiation codon in tumor cells, further broadens the translational initiation repertoire that cancer cells exploit to gain an advantage (24). *Cis*-acting mRNA control elements also play critical roles in regulating translation at many levels. For example, cap-independent translation via internal ribosomal entry site (IRES) elements is emerging as an important mechanism in tumorigenesis (25), particularly in neovascularization (reviewed in ref. 26). RNA G-quadruplex structures (G-rich RNA sequences that fold into a four-stranded conformation) located in 5' untranslated leader sequences inhibit translation initiation of oncogenes; overexpression of RNA helicases such as EIF4A can overcome these barriers to promote overexpression of G-quadruplex-containing mRNAs (27). Furthermore, 5' terminal oligopyrimidine tract motifs regulate translation of mRNAs encoding ribosomal proteins; their dysregulation enables uncontrolled ribosome synthesis, a critical requirement for uncontrolled cellular proliferation (reviewed in ref. 28). Finally, additional recently discovered mRNA regulons such as the translation inhibitor element (29), pyrimidine-rich translational element (30), and cytosine-enriched regulator of translation (31), are thought

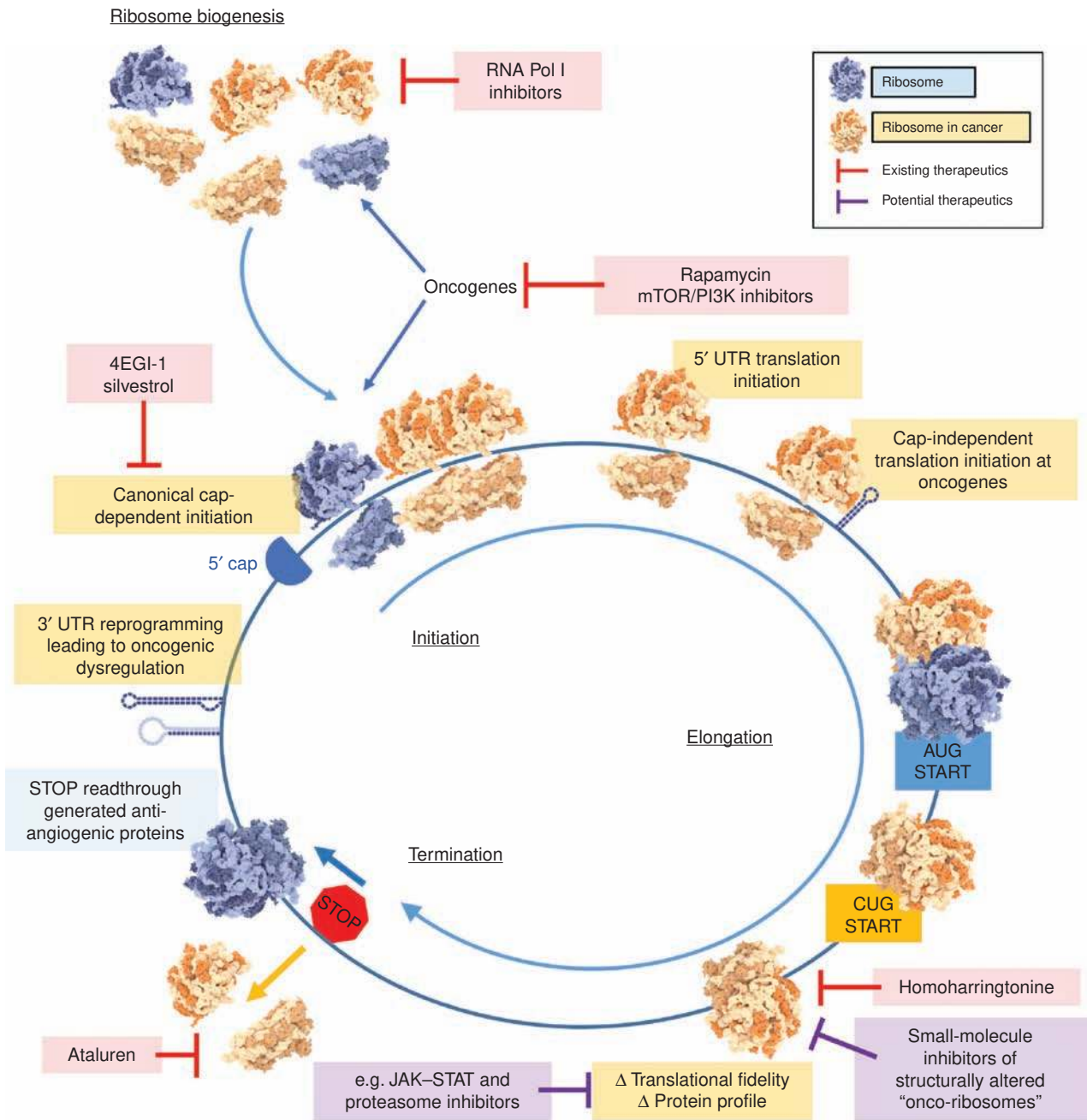


**Figure 1.** RPs implicated in ribosomopathies and/or human cancer. All RPs that have been implicated in ribosomopathies (blue), in cancer (red), or in both (purple) indicated on a structural model of the human ribosome for the small 40S subunit (**A**) and the large 60S subunit (**B**). This figure was generated in PyMOL and is based on the human X-ray structure with a resolution of 3 Å (PDB entry 4V6X).

to interface with translation initiation factors on distinct protumorigenic mRNAs.

Regulation of gene expression during the translation elongation cycle is equally important for maintaining cellular homeostasis. For example, programmed -1 ribosomal frameshifting (-1 PRF), a molecular mechanism in which *cis*-acting elements cause elongating ribosomes to slip backward on mRNAs by one base, is emerging as a potentially important regulatory mechanism (reviewed in ref. 32). Key to regulation by -1 PRF is the finding that frameshift events on

cellular mRNAs direct elongating ribosomes to premature termination codons, stimulating their rapid degradation by the nonsense-mediated mRNA decay pathway (33). Thus, dysregulation of -1 PRF can result in changes in gene expression. KEGG analysis of the predicted ribosomal frameshift signal database (PRFdb; <http://prfdb.umd.edu/>; ref. 34) reveals a significant enrichment for genes in particular pathways. For example, although approximately 10% of human protein coding genes harbor predicted -1 PRF signals, 30% of the genes in the oncogenic JAK-STAT pathway contain



Downloaded from <http://aacrjournals.org/cancerdiscovery/article-pdf/7/10/1069/1837824/1069.pdf> by guest on 26 August 2022

**Figure 2.** Ribosome dysfunction in cancer. The main differences in the function of ribosomes in cancer cells compared with healthy cells throughout the ribosomal lifecycle are shown. These include upregulation of both ribosome biogenesis and canonical translation initiation by oncogenic factors such as mTOR, c-MYC, and RAS; noncanonical translation initiation at oncogenes such as c-MYC; the use of unconventional start codons; and altered translational fidelity and translational profiles. Current and potential promising therapeutic intervention points at key nodes are indicated.

these elements (35). The potential role of -1 PRF deregulation on mRNAs encoding oncogenic and tumor suppressor proteins is an exciting avenue for future research. Regulation of the translocation step of elongation through EEF2 phosphorylation by EEF2 kinase has also been implicated in cancer by exerting cytoprotective effects (reviewed in ref. 36).

Lastly, the termination step of translation also provides opportunities for the regulation of gene expression. For example, programmed read-through of the termination codon in the *VEGF* mRNA results in a C-terminally extended isoform of the protein (VEGF<sub>AX</sub>) with antiangiogenic activity, i.e., it antagonizes the normal angiogenic activity of VEGF (reviewed in ref. 37). Beyond termination codons,

mammalian mRNAs tend to have long 3' UTRs that have been shown to contain many different families of *cis*-acting regulatory elements which function to regulate gene expression through their interactions with both protein- and non-coding-based *trans*-acting factors. As it is beyond the scope of this review, the reader is directed to other recent reviews on this topic (38–42).

Dysregulation of translational control, particularly at the step of initiation and briefly summarized above, has been well studied in past years (20, 43). Dysregulation of ribosomal function due to mutations in ribosomal proteins, particularly those that are somatically acquired, represents a novel topic warranting increased exploration. We focus on this growing field of study in the following section.

## DEFECTS IN RIBOSOME BIOGENESIS, RIBOSOMAL PROTEINS AND RIBOSOMAL RNA MODIFICATION AND DYSREGULATION OF RIBOSOMAL FUNCTION

In the 19th century, scientists discovered a visually distinguishable characteristic of cancer cells: an increased number and size of nucleoli visible in the nucleus. Nucleoli are the initial sites of ribosome production, a complex process known as ribosome biogenesis. They are dense structures organized around ribosomal DNA sequences (rDNA), the DNA transcriptional units that encode the rRNA components of the ribosome which co- and posttranscriptionally associate with RPs and other nonstructural factors to assemble the mature ribosome. The individual assembly of the subunits occurs in several steps and cellular compartments. First, the transcription of precursor rRNA by RNA polymerase I (RNA Pol I) occurs in the nucleolus. Here, this pre-rRNA undergoes a series of modifications along with processing by small nucleolar ribonucleoproteins (snoRNP), followed by early assembly with RPs to form the subunit cores. A large number of *trans*-acting factors contribute to further processing in the nucleoplasm and eventual export of the preribosomal subunits into the cytoplasm where the final steps of maturation occur. Lastly, 80S formation is achieved on target mRNAs (44, 45). Up to 2,000 ribosomes per minute are estimated to be produced in growing eukaryotic cells (46), and a highly proliferating cancer cell relies extensively on this process. Consequently, several tumor suppressors involved in the negative control of rDNA transcription (e.g., *CDKN2A*, *TP53*, *RBI*, and *PTEN*) are frequently deleted in cancer. For example, p53 has a well-characterized role in inhibiting the RNA Pol I transcription machinery to repress rRNA synthesis (47). Loss of p53 in cancer, therefore, provides a mechanism for bypassing this control. For more than 100 years, the site of ribosome production in the cell has thus been linked to cancer. Mutations in RPs were not suspected to be found in transformed cells because they were thought to be incompatible with viability. However, the recent discovery of both congenital and somatic mutations in RPs as well as ribosome biogenesis factors has overturned that view and has become an increasingly important topic in cancer biology. In this section, we present an overview of ribosomopathies with elevated cancer risks and of the somatic ribosome defects that have been described in cancer to date (Tables 1 and 2; Fig. 1). We first cover defects

in ribosome biogenesis factors, followed by ribosomal protein and ribosomal RNA modification alterations, and end with a discussion of the emerging molecular insights on how these defects might promote cancer.

## Ribosomopathies with Elevated Cancer Risk

Ribosomopathies are a collection of syndromes with the shared underlying feature of defective ribosomal function or production. The majority of these disorders are congenital, with the notable exception of 5q-syndrome, an acquired ribosomopathy that usually emerges at advanced age. Many of these syndromes initially present clinical phenotypes that can be categorized as cellular hypoproliferative defects: bone marrow failure, malformations, mental and motor deficiency. Intriguingly, some of these diseases are also linked with higher incidences of cancer later in life (6, 48, 49). This review will focus on this latter subset of ribosomopathies, and for more extensive reviews we refer to other literature (50, 51).

### Shwachman–Diamond Syndrome

SDS is characterized by pancreatic insufficiency, ineffective hematopoiesis, and frequent serious infections due to neutropenia (52, 53). Approximately 90% of patients have biallelic mutations in the Shwachman–Bodian–Diamond syndrome (*SBDS*) gene (54). The SBDS protein was observed to be localized in the nucleolus in human cells, implying a role in ribosome biogenesis (55). This idea was strengthened by the observations that SBDS associates with rRNA and several RPs (56, 57). SBDS plays an essential role in the final steps of ribosome biogenesis by facilitating the release of the anti-association factor EIF6 from the pre-60S particle, allowing the association of the 60S and 40S subunits into mature and translationally competent 80S ribosomes (58, 59). SDS patients are at increased risk of developing myelodysplastic syndrome (MDS) and subsequently acute myeloid leukemia (AML), with a cumulative risk for malignancy of 19% at 20 years and up to 36% at 30 years of age (53). This is remarkable, considering that the average risk of developing MDS or AML under the age of 50 years in the general population is less than 0.1% (60, 61).

### Dyskeratosis Congenita

DC carries one of the highest risks of malignancy among all ribosomopathies and is characterized by abnormal skin pigmentation, white patches inside the mouth (oral leukoplakia), and nail malformation. Additionally, 86% of patients develop bone marrow failure, which is reported as the major cause of premature mortality at the median age of 44 (62, 63).

Genetically, DC can present as X-linked recessive, autosomal dominant, or autosomal recessive. The X-linked variant (X-DC) is the most common and has been associated with defects in *DKC1* (64, 65). *DKC1* encodes dyskerin, which has two important nucleolar functions during ribosome biogenesis. First, it binds to a group of small nucleolar RNAs (snoRNA) containing the H-box (ANANNA) and ACA box (ACA) sequence motifs (H/ACA; ref. 66). These H/ACA snoRNAs are involved in rRNA pseudouridylation, a type of rRNA modification that is required for proper ribosome biogenesis and function (67). Secondly, dyskerin also

binds to the RNA component of the telomerase complex (TERC), through the same sequences as present in the H/ACA snoRNAs. This interaction stabilizes TERC, allowing telomere lengthening (68). The latter function of dyskerin links the X-linked form of the disease to the autosomal forms, which are manifested by either recessive or dominant mutations of various components important for telomere maintenance, including *TERC* and telomerase (*TERT*; refs. 69–71). Indeed, short telomeres are a hallmark of DC cells and can be used to identify patients in the absence of other symptoms (72). Cancer incidence in patients with DC is 11 times higher than in the general population. The cumulative risk of malignancy is 40% to 50% by the age of 50, with a median age of 29. The elevated risk is highest for head and neck squamous cell carcinoma and is elevated by 1,154-fold [observed over expected ratio (O/E)], followed by AML at 196 O/E. Importantly, the risk to develop MDS is an astounding 2,500-fold higher than the expected risk (48).

### MDS with Chromosome 5q Deletion

Although the risk of developing solid tumors varies between the disorders described above, they share an elevated risk for MDS, a syndrome that progresses to AML in up to 40% of cases. It is therefore not surprising that a subtype of MDS has been identified as a ribosomopathy.

The World Health Organization has divided MDS into subtypes, one of which is “MDS associated with isolated del(5q).” The long arm of chromosome 5 is deleted [del(5q)] in 10%–20% of patients with MDS (73–75), suggesting that the 5q region contains genes whose loss contributes to the initiation and/or progression of the syndrome. The commonly deleted region (CDR) delineates a 1.5-kb region encompassing 40 genes at 5q32–33 that includes, among other candidates, ribosomal protein *RPS14* and the kinase *CSNK1A1* genes (76). Although mutations of genes in the CDR are rare, 7% of del(5q) patients carry *CSNK1A1* mutations on the nondeleted allele (77).

### Diamond-Blackfan Anemia

The first identified and best-studied congenital ribosomopathy is DBA, a disease characterized by red blood cell aplasia (78). Patients typically develop anemia before the first birthday and some also present with birth defects such as craniofacial and digit abnormalities (79).

The *RPS19* gene was discovered as the target of chromosomal translocation, mutation, and deletion in approximately 25% of patients with DBA (4, 80). Subsequent research identified additional RPs, of both the small and large subunit, that are mutated or deleted in another 30% of patients with DBA [*RPL5* (7%), *RPS10* (6%), *RPL11* (5%), *RPS26* (3%), *RPL35A* (3%), *RPS24* (2%), *RPS17* (1%), *RPS7* (1%), *RPL26*, *RPL27*, *RPS27*, *RPL15*, *RPS28*, *RPL31*, *RPS29*; Fig. 1; refs. 81–90]. Apart from mutations in the ribosome, some patients carry mutations in the hematopoietic transcription factor *GATA1* (91). Reduction of *RPS19*, *RPL5*, *RPL11*, or *RPS24* can also affect *GATA1* levels, thereby linking these defects mechanistically (92, 93).

DBA is a classic example of “Dameshek’s Riddle” (94): Patients initially develop anemia, but those who survive to adulthood are at increased risk of developing cancer. An average elevated cancer risk of 5.4-fold has been described for

patients with DBA, with a cumulative risk of 22% of developing malignancy by the age of 46 years. Patients present with a variety of malignancies such as AML (O/E = 28), colon carcinoma (O/E = 36), and osteogenic sarcoma (O/E = 33). Additionally, patients with DBA have a 287-fold higher risk of developing MDS, which in turn predisposes to AML (49).

### Somatic Ribosomal Protein Defects in Cancer

After the initial correlation between congenital defects in ribosomal proteins or biogenesis factors and increased cancer risks, more evidence for a direct link between ribosomal perturbation and cancer followed. In 2004, the observation of tumors in heterozygous RP mutant zebrafish provided the first indication that ribosome deficiency is sufficient to cause cancer (7). Several years later, next-generation sequencing–based genome-wide screens for mutations in cancer samples revealed previously unanticipated somatic mutations in various ribosomal protein genes. This section explores the most well-understood mutations affecting ribosomes that are implicated in cancer.

#### 60S Proteins

***RPL5*.** In addition to its established role in DBA, *RPL5* is also a target for somatic mutations in cancer. In T-cell acute lymphoblastic leukemia (T-ALL), 2% of patients carry heterozygous inactivating mutations in *RPL5* (8). Moreover, *RPL5* is located at a significant peak of heterozygous deletion, and it is deleted or mutated in 11% of glioblastoma, 28% of melanoma, 34% of breast cancer, and  $\geq 20\%$  of multiple myeloma tumors (11, 12). A haploinsufficient tumor suppressor role for *RPL5* is further supported by the observation that a 50% knockdown of *RPL5* in breast cancer cell lines enhances G<sub>2</sub>–M cell-cycle progression and accelerates tumor progression in a xenograft mouse model (12). Of therapeutic relevance, in the context of multiple myeloma, patients with low *RPL5* expression have poor prognoses, which can be overcome by including the proteasome inhibitor bortezomib in the treatment regimen (11).

***RPL10*.** *RPL10* is mutated in 8% of pediatric patients with T-ALL. The *RPL10* gene contains a strong mutational hotspot with nearly all patients displaying the same arginine-98-serine (R98S) missense mutation in T-ALL (8). *RPL10* is functionally linked to *SBDS*, as they work in conjunction to promote the release of EIF6 from the pre-60S particle in the final 60S maturation steps. It is thus not surprising that mutations in *SBDS* and the *RPL10-R98S* allele result in similar ribosome biogenesis defects (8, 59, 95). Rare somatic mutations in *RPL10* have also been described in multiple myeloma. Interestingly, the *RPL10* mutations in this disease entity cluster in a different region as compared with the described mutational hotspot in T-ALL (96).

***RPL11*.** Whereas *RPL11* is an established DBA gene, somatic mutations in this gene are less common in cancer samples. Rare somatic mutations in patients with relapsed T-ALL have been described, and 1.4% of melanoma cases show mutations (12, 97). Besides displaying a DBA phenotype, heterozygous *Rpl11* knockout mice present increased susceptibility to radiation-induced lymphomagenesis (98), making this mouse the only model fully recapitulating DBA to date, including its cancer predisposition.



**RPL22.** *RPL22* is somatically inactivated by mutations and deletions in 10% of patients with T-ALL (9). A recurrent mutation in the *RPL22* gene resulting in a truncated protein has been described in ~10% of gastric, endometrial, and colorectal cancer samples (99–101). This *RPL22* mutation is a single base deletion in a homopolymeric stretch of eight A nucleotides, which is consistent with the exclusive presence of this mutation in microsatellite-unstable tumors. Although this may indicate a passenger role for these defects, a haploinsufficient tumor suppressor role for *RPL22* is supported by the observation that heterozygous inactivation of *RPL22* can accelerate lymphoma development driven by myristoylated AKT2 (9). Homozygous inactivation also accelerates generation of a lymphoma that limits the migration of the lymphoma cells to peripheral tissues such as the spleen, lymph nodes, and liver. Consequently, *Rpl22*<sup>-/-</sup> lymphoma mice have an increased size and angiogenesis of the thymic tumors (102).

**RPL23A.** *RPL23A* is amplified in 12.5% of uterine cancers, where it is part of a distinct amplification peak. These *RPL23A* amplifications are more frequent among the serous endometrial tumors, a more rare and aggressive subtype of uterine cancers (12).

#### 40S Proteins

The most frequent somatic defects in 40S proteins identified so far are the defects in *RPS15*. This gene is a targeted by somatic missense mutations that mainly cluster in a 7 amino-acid region of exon 4 in 10% to 20% of patients with chronic lymphocytic leukemia (CLL; refs. 10, 13). Additionally, *RPS27* contains an identical mutation in its 5' UTR in 10% of patients with melanoma (103). The impact of this noncoding variant on *RPS27* expression remains to be determined. Finally, *RPSA* is significantly mutated in 3% of patients with stomach cancer, with clustering of the mutations pointing to a possible role in the disease (12).

It is important to comparatively consider the incidence of somatic ribosomal protein mutations in the crowded world of oncogenic mutations. The total number of somatic point mutations is highly variable among cancers, ranging from 4 to as much as 1,600 per cancer type (104), and incidences of copy-number changes are generally much lower than those of point mutations (104, 105). Interestingly, in contrast to this general trend in cancer, alterations of ribosomal protein genes occur more often through copy-number changes than point mutations. Moreover, the cancer types with the highest incidences of ribosomal protein mutations are not correlated with high general mutation incidences. For example, pediatric T-ALL contains ribosomal mutations in 15% to 20% of cases, but is characterized by a very low load of copy-number changes and somatic point mutations (8, 106, 107). Other cancer types in which ribosomal protein mutations have been described have similarly low-to-moderate mutational loads, with the exception of UV exposure-driven melanoma (104).

Moreover, the currently known incidences of ribosomal protein defects are lower than those in other functional protein classes. For example, all pediatric patients with T-ALL have mutations in transcription factors, the large majority have cell-cycle regulator lesions, 60% have mutations in signaling pathways, and 40% have mutations in epigenetic regulators

(108). A similar trend applies to other cancer types with a significant incidence of ribosomal protein lesions: mutational categories such as the p53 pathway, signaling, transcription factors, and epigenetic modulators are often represented at higher frequencies than the ribosome. This could be due to the stringent filtering in the detection of ribosomal mutations: All ribosomal protein genes with copy-number changes that coincided with copy-number changes in known cancer genes were excluded from the analysis (12). Although this eliminates the possibility of false positive detection of ribosomal protein genes due to their proximity to other known cancer drivers, it also likely causes an underestimation of the incidence of ribosomal gene mutations. Alternatively, ribosomal protein mutations could influence a wide variety of downstream pathways. This is exemplified by the RPL10-R98S mutation in T-ALL, which functionally mimics activation of the oncogenic JAK-STAT signaling pathway, thereby eliminating the need for JAK-STAT-activating lesions (35). Finally, the lower incidences of ribosomal gene mutations might be due to the incompatibility of such mutations with viability. Indeed, only a fraction of the 81 ribosomal proteins show congenital and/or somatic defects in cancer and ribosomopathies. This may suggest that cells cannot survive with defects in particular critical RPs, as supported by the fact that many of the cancer-associated RPs are incorporated into the ribosome late in the biogenesis process and are not involved in the formation of the ribosomal core. Alternatively, modulation of certain extraribosomal functions, discussed in more detail in the next section, may be needed for carcinogenesis, and the differential implication of distinct RPs in cancer could be a reflection of their different involvement in these functions.

Somatic ribosomal protein mutations form a novel functional class of defects in cancer, and their importance in oncogenesis may still be underestimated. Additional RPs, besides the ones described above, show differential expression in cancer, often because of copy-number changes (86, 87). Thus, further investigations are needed to elucidate the role of the deletions and amplifications of ribosomal protein genes in cancer. In contrast to the congenital syndromes in which components of the 60S and 40S subunits have both been heavily implicated in disease pathogenesis, the somatic defects identified in cancer samples are more common in ribosomal proteins of the large subunit at this time, with heterozygous inactivating lesions in *RPL5* being the most common. *RPL5* moreover displays congenital defects in DBA, whereas other ribosomal protein defects seem to display specificity for either congenital or somatic disease, or even a single particular disease entity. A striking example of the latter is the RPL10-R98S point mutation, which has been described only in childhood T-ALL so far. An explanation for this could be the concept of “specialized ribosomes”: tissue-specific phenotypes might stem from cell-specific differences in ribosomal composition (rRNA, RPs, and/or their modifications), or be mediated through binding of cell-specific and developmentally regulated RNA or ribosome-associated factors (29, 109–113). In support of this, a quarter of human ribosomal proteins were recently found to exhibit tissue-specific expression, with primary hematopoietic cells displaying the most complex expression patterns (114). Moreover, extensive analysis of ribosomes in mouse embryonic stem

cells revealed heterogeneity in ribosomal protein composition that is associated with translation of distinct subsets of mRNAs by different ribosomal subpopulations (113), suggesting that ribosomes can function in the absence of specific ribosomal proteins (115). Yet another layer of heterogeneity is added by ribosome-associated proteins that are differentially expressed in subcellular locations, enabling another mechanism for transcript-specific translation and regulatory potential (112).

### rRNA Modification Defects

The importance of rRNA modification in gene expression and cancer is also becoming apparent. rRNA is highly modified, containing over 100 modifications, the most common of which are base and ribose 2'-hydroxyl methylation and pseudouridylation (116). rRNA hypomodification has been linked to numerous diseases including X-DC, several cancers, and aging (1, 116–118). Although none of the close to 100 known pseudouridylation events are essential, it is thought that these modifications provide an additional layer for fine-tuning ribosome structure, enabling it to equally distinguish between 61 different tRNAs and other ligands (104). Interestingly, quantitative mass spectroscopic analyses have revealed sub-stoichiometric rRNA base modification in normal populations of ribosomes (119, 120). That cells may normally harbor mixed populations of ribosomes represents a radical departure from the general concept of “the” ribosome. Rather, diversity among ribosomes may confer a means to “buffer” translational capacity, enabling cells to maximize their ability to interact with many different *trans*-acting partners and translate many different mRNAs (14). It also presents a potential modality for ribosome specialization (14). As discussed below, biochemical and genetics analyses have shown that mutations that result in either base-specific or general hypomodification of rRNA alter translational fidelity, resulting in decreased translational accuracy (121–126). Additional ribosome heterogeneity at the rRNA level could be ascribed to the function of snoRNAs and snoRNPs, the expression of which is altered in various cancers (reviewed in ref. 127). It is thus tempting to speculate that expression of different rRNA forms may be regulated during normal cell growth and differentiation, and that its dysregulation may result in dysmorphisms and cancers.

### Mechanistic Insights into the Oncogenic Potential of Ribosomal Defects

The work described above established the involvement of RPs and ribosome biogenesis factors in ribosomopathies and malignancies. The underlying molecular mechanisms by which these factors promote oncogenesis are beginning to emerge and are summarized below.

#### Ribosomal Functions

It is reasonable to speculate that the ribosomopathy-associated phenotypes, including increased cancer susceptibility, might be due to altered translation potential of the ribosome. Several lines of evidence support this hypothesis and suggest that certain phenotypes associated with RP and biogenesis factor defects are caused by highly specific changes in translation. For example, the *Rps19* and *Rpl11* mutant zebrafish lines

show a decrease in globin translation in erythroid cells, possibly explaining their dysfunction (128). Additionally, reduced expression of RPS19, RPL5, RPL11, or RPS24 in DBA cells leads to a specific reduction of GATA1 translation, raising the possibility that this link between RPs with GATA1 translation contributes to the anemia phenotype in RP-mutated DBA cases (93). Moreover, cells derived from nerve sheath tumors developed in heterozygous RP mutant zebrafish display a specific defect in translation of p53 (129). Knockdown or mutation of SBDS impairs translation reinitiation of the *CEBPA* and *CEBPB* mRNAs, indispensable regulators of granulocytic differentiation (130).

Altered ribosome function can also be attributed to differences in rRNA modification. Changes in rRNA 2'-O-methylation patterns due to p53 control of fibrillarin expression result in changes in termination codon read-through and increased translation of IRES-containing cellular mRNAs (131). Conversely, fibrillarin overexpression contributes to tumorigenesis and is associated with poor survival in patients with breast cancer (131). Hypo-pseudouridylation of rRNA renders ribosomes unable to directly translate of IRES-containing mRNAs, including the tumor suppressor genes *TP53* (132) and *CDKN1B*, and the antiapoptotic factors *BCL2L1* and *XIAP* (133). Indeed, the demonstration that loss of IRES-mediated p27 translation contributed to pituitary tumorigenesis in mice established *DKC1*, the gene responsible for rRNA pseudouridylation, as a tumor suppressor (134). In the context of DC, expression of CBF5P-D95A (a catalytically impaired mutant of CBF5P, the yeast homolog of *DKC1*) reduces rRNA pseudouridylation, resulting in reduced ribosomal affinity for tRNAs and certain viral IRES elements. These biochemical impairments in ribosome activity manifest as decreased translational fidelity and IRES-dependent translational initiation, which are also evident in mouse and human cells deficient in *DKC1* (126). Additionally, decreased affinities for tRNAs result in increased rates of -1 PRF (126). As discussed above, given the overrepresentation of -1 PRF signals in cancer pathways, this observation may provide a mechanistic explanation for increased cancer incidence in patients with X-DC. Altered biochemical properties and decreased translation fidelity of the ribosome have also been attributed to the T-ALL-associated RPL10-R98S mutation in yeast (95). Interestingly, in the context of lymphoid cells, RPL10-R98S expression results in overexpression of the JAK-STAT oncogenic signaling cascade, a pathway that is highly enriched for -1 PRF signals (35).

#### Extraribosomal Functions

Several RPs have been shown to function outside the ribosome. These “extraribosomal” roles might also be relevant to understanding the cancer-promoting action of ribosome defects, particularly because some of these functions relate to major cancer genes such as *TP53* and *MYC*.

MDM2, an inhibitor of p53, is directly regulated by several RPs in an essential pathway in response to nucleolar stress (135–137). In growing cells, ribosome biogenesis is fully active, and free RPs are incorporated into the ribosome. In particular, RPL5 and RPL11 first assemble into a complex with the 5S rRNA before being added at the late stages of large subunit assembly. Under these conditions, MDM2 is free to bind p53, promoting p53 ubiquitination and

degradation (Fig. 3A). Conversely, reduction of RP synthesis in response to starvation, stress or antigrowth signals stalls the ribosome biogenesis process, inducing nucleolar stress. This results in free RPL5/RPL11/5S rRNA complexes that sequester MDM2, thereby stabilizing p53 (138, 139). Activated p53 can then turn on transcriptional programs inducing cell-cycle arrest and apoptosis (Fig. 3B). Although only RPL5, RPL11, and RPS27A have been demonstrated to be essential for p53 activation in response to nucleolar stress, many other RPs have also been linked to this direct regulation of p53 (140–143). Of these, RPL26 is of particular interest, as it has also been shown to bind *TP53* mRNA to enhance its own translation (144, 145). The linkage of RP-associated malignancy to the p53 pathway has also been explored in RP mouse and cellular models. For example, *RPL11* heterozygous knockout mouse lymphomas show an impaired p53 response (98). The same applies for the *RPS15* mutants recently found in CLL, although modeled in a colorectal cancer cell line (13). The relevance of the RP–MDM2 interaction in hematopoietic failure and malignancy is also supported by mouse models with *Mdm2* mutations abrogating its ability to bind RPs. These animals develop hematopoietic deficiencies similar to those of DBA models while accelerating MYC-induced lymphomagenesis (146). RPL22 deficiency-associated T-cell development phenotypes have also been linked to p53 via an indirect mechanism. RPL22-deficient mice display selective apoptosis of the  $\alpha\beta$  T-cell lineage (9), causing endoplasmic reticulum (ER) stress in this subset of T cells. Whereas increased ER stress normally inhibits protein synthesis, RPL22 deficiency appears to aggravate ER stress by interfering with the ability of ER stress signals to block protein synthesis, resulting in p53 induction and apoptosis of  $\alpha\beta$  T cells (102). It has been suggested that p53-induced apoptosis in distinct tissues can lead to both the morphologic and the hematopoietic defects of the DBA models.

A second extraribosomal role of RPs involves a negative feedback loop with c-MYC. This factor enhances ribosome biogenesis by inducing both rRNA and RP transcription (147–149), and certain RPs in turn inhibit c-MYC levels and function (150). RPL11 interacts with c-MYC at promoter regions of c-MYC target genes, inhibiting c-MYC-dependent transcription (Fig. 3C; refs. 150, 151). In addition, RPL5 and RPL11 cooperatively bind to the c-MYC mRNA and guide it to the RNA-induced silencing complex (RISC) for degradation (Fig. 3D; refs. 152, 153). A similar mechanism has also been described for RPS14 (154). Regulation of c-MYC may also be relevant in a cancer setting, as RPL11-deleted mouse lymphomas show c-MYC upregulation (98). RPL22 inactivation also leads to c-MYC activation, albeit indirectly via the NF $\kappa$ B–LIN28B–LET7 miRNA axis (9). On the other hand, MYC-induced lymphomas can be suppressed by heterozygous deletion of *Rpl24* and *Rpl38* in mouse models (155). Following this observation, overexpression of *RPL24* has been implicated in breast cancer progression (156). Collectively these findings indicate that RP defects could be regulating oncogenic c-MYC in a context-dependent manner—an interesting concept requiring further exploration.

Whereas the extraribosomal links with c-MYC and p53 are by far the most well described, several other findings are worth mentioning. RPSA has an extraribosomal role on the

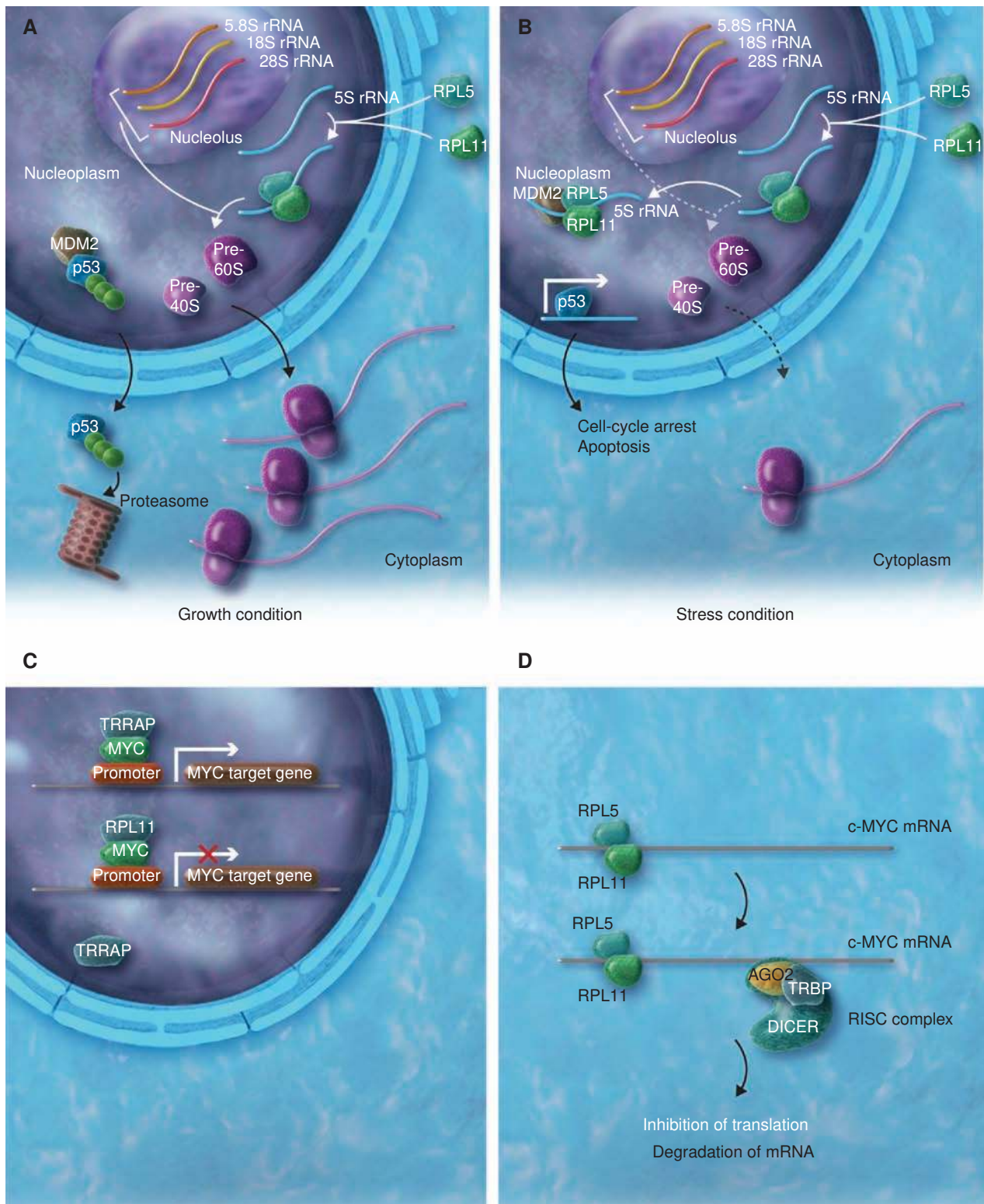
cellular membrane as a laminin receptor and transduces extracellular signals regulating cancer-related pathways including apoptosis and cell migration (157, 158). Rare mutations implicated in stomach cancer affect residues that might be essential for this RPSA function (12). Additionally, the recent observation of elevated STAT3 and mTOR phosphorylation levels in leukocytes from patients with SDS reflects a potential novel extraribosomal link between SBDS and mTOR/STAT3 (159). The increased ROS levels and autophagy phenotypes observed in zebrafish models of several DBA RPs including RPS19 may also reflect novel extraribosomal functions (160).

## THERAPEUTIC STRATEGIES

The recent insights into the role of dysregulation of protein synthesis in cancer provide opportunities for therapeutic intervention. Such approaches comprise (i) “starving” cancer cells of ribosomes and preventing translation initiation, (ii) targeting the consequences of a translational defect, and (iii) specifically targeting the “onco-ribosome.” Considering the low efficacy of single-agent cancer therapies due to resistance development, multiagent combination therapy cocktails should be developed to treat cancer with high efficacy and low toxicity. Such drug cocktails may contain a combination of several agents targeting translation.

Current drug therapies primarily address the first point by inhibiting targets including RNA Pol I, EIF4A, EIF4e, EIF2S1, mTOR, and dual PI3K–mTOR, resulting in inhibition of ribosome production and initiation (Fig. 2). Given its mechanistic and regulatory complexity, 5' 7MeGppp cap-dependent initiation is a target-rich environment for therapeutic intervention. Indeed, one of the earliest effective therapeutics, rapamycin, targets mTOR, a critical protein in the PI3K–mTOR–EIF4 axis. With the advent of high-resolution structural information, newer small-molecule therapeutics are being designed to target other key regulatory proteins and pathways in this process, including components of the MEK/ERK/MNK pathway, the cap binding protein EIF4E and its regulation by EIF4E-BP, and formation of the EIF2 complex. For a more extensive description of available agents interfering with these steps, we refer to other excellent reviews (43, 161, 162).

Regarding inhibition of other steps of translation, several recently described antibiotics interfere with ribosomal function through direct interactions. For example, homoharringtonine (also known as omacetaxine or Synribo) is an inhibitor of the first round of peptide bond formation by the ribosome that has been approved for treatment of tyrosine kinase inhibitor-resistant chronic myeloid leukemia (163) and is currently in clinical trials for AML (43, 164, 165). Several eukaryotic-specific inhibitors that bind the ribosomal E-site, e.g., cycloheximide, show promising antiproliferative effects in a panel of leukemia cell lines (166). Conversely, administration of L-leucine, a known activator of mRNA translation, shows promising results for correcting the anemia and developmental defects in DBA and 5q- syndrome (167, 168). It is however unclear if L-leucine is also able to reduce the risk of transition to the cancer phenotype associated with these diseases. Although L-leucine dampens activation of p53 target genes in a DBA RPS19 mouse model (168), it does not impair ribosomal stress-induced p53 response in



**Figure 3.** Oncogenic potential of the extraribosomal functions of RPs. **A**, Under growth conditions, the cell is actively translating. Ribosome biogenesis is highly efficient, and the RPL5-5S rRNA-RPL11 complex is rapidly incorporated into mature ribosomes. In this situation, MDM2 is free to bind p53 and promote its degradation. **B**, Under stress conditions, translation and ribosome biogenesis decrease, leaving the RPL5-5S rRNA-RPL11 complex free to sequester MDM2. p53 is therefore stabilized, suppressing the cell cycle and eventually promoting apoptosis. **C**, RPL11 has been shown to negatively regulate c-MYC by binding c-MYC at the promoter regions of its target genes, thereby inhibiting the recruitment of coactivators such as TRRAP. **D**, RPL5 and RPL11 have both been shown to associate with c-MYC mRNA to promote its degradation through recruitment of the RISC complex, which includes DICER, AGO2, and TRBP.

Downloaded from <http://aacrjournals.org/cancerdiscovery/article-pdf/7/10/1069/1837824/1069.pdf> by guest on 26 August 2022

RPS19 and RPS14 morpholino zebrafish and human CD34<sup>+</sup> cells (169). An intact p53 response upon administration of L-leucine may thus protect the cells from transformation. However, because L-leucine does not correct the actual ribosomal defect in these diseases, it might promote usage of defective, cancer-promoting ribosomes. It will be interesting to determine to what extent translational fidelity is perturbed by the ribosomal protein defects in DBA and MDS. If fidelity is impaired, combining L-leucine with a drug that corrects fidelity defects may be required. Indeed, drugs targeting ribosomal fidelity are an active field of research: Drug screens have identified compounds that decrease the fidelity of start codon initiation (170). Moreover, ataluren (Translarna), a drug promoting premature stop-codon read-through (171), has received market authorization from the European Commission and is in clinical trials for treatment of diseases caused by nonsense mutations, such as Duchenne muscular dystrophy and cystic fibrosis (172, 173). Because stop-codon read-through is emerging as a relevant cellular antiangiogenic mechanism (174), similar drugs could also find applications in cancer treatments in the future.

Targeting the consequences of a translational defect requires thorough knowledge of the molecular biological implications of the lesion and requires availability of drugs to target these downstream consequences. For example, the RPL10-R98S mutation in T-ALL enhances JAK-STAT signaling and alters cellular proteasome activity, sensitizing the cells to clinically used JAK-STAT and proteasome inhibitors (35). Notably, lesions in RPL5 have also been linked to increased sensitivity to proteasome inhibitors (11), suggesting that proteasome inhibitors might benefit patients with cancer with various ribosomal protein defects.

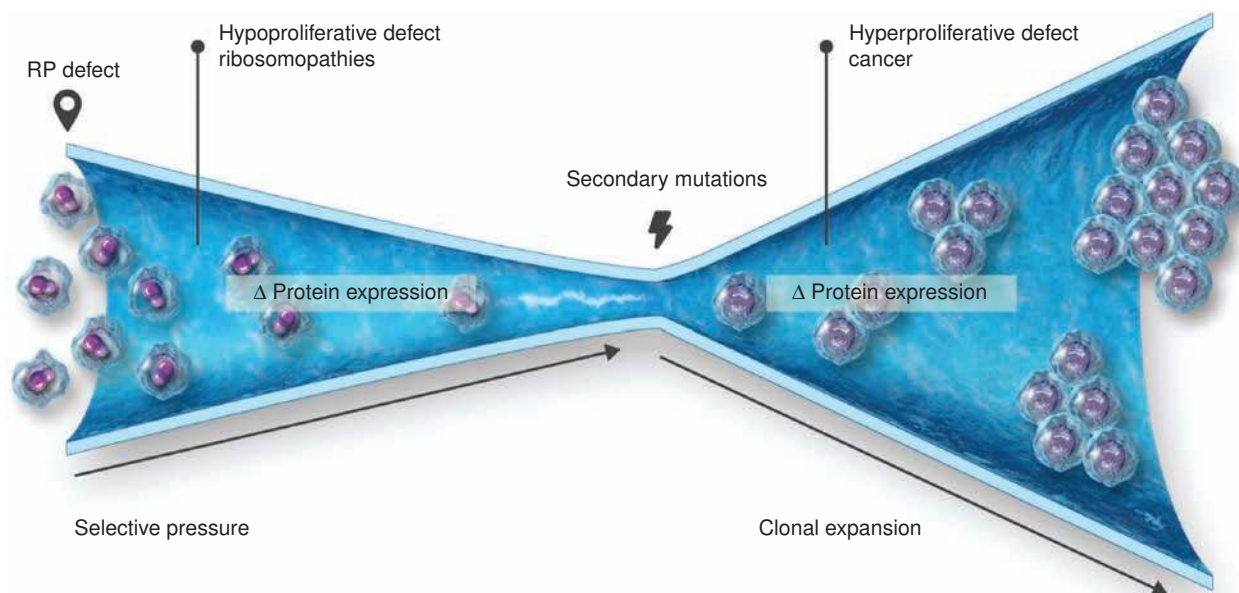
Although the strategy of specifically targeting defective “oncogenic” ribosomes in somatic cancers is in its infancy, it is perhaps the most promising. Selective inhibition of defective ribosomes in tumors could be achieved with small-molecule inhibitors and antibiotics. Because of the central role of translation in the cell, as well as the structural and functional differences between prokaryotic and eukaryotic ribosomes, protein synthesis inhibitors have been the most successful clinical antibiotics to date. As the ribosome exceeds the size of an average antibiotic by four orders of magnitude, it provides a multitude of targets: Approximately 50% of all existing antibiotics inhibit ribosome function, and antibiotics interfering with almost every step of translation are clinically available (175, 176). The past decade has seen the advent of high-resolution bacterial ribosomal structures bound with antibiotics, which led to invaluable insights into the mode of antibiotic interactions and exact mechanisms of action. High-resolution structures of human ribosomes bound to various antibiotics (166) are paving the way toward a new era of ribosomal inhibitors which specifically interact with defective human ribosomes. Recent breakthroughs in single-particle molecular imaging, for example, in cryo-EM (177) and femtosecond high-energy electron X-ray technologies (178), promise to reveal novel structural features of mutant ribosomes that can be targeted by computer-aided “designer” small molecules, which should allow development of new drugs that bind only “onco-ribosomes.” Such drugs can be exploited to specifically eliminate cells with acquired ribo-

some defects and would minimize unwanted side effects by minimizing their ability to target healthy ribosomes. Additionally, identifying such new classes of ribosomal inhibitors could be accomplished by the screening and repurposing of existing prokaryotic-specific antibiotics which could display specificity for the distinct structural differences of cancer-mutant ribosomes.

## CONCLUSIONS AND FUTURE PERSPECTIVES

Translational dysregulation and tumor protein biology have been largely overlooked in cancer biology. This is likely a function of technological necessity: Proteomics technologies have lagged for several decades as compared with other -omics technologies. Although interrogation of nucleic acid sequences and their regulation was amenable to the earliest tools of molecular biology, and genomes and transcriptomes of tumors have been catalogued in detail for many years thanks to copy-number and gene expression arrays and next-generation DNA and RNA sequencing, analyzing entire proteomes of cancer cells is not yet within reach of many research labs. The Cancer Genome Atlas (TCGA) contains full transcriptome, exome, and genome data for thousands of tumor samples. In contrast, protein data are often restricted to reverse phase protein array data measuring abundances and phosphorylation of only 200 to 300 proteins and post-translational modifications for which reliable antibodies are available (179). The first full quantitative mass spectrometry-based proteomic and phosphoproteomic descriptions of TCGA cancer datasets are only now emerging (180, 181). We are just beginning to build correlations between tumor transcriptomes and proteomes, enabling the identification and categorization of significant RNA-protein discordances, indicative of extensive translational deregulation. We thus envision that in the coming years increased understanding of the translateome will complement transcriptomic and genomic data, and that such studies may reveal additional tumor samples with extensive translational dysregulation due to yet undiscovered mechanisms.

Many questions and challenges remain. For instance, is there a single cause or multiple causes for the paradoxical transition from hypoproliferation to hyperproliferation phenotypes in congenital ribosomopathies? Why are the hematopoietic lineages so heavily affected by these phenotypes? Models addressing the latter question have recently been proposed. One posits that because some blood lineages lose the ribosomal recycling factor ABCE1 during terminal differentiation, they may be sensitized to ribosomal protein and biogenesis mutations that further imbalance ribosome homeostasis (182). Alternatively, specialized composition of ribosomes in hematopoietic lineages might make these cells more vulnerable (14). Yet another potential explanation proposed for heterozygous defects could be that the balance between expression of the wild-type and mutant protein might differ between tissues (183). Eventually, however, the same cells gain a hyperproliferative phenotype. Therefore, we postulate that RP defects mainly create a selective pressure on cells to compensate for the proliferation defect. This would eventually be achieved through the acquisition of secondary mutations that, in cooperation



**Figure 4.** Model of oncogenesis after initial RP defect. RP defects generally lead to hypoproliferative phenotypes such as anemia presented in ribosomopathies. In this model, the hypoproliferation leads to selective pressure on the cells to acquire secondary mutations. These rescuing mutations could then cause a hyperproliferative phenotype, either singularly or in cooperation with the initial RP defect, leading to clonal expansion of cells with an altered translational profile. This figure is adapted from ref. 95.

with the RP defect, lead to oncogenesis (Fig. 4; refs. 6, 95). It is important to note that not all ribosomopathies are linked to elevated cancer risks. For example, Treacher Collins syndrome is characterized by craniofacial deformities but is not associated with hematologic abnormalities nor an increased incidence of malignancy. It is caused by defects in the ribosome biogenesis factor TCOF1, which is implicated in the transcription and methylation of rRNA (184). Among the multitude of ribosomal variations implicated in cancer, rRNA alterations are currently underrepresented compared with the growing list of ribosomal protein defects. This may reflect (i) a different threshold for the development of malignancy driven by rRNA alteration, (ii) a higher need for cooperating defects, and/or (iii) the fact that no systematic analyses of rRNA modifications in cancer have been performed to date. The possible contribution of changes in rRNA modification in cancers is therefore an important area for future research.

Cancers with somatic RP defects might also go through a hypoproliferative phase. Regarding hematologic cancers, one can imagine the existence of a niche in the bone marrow in which cells with fewer functional ribosomes could survive until they acquire the necessary compensatory/cooperating mutations. Such secondary mutations have not yet been identified in a cancer setting. However, research on the leukemia-associated RPL10-R98S mutation in yeast has provided some insights. RPL10-R98S alters translation fidelity by the ribosome and impairs ribosome biogenesis and proliferation (8, 95). Whereas ribosome production and cell proliferation could be rescued by acquisition of mutations in ribosome biogenesis factors NMD3 and TIF6 (human EIF6), the altered

translational fidelity was not (95), resulting in cells with restored proliferation capacity but altered protein synthesis properties. A mechanism of the cooperative effect of these rescuing mutations lies in their ability to circumvent ribosome production control. Because ribosome biogenesis and protein synthesis are two of the most energy-consuming processes in a growing cell, cells have evolved critical surveillance systems for monitoring proper assembly of the translational machinery: Final proofreading steps during late ribosome assembly have been described for both the pre-40S (185) and pre-60S (186, 187). These translation-like “test-drives” serve as final quality control steps in which major functions of the maturing subunits are tested before they are released into the translationally active ribosome pool. The NMD3 and TIF6 mutations can bypass these quality control checkpoints in RPL10-R98S cells (95). Given the overexpression of ribosome assembly factors in cancer cells, it is likely, though it still remains to be determined, that cancer cells exploit similar mechanisms.

In summary, although the past decade has seen great progress in elucidating the role of translational dysregulation and the ribosome in cancer, the next promises tremendous new breakthroughs that will be translated to the clinical setting.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Acknowledgments

We thank all researchers and clinicians for their contributions to the field and apologize to those whose work we did not describe or cite. Figures 3 and 4 were prepared by Somersault18:24.

## Grant Support

S.O. Sulima is the recipient of an EMBO long-term postdoctoral fellowship and an EHA José Carreras junior research grant. I.J.F. Hofman was the recipient of a fellowship of the Flemish Agency for Innovation through Science and Technology (IWT). The laboratory of K. De Keersmaecker is funded by an ERC starting grant (334946), FWO-Vlaanderen funding (G067015N), and funding from Stichting Tegen Kanker (2016-775 and 2016-801). J.D. Dinman was funded by grants from the Department of Health and Human Services (NIHR01GM117177 and R01HL119439).

Received May 22, 2017; revised July 18, 2017; accepted July 31, 2017; published OnlineFirst September 18, 2017.

## REFERENCES

- Ruggero D, Pandolfi PP. Does the ribosome translate cancer? *Nat Rev Cancer* 2003;3:179–92.
- Fox GE. Origin and evolution of the ribosome. *Cold Spring Harb Perspect Biol* 2010;2:a003483.
- Claude A. The constitution of protoplasm. *Science* 1943;97:451–6.
- Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, et al. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. *Nat Genet* 1999; 21:169–75.
- Liu JM, Ellis SR. Ribosomes and marrow failure: coincidental association or molecular paradigm? *Blood* 2006;107:4583–8.
- De Keersmaecker K, Sulima SO, Dinman JD. Ribosomopathies and the paradox of cellular hypo- to hyperproliferation. *Blood* 2015;125: 1377–82.
- Amsterdam A, Sadler KC, Lai K, Farrington S, Bronson RT, Lees JA, et al. Many ribosomal protein genes are cancer genes in zebrafish. *PLoS Biol* 2004;2:E139.
- De Keersmaecker K, Atak ZK, Li N, Vicente C, Patchett S, Girardi T, et al. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. *Nat Genet* 2013;45:186–90.
- Rao S, Lee SY, Gutierrez A, Perrigou J, Thapa RJ, Tu Z, et al. Inactivation of ribosomal protein L22 promotes transformation by induction of the stemness factor, Lin28B. *Blood* 2012;120:3764–73.
- Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature* 2015;526:525–30.
- Hofman IJF, van Duin M, De Bruyne E, Fancello L, Mulligan G, Geerdens E, et al. RPL5 on 1p22.1 is recurrently deleted in multiple myeloma and its expression is linked to bortezomib response. *Leukemia* 2017;31:1706–14.
- Fancello L, Kampen KR, Hofman IJF, Verbeeck J, De Keersmaecker K. The ribosomal protein gene RPL5 is a haploinsufficient tumor suppressor in multiple cancer types. *Oncotarget* 2017;8:14462–78.
- Ljungström V, Cortese D, Young E, Pandzic T, Mansouri L, Plevova K, et al. Whole-exome sequencing in relapsing chronic lymphocytic leukemia: clinical impact of recurrent RPS15 mutations. *Blood* 2016; 127:1007–16.
- Dinman JD. Pathways to specialized ribosomes: the Brussels lecture. *J Mol Biol* 2016;428:2186–94.
- Ban N, Beckmann R, Cate JH, Dinman JD, Dragon F, Ellis SR, et al. A new system for naming ribosomal proteins. *Curr Opin Struct Biol* 2014;24:165–9.
- Zaher HS, Green R. Fidelity at the molecular level: lessons from protein synthesis. *Cell* 2009;136:746–62.
- Khatter H, Myasnikov AG, Natchiar SK, Klaholz BP. Structure of the human 80S ribosome. *Nature* 2015;520:640–5.
- Schultze H, Nierhaus KH, Schulze H. Minimal set of ribosomal components for reconstitution of the peptidyltransferase activity. *EMBO J* 1982;5:609–13.
- Melnikov S, Ben-Shem A, Garreau de Loubresse N, Jenner L, Yusupova G, Yusupov M. One core, two shells: bacterial and eukaryotic ribosomes. *Nat Struct Mol Biol* 2012;19:560–7.
- Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 2009;136: 731–45.
- Ruggero D. Translational control in cancer etiology. *Cold Spring Harb Perspect Biol* 2013;5:a012336.
- Truitt ML, Ruggero D. New frontiers in translational control of the cancer genome. *Nat Rev Cancer* 2016;16:288–304.
- Miluzio A, Beugnet A, Grosso S, Brina D, Mancino M, Campaner S, et al. Impairment of cytoplasmic eIF6 activity restricts lymphomagenesis and tumor progression without affecting normal growth. *Cancer Cell* 2011;19:765–75.
- Sendoel A, Dunn JG, Rodriguez EH, Naik S, Gomez NC, Hurwitz B, et al. Translation from unconventional 5' start sites drives tumour initiation. *Nature* 2017;541:494–9.
- Walters B, Thompson SR. Cap-independent translational control of carcinogenesis. *Front Oncol* 2016;6:128.
- Morfoisse F, Renaud E, Hantelys F, Prats A-C, Garmy-Susini B. Role of hypoxia and vascular endothelial growth factors in lymphangiogenesis. *Mol Cell Oncol* 2015;2:e1024821.
- Wolfe AL, Singh K, Zhong Y, Drewe P, Rajasekhar VK, Sanghvi VR, et al. RNA G-quadruplexes cause eIF4A-dependent oncogene translation in cancer. *Nature* 2014;513:65–70.
- Meyuhas O, Dreazen A. Chapter 3 ribosomal protein S6 kinase: from TOP mRNAs to cell size. *Prog Mol Biol Transl Sci* 2009;90:109–53.
- Xue S, Tian S, Fujii K, Kladwang W, Das R, Barna M. RNA regulons in Hox 5'UTRs confer ribosome specificity to gene regulation and body plan formation. *Nature* 2015;517:33–8.
- Hsieh AC, Liu Y, Edlind MP, Ingolia NT, Janes MR, Sher A, et al. The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature* 2012;485:55–61.
- Truitt ML, Conn CS, Shi Z, Pang X, Tokuyasu T, Coady AM, et al. Differential requirements for eIF4E dose in normal development and cancer. *Cell* 2015;162:59–71.
- Dinman JD. Mechanisms and implications of programmed translational frameshifting. *Wiley Interdiscip Rev RNA* 2012;3:661–73.
- Advani VM, Dinman JD. Reprogramming the genetic code: the emerging role of ribosomal frameshifting in regulating cellular gene expression. *BioEssays* 2016;38:21–6.
- Belew AT, Hepler NL, Jacobs JL, Dinman JD. PRFdb: a database of computationally predicted eukaryotic programmed -1 ribosomal frameshift signals. *BMC Genomics* 2008;9:339.
- Girardi T, Vereecke S, Sulima SO, Khan U, Fancello L, Briggs JW, et al. The T-cell leukemia associated ribosomal RPL10 R98S mutation enhances JAK-STAT signalling. *Leukemia* 2017; DOI: 10.1038/leu.2017.225. [Epub ahead of print]
- Proud CG. Regulation and roles of elongation factor 2 kinase. *Biochem Soc Trans* 2015;43:328–32.
- Eswarappa SM, Fox PL. Antiangiogenic VEGF-Ax: a new participant in tumor angiogenesis. *Cancer Res* 2015;75:2765–9.
- Li J, Lu X. The emerging roles of 3' untranslated regions in cancer. *Cancer Lett* 2013;337:22–5.
- White EJJ, Matsangos AE, Wilson GM. AUF1 regulation of coding and noncoding RNA. *Wiley Interdiscip Rev RNA* 2017;8:e1393.
- Diederichs S, Bartsch L, Berkmann JC, Fröse K, Heitmann J, Hoppe C, et al. The dark matter of the cancer genome: aberrations in regulatory elements, untranslated regions, splice sites, non-coding RNA and synonymous mutations. *EMBO Mol Med* 2016;8:442–57.
- Zhang S, Lu Z, Unruh AK, Ivan C, Baggerly KA, Calin GA, et al. Clinically relevant microRNAs in ovarian cancer. *Mol Cancer Res* 2015;13:393–401.
- Guz M, Rivero-Müller A, Okoń E, Stenzel-Bembenek A, Polberg K, Slomka M, et al. MicroRNAs-role in lung cancer. *Dis Markers* 2014;2014:218169.
- Bhat M, Robichaud N, Hulea L, Sonenberg N, Pelletier J, Topisirovic I. Targeting the translation machinery in cancer. *Nat Rev Drug Discov* 2015;14:261–78.

44. Lafontaine DLJ, Tollervey D. The function and synthesis of ribosomes. *Nat Rev Mol Cell Biol* 2001;2:514–20.
45. Strunk BS, Karbstein K. Powering through ribosome assembly. *RNA* 2009;15:2083–104.
46. Warner JR, Vilarde J, Sohn JH. Economics of ribosome biosynthesis. *Cold Spring Harb Symp Quant Biol* 2001;66:567–74.
47. Zhai W, Comai L. Repression of RNA polymerase I transcription by the tumor suppressor p53. *Mol Cell Biol* 2000;20:5930–8.
48. Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in dyskeratosis congenita. *Blood* 2009;113:6549–57.
49. Vlachos A, Rosenberg PS, Afsaroglu E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Blood* 2012;119:3815–9.
50. Narla A, Ebert BL. Ribosomopathies: human disorders of ribosome dysfunction. *Blood* 2010;115:3196–205.
51. Farley KI, Baserga SJ. Probing the mechanisms underlying human diseases in making ribosomes. *Biochem Soc Trans* 2016;44:1035–44.
52. Shwachman H, Diamond L, Oski F, Khaw K. The syndrome of pancreatic insufficiency and bone marrow dysfunction. *J Pediatr* 1964;65:645–63.
53. Burroughs L, Woolfrey A, Shimamura A. Shwachman-Diamond syndrome: a review of the clinical presentation, molecular pathogenesis, diagnosis, and treatment. *Hematol Oncol Clin North Am* 2009;23:233–48.
54. Boocock GRB, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. *Nat Genet* 2002;33:97–101.
55. Austin KM, Leary RJ, Shimamura A. The Shwachman-Diamond SBDS protein localizes to the nucleolus. *Blood* 2005;106:1253–8.
56. Ganapathi KA, Austin KM, Lee CS, Dias A, Malsch MM, Reed R, et al. The human Shwachman-Diamond syndrome protein, SBDS, associates with ribosomal RNA. *Blood* 2007;110:1458–65.
57. Ball HL, Zhang B, Riches JJ, Gandhi R, Li J, Rommens JM, et al. Shwachman-Bodian Diamond syndrome is a multi-functional protein implicated in cellular stress responses. *Hum Mol Genet* 2009;18:3684–95.
58. Finch AJ, Hilcenko C, Basse N, Drynan LF, Goyenechea B, Menne TF, et al. Uncoupling of GTP hydrolysis from eIF6 release on the ribosome causes Shwachman-Diamond syndrome. *Genes Dev* 2011;25:917–29.
59. Weis F, Giudice E, Churcher M, Jin L, Hilcenko C, Wong CC, et al. Mechanism of eIF6 release from the nascent 60S ribosomal subunit. *Nat Struct Mol Biol* 2015;22:914–9.
60. Ma X. Epidemiology of myelodysplastic syndromes. *Am J Med* 2012;125:S2–5.
61. Deschler B, Lübbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer* 2006;107:2099–107.
62. Kirwan M, Dokal I. Dyskeratosis congenita: a genetic disorder of many faces. *Clin Genet* 2008;73:103–12.
63. Savage SA, Alter BP. Dyskeratosis congenita. *Hematol Oncol Clin North Am* 2009;23:215–31.
64. Connor J, Gatherer D, Gray F, Pirrit L, Affara N. Assignment of the gene for dyskeratosis congenita to Xq28. *Hum Genet* 1986;72:348–51.
65. Heiss NS, Knight SW, Vulliamy TJ, Klauk SM, Wiemann S, Mason PJ, et al. X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. *Nat Genet* 1998;19:32–8.
66. Ganot P, Caizergues-Ferrer M, Kiss T. The family of box ACA small nucleolar RNAs is defined by an evolutionarily conserved secondary structure and ubiquitous sequence elements essential for RNA accumulation. *Genes Dev* 1997;11:941–56.
67. Meier UT. The many facets of H/ACA ribonucleoproteins. *Chromosoma* 2005;114:1–14.
68. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* 1999;402:551–5.
69. Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. *N Engl J Med* 2005;352:1413–24.
70. Marrone A, Walne A, Tamary H, Masunari Y, Kirwan M, Beswick R, et al. Telomerase reverse-transcriptase homozygous mutations in autosomal recessive dyskeratosis congenita and Hoyeraal-Hreidarsson syndrome. *Blood* 2007;110:4198–205.
71. Yamaguchi H, Baerlocher GM, Lansdorp PM, Chanock SJ, Nunez O, Sloand E, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. *Blood* 2003;102:916–8.
72. Alter BP, Baerlocher GM, Savage SA, Chanock SJ, Weksler BB, Willner JP, et al. Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenita. *Blood* 2007;110:1439–47.
73. Boultonwood J, Pellagatti A, McKenzie ANJ, Wainscoat JS. Advances in the 5q- syndrome. *Blood* 2010;116:5803–11.
74. Kawankar N, Rao Vundinti B. Cytogenetic abnormalities in myelodysplastic syndrome: an overview. *Hematology* 2011;16:131–8.
75. Bernasconi P, Klersy C, Boni M, Cavigliano PM, Calatroni S, Giardini I, et al. Incidence and prognostic significance of karyotype abnormalities in de novo primary myelodysplastic syndromes: a study on 331 patients from a single institution. *Leukemia* 2005;19:1424–31.
76. Boultonwood J, Fidler C, Strickson AJ, Watkins F, Gama S, Kearney L, et al. Narrowing and genomic annotation of the commonly deleted region of the 5q- syndrome. *Blood* 2002;99:4638–41.
77. Schneider RK, Ademà V, Heckl D, Järås M, Mallo M, Lord AM, et al. Role of casein kinase 1A1 in the biology and targeted therapy of del(5q) MDS. *Cancer Cell* 2014;26:509–20.
78. Diamond L, Blackfan K. Hypoplastic anemia. *Am J Dis Child* 1938;56:464–7.
79. Lipton JM, Ellis SR. Diamond-Blackfan anemia: diagnosis, treatment, and molecular pathogenesis. *Hematol Oncol Clin North Am* 2009;23:261–82.
80. Quarello P, Garelli E, Brusco A, Carando A, Pappi P, Barberis M, et al. Multiplex ligation-dependent probe amplification enhances molecular diagnosis of Diamond-Blackfan anemia due to RPS19 deficiency. *Haematologica* 2008;93:1748–50.
81. Farrar JE, Nater M, Caywood E, McDevitt MA, Kowalski J, Takemoto CM, et al. Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia. *Blood* 2008;112:1582–92.
82. Cmejla R, Cmejlova J, Handrkova H, Petrak J, Pospisilova D. Ribosomal protein S17 gene (RPS17) is mutated in Diamond-Blackfan anemia. *Hum Mutat* 2007;28:1178–82.
83. Gazda HT, Preti M, Sheen MR, O'Donohue M-F, Vlachos A, Davies SM, et al. Frameshift mutation in p53 regulator RPL26 is associated with multiple physical abnormalities and a specific pre-ribosomal RNA processing defect in Diamond-Blackfan anemia. *Hum Mutat* 2012;33:1037–44.
84. Gazda HT, Sheen MR, Vlachos A, Choessel V, O'Donohue M-F, Schneider H, et al. Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. *Am J Hum Genet* 2008;83:769–80.
85. Doherty L, Sheen MR, Vlachos A, Choessel V, O'Donohue M-F, Clinton C, et al. Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. *Am J Hum Genet* 2010;86:222–8.
86. Gripp KW, Curry C, Olney AH, Sandoval C, Fisher J, Chong JX-L, et al. Diamond-Blackfan anemia with mandibulofacial dysostosis is heterogeneous, including the novel DBA genes TSR2 and RPS28. *Am J Med Genet A* 2014;164A:2240–9.
87. Mirabello L, Macari ER, Jessop L, Ellis SR, Myers T, Giri N, et al. Whole-exome sequencing and functional studies identify RPS29 as a novel gene mutated in multicase Diamond-Blackfan anemia families. *Blood* 2014;124:24–32.
88. Landowski M, O'Donohue M-F, Buros C, Ghazvinian R, Montel-Lehry N, Vlachos A, et al. Novel deletion of RPL15 identified by array-comparative genomic hybridization in Diamond-Blackfan anemia. *Hum Genet* 2013;132:1265–74.



89. Farrar JE, Quarello P, Fisher R, O'Brien KA, Aspesi A, Parrella S, et al. Exploiting pre-rRNA processing in Diamond-Blackfan anemia gene discovery and diagnosis. *Am J Hematol* 2014;89:985-91.
90. Gazda HT, Grabowska A, Merida-Long LB, Latawiec E, Schneider HE, Lipton JM, et al. Ribosomal protein S24 gene is mutated in Diamond-Blackfan anemia. *Am J Hum Genet* 2006;79:1110-8.
91. Sankaran VG, Ghazvinian R, Do R, Thiru P, Vergilio J-A, Beggs AH, et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. *J Clin Invest* 2012;122:2439-43.
92. Bibikova E, Youn M-Y, Danilova N, Ono-Uruga Y, Konto-Ghiorgi Y, Ochoa R, et al. TNF-mediated inflammation represses GATA1 and activates p38 MAP kinase in RPS19-deficient hematopoietic progenitors. *Blood* 2014;124:3791-8.
93. Ludwig LS, Gazda HT, Eng JC, Eichhorn SW, Thiru P, Ghazvinian R, et al. Altered translation of GATA1 in Diamond-Blackfan anemia. *Nat Med* 2014;20:748-53.
94. Dameshek W. Riddle: what do aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH) and "hypoplastic" leukemia have in common? *Blood* 1967;30:251-4.
95. Sulima SO, Patchett S, Advani VM, De Keersmaecker K, Johnson AW, Dinman JD. Bypass of the pre-60S ribosomal quality control as a pathway to oncogenesis. *Proc Natl Acad Sci* 2014;111:5640-5.
96. Hofman IJ, Patchett S, van Duin M, Geerdens E, Verbeeck J, Michaux L, et al. Low frequency mutations in ribosomal proteins RPL10 and RPL5 in multiple myeloma. *Haematologica* 2017;102:e317-20.
97. Tzoneva G, Perez-Garcia A, Carpenter Z, Khiabani H, Tosello V, Allegratta M, et al. Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. *Nat Med* 2013;19:368-71.
98. Morgado-Palacin L, Varetti G, Llanos S, Gómez-López G, Martínez D, Serrano M. Partial Loss of Rpl11 in adult mice recapitulates Diamond-Blackfan anemia and promotes lymphomagenesis. *Cell Rep* 2015;13:712-22.
99. Ferreira A, Tuominen I, van Dijk-Bos K, Sanjabi B, van der Sluis T, van der See A, et al. High frequency of RPL22 mutations in microsatellite-unstable colorectal and endometrial tumors. *Hum Mutat* 2014;35:1442-5.
100. Nagarajan N, Bertrand D, Hillmer AM, Zang ZJ, Yao F, Jacques P-É, et al. Whole-genome reconstruction and mutational signatures in gastric cancer. *Genome Biol* 2012;13:R115.
101. Novetsky AP, Zigelboim I, Thompson DM, Powell MA, Mutch DG, Goodfellow PJ. Frequent mutations in the RPL22 gene and its clinical and functional implications. *Gynecol Oncol* 2013;128:470-4.
102. Rao S, Cai KQ, Stadanlick JE, Greenberg-Kushnir N, Solanki-Patel N, Lee S-Y, et al. Ribosomal protein Rpl22 controls the dissemination of T-cell lymphoma. *Cancer Res* 2016;76:3387-96.
103. Dutton-Regester K, Gartner JJ, Emmanuel R, Qutob N, Davies MA, Gershenwald JE, et al. A highly recurrent RPS27 5'UTR mutation in melanoma. *Oncotarget* 2014;5:2912-7.
104. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. *Science* 2013;339:1546-58.
105. Zack TI, Schumacher SE, Carter SL, Cherniack AD, Saksena G, Tabak B, et al. Pan-cancer patterns of somatic copy number alteration. *Nat Genet* 2013;45:1134-40.
106. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* 2012;481:157-63.
107. Van Vlierberghe P, Ambesi-Impombato A, De Keersmaecker K, Hadler M, Paietta E, Tallman MS, et al. Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia. *Blood* 2013;122:74-82.
108. Girardi T, Vicente C, Cools J, De Keersmaecker K. The genetics and molecular biology of T-ALL. *Blood* 2017;129:1113-23.
109. Xue S, Barna M. Specialized ribosomes: a new frontier in gene regulation and organismal biology. *Nat Rev Mol Cell Biol* 2012;13:355-69.
110. Kondrashov N, Pusic A, Stumpf CR, Shimizu K, Hsieh AC, Xue S, et al. Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell* 2011;145:383-97.
111. Lopes AM, Miguel RN, Sargent CA, Ellis PJ, Amorim A, Affara NA. The human RPS4 paralogue on Yq11.223 encodes a structurally conserved ribosomal protein and is preferentially expressed during spermatogenesis. *BMC Mol Biol* 2010;11:33.
112. Simsek D, Tiu GC, Flynn RA, Byeon GW, Leppik K, Xu AF, et al. The mammalian ribo-interactome reveals ribosome functional diversity and heterogeneity. *Cell* 2017;169:1051-1065.
113. Shi Z, Fujii K, Kovary KM, Genuth NR, Röst HL, Teruel MN, et al. Heterogeneous ribosomes preferentially translate distinct subpools of mRNAs genome-wide. *Mol Cell* 2017;67:71-83.
114. Guimaraes JC, Zavolan M. Patterns of ribosomal protein expression specify normal and malignant human cells. *Genome Biol* 2016;17:236.
115. Briggs JW, Dinman JD. Subtractional heterogeneity: a crucial step toward defining specialized ribosomes. *Mol Cell* 2017;67:3-4.
116. Sharma S, Lafontaine DLJ. "View From A Bridge": a new perspective on eukaryotic rRNA base modification. *Trends Biochem Sci* 2015;40:560-75.
117. Penzo M, Galbiati A, Treré D, Montanaro L. The importance of being (slightly) modified: The role of rRNA editing on gene expression control and its connections with cancer. *Biochim Biophys Acta-Rev Cancer* 2016;1866:330-8.
118. Schosserer M, Minois N, Angerer TB, Amring M, Dellago H, Harreither E, et al. Methylation of ribosomal RNA by NSUN5 is a conserved mechanism modulating organismal lifespan. *Nat Commun* 2015;6:6158.
119. Yang J, Sharma S, Watzinger P, Hartmann JD, Kötter P, Entian K-D. Mapping of complete set of ribose and base modifications of yeast rRNA by RP-HPLC and mung bean nuclease assay. *PLoS One* 2016;11:e0168873.
120. Birkedal U, Christensen-Dalsgaard M, Krogh N, Sabarinathan R, Gorodkin J, Nielsen H. Profiling of ribose methylations in RNA by high-throughput sequencing. *Angew Chem Int Ed Engl* 2015;54:451-5.
121. Piekna-Przybylska D, Przybylski P, Baudin-Baillieu A, Rousset JP, Fournier MJ. Ribosome performance is enhanced by a rich cluster of pseudouridines in the A-site finger region of the large subunit. *J Biol Chem* 2008;283:26026-36.
122. King TH, Liu B, McCully RR, Fournier MJ. Ribosome structure and activity are altered in cells lacking snoRNPs that form pseudouridines in the peptidyl transferase center. *Mol Cell* 2003;11:425-35.
123. Mereau A, Fournier R, Gregoire A, Mougou A, Fabrizio P, Luhrmann R, et al. An in vivo and in vitro structure-function analysis of the Saccharomyces cerevisiae U3A snoRNP: protein-RNA contacts and base-pair interaction with the pre-ribosomal RNA. *J Mol Biol* 1997;273:552-71.
124. Liang XH, Liu Q, Fournier MJ. Loss of rRNA modifications in the decoding center of the ribosome impairs translation and strongly delays pre-rRNA processing. *RNA* 2009;15:1716-28.
125. Baxter-Roshek JL, Petrov AN, Dinman JD. Optimization of ribosome structure and function by rRNA base modification. *PLoS One* 2007;2:e174.
126. Jack K, Bellodi C, Landry DM, Niederer RO, Meskauskas A, Musalgaonkar S, et al. rRNA Pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells. *Mol Cell* 2011;44:660-6.
127. Lafontaine DLJ. Noncoding RNAs in eukaryotic ribosome biogenesis and function. *Nat Struct Mol Biol* 2015;22:11-9.
128. Zhang Y, Ear J, Yang Z, Morimoto K, Zhang B, Lin S. Defects of protein production in erythroid cells revealed in a zebrafish Diamond-Blackfan anemia model for mutation in RPS19. *Cell Death Dis* 2014;5:e1352.
129. MacInnes AW, Amsterdam A, Whittaker CA, Hopkins N, Lees JA. Loss of p53 synthesis in zebrafish tumors with ribosomal protein gene mutations. *Proc Natl Acad Sci U S A* 2008;105:10408-13.
130. In K, Zaini MA, Müller C, Warren AJ, von Lindern M, Calkhoven CF. Shwachman-Bodian-Diamond syndrome (SBDS) protein deficiency impairs translation re-initiation from C/EBP $\alpha$  and C/EBP $\beta$  mRNAs. *Nucleic Acids Res* 2016;44:4134-46.

131. Marcel V, Ghayad SE, Belin S, Therizols G, Morel A-P, Solano-González E, et al. p53 acts as a safeguard of translational control by regulating fibrillar and rRNA methylation in cancer. *Cancer Cell* 2013;24:318–30.
132. Bellodi C, Kopmar N, Ruggero D. Deregulation of oncogene-induced senescence and p53 translational control in X-linked dyskeratosis congenita. *EMBO J* 2010;29:1865–76.
133. Yoon A, Peng G, Brandenburger Y, Zollo O, Xu W, Rego E, et al. Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. *Science* 2006;312:902–6.
134. Bellodi C, Krasnykh O, Haynes N, Theodoropoulou M, Peng G, Montanaro L, et al. Loss of function of the tumor suppressor DKC1 perturbs p27 translation control and contributes to pituitary tumorigenesis. *Cancer Res* 2010;70:6026–35.
135. Pelava A, Schneider C, Watkins NJ. The importance of ribosome production, and the 5S RNP-MDM2 pathway, in health and disease. *Biochem Soc Trans* 2016;44:1086–90.
136. Chakraborty A, Uechi T, Kenmochi N. Guarding the “translation apparatus”: defective ribosome biogenesis and the p53 signaling pathway. *Wiley Interdiscip Rev RNA* 2011;2:507–22.
137. Golomb L, Volarevic S, Oren M. p53 and ribosome biogenesis stress: The essentials. *FEBS Lett* 2014;588:2571–9.
138. Donati G, Peddigari S, Mercer CA, Thomas G. 5S ribosomal RNA is an essential component of a nascent ribosomal precursor complex that regulates the Hdm2-p53 checkpoint. *Cell Rep* 2013;4:87–98.
139. Sloan KE, Bohnsack MT, Watkins NJ. The 5S RNP couples p53 homeostasis to ribosome biogenesis and nucleolar stress. *Cell Rep* 2013;5:237–47.
140. Bursac S, Brdovcak MC, Donati G, Volarevic S. Activation of the tumor suppressor p53 upon impairment of ribosome biogenesis. *Biochim Biophys Acta - Mol Basis Dis* 2014;1842:817–30.
141. Sun X-X, DeVine T, Challagundla KB, Dai M-S. Interplay between ribosomal protein S27a and MDM2 protein in p53 activation in response to ribosomal stress. *J Biol Chem* 2011;286:22730–41.
142. Kim T-H, Leslie P, Zhang Y. Ribosomal proteins as unrevealed caretakers for cellular stress and genomic instability. *Oncotarget* 2014;5:860–71.
143. He X, Li Y, Dai M-S, Sun X-X, He X, Li Y, et al. Ribosomal protein L4 is a novel regulator of the MDM2-p53 loop. *Oncotarget* 2016;7:16217–26.
144. Ofir-Rosenfeld Y, Boggs K, Michael D, Kastan MB, Oren M. Mdm2 regulates p53 mRNA translation through inhibitory interactions with ribosomal protein L26. *Mol Cell* 2008;32:180–9.
145. Takagi M, Absalon MJ, McLure KG, Kastan MB. Regulation of p53 Translation and induction after DNA damage by ribosomal protein L26 and nucleolin. *Cell* 2005;123:49–63.
146. Kamio T, Gu B, Olson TS, Zhang Y, Mason PJ, Bessler M. Mice with a mutation in the Mdm2 gene that interferes with MDM2/ribosomal protein binding develop a defect in erythropoiesis. *PLoS One* 2016;11:e0152263.
147. Boon K, Caron HN, van Asperen R, Valentijn L, Hermus MC, van Sluis P, et al. N-myc enhances the expression of a large set of genes functioning in ribosome biogenesis and protein synthesis. *EMBO J* 2001;20:1383–93.
148. Menssen A, Hermeking H. Characterization of the c-MYC-regulated transcriptome by SAGE: identification and analysis of c-MYC target genes. *Proc Natl Acad Sci U S A* 2002;99:6274–9.
149. Coller HA, Grandori C, Tamayo P, Colbert T, Lander ES, Eisenman RN, et al. Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. *Proc Natl Acad Sci U S A* 2000;97:3260–5.
150. Dai MS, Sears R, Lu H. Feedback regulation of c-Myc by ribosomal protein L11. *Cell Cycle* 2007;6:2735–41.
151. Dai M-S, Sun X-X, Lu H. Ribosomal protein L11 associates with c-Myc at 5 S rRNA and tRNA genes and regulates their expression. *J Biol Chem* 2010;285:12587–94.
152. Liao J-M, Zhou X, Gatignol A, Lu H. Ribosomal proteins L5 and L11 co-operatively inactivate c-Myc via RNA-induced silencing complex. *Oncogene* 2014;33:4916–23.
153. Challagundla KB, Sun X-X, Zhang X, DeVine T, Zhang Q, Sears RC, et al. Ribosomal protein L11 recruits miR-24/miRISC to repress c-Myc expression in response to ribosomal stress. *Mol Cell Biol* 2011;31:4007–21.
154. Zhou X, Hao Q, Liao J-M, Liao P, Lu H. Ribosomal protein S14 negatively regulates c-Myc activity. *J Biol Chem* 2013;288:21793–801.
155. Barna M, Pusic A, Zollo O, Costa M, Kondrashov N, Rego E, et al. Suppression of Myc oncogenic activity by ribosomal protein haploinsufficiency. *Nature* 2008;456:971–5.
156. Wilson-Edell KA, Kehasse A, Scott GK, Yau C, Rothschild DE, Schilling B, et al. RPL24: a potential therapeutic target whose depletion or acetylation inhibits polysome assembly and cancer cell growth. *Oncotarget* 2014;5:165–76.
157. Scheiman J, Jamieson K V, Ziello J, Tseng J-C, Meruelo D. Extraribosomal functions associated with the C terminus of the 37/67 kDa laminin receptor are required for maintaining cell viability. *Cell Death Dis* 2010;1:e42.
158. Venticinque L, Jamieson K V, Meruelo D, Meruelo D, Hada A. Interactions between laminin receptor and the cytoskeleton during translation and cell motility. *PLoS One* 2011;6:e15895.
159. Bezzeri V, Vella A, Calcaterra E, Finotti A, Gasparello J, Gambari R, et al. New insights into the Shwachman-Diamond Syndrome-related haematological disorder: hyper-activation of mTOR and STAT3 in leukocytes. *Sci Rep* 2016;6:33165.
160. Heijnen HF, van Wijk R, Pereboom TC, Goos YJ, Seinen CW, van Oirschot BA, et al. Ribosomal protein mutations induce autophagy through S6 kinase inhibition of the insulin pathway. *PLoS Genet* 2014;10:e1004371.
161. Siddiqui N, Sonenberg N. Signalling to eIF4E in cancer. *Biochem Soc Trans* 2015;43:763–72.
162. Pelletier J, Graff J, Ruggero D, Sonenberg N. Targeting the eIF4F translation initiation complex: a critical nexus for cancer development. *Cancer Res* 2015;75:250–63.
163. Alvandi F, Kwitkowski VE, Ko CW, Rothmann MD, Ricci S, Saber H, et al. U.S. Food and Drug Administration approval summary: omacetaxine mepesuccinate as treatment for chronic myeloid leukemia. *Oncologist* 2014;19:94–9.
164. Jin J, Wang JX, Chen FF, Wu DP, Hu J, Zhou J-F, et al. Homoharringtonine-based induction regimens for patients with de-novo acute myeloid leukaemia: a multicentre, open-label, randomised, controlled phase 3 trial. *Lancet Oncol* 2013;14:599–608.
165. Lam SSY, Ho ESK, He BL, Wong WW, Cher CY, Ng NKL, et al. Homoharringtonine (omacetaxine mepesuccinate) as an adjunct for FLT3-ITD acute myeloid leukemia. *Sci Transl Med* 2016;8:359ra129.
166. Myasnikov AG, Kundhavi Natchiar S, Nebout M, Hazemann I, Imbert V, Khatter H, et al. Structure–function insights reveal the human ribosome as a cancer target for antibiotics. *Nat Commun* 2016;7:12856.
167. Payne EM, Virgilio M, Narla A, Sun H, Levine M, Paw BH, et al. L-leucine improves the anemia and developmental defects associated with Diamond–Blackfan anemia and del(5q) MDS by activating the mTOR pathway. *Blood* 2012;120:2214–24.
168. Jaako P, Debnath S, Olsson K, Bryder D, Flygare J, Karlsson S. Dietary L-leucine improves the anemia in a mouse model for Diamond–Blackfan anemia. *Blood* 2012;120:2225–8.
169. Narla A, Payne EM, Abayasekara N, Hurst SN, Raiser DM, Look AT, et al. L-Leucine improves the anaemia in models of Diamond Blackfan anaemia and the 5q- syndrome in a TP53-independent way. *Br J Haematol* 2014;167:524–8.
170. Takacs JE, Neary TB, Ingolia NT, Saini AK, Martin-Marcos P, Pelletier J, et al. Identification of compounds that decrease the fidelity of start codon recognition by the eukaryotic translational machinery. *RNA* 2011;17:439–52.
171. Siddiqui N, Sonenberg N. Proposing a mechanism of action for ataluren. *Proc Natl Acad Sci U S A* 2016;113:12353–5.
172. Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sermet-Gaudelus I, Wilschanski M, et al. Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Respir Med* 2014;2:539–47.

173. Finkel RS, Flanigan KM, Wong B, Bönnemann C, Sampson J, Sweeney HL, et al. Phase 2a study of ataluren-mediated dystrophin production in patients with nonsense mutation duchenne muscular dystrophy. *PLoS One* 2013;8:e81302.
174. Eswarappa SM, Potdar AA, Koch WJ, Fan Y, Vasu K, Lindner D, et al. Programmed translational readthrough generates antiangiogenic VEGF-Ax. *Cell* 2014;157:1605–18.
175. Sohmen D, Harms JM, Schlünzen F, Wilson DN. SnapShot: Antibiotic inhibition of protein synthesis I. *Cell* 2009;138:1248–1248.
176. Sohmen D, Harms JM, Schlünzen F, Wilson DN. Enhanced SnapShot: Antibiotic inhibition of protein synthesis II. *Cell* 2009;139:212.
177. Elmlund D, Le SN, Elmlund H. High-resolution cryo-EM: the nuts and bolts. *Curr Opin Struct Biol* 2017;46:1–6.
178. Martin-Garcia JM, Conrad CE, Coe J, Roy-Chowdhury S, Fromme P. Serial femtosecond crystallography: a revolution in structural biology. *Arch Biochem Biophys* 2016;602:32–47.
179. Akbani R, Ng PKS, Werner HMJ, Shahmoradgoli M, Zhang F, Ju Z, et al. A pan-cancer proteomic perspective on The Cancer Genome Atlas. *Nat Commun* 2014;5:4.
180. Mertins P, Mani DR, Ruggles KV, Gillette MA, Clauser KR, Wang P, et al. Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature* 2016;534:55–62.
181. Zhang B, Wang J, Wang X, Zhu J, Liu Q, Shi Z, et al. Proteogenomic characterization of human colon and rectal cancer. *Nature* 2014;513:382–7.
182. Mills EW, Wangen J, Green R, Ingolia NT. Dynamic regulation of a ribosome rescue pathway in erythroid cells and platelets. *Cell Rep* 2016;17:1–10.
183. Paoletti NA, Attwood M, Sondalle SB, Vieira CM, van Adrichem AM, di Summa FM, et al. A ribosomopathy reveals decoding defective ribosomes driving human dysmorphism. *Am J Hum Genet* 2017;100:506–22.
184. Ciccia A, Huang J-W, Izhar L, Sowa ME, Harper JW, Elledge SJ. Treacher Collins syndrome TCOF1 protein cooperates with NBS1 in the DNA damage response. *Proc Natl Acad Sci U S A* 2014;111:18631–6.
185. Strunk BS, Novak MN, Young CL, Karbstein K. A translation-like cycle is a quality control checkpoint for maturing 40S ribosome subunits. *Cell* 2012;150:111–21.
186. Bussiere C, Hashem Y, Arora S, Frank J, Johnson AW. Integrity of the P-site is probed during maturation of the 60S ribosomal subunit. *J Cell Biol* 2012;197:747–59.
187. Sulima SO, Gülay SP, Anjos M, Patchett S, Meskauskas A, Johnson AW, et al. Eukaryotic rplL10 drives ribosomal rotation. *Nucleic Acids Res* 2014;42:2049–63.
188. Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. *Nature* 2008;451:335–9.
189. Ear J, Hsueh J, Nguyen M, Zhang Q, Sung V, Chopra R, et al. A zebrafish model of 5q-syndrome using CRISPR/Cas9 targeting RPS14 reveals a p53-independent and p53-dependent mechanism of erythroid failure. *J Genet Genomics* 2016;43:307–18.
190. Danilova N, Sakamoto KM, Lin S. Ribosomal protein S19 deficiency in zebrafish leads to developmental abnormalities and defective erythropoiesis through activation of p53 protein family. *Blood* 2008;112:5228–37.
191. Uechi T, Nakajima Y, Chakraborty A, Torihara H, Higa S, Kenmochi N. Deficiency of ribosomal protein S19 during early embryogenesis leads to reduction of erythrocytes in a zebrafish model of Diamond-Blackfan anemia. *Hum Mol Genet* 2008;17:3204–11.
192. Mattsson H, Davey EJ, Draptchinskaia N, Hamaguchi I, Ooka A, Levee P, et al. Targeted disruption of the ribosomal protein S19 gene is lethal prior to implantation. *Mol Cell Biol* 2004;24:4032–7.
193. Devlin EE, DaCosta L, Mohandas N, Elliott G, Bodine DM. A transgenic mouse model demonstrates a dominant negative effect of a point mutation in the RPS19 gene associated with Diamond-Blackfan anemia. *Blood* 2010;116:2826–35.
194. Jaako P, Flygare J, Olsson K, Quere R, Ehinger M, Henson A, et al. Mice with ribosomal protein S19 deficiency develop bone marrow failure and symptoms like patients with Diamond-Blackfan anemia. *Blood* 2011;118:6087–96.
195. McGowan KA, Li JZ, Park CY, Beaudry V, Tabor HK, Sabnis AJ, et al. Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. *Nat Genet* 2008;40:963–70.
196. Kazerounian S, Ciarlini PDSC, Yuan D, Ghazvinian R, Alberich-Jorda M, Joshi M, et al. Development of soft tissue sarcomas in ribosomal proteins L5 and S24 heterozygous mice. *J Cancer* 2016;7:32–6.
197. Brooks SS, Wall AL, Golzio C, Reid DW, Kondyles A, Willer JR, et al. A novel ribosomopathy caused by dysfunction of RPL10 disrupts neurodevelopment and causes X-linked microcephaly in humans. *Genetics* 2014;198:723–33.
198. Ear J, Huang H, Wilson T, Tehrani Z, Lindgren A, Sung V, et al. RAP-011 improves erythropoiesis in zebrafish model of Diamond-Blackfan anemia through antagonizing lefty1. *Blood* 2015;126:880–90.
199. Toki T, Ito E. Molecular mechanisms underlying the pathology of Diamond-Blackfan anemia. *Rinsho Ketsueki* 2015;56:867–76.
200. Zhang Y, Duc A-CE, Rao S, Sun X-L, Bilbee AN, Rhodes M, et al. Control of hematopoietic stem cell emergence by antagonistic functions of ribosomal protein paralogs. *Dev Cell* 2013;24:411–25.
201. Danilova N, Sakamoto KM, Lin S. Ribosomal protein L11 mutation in zebrafish leads to haematopoietic and metabolic defects. *Br J Haematol* 2011;152:217–28.
202. Wan Y, Zhang Q, Zhang Z, Song B, Wang X, Zhang Y, et al. Transcriptome analysis reveals a ribosome constituents disorder involved in the RPL5 downregulated zebrafish model of Diamond-Blackfan anemia. *BMC Med Genomics* 2016;9:13.
203. Taylor AM, Humphries JM, White RM, Murphey RD, Burns CE, Zon LI. Hematopoietic defects in rps29 mutant zebrafish depend upon p53 activation. *Exp Hematol* 2012;40:228–37.
204. Hockemeyer D, Palm W, Wang RC, Couto SS, de Lange T. Engineered telomere degradation models dyskeratosis congenita. *Genes Dev* 2008;22:1773–85.
205. Gu B-W, Bessler M, Mason PJ. A pathogenic dyskerin mutation impairs proliferation and activates a DNA damage response independent of telomere length in mice. *Proc Natl Acad Sci U S A* 2008;105:10173–8.
206. Ruggero D, Grisendi S, Piazza F, Rego E, Mari F, Rao PH, et al. Dyskeratosis congenita and cancer in mice deficient in ribosomal RNA modification. *Science* 2003;299:259–62.
207. Provost E, Wehner KA, Zhong X, Ashar F, Nguyen E, Green R, et al. Ribosomal biogenesis genes play an essential and p53-independent role in zebrafish pancreas development. *Development* 2012;139:3232–41.
208. Oyarbide U, Kell MJ, Farinas J, Topczewski J, Corey S. Gene disruption of zebrafish Sbds phenocopies human shwachman-diamond syndrome but suggests more global and lineage defects. *Blood* 2016;128:226.
209. Zambetti NA, Bindels EMJ, Van Strien PMH, Valkhof MG, Adisty MN, Hoogenboezem RM, et al. Deficiency of the ribosome biogenesis gene Sbds in hematopoietic stem and progenitor cells causes neutropenia in mice by attenuating lineage progression in myelocytes. *Haematologica* 2015;100:1285–93.
210. Zhang S, Shi M, Hui C-C, Rommens JM. Loss of the mouse ortholog of the shwachman-diamond syndrome gene (Sbds) results in early embryonic lethality. *Mol Cell Biol* 2006;26:6656–63.
211. Tourlakis ME, Zhong J, Gandhi R, Zhang S, Chen L, Durie PR, et al. Deficiency of Sbds in the mouse pancreas leads to features of Shwachman-Diamond syndrome, with loss of zymogen granules. *Gastroenterology* 2012;143:481–92.