Rare ribosomopathies: insights into mechanisms of cancer

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Abstract | Long thought to be too big and too ubiquitous to fail, we now know that human cells can fail to make sufficient amounts of ribosomes, causing a number of diseases collectively known as ribosomopathies. The best characterized ribosomopathies, with the exception of Treacher Collins syndrome, are inherited bone marrow failure syndromes, each of which has a marked increase in cancer predisposition relative to the general population. Although rare, emerging data reveal that the inherited bone marrow failure syndromes may be underdiagnosed on the basis of classical symptomology, leaving undiagnosed patients with these syndromes at an elevated risk of cancer without adequate counselling and surveillance. The link between the inherited ribosomopathies and cancer has led to greater awareness that somatic mutations in factors involved in ribosome biogenesis may also be drivers in sporadic cancers. Our goal here is to compare and contrast the pathophysiological mechanisms underpinning ribosomopathies to gain a better understanding of the mechanisms that predispose these disorders to cancer.

Ribosomopathies are generally defined as diseases linked to defects in ribosome synthesis and function. The origins of the term ribosomopathy can be traced back to a commentary in 1998 on the emerging role for a defect in ribosome biogenesis in the pathophysiology of the inherited bone marrow failure syndrome dyskeratosis congenita[1]. The term was expanded to include Diamond–Blackfan anaemia (DBA) in 2008 (REF.[2]), and since then the number of putative ribosomopathies has grown considerably (TABLE 1).

Diseases are typically classified as ribosomopathies if the genes affected encode any of the myriad of factors known to have a role in the synthesis of ribosomes. However, the extent to which defects in ribosome synthesis contribute to clinical phenotypes is not always evident, and, thus, it remains unclear whether classification of all of these disorders as ribosomopathies is appropriate. For example, genes encoding factors involved in different aspects of ribosome biogenesis are the primary targets of pathogenic mutations in DBA, Shwachman–Diamond syndrome (SDS) and Treacher Collins syndrome (TCS). For each of these diseases, multiple genes have been identified that are tied together mechanistically, giving confidence that specific yet distinct defects in ribosome biogenesis play a primary role in disease pathophysiology. By contrast, for diseases such as X-linked dyskeratosis congenita (XL-DC) and cartilage–hair hypoplasia–anauxetic dysplasia (CHH–AD), the products of genes affected have functions in addition to their roles in ribosome synthesis. In these cases, the defects in ribosome biogenesis appear to contribute as disease modifiers, generally increasing disease severity compared with that in patients in whom ribosome biogenesis is minimally affected. Finally, there are a number of diseases in which a single gene linked to ribosome biogenesis has been identified but the degree to which defects in ribosome synthesis contribute to disease pathology is unknown, leaving some degree of uncertainty as to whether they should be classified as ribosomopathies.

Our focus in this Review is on those diseases in which factors involved in ribosome synthesis are the primary target of disease-causing mutations or in which defects in ribosome maturation appear to modify disease presentation and as such are biased towards inherited bone marrow failure syndromes (IBMFSs). Notably, these syndromes all have a predisposition to cancer[3]. Moreover, recent studies have also revealed that mutations in factors involved in ribosome synthesis appear to be drivers of tumorigenesis in sporadic cancers[4]. Thus, the pathophysiological mechanisms underpinning cancer predisposition in the rare congenital disorders will provide insights into mechanisms that contribute to the evolution of more prevalent sporadic cancers.

Ribosome synthesis

The human ribosome is composed of 4 RNAs and 80 ribosomal proteins. RNA polymerases I, II and III are all involved in the synthesis of structural components of the ribosome. RNA polymerase I is needed for the synthesis of a large polycistronic RNA that gives rise to 18S rRNA of the 40S ribosomal subunit and 5.8S and 28S rRNAs

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Table 1 | **Ribosomopathies gene defects and clinical features**

Pathology	Responsible genes[a]	Inheritance	Clinical features	Associated tumours or preneoplastic conditions
Inherited ribosomopathies with ribosome synthesis gene defects as the primary pathogenic mechanism				
Diamond–Blackfan anaemia	*RPS7*, *RPS10*, *RPS15A*, *RPS17*, *RPS19*, *RPS24*, *RPS26*, *RPS27*, *RPS28*, *RPS29*, *RPL5*, *RPL11*, *RPL15*, *RPL18*, *RPL26*, *RPL27*, *RPL31*, *RPL35*, *RPL35A* and *TSR2* (REFS[11,13,110])	Autosomal dominant (X-linked for *TSR2*)	Macrocytic anaemia, growth retardation, short stature and congenital malformations (craniofacial, upper limb, genitourinary and heart)	MDS, AML and solid tumours (for example, osteosarcoma and colon carcinoma)
Shwachman–Diamond syndrome	*SBDS*, *DNAJC21* and *EFL1* (REFS[19,21,22])	Autosomal recessive	Neutropenia, exocrine pancreatic insufficiency and short stature	MDS and AML
Treacher Collins syndrome	*TCOF1*, *POLR1C* and *POLR1D*[107,108]	Autosomal dominant (*TCOF1* and *POLR1D*) and autosomal recessive (*POLR1C*)	Craniofacial abnormalities	NA
Acquired ribosomopathy with the loss of a ribosome synthesis gene contributing to the pathogenic mechanism				
5q$^-$ syndrome	*RPS14* (REF.[18])	Acquired condition	Macrocytic anaemia and hypolobated megakaryocytes	MDS and AML
Ribosomopathies with ribosome synthesis gene defects as disease modifiers				
Dyskeratosis congenita	*DKC1* and *PARN*[24,111]	X-linked (*DKC1*) and autosomal recessive (*PARN*)	Bone marrow failure, skin hyperpigmentation, nail dystrophy, mucosal leukoplakia and pulmonary fibrosis	MDS, AML and head and neck tumours
Cartilage–hair hypoplasia–anauxetic dysplasia	*RMRP*[29]	Autosomal recessive	Dwarfism, anaemia, hair growth abnormalities and immunodeficiency	Non-Hodgkin lymphoma and basal cell carcinoma
Suspected ribosomopathies				
Alopecia and neurological and endocrinopathy syndrome	*RBM28* (REF.[112])	Autosomal recessive	Hair loss, neurological defects and hypogonadism	NA
Aplasia cutis congenita	*BSM1* (REF.[113])	Autosomal dominant	Skin defects, mostly affecting the scalp	NA
Bowen–Conradi syndrome	*EMG1* (REF.[114])	Autosomal recessive	Mental and psychomotor retardation, rockerbottom feet and death in the first months of life	NA
Congenital asplenia	*RPSA*[115]	Autosomal dominant	Absence of spleen	NA
Leukoencephalopathy, brain calcifications and cysts	*SNORD118* (REF.[116])	Autosomal recessive	Central nervous system abnormalities (leukoencephalopathy, intracranial calcifications and cysts)	NA
RPS23-related ribosomopathy	*RPS23* (REF.[117])	Autosomal dominant	Microcephaly, hearing loss and dysmorphic features	NA

AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; NA, not applicable. [a]Only genes encoding factors involved in ribosome synthesis are included in this table.

of the 60S ribosomal subunit. RNA polymerase II produces mRNAs for the 80 ribosomal proteins, including 33 proteins of the 40S subunit and 47 proteins of the 60S subunit. Finally, RNA polymerase III produces 5S rRNA of the 60S ribosomal subunit. In addition to the structural components of the ribosome, hundreds of extraribosomal factors are required for numerous steps in the maturation of ribosomal subunits from nascent RNA polymerase I transcripts in the nucleolus to functionally mature ribosomal subunits in the cytoplasm (FIG. 1).

Ribosome biogenesis begins co-transcriptionally in the nucleolus with a subset of ribosomal proteins and extraribosomal factors assembling on nascent RNA polymerase I transcripts in a hierarchical fashion to form the 90S pre-ribosome[5]. A series of small nucleolar RNA–protein complexes, referred to as C/D and H/ACA box snoRNPs, modify pre-rRNA by 2′-*O*-methylation and pseudouridylation, respectively[6]. Early cleavages of the nascent pre-rRNA transcript within the 90S pre-ribosome release the pre-40S particle from the remaining particle, which matures to the 60S ribosomal subunit. The preassembled 5S ribonucleoprotein complex (5S-RNP), including 5S rRNA and ribosomal proteins RPL5 (also known as uL18, according to the new system of nomenclature for ribosomal proteins[7]) and RPL11 (also known as uL5), is incorporated into the assembling 60S subunit early[8] but undergoes a considerable structural rearrangement as the 60S continues

2′-*O*-methylation
The addition, mostly found in pre-rRNA, of a methyl group at the 2′ position of the ribose by ribonucleoprotein complexes containing C/D box family small nucleolar RNA.

Pseudouridylation
The isomerization of uridine due to a 180° rotation for which the uracil is attached to the ribose via a carbon–carbon instead of a nitrogen–carbon glycosidic bond.

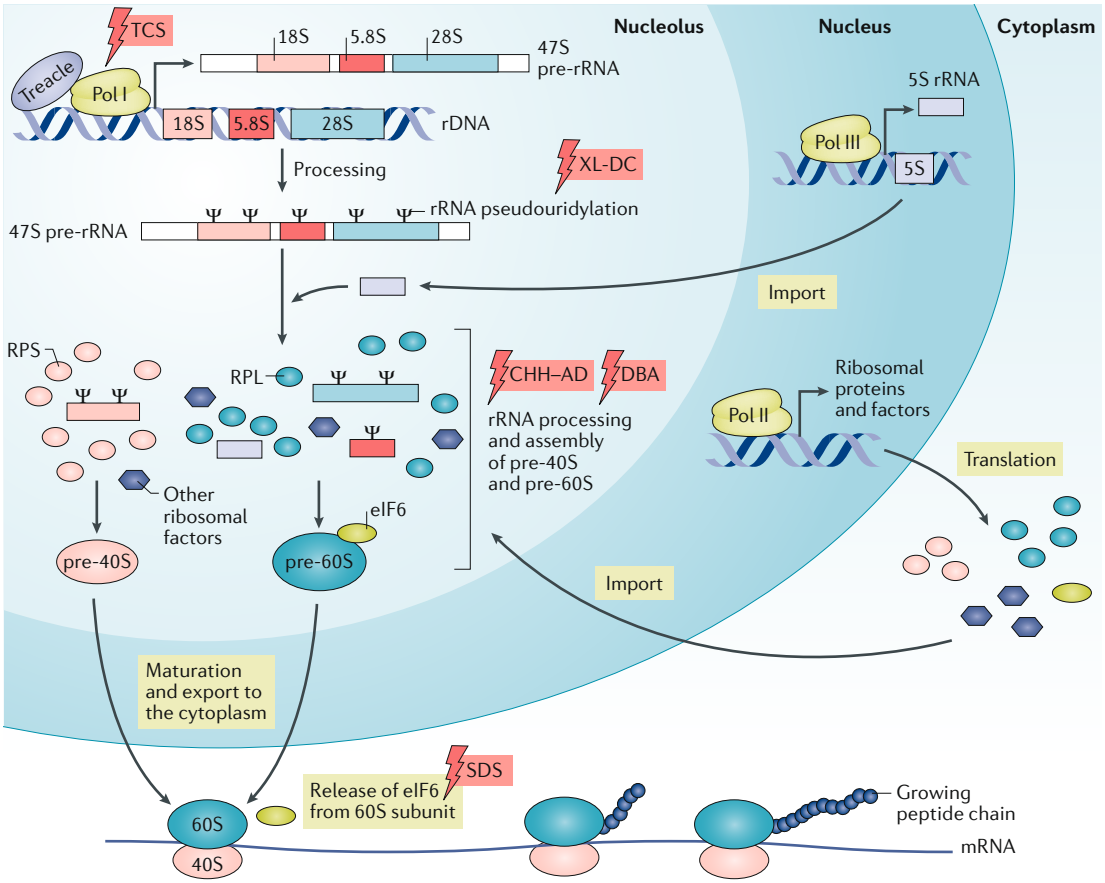

Fig. 1 | **Ribosomopathies affect different steps of ribosome synthesis.** In the nucleolus, RNA polymerase I (Pol I) transcribes the 47S pre-rRNA, which includes the sequences of 18S, 5.8S and 28S rRNAs. Mutations in *POLR1C* and *POLR1D*, encoding two Pol I subunits, and *TCOF1*, encoding the protein treacle, lead to decreased transcription of the 47S pre-rRNA and cause Treacher Collins syndrome (TCS). Mature rRNAs are processed from the 47S precursor after sequential nucleolytic cleavages and chemical modifications, typically pseudouridylation and methylation. The X-linked form of dyskeratosis congenita (XL-DC) is caused by mutations in the *DKC1* gene, encoding dyskerin, a component of a ribonuclear complex required for rRNA pseudouridylation. Thirty-three small subunit ribosomal proteins (RPS) are assembled with the 18S rRNA in the 40S ribosomal subunit, while 47 large subunit ribosomal proteins (RPLs), 5.8S and 28S rRNAs and the 5S rRNA, which is transcribed by RNA polymerase III, are assembled in the 60S ribosomal subunit. In Diamond–Blackfan anaemia (DBA), the deficiency of any one of a number of different ribosomal proteins impairs pre-rRNA processing and ribosomal subunit synthesis. A similar mechanism has been proposed for cartilage–hair hypoplasia–anauxetic dysplasia (CHH–AD), in which the responsible gene, *RMRP*, encodes the non-coding RNA subunit of the RNase MRP complex, which is required for pre-rRNA processing. Numerous non-ribosomal factors contribute to the stepwise assembly and maturation of pre-60S and pre-40S particles, which are eventually exported to the cytoplasm. The final steps of 60S maturation, and in particular the release of eIF6 from the 60S subunit, are impaired in Shwachman–Diamond syndrome (SDS).

its maturation[9]. These additional steps in the maturation of ribosomal subunits occur as nascent particles exit the nucleolus to the nucleoplasm, where they become competent for transport through nuclear pores by the acquisition of additional ribosomal proteins and the gain and loss of extraribosomal factors. Finally, late stages of subunit maturation occur in the cytoplasm, which include quality control steps monitoring the functionality of newly made subunits. The 40S and 60S subunits are then capable of joining together during translational initiation to form the functional 80S ribosome. Genes affected in the ribosomopathies encode factors functioning at various stages in this pathway from the transcription of ribosomal RNAs in the nucleolus to final quality control steps in subunit maturation in the cytoplasm.

Inherited ribosomopathies

Clinical presentation and genetics

Diamond–Blackfan anaemia. DBA is an inherited bone marrow failure syndrome that classically presents in the first year of life as a red cell aplasia[10]. These patients also display a heterogeneous array of congenital anomalies including craniofacial, cardiac, genitourinary, limb and hand malformations.

The vast majority of genes affected in DBA patients encode ribosomal proteins, which are structural components of mature ribosomes (TABLE 1). To date, patients with DBA have been shown to have mutations in one of 19 genes encoding proteins of both the small 40S ribosomal subunit (RPS) and large 60S ribosomal subunit (RPL)[11–13]. Mutations in ribosomal protein genes

causing DBA exhibit autosomal dominant inheritance and lead to haploinsufficiency for their respective ribosomal protein. The respective gene variants have variable penetrance, as unaffected carriers have been observed in certain families[14]. Loss of ribosomal protein function preferentially affects the maturation of the ribosomal subunit containing that protein and ultimately reduces the amount of functional 80S ribosomes within a cell[15–17] (FIG. 1). The relationship between haploinsufficiency for ribosomal protein genes and defects in erythropoiesis was reinforced by the finding that the defective erythropoiesis and macrocytic anaemia observed in 5q$^-$ syndrome, a subtype of myelodysplastic syndrome (MDS), were caused by the loss of the *RPS14* (also known as *uS11*) gene on the long arm of chromosome 5 (REF.[18]).

SDS. SDS is an IBMFS characterized by neutropenia or multilineage cytopenias, exocrine pancreatic dysfunction and metaphyseal chondrodysplasia. Approximately 90% of patients with SDS have biallelic mutations in the *SBDS* gene[19,20]. Recently, two new genes have been implicated in SDS, *EFL1* (REF.[21]) and *DNAJC21* (REF.[22]), both of which cooperate with *SBDS* in late steps in the maturation of 60S ribosomal subunits (FIG. 1).

Dyskeratosis congenita. Dyskeratosis congenita is an IBMFS that is often associated with a number of other clinical findings including abnormal skin pigmentation, nail dystrophy and leukoplakia of oral mucosa[23]. XL-DC is often classified as a ribosomopathy because the affected *DKC1* gene encodes dyskerin, a pseudouridine synthase involved in ribosome biogenesis and function[24–26] (FIG. 1). Dyskerin is a component of H/ACA ribonuclear protein complexes that are required for the modification of not only rRNAs but also a number of other RNAs including spliceosomal RNAs and the RNA component of telomerase.

As additional genes were identified in dyskeratosis congenita, it was soon realized that the primary target for pathogenic mutations were genes encoding components of telomerase (*TERC* and *TERT*) and other factors involved in telomere maintenance (*TINF2*, *POT1, ACD, RTEL1, NAF1, NOP10*, *NHP2, WRAP53, CTC1* and *PARN*)[27]. Sorting out how defects in ribosome synthesis and function contribute to the clinical features of dyskeratosis congenita-related disorders has been challenging[28]. However, it has become increasingly clear that any contributions made by defective ribosome synthesis and function to the clinical features of XL-DC serve as a disease modifier as opposed to the primary pathogenic target (see discussion below under Pathophysiological mechanisms and Translational alterations).

CHH–AD. CHH–AD is a continuum of skeletal dysplasias. These disorders may also include hair hypoplasia, bone marrow failure and immunodeficiency[29]. CHH–AD is caused by biallelic mutations in *RMRP*[30]. *RMRP* encodes the non-coding RNA subunit of the ribonuclease MRP complex required for the maturation of early pre-rRNA species by promoting a cleavage event in ITS1 that separates precursors, giving rise to mature 18S rRNA from downstream precursors that form mature 5.8S and 28S rRNAs[31] (FIG. 1).

Like XL-DC, the gene affected in CHH–AD is multifunctional, with ribosome synthesis being one of several processes disrupted in these patients. In addition to its role in ribosome synthesis, the ribonuclease MRP complex is required for cyclin B2 mRNA cleavage during cell cycle progression[32] and for processing of mitochondrial RNA[33]. Here again, identifying how these different activities contribute to clinical phenotypes and cancer risk has been extremely challenging[34].

Pathophysiological mechanisms

Two non-mutually exclusive mechanisms have been proposed to be involved in the pathophysiology of ribosomopathies. These mechanisms include nucleolar stress and subsequent p53 activation caused by abortive ribosome assembly and downstream alterations in translation caused by a reduction in ribosome number and/or function.

Nucleolar stress and p53 activation. p53 activation in response to defects in ribosome synthesis was first demonstrated in a study in which the term nucleolar stress was coined to describe pathways involved in transmitting signals from abortive ribosome synthesis to p53 activation and subsequent cell cycle arrest or apoptosis[35]. Studies in a zebrafish model of DBA were the first to show that p53 activation could also play a role in the pathophysiology of ribosomopathies[36]. Importantly, these studies also showed that null mutations in *tp53* could rescue associated phenotypes. Similar results have subsequently been obtained in mouse and human cellular models of DBA[37–39].

The emerging role for p53 activation in the pathophysiology of DBA soon converged with studies from investigators in the field of cancer biology who had discovered a role for ribosomal proteins in modulating the activity of MDM2, a critical regulator of p53 levels[40–43]. MDM2 is an E3 ubiquitin ligase that functions in targeting p53 for proteasomal destruction, keeping p53 levels low in unstressed cells. While numerous ribosomal proteins have been shown to bind MDM2 and inhibit its ability to ubiquitylate p53 (REF.[44]), two ribosomal proteins, RPL5 and RPL11, in complex with 5S rRNA appear to be the major factors involved in p53 activation in response to nucleolar stress[45,46] (FIG. 2).

According to the nucleolar stress model, abortive ribosome assembly interferes with the assembly of the 5S-RNP complex into pre-ribosomes, leaving it free to inhibit MDM2, thereby resulting in p53 activation[47] (FIG. 2). Consistent with this model, a study in which each of the 80 ribosomal proteins was depleted in HeLa cells revealed that p53 induction was greatest for a number of large subunit ribosomal proteins that were also shown to be required for nucleolar integrity and that this induction was dependent on RPL5 and RPL11 (REF.[48]). While this model fits well with the idea that the 5S-RNP could accumulate in a free state when the assembly of 60S ribosomal subunits is disrupted, it was less clear how 5S-RNP could accumulate in patients haploinsufficient for 40S ribosomal proteins. This concern was resolved

Myelodysplastic syndrome
A heterogeneous group of clonal disorders characterized by ineffective haematopoiesis and cytopenias that may evolve into acute myeloid leukaemia.

Metaphyseal chondrodysplasia
A defective bone development causing short stature.

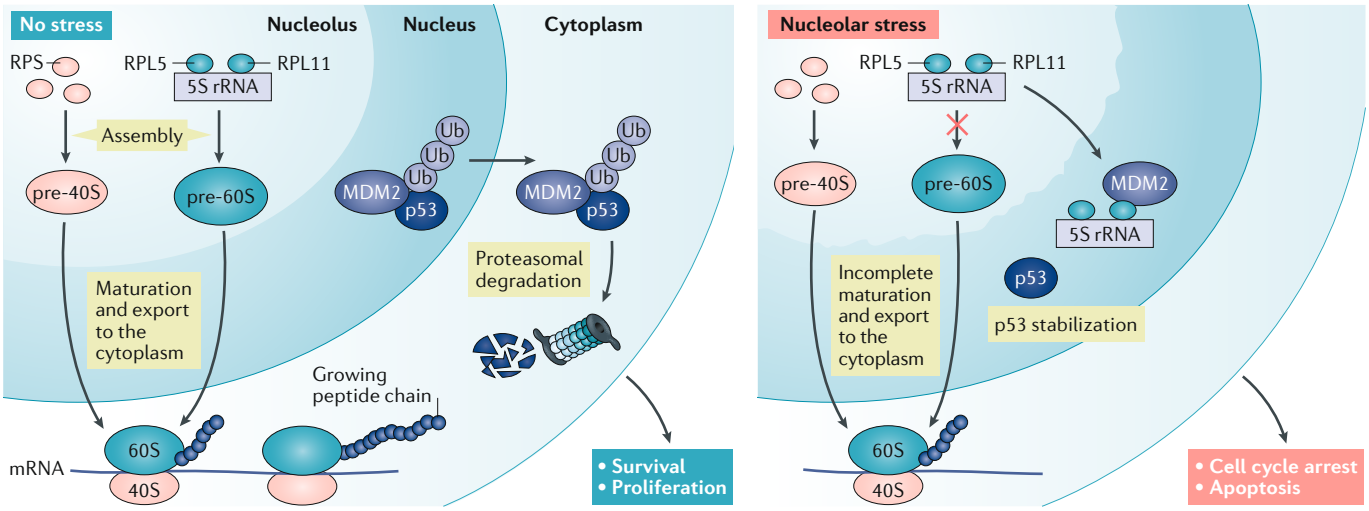

Fig. 2 | **Nucleolar stress response is induced by defective ribosome assembly.** In absence of nucleolar stress, the 5S-RNP that includes RPL5, RPL11 and 5S rRNA is incorporated into 60S ribosomal subunits and as such is unavailable for interactions with MDM2. MDM2, in turn, ubiquitylates p53, resulting in its degradation by the proteasome. If ribosome biogenesis is defective, the 5S-RNP complex that is not assembled into 60S subunit is available for interaction with MDM2. This interaction prevents MDM2 binding to p53, resulting in p53 stabilization, cell cycle arrest and apoptosis.

by the finding that a general upregulation in the translation of ribosomal proteins in response to insufficiency for ribosomal proteins of either ribosomal subunit could create an environment in which components of 5S-RNP could transiently accumulate regardless of which ribosomal subunit was being affected[49]. A role for 5S-RNP in nucleolar stress signalling in SDS seems less likely, as the defect in ribosome biogenesis occurs at a stage at which 5S-RNP has already been incorporated into the pre-60S subunit[8].

A central role for the 5S-RNP in the pathophysiology of DBA is complicated by the fact that both *RPL5* and *RPL11* have been shown to harbour pathogenic mutations in patients with DBA[50]. Loss-of-function mutations in either *RPL5* or *RPL11* interfere with subcomplex signalling and p53 activation, suggesting that alternative mechanisms must give rise to clinical phenotypes in patients with DBA who have mutations in either of these genes[50]. Indeed, mutations in *RPL5* and *RPL11* do not activate cell cycle arrest by p53 activation but instead reduce cell cycle progression by limiting translation[51].

Translational alterations. While ample evidence for a role for p53 in the pathophysiology of DBA and other ribosomopathies exists, there is also considerable evidence that limiting translation through defects in ribosome synthesis also plays a role in disease pathology. The finding that numerous ribosomal proteins of either ribosomal subunit are affected in DBA argues for a general reduction in translational capacity as a factor in the pathophysiology of this disorder as opposed to highly specialized effects on ribosome function linked to a specific ribosomal protein. Indeed, reductions in the total levels of functional ribosomes can selectively affect the translation of certain mRNA populations relative to others, potentially influencing cell fate decisions[15,52] (FIG. 3a). For example, reduced levels of RPS19 (also known as eS19), RPL5 and RPL11 selectively reduce the translation of *GATA1* mRNA, which is relevant for the pathophysiology of DBA[53]. The *GATA1* mRNA has a highly structured 5′ end, which is thought to interfere with efficient translational initiation and to be the reason for its heightened sensitivity to reductions in the levels of functional ribosomes. This observation mechanistically linking ribosomal protein haploinsufficiency with GATA1 deficiency provided the molecular underpinnings for how reduced ribosome numbers influence erythropoiesis by interfering with translation of a major transcription factor involved in this lineage specification[53] (FIG. 3b). Other targets downstream of the translational defect in cells haploinsufficient for ribosomal proteins that may factor into the erythroid tropism of DBA include HSP70 (also known as HSPBP1)[54], BAG1 and/or CSDE1 (REF.[55]) and globin polypeptides[56].

Specific alterations in ribosome function also play a role in the pathophysiology of congenital ribosomopathies, as has been observed in XL-DC. Several studies have shown that a reduction in pseudouridine levels in rRNA caused by mutations in *DKC1* interferes with the translation of internal ribosome entry site (IRES)-containing mRNAs and the accuracy of the translating ribosomes[26,57] (FIG. 3c). Some of these mRNAs encode critical cell cycle regulators, and their dysregulation could promote tumorigenesis and modify phenotypes in XL-DC[58].

Cancer predisposition

Much of the information on the incidence of cancer in ribosomopathies comes from registries for the different IBMFSs[59–62]. Quantification of cancer risk in ribosomopathies is complicated by a number of factors including small samples sizes, bias towards younger patient cohorts and competing risks. The competing risks of bone marrow failures typically include patients who receive bone

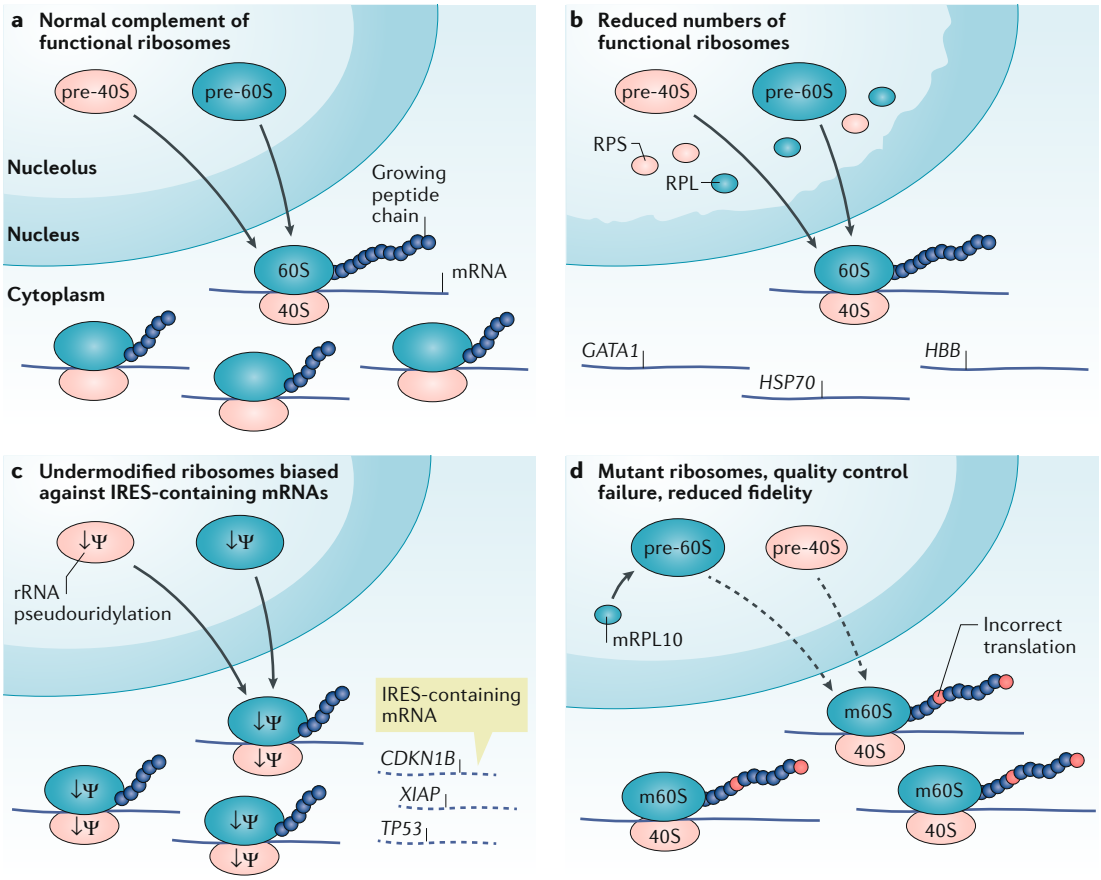

Fig. 3 | **Translational alterations in ribosomopathies. a** | Healthy cells with an adequate number of functional ribosomes necessary for the translation of mRNAs required for appropriate cell function. **b** | Cells lacking a sufficient number of ribosomes to adequately translate mRNAs required for appropriate cell function. When mRNAs compete for a suboptimal number of ribosomes, certain mRNAs fail to adequately compete for translation, disrupting cell fate determination and other cellular functions. mRNAs known to be inefficiently translated in cells from DBA patients are listed. **c** | Cells with compromised pseudouridylation of rRNA produced ribosomes with altered function, including reduced translational fidelity and inefficient translation of certain internal ribosome entry site (IRES)-containing mRNAs. IRES-containing mRNAs known to be affected by suboptimal pseudouridylation are listed. **d** | Cells with mutations in *RPL10* (mRPL10) which affects ribosome biogenesis and function. Failures in quality control mechanisms linked to a network of proteins involved in the pathogenesis of SDS allow dysfunctional 60S subunits with reduced translational fidelity to participate in translation. RPL, large ribosomal subunit; RPS, small ribosomal subunit.

marrow transplants, which in themselves carry an attendant risk of malignancy and death caused by complications of disease treatment or progression[59,60]. As the management of disease complications has improved over the past 20 years and as patients live longer, it is anticipated that the cancer incidence will likely rise for these populations.

Cancer risk in Diamond–Blackfan anaemia

Three studies have addressed the cancer incidence in different cohorts of patients with DBA[59,60,62]. The frequency for all malignancies in these cohorts with DBA ranged from 3% to 5% for patients who had not received a haematopoietic stem cell transplantation. After accounting for factors such as age and sex, the ratio of observed to expected cancers (odds ratio) in patients with DBA relative to the general population ranged from 2.5 to 5.4 for any malignancy in patients who had not received a transplantation. The odds ratios for specific types of cancer were significantly higher at 45 and 42 for colon cancer and cervical cancer, respectively. The overall cumulative incidence of cancer by age 45 years was 13.7%. These values do not include myelodysplastic syndrome (MDS), which was analysed separately as a non-competing risk and had an odds ratio of 352. To date, there have been no significant genotype and phenotype relationships identified between affected genes and cancer incidence in DBA[59,60,62].

DBA is known for its phenotypic variability. Importantly, a recent study evaluating a small cohort of patients with cardiac abnormalities and no history of anaemia revealed that one of these patients had occult DBA without classic haematological findings[63]. This study indicates that syndromic DBA may be underdiagnosed, especially because the types of congenital anomaly observed in DBA are relatively common in the general population. The extent to which these occult patients have a risk of cancer similar to the risk in those presenting with more classical haematological features of DBA remains to be determined.

Cancer risk in SDS

Data on the incidence of cancer in patients with SDS indicated a frequency of 6% for all malignancies with an odds ratio of 8.5 (REF. [60]). Furthermore, the French Severe Chronic Neutropenia registry reported the risk of evolution to MDS or AML in patients with SDS to be 19% and 36% at 20 years and 30 years, respectively[61]. Recent studies on genomic changes in a large cohort of patients with MDS before bone marrow transplantation revealed several patients who apparently had undiagnosed forms of SDS[64]. Thus, occult forms of SDS may remain undiagnosed until a patient progresses to MDS. Thus, like DBA, SDS may be underdiagnosed, leaving occult patients at risk of cancer without adequate counselling or surveillance.

Cancer risk in dyskeratosis congenita

Patients with dyskeratosis congenita who had not been transplanted had a cancer frequency of almost 10%, which is somewhat higher than the cancer frequencies reported for DBA and SDS[60]. The odds ratio for all cancers in dyskeratosis congenita was 4.2. The cumulative incidence of all cancers by the age of 50 years for dyskeratosis congenita was approximately 20%. The frequency of cancers in patients with dyskeratosis congenita was similar among patients with mutations in *DKC1* and those with mutations in genes solely involved in telomere maintenance[60]. At present, there is no evidence to suggest that defects in ribosome synthesis increase the risk of cancer over and above that attributable to effects on telomere maintenance, although the numbers remain small. Here again, MDS was considered separately and had an odds ratio of 578 (REF. [60]).

Cancer risk in CHH–AD

The incidence of cancer in CHH–AD is 11% and includes both haematological malignancies and solid tumours[65]. The standardized incidence ratio (similar to odds ratio) for all malignancies in patients with CHH–AD was 7. The probability of cancer by age 65 years in the Finnish cohort of patients with CHH–AD is 41%.

Ribosome synthesis in sporadic cancers

Intriguingly, somatic mutations in ribosomal protein genes, including some of the genes affected in DBA, have been identified as possible drivers in a number of sporadic cancers. Initial studies identified mutations in *RPL5* and *RPL10* (also known as *uL16*) in approximately 10% of T cell acute lymphoblastic leukaemias (T-ALLs)[4]. *RPL5* deletions have also been identified in patients with multiple myeloma and other cancers, suggesting that *RPL5* is a haploinsufficient tumour suppressor[66,67]. Intriguingly, RPL5 stands out among the ribosomal proteins affected in sporadic cancers both in terms of the frequency of loss-of-function mutations and in the number of different types of cancer in which these mutations occur. Recent studies have revealed loss-of-function mutations in *RPL5* in patients with paediatric T-ALL (2%), glioblastoma (11%), melanoma (28%), breast cancer (34%) and multiple myeloma (>40%)[66]. By contrast, other ribosomal proteins shown to have reoccurring mutations in sporadic cancers have considerably lower frequencies. Given the preponderance of mutations in *RPL5* in sporadic cancers, it is surprising that cancer is not overrepresented in patients with DBA who have mutated *RPL5*. Instead, the cancer incidence in DBA appears to parallel the relative frequency with which genes are mutated in patient cohorts[59,60,62].

Other genes encoding ribosomal proteins shown to have reoccurring somatic mutations in sporadic cancers include *RPSA (*also known as *uS2), RPS5 (*also known as *uS7), RPS20* (also known as *uS10), RPS27* (also known as *eS27), RPL11*, *RPL22* (also known as *eL22*) and *RPL23A* (also known as *uL23*)[66,68]. Surprisingly, germline mutations in *RPS20* have been identified as a risk factor for colon cancer[69]. While patients with DBA show an elevated risk of colon cancer (OR = 45), patients with mutations in *RPS20* with a detectable defect in ribosome biogenesis somehow escape the bone marrow failure seen with germline mutations in other genes of the small ribosomal subunit. Furthermore, RPL22 is unique among the protein products of the genes listed above in that its absence does not appear to affect ribosome biogenesis or general translation[70]. Instead, RPL22 and other non-essential ribosomal proteins appear to function in specialized regulatory capacities either through the creation of specialized ribosomes lacking these proteins[71] or via specific extraribosomal functions for these proteins[72]. These latter ribosomal proteins, while fascinating, have not as yet been shown to be affected in inherited ribosomopathies and therefore are beyond the scope of the current Review.

Carcinogenic mechanisms

The two major mechanisms underlying disease pathology for the ribosomopathies also likely account for the increased incidence of cancer in these disorders. Moreover, like disease pathology, cancer incidence likely involves a synthesis of both mechanisms.

Selection for loss of p53 function

The most obvious link between the ribosomopathies and cancer relates to a role for p53 activation in the proapoptotic phenotype of different cell lineages affected in these disorders. Activation of p53 has been implicated in the pathophysiology of DBA[38], SDS[73], TCS[74] and XL-DC[75]. The importance of p53 activation in the pathophysiology of these diseases is most evident based on the rescue of phenotypes in various disease models by the inactivation of p53 originally observed in zebrafish[36]. These observations suggested that there may be a selective pressure for a loss of p53 function as a means of cell survival in ribosomopathies and potentially provide a mechanism through which patients could enter into haematological remission or be unaffected carriers. Loss of p53 function would in turn subvert its function as a major tumour suppressor, thereby increasing cancer risk[76]. Evidence for this selective pressure was recently observed in a study correlating haploinsufficiency for ribosomal protein genes with loss-of-function mutations in *TP53* in over 10,000 cancer specimens and cell lines[77]. Moreover, a recent study on haematopoietic stem cell populations in patients with SDS revealed clonal haematopoiesis with an associated high level of p53 mutations, again suggesting

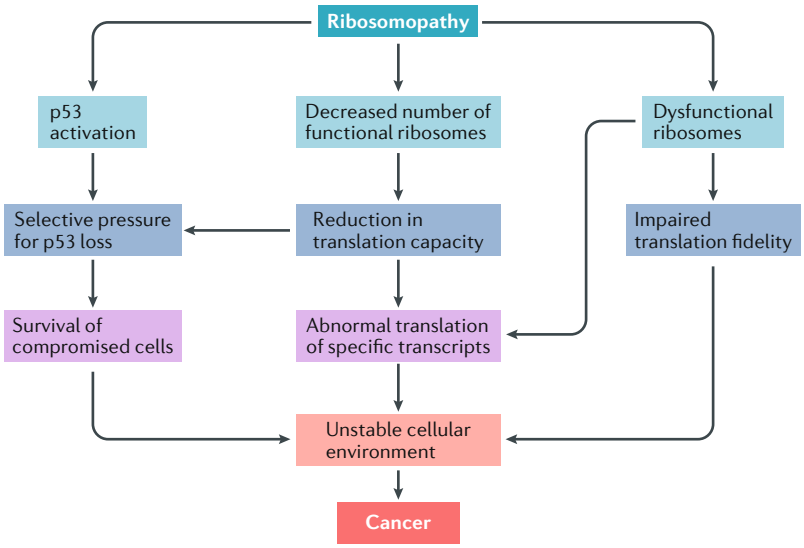

Fig. 4 | **Disruption of ribosome synthesis and function can promote tumorigenesis.** Disruption of ribosome synthesis results in reduced global protein synthesis, as the number of ribosomes is decreased, and activation of p53. These features lead to a hypoproliferative phenotype and exert a selective pressure for p53 abrogation to overcome cell cycle arrest and apoptosis. This selective pressure may promote the expansion of mutant clones with higher proliferation rate, driving tumorigenesis. Cell survival may also be influenced by alterations in the translation of specific transcripts owing to the reduced number of ribosomes. Moreover, changes in the composition or chemical modification of ribosomes may produce aberrant ribosomes, which may escape degradation by quality control mechanisms and lead to decreased translation fidelity. Some or all of these mechanisms may act in concert to drive tumour initiation and/or progression.

a selective pressure for initial events that may be driving disease progression in these patients to MDS and AML[78]. Finally, although patients with $5q^-$ MDS generally have a relatively low progression to AML and a favourable prognosis, the presence of mutations in *TP53* in approximately 20% of these patients significantly increases their risk of progression to AML[79,80].

Translating cancer

The first manuscript to suggest that the ribosome might have a role in translating cancer stated that defects in ribosome synthesis and function could promote cancer by erroneously translating mRNAs that encode oncogenes or tumour suppressors, thereby contributing to carcinogenesis[81]. These effects could be qualitative in nature, whereby alterations in ribosome structure and function as seen in XL-DC interfere with the translation of IRES-containing mRNAs, some of which encode tumour suppressors[26,58] (FIG. 3c). Alternatively, these effects could be quantitative in nature, whereby mRNA translation is impaired owing to a reduction in ribosome number, which alters the milieu in which mRNAs compete with one another for translation[82] (FIG. 3b).

In extending these mechanisms to other ribosomopathies, it was suggested that defects in ribosome assembly linked to ribosomal protein haploinsufficiency could lead to oncoribosomes with altered function that contribute to tumorigenesis[83]. Specifically, given the high incidence of *RPL5* mutations in sporadic cancers, it was proposed that specialized oncoribosomes may arise that lack the central protuberance, a critical structural and functional element of 60S ribosomal subunits formed in part by the 5S-RNP[9]. As yet, there is little evidence to support the view that partially assembled ribosomal subunits, which transiently accumulate in cells haploinsufficient for RPL5 and other DBA proteins, can escape degradation and function as specialized ribosomes. In this regard, a mass spectroscopy analysis on translating ribosomes in cellular models of DBA has shown no evidence of ribosome heterogeneity in functional polysomes[15].

While the pathophysiology and associated cancer incidence in DBA appear to reside with a general reduction in ribosome levels, there is additional evidence to suggest that more specific effects on ribosome function may have a role in the pathophysiology of other ribosomopathies. Remarkably, a high incidence of specific point mutations was found in the gene encoding RPL10 in T-ALL. In one study, recurrent missense mutations were found at codon Arg98 in 8.2% of a cohort of paediatric patients with T-ALL[4]. In yeast models, these same mutations were shown to affect late steps in the biogenesis of 60S subunits involving genes affected in SDS[84]. Specifically, Arg98 of RPL10 contacts critical structural features of the peptidyltransferase centre of the ribosome. The SBDS protein together with EFL1 and other factors act to monitor the functional integrity of the peptidyltransferase centre before certifying the 60S subunit for participation in translation[85]. Intriguingly, while the recurrent mutations in *RPL10* affect ribosome biogenesis and reduce the amount of functional ribosomes in cells, subunits that escape quality control destruction have reduced translational accuracy[84] (FIG. 3d). These results suggested a model in which mutations in certain ribosomal proteins exert a powerful selective pressure for escape mechanisms that involve quality control genes such as *SBDS*, which may allow considerable numbers of dysfunctional ribosomes to escape destruction and alter the translational milieu of the cell in such a way to promote cancer[84].

Synthesis

The mechanisms by which disruption of ribosome synthesis predisposes to cancer likely include signalling mechanisms set in motion by both nucleolar stress and associated translational alterations caused by quantitative or qualitative changes in ribosome output. The combined effect of reductions in ribosome numbers and increased levels of p53 in creating a hypoproliferative phenotype and reduced competitive advantage for cells imposes a strong selection for mutations in p53 as a means of survival (FIG. 4). Survival of these compromised cells sets the stage for additional events that overcome impediments to growth, transitioning to more hyperproliferative phenotypes associated with tumorigenesis. These observations provide a solution to Dameshek's riddle for how diseases with hypoproliferative phenotypes can transition to cancer and a hyperproliferative phenotype[86,87].

Events that overcome these impediments to growth could involve activation of oncogenes and loss of suppression of additional tumour suppressors, both of

which lead to an upregulation of ribosome synthesis[81]. These events could be fostered by specific changes to the translational machinery, including reduced translational fidelity and subsequent errors transiently compromising the function of all components of the proteome and/or a general reduction in ribosome numbers and an altered milieu of mRNAs being translated within a cell (FIG. 4). These events set in motion by changes to the ribosome and the subsequent readout of the genetic programme of a cell could in principle be consistent with a modified version of the error catastrophe model[88]. According to this model, errors in protein synthesis or mRNA selection could lead to the progressive deterioration of a cell and create an unstable intracellular environment, which, in turn, could make conditions favourable for tumorigenesis (FIG. 4).

Treacher Collins conundrum

TCS, the only known well-characterized ribosomopathy that does not show bone marrow failure or an increased incidence of cancer (BOX 1), presents a conundrum when considering the pathophysiology of ribosomopathies, including the predisposition of many of these disorders to cancer. There are clear parallels between the mechanisms involved in TCS and DBA pathogenesis. Like DBA, a role for p53 activation has also been demonstrated in mouse models of TCS, in which loss of p53 function has again been shown to rescue phenotypes[74]. While not directly studied in animal models of TCS, numerous studies in cellular systems have documented a role for 5S-RNP in signalling nucleolar stress caused by inhibition of transcription by RNA polymerase I[89,90]. Thus, a reduction in RNA polymerase I activity decreases the amount of rRNA, limiting the incorporation of 5S-RNP into ribosomes and making it available for inhibitory interactions with MDM2 leading to p53 activation. Furthermore, similar to other ribosomopathies, defective polymerase I transcription limits the number of ribosomes per cell, with subsequent effects on translational output.

Given the parallels between the pathophysiological mechanisms proposed for TCS, DBA and 5q$^-$ syndrome, particularly with respect to potential selective pressures for loss of p53 activity as a means of cell survival, the observation that patients with TCS do not have a predisposition to cancer raises intriguing prospects for further investigation. While TCS, DBA and 5q$^-$ syndromes all affect ribosomal synthesis, TCS does so by reducing the nascent 47S-pre-rRNA transcript on which ribosome assembly begins, whereas DBA and 5q$^-$ syndrome do so after ribosome assembly has begun by interfering with the maturation of partially assembled pre-ribosomes.

Transcription by RNA polymerase I has been shown to be the primary control point that regulates ribosome synthesis in response to physiological and environmental cues[91]. Changes in ribosome levels at the level of RNA polymerase I are interconnected with the synthesis of ribosomal proteins and 5S rRNA transcribed by RNA polymerase III and need to be coordinated to bring about adjustments in ribosome synthesis without triggering the pathophysiological mechanisms outlined in this Review. For example, a molecular titration system has been identified that coordinates RNA polymerase I transcription with the transcription of ribosomal protein genes by RNA polymerase II[92]. Activation of this system by inhibition of RNA polymerase I leads to reduction of the transcription of ribosomal protein genes by RNA polymerase II and thus a coordinated reduction of ribosomal components. These regulatory mechanisms may assist in tempering nucleolar stress signalling brought about by changes in RNA polymerase I transcription, though it still should be noted that at particular times during the development of cell types such as neural crest cells, other stressors may interact with tempered nucleolar stress signalling to promote cell death and trigger craniofacial phenotypes[93].

Although ribosomal protein deficiencies can ultimately affect RNA polymerase I transcription by growth-rate-dependent regulatory mechanisms[91,94], these controls manifest later after abortive assembly intermediates have accumulated and stress pathways have been triggered. Moreover, disposal of abortive assembly intermediates may also factor into pathophysiological mechanisms, particularly as inducers of autophagy have been shown to ameliorate phenotypes in induced pluripotent stem cells from patients with DBA[95]. Presumably, mechanisms coordinating RNA polymerase I transcription with ribosomal protein synthesis limit flux through the ribosome synthesis pathway and reduce the demands for eliminating abortive complexes. The observation that defects in ribosome synthesis at the level of transcription by RNA polymerase I seen in TCS somehow escape bone marrow failure, as well as the increased cancer incidence observed with other ribosomopathies, raises the intriguing possibility that an inhibition of ribosome synthesis upstream of later events in ribosome maturation may be a means of treating certain bone marrow failure syndromes and perhaps even reducing the increased incidence of cancer observed in patients with these syndromes.

Box 1 | **Treacher Collins syndrome**

Treacher Collins syndrome (TCS) is a well-characterized ribosomopathy without bone marrow failure or an increased incidence of cancer and affects craniofacial development. The clinical features of TCS are variable, with most children exhibiting bilateral mandibular and malar hypoplasia, downward slanting palpebral fissures and microtia[106]. To date, genes reported to be mutated in TCS are *TCOF1* (REF.[107]), *POLR1C* and *POLR1D*[108]. These mutations all reduce RNA polymerase I activity, with *POLR1C* and *POLR1D* also being subunits of RNA polymerase III. Defects in RNA polymerase I transcription disrupt the production of both ribosomal subunits, as the polycistronic transcript produced includes 18S of the 40S subunit and 5.8S and 28S rRNAs of the 60S subunit. TCS is therefore a well-defined ribosomopathy clinically characterized by craniofacial anomalies. While there appears to be some phenotypic and pathophysiological overlap between patients with TCS and DBA[109], it is notable that patients with TCS have normal bone marrows and no evidence of an increased risk of cancer.

Concluding remarks

The sense that factors involved in ribosome biogenesis are often overlooked in contributing to mechanisms of tumorigenesis seems a matter of not being able to see the forest for the trees given the ubiquitous nature of ribosomes, their abundance and their central role in

translating the proteome of each and every cell. Studies correlating the growth rate of cells and ribosome content can trace their roots to work from over 60 years ago[96]. These and numerous other studies since then suggest that upregulation of ribosome synthesis is a critical factor in fast-growing cancers[97,98]. Indeed, oncogenes such as *MYC* are critical regulators of ribosome synthesis[99]. Nevertheless, the question of whether the ribosome is a passive downstream mediator of tumorigenesis or whether factors involved in ribosome synthesis and function could have more active roles as drivers of tumorigenesis remains. Because many of the ribosomopathies are cancer predisposition syndromes, factors involved in ribosome synthesis could indeed serve as drivers of tumorigenesis, a view supported by the increased identification of somatic mutations in ribosomal protein genes in sporadic cancers.

Mutational changes that interfere with ribosome biogenesis may be relatively prevalent in human cancers, which provides a potential therapeutic avenue whereby drugs targeting ribosome synthesis could work synergistically with these intrinsic defects in targeting certain cancers[100,101]. In this regard, CX-5461, a selective RNA polymerase I inhibitor is in a phase I clinical trial for haematological malignancies[102] and is also being tested in a number of model systems for a wide range of human malignancies[103–105]. Thus, rare congenital ribosomopathies have provided insights into an oft-overlooked facet of cancer biology.

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