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REVIEW

Emerging roles of nucleolar and ribosomal proteins in cancer, development, and aging

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Abstract Changes in nucleolar morphology and function are tightly associated with cellular activity, such as growth, proliferation, and cell cycle progression. Historically, these relationships have been extensively examined in cancer cells, which frequently exhibit large nucleoli and increased ribosome biogenesis. Recent findings indicate that alteration of nucleolar activity is a key regulator of development and aging. In this review, we have provided evidences that the nucleolus is not just a housekeeping factor but is actively involved in the regulation of cell proliferation, differentiation, and senescence both in vitro and in vivo. In addition, we have discussed how alteration of nucleolar function and nucleolar proteins induces specific physiological effects rather than widespread effects.

Keywords Nucleolus · Ribosome biogenesis · Stem cells · Differentiation · Longevity

Abbreviations

ES cells	Embryonic stem cells
pre-rRNA	Precursor ribosomal RNA
Pol I	RNApolymerase I
rDNA	Ribosomal DNA
snoRNPs	Small nucleolar ribonucleoprotein particles
TIF-IA	Transcription initiation factor IA
UBF	Upstream binding factor

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Introduction

The nucleolus is the most prominent subnuclear structure where ribosomal RNA (rRNA) and ribosomal subunits are synthesized. These events are highly coordinated and the most energy-consuming cellular processes. Within the nucleolus, ribosomal DNA (rDNA) is transcribed by RNA polymerase I (Pol I). The precursor rRNA (pre-rRNA) obtained is then processed and modified to generate 28S, 18S, and 5.8S rRNAs. The mature rRNAs are then assembled with ribosomal proteins and are exported to the cytoplasm for protein synthesis [1, 2]. The size of the nucleolus varies among cells and reflects the rate of ribosome biogenesis. Large nucleoli are found in actively proliferating cells that require continuous ribosome biosynthesis, whereas the size of the nucleolus decreases upon cell cycle arrest [3].

The relationship between nucleolar size and cell activity has been extensively examined in cancer biology for many years. In as early as the late 19th century, a study reported that the large, irregular nucleoli were the hallmarks of progressive cancer cells [2]. Since then, many studies have been performed to determine the molecular mechanisms underlying nucleolar dynamics in cancer progression. Some studies suggest that nucleolar changes are just the consequence of enhanced ribosome biogenesis in cancer cells [4]. This view is supported by the evidence that the rate of ribosome biogenesis is regulated by oncogenes and tumor suppressors [5]. According to this view, changes in oncogenes or tumor suppressors increase ribosome biogenesis to satisfy the increasing demand for protein synthesis in rapidly proliferating cancer cells, thus resulting in hypertrophic nucleoli. In contrast, many studies indicate that nucleolar dynamics play a causative role in tumorigenesis. In fact,

some studies have shown that mutations in genes encoding ribosomal proteins, which are observed in patients with inherited genetic disease, increase the incidence of cancer [4]. Moreover, in vitro studies have shown that dysregulation of nucleolar function induces malignant transformation [6, 7]. Thus, nucleolar dynamics is not only the consequence of malignant transformation but also is actively involved in cancer progression.

Recent studies have shown that nucleoli are actively involved in stem cell maintenance [8–11, 75] and lifespan regulation in model organisms [12–14] and play a role in various cellular functions in addition to cancer progression. In this review, we discuss the functions of nucleoli in cancer cell progression, stem cell maintenance, and lifespan regulation, with an emphasis on how nucleoli regulate specific cellular processes rather than the overall cellular homeostasis. In addition, we discuss the findings of recent studies highlighting the specific roles of ribosomal proteins in the regulation of gene expression, which challenges the classical view that nucleoli and ribosome only perform housekeeping functions.

Ribosome biogenesis and cancer

Cancer cells require continuous ribosome biogenesis and protein translation to maintain their high proliferation rate. Therefore, nucleolar hypertrophy with enhanced ribosome biogenesis is one of the characteristic features of cancer cells. In rapidly proliferating cancer cells, nucleolar proteins, which are involved in rRNA synthesis and processing, become more abundant, leading to nucleolar hypertrophy (Fig. 1). In fact, the size of the nucleolus, the amount of nucleolar proteins, and rate of rRNA synthesis are closely associated with the doubling time of various cancer cells [3].

Changes in oncogenes and tumor suppressors in cancer cells result in the hyperactivation of ribosome biogenesis. The c-Myc proto-oncogene enhances the transcription of rDNA by directly controlling the expression of upstream binding factor (UBF), an essential component of Pol I transcription machinery, and by interacting with rDNA [15]. Moreover, c-Myc directly regulates the expression of genes involved in pre-rRNA processing and increases 5S rRNA biosynthesis by activating the transcription of RNA polymerase III [16]. These data clearly indicate that overexpression of c-Myc enhances ribosome biogenesis in cancer cells. RAS/RAF/ERK signaling pathway, an oncogenic signaling pathway, activates rRNA synthesis by regulating the phosphorylation and activation of UBF and transcription initiation factor IA (TIF-IA) [17]. Similarly, PI3 K/AKT/mTORC1 signaling pathway also modulates the phosphorylation of UBF and TIF-IA [18]. These data illustrate that activating mutations in these signaling pathways could enhance ribosome biogenesis and trigger cancer cell proliferation. In contrast, tumor suppressor protein p53 inhibits the transcriptional activity of Pol I by binding to SL-1 complex, which is necessary for the formation of the Pol I complex on the rDNA promoter [19]. In addition, retinoblastoma-associated protein represses the transcriptional activity of Pol I by preventing the recruitment of UBF to the rDNA promoter [20]. Thus, inactivation of these tumor suppressors, which is frequently observed in cancer cells, prevents the repression of rRNA transcription, leading to the hyperactivation of ribosome biogenesis.

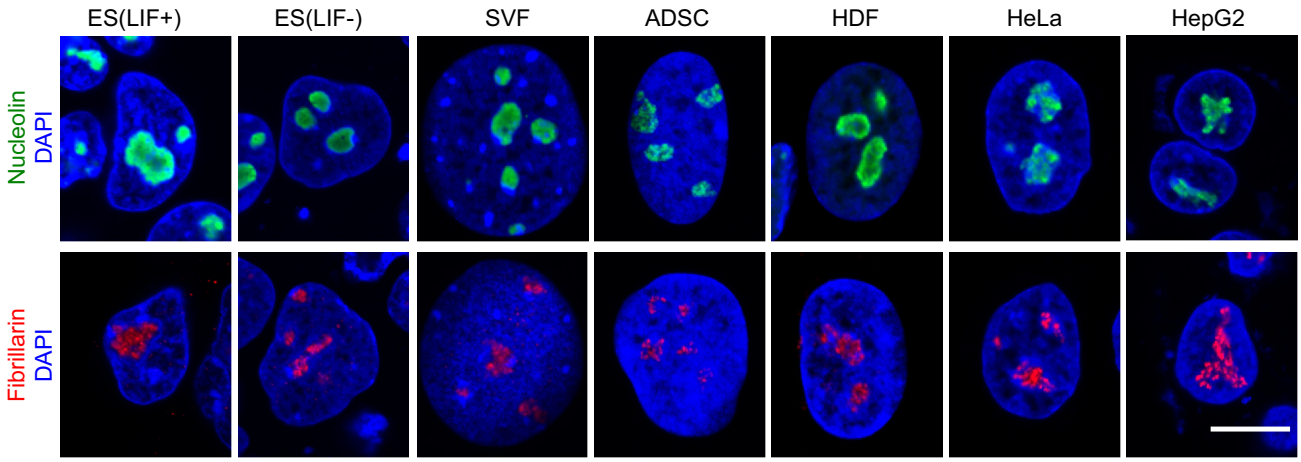

Fig. 1 Differently sized nucleoli of pluripotent, differentiated, and cancer cells. Mouse ES cells cultured in the presence or absence of LIF, mouse SVF (stromal vascular fraction), human ADSCs (adipose derived stem cells), HDF (human dermal fibroblasts), HeLa cells, and HepG2 cells were stained with antibody to nucleolin (*green*), fibrillarin (*red*), and DAPI (*blue*). Note that pluripotent ES cells (+LIF) and cancer cells (HeLa and HepG2) have large nucleoli compared to differentiated cells (−LIF ES cells, SVF, ADSC, and HDF). *Scale bar* 10 μm

In addition to transcriptional regulation, epigenetic networks control rRNA expression in cancer cells. The promoter region of the human rRNA gene contains a CpG island encompassing 19 CpGs in the upstream control element and six CpGs in the core promoter region. Bisulfite genomic sequencing has shown that most CpGs in the upstream control element are significantly hypomethylated in human hepatocellular carcinoma cells compared with those in pair-matched liver tissue cells. Luciferase reporter assay using the rRNA promoter showed that hypermethylation of CpG islands inhibits the activity of the rRNA promoter and that methyl-CpG-binding protein MBD2 mediates the silencing of the rRNA promoter, suggesting that CpG methylation density is inversely correlated with the activity of the rRNA promoter [21]. These data imply that hypomethylation of the rRNA promoter enhances the transcription of rRNA genes, thus increasing ribosome biosynthesis in cancer cells.

Although the above results suggest that alteration of ribosome biogenesis can be explained as a consequence of malignant transformation, many evidences show that dysregulation of ribosomal proteins itself increases the susceptibility to tumorigenesis. Several ribosomal proteins are overexpressed in tumor cells and clinical tissue samples obtained from cancer patients [22–24]. For example, RPL36A, a tumor-associated ribosomal protein, is highly expressed in hepatocellular carcinoma. Ectopic overexpression of RPL36A in Chang liver cells enhances colony formation and increases cell proliferation by accelerating the cell cycle [7], suggesting that increased expression of ribosomal proteins plays a key role in tumor cell proliferation. Similarly, overexpression of another ribosomal protein RPS3a in NIH3T3 cells induces the characteristic features of malignant transformation, such as foci formation, anchorage independent growth, and tumorigenicity in nude mice, suggesting that increased expression of ribosomal proteins may lead to malignant transformation [6]. In addition to enhanced production of ribosomal subunits, reduced expression of ribosomal proteins also leads to tumor formation, which has long been examined in patients with inherited genetic diseases such as ribosomopathies. For example, heterozygous mutations in *RPS19* encoding a small ribosomal subunit protein RPS19 are observed in 25 % patients with Diamond–Blackfan anemia (DBA), a bone marrow failure syndrome associated with increased incidence of acute myeloid leukemia and osteogenic sarcoma. These mutations inhibit the processing of pre-rRNA to 18S rRNA and formation of the 40S rRNA subunit, suggesting that defects in ribosome biogenesis are associated with DBA pathogenesis [25]. Other ribosomal proteins, including RPS24, RPS7, RPS15, RPS17, RPL35A, RPL5, and RPL11, are also mutated in patients with DBA [26]. The 5q-syndrome is a type of myelodysplastic syndrome characterized by severe anemia, dysmegakaryopoiesis, and increased susceptibility to hematopoietic tumors [27]. In patients with the 5q-syndrome, an allele of the gene encoding ribosomal protein S14 (*RPS14*) is deleted, resulting in the haploinsufficient expression of RPS14. Knockdown experiments showed that decreased expression of RPS14 inhibited pre-rRNA processing and decreased the level of mature rRNA, which was similar to the RPS19 deficiency observed in patients with DBA, thus supporting the association between dysregulation of rRNA synthesis and tumor formation [27]. Besides mutations in genes encoding ribosomal proteins, mutations in genes encoding nucleolar proteins responsible for pre-rRNA maturation cause inherited genetic diseases associated with an increased risk of tumor formation. X-linked dyskeratosis congenita (DC) is a bone marrow failure syndrome characterized by ectodermal dysplasia, hematopoietic failure, and increased tumor susceptibility. Pathogenesis of DC is attributed to a mutation in *DKC1* that encodes dyskerin, a component of small nucleolar ribonucleoprotein particles (snoRNPs) that are involved in pre-rRNA processing [28, 29]. In addition, mutations in the gene encoding RMRP, an RNase involved in pre-rRNA cleavage, are observed in patients with cartilage–hair hypoplasia that predisposes these patients to cancer [30]. Although molecular mechanisms underlying increased susceptibility to tumor formation remain poorly understood, the above evidences strongly suggest that defects in ribosome biogenesis actively contribute to tumorigenesis.

Non-ribosomal nucleolar proteins in cancer cells

The nucleolus acts as a key stress sensor and controls the cellular level of p53. Under normal condition, p53 is maintained at a low level through continuous degradation by MDM2-mediated ubiquitination. In response to cellular stress, nucleolar proteins, including ribosomal proteins L11 and ARF, are released from the nucleolus into the cytoplasm where they interact with MDM2 and inhibit MDM2-mediated ubiquitination of p53, stabilizing the level of p53. Thus, the nucleolus is a crucial regulator that links stress signaling with cellular p53 level and allows cells to respond to stress by inducing p53-mediated cell cycle arrest [31, 32]. Furthermore, the nucleolus is involved in multiple biological processes, including cell cycle regulation, DNA repair, and telomere replication [33]. Nucleolar proteins that facilitate these cellular processes other than ribosome biogenesis are also involved in cancer progression. Nucleophosmin (NPM) is a non-ribosomal protein, and its dysregulation is tightly associated with tumorigenesis. NPM shuttles between the nucleolus and the cytoplasm during cell cycle and performs diverse cellular

functions, including centrosome duplication, protein folding, and genome stability maintenance [34]. Because of its multifunctionality, it is unclear whether NPM has oncogenic or tumor suppressive properties even though its functions have been extensively studied [35]. In fact, NPM is frequently overexpressed in various cancers, whereas mutations in the gene encoding NPM are also observed in patients with acute myeloid leukemia [36]. In NPM-mutant cells, ARF, a tumor suppressor protein, is mislocalized to the cytoplasm where it cannot bind to MDM2, leading to the impairment in ARF-dependent p53 activation [35, 37]. In addition, NPM inactivation induces DNA damage and unrestricted centrosome duplication, resulting in aberrant oncogenesis [38]. These data indicate that NPM is critical for maintaining proper p53 function and genome stability, thus implying its tumor suppressive role. In contrast, some studies reported that NPM overexpression is associated with cell proliferation and tumorigenesis. Clinical samples show that cancer tissues express extremely high levels of NPM compared with normal tissues, and these levels are closely correlated with cancerous growth [39]. In chronic myelogenous leukemia-derived cells, NPM interacts with a tumor suppressor protein IRF-1 and interferes with its DNA-binding and transcriptional activity. Furthermore, overexpression of NPM in NIH3T3 cells induces malignant transformation in vivo and tumorigenicity in nude mice by possibly inhibiting the activity of IRF-1 [40]. These findings imply the oncogenic role of NPM. Nucleostemin, a GTP-binding protein, is another non-ribosomal protein that is highly expressed in various cancer cells and cancer stem cells. Nucleostemin regulates the cell cycle and proliferation by interacting with multiple proteins, including p53, MDM2, and ARF [41]. In cancer cells, overexpression of nucleostemin increases the expression of several markers of tumor-initiating cells and tumorigenicity thorough the formation of a complex containing a telomerase catalytic subunit and Brg1 [42]. Together, these findings illustrate that non-ribosomal nucleolar proteins contribute to tumorigenesis.

Nucleolar dynamics in stem cells

In addition to cancer cells, a large nucleolus is a characteristic feature of stem cells and progenitor cells. For example, embryonic stem (ES) cells possess large, condensed nucleoli, which are the hallmark of pluripotency (Fig. 1). These nucleoli change their morphology from large to small foci during differentiation [43]. In addition, during *Drosophila* eye disc development, nucleoli are much larger in proliferating progenitor cells of the anterior eye disc than in differentiating and post-mitotic cells of the posterior eye disc [44]. These data show that nucleolar size reflects the stemness and proliferation potential of the cells, which are consistent with the finding that proliferating cells require active nucleolar function with increased ribosome biogenesis.

The proto-oncogene c-Myc plays a critical role in the regulation of nucleolar size during transition from progenitor to post-mitotic differentiated state in stem cells as well as in cancer cells. Actively proliferating stem cells and progenitor cells express high levels of c-Myc. During cell differentiation, downregulation of c-Myc leads to the loss of Pol I transcription initiation factor UBF, which represses rRNA synthesis [45]. Furthermore, lineage-specific differentiation factors such as MyoD, myogenin, Runx2, and C/EBP-β bind to the rDNA loci and decrease c-Myc binding, thus downregulating rRNA transcription [46]. These findings clearly indicate that cell growth regulatory factors and lineage commitment factors regulate nucleolar activity and rRNA synthesis in a mutually exclusive manner.

Novel nucleolar function in stem cell maintenance and differentiation

One fundamental question is whether the change in nucleolar size is just an indicator of stem cell properties or nucleolar activity itself controls the proliferation and differentiation of stem cells. Recent studies by us and by other researchers showed that the nucleolus is a critical regulator of the unique properties of stem cells. In ES cells, non-ribosomal nucleolar proteins such as nucleolin and nucleostemin are highly expressed, which is similar to that observed in cancer cells. Depletion of these nucleolar proteins results in reduced cell proliferation, abnormal cell cycle, and enhanced differentiation, suggesting that proper nucleolar function is required for the self-renewal of ES cells [47, 48]. Mechanistically, knockdown of nucleostemin in ES cells downregulates the expression of cell cycle regulators and decreases the progression through the G1 phase of the cell cycle [48]. This elongated G1 phase allows ES cells to respond to MEK/ERK signaling and promotes the differentiation of ES cells. Depletion of nucleolin in ES cells increases the stability of p53 and activates p53 signaling, resulting in the elongation of the G1 phase and induction of differentiation [47]. These data imply that nucleostemin and nucleolin have similar molecular functions and contribute to the maintenance of ES cell identity by sustaining its unique cell cycle profiles.

Nucleolar proteins involved in ribosome biogenesis are also the critical regulators of stem cell maintenance. Fibrillarin (FBL), a critical methyltransferase involved in

rRNA processing, is highly expressed in ES cells and its expression rapidly decreases upon the differentiation of ES cells. Stable expression of FBL prolonged the pluripotent state of mouse ES cells cultured without leukemia inhibitory factor (LIF). Partial knockdown of the gene encoding FBL decreases rRNA synthesis and inhibits the growth of ES cells. This in turn promotes the differentiation of ES cells even in the presence of LIF. Microarray analysis showed that abnormal differentiation caused by a decrease in FBL expression is attributed to the activation of p53 signaling [11]. These data suggest that proper ribosome biogenesis is a critical regulator for the pluripotency of ES cells and that alteration of ribosome production induces the differentiation of ES cells in a p53-dependent manner. In mouse hematopoietic stem cells, inhibition of rRNA production by actinomycin D or siRNA against the gene encoding TIF-IA, an essential factor for Pol I transcription, decreases pre-rRNA levels and induces cell differentiation [10]. Importantly, inhibition of cell cycle without the inhibition of ribosome biogenesis does not induce cell differentiation, suggesting that changes in ribosome biogenesis positively regulate cell differentiation independently of cell cycle progression. Deletion of Notchless, which is known to be involved in pre-60S maturation, leads to defective ribosome biogenesis and p53 activation in hematopoietic stem cells and in turn causes their rapid exhaustion [75]. The role of ribosome biogenesis in stem cells was also investigated in vivo in the development of *Drosophila* ovary. While screening for mutations that affected early oogenesis, Fichelson el al. observed that wicked (*wcd*) mutant induced the premature differentiation of germ-line stem cells. *Wcd* encodes a yeast homolog of U3 snoRNA-associated protein UTP18, a nucleolar protein responsible for pre-rRNA maturation. Consistent with this, knockdown of *wcd* in S2 cells induced the accumulation of long forms of pre-rRNA, suggesting the role of *wcd* in rRNA maturation. Intriguingly, Wcd was asymmetrically segregated into the daughter cells during germ stem cell mitosis, and the daughter cells that inherited *wcd* maintained their stem cell property [8]. These data indicate that modulation of ribosome biosynthesis mediated by the asymmetrical segregation of Wcd is required for stem cell maintenance and germ cell differentiation. Recently, another group showed the requirement of ribosome biogenesis for germ cell maintenance and identified the underlying molecular mechanisms. They searched for mutants on the basis of sterile phenotypes and identified a mutant *under-developed* (*udd*) which exhibits germ cell loss. Decreased rRNA production induced by the disruption of *udd*, a component of Pol I regulatory complex, promoted germ cell differentiation into multicellular cysts while overexpression of TIF-IA, a mediator of rRNA transcription by Pol I, delayed germ cell differentiation. Interestingly, reduction in rRNA production specifically downregulated the level of Mad protein, a component of BMP signaling, but did not affect the level of another BMP signaling component Medea or histone H2B [9]. Thus, this study suggests that alteration of rRNA production regulates cell differentiation by modulating specific proteins but not by reducing the overall protein synthesis. It will be interesting to examine whether downregulation of Mad proteins is dependent on p53 activation, as observed during ES cell differentiation and, if it is not, then how does ribosome biogenesis regulate the expression of specific proteins.

Nucleolar proteins in development

Ribosome biogenesis not only regulates stem cell differentiation, but also controls cell survival and growth during the development of an organism. Deletion or mutation of nucleolar proteins involved in ribosome biogenesis leads to growth defect phenotypes. For example, in *Drosophila*, RNAi knockdown of NS1, which is the closest homolog of human nucleostemin, inhibits the release of the large ribosomal subunit from the nucleolus, induces loss of adult precursor cells in the midgut epithelium, and retards cell growth in the salivary gland [49]. Loss of Nopp140, which functions as a molecular chaperon for snoRNA [50], disrupts ribosome biogenesis and induces apoptosis in wing discs during larval development [51]. The apoptotic phenotype cannot be rescued by a *p53* gene deletion, indicating that depletion of Nopp140 induces apoptosis in a p53-independent manner. Instead of p53, activated JNK and its downstream pro-apoptotic factor Hid accumulate in Nopp140-depleted larvae, suggesting that reduced ribosome biogenesis activates the JNK signaling pathway. In zebrafish, mutation in the nucleolar protein NOM1 results in decreased proliferation of pancreatic cells during the development of pancreas. RNA-seq analysis revealed that NOM1 deficiency affects the expression of ribosomal-related genes, leading to the decreased production of 18S rRNA [52]. These data suggest that the regulation of ribosome biogenesis is essential for the proper development of various organs.

Several studies have revealed that Myc and Tor are involved in the regulation of ribosome biogenesis during *Drosophila* development. Overexpression of dMyc in larvae increases ribosome biogenesis, whereas the level of rRNA is decreased in dMyc mutant larvae. Using microarray analysis, Grewal et al. revealed that dMyc upregulates the expression of genes encoding Pol I regulators in the developing larvae. Expression of dMyc increases cell size in the wing disc, but the cell size effect is diminished in cells carrying a mutation in Rpl135, a component of the Pol I complex. This suggests that dMyc

controls cell growth through the regulation of ribosome biogenesis [53]. Demontis et al. revealed the role of dMyc in skeletal muscles. While inhibition of dMyc activity leads to smaller muscles, dMyc overexpression promotes endoreplication, induces enlarged nucleoli, and upregulates the expression of genes involved in ribosome biogenesis. Under conditions that inhibit Insulin receptor (InR)/Tor signaling, dMyc expression does not promote endoreplication, suggesting that InR/Tor signaling is required for dMyc activity [54]. Grewal et al. also reported the involvement of the Tor pathway in the regulation of ribosome biogenesis in vivo. *Drosophila* TIF-IA regulates pre-rRNA synthesis through the recruitment of Pol I to the rDNA promoter. While Tif-IA$^{-/-}$ mutants exhibit a growth arrest phenotype, TIF-IA overexpression increases ribosome biogenesis in larvae. Treatment with rapamycin, an inhibitor of Tor, inhibits the association of TIF-IA with rDNA. In addition, TIF-IA overexpression can increase the levels of pre-RNA even in rapamycin-treated larvae, suggesting that TIF-IA functions downstream of Tor [55]. These studies suggest a link between the Tor pathway, Myc, and ribosome biogenesis, which plays an important role in controlling cell growth during development.

Nucleolar proteins in aging

Because the nucleolus functions as a regulator of cell proliferation, cell cycle progression, and telomerase replication, the nucleolus could also be associated with senescence. Sir2, a key regulator of replicative senescence in yeast, is localized in the nucleolus [56] where it regulates rDNA silencing [57, 58] and inhibits homologous recombination at the rDNA locus [59]. Sirt1, the mammalian homolog of Sir2, complexes with nucleomethylin and SUV39H1 to form energy-dependent nucleolar silencing complex that inhibits the transcription of rRNA during nutrient starvation [60, 61]. TORC1 signaling, whose reduction is associated with the extension of lifespan in *Drosophila* and *Caenorhabditis elegans* [62], also regulates ribosome biogenesis [63]. These data imply that changes in nucleolar function regulate lifespan. Consistent with these findings, recent in vivo studies have clearly shown that ribosome biogenesis modulates aging and longevity of organisms. For example, yeast strains lacking genes encoding different 60S ribosomal proteins show extended replicative lifespan. Moreover, deletion of 60S-specific ribosomal processing factors or inhibition of 60S subunit biogenesis by treatment with small molecule inhibitors increased the replicative lifespan of yeast [13], suggesting that 60S ribosome biogenesis is a negative regulator of longevity. Using *C. elegans* as a model organism, Hansen et al. showed that reducing the levels of ribosomal proteins by RNAi inhibited protein synthesis and increased lifespan [12]. Furthermore, *C. elegans* subjected to RNAi knockdown of *nog-1*, which encodes a nucleolar GTPase required for 60S ribosome biogenesis, show increased lifespan, whereas those overexpressing *nog-1* show decreased adult longevity [14]. These data indicate that ribosome biogenesis regulates lifespan in vivo. A very recent study by Demontis et al. showed a fascinating association among myokine, nucleolar function, and aging in *Drosophila*. Mnt, a basic helix-loop-helix transcription factor, regulates the expression of genes involved in ribosome biogenesis. Overexpression of Mnt in the skeletal muscle decreases age-related climbing defects and prolongs lifespan. Gene expression analysis showed that Mnt overexpression downregulated the expression of genes encoding nucleolar proteins, which in turn decreased rRNA levels. RNAi knockdown of proteins involved in rRNA synthesis and ribosome biogenesis extended the medial lifespan, suggesting that decreased nucleolar function regulates longevity. Interestingly, changes in nucleolar function by Mnt overexpression were observed not only in the skeletal muscle but also in the adipose tissue. This intertissue control of nucleolar function depends on the activity of myoglianin, a myokine whose expression is induced by Mnt. Similar to Mnt, myoglianin overexpression in the skeletal muscle extends the lifespan and decreases nucleolar size in the adipose tissue [64]. These data suggest that myokine-mediated intertissue connection between the skeletal muscle and adipose tissue regulates lifespan at a whole-body level by modulating ribosome biogenesis.

Although these findings clearly demonstrate the relationship between the rate of ribosome biogenesis and aging, it is unclear how the reduction in ribosome biogenesis extends lifespan. Because increased levels of Arf and p53 induce cancer resistance and extend the lifespan of mice [65], it can be suggested that p53 activation induced by reduced ribosome biogenesis, which is observed in stem cells (see above), may be involved in longevity. Moreover, reduced metabolic rate with a corresponding decrease in free radical production is associated with aging and lifespan [66], suggesting an association between ribosome biogenesis and aging. It will be interesting to examine whether p53 activation or decreased oxidative stress contributes to lifespan extension induced by reduced ribosome biogenesis.

Specific functions of ribosomal proteins

Although ribosome is an essential cellular component that facilitates protein synthesis in all cells, recent evidences indicate that changes in the rate of ribosome biogenesis regulate tumorigenesis, stem cell maintenance, and aging.

This raises a question as to why dysregulation of ribosomal proteins induces cell type-specific phenotypes rather than widespread dysfunction. For example, decreased expression of ribosomal proteins in ribosomopathies mainly affects hematopoietic cells in the bone marrow but not other cells in the body (described above). Moreover, knockdown of proteins involved in pre-rRNA processing induces the apoptosis of ES cells but does not significantly affect differentiated cells [11]. Furthermore, in some cases reduced ribosomal biogenesis delays aging but in other cases they induce tumorigenesis. Given the increased demand for protein synthesis in proliferating cells, this specific phenomenon could be attributed to intrinsic differences in cellular activity. Rapidly proliferating ES cells are more sensitive to changes in ribosome biogenesis than differentiated cells. However, the mechanisms underlying cell type-specific dysfunction induced by ribosome dysregulation are poorly understood thus far.

Increasing evidences of unique functions of multiple ribosomal proteins in a wider context have important implications for understanding the cell type-specific roles of ribosome biogenesis. Two studies showed a transient increase in the expression of specific ribosomal proteins in a developmental stage- and stimulus-dependent manner. In the context of sexual differentiation of zebra finch song system, Tang et al. showed that genes encoding ribosomal proteins L17 and L37 were specifically upregulated in the song control nuclei of the forebrain of developing males [67]. To determine the genes involved in freeze tolerance of wood frog, which can survive after freezing in winter, Wu et al. analyzed the gene expression profiles of cold- and warm-acclimated frogs and observed that expression of the gene encoding ribosomal large subunit protein 7 (RPL7) was specifically upregulated in the skin of cold-acclimated frogs [68].

In addition to gene expression analysis, knockdown experiments have clearly shown the requirement of specialized functions of ribosomal proteins for tissue regeneration and development. Translation of ribosomal protein L4 (Rpl4) is increased in PC12 cells during neurite regeneration after neurite injury but is inefficient in differentiated PC12 cells [69]. Knockdown of *Rpl4* blocks neurite regeneration, suggesting that translational control of Rpl4 is required for rapid axonal regeneration. In addition to in vitro experiments, in vivo studies have shown that ribosomal proteins perform distinct functions during specific developmental processes. In zebrafish, knockdown of multiple ribosomal proteins gives rise to phenotypes specific to each gene rather than non-specific defects, with abnormalities in the brain, body trunk, eye, and ears [70], suggesting the specific functions of ribosomal proteins. Similarly, loss-of-function analysis showed that ribosomal protein L22 (Rpl22) and its paralog Rpl22l1 have distinct functions in zebrafish. Rpl22 morphants exhibited arrested T cell development in a p53-dependent manner, and knockdown of Rpl22l1 impaired the emergence of HSCs in a p53-independent manner. Mechanistically, both Rpl22 and Rpl22l1 bind to *smad1* mRNA, which is an important regulator for HSC emergence, but play distinct roles in smad1 expression, with Rpl22l1 facilitating smad1 expression and Rpl22 repressing it [71]. In yeast, mutant analysis showed that cells lacking ribosomal protein paralogs exhibited different phenotypes with distinct gene expression changes and that translation of specific mRNAs required a specific subset of ribosomal proteins [72]. A very recent study showed an unexpected role of ribosomal proteins in mouse embryonic development. Mice with mutation in *Rpl38* exhibited homeotic transformation along the anterior–posterior axis of skeletal patterning. In *Rpl38*-mutant embryos, global protein synthesis was not changed but translation of 8 out of 39 Hox-encoding genes was inhibited, suggesting that Rpl38 facilitated the expression of a specific subset of Hox proteins [73]. This specialized translation was mediated by structured RNA elements embedded in the 5′-UTR of *Hox*, through which RPL38 regulated 80S ribosome complex formation and facilitated cap-independent translation. An additional regulatory element in the 5′-UTR of *Hox* blocked cap-dependent translation and enabled Rpl38-dependent specialized translation [74], suggesting that Rpl38 and 5′-UTR elements control gene expression. These data suggest that ribosomal proteins are expressed in a tissue-specific manner and could regulate developmental processes.

Concluding remarks

Recent evidences suggest that the nucleolus functions not only as a housekeeping factor but also as an active regulator of disease progression, stem cell maintenance, and longevity both in vitro and in vivo (Table 1). Although the exact molecular mechanisms underlying the regulation of these cellular processes by the nucleolus are largely unknown, increasing evidences suggest that nucleoli and ribosome mediate these cellular processes by regulating the expression of specific genes in a cell type-dependent manner. We anticipate that advancements in our understanding of nucleolar functions will not only reveal the novel molecular mechanisms underlying tumorigenesis, stem cell maintenance, and aging but also offer new

Table 1 Novel functions of nucleolar proteins

Protein name	Protein function	Change in protein expression	Organism	Condition	Newly identified phenotype	References
BRF	Involved in the transcription of 5S rRNA	Knockdown	*Drosophila*	In vivo	Extended lifespan	[64]
CG5033	Involved in pre-rrna processing and ribosome maturation	Knockdown	*Drosophila*	In vivo	Extended lifespan	[64]
Dyskerin	A component of small nucleolar ribonucleoprotein particles involved in pseudouridylation	Mutation	Human	In vivo	Bone marrow failure, abnormal skin pigmentation, cancer (X-linked dyskeratosis congenita)	[28, 29]
Fibrillarin	A methyltransferase for ribosomal RNA processing	Overexpression	Mouse	In vitro	Prolongation of pluripotent state of embyonic stem cell	[11]
		Knockdown	Mouse	In vitro	Embryonic stem cell differentiation, decreased self-renewal ability	
Multiple ribosomal proteins	Components of 40S and 60S subunit	Knockdown	*C. elegans*	In vivo	Increased lifespan	[12]
Multiple ribosomal proteins	Components of 60S subunit	Deletion	Yeast	In vivo	Increased replicative lifespan	[13]
Multiple ribosomal proteins	Ribosomal protein	Knockdown	Zebrafish	In vivo	Abnormalities in the brain, body trunk, eyes, and ears	[70]
NOG-1	A nucleolar GTPase required for 60S ribosome biogenesis	Knockdown	*C. elegans*	In vivo	Increased lifespan	[14]
		Overexpression			Decreased adult longevity	
Nom1	Involved in pre-rRNA processing	Mutation	zebrafish	In vivo	Defects in endoderm development	[52]
Nopp140	A nucleolar phosphoprotein which functions as a snoRNP chaperone	Knockdown	*Drosophila*	In vivo	Apoptosis induction in larval wing discs	[51]
		Knockdown	*Drosophila*	In vivo	Extended lifespan	[64]
Nucleolin	Involved in various cellular processes (e.g., ribosome biogenesis, cell cycle, proliferation)	Knockdown	Mouse	In vitro	Embryonic stem cell differentiation, decreased self-renewal ability	[47]
Nucleophosmin	Involved in various cellular processes (e.g., centrosome duplication, protein folding)	Overexpression of mutant protein	Mouse	In vitro	p53 activation	[37]
		Heterozygous knockout	Mouse	In vitro, in vivo	Increased tumorigenesis	[38]
		Overexpression	Human	In vivo	Malignant transformation	[40]
Nucleostemin	A GTP-binding protein involved in the regulation of cell cycle and proliferation	Overexpression	Human	In vitro	Increase in tumor-initiating properties of cells	[42]
		Knockdown	Mouse	In vitro	Embryonic stem cell differentiation, decreased self-renewal ability	[48]
		Knockdown	*Drosophila*	In vivo	Loss of imaginal island cells	[49]
RMRP	A component of the RNase MRP complex involved in pre-rRNA processing	Mutation	Human	In vivo	Cartilage/skeletal defects, hypoplastic anemia, cancer (cartilage-hair hypoplasia)	[30]
RPL4	Ribosomal protein	Knockdown	Rat	In vitro	Impairment of neurite regeneration	[69]
RPL7	Ribosomal protein	Upregulation	Wood frog	In vivo	Involved in natural freezing survival?	[68]
RPL17, RPL37	Ribosomal protein	Upregulation	Zebra finches	In vivo	Involved in induction of song circuit?	[67]
RPL22	Ribosomal protein	Knockdown	Zebrafish	In vivo	Impairment of the development of T lineage progenitors	[71]

Table 1 continued

Protein name	Protein function	Change in protein expression	Organism	Condition	Newly identified phenotype	References
RPL22L1	Ribosomal protein	Knockdown	Zebrafish	In vivo	Impairment of the emergence of hematopoietic stem cells	[71]
RPL36A	Ribosomal protein	Overexpression	Human	In vitro	Enhanced colony formation	[7]
RPL38	Ribosomal protein	Deletion	Mouse	In vivo	Homeotic transformations of the axial skeleton	[73], [74]
RpLP0	Ribosomal protein	Knockdown	*Drosophila*	In vivo	Extended lifespan	[64]
RPS14	Ribosomal protein	One allele deletion	Human	In vivo	Macrocytic anemia, acute myeloid leukemia (5q- syndrome)	[27]
RPS19	Ribosomal protein	Heterozygous mutation	Human	In vivo	Bone marrow failure, congenital anomalies, cancer (Diamond-Blackfan anemia)	[25]
SSRP	Involved in rDNA transcription	Knockdown	*Drosophila*	In vivo	Extended lifespan	[64]
TIF-IA	RNA polymerase I transcription initiation factor	Knockdown	Mouse	In vitro	Differentiation of hematopoietic stem cells	[10]
		Knockdown	*Drosophila*	In vivo	Growth arrest	[55]
Udd	A component of the Pol I regulatory complex	Heterozygous deletion	*Drosophila*	In vivo	Germ cell loss	[9]
Wicked	A U3 snoRNA-associated protein required for pre-rRNA maturation	Mutation	*Drosophila*	In vivo	Premature differentiation of germline stem cells	[8]

therapeutic targets for diseases such as cancer, ribosomopathies, and age-associated diseases.

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