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Review

Activation of the tumor suppressor p53 upon impairment of ribosome biogenesis☆

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ABSTRACT

Errors in ribosome biogenesis can result in quantitative or qualitative defects in protein synthesis and consequently lead to improper execution of the genetic program and the development of specific diseases. Evidence has accumulated over the last decade suggesting that perturbation of ribosome biogenesis triggers a p53-activating checkpoint signaling pathway, often referred to as the ribosome biogenesis stress checkpoint pathway. Although it was originally suggested that p53 has a prominent role in preventing diseases by monitoring the fidelity of ribosome biogenesis, recent work has demonstrated that p53 activation upon impairment of ribosome biogenesis also mediates pathological manifestations in humans. Perturbations of ribosome biogenesis can trigger a p53-dependent checkpoint signaling pathway independent of DNA damage and the tumor suppressor ARF through inhibitory interactions of specific ribosomal components with the p53 negative regulator, Mdm2. Here we review the recent advances made toward understanding of this newly-recognized checkpoint signaling pathway, its role in health and disease, and discuss possible future directions in this exciting research field. This article is part of a Special Issue entitled: Role of the Nucleolus in Human Disease.

1. Introduction

The mammalian ribosome is a complex structure composed of four RNAs (rRNAs) and 80 distinct proteins (RPs) [1,2]. Increased capacity for mRNA translation by way of ribosome biogenesis dictates the capacity of cells to grow, proliferate and differentiate [3–6]. Most steps in ribosome biogenesis are temporally and spatially organized within the nucleolus, where a 47S rRNA precursor is transcribed by RNA polymerase I, processed and modified by more than 150 non-ribosomal proteins and 100 small non-coding RNAs into the mature 18S rRNA of the 40S ribosomal subunit and to 5.8S and 28S rRNAs of the 60S ribosomal subunit [7–9]. Although the genes for 5S rRNA are transcribed in the nucleus by RNA polymerase III, 5S rRNA is assembled into the 60S ribosomal subunit in the nucleolus [8,10]. The RP mRNAs are transcribed in the nucleus by RNA polymerase II and translated on cytoplasmic ribosomes, imported into the nucleus, and assembled with the rRNA while the rRNA is processed in the nucleolus [11–15]. Specific RPs join nascent 60S and 40S subunits during their path from the nucleolus to the cytoplasm [11,14,15]. In addition to their roles in stabilization and promotion of correct folding of rRNAs for ribosome assembly, RPs are involved in export of ribosomal precursors and regulation of specific steps in protein synthesis [11,14,15]. However, the exact requirement of individual RPs for different stages of ribosome biogenesis and/or distinct steps of mRNA translation process in mammals is just beginning to be understood [11,14,15].

Given the enormous energy investment in ribosome biogenesis, the proper execution of this component of the genetic program has high importance. Errors in this process can result in the development of a number of pathological conditions. We and others have hypothesized that molecular mechanisms must have evolved to sense the fidelity of this critical cellular process to prevent the development of disease [6,16,17]. The first indication of this came from a study using an inducible model for deleting the gene encoding the S6 ribosomal protein, *Rps6*, in the liver of adult mice, which led to abrogation of nascent 40S ribosome biogenesis and abolition of cell proliferation in the liver following partial hepatectomy [17]. These results could not be simply attributed to the loss of protein synthetic capacity in these cells as pre-existing ribosomes provided sufficient translational capacity to increase liver size in response to a fasting/re-feeding regimen, a process that primarily involves an increase in cell mass but not in cell number. These observations suggested the existence of a novel cell cycle checkpoint triggered by impaired ribosome biogenesis [17], in a manner analogous to of checkpoints triggered by DNA damage [18]. Studies over the last decade have convincingly demonstrated that perturbation of ribosome biogenesis activates the tumor suppressor p53 *via* binding of several ribosomal components to its negative regulator, Mdm2, independent of DNA damage [19–22]. The p53 is best known for its role as

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a tumor suppressor. The exposure of cells to various stressors activates p53, which regulates transcription of many coding and noncoding genes, with ensuing multiple outcomes including cell cycle arrest, senescence, apoptosis, changes in metabolism, and DNA repair [23,24]. Disruption of these functions allows continuous proliferation, genomic instability and evolution of stress-damaged cells, resulting in their illegitimate survival and malignancy [24,25]. Given that the loss of wild-type p53 provides many selective advantages to cells, it comes as no surprise that half of all human malignant tumors have mutations within the *TP53* gene [26]. In cancers retaining wild-type p53, the functions of p53 are likely inactivated by defects in upstream or downstream p53 network components [23]. As an abundance of evidence has shown, inherited and acquired abnormalities in ribosome function can lead to tumorigenesis [27,28] and thus it can be speculated that induction of a p53-dependent checkpoint response might prevent expansion of such potentially hazardous cells [29]. Although there are some indications in support of this idea, definitive evidence has not yet been provided [30]. Recent evidence has shown that p53 activation upon impairment of ribosome biogenesis can also be responsible for certain pathological manifestations in mice and humans [31–35].

2. Impairment of ribosome biogenesis at various stages can activate the p53 tumor suppressor

2.1. Evidence from cell culture studies

Following the study of mice with liver-specific inducible deletion of the *Rps6* gene that demonstrated the existence of a previously unrecognized checkpoint triggered as a result of deficiency in ribosome biogenesis [17], Pestov and co-workers provided the first evidence that the p53 tumor suppressor is a component of this pathway [20]. They demonstrated that the expression of dominant negative mutants of Bop1, which inhibit rRNA processing, prevented cell cycle progression in a p53-dependent manner. A number of subsequent investigations have demonstrated that other perturbations of ribosome biogenesis in cell culture can also trigger the p53 response [22]. That the inhibition of rRNA transcription can lead to functional alterations of the nucleolus and upregulation of p53 protein has been demonstrated in many different ways including: genetic inactivation of the RNA polymerase I (Pol I) transcription factor TIF-1A [36], blockage of Pol I transcription factor UBF by microinjection of specific monoclonal antibodies [37], silencing the *POLR1A* gene coding for the Pol I catalytic subunit [38], treatment with the immunosuppressant mycophenolic acid [39,40], low concentrations (<10 nM) of actinomycin D, which intercalates into the GC-rich regions of rDNA [37,41] or the small molecule compound CX-3543 (quarfloxin) that impairs binding of SL1/TIF-1B to the rDNA promoter leads to functional and morphological alterations of the nucleolus and stabilization of p53 protein levels [42]. Furthermore, the inhibition of rRNA processing by treatment with a chemotherapeutic compound 5-fluorouracil [43,44] or decreased expression of proteins required for maturation of 18S and 28S rRNA such as hUTP18 [45], PAK1IP1[46], WDR3[47], WDR12 [45], WDR36 [48], nucleophosmin (NPM, B23) [49], nucleostemin [50] as well as specific RPs of either 40S or 60S [51] including RPS6 [51], RPS9 [52], RPL23 [53–55], RPL7a [51], RPS7 [51,53,54], RPL24 [31], RPL26 [53], RPL29 [56], RPL30 [56], RPL37 [57], RPS14 [58,59], RPS19 [58], RPS15, RPS20 and RPL37 [60] can also induce a p53-mediated stress signal. Additionally, it has been recently demonstrated that the inhibition of RP nuclear import or nuclear export of ribosomal subunits by depletion of importin 7 (IPO7) or exportin 1 (XPO1), respectively, perturbs ribosome biogenesis, and consequently triggers the p53 response [61]. Taken together, inhibition of ribosome biogenesis at various levels consistently leads to p53 accumulation (Fig. 1). However, it remains to be determined how various lesions in ribosome biogenesis are sensed by this p53-dependent checkpoint mechanism.

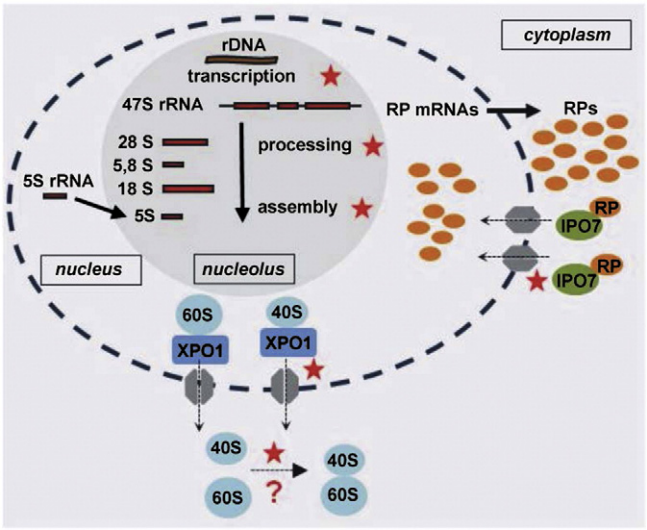

Fig. 1. Impairment of ribosome biogenesis at various steps can trigger p53 upregulation. Inhibition of Pol I transcription, rRNA processing, assembly of ribosomal subunits, RP nuclear import by depletion of importin 7 (IPO7) or nuclear export of 40S and 60S ribosomal subunits by the depletion of exportin 1 (XPO1) can lead to p53 upregulation (indicated by red stars). p53 upregulation by impaired association of the 40S and 60S subunits has not been unambiguously demonstrated (question mark).

2.2. Evidence from in vivo studies

The first *in vivo* evidence implicating p53 as the critical checkpoint component triggered by deficiency in ribosome biogenesis was obtained by studies of mouse lines employing T cell-specific [21] and ubiquitous deletions of one *Rps6* allele [34]. Conditional deletion of one *Rps6* allele in T cells inhibited their accumulation in the spleen and lymph nodes, because of decreased survival. Additionally, T cell receptor-mediated stimulation of *Rps6*-heterozygous T cells induced a normal increase in their size, but cell cycle progression was impaired. Genetic inactivation of p53 in *Rps6*-haploinsufficient T cells rescued this proliferative defect and restored normal numbers of T lymphocytes in the peripheral lymphoid organs, suggesting that the defect in ribosome biogenesis activated a p53-dependent apoptosis and cell cycle checkpoints to prevent the survival and proliferation of defective T lymphocytes [21]. To investigate the response to *Rps6*-deficiency in the whole organism, one *Rps6* allele was conditionally deleted in growing mouse oocytes and *Rps6*-heterozygous embryos generated [34]. Embryonic development up to embryonic day 5.5 (E5.5) was unaffected. However, gastrulating *Rps6*-heterozygous embryos (E5.5–E7.5) displayed a dramatic increase in p53 protein levels, inhibition of cell cycle progression and apoptosis, which resulted in embryonic lethality at this developmental period, at which under normal conditions ribosome biogenesis and cell proliferation are dramatically upregulated. Inactivation of *p53* in *Rps6*-heterozygous embryos bypassed this gastrulation checkpoint and allowed development until E12.5, when they died with diminished fetal liver erythropoiesis and severe placental defects, most likely because a defective translation of specific mRNAs or an uncharacterized p53-independent checkpoint response [34].

In contrast to *Rps6*-heterozygous mice, *Rpl22*-heterozygous mice showed no obvious pathological phenotype. However, *Rpl22*-null mice displayed a selective defect in the development of αβ-lineage but not γδ T cells, which was rescued in a p53-negative genetic background [62]. More recently, the role of p53 in the phenotype of the Belly Spot and Tail (*Bst*) mouse that carries a hypomorphic mutation in one allele of the *Rpl24* gene has been analyzed [31]. *Rpl24*$^{Bst/+}$ mice reach adulthood and display a number of pathological phenotypes including reduced body size, a white ventral middle spot, retinal abnormalities, a kinked tail, and other skeletal abnormalities. It was demonstrated