Ribosome Biogenesis and Control of Cell Proliferation: p53 Is Not Alone

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Abstract

Cell growth is a prerequisite for cell proliferation, and ribosome biogenesis is a limiting factor for cell growth. In mammalian cells, the tumor suppressor p53 has been shown to induce cell-cycle arrest in response to impaired ribosome biogenesis. Recently, p53-independent mechanisms of cell-cycle arrest in response to alterations of ribosome biogenesis have been described. These findings provide a rational basis for the use of drugs that specifically impact ribosome biogenesis for the treatment of cancers lacking active p53 and extend the scenario of mechanisms involved in the relationship between cell growth and cell proliferation. Cancer Res; 72(7): 1602–7.

Introduction

Cell growth (increase in cell mass) and cell proliferation (increase in cell number) are two tightly linked phenomena. In cells stimulated to proliferate, a progressive increase in cell constituents occurs before division ensuring appropriately sized daughter cells (1). Cell growth is the consequence of an enhanced stimulation of protein synthesis that characterizes proliferating cells. The increased demand for protein synthesis is accomplished by changes in the rate of ribosome biogenesis, and ribosome biogenesis is the major metabolic effort in a proliferating cell (2). Ribosome biogenesis is the result of a series of coordinated steps occurring in the nucleolus. These include the transcription of ribosomal genes by RNA polymerase I (Pol I) to produce the 47S rRNA precursor, the modification and processing of this transcript to generate the mature 18S, 5.8S, and 28S rRNA, the import to the nucleolus of 5S rRNA and ribosomal proteins, the assembly of the rRNAs with the ribosomal proteins to form the large 60S and the small 40S subunits of the mature ribosome, and the export of ribosomal subunits to the cytoplasm (3). Transcription of ribosomal genes requires the assembly of a specific multiprotein complex at the rDNA promoter containing Pol I and, in mammals, at least 3 other basal factors: the transcription initiation factor I (TIF-I) A, the selectivity factor I (SL1), and the upstream binding factor (UBF; reviewed in ref. 4; see Fig. 1).

It is well established that at the end of G1 phase, the so-called restriction point defines a limit beyond which the cell is committed to divide, independent of growth (5). Therefore, it is within the G1 phase that a proliferating cell has to carry out its effort of supplying the components necessary for the transit from G1 to S-phase, and it is at the end of G1 phase that the cell must determine whether this effort has been successfully accomplished. Data indicate that it is the amount of ribosomes produced that controls the G1–S-phase transition, thus regulating the cell-cycle progression. In partially hepatectomized mice with induced conditional deletion of 40S ribosomal protein S6, hepatocytes with hindered ribosome production but not hindered protein synthesis failed to enter the S-phase (6). Drug-induced stimulation or inhibition of rRNA synthesis caused an accelerated or delayed G1–S-phase progression, respectively, in rat hepatoma cells, due to an accelerated or delayed achievement of the appropriate amount of ribosomes during the G1 phase (7). Regarding the mechanism that regulates the cross-talk between ribosome biogenesis and cell-cycle progression, there is a general consensus on the key role played by the tumor suppressor p53. In fact, as described more extensively later, it has been clearly shown that altered ribosome biogenesis is responsible for p53 stabilization and, therefore, for the arrest of cell-cycle progression (reviewed in refs. 8–10). On the other hand, very recent data indicate that p53-independent mechanisms are also activated that hinder proliferation as a consequence of altered ribosome biogenesis (11–15).

Ribosome Biogenesis and the Control of Cell-Cycle Progression: the p53 Paradigm

Defects in ribosome production cause p53 stabilization and induce a marked p53-dependent inhibition of cell proliferation (10). It is worth recalling that in proliferating mammalian cells, the G1–S-phase restriction point can be overwhelmed by the activation of the E2F transcription factor family (16), which regulates the expression of genes whose products are necessary for the synthesis of DNA, and therefore for the progression...
from G₁ to S-phase. The retinoblastoma protein (pRB), the product of the tumor suppressor RB1 gene, in its active hypophosphorylated form, is bound to E2Fs and prevents them from inducing E2F target genes. In the hyperphosphorylated form, pRB no longer binds to E2Fs, which can then activate their target genes. Phosphorylation of pRB is triggered in the early G₁ phase by cyclin D–cyclin-dependent kinase (CDK)-4 and -6 complexes and is completed at the end of the G₁ phase by cyclin E–CDK-2 complexes. pRB phosphorylation is hindered by CDK inhibitors: CDK-4 and CDK-6 are inhibited mainly by p16Ink4a, whereas p21(Cip1) and p27(Kip1), as shown in Fig. 2A, p53 stabilization, occurring in response to a variety of cellular stresses, leads to induction of the CDK-2 inhibitor p21, inhibition of cyclin E–CDK-2 complexes, and pRB-dependent cell-cycle arrest at the G₁–S-phase checkpoint (16).

The mechanism of p53 stabilization after perturbation of ribosome biogenesis is apparently the consequence of changes in functional and physical interactions of the tumor suppressor with MDM2. MDM2 negatively controls p53 activity in 2 ways: by binding to the protein and interfering with its transactivation activity and by facilitating p53 proteasomal degradation thereby acting as an E3 ubiquitin ligase (17). As a consequence of altered ribosome biogenesis, several ribosomal proteins no longer used for ribosome construction bind to MDM2 and relieve its inhibitory activity toward p53. In fact, it was shown that the ribosomal proteins RPL5 (18), RPL11 (19–21), RPL23 (22, 23), and RPS7 (24) bind MDM2, thus inducing p53 stabilization by inhibiting MDM2’s E3 ubiquitin ligase function (also reviewed in ref. 10). The inhibition of rRNA transcription, induced either by drugs such as actinomycin D, 5-fluorouracil, or mycophenolic acid, or by depletion of essential Pol I complex components (10, 15, 25), stabilizes p53 by causing ribosomal protein–induced inactivation of MDM2. These data are consistent with a mechanism by which altered ribosome biogenesis inhibits cell proliferation in a strictly p53-dependent manner through the activation of the ribosomal protein–MDM2–p53 pathway.

**p53-Independent Mechanisms**

The regulation of the cell cycle in response to defects in ribosome biogenesis, far from being exclusive to mammalian cells, also occurs in organisms where p53 does not regulate the cell cycle or is not even present. In the metazoan Drosophila melanogaster, p53 does not regulate p21 transcription, nor does it regulate the cell cycle (26). In this organism, haploinsufficiency of several ribosomal protein genes gives rise to the minute phenotype. Cells from minute mutants are the same size as wild type, but their proliferation rate is reduced (27).
implying the existence of a mechanism controlling cell-cycle progression in response to hampered ribosome biogenesis. Depletion of the RNA polymerase I cofactor TIF-IA induces a p53-independent proliferation arrest in Drosophila cells (28). Furthermore, in the yeast Saccharomyces cerevisiae, where no p53 homolog has been found, defective ribosome biogenesis delays the cell-cycle progression to S-phase before any variation in protein synthesis capacity ensues (29).

The presence of a p53-independent pathway regulating the relationship between ribosome biogenesis and cell proliferation in mammalian cells was first suggested by the observation that selective inhibition of rRNA synthesis by actinomycin D was able to induce a perturbation of cell-cycle progression also in cells silenced for p53 expression, although milder than that occurring in the presence of p53 (30). In the past 2 years, some light has been shed on how
altered ribosome biogenesis can hinder cell proliferation in a p53-independent way. A good example is constituted by pescadillo depletion. The pescadillo nucleolar protein plays a role in the processing of pre-rRNA molecules during assembly of 60S ribosomal subunits, through formation of the PeBoW complex with Bop1 and WDR12 proteins (11). Depletion of pescadillo inhibits ribosome biogenesis, and, similar to other mechanisms that hinder ribosome production, stabilizes p53, leading to cell-cycle arrest (11). However, pescadillo gene knockdown impaired cell proliferation in cells harboring mutated p53, indicating that in these cells, the observed cell-cycle control mediated by pescadillo protein was p53 independent (13). In fact, pescadillo depletion resulted in decreased expression of cell-cycle protein cyclin D1 and upregulation of the CDK inhibitor p27, with the consequent marked reduction of pRB phosphorylation (13).

Another p53-independent mechanism of cell proliferation arrest induced by impaired ribosome biogenesis was shown by the downregulation of PIM1 expression caused by ribosomal protein deficiency and various other ribosomal stressors (14). PIM1 is a constitutively active serine–threonine kinase regulated by cytokines, growth factors, and hormones that has been shown to reduce the activity of p27Kip1 and increase its degradation (31). Furthermore, PIM1 kinase has been shown to interact with the ribosomal protein RPS19, and it has been shown that depletion of RPS19 or other ribosomal proteins caused increased PIM1 kinase proteasome degradation (14). The downregulation of PIM1 kinase stabilized and activated p27Kip1, thus causing a block in cell-cycle progression regardless of p53 status (14).

The data reported above indicate that changes in the biogenesis of single ribosome subunit hinder cell proliferation in cells with activated or inactivated p53. Moreover, data have recently been reported showing that specific inhibition of rRNA transcription by depletion of POLR1A hindered cell-cycle progression in cancer cell lines with inactivated p53 (15). This was due to the fact that the inhibition of rRNA synthesis decreased the expression of E2F-1. Normally, E2F-1 is protected from proteasome-mediated degradation by the interaction with MDM2, which prevents the binding of other E3 ligases responsible for E2F-1 ubiquitination (32). The inhibition of rRNA synthesis releases the ribosomal protein L11, which, by binding to MDM2, prevents its stabilizing function on the E2F-1 protein (15). The downregulation of the E2F-1 protein caused a reduction in the expression of the E2Fs target genes that are necessary for the entry and progression through the S-phase. RB1 silencing rescued the expression of these genes completely and prevented the effect of the rRNA synthesis inhibition on cell proliferation. The inhibition of rRNA synthesis is not always associated with a downregulation of E2F-1. In fact, actinomycin D, cisplatin, and etoposide—drugs that inhibit rRNA transcription—caused an accumulation of E2F-1 protein (33). However, these drugs are also DNA-damaging agents, and it has been shown that these agents increase E2F-1 half-life and its transcriptional activity (33). Importantly, reducing the synthesis of rRNA by TIF-IA silencing, that is, a non–DNA-damaging procedure to inhibit rRNA transcription, reduced the expression of the E2F-1 protein (15).

Finally, another mechanism defining the relationship between ribosome biogenesis and cell proliferation in cells with inactive p53 is the downregulation of c-Myc in response to ribosome stress. c-Myc, in addition to stimulating cell proliferation, controls all the steps of ribosome biogenesis: It increases Pol I activity by facilitating the recruitment of SL1 to promoters, stimulates ribosomal protein synthesis by increasing Pol II transcription, and enhances Pol III transcription by activating TFIIIB (34). It was found that, in response to ribosome biogenesis inhibition, RPL11 binds to c-Myc, reducing its transcriptional activity and to c-Myc mRNA, promoting its degradation (12). The RPL11-mediated downregulation of c-Myc reduced cell proliferation, and this effect was also observed in p53-null cells, thus showing another p53-independent mechanism of proliferation control (12).

In conclusion, there is now evidence that ribosomal stress hinders cell proliferation in mammalian cells with and without p53. The p53-dependent and -independent mechanisms by which ribosome biogenesis controls cell-cycle progression are schematically summarized in Fig. 2.

Ribosome Biogenesis Inhibition and Chemotherapy of p53-Deficient Cancer

The disruption of p53 function occurs in more than 50% of human tumors, thus representing the most frequent gene alteration in cancers (35). Commonly used chemotherapeutic agents in human cancer induce some cell stress, which activates the p53-mediated cell-cycle blockage and/or apoptosis. Therefore, tumors without functioning p53 are less sensitive to cytostatic and cytotoxic drug treatment than those harboring wild-type p53 (36). The p53-independent control of ribosome biogenesis on cell proliferation may explain some recent data showing an antiproliferative effect of Pol I complex inhibitors on cancer lacking functional p53. Indeed, a study conducted by Drygin and colleagues (37) showed that targeting factors of the rDNA transcription complex represents a promising strategy for chemotherapeutic treatment of cancers, both p53 proficient and deficient. During a screening assay for agents that selectively inhibit Pol I transcription relative to Pol II transcription, CX-5461 (Cylene Pharmaceuticals Inc.), a potent small molecule that selectively inhibits Pol I–driven transcription, was identified (37). It was shown that CX-5461 disrupts the binding of the SL1 transcription factor to the rDNA promoter, thus preventing the initiation of rRNA synthesis by the Pol I multiprotein complex. In fact, SL1 mediates specific interactions between the rDNA promoter region and the Pol I enzyme complex by recruiting Pol I, together with a series of Pol I–associated factors, to rDNA. CX-5461 exhibited antiproliferative activity in numerous cancer cells in vitro, and, importantly, this occurred in wild-type and mutant p53 cell lines. In this study, the authors found that the mechanisms leading to cell death after inhibition of rRNA synthesis involved autophagy and senescence. Unfortunately, potential mechanisms affecting the control of cell-cycle progression were not investigated. However, according to the results obtained by Pol I and TIF-IA
depletion, it is likely that the inhibitory effect on proliferation of cells lacking active p53 observed after CX-5461 treatment might be mediated by a downregulation of E2F-1.

Perspectives

Studies on p53-independent mechanisms regulating the relationship between cell growth and cell-cycle progression are expected not only to increase our knowledge of the homeostatic control of cell proliferation but also to reveal new aspects of the process of tumorigenesis and potential chemotherapeutic therapies for p53-negative cancers.

The stabilization of p53 following ribosomal stress plays an important role against neoplastic transformation (38). An altered ribosome biogenesis may be responsible for a failure in the p53 activation pathway, and this may facilitate tumor onset (39). Similarly, functional inhibition of p53-independent mechanisms for opposing cell-cycle progression after ribosomal stress could downregulate the tumor suppressor potential of the cell. Experimental studies of the effect of alterations of factors involved in these mechanisms (such as Pim1, pescadillo, Bop1) on tumorigenesis, together with the analysis of the integrity of their function in human tumors could possibly point out their involvement in the process of neoplastic transformation and identify new targets for therapeutic applications.

The results reported in this review strongly encourage studies to develop inhibitors of ribosome biogenesis that downregulate E2F-1 expression. Furthermore, considering the role of pRB in controlling E2F-1 function, it might be of interest to combine the ribosome biogenesis inhibitors with drugs that hinder the activity of the CDKs. The resulting reduction of E2F-1 expression, together with the increased E2F-1 binding to hypophosphorylated pRB can be reasonably expected to have a very strong impact on cell proliferation in cancers lacking active p53. A similar chemotherapeutic approach should be highly effective on those cancers in which this transcription factor is known to exert a major role in the biology of the transformed cells. For example, aberrant E2F-1 activity is indeed a critical factor for the initiation and progression of B-cell Burkitt lymphoma (40) and for the emergence of castration-resistant prostate cancer (41).

The use of drugs targeting the CDKs in combination with ribosome biogenesis inhibitors that downregulate E2F-1 expression may also be very useful for treatment of cancers characterized by Myc overexpression, which is frequently observed in human tumors of different origin. In fact, these drugs may exert a p53-independent antiproliferative activity by both downregulating c-Myc mRNA expression as a consequence of an increased availability of RPL11 after the inhibition of rRNA synthesis and by reducing E2F-1 expression and activity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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