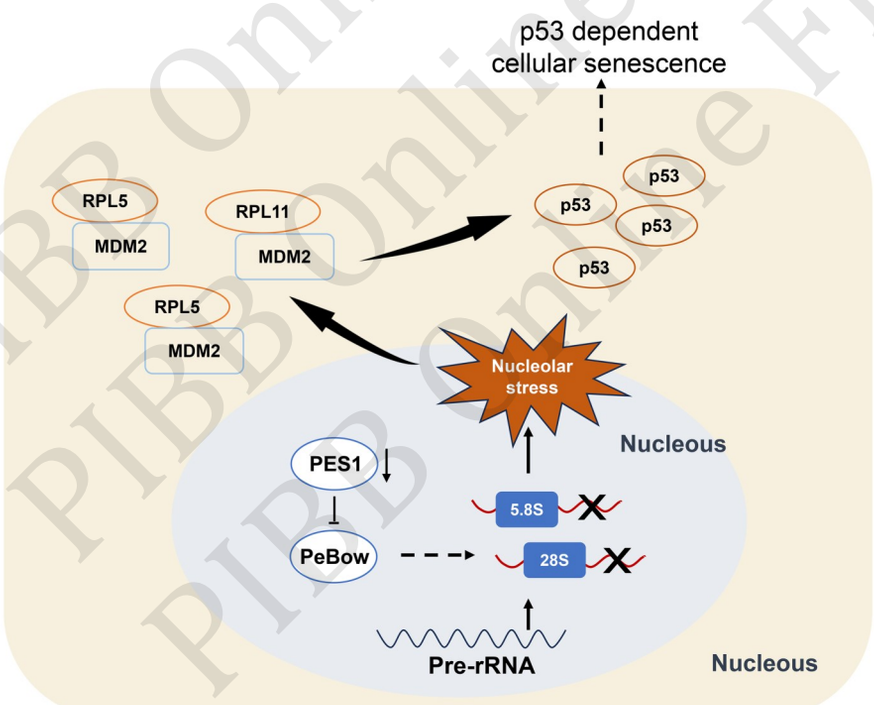
PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
XXXX, XX(XX): 1~12
www.pibb.ac.cn

PES1 Repression Triggers Ribosomal Biogenesis Impairment and Cellular Senescence Through p53 Pathway Activation*

ZHANG Chang-Jian[1,2], LI Yu-Fang[1,3], WU Feng-Yun[1,3], JIN Rui[1], NIU Chang[3]**, YE Qi-Nong[1]**, CHENG Long[4]**

([1]*Laboratory of Advanced Biotechnology, Academy of Military Medical Sciences, Beijing* 100071, China;
[2]*Senior Department of Otolaryngology-Head & Neck Surgery, the Sixth Medical Center, Chinese People's Liberation Army (PLA) General Hospital, Beijing* 100048, China;
[3]*College of Life Sciences, Capital Normal University, Beijing* 100069, China;
[4]*Beijing Institute of Geriatrics, Beijing Hospitall , Beijing* 100005, China)

Graphical abstract



* This work was supported by grants from China Postdoctoral Science Foundation (2023M744316), Special Scientific Research Project of Laboratory Animals (SYDW_KY[2021]07), Beijing Natural Science Foundation (M23003), and National High Level Hospital Clinical Research Funding (LYS-2023-16, BJ-2024-219).

** Corresponding author.
CHENG Long. Tel: 86-10-58115045, E-mail: biolongcheng@outlook.com
YE Qi-Nong. Tel: 86-10-66948841, E-mail: yeqn66@yahoo.com
NIU Chang. Tel: 86-10-68901494, E-mail: niuchang@cnu.edu.cn

Received: January 8, 2025 Accepted: April 14, 2025

Abstract **Objective** This study investigates the regulatory role of pescadillo ribosomal biogenesis factor 1 (PES1) in cellular senescence and elucidates its underlying molecular mechanisms. **Methods** Using replicative senescence models of mouse embryonic fibroblasts (MEFs) and doxorubicin-induced senescence in human hepatocellular carcinoma HepG2 cells, we first quantified PES1 expression dynamics through immunoblotting. Subsequently, siRNA-mediated PES1 knockdown in HepG2 or other cells was employed to assess senescence phenotypes *via* β-galactosidase staining and immunodetection of senescence-associated markers. Mechanistic exploration involved Northern blot for pre-rRNA processing analysis and fluorescence microscopy for nucleolar morphology observation. **Results** PES1 expression was significantly downregulated in both replicatively senescent MEFs and doxorubicin-induced senescent HepG2 cells. siRNA-mediated PES1 depletion triggered premature senescence characterized by increased SA-β-gal positivity and upregulated p53/p21 signaling, while Rb pathway components remained unaltered. Notably, PES1 deficiency impaired 28S rRNA biogenesis and induced nucleolar fragmentation, indicative of nucleolar stress. **Conclusion** Inhibition of PES1 expression can induce nucleolar stress and activate p53-dependent rather than Rb-dependent senescence signals within cells.

Key words PES1, cellular senescence, ribosomal biogenesis, p53
DOI：10.16476/j.pibb.2025.0005　　**CSTR**：32369.14.pibb.20250005

Cellular senescence is a state of irreversible cell cycle arrest that results from a variety of stresses[1-3]. Senescent cells accumulate across tissues during aging, contributing to the functional decline of organs and aging-related diseases[4-7]. The nucleolus is the most prominent subnuclear structure involved in ribosomal RNA (rRNA) synthesis and ribosome biogenesis in cells and thus linked to the regulation of cellular homeostasis and senescence[8-10]. The nucleolus has a typical tripartite organization and morphology[11], and pathologists have recognized the correlation between changes in nucleolar size and/or number and senescence. For example, small nucleolar size and transcriptionally refractive heterochromatic rDNA are often regarded as cellular hallmarks of longevity in diverse model organisms[12]. By contrast, increased nucleolar size is observed in premature aging cells in humans[13].

In the process of ribosome biogenesis, rRNAs are transcribed from 47S rRNA genes and processed by many cofactors and finally cleaved to form 5.8S, 18S and 28S rRNAs, while 5S rRNA is synthesized in the nucleoplasm[14]. Several lines of evidence suggest that senescent cells exhibit reduced ribosome biogenesis, an extremely energy-demanding process, due to an ageing related reduced energy metabolism. Conversely, inhibition of ribosome biogenesis through knockdown of processing factors can trigger cellular senescence which can be explained by a delay in rRNA processing eventually leading to the accumulation of the ribosomal proteins L5 (uL18) and L11 (uL5), which form a complex with the 5S rRNA and disable the E3 ligase MDM2 (or HDM2), thus activating p53[15]. Lessard *et al*.[16] also revealed that overexpression of the ribosomal protein S14 (RPS14 or uS11) is sufficient to inhibit Rb phosphorylation, inducing cell cycle arrest and senescence.

Pescadillo ribosomal biogenesis factor 1 (PES1) is a nucleolar protein of 588 amino acids that involved in rRNA processing and 60S subunit assembly, and it can form a complex with block of proliferation 1 (BOP1) and WD repeat domain 12 (WDR12) that plays an important role in the procession of the 32S pre-rRNA into mature 28SrRNA [17]. Numerous studies have shown that PES1 is associated with tumor cell proliferation, invasion and metastasis in many types of cancers[18], including prostate cancer[19], liver cancer[20], pancreatic cancer[21], thyroid cancer[22], breast cancer[23], ovarian cancer[24], gastric cancer[25]and colon cancer[26]. Our previously study revealed that PES1 can directly interact with telomerase to control telomerase activity, maintain telomere length, and regulate cellular senescence[27]. Whether and how PES1 regulates cellular senescence through rRNA processing remains largely unknown.

In this study, we reported that PES1 was down-regulated in senescent mouse embryonic fibroblast (MEF) cells and HepG2 cells. Knockdown of PES1 in HepG2 cells led to retardation in rRNA processing, which in turn activates p53 rather than Rb pathway, leading to cellular senescence. We demonstrated that PES1 affected cellular senescence by regulating ribosomal biogenesis, providing a novel perspective on the mechanisms involved in the senescence regulated by PES1.

1 Materials and methods

1.1 Reagents

Doxorubicin (DOX, 23214-92-8, MedChemExpress) was dissolved in dimethyl Sulfoxide (DMSO, D8371, Solarbio) to a concentration of 10 g/L and stocked at －20℃ until use.

1.2 Cell culture

HepG2 cells, HCT116 cells (wild type (wt) and $p53^{-/-}$) and DU145 cells were purchased from the American Type Culture Collection. MEFs were isolated from mouse embryos at day 14.5 of gestation as previously described[27]. Briefly, embryos were surgically extracted and separated from maternal tissues and the yolk sac. The tissues were then finely minced and incubated in a trypsin-EDTA solution while shaking at 37° C for 30 min. Following incubation, the solution was allowed to settle for 2 min, after which the supernatant was centrifuged at 1 000*g* for 3 min. The resulting pellet was resuspended in culture medium to obtain MEFs. Cells were maintained in DMEM containing 1 000 mg/L glucose supplemented with 10% fetal bovine serum (FBS). The cells were cultured in 5%CO_2 and 20% oxygen conditions.

1.3 SA−β−gal staining

senescence-associated β -galactosidase (SA- β -gal) staining was carried out according to the manufacturer's instructions (Byeotime, C0602). Cells were washed once with PBS and fixed with 0.5% glutaraldehyde in PBS at pH7.2 for 15 min. After washing in PBS, cells were stained in X-gal solution (100 mmol/L sodium phosphate, 2 mmol/L $MgCl_2$, 150 mmol/L NaCl, 0.01% sodium deoxycholate, 0.02% NP40, 5 mmol/L potassium ferricyanide, 5 mmol/L potassium ferrocyanide, and X-gal (1 g/L) at pH6.0) overnight at 37° C. Tissue sections were then stained with eosin.

1.4 siRNA transfection

One hour after their plating, cells were transfected with a final concentration of 10 nmol/L siRNA (a mix of 3 siRNA sequences) using Lipofectamine RNAiMax transfection reagent (Thermo Scientific, CA) according to the manufacturer's instructions. Cells treated under the same conditions with transfection reagent without siRNA were used as controls (mock-depleted cells). Six hours after transfection, the medium was replaced by fresh medium supplemented with 5% FBS, and the next day, by complete MEM. Phenotypes were analyzed and quantified 72 h posttransfection. The sequences of all siRNAs used in this study are as follows. sihPES1: 5'-ACCCAAACACAAGAAGAAGGUUAAC-3', 5'-UGUCAACAAGUUCCGUGAAUACAAG-3', 5'-GGCAGGCCAGAGGACCUAAGUGUGA-3'; simPES1: 5'-GGTGTTCTGGCTGCCTTGAATTTCT-3', 5'-GCGCAAGGTCTTCCTGTCCATTAAA-3', 5'-GCTCTACTCTGAACCTCCTTCCTCA-3'; sihp53 5'-GCAUCUUAUCCGAGUGGAA-3', 5'-CCCGGACGAUAUUGAACAA-3', 5'-CACUACAACUACAUGUGUA-3'.

1.5 Western blot

Cells were seeded in a 6-well plate (8×10^5 cells/well) and cultured overnight. After exposure to gliotoxin with or without the indicated pharmacological agents pretreatment, the cells were washed with cold PBS and then lysed in cold RIPA lysis buffer. Equal amounts of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% gradient) and then transferred to a polyvinylidene fluoride membrane. Primary antibodies targeting human PES1 (1∶200, sc-166300, Santa-Cruz), p53 (1∶2 000, 10442-1-AP, Proteintech), p21 (1∶2 000, 10355-1-AP, proteintech), p16 (1∶1 000, 10883-1-AP, Proteintech), Rb (1∶100, 554164, BD Pharmingen), p-Rb (1∶500, #3590, CST), IL-1β (1∶1 000, 16806-1-AP, Proteintech), IL-8 (1∶1 000, 27095-1-AP, Proteintech), IL-6 (1∶1 000, 10395-MM19, Sino biological), β -actin (1∶3 000, sc-47778, Santa-Cruz) were employed at room temperature for 2 h or at 4℃ overnight, and subsequently with the HRP-conjugated secondary antibody at room temperature for 1 h. The proteins were visualized using enhanced chemiluminescence (Santa Cruz Biotechnology).

1.6 Immunostaining

Cells were fixed with 4% paraformaldehyde at room temperature for 20 min and permeabilized with 0.4% Triton X-100 in PBS for 20 min at room temperature. After incubation with 10% donkey serum in PBS for 1 h, the cells were incubated with primary anti-bodies (PES1, 13553-1-AP, Proteintech, 1∶200; NPM1, PTM-61291, PTMBIO, 1∶200) at 4℃ overnight. Subsequently, cells were incubated with secondary antibodies at room temperature for 20 min.

Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). Confocal images were collected using an LSM 780 confocal microscope (Zeiss).

1.7 EdU incorporation assay

To assess the proliferative activity of cells, 5-ethynyl-2'-deoxyuridine (EdU) (20 μmol/L; Invitrogen, Darmstadt, Germany) was added to cells for 3 h before each time point monitored. Coverslips were fixed in 4% paraformaldehyde (PFA) for 15 min, washed with 10% FBS, and permeabilized with 0.3% Triton X-100 in PBS for 7 min. Instructions from the Click-iT EdU Alexa Fluor 594 imaging kit (Invitrogen) were then followed. Subsequently, cell nuclei were stained with DAPI. For each coverslip, 6 fields were randomly taken using LSM 780 confocal microscope (Zeiss). The numbers of EdU-labled and DAPI-stained cells were counted by Image-Pro Plus 6.0.

1.8 Quantitative RT−PCR

Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions (Invitrogen). First-strand cDNA was reverse-transcribed with random primers using moloney murine leukemia virus reverse transcriptase (Promega). The first-strand cDNA was used for PCR amplification with the following primers. IL-6 forward, 5'-AGGAGAAGATTCCAAAGATGTAGCCGCCC-3'; IL-6 reverse, 5'-TCTGCCAGTGCCTCTTTGCTGCT-3'. IL-8 forward, 5'-ACACTGCGCCAACACAGAAATTA-3'; IL-8 reverse, 5'-TTTGCTTGAAGTTCACTGGCATC-3'. IL-1β forward, 5'-ACGATGCACCTGTACGATCACT-3'; IL-1β reverse, 5'-CACCAAGCTTTTTTGCTGTGAGT-3'. hPES1 forward, 5'-GTGTTTAGAGCCACAAGAGGT-3'; hPES1 reverse, 5'-GGTCTTGTCTTCCCGTTTCTC-3'. mPES1 forward, 5'-CACATATGCATTGGACTCTGAG-3'; mPES1 reverse, 5'-TCCAGCTCTTTCTTCCGATCTT-3'. mβ-actin forward, 5'-ACCCACACTGTGCCCATCTA-3'; mβ-actin reverse, 5'-CACGCTCGGTCAGGATCTTC-3'. hβ-actin forward, 5'-AGAAGAGCTACGAGCTGCCTGA-3'; hβ-actin reverse, 5'-CAATGATCTTGATCTTCATTGTGCT-3'. β-actin was used as an internal control. The relative expression was calculated by the comparative Ct method.

1.9 Northern blot

Total cellular RNA was extracted using Trizol extraction according to the manufacturer's protocols. 5 μg of total RNA per lane was resolved on agarose denaturing gels (6% formaldehyde/1% agarose in HEPES-EDTA buffer), and gels were migrated for 4 h at 75 V. After washing, the gels were transferred to nylon membranes by capillarity overnight in 10× saline sodium citrate (SSC). The membranes were UV cross-linked (120 mJ/cm^2), followed prehybridized for 1 h at 65℃ in 50% formamide, 5×SSPE, 5× Denhardt's solution, 1% *w*/*v* SDS, 200 g/L fish sperm DNA solution (Roche). The ^{32}P-labeled oligonucleotide probe was added and incubated for 1 h at 65℃ and then overnight at 37℃. Sequences of the probes are as follows. mITS2: 5'-ACCCACCGCAGCGGGTGACGCGATTGATCG-3'; 7564: 5'-GCGCGACGGCGGACGACACCGCGGCGTC-3'.

1.10 Statistical analysis

Charting and statistical analyses were conducted utilizing the Prism software. To assess distinctions in means between groups, Welch's *t*-test was deployed, while analysis of variance (ANOVA) was employed to examine disparities in means across three or more groups. A significance threshold of $P<0.05$ was adopted to ascertain statistical significance.

2 Results

2.1 PES1 was downregulated in senescent cells

To investigate the potential association between PES1 and cellular senescence, we examined the levels of PES1 in senescent MEFs and senescent HepG2 cells. Cellular senescence is characterized by typical alterations in cell morphology (flattened cells with enlarged cytoplasm) and SA- β -gal activity[28]. Initially, we monitored the proliferation dynamics of HepG2 cells, which revealed a time-dependent increase in cell growth without observable signs of senescence (Figure 1b). To induce senescence, HepG2 cells were treated with DOX, a topoisomerase II inhibitor and widely used chemotherapeutic agent known for inducing senescence across various cancer cell lines[29-30]. HepG2 cells were exposed to 100 μg/L DOX for 17.5 h, followed by 24, 48, 72 h without drug. Results indicated that the growth rates of P9 MEF cells (Figure 1a) and DOX-treated HepG2 cells

(72 h group) (Figure 1c) displayed a marked increase in SA-β-gal activity and flattened morphology, suggesting that these stresses induced cellular senescence. Additionally, Western blot analysis revealed a significant reduction in PES1 expression levels in P9 MEF cells and DOX-treated HepG2 cells (Figure 1a, c). These findings suggested that the downregulation of PES1 may contribute to the process of cellular senescence.

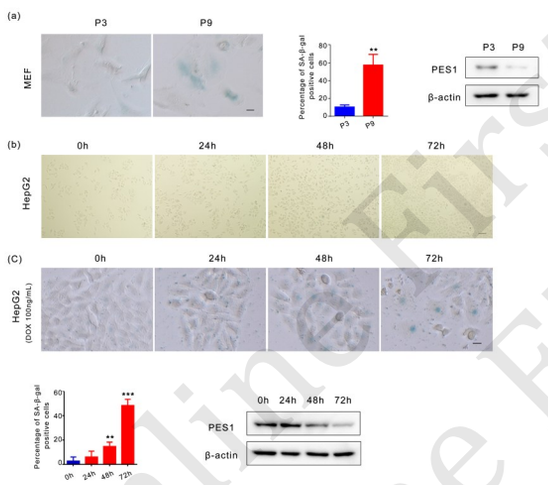


Fig. 1　PES1 is downregulated in senescent cells

（a） SA-β-gal staining in MEFs derived from different growth rates （P3， P9）， Scar bar=20 μm. Percentage of SA-β-gal positive cells were presented as the mean±SEM （*n*=5）. Western blot analysis of PES1 protein in MEFs. β-actin was used as the loading control. （b） SA-β-gal staining in HepG2 cells cultured for varying durations， Scar bar=100 μm. （c） SA-β-gal staining and Western blot analysis of PES1 protein in DOX-treated HepG2 cells. Scar bar=20 μm. Percentage of SA-β-gal positive cells were presented as the mean±SEM （*n*=5）. ***P*<0.01， ****P*< 0.001.

2.2　PES1 knockdown induces cellular senescence

To elucidate the role of PES1 in cellular senescence, we generated PES1 knockdown (PES1-KD) MEFs and HepG2 cells by transfecting small interfering RNAs targeting PES1 (siPES1). Notably, the number of SA-β-gal positive cells in PES1-KD MEFs and HepG2 cells was significantly elevated compared to control cells transfected with a negative control siRNA (siNC) (Figure 2a, b). Furthermore, we transfected PES1-KD HepG2 cells with a siRNA-resistant PES1 plasmid (Flag-pes1) to restore PES1 levels. Remarkably, the proportion of SA-β-gal positive cells in this restoration group was decreased to the basal level (Figure 2b). Moreover, the proportion of cells undergoing DNA synthesis, as measured by EdU incorporation, reduced in PES1-KD cell cultures (Figure 2c).

We also examined senescence-related marker proteins, Western blot analysis revealing that p53 and p21 were upregulated in PES1-KD HepG2 cells (Figure 2d). Furthermore, we conducted reverse transcription-quantitative PCR (RT-qPCR) and Western blot analyses to assess the expression of senescence-associated secretory phenotype (*SASP*) genes. Notably, the transcription and expression levels of inflammatory cytokines (IL-1β, IL-6, IL-8) were elevated in PES1-KD HepG2 cells (Figure 2e, f), highlighting the role of PES1 inhibition in the regulation of cellular senescence.

2.3　Downregulation of PES1 expression induces p53−dependent cellular senescence

Recent studies have revealed that the nucleolus senses various stressors and activates p53. Next, we examined whether PES1-KD-mediated cellular senescence is p53-dependent. we transfected HepG2 cells with siPES1 and sip53, and found that knockdown of p53 dramatically decreased siPES1

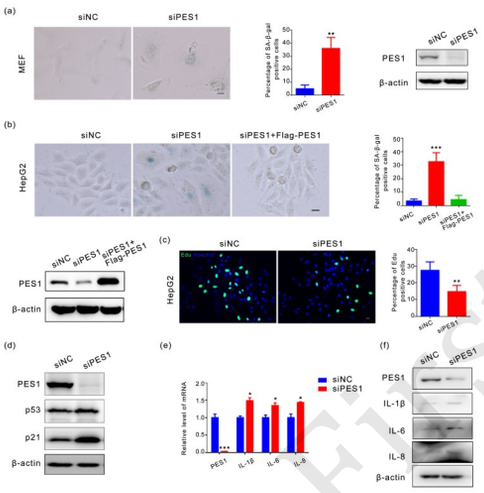


Fig. 2　Knockdown of PES1 induces cellular senescence

（a） SA-β-gal of MEFs treated with the indicated siRNA （siNC， siPES1）， Scar bar=20 μm. Data are presented as the mean±SD （*n*=5）. Western blot analysis of PES1 protein in MEFs. β-actin was used as the loading control. （b） SA-β-gal of HepG2 cells treated with the indicated combinations （siNC， siPES1， siPES1+Flag-PES1）， Scar bar=20 μm. Data are presented as the mean±SD （*n*=5）. Western blot analysis of PES1 protein in HepG2 cells. β-actin was used as the loading control. （c） HepG2 cells treated with the indicated siRNA （siNC， siPES1） labeled for EdU （green） and Hoechst （blue）， Scar bar=20 μm. Percentage of EdU^+ cells wre presented as the mean±SD （*n*=5）. （d） HepG2 cells treated with indicated siRNA （siNC， siPES1） were immunoblotted using indicated antibodies （PES1， p53， p21）. β-actin was used as the loading control. （e） HepG2 cells treated with indicated siRNA （siNC， siPES1） were analyzed for the expression of SASP genes （*IL-1β*， *IL-6*， *IL-8*） by RT-qPCR. （f） HepG2 cells treated with indicated siRNA （siNC， siPES1） were analyzed for the expression of IL-1β， IL-6 and IL-8 by Western blot. **P*<0.05， ***P*<0.01， ****P*<0.001.

induced cellular senescence (Figure 3a). Next, wt HCT116 cells (HCT116 wt) and p53-deficient HCT116 cells (HCT116 $p53^{-/-}$) were used to further determine the role of p53 in siPES1 induced cellular senescence. SA-β-gal staining assay (Figure 3b) and EdU staining assay (Figure 3c) showed that knockdown of PES1 significantly inhibited EdU incorporation and induced cellular senescence in wild type HCT116 cells but not in HCT116 $p53^{-/-}$ cells. Additionally, Western blot showed that the expression levels of p53, p21 and p16 in HCT116 wt cells were significantly up-regulated after PES1 silenced, while the expression levels of p21 and p16 in HCT116 $p53^{-/-}$ cells did not change significantly (Figure 3d). These results indicated that PES1 inhibition induced cellular senescence dependent on p53. Since ribosomal biogenesis inhibition induces cellular senescence can also be mediated by Rb signaling[16], the expressions of Rb, p-Rb, in normal HCT116 cells and HCT116 $p53^{-/-}$ cells were also detected in this study. Results showed no significant changes in the levels of Rb, P-Rb, suggesting that Rb was not be involved in PES1 mediated cellular senescence (Figure 3d).

To further confirm this conclusion, we knockdown of PES1 in Rb-deficient cells Du145, prostate cancer cells. SA-β-gal staining assay (Figure 3e) and EdU staining assay (Figure 3e) showed that PES1-KD inhibited Du145 cell proliferation and increased senescence. Western blot results showed that transfection of siPES1 up-regulated the expression of p53 and p16 (Figure 3f). These results indicated that inhibition of PES1 expression induced cellular senescence independent of Rb pathway.

2.4　Down−regulation of PES1 expression inhibits rRNA processing

It is known that PES1 plays an important role in the processing of 32S pre-rRNA to mature 28S rRNA, blocking this process leads to the accumulation of intracellular 47S pre-rRNA and rRNA intermediates (such as 45S, 41S, 32S, 17S, *etc*.). In this study, Northern blot was used to detect the level of rRNA intermediates to explore the effect of PES1 down-

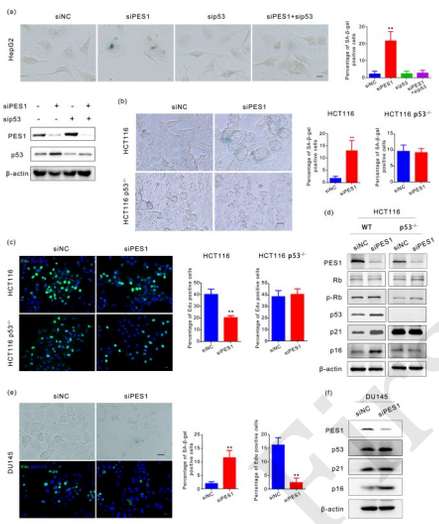


Fig. 3　PES1 regulates cellular senescence in p53−dependent manner

（a） SA-β-gal of HepG2 cells treated with the indicated combinations （siNC， siPES1， sip53， siPES1+sip53）， Scar bar=20 μm. Data were presented as the mean±SD （*n*=5）. Western blot analysis of PES1 and p53 protein in HepG2 cells. β-actin was used as the loading control. （b） SA-β-gal of HCT116 and HCT116 $53^{-/-}$ cells treated with the indicated siRNA （siNC， siPES1）， Scar bar=20 μm. Data were presented as the mean±SD （*n*=5）. （c） HCT116 and HCT116 $p53^{-/-}$ cells treated with the indicated siRNA （siNC， siPES1） labeled for EdU （green） and Hoechst （blue）， Scar bar=20 μm. Percentage of EdU^{+} cells were presented as the mean±SD （*n*=5）. （d） HCT116 and HCT116 $p53^{-/-}$ cells treated with indicated siRNA （siNC， siPES1） were immunoblotted using indicated antibodies （PES1， p53， p21， p16， Rb， p-Rb）. β-actin was used as the loading control. （e） SA-β-gal and EdU staining assay of DU145 cells treated with the indicated siRNA （siNC， siPES1）， Scar bar=20 μm. Data were presented as the mean±SD （*n*=5）. （f） DU145 cells treated with indicated siRNA （siNC， siPES1） were immunoblotted using indicated antibodies （PES1， p53， p21， p16）. β-actin was used as the loading control. ***P*<0.01.

regulation on pre-rRNA processing, by using oligonucleotide probes that hybridize to specific sites within the pre-rRNA transcript (Figure 4a). Results showed that the 45S/47S, 32S pre-rRNAs were significantly accumulated, accompanied by a decrease of 28S rRNAs in the PES1-KD MEFs and HepG2 cells (Figure 4b). These results indicated that the down-regulation of PES1 expression inhibited rRNA synthesis process.

Impaired ribosomal biogenesis could induce noticeable structural alterations in the nucleolus, including enlarged and irregular changes in its structure and morphology. when the ribosomal organisms are obstructed, the most noticeable outcome is the alteration in the nucleolus's structure, including morphological changes in the nucleolar structure (resulting in an enlargement and irregular shape). To assess whether the knockdown of PES1 induces structural alterations, we employed siPES1 to reduce PES1 expression in HepG2 cells and observed the resulting fragmentation of the nucleoli (Figure 4c). Furthermore, immunostaining also shown that PES1 knockdown induced the nucleoplasmic translocation of nucleolar proteins NPM1 (Figure 4d), suggesting a morphological change of nucleoli caused by PES1 silencing.

3　Discussion

In higher eukaryotes, the nucleolus displays concentric arrangement of 3 structural components: fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC) [31]. The most well-known function of the nucleolus is ribosome biogenesis, which included pre-rRNA transcription, processing, and mature rRNA assembly with ribosomal proteins.

Nucleolar stress reflects a misfunction of the nucleolus caused by a failure in ribosome biogenesis and defective nucleolar architecture[32]. It can be triggered by multiple conditions interfering with nucleolar integrity: Mutation of ribosome biogenesis factors or processing factors, intracellular stress, such

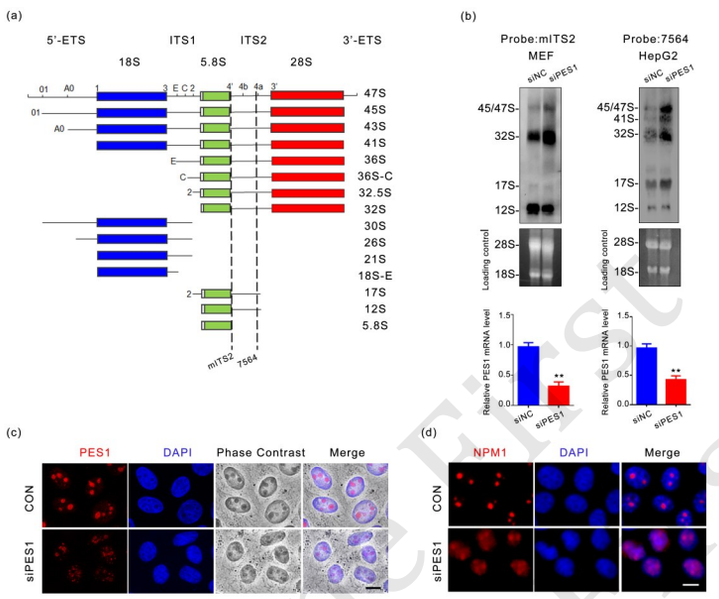


Fig. 4　Down-regulation of PES1 expression inhibits rRNA processing

（a） The main pre-rRNA processing intermediates in eukaryotic cells. Major cleavage sites in the primary 47S pre-rRNA and oligonucleotide probes that hybridize to specific sites within the pre-rRNA transcript were shown at the top. （b） Northern blot analysis of pre-rRNA processing in MEFs and HepG2 cells treated with indicated siRNA （siNC， siPES1）. Data were presented as the mean±SEM （n=3）. $^{**}P$<0.01. （c） Immunostaining of HepG2 cells treated with the indicated siRNA （siNC， siPES1） labeled for PES1 （red） and DAPI （blue）， Scar bar=20 μm. （d） Immunostaining of HepG2 cells treated with the indicated siRNA （siNC， siPES1） labeled for NPM1 （red） and DAPI （blue）， Scar bar=20 μm.

as reactive oxygen species (ROS) and extrinsic stress, such as UV irradiation or chemotherapeutic drugs[32]. Activation of the classical nucleolar stress response pathway triggers the release of ribosomal proteins from the nucleolus into the nucleoplasm. This event inactivates the E3-ubiquitin ligase MDM2, which normally keeps p53 levels low *via* proteasomal degradation[33-35]. As a result, the tumor suppressor p53 is stabilized and mediates p53-dependent nucleolar stress responses, such as cell cycle arrest, senescence, DNA damage or apoptosis[36-38]. In addition, nucleolar stress could also mediate tumor suppression through p53-independent signaling pathways. Specific ribosomal proteins, including RPL5, RPL11, and RPS14, have been demonstrated to activate TAp73, a p53 homologue[39], and inactivate the oncoprotein c - Myc in response to nucleolar stress[40-42].

PES1 is a nucleolar protein located in GC layer of nucleolus and associates with BOP1 and WDR12 to form the PeBoW complex in mammalian cells, which modulated pre-rRNA processing for the synthesis of 28S and 5.8S rRNAs. Dysfunction of this complex disrupts the assembly of the 60S large ribosomal subunit, leading to perturbations in ribosome biogenesis[17]. Notably, the perturbations in rRNA synthesis or ribosome assembly results in the release of an excess of ribosome proteins[43-44]. Ribosomal proteins like RPL11, RPL5, RPL23, and RPS7 are accumulated as free proteins and bind the E3 ubiquitin ligase MDM2 that targets the tumor suppressor p53 for degradation[15, 33-35]. This mechanism is consistent with our findings that PES1 knockdown elevates p53 levels, as impaired rRNA processing diminishes the production and export of mature ribosomes, paralleling the demand for ribosomal proteins RPL5, RPL11, and RPL23. Meanwhile, the dominant-negative mutants of WDR12 and BOP1 could also induce significant nuclear accumulation of p53 and transcriptional

activation of its target gene *p21*[45], suggesting that PES1, BOP1 or WDR12 is required for pre-rRNA processing by regulating the stability of the PeBoW complex. Additionally, BRIX1 (ribosome biogenesis protein BRX1 homolog) facilitated the processing of pre-rRNA by supporting the formation of the PeBoW complex[46]. Depletion of BRIX1 triggered nucleolar stress by impairing the processing of pre - rRNA resulting in increased interactions between RPL5/ RPL11 and MDM2 and the consequent stabilization and activation of p53[46]. Collectively, these findings suggest PES1 dysfunction as a potent activator of p53 signaling. Conversely, we observed no alterations in Rb signaling following PES1 knockdown. This pathway's activation depends on the accumulation of the 40S ribosomal subunit protein RPS14, which was likely unaffected by PES1 knockdown.

Recent study had revealed that some nucleolar proteins, such as ribosomal L1 domain containing 1 (RSL1D1), nucleostemin (NS), DExD-Box helicase 21 (DDX21) and EBNA1 binding protein 2 (EBP2) were downregulated at the protein level in senescent cells[16]. Depletion of these factors induced a proliferation arrest, a decrease in the proliferation markers KI67 and CENPA, and an increase in SA-β -gal[16]. Other nucleolar protein, like WDR3, BMS1 ribosome biogenesis factor, UTP6 small subunit processsome component, G-patch domain containing 4 (GPATCH4) deficient could also induce cellular senescence, developed an enlarged nucleolus, accumulated p53, p21, and p16, increased SA-β-gal activity[15, 47], which was similar to results observed in PES1 KD cells. Furthermore, in contrast to the decreased expression of nucleolar proteins, the selective overexpression of certain nucleolar proteins has been observed in multiple types of cancer[48], suggesting a high rate of ribosome biogenesis. This was associated with the upregulation of PES1 in tumor cells.

Previous investigations concerning nucleolar protein implicated in cellular senescence have examined the morphological shifts within the nucleolus. The phenomenon observed in senescent cells was a decline in nucleolus number and an increase in individual nucleolus size[47, 49-50], However, the phenomenon was the fragmentation of the nucleolus in cells with PES1 knockdown. Further research shown that the nucleoli of aging cells could be enlarge or fragment, and fragment was the final form[51]. PES1 is a nucleolar protein located within the GC layer of the nucleolus. Its expression level plays a critical role in maintaining the morphology and function of nucleoli. Consequently, PES1 knockdown represents a significant stressor for nucleolar integrity, which may explain the observed fragmentation of the nucleolus in cells with PES1 knockdown.

PES1 is necessary for the processing of the 32S precursor rRNA, and prominent accumulation of the 32S pre-rRNA indicates defective processing in the rRNA internal transcribed spacer 2 (ITS2), which was crucial for functional ribosome production. However, the specific role of PES1 on ITS2 processing remains largely unknown. Previous investigations have revealed that the Las1L-Nol9 endonuclease—kinase complex was essential for the cleavage of the ITS2[52]. The endonuclease Las1L cleaves the 32S rRNA at ITS2[53]. Additionally, Nol9 phosphorylates the 5' -hydroxyl end of ITS2[54], thereby facilitating degradation by the 5' to 3'-exonuclease XRN2[55-56] and 28S rRNA maturation. Nonetheless, whether the regulation of the Las1L-Nol9 complex by PES1 occurred in cellular contexts remains unclear.

PES1 could directly interact with TERT to control telomerase activity, maintain telomere length, and regulate cellular senescence[27]. However, critically short telomeres trigger DNA damage responses and activate p53 which leads to apoptosis or replicative senescence[57-60], our results could not exclude the function of telomers on PES1-KD induced p53. Additionally, numerous studies have emphasized the pivotal role of p53 in tumor suppression, with its dysfunction implicated in a broad spectrum of cancers[61]. Notably, mutations in the p53 gene are observed in over 50 % of human tumors[62], indicates that PES1 deficient could not induce senescence in these tumors.

4 Conclusion

We reported that the nucleolar protein PES1 was down-regulated in senescent cells, and knockdown of PES1 led to abnormalities in ribosomal biogenesis, which in turn activates p53 rather than Rb pathway, leading to cellular senescence, providing a novel perspective on the mechanisms involved in the senescence regulated by PES1.

References

[1] Wu Z, Qu J, Zhang W, *et al*. Stress, epigenetics, and aging: unraveling the intricate crosstalk. Mol Cell, 2024, **84**(1): 34-54

[2] Lu H, Jing Y, Zhang C, *et al*. Aging hallmarks of the primate ovary revealed by spatiotemporal transcriptomics. Protein Cell, 2024, **15**(5): 364-384

[3] Aging Biomarker C, Bao H, Cao J, *et al*. Biomarkers of aging. Sci China Life Sci, 2023, **66**(5): 893-1066

[4] Sun Y, Li Q, Kirkland J L. Targeting senescent cells for a healthier longevity: the roadmap for an era of global aging. Life Med, 2022, **1**(2): 103-119

[5] van Deursen J M. The role of senescent cells in ageing. Nature, 2014, **509**(7501): 439-446

[6] Schosserer M. The role and biology of senescent cells in ageing-related tissue damage and repair. Mech Ageing Dev, 2022, **202**: 111629

[7] Ren J, Song M, Zhang W, *et al*. The Aging Biomarker Consortium represents a new era for aging research in China. Nat Med, 2023, **29**(9): 2162-2165

[8] Hansen M, Taubert S, Crawford D, *et al*. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. Aging Cell, 2007, **6**(1): 95-110

[9] Grummt I. The nucleolus—guardian of cellular homeostasis and genome integrity. Chromosoma, 2013, **122**(6): 487-497

[10] Steffen K K, MacKay V L, Kerr E O, *et al*. Yeast life span extension by depletion of 60s ribosomal subunits is mediated by Gcn4. Cell, 2008, **133**(2): 292-302

[11] Thiry M, Lafontaine D L J. Birth of a nucleolus: the evolution of nucleolar compartments. Trends Cell Biol, 2005, **15**(4): 194-199

[12] Tiku V, Jain C, Raz Y, *et al*. Small nucleoli are a cellular hallmark of longevity. Nat Commun, 2017, **8**: 16083

[13] Buchwalter A, Hetzer M W. Nucleolar expansion and elevated protein translation in premature aging. Nat Commun, 2017, **8**(1): 328

[14] Kinoshita Y, Jarell A D, Flaman J M, *et al*. Pescadillo, a novel cell cycle regulatory protein abnormally expressed in malignant cells. J Biol Chem, 2001, **276**(9): 6656-6665

[15] Nishimura K, Kumazawa T, Kuroda T, *et al*. Perturbation of ribosome biogenesis drives cells into senescence through 5S RNP-mediated p53 activation. Cell Rep, 2015, **10**(8): 1310-1323

[16] Lessard F, Igelmann S, Trahan C, *et al*. Senescence-associated ribosome biogenesis defects contributes to cell cycle arrest through the Rb pathway. Nat Cell Biol, 2018, **20**(7): 789-799

[17] Rohrmoser M, Hölzel M, Grimm T, *et al*. Interdependence of Pes1, Bop1, and WDR12 controls nucleolar localization and assembly of the PeBoW complex required for maturation of the 60S ribosomal subunit. Mol Cell Biol, 2007, **27**(10): 3682-3694

[18] He Y, Xiang J, Li Y, *et al*. PES1 is a biomarker of head and neck squamous cell carcinoma and is associated with the tumor microenvironment. Cancer Med, 2023, **12**(11): 12622-12638

[19] Jiang Z, Zhang Y, Chen X, *et al*. microRNA-1271 impedes the development of prostate cancer by downregulating PES1 and upregulating ERβ. J Transl Med, 2020, **18**(1): 209

[20] Fu Z, Jiao Y, Li Y Q, *et al*. PES1 in liver cancer: a prognostic biomarker with tumorigenic roles. Cancer Manag Res, 2019, 11: 9641-9653

[21] Jin X, Fang R, Fan P, *et al*. PES1 promotes BET inhibitors resistance and cells proliferation through increasing c-Myc expression in pancreatic cancer. J Exp Clin Cancer Res, 2019, **38**(1): 463

[22] Qiu Y B, Liao L Y, Jiang R, *et al*. PES1 promotes the occurrence and development of papillary thyroid cancer by upregulating the ERα/ERβ protein ratio. Sci Rep, 2019, **9**(1): 1032

[23] Li S, Wang M, Qu X, *et al*. SUMOylation of PES1 upregulates its stability and function *via* inhibiting its ubiquitination. Oncotarget, 2016, **7**(31): 50522-50534

[24] Li J, Zhuang Q, Lan X, *et al*. PES1 differentially regulates the expression of ERα and ERβ in ovarian cancer. IUBMB Life, 2013, **65**(12): 1017-1025

[25] Li J, Zhou X, Lan X, *et al*. Repression of PES1 expression inhibits growth of gastric cancer. Tumor Biol, 2016, **37**(3): 3043-3049

[26] Xie W, Feng Q, Su Y, *et al*. Transcriptional regulation of PES1 expression by c-Jun in colon cancer. PLoS One, 2012, **7**(7): e42253

[27] Cheng L, Yuan B, Ying S, *et al*. PES1 is a critical component of telomerase assembly and regulates cellular senescence. Sci Adv, 2019, **5**(5): eaav1090

[28] Hernandez-Segura A, Nehme J, Demaria M. Hallmarks of cellular senescence. Trends Cell Biol, 2018, **28**(6): 436-453

[29] Yang F, Teves S S, Kemp C J, *et al*. Doxorubicin, DNA torsion, and chromatin dynamics. Biochim Biophys Acta, 2014, **1845**(1): 84-89

[30] Hsu C H, Altschuler S J, Wu L F. Patterns of early p21 dynamics determine proliferation-senescence cell fate after chemotherapy. Cell, 2019, **178**(2): 361-373.e12

[31] Frottin F, Schueder F, Tiwary S, *et al*. The nucleolus functions as a phase-separated protein quality control compartment. Science, 2019, **365**(6451): 342-347

[32] Pfister A S. An update on nucleolar stress: the transcriptional control of autophagy. Cells, 2023, **12**(16): 2071

[33] Zhang Y, Wolf G W, Bhat K, *et al*. Ribosomal protein L11 negatively regulates oncoprotein MDM2 and mediates a p53-dependent ribosomal-stress checkpoint pathway. Mol Cell Biol, 2003, **23**(23): 8902-8912

[34] Lohrum M A E, Ludwig R L, Kubbutat M H G, *et al*. Regulation of HDM2 activity by the ribosomal protein L11. Cancer Cell, 2003, **3**(6): 577-587

[35] Manfredi J J. The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. Genes Dev, 2010, **24**(15): 1580-1589

[36] Rubbi C P, Milner J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. EMBO J, 2003, **22**(22): 6068-6077

[37] Lindström M S, Jurada D, Bursac S, *et al*. Nucleolus as an emerging hub in maintenance of genome stability and cancer

pathogenesis. Oncogene, 2018, **37**(18): 2351-2366

[38] Han X, Xing L, Hong Y, *et al*. Nuclear RNA homeostasis promotes systems-level coordination of cell fate and senescence. Cell Stem Cell, 2024, **31**(5): 694-716.e11

[39] Zhou X, Hao Q, Zhang Q, *et al*. Ribosomal proteins L11 and L5 activate TAp73 by overcoming MDM2 inhibition. Cell Death Differ, 2015, **22**(5): 755-766

[40] Challagundla K B, Sun X X, Zhang X, *et al*. Ribosomal protein L11 recruits miR-24/miRISC to repress c-Myc expression in response to ribosomal stress. Mol Cell Biol, 2011, **31**(19): 4007-4021

[41] Zhou X, Hao Q, Liao J M, *et al*. Ribosomal protein S14 negatively regulates c-Myc activity. J Biol Chem, 2013, **288**(30): 21793-21801

[42] Liao J M, Zhou X, Gatignol A, *et al*. Ribosomal proteins L5 and L11 co-operatively inactivate c-Myc *via* RNA-induced silencing complex. Oncogene, 2014, **33**(41): 4916-4923

[43] Kressler D, Linder P, Cruz J D L. Protein trans-acting factors involved in ribosome biogenesis in *Saccharomyces cerevisiae*. Mol Cell Biol, 1999, **19**(12): 7897-7912

[44] Rothstein J D, Patel S, Regan M R, *et al*. Nucleolar proteome dynamics. Nature, 2005, **433**(7021): 73-77

[45] Hölzel M, Rohrmoser M, Schlee M, *et al*. Mammalian WDR12 is a novel member of the Pes1-Bop1 complex and is required for ribosome biogenesis and cell proliferation. J Cell Biol, 2005, **170**(3): 367-378

[46] Gan Y, Hao Q, Han T, *et al*. Targeting BRIX1 *via* engineered exosomes induces nucleolar stress to suppress cancer progression. Adv Sci (Weinh), 2024, **11**(47): e2407370

[47] Kodera K, Hishida R, Sakai A, *et al*. $GPATCH_4$ contributes to nucleolus morphology and its dysfunction impairs cell viability. Biochem Biophys Res Commun, 2024, **693**: 149384

[48] Turi Z, Lacey M, Mistrik M, *et al*. Impaired ribosome biogenesis: mechanisms and relevance to cancer and aging. Aging (Albany NY), 2019, **11**(8): 2512-2540

[49] Lafita-Navarro M C, Conacci-Sorrell M. Nucleolar stress: from development to cancer. Semin Cell Dev Biol, 2023, 136: 64-74

[50] Kasselimi E, Pefani D E, Taraviras S, *et al*. Ribosomal DNA and the nucleolus at the heart of aging. Trends Biochem Sci, 2022, **47**(4): 328-341

[51] Li Y, Jiang Y, Paxman J, *et al*. A programmable fate decision landscape underlies single-cell aging in yeast. Science, 2020, **369**(6501): 325-329

[52] Li Y, Yang Y, Sears R C, *et al*. USP36 SUMOylates Las1L and promotes its function in pre-ribosomal RNA ITS2 processing. Cancer Res Commun, 2024, **4**(10): 2835-2845

[53] Gasse L, Flemming D, Hurt E. Coordinated ribosomal ITS2 RNA processing by the Las1 complex integrating endonuclease, polynucleotide kinase, and exonuclease activities. Mol Cell, 2015, **60**(5): 808-815

[54] Gordon J, Pillon M C, Stanley R E. Nol9 is a spatial regulator for the human ITS2 pre-rRNA endonuclease-kinase complex. J Mol Biol, 2019, **431**(19): 3771-3786

[55] Fang F, Phillips S, Butler J S. Rat1p and Rai1p function with the nuclear exosome in the processing and degradation of rRNA precursors. RNA, 2005, **11**(10): 1571-1578

[56] Pillon M C, Lo Y H, Stanley R E. IT'S 2 for the price of 1: multifaceted ITS2 processing machines in RNA and DNA maintenance. DNA Repair (Amst), 2019, **81**: 102653

[57] Şerifoğlu N, Lopes-Bastos B, Ferreira M G. Lack of telomerase reduces cancer incidence and increases lifespan of zebrafish $tp53^{M214K}$ mutants. Sci Rep, 2024, **14**(1): 5382

[58] Herbig U, Jobling W A, Chen B P C, *et al*. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol Cell, 2004, **14**(4): 501-513

[59] Lechel A, Satyanarayana A, Ju Z, *et al*. The cellular level of telomere dysfunction determines induction of senescence or apoptosis *in vivo*. EMBO Rep, 2005, **6**(3): 275-281

[60] D'Alcontres M S, Mendez-Bermudez A, Foxon J L, *et al*. Lack of TRF2 in ALT cells causes PML-dependent p53 activation and loss of telomeric DNA. J Cell Biol, 2007, **179**(5): 855-867

[61] Wang X, Yang J, Yang W, *et al*. Multiple roles of p53 in cancer development: regulation of tumor microenvironment, m^6A modification and diverse cell death mechanisms. J Adv Res, 2024: **S2090**-1232(24)**00481**-8

[62] Dolgin E. The most popular genes in the human genome. Nature, 2017, **551**(7681): 427-431

PES1表达缺陷介导核糖体生成抑制并通过激活p53信号诱导细胞衰老*

张常建[1,2)]　李玉芳[1,3)]　武凤云[1,3)]　金　蕊[1)]　牛　畅[3)**]　叶棋浓[1)**]　程　龙[4)**]

([1)] 军事科学院军事医学研究院前沿生物技术实验室，北京 100071；

[2)] 解放军总医院第六医学中心耳鼻咽喉头颈外科医学部研究所，北京 100048；

[3)] 首都医科大学生命科学学院，北京 100069；[4)] 北京医院，国家卫生健康委北京老年医学研究所，北京 100005）

摘要　目的　本研究旨在探讨核仁蛋白PES1表达抑制对细胞衰老的影响及其分子机制。**方法**　首先检测复制性衰老的小鼠胚胎成纤维细胞（MEFs）及阿霉素诱导衰老的人肝癌细胞系HepG2内PES1的表达情况。然后利用外源siPES1干扰HepG2及其他细胞内PES1的表达，观察细胞衰老情况，并检测衰老相关蛋白的表达情况。最后利用Northern blot及荧光技术检测PES1表达抑制对pre-rRNA成熟及核仁形态的影响。**结果**　衰老的MEF细胞及HepG2细胞内PES1表达下调。细胞内PES1表达抑制可激活细胞内p53而非Rb依赖的衰老信号。进一步研究发现，抑制PES1的表达可影响pre-rRNA的成熟并诱导核仁应激的发生。**结论**　核仁蛋白PES1的表达抑制可诱导核仁应激，并激活细胞内依赖于p53而非Rb信号的衰老信号。

关键词　PES1，细胞衰老，核糖体生物合成，p53

中图分类号　Q26；Q75　　**DOI**：10.16476/j.pibb.2025.0005　　**CSTR**：32369.14.pibb.20250005

* 中国博士后科学基金（2023M744316），军队实验动物专项课题（SYDW_KY［2021］07），北京市自然科学基金（M23003）和国家高层次医院临床科研基金（LYS-2023-16，BJ-2024-219）资助项目。

** 通讯联系人。

程龙　Tel：010-58115045，E-mail：biolongcheng@outlook.com

叶棋浓　Tel：010-66948841，E-mail：yeqn66@yahoo.com

牛畅　Tel：010-68901494，E-mail：niuchang@cnu.edu.cn

收稿日期：2025-01-08，接受日期：2025-04-14