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Mitotic centromere-associated kinase (MCAK/Kif2C) regulates cellular senescence in human primary cells through a p53-dependent pathway

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ABSTRACT

Mitotic centromere-associated kinase (MCAK/Kif2C) plays a critical role in chromosome movement and segregation with ATP-dependent microtubule depolymerase activity. However, its role in cellular senescence remains unclear. MCAK/Kif2C expression decreased in human primary cells under replicative and premature senescence. MCAK/Kif2C down-regulation in young cells induced premature senescence. MCAK/Kif2C overexpression in old cells partially reversed cell senescence. Senescence phenotypes by MCAK/Kif2C knockdown were observed in p16-knockdown cells, but not in p53-knockdown cells. These results suggest that MCAK/Kif2C plays an important role in the regulation of cellular senescence through a p53-dependent pathway and might contribute to tissue/organism aging and protection of cellular transformation.

1. Introduction

Cellular senescence is an irreversible arrest state of cell proliferation [1], which is caused by telomere shortening, activation of oncogene and tumor suppressor genes, oxidative stress, DNA damage, inflammation, chemotherapeutic agents and ultraviolet (UV) or gamma-irradiation [2]. Accumulating evidence suggests that cellular senescence is one of the intrinsic safeguards against cancer progression as well as contributes to tissue/organism aging and age-related disease [3]. In addition to growth arrest, senescent cells exhibit senescence-associated β-galactosidase activity (SA-β-gal), DNA scars or damage foci, and senescence-messaging secretome or senescence-associated secretory phenotypes [4]. Although different triggers induce cellular senescence, p53 and p16/Rb tumor suppressor pathways are well documented to play a critical role in cell senescence program [5].

Senescent cells manifest multiple defects in chromatin structure [6]. Increasing errors in the mitotic machinery of dividing cells and resulting persistent DNA damage during cell senescence progress are suggested to induce global changes in chromatin structure of senescent cells, contributing to physiological and premature aging, as well as defense mechanism against tumor transformation [7]. A variety of genes involved in the regulation of chromosomal processing and assembly including CENP-F, mitotic kinesin-like protein-1, CENP-A, kinesin-like spindle protein, and mitotic centromere-associated kinase (MCAK) are down-regulated in cells from middle age, old age, and progeria versus young human subjects, suggesting that chromosomal pathologies led by misregulation of mitotic genes might be involved in the aging process [8].

Hutchinson–Gilford progeroid syndrome (HGPS), a premature aging disorder, is caused by a point mutation in the lamin A/C gene (LMNA) and shows multiple chromosomal defects and continual DNA damage [9]. Aging-related chromatin defects are mediated via loss of nucleosome remodeling and deacetylase chromatin remodeling (NuRD) complex in premature and physiological aging [10]. In addition, other genes such as CENP-A [11], aurora B kinase (Aurora B) [12], and Bub1 [13], were involved in chromosome movement and segregation during the mitotic process. However, the molecular mechanism of aging-related chromatin abnormalities and its relationship to cellular senescence remain to be established.

Mitotic centromere-associated kinase or kinesin family member 2C (MCAK/Kif2C) is a member of the kinesin superfamily of microtubule motor proteins [14]. Instead of carrying cargo along microtubules, it associates with the microtubule surface and depolymerizes microtubules with ATPase activity [15], which is a critical process in normal chromosome movement and segregation. MCAK/Kif2C is localized at the centromeres and kinetochores and in the spindle poles, and functions as a key regulator of mitotic spindle assembly and dynamics [16,17]. Both localization of MCAK

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to the centromere and its microtubule depolymerase activity are regulated by phosphorylation by Aurora B [18,19], Aurora A [20], and polo-like kinase-1 (Plk1) [21], and dephosphorylation by type I protein phosphatase [19]. Depletion or down-regulation of MCAK/Kif2C revealed chromosomal congression and segregation defects due to improper kinetochore attachments in potoroo kidney (PtK) cells [22] and in normal diploid RPE-1 cells [23]. In contrast, overexpression of MCAK/Kif2C enhances microtubule depolymerization, resulting in microtubule detachment from centromeres [24]. The higher expression of MCAK level was found in gastric cancer tissue [25], colorectal and other epithelial cancers [26], and breast cancer [27]. Considerable studies on MCAK/Kif2C have focused mainly on its role in microtubule depolarization and chromosome segregation and suggested that alteration of MCAK/Kif2C might be involved in chromosome instability related to cancer or aging, and thus, may contribute to cell proliferation as well as senescence. However, the role of MCAK/Kif2C in cellular senescence has not been reported.

In the present study, we reveal the causative effect of MCAK/Kif2C on cellular senescence in human primary cells. Down-regulation of MCAK/Kif2C in young cells induced premature senescence. In contrast, ectopic expression of MCAK/Kif2C in old cells partially reversed cell senescence. Senescence phenotypes induced by MCAK/Kif2C repression were observed in p16-knockdown cells but not in p53-knockdown cells. These results suggest that MCAK/Kif2C plays an important role in the regulation of cellular senescence through a p53-dependent pathway and might contribute to tissue/organism aging as well as protection of cell transformation.

2. Materials and methods

2.1. Materials

Human dermal fibroblasts (HDFs), human umbilical vein endothelial cells (HUVECs), and endothelial cell basal medium-2 (EBM-2) containing several growth factors and supplements (EGM-2) were purchased from Lonza (Walkersville, MD). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin were obtained from WelGene (Daegu, Republic of Korea). Bromo-chloro-indolyl-galactopyranoside (BCIG, X-gal) was purchased from Sigma–Aldrich (St. Louis, MO). Antibodies against caspase 3, p53, cyclin A, MCAK/Kif2C, and poly ADP ribose polymerase (PARP)-1/2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody against phospho-Rb at serine 807 and 811 ($pRb^{ser807/811}$) was obtained from Cell Signaling Technology (Danvers, MA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was kindly provided by KS Kwon (KRIBB, Daejeon, Republic of Korea). The Topo-TA cloning kit, Lipofectamine 2000, Alexa Fluor® 647-conjugated annexin V, and horseradish peroxidase (HRP)-conjugated secondary rabbit or mouse polyclonal antibodies were purchased from Invitrogen Life Technologies (Carlsbad, CA). The oligonucleotides for polymerase chain reaction (PCR) primers of MCAK/Kif2C, p53, p16 and GAPDH were obtained from Bioneer (Daejeon, Republic of Korea) (Table 1). siRNAs used in the present study were listed in Table 1. Total RNA isolation (TRI) reagent was from Bio Science Technology (Daegu, Republic of Korea) and pAdEasy-1 adenoviral vector was purchased from Stratagene (Santa Clara, CA).

2.2. Cell culture and treatment

HDFs in DMEM containing 10% FBS and 1% antibiotics, and HUVECs in EBM-2 with EGM-2 were plated at 1×10^5 cells per 100 mm-diameter culture plate and cultured at 37 °C in 5% CO_2 humidified air. The number of population doublings (PDs) during cell culture was monitored and cells were used in either PD < 24 (young) or PD > 50 (old). For induction of premature cell senescence by adriamycin, cells (5×10^4) were seeded in wells of six-well culture plates and incubated overnight and treated with 0.5 μM adriamycin for 4 h. After washing, cells were incubated for the indicated times. Adriamycin-induced cellular senescence was confirmed by SA-β-gal staining as described immediately below.

Table 1
PCR primer sequences and siRNA sequences.

Name	Sequence
MCAK/Kif2C	Forward: ATGGCCATGGACTCGTCGCTT Reverse: TCGCTGGGGCCGTTTCTTGCT
p53	Forward: CCCTGAGGTTGGCTCTGA Reverse: GTGCTGAGCCTCCCCTTT
p16	Forward: CTTCCTGGACACGCTGGT Reverse: ACCTTCCGCGGCATCTAT
GAPDH	Forward: CGACCACTTTGTCAAGCTCA Reverse: AGGGGTCTACATGGCAACTG
Negative control siRNA	dTdTUUCUCCGAACGUGUCACGU dTdTACGUGACACGUUCGGAGAA
MCAK/Kif2C Stealth RNAi duplex Oligonucleotides	AAUUGGAGUUGGCAAAUGUCUCGGC GGGCAGACAUUUGCCAACUCCAAUU
p53 Stealth RNAi duplex Oligonucleotides	UCCACACGCAAAUUUCCUUCCACUC GAGUGGAAGGAAAUUUGCGUGUGGA
p16 *Silencer*® Select siRNA	CUACCGUAAAUGUCCAUUUTT AAAUGGACAUUUACGGUAGTG

2.3. Senescence associated β-galactosidase (SA-β-gal) staining

SA-β-gal activity in cells was observed as described previously [28]. After SA-β-gal staining, cells were counterstained with 1% eosin for 3 min and then washed twice with phosphate buffered saline (PBS). The percentage of blue cells observed in 100 cells examined by light microscopy was determined.

2.4. Immunofluorescence staining

Cells were fixed with 3.7% para-formaldehyde in PBS for 10 min and permeabilized in PBS containing 0.5% Triton X-100 for 30 min. An antibody specific to MCAK/Kif2C (1:200) was applied for 1 h and then Alexa Fluor® 488 goat anti-mouse IgG (1:250) (Invitrogen Life Technologies) was applied for 30 min. The nuclei were stained with 0.1 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI) for 5 min. Images were obtained using a fluorescence microscope.

2.5. Total RNA extraction and reverse transcriptase (RT)-PCR

RNA was extracted from cells using Tri-RNA isolation reagent according to the manufacturer's protocols. Total RNA was incubated with 2.5 μM oligo-dT primer, 2.5 mM dNTPs, moloney murine leukemia virus (MMLV) reverse transcriptase, and RNase inhibitor. MCAK/Kif2C, p53, and p16 were amplified from resulting cDNAs with Super-Therm DNA polymerase (JMR Holdings, London, UK) and gene specific primers. The PCR protocol consisted of an initial denaturation for 5 min at 95 °C; 30 cycles of 15 s at 95 °C, 15 s at 60 °C, and 30 s at 72 °C; and a final incubation for 1 min at 72 °C. GAPDH primers were used to standardize the amount of RNA in each sample. PCR products were resolved on 1% agarose gels and visualized by SYBR Green staining (Applied Biosystems, Carlsbad, CA).

2.6. Real-time PCR

Real-time PCR was performed using SYBR Green PCR master mix (Applied Biosystems) and a LightCycler 2.0 Real-Time PCR system (Roche Diagnostic, Indianapolis, IN). The PCR protocol was

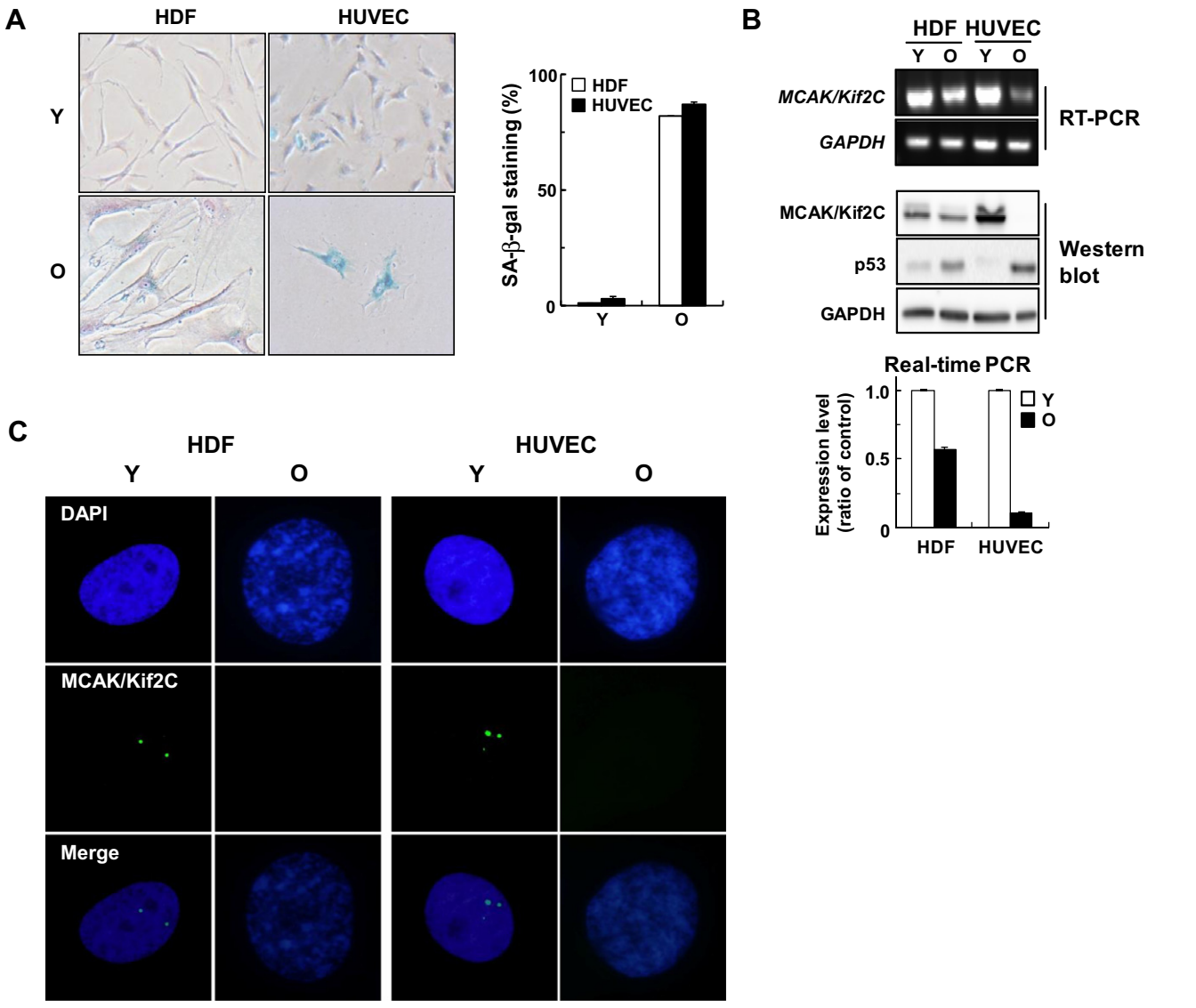

Fig. 1. Expression levels of MCAK/Kif2C in young and old cells under replicative senescence and in adriamycin-treated cells. (A) SA-β-gal staining of young and old cells (×100). The percentages of SA-β-gal-positive cells were determined by counting of 100 cells in five different fields. Values are means ± S.D. of three independent experiments. (B) Semi-quantitative RT-PCR, Western blotting, and real-time PCR. (C) Immunostaining of MCAK/Kif2C in young and old cells. (D) Immunostaining of MCAK/Kif2C in the nuclei of young cells treated with nocodazole (50 ng/ml). Cells were stained with an MCAK/Kif2C antibody (green) and DAPI (blue) for the nuclei. (E) Expression levels of MCAK/Kif2C protein in adriamycin-treated cells. The MCAK/Kif2C and p53 protein level was detected by Western blotting. Representative data from three independent experiments are shown. Y, young cells; O, old cells; ADR, adriamycin.

2 min at 50 °C, 10 min at 94 °C, and 38 cycles of 15 s at 95 °C and 2 min at 54 °C. Expression levels were analyzed with the LightCycler software (Roche Diagnostic).

2.7. Protein extraction and Western blot analysis

Proteins (50 μg) were separated by 10% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose membranes. The membranes were incubated overnight with one of the specific primary antibodies (1:1000). Then, HRP-conjugated goat anti-mouse or goat anti-rabbit (1:3000) antibody were applied for 1.5 h. Antigen–antibody complex was detected using Western Blotting Luminol solution (Santa Cruz Biotechnology) with a LAS-3000 image system (Fujifilm).

2.8. Preparation of recombinant MCAK/Kif2C adenovirus

Full length human MCAK/Kif2C cDNA was PCR-amplified and cloned into a TOPO-TA cloning vector. Nucleotide sequences of MCAK/Kif2C cDNA (TOPO/MCAK/Kif2C) were confirmed by dideoxy sequencing (SolGent, Daejeon, Republic of Korea). Recombinant MCAK/Kif2C adenoviral vector (pAd/MCAK/Kif2C) was prepared using the pAdEasy-1 adenoviral vector. The recombinant pAd/MCAK/Kif2C vector was linearized with *Pac*I digestion and transfected into AD293 cells using Fugene® HD transfection reagent (Promega, Madison, WI). Recombinant adenovirus was amplified in AD293 cells and purified by an adenovirus purification kit (CellBiolabs, San Diego, CA). Virus titers were determined using the pAdEasy titer kit in AD293 cells.

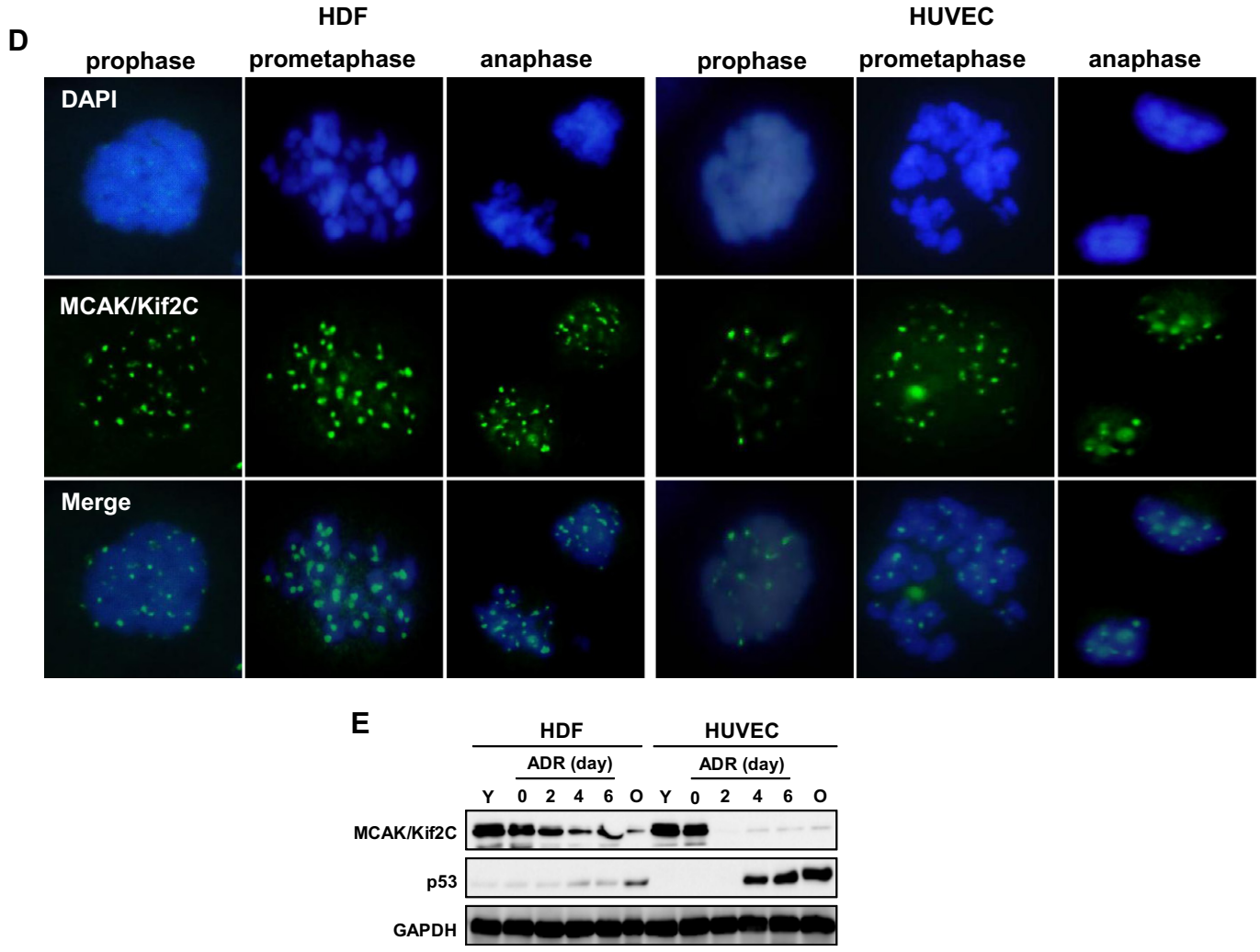

Fig. 1. (*continued*)

2.9. Transduction of recombinant MCAK/Kif2C adenovirus

Old cells (PD > 50, 1×10^5 cells) were seeded in 60 mm-diameter culture plate and incubated overnight. Cells were treated at a multiplicity of infection (MOI) of 3, 6, and 12 of recombinant MCAK/Kif2C adenovirus for 24 h. Cell proliferation and SA-β-gal activity were measured at 4 days post-transduction.

2.10. Transfection of MCAK/Kif2C, p53, and p16 small interfering RNAs (siRNAs)

Young cells (1×10^5) were seeded in 60 mm-diameter culture plates and incubated overnight. siRNAs (5 pmol) against MCAK/Kif2C, p53, or p16 were transfected into cells using Lipofectamine 2000 transfection reagent (Invitrogen Life Technologies). After 24 h incubation, media were changed and their expression levels were observed by RT-PCR or Western blotting. Cell proliferation and SA-β-gal activity were measured at 4 days post-transfection.

2.11. Annexin V staining

Apoptosis was observed in cells transfected with MCAK or negative control siRNAs using Alexa Fluor® 647-conjugated annexin V according to the manufacturer's suggestion. The samples were analyzed using the Becton–Dickinson FACS Canto II flow cytometer (Becton–Dickinson, San Jose, CA).

2.12. Statistical analysis

The results are represented as means ± S.D. *P* values for determining statistical significance were calculated using an unpaired two-tailed Student's *t*-test.

3. Results

3.1. Expression levels of MCAK/Kif2C in young and old cells

While screening for genes differentially expressed in human primary cells during replicative senescence by cDNA microarray technology, we found that MCAK/Kif2C expression was down-regulated in senescent cells compared to young cells (data not shown). The levels of many genes associated with mitotic process and chromosomal segregation were decreased [12]. Since MCAK/Kif2C functions as a key regulator of mitotic spindle assembly and dynamics [16,17] and chromosomal pathologies led by misregulation of mitotic genes might be involved in the aging process [8], we tried to investigate the role of MCAK/Kif2C in cellular senescence. Cellular senescence of HDFs and HUVECs was confirmed by increases in SA-β-gal activity (Fig. 1A) and p53 levels (Fig. 1B). Decreased expression levels of MCAK/Kif2C were shown in old cells by RT-PCR, real-time PCR, and Western blotting (Fig. 1B). Immunostaining analysis revealed that MCAK/Kif2C was localized in the nucleus of young and old cells, and that its level was reduced in

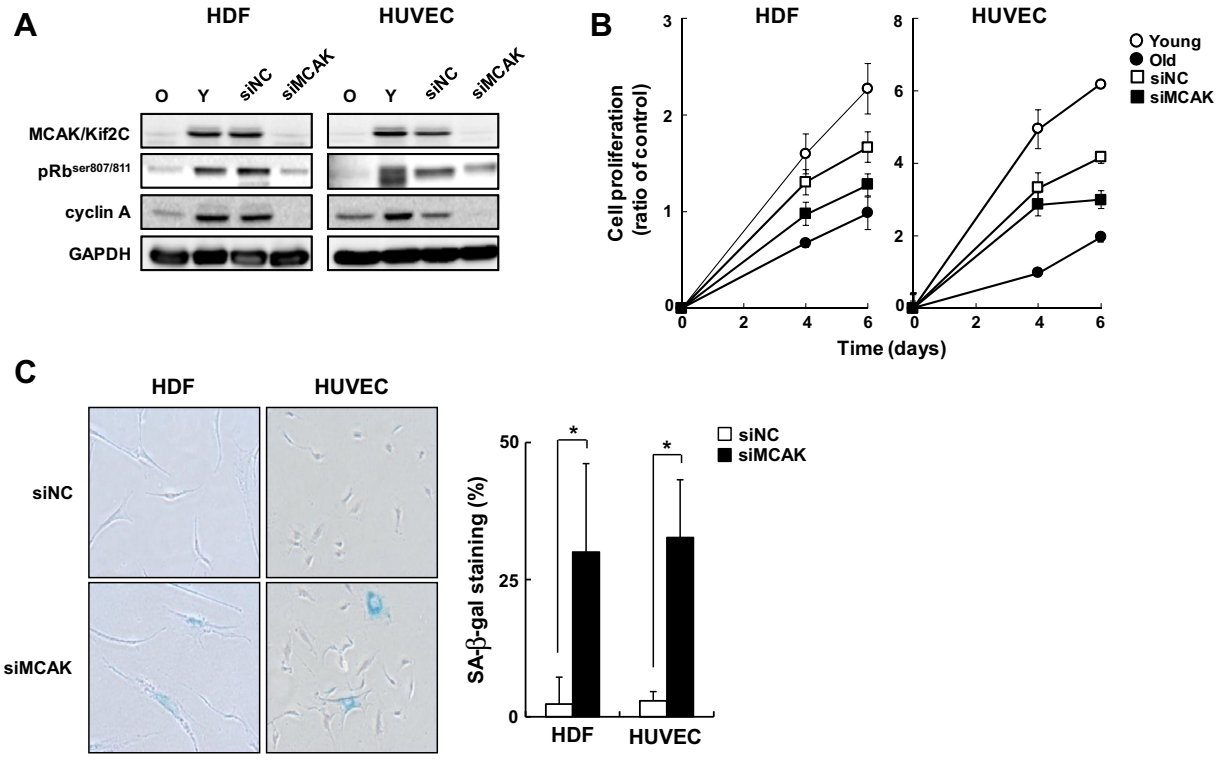

Fig. 2. Effects of MCAK/Kif2C knockdown on cellular senescence in young HDFs and HUVECs. Young cells were transfected with either control siRNA or MCAK/Kif2C siRNA for 24 h. After further incubation for 4 days, cells were harvested. (A) Western blot analysis for the levels of MCAK/Kif2C, pRb$^{ser807/811}$, and cyclin A proteins. (B) Cell proliferation measured by cell counting at the indicated times. (C) SA-β-gal staining (×100) and the percentages of SA-β-gal-positive cells. Representative data from four independent experiments are shown. Values are mean ± S.D. of four independent experiments. *$P < 0.05$; Y, young cells; O, old cells; siNC, control siRNA; siMCAK, MCAK/Kif2C siRNA.

old cells compared to young cells (Fig. 1C). When treated with nocodazole, an inhibitor of microtubule polymerization, MCAK/Kif2C was confirmed to be localized in chromosomes of the prometaphase, metaphase, and anaphase in young cells (Fig. 1D). In addition, we tested whether stress-induced premature senescence also causes a decrease in MCAK/Kif2C expression level. When cells were exposed to adriamycin, which induces premature senescence [29], the level of MCAK/Kif2C protein declined in a time-dependent manner (Fig. 1E). These results showed that MCAK/Kif2C was down-regulated under replicative senescence as well as stress-induced premature senescence.

3.2. MCAK/Kif2C knockdown induces premature cellular senescence

To determine role of MCAK/Kif2C in the regulation of cellular senescence of human primary cells, MCAK/Kif2C expression was knocked-down with MCAK/Kif2C siRNA and the resulting senescence phenotypes were assessed. Down-regulation of MCAK/Kif2C was confirmed by Western blotting (Fig. 2A). MCAK/Kif2C reduction in young cells was associated with decreases in the levels of phosphorylated Rb and cyclin A, which have been reported to be decreased in cellular senescence [30] (Fig. 2A). Whereas cell proliferation was decreased, SA-β-gal activity was enhanced by MCAK/Kif2C knockdown (Fig. 2B and C). To examine whether inhibition of cell proliferation in MCAK/Kif2C siRNA cells is caused by apoptosis, Annexin V staining and the levels of PARP1/2 and caspase 3 were measured. Flow cytometry revealed that Annexin V staining was not increased by MCAK/Kif2C knockdown (Fig. 3A and B). No cleavages of PARP-1/2 and caspase 3 were observed (Fig. 3C). These results suggested that knockdown of MCAK/Kif2C stimulated premature cellular senescence in human primary cells.

3.3. MCAK/Kif2C overexpression partially reverses senescence phenotypes

In contrast, to investigate the effects of MCAK/Kif2C overexpression on cellular senescence in old cells, old cells were transduced with MCAK/Kif2C recombinant adenovirus and the senescence features were assessed. An increase in MCAK/Kif2C level was confirmed by RT-PCR (Fig. 4A). MCAK/Kif2C up-regulation significantly increased cell proliferation (Fig. 4B), decreased SA-β-gal activity, and converted flattened and enlarged cell morphology of senescence cells to young cell-like morphology (Fig. 4C). These data implicated that up-regulation of MCAK/Kif2C level in old cells resulted in partial reversion of senescence phenotype.

3.4. Induction of premature cell senescence by MCAK/Kif2C down-regulation via a p53-dependent pathway

Since cellular senescence is regulated by p53- and p16/Rb-dependent tumor suppressor pathways [31], it was presently appropriate to investigate which pathway might be involved in premature senescence induced by MCAK/Kif2C knockdown. Knockdown of p53, p16, and MCAK/Kif2C were confirmed by RT-PCR and Western blotting (Fig. 5A). A decrease in cell proliferation and an increase in SA-β-gal activity by MCAK/Kif2C knockdown were not observed in p53 siRNA cells, but were evident in p16 siRNA cells (Fig. 5B and C). Senescent-like cell morphology was also shown in p16 siRNA cells, but not p53 siRNA cells (Fig. 5D). These results support the view that premature cellular senescence induced by MCAK/Kif2C knockdown might be regulated via a p53-dependent pathway.

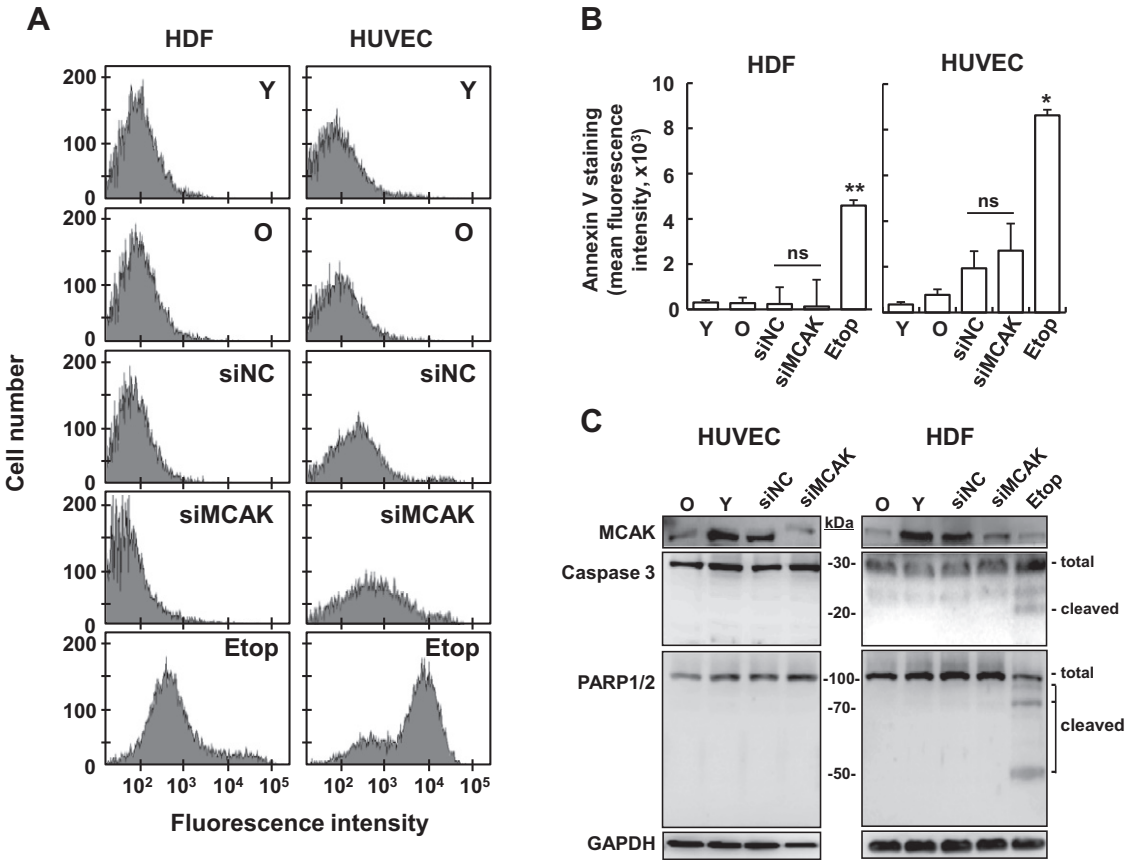

Fig. 3. Effects of MCAK/Kif2C knockdown on apoptosis in young HDFs and HUVECs. Young cells were transfected with either control siRNA or MCAK/Kif2C siRNA for 24 h. After further incubation for 4 days, cells were harvested. Cells were also treated with 100 μg/ml etoposide for 1 day and harvested. (A) Flow cytometry of cells stained with Annexin V. (B) Mean fluorescence intensity of cells with stained with Annexin V. Values are means ± S.D. of three independent experiments. (C) Western blot analysis of MCAK, caspase 3 and PARP1/2 proteins. Representative data from three independent experiments are shown. $^{**}P < 0.01$ or $^{*}P < 0.05$ vs siNC; ns, not significant; Y, young cells; O, old cells; siNC, control siRNA; siMCAK, MCAK/Kif2C siRNA; Etop, etoposide.

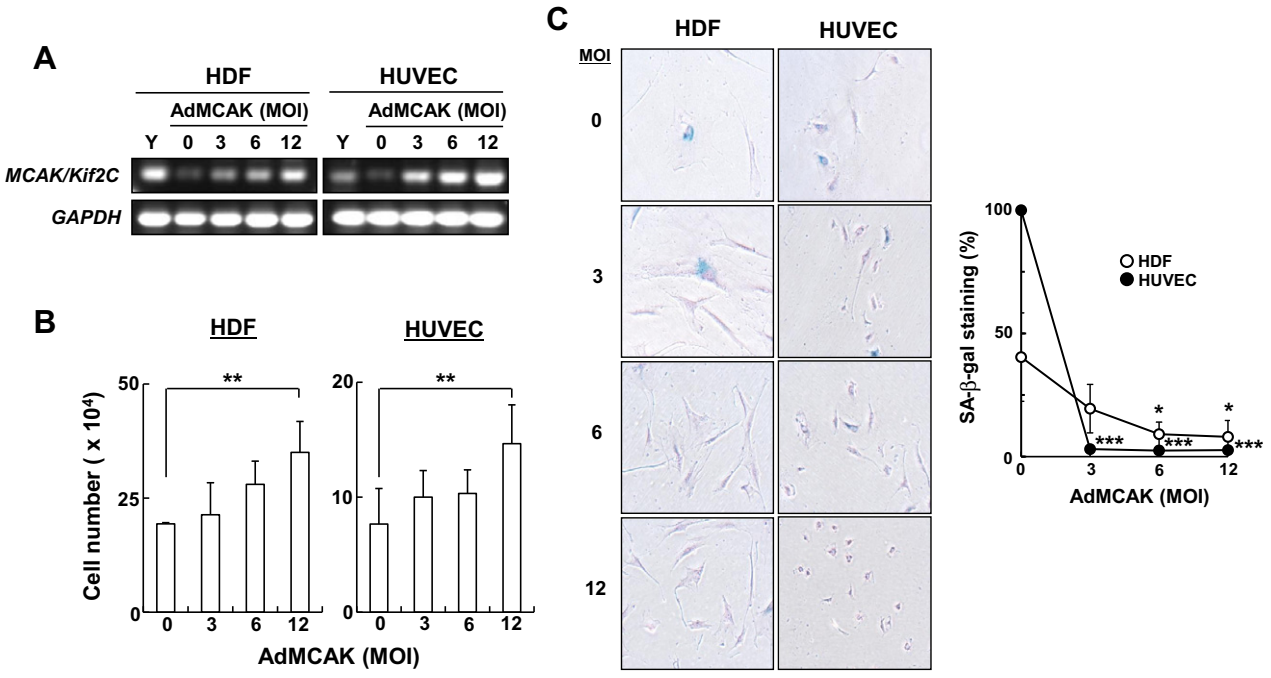

Fig. 4. Effects of MCAK/Kif2C up-regulation on cellular senescence in old HDFs and HUVECs. Old cells were transduced with recombinant MCAK/Kif2C adenovirus for 24 h. After further incubation for 4 days, cells were harvested. (A) RT-PCR. (B) Data on cell proliferation. (C) SA-β-gal staining (×100) and the percentages of SA-β-gal-positive cells. Representative data from five or six independent experiments are shown. $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$; Y, young cells; O, old cells; MOI, multiplicity of infection; AdMCAK, recombinant MCAK/Kif2C adenovirus.

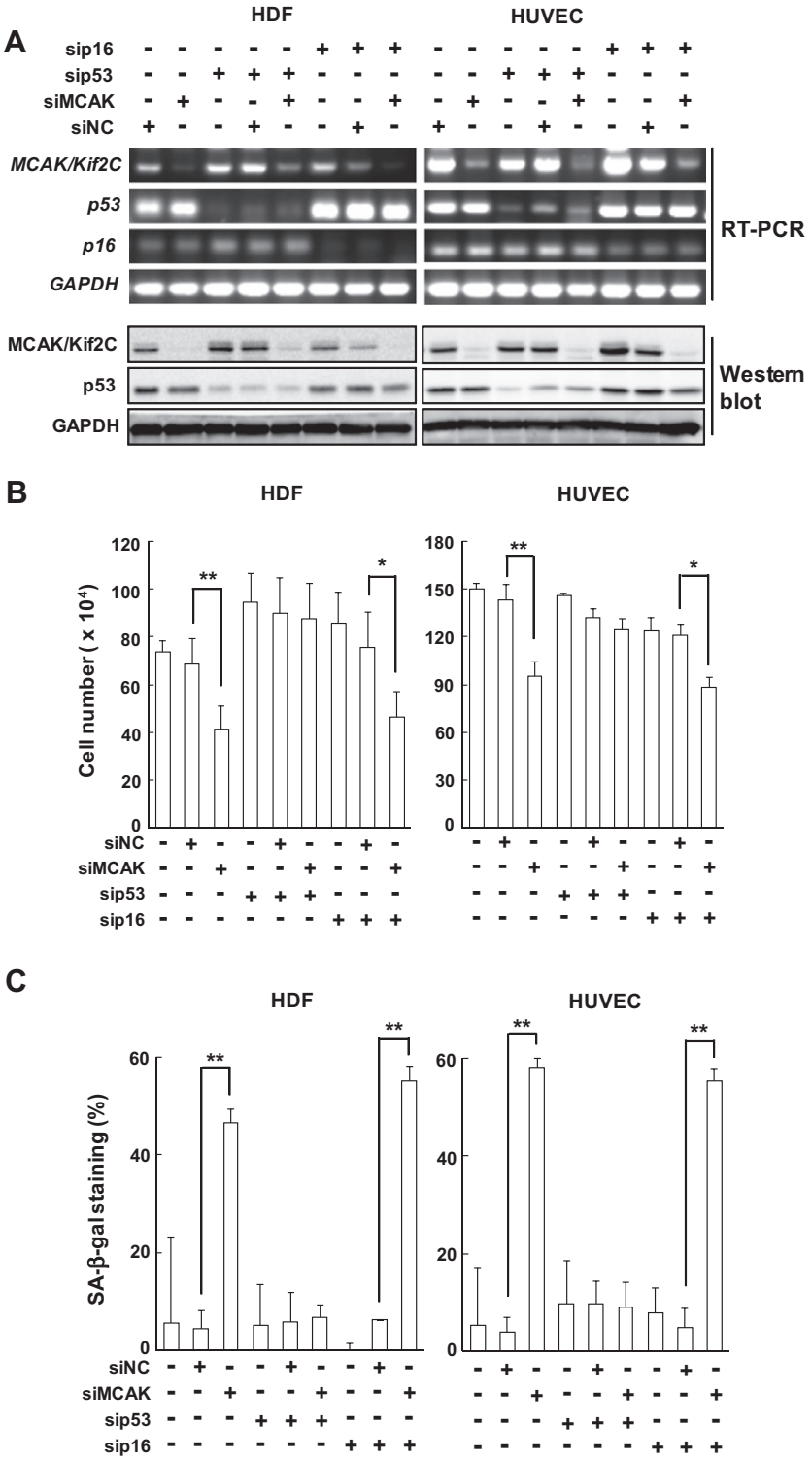

Fig. 5. Effect of p53 or p16 knockdown on cellular senescence induced by MCAK/Kif2C down-regulation in HDFs and HUVECs. Cells were transfected with p53 or p16 siRNAs and incubated for 24 h. Then cells were transfected with either control or MCAK/Kif2C siRNA and further incubated for 24 h. After 4-day incubation, cells were harvested. (A) RT-PCR and Western blot analyses. (B) Cell counting. (C) The percentages of SA-β-gal-positive cells. (D) SA-β-gal staining (×100). Representative data from three independent experiments are shown. Values are means ± S.D. of three independent experiments. $^{*}P < 0.05$ or $^{**}P < 0.01$. Y, young cells; O, old cells; siNC, control siRNA; siMCAK, MCAK/Kif2C siRNA; sip53, p53 siRNA; sip16, p16 siRNA.

4. Discussion

The present study clearly shows that MCAK/Kif2C is involved in the regulation of cellular senescence in human primary cells. This conclusion is based on the following observations: (i) the expression levels of MCAK/Kif2C decreased in cells under replicative or premature senescence induced by adriamycin (Fig. 1B–E), (ii) down-regulation of MCAK/Kif2C in young cells induced premature senescence (Fig. 2), and (iii) ectopic expression of MCAK/Kif2C in senescent cells partially rescued senescence phenotypes (Fig. 4).

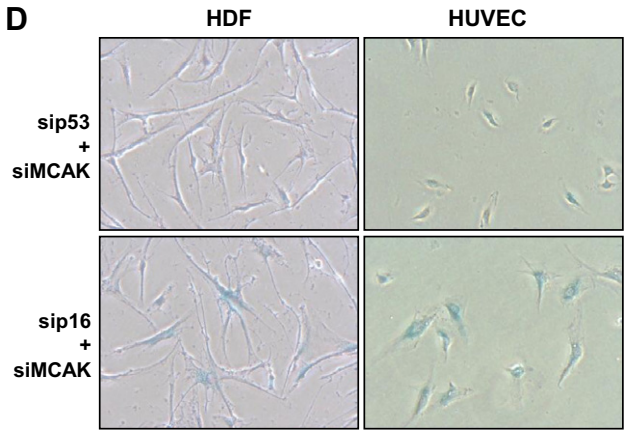

Fig. 5. (*continued*)

To our knowledge, this is the first report to reveal the involvement of MCAK/Kif2C in cellular senescence in human primary cells. However, some indirect evidence has suggested that MCAK/Kif2C might be involved in cellular senescence by regulating proliferation. The expression levels of MCAK/Kif2C gene are higher in gastric cancer tissue [25], breast cancer [27], prostate cancer [32], and in a variety of solid tumors [26]. Moreover, its expression levels correlate with the proliferative activity of the tumor, lymph node metastasis, and overall survival [25,26]. Consistent with clinical findings, ectopic expression of MCAK/Kif2C in gastric cancer cells increases cell proliferation as well as migratory ability [25]. Altered expression of MCAK/Kif2C might be caused by decreased methylation of CpG island of its promoter in breast cancer [33]. In contrast, down-regulation of MCAK/Kif2C effectively inhibits the growth of the breast cancer cells [27] and prostate cancer cells [32], and decreases the mitotic index in HeLa cells by blocking the prometaphase-to-metaphase transition with misaligned or malorientated chromosomes, suggesting the inhibition of cell proliferation [16]. The absence of MCAK/Kif2C in breast cancer cells resulted in enlarged cell morphology changes due to the inhibition of cytokinesis [27]. These lines of evidence point to MCAK/Kif2C's role in cell proliferation and growth, indirectly supporting its association with cellular senescence.

MCAK/Kif2C as an ATP-dependent microtubule (MT) depolymerase affects diverse aspects in mitosis during cell division, such as spindle assembly, MT dynamics, correct kinetochore-MT attachment, and chromosome positioning and segregation [14]. Therefore, MCAK/Kif2C activity should be regulated in a precisely-coordinated and finely-tuned manner. Several protein kinases involved in mitosis and the cell cycle, such as Aurora B [18] and A [20], and Plk1 [21], are pivotal to the complex spatiotemporal phosphorylation-mediated regulation of MCAK/Kif2C activity. Among these kinases, Aurora B is down-regulated during cellular senescence and regulates cellular senescence in human fibroblasts and endothelial cells through a p53-dependent pathway [12]. Aurora A overexpression induces cellular senescence in mammary gland hyperplastic tumors developed in p53-deficient mice [34]. Bub1 mutant murine embryonic fibroblasts (MEFs) underwent premature senescence displaying defects in chromosome congression to the metaphase plate, severe chromosome missegregation, and aneuploidy [13]. Down-regulation of BubR1 levels in MEFs resulted in aneuploidy and premature senescence, and BubR1-null mice revealed early aging-associated phenotypes along with progressive aneuploidy [35]. Our results are consistent with these studies in supporting the view that alteration of MCAK/Kif2C activity might be involved in the regulation of cellular senescence.

p53 and pRb/p16INK4A tumor suppressor pathways play a critical role in the regulation of cellular senescence induced by diverse factors [5]. Therefore, we tried to elucidate the tumor suppressor pathway involved in cell senescence induced by MCAK/Kif2C down-regulation. The present data confirm that the p53-dependent pathway might govern the regulation of cellular senescence induced by MCAK/Kif2C knock-down. Two findings support this suggestion. Firstly, MCAK/Kif2C knockdown decreased cell proliferation in p16-shRNA-treated cells, but not in p53-shRNA-treated cells (Fig. 5B). Secondly, MCAK/Kif2C knockdown increased SA-β-gal activity in p16-shRNA-treated cells but not in p53-shRNA-treated cells (Fig. 5C and D). MCAK/Kif2C expression has been reported to be regulated by a p53 dependent pathway. MCAK/Kif2C levels are increased by ectopic expression of p53 but decreased by depletion of p53 in breast cancer cells [27] and the promoter sequence of MCAK/Kif2C was predicted to contain a putative p53 binding site [36], suggesting the involvement of p53 in the regulation of MCAK/Kif2C level. Further study is necessary to investigate the complex regulatory mechanism between MCAK/Kif2C and p53 involved in the regulation of cellular senescence.

In conclusion, our results reveal a causative role of MCAK/Kif2 down-regulation in cellular senescence of human primary cells, thus contributing to tissue homeostasis, organism aging, and age-related diseases. MCAK/Kif2C down-regulation during cellular senescence seems to function in the growth arrest of cells with aberrant chromosomes, as well as in protection of cellular transformation.

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References

[1] Hayflick, L. and Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. Exp. Cell Res. 25, 585–621.
[2] Kuilman, T., Michaloglou, C., Mooi, W.J. and Peeper, D.S. (2010) The essence of senescence. Genes Dev. 24, 2463–2479.
[3] Adams, P.D. (2009) Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Mol. Cell 36, 2–14.
[4] Rodier, F. and Campisi, J. (2011) Four faces of cellular senescence. J. Cell. Biol. 192, 547–556.
[5] Collado, M., Blasco, M.A. and Serrano, M. (2007) Cellular senescence in cancer and aging. Cell 130, 223–233.
[6] Aranda-Anzaldo, A. (2009) A structural basis for cellular senescence. Aging 1, 598–607.
[7] Campisi, J. (2005) Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 120, 513–522.
[8] Ly, D.H., Lockhart, D.J., Lerner, R.A. and Schultz, P.G. (2000) Mitotic misregulation and human aging. Science 287, 2486–2492.
[9] Reddy, S. and Comai, L. (2012) Lamin A, farnesylation and aging. Exp. Cell Res. 318, 1–7.
[10] Pegoraro, G., Kubben, N., Wickert, U., Gohler, H., Hoffmann, K. and Misteli, T. (2009) Ageing-related chromatin defects through loss of the NURD complex. Nat. Cell Biol. 11, 1261–1267.
[11] Maehara, K., Takahashi, K. and Saitoh, S. (2010) CENP-A reduction induces a p53-dependent cellular senescence response to protect cells from executing defective mitoses. Mol. Cell. Biol. 30, 2090–2104.
[12] Kim, H.J., Cho, J.H., Quan, H. and Kim, J.R. (2011) Down-regulation of Aurora B kinase induces cellular senescence in human fibroblasts and endothelial cells through a p53-dependent pathway. FEBS Lett. 585, 3569–3576.
[13] Schliekelman, M., Cowley, D.O., O'Quinn, R., Oliver, T.G., Lu, L., Salmon, E.D. and Van Dyke, T. (2009) Impaired Bub1 function in vivo compromises tension-dependent checkpoint function leading to aneuploidy and tumorigenesis. Cancer Res. 69, 45–54.
[14] Sanhaji, M., Friel, C.T., Wordeman, L., Louwen, F. and Yuan, J. (2011) Mitotic centromere-associated kinesin (MCAK): a potential cancer drug target. Oncotarget 2, 935–947.
[15] Hunter, A.W., Caplow, M., Coy, D.L., Hancock, W.O., Diez, S., Wordeman, L. and Howard, J. (2003) The kinesin-related protein MCAK is a microtubule depolymerase that forms an ATP-hydrolyzing complex at microtubule ends. Mol. Cell 11, 445–457.

[16] Zhu, C., Zhao, J., Bibikova, M., Leverson, J.D., Bossy-Wetzel, E., Fan, J.B., Abraham, R.T. and Jiang, W. (2005) Functional analysis of human microtubule-based motor proteins, the kinesins and dyneins, in mitosis/cytokinesis using RNA interference. Mol. Biol. Cell 16, 3187–3199.
[17] Ganguly, A., Bhattacharya, R. and Cabral, F. (2008) Cell cycle dependent degradation of MCAK: evidence against a role in anaphase chromosome movement. Cell Cycle 7, 3187–3193.
[18] Andrews, P.D., Ovechkina, Y., Morrice, N., Wagenbach, M., Duncan, K., Wordeman, L. and Swedlow, J.R. (2004) Aurora B regulates MCAK at the mitotic centromere. Dev. Cell 6, 253–268.
[19] Gorbsky, G.J. (2004) Mitosis: MCAK under the aura of Aurora B. Curr. Biol. 14, R346–R348.
[20] Zhang, X., Ems-McClung, S.C. and Walczak, C.E. (2008) Aurora A phosphorylates MCAK to control ran-dependent spindle bipolarity. Mol. Biol. Cell 19, 2752–2765.
[21] Zhang, L. et al. (2011) PLK1 phosphorylates mitotic centromere-associated kinesin and promotes its depolymerase activity. J. Biol. Chem. 286, 3033–3046.
[22] Stout, J.R., Rizk, R.S., Kline, S.L. and Walczak, C.E. (2006) Deciphering protein function during mitosis in PtK cells using RNAi. BMC Cell Biol. 7, 26.
[23] Bakhoum, S.F., Genovese, G. and Compton, D.A. (2009) Deviant kinetochore microtubule dynamics underlie chromosomal instability. Curr. Biol. 19, 1937–1942.
[24] Ganguly, A., Yang, H. and Cabral, F. (2011) Overexpression of mitotic centromere-associated Kinesin stimulates microtubule detachment and confers resistance to Paclitaxel. Mol. Cancer Ther. 10, 929–937.
[25] Nakamura, Y., Tanaka, F., Haraguchi, N., Mimori, K., Matsumoto, T., Inoue, H., Yanaga, K. and Mori, M. (2007) Clinicopathological and biological significance of mitotic centromere-associated kinesin overexpression in human gastric cancer. Br. J. Cancer 97, 543–549.
[26] Gnjatic, S. et al. (2010) NY-CO-58/KIF2C is overexpressed in a variety of solid tumors and induces frequent T cell responses in patients with colorectal cancer. Int. J. Cancer 127, 381–393.
[27] Shimo, A. et al. (2008) Involvement of kinesin family member 2C/mitotic centromere-associated kinesin overexpression in mammary carcinogenesis. Cancer Sci. 99, 62–70.
[28] Dimri, G.P. et al. (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. USA 92, 9363–9367.
[29] Kim, K.S., Seu, Y.B., Baek, S.H., Kim, M.J., Kim, K.J., Kim, J.H. and Kim, J.R. (2007) Induction of cellular senescence by insulin-like growth factor binding protein-5 through a p53-dependent mechanism. Mol. Biol. Cell 18, 4543–4552.
[30] Byun, H.O. et al. (2009) Cathepsin D and eukaryotic translation elongation factor 1 as promising markers of cellular senescence. Cancer Res. 69, 4638–4647.
[31] Campisi, J. and d'Adda di Fagagna, F. (2007) Cellular senescence. when bad things happen to good cells. Nat. Rev. Mol. Cell Biol. 8, 729–740.
[32] Sircar, K. et al. (2012) Mitosis phase enrichment with identification of mitotic centromere-associated kinesin as a therapeutic target in castration-resistant prostate cancer. PLoS ONE 7, e31259.
[33] Kamalakaran, S. et al. (2011) DNA methylation patterns in luminal breast cancers differ from non-luminal subtypes and can identify relapse risk independent of other clinical variables. Mol. Oncol. 5, 77–92.
[34] Zhang, D. et al. (2008) Aurora A overexpression induces cellular senescence in mammary gland hyperplastic tumors developed in p53-deficient mice. Oncogene 27, 4305–4314.
[35] Baker, D.J. et al. (2004) BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat. Genet. 36, 744–749.
[36] Jun, D.Y., Park, H.S., Lee, J.Y. and Kim, Y.H. (2008) Regulation of the human mitotic centromere-associated kinesin (MCAK) promoter by the transcription factors Sp1 and E2F1. Biochim. Biophys. Acta 1779, 356–361.