Mitochondrial Fission and Fusion Mediators, hFis1 and OPA1, Modulate Cellular Senescence

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The number and morphology of mitochondria within a cell are precisely regulated by the mitochondrial fission and fusion machinery. The human protein, hFis1, participates in mitochondrial fission by recruiting the Drp1 into the mitochondria. Using short hairpin RNA, we reduced the expression levels of hFis1 in mammalian cells. Cells lacking hFis1 showed sustained elongation of mitochondria and underwent significant cellular morphological changes, including enlargement, flattening, and increased cellular granularity. In these cells, staining for acidic morphological changes, including enlargement, flattening, and hFis1 in mammalian cells. Cells lacking hFis1 were found to induce mitochondrial fragmentation (1, 2). The Drp1 is predominantly distributed in the cytoplasm and partially associates with the mitochondrial outer membrane (3). A portion of cytosolic Drp1 can be recruited to mitochondria through an interaction with hFis1 (4–6). The opposing process, mitochondrial fusion, is controlled in mammalian cells by Mitofusins (Mfn) and OPA1. Mitofusin1 and -2 (Mfn1 and Mfn2) localize on the outer membrane of mitochondria and may directly mediate mitochondrial fusion (7–9). OPA1 (optic atrophy 1) is a dynamin family GTPase that resides in the intermembrane space of mitochondria and is essential for mitochondrial fusion (10, 11). However, the functional mechanism by which these proteins cooperate to induce mitochondrial fission and fusion remains unidentified.

Despite relatively intensive studies on the components of the mitochondrial fission and fusion machineries, a link between mitochondrial dynamics and cellular function is only partly established. Mitochondria undergo morphological change during mating (12), meiosis in Saccharomyces cerevisiae (13), and during human spermatogenesis (14) and oogenesis (15). Furthermore, dramatic shape changes of mitochondria occur during cell death. During the early step of apoptosis, the mitochondrial network disintegrates, resulting in mitochondrial fragmentation and condensation (16–19). Upon induction of apoptosis, Drp1 translocates to the potential scission sites of mitochondria (20) and Bax co-localizes with Drp1 and Mfn2 at distinct foci on mitochondria (21, 22). Recently, OPA1, along with rhomboid PARL, has been shown to protect cells from apoptosis by controlling the remodeling of mitochondrial cristae (23, 24). In addition, Bak and Bad (25) are essential for mitochondrial morphogenesis in healthy cells. These findings suggest that

Mitochondria are dynamic organelles that can change in number and morphology within a cell during development, the cell cycle, or when challenged with various cytotoxic conditions. Size, shape, and interconnectivity of mitochondria are determined by fusion and fission. In mammals, the key molecules for mitochondrial fission are hFis1 and Drp1. The hFis1 protein is anchored to the outer mitochondrial membrane via a C-terminal transmembrane domain, and overexpression of hFis1 was found to induce mitochondrial fragmentation (1, 2). The Drp1 is predominantly distributed in the cytoplasm and partially associates with the mitochondrial outer membrane (3). A portion of cytosolic Drp1 can be recruited to mitochondria through an interaction with hFis1 (4–6). The opposing process, mitochondrial fusion, is controlled in mammalian cells by Mitofusins (Mfn) and OPA1. Mitofusin1 and -2 (Mfn1 and Mfn2) localize on the outer membrane of mitochondria and may directly mediate mitochondrial fusion (7–9). OPA1 (optic atrophy 1) is a dynamin family GTPase that resides in the intermembrane space of mitochondria and is essential for mitochondrial fusion (10, 11). However, the functional mechanism by which these proteins cooperate to induce mitochondrial fission and fusion remains unidentified.

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The abbreviations used are: Mfn, mitofusin; JC-1, 5,5′,6,6′-tetrachloro-1,10,3,30-tetrathylbenzimidazole carboxyanide iodide; Δψm, mitochondrial membrane potential; TGase 2, transglutaminase 2; ROS, reactive oxygen species; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RNAi, RNA interference; shRNA, short hairpin RNA; XTT, sodium 3′-1-(phenylaminocarbonyl)-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate.

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Mitochondrial dynamics plays an important role in both cellular functions involved in cell survival and cell death. Inhibition of mitochondrial fusion significantly affects cellular function. Disruption of mitochondrial fusion by knockdown of Mfn1 or OPA1 leads to mitochondrial fragmentation, and targeted null mutations of either Mfn1 or Mfn2 in mice triggered mid-gestational lethality (9). Mfn-null or OPA1 RNAi cells show severe defects in mitochondrial fusion as well as cellular dysfunctions, including poor cell growth and decreased cellular respiration (26). Notably, mutations in genes controlling mitochondrial fusion have been directly associated with several human diseases. Mutations in Mfn2 were found in patients with Charcot-Marie-Tooth neuropathy type 2A (27, 28), and mutations in the OPA1 gene have been shown to cause dominant optic atrophy (29, 30). Thus, defects in mitochondrial fusion cause cellular dysfunctions that relate to different human diseases. So far, however, it is unclear how defects in mitochondrial fission, which result in the formation of elongated net-like structures, affect cellular function.

In this study, we have focused on clarifying the cellular functions of mitochondrial fission by efficiently blocking the fission event using hFis1 RNAi. Our results reveal that hFis1 knockdown leads to sustained mitochondrial elongation and senescence-associated phenotypic changes, and further show that these changes could be suppressed by reconstitution of mitochondrial fission, suggesting that mitochondrial fission opposing mitochondrial fission is required for normal cell growth in mammalian cells. Here we report and discuss a novel cellular function of mitochondrial fission, a defense role against stress-induced cellular senescence.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—MitoTracker RedTM CMXRos, 4',6-diamidine-2-phenylindole, 2',7'-dichlorodihydrofluorescein diacetate, and 5,50,6,60-tetrachloro-1,10,3,30-tetrathylbenzimidazole carbocyanide iodide (JC-1) were obtained from Molecular Probes (Eugene, OR). The polyethyleneimine used for DNA transfection was purchased from Polysciences (Warrington, PA), and hygromycin B was from Roche Applied Science. The polyethyleneimine, Chang or HeLa cells were grown in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen), 1% antibiotic-antimycotic (Invitrogen), 10% fetal bovine serum (Invitrogen), 2,7'-dichlorodihydrofluorescein diacetate, and 5,50,6,60-tetrachloro-1,10,3,30-tetrathylbenzimidazole carbocyanide iodide (JC-1) were obtained from Molecular Probes (Eugene, OR). The polyethyleneimine used for DNA transfection was purchased from Polysciences (Warrington, PA), and hygromycin B was from Roche Applied Science. The anti-hFis1 antibody was kindly provided by Mark A. McNiven (Mayo Clinic and Foundation, Rochester, MN). The antibody against γ-H2AX and α-tubulin was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY), and Calbiochem. The anti-β-actin and anti-c-Myc antibodies were obtained from Sigma.

Cell Culture, RNAi by shRNA Gene Silencing System, and Transfection—Chang (CCL-13, ATCC) and HeLa cells were grown in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 1% antibiotic-antimycotic (Invitrogen) and maintained in 5% CO₂ at 37 °C. RNAi was performed using the short hairpin-activated gene silencing system (31). The plasmids containing hFis1 shRNA or OPA1 shRNA mRNA sequences (31) were expressed under the pRE4 (Invitrogen), which allows for long term suppression of gene expression. These shRNA systems were shown to specifically suppress hFis1 and OPA1 gene expression in HeLa cells (31). One day after transfection with these pRE4 constructs using polyethyleneimine, Chang or HeLa cells were grown in the presence of 200 μg/ml hygromycin B for 2 days. Any dying cells were removed by brief centrifugation, and the cells selected were reseeded on the plates, which is designated as day 0, and further grown in DMEM containing 30 μg/ml hygromycin B for the indicated times.

Immunofluorescence Staining and Confocal Microscopy—Chang cells expressing appropriate RNAi constructs were seeded onto coverslips and grown in the DMEM containing 30 μg/ml hygromycin B. For visualization of mitochondria, cells were stained for 30 min with 125 nm MitoTracker RedTM and fixed in a solution of methanol-acetone (1:1) for 10 min. Fixed cells were permeabilized for 5 min in PBS containing 0.075% Triton X-100, preincubated in blocking solution (1% bovine serum albumin in PBS), and then incubated overnight with appropriate primary antibodies at 4 °C. The cells were then washed, probed with a fluorescence-conjugated secondary antibody, and mounted for microscopic observation. For analysis of mitochondrial membrane potential, cells were incubated for 20 min in culture medium containing 5 μg/ml JC-1 and analyzed by microscopy. All images were captured with an LSM510 Zeiss confocal microscopy (Carl Zeiss, Thornwood, NY).

Senescence-associated β-Galactosidase Assay—Senescence-associated β-galactosidase was assayed at pH 6.0 (32). Briefly, cells were washed with PBS and fixed in 2% formaldehyde, 0.2% glutaraldehyde (Sigma) for 10 min. After fixation, cells were washed with PBS and then incubated overnight in freshly prepared staining solution (40 mM citrate-phosphate buffer, pH 6.0, containing 1 mg/ml 5-bromo-4-chloro-3-indolyβ-D-galactopyranoside (X-gal) (Roche Applied Science), 5 mM potassium ferrocyanide (Sigma), 5 mM potassium ferricyanide (Sigma), 150 mM NaCl, and 2 mM MgCl₂). After incubation for 48 h, senescence-associated β-galactosidase-positive cells were counted. The results are the average of at least three independent experiments.

Cell Proliferation Assay—Cell proliferation was determined by evaluating metabolically active cells using an XTT assay kit (Roche Applied Science) according to the manufacturer’s protocol. Briefly, RNAi-transfected cells were plated at a density of 1 × 10⁵ cells/well in a 96-well plate in the presence of 30 μg/ml hygromycin B. After incubation for indicated days, 50 μl containing the XTT labeling mixture, XTT labeling reagent, and electron coupling reagent was added to each well, and the plate was incubated for 4 h at 37 °C. Absorbance generated by the formazan product was measured at 450 nm using an automatic microplate reader. The presented results are the average of at least three independent experiments. For viable cell counting, cells were plated in a density of 1 × 10⁵ cells/well in a 12-well plate in the presence of 30 μg/ml hygromycin B. Each indicated day, cell numbers were counted using hemocytometer after trypan blue staining in four independent experiments.

Analysis of Cellular Granularity, Mitochondrial Transmembrane Potential (ΔΨₘ), and Intracellular ROS Levels by Flow Cytometry—Cellular granularity was quantified by analyzing the 90° side light scatter under flow cytometry. To assess changes in mitochondrial membrane potential (ΔΨₘ), cells transfected with the shRNA constructs were incubated for 20
min with 5 μg/ml of JC-1 fluorescent dye and washed with PBS. For quantification of Δψ<sub>m</sub> disruption, JC-1-stained cells were collected by trypsinization, and the green fluorescence intensities (representing the degree of Δψ<sub>m</sub> disruption) were measured by flow cytometry (FACS Vantage, BD Biosciences). Intracellular ROS levels were determined by staining cells with 10 mM of DCFH-DA fluorescence dye for 20 min, and the fluorescent intensities were also quantified by flow cytometry. The presented results are the average of at least three independent experiments.

**Immunoblotting**—Cells were trypsinized, washed twice with PBS, and lysed with lysis buffer (50 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 1 mM EDTA, 1% Triton X-100, 10 mg/ml each of aprotinin and leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Equivalent amounts of proteins were run on polyacrylamide gels, transferred to nitrocellulose membrane, and immunoblotted. The immunoblots were visualized by the enhanced chemiluminescence system (ECL, Amersham Biosciences).

**RNA Isolation and Reverse Transcription-PCR**—Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. To synthesize first strand cDNA, 1 μg of RNA was reverse-transcribed using reverse transcriptase from avian myeloblastosis virus (Takara, Japan). The synthesized cDNAs were amplified in triplicate using specific primers. The primers used were as follows: TGase 2 sense primer, 5’-CTCGTGGAGCCAGTTATCAACAGCTAC-3’, TGase 2 antisense primer, 5’-TCTCGAAGTTTACCCACCCAGCTTGTG-3’; hFis1 sense primer, 5’-GTCGACATGGAGCCGTCGCTGAAC-3’; hFis1 antisense primer, 5’-GGGCGTGACGGATTCTGCAGTTGGA-3’; GAPDH sense primer, 5’-CCATGGAGAACGTGGGG-3’; and GAPDH antisense primer, 5’-CAGGTTGTCCATGAGTGAC-3’.

**Statistical Analyses**—The error bars on figures represent the mean ± S.D. of all determinations. All the experiments were repeated at least three times. Two-sided unpaired <i>t</i> tests were used to assess statistical significance.

**RESULTS**

**Knockdown of hFis1 Induces Senescence-associated Phenotypic Changes**—To investigate the cellular consequences of hFis1 depletion, we used the pREP4 plasmid harboring U6-hFis1 shRNA inserts for RNA interference of endogenous hFis1 mRNA levels in Chang and HeLa cells (31). Transfected cells were initially selected with 200 μg/ml hygromycin B and then maintained in the presence of 30 μg/ml hygromycin B. Western blotting confirmed the diminished level of hFis1 in cells (Fig. 1B) where the 1st day of incubation with the lower concentration of hygromycin B was designated as day 0. Most cells transfected with the control shRNA showed a normal short tubular mitochondrial structure (Fig. 1A, upper panel), whereas more than 70% of the hFis1 knockdown cells had an elongated, net-like structure of mitochondria (Fig. 1A, lower panel) as observed previously (31), and the remaining cells showed a normal short tubular mitochondrial structure as control cells (Fig. 1C). Both Chang and HeLa cells displayed the same morphological changes of mitochondria after hFis1 RNAi (data not shown).

Notably, the hFis1 knockdown cells exhibited significant cellular morphological changes. The hFis1 knockdown cells started becoming progressively flattened on day 2 and were obviously flattened and enlarged on day 4 (Fig. 2A), showing a phenotype similar to that of cells undergoing senescence. Typically, cells entering senescence remain in a nondividing state and undergo dramatic changes in cellular morphology with increased cellular granularity (33–35). The changes in cell granularity were assessed by flow cytometry. The 90° side light scatter values were significantly higher in the hFis1 knockdown cells than in control cells, showing 2-fold increase on day 2 and 3-fold increase on day 4 (Fig. 2B). Microscopic evaluations revealed that the proliferation of hFis1-depleted cells was noticeably slow. Indeed, cell growth of the hFis1-depleted cells...
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**FIGURE 2.** The hFis1 depletion induces senescence-associated phenotypic changes. Chang cells were transfected and selected as described in Fig. 1. A, microscopic observation of cells under the same magnification on day 4. B, cellular granularity was evaluated by analyzing the 90° side light scatter (90° LS) values in flow cytometry. Histogram of flow cytometry is shown at the right. C, cells were plated in a density of 1 × 10⁴ cells/well in a 12-well plate in the presence of 30 μg/ml hygromycin B. Each indicated day, cell numbers were counted using hemocytometer after trypan blue staining in four independent experiments. D, Chang cells were fixed and incubated in freshly prepared staining solution for 2 days. E, quantification of senescence-associated β-galactosidase-positive cells and ~300 cells in several fields were counted in four independent experiments. F, levels of TGase 2 mRNA expression were detected by reverse transcription-PCR. GAPDH was used as an internal control. *, p < 0.05; **, p < 0.01 versus control shRNA by Student’s t test.

was found to be significantly retarded in measuring cell numbers (Fig. 2C) and by XTT assays (data not shown). To assess whether these senescence phenotypes are caused by an hFis1 depletion-specific event, we established the cell lines stably overexpressing the Mfn1 or Drp1K38A gene. These cells exhibited mild phenotypes in mitochondrial elongation as well as in morphological changes of senescence (data not shown). Collectively, these findings indicate that the sustained extensive elongation of mitochondria accompanies the senescence-associated morphological changes.

Senescence-associated-β-galactosidase activity has been used as a reliable biochemical marker for cellular senescence (33–35). On days 4 and 5, ~30% of the hFis1 RNAi cells stained positive for senescence-associated β-galactosidase (Fig. 2, D and E; blue-colored cells), whereas no staining was observed in control cells. Reverse transcription-PCR analysis of hFis1 RNAi cells for expression of TGase 2, being up-regulated in senescent cells (36), revealed the increased TGase 2 mRNA expression (Fig. 2F). Together, these results indicate that prolonged depletion of hFis1 can induce senescence-associated phenotypic changes.

Reconstitution of hFis1 to the hFis1-depleted Cells Reduces Positive Senescence-associated β-Galactosidase Staining—To confirm that the observed senescence-associated phenotypic introduction of hFis1ΔTM (Fig. 3B). In addition, overexpression of Drp1 neither induced mitochondrial fragmentation (data not shown) nor rescued cells from senescence-associated changes in hFis1 RNAi cells (Fig. 3B), suggesting that additional Drp1 overexpression without appropriate interaction with hFis1 cannot induce mitochondrial fission. Because hFis1 knockdown by itself did not affect endogenous cellular levels of mitochondrial fission and fusion molecules (data not shown), including Drp1 (Fig. 3D), we conclude that hFis1 depletion did indeed cause the observed senescence-associated phenotypic changes.

OPA1 Knockdown Reverses the hFis1 Knockdown-induced Senescence-associated Phenotypic Changes—We next speculated that if the prolonged mitochondrial elongation induced by hFis1 depletion caused senescence-associated changes, then inhibition of mitochondrial fusion leading to mitochondrial fragmentation in these cells might counteract those changes. Inhibition of mitochondrial fusion can be induced either by depletion of Mfns or OPA1 (26). Cells depleted of OPA1 showed severe mitochondrial fragmentation, and simultaneous silencing of the genes encoding OPA1 and hFis1 also triggered extensive mitochondria fragmentation in Chang cells (Fig. 4A), consistent with the previous observation in HeLa cells (31). Notably, the senescent cell morphology (flattening) was signif-
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likely that the intracellular level of OPA1 is not a limiting factor in the control of the mitochondrial fusion process, and under those conditions mitochondrial fission molecules still counteract mitochondrial elongation. Contrary to previous reports (26, 37), we did not observe cell growth inhibition in OPA1 RNAi cells (Fig. 4E; supplemental Fig. 2). This is likely due to the fact that our selection protocol included the removal of early apoptotic cells from the OPA1 RNAi cultures, and we analyzed the cells capable of surviving under the low concentration of hygromycin B. These findings collectively indicate that sustained mitochondrial elongation, not just inhibition of mitochondrial fission per se, may be the primary cause of senescence-associated changes in hFis1 knockdown cells.

The hFis1 Depletion Leads to Changes in Mitochondrial Membrane Potential and ROS Production and Induces DNA Damage—We sought to identify the signals involved in triggering hFis1 depletion-induced senescence-associated changes. Cellular senescence can be induced by sub-lethal stresses, such as accumulation of ROS and DNA damage (35). Therefore, we examined ROS levels in hFis1-depleted cells, using 2',7'-dichlorodihydrofluorescein diacetate staining and flow cytometric measurement of changes in fluorescent intensity. We found that prolonged depletion of hFis1 in fact increased ROS production on day 4 (Fig. 4A). Moreover, sustained depletion of hFis1 caused a significant loss of mitochondrial membrane potential (Δψm; Fig. 5B). In control RNAi cells, high (red fluorescence) and low (green) Δψm values were equally abundant (Fig. 5B, right) after staining with the Δψm-sensitive JC-1 fluorescent dye. In contrast, mitochondria in the hFis1-depleted cells showed a significant reduction of red fluorescence but an increase of green fluorescence. Flow cytometric increase of green fluorescent intensity in hFis1-depleted cells indicated an increased population of mitochondria with low Δψm in these cells. Although both TMRE/TMRM and JC-1 reflect the status of Δψm, we experienced that quantification of fluorescent intensities after TMRE/TMRM staining can be interfered with by extensive mitochondrial elongation or by increased mitochondria mass (data not shown). Finally, we examined the possibility of DNA damage in hFis1 RNAi cells. It is known that γ-H2AX, a histone H2A variant, is phosphorylated upon DNA damage and moves to the DNA damage foci (38). Likewise, the phosphorylated γ-H2AX foci in the nuclei of hFis1-depleted cells were significantly elevated with ~40% of the cells on day 4 (Fig. 5C), indicating that DNA strand breakage occurs in these cells. Thus, prolonged depletion of hFis1 induces loss of Δψm, accumulation of ROS, and DNA strand breakage, which may at least partly contribute to the observed senescence-associated phenotypic changes.

DISCUSSION

One major mystery of mitochondrial biology is the cellular roles of mitochondrial dynamics, and researchers have yet to firmly establish a link between mitochondrial dynamics and cellular function. Here we demonstrate that the sustained extensive mitochondrial elongation caused by hFis1 depletion induces senescence-associated phenotypic changes. To our knowledge, this is the first indication that mitochondrial fission, the opposing force that counters mitochondrial fusion, is
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FIGURE 4. Depletion of both hFis1 and OPA1 reverses the cellular senescence phenotype caused by hFis1 knockdown. Chang cells were transfected with pREP4 constructs containing shRNA targeted against hFis1 and/or OPA1, and transfectants were selected in media containing hygromycin B. A, elongated net-like mitochondrial morphology in hFis1-depleted cells was reversed by simultaneous depletion of OPA1. Mitochondria were visualized with MitoTracker Red staining under confocal microscopy. Scale bars represent 10 μm. Magnification of the inset (**, **) is shown at right. B, expression levels of OPA1 and hFis1 were determined by immunoblotting. C, quantification of senescence-associated β-galactosidase-positive cells; D, cellular granularity by analyzed in flow cytometry in four independent experiments. E, cell proliferation rates were determined by XTT assay in three independent experiments. **, p < 0.01 versus control shRNA by Student’s t test.

FIGURE 5. The hFis1 depletion leads to changes in ∆Ψm and ROS production. Chang cells were transfected and selected as described in Fig. 1. A, intracellular ROS levels were determined by incubation of cells with 10 μM DCFH-DA for 20 min at 37 °C, followed by flow cytometry. B, transfected cells were incubated for 20 min with 5 μg/ml of the JC-1 fluorescent dye. For quantification, JC-1-stained cells were washed, collected by trypsinization, and resuspended in PBS. The green fluorescence intensity (representing the degree of decreased ∆Ψm) was analyzed by flow cytometry in four independent experiments. C, DNA damage was assessed by immunostaining against γ-H2AX. On day 4, cells were fixed and stained with a γ-H2AX-specific antibody and visualized with a fluorescein isothiocyanate-conjugated secondary antibody (green). Nuclei were stained with 4′,6-diamidine-2-phenylindole (blue). The γ-H2AX-positive cells were counted from three independent experiments. **, p < 0.01; ***, p < 0.005 by Student’s t test.

indispensable for normal cell growth and function in mammalian cells. Two recent studies suggested that giant mitochondria might involve cellular senescence in mammalian and plant cells (39, 40). In particular, elongated giant mitochondria have been observed in senescence models induced by treatment with desferoxamine or H2O2, and their emergence has been correlated with reduction in hFis1 levels during senescent progression (39). These results strongly support our findings that balanced fission mediated by hFis1 is important for proper cell growth.

Senescent cells are defined by their characteristic phenotypes. They remain in a nondividing state accompanied by dramatic cellular and morphological changes (35, 41). Replicative senescence refers to a state that occurs following extended cell proliferation and division. Alternatively, cells subjected to sublethal stresses such as accumulation of ROS and oxidative stress, hyperoncogenic activation, DNA damage, and telomere shortening enter a state of stress-induced premature senescence (35, 42, 43). Our data indicate that sustained inhibition of mitochondrial fission behaves as a sublethal stress. Notably, hFis1-depleted cells showed prolonged mitochondrial elongation and progressive senescence, in association with a series of dramatic phenotypic changes. This result differs from the previous report that mitochondrial fusion complements mtDNA mutations (44) and thus could be seen as a defense against senescence. It is thought that fused mitochondrial filaments may compensate for uneven oxygen supplies and may distribute energy more evenly throughout the cell (45). However, the prolonged mitochondrial elongation (fusion) in hFis1-depleted cells appears to differ from normal mitochondrial fusion events. Indeed, our results indicate that cells harboring prolonged mitochondrial elongation show increased ROS levels and decreased ∆Ψm.

Interestingly, in cells depleted of both hFis1 and OPA1, senescent-as-
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