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Actin in Senescence

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Abstract

Actin can be polymerized into actin filaments, which are strictly regulated in spatio-temporal dimension. Actin filaments support the functions of cell morphology, movement, cargo transport, signal transduction and cell division. In yeast, knocking down Scp1p to increase actin turnover, affects mitochondrial function and increase cellular ROS, which result in 65 percent improvement on yeast life span. SM22 is homologous to Scp1p in mammalian cells. We found that actin cytoskeleton turnover was significantly weaker in senescent IMR-90 cells than in young cells. It is known that mitochondria are abnormal in senescent cells. Our results suggest that actin and/or actin binding proteins in mammalian cells may regulate senescence with the similar mechanism as Scp1p in yeast.

Keywords

Cellular senescence; Actin cytoskeleton; FARP.

1. Introduction

Normal human diploid cells have a limited number of cell divisions. When the cells lose the ability of proliferation, the limited number of cell divisions is known as the Hayflick limit[1,2]. Aging is closely related to cellular senescence, and age-related functional declines can lead to muscle disease, atherosclerosis, heart failure, osteoporosis, macromolecule degeneration, lung dysfunction, kidney failure, neurodegenerative diseases and cancer[3]. Cellular senescence usually has some common characteristics, such as large cell size, adherence with a flat shape, and permanent cell cycle arrest. Molecular markers of senescent cells include positive histochemical staining of SA-β-gal[4], high expression of $p16^{INK4a}$ [5], significant down-regulation of Lamin B1 [6], secretion of SASP[7]. Metabolic dysfunction, telomere shortening, DNA damage, ROS, and mutations of Lamin B1 will cause cellular senescence[8]. Generally, senescent cells cannot re-enter the cell cycle, but under special conditions, they are reversible[9,10]. Recent studies have found that selective clearance of senescent cells in experimental animals can delay the onset of age-related diseases and prolong lifespan and healthspan[11,12].

Actin microfilaments perform important functions in diverse cellular functions. Actin monomer was polymerized into filaments (microfilaments) with the assistance of nucleation-related proteins (Formin-2, Arp2/3 complex and Spire-1/2)[13], and could also be depolymerized into monomer actin under the action of cleavage/depolymerization proteins (Cofilin and Mical)[14]. The balance between monomer and filamentous actin is crucial to the execution of its physiological functions. Actin assembly and disassembly are strictly spatio-temporal regulated, supporting life activities such as cell morphology, structure, movement, material transport, signal transduction and cell division[15,16]. Microfilaments and actin monomers are present in the cytoplasm and nucleus. In recent years, nuclear actin shows strong potential in regulating cell functions. Nuclear actin monomer and Arps form a conserved subunit of complex to participate in chromatin remodeling[17]. Actin directly binds to all

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three RNA polymerases I, II, and III and promotes transcription [18,19,20]. The formation of nuclear actin filaments leads to the release of MRTF-A and promotes SRF-mediated transcription[21]. During DNA damage, nuclear actin monomer will polymerize into nuclear F-actin to promote DNA damage repair[22,23].

The dynamic and function of actin in senescent cells are not well studied. It has been reported that the monomer actin and dephosphorylated cofilin significantly accumulate in the senescent diploid nucleus, accompanied by inhibition of LIMK-1 activity[24]. Knocking down Scp1p to increase actin turnover in yeast, affects mitochondrial function and increase cellular ROS, resulting in 65 percent improvement in yeast lifespan[25]. SM22 is homologous to Scp1p in mammalian cells. It is possible that actin and actin binding proteins in mammalian cells may regulate senescence with similar mechanism as Scp1p in yeast. However, the association between actin and aging in mammalian cells are not well characterized. Here, we investigated the relationship between actin turnover and senescence in eukaryotic cells, which helps to reveal the detailed molecular mechanism of senescence and provides potential for delaying cellular senescence.

2. Materials and Methods

2.1 Cell culture and transfection

IMR-90 (CAS，SCSP-5013) was kindly provided by the Stem Cell Bank, Chinese Academy of Sciences. IMR-90 cells were cultured in Minimum Essential Media (Hyclone, SH30265.01) with 10% FBS (Gibco, 10099141C), 1% Gluta-max (Gibco, A12860-01), 1% MEM Non-Essential Amino Acids (Gibco, 11140-050), 1% Sodium pyruvate solution (Sigma, S8636), and 1% Penicillin-Streptomycin (Gibco, 15140122). Cells were grown in an incubator maintained at 37°C and 5% CO_2. Proliferating IMR-90 cells were used at population doubling level (PDL)16~PDL25, and for replicative senescence, cells were cultured until replicative exhaustion (PDL40). IMR-90 cells were transfected with pcDNA3.1(+)-β-actin-EGFP-puro by Lipofectamine™ 3000 Reagent (Invitrogen, L3000015), according to the manufacturer's instructions.

2.2 SA-β-galactosidase (SA-β-Gal) activity

Senescence-associated β-galactosidase activity was assessed using a Senescence β-galactosidase staining kit (Biyotime).

2.3 Fluorescence recovery after photobleaching (FRAP)

IMR-90 proliferation and senescence cells were transfected with pcDNA3.1(+)-β-actin-EGFP-puro. Living cells were photobleached in 2-μm-diameter circles using LSM 880 (Zeiss) and a 488-nm laser line at 100% intensity for 20 iterations. Photobleaching was completed in <250 ms. The recovery of the fluorescence signal over time was then monitored. Recovery curves were generated from between 20 and 30 individual cells recorded from at least three separate experiments.

2.4 Image analysis and processing

Images were analyzed using Fiji (http://fiji.sc) and Imaris (Bitplane).

3. Results

3.1 Establishment of a cellular senescence model

To study cellular senescence, we first established a replicative cellular senescence model. Cellular senescence was confirmed by an increase staining of senescence-associated beta-galactosidase (SA-β-gal), young cells were rarely stained but senescent cells were stained blue (Fig. 1).

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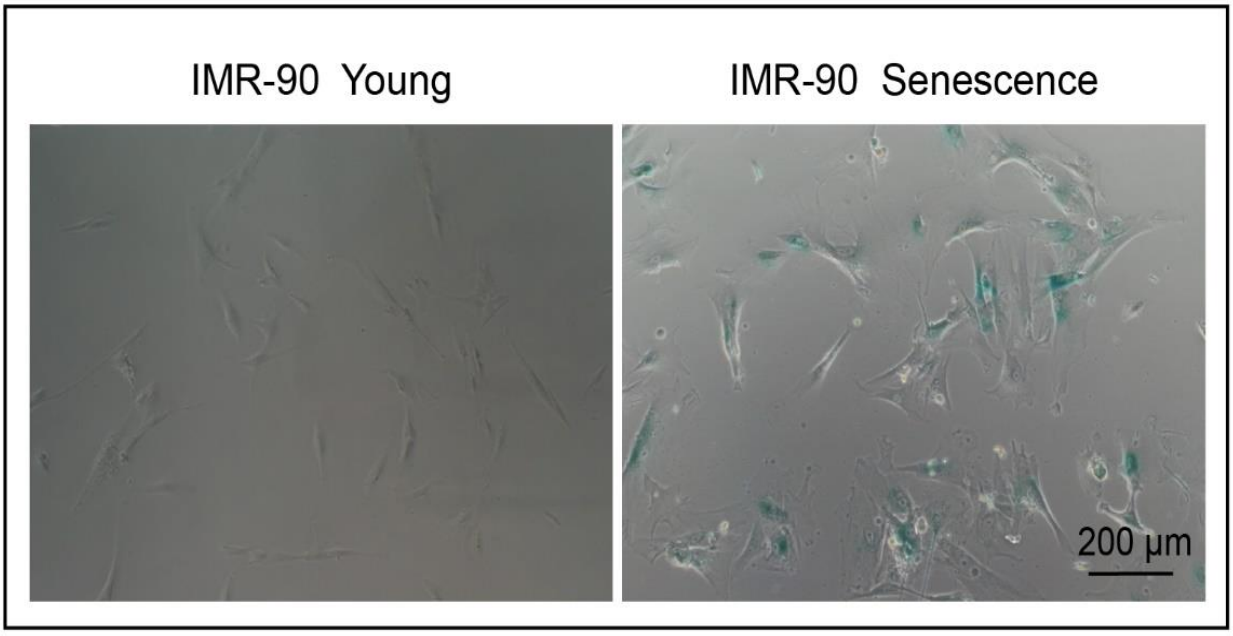

Fig. 1 Young and senescent cells are detected by SA-β-Gal staining. Young cells are rarely stained but senescent cells are stained blue.

3.2 The actin turnover is more efficient in young cells than senescent cells.

Next, to understand the actin turnover efficiency between young cells and senescent cells, both young and senescent IMR-90 cells were transiently transfected with pcDNA3.1(+)-β-actin-EGFP-puro. The actin turnover rate was detected by FRAP using Zeiss LSM 880 microscope (Fig. 2). We analyzed the recovery efficiency of fluorescence fusion proteins in young and senescent cells. We found that the recovery efficiency of fluorescence in young cells was significantly faster than that in senescent cells, indicating that the actin turnover efficiency in young cells was higher than that in senescent cells (Fig. 3).

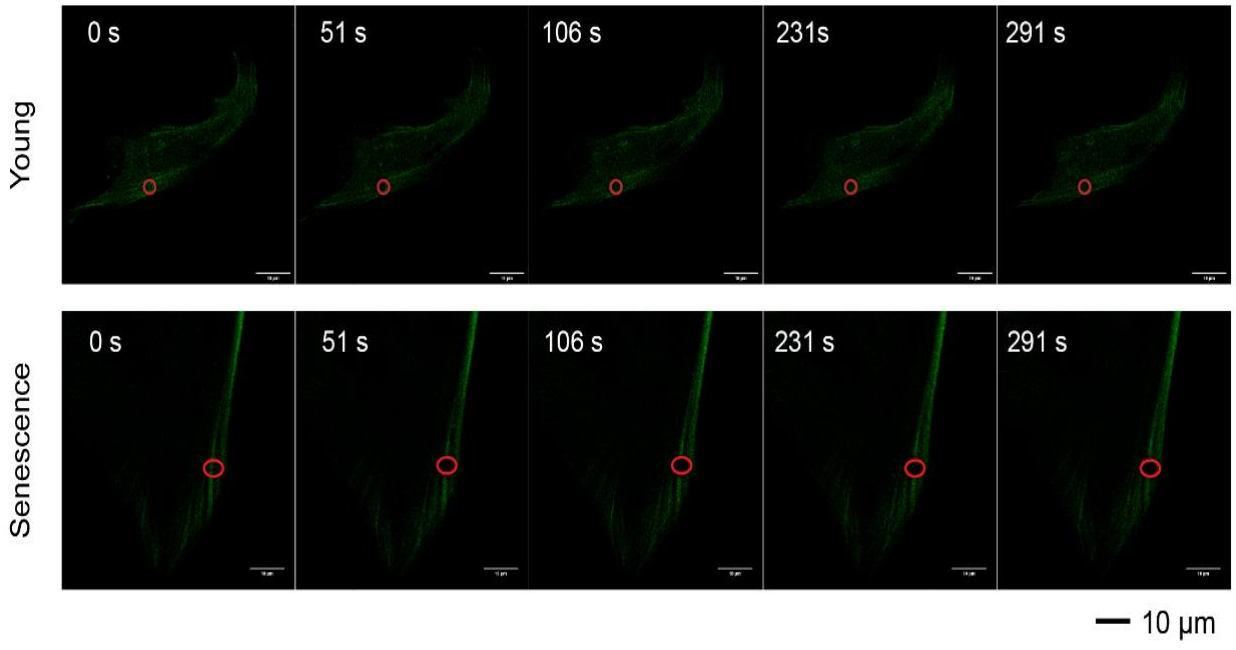

Fig. 2 Young and senescent IMR-90 cell were transient transfected pcDNA3.1(+)-β-actin-EGFP-puro. The actin turnover rate was detected by FRAP using LSM 880 microscope.

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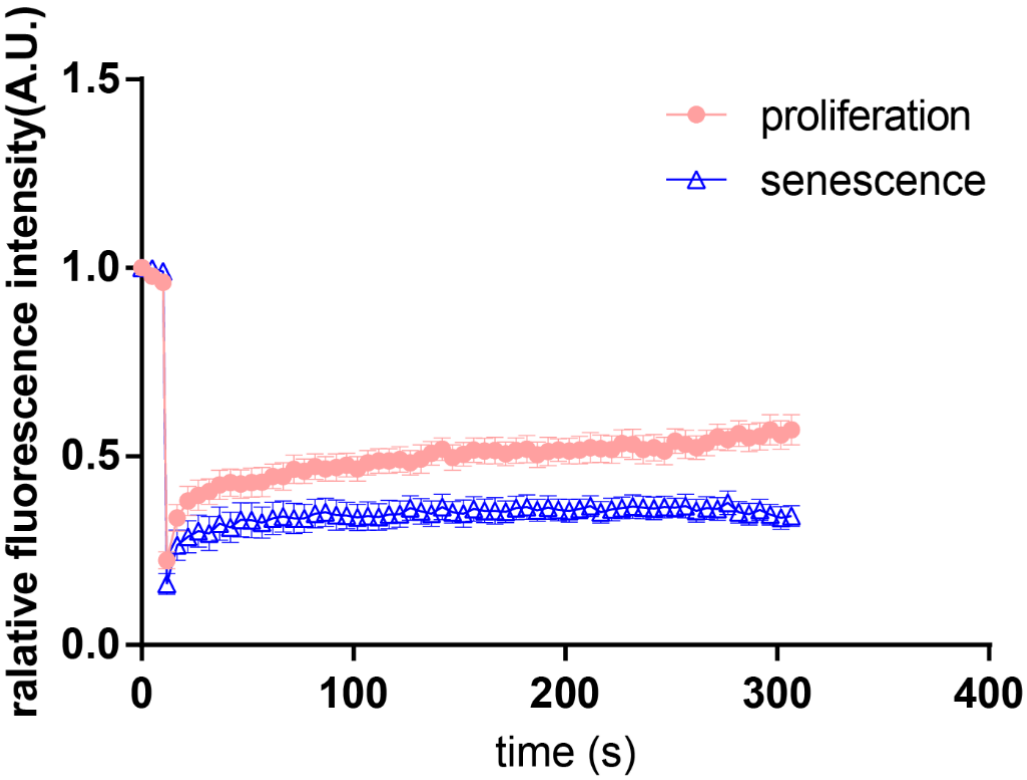

Fig. 3 The actin turnover efficiency in young cells is higher than that in senescent cells.

4. Discussion

Dissecting the fundamental mechanisms and effects of cellular senescence is beneficial for the basic aging researches and the development of anti-aging pharmaceutics. In yeast, the relationship between actin turnover and aging has been studied, and actin turnover is closely related to mitochondrial function and aging[26]. In mammal cells, senescence causes mitochondrial damage[27]. Through establishing an IMR-90 cellular senescence model, our subsequence findings suggest that actin turnover in senescent cells is weaker than in younger cells. In general, our results suggest that in mammalian cells, actin may regulate/associate with cellular senescence in the similar way as in yeast, regulating mitochondrial function and aging through changes in actin turnover.

Acknowledgements

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