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Young and Senescent Cells: Distinct Nuclear F-actin Patterns Upon Latrunculin B Induction

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Abstract: Both cellular senescence and cytoskeleton are involved in the formation of many diseases and cell signaling pathways. Although recent studies have shown that F-actin is involved in DNA damage repair, chromatin decompression, gene transcription regulation, and cell fate determination. But studies on F-actin and aging are still absence. It is unclear whether nuclear F-actin is present during cellular senescence. Here, by confocal optical sectioning and time-lapse imaging, we found acitn chrommobody-TagGFP2-NLS shows the beneficial on investigating senescent human fibroblast IMR-90 cells. To induce the nuclear F-actin assembly in single cell, we uesd Latrunculin B (latB) which a cytoplasmic F-actin polymerization inhibitor. It is currently unknown whether the nuclear F-actin cytoskeleton in young and senescent cells responds differently to latB treatment. Here, latB application induces distinct nuclear F-actin patterns and dynamics in young and senescent cells. Thus, after analyzing the results of actin dynamic we demonstrate a diverse effect of latB on the nuclear F-actin cytoskeleton in young and senescent cells.

Keywords: Cellular Senescence; Actin Cytoskeleton; Latrunculin B; Nucleus; F-actin.

1. Introduction

A large number of studies have shown that human aging is related to unhealthy eating habits and lifestyle habits [1-3]. As individuals age, the incidence of diseases such as Parkinson's and cancer increases. Therefore, it's important to study what kind of physiological pathway changes individual cells exhibit in aging models.

Cytoskeleton, a complex structural network of filamentous polymers and regulatory proteins, performs important functions in keeping ingetrity of independent cells [4]. It plays crucial and fundamental roles for many cellular activities such as cell morphogenesis, cell division, regulation of glycolysis, vesicular trafficking, chromatin remodeling and gene transcription etc [5-7]. Actin, being one of the three major components of the cytoskeletal proteins, is evolutionarily conserved across biological kingdoms. Notably, the dynamics of the filamentous actin are crucial for the execution of diverse cellular functions [8].

Whether the nuclear actin cytoskeleton in young and senescent cells responds differently to external pharmaceutical applications remains obscure. Latrunculin B(LatB) was initially identified as a cell-permeable cytoplasmic F-actin polymerization inhibitor. LatB destabilizes the cytoplasmic F-actin networks by binding to free G-actin in a molar ratio of 1:1 at the site adjacent to the ATP-binding cleft [9]. Interestingly, LatB treatment can also induce the assembly of nuclear F-actin [10]. However, little is known about the dynamics of the nuclear F-actin upon latB treatment and whether the nuclear actin cytoskeleton in young and senescent cells responds differently to latB. For future drug discovery and drug application, it is necessary to study how the cellular structure changes in aging cells.

2. Results

2.1. Application of a Probe for Visualizing Nuclear F-actin in IMR-90 Cells

Recently, nuclear F-actin is found to play essential roles in different physiological or stressed conditions. We therefore asked whether nuclear F-actin participates in the cellular senescence process. To visualize nuclear F-actin in young and senescent cells under confocal fluorescence microscopy, a proper probe was essential for labeling nuclear F-actin faithfully in IMR-90 cells. Although cytoplasmic F-actin can be clearly visualized using conventional phalloidin conjugates, many researches have reported that it is technically challenging to detect nuclear F-actin in fixed cells. Moreover, GFP- or derivatives-tagged actin is not fully functional in live cells [11,12]. The caveats led to the development of several actin-binding surrogates to facilitate visualizing nuclear F-actin in live cells [13-15]. LifeAct is a well-proven probe for F-actin visualization [8,16-20]. However, we found it is not suitable for nuclear F-actin visualization in IMR-90 because the overexpression of LifeAct-tdTomato-3NLS artificially induced a high frequency of aberrant nuclear F-actin assembly with nearly unchanged dynamics (Figure 1A). Immunofluoresence staining of nuclear F-actin binding proteins including Cofilin, N-WASP, ARPC2 and Formin 2 in senescent cells did not show such aberrant structures (Figure 1B). These observations, consistent with the previous knowledge about LifeAct and F-actin dynamics, suggested that LifeAct is not an ideal tool for nuclear F-actin visualization in IMR-90 cells. On the contrary, actin chromobody, another widely used F-actin probe [13,14,21], appeared a better tool for nuclear actin visualization when tagged with TagGFP2-NLS (Figure 1C).

Thus, we used actin-chromobody-TagGFP2-NLS overexpressed IMR-90 to detect nuclear actin in the following study.

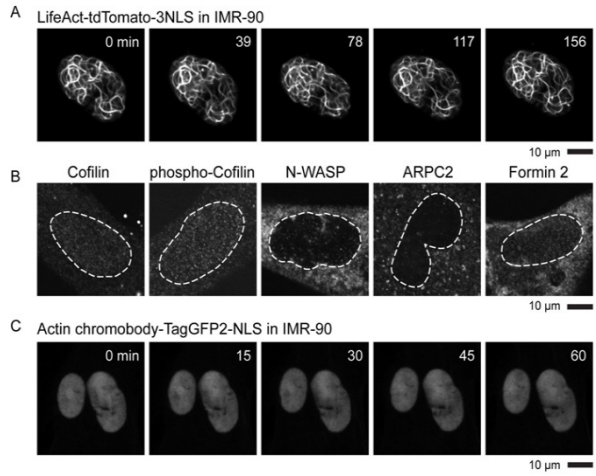

Figure 1. Actin chromobody is a suitable probe for visualizing nuclear F-actin in IMR-90 cells. (**A**) Expression of LifeAct-tdTomato-3NLS artificially induces aberrant nuclear F-actin assembly in IMR-90 cells. (**B**) Immunofluorescence staining images of some nuclear F-actin regulating proteins in senescent IMR-90. (**C**) Expression of actin chromobody-TagGFP2-NLS showed no aberrant nuclear F-actin structure in young IMR-90 cells. Scale bars, 10 μm.

2.2. Distinct Nuclear F-actin Structure and Dynamics Induced Upon latB Treatment.

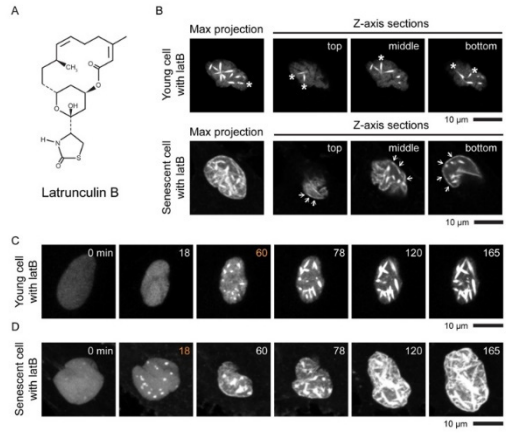

Figure 2. Distinct nuclear F-actin structures and dynamics are induced upon latB treatment in young and senescent cells. (**A**) Molecular structure of latB. (**B**) Confocal microscopy maximum intensity projected images and Z-axis images of young and senescent cell nuclei upon latB treatment. White asterisks show the straight F-actin in a young cell nucleus. White arrows show F-actin adjacent to the nuclear periphery in a senescent cell nucleus. (**C, D**) Maximum intensity projected time-lapse confocal microscopy images of nuclear F-actin in young and senescent cells. Orange time points indicate the initial assembly time of nuclear F-actin in young and senescent cells. Scale bars, 10 μm.

To more specifically address the differences of the nuclear F-actin cytoskeleton in young and senescent cells, we treated IMR-90 cells with the latB (Figure 2A). Treatment with latB on both young and senescent cells induced massive assembly of nuclear F-actin (Figure 2B). Confocal microscopy Z-axis optical sectioning images showed that distribution angle of F-actin within the nucleoplasm was random in both young and senescent cells (Figure 2B, Z-axis sections of the nuclei). Appealingly, the distribution pattern and dynamics of nuclear F-actin were different. After latB treatment, nuclear F-actin in the young cells was straight and away from the nuclear boundary (Figure 2B, white asterisks in the upper panel) but nuclear F-actin in senescent cells was more in number and some nuclear F-actin appeared adjacent to the inner nuclear boundary (Figure 2B, white arrows in the lower panel). To analyze the nuclear F-actin dynamics in detail, confocal microscopy time-lapse imaging was performed. Results showed that, upon latB treatment in both young and senescent cells, multiple F-actin assembled simultaneously within the same nucleus (Figure 2C, 60 min time point and Figure 2D, 18 minutes time point). However, nuclear F-actin assembled faster in the senescent cells than nuclear F-actin in the young cells (Figure 2C, 60 min time point and Figure 2D, 18 minutes time point, both time points indicated the initial appearance of nuclear F-actin). Unlike the transient existence of nuclear F-actin in reported contexts [12,14,22,23], the latB-induced nuclear F-actin structures in both young and senescent cells lasted for hours throughout the whole imaging periods (Figure 2C, 2D, 3A~3C).

2.3. Examples of Nuclear F-actin Dynamics in IMR-90 Cells after latB Treatment

Dynamics of F-actin correlates with diverse cellular functions. Merging (Figure 3A, yellow arrows) and splitting (Figure 3B, yellow arrows) of the nuclear F-actin upon latB applications were observed. In IMR-90 cells treated with latB, while some nuclear F-actin underwent directional movement (Figure 3C, blue asterisks), the majority of nuclear F-actin remained relatively stable (Figure 3C, Figure 2C and 2D). After latB treatment for a longer period, some nuclear F-actin was disassembled (Figure 3C, yellow arrows).

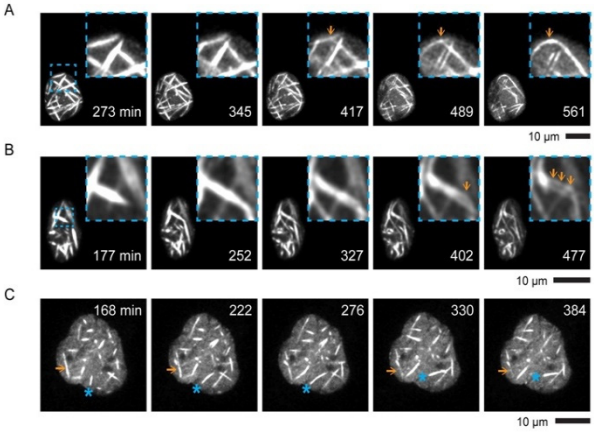

Figure 3. Examples of nuclear F-actin dynamics upon latB treatment. (**A**) Merging of the nuclear F-actin structures (yellow arrows). (**B**) Splitting of the nuclear F-actin structures (yellow arrows). (**C**) Directional movement of the nuclear F-actin structure (blue asterisks) and disassembly of the nuclear F-actin structure (yellow arrows). All representative examples from young IMR-90. Scale bars, 10 μm.

3. Discussion

we established that there is a significant difference of nuclear actin response between young and senescent cells upon latB application. Leveraging confocal optical sectioning and time-lapse imaging, we found that when treated with latB, nuclear F-actin assembly patterns and dynamics differed between the young cells and the senescent cells. It is possible that nuclear actin in the senescent cells is more in quantity compared to the nuclear actin in the young cells. We also demonstrated that the majority of nuclear F-actin is relatively stable after latB-induced assembly, with a small portion of nuclear F-actin splits, merges or moves around.

Our results support the following conclusions: Young and senescent cells respond differently nuclear F-actin dynamics to latB application. Collectively, this study reflects the dynamic changes of nuclear actin in cellular senescence models. And it would be a positive impact on the future drug research and application of latB as well as the study on how cytoskeleton changing affects aging.

4. Materials and Methods

4.1. Cell Culture and Transfection

IMR-90 cells were obtained from Stem Cell Bank, Chinese Academy of Sciences. The cells were cultured in in minimum essential medium (Hyclone, SH30265.01) with 10% FBS (Gibco, 10099141C), 1% GlutaMAX (Gibco, A12860-01), 1% MEM non-essential amino acids (Gibco, 11140-050), 1% sodium pyruvate solution (Sigma, S8636), and 1% penicillin-streptomycin (Gibco, 15140122). Growed in ESCO incubator maintained at 37°C and 5% CO2.To construct an IMR-90 cell senescence model, IMR-90 (PD25) cells were treated with 1 μM Doxorubicin (MCE) for 48 h and harvested 10 days later.

4.2. Drug Treatment

For living cell imaging, IMR-90 cells were treated with Latrunculin B (Cayman, 1 μM).

4.3. Sample Preparation and Immunofluorescence Staining

Cells were incubated in a confocal dish. After drug treatment, cells were washed and fixed with 1 mL 4% paraformaldehyde for 15 min. After washing 3 times with 1 mL PBS 0.1% Triton X-100 was added for 3 min. After washing, add 1 mL 5% BSA for 1 h at room temperature. The primary antibody was diluted with PBS according to the antibody instructions and incubated at room temperature for 2 h. The cells were washed 3 times and added secondary antibodies which diluted with PBS at 1:2000 and incubated for 1 h in the dark at room temperature. The following primary antibodies were used in Figure 1B: Cofilin, CST (5175S); phospho-Cofilin (ser3)77G2, CST (3313); N-WASP, abcam (ab126626); Formin 2, proteintech (11259-1-AP); ARPC2, abcam (ab133315).

4.4. Confocal Microscopy Imaging

Microscopy imaging was performed using Zeiss LSM 880 equipped with a definite focus system, a 20x air objective and a 63x oil objective. General acquisition settings were as follows: frame size 1,024 × 1,024 pixels, gain 600, 0.2~0.5 μm Z step size. Cells were imaged within a heated, humidified incubation chamber with CO2 control.

4.5. Image Analysis and Processing

Confocal microscopy images were analyzed and processed using Fiji (http://fiji.sc).

Abbreviations

Table 1. The Abbreviations

LatB	Latrunculin B
F-actin	micron-scaled actin filaments

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