**International Journal of Biology and Life Sciences**
ISSN: 2957-9511 | Vol. 2, No. 3, 2023

# Nuclear Actin Puncta in Senescent Cells

**Haiying Mai ***

Key Laboratory for Regenerative Medicine, Ministry of Education, College of Life Science and Technology, Jinan University, Guangzhou, 510632, China

* **Corresponding author Email:** 2064210442@qq.com

**Abstract:** Both cellular senescence and the nuclear actin cytoskeleton participate in a plethora of physiological and pathological activities. However, their connection remains elusive. Recently, it is becoming increasingly apparent that filamentous actin plays important functions within the nucleus. Particularly, micron-scaled actin filaments (hereinafter referred to as F-actin) in the nucleus are of the essence in DNA damage repair, cell fate determination and age-related diseases etc. Latrunculin B (latB), a pharmaceutically applicable macrolide, can induce nuclear F-actin assembly. Now it is unclear how the nuclear actin skeleton responds to latB stimulation in senescent cells. Here, by time-lapse images, Doxo-induced senescent IMR-90 cells showed a large number of nuclear actin puncta, then the nuclear actin puncta become brighter and larger in size upon latB treatment. Some actin puncta even served as the elongation bases for the nuclear F-actin. Therefore, our results link the nuclear actin skeleton to cell aging and reveal the effect of latB treatment on the nuclear actin skeleton in aging cells.

**Keywords:** Cellular Senescence; Aging; Actin Cytoskeleton; Nuclear Actin Puncta.

## 1. Introduction

Tremendous researches have shown that human health and biological systems change with aging [1–3]. Many diseases such as cardiovascular disease, cancer, diabetes, osteoarthritis and Parkinson's disease, are closely connected with organismal aging [4–7]. Therefore, for better medication and drug discovery, it is important to understand the mechanism of aging and find out the effects of aging at the cellular level.

Recently, researches have been focusing on dissecting the roles of micron-scaled nuclear filamentous actin under different physiological or stress conditions [9–14]. Although F-actin is widely studied in the cytosol in most cells, the presence of nuclear F-actin, only recently, has been associated with diverse nuclear functions [10,13,15–18]. Transient (usually appears and exists in the order of minutes, disassembles afterwards) assembly of nuclear F-actin is found during serum stimulation, cell spreading, mitotic exit, DNA damage repair and T cell activation [15–20]. In contrary, persistent nuclear F-actin has been proved to correlate with some diseases/stress statuses (such as human skeletal muscle disease intranuclear rod myopathy, Parkinson's disease and Huntington's disease) [21]. The existence of persistent nuclear F-actin inhibits global transcription by RNA Polymerase II and reduces cell proliferation [22]. Therefore, it is interesting and important to unravel the relationship between cellular senescence and the nuclear actin cytoskeleton.

Latrunculin B (latB), a bioactive macrolide with potential pharmaceutical and extensive basic biomedical applications, is firstly isolated from the sponge *Latrunculia magnifica* [23–28]. Human clinical trials were conducted with latB on the treatment of ocular hypertension, early primary open-angle glaucoma and facilitating intracytoplasmic sperm injection (Clinicaltrials.gov Identifier NCT03678597) [23]. In addition to inhibiting actin polymerization, latB can induce nuclear F-actin assembly.

However, the relationship between nuclear actin dynamics and cellular senescence and its role in latB trratment in senescent cells are unknown. In this study, we aimed to solve this problem.

## 2. Results

### 2.1. Establishment of a Cellular Senescence Model

To study cellular senescence, we first established a doxorubicin-induced cellular senescence model[8]. Cellular senescence was confirmed by an increase staining of senescence-associated beta-galactosidase (SA-β-gal) and decrease staining of the cell proliferation marker Ki-67 (Figure 1 A and 1B) [8,29].

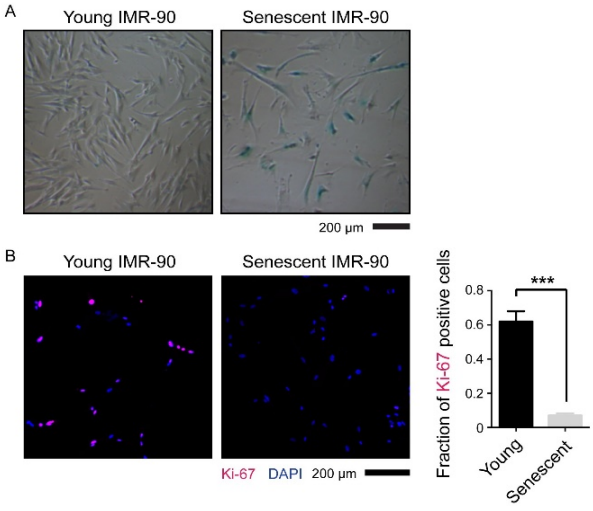


**Figure 1.** Establishment of a senescent IMR-90 cell model. (**A**) Young and senescent cells are detected by SA-β-Gal staining. Young cells are rarely stained but senescent cells are stained blue. (**B**) Ki-67 immunofluorescence staining of young and senescent cells. Graph shows mean ± SEM, *** p<0.001. Scale bars, 200 μm.

### 2.2. RNA Sequencing Comparing Young and Senescent Cells

Next, to understand the potential changes during cellular senescence, we performed RNA sequencing experiments comparing the transcriptomes of the early passage young cells

and the doxorubicin-induced senescent cells. Curiously, the KEGG enrichment analysis showed the enrichment of actin cytoskeleton-related genes among other pathways, suggesting changes of actin regulation or the actin cytoskeleton during cellular senescence (Figure 2).

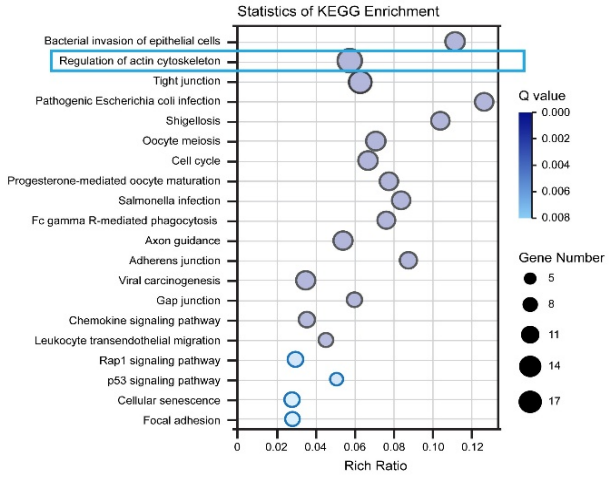


**Figure 2.** KEGG Enrichment of RNA sequencing results shows a change in some actin regulation genes. RNA sequencing was done comparing the young and senescent IMR-90 cells

## 2.3. Nuclear Actin Puncta in Senescent IMR-90 Cells

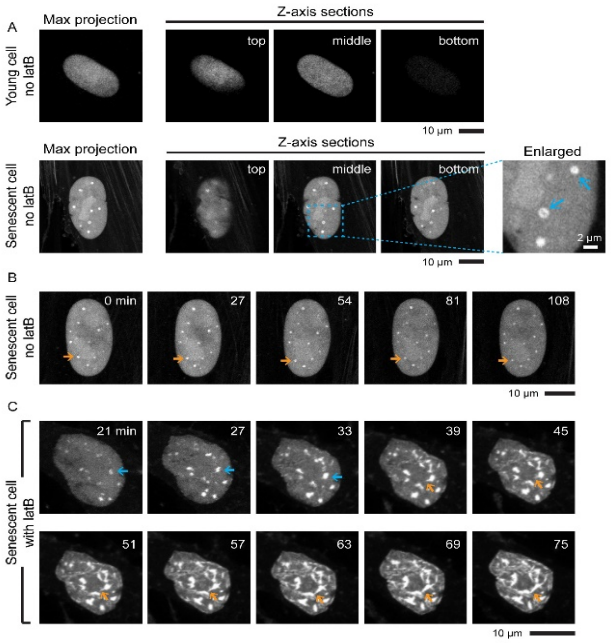


**Figure 3.** Nuclear actin puncta in senescent IMR-90 cells. (**A**) Nuclear actin puncta structures are obvious in senescent cells rather than in young cells. Blue arrows indicate hollow actin puncta. (**B**) Time-lapse images of nuclear actin puncta in senescent IMR-90 without latB treatment. Yellow arrows indicate a relatively stable actin punctum. (**C**) Nuclear actin puncta upon latB treatment. Blue arrows show the nuclear actin puncta become brighter and larger in size. Yellow arrows show F-actin elongation from the nuclear actin puncta. Scale bars, 10 μm.

It is noteworthy that, without latB treatment, a relatively high percentage of dot-like actin structures (hereinafter referred to as actin puncta) was observed in senescent cells rather than in young cells, suggesting a sub-population of actin structures exist during cellular senescence (Figure 3A, lower panel and Figure 3B). Enlargement of the senescent cell nucleus showed that some actin puncta displayed hollow shapes (Figure 3A, second panel enlarged image, blue arrows), sizes of which were close to the microscopy diffraction limit. Confocal time-lapse microscopy imaging showed that nuclear actin puncta were relatively stable without latB treatment (Figure 3B, yellow arrows). After latB treatment, many actin puncta in the senescent cells became brighter and larger in size (Figure 3C, blue arrows). Some actin puncta served as the elongation bases for the nuclear F-actin (Figure 3C, yellow arrows).

# 3. Discussion

Dissecting the fundamental mechanisms and effects of cellular senescence is beneficial for the basic aging researches and the development of anti-aging pharmaceutics. The relationship between cellular senescence and the nuclear F-actin cytoskeleton in mammalian cells is unclear. Most previous researches focused on the cytoplasmic F-actin rather than the nuclear actin counterpart. Although a number of works suggested a link between the cytoplasmic F-actin and cellular senescence [30,31], there were limited pieces of evidence suggesting a direct relationship between nuclear F-actin and cellular senescence [8,32].

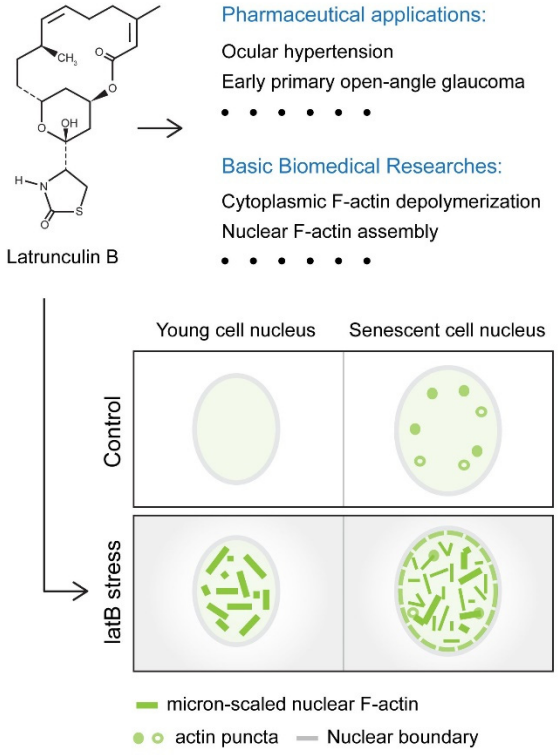


**Figure 4.** Schematic of distinct nuclear F-actin response upon latB treatment

In this study, we have addressed a new link between cellular senescence and micron-scaled nuclear F-actin assembly under latB treatment (Figure 4). By using doxorubicin-induced senescent human diploid fibroblast IMR-90 as a cellular senescence model, we found an enrichment of the transcriptional change of the actin cytoskeleton regulation genes during cellular senescence. By time-lapse images, we also found nuclear actin puncta that participate in nuclear actin reorganization upon latB treatment in the senescent cells. Our results showed that some actin

puncta displayed hollow shapes. Superresolution microscopy will be needed to further decipher the structure and function of these nuclear actin puncta. Collectively, this study opens a new perspective to understand the nuclear actin changes during cellular senescence and demonstrates a new link between cellular senescence, nuclear actin cytoskeleton and latB application.

# 4. Materials and Methods

## 4.1. 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 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). Cells were grown in an incubator (Esco) maintained at 37°C and 5% $CO_2$. Proliferating IMR-90 cells were used at population doubling (PD)16~PD25. For doxorubicin-induced senescent cells, IMR-90 cells (PD25) were treated with 1 μM Doxorubicin (MCE) for 48 h and harvested after 10 days.

## 4.2. RNA Sequencing and Data Analysis

Total RNAs of young and doxorubicin-induced senescent IMR-90 cells were isolated for RNA sequencing experiments and analysis. cDNA library construction and sequencing were performed by Shenzhen B.G.I. company using the BGISEQ-2000 platform. The expression levels for each gene were normalized to fragments per kilobase of exon model per million mapped reads (FPKM) using RNA-seq by Expectation Maximization (RSEM). Data analysis was done by using the online Dr. Tom system.

## 4.3. Lentivirus Preparation and Transduction

For lentivirus packaging, HEK293T cells were cotransfected with 5 μg pMD2.G, 10 μg psPAX2 and 10 μg pHBLV-actin chromobdy-NLS-puro. Lentivirus was harvested around 48 h~72 h after transfection. Lentivirus was purified by centrifugation (70,000 *g* for 2 h at 4°C). IMR-90 cells were transduced with 30 MOI lentiviral particles in the presence of 10 μg/mL Polybrene (Sigma). Transfected IMR-90 cells were selected by 5 μg/ml puromycin (Thermo) for one week.

## 4.4. Sample Preparation and Immunofluorescence Staining

IMR-90 cells were seeded in the glass-based 35 mm confocal dishes (NEST). After cells reached ~70% confluence, cells were fixed for 10 min by 4% paraformaldehyde (freshly prepared). Fixation solution was decanted and IMR-90 cells were washed three times in PBS. IMR-90 cells were permeabilized for around 5 min by 0.1% Triton X-100 solution. Permeabilization solution was decanted and IMR-90 cells were washed three times in PBS. Then IMR-90 cells were blocked for around 1 h by 5% BSA at room temperature and further incubated with primary antibody for 2 h at room temperature. Solution was decanted and IMR-90 cells were washed three times (5 min each wash) in PBS. IMR-90 cells were Incubated with the secondary antibody goat anti-rabbit IgG Alexa Fluor-488 (Invitrogen, A-11034) in 1% BSA for 1 h at room temperature in dark. Solution was decanted and cells were washed three times (5 min each wash) in PBS. Nuclear DNA was labeled by DAPI. The following primary antibodies were used in Figure 1B: Ki-67, abcam (ab15580).

## 4.5. Confocal Microscopy Imaging

IMR-90 cells were seeded in the glass-based 35 mm confocal dishes (NEST) at appropriate cell density overnight. After changing media with Latrunculin B (Cayman, 1 μM), cells were maintained in a 37°C chamber with 5% CO2 during the entire imaging process. Confocal microscopy systems were utilized for capturing fluorescent images.

## 4.6. Quantification and Statistical Analysis

Statistical analysis was performed using an unpaired Student's t-test. All summarized data are reported as means ± SEM (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ns, not significant). Analyses were performed using GraphPad Prism 6 software.

# References


[1] Kennedy, B.K.; Berger, S.L.; Brunet, A.; Campisi, J.; Maria, A.; Epel, E.S.; Franceschi, C.; Lithgow, G.J.; Richard, I. Aging: a common driver of chronic diseases and a target for novel interventions. 2014, 159, 709–713.

[2] Campisi, J.; Kapahi, P.; Lithgow, G.J.; Melov, S.; Newman, J.C.; Verdin, E. From discoveries in ageing research to therapeutics for healthy ageing. Nature 2019, 571, 183–192.

[3] Zhang, W.; Qu, J.; Liu, G.-H.; Belmonte, J.C.I. The ageing epigenome and its rejuvenation. Nat. Rev. Mol. Cell Biol. 2020, 21, 1–14.

[4] Xiao, F.; Zheng, X.; Cui, M.; Shi, G.; Chen, X.; Li, R.; Song, Z.; Rudolph, K.L.; Chen, B.; Ju, Z. Telomere dysfunction-related serological markers are associated with type 2 diabetes. Diabetes Care 2011, 34, 2273–2278.

[5] Luo, J.; Mills, K.; le Cessie, S.; Noordam, R.; van Heemst, D. Ageing, age-related diseases and oxidative stress: What to do next? Ageing Res. Rev. 2020, 57.

[6] Teder-Braschinsky, A.; Märtson, A.; Rosenthal, M.; Taba, P. Parkinson's Disease and Symptomatic Osteoarthritis Are Independent Risk Factors of Falls in the Elderly. Clin. Med. Insights Arthritis Musculoskelet. Disord. 2019, 12.

[7] Campisi, J. Aging, Cellular Senescence, and Cancer. Annu. Rev. Physiol. 2013, 75, 685–705.

[8] Gorgoulis, V.; Adams, P.D.; Alimonti, A.; Bennett, D.C.; Bischof, O.; Bishop, C.; Campisi, J.; Collado, M.; Evangelou, K.; Ferbeyre, G.; et al. Cellular Senescence: Defining a Path Forward. Cell 2019, 179, 813–827.

[9] Kelpsch, D.J.; Tootle, T.L. Nuclear Actin: From Discovery to Function. Anat. Rec. 2018, 301, 1999–2013.

[10] Plessner, M.; Grosse, R. Dynamizing nuclear actin filaments. Curr. Opin. Cell Biol. 2019.

[11] Patrick, C.C.; Matthias, P.; Robert, G.; Irene, C. Role of nuclear actin filaments in DNA repair dynamics. Nat. Cell Biol. 2019, 21.

[12] Hurst, V.; Shimada, K.; Gasser, S.M. Nuclear Actin and Actin-Binding Proteins in DNA Repair. Trends Cell Biol. 2019, 29, 462–476.

[13] Belin, B.J.; Lee, T.; Mullins, R.D. DNA damage induces nuclear actin filament assembly by formin-2 and spire-1/2 that promotes efficient DNA repair. Elife 2015.

[14] Moore, H.M.; Vartiainen, M.K. F-actin organizes the nucleus. Nat. Cell Biol. 2017.

[15] Tsopoulidis, N.; Kaw, S.; Laketa, V.; Kutscheidt, S.; Baarlink, C.; Stolp, B.; Grosse, R.; Fackler, O.T. T cell receptor–triggered nuclear actin network formation drives CD4+ T cell effector functions. Sci. Immunol. 2019.

[16] Baarlink, C.; Plessner, M.; Sherrard, A.; Morita, K.; Misu, S.; Virant, D.; Kleinschnitz, E.M.; Harniman, R.; Alibhai, D.; Baumeister, S.; et al. A transient pool of nuclear F-actin at mitotic exit controls chromatin organization. Nat. Cell Biol. 2017.

[17] Schrank, B.R.; Aparicio, T.; Li, Y.; Chang, W.; Chait, B.T.; Gundersen, G.G.; Gottesman, M.E.; Gautier, J. Nuclear ARP2/3 drives DNA break clustering for homology-directed repair. Nature 2018, 559, 61–66.

[18] Ryu, T.; Amaral, N.; D'Agostino, C.; Khodaverdian, V.Y.; Li, X.; Caridi, C.P.; Zapotoczny, G.; Chiolo, I.; Lin, E.; Rau, A.R.; et al. Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. Nature 2018, 559, 54–60.

[19] Baarlink, C.; Wang, H.; Grosse, R. Nuclear actin network assembly by formins regulates the SRF coactivator MAL. Science (80-. ). 2013.

[20] Plessner, M.; Melak, M.; Chinchilla, P.; Baarlink, C.; Grosse, R. Nuclear F-actin formation and reorganization upon cell spreading. J. Biol. Chem. 2015, 290, 11209–11216.

[21] Serebryannyy, L.A.; Yuen, M.; Parilla, M.; Cooper, S.T.; de Lanerolle, P. The effects of disease models of nuclear actin polymerization on the nucleus. Front. Physiol. 2016, 7, 1–11.

[22] Serebryannyy, L.A.; Parilla, M.; Annibale, P.; Cruz, C.M.; Laster, K.; Gratton, E.; Kudryashov, D.; Kosak, S.T.; Gottardi, C.J.; de Lanerolle, P. Persistent nuclear actin filaments inhibit transcription by RNA polymerase II. J. Cell Sci. 2016, 129, 3412–25.

[23] Rasmussen, C.A.; Kaufman, P.L.; Ritch, R.; Haque, R.; Brazzell, R.K.; Vittitow, J.L. Latrunculin B Reduces Intraocular Pressure in Human Ocular Hypertension and Primary Open-Angle Glaucoma. Transl. Vis. Sci. Technol. 2014, 3, 1.

[24] Roeles, J.; Tsiavaliaris, G. Actin-microtubule interplay coordinates spindle assembly in human oocytes. Nat. Commun. 2019, 10, 1–10.

[25] Pfitzer, L.; Moser, C.; Gegenfurtner, F.; Arner, A.; Foerster, F.; Atzberger, C.; Zisis, T.; Kubisch-Dohmen, R.; Busse, J.; Smith, R.; et al. Targeting actin inhibits repair of doxorubicin-induced DNA damage: a novel therapeutic approach for combination therapy. Cell Death Dis. 2019, 10, 302.

[26] Okka, M.; Tian, B.; Kaufman, P.L. Effects of latrunculin B on outflow facility, intraocular pressure, corneal thickness, and miotic and accommodative responses to pilocarpine in monkeys. Trans. Am. Ophthalmol. Soc. 2004, 102, 251–259.

[27] Peterson, J.A.; Tian, B.; Geiger, B.; Kaufman, P.L. Effect of Latrunculin-B on outflow facility in monkeys. Exp. Eye Res. 2000, 70, 307–313.

[28] Gil-Krzewska, A.; Saeed, M.B.; Oszmiana, A.; Fischer, E.R.; Lagrue, K.; Gahl, W.A.; Introne, W.J.; Coligan, J.E.; Davis, D.M.; Krzewski, K. An actin cytoskeletal barrier inhibits lytic granule release from natural killer cells in patients with Chediak-Higashi syndrome. J. Allergy Clin. Immunol. 2018, 142, 914-927.e6.

[29] Baker, D.J.; Sedivy, J.M. Probing the depths of cellular senescence. J. Cell Biol. 2013, 202, 11–13.

[30] Kasper, G.; Mao, L.; Geissler, S.; Draycheva, A.; Trippens, J.; Kühnisch, J.; Tschirschmann, M.; Kaspar, K.; Perka, C.; Duda, G.N.; et al. Insights into mesenchymal stem cell aging: Involvement of antioxidant defense and actin cytoskeleton. Stem Cells 2009.

[31] Wu, C.; Haynes, E.M.; Asokan, S.B.; Simon, J.M.; Sharpless, N.E.; Baldwin, A.S.; Davis, I.J.; Johnson, G.L.; Bear, J.E. Loss of Arp2/3 induces an NF-κB-dependent, nonautonomous effect on chemotactic signaling. J. Cell Biol. 2013, 203, 907–916.

[32] Choi, O.R.; Kwak, I.H.; Kim, H.S.; Lim, I.K.; Ryu, M.S. Nuclear Accumulation of Globular Actin as a Cellular Senescence Marker. Cancer Res. 2005, 64, 572–580.