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Original Research Article

LncRNA NEAT1-206 regulates autophagy of human umbilical cord mesenchymal stem cells through the WNT5A/Ca^{2+} signaling pathway under senescence stress

Weili Wang [a,b], Yongyu Wang [a,b], Chunchun Duan [a,b], Wenjing Tian [a,b], Liyang Gao [a,b,*]

[a] Life Science School, Ningxia University, Yinchuan, China
[b] Key Laboratory of Ministry of Education for Conservation and Utilization of Special Biological Resources in the Western, Ningxia University, Yinchuan, China

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ABSTRACT

Stem cells are crucial for maintaining bodily stability, but their regenerative abilities decline with age. This decline is marked by reduced proliferation and differentiation capacities of stem cells, as well as exhaustion of the stem cell pool. The accumulation of aged mesenchymal stem cells (MSCs) can reduce the tissue regeneration, but the molecular mechanisms influencing MSCs aging remain unclear. Moreover, collecting MSCs from elderly individuals is not suitable for observing the early response of MSCs to senescence stress, and the factors involved in early senescence remain unclear. In our previous study, we established a fast MSC aging model using D-galactose. We discovered that, while not affecting the "stemness" markers of mesenchymal stem cells, the expression of LncRNA NEAT1-206 was notably increased during the early stages of aging induction (within 4 days). And LncRNA NEAT1-206 was observed to be localized in the cytoplasmic matrix due to enhanced nuclear export. We found that the LncRNA NEAT1-206 could trigger autophagy through the WNT5A/Ca^{2+} signaling pathway, thereby decreasing senescence markers and enhancing the osteogenic differentiation of MSCs. This study elucidated the role that LncRNA NEAT1-206 as a potential key factor in conferring resistance to D-galactose-induced cell senescence at the early stage and promoting the osteogenic differentiation of MSCs. This study may provide a foundational understanding for delaying the MSCs aging process.

1. Introduction

Mesenchymal stem cells (MSCs) are adult stem cells originating from the mesoderm and are found in various tissues such as bone marrow, placenta, umbilical cord, and umbilical cord blood [1]. Among these, human umbilical cord mesenchymal stem cells (umbilical cord mesenchymal stem cells, UC-MSCs) are versatile stem cells existed in newborn umbilical cord tissue, capable of proliferation and differentiation into different cell types under specific conditions [2]. Recently, hUC-MSCs have demonstrated promising efficacy in treating various diseases, including autoimmune diseases (autoimmune disease, AID) [3], neurodegenerative disorders (neurodegenerative disorders, NS) [4], metabolic diseases [5] and cardiovascular diseases (cardiovascular diseases, CVDs) [6], highlighting their immense therapeutic potential.

MSCs senescence refers to the irreversible cell cycle arrest due to accumulated damage within the cells, often associated with the exhaustion of the stem cell pool [7,8]. The senescence of stem cells can drive body aging and dysfunction, which is related to the occurrence of many diseases. For example, patients with idiopathic pulmonary fibrosis exhibit senescence of MSCs, alongside reduced mitochondrial function and DNA damage [9,10]. Additionally, the senescence of MSCs is an important cause of transforming myelodysplastic syndromes into leukemia [11,12]. Moreover, stem cell aging hinders their effectiveness as seed cells in clinical treatments. In cell therapy, extensive in vitro expansion of stem cells is necessary for transplantation, but long-term culture in vitro easily induces the aging of UC-MSCs, compromising their reparative capacity [13]. Therefore, elucidating the molecular mechanisms underlying stem cell aging holds significant importance in advancing clinical research on stem cells.

However, there are some obstacles to study early cell senescence of MSCs. For example, the DEGs data from primary cells isolated from old donors were different from cells isolated from young donors, so MSCs collected from old donors might not suitable for exploring the early stage of senescence. To investigate regulatory factors in MSCs'

* Corresponding author. Life Science School, Ningxia University, Yinchuan, China.
E-mail address: pandarun@nxu.edu.cn (L. Gao).

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senescence, our previous research used hUC-MSCs as a tool to establish an early cell senescence model in short time. D-galactose was widely used to induce senescence in vivo as well as adult cells, it has been used to induce cell senescence of human red blood cells [14], C2C12 myoblast [15] cardiomyocyte [16], and it also been used to establish aging mouse model, [17–19]. Some aging related disease such as bone dyschondrosteosis [21], learning and memory impairment [22], and Alzheimer's disease [23]. It has reported that the senescence of stem/progenitor cell could be induced by D-galactose [20]. In our previous research, we treated hUC-MSCs with 20 mg/mL D-galactose for a brief period of 4 days. Interestingly, we observed that crucial "stemness" markers like OCT4, CD105, NANOG, and CD117 did not decrease, indicating that short-term D-galactose treatment may not undermine the potency of these stem cells. However, we also noted a decrease in cell proliferation and ATP levels in individual cells. Moreover, we noticed a significant increased expression of senescence-related genes and proteins in D-galactose induced hUC-MSCs. These results suggested that D-galactose could be used to establish early senescence model of hUC-MSCs.

Long non-coding RNA (LncRNA) denotes a class of linear RNA molecule exceeding 200 nt in length, lacking protein-coding capability [24]. Despite this, LncRNA plays crucial roles in regulating important phenotypes in mammals. It participates in various biological processes such as epigenetics, chromosome measurement compensation, autophagy, cellular senescence, cell differentiation, and cell cycle [25,26]. A specific LncRNA, Nuclear Enriched Transcript 1 (NEAT1), originated from the familial neoplastic syndrome multiple endocrine neoplasia type 1 locus on chromosome 11 [27]. According to the Ensembl database, LncRNA NEAT1 has a total number of 9 transcripts. LncRNA NEAT1-206 (Ensembl: ENST00000642367) is one of the transcripts of LncRNA NEAT1 and the longth of is transript is 1145bp. LncRNA NEAT1 significantly contributes to the formation, integrity, and assembly of paranuclear plaques [27]. Previous studies have implicated LncRNA NEAT1 in processes of invasion, proliferation, metastasis, and autophagy. For example, it has been observed to suppress ovarian granulosa cell apoptosis and autophagy through the MAPK signaling pathway mediated by miR-654/STC2 [28]. Additionally, LncRNA NEAT1 induces autophagy by epigenetically regulating the expression of autophagy-related genes in glial cells [29]. However, there exists a gap in research regarding the involvement of LncRNA NEAT1 in the processes of autophagy and cellular senescence.

In conclusion, aging represents a multifaceted process marked by the gradual decline in cellular and bodily functions over time [30]. Hence, it becomes crucial to precisely dissect the mechanism through which LncRNA regulates cellular senescence to enhance the preparation of MSCs and augment their clinical therapeutic efficacy. In this study, we identified the aging-related LncRNA NEAT1-206 for the first time. Our finding shows that LncRNA NEAT1-206 regulates stem cell autophagy via WNT5A/Ca^{2+} signaling pathway, thereby delaying cell senescence.

2. Materials and methods

2.1. Bioinformatics analysis

Single-cell RNA sequencing data (GSE157773) we were collected from GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE157773). Utilizing the Seurat R package v4.0.2 and RStudio-1.1.453, the RNA-seq data from human mesenchymal stem cells (GSM4774851) underwent filtering, normalization, dimensionality reduction, and then analyzed differential expression genes (DEGs) among 4 different clusters. To ensure data quality, the threshold for mitochondrial RNA was set at 10%, and gene expression was limited to <2500 to exclude low-quality cells. Following application of the "RunPCA" function, the top 40 principal components were selected for input into UMAP to generate 2D projections, facilitating sub-clustering of human mesenchymal stem cells.

The data of mesenchymal stem cells with different donor age, was collected from GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE12274). We selected four samples which from older donors(GSM308234, GSM308233, GSM308231, GSM308228), and 3 samples which from younger donors(GSM308224, GSM308225, GSM308226). All results were analyzed by Seurat R package v4.0.2 and RStudio-1.1.453.

The gene ontology (GO) analysis (including biologic process, cellular component, and molecular function categories) and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis [59,60], were performed by DAVID (http://david.abcc.ncifcrf.gov).

2.2. Cell culture and osteogenic differentiation

The human umbilical cord-derived mesenchymal stem cells used in this study were obtained from the Key Laboratory of Western Biological Resources Protection and Utilization at Ningxia University, Ministry of Education, China. Two types of medium, basic DMEM (Gibco, USA) contain 15% FBS (Gibco, USA) and serum-free medium (TBD, China) were used to maintain the hUC-MSCs, then cells were cultured in incubator at 37°C and 5% CO_2. For osteoblast differentiation, hUC-MSCs were cultured in hUC-MSCs osteogenic differentiation medium (Procell, China) and Alizarin Rad staining (Procell, China) was performed to examine the formation of osteoblasts after 21 days.

2.3. Establishing an early senescence model

Weigh 1g of D-galactose (Solarbio, China) and dissolve it in 20 mL of serum-free culture medium. Mix thoroughly by inverting the solution, or accelerate dissolution by using a water bath at 37 °C. Filter the solution through a 0.22 μm filter. After filtration, prepare serum-free complete culture medium containing 20 mg/mL D-galactose. The hUC-MSCs were exposed to D-galactose for 4 days, with sub-culturing of the cells performed every two days.

2.4. β-Galactosidase staining

SA-β-gal staining was performed using the Senescence β-Galactosidase Staining Kit obtained from Beyotime Biotechnology in Shanghai, China. Cells were cultured in a 35-mm dish until reaching 80 % confluence, then washed with PBS and staining with this kit according to protocol. Subsequently, these cells were incubated overnight at 37 °C in the β-galactosidase staining solution. After the color development, the samples were rinsed to stop the reaction. Then the stained samples were examined under a microscope to visualize the blue color indicative of β-galactosidase activity.

2.5. Cell counting Kit-8 (CCK-8) assay

The CCK-8 test was conducted using the Beyotime Biotechnology kit (Shanghai, China) following the manufacturer's instructions. A total cells number of 9×10^3 cells were seeded in 96-well plate and cultured all cells for 4 days inside cell culture incubator. Then, 10 μL of CCK-8 solution was added to each well, followed by an incubation period at 37 °C for 2 hours. Absorbance at 490 nm was measured using a microplate reader after the incubation period.

2.6. Western blotting

Proteins were extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) with PMSF (Servicebio, China). Protein concentration was quantified using the enhanced BCA protein assay kit (Beyotime Biotechnology, Shanghai, China). Each sample was loaded with 35 μg of protein per lane, and the proteins were separated via a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis stacking gel. Separated proteins were transferred onto a 0.22 μm PVDF membrane. The PVDF membranes were blocked with 5% non-fat dry

milk in 1 × TBST (Sevenbio, China) at room temperature for 2 hours, followed by three washes with 1 × TBST. Subsequently, these membranes were incubated overnight at 4°C with primary antibodies against P21 (dilution, 1:1000; proteintech, USA), actin (dilution, 1:3000; proteintech, USA), P16 (dilution, 1:1500; proteintech, USA), GLB1 (dilution, 1:1500; proteintech, USA), ATG5 (dilution, 1:1500; proteitech, USA), BECN1 (dilution, 1:1500; proteintech, USA), and LC3 (dilution, 1:1500; proteintech, USA),WNT5A (dilution, 1:1500; proteintech, USA), CAMKII(dilution, 1:1500; proteintech, USA) and p-CAMKII(dilution, 1:1500; proteintech, USA). After this, the membranes were washed three times and incubated with horseradish peroxidase-conjugated secondary antibodies (dilution, 1:8000; proteintech, USA) at room temperature for 1 hours. Finally, Immobilon Western Chemiluminescent HRP Substrate (Beyotime Biotechnology, Shanghai, China) was utilized to visualize the signals.

2.7. Viability assays

The hUC-MSCs were adjusted to a concentration of 9×10^3 cells/mL in complete medium with 20 mg/mL D-galactose. Subsequently, the cell suspension was dispensed in 100 μL aliquots into 96 well plates and incubated for 2 hours to allow cell attachment, then 100 μL of CellTiter-LumiTM Cell Viability Assay (Beyotime Biotechnology, Shanghai, China) was mixed with cells, and Relative Luminescence Units (RLUs) were measured by multi-functional microplate reader.

2.8. RNA extraction and RT-qPCR

The hUC-MSCs total RNA extraction using Trizol reagent (Beyotime Biotechnology, China).Added 1 mL Trizol in a 6-well-plate after aspirating culture medium then transfer the well-mixed lysate to a 1.5 mL centrifuge tube. Incubated the lysate at RT for 5 mins, and added 0.2 mL chloroform into the same tube. After vortex and 2–3 mins incubating, the lysate was centrifuged at 12,000g for 15 mins at 4 °C. Then transferred the upper phase to a new 1.5 mL centrifuge tube, and 0.5 mL isopropanol was mixed with aqueous phage. After centrifuge at 12,000g for 10 mins at 4 °C, supernatant was discarded and 1 mL 75 % ethanol was added. Then centrifuge at 7,500g for 5 mins, the RNA pellet was finally dissolved in 40μ DEPC water (Beyotime Biotechnology, China) and store at −80 °C. The mRNA expression for both D-galactose treated samples and control samples was profiled using RNA sequencing (RNA-Seq, Majorbio, China).

Real-time PCR analysis to evaluate the expression of target mRNA was performed using the SYBR Green PCR kit (Takara in Kyoto, Japan). The reaction protocol consisted of the following conditions: 95 °C for 30 s; 95 °C for 15 s; and 60 °C for 30 s, for a total of 40 cycles. Primers for real-time PCR were obtained from Sangon Biotech in Shanghai, China. Normalization of relative mRNA expression was conducted utilizing ACTIN and calculated based on the $2^{-\Delta\Delta CT}$ method. The primer sequences are detailed below S1.

2.9. Immunofluorescent staining

The sample was rinsed with PBS and then fixed in a 4% paraformaldehyde solution (Servicebio, China) for 20 mins at room temperature. Then the cells climbing sheets were washes with PBS for three times. The cells were permeabilized with 0.3% Triton X-100 for 15 mins and sealed with 3 % bovine serum albumin (Solarbio, China) at room temperature for 30 mins. Following this, the cells were incubated overnight at 4°C with the following antibodies: P21 (dilution 1:200; proteintech, USA), P16 (dilution 1:200; proteintech, USA), GLB1 (dilution 1:200; proteintech, USA), WNT5A (dilution 1:200; proteintech, USA), and p-CAMKII (dilution 1:200; proteintech, UAS). The following day, the primary antibody was recovered and washed with PBS. Goat anti-rabbit-IgG antibody or anti-mouse-IgG antibody (dilution 1:500; Thermofisher, USA) conjugated with fluorescein isothiocyanate was then incubated at room temperature in a dark environment for at least 1 hour. Finally, the sample was sealed with an anti-fluorescence quenching solution (including DAPI), and fluorescence was observed under an inverted fluorescence microscope.

2.10. MDC

MDC staining is commonly employed to measure autophagosome vesicle formation. To prepare a 1 × Wash buffer, 10 × Wash buffer was diluted tenfold with distilled water. The cells were then collected in a tube and then were washed once with 500 μL of 1 × Wash buffer. Following the removal of the supernatant, an appropriate volume of 1 × Wash buffer was added to suspend the cells and adjust the cell concentration to 1×10^6/mL. Then, the cells were mixed with 10 μL MDC Stain and incubated for 15–45 mins at room temperature in the absence of lights. Afterwards, the cells were collected in a tube and washed once with 500 μL of 1 × Wash buffer. Upon removing the supernatant, 100 μL of Collection buffer was added to suspend the cells. The suspension was then dropped onto a slide, and the slide was covered. Finally, the cells were observed under a fluorescence microscope.

2.11. Cell transfection

The hUC-MSCs seeded into a 6 cm cell culture dish and transfected the cell the day after seeding, once the cell density reached approximately 60–80%. Following the instructions in the ZETA transfection reagent manual (ZETA LIFE INC, USA), The nucleic acid and transfection reagent were mixed in a ratio of 1:1, and then incubated at room temperature for 15 mins. Then, the mixture was added into the cell culture plate with a moderate shaking, and continual culture the cells in the incubator and replaced the cell culture medium after 6 hours. 48 hours after transfection, total RNA and protein were extracted through digestion and centrifugation for subsequent experiments. This experiment established 4 cell lines: cells transfected with an pCDNA3.1 empty vector (OENC); the LncRNA NEAT1 overexpression group transfected with the pCDNA-3.1-NEAT1-206 plasmid (NEAT1OE); cells transfected with a negative control (SiNC); and the knockdown group transfected with NEAT1RNAi (NEAT1RNAi). Additionally, the transfection also conducted on cells which treated with D-galactose for 4 days. These cells referred as two pairs: OENC (aging) vs NEAT1OE (aging), and SiNC (aging) vs NEAT1RNAi (aging).

2.12. Autophagic flow analysis

The mRFP-GFP-LC3 assay was used to evaluate autophagic flow. Initially, hUC-MSCs were seeded on laser confocal culture dishes at a density of 1×10^5 cells/dish. Upon reaching 30% confluence, the cells were exposed to the mRFP-GFP-LC3 adenovirus (1×10^{10} pfu/mL, Genechem, Chian) at 100 MOI for a period of 24 hours. After an additional 48 hours incubation period with opti-MEM medium, the cells treated with pCDNA-3.1-NEAT1 and LncRNA NEAT1-206 smart silencer (RiboBio, China) at 37 °C for 48 hours. To examine the autophagic flux, the cells were fixed with 4% paraformaldehyde for 20 mins at room temperature, and then autophagosomes and autolysosomes were observed under the laser confocal. Autophagosomes were identified by yellow puncta (GFP/mRFP), while autolysosomes were represented by red puncta (GFP/mRFP).

2.13. Fluorescence in situ hybridization (FISH)

To detect the localization of LncRNA NEAT1-206 in cells, FISH assay was conducted. FITC-labeled LncRNA NEAT1-206 probe was synthesized (Servicebio, China). LncRNA NEAT1-206 transduced cells were fixed with 4% paraformaldehyde for 20 min. After permeation with 0.3% TritonX-100, the cells were blocked at 37 °C for 30 mins with prehybridization solution. Subsequently, the cells were incubated with the

probe mix at 37°C overnight. Finally, the distribution of LncRNA NEAT1-206 was observed using a confocal laser scanning microscope and sealed with an anti-fluorescence quenching sealing solution containing DAPI (Beyotime, Shanghai, China).

2.14. Subcellular fractionation

The hUC-MSCs were lysed on ice using 200 μL of pre-chilled lysis buffer (Norgen, Thorold, ON, Canada). The lysate was collected by centrifugation at a speed of 12000g. The supernatant, which containing cytoplasmic RNA, was transferred to a RNase-free tube. Meanwhile, the cell precipitate containing nuclear RNA was transferred to another tube. Then a total volume of 200 μL of buffer SK was added to each separation column to wash the membrane. The column was washed with Wash buffer A, followed by elution with Elution buffer E. Then eluted RNA was examined by nanodrop.

2.15. Flow cytometry

A total number of 2×10^5 cells were seeded in 6-well plate, when the cell reached 80–90 % confluence. The complete medium was aspirated and the cells were rinsed with PBS for 2–3 times. Then, 1 mL of fresh complete medium was added to each well, along with Fluo-4AM (Beyond Biotechnology, Shanghai, China) at a concentration of 5 μM. The cells were subsequently cultured at 37°C in the absence of light for 60 mins. After removal of the staining solution, the cells were centrifuged at 1000g for 5 mins for collection. Following 2–3 washes with PBS, the cells were resuspended in 500μL of PBS and analyzed using flow cytometry (FACSCanto, USA).

2.16. Statistical analysis

All biological experiments were performed in triplicate, and the presented data are representative of at least three independently performed experiments. Data are displayed as mean ± standard deviation (SD). Statistical analyses were proceeded using GraphPad Prism software version 9 (GraphPad Software Inc., La Jolla, CA, USA). Paired comparisons were computed using paired *t*-test or repeated measures ANOVA, with *p* value less than 0.05 indicating significance.

3. Results

3.1. LncRNA NEAT1 might a marker for a subpopulation of MSCs

The single-cell RNA sequencing data GSM4774851 was retrieved from GEO database, we further identified four cell subpopulations in MSC sample, and found LncRNA NEAT1 was highly expressed in the subpopulation group 1 (Fig. 1A), and LncRNA NEAT1 was significantly expressed only in group 1 (Fig. 1B). Additionally, differential expressed genes (DEGs) of subpopulation group 1 were used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, which identified enrichment in some pathways such as AGE-RAGE signaling pathway, cellular senescence, lysosome, osteoclast differentiation and signaling pathways regulating pluripotency of stem cells (Fig. 1C). This KEGG enrichment result suggested that LncRNA NEAT1 might potentially serve as a marker for specific subpopulation of MSCs, which may be associated with stemness and senescence. However, the function of LncRNA NEAT1 in MSCs remain to be elucidated. Thus, our research focus on the role of LncRNA NEAT1 in MSC biology and its potential implications in stem cell regulation.

3.2. The highly expression of LncRNA NEAT1-206 in D-galactose-induced hUC-MSCs senescence model

In our research, 20 mg/mL D-galactose was used to established an early cell senescence model by hUC-MSCs.After treating the cells with 20 mg/mL D-galactose for 4 days, cell growth was assessed using CCK-8 and the ATP level in cells was assessed using CellTiter-Lumi luminescence assays. We observed a significant decrease in cell growth and viability in hUC-MSCs, which treated with D-galactose for 4 days compared to normal cells (Fig. 2A–B). To investigated the senescence in hUC-MSCs, the senescence-associated β-galactosidase (SA-β-gal) staining was utilized. The staining revealed a distinct blue-green coloration in hUC-MSCs treated with D-galactose for 4 days compared to untreated

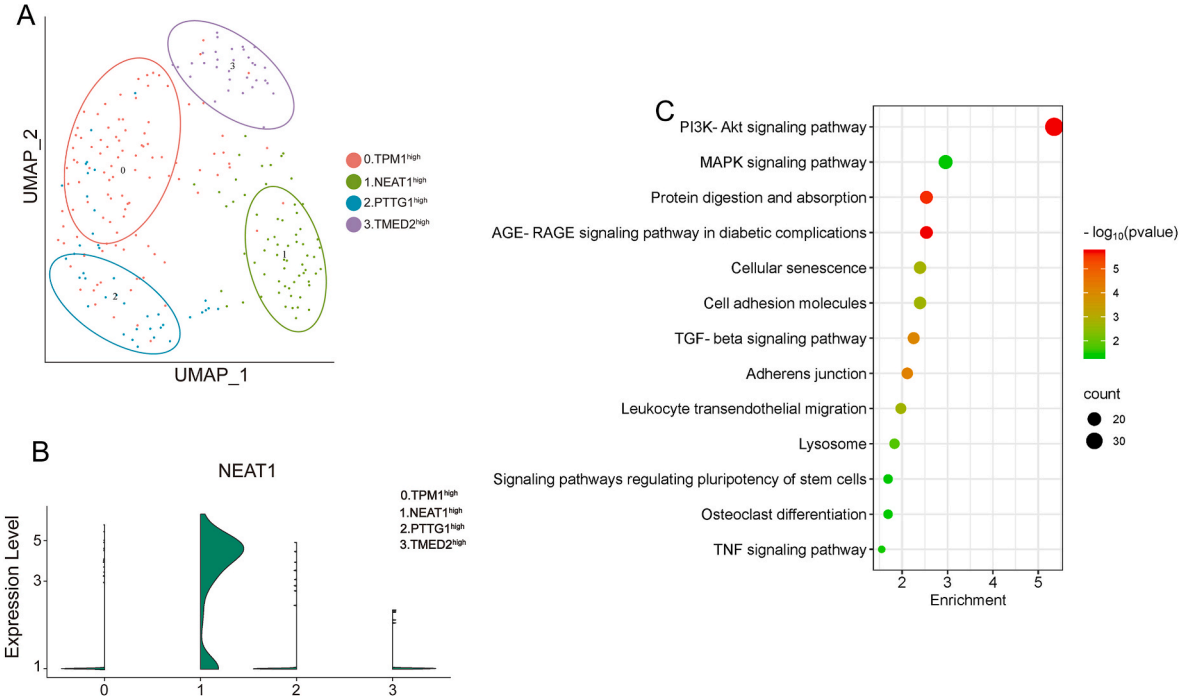

Fig. 1. ***LncRNA NEAT1 was a potential marker for a subpopulation of MSCs.*** (**A**) Cell grouping by UMAP method, and different cell groups manually annotated; (**B**) Expression of LncRNA NEAT1 gene in different groups, expressed in green; (**C**) KEGG enrichment analysis of differentially expressed genes (DEGs) in subgroup 1.

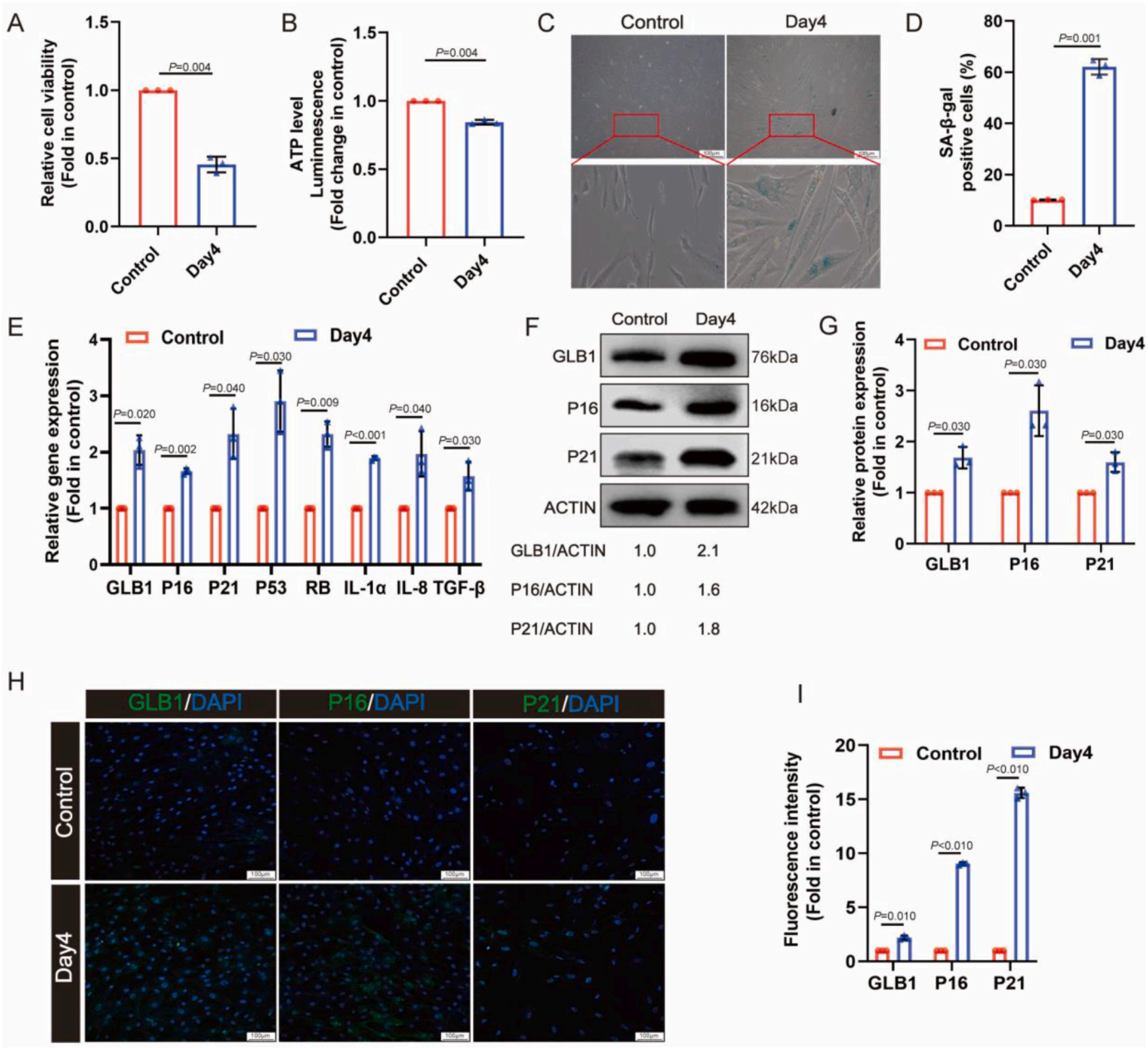

Fig. 2. ***Identification of aging hUC-MSCs.*** (**A**) CCK-8 method to detect D-galactose effect of cell proliferation ability; (**B**) CellTiter-Lumi™ luminescence method to detect the cell viability; (**C**) SA-β-gal staining for senescence cells (blue); (**D**) Cell counting after SA-β-gal staining; (**E**) RT-qPCR to detect the expression of aging-related genes; (**F**) Western Blot detection of the expression of senescence proteins GLB1, P16 and P21; (**G**) usingThe protein levelsof GLB1, P16, P21 were quantified by ImageJ; (**H**) Immunofluorescence detection of GLB1, P16, P21. Control: untreated hUC-MSCs; Day4: cells treated with D-galactose for 4 days.

normal cells (Fig. 2C–D). Additionally, RT-qPCR analysis reveraled a significant increase in senescence-related gene *GLB1*, *P16*, *P21*, *P53*, *RB*, *IL-1α*, *IL-8* and *TGF-β* expression in hUC-MSCs treated with D-galactose for 4 days compared to untreated cells (Fig. 2E). Similarly, Western Blot and immunofluorescence results demonstrated a significant increase in expressiong aging-related markers, such as GLB1, P16, and P21 (Fig. 2F–I). These results provide evidence of the senescence-inducing effects of D-galactose on hUC-MSCs on both reducing cell viability and increasing senescence-related markers.

The RNA sequencing was performed to screen the differential expressed genes (DEGs) in D-galactose treated hUC-MSCs. Interestingly, the expression of the LncRNA NEAT1-206 was found to be significantly increased in the D-galactose treated cells compared to untreated cells (Fig. 3A), and this result was confirmed by RT-qPCR (Fig. 3B). Subsequent investigation into the subcellular localization of LncRNA NEAT1-206 revealed that it is highly enriched in the cytoplasm, as observed through nucleoplasm RNA isolation (Fig. 3C). To further confirm the nuclear export of LncRNA NEAT1-206, RNA in situ hybridization (RISH) was performed to monitor the subcellular localization of LncRNA NEAT1-206 (Fig. 3D). All these results showed a significant transport of LncRNA NEAT1-206 from nucleus to cytoplasm during D-galactose induced cell aging process.

3.3. *Autophagy induced by D-galactose might regulate by LncRNA NEAT1-206*

We observed an increase in the expression of autophagy-related genes in cells treated with 20 mg/mL D-galactose by RT-qPCR (Fig. 4A). Western blot analysis further confirmed a significant upregulation of ATG5, BECN1, and LC3 in hUC-MSCs treated with D-

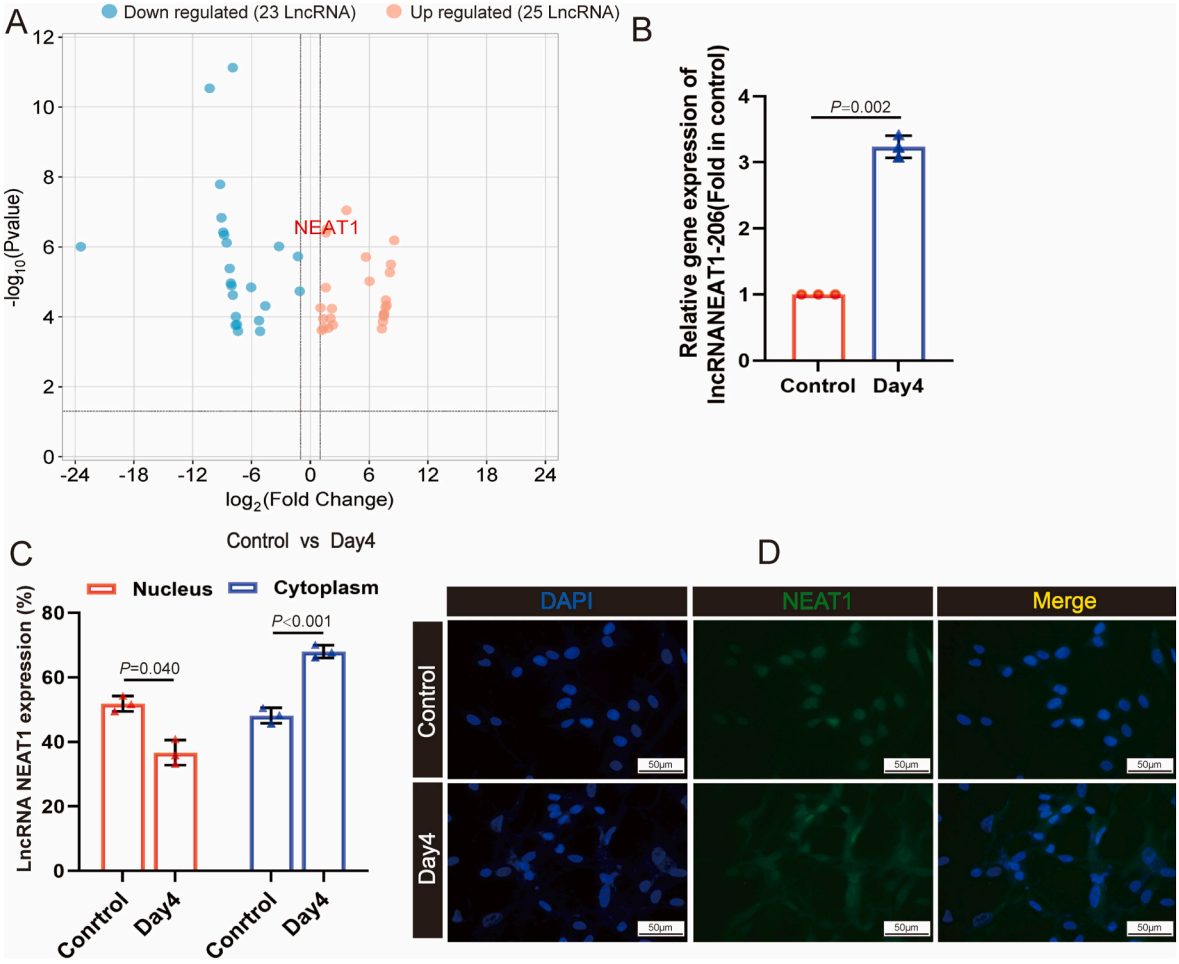

Fig. 3. ***Expression and Localization of LncRNA NEAT1-206 in normal and senescent cells.*** (**A**) RNA-sequencing result of *LncRNA NEAT1-206* in untreated and D-galactose treated cells; (**B**) *LncRNA NEAT1* expression was confirmed by RT-qPCR (**C**) RT-qPCR was used to detect the *LncRNA NEAT1-206* expression in the nucleus and cytoplasm of hUC-MSCs; (**D**) The subcellular localization of *LncRNA NEAT1-206* in hUC-MSCs was detected via RISH. Control: untreated hUC-MSCs; Day4: cells treated with D-galactose for 4 days.

galactose to induce early senescence (Fig. 4B–C). To assess the dynamic changes in autophagy, monodansylcadaverine (MDC) staining was employed to detect autophagosomes, revealing an increase in the fluorescence intensity of MDCs in D-galactose-treated hUC-MSCs (Fig. 4D).

Additionally, Adenovirus mRFP-GFP-LC3 was used to monitor and quantify the autophagic flux rate. The results indicated that RFP-positive (RFP+) GFP + puncta represented autophagosomes, whereas RFP + GFP- puncta indicated the quenched signal of RFP in acidic autolysosomes. A notable increase was observed in both yellow spots (autophagosomes) and red spots (autolysosomes) in D-galactose-treated hUC-MSCs compared to untreated cells (Fig. 4E). These findings suggest that D-galactose induces cell senescence accompanied by the autophagy in hUC-MSCs. Over the course of D-galactose treatment (0–6 days), the senescence marker GLB1 and the autophagy marker LC3 showed a gradual increase, followed by a slight decrease on day 6 (Fig. 4F–H).

To investigate the potential LncRNA NEAT1-206 to activate autophagy in hUC-MSCs, we utilized RT-qPCR and Western Blot analysis to assess changes in the expression of ATG5, BECN1, and LC3 in hUC-MSCs upon overexpression or inhibition LncRNA NEAT1-206. First, the pCDNA3.1-NEAT1 (Hygro) vector was constructed then transfected into hUC-MSCs. The result demonstrated a significant enhancement in the expression of LncRNA NEAT1-206 in hUC-MSCs following transfection with the overexpression vector, compared to the control group (Fig. 5A). Gene and protein levels of ATG5, BECN1, and LC3 were significantly upregulated in LncRNA NEAT1-206 overexpression cells (Fig. 5B–C). Additionally, we designed small interfering RNA to down-regulate the gene expression of LncRNA NEAT1-206 in both nucleus and cytoplasm (Fig. 5D). A notable decrease was noticed in the gene and protein expression levels of ATG5, BECN1, and LC3 after downregulating the expression of LncRNA NEAT1-206 in hUC-MSCs (Fig. 5E–F). These findings collectively suggest that a regulatory functionof LncRNA NEAT1-206 in autophagy in hUC-MSCs.

3.4. LncRNA NEAT1-206 regulate the expression of WNT5A in hUC-MSCs

The RNA-sequencing result showed that the *WNT5A* expression increased in D-galactose treated cells (Fig. 6A). Utilizing the GEO database (GSE12274), we selected four human bone marrow MSCs isolated from elderly donors, and three MSCs from young donors for bioinformatics analysis. We found the mRNA expression of *WNT5A* ($P = 0.03$) was significantly higher in the elderly group then in the younger group (Fig. 6B). Furthermore, through both overexpression and inhibition of LncRNA NEAT1-206 in hUC-MSCs, we observed a correlation between the expression of *WNT5A* mRNA and LncRNA NEAT1-206 gene expression (Fig. 6C–D). This result suggested that *WNT5A* was highly expressed in senescence MSCs, and it mRNA expression was regulated by LncRNA NEAT1.

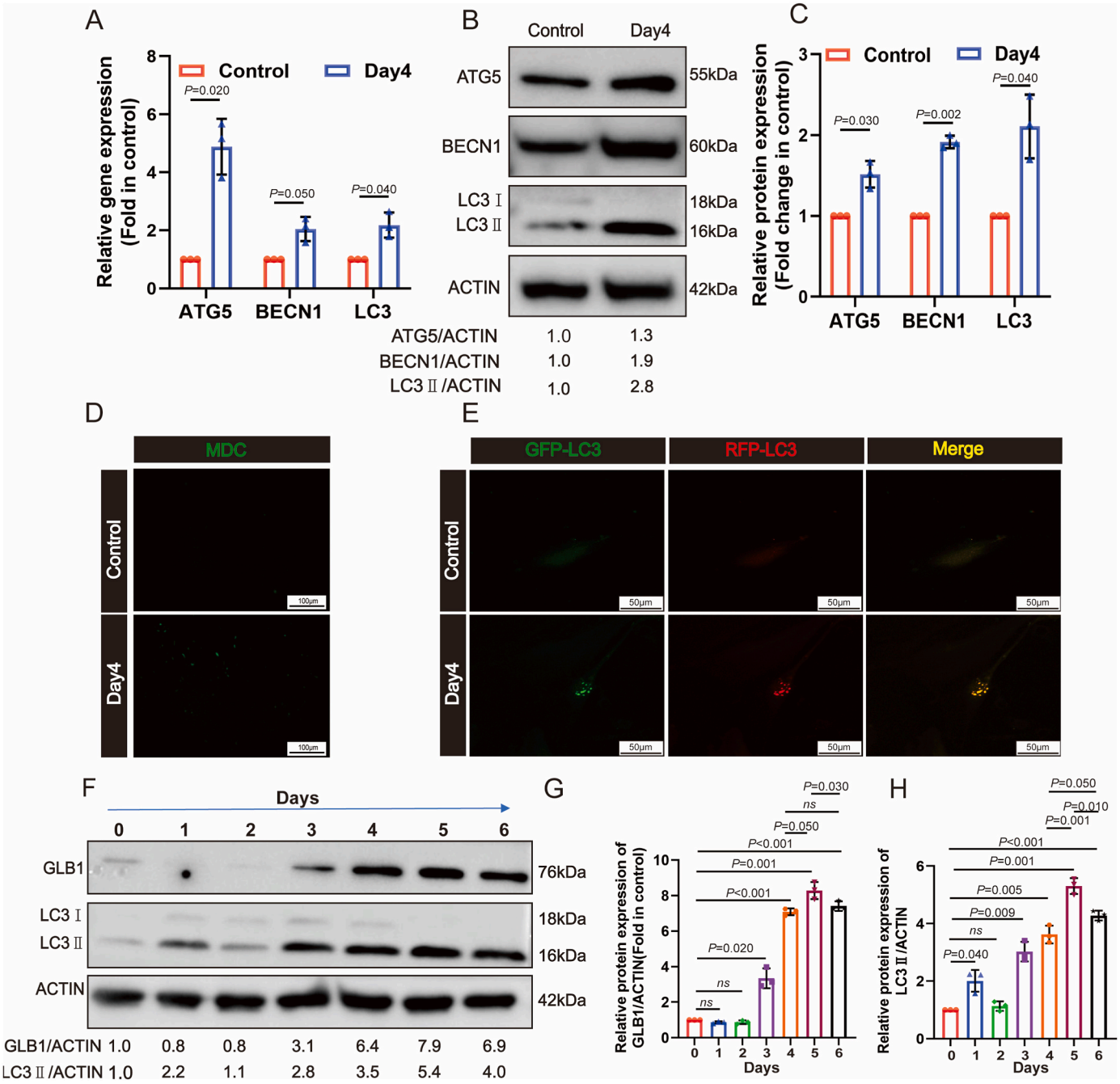

Fig. 4. ***Autophagy caused by D-galactose induced cellular aging.*** (**A**) RT-qPCR detects the expression of autophagy genes *ATG5, BECN1* and *LC3*in hUC-MSCs; (**B**) Western Blot detects the expression of autophagy proteins ATG5, BECN1 and LC3 in hUC-MSCs; (**C**) The autophagy protein levelswere quantified by ImageJ; (**D**) Monodansylcadaverine (MDC) staining to detect autophagy lysosomes; (**E**) Adenovirus mRFP-GFP-LC3 to detect changes in autophagy flow; (**F**) Western blot result for the expression levels of GLB1 and LC3 in hUC-MSCs; (**G**) Statistical method to calculate the difference in GLB1 protein levels in hUC-MSCs treated with D-galactose at different days; (**H**) Statistical method to calculate the difference in LC3 protein levels in hUC-MSCs treated with D-galactose at different dyas. Control: untreated hUC-MSCs; Day4: cells treated with D-galactose for 4 days.

3.5. LncRNA NEAT1-206 might regulate autophagy via WNT5A/Ca^{2+} pathway as a response to senescence stress

Western blot and immunofluorescence results demonstrated increased protein levels of WNT5A, p-CAMK II and LC3 following overexpression of LncRNA NEAT1-206 (Fig. 7A–C). In contrast, upon knockdown of LncRNA NEAT1-206, the expression level of WNT5A, p-CAMK II and LC3 were significantly reduced (Fig. 7E–F). These findings suggest that LncRNA NEAT1-206 might regulate autophagy through the WNT non-canonical signaling pathway in hUC-MSCs. Additionally, our observations revealed an increase in intracellular calcium flux and Ca^{2+} release in hUC-MSCs after overexpressing LncRNA NEAT1-206 (Fig. 7D). Conversely, downregulating LncRNA NEAT1-206 led to an reduction in intracellular calcium flux and Ca^{2+} release in hUC-MSCs (Fig. 7H). These results provide additional support for the role of LncRNA NEAT1-206 in modulating autophagy in hUC-MSCs through the WNT5A/Ca^{2+} signaling pathway.

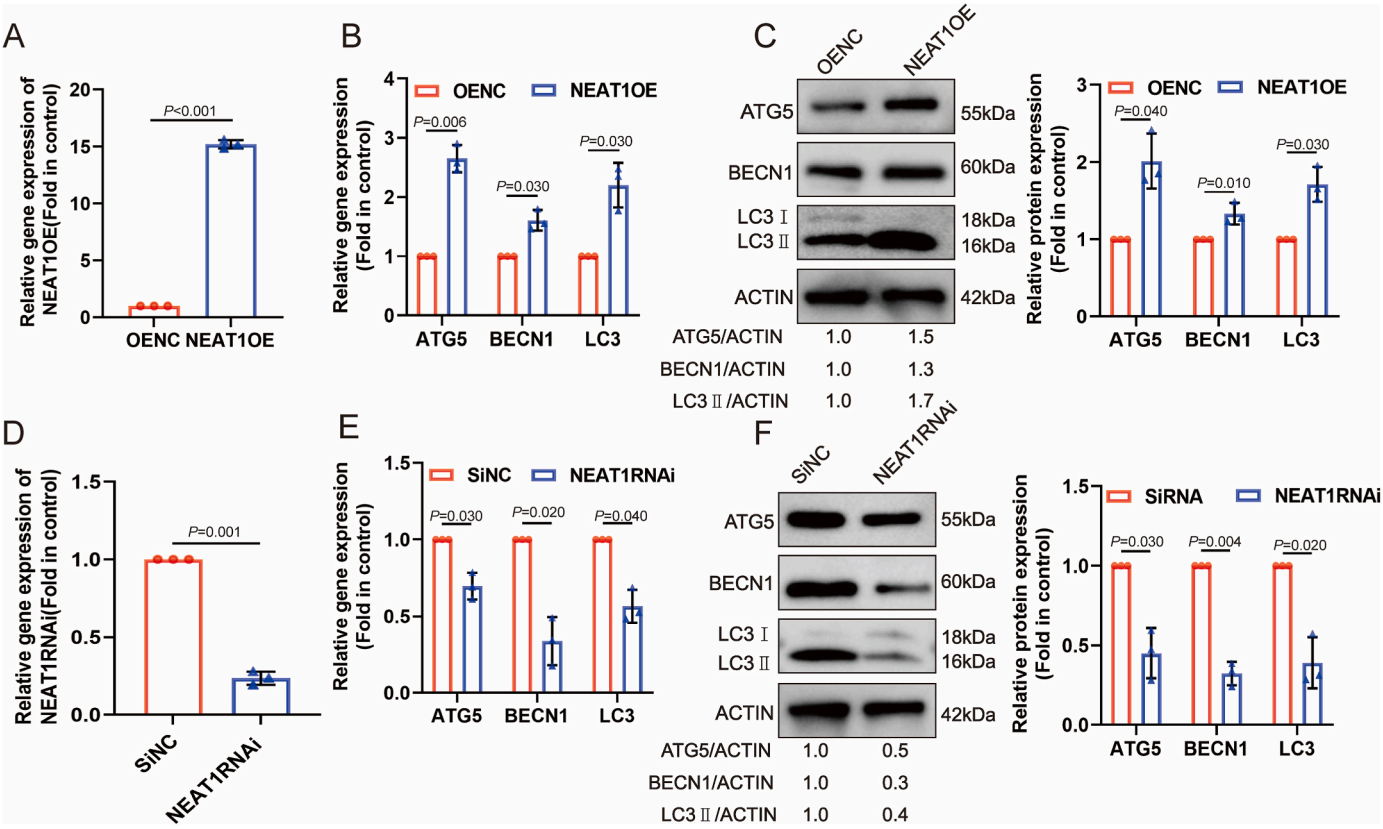

Fig. 5. ***LncRNA NEAT1-206 activates autophagy in hUC-MSCs.*** (**A**) RT-qPCR detects the transfection efficiency after overexpression of LncRNA NEAT1-206; (**B**) RT-qPCR detects the expression of autophagy genes *ATG5*, *BECN1* and *LC3* after overexpression of LncRNA NEAT1-206; (**C**) Western Blot detects the autophagy protein ATG5, BECN1 and LC3 after overexpression of *LncRNA NEAT1-206* and statistical methods to calculate the difference in protein levels of autophagy protein ATG5, BECN1 and LC3 between the Control group (empty load) and the overexpression group; (**D**) RT-qPCR to detect LncRNA NEAT1-206 knockdown Transfection efficiency after; (**E**) RT-qPCR detects the expression of autophagy genes *ATG5*, *BECN1* and *LC3* after knocking down LncRNA NEAT1-206; (**F**) Western Blot detects the expression of autophagy protein ATG5, BECN1 and LC3 after knocking down LncRNA NEAT1-206. Statistical methods were used to calculate the difference in protein levels of autophagy protein ATG5, BECN1 and LC3 between the Control group (no load) and the knockdown group. OENC: empty vector transfected cells; NEAT1OE: LncRNA NEAT1-206 overexprssion cells; SiNC: negative control; NEAT1RNAi: lncRNA NEAT1-206 knockdown cells.

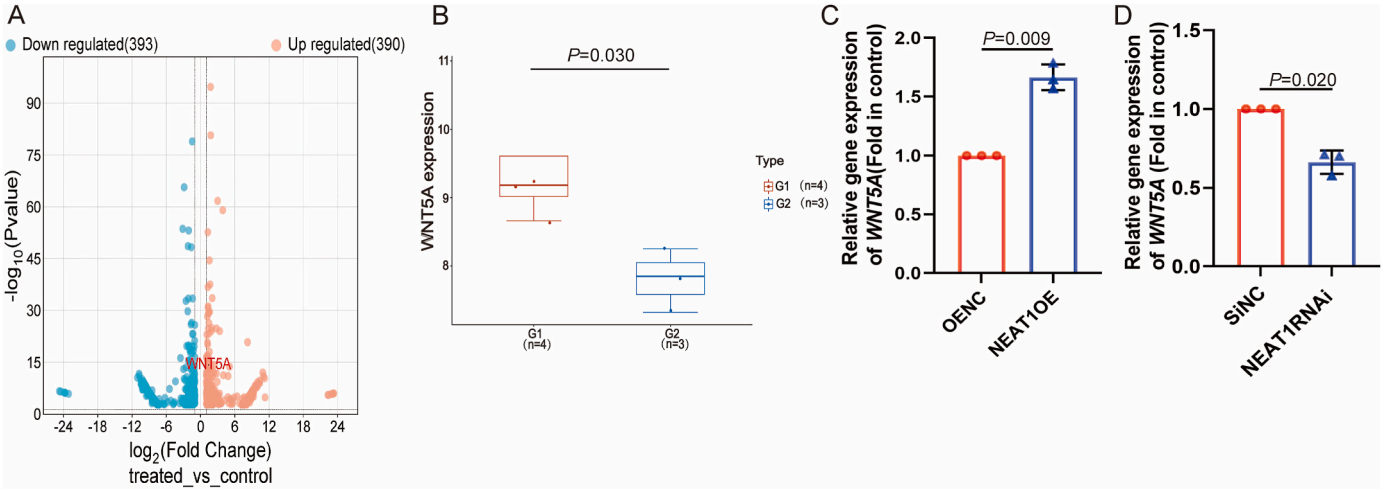

Fig. 6. ***WNT5A expression level in MSCs.*** (A) Expression of *WNT5A* in D-galactose-induced (4 days) senescent hUCMSCs; (B) WNT5A expressed higher in BMSCs from old done; (**C**) RT-qPCR detects the *WNT5A* expression after overexpression of *LncRNA NEAT1-206*; (**D**) RT-qPCR detects the *WNT5A* expression after of knocking down LncRNA NEAT1-206. OENC: empty vector transfected cells; NEAT1OE: LncRNA NEAT1-206 overexprssion cells; SiNC: negative control; NEAT1RNAi: LncRNA NEAT1-206 knockdown cells.

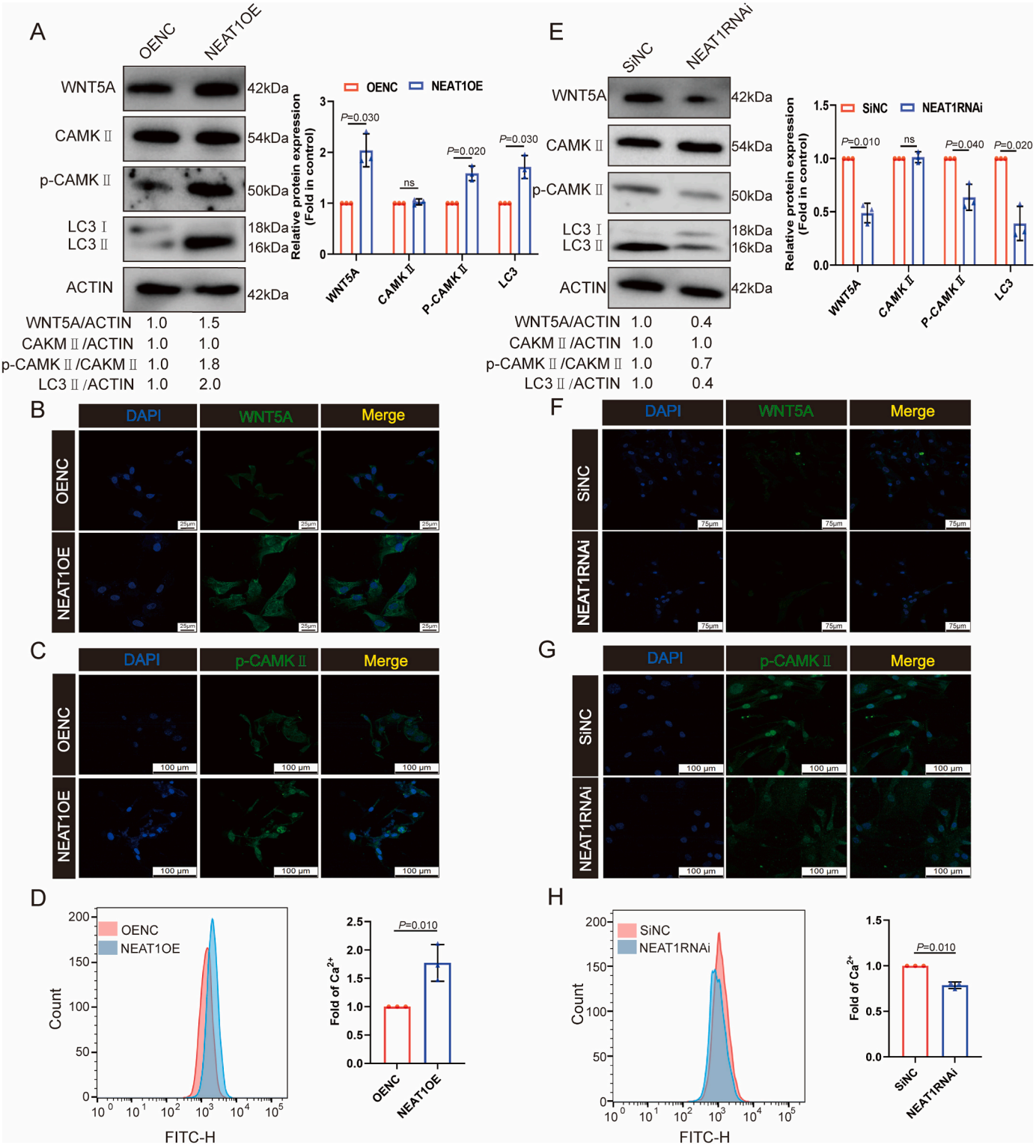

Fig. 7. ***LncRNA NEAT1-206*** **activates signal transduction of WNT5A/Ca^{2+}in hUC-MSCs.** (**A**) Western Blot detects the protein expression of WNT5A, CaMKII,LC3 and p-CaMKII after overexpressing LncRNA NEAT1-206 and statistically calculates the difference in protein levels of WNT5A, CaMKII,LC3 and p-CaMKII; (**B**) Immunofluorescence Technology detects the expression level of WNT5A protein fluorescence intensity; (**C**) Immunofluorescence technology detects the expression level of p-CaMKII protein fluorescence intensity; (**D**) The Control group was detected by flow cytometry Changes in Ca^{2+} concentration, and statistical methods to calculate the difference in Ca^{2+} concentration levelsusing ImageJ; (**E**) Western Blot detection of knockout The protein expression of WNT5A, CaMKII,LC3 and p-CaMKII after low LncRNA NEAT1-206 and the statistical method to calculate the difference in protein levels of autophagy genes WNT5A, CaMKII,LC3 and p-CaMKII; (**F**) Control detected by immunofluorescence technology The expression of WNT5A protein fluorescence intensity; (**G**) The immunofluorescence images of p-CaMKII protein; (**H**) Flow cytometry detection of Ca^{2+} concentration, and statistical methods were used to calculate the difference in Ca^{2+} concentration levels. OENC: empty vector transfected cells; NEAT1OE: lncRNA NEAT1-206 overexprssion cells; SiNC: negative control; NEAT1RNAi: lncRNA NEAT1-206 knockdown cells.

3.6. Overexpression LncRNA NEAT1 promoted osteogenic differentiation of D-galactose-induced hUC-MSCs

To investigate the potential of LncRNA NEAT1-206 in delaying cell senescence and enhancing the differentiation of hUC-MSCs, osteogenic differentiation was induced in hUC-MSCs using an osteogenic differentiation medium. First, hUC-MSCs were treated with D-galactose to establish an early senescent cell model, and then the pCDNA-3.1-NEAT1 and NEAT1RNAi were utilized to upregulate/downregulate the gene expression of LncRNA NEAT1-206 in these cells. Following 21 days of osteogenic induction, osteoblast formation was observed under a microscope after alizarin red staining (Fig. 8A). The LncRNA NEAT1-206 overexpression group exhibited increased calcium deposition compared to the empty vector transfected NC group and senescence group, in contrast, all cells dead after knockdown the expression of NEAT1 (Fig. 8B). This result suggested that LncRNA NEAT1-206 might play an important role in rescuing the differentiation potential of hUC-MSCs, thus preventing MSCs from senescence.

3.7. LncRNA NEAT1 reduced the expression of aging-related markers in D-galactose-induced hUC-MSCs

Our study elucidates the role of LncRNA NEAT1-206 in regulating autophagy in hUC-MSCs via the WNT5A/Ca^{2+} signaling pathway. However, the effects of LncRNA NEAT1-206 on cell senescence of MSCs remains unclear. To address this, we induced senescence in hUC-MSCs using D-galactose to establish an early senescent cell model. Subsequently, the gene expression of LncRNA NEAT1-206 in this model was manipulated through gene overexpressing and inhibiting techniques. We then utilized RT-qPCR and Western Blot analyses to evaluate changes in the expression of senescence-related proteins (GLB1, P16, and P21). The results demonstrated that the overexpression of LncRNA NEAT1-206 resulted in a significant decrease in the gene and protein expression levels of GLB1, P16, and P21 in senescent hUC-MSCs (Fig. 9A–C). In contrast, downregulated LncRNA NEAT1-206 led to a significant increase in the expression of GLB1, P16, and P21 (Fig. 9D–F).

4. Discussion

Aging, or senescence, denotes the progressive deterioration of tissue homeostasis and functional decline over time, manifesting in age-related pathological changes [31]. Alongside functional decline, aging correlates with the onset of various age-related diseases, including Parkinson (PD [32], Alzheimer (AD [33], cardiovascular [34], osteoporosis [35], diabetes [36], and cancer [37]. The aging process is influenced by multiple factors, such as oxidative stress, genome stability, energy metabolism, and telomerase [38]. Stem cell aging is a key contributor to various aging-related phenomena, leading to diminished cell replenishment in tissues and a decline in tissue and organ functions, culminating in systematic aging of the body [39]. The aging and depletion of adult stem cells are pivotal drivers of tissue and organ aging, as well as aging-related diseases. MSCs possess desirable characteristics for tissue repair, including high self-renewal ability, multi-lineage differentiation potential, and low immunogenicity [40]. Therefore, comprehending MSCs aging biology and devising interventions for aging-related diseases are imperative.

4.1. LncRNA NEAT1 highly expressed in early senescence MSCs

In this model of early cell senescence, we observed an increase in the expression of lncRNA NEAT1 compared to another study which reported reduced expression of lncRNA NEAT1 in senescent hepatoma cells [41]. These contrasting findings suggest that MSCs might exhibit resistance to induced senescence by upregulating lncRNA NEAT1 at early stage.

However, MSCs display cellular heterogeneity characteristics in vitro, including differentiated cells, senescent cells, and high-quality cells. To delineate these subsets, we gathered single-cell data of MSCs from the GEO database and discovered that LncRNA NEAT1 served as a marker for a specific subpopulation within MSCs. These findings suggest that LncRNA NEAT1 may function as a regulatory factor in MSCs. LncRNA NEAT1 is one of the most examined genes, it has been found in various cell types. It is enriched in UC-MSCs as well as in other types of MSCs, including bone mesenchymal stem cells, bone marrow-derived

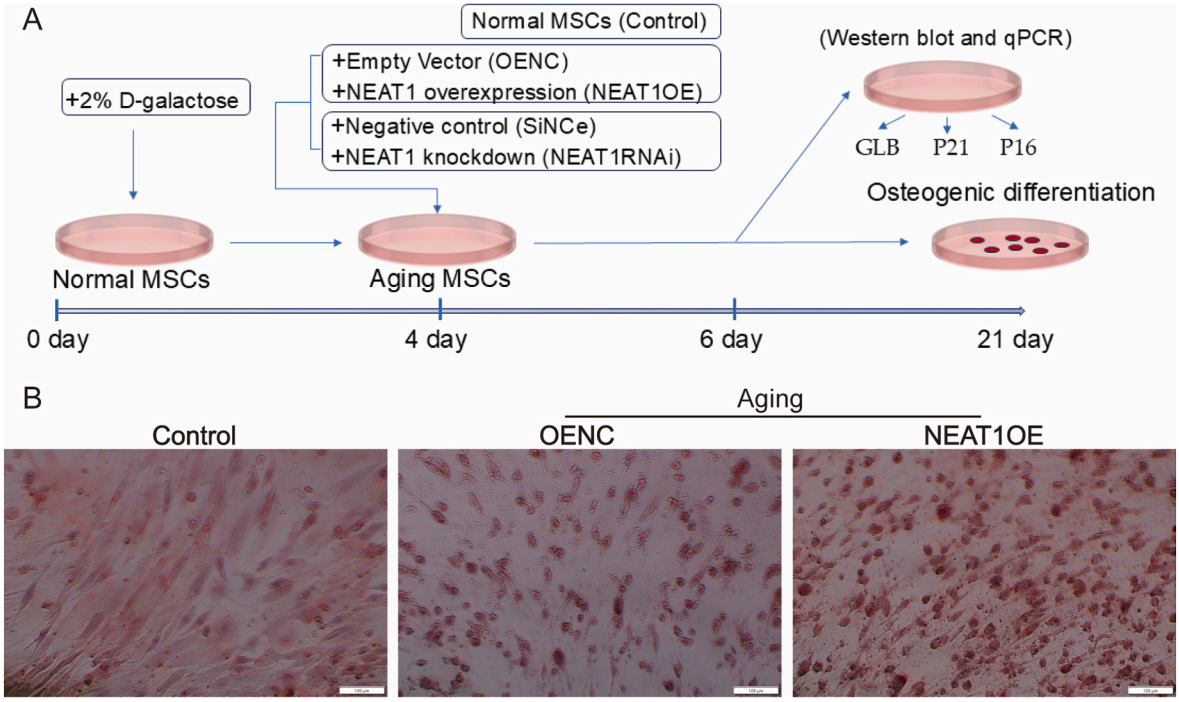

Fig. 8. ***The effect of overexpression LncRNA NEAT1-206 on osteogenic differentiation of D-galactose-induced hUC-MSCs.*** (A) schematic diagram of the experimental process (**B**) Detection of osteogenic differentiation ability. Control: normal cells; OENC: senescent cells + *pcDNA3.1*; *LncRNA NEAT1-206OE*: senescent cells + *pcDNA3.1-NEAT1-206* plasmid.

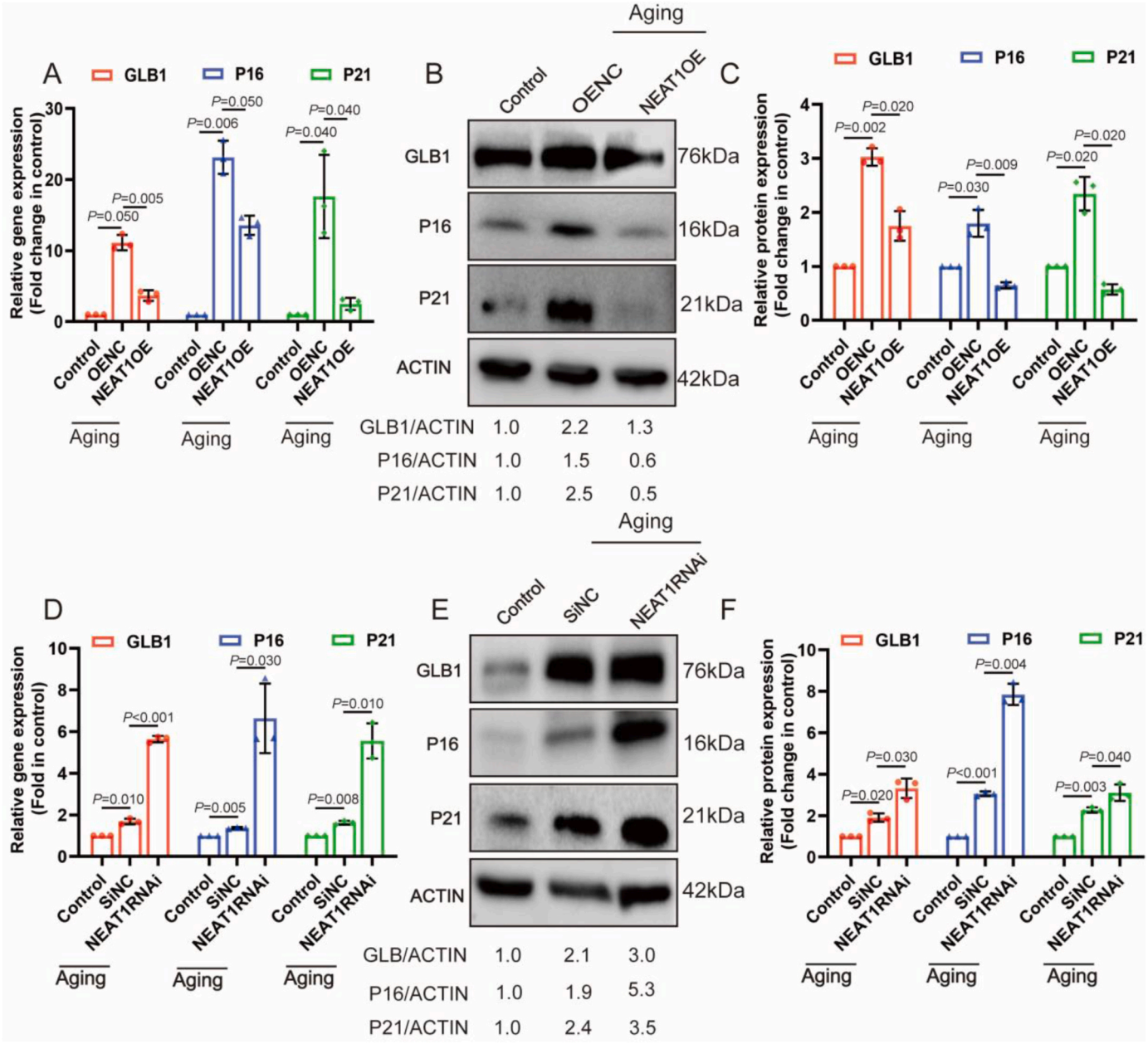

Fig. 9. ***The effect of LncRNA NEAT1-206 on the senescence-related markers of induced hUC-MSCs.*** (A) RT-qPCR detects the expression changes of GLB1, P16 and P21 after overexpression of LncRNA NEAT1-206 in aging cells; (B) Western blot detects the protein expression of GLB1, P16 and P21 after overexpression of LncRNA NEAT1-206 in aging cells; (C) Statistical method to calculate the GLB1, P16 and P21 protein levels by ImageJ; (D) RT-qPCR detection of the expression changes of GLB1, P16 and P21 after knockdown of LncRNA NEAT1-206 in aging cells; (D) Western blot detection the expression levels of GLB1, P16 and P21 proteins after knockdown of LncRNA NEAT1-206 in aging cells; (F) Statistical method to calculate the differences in GLB1, P16 and P21 protein levels using ImageJ. The transfection conducted on cells which treated with D-galactose for 4 days. These cells referred as two pairs: OENC (aging) vs NEAT1OE (aging), and SiNC (aging) vs NEAT1RNAi (aging). OENC: empty vector transfected cells; NEAT1OE: lncRNA NEAT1-206 overexprssion cells; SiNC: negative control; NEAT1RNAi: lncRNA NEAT1-206 knockdown cells.

mesenchymal stem cells, and adipose-derived mesenchymal stem cells [42–44].

Some reports showed that LncRNA NEAT1 acts as a 'sponge' for miRNA, known as ceRNA, and can also function as a scaffold to bind with proteins. It has been was reported to regulate the gemcitabine resistance in pancreatic cancer cells, by binding with miR-491-5p and Snail [43]. In osteoarthritis, lncRNA NEAT1 binds to miR-122-5p to increase the expression of Sesn 2 [45]. In prostate cancer, NEAT1 was found to up-regulated the RUNX2 expression by binding with miR-205-5p [46]. In osteosarcoma cells, NEAT1 up-regulated the expression of STAT3 by sponging miR-483 [47]. Additionally, lncRNA NEAT1 could up-regulate the expression of CYP1A2 by sponging miR-23b-3p [48] lncRNA NEAT1 could protecting cardiomyocytes from apoptosis by miR-142-30/FOXO1 axis [49]. lncRNA NEAT1 has also been reported to work as a key bone-fat switch, which regulated the adipocytes differentiation of BMSCs by impairing SOX2/OCT4 complex [42].

According to ensembl genome browser, LncRNA NEAT1 has 9 transcripts, but which one involve in the senescence process of MSCs, is not clear. Using RNA-sequencing and RT-qPCR, we noticed that LncRNA NEAT1-206 was a significant increased transcript in MSCs. Moreover, we found that LncRNA NEAT1-206 expression in both nucleus and

cytoplasm after treating with D-galactose, these results suggested LncRNA NEAT1-206 might serve as a marker of cells with aging tendencies.

4.2. LncRNA NEAT1 responds to D-galactose-induced senescence in mesenchymal stem cells and promotes autophagy through the WNT5A/Ca^{2+} pathway

The RISH images showed that LncRNA NEAT1-206 was transferred from nucleus to cytoplasm, suggests a biological regulatory function of LncRNA NEAT1-206 in cytoplasm during cell aging process. After overexpressing LncRNA NEAT1, we conducted RNA-sequencing on both the overexpressing cells and the control cells; however, no DEGs directly associated with the autophagy pathway were identified. However, autophagy was observed in D-galactose-induced cells. Moreover, we found that the modulation of autophagy was influenced by both the overexpression and inhibition of LncRNA NEAT1. These results suggest that LncRNA NEAT1 might not directly regulate gene expression associated with autophagy but act through alternative pathways. Subsequently, we identified that autophagy is controlled by the NEAT1/WNT5A/Ca^{2+} axis. The WNT signaling pathway is primarily divided into three categories: the classical WNT pathway (WNT/β-catenin), the non-classical WNT/planar cell polarity pathway (WNT/PCP), and the non-classical WNT/Ca^{2+} pathway (WNT/Ca^{2+}). To active the WNT/Ca^{2+} pathway, WNT5A activates the Dishevelled protein (Dvl), which subsequently activates the phosphodiesterase (PDE), leading to the release of Ca^{2+} from unstimulated cells and an increase in intracellular Ca^{2+} levels. In our research, we found the expression of WNT5A highly expressed in aging UCMSCs, it suggested that non-classical WNT signaling pathway might play a role in this process. Furthermore, the level of Ca^{2+} increased in LncRNA NEAT1 overexpression cells, and the p-CAMK II level increased as well. In contrast, inhibited LncRNA NEAT1 could reduce the level of Ca^{2+} and expression of p-CAMK II. These results indicated that LncRNA NEAT1 could regulate WNT/Ca^{2+} pathway during aging process of UCMSCs.

Autophagy levels increased with prolonged D-galactose treatment, reaching a peak on day 5 and showing a slight decrease on day 6. These Western blot results suggest that NEAT1-regulated autophagy might help rescue cells at the early stages of induced senescence by reducing the expression of GLB, but this effect may not be sustained long enough to confer resistance to D-galactose-induced senescence. However, reducing the expression of WNT5A in cells overexpressing NEAT1 resulted in a rapid decrease in cell growth and cell numbers. Consequently, we could not collect sufficient data to confirm that NEAT1 exclusively influences early cell senescence through the WNT5A pathway.

Autophagy, a fundamental cellular metabolic process, plays a crucial role in maintaining cell homeostasis, differentiation, development, and survival [50]. Autophagy entails the degradation of diverse molecular and subcellular components, encompassing nucleic acids, proteins, lipids, and organelles, through lysosomes. Despite its close association with overall health, the intricate interplay between autophagy, aging, and disease remains incompletely elucidated. Autophagy serves as a highly selective pathway for clearing cells and maintaining tissue homeostasis, with recent preclinical studies highlighting its potential in thwarting age-related diseases, such as neurodegenerative disorders [51]. Notably, inhibiting mTOR, such as by using rapamycin, can dampen protein synthesis and promote autophagy, thus prolonging the lifespan of various organisms including yeast, nematodes, fruit flies, and mice [52]. Evidence indicates a decline in the self-renewal and tri-lineage differentiation capabilities of BMSCs derived from elderly donors [53]. Senescence in BMSCs, along with increased bone loss, can result from defects in the autophagy receptor OPTN [54]. However, activating autophagy in BMSCs can reverse these aging-related effects and restore the biological characteristics, highlighting the potential utility of the autophagy pathway in preserving MSCs' stemness. These findings underscore the interaction between autophagy and cellular senescence. Additionally, this study observed the induction of autophagy in hUC-MSCs' aging process. However, given the complexity of intracellular autophagy processes and pathways, it is crucial to elucidate the optimal range and precise mechanism of autophagy. This understanding can facilitate the precise regulation of autophagy levels in MSCs, thereby optimizing their therapeutic efficacy. The application of MSCs in research holds great significance.

In human genes, approximately 2% of RNA consists of protein-coding mRNAs, with the remaining 98% being non-coding RNAs. Among these, LncRNAs are representatives of non-coding RNAs, characterized by their length exceeding 200 nucleotides. Despite of being encoding proteins, LncRNAs exert pivotal regulatory roles in gene expression through diverse pathways. Their impact spans cell growth, aging, and the development of age-related diseases such as cancer, neurodegenerative diseases, and cardiovascular diseases [55]. Many LncRNAs participate in the regulation of cell proliferation, differentiation, apoptosis, and aging, thereby influencing the cellular aging process. For instance, LncRNA ANRIL has been demonstrated to boost cell activity and inhibit the aging of vascular smooth muscle cells by regulating miR-181a/sirtuin1 [56]. This mechanism involves the suppression of the p53/p21 signaling pathway and the reduction of cell cycle arrest, underscoring the pivotal role of autophagy in aging [57]. Nevertheless, the impact of autophagy on the long-term health of cells and tissues is an underexplored question. Therefore, it is to comprehensively comprehend the role of LncRNAs in cellular aging, together with their impact on aging and related diseases, and their utility as biological markers for the prevention, screening, diagnosis, and prognosis of aging-related diseases. Our study underscores the role of LncRNA NEAT1-206 in regulating hUC-MSCs autophagy and delaying senescence through the WNT5A/Ca^{2+} signaling pathway (Fig. 10).

4.3. LncRNA NEAT1 might promote osteogenic differentiation and reduce senescence markers during D-galactose-induced early cell senescence of MSCs

In order to validate the role of LncRNA NEAT1-206 in the early senescence process of mesenchymal stem cells, we designed a rescue experiment. Firstly, cells were induced to senescence by D-galactose and then overexpressed or inhibited LncRNA NEAT1-206. We found that overexpression of LncRNA NEAT1-206 significantly increased the osteogenic differentiation capability of induced-senescent MSCs. Moreover, the expression of senescence markers, such as GLB, P21, P16 were found to related with LncRNA NEAT1-206. This finding supports the association of *LncRNA NEAT1-206* with the cellular aging process through its influences on aging-related signaling pathways. It suggests that LncRNA NEAT1-206 may exert anti-aging effects at the onset of senescence induced by metabolic alterations. This implies the prospect of using LncRNA NEAT1 as a target for the prevention and treatment of aging-related diseases and as a potential biomarker of aging. Furthermore, it may serve as a drug option to delay cell senescence.

However, the intricacies of cell senescence, particularly in hUC-MSCs, are multifaceted and dynamic [58]. Currently, the mechanism underlying the transfer of LncRNA NEAT1 from the nucleus to the cytoplasm remains insufficiently understood. Additionally, it is unclear whether LncRNA NEAT1 elicits a comparable anti-aging effect in animal models. Moreover, the precise mechanism by which LncRNA NEAT1 regulates the WNT5A/Ca^{2+} signaling pathway to impact autophagy remains elusive. Future research endeavors are anticipated to leverage cutting-edge technologies and robust disease models to elucidate the role and mechanism of LncRNA NEAT1 in cellular aging and related diseases. These investigations will critically evaluate the potential of LncRNA NEAT1 as a novel biomarker, facilitating the development of more accurate and safe treatment strategies, ultimately leading to a delay of aging, reduced incidence of aging-related diseases, alleviated social burdens, and patients' high quality of life.

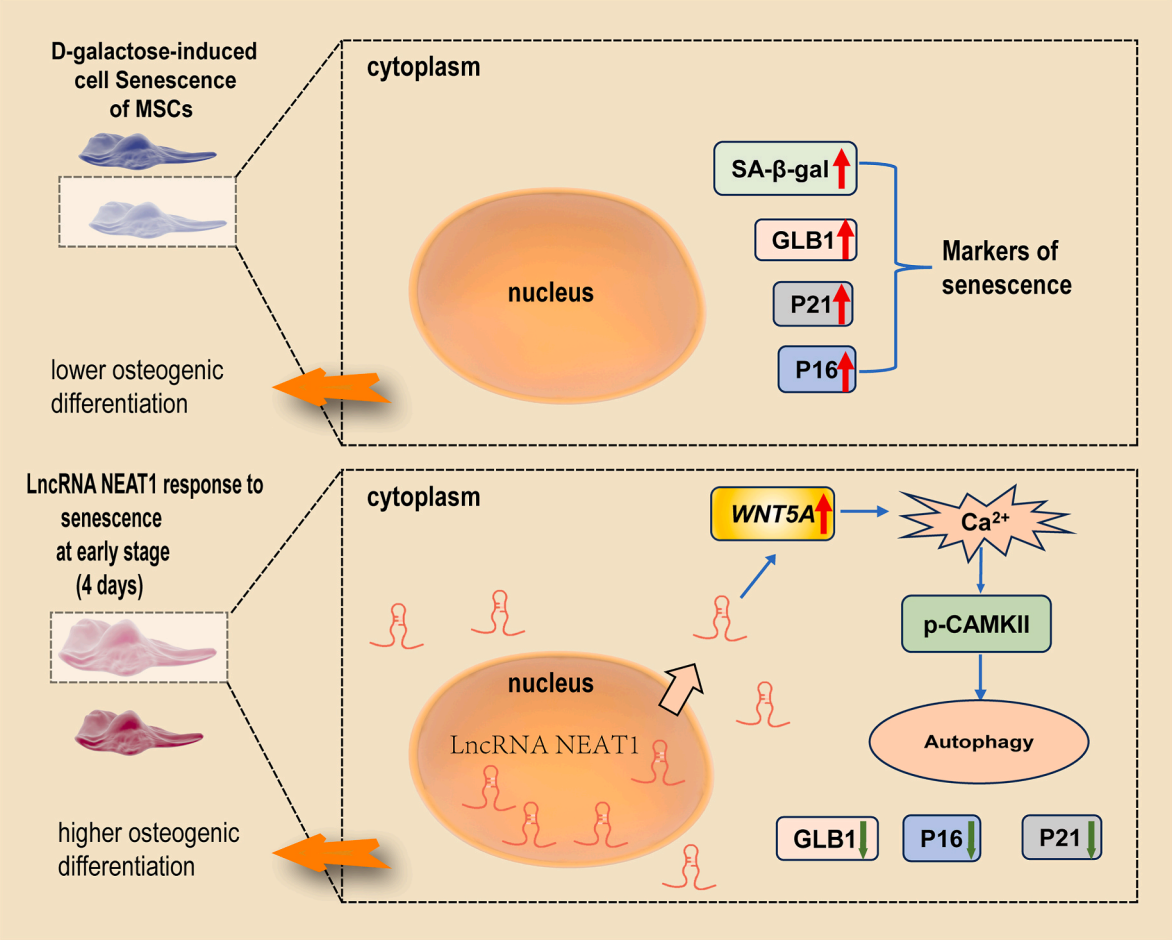

Fig. 10. Schematic diagram of the mechanism of LncRNA NEAT1-206 regulating the early senescence process of hUCMSCs.

5. Conclusion

In our previous research, we observed that D-galactose could act as a stressor to promote the aging process in hUC-MSCs. During the early stage of cellular senescence, we noted an increase in the expression of LncRNA NEAT1-206 transcript in hUC-MSCs. Furthermore, due to the heterogeneity of stem cells, a subset of cells within the normal cells exhibited higher LncRNA NEAT1 level than the rest. These observations suggest that LncRNA NEAT1-206 might play an important role during cellular aging process. We subsequently identified that autophagy, regulated by the LncRNA NEAT1/WNT5A/Ca^{2+} axis, could decrease the expression of senescence-associated markers in hUC-MSCs under senescent stress. Additionally, the overexpression LncRNA NEAT1-206 was found to promote osteogenic differentiation in D-galactose-induced hUC-MSCs. Therefore, LncRNA NEAT1-206 might function as a protective factor against senescence stress in hUC-MSCs.

CRediT authorship contribution statement

Weili Wang: Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Yongyu Wang:** Writing – original draft, Validation, Formal analysis, Data curation. **Chunchun Duan:** Validation, Data curation. **Wenjing Tian:** Writing – review & editing, Validation. **Liyang Gao:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material.

Author declaration

We confirm that the manuscript has read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the authors listed in the manuscript has been approved by all of us.

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, " **LncRNA NEAT1-206 regulates autophagy of human umbilical cord mesenchymal stem cells through the WNT5A/Ca^{2+} signaling pathway under senescence stress**".

Conflicts of interest

The authors declare that this study does not present any conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ncrna.2024.12.013.

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