PBX1-SIRT1 positive feedback loop attenuates ROS-mediated HF- MSC senescence and apoptosis

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Research Article

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Abstract

Background: Stem cell senescence and depletion are major causes of organismal aging and aging-related diseases. The NAD–SIRT1–PARP1 axis has garnered remarkable interest owing to its significant role in regulating stem cell senescence and organismal aging. Event though our recent study showes that PBX1 overexpression attenuates hair follicle-derived mesenchymal stem cell (HF-MSC) senescence and apoptosis by regulating ROS-mediated DNA damage via downregulation of PARP1 expression, suggesting PARP1 downregulation is a common manifestation of the roles of both PBX1 and SIRT1 in the attenuation of HF-MSC senescence, and implying a potential link between PBX1 and SIRT1 via PARP1.

Methods: HF-MSCs overexpressing PBX1, overexpressing both PBX1 and PARP1, downregulating SIRT1, and overexpressing PBX1 and downregulating SIRT1 were generated, and biomarkers related to cell senescence, apoptosis, DNA damage, and repair were detected at the cellular and protein levels.

Results: (1) PBX1 overexpression alleviated HF-MSC senescence and apoptosis accompanied by upregulation of SIRT1 expression, downregulation of PARP1 expression, and increased intracellular NAD and ATP levels. (2) SIRT1 knockdown or PARP1 overexpression enhanced cellular senescence and apoptosis, accompanied by increased ROS accumulation and DNA damage aggravation and decreased intracellular NAD and ATP levels. (3) PBX1 overexpression rescued HF-MSC senescence and apoptosis induced by SIRT1 knockdown or PARP1 overexpression. (4) Dual luciferase reporter showed PBX1 enhanced SIRT1 expression by activating SIRT1 promoter.

Conclusions: Our results reveal that a positive interaction feedback loop exists between PBX1 and SIRT1, that PBX1 is the upstream of SIRT1. To the best of our knowledge we are the first to report that there is a PBX1-SIRT1-PARP1 axis and this axis plays a critical role in alleviation of HF-MSC senescence and apoptosis. The results provide a new perspective on the mechanism of stem cell senescence and age-related disease prevention and treatment.

Introduction

Tissue homeostasis is regulated by stem cells, and age-related tissue degeneration is closely associated with a decline in stem cell function and number [1]. Stem cells are characterized, in general, by their ability to self-renew and differentiate into multiple lineages [2, 3]. By committing to tissue-specific cells through proliferation and differentiation and consequently replacing, repairing, and regenerating damaged and degenerated tissues, stem cells participate in maintaining tissue homeostasis and preventing and treating aging-related diseases. Hence, stem cell senescence and depletion are regarded as major risk factors for organismal aging and age-related diseases. Cellular senescence is a state of permanent cell cycle arrest, leading to stable and long-term loss of proliferative capacity, despite preservation of cell viability and metabolic activity [4, 5]. DNA damage is a major cause of stem cell senescence. Cells maintain genomic stability via the DNA damage response (DDR) [6]. Besides the repair mechanisms,

another essential factor which influences DNA damage is the state of chromatin, which may affect the sensitivity of DNA to DNA-genotoxic agents and interfere with access to DDR signaling and DNA repair factors [6]. Histone acetylases (HATs) and deacetylases (HDACs) can identify DNA damage sites, contribute to the recruitment of DNA repair proteins, silence transcription during the repair process, and restore the chromatin state after repair [7, 8].

SIRT1 is a highly conserved NAD-dependent lysine deacetylase and ADP-ribosyl transferase. It is widely recognized as a crucial epigenetic regulator [9, 10] and participates in numerous biological processes, including gene silencing, DNA repair, metabolic regulation, cell cycle regulation, apoptosis, inflammation [11, 12], autophagy, cellular senescence [13], and heterochromatin formation, and in protection against various human diseases. SIRT1 is mainly located in the nucleus and is involved in the prevention of DNA damage via the suppression of reactive oxyden species (ROS) in the mitochondria or the activation of ROS-removing enzymes [14] and the promotion of DNA repair by many different pathways [15, 16]. SIRT1 can contribute to decreased levels of 8-OHdG, which is a critical oxidative stress biomarker that affects DNA repair [17] and can dissociate from transcriptionally repressed DNA loci and relocate to DNA breaks to promote DNA damage repair via the ATM signaling pathway [18]. SIRT1 can also recruit repair-related proteins to DNA damage sites to regulate their activity by deacetylation and can regulate the formation of γH2AX, Rad51, BRCA1, and NBS1 foci upon DNA damage. Hence, it aids in promoting DNA damage repair and regulating cell cycle checkpoints [19]. Repair protein KU70, which is a crucial protein belonging to non-homologous end joining (the major DNA repair mechanism for double-stranded breaks in mammalian cells), and the FOXO family, which forms a complex that induces cell cycle arrest and resistance to oxidative stress, are deacetylated and activated by SIRT1 upon DNA damage [8, 16, 20]. In addition, SIRT1 deacetylates non-histone substrates, including p53, Ku70, FoxOs, PGC1-a, PPAR-γ, AMPK, mTOR, MyoD, and NF-κB [21, 22], which are related to delayed cellular senescence. SIRT1 is thus widely involved in the regulation of cellular senescence and contributes to organism longevity via acetylation and deacetylation of these substrates, altering their transcriptional and enzymatic activities as well as corresponding protein levels [11, 23]. Thus, SIRT1 upregulation can affect the regulation of cellular senescence in aging and aging-related diseases.

A strategy to activate sirtuins relies on increasing NAD levels. An increase in NAD is mediated via the repression of major cellular NAD-consuming enzymes, such as PARP-1 and CD38, or the direct supplementation of NAD precursors, such as nicotinamide mononucleotide (NNM) and nicotinamide riboside (NR). In addition, increasing NAD levels can help maintain telomere integrity and promote the DNA damage response in a SIRT1-dependent manner, including activation of genes involved in DNA damage repair and direct chromatin modifications at DNA breaks through the recruitment of DNA damage repair proteins [24, 25]. Therefore, activation or upregulation of SIRT1 is expected to be an effective measure for the maintenance of tissue homeostasis, attenuation of stem cell senescence, prolongation of lifespan, and amelioration of aging-related diseases.

PBX1 is a key transcription factor that regulates cell fate in non-tumoral conditions, particularly stem cell proliferation, apoptosis, senescence, and differentiation [26–28]. Hair follicles (HFs) are epithelial mini-

organs of the skin that sustain cyclic hair regrowth and maintain homeostasis over repeated hair cycles [29]. Moreover, because of the easy access, rich source, and low immunogenicity of autologous stem cells, as well as less ethical dilemmas regarding them [30], HF- MSCs offer distinct advantages over other stem cell sources in stem cell-based regenerative medicine, particularly in hair regeneration and skin repair. HF-MSCs coordinate synergistically in a spatiotemporal manner to maintain hair cycles, HF genesis, hair repair, and hair regeneration [31]. Senescence of HF-MSCs not only disrupts HF homeostasis, leading to hair loss, but also compromises the therapeutic potential of HF-MSCs, even raising safety concerns regarding HF-MSC-based regenerative medicine. Our previous study showed that PBX1 overexpression significantly attenuates HF-MSC senescence and apoptosis, accompanied by a downregulation of PARP1 expression. In injury and inflammation, PARP1 inhibition exerts marked protective effects [32].

PARP1 can be activated by oxidative, metabolic, and genotoxic stresses and directs cells to specific fates depending on the type and intensity of stress [33]. Although PARP1 is typically regarded as a DNA damage repair enzyme, it also has equally important functions in the regulation of apoptosis or necrotic cell death, which depletes large amounts of NAD and ATP [34–36]. However, post-translational modification–derived PARP-1 overactivation by excessive DNA damage may alter the function of many enzymes and structural proteins and initiate caspase-independent cell death, which depletes large amounts of NAD and ATP [36]. Considering that PARP1 is a major cellular NAD consumer, PARP1 levels can directly affect the activity of other NAD-dependent enzymes, such as SIRT1, which plays a crucial role in protection against oxidative injury. NAD depletion mediated by PARP-1 overactivation attenuates the deacetylase activity of SIRT1, which is implicated in longevity and cellular protection since NAD is a replaceable substrate of SIRT1.

Whether PBX1 attenuates HF-MSCs senescence and apoptosis by regulating the SIRT1-PARP1 axis remains unknown. To this end, this study aimed to develop HF-MSCs overexpressing PBX1, overexpressing both PBX1 and PARP1, downregulating SIRT1, or overexpressing PBX1 and downregulating SIRT1 and to examine biomarkers related to senescence, apoptosis, DNA damage, and repair.

Materials And Methods

This work was approved by the Ethics Committee of the School of Public Health, Jilin University, China.

Cell culture

The isolation and identification of HF-MSCs were carried out as in our previous study [28, 37]. Isolated HF-MSCs were cultured in Dulbecco's modified Eagle's medium (Life Technologies, USA) containing 10% fetal bovine serum (FBS; Hyclone, Liogan, UT, USA), 2 ng/mL of basic fibroblast growth factor (Sino Biological Inc., China) and 100 U/mL penicillin–streptomycin (Hyclone). HEK 293T cells were cultured in DMEM containing 10% FBS and 100 U/mL penicillin-streptomycin. All cells were cultured at 37°C and 5%

CO_2.When the cells proliferated to 80–90% confluence, they were digested and subcultured under similar individual conditions.

Generation of HF-MSCs overexpressing PBX1, PARP1, and both PBX1 and PARP1

The overexpression of PBX1, PARP1, and PBX1 + PARP1 in HF-MSCs was achieved as in similar to our previous work [28, 37, 38].

SIRT1 silencing

Small interfering RNA (siRNA) oligonucleotides were purchased from RiboBio (China) with sequences targeting SIRT1. HF-MSCs were transfected with 100 nM of the indicated siRNA or scrambled RNA (scRNA) using riboFECT CP Transfection Kit (RiboBio) according to the manufacturer's instructions. The effects of siRNA on the indicated protein levels were examined by western blot analysis.

ATP production measurement

Approximately 1 × 10^6 HF-MSCs were harvested in 200 μL cell lysis solution and centrifuged at 12,000 g for 5 min. The intracellular ATP production was assessed using an Enhanced ATP Assay kit (Beyotime Institute of Biotechnology), adhering to the manufacturer's guidelines. Moreover, luminescence was measured using Cytation 3 (BioTek, USA).

NAD/NADH production measurement

Approximately 1 × 10^6 HF-MSCs were harvested in 200 μL NAD/NADH extraction solution and centrifuged at 12,000 g for 5 min. The intracellular NAD/NADH production was assessed using an NAD/NADH Assay Kit (Beyotime) according to the manufacturer's instructions and absorbance using Cytation 3 (BioTek).

Senescence-associated-*β*-galactosidase and apoptosis assays

The cellular senescence staining kit (Beyotime) was used to detect senescence-associated (SA)-*β*-gal activity-positive cells according to the manufacturer's instructions. When the HF-MSCs reached 75–80% confluence in a 24-well plate, they were fixed for 15 min at room temperature and washed using phosphate-buffered saline (PBS). Then, the HF-MSCs were incubated in Staining Solution Mix overnight at 37°C. The next day, the cells were washed three times with PBS and observed using an optical microscope (Leica, Germany). The number of β-gal–positive cells and total cells were counted from randomly selected three fields of view.

The Annexin V-FITC/7-AAD Apoptosis Detection Kit (Sungene, China) was used to detect apoptotic-positive cells according to the manufacturer's instructions. Approximately 1 ×10^5 HF-MSCs were suspended in 100 μL binding buffer containing 5 μL Annexin V-FITC and incubated for 15 min in the dark at room temperature. After incubation, 5 μL 7-AAD was added and co-incubated for 5 min at similar conditions. The HF-MSCs were detected by flow cytometry (BD, USA).

ROS Assay

For ROS probing, HF-MSCs were collected by centrifugation, washed with PBS, and incubated with DCFH-DA. HF-MSCs were then detected by flow cytometry (BD, USA) and analyzed by FlowJo software (Treestar. USA).

Western Blotting Assay

Approximately 8 × 10^5 HF-MSCs were plated in a 100-mm cell culture dish in DMEM medium containing 10% FBS and 2 ng/mL bFGF. When HF-MSCs reached 80% confluence, they were harvested and lysed in 200 μL RIPA buffer (Beyotime) supplemented with 1% protease inhibitor cocktail (CoWin Biosciences, China) and 1% phosphatase inhibitor cocktail (CoWin Biosciences) at 4℃ for 40 min and centrifuged at 13,000 g for 20 min at 4℃. The supernatant was collected and protein concentration was analyzed using an Enhanced BCA Protein Assay Kit (Beyotime). Twenty-five micrograms of protein per sample were loaded in each well and separated by 10% SDS polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membranes (Millipore, USA). The membranes were incubated in 5% nonfat milk (Anchor, New Zealand) at room temperature for 45 min. The membranes were then incubated with primary antibodies: PBX1 rabbit mAb (1:1000, Cell Signaling Technologies [CST]), p16 rabbit mAb (1:1000; ProteinTech Group, USA), p21Waf1/Cip1 (12D1) rabbit mAb (1:1000; CST), p53 mouse mAb (1:1000; Santa Cruz Biotechnology), PARP1 rabbit mAb (1:1000; CST), AIF rabbit mAb (1:1000, CST), Sirtuin 1 mouse mAb (1:1000, CST), Cleaved Caspase 3 rabbit mAb (1:1000, CST), γH2AX rabbit mAb (1:1000, CST), GAPDH mouse mAb (1:10000; ProteinTech Group), HRP-conjugated AffiniPure Goat Anti-Rabbit IgG (H + L) (1:5000; ProteinTech Group), and HRP-conjugated AffiniPure Goat Anti-Mouse IgG (H + L) (1:5000; Protein-Tech Group). The proteins were visualized using a chemiluminescence imaging analysis system (ECL; Tanon 5200; Shanghai Tianneng Technology Co., Shanghai, China), and band intensity was analyzed with ImageJ.

Statistical Analysis

Data were statistically analyzed using SPSS software. Results are expressed as mean ± standard deviation and are representative of at least three independent experiments. Comparisons between the two groups were performed with independent sample t tests, and differences among multiple groups were compared with one-way analysis of variance. The results were considered significant at $P < 0.05$.

Results

SIRT1 expression decreased with subculturing

SIRT1 expression decreases with age at the protein and transcriptional levels in mammalian tissues, including the liver, kidney, and brain [39–41]. As expected, HF-MSCs entered an apoptotic and senescent state with cell passaging (Figs. 1g–k). Western blot results showed that the expression of SIRT1 in HF-MSCs decreased with subculturing ($P < 0.05$; Fig. 1a,b). Moreover, the levels of total NAD, NADH, NAD, and ATP in HF-MSCs decreased with subculturing ($P < 0.05$; Fig. 1c–f), with P7 (seventh-generation passage cells) showing 0.62, 0.22, 0.81, and 0.53 times less than those of P3 (third-generation passage cells), respectively ($P < 0.05$; Fig. 1c–f). The percentage of SA-β-gal-positive cells and 7-AAD Annexin V-FITC-positive cells at P7 were significantly higher than those at P3 ($P < 0.05$; Figs. 1g–k).

SIRT1 knockdown enhanced cellular senescence and apoptosis, accompanied by increased DNA damage aggravation and NAD and ATP depletion

HF-MSCs enter replicative senescence and apoptosis, which was accompanied by downregulation of SIRT1 expression. To further confirm whether the inhibition of SIRT1 promotes cellular senescence and apoptosis, we knocked down SIRT1 in HF-MSCs. As expected, western blotting results showed that SIRT1 knockdown upregulated the expression of 1) senescence-related proteins such as p53, p21, and p16 ($P < 0.05$; Fig. 2a,h); 2) apoptosis-related proteins such as cleaved caspase 3, Cyt C, and 57-kDa AIF ($P < 0.05$; Fig. 2a,e,f); and 3) DNA damage-related protein γH2AX ($P < 0.05$; Fig. 2a,i) compared to SiNC. Thus, the western blotting results of senescence and apoptosis-related proteins were in agreement with the results of 7-AAD Annexin V-FITC ($P < 0.05$; Fig. 2p,q) and SA-β-gal ($P < 0.05$; Fig. 2n,o). The percentage of SA-β-gal-positive (Fig. 2n,o), 7-AAD Annexin V-FITC-positive (Fig. 2p,q), and ROS-positive (Fig. 2r,s) cells increased from 27.11%, 7.46%, and 3.18% in the SiNC group to 66.04%, 28.47%, and 21.12% in the SiSIRT1 group ($P < 0.05$; Fig. 2n-s), respectively. SIRT1 knockdown increased PARP1 expression ($P < 0.05$; Fig. 2a,d), decreased the expression of PBX1 ($P < 0.05$; Fig. 2a,b), inhibited the expression of PGC1α and FOXO1 ($P < 0.05$; Fig. 2a,g), and reduced the total levels of intracellular total NAD, NADH, NAD, and ATP compared to those observed in the SiNC ($P < 0.05$ Fig. 2t,u).

PBX1 rescued SIRT1 knockdown-mediated HF-MSC senescence and apoptosis by alleviating ROS-mediated DNA damage and intracellular NAD depletion

As described, SIRT1 positively regulates cell survival. Therefore, regulation of SIRT1 may be a plausible strategy for the treatment of aging and aging-related diseases. Our previous studies have shown that PBX1 promotes HF-MSC proliferation and attenuates cellular senescence and apoptosis by upregulating SIRT1 expression. To further confirm whether PBX1 could attenuate HF-MSCs senescence and apoptosis by upregulating SIRT1 and whether PBX1 could rescue SIRT1-knockdown-mediated HF-MSC senescence and apoptosis, we overexpressed PBX1 and knocked down SIRT1 in HF-MSCs. A dual-luciferase reporter gene assay showed that the luciferase activity of the SIRT1 promoter in the PBX1 group was significantly higher than that in the vector group ($P < 0.05$; Fig. 3b). We found that compared with the control, SIRT1

knockdown group showed significant increase in the percentage of apoptotic ($P < 0.05$; Fig. 3l, m), senescent ($P < 0.05$; Fig. 3j, k), and ROS-positive cells ($P < 0.05$; Fig. 3n,o). In contrast, PBX1-overexpressing group showed decrease in the percentages of apoptotic ($P < 0.05$; Fig. 3l, m), senescent ($P < 0.05$; Fig. 3j, k), and ROS-positive cells ($P < 0.05$; Fig. 3n, o) compared to those observed in the empty vector group. Furthermore, the percentage of SA-β-gal-positive cells ($P < 0.05$; Fig. 3j, k), 7-AAD Annexin V-FITC-positive cells ($P < 0.05$; Fig. 3l, m), and ROS-positive cells ($P < 0.05$; Fig. 3n, o) decreased from 79.01%, 13.15%, and 41.83% in the vector + SiSIRT1 group to 44.69%, 4.62%, and 12.50% in the PBX1 + SiSIRT1 group ($P < 0.05$; Fig. 3j-o), respectively.

Western blot assay results showed that SIRT1 knockdown significantly upregulated the expression of p53, p21, and p16 ($P < 0.05$; Fig. 3a,h); 57-kDa AIFs, cleaved caspase 3, and Cyt C ($P < 0.05$; Fig. 3a,f); and γH2AX ($P < 0.05$; Fig. 3a,g) compared to control. Moreover, PBX1 overexpression significantly downregulated the expression levels of p53, p21, and p16 ($P < 0.05$; Fig. 3a,h) and 57-kDa AIF, cleaved caspase 3, and Cyt C ($P < 0.05$; Fig. 3a,f) compared to those by the empty vector. Further, PBX1 + SiSIRT1 downregulated the expression of p53, p21, and p16 ($P < 0.05$; Fig. 3a,h); 57-kDa AIF, cleaved caspase 3, and Cyt C ($P < 0.05$; Fig. 3a,f); and γH2AX ($P < 0.05$; Fig. 3a,g) compared to those by the vector + SiSIRT1. The percentage of ROS-positive cells was also decreased ($P < 0.05$; Fig. 3n, o). PBX1 + SiSIRT1 downregulated the expression of PARP1 and PAR ($P < 0.05$; Fig. 3a,e); upregulated the expression of SIRT1, PGC1α, and FOXO1 ($P < 0.05$; Fig. 3a, d, i); and increased the levels of intracellular total NAD, NADH, NAD, and ATP ($P < 0.05$; Fig. 3p) in comparison to vector + SiSIRT1. These data suggested that PBX1 rescues SIRT1-knockdown-mediated HF-MSCs senescence and apoptosis by alleviating ROS-mediated DNA damage and intracellular NAD depletion and that the SIRT1–PARP1 axis plays a critical role in PBX1-alleviated HF-MSC senescence and apoptosis.

PBX1 rescued PARP1 overexpression-mediated HF-MSC senescence and apoptosis, accompanied by increased SIRT1 expression and intracellular NAD and ATP levels

Our previous studies demonstrated that HF-MSCs undergo replicative senescence and apoptosis, accompanied by decreased PARP1 expression. SIRT1 knockdown significantly upregulated the expression levels of PARP1. In contrast, PBX1 overexpression downregulated the expression levels of PARP1. Considering that NAD is a major shared substrate between SIRT1 and PARP1, we generated PARP1-, PBX1-, and PARP1 + PBX1-overexpressing HF-MSCs to ascertain whether PARP1–SIRT1 axis plays a role in PBX1-mediated alleviation of cellular senescence and apoptosis (Figs. 4). Western blot results showed that both PARP1 and PBX1 overexpression increased SIRT1 levels ($P < 0.05$; Fig. 4a, d) and decreased the expression of p53, p21, and p16 ($P < 0.05$; Fig. 4a, e); 57-kDa AIF, cleaved caspase 3, and Cyt C ($P < 0.05$; Figs. 4a, g); and γH2AX, Ku 70, Ku 80, and Rad 51 ($P < 0.05$; Figs. 4a,f), compared to PARP1 vector overexpression.

Our results showed that PARP1-overexpressing group significantly decreased the expression of SIRT1 ($P < 0.05$; Fig. 4a,d) and total NAD ($P < 0.05$; Fig. 4k), NADH ($P < 0.05$; Fig. 4k), and ATP ($P < 0.05$; Fig. 4j) levels compared to that observed in the empty vector group. In contrast, PBX1-overexpressing group

increased the expression of SIRT1 ($P < 0.05$; Fig. 4a,d) and total NAD ($P < 0.05$; Fig. 4k), NADH ($P < 0.05$; Fig. 4k), and ATP ($P < 0.05$; Fig. 4j) levels compared to that in the empty vector group. Furthermore, PARP1 + PBX1 overexpression increased the expression of SIRT1 ($P < 0.05$; Fig. 4a,d) and total NAD ($P < 0.05$; Fig. 4k), NADH ($P < 0.05$; Fig. 4k), and ATP ($P < 0.05$; Fig. 4j) levels compared to vector + PARP1 overexpression. Surprisingly, compared to the empty vector a group, PBX1-overexpressing group downregulated PARP1 expression ($P < 0.05$; Fig. 4a,c) and upregulated SIRT1 expression ($P < 0.05$; Fig. 4a,d), suggesting that the PARP1–SIRT1 axis plays a role in PBX1-mediated alleviation of cellular senescence and apoptosis.

Discussion

In this study, we demonstrated that PBX1 alleviates HF-MSCs senescence and apoptosis by reducing oxidative stress-mediated DNA damage by regulating the SIRT1–PARP1 axis. In this study, subculturing HF-MSCs decreased SIRT1 expression and increased ATP and intracellular NAD depletion. This was consistent with the observation that SIRT1 expression diminishes with aging in mammals. The degradation of SIRT1, which is commonly considered an autophagy substrate by the autophagosome-lysosome, contributes to the loss of SIRT1 during cellular senescence [35]. SIRT1 is regarded as a crucial epigenetic regulator that facilitates DNA repair and genome stability preservation by depleting a large amount of NAD. The levels of SIRT1, which is a major cellular NAD consumer, can directly affect the activity of other NAD-dependent enzymes, such as PARP1. SIRT1 is widely involved in the anti-aging process in various organisms, including yeast, worms, and mammals [11]. Our previous studies demonstrated that HF-MSCs enter replicative senescence and apoptosis, which is accompanied by decreased PARP1 expression. Accumulating evidence suggests that diminished NAD levels during aging result in mitochondrial and stem cell dysfunction as well as accumulation of DNA damage by induction of the binding of DBC1 to PARP1 and SIRT1 and their consequent inhibition [42]. Therefore, various novel strategies to regulate the SIRT1–PARP1 axis either directly or indirectly may demonstrate therapeutic potential in attenuating senescence and apoptosis involved in aging and aging-related diseases.

Furthermore, SIRT1 catalyzes the deacetylation of the acetyl-lysine residues of histone proteins H1, H3, and H4. In addition, SIRT1 deacetylates non-histone substrates, including p53, Ku70, FoxOs, PGC1-a, PPAR-γ, AMPK, mTOR, MyoD, and NF-κB [21, 22], which are all related to delayed cellular senescence. SIRT1 is widely involved in the regulation of cellular senescence and organismal longevity via the acetylation and deacetylation of these substrates, and by the alteration of their transcriptional and enzymatic activities as well as protein levels [11, 23]. SIRT1 upregulation can affect the regulation of cellular senescence in aging and aging-related diseases. To further confirm whether the inhibition of SIRT1 promotes cellular senescence and apoptosis, we knocked down SIRT1 in HF-MSCs. As expected, the inhibition of SIRT1 led to intracellular NAD depletion and increased PARP1 activity accompanied by increased DNA damage aggravation, cellular senescence, and apoptosis, which was correlated with the increased expression of proteins associated with cellular senescence (p53, p16, and p21), apoptosis (57 kDa AIF, cleaved caspase 3, and Cyt C), and DNA damage (γH2AX). SIRT1 deletion in hematopoietic stem cells (HSCs) induces characteristic changes normally associated with HSC senescence, such as genomic

instability and increased sensitivity to DNA damage [43]. The data also showed that inhibition of SIRT1 decrease FOXO1 and PGC1α expression, which implies that SIRT1 knockdown could influence mitochondrial biogenesis and respiratory function.

The pivotal function of SIRT1 is displayed through specific interactions with p53, namely p53 deacetylation at the C-terminal lysine-382 residue in a NAD-dependent manner [44]. This interaction reduces p53-mediated transcriptional and translational levels and the expression of its downstream proteins, including the cyclin-dependent kinase inhibitors p21 and p16, which are known to participate in the regulation of cell cycle arrest, cellular senescence, and apoptosis [44]. Therefore, SIRT1 can inhibit p53-dependent cell cycle arrest, cellular senescence, and apoptosis while also activating the genes involved in DNA damage repair to promote cell survival and cell proliferation. This implies that regulation of the SIRT1–p53 axis can regulate stem cell fate, which influences tissue homeostasis, regeneration, and repair. In our study, SIRT1 knockdown decreased PBX1 expression. SIRT1 deficiency reduces PBX1 binding to the apoptotic cell response element of the IL-10 promoter [45]. Conversely, the activation of SIRT1 ultimately activates PBX1 [45]. PBX1 is a key transcription factor that participates in the regulation of stem cell fate by cooperating with Oct 4, Nanog, and Sox2 [46, 47] and participates in stem cell proliferation, apoptosis, senescence, and differentiation to maintain them in a pluripotent and undifferentiated state [26–28]. Our recent studies showed that PBX1 enhances HF-MSCs proliferation and their reprogramming into induced pluripotent stem cells, and attenuates their senescence [28]. Here, we found that increased expression of PBX1 attenuates HF-MSC senescence and apoptosis, suggesting that PBX1 may be involved in HF-MSC senescence, which is consistent with the findings of our previous study [38].

To further confirm whether PBX1 could attenuate HF-MSC senescence and apoptosis by upregulating SIRT1, and whether PBX1 could rescue SIRT1 knockdown-mediated HF-MSC senescence and apoptosis, we overexpressed PBX1 and knocked down SIRT1 in HF-MSCs. The results showed that the increased expression of PBX1 rescued HF-MSC senescence and apoptosis caused by SIRT1 knockdown. Increased expression of PBX1 enhanced the luciferase activity of the SIRT1 promoter in a dual-luciferase reporter gene assay, which implies that PBX1 promotes transcription of SIRT1 by binding to the SIRTI promoter. Furthermore, increased PBX1 expression attenuated ROS accumulation, DNA damage aggravation, and intracellular NAD depletion with or without SIRT1 knockdown. PBX1 thus attenuates intrinsic ROS-mediated HF-MSCs senescence and apoptosis by regulating the NAD-SIRT1-PARP1 axis.

Both SIRT1 and PARP1 utilize NAD for their activities. PARP1 uses poly(ADP-ribose)s (PARs) converted from NAD, and SIRT1 uses NAD as its cofactor [48]. Considering that SIRT1 consumes the highest levels of cellular NAD, the level of SIRT1 can directly affect the level and activation of other NAD-dependent enzymes, such as PARP1, which plays a crucial role in protection against oxidative injury. PARP1, which accounts for approximately 90% of the PARP enzyme family protein activity [49], catalyzes the transfer of ADP-ribose onto target proteins using NAD [50, 51], and plays a key role in PAR-mediated cellular physiological reactions [52]. However, PARP1 has dual effects, depending on the cellular environment. PARP1 is typically regarded as a DNA damage repair enzyme that exerts a protective effect by recruiting

repair-related proteins to sites with minor DNA damage to facilitate DNA damage repair [51]. In contrast, PARP1 overactivation due to depletion of large amounts of NAD and ATP leads to an energy crisis and parthanatos, a regulated type of necrosis that occurs in response to extensive DNA damage [53]. Upon PARP1 activation, poly(ADP-ribosyl)ation dissociates histone H1 from the FoxO3a target gene promoter and facilitates FoxO3a nuclear accumulation and binding to its target promoters, resulting in improved expression of autophagy-related genes. Autophagy activated by PARP1 impairs mitochondrial metabolism and accelerates cellular senescence and death [54]. Our previous study also showed that overexpression of PARP1 significantly aggravated HF-MSC senescence and apoptosis and decreased the expression of SIRT1, suggesting that regulating the SIRT1-PARP1 axis would be advantageous for attenuating senescence and apoptosis involved in aging and aging-related diseases. To explore whether PBX1 participates in attenuation of cellular senescence and apoptosis in HF-MSCs, and to elucidate the underlying mechanism, we generated HF-MSCs overexpressing PBX1, PARP1, or both. As expected, increased PBX1 expression significantly reduced DNA damage aggravation, intracellular NAD depletion, cellular senescence, and apoptosis, and increased SIRT1 expression in HF-MSCs, suggesting that PBX1 participates in the attenuation of cellular senescence and apoptosis in HF-MSCs, possibly by interfering with the SIRT1-PARP1 axis. In contrast, PARP1 overexpression increased cellular senescence and apoptosis in HF-MSCs, which correlated with increased expression of proteins related to cellular senescence (p53, p16, and p21), apoptosis (57 kDa AIF, cleaved caspase 3, and Cyt C), and DNA damage (γH2AX). Interestingly, compared to PARP1 + vector overexpression, both PBX1 and PARP1 overexpression reduced DNA damage accumulation, cellular senescence, and apoptosis in HF-MSCs, which was consistent with the western blotting results. The data showed that PBX1 overexpression downregulated PARP1 expression and upregulated SIRT1 expression, suggesting that SIRT1 inhibits PARP1 activity. This implies that the SIRT1-PARP1 axis plays a role in cellular senescence and apoptosis.

These data demonstrate that the NAD-SIRT1-PARP1 axis plays a critical role in PBX1-alleviated HF-MSC senescence and apoptosis. PBX1 alleviates HF-MSCs senescence and apoptosis by reducing oxidative stress-mediated DNA damage via the SIRT1-PARP1 axis. This provides a new perspective on the mechanism of stem cell senescence and lays the foundation for age-related disease prevention and treatment.

However, SIRT1 mediates positive regulatory influences on cell survival, whereas PARP1 kills or protects cells, depending on the severity of the insult. Therefore, manipulation of the SIRT1-PARP1 axis seems to be a plausible strategy for the treatment of aging and age-related diseases. However, PARP1 inhibitory drugs in the market, which target the catalytic activity of PARP1, possibly affect diverse biochemical functions of PARP1 [55]. In contrast, drugs selectively affecting the SIRT1-PARP1 interaction would not exert unnecessary side effects on desirable functions mediated by PARP1. Therefore, the development of a specific regulator targeting the SIRT1–PARP1 axis would be advantageous for the treatment of aging and aging-related diseases.

Conclusion

In this study, we demonstrated that PBX1 attenuates intrinsic ROS-mediated HF-MSC senescence and apoptosis by regulating the NAD–SIRT1–PARP1 axis(Figure 5). This study provides support for the development of a specific regulator targeting the SIRT1–PARP1 axis, which would be advantageous for the prevention and treatment of aging and aging-related diseases, especially for hair follicle repair and regeneration.

Abbreviations

DDR: DNA damage response, HDACs: Histone deacetylases, HATs: Histone acetylases, HFs: Hair follicles, HF-MSC: Hair follicle-derived mesenchymal stem cell, NNM: Nicotinamide mononucleotide, NR: Nicotinamide riboside, HSCs: Hematopoietic stem cells, P3: Third-generation passage cells, P7: Seventh-generation passage cells, PARs: Poly(ADP-ribose)s, PBX1: Pre-B-cell leukemia transcription factor1, ROS: Reactive oxyden species

Declarations

Acknowledgments

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Author Contributions

Yuan Wang contributed to the study. Jinyu Liu is the corresponding author. This study was designed by Liu et al. Yuan Wang carried out most of the experiments, Xiaomei Liu and Jiahong Shi instructed us to finish the paper, Yutong Sui and Ye Niu performed the statistical analysis, and Feilin Liu drafted the manuscript. Liu, Xu, Zuo, Liu, Liu, Zou, Sun, Wang and Liu conducted experiments. All the authors have read and approved the final manuscript.

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Ethics approval statement

All experiments were approved by the Jilin University Public Health Department, Jilin University Ethics Committee (2022-02-22).

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Applicable. All experiments were approved by the Jilin University Public Health Department, Jilin University Ethics Committee.

Consent for publication

Not applicable.

Competing interests

There are no conflicts of interest. The authors declare that they have no conflicts of interest.

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Figures

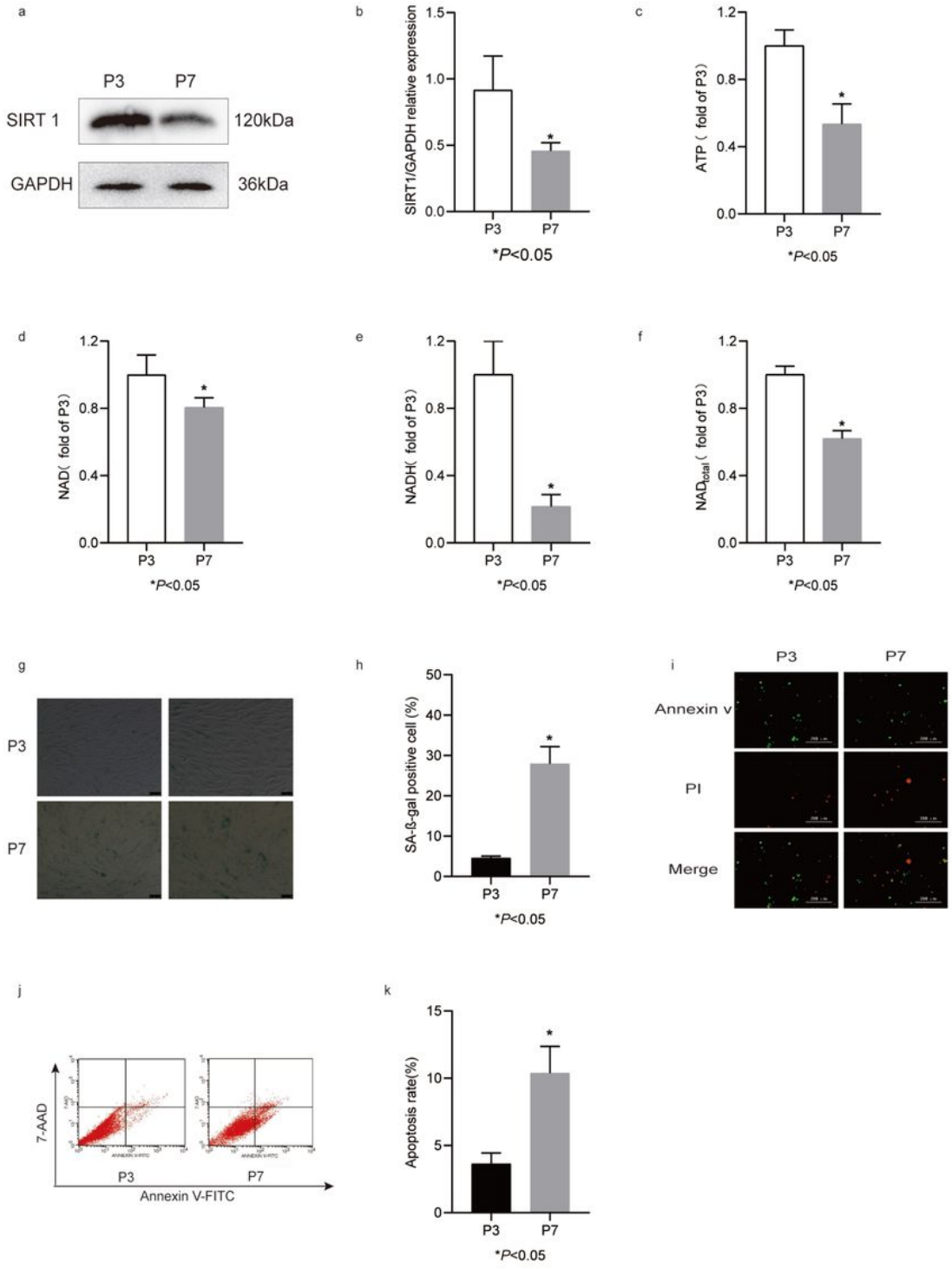

Figure 1

SIRT1 expression decreases with subculture. (a, b) Western blotting results showing the protein expression levels of SIRT1 in HF-MSCs at P3 and P7. (c) The ATP levels of HF-MSCs at P3 and P7. (d, e, f) The levels of NAD, NADH, total NAD in HF-MSCs at P3 and P7. (g, h) SA-β-gal staining results of HF-MSCs at P3 and P7 (Scale bar = 200 μm). (i) Annexin V/PI staining results of HF-MSCs at P3 and P7 (scale bar, 200 μm). (j, k) Flow cytometry results of HF-MSC apoptosis at P3 and P7.

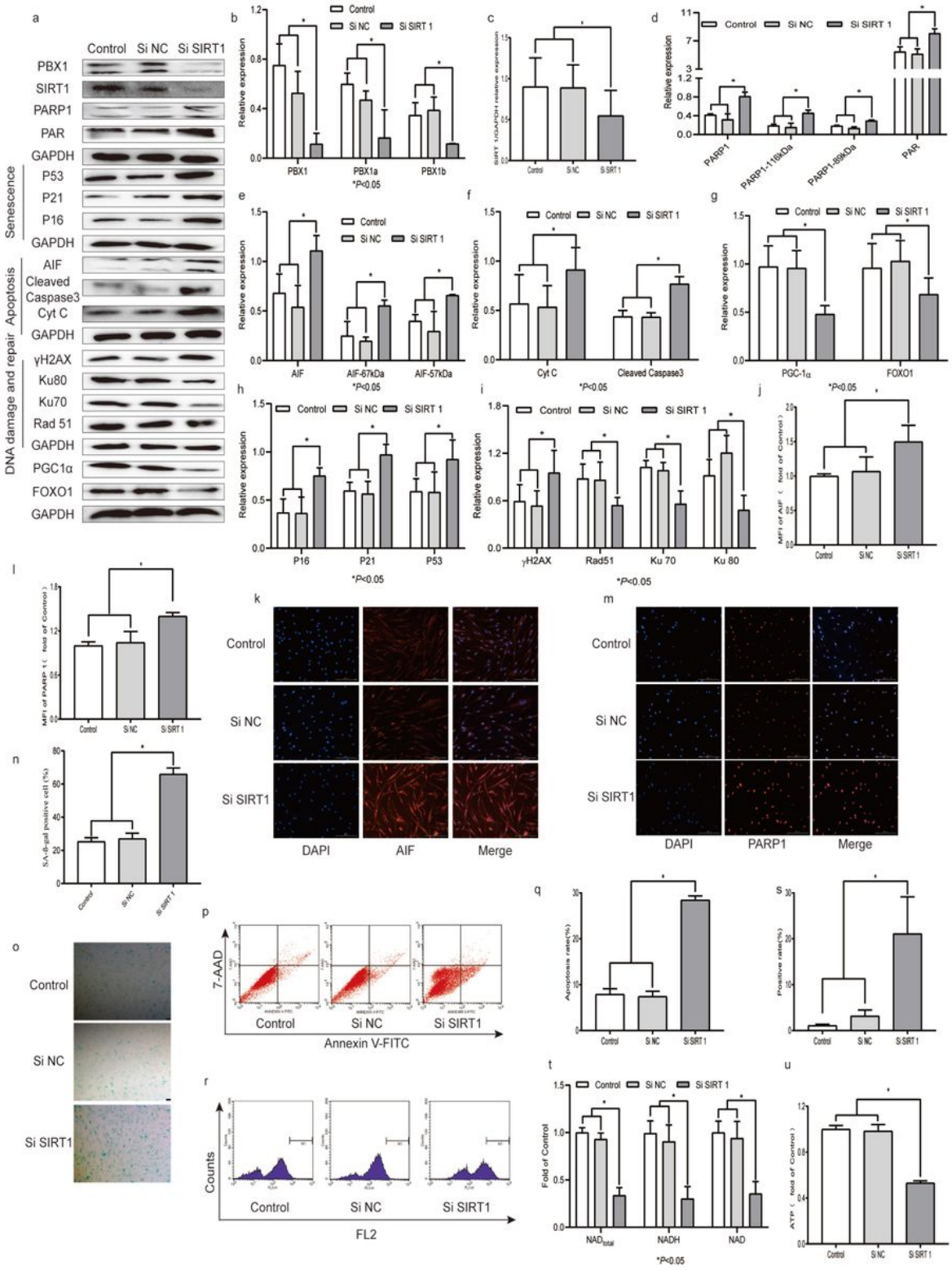

Figure 2

SIRT1 knockdown enhances cellular senescence and apoptosis, which is accompanied by increased DNA damage aggravation and NAD and ATP depletion. (a, b, c, d, e, f, g, h, i) Western blotting analysis of the protein expression levels of PBX1, P53, P21, P16, AIF, cleaved caspase 3, Cyt C, Ku70, Ku80, Rad 51, γH2AX, SIRT1, PAR, PARP1, PGC1α and FOXO1after SIRT1 knockdown in HF-MSCs. (j, k, l, m) Immunofluorescence analysis of AIF1 and PARP1 expression and localization following SIRT1

knockdown in HF-MSCs. (Scale bar = 200 μm). (n, o) HF-MSC SA-β-gal staining results after SIRT1 knockdown (Scale bar = 200 μm). (p, q) Flow cytometry results of HF-MSC apoptosis after SIRT1 knockdown. (r, s) Flow cytometry results of the ROS levels in HF-MSCs after SIRT1 knockdown. (t, u) Levels of ATP, NAD, NADH, and total NAD in HF-MSCs after SIRT1 knockdown.

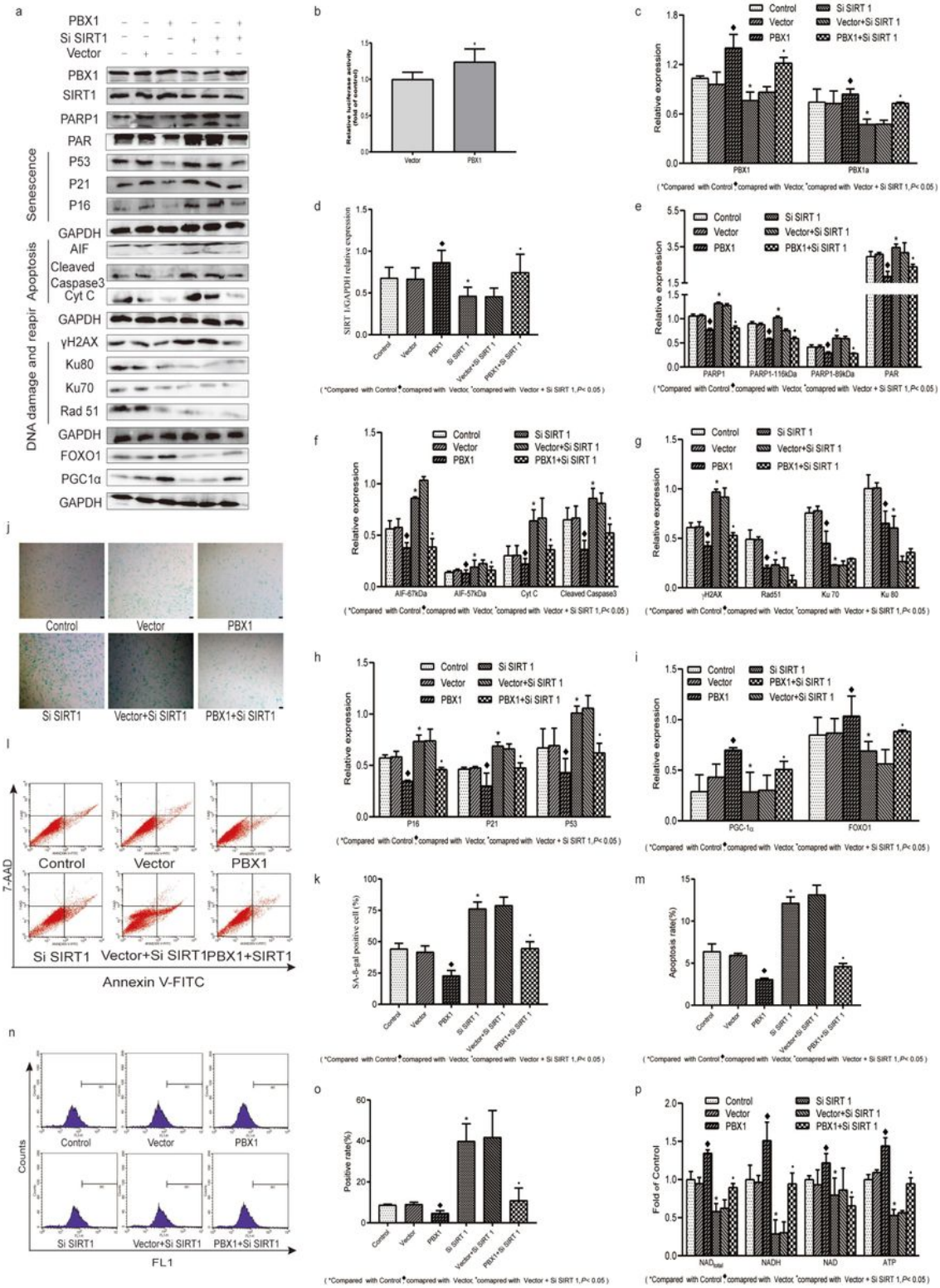

Figure 3

PBX1 rescues SIRT1 knockdown-mediated HF-MSC senescence and apoptosis by alleviating ROS-mediated DNA damage and intracellular NAD depletion. (a, c, d, e, f, g, h,i) Western blotting analysis of the protein expression levels of PBX1, P53, P21, P16, AIF, cleaved caspase 3, Cyt C, Ku70, Ku80, Rad 51, γH2AX, SIRT1, PAR, PARP1, PGC1α and FOXO1after PBX1 overexpression in the SIRT1 knockdown group. (b) Dual-luciferase reporter gene results of the luciferase activity of the SIRT1 promoter after PBX1 overexpression. (j, k) HF-MSC SA-β-gal staining results after PBX1 overexpression in the SIRT1 knockdown group (Scale bar = 200 μm). (l, m) Flow cytometry results of HF-MSC apoptosis after PBX1 overexpression in the SIRT1 knockdown group. (n, o) Flow cytometry results of ROS levels in HF-MSCs after PBX1 overexpression in the SIRT1 knockdown group. (p) The level of ATP, NAD, NADH, and total NAD in HF-MSCs after PBX1 overexpression in the SIRT1 knockdown group.

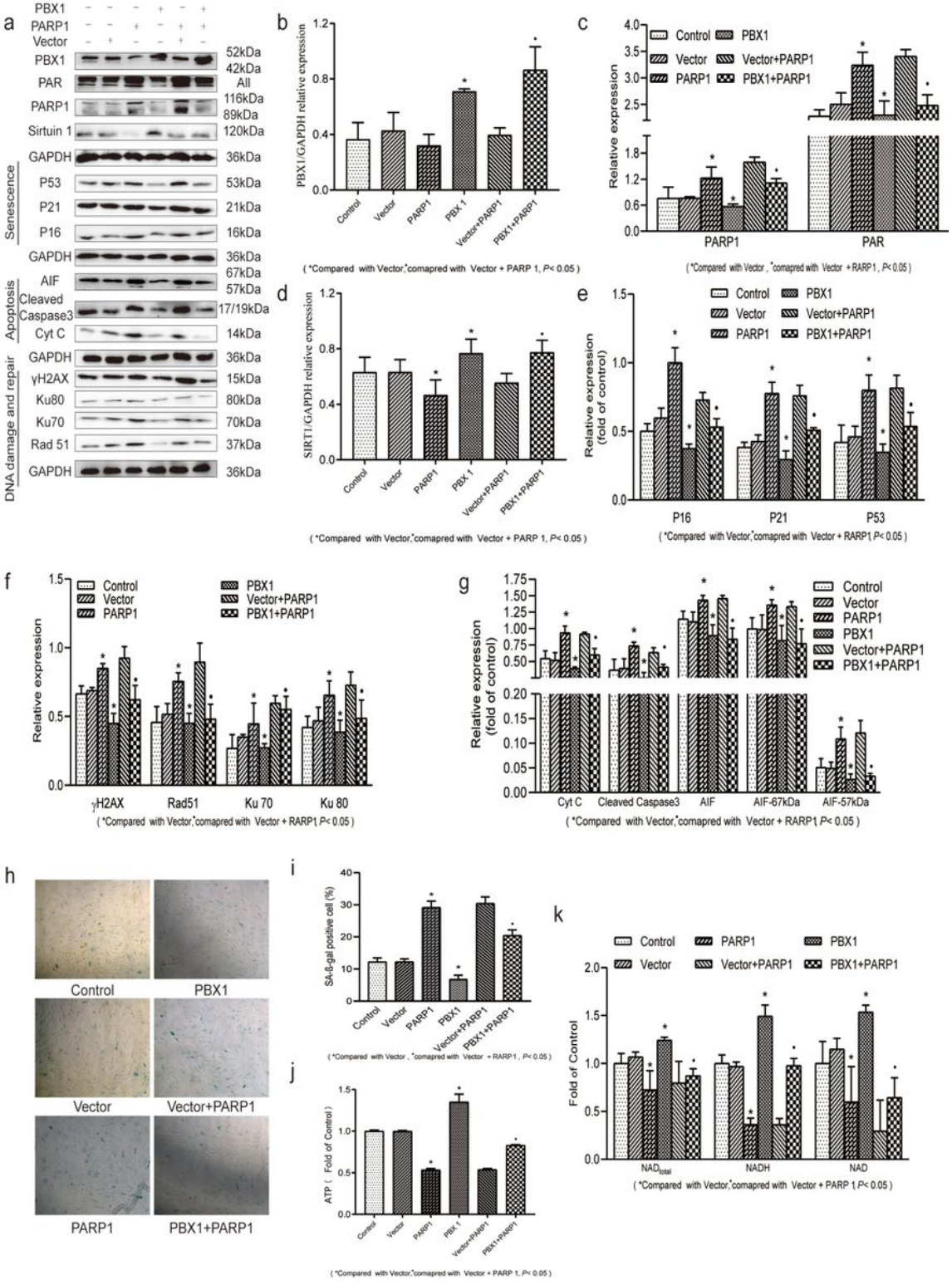

Figure 4

PBX1 rescues PARP1 overexpression-mediated HF-MSCs senescence and apoptosis, accompanied by increased SIRT1 expression and intracellular NAD and ATP levels. (a, b, c, d, e, f, g) Western blotting analysis of the protein expression levels of PBX1, P53, P21, P16, AIF, cleaved caspase 3, Cyt C, Ku70, Ku80, Rad 51, γH2AX, SIRT1, PAR, and PARP1 after PBX1 overexpression in PARP1-overexpressing cells. (h, i) The SA-β-gal staining results after PBX1 overexpression in PARP1-overexpressing HF-MSC cells

(Scale bar = 200 μm). (j, k) Levels of ATP, NAD, NADH, total NAD in HF-MSCs after PBX1 overexpression in PARP1-overexpressing cells.

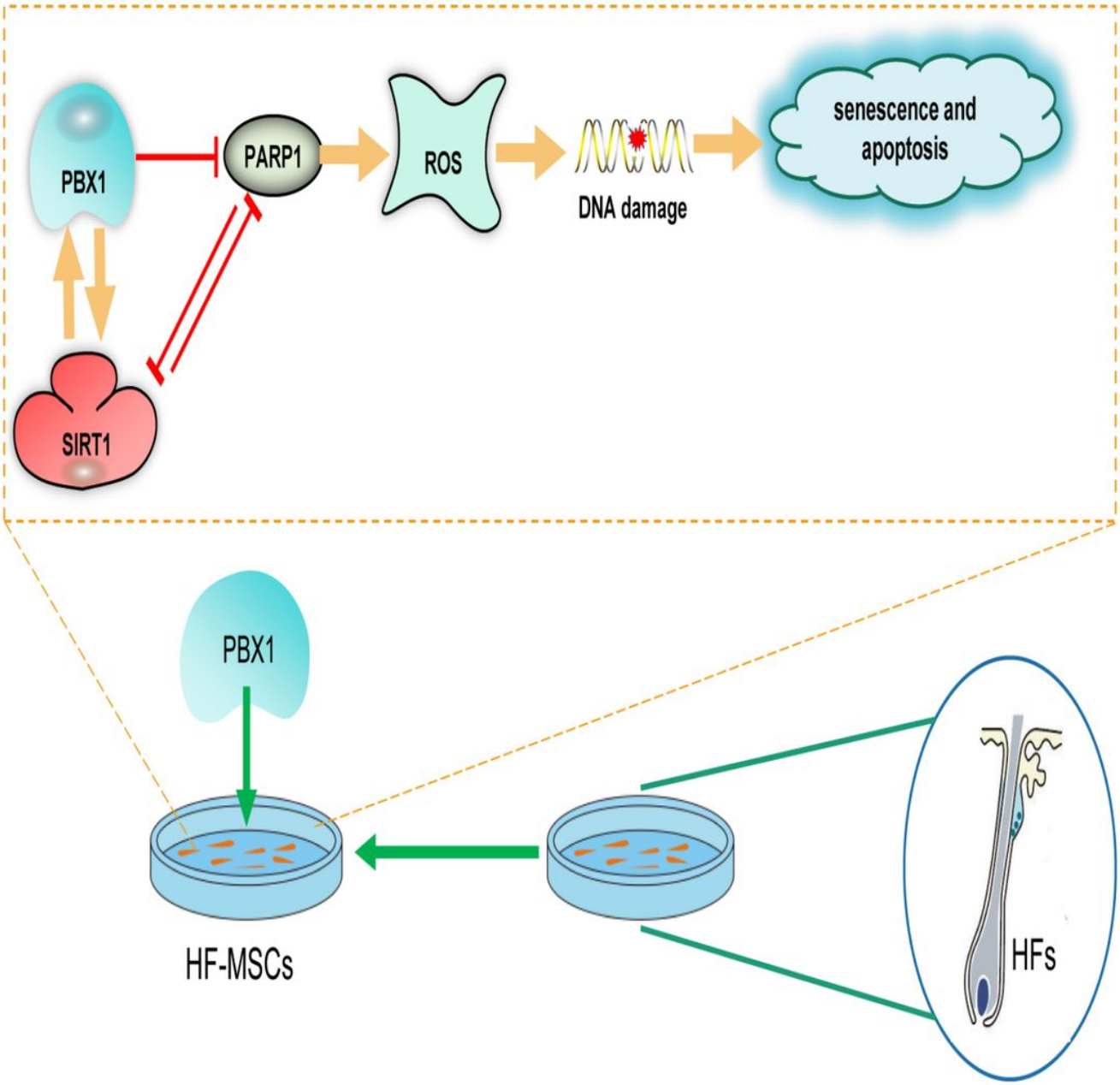

Figure 5

PBX1 alleviates HF-MSCs senescence and apoptosis by reducing oxidative stress-mediated DNA damage via interaction with the PARP1-SIRT1 axis.