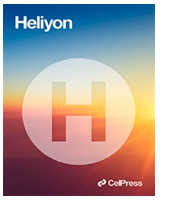
Heliyon 10 (2024) e32108

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Nesfatin-1 attenuated lipopolysaccharide-induced inflammatory response and senescence in human dental pulp cells

Lili Zhang [a,*], Bo Pang [b], Rong Wang [a], Bin Yang [a], Xubei Jia [a]

[a] Department of Stomatology, Shijingshan Teaching Hospital of Capital Medical University, Beijing Shijingshan Hospital, Beijing, 100043, China
[b] Department of Big Data, Computer Network Information Center, Chinese Academy of Sciences, Beijing, 100190, China

ARTICLE INFO

Keywords:
Gingivitis
LPS
SIRT1
SA-β-gal
Human dental pulp cells

ABSTRACT

Lipopolysaccharide (LPS)-triggered damage in human dental pulp cells (hDPCs) is associated with the progression of gingivitis, which is inflammation of the gingival tissue. Nesfatin-1 is a peptide secreted by neurons and peripheral tissues. Here, we report a novel property of Nesfatin-1 in ameliorating LPS-induced inflammatory response and senescence in hDPCs. First, we demonstrate that Nesfatin-1 repressed LPS-triggered expression of inflammatory factors. Secondly, Nesfatin-1 restored telomerase activity and the expression of human telomerase reverse transcriptase (hTERT) and telomeric repeat binding factor 2 (TERF2) against LPS. Senescence-associated β-galactosidase (SA-β-gal) staining assay revealed that Nesfatin-1 attenuated LPS-induced cellular senescence in hDPCs. We also found that Nesfatin-1 increased telomerase activity in LPS-challenged hDPCs. It is also shown that Nesfatin-1 reduced the expression of plasminogen activator inhibitor-1 (PAI-1) and p16. Additionally, LPS stimulation reduced the expression of SIRT1, which was rescued by Nesfatin-1. However, the silencing of sirtuin1 (SIRT1) abrogated the protective property of Nesfatin-1 in preventing cellular senescence, implying that the function of Nesfatin-1 is regulated by SIRT1. Taken together, our findings suggest that Nesfatin-1 might possess a protective effect against gingivitis.

1. Introduction

Gingivitis is the most common complication in orthodontic treatment, resulting in gingival swelling, hyperplasia and bleeding, thereby seriously affecting the quality of life of orthodontic patients [1–3]. Toll-like receptors (TLRs) are pattern recognition receptors that recognize pathogenic-associated molecular patterns (PAMPs) as part of innate immunity. These receptors specifically recognize LPS, which leads to the induction of inflammation. It is widely reported that LPS can be used as an important inducer to stimulate the inflammatory response of dental pulp cells *in vitro*, which is useful for screening drugs for the early treatment of gingivitis. Kim [4] introduced glutamate to LPS-stimulated human dental pulp cells (hDPCs) and found that glutamate inhibited the production of inflammatory factors and exhibited an anti-inflammatory property. Steroids and non-steroidal anti-inflammatory drugs (NSAIDS), two commonly used anti-inflammatory agents, are reported to reduce the release of inflammatory factors in LPS-triggered gingivitis [5,6]. Similar to other tissues, senescence-related changes are induced in the gingival tissue under certain conditions, such as reduced volume, decreased numbers of blood vessels and nerves, etc. [7]. Feng et al. [8] found that repeated stimulation with continuous

* Corresponding author. Department of stomatology, Shijingshan Teaching Hospital of Capital Medical University, Beijing Shijingshan Hospital, No. 24 Shijingshan Road, Shijingshan District, Beijing, 100043, China.
E-mail address: dr_lilizhang96522@126.com (L. Zhang).

https://doi.org/10.1016/j.heliyon.2024.e32108
Received 10 February 2023; Received in revised form 19 May 2024; Accepted 28 May 2024
Available online 31 May 2024

Abbreviations

Lipopolysaccharide (LPS)
human dental pulp cells (hDPCs)
human telomerase reverse transcriptase (hTERT)
telomeric repeat binding factor 2 (TERF2)
Senescence-associated β-galactosidase (SA-β-gal)
plasminogen activator inhibitor-1 (PAI-1)
sirtuin1 (SIRT1)
toll-like receptors (TLRs)
pathogenic-associated molecular patterns (PAMPs)
non-steroidal anti-inflammatory drugs (NSAIDS)
reactive oxygen species (ROS)
toll-like receptor 4 (TLR4)
ventral tegmental area (VTA)
phosphate buffer solution (PBS)
Eppendorf (Ep)
fetal bovine serum (FBS)
polymerase chain reaction (PCR)
Enzyme-linked immunosorbent assay (ELISA)
interleukin-1β (IL-1β)
tumour necrosis factor-α (TNF-α)
interleukin-6 (IL-6)
bicinchoninic acid (BCA)
3,3′,5,5′-tetramethyl-[1,1′-Biphenyl]-4,4′-diamine (TMB)
sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)
polyvinylidene fluoride (PVDF)
standard deviation (S.D.)
analysis of variance (ANOVA)
Student Newman Keuls (S–N–K)

low-concentration LPS results in the generation of reactive oxygen species (ROS) and elevated expression of deoxyribonucleic acid (DNA) double-strand break marker (γ-H2AX) in hDPCs. This is accompanied by upregulation of p16 mediated by the activation of toll-like receptor 4 (TLR4) signaling, which ultimately contributes to cellular senescence. Additionally, LPS has been reported to possess a senescence-inducing effect on various cell types including human dental pulp stem cells by regulating senescence-associated β-galactosidase (SA-β-gal) activity and the expression of SIRT1 and p16 [9]. Additionally, exposure to LPS may reduce the activity of telomerase [10]. Therefore, LPS-associated cellular senescence is an important target for the treatment of gingivitis.

Nesfatin-1 was discovered as a secreted protein expressed in the hypothalamic nuclei that controls appetite in rats [11]. It is expressed in the gastric gland, duodenum, islet, cortex, limbic system, thalamus and hypothalamus in peripheral tissues [12]. Nesfatin-1 is reported to inhibit food intake, reduce weight gain and regulate blood glucose in rats, which directly acts on dopamine neurons in the ventral tegmental area (VTA). The food intake of mice is reduced when Nesfatin-1 is injected into the VTA [13]. A recent study revealed that Nesfatin-1 showed a promising inhibitory property on LPS-induced inflammation [14]. Interestingly, Nesfatin-1 has been reported to exert a protective effect on tissues by increasing the expression of SIRT1 [15]. However, it is unknown whether Nesfatin-1 might be able to protect against LPS-induced injury in hDPCs. The present study examines the potential regulatory effect of Nesfatin-1 on inflammation and cellular senescence in LPS-treated hDPCs and aims to explore multiple approaches for the clinical treatment of gingivitis.

2. Materials and methods

2.1. Cell isolation, treatment and transduction

Informed consent was obtained from all individual donors involved in the study. All experiments were carried out according to the guidelines of the Declaration of Helsinki. Healthy third molars were collected from patients aged 18–25 years and were immediately placed in a cooling phosphate buffer solution (PBS). The teeth were rinsed repeatedly with PBS, and gingival tissues were gently removed and transferred to a 1.5 mL Eppendorf (Ep) tube containing 5 % penicillin-streptomycin (Cat#U31–301C, Yobibio, Shanghai, China). The teeth were then cut into 0.2 to 0.5 mm-diameter sections with ophthalmic scissors and cleaned by forced air. An appropriate amount of 3 % type I collagenase (Cat#C8140, Solarbio, Beijing, China) was added, and the samples were placed in a culture box at 37 °C for digestion for 30–40 min until the tissue was flocculent. Digestion was terminated by adding an equal volume of complete medium (Cat#SNM-004E, Sunncell, Wuhan, China) including 10 % fetal bovine serum (FBS) and 1 % penicillin-

streptomycin. The supernatant was discarded following centrifugation, and the precipitation was resuspended with α-Minimum Essential Medium (α-MEM, Cat#YC-3007, Yuchun Biology, China) supplemented with 20 % FBS (Cat#S9020, Solarbio, Beijing, China) and 2 % penicillin-streptomycin (Cat#P1400, Solarbio, Beijing, China), which was evenly inoculated in a 6 cm Petri dish and cultured at 37 °C in a 5 % CO_2 incubator. After the cells were obtained from whole dental pulp on days 3–4, the medium was changed every other day. On days 7–8, when confluence reached 70%–80 %, trypsin (Cst#T1300, Solarbio, Beijing, China) was utilized for digestion, and the isolated hDPCs were obtained. To achieve SIRT1-knockdown in hDPCs, cells from passages 2–3 were transduced with a designed lentiviral shRNA targeting SIRT1 (lentiviral SIRT1 shRNA) for 48 h. Knockdown efficacy was analyzed by Western blot. For the experiment, cells at passages 3–4 were stimulated with 1 μg/mL LPS (CS0158, G-clone, Beijing, China) for 24 h, after which Nesfatin-1(Cat#SRP6226, Singma-Aldrich, USA) (30, 60 nM) was added for 6 h.

2.2. Real-time polymerase chain reaction (PCR)

RNA was extracted from hDPCs using TRIzol reagent following different treatments, then transcribed to cDNA using a 1st-Strand cDNA Synthesis Kit (LMAI Bio, Shanghai, China). SYBR Green Real-time PCR Master Mix (Roche Diagnostics, Basel, Switzerland) was used while conducting the PCR reaction. The expression of IL-6, IL-1β, TNF-α, hTERT, TERF2, PAI-1, p16, and SIRT1 genes was normalized to the housekeeping gene GAPDH. The $2^{-\Delta\Delta Ct}$ method was used to calculate the gene expression levels.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The release of interleukin-1β (IL-1β) (MEIMIAN, Jiangsu, China), tumour necrosis factor-α (TNF-α) (Elabscience, Wuhan, China), and interleukin-6 (IL-6) (Elabscience, Wuhan, China) in hDPCs was determined by ELISA. The supernatant was obtained following centrifugation and then seeded in a 96-well plate. After incubation, the solution was discarded and conjugate reagents were introduced into the wells, followed by further incubation at 37 °C for 60 min. Subsequently, 3,3′,5,5′-tetramethyl-[1,1′-Biphenyl]-4,4′-diamine (TMB) was added to the wells and incubated for 15 min, after which the stop solution was introduced. Lastly, a microplate reader (Molecular Devices, Shenzhen, China) was utilized to obtain the OD value at 450 nm.

2.4. SA-β-gal staining

hDPCs were seeded in 6-well plates at a density of 800 cells/well and stimulated with LPS with or without Nesfatin-1 (30, 60 nM). After media removal, the fixative solution (1 ×) was added to each well and incubated for 15 min at room temperature. Cells were then stained with the β-Galactosidase staining solution (Cat#CBA-231, Cell Biolabs, USA) overnight at 37 °C. The senescent cells were observed using a light microscope (WUMO, Shanghai, China).

2.5. Telomerase activity

hDPCs were lysed using CHAPS buffer, followed by centrifugation at 15000×*g* for 30 min, the concentration of which was determined using a bicinchoninic acid (BCA) kit. The activity of telomerase in hDPCs was then detected using the TeloTAGGG Telomerase PCR ELISA Plus Kit (Roche, Basel, Switzerland), followed by introduction of the target RNA arbitrarily-primed polymerase chain reaction (TRAP-PCR) system along with samples and primers. Finally, quantification was performed using a reverse-transcription PCR (RT-PCR) assay.

2.6. Western blot analysis

Cells were isolated with lysis buffer and were further quantified using the BCA method (pc0020, Solarbio, Beijing, China). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins at 12 % concentration, followed by transferance of the proteins to a polyvinylidene fluoride (PVDF) membrane (10600023, GE Healthcare Life, Pittsburgh, USA). After blocking, primary antibodies for PAI-1 (Cat#27535, 1:1000, CST, USA), p16 (Cat#80772, 1:1000, CST, USA), SIRT1 (Cat#2028, 1:1000, CST, USA) and GAPDH (Cat#5174, 1:1000, CST, USA) were introduced and incubated at 4 °C for 12 h, followed by introduction of secondary antibody (Cat #7074, 1:2000, CST, USA) for 90 min. Lastly, the levels of proteins were quantified by analyzing the bands with Image J software.

2.7. Statistical analysis

Data are expressed as means ± standard deviation (S.D.), and the normality of data was assessed using the software SPSS. Results were analyzed using the analysis of variance (ANOVA) method, followed by the post-hoc Student Newman Keuls (S–N–K) test. Statistical significance was determined by the value of P being lower than 0.05.

3. Results

3.1. Nesfatin-1 inhibited LPS-triggered secretion of cytokines in hDPCs

The mRNA levels of IL-6, IL-1β and TNF-α (Fig. 1A–C) were upregulated in LPS-incubated hDPCs but were repressed by 3.2–1.2 times in response to 30 and 60 nM Nesfatin-1, respectively. Furthermore, the release of IL-6 (Fig. 1D) was raised from 123.5 ± 14.6 ng/mL to 354.1 ± 39.3 ng/mL by LPS in hDPCs, which was reduced to 261.7 ± 23.8 and 135.9 ± 15.2 ng/mL by 30 and 60 nM Nesfatin-1, respectively. The generation of IL-1β (Fig. 1E) in the control group and the LPS, 30 nM Nesfatin-1, and 60 nM Nesfatin-1 groups was 166.5 ± 19.3, 521.6 ± 66.3, 335.8 ± 43.2 and 221.5 ± 32.6 ng/mL, respectively. Moreover, the generation of TNF-α (Fig. 1F) was elevated from 141.3 ± 32.6 ng/mL to 434.2 ± 32.6 ng/mL in LPS-incubated hDPCs, which was decreased to 282.5 ± 31.3 and 181.3 ± 21.5 ng/mL by 30 and 60 nM Nesfatin-1, respectively. These data suggest that the LPS-triggered release of cytokines was dramatically repressed by Nesfatin-1.

3.2. Nesfatin-1 restored telomerase activity against LPS in hDPCs

Cells were stimulated with LPS (1 μg/mL), followed by the addition of Nesfatin-1 (30, 60 nM) for 7 days. A telomerase activity assay was then conducted. We found that telomerase activity (Fig. 2) in LPS-incubated hDPCs declined from 23.6 ± 2.56 IU/L to 13.6 ± 2.56 IU/L, but was elevated to 17.2 ± 2.56 IU/L and 20.5 ± 2.33 IU/L by 30 and 60 nM Nesfatin-1, respectively, suggesting that telomerase activity in LPS-incubated hDPCs was rescued by Nesfatin-1.

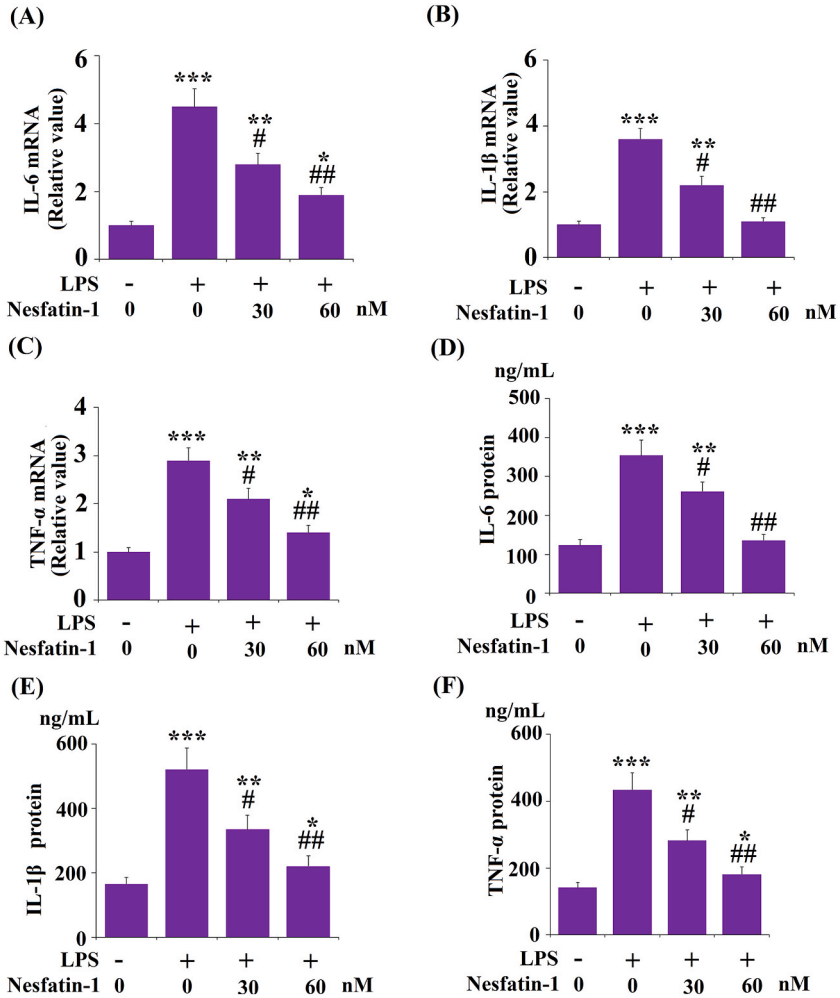


Fig. 1. Nesfatin-1 inhibited LPS-induced expression and secretion of IL-6, IL-1β and TNF-α in human dental pulp cells (hDPCs). Cells were stimulated with LPS with or without Nesfatin-1 (30, 60 nM) for 6 h. (A–C). mRNA of IL-6, IL-1β and TNF-α; (D–F). Secretions of IL-6, IL-1β and TNF-α (*, **, ***, $P < 0.05, 0.01, 0.001$ vs. vehicle group; #, ##, $P < 0.05, 0.01$ vs. LPS group).

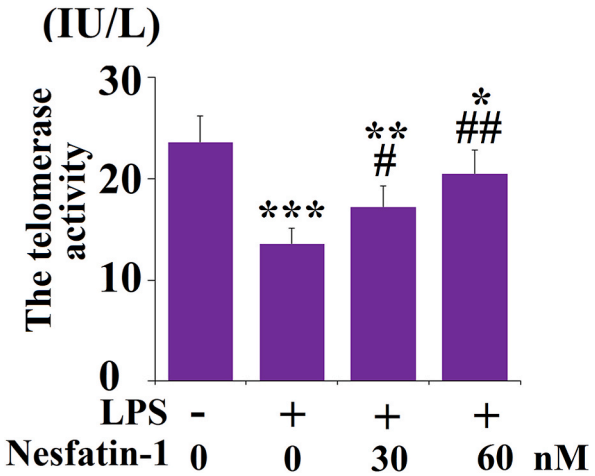


Fig. 2. Nesfatin-1 restored the telomerase activity against LPS in human dental pulp cells (hDPCs). Cells were stimulated with LPS with or without Nesfatin-1 (30, 60 nM) for 7 days. The telomerase activity was measured (*,**, ***, P < 0.05, 0.01, 0.001 vs. vehicle group; #, ##, P < 0.05, 0.01 vs. LPS group).

3.3. Effects of Nesfatin-1 on the gene expression of human telomerase reverse transcriptase (hTERT) and telomere repeat binding factor 2 (TERF2)

hTERT and TERF2 are two important biomarkers representing the activity of telomerase [16]. Dramatic downregulation of hTERT and upregulation of TERF2 were observed in LPS-incubated hDPCs, but hTERT expression was reversed from 0.53 ± 0.04 to 0.78 ± 0.09 and 0.93 ± 0.12 by 30 and 60 nM Nesfatin-1 (Fig. 3A), respectively, and TERF2 expression was reversed from 3.63 ± 0.43 to 2.21 ± 0.26 and 1.52 ± 0.17 by 30 and 60 nM Nesfatin-1 (Fig. 3B), respectively, suggesting that Nesfatin-1 might rescue telomerase activity in LPS-incubated hDPCs by regulating the expression levels of hTERT and TERF2.

3.4. Nesfatin-1 attenuated LPS-induced cellular senescence against LPS in hDPCs

The status of cellular senescence was evaluated using senescence-associated β-galactosidase (SA-β-gal) staining. As shown in Fig. 4A and B, the percentage of SA-β-Gal staining-positive cells was increased 3.4 times in LPS-incubated hDPCs, which was dramatically decreased by the introduction of 30 and 60 nM Nesfatin-1, indicating a promising inhibitory property of Nesfatin-1 against LPS-induced cellular senescence in hDPCs.

3.5. Nesfatin-1 reduced the expression of PAI-1 and p16 in LPS-challenged hDPCs

We found that the expression levels of PAI-1 and p16, the biomarkers of cellular senescence, were elevated by 3.7 and 3.2 times, respectively, at the mRNA level, and by 2.8 and 2.7 times at the protein level in LPS-challenged hDPCs. PAI-1 mRNA expression was inhibited from 3.7 ± 0.41 to 2.5 ± 0.22 and 1.7 ± 0.15 by 30 and 60 nM Nesfatin-1, respectively, while p16 mRNA expression was inhibited from 3.2 ± 0.33 to 2.5 ± 0.22 and 1.7 ± 0.15 by 30 and 60 nM Nesfatin-1, respectively (Fig. 5A). Likewise, the PAI-1 protein

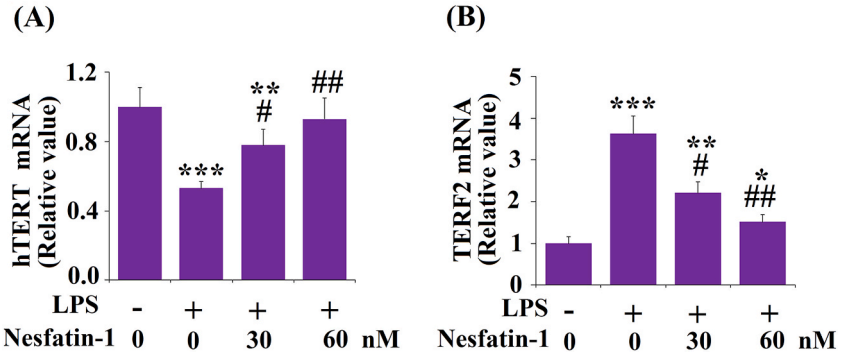


Fig. 3. The effects of Nesfatin-1 on the gene expression of hTERT and TERF2. (A). Gene levels of hTERT; (B). Gene levels of TERF2 (*,**, ***, P < 0.05, 0.01, 0.001 vs. vehicle group; #, ##, P < 0.05, 0.01 vs. LPS group).

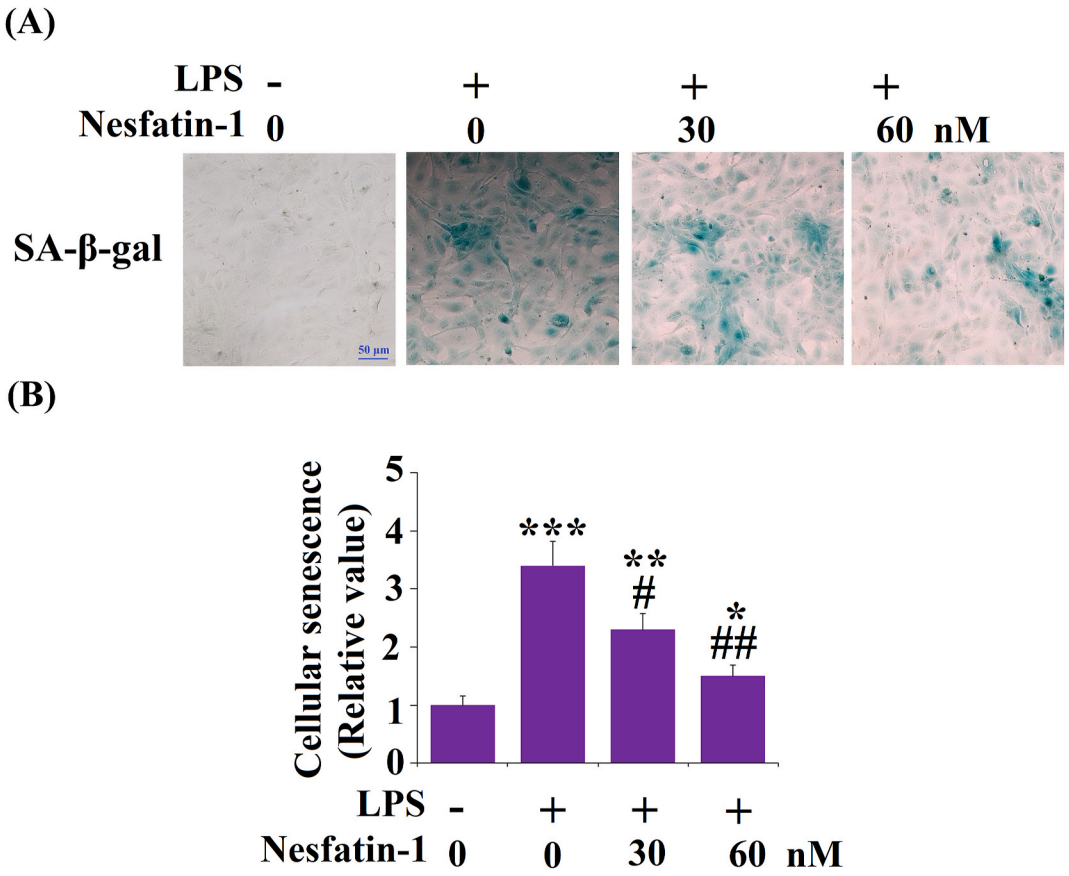


Fig. 4. Nesfatin-1 attenuated LPS-induced cellular senescence against LPS in human dental pulp cells (hDPCs). Cells were stimulated with LPS with or without Nesfatin-1 (30, 60 nM) for 7 days. (A). Cellular senescence was measured by senescence-associated-β-galactosidase (SA-β-Gal) staining (400 ×). Scale bar, 50 μm; (B). Quantification of SA-β-Gal staining (*,**, ***, $P < 0.05, 0.01, 0.001$ vs. vehicle group; #, ##, $P < 0.05, 0.01$ vs. LPS group).

level was inhibited from 2.8 ± 0.27 to 2.2 ± 0.23 and 1.6 ± 0.15 by 30 and 60 nM Nesfatin-1, and that of p16 from 2.7 ± 0.28 to 2.1 ± 0.21 and 1.3 ± 0.12 by 30 and 60 nM Nesfatin-1, respectively (Fig. 5B), thereby confirming the inhibitory effect of Nesfatin-1 on LPS-triggered cellular senescence in hDPCs.

3.6. Nesfatin-1 restored the level of SIRT1 in LPS-challenged cells

SIRT1 is an important transcriptional factor that regulates cellular senescence [17]. We found that the mRNA (Fig. 6A) and protein levels (Fig. 6B) of SIRT1 were downregulated by 44 % and 48 %, respectively, in LPS-challenged hDPCs. The mRNA of SIRT1 was upregulated from 0.56 ± 0.05 to 0.77 ± 0.07 and 0.95 ± 0.1, and its protein from 0.52 ± 0.07 to 0.82 ± 0.08 and 0.96 ± 0.11 by 30 and 60 nM Nesfatin-1, suggesting that SIRT1 may be involved in the regulatory function of Nesfatin-1 against LPS- induced cellular senescence in hDPCs.

3.7. Silencing of SIRT1 abrogated the protective effects of Nesfatin-1 on cellular senescence

To further verify the mechanism of Nesfatin-1, hDPCs were transduced with lentiviral SIRT1 shRNA, followed by treatment with 1 μg/mL LPS with or without Nesfatin-1 (60 nM). Firstly, the efficacy was determined using the results of Western blotting (Fig. 7A). Telomerase activity in LPS-challenged hDPCs was greatly elevated from 14.2 ± 1.3 IU/L to 20.3 ± 2.4 IU/L by 60 nM Nesfatin-1, which declined to 15.5 ± 1.53 IU/L as a result of SIRT1 knockdown (Fig. 7B). Furthermore, an increase in the percentage of SA-β-Gal staining positive cells in LPS-challenged hDPCs of 3.1 times was repressed to 1.8 times by 60 nM Nesfatin-1, which was abolished by the silencing of SIRT1 (Fig. 7C). Lastly, the upregulation of PAI-1 and p16 in LPS-incubated hDPCs was suppressed by 60 nM Nesfatin-1, which was abrogated by transfection with lentiviral SIRT1 shRNA (Fig. 7D). These data reveal that SIRT1 mediated the regulatory function of Nesfatin-1 against LPS-induced cellular senescence in hDPCs.

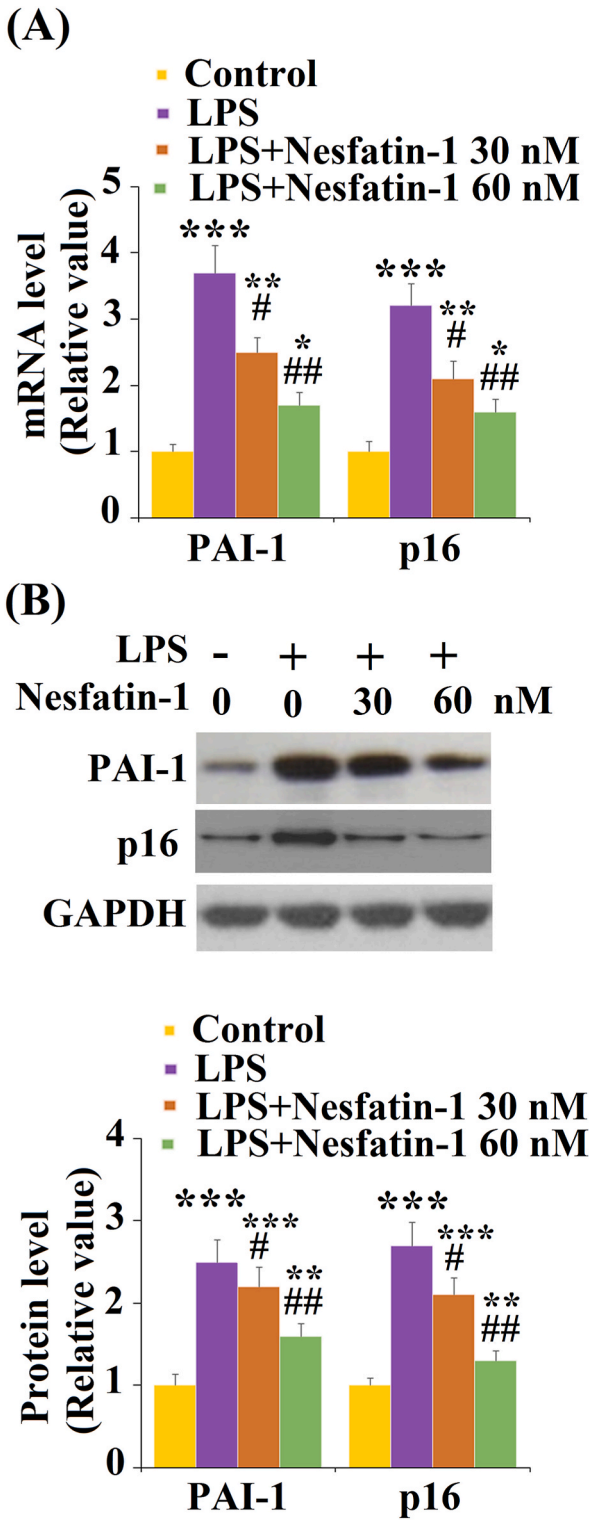


Fig. 5. Nesfatin-1 reduced the expression of PAI-1 and p16 in LPS-challenged cells. Cells were stimulated with LPS with or without Nesfatin-1 (30, 60 nM) for 24 h. (A). mRNA of PAI-1 and p16; (B). Protein of PAI-1 and p16 (*,**, ***, $P < 0.05$, 0.01, 0.001 vs. vehicle group; #, ##, $P < 0.05$, 0.01 vs. LPS group).

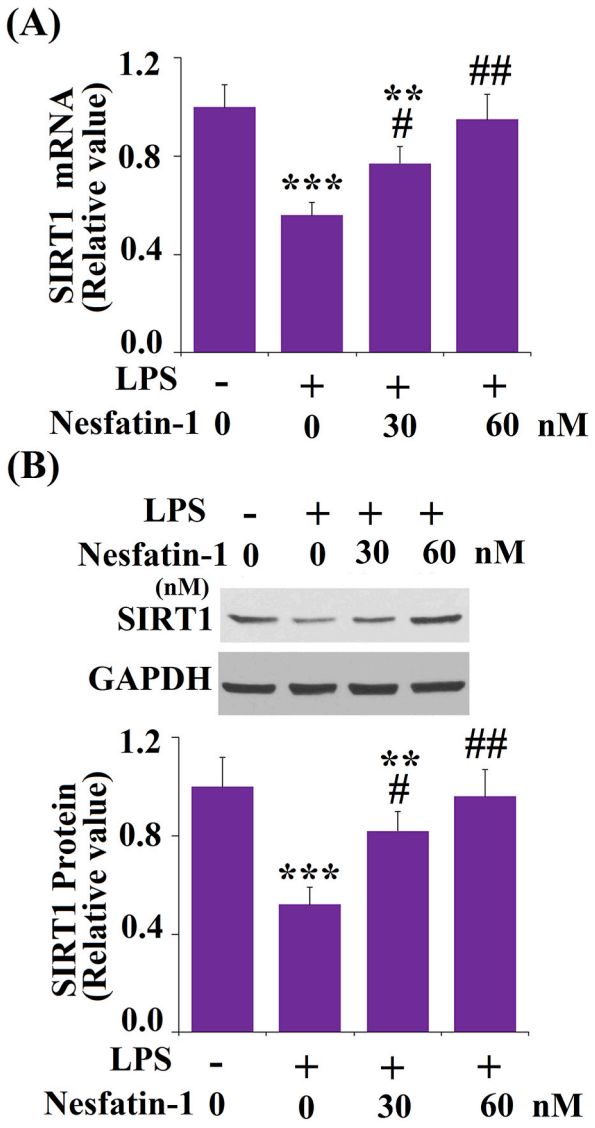


Fig. 6. Nesfatin-1 restored the expression of SIRT1 in LPS-challenged cells. Cells were stimulated with LPS with or without Nesfatin-1 (30, 60 nM) for 24 h. (A). mRNA of SIRT1; (B). Protein of SIRT1 (**, ***, $P < 0.01$, 0.001 vs. vehicle group; #, ##, $P < 0.05$, 0.01 vs. LPS group).

4. Discussion

Cellular senescence is the process by which cell division is terminated and obvious phenotypic changes are observed [18]. In the aging process, the continuous shortening of telomeres leads to chromosomal instability, which triggers apoptosis. Telomeres are structures located at the ends of chromosomes in eukaryotic cells. They regulate gene expression, participate in chromosome replication, and protect chromosomes from nuclease degradation. In somatic cells, telomeres shorten gradually with cell division, and their progressive shortening is an important mechanism of human cellular senescence [19]. With the division and proliferation of cells, carcinogenic signals of DNA damage are constantly strengthened. When telomere shortening reaches a critical value, tumor suppressor genes, such as P16 and P21, are upregulated, cellular senescence develops, and the cell cycle is blocked [20,21]. Telomerase is a ribonucleic protein responsible for maintaining the length of telomeres, the activity of which is rarely detectable in most normal human cells. However, telomerases are expressed in cells during early development and are active in germ/embryonic stem cells and cancer cells. Furthermore, high levels of telomerase activity have been detected in proliferating tissues with high regeneration potential, such as bone marrow, skin, the gastrointestinal tract and testes, as well as activated lymphocytes [22,23].

It has been reported that growth arrest is observed in hTERT-overexpressed cells [24]. The hTERT gene has been found to promote cell survival and proliferation, as well as antagonize apoptosis induced by p53 and Bcl-2. Inhibition of hTERT induces rapid cell cycle arrest by affecting the activation of p53 and CHK1 [25]. TERF2 also plays a certain role in regulating telomere length by participating in chromosomal telomere protection and preventing end-to-end fusion of chromosomes. TERF2 exerts a protective effect on telomeres,

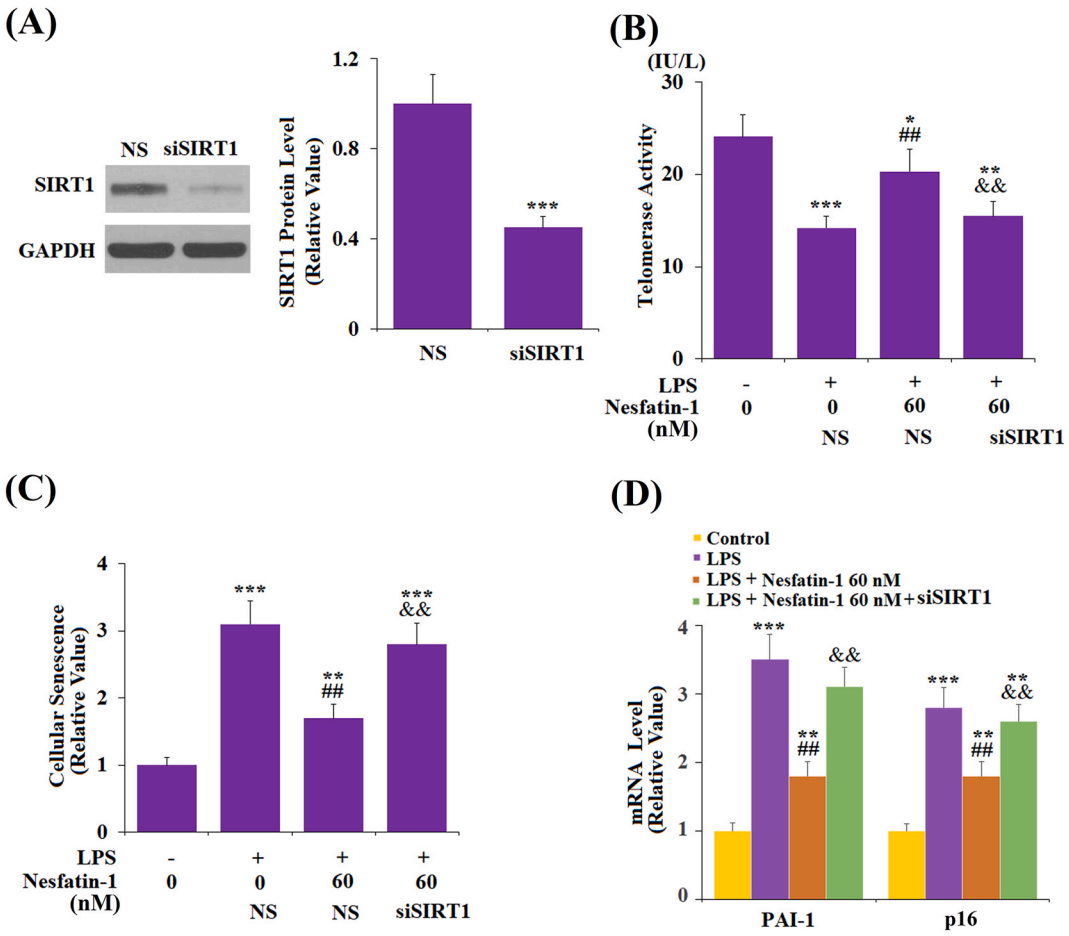


Fig. 7. Knockdown of SIRT1 abolished the protective effects of Nesfatin-1 on cellular senescence. Cells were transduced with lentiviral SIRT1 shRNA, followed by stimulation with LPS with or without Nesfatin-1 (60 nM). (A). Western blot analysis revealed successful knockdown of SIRT1. (B). The telomerase activity was measured; (C). Cellular senescence was measured by senescence-associated-β-galactosidase (SA-β-Gal) staining; (D). mRNA of PAI-1 and p16 (*,**, ***, $P < 0.05$, 0.01, 0.001 vs. vehicle group; ##, $P < 0.01$ vs. LPS group; &&, $P < 0.01$ vs. LPS + Nesfatin-1 group).

the mechanism of which may be related to its interactions with DNA damage response signal factors and damage repair factors [26]. The results of the present study show that a significant increase in the release of inflammatory factors was observed in LPS-stimulated hDPCs, accompanied by an enhanced state of cellular senescence, both of which in accordance with observations reported by Cao [27] and Feng [8]. Recently, substantial research has reported on the effects of Nesfatin-1 on inflammation and its ability to suppress inflammation under different conditions [28,29]. Similarly, our results indicate that under Nesfatin-1 treatment, inflammation was greatly alleviated and cellular senescence was reversed. Furthermore, the inhibitory effect of Nesfatin-1 on cellular senescence in LPS-challenged hDPCs were further confirmed by the downregulation of cellular senescence biomarkers (PAI-1 and p16), upregulation of hTERT, and declined expression levels of TERF2.

Sirtuins are a class of NAD^+-dependent enzymes that affect different cellular processes through deacetylation of multiple downstream targets and other post-translational modifications. SIRT1 deficiency is highly correlated with susceptibility to age-related diseases and metabolic diseases [30], the depletion of which has been proven to contribute to telomere dysfunction [31]. Activation of SIRT1 by resveratrol leads to an increase in extrachromosomal telomere DNA and the co-localization of telomere TRF1 with WRN helicase and BRCA1 in cells [32]. Swami R. Narala et al. found that in hematopoietic stem cells and primary human diploid fibroblasts, SIRT1 negatively regulates cell growth. After SIRT1 knockdown, hTERT was found to be significantly upregulated, which is related to the increase in telomerase activity [33]. Several studies have shown that SIRT1 participates in the regulation of senescence by regulating the expression of key molecules such as P53, Ku70, FOXO, E2F1, NF-κB and PGC-1α [34,35]. Furthermore, a recent study demonstrated that Nesfatin-1 inhibits oxidative stress through the SIRT1/PGC-1α pathway in hyperoxia-induced lung injury mice [36]. Similarly, we found that SIRT1 was downregulated in LPS-stimulated hDPCs, which was abrogated by Nesfatin-1, implying the potential involvement of SIRT1 in the anti-senescence function of Nesfatin-1. Moreover, the protective effects of Nesfatin-1 on cellular senescence in LPS-stimulated hDPCs were greatly abrogated by the silencing of SIRT1, which confirmed that Nesfatin-1 exerted its anti-senescence property in LPS-incubated hDPCs by increasing the level of SIRT1. The function of Nesfatin-1 on gingivitis will be further verified using an animal model in our subsequent experiments, which will provide more convincing evidence for the treatment

of gingivitis using Nesfatin-1.

Collectively, our data reveal that Nesfatin-1 attenuated the inflammatory response and senescence in hDPCs triggered by LPS by regulating SIRT1. This study provides a novel insight and potential therapeutic strategy for the treatment of gingivitis. However, there are several limitations of the current study. Firstly, cellular senescence and telomerase activity were only evaluated at one time point. More time points and kinetics of the cell cycle should be investigated in our future research. Secondly, the experiments were only performed in hDPCs, and *in vivo* experiments with animals are necessary.

Data availability statement

The supporting data is available upon reasonable request from the corresponding author.

Patient consent for publication

Not applicable.

Ethics statement

This study was designed in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of Beijing Shijingshan Hospital (No. 20162135).

CRediT authorship contribution statement

Lili Zhang: Writing – original draft, Investigation, Data curation, Conceptualization. **Bo Pang:** Investigation, Data curation. **Rong Wang:** Resources, Investigation, Data curation. **Bin Yang:** Resources, Investigation. **Xubei Jia:** Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding and acknowledgments

This study was supported by “Shijingshan teaching hospital of Capital Medical University”.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32108.

References

[1] M. Trimarchi, et al., Mast cell cytokines in acute and chronic gingival tissue inflammation: role of IL-33 and IL-37, Int. J. Mol. Sci. 23 (21) (2022) 13242.
[2] N. Kantrong, et al., Gingival epithelial cell recognition of lipopolysaccharide, Adv. Exp. Med. Biol. 1197 (2019) 55–67.
[3] R. Lira-Junior, et al., Severe chronic periodontitis is associated with endothelial and microvascular dysfunctions: a pilot study, J. Periodontol. 85 (12) (2014) 1648–1657.
[4] D.S. Kim, et al., Anti-inflammatory effects of glutamine on LPS-stimulated human dental pulp cells correlate with activation of MKP-1 and attenuation of the MAPK and NF-kappaB pathways, Int. Endod. J. 48 (3) (2015) 220–228.
[5] E.K. Choi, et al., Ketoprofen inhibits expression of inflammatory mediators in human dental pulp cells, J. Endod. 39 (6) (2013) 764–767.
[6] Z. Liu, et al., Fluocinolone acetonide partially restores the mineralization of LPS-stimulated dental pulp cells through inhibition of NF-kappaB pathway and activation of AP-1 pathway, Br. J. Pharmacol. 170 (6) (2013) 1262–1271.
[7] I. Iezzi, et al., Dental pulp stem cells senescence and regenerative potential relationship, J. Cell. Physiol. 234 (5) (2019) 7186–7197.
[8] X. Feng, et al., Repeated lipopolysaccharide stimulation promotes cellular senescence in human dental pulp stem cells (DPSCs), Cell Tissue Res. 356 (2) (2014) 369–380.
[9] M. Sattari, et al., Evaluating the effect of LPS from periodontal pathogenic bacteria on the expression of senescence-related genes in human dental pulp stem cells, J. Cell Mol. Med. 26 (2) (2022) 5647–5656.
[10] S. Li, et al., Regulation of PINX1 expression ameliorates lipopolysaccharide-induced lung injury and alleviates cell senescence during the convalescent phase through affecting the telomerase activity, Aging (Albany NY) 13 (7) (2021) 10175–10186.
[11] I.S. Oh, et al., Identification of nesfatin-1 as a satiety molecule in the hypothalamus, Nature 443 (7112) (2006) 709–712.
[12] G. Wang, et al., RYGB increases postprandial gastric nesfatin-1 and rapid relieves NAFLD via gastric nerve detachment, PLoS One 15 (12) (2020) e0243640.
[13] X. Chen, et al., Nesfatin-1 acts on the dopaminergic reward pathway to inhibit food intake, Neuropeptides 53 (2015) 45–50.
[14] Z.Z. Wang, et al., Nesfatin-1 alleviates acute lung injury through reducing inflammation and oxidative stress via the regulation of HMGB1, Eur. Rev. Med. Pharmacol. Sci. 24 (9) (2020) 5071–5081.
[15] X. Yang, et al., Nesfatin-1 alleviates hyperoxia-induced lung injury in newborn mice by inhibiting oxidative stress through regulating SIRT1/PGC-1α pathway, Cytokine 169 (2023) 156239.

[16] F.Z. Darvishi, et al., Morphine treatment is associated with diminished telomere length together with down-regulated TERT and TERF2 mRNA levels, Drug Alcohol Depend. 227 (2021) 108982.
[17] J.W. Hwang, et al., Redox regulation of SIRT1 in inflammation and cellular senescence, Free Radic. Biol. Med. 61 (2013) 95–110.
[18] E. Sikora, et al., What is and what is not cell senescence, Postepy Biochem. 64 (2) (2018) 110–118.
[19] W. Lu, et al., Telomeres-structure, function, and regulation, Exp. Cell Res. 319 (2) (2013) 133–141.
[20] R. Bartenschlager, et al., The molecular and structural basis of advanced antiviral therapy for hepatitis C virus infection, Nat. Rev. Microbiol. 11 (7) (2013) 482–496.
[21] B.G. Childs, et al., Cellular senescence in aging and age-related disease: from mechanisms to therapy, Nat. Med. 21 (12) (2015) 1424–1435.
[22] J. Yui, et al., Telomerase activity in candidate stem cells from fetal liver and adult bone marrow, Blood 91 (9) (1998) 3255–3262.
[23] K. Liu, et al., Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes, Proc. Natl. Acad. Sci. U. S. A. 96 (9) (1999) 5147–5152.
[24] S.T. Wang, et al., Atorvastatin-induced senescence of hepatocellular carcinoma is mediated by downregulation of hTERT through the suppression of the IL-6/STAT3 pathway, Cell. Death, Discover 6 (2020) 17.
[25] R. Jahanban-Esfahlan, et al., The herbal medicine Melissa officinalis extract effects on gene expression of p53, Bcl-2, Her2, VEGF-A and hTERT in human lung, breast and prostate cancer cell lines, Gene 613 (2017) 14–19.
[26] Z. Deng, et al., TERRA RNA binding to TRF2 facilitates heterochromatin formation and ORC recruitment at telomeres, Mol. Cell 35 (4) (2009) 403–413.
[27] R. Cao, et al., Nell-1 attenuates lipopolysaccharide-induced inflammation in human dental pulp cells, J. Mol. Histol. 52 (4) (2021) 671–680.
[28] S. Gharanei, et al., NUCB2/Nesfatin-1 reduces obesogenic diet induced inflammation in mice subcutaneous white adipose tissue, Nutrients 14 (7) (2022) 1409.
[29] H. Sun, et al., Protective role and molecular mechanism of action of Nesfatin-1 against high glucose-induced inflammation, oxidative stress and apoptosis in retinal epithelial cells, Exp. Ther. Med. 22 (2) (2022) 833.
[30] J. Perla-Kajan, et al., Mechanisms of homocysteine toxicity in humans, Amino Acids 32 (4) (2007) 561–572.
[31] C. Chen, et al., SIRT1 and aging related signaling pathways, Mech. Ageing Dev. 187 (2020) 111215.
[32] J.A. Palacios, et al., SIRT1 contributes to telomere maintenance and augments global homologous recombination, J. Cell Biol. 191 (7) (2010) 1299–1313.
[33] S.R. Narala, et al., SIRT1 acts as a nutrient-sensitive growth suppressor and its loss is associated with increased AMPK and telomerase activity, Mol. Biol. Cell 19 (3) (2008) 1210–1219.
[34] M. Kilic Eren, et al., Resveratrol induced premature senescence is associated with DNA damage mediated SIRT1 and SIRT2 down-regulation, PLoS One 10 (4) (2015) e0124837.
[35] M.R. Ramis, et al., Caloric restriction, resveratrol and melatonin: role of SIRT1 and implications for aging and related-diseases, Mech. Ageing Dev. 146–148 (2015) 28–41.
[36] X. Yang, et al., Nesfatin-1 alleviates hyperoxia-induced lung injury in newborn mice by inhibiting oxidative stress through regulating SIRT1/PGC-1α pathway, Cytokine 169 (2023) 156239.