

Liraglutide Attenuates Endothelial Dysfunction by Inhibiting Hyperglycemia-Induced Endothelial Cell Senescence Through the LARP7/SIRT1 Pathway

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Abstract

Vascular endothelial cells (ECs) are pivotal in maintaining vascular homeostasis. Liraglutide (LIR) can prevent and reverse hyperglycemia-induced cell dysfunction. However, the mechanism by which it improves hyperglycemia-induced EC senescence remains unclear. This study investigates whether the La Ribonucleo-protein 7/Sirtuin 1 (LARP7/SIRT1) signaling axis is essential for LIR efficacy in mitigating EC senescence and dysfunction. We treated senescent human umbilical vein ECs induced by high glucose levels with LIR and evaluated cell viability cell counting kit-8 (CCK-8 assay), senescence (SA- β -gal staining), and SASP alterations (qPCR). We also investigated the expression of senescence-related proteins and changes in the LARP7/SIRT1 signaling pathway using Western blot analysis. Additionally, reactive oxygen species levels were measured with 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA), and changes in oxidative stress-related factors were assessed using specific assay kits. The effect of LIR on endothelial dysfunction was examined by cellular tube-forming assay and transwell assay after LARP7 knockdown/overexpression. LIR markedly improved cell vitality and the senescence phenotype while reducing oxidative stress. The LARP7/SIRT1 pathway emerged as crucial for its effectiveness. Following LARP7 knockdown, the therapeutic efficacy of LIR was notably attenuated. The overexpression of LARP7 enhanced the therapeutic efficacy of LIR. The tube formation and transwell assays further supported the hypothesis that LIR's beneficial impact on endothelial dysfunction depends on the LARP7/SIRT1 signaling axis. Our study reveals a novel aspect of LIR as an antidiabetic agent in delaying vascular aging driven by high glucose through targeting the LARP7/SIRT1 pathway. This discovery enhances the therapeutic value of LIR and proposes a new strategy for addressing vascular aging in treatments for elderly patients with diabetes.

Keywords

Liraglutide · Endothelial cell senescence · Oxidative stress · LARP7 · SIRT1

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1. Introduction

Diabetes mellitus is a chronic global health threat and a leading cause of mortality and disability worldwide (Bako et al. 2024). Among these cases, over 90% are classified as Type 2 diabetes mellitus (T2DM), where the risk of cardiovascular diseases (CVD) and mortality is more than double compared with non-T2DM subjects (Zhang et al. 2024). Clinical studies have demonstrated that vascular aging has been proven to be an important independent predictor of CVD events (Paneni et al. 2017). Assessment measures for vascular aging, such as flow-mediated dilation, can facilitate early diagnosis and prediction of CVD outcomes (Thijssen et al. 2019). Recent studies have identified age-related endothelial cell (EC) dysfunction as a crucial risk factor for cardiovascular complications associated with T2DM (Palanca et al. 2024). ECs, which are the primary component of the vascular endothelium, are in direct contact with circulating blood and play

an essential role in maintaining vascular functional homeostasis (Miao et al. 2023). EC dysfunction, predominantly attributed to EC senescence, disrupts vascular endothelial integrity and function due to the progressive accumulation of senescent ECs (Ren et al. 2024; Santinha et al. 2024). High-glucose (HG)-induced reactive oxygen species (ROS) have been implicated in abnormal mitochondrial fission and DNA damage, thereby impairing the self-repair capabilities of ECs, which could be a critical factor in EC senescence (Wang et al. 2024). However, the precise role and mechanism of EC aging in diabetic CVD, as well as the impact of EC dysfunction on the progression of cardiovascular complications, remain inadequately elucidated.

Liraglutide (LIR), an approved therapeutic for T2DM, is a glucagon-like peptide-1 (GLP-1) analog. It exhibits anti-inflammatory properties, activates protective autophagy, and exerts anti-apoptotic effects, primarily through the AMP-activated Protein Kinase (AMPK)/SIRT1 signaling pathway (Lord et al. 2023; Daniali et al. 2024). In diabetic myocardial injury mouse models, LIR mitigated myocardial pathological changes, inflammation, and cardiomyocyte pyroptosis by activating SIRT1 and AMPK (Inoue et al. 2015). Similarly, in a focal cerebral cortical

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ischemia mouse model, LIR significantly enhanced cell survival and restored neuronal activity in the cortex, mediated by improvements in mitochondrial function through SIRT1 activation (He et al. 2020).

SIRT1, a member of the sirtuin family, plays a vital role in cell cycle regulation, oxidative stress response, and aging. Echinacoside alleviates oxidative stress and the progression of aging in human umbilical vein endothelial cells (HUVECs) via SIRT1 activation (Li et al. 2023). In diabetic cardiomyopathy mouse models, long-term supplementation with exogenous SIRT1 partially restored cardiovascular function. Furthermore, SIRT1 knockdown in hair follicle-derived mesenchymal stem cells enhances aging and apoptosis, accompanied by increased ROS accumulation, DNA damage, and reduced levels of Nicotinamide adenine dinucleotide (NAD) and Adenosine triphosphate (ATP) (Wang et al. 2023). These findings have fueled our interest in exploring the mechanisms by which LIR and SIRT1 operate in EC aging.

Recent advances in aging research have highlighted the pivotal role of LARP7 in cellular senescence. As an RNA-binding protein, it primarily codes for proteins in the 7SK small nuclear ribonucleoprotein complex, which is closely associated with cell cycle cyclin-dependent kinases (Páez-Moscoso et al. 2022; Yang et al. 2022). It has been observed that the absence of LARP7 leads to the inhibition of SIRT1 deacetylase activity, consequently accelerating cellular aging (Yan et al. 2021), potentially linked to LARP7's role in promoting telomerase assembly (Mennie et al. 2018). Some researchers have proposed that LARP7 is one of the primary regulators of cellular aging, playing a significant role in DNA damage response (DDR)-mediated cellular senescence and atherosclerosis through the SIRT1-p53/p65 pathway (Liang et al. 2021). However, the involvement of LARP7 in EC aging and its role and mechanism in CVD remain to be fully understood.

This study reports that LIR significantly delays oxidative stress and the aging process in high-glucose-induced HUVECs. Our findings indicate that LIR decelerates EC senescence and improves endothelial dysfunction through the LARP7-SIRT1 signaling pathway.

2. Materials and Methods

2.1. Cell culture

HUVECs, procured from Shanghai FuHeng (FH1122), were cultured in an ECM-specific medium (FH-HUVEC, FuHeng, Shanghai, China). The cells were passaged in a controlled environment at 37°C with 5% CO₂, ensuring that HUVECs used for experiments were within 25 passages. Cells were divided into three groups and cultured for 72 hours (the NG group (exposed to 5.5 mM normal glucose), the HG group (exposed to 30 mM high glucose), and the HG+LIR group

(30 mM high glucose + 500 µM LIR. LARP7 knockdown and overexpression vectors acquired from GenePharma (Shanghai, China) with their RNA sequences are detailed in Table S1 in Supplementary Materials. These vectors were transfected using Lipofectamine™ 3000 (L3000150, Thermo, Waltham, USA) at 50 nM for 4 h at 37°C. Cells were harvested 72 h post-transfection for subsequent RNA and protein analysis.

2.2. CCK-8 assay

HUVECs, at a density of 2×10^3 cells per well, were seeded in 96-well plates. Cell viability was determined 3 h post CCK-8 solution treatment (CA1210, Solarbio, Beijing, China), adhering to the manufacturer's guidelines. Absorbance readings at 450 nm were obtained using a microplate reader, and the optical density (OD) values of the normal glucose group (NG group) served as the reference for percentage calculations.

2.3. β-Galactosidase (SA-β-gal) staining

Following the manufacturer's protocol, cell senescence was ascertained using an SA-β-gal staining kit (K146501, Thermo). Briefly, 5×10^4 cells were incubated per well in a 6-well plate for 72 h. Post-culture medium removal, cells were washed once with 1× phosphate buffered saline (PBS) and fixed with 1 mL of β-Gal Fixative Solution A for 15 min at ambient temperature. The fixed cells were washed thrice with 1 × PBS, each lasting 3 min. The staining solution was formulated in a 5:1:1:93 ratio using components B, C, D, and E. After removing the β-Gal wash solution, 1 mL of the prepared staining solution was added to each well and incubated overnight at 37°C. Senescent cells were identified under an optical microscope (CKX41, Olympus, Tokyo, Japan) by blue cytoplasmic staining.

2.4. Western blot

Total protein from HUVECs was extracted utilizing the Whole Protein Extraction Kit (90079, Thermo). Proteins were separated on a Bis-Tris gel system (NW00107BOX, Thermo) and transferred to a polyvinylidene fluoride (PVDF) membrane (PB0117, Thermo). The membranes were blocked with 5% non-fat milk for 2 h at 37°C and incubated sequentially with primary antibodies overnight at 4°C and secondary antibodies for 2 h at 37°C. Immunoblot images were captured using the iBright™ CL750 Imaging System (A44116, Thermo, USA) and analyzed quantitatively with ImageJ 6.0 (Bethesda, USA). Employed antibodies included: Anti-LARP7 (17067-1-AP, Proteintech, Rosemont, USA, 1:1000), Anti-p53 (60283-2-Ig, Proteintech, 1:5000), Anti-p21 (10355-1-AP, Proteintech, 1:1000), Anti-p16 (10883-1-AP, Proteintech, 1:1000), Anti-SIRT1 (13161-1-AP, Proteintech, 1:1000),

Anti-EP300 (20695-1-AP, Proteintech, 1:500), Anti-p-AMPK (Phosphorylated AMPK) (Thr172) (D4D6D, CST, Danvers, USA, 1:1000), Anti-AMPK (10929-2-AP, Proteintech, 1:2000), Anti-IgG (PR30012, Proteintech).

2.5. Quantitative PCR (qPCR)

Total RNA was extracted from HUVEC cells using the RNA Extraction Kit (R1200, Solarbio), and its concentration was quantified with a BioPhotometer Plus spectrophotometer (Eppendorf Ltd., Hamburg, Germany). Complementary DNA (cDNA) synthesis was performed using the Reverse Transcription Kit (T2240, Solarbio). The messenger ribonucleic acid (mRNA) expression levels were assessed utilizing the One-Step SYBR Green RT-qPCR Kit (11736051, Thermo). Primer sequences are detailed in Table S2 in the Supplementary Materials. All results were normalized against the glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.6. DCFH-DA fluorescence staining

Intracellular ROS levels were detected using DCFH-DA fluorescence staining per the manufacturer's protocol (CA1410, Solarbio). Cells were cultured in dulbecco's modified eagle's medium (DMEM) supplemented with 1% fetal bovine serum (FBS) for 24 h, incubated with DCFH-DA at 37°C for 60 min, and visualized under a fluorescence microscope (SZ51, Olympus).

2.7. MDA content assay

HUVEC cells were lysed and processed for malondialdehyde (MDA) content analysis as described in the MDA Assay Kit (EY-01H360, Yansheng, China). The supernatant (1 mL) was combined with MDA detection reagents (Dilution Work Solution A, Storage Work Solution B, and Antioxidant Work Solution C at a 10:3:1 ratio), thoroughly dissolved at 70°C, heated in a boiling water bath for 20 min, and then cooled to room temperature. After centrifugation (1000 rpm, 10 min), the absorbance of the supernatant was measured at 532 nm.

2.8. Superoxide dismutase activity measurement (WST-1 method)

As instructed by the manufacturer (BL902A, Bioshrap, Hefei, China), 1×10^6 cells were cultured in a 6-well plate for 72 h, followed by a cold bath (4°C, 10 min). After adding 200 μ L of superoxide dismutase (SOD) sample preparation solution and centrifuging (4°C, 12,000 rpm), the supernatant was collected for protein quantification using the BCA Protein Assay Kit (P0011, Beyotime, Shanghai, China). A mixture of 160 μ L WST-1/enzyme working solution (ratio of 150:8:1 of SOD

Assay Buffer, WST, and enzyme solution) and 40 μ L reaction initiation solution (ratio of 39:1 of SOD Buffer and reaction initiation solution) was prepared. The assay involved the sequential addition of the test samples, SOD Assay Buffer, WST-1/enzyme working solution, and reaction initiation solution, incubated at 37°C for 30 min. Absorbance at 450 nm was measured using a spectrophotometer, and SOD activity was calculated according to the formula provided in the kit.

2.9. Nitric oxide content measurement

Nitric oxide (NO) levels were determined following the protocol of the NO Assay Kit (AAT-B16350, Qiyuan, Shanghai, China). As described earlier, HUVEC cell supernatants were incubated with Work Solutions A–B (37°C, 60 min), followed by Work Solutions C–D (37°C, 40 min) and centrifugation (1000 rpm, 10 min). After adding 1 mL of color-developing reagent and incubating (37°C, 10 min), absorbance at 550 nm was measured using a spectrophotometer. The NO content was calculated based on the established standard curve.

2.10. Tube formation assay

Matrigel (BD Biosciences, Franklin Lakes, USA) was dissolved at 4°C for 24 h, diluted, and mixed with pre-cooled serum-free medium in a 1:3 ratio. After allowing Matrigel to solidify for 2 h at 37°C, HUVECs were seeded to overlay the Matrigel. Post 6-h incubation, tube formation by the cells was observed and quantified in terms of number and length using a CKX41 microscope (Olympus).

2.11. Transwell migration assay

The migratory capacity of HUVECs was assessed using a Transwell assay, as described in Abdel Ghafar et al. (2020). Briefly, cells were placed in the upper chamber of the Transwell (in DMEM without FBS), while the lower chamber contained 10% FBS. After 72 h of incubation, non-migrating cells in the upper chamber were carefully removed. Cells that migrated were stained with crystal violet and counted under a microscope.

2.12. Statistical analysis

Statistical analyses and graph plotting were performed using GraphPad, San Diego, USA, Prism 9 software (GraphPad Software Inc.). All experiments were replicated at least three times, with results expressed as mean $\pm$ standard deviation. Two-sample comparisons were conducted using *t*-tests, while analysis of variance (ANOVA) was employed for analyses involving three or more groups. A *p*-value of <0.05 was considered statistically significant (**p* < 0.05, ***p* < 0.01, #*p* < 0.05, ###*p* < 0.01).

3. Results

3.1. LIR upregulates LARP7 expression and mitigates HG-induced senescence in HUVECs

To ascertain the induction of cellular senescence by HG and the counteracting effects of LIR, HUVECs were treated with a high concentration of glucose (30 mM) and LIR. The CCK-8 assay results demonstrated a marked decline in cell viability under HG conditions, which was effectively reversed by LIR (Figure 1a). Following 25 generations of culture under high-glucose conditions and subsequent SA- β -gal staining, pronounced senescence features were evident in HUVECs, whereas LIR treatment slowed cellular aging (Figure 1b). To

elucidate the role of LARP7 in HG-induced HUVEC senescence, we examined the protein and mRNA expression levels of LARP7 and aging-related genes, including cellular tumor antigen p53 (p53), cyclin-dependent kinase inhibitor 1A (p21), and cyclin-dependent kinase inhibitor 2A (p16), using Western blot and qPCR. LARP7 expression decreased in senescent HUVECs, showing a negative correlation with age-related gene expression (Figure 1c). Furthermore, quantitative polymerase chain reaction (qPCR) analysis of senescence-associated secretory phenotype (SASP) genes, including interleukin (IL)-1 α , IL-1 β , C-X-C motif chemokine ligand 8 (IL-8), C-C motif chemokine ligand 2 (MCP-1), and intercellular cell adhesion molecule 1 (ICAM-1), indicated elevated secretion of SASP proteins in HUVECs under HG

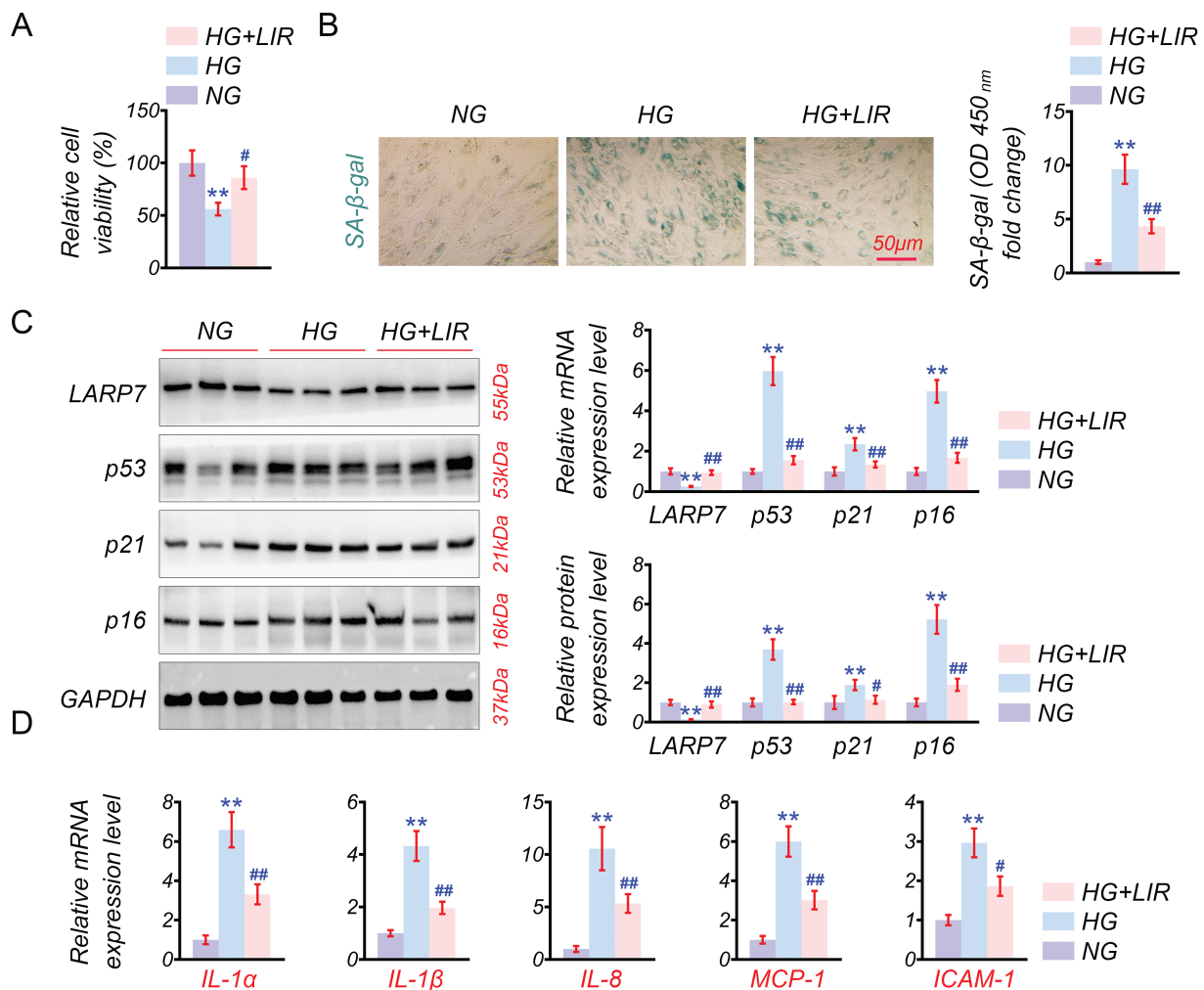


Fig 1. LIR augments LARP7 expression and inhibits HG-induced HUVEC senescence. Cells were categorized into four groups: NG group (5.5 mM glucose), HG group (30 mM glucose), and HG + LIR group (30 mM glucose, 500 μ M LIR). (A) CCK-8 assay to assess cell viability. (B) SA- β -gal staining to evaluate cellular senescence. (C) Western blot and qPCR analysis for changes in LARP7, p53, and p21 expression levels. (D) qPCR to quantify SASP gene expression levels of IL-1 α , IL-1 β , IL-8, MCP-1, and ICAM-1. $n = 3$ for each group. Scale bar: 50 μ m. ** $p < 0.01$ vs. NG, * $p < 0.05$, ## $p < 0.01$ vs. HG. HG, high glucose; HUVECs, human umbilical vein endothelial cells; LIR, Liraglutide; MCP-1, motif chemokine ligand 2; SASP, senescence-associated secretory phenotype.

conditions. LIR treatment effectively reduced SASP secretion (Figure 1d).

3.2. LIR suppresses oxidative stress in aging ECs via LARP7

To elucidate LARP7's role in HG-induced HUVEC senescence, we developed a LARP7 knockdown HUVEC model. LARP7 mRNA and protein levels in this model were confirmed to be substantially reduced through qPCR (Figure 2a) and Western blot (Figure 2b) analyses. Oxidative stress is commonly associated with cellular aging. Studies have highlighted oxidative stress and DNA damage accumulation as critical contributors to cellular senescence (Lee et al. 2024). Therefore, HG-induced HUVECs were divided into four treatment groups: HG induction alone, LIR treatment, and LIR treatment with concurrent LARP7 knockdown or overexpression. ROS levels in these groups were measured using the DCFH-DA fluorescent probe. Results indicated that HG treatment significantly increased ROS levels in cells, effectively mitigated by LIR. Intriguingly, the therapeutic

effect of LIR was almost nullified following LARP7 knockdown, while the overexpression of LARP7 had the opposite effect (Figure 2c). This suggests that LIR's inhibition of HG-induced oxidative stress in HUVECs depends on LARP7. Similarly, assay kit measurements of oxidative stress-related factors, including MDA, SOD, catalase, and NO, revealed that LIR effectively controlled the onset of oxidative stress in HUVECs under HG conditions. However, this effect was significantly compromised after LARP7 knockdown, but LARP7 overexpression amplified the therapeutic effects of LIR (Figure 2d). This suggests that LARP7's presence is essential for LIR to reduce oxidative stress in HG-induced HUVECs effectively, and its absence significantly impairs LIR's therapeutic efficacy, with enhanced LARP7 expression boosting LIR's therapeutic effects.

3.3. LIR regulates EC senescence through the LARP7/SIRT1 pathway

To delve deeper into the specific mechanisms by which LIR functions via LARP7, we employed Western blot analysis to

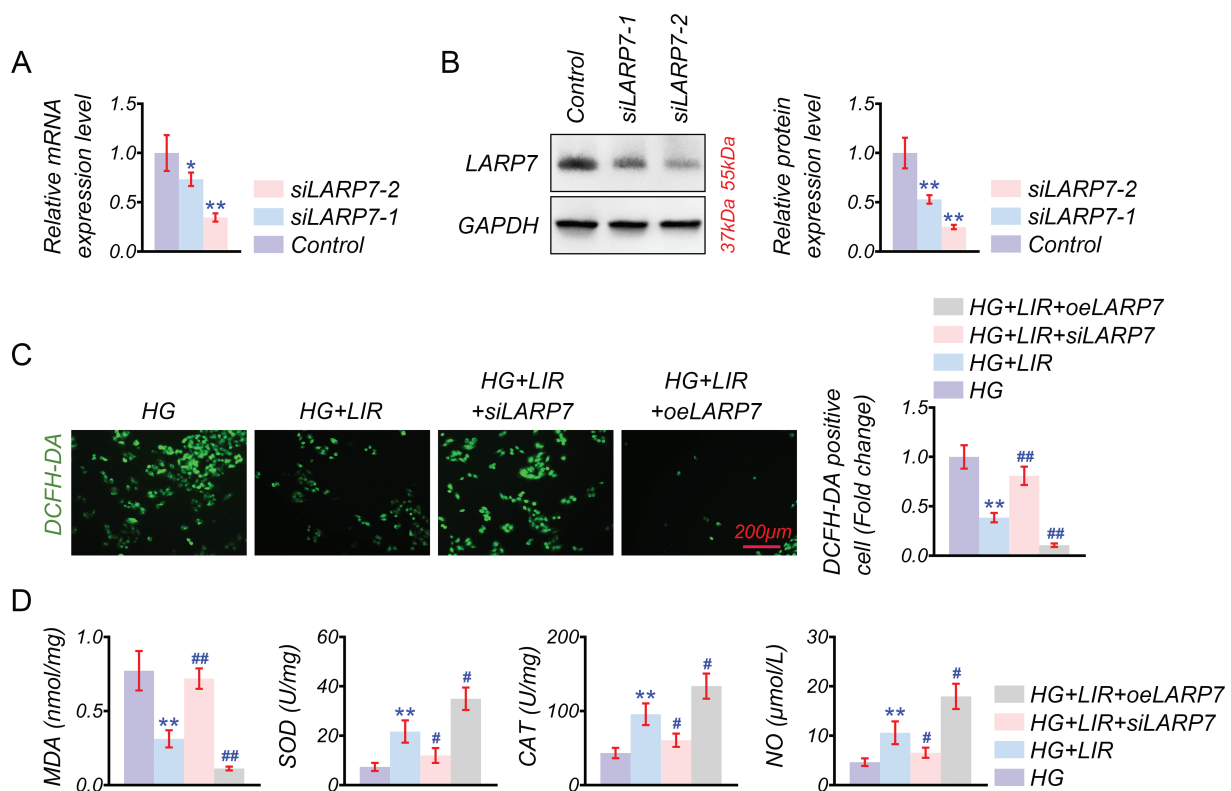


Fig 2. LIR alleviates oxidative stress in senescent ECs via LARP7. Two distinct LARP7 knockdown vectors were constructed to mitigate off-target responses, with knockdown efficiency subsequently validated. (A,B) qPCR and Western blot for assessing LARP7 mRNA and protein expression post-transfection. The impact of LARP7 knockdown on HUVECs was examined in four groups: HG, HG + LIR, HG + LIR + siLARP7, and HG + LIR + oeLARP7. (C) DCFH-DA fluorescent probe for measuring cellular ROS content. (D) Assay kits to determine oxidative stress-related factors, specifically MDA/NO content and SOD and CAT activities. $n = 3$ for each group. Scale bar: 200 μ M. * $p < 0.05$, ** $p < 0.01$ vs. Control or HG, * $p < 0.05$, *** $p < 0.01$ vs. HG + LIR. CAT, catalase; ECs, endothelial cells; HG, high glucose; HUVECs, human umbilical vein endothelial cells; LIR, Liraglutide; MDA, malondialdehyde; NO, nitric oxide; ROS, reactive oxygen species; SOD, superoxide dismutase.

assess the downstream genes of LARP7, namely, SIRT1 and age-related signaling molecule EP300 (1A Binding Protein P300) and phosphorylated AMP-activated protein kinase (p-AMPK; Figure 3a) (Kalinin et al. 2023). Our findings revealed that in HUVECs treated with HG, there was a notable decrease in SIRT1 expression, a significant upregulation of EP300, and inhibition of AMPK phosphorylation. After we knocked down or overexpressed LARP7, we examined the above protein expression levels again. The results showed that SIRT1 expression was restored after LIR treatment, EP300 expression was significantly inhibited, and AMPK phosphorylation was reactivated. However, alterations induced by LIR treatment were almost completely nullified following LARP7 knockdown, and LARP7 overexpression enhanced the effects of LIR (Figure 3b). To ascertain if LIR's impact on cellular senescence is associated with LARP7, we conducted CCK-8 assays on cells from these

varied treatment groups. The results indicated that LIR's protective effect on cell viability was partially diminished due to LARP7 knockdown and enhanced by LARP7 overexpression (Figure 3c). Similarly, SA- β -gal staining demonstrated that LIR effectively mitigated the cellular senescence phenotype; however, it could not be effectively controlled following LARP7 knockdown. Critically, LARP7 overexpression further promoted the mitigation of cellular senescence by LIR (Figure 3d). Through qPCR analysis of SASP genes, including IL-1 α , IL-1 β , IL-8, MCP-1, and ICAM-1 mRNA expression, it was observed that SASP mRNA levels in HUVECs under HG conditions were significantly reduced by LIR, an effect also hindered by LARP7 knockdown, and conversely, it was enhanced by LARP7 overexpression (Figure 3e). These findings suggest that the efficacy of LIR in enhancing HUVEC cell vitality and inhibiting cellular senescence necessitates the involvement of LARP7. SIRT1 and AMPK

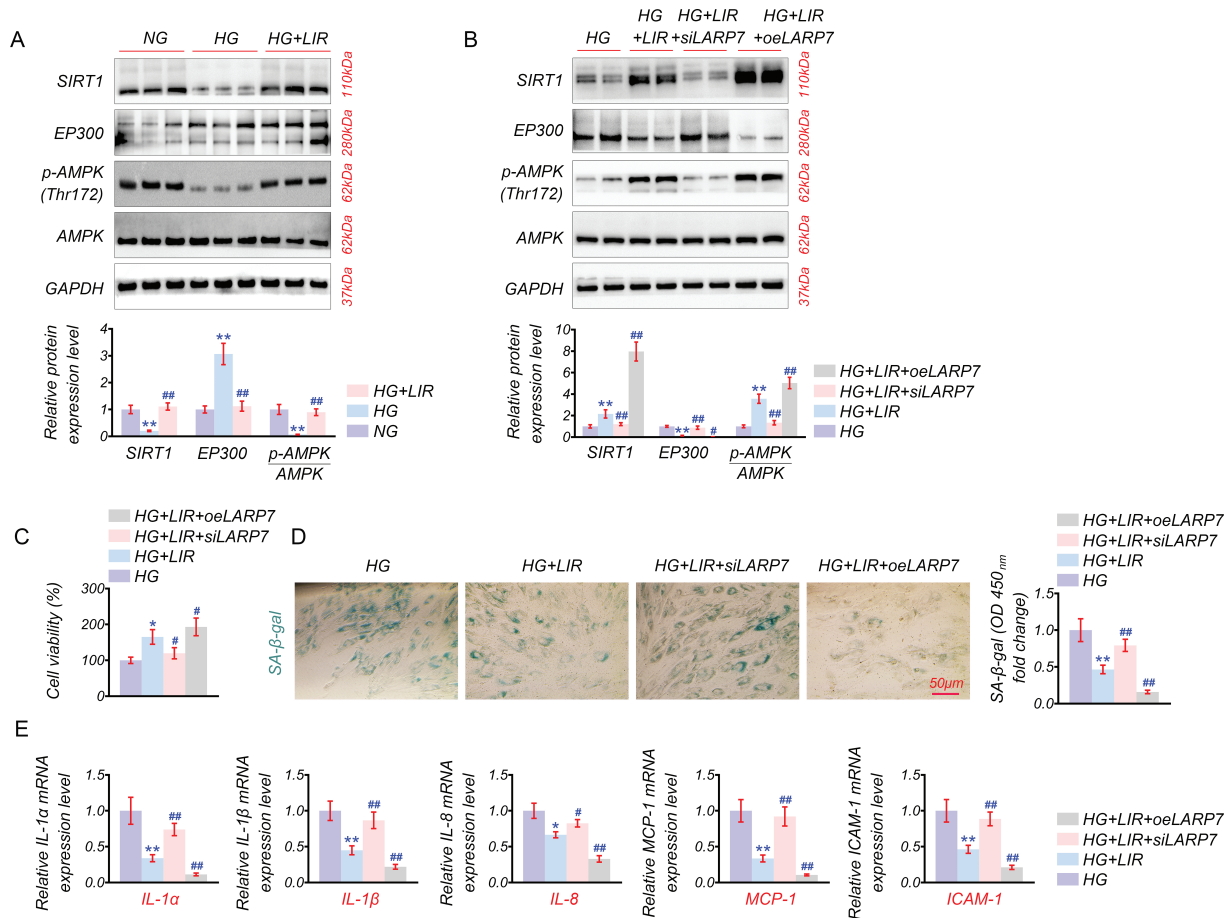


Fig 3. LIR regulates EC senescence through the LARP7/SIRT1 pathway. Cells were initially segregated into NG, HG, and HG + LIR groups. (A) Western blot analysis for alterations in the known senescence signaling axes, focusing on SIRT1, EP300, p-AMPK (Thr172), and AMPK. Subsequent assessments in the HG, HG + LIR, HG + LIR + siLARP7, and HG + LIR + oeLARP7 groups were conducted to determine the effects on the senescence signaling axis and cellular phenotype. ** $p < 0.01$ vs. NG, *** $p < 0.01$ vs. HG. (B) Western blot analysis for SIRT1, EP300, p-AMPK (Thr172), and AMPK. (C) CCK-8 assay for evaluating cell viability. (D) SA- β -gal staining to determine cellular senescence. (E) qPCR to measure SASP gene expression levels of IL-1 α , IL-1 β , IL-8, MCP-1, and ICAM-1. $n = 3$ for each group. Scale bar: 50 μ m. * $p < 0.05$, ** $p < 0.01$ vs. HG, # $p < 0.05$, ### $p < 0.01$ vs. HG + LIR. EC, endothelial cell; HG, high glucose; LIR, Liraglutide; MCP-1, motif chemokine ligand 2; SASP, senescence-associated secretory phenotype.

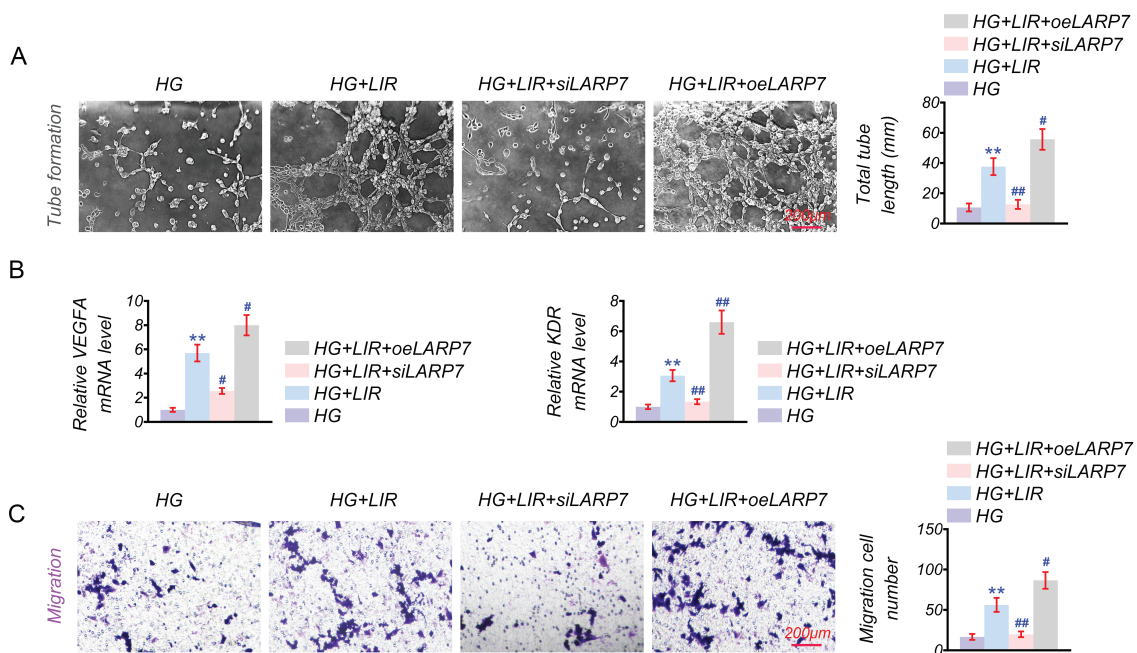


Fig 4. LIR restores endothelial function impaired by LARP7 deficiency. Further investigation into the impact of LIR and LARP7 on HUVEC function was conducted in the HG, HG + LIR, HG + LIR + siLARP7, and HG + LIR + oeLARP7 groups. **(A)** Tube formation assay to measure angiogenic capacity. **(B)** qPCR is used to evaluate mRNA changes in VEGFA and KDR. **(C)** Transwell assay for assessing HUVEC migration capabilities. $n = 3$ for each group. Scale bar: 200 μ m. ** $p < 0.01$ vs. HG, * $p < 0.05$, ## $p < 0.01$ vs. HG + LIR. HG, high glucose; HUVECs, human umbilical vein endothelial cells; KDR, kinase insert domain receptor; LIR, Liraglutide; VEGFA, vascular endothelial growth factor A.

phosphorylation are activated in this context, while EP300 is inhibited. The significant attenuation of LIR's effect following LARP7 knockdown, coupled with the re-inhibition of SIRT1 and AMPK phosphorylation and partial reactivation of EP300, and the opposite effect following LARP7 overexpression. It is further elucidated that LIR may regulate the senescence process of HUVEC cells through LARP7. The involvement of LARP7 in this process was significantly correlated with the SIRT1/EP300/AMPK signaling axis.

3.4. LIR restores endothelial dysfunction through LARP7

Having established LIR's positive impact on HUVEC cell vitality and senescence, we extended our investigation to explore its role in facilitating endothelial dysfunction. We evaluated EC functional characteristics, including tube formation and migration capabilities. The tube formation assay results demonstrated that LIR treatment significantly enhanced the tube-forming ability of HUVEC cells. However, the extent of vascular formation was negated mainly by LARP7 knockdown, and the upregulation of LARP7 similarly improved the effects of LIR in promoting vascular formation (Figure 4a). We then assessed the mRNA expression levels of vascular endothelial growth factor A (VEGFA) and kinase insert domain receptor (KDR), both

critical in tube formation processes. The results indicated that LIR effectively boosted the mRNA expression levels of VEGFA and KDR, which, however, declined again following LARP7 knockdown, but the overexpression of LARP7 had the opposite effect (Figure 4b). Furthermore, the results from the transwell assay, which assessed HUVEC cell migration capabilities, revealed that LIR significantly promoted HUVEC cell migration, a capability that was suppressed upon LARP7 knockdown, and the upregulation of LARP7 similarly enhanced the ability of LIR to improve cell migration (Figure 4c). The above results demonstrated that LIR could effectively promote the functional properties of ECs and that the upregulation of LARP7 effectively enhanced the efficacy of LIR.

4. Discussion

Cellular senescence represents a multifaceted process characterized by an indefinite arrest in the cell cycle and significant changes in the SASP (Kalinin et al. 2023). In younger individuals, normal cellular metabolic activities contribute positively to developmental processes. However, in the elderly, the persistent and irreversible accumulation of senescent cells, which are not replaced by healthy cells, leads to a progressive decline in organ function, exerting profound multifactorial effects on the organism (Chin et al. 2023; Molnár

et al. 2023). Notably, in the diabetic model, vascular aging driven by EC senescence exemplifies a classical manifestation of cellular senescence (Zhu et al. 2022; Liu et al. 2023). A plethora of evidence correlates diabetic vascular complications, such as renal dysfunction, retinopathy, and CVD like atherosclerosis, with prolonged hyperglycemia, identifying EC senescence as a primary contributor to these complications (El-Osta 2023; Savić et al. 2023).

Our study, involving the treatment of HUVECs with HG, revealed a significant decline in cell vitality, cellular senescence induction, and the onset of oxidative stress. Studies have shown that essential oil of fructus alpiniae zerumbet (EOFAZ) and rosiglitazone reverse HG-induced cellular senescence, improving abnormal lipid metabolism and vascular aging in diabetic mice (Liao et al. 2024). Similarly, metformin treatment, by activating the AMPK-SIRT1/SIRT6 axis, regulates vascular aging, consequently delaying the onset of age-related atherosclerosis (Pulipaka et al. 2024). These findings suggest that targeting the inhibition of EC senescence might be a critical pathway for improving vascular aging in diabetic patients.

In our research, treatment of HUVECs under HG conditions with the antidiabetic agent LIR effectively mitigated oxidative stress and signs of aging. LIR, a medication for T2DM, has gained increased attention for its antioxidative and anti-inflammatory actions in CVDs. For instance, in rat models with isoproterenol-induced myocardial injury, LIR treatment improved oxidative stress and inflammation markers while also ameliorating cardiac pathological damage (Abdel-Reheim et al. 2024). In diabetic mice lacking GLP-1 receptors, renal damage was evident, and activation of the GLP-1 receptor pathway with LIR, including the downregulation of renal receptor for advanced glycation end-products (RAGE), facilitated macrophage M2 polarization, reducing pathological kidney damage in diabetic mice (Sourris et al. 2023). In cases of diabetic osteoporosis, LIR showed therapeutic efficacy by promoting bone growth and inhibiting bone resorption, thus alleviating trabecular bone loss (Fathy et al. 2023). Furthermore, in a Type 2 diabetes-induced fatty liver mouse model, LIR treatment improved glucose metabolism, liver tissue damage, and liver function by activating the AMPK/ Acetyl-CoA Carboxylase (ACC) pathway and inhibiting ferroptosis (Guo et al. 2023). These results demonstrate LIR's complex pharmacological actions and therapeutic potential in diabetic complications. Distinctively, our study identified the critical LARP7/SIRT1 signaling axis through which LIR regulates oxidative stress and aging in ECs. Notably, following LARP7 knockdown, LIR was nearly ineffective against HG-induced aging in HUVECs, but the overexpression of LARP7 significantly enhanced the therapeutic effects of LIR.

LARP7 has been identified as a novel anti-aging gene; its deficiency in rodent models leads to the accumulation of senescent cells and premature aging. Furthermore, inhibition

of the active LARP7-SIRT1-p53/p65 signaling axis in vascular aging significantly reduces cellular senescence (Yan et al. 2021). LARP7 plays a crucial role in cellular aging by regulating SIRT1 stability and activity, which are essential for mitochondrial biogenesis, energy production, and cardiac function.

Cardiac-specific LARP7 knockout mice exhibit defects in mitochondrial biogenesis, elevated oxidative stress, impaired SIRT1-mediated transcription of energy metabolism genes, and altered cardiac function (Yu et al. 2021). However, restoration of LARP7 levels can provide cardioprotective effects. These findings underscore LARP7's significance in cellular aging, consistent with the findings of the present study.

A noteworthy clinical study funded by the Health Research Board of Ireland discovered that the combined administration of Continuous Positive Airway Pressure with LIR significantly reduces vascular inflammation in patients with obstructive sleep apnea. This combination was also correlated with improvements in endothelial function and a concurrent decrease in intravascular coronary plaque volume. Such outcomes were not achieved with LIR monotherapy (O'Donnell et al. 2024). This finding appears to diverge from current mainstream perspectives and our results. We hypothesize that this may be attributed to LIR's complex pharmacological actions and mechanisms, highlighting the critical importance of delving into LIR's mode of action. Identifying key targets for LIR's efficacy and developing combination therapies to enhance its therapeutic impact could be an effective clinical strategy for treating vascular diseases.

In summary, our study delineates LIR's role in the senescence of HG-induced HUVECs. It modulates oxidative stress and aging characteristics in HUVECs through the LARP7/SIRT1 signaling axis, ultimately ameliorating endothelial dysfunction. LARP7 emerges as a pivotal element in LIR's mechanism of action, where its absence markedly diminishes LIR's therapeutic efficacy.

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Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Author Contributions

Weili Zhong, Ying Yang, and Yanru Wang designed the study and carried them out, supervised the data collection, analyzed the data, interpreted the data, prepared the manuscript for publication, and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

Competing Interests

The authors state that there are no conflicts of interest to disclose.

Consent to Participate Statement

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Data Availability

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

References

- Abdel Ghafar MT, Gharib F, Abdel-Salam S et al. (2020) Role of serum metadherin mRNA expression in the diagnosis and prediction of survival in patients with colorectal cancer. *Mol Biol Rep* 47: 2509–2519. <https://doi.org/10.1007/s11033-020-05334-5>
- Abdel-Reheim MA, Zaafer D, El-Shoura EAM et al. (2024) Unlocking the miRNA-34a-5p/TGF- β and HMGB1/PI3K/Akt/mTOR cross-talk participate in the enhanced cardiac protection of liraglutide against isoproterenol-induced acute myocardial injury rat model. *Int Immunopharmacol* 127:111369. <https://doi.org/10.1016/j.intimp.2023.111369>
- Bako KR, Mohammadnezhad M, Sika-Paotonu D et al. (2024) Diabetes cam: an objective methodology to study diabetes self-management. *Am J Prev Med* 66:909–913. <https://doi.org/10.1016/j.amepre.2023.12.016>
- Chin T, Lee XE, Ng PY et al. (2023) The role of cellular senescence in skin aging and age-related skin pathologies. *Front Physiol* 14:1297637. <https://doi.org/10.3389/fphys.2023.1297637>
- Daniali M, Nikfar S, Abdollahi M (2024) Advancements in pharmacotherapy options for treating diabetes in children and adolescents. *Expert Rev Endocrinol Metab* 19:37–47. <https://doi.org/10.1080/17446651.2023.2290491>
- El-Osta A (2023) Transcriptional control of endothelial senescence and vascular repair. *Circ Res* 133:858–860. <https://doi.org/10.1161/CIRCRESAHA.123.323716>
- Fathy MA, Anbaig A, Aljafil R et al. (2023) Effect of liraglutide on osteoporosis in a rat model of type 2 diabetes mellitus: a histological, immunohistochemical, and biochemical study. *Microsc Microanal* 29:2053–2067. <https://doi.org/10.1093/micmic/ozad102>
- Guo T, Yan W, Cui X et al. (2023) Liraglutide attenuates type 2 diabetes mellitus-associated non-alcoholic fatty liver disease by activating AMPK/ACC signaling and inhibiting ferroptosis. *Mol Med* 29:132. <https://doi.org/10.1186/s10020-023-00721-7>
- He W, Wang H, Zhao C et al. (2020) Role of liraglutide in brain repair promotion through Sirt1-mediated mitochondrial improvement in stroke. *J Cell Physiol* 235:2986–3001. <https://doi.org/10.1002/jcp.29204>
- Inoue T, Inoguchi T, Sonoda N et al. (2015) GLP-1 analog liraglutide protects against cardiac steatosis, oxidative stress and apoptosis in streptozotocin-induced diabetic rats. *Atherosclerosis* 240: 250–259. <https://doi.org/10.1016/j.atherosclerosis.2015.03.026>
- Kalinin A, Zubkova E, Menshikov M (2023) Integrated stress response (ISR) pathway: unraveling its role in cellular senescence. *Int J Mol Sci* 24:17423. <https://doi.org/10.3390/ijms242417423>
- Lee YS, Park YH, Hwang G et al. (2024) Cpne7 deficiency induces cellular senescence and premature aging of dental pulp. *Aging Cell* 23:e14061. <https://doi.org/10.1111/acer.14061>
- Liang X, Wu S, Geng Z et al. (2021) LARP7 suppresses endothelial-to-mesenchymal transition by coupling with TRIM28. *Circ Res* 129:843–856. <https://doi.org/10.1161/CIRCRESAHA.121.319590>
- Liao J, Fu L, Tai S et al. (2024) Essential oil from *Fructus Alpiniae zerumbet* ameliorates vascular endothelial cell senescence in diabetes by regulating PPAR- γ signalling: a 4D label-free quantitative proteomics and network pharmacology study. *J Ethnopharmacol* 321:117550. <https://doi.org/10.1016/j.jep.2023.117550>
- Liu H, Ghosh S, Vaidya T et al. (2023) Activated cGAS/STING signaling elicits endothelial cell senescence in early diabetic retinopathy. *JCI Insight* 8:e168945. <https://doi.org/10.1172/jci.insight.168945>
- Li Y, Wu Y, Ning Z et al. (2023) Echinacoside ameliorates 5-fluorouracil-induced endothelial injury and senescence through SIRT1 activation. *Int Immunopharmacol* 120:110279. <https://doi.org/10.1016/j.intimp.2023.110279>
- Lord SM, Bahnson HT, Greenbaum CJ et al. (2023) Testing a new platform to screen disease-modifying therapy in type 1 diabetes. *PLoS One* 18:e0293268. <https://doi.org/10.1371/journal.pone.0293268>
- Mennie AK, Moser BA, Nakamura TM (2018) LARP7-like protein Pof8 regulates telomerase assembly and poly(A)+TERRA expression in fission yeast. *Nat Commun* 29:586. <https://doi.org/10.1038/s41467-018-02874-0>

- Miao L, Zhou C, Zhang H et al. (2023) *Portulaca oleracea* L. (Purslane) extract protects endothelial function by reducing endoplasmic reticulum stress and oxidative stress through AMPK activation in diabetic obese mice. *Antioxidants* 12:2132. <https://doi.org/10.3390/antiox12122132>
- Molnár AÁ, Pásztor DT, Tarcza Z et al. (2023) Cells in atherosclerosis: focus on cellular senescence from basic science to clinical practice. *Int J Mol Sci* 24:17129. <https://doi.org/10.3390/ijms242417129>
- O'Donnell C, Crilly S, O'Mahony A et al. (2024) Continuous positive airway pressure but not GLP1-mediated weight loss improves early cardiovascular disease in obstructive sleep apnea: a randomized proof-of-concept study. *Ann Am Thorac Soc* 21:464–473. <https://doi.org/10.1513/AnnalsATS.202309-821OC>
- Páez-Moscoso DJ, Ho DV, Pan L et al. (2022) A putative cap binding protein and the methyl phosphate capping enzyme Bin3/MePCE function in telomerase biogenesis. *Nat Commun* 13:1067. <https://doi.org/10.1038/s41467-022-28545-9>
- Palanca A, Ampudia-Blasco FJ, Calderón JM et al. (2024) Comparison of GLP-1 receptor agonists and other glucose-lowering agents on cardiovascular outcomes in individuals with type 2 diabetes and obesity: a Spanish real-world population-based study. *Diabetes Res Clin Pract* 207:111071. <https://doi.org/10.1016/j.diabres.2023.111071>
- Paneni F, Diaz Cañestro C, Libby P et al. (2017) The aging cardiovascular system: understanding it at the cellular and clinical levels. *J Am Coll Cardiol* 69:1952–1967. <https://doi.org/10.1016/j.jacc.2017.01.064>
- Pulipaka S, Singuru G, Sahoo S et al. (2024) Therapeutic efficacies of mitochondria-targeted esculetin and metformin in the improvement of age-associated atherosclerosis via regulating AMPK activation. *Geroscience* 46:2391–2408. <https://doi.org/10.1007/s11357-023-01015-w>
- Ren X, Cui Z, Zhang Q et al. (2024) JunB condensation attenuates vascular endothelial damage under hyperglycemic condition. *J Mol Cell Biol* 15:mjad072. <https://doi.org/10.1093/jmcb/mjad072>
- Santinha D, Vilaça A, Estronca L et al. (2024) Remodelling of the cardiac extracellular matrix proteome during chronological and pathological ageing. *Mol Cell Proteomics* 23:100706. <https://doi.org/10.1016/j.mcpro.2023.100706>
- Savić R, Yang J, Koplev S et al. (2023) Integration of transcriptomes of senescent cell models with multi-tissue patient samples reveals reduced COL6A3 as an inducer of senescence. *Cell Rep* 42:113371. <https://doi.org/10.1016/j.celrep.2023.113371>
- Sourris KC, Ding Y, Maxwell SS et al. (2023) Glucagon-like peptide-1 receptor signaling modifies the extent of diabetic kidney disease through dampening the receptor for advanced glycation end products-induced inflammation. *Kidney Int* 105:132–149. <https://doi.org/10.1016/j.kint.2023.09.029>
- Thijssen DHJ, Bruno RM, van Mil ACCM et al. (2019) Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* 2019:2534–2547. <https://doi.org/10.1093/eurheartj/ehz350>
- Wang Y, Sui Y, Niu Y et al. (2023) PBX1-SIRT1 positive feedback loop attenuates ROS-mediated HF-MSC senescence and apoptosis. *Stem Cell Rev Rep* 19:443–454. <https://doi.org/10.1007/s12015-022-10425-w>
- Wang L, Zhang X, Huang X et al. (2024) Homoplagitinin alleviates high glucose-induced vascular endothelial senescence by inhibiting mtDNA-cGAS-STING pathway via blunting DRP1-mitochondrial fission-VDAC1 axis. *FASEB J* 38:e70127. <https://doi.org/10.1096/fj.202401299RR>
- Yang Y, Liu S, Egloff S et al. (2022) Structural basis of RNA conformational switching in the transcriptional regulator 7SK RNP. *Mol Cell* 82:1724–1736.e1727. <https://doi.org/10.1016/j.molcel.2022.03.001>
- Yan P, Li Z, Xiong J et al. (2021) LARP7 ameliorates cellular senescence and aging by allosterically enhancing SIRT1 deacetylase activity. *Cell Rep* 37:110038. <https://doi.org/10.1016/j.celrep.2021.110038>
- Yu H, Zhang F, Yan P et al. (2021) LARP7 protects against heart failure by enhancing mitochondrial biogenesis. *Circulation* 143:2007–2022. <https://doi.org/10.1161/CIRCULATIONAHA.120.050812>
- Zhang C, Gu L, Xie H et al. (2024) Glucose transport, transporters and metabolism in diabetic retinopathy. *Biochim Biophys Acta Mol Basis Dis* 1870:166995. <https://doi.org/10.1016/j.bbadis.2023.166995>
- Zhu SL, Wang ML, He YT et al. (2022) Capsaicin ameliorates intermittent high glucose-mediated endothelial senescence via the TRPV1/SIRT1 pathway. *Phytomedicine* 100:154081. <https://doi.org/10.1016/j.phymed.2022.154081>

Supplementary Materials

Table S1. Sequences of transfection plasmid constructs used in this study

Name	Sequence
LARP7 shRNA-1	5'- GGATGAACGCACAGTGTATGT -3'
LARP7 shRNA-2	5'- GGACAGAGTTGAAGCATCTAG -3'
Ctrl shRNA	5'- GTTCAGATGTGCGGCGAGTAA -3'

Table S2. qPCR primers used in the study

Name	Sequence
GAPDH	Forward: 5'-CAGCCTCAAGATCATCAGCA-3'
	Reverse: 5'-TGTGGTCATGAGTCCTTCCA-3'
LARP7	Forward: 5'-TGGAAGCTCGAGATCCTTTCTG-3'
	Reverse: 5'-GTTTTGCCTGTCTATCAACCAA-3'
ICAM-1	Forward: 5'-GTGATGCTCAGGTATCCATCCA-3'
	Reverse: 5'-CACAGTTCTCAAAGCACAGCG-3'
IL-1 β	Forward: 5'-GCAACTGTTCTGAACTCAACT-3'
	Reverse: 5'-ATCTTTTGGGGTCCGTCAACT-3'
IL-1 α	Forward: 5'-TCTGCAAGATGCCACAAGGT-3'
	Reverse: 5'-TGAAGCCCACTTGGTTCAGG-3'
IL-8	Forward: 5'-TTGGCAGCCTTCCTGATTTTC-3'
	Reverse: 5'-AACTTCTCCACAACCCTCTGCA-3'
MCP-1	Forward: 5'- GCCCCACTCACCTGCTGCTACT-3'
	Reverse: 5'- CCTGCTGCTGGTGATCCTCTTGT-3'