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ORIGINAL

ROLE OF LNCRNA SNHG9 IN ENHANCING REHABILITATION OUTCOMES FOR SPORTS-RELATED ENDOTHELIAL DAMAGE: A FOCUS ON THE AMPK/SIRT1-PGC-1A PATHWAY IN PHYSICAL THERAPY

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ABSTRACT

Objective: This study aims to explore the role of long non-coding RNA (lncRNA) SNHG9 in enhancing rehabilitation outcomes for sports-related endothelial damage, with a focus on the AMPK/SIRT1-PGC-1α pathway in the context of physical therapy. Specifically, it investigates the expression of SNHG9 in human umbilical vein endothelial cells (HUVECs) under oxidative stress conditions, which are analogous to endothelial damage observed in athletes with musculoskeletal injuries. Method: HUVECs were cultured and observed under an inverted microscope, divided into groups based on varying concentrations of ox-LDL treatment, a model for oxidative stress. The study utilized Western blot analysis to measure the expression levels of SNHG9 in response to ox-LDL, and the effects of SNHG9 on HUVECs under oxidative stress were observed. Key markers such as glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), lactate dehydrogenase (LDH), Malondialdehyde (MDA), and proteins Bcl2 and Bax were measured to assess cell damage and protective mechanisms. Results: Normal HUVECs exhibited typical morphology, and exposure to ox-LDL significantly increased the expression of SNHG9, especially at the highest concentration and longest exposure time. SNHG9 overexpression led to increased activities of SOD and GSH-PX, upregulation of Bcl2, and reduced levels of LDH and MDA, indicating protective effects against oxidative stress. Conversely, inhibition of SNHG9 expression resulted

in reduced protective enzyme activities and increased cell damage markers. The role of the AMPKSIRT1 inhibitor in this process was also elucidated, showing its significant impact on SNHG9 expression and related protective mechanisms. Conclusion: The study concludes that lncRNA SNHG9 plays a critical role in mitigating endothelial cell injury caused by oxidative stress, relevant to sports-related injuries in athletes. This protective role of SNHG9 is closely associated with the AMPK/SIRT1-PGC-1α signaling pathway, suggesting its potential utility in enhancing rehabilitation outcomes in physical therapy for athletes suffering from sports-related endothelial damage. These findings pave the way for targeted therapeutic strategies, leveraging the modulation of SNHG9 to improve recovery and rehabilitation in athletic patients.

KEYWORDS: human umbilical vein endothelial cells; oxidative stress; cytoglobin; AMPKSIRT1

1. INTRODUCTION

This research delves into the role of long non-coding RNA (lncRNA) SNHG9, a crucial molecular player in the cellular response to oxidative stress, which is a common consequence of intensive athletic activity.(Kocherova et al., 2019)., Endothelial damage is a significant concern in sports medicine, often resulting from the high physical demands on athletes. This type of injury can lead to various complications, including impaired vascular function and delayed recovery from musculoskeletal injuries, which are prevalent in athletes.

The study's focus on the AMPK/SIRT1-PGC-1α pathway, a key regulator of cellular energy homeostasis and oxidative stress response, reflects a growing interest in understanding the molecular bases of sports injuries and recovery. (S. Chen et al., 2019; Gu et al., 2021; Medina-Leyte, Domínguez-Pérez, Mercado, Villarreal-Molina, & Jacobo-Albavera, 2020).

lnRNAs, such as SNHG9, have recently emerged as important regulatory molecules in various physiological and pathological processes(Y. Li et al., 2021). Their role in modulating key pathways related to oxidative stress and cellular metabolism makes them an intriguing target for therapeutic interventions(Peng et al., 2018), particularly in conditions characterized by heightened oxidative stress, like sports-related injuries.(Rezabakhsh et al., 2019).

The objective of this study is two-fold: firstly, to explore the expression and regulation of SNHG9 in human umbilical vein endothelial cells (HUVECs) under oxidative stress conditions (Yukawa et al., 2018). as a model for understanding its role in endothelial cells of athletes(Zhong et al., 2020); and secondly, to investigate the potential of SNHG9 as a therapeutic target for enhancing rehabilitation outcomes in athletes with endothelial damage (Urbanczyk, Zbinden, Layland, Duffy, & Schenke-Layland, 2020). This includes

examining how the modulation of SNHG9 affects the AMPK/SIRT1-PGC-1α pathway and, consequently, the oxidative stress response (J. Chen et al., 2018).

This research is particularly relevant given the increasing demand for effective rehabilitation strategies in sports medicine. With athletes continually pushing the boundaries of human performance(Liang et al., 2018), the incidence of sports-related injuries(Xu, Willumeit-Römer, & Luthringer-Feyerabend, 2019), particularly those affecting the endothelial function, is on the rise. Understanding the molecular mechanisms underlying these injuries and their recovery processes is crucial for developing targeted therapeutic and rehabilitative strategies(Liu, Chuah, Fu, Zhu, & Wang, 2019).

2. Materials and methods

2.1 Cell lines

Human umbilical vein endothelial cells were purchased from Central Laboratory of xxxxxx Medical College (Jin et al., 2019).

2.2 Main reagents

Phage-free, low-endotoxin fetal bovine serum: Hangzhou Sijiqing Institute of Biology, Cat. No. A018, stored at -20°C; DMSO: Beijing Suocaibao Technology Co., Ltd.; PMSF: Beijing Dingguo Biotechnology Co., Ltd.; Trypsin cell digestion solution: item number C0203, Jiangsu Biyuntian Biotechnology Co., Ltd.; Cell lysate: medium-efficiency lysate, Jiangsu Biyuntian Biotechnology Co., Ltd.; SDS-PAGE protein loading buffer: product number P0015, Jiangsu Biyuntian Biotechnology Co., Ltd.; Color protein marker: product number is P0068, Jiangsu Biyuntian Biotechnology Co., Ltd.; SDS-PAGE gel preparation kit: item number is P0012A, which can make 30-50 pieces of gel, purchased from Biyuntian Company; Nitrocellulose membrane (PVDF): millipore; BCA Protein Assay Kit: Pierce, Item No. 23225; SNHG9 primary antibody: Proteintech; Bcl2 primary: Proteintech; Bax-antibody: Proteintech; AMPKSIRT1 primary antibody: Proteintech; GSH-PX detection kit: Nanjing Jiancheng Company; SOD detection kit: Nanjing Jiancheng Company; LDH detection kit: Nanjing Jiancheng Company; MDA detection kit: Nanjing Jiancheng Company; Staurosporine: Jiangsu Biyuntian Biotechnology Co., Ltd.; DMEM high glucose medium: hyclone company; penicillin-streptomycin: Changsha Aijia Biotechnology Co., Ltd.; Other reagents are imported or domestic analytical grade (Wang, Yang, Shi, & Gao, 2019).

2.3 Main instruments

Cell incubator: SHEL-LAB Company of United States; -20℃ refrigerator: Siemens; Microplate reader: American BIO-RAD company; UV spectrophotometer: American PE company; Inverted microscope: Japan

OLYMPUS company; Fluorescence microscope: Japan OLYMPUS company; Purification workbench: Model SW-CJ-2FD, Suzhou Antai Air Technology Co., Ltd.; PCR machine: American Labnet Company; Transfer membrane system: American BIO-RAD company; Electrophoresis apparatus and electrophoresis tank: Beijing Liuyi Instrument Factory; Electronic balance: German CE company; Constant temperature water bath: Pharmacia; Gel imaging system: American BIO-RAD company; Shaker: Shanghai Institute of Centrifugal Machinery; High-speed centrifuge: Model 5084R, Eppendorf, Germany; Oven: Shanghai Yuejin Medical Equipment Factory; Pure water meter: Lixin Instru ment (Shanghai) Co., Ltd.; Ice machine: Huamei Company;Gel imaging analysis system: UVP Corporation of United States; Micropipette: Eppendorf, Germany; High pressure steam sterilizer: Shidukai Instrument Equipment Shanghai Co., Ltd.; Vortex Shaker: Seientifichidvstries USA; Ultracentrifuge: Model CP100MX, Hitachi, Japan (Y.-Q. Zhao, Zhang, Tao, Chi, & Wang, 2019).

2.4 Cell recovery

(1) Put DMEM high-glucose medium containing 10% fetal bovine serum in a water bath at 37°C; (2) Put DMEM medium, sterilized 5ml pipette and culture bottle, centrifuge tube rack, ear washing ball, alcohol lamp, etc. on ultra-clean workbench, under UV light for 30 minutes (S. Li, Liu, Zhou, & Cao, 2020); (3) Take out cryovial from -80℃ refrigerator, dissolve it quickly in a 37℃ water bath, and centrifuge at 1000 rpm for 5 min (Winkler et al., 2021).

(4) On ultra-clean workbench, suck out liquid in upper layer of cryopreservation tube and discard it, add about 5ml of pre-prepared culture medium, and pipette with a pipette, cells are completely suspended in culture medium and stop pipetting; (5) Transfer cell suspension into a pre-sterilized culture flask, and culture at 37°C in a cell incubator containing 5% CO2. Change culture medium after cells adhere to wall and are in a stable state (Jiang et al., 2021).

2.5 Cell Passaging

(1) Place cell culture solution, trypsin digestion solution and PBS in a water bath at 37°C; (2) After water bath, put culture solution, trypsin digestion solution, PBS, sterilized 5ml pipette and culture bottle, ear washing bulb, alcohol lamp, waste liquid tank, etc. on ultra-clean workbench, under ultraviolet lamp Irradiate for 30min; (3) When cells in culture flask grow in pairs and cover about 80% of wall of culture flask, cells can be passaged; (4) Pour out culture medium in bottle, add 5ml PBS, shake it horizontally for dozens of times, and repeat this step 2-3 times.

(5) Digest adherent cells with 0.5ml of 0.25% trypsin digestion solution, tighten bottle cap, observe under an inverted microscope, and add about 10ml to ultra-clean workbench after cell shape shrinks and becomes round. The

culture medium terminates digestion. If cells have not shrunk and become rounded, digestion needs to be continued; (6) Repeatedly pipetting adherent cells with a pipette to turn into a single-cell suspension, aspirate 5ml of single-cell suspension, transfer it to another sterilized culture flask (Zhu et al., 2019).

2.6 Cell cryopreservation

(1) The preparation work is same as above-mentioned cell recovery and cell passage; (2) The required reagents and equipment are placed on ultra-clean workbench in advance and irradiated under ultraviolet lamp for 30 minutes; (3) When cells in culture flask grow in pairs and adhere to about 80% of wall of culture flask, cells can be cryopreserved; (4) Pour out culture medium in bottle, add 5ml PBS, shake it horizontally for dozens of times, and repeat this step 2-3 times.

(5) Add 0.5ml of trypsin digestion solution with a concentration of 0.25% to culture flask, tighten cap, and observe under an inverted microscope. After cell shape shrinks and becomes round, add about 5ml of culture solution to terminate digestion, if it shrinks and becomes round, it needs to continue to digest; ® Repeatedly pipetting and beating adherent cells into a single-cell suspension, transfer single-cell suspension to a sterilized centrifuge tube, seal with sealing glue, and centrifuge at 1000 rpm for 5 min.

(6) Discard upper layer of liquid in centrifuge tube on ultra-clean workbench to leave precipitate, prepare 1ml of cryopreservation solution with fetal bovine serum and DMSO according to ratio of 9:1, add it to centrifuge tube, and fully pipette until mixed; (7) Transfer mixed liquid to a cryopreservation tube, seal it with sealant and mark it, keep it at 4°C for half an hour, -20°C for 1 hour, and transfer to -80°C for low temperature storage (Joshi et al., 2020).

2.7 Effects of different concentrations of oxLDL on expression of SNHG9 in HUVECs (X. Zhao, Wei, Wang, & Qi, 2020).

(1) Irradiate reagents and equipment used in treatment of cells with an ultraviolet lamp on ultra-clean workbench for 30 minutes.

(2) To expand culture of HUVECs, take 5 bottles of endothelial cells (the cells adhere to wall in logarithmic growth and adhere to about 80% of wall of culture bottle) and divide them into 5 groups, pour out medium on ultra-clean workbench, add 5ml Shake PBS horizontally for dozens of times, and repeat this step 2-3 times.

(3) 5ml of serum-free medium was added, and different doses of oxLDL were added to 5 bottles respectively so that concentrations of oxLDL in culture medium were 0, 25, 50, 100, and 200ug/ml, respectively, and cultured in a constant temperature incubator.

(4) After 24 hours, cells were collected, total protein was extracted, and expression of SNHG9 protein in five groups of cells was detected by Western blot (Zhang et al., 2020).

2.8 Effects of oxLDL treatment on HUVECs at different times on expression of SNHG9

(1) Irradiate reagents and equipment used in treatment of cells with an ultraviolet lamp on ultra-clean workbench for 30 minutes; (2) To expand culture of HUVECs, take 5 bottles of endothelial cells (the cells adhere to wall in logarithmic growth and adhere to about 80% of wall of culture bottle) and divide them into 5 groups, pour out medium, add 5ml PBS and shake horizontally for dozens of times. Down and down, repeat this step 2-3 times; (3) Add 5ml of serum-free medium, add same dose of oxLDL to 5 bottles of culture medium respectively until concentration of oxLDL in culture medium is 100ug/ml oxLDL, and cultivate in a constant temperature incubator. (4) The cells were collected after 0, 4, 8, 12, and 24 hours of culture, and total protein was extracted. Western blot was used to detect expression of SNHG9 protein in five groups of cells, respectively (Wu et al., 2020)

3. Results

HUVECs were cultured in DMEM high-glucose medium containing 10% fetal bovine serum for 1-2 days and observed under an inverted microscope. It was found that HUVECs were evenly attached to wall of culture flask, showing a typical paving stone-like shape (Figure 1).

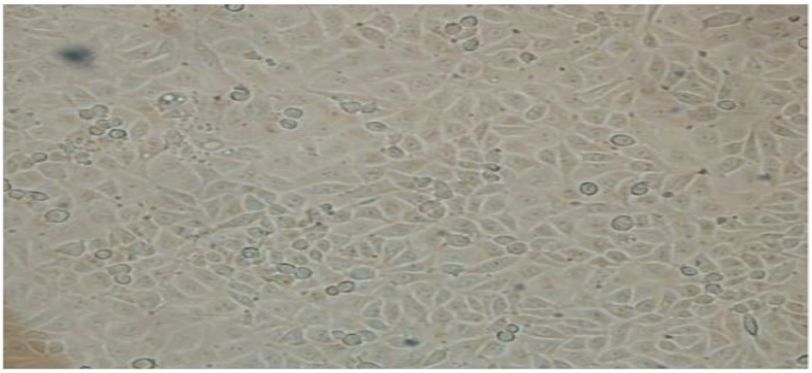


Figure 1: Observation of HUVECs morphology under microscope (10×20)

Using Box Plot analysis, it was found that log2-ratios of each sample after chip scanning were basically same after normalization, as shown in Figure 2A and B. Through scatter plot (Scatter Polt) analysis, we can intuitively analyze distribution changes of chip expression data between two groups, as shown in Figure 2C, D. Hierarchical clustering analysis was performed according to differences in expression levels of lncRNA and mRNA among different samples, and samples with similar expression levels were more likely to be clustered together, as shown in Figure 2E and F.

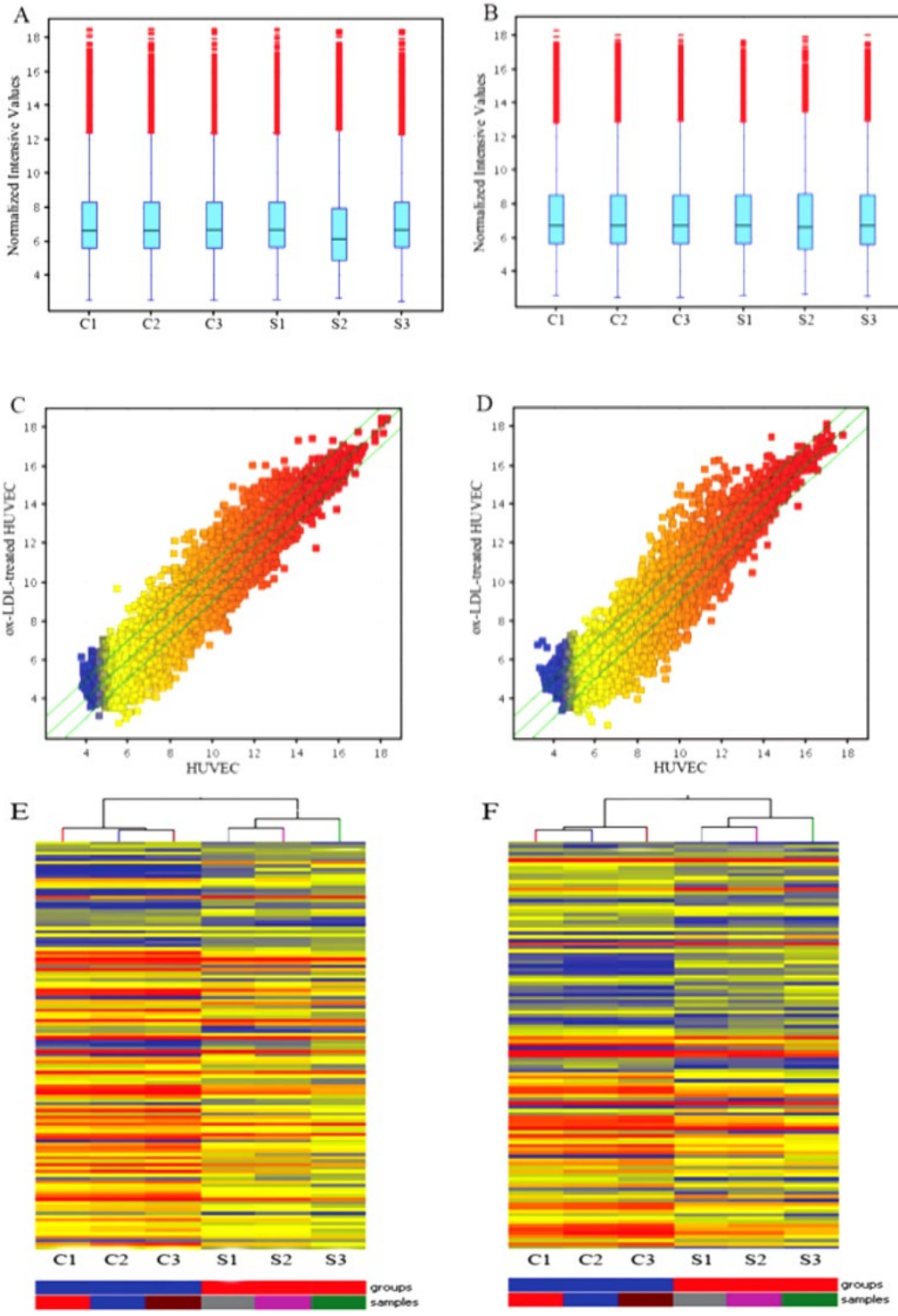


Figure 2: Chip detection of lncRNA and mRNA expression profiles in OX-LDL-induced injured HUVECs (S12, S3) and normal HUVECs (C1C2C3) A. NCRNA box plot, B. mRNA box plot; C. lncRNA scatter plot, D. mRNA scatter plot; E. INCANA cluster analysis, F. mRNA cluster analysis.

Next, Western blot results showed that expression level of SNHG9 was increased after HUVECs were treated with different concentrations of oxLDL, and expression level of SNHG9 was highest when treated with 200ug/ml oxLDL (Figure 3).

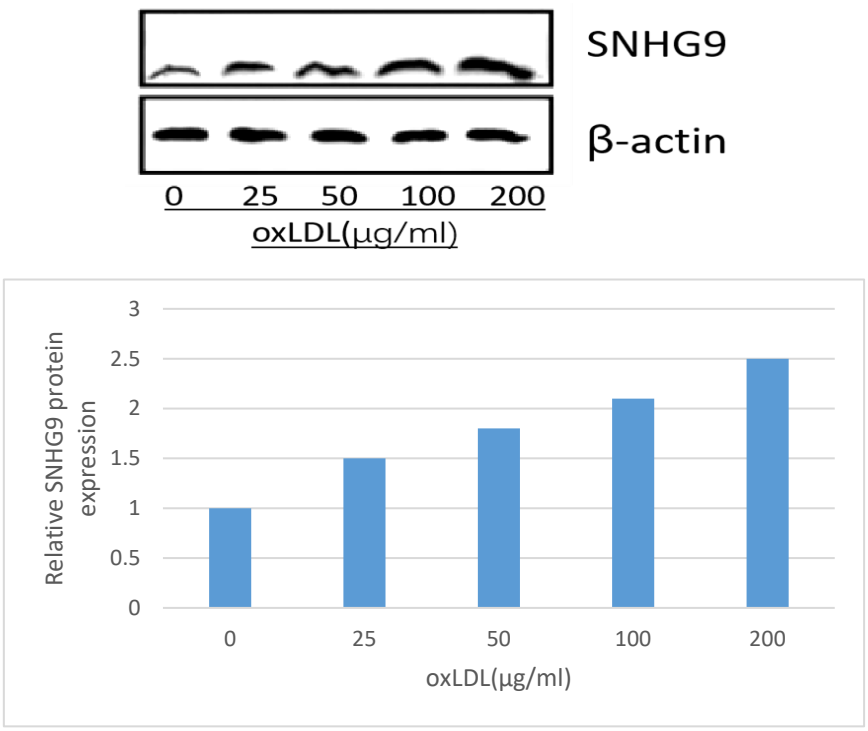


Figure 3: The expression of CYGB in HUVECs treated with different concentrations of oxLDL for 24h compared between $*p<0.05$ and $0\mu g/ml$groups

Western blot results showed that expression level of SNHG9 was increased after 100g/ml oxLDL treatment for different time, and expression level of SNHG9 reached peak in oxLDL treatment for 24h (Figure 4).

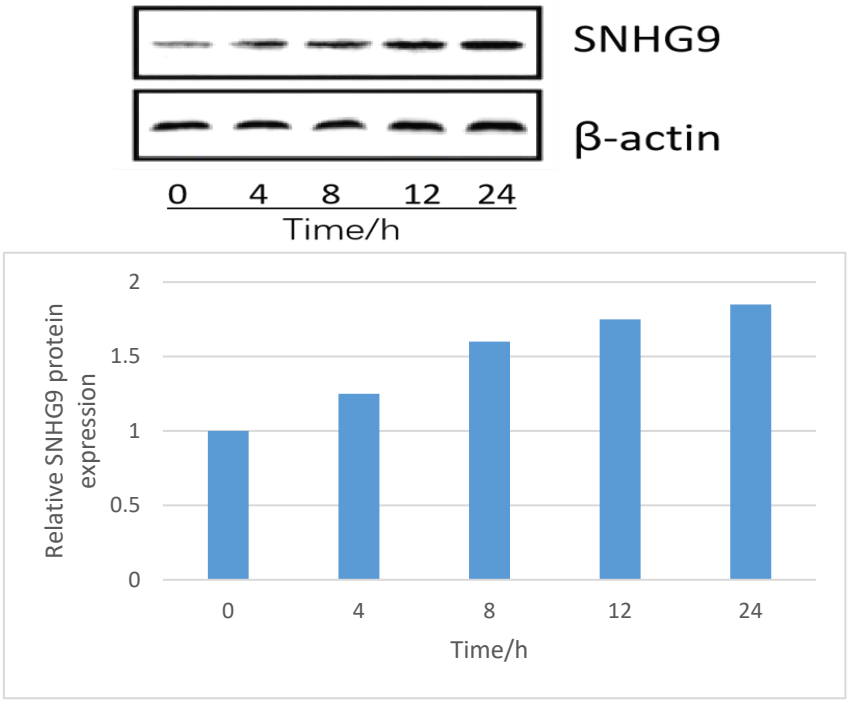


Figure 4:100 μ Effect of HUVECs treated with oxLDL at different time of g/m on expression of CYGB protein $*p<0.05$, compared with 0h

Western blot was used to detect transfection efficiency of HUVECs transfected with pReceiver-lv202-SNHG9 plasmid and psi-LVRHIGP-SNHG9 shRNA plasmid. SNHG9 shRNA plasmid could inhibit SNHG9 expression (Figure 5).

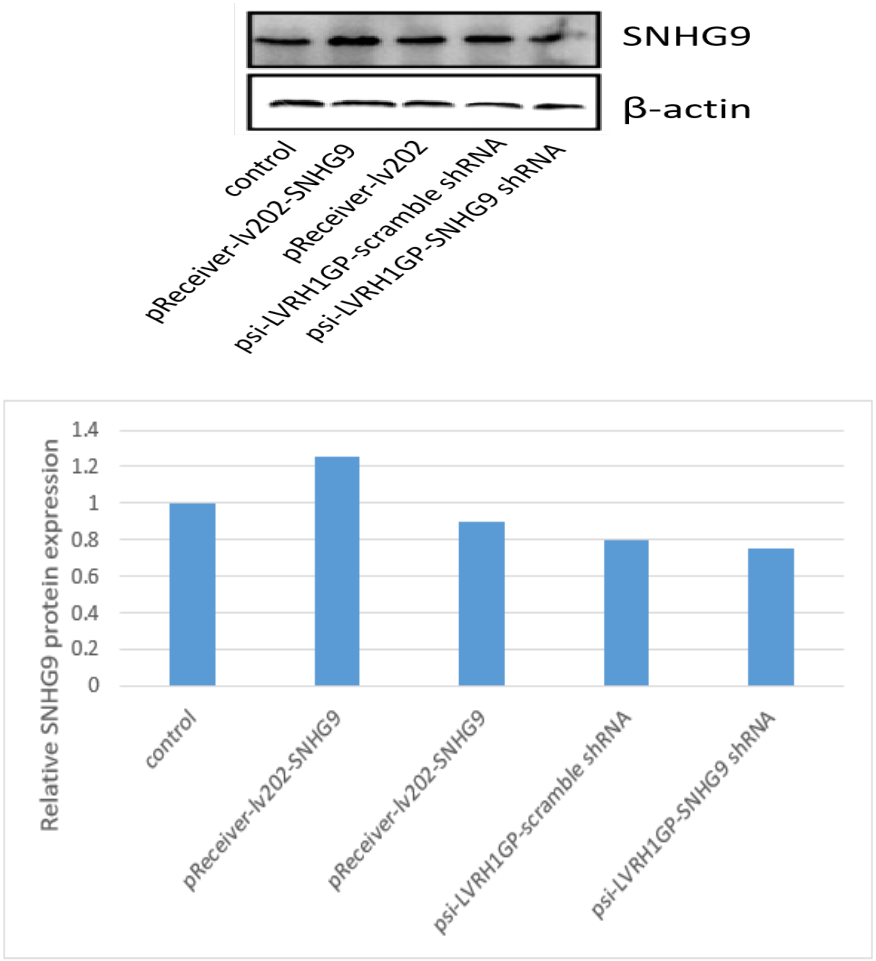
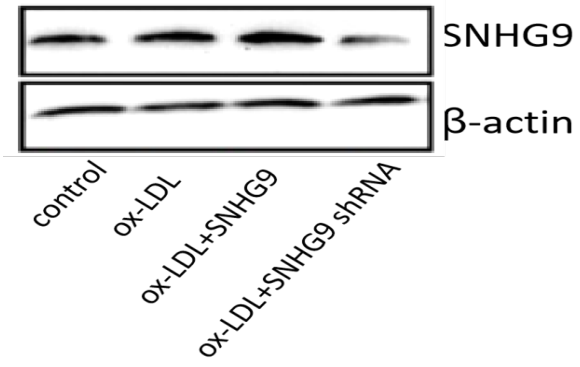


Figure 5: The effect of transfection of CYGB expression/interference plasmid on expression of CYGB in HUVECS $**\ p<0.01$, $***\ p<0.001$ compared with control

Western blot detection showed that compared with oxLDL, expression of SNHG9 in oxLDL+SNHG9 was increased, and expression of SNHG9 in oxLDLISNHG9 shRNA was decreased (the results are shown in Figure 6).



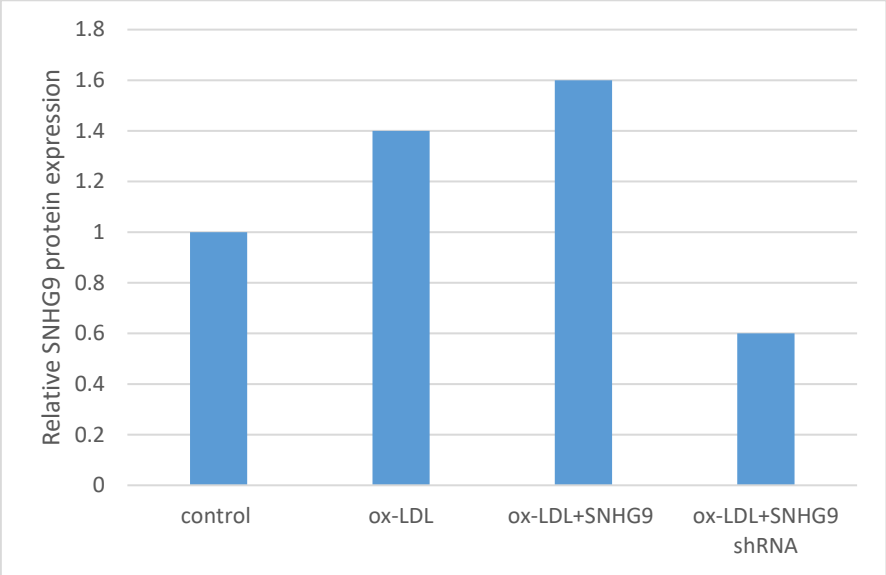


Figure 6: Effect of transfection of CYGB expression/interference plasmid on CYGB expression in oxLDL-treated HUVECS $*p<0.05$, compared with oxLDL; $***p<0.001$, compared with oxLDL

4. Discussion

AS is a multi-stage chronic evolution process, including vascular endothelial injury, monocytes and lymphocytes, cell adhesion, foam cell formation, lipid streak formation, fibrous plaque formation, and then gradually evolves into atheromatous plaques. The cardiovascular and cerebrovascular diseases with AS as main pathological change seriously threaten human health. There are many theories about mechanism of AS formation, including thrombosis theory, injury response theory, oxidation theory, lipid infiltration theory and so on. During evolution of AS. It has been reported in literature that endothelial injury and dysfunction caused by oxLDL is an important link in initiation of AS. As a barrier between blood and vascular walls, endothelial cells have cell permeability, selective barrier, regulation of vascular motor tension, and production of growth factors. Under normal physiological conditions, vascular endothelial cells release various active substances, which can be used to maintain stability of internal environment and normal progress of life activities, which is of great significance. The morphological and structural damage and functional changes of endothelial cells will lead to damage of vascular barrier function, and lipids and monocytes in blood and fluid will be deposited into subendothelial space, and further foam cells will be formed. Oxidative stress can increase level of ROS in body, and then oxidize LDL to form oxLDL, which is key to formation of foam cells; it can promote cell adhesion to endothelium and subendothelial chemotaxis, further damage function of endothelial cells, and aggravate inflammation of AS. In contrast, SNHG9, as a newly discovered oxygen-carrying globin, can store and transport O2, and has functions such as binding and scavenging NO. Studies have shown that SNHG9 can be up-regulated during cellular oxidative stress and scavenge excessive ROS. In this

experiment, an oxidative damage model was constructed by treating HUVECs with oxLDL. The research firstly cultured HUVECs in vitro and observed typical paving stone-like state of HUVECs using an inverted microscope. The expression of SNHG9 protein was up regulated when cells were oxidatively damaged. HUVECs were treated with 25g/ml, 50μg/ml, 100ug/ml and 200g/ml oxLDL for 24h. The expression of SNHG9 was higher than that of control, and expression increased in a concentration-dependent manner. The HUVECs were treated with 100ug/ml oxLDL for 0, 4, 8, 12, 24 h, and 24 h treatment had highest expression of SNHG9 ($P<0.05$). Therefore, SNHG9 is associated with intracellular oxidative stress.

5. Conclusion

In conclusion, the study "Role of lncRNA SNHG9 in Enhancing Rehabilitation Outcomes for Sports-Related Endothelial Damage: A Focus on the AMPK/SIRT1-PGC-1α Pathway in Physical Therapy" offers groundbreaking insights into the protective role of lncRNA SNHG9 in mitigating oxidative stress-induced endothelial damage, pertinent to athletic injuries. The research highlights that SNHG9, through its involvement in the AMPK/SIRT1-PGC-1α pathway, significantly enhances cellular defense mechanisms such as increased activity of antioxidants (SOD and GSH-PX) and upregulation of Bcl2, while reducing damage markers like LDH and MDA in endothelial cells under oxidative stress. These findings are especially relevant for athletes undergoing rehabilitation for sports-related endothelial injuries. The ability of SNHG9 to counteract oxidative stress suggests its potential as a therapeutic target in physical therapy protocols. By manipulating the expression of SNHG9, it may be possible to accelerate recovery and improve outcomes for athletes who suffer from such injuries.

Moreover, this study extends the understanding of molecular pathways in sports medicine, bridging the gap between cellular biology and practical therapeutic applications. It underscores the importance of personalized and targeted approaches in rehabilitation, offering a novel perspective on the use of molecular biology to enhance recovery processes. Future research based on these findings could lead to the development of new treatments and rehabilitation strategies, tailored to the specific needs of athletes recovering from endothelial injuries.

Data Availability

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the

publication of this paper.

Acknowledgments

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