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Targeting the E3 ligase RLIM to regulate VSMC phenotypic switching in vascular aging: implications for cold stress

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Abstract

Objective: Cold exposure may impair vascular function and promote cardiovascular diseases (CVDs) by causing vasoconstriction, hemodynamic changes, and sympathetic activation. Vascular aging, a key factor in CVDs, is linked to phenotypic switching of vascular smooth muscle cells (VSMCs), but its regulatory mechanisms are not fully understood. **Materials and methods:** We used aged C57BL/6 mice and D-galactose-induced senescent VSMCs to investigate the role of the E3 ligase RLIM in arterial aging. RLIM knockdown and overexpression *in vivo* were achieved using adeno-associated virus (AAV) vectors. Vascular aging and stiffness were assessed using β-galactosidase staining, pulse wave velocity (PWV) measurements, and histological staining. Proteomic profiling was conducted to identify key protein alterations associated with vascular dysfunction and to elucidate underlying mechanisms. **Results:** RLIM expression was significantly upregulated in the aortae of aged mice and D-galactose-induced senescent VSMCs. AAV-mediated RLIM knockdown significantly attenuated vascular aging, as evidenced by vascular ultrasound and histological assessments. Conversely, RLIM overexpression exacerbated vascular damage. Proteomic analysis revealed that RLIM knockdown in VSMCs from aged mice resulted in increased expression of smooth muscle contractile proteins and decreased levels of inflammatory markers, indicating a phenotypic shift toward a more contractile state. **Conclusion:** These findings identify RLIM as a key regulator of arterial aging and a promising therapeutic target for age-related cardiovascular diseases.

Keywords

arterial aging; vascular remodeling; ubiquitination; vascular smooth muscle cell; phenotypic switching

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1 Introduction

The impact of cold environments on human health is gaining increasing attention, particularly among the elderly[1-2]. Cold exposure adversely affects the vascular system, leading to vasoconstriction, increased blood pressure, and altered hemodynamics[3-5]. Prolonged exposure to low temperatures has been shown to exacerbate vascular dysfunction, with older adults especially susceptible to age-related vascular decline[6]. Cold-induced vasoconstriction and increased cardiac workload place additional stress on the vasculature, potentially accelerating the development of vascular diseases such as atherosclerosis and hypertension[7-9].

As global life expectancy rises, the aging population expands, posing challenges to healthcare systems[10-12]. This demographic shift, coupled with the heightened vulnerability of older individuals to environmental and physiological stressors, has led to a surge in age-related diseases, most notably cardiovascular diseases (CVDs), which remain the leading cause of mortality in the elderly[13]. Age-associated structural and functional alterations in arteries include endothelial dysfunction, VSMC phenotypic switching, and extracellular matrix (ECM) remodeling[14-16]. These alterations result in increased vascular

stiffness, diminished elasticity, and compromised compliance, thereby elevating the risk of chronic vascular conditions such as atherosclerosis, aortic aneurysms, renal artery disease, and peripheral artery disease[17-20].

Aging promotes the phenotypic transition of vascular smooth muscle cells (VSMCs) from a contractile state to a synthetic or senescence-associated secretory phenotype (SASP)[21-22]. This transition involves the loss of contractile proteins, leading to reduced contractile capacity and vascular compliance. Concurrently, pro-inflammatory cytokines and matrix metalloproteinases (MMPs) are upregulated, contributing to ECM remodeling characterized by elastic fiber degradation and excessive collagen deposition[23]. These pathological changes contribute to arterial stiffening and functional deterioration.

Recent studies have shown that VSMC senescence is regulated by multiple molecular pathways, with disruption of protein homeostasis plays a central role[24-25]. Ring Finger protein-LIM domain interacting (RLIM), an E3 ubiquitin ligase, is involved in cell cycle regulation, telomere maintenance, and modulation of the TGF-β signaling, all relevant to vascular aging[26]. RLIM inhibits c-MYC activity and upregulates p15 and p21 by interacting with MIZ1. It also promotes the ubiquitination and degradation of MDM2, thereby activating p53 and inducing cell cycle arrest[27-29]. Furthermore, RLIM facilitates the ubiquitination of the telomere-binding protein (TRF1), leading to telomere shortening and suppression of cell proliferation[30]. It also activates TGF-β signaling by targeting Smad7, a negative regulator of the pathway, for degradation[31].

This study investigates RLIM expression and its role in vascular aging by evaluating its effects on aging-related vascular changes in young and aged mice. Proteomic analysis of aortic tissue from aged mice with VSMC-specific RLIM knockdown helps identify molecular mechanisms involved, aiming to uncover potential therapeutic targets for age-related cardiovascular diseases.

2 Materials and methods

2.1 Animals

All animal procedures were conducted by the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Review Board of Harbin Medical University (IRB3061724). Female C57BL/6J mice were purchased from Nanjing Cavans Biotechnology Company. To investigate RLIM expression during arterial aging, 3-month-old (young) and 24-month-old (aged) mice were used. For adeno-associated virus (AAV)-mediated gene intervention experiments, 3-month-old (young) and 18-month-old (aged) mice were utilized. Mice were housed under specific pathogen-free (SPF) conditions with controlled temperature (21 ± 2 °C), humidity (55 ± 10%), and a 12-hour light/dark cycle. Animals were provided with standard pellet feed and housed in groups of no more than five mice per cage. To eliminate the confounding effects of male aggression and associated mortality, only female mice were used in this study. Group allocation was performed randomly using a random number table. The technician responsible for daily care and evaluation was blinded to the group assignments and physical characteristics of the mice.

2.2 Cell culture

Mouse vascular smooth muscle cells (MOVAS) were obtained from American Type Culture Collection (ATCC) and cultured in DMEM (11965092, Gibco, California, USA) supplemented with 10% fetal bovine serum (C2910-0500, VivaCell, Shanghai, China), 0.2 mg/mL G418 (A1720, Sigma, Missouri, USA), and 1% penicillin-streptomycin. Cells were maintained in a humidified incubator at 37 °C with 5% CO_2. The culture medium was refreshed every 2-3 days, and subculturing was performed once cell confluence reached 80%-90%.

2.3 Statistical analysis

Data are presented as mean ± standard deviation (SD). Normality was assessed using the Shapiro-Wilk test. Group comparisons were made using unpaired Student's *t*-test, with Bonferroni correction applied for multiple comparisons. Statistical analyses were performed using GraphPad Prism 9.5 and SPSS 26.0. A P value < 0.05 was considered statistically significant. Detailed methods are available in the Supplementary Methods.

3 Result

3.1 Elevated expression of RLIM in aging aorta

To explore the potential involvement of E3 ubiquitin ligases in vascular aging, we analyzed data from the *Tabula Muris Senis* single-cell transcriptomic atlas[32], which includes comprehensive gene expression profiles from multiple tissues of young and aged mice. In VSMCs of the aorta, six E3 ligases showed differential expression, with RLIM exhibiting the most significant age-related upregulation (Fig. 1A). To validate these findings, we performed quantitative real-time PCR (qRT-PCR) on aortic tissues from young and aged mice. The results confirmed a marked increase in Rlim mRNA expression in aged aortae, whereas Rnf130 expression was only slightly elevated (Fig. 1B). Consistently, Western blot analysis revealed higher RLIM protein levels in the aortic tissue of aged mice compared to young controls (Fig. 1C). Immunohistochemistry confirmed increased RLIM expression in

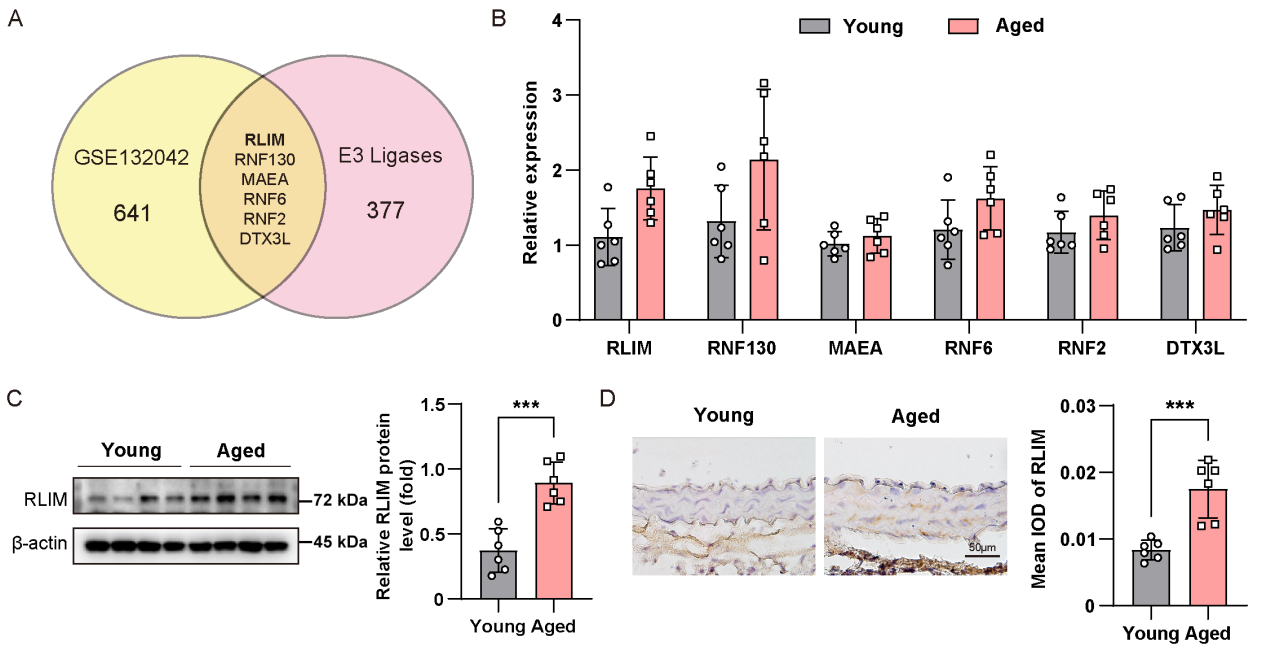

Fig. 1 Elevated expression of ring finger protein-LIM domain interacting (RLIM) in aortic tissues from aged mice

(A) Venn diagram showing six differentially expressed E3 ligases in aortic vascular smooth muscle cells (VSMCs) from young and aged mice based on Tabula Muris Senis single-cell RNA-seq data. (B) qRT-PCR analysis of selected E3 ligases in aortae of young and aged mice. (C) Western blot analysis of RLIM protein levels in aortic tissues. (D) Immunohistochemistry showing increased RLIM expression in the medial layer of aged aortic sections. Scale bars, 50 μm. Statistical differences were analyzed using an unpaired Student's *t*-test. $N = 6$ per group. Data are expressed as mean ± SD. $^{***}P < 0.001$.

aged aortae, particularly localized to the medial layer, which is enriched in VSMCs (Fig. 1D). Collectively, these results suggest that RLIM is upregulated in the aging aorta and may contribute to the molecular processes underlying vascular aging.

3.2 Elevated RLIM expression in D-galactose-induced senescent MOVAS

To determine whether RLIM expression is associated with cellular senescence *in vitro*, we established a senescence model using MOVAS cells treated with D-galactose. Cells were exposed to various concentrations of D-galactose (0, 5, 25, and 50 mmol/L) for different durations (12, 24, 36, and 48 hours). SA-β-galactosidase (SA-β-gal) staining revealed a significant increase in the proportion of senescent cells at 36- and 48-hours following treatment with 25 mmol/L and 50 mmol/L D-galactose (Fig. 2A). Cell proliferation was significantly reduced, as demonstrated by cell counting kit-8 (CCK-8) and EdU incorporation assays (Fig. 2B and 2C). Western blot analysis showed increased levels of senescence markers p21 and p16, along with elevated RLIM protein after 36 hours of treatment with 25 mmol/L D-galactose (Fig. 2D and S1A), suggesting RLIM's involvement in VSMC senescence.

3.3 RLIM knockdown in VSMCs alleviates vascular aging

To investigate RLIM's role in vascular aging, we constructed a VSMC-specific AAV9 vector (AAV9-SM22α-shRLIM) for *in vivo* gene knockdown. Young (3-month-old) and aged (18-month-old) mice received tail vein injections of AAV9-SM22α-shRLIM or control vector (Fig. 3A). RLIM knockdown in the aortic VSMCs of aged mice was confirmed by immunohistochemistry (Fig. S2A). No significant changes in blood pressure or heart rate were observed between RLIM-knockdown and control mice (Fig. S2B). The intensity of SA-β-gal staining in the aortae of aged AAV9-SM22a-shRLIM-injected mice was significantly lower than that in aged control mice (Fig. 3B). Pulse wave velocity (PWV) in the aortic arch was significantly reduced, with a trend in the carotid artery (Fig. 3C and S2C). Histological analysis showed reduced medial thickening, fewer elastic fiber breaks, and less collagen deposition (Fig. 3E). Collectively, these findings demonstrate that VSMC-specific RLIM knockdown attenuates age-related vascular remodeling and stiffness, suggesting a protective role against vascular aging.

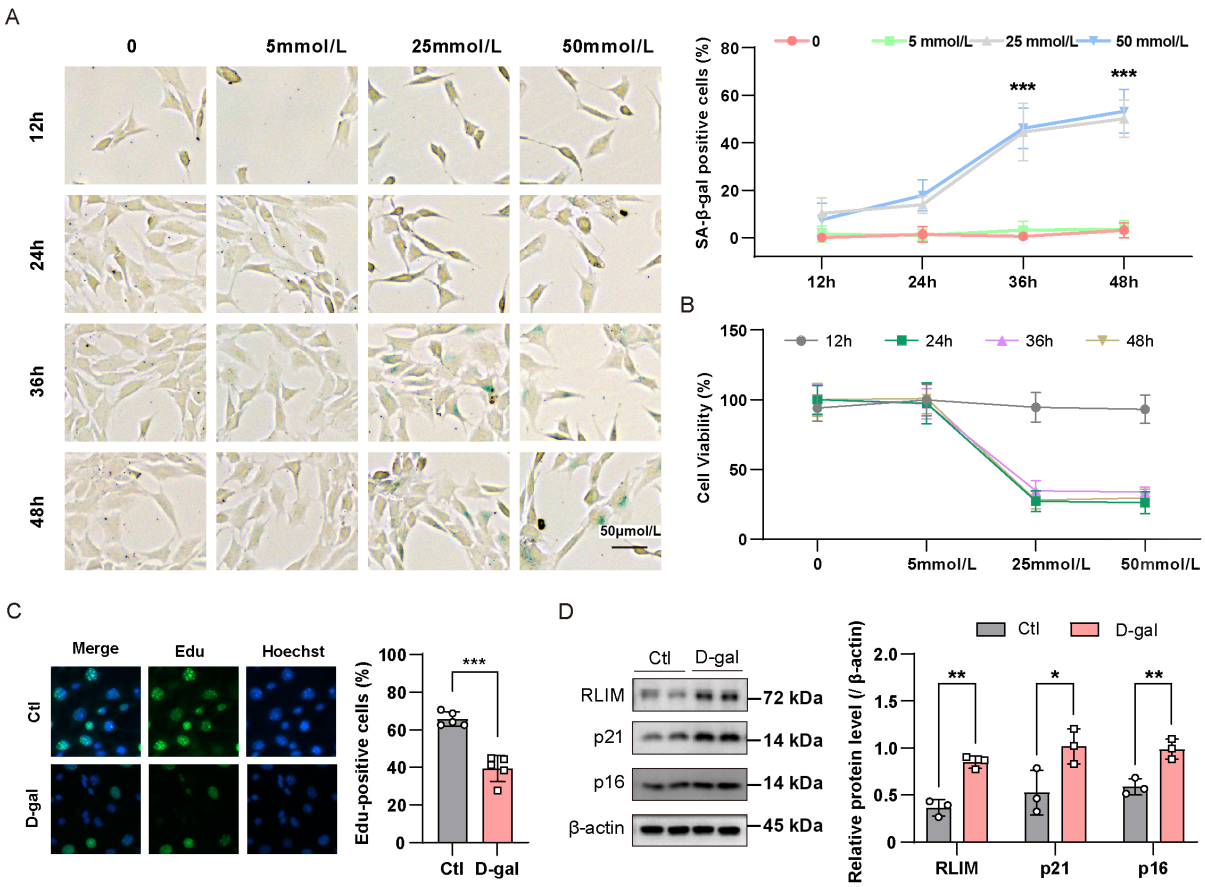

Fig. 2 Upregulation of ring finger protein-LIM domain interacting (RLIM) in D-galactose-induced senescence of mouse vascular smooth muscle cells (MOVAS) cells (A) SA-β-gal staining of MOVAS cells treated with 0, 5, 25, or 50 mmol/L D-galactose for 12, 24, 36, or 48 hours. Quantification of SA-β-gal-positive cells is shown in the line graph. Scale bars, 50 μm. (B) Cell counting kit-8 (CCK-8) assay at different D-galactose concentrations and time points. (C) EdU incorporation assay assessing cell proliferation in control and D-galactose-treated MOVAS cells. Scale bars, 50 μm. (D) Western blot analysis of RLIM, p21, and p16 expression in MOVAS cells treated with D-galactose compared to control. Statistical comparisons were performed using unpaired Student's *t*-test. $N = 3$-5 per group. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.4 RLIM overexpression in VSMCs aggravates vascular aging

To investigate RLIM's role in aortic aging, we constructed an AAV9-SM22α-RLIM vector to overexpress RLIM in VSMCs. Young (3-month-old) and aged (18-month-old) mice received three injections at two-month intervals (Fig. 4A). RLIM expression in aortic VSMCs was significantly elevated in young mice after injection (Fig. S3A), with no significant changes in blood pressure or heart rate (Fig. S3B). SA-β-gal staining intensity was higher in RLIM-overexpressing young mice than in controls (Fig. 4B). PWV increased in both the aortic arch and left carotid artery (Fig. 4C). RLIM overexpression also reduced running distance, work output (Fig. 4D), and grip strength (Fig. S3C). Young AAV9-SM22a-RLIM-treated mice exhibited increased medial thickness, more elastic fiber breaks, and greater collagen deposition in the thoracic aorta compared to young controls (Fig. 4E). These findings suggest that RLIM promotes vascular aging and remodeling.

3.5 RLIM knockdown regulates VSMC phenotypic switching during arterial aging

Protein homeostasis is a hallmark of healthy aging, and its disruption contributes to vascular dysfunction. E3 ubiquitin ligases, through the ubiquitin-proteasome system, play a pivotal role in regulating protein turnover. Dysregulation of E3 ligases during aging may impair protein degradation, leading to cellular dysfunction. Prior studies have linked E3 ligases to vascular aging, for instance, HRD1 deficiency accelerates VSMC senescence under cholesterol-induced stress, and impaired Mdm2 function enhances p53 stability, promoting senescence.

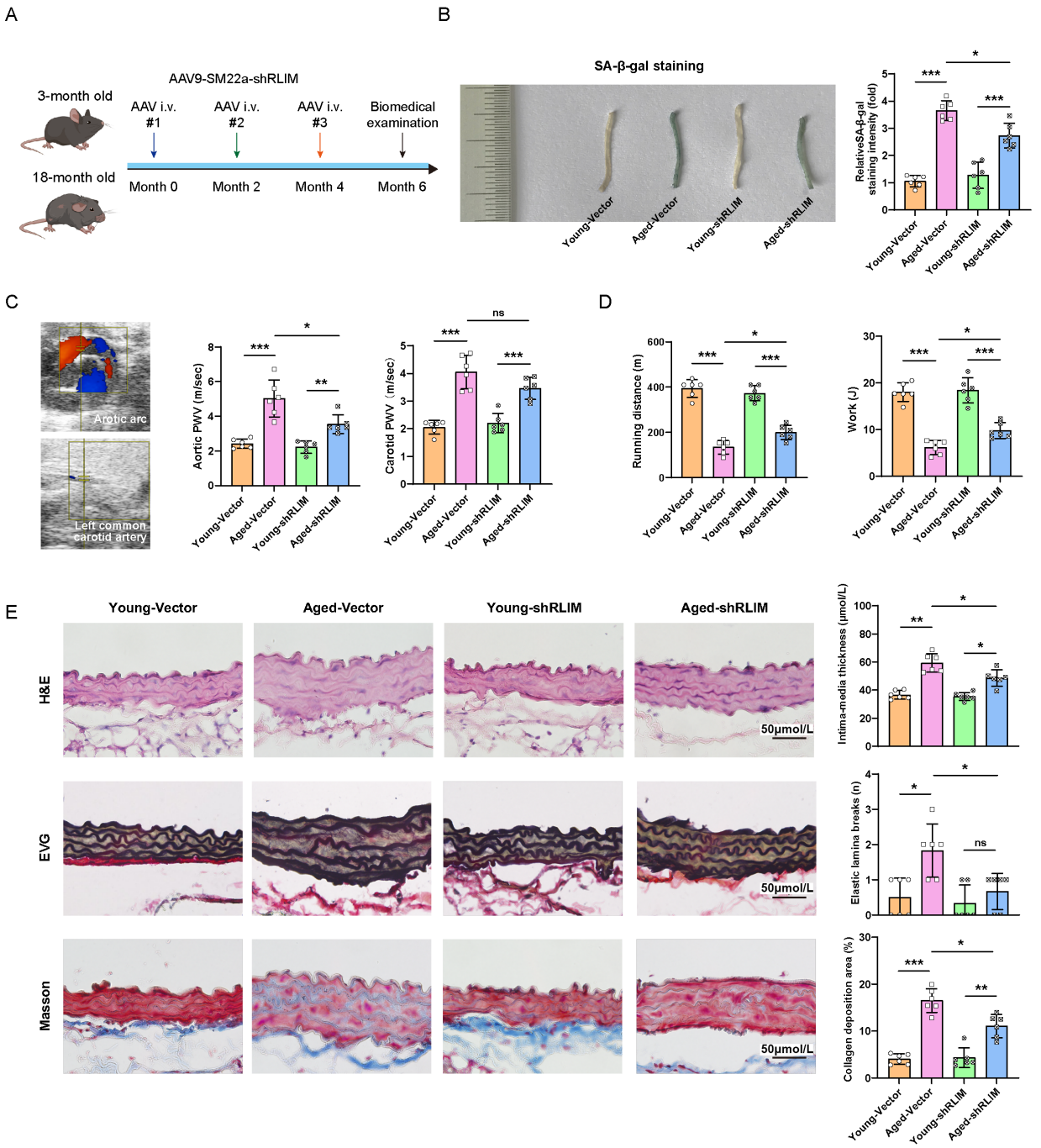

Fig. 3 Effects of ring finger protein-LIM domain interacting (RLIM) knockdown on vascular aging in young and aged mice

(A) Experimental timeline for adenoassociated virus (AAV)9-SM22α-shRLIM injection in young (3-month) and aged (18-month) mice. (B) SA-β-gal staining of aortae from young and aged mice treated with AAV9-SM22α-shRLIM or vector control. (C) Pulse wave velocity (PWV) measurements in the aortic arch and left common carotid artery of young and aged mice following RLIM knockdown in vascular smooth muscle cells (VSMCs). (D) Treadmill performance in young and aged mice with or without RLIM knockdown in VSMCs. (E) Histological images of aortic sections stained with hematoxylin and eosin (H&E), elastica-van-gieson (EVG), and Masson's trichrome in RLIM knockdown and control mice. Scale bars, 50 μm. Statistical analysis was performed using unpaired Student's *t*-test. $N = 6$ per group. Data are presented as mean ± SD. $^{*}P < 0.017$, $^{**}P < 0.003$, $^{***}P < 0.0003$ after Bonferroni correction.

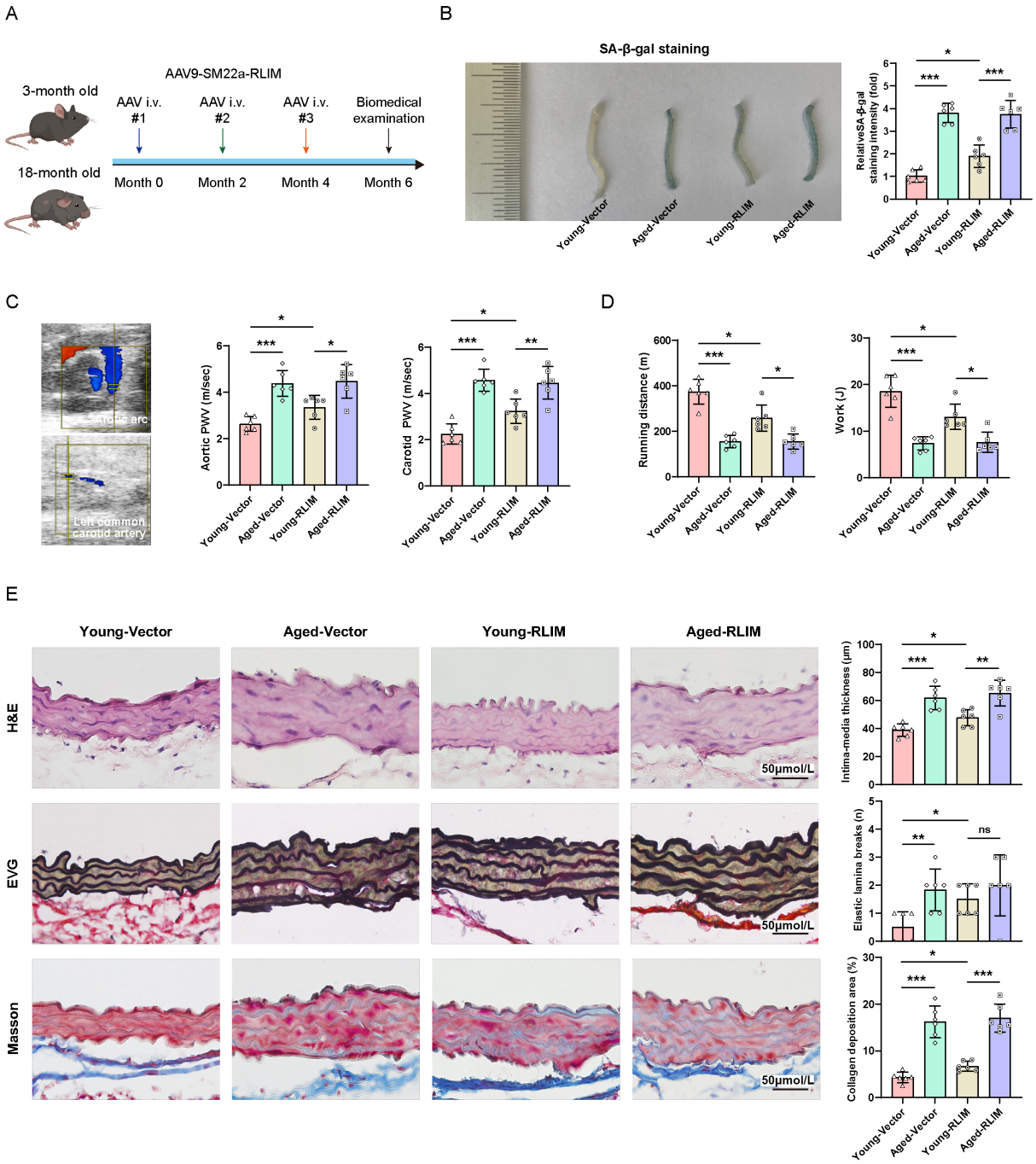

Fig. 4 Effects of ring finger protein-LIM domain interacting (RLIM) overexpression on vascular aging and function in young and aged mice

(A) Experimental timeline of adenoassociated virus (AAV)9-SM22α-RLIM injections in young (3-month) and aged (18-month) mice. (B) Representative SA-β-gal staining of aortic tissues from young and aged mice treated with AAV9-SM22α-RLIM or vector control. (C) Pulse wave velocity (PWV) measurements in the aortic arch and left common carotid artery of young and aged mice with or without RLIM overexpression in vascular smooth muscle cells (VSMCs). (D) Treadmill performance in young and aged mice following RLIM overexpression in VSMCs. (E) Histological staining hematoxylin and eosin (H&E), elastica-Van-Gieson (EVG), Masson's trichrome of aortic sections in RLIM-overexpressing and control mice. Scale bars, 50 μm. Statistical analysis was performed using unpaired Student's *t*-test. $N = 6$ per group. Data are expressed as mean ± SD. $^{*}P < 0.017$, $^{**}P < 0.003$, $^{***}P < 0.0003$ after Bonferroni correction for multiple comparisons.

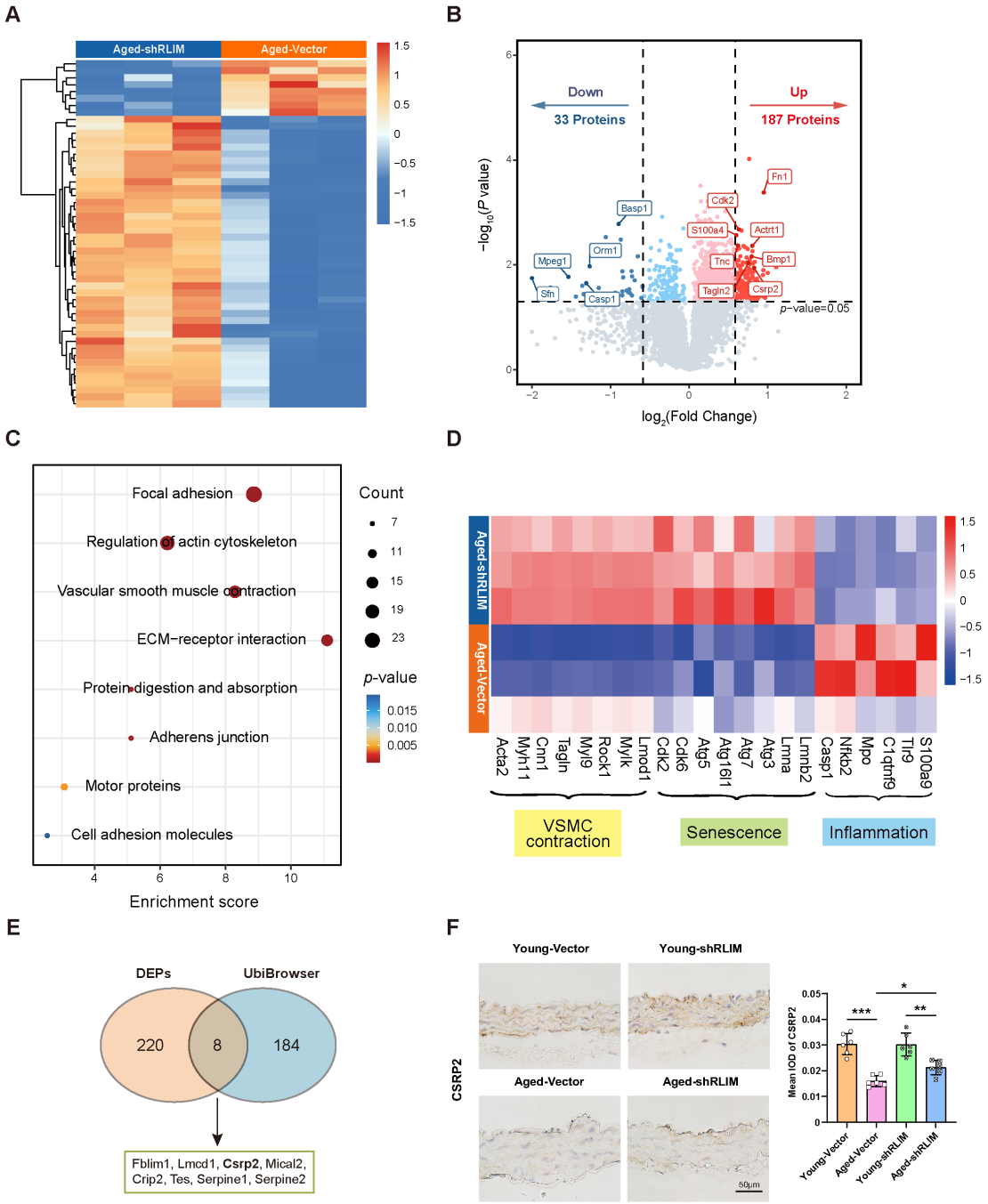

Fig. 5 Proteomic analysis of aortic tissue from aged mice with vascular smooth muscle cell (VSMC)-specific ring finger protein-LIM domain interacting (RLIM) knockdown (A-B) Heatmap (A) and volcano plot (B) showing differentially expressed proteins (DEPs) in the aortae of aged mice treated with adenoassociated virus (AAV)9-SM22α-shRLIM compared to control mice, based on $P < 0.05$ and $|\log_2 \text{fold change}| \geq 0.585$. (C) Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis of DEPs. Bubble size indicates the number of proteins involved in each pathway; color gradient represents statistical significance. (D) Heatmap of DEPs involved in smooth muscle contraction, cell cycle regulation, autophagy, and inflammation. (E) Venn diagram illustrating the overlap between DEPs and predicted RLIM ubiquitination substrates from the Ubibrowser 2.0 database. (F) Immunohistochemical staining of CSRP2 in aortic sections from young and aged mice with or without RLIM knockdown in VSMCs. Scale bars, 50 μm. Statistical analysis was performed using unpaired Student's *t*-test. $N = 6$ per group. Data are expressed as mean ± SD. $^{*}P < 0.017$, $^{**}P < 0.003$, $^{***}P < 0.0003$ after Bonferroni correction.

To explore RLIM's role in arterial aging, we performed proteomic analysis of aortae from aged mice with VSMC-specific RLIM knockdown versus controls. A total of 187 proteins were upregulated and 33 downregulated in AAV9-SM22α-shRLIM-treated mice (Fig. 5A-B). KEGG pathway analysis revealed enrichment in cytoskeletal regulation, smooth muscle function, and cell adhesion (Fig. 5C). Heatmap analysis showed increased expression of contractile proteins, cell cycle regulators, and autophagy markers, while inflammatory proteins were reduced, indicating a shift toward a contractile phenotype (Fig. 5D). Cross-referencing differentially expressed proteins with predicted RLIM ubiquitination targets (Ubibrowser 2.0) identified eight candidates (Fig. 5E). Among them, CSRP2, a regulator of VSMC phenotype, was significantly upregulated, as confirmed by immunohistochemistry (Fig. 5F). These findings suggest that RLIM knockdown may promote a contractile VSMC phenotype *via* CSRP2 upregulation, potentially mitigating arterial aging.

4 Discussion

This study identified RLIM, an E3 ubiquitin ligase, as a key regulator of VSMC phenotypic switching during arterial aging. RLIM expression was significantly upregulated in the aortae of aged mice. Knockdown of RLIM alleviated aging-induced vascular structural and functional deterioration, whereas its overexpression exacerbated vascular damage. Proteomic profiling revealed that RLIM knockdown increased contractile protein expression and reduced inflammation-related proteins, consistent with a shift toward a more contractile VSMC phenotype. These findings provide new insights into the role of RLIM in vascular aging and suggest RLIM as a potential therapeutic target for age-related cardiovascular diseases.

VSMCs are essential for maintaining arterial tone and structural integrity[33]. Under physiological conditions, they exhibit a contractile phenotype characterized by high levels of contractile proteins, low proliferation, and limited protein synthesis[34]. In response to aging or pathological stimuli, VSMCs undergo phenotypic switching to a synthetic and senescence-associated secretory phenotype (SASP), marked by increased cell size, reduced contractile protein expression, enhanced extracellular matrix (ECM) production, and secretion of pro-inflammatory cytokines and matrix metalloproteinases (MMPs)[21-22]. This phenotypic transition contributes to arterial stiffening, loss of vascular compliance, and increased risk of hypertension and vascular injury[35-36].

Loss of protein homeostasis is a hallmark of aging and contributes to vascular dysfunction[37]. E3 ubiquitin ligases are key regulators of protein turnover via the ubiquitin-proteasome system[38]. For instance, loss of HRD1 accelerates VSMC senescence under cholesterol stress, while SM22α accumulation blocks Mdm2-mediated p53 degradation, promoting Ang II-induced senescence[24-25].

Single-cell RNA sequencing of aortic VSMCs from young and aged mice identified six differentially expressed E3 ligases, among which RLIM was notably upregulated in aged vessels (Fig. 1A). This upregulation was validated by qRT-PCR, Western blot, and immunohistochemistry. (Fig. 1B-D). *In vitro*, D-galactose-induced senescence in MOVAS cells was confirmed by SA-β-gal staining, and further validated by CCK-8 and EdU assays, as well as increased p21 and p16 expression (Fig. 2A-D).

To investigate the role of RLIM in VSMC senescence, we used AAV vectors to knock down or overexpress RLIM in young and aged mice. We demonstrated that VSMC-specific knockdown of RLIM significantly alleviated vascular aging, as evidenced by reduced PWV, improved exercise performance, and less elastic fiber damage. In contrast, overexpression of RLIM had detrimental effects on vascular aging. These findings suggest that RLIM plays a key regulatory role in the progression of vascular senescence.

To further investigate the mechanisms of RLIM in vascular aging, we performed proteomic analysis on the aortae of naturally aged mice with VSMC-specific RLIM knockdown and controls. Enrichment analysis highlighted pathways associated with cytoskeletal remodeling, cell adhesion, and inflammation. RLIM knockdown led to the upregulation of smooth muscle contraction proteins and downregulation of inflammatory proteins, promoting a more contractile VSMC phenotype.

Our findings not only shed light on the molecular mechanisms of RLIM in vascular aging but also open up new therapeutic avenues for treating age-related cardiovascular diseases. AAV vectors for gene therapy offer excellent safety and low immunogenicity, enabling long-term stable gene expression without triggering significant immune responses[39]. Therefore, targeting RLIM could represent a promising therapeutic strategy to delay or reverse vascular aging and improve cardiovascular health. Future studies could explore genetic editing approaches or small-molecule inhibitors to regulate RLIM expression or activity, providing a foundation for developing novel treatments for age-related vascular diseases.

5 Conclusion

In summary, we identified RLIM as a key regulator of VSMC phenotypic switching and vascular aging. Targeted inhibition of RLIM in VSMCs alleviates structural and functional deterioration of the aorta, highlighting its therapeutic potential in

combating age-related vascular diseases. These findings advance our understanding of vascular aging and lay the groundwork for future therapeutic developments aimed at improving cardiovascular health in the elderly.

Acknowledgements

Not applicable.

Research ethics

Not applicable.

Informed consent

Not applicable.

Author contributions

Jiang W Q performed the experiments and collected data. Guo M D assisted with the experiments. Wang X and Liu X contributed to the study design and methodology. Zhang Y supervised the project and provided funding support.

Use of Large Language Models, AI and Machine Learning Tools

No LLM, AI or machine learning tool was used for any part of the present study.

Research funding

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Conflict of interest

Zhang Y is an Editorial Board Member of Frigid Zone Medicine. The article was subject to the journal's standard procedures, with peer review handled independently of this Member and his research groups.

Data availability

All data used during the study are available from the corresponding author by request.

References

[1] Song X, Wang S, Hu Y, *et al.* Impact of ambient temperature on morbidity and mortality: An overview of reviews. Sci Total Environ, 2017; 586: 241-254.
[2] Bunker A, Wildenhain J, Vandenbergh A, *et al.* Effects of air temperature on climate-sensitive mortality and morbidity outcomes in the elderly; a systematic review and meta-analysis of epidemiological evidence. EBioMedicine, 2016; 6: 258-268.
[3] Liu C, Yavar Z, Sun Q. Cardiovascular response to thermoregulatory challenges. Am J Physiol Heart Circ Physiol, 2015; 309(11): H1793-H1812.
[4] Fares A. Winter cardiovascular diseases phenomenon. N Am J Med Sci, 2013; 5(4): 266-279.
[5] Shepherd J T, Rusch N J, Vanhoutte P M. Effect of cold on the blood vessel wall. Gen Pharmacol, 1983; 14(1): 61-64.
[6] Hess K L, Wilson T E, Sauder C L, *et al.* Aging affects the cardiovascular responses to cold stress in humans. J Appl Physiol (1985), 2009; 107(4): 1076-1082.
[7] Dong M, Yang X, Lim S, *et al.* Cold exposure promotes atherosclerotic plaque growth and instability via UCP1-dependent lipolysis. Cell Metab, 2013; 18(1): 118-129.
[8] van Bergen P, Fregly M J, Rossi F, *et al.* The effect of intermittent exposure to cold on the development of hypertension in the rat. Am J Hypertens, 1992; 5(8): 548-555.
[9] Fu S, Cao Y, Li Y. [Epidemiological study of hypertension in Heilongjiang province]. Zhonghua Nei Ke Za Zhi, 2022; 41: 114-116.
[10] Kontis V, Bennett J E, Mathers C D, *et al.* Future life expectancy in 35 industrialised countries: projections with a Bayesian model ensemble. Lancet, 2017; 389(10076): 1323-1335.
[11] Department of Economic and Social Affairs. Leaving no one behind in an ageing world (World Social Report 2023). New York: World Population Ageing, 2019.
[12] Yazdanyar A, Newman A B. The burden of cardiovascular disease in the elderly: morbidity, mortality, and costs. Clin Geriatr Med, 2009; 25(4): 563-vii.
[13] Fajemiroye J O, da Cunha L C, Saavedra-Rodríguez R, *et al.* Aging-induced biological changes and cardiovascular diseases. Biomed Res Int, 2018; 2018: 7156435.
[14] Laurent S. Defining vascular aging and cardiovascular risk. J Hypertens, 2012; 30 Suppl: S3-S8.
[15] Ungvari Z, Tarantini S, Kiss T, *et al.* Endothelial dysfunction and angiogenesis impairment in the ageing vasculature. Nat Rev Cardiol, 2018; 15(9): 555-565.
[16] Lacolley P, Regnault V, Nicoletti A, *et al.* The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles. Cardiovasc Res, 2012; 95(2): 194-204.
[17] Abdellatif M, Sedej S, Carmona-Gutierrez D, *et al.* Autophagy in cardiovascular aging. Circ Res, 2018; 123(7): 803-824.
[18] Wang J C, Bennett M. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. Circ Res, 2012; 111(2): 245-259.

[19] Mian O, Santi N, Boodhwani M, *et al.* Arterial age and early vascular aging, but not chronological age, are associated with faster thoracic aortic aneurysm growth. J Am Heart Assoc, 2023; 12(16): e029466.
[20] Matsumoto T, Watanabe S, Ando M, *et al.* Diabetes and age-related differences in vascular function of renal artery: possible involvement of endoplasmic reticulum stress. Rejuvenation Res, 2016; 19(1): 41-52.
[21] Lacolley P, Regnault V, Avolio A P. Smooth muscle cell and arterial aging: basic and clinical aspects. Cardiovasc Res, 2018; 114(4): 513-528.
[22] Owens G K, Kumar M S, Wamhoff B R. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev, 2004; 84(3): 767-801.
[23] Monk B A, George S J. The Effect of ageing on vascular smooth muscle cell behaviour--a mini-review. Gerontology, 2015; 61(5): 416-426.
[24] Wang L, Wang M, Niu H, *et al.* Cholesterol-induced HRD1 reduction accelerates vascular smooth muscle cell senescence via stimulation of endoplasmic reticulum stress-induced reactive oxygen species. J Mol Cell Cardiol, 2024; 187: 51-64.
[25] Miao S B, Xie X L, Yin Y J, *et al.* Accumulation of smooth muscle 22α protein accelerates senescence of vascular smooth muscle cells via stabilization of p53 *in vitro* and *in vivo*. Arterioscler Thromb Vasc Biol, 2017; 37(10): 1849-1859.
[26] Wang F, Bach I. Rlim/Rnf12, Rex1, and X chromosome inactivation. Front Cell Dev Biol, 2019; 7: 258.
[27] Huang Y, Nie M, Li C, *et al.* RLIM suppresses hepatocellular carcinogenesis by up-regulating p15 and p21. Oncotarget, 2017; 8(47): 83075-83087.
[28] Gao K, Wang C, Jin X, *et al.* RNF12 promotes p53-dependent cell growth suppression and apoptosis by targeting MDM2 for destruction. Cancer Lett, 2016; 375(1): 133-141.
[29] Gao R, Wang L, Cai H, *et al.* E3 ubiquitin ligase RLIM negatively regulates c-Myc transcriptional activity and restrains cell proliferation. PLoS One, 2016; 11(9): e0164086.
[30] Her Y R, Chung I K. Ubiquitin ligase RLIM modulates telomere length homeostasis through a proteolysis of TRF1. J Biol Chem, 2009; 284(13): 8557-8566.
[31] Zhang L, Huang H, Zhou F, *et al.* RNF12 controls embryonic stem cell fate and morphogenesis in zebrafish embryos by targeting Smad7 for degradation. Mol Cell, 2012; 46(5): 650-661
[32] Tabula Muris Consortium. A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. Nature, 2020; 583(7817): 590-595.
[33] Frismantiene A, Philippova M, Erne P, *et al.* Smooth muscle cell-driven vascular diseases and molecular mechanisms of VSMC plasticity. Cell Signal, 2018; 52: 48-64.
[34] Zhang M J, Zhou Y, Chen L, *et al.* An overview of potential molecular mechanisms involved in VSMC phenotypic modulation. Histochem Cell Biol, 2016; 145(2): 119-130.
[35] Chi C, Li D J, Jiang Y J, *et al.* Vascular smooth muscle cell senescence and age-related diseases: State of the art. Biochim Biophys Acta Mol Basis Dis, 2019; 1865(7): 1810-1821.
[36] Uryga A K, Bennett M R. Ageing induced vascular smooth muscle cell senescence in atherosclerosis. J Physiol, 2016; 594(8): 2115-2124.
[37] Ungvari Z, Tarantini S, Donato A J, *et al.* Mechanisms of vascular aging. Circ Res, 2018; 123(7): 849-867.
[38] Callis J. The ubiquitination machinery of the ubiquitin system. Arabidopsis Book, 2014; 12: e0174.
[39] Pupo A, Fernández A, Low S H, *et al.* AAV vectors: the Rubik's cube of human gene therapy. Mol Ther, 2022; 30(12): 3515-3541.