Redox Biology 70 (2024) 103063

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

The mechanism of selenium regulating the permeability of vascular endothelial cells through selenoprotein O

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ARTICLE INFO

Keywords:
Selenoprotein
Cardiovascular disease
Vascular injury
Adhesion
Intercellular junctions

ABSTRACT

Vascular diseases, a leading cause of death in human, are strongly associated with pathological damage to blood vessels. The selenoprotein (Sel) have been reported to play important roles in vascular disease. However, the role of SelO in vascular disease has not been conclusively investigated. The present experiment was to investigate the regulatory mechanism of the effect of SelO on the permeability of vascular endothelial. The H.E staining, FITC-Dextran staining, Dil-AC-LDL staining and FITC-WGA staining showed that vascular structure was damaged, and intercellular junctions were disrupted with selenium (Se)-deficient. Immunohistochemistry, qPCR and Western blot revealed decreased expression of the adhesion plaque proteins vinculin, talin and paxillin, decreased expression of the vascular connectivity effector molecules connexin, claudin-1 and E-cadherin and increased expression of JAM-A and N-cadherin, as well as decreased expression of the ZO-1 signaling pathways ZO-1, Rock, rhoGEF, cingulin and MLC-2. In a screening of 24 Sel present in mice, SelO showed the most pronounced changes in vascular tissues, and a possible association between SelO and vascular intercellular junction effectors was determined using IBM SPSS Statistics 25. Silencing of SelO, vascular endothelial intercellular junction adverse effects present. The regulatory relationship between SelO and vascular endothelial intercellular junctions was determined. The results showed that Se deficiency lead to increased vascular endothelial permeability and vascular tissue damage by decreasing SelO expression, suggesting a possible role for SelO in regulating vascular endothelial permeability.

1. Introduction

Selenium (Se), an essential trace element for the body, plays a crucial role in many physiological functions, including thyroid hormone metabolism, the antioxidant defence system, the adaptive and acquired immune systems, and the prevention of certain cancers [1–4]. A large number of studies have revealed that Se deficiency is closely related to the occurrence of many diseases. Se deficiency is associated with inflammation and Cardio-metabolic dysregulation in women with Hypertensive disorders of Pregnancy [5]. Se deficiency induced myocardial injury and exudative diathesis were more widely recognized in humans and animals [6,7]. In addition, selenium deficiency exacerbates the inflammatory response in lipopolysaccharide (LPS)-induced mastitis [8]. Se deficiency in the small intestine, an important site for selenium absorption, exacerbates bisphenol A-induced intestinal damage in chickens via reactive oxygen species/cellular tumor antigen p53 [9]. At the same time, numerous studies have shown Se was closely associated with inflammatory diseases, especially in the cardiovascular system [10]. Se palyed an anti-inflammatory role to impede systemic inflammatory cytokine axes to circumvent aggravating atherosclerosis in the post-surgical period [11]. The high serum selenium levels may reduce serum C-reactive protein levels in individuals with HIV to inhibit the inflammation [12].Se supplementation provides potent neuroprotection and reduced inflammation in cerebral ischemia [13].In contrast, Se deficiency induces inflammation in porcine aorta via miR-223/nucleotide-binding oligomerization domain-like receptor family 3 (NLRP3) [14]. There is increasing evidence that Se was also important for vascular function. Se deficiency or low serum Se levels are considered a risk factor for vascular disease. Reduced serum Se levels may lead to an inability to prevent oxidation of low-density lipoproteins, thereby accelerating the development of atherosclerotic plaques [15]. Selenium-stimulated exosomes showed significant effects in inhibiting inflammation and improving pro-angiogenesis in human umbilical vein endothelial cells [16]. In conclusion, selenium inhibits vascular

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https://doi.org/10.1016/j.redox.2024.103063
Received 26 December 2023; Received in revised form 25 January 2024; Accepted 25 January 2024
Available online 1 February 2024
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endothelial inflammation.

Se acts mainly through the incorporation of various selenoproteins (Sel), 24 of which, including selenocysteine (Sec), are known to exist in mice and rats. The substitution of selenium atoms for sulphur atoms and the tighter valence electrons of the selenium atoms have made the Sel a family of enzymes involved in various redox reactions [17]. The functions of some Sel were explored with inflammation. Sel shown a role on the oxidative stress-induced inflammatory tumorigenesis [18]. SelS shown an effect on the inflammation and coronary artery disease [19,20]. SelW was an anti-inflammatory role in the skeletal muscle and intestinal tract [21,22]. However, the function of most Sel still needs to be further explored. Some studies have found that certain Sel (e.g. Glutathione peroxidase 1 (GPX1), GPX4, SelS and SelP) also bind to perform a variety of reactions during the wound healing phase, such as antioxidant activity, inhibition of inflammatory cytokines, and elimination of peroxynitrates during the inflammatory phase [23,24]. SelO, as one of the largest mammalian Sel, contains the rare amino acid selenocysteine (Sec), which is thought to have redox properties, but its exact biological function is not known and its effects on the vasculature have never been reported [25]. Nevertheless, there is considerable evidence that Se and Sel play an important role in the vascular system. It has been found that GPX1 overexpression in a transgenic mouse model better protects the myocardium from ischemia/reperfusion injury [26]. The redox-regulatory proteins thioredoxin, in particular thioredoxin 1/thioredoxin reductase 1, are thought to reversibly reduce signaling molecules that regulate cardiac function and are implicated in vascular disease [27]. GPX, thioredoxin reductase and methionine sulphoxide reductase B1 have also been implicated in vascular stress [28]. SelO may play a crucial role in maintaining the health of vascular system.

The vascular endothelium plays a critical role in maintaining normal vascular permeability. Impaired endothelial function is a key component of many cardiovascular diseases. However, endothelial integrity is closely to intercellular junctions. The gap junction (GJ), as a transmembrane transport channel between two adjacent cells, can regulate the cellular gap junction communication function for various physiological processes. Connexin (Cx) has gap junction and communication functions and plays an important physiological role in vascular disorders. Abnormal Cx expression has been found in early plaques of atherosclerosis, and abnormal proliferation of the neoplastic intima after carotid injury has been associated with abnormal Cx protein expression [29].

The tight junction (TJ), generally represented as a tight fit of the plasma membrane between adjacent cells, has an important function in forming a permeability barrier that limits the free diffusion of molecules between cells [30,31]. It has been shown that the expression of the TJ proteins claudin-1, occludin-1 and ZO-1 is significantly upregulated in atherosclerosis and that the tight junction pathway is activated in the pathogenesis of atherosclerosis [32]. The adherens junction (AJ) is closer to the base than the TJ and is essential for initiating and maintaining cell-cell adhesion [33].Li et al. demonstrated that LPS reduced the progression of endothelial cell adhesion junction-mediated coronary artery disease by decreasing the expression of adhesion junction proteins [34]. Abnormal endothelial cell permeability and reduced expression of adhesion proteins can lead to haemangiomas, scleroderma and other forms of vascular inflammation. The structure of vascular intercellular adhesion junctions can be visualised using wheat germ agglutinin (WGA) [35]. Cell adhesion is a process in which activated clusters of integrins recognise external adhesion sites and recruit into the intracellular region of their β-subunit to form adhesion plaques. Tyrosine phosphorylation of the paxillin has been shown to increase 5-fold in response to angiotensin II stimulation, thereby mediating vascular hyperproliferative disorders such as restenosis after angioplasty [36]. Permeability and integrity of intercellular junctions can be assessed using dextran staining (FITC-Dextran) and human-derived acetylated low-density lipoprotein (Dil-Ac-LDL). ZO-1 is often used as one of the indicators to measure permeability and integrity of tight junctions and to assess barrier function [37]. Deficiency of the tight junction protein ZO-1 and the cytoskeletal regulator Rho-associated protein kinase (rock) results in increased tissue permeability [38]. However, downregulation of cingulin at endothelial cell junctions leads to increased rhoGEF activation and permeability [39]. Disruption of vascular endothelial cell junctions leads to an increase in endothelial permeability, which was a key trigger of vascular disease.

With the progressive advancement of research, the understanding of the detrimental effects of Se deficiency on other organs has gradually expanded, and numerous studies have reported on the pathology of various Se deficiency diseases. However, limited research exists regarding vascular damage caused by Se deficiency. Notably, SelO, a conserved and one of the largest Sel in living organisms, remains poorly understood in terms of its biological significance and its impact on vascular [25]. Based on this, the present study established a mouse model with different concentrations of selenium treatment and observed the changes in vascular structure as well as the expression of vascular adhesion plaque proteins, the structure of vascular intercellular junctions and vascular permeability by immunohistochemical staining and pathological histology. To select key Sel with association between vascular and Sel when selenium concentration change, screening of 24 Sel in model mice. In addition, the molecular expression of the adhesion plaque proteins vinculin, talin and paxillin, the junctional effector molecules connexin, JAM-A, claudin and cadherin, and the tight junction ZO-1 pathway molecules ZO-1, rock, rhoGEF, cingulin and MLC-2 were monitored to determine the relationship between selenium and adhesion junction proteins and intercellular junction associated factors. Further validation was performed at the molecular level for the screened key target protein SelO. The siRNA-SelO interference model was established using transfection siRNA technology to explore the mechanism of SelO in maintaining vascular endothelial permeability by affecting the expression of adhesion plaque proteins, intercellular junction effectors and tight junction ZO-1 related signaling pathway molecules. The present study aims to elucidate the potential mechanisms of selenium deficiency-induced vascular injury and to elucidate the beneficial role and mechanism of action of SelO in the vascular endothelial cell permeability barrier, thus enriching the theoretical basis of SelO related studies.

2. Materials and methods

2.1. Animal model construction and sample collection

The total 120C57BL/6 mice (60 females and 60 males) aged between 5 and 7 weeks, which were randomly divided into three groups (40 mice per group): The low selenium group (L) was fed selenium-deficient diet (0.01 mg/kg selenium in the diet), the control group (C) was fed normal selenium diet (0.18 mg/kg selenium in the diet), and the high selenium group (H) was fed selenium-supplemented diet (0.6 mg/kg selenium in the diet). The feed for each group was purified standard feed provided by Trophic Feed Technology Co. (Nantong, Jiangsu Province, China), which contained the same nutrients except selenocysteine. Once plasma exudate and hemorrhagic spots were observed in the thorax and abdomen of the mice, indicating successful establishment of the animal model, euthanasia was performed to collect vascular tissue from the hepatic aorta. Some tissues were stored at -80 °C for subsequent experiments while others were processed to appropriate sizes before being placed in 4 % paraformaldehyde fixative away from light for future use as previously described [40]. All animal procedures conducted in this study received approval from the Animal Ethics Committee of Northeastern Agricultural University.

2.2. Histopathological analysis

After adequate fixation, dehydration and transparency of mouse vascular tissues using different concentrations of ethanol and

embedding in paraffin, 5 μm tissue sections were cut and stained with hematoxylin-eosin (H&E) as previously described [41]. The staining results were examined under a light microscope (Olympus, Japan) and corresponding changes were recorded through photography for analysis.

2.3. Red fluorescence labeled Dil-Ac-LDL staining

Red fluorescence labeled Dil-Ac-LDL (Maokang Biotechnology, Shanghai, China) staining was performed to observe intercellular junctions as Dil-Ac-LDL binds to endothelial cells. The samples were incubated with Dil-Ac-LDL at 37 °C for 1 h, washed, and fixed with 2 % paraformaldehyde before being sectioned and observed using a fluorescence microscope (Olympus, Japan) at wavelengths of Ex: 514/549 nm; Em: 565 nm.

2.4. FITC-WGA staining

WGA (Sigma-Aldrich, Germany), one of the lectins widely used in cell biology, recognises N-acetylglucosamine and binds to its dimer and trimer. It specifically recognises adhesion proteins. In this experimental study, WGA was utilized to interact with vascular endothelial glycoproteins for plasma membrane presentation. Tissue sections were deparaffinized, followed by rinsing with distilled water and two subsequent washes (each lasting 5 min) using PBS containing 1 % bovine serum albumin. Subsequently, the sections were incubated with diluted FITC-WGA within an incubator box at room temperature for a duration of 1 h. Afterward, the sections underwent three rounds of washing with PBS (each lasting 5 min), sealing of slices with a water-soluble sealer, and observation under a fluorescence microscope at Ex/Em: 495/515 nm to capture green fluorescence images.

2.5. FITC-dextran staining

FITC-Dextran is a fluorescently labeled polysaccharide composed of branched glucose molecules with varying lengths. FITC-Dextran is commonly used to measure the permeability of the blood-brain barrier as it can be observed due to its ability to embed itself in cell membrane fibronectin hydrogels and release 250 kDa of FITC. In this study, we utilized this principle to evaluate vascular endothelial permeability. Anesthetized mice were intravenously injected with FITC-Dextran (50 mg/kg) via the tail vein, and after 1 min, the vascular tissue was excised and fixed in 4 % paraformaldehyde fixative at 4 °C for 24 h. Subsequently, sections of the fixed tissue were prepared and observed.

2.6. Immunohistochemical staining

The sections were dried in an oven at a constant temperature of 70 °C for 120 min. Dewaxing samples. Antigen retrieval was performed using an autoclave,.pour the EDTA repair solution into the autoclave, heat the autoclave until the liquid boils and immerse the sections in the repair solution. After removal, the samples were rinsed with PBS and treated dropwise with 3 % inactivated endogenous peroxidase at 37 °C for 15 min. The sections were washed with PBS and incubated overnight at 4 °C with the primary antibody. On the following day, after returning to room temperature, the sections were rinsed again in PBS and incubated with secondary antibody at 37 °C for half an hour. Subsequently, the sections were rinsed repeatedly in PBS before adding color development solution drop by drop and observing under a microscope; color development was stopped timely and washed afterward.The sections underwent dehydration using sequential ethanol solutions (75 %, 95 %, and anhydrous), followed by clearing in xylene, and finally sealed.

2.7. Cell culture of HUVECs

HUVEC cells (Binsui Biotechnology, Shanghai, China) were cultured in T25 culture flasks with 95 % ECM medium supplemented with 5 % fetal bovine serum, 1 % endothelial cell growth supplement, and 1 % penicillin-streptomycin. The cultures were maintained at a constant temperature of 37 °C in a incubator containing 5 % CO_2. Cells were passaged when the confluence reached 90-100 %.

Table 1
Selenium treatment medium configuration.

	RPMI-1640	1 % FBS	Transferrin (5 μg/mL)	Insulin (10 μg/mL)	Selenocysteine
L	46.5 mL	2.5 mL	250 μL	250 μL	0 ng/mL
M	46.25 mL	2.5 mL	250 μL	250 μL	5 ng/mL
H	47 mL	2.5 mL	250 μL	250 μL	10 ng/mL

Table 2
Interference test medium configuration.

	RPMI-1640	1 % FBS	Transferrin (5 μg/mL)	Insulin (10 μg/mL)	Selenocysteine
siRNA-L	46.5 mL	2.5 mL	250 μL	250 μL	0 ng/mL
siRNA-M	46.25 mL	2.5 mL	250 μL	250 μL	5 ng/mL
siRNA-H	47 mL	2.5 mL	250 μL	250 μL	10 ng/mL

Table 3
The sequences of siRNA.

Name	Sequences
siRNA-SelO	F (5'→3') :GGUCAAGCAUCCGUGAGUUTT R (5'→3') :AACUCACGGAUGCUUGACCTT

2.8. Culture of HUVECs treated with Se

HUVECs were seeded into 6-well plates at a density of 1×10^6 cells/well and cultured in regular medium until reaching approximately 80 % confluence (usually after 48 h). Then, the regular medium was replaced with Se-containing medium to establish different treatment groups: the Se-deficient group (Low Se group, L) was cultured with selenocysteine-free medium (0 ng/mL), the middle Se group (Middle Se group, M) was cultured with selenocysteine at a concentration of 5 ng/mL, and the Se-supplemented group (High Se group, H) was cultured with selenocysteine at a concentration of 10 ng/mL (the composition of cell culture medium is shown in Table 1, and selenocysteine was used as it was prepared) . After treatment for 24 h, subsequent experiments could be performed based on specific requirements.

2.9. siRNA transfection

siRNAs targeting SelO and its negative control siRNA-NC were designed and synthesized by Wuhan Jintosi Biotechnology Co. HUVEC cells were grown in six-well plates until reaching 60-70 % confluence before introducing siRNA (Table 3) using liposomal RNAi Max transfection agent (invitrogen, USA) as previously described [20]. The siRNA-NC group was then maintained in regular medium while the siRNA-L, siRNA-M, and siRNA-H groups were treated with selenocysteine at 0 ng/mL, 5 ng/mL, and 10 ng/mL (the composition of the cell culture medium for the interference experiment is shown in Table 2), respectively, for 24 h.

Table 4
mRNAs primer sequences.

Name	F (5'→3')	R (5'→3')
GAPDH	GGCAAATTCAACGGCACAGTCAAG	TCGCTCCTGGAAGATGGTGATGG
vinculin	GCATTGCAAAGAAGATTGATGC	TAATTCTGCAATCTTCCGAGCT
talin	AAGAGACGTGGATAATGCCCTA	CTCTGTGCTTCTTGAAATGTCC
paxillin	CAAAGCGTACTGTCGTAAAGAT	TTGAGGGCTGAAATGTAGTTCT
connexin	GTGACAGAAACAATTCCTCCTG	ATTTTGCTCTGCGCTGTAATTC
JAM-A	CATCAAATTGACCTGCACCTAC	AGCTGTGATCTGGCTGTTATAA
claudin-1	AGATACAGTGCAAAGTCTTCGA	CAGGATGCCAATTACCATCAAG
cadherin	CTCAGAAGACAGAAACGAGACT	AACCAGGTTCTTTGGAAATTCG
ZO-1	CTGGTGAAGTCTCGGAAAAATG	CATCTCTTGCTGCCAAACTATC
rock	AGAAGCTAGAACATTTGACCGA	CTGCTTCAAATGCTTGAGTCTT
rhoGEF	GCCATCAGTGATTTCCCTCCAGAAG	CATCCAGTTATTCCGCTCCTCCTTG
cingulin	GAGACACAAGAGGAAAATGACG	CTTCCAGCTGCTGTTTCTCTA
MLC-2	GACGAGTGAACGTGAAAAATGA	TGAGGAACACGGTGAAGTTAAT
GPx1	GCAATCAGTTCGGACACCAGGAG	TCTCACCATTCACTTCGCACTTCTC
GPx2	AGGGCTGTGCTGATTGAGAATGTG	CTCCTGATGTCCGAACTGGTTGC
GPx3	GGCTTGGTCATTCTGGGCTTCC	CCACCTGGTCGAACATACTTGAGAC
GPx6	ACGTACCCTGAGCTGAACACATTG	CGCCTGGACGCACATACTTGAG
TrxR1	CATCTACGCCATCGGTGACATCC	CATACAGCCTCTGAGCCAGCAATC
TrxR2	GGTGTGGACAAAGGCGGGAAG	TTCAGCCAGAAGATGTCGTCACTTG
TrxR3	CATCATCATCGGTGGTGGTTCTGG	CCTGAGGTGACGGGACAACAAAG
DIO1	ACTTTGCCTCCACAGCCGATTTC	CGTTGTTCTTAAAAGCCCAGCCATC
DIO2	TTCTCCTCGGTGGCTGACTTCC	GCACATCGGTCCTCTTGGTTCC
DIO3	TGGTGGTCGGAGAAGGTGAAGG	CAGCGAGTGAAGCAGCAGAGAG
Sel M	AGCCACCTCCACCACCAACTAC	GGCGATTCAACTGTCATCCTCCAC
Sep 15	TGGACGACAACGGGAACATTGC	CCAACTTCTCGCTCAGGAACTCTTC
Sel S	GCTATGGCTGGTACATCCTCTTCAG	CCTCTTGCCGCTTAACAACAACATC
Sel K	CCAGGAAACCCTCCACGAAGAATG	CCTTCCTCATCCACCAGCCATTG
Sel W	ATCTGCGGCGAGGGAACTCC	CTTTCTGTGTCCACGTAGCCATCG
Sel H	CGCCATGCTGCTGCCTTGAG	CGCGGTTTGGACGGGTTCAC
Sel T	CAGTGTATGTCAACGGGTGCATTTG	TACAAGCTGCTGCATGGAAGGAAG
Sel V	GTGTGATGTACTGTGGCCTCTGAAG	AACCTGGGTAGCTCTTTCCTCCTC
Sel P	CTGTGATGAAGGCTCGCTCTTGG	GGCTGAACGCAGGTCATGGATC
Sel R	TCCTGTGGCAAGTGTGGCAATG	GCCTTTAGGGACGAACTTCAGTGAG
Sel N	TCCATCGCCTGTTAAGCATGTTCC	CACGGTGTAGTAGGAATCGCTGATG
Sel I	ATGTGCCTGACTGGGTTTGGATTG	TTGGTTCTCCGTGCTTGCTTTCC
Sel O	TGTCCTGAAGCTGCTAGAGTCTCC	GAAGATTGCTCCTCAGTGCTCCTTG

2.10. qPCR

The total RNA was isolated from mouse vascular tissues and HUVEC cells using the conventional Trizol (invitrogen,USA) method, and the concentration of RNA was determined using Nano-Drop (Thermo Fisher Scientific) as previously described [42]. For each sample, reverse transcription was performed separately following the cDNA First Strand Synthesis Kit instructions (Bioer Technology, China). Subsequently, qPCR analysis was carried out on a Light Cycler@480 System (Roche, Switzerland) following the BioEasy Master Mix kit instructions. The relative expression of target genes was calculated by comparing them to the expression of housekeeping gene GAPDH, and final results were obtained using the $2^{-\Delta\Delta Ct}$ method. The sequences of mRNAs used for qPCR are provided in Table 4.

2.11. Western blot

Proteins were extracted from mouse vascular tissues and HUVEC cells by lysing them with RIPA protein lysate (RIPA: PMSF = 100:1). The protein concentrations were determined using a BCA kit (Beyotime, Shanghai, China) as previously described [43]. Samples from each group were adjusted to the same concentration, and then the protein samples were boiled for 10-15 min after adding 5 × SDS loading buffer. Depending on the size of the proteins, an equal amount of protein from each group was loaded onto a 6-15 % SDS-PAGE gel based on their respective sizes, followed by electrophoresis and subsequent transfer to an NC membrane (Sigma, Germany). The membranes were incubated in an incubation box containing 5 % skimmed milk at 37 °C for 2 h. After the incubation period, they were washed with TBST, excess liquid was removed, and primary antibody dilution was applied to the NC membrane which was refrigerated overnight at 4 °C. On the following day, another round of TBST washes was performed before incubating with HRP-conjugated secondary antibody at room temperature for 2 h while protected from light exposure. Chemiluminescence detection was carried out using ECL chemiluminescence solution (Gibco, USA) followed by imaging on a gel imager. Finally, greyscale values of the protein bands were analyzed using Image J software. The relative expression levels were calculated by comparing them to the expression of the GAPDH. The protein antibody information used in this study are shown in Table 5.

Table 5
The information of antibodies used in Western Blot.

Name	Source	Dilution Ratio
GAPDH	Cell Signaling Technology	1:1000
vinculin	Cell Signaling Technology	1:1000
talin	Cell Signaling Technology	1:1000
paxillin	Cell Signaling Technology	1:1000
connexin	Cell Signaling Technology	1:1000
JAM-A	Cell Signaling Technology	1:1000
claudin-1	Cell Signaling Technology	1:1000
cadherin	Cell Signaling Technology	1:1000
ZO-1	Cell Signaling Technology	1:1000
rock	Cell Signaling Technology	1:1000
rhoGEF	Cell Signaling Technology	1:1000
cingulin	Cell Signaling Technology	1:1000
MLC-2	Cell Signaling Technology	1:1000
Sel O	Cell Signaling Technology	1:1000
Goat anti-mouse IgG-HRP	Cell Signaling Technology	1:2000
Goat anti-rabbit IgG-HRP	Cell Signaling Technology	1:2000

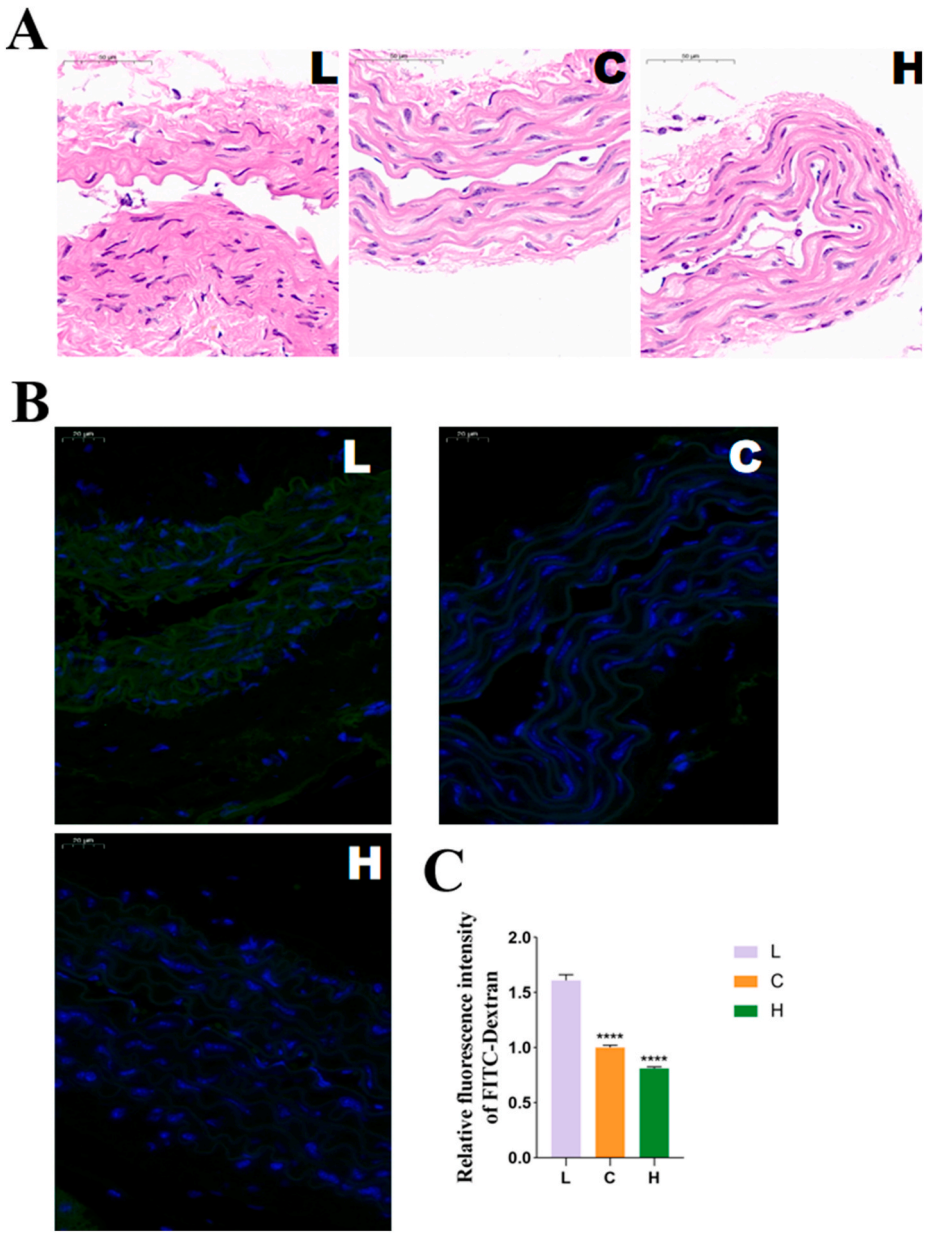

Fig. 1. Se effects on vascular structure. (A) Histopathological changes of the vascular. L, C, H, were mice fed with selenium at 0 mg/kg, 0.18 mg/kg, and 0.6 mg/kg (N = 10, scale bar, 50 μm). (B)FITC-Dextran staining of the vascular (N = 10, scale bar, 20 μm). (C) Quantification of relative fluorescence intensity of FITC-Dextran staining (N = 10). Results are presented as mean ± SEM. Statistical significance was obtained by one-way ANOVA. *P < 0.05, significant difference; **P < 0.01, more significant difference; ***P < 0.001, very significant difference; ****P < 0.0001, very significant difference.

2.12. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 25 (Armonk, NY, USA), GraphPad prism 9.5(New York, NY, USA). Differences between groups were analyzed using the one-way ANOVA method, and all data were presented as "mean ± SEM". $p < 0.05$ indicates a statistical difference compared.

3. Results

3.1. Se effects on vascular structure

To determine the relationship between vascular injury and dietary Se content, pathological examination was conducted to observe the vascular structure of the hepatic aorta in groups of mice fed different diets. H.E. staining revealed that compared to the normal control group, Se-deficient mice exhibited thickened walls of vascular tissue, irregular arrangement of endothelial cells, disorganized orientation of smooth muscle fibers, and defects in the outer membrane (Fig. 1A). These findings indicate that Se deficiency leads to damage in the vascular tissue of mice. Additionally, FITC-dextran is a labeled polysaccharide that remains stable and does not release fluorescein moieties under normal conditions. Only when embedded within cells and releasing fibrin hydrogels does thiocarbamoyl bond hydrolysis produce 4- or 5-aminofluorescein, which can be quantified immediately using high performance liquid chromatography (HPLC). After FITC-Dextran staining, it was observed that the fluorescence intensity in group L was significantly higher than that in group C, while groups C and H showed similar fluorescence levels (Fig. 1B and C). This indicates that endothelial permeability of blood vessels is significantly increased in group L compared to groups C and H due to Se deficiency. Taken together, these results demonstrate that Se deficiency damages the vascular structure

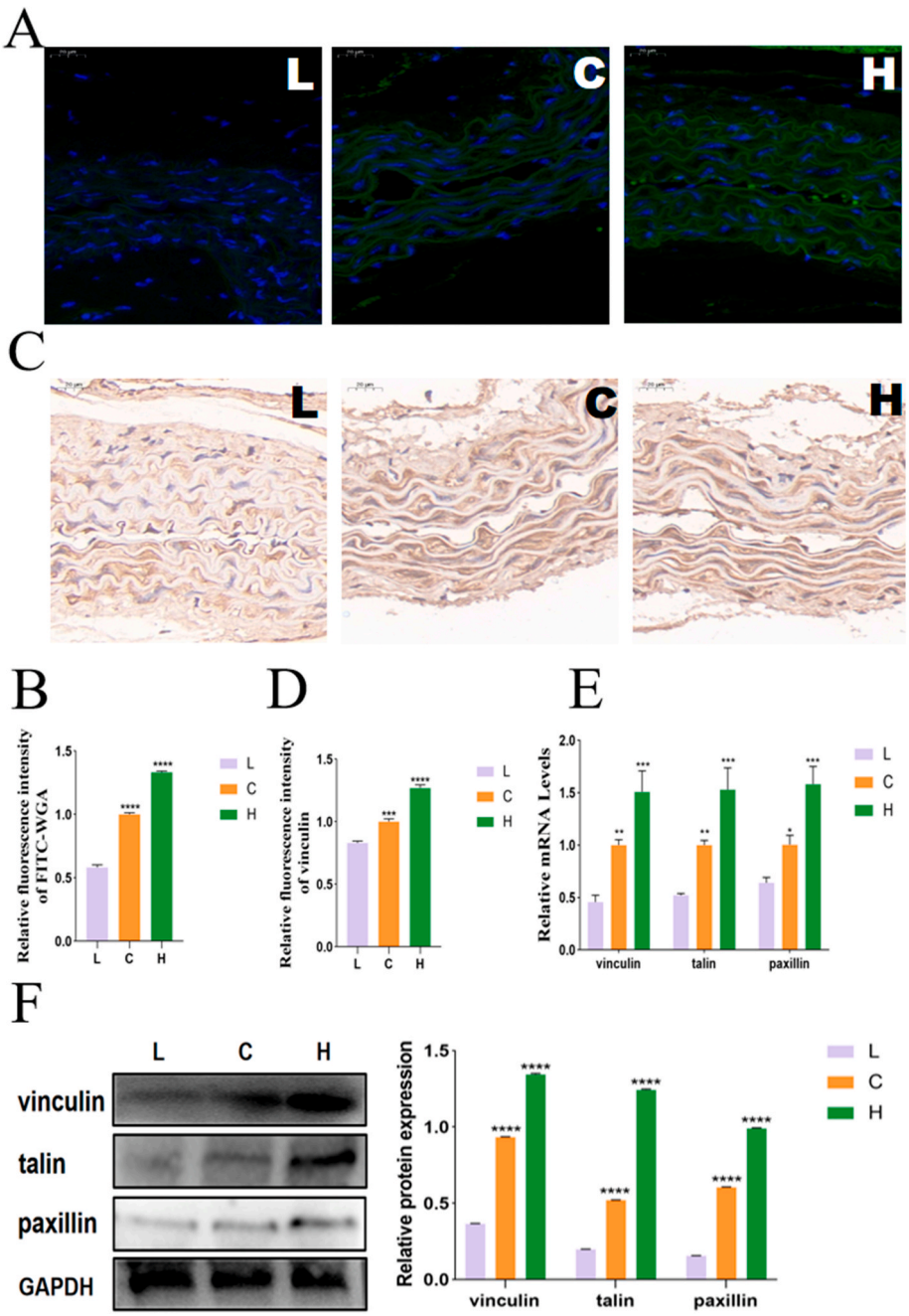

Fig. 2. Se impacts the expression of vascular adhesive plaque proteins. (A) FITC-WGA staining of the vascular (N = 10, scale bar, 20 μm). (B) Quantification of relative fluorescence intensity of FITC-WGA staining (N = 10). (C) Immunohistochemical detection of vinculin protein staining in the vascular (N = 6, scale bar, 20 μm). (D) Quantification of relative fluorescence intensity of vinculin protein (N = 6). (E) The mRNA levels of adhesive plaque protein (vinculin, talin, and paxillin) in the vascular (N = 3). (F) The protein levels of adhesive plaque protein (vinculin, talin, and paxillin) in the vascular(N = 3). Results are presented as mean ± SEM. Statistical significance was obtained by one-way ANOVA. *P < 0.05, significant difference; **P < 0.01, more significant difference; ***P < 0.001, very significant difference; ****P < 0.0001, very significant difference.

and increases vascular endothelial permeability.

3.2. Se impacts the expression of vascular adhesive plaque proteins

To further investigate the detrimental effects of dietary Se deficiency on vascular endothelial permeability, we assessed the expression of vascular adhesion plaque proteins. Initially, FITC-WGA staining was employed to evaluate the expression of adhesion plaque proteins, revealing significantly reduced fluorescence values in group L and elevated fluorescence intensity in group H compared to group C (Fig. 2A and B). This indicates a decrease in adhesion plaque protein expression in group L and an increase in adhesion plaque protein expression in group H, suggesting a potential association between Se and adhesive protein expression correlated molecules. As shown in Fig. 2C and D, the expression of vinculin in blood vessels was detected by immunohistochemistry, and the protein expression level of vinculin increased with the increase of Se content, in which the expression of group L was lower than that of group C, and the expression of group H was higher than that of group C, which strongly proved that selenium deficiency reduced the expression of vinculin in blood vessels. qPCR and Western blot analysis demonstrated that both mRNA and protein expressions of adhesion plaque proteins vinculin, talin, and paxillin increased with higher Se

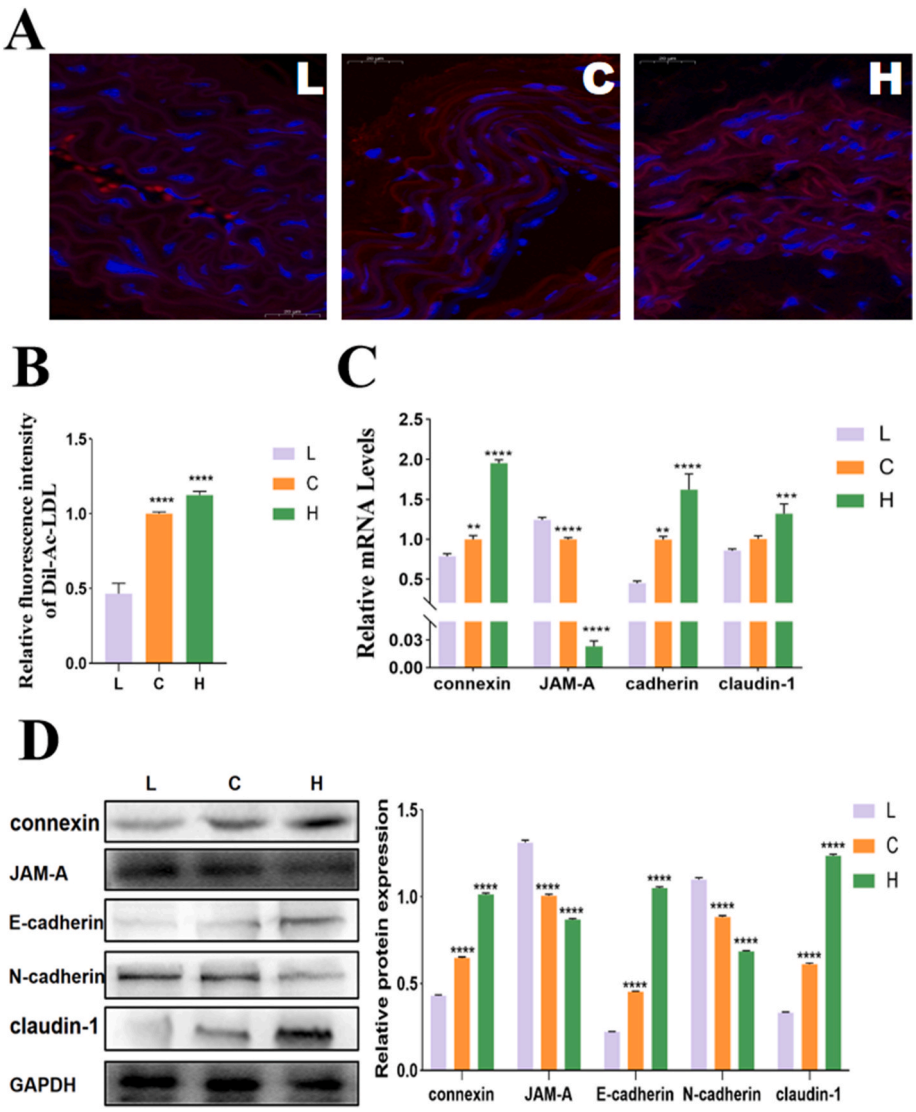

Fig. 3. Effect of Se on vascular endothelial intercellular junctions. (A) Dil-Ac-LDL staining of the vascular (N = 10, scale bar, 20 μm). (B) Quantification of relative fluorescence intensity of Dil-Ac-LDL staining (N = 10). (C) The mRNA levels of intercellular junctions (connexin, JAM-A, cadherin, and claudin-1) in the vascular (N = 3). (D) The protein levels of intercellular junctions (connexin, JAM-A, E-cadherin, N-cadherin and claudin-1) in the vascular (N = 3). Results are presented as mean ± SEM. Statistical significance was obtained by one-way ANOVA. *P < 0.05, significant difference; **P < 0.01, more significant difference; ***P < 0.001, very significant difference; ****P < 0.0001, very significant difference.

content within vascular (Fig. 2E and F). This suggests that selenium has a positive effect on the production of adhesion plaque proteins and that a certain dose of selenium can maintain the content of adhesion plaque proteins among vascular endothelial cells.

3.3. Effect of Se on vascular endothelial intercellular junctions

The effect of dietary Se deficiency on intercellular junctions in vascular endothelial was further investigated. Dil-Ac-LDL staining revealed clearly labeled orange-red fluorescence in the endothelial cell layer of group C. Group H exhibited stronger fluorescence values compared to group C, while group L showed significantly reduced fluorescence values than both groups C and H, indicating impaired vascular endothelial intercellular junctions in group L (Fig. 3A and B). These results suggest that Se deficiency disrupts intercellular junctions in vascular tissues of mice. Subsequently, we examined the expression of vascular endothelial junctional effector molecules (connexin, JAM-A, claudin-1, cadherin) in vascular tissue. The mRNA expression of connexin, claudin-1, and cadherin gradually increased from group L to group C to group H. Conversely, the mRNA expression of JAM-A decreased with increasing Se content (Fig. 3C). Western blot analysis also demonstrated an increase in protein expression for connexin, claudin-1, and E-cadherin with increasing Se content but a decrease for N-cadherin and JAM-A protein expression (Fig. 3D). These findings indicate that Se can regulate the expression of junctional effector molecules within vascular.

3.4. Effect of Se on the ZO-1 signaling pathway in the vasculature

We utilized qPCR to detect changes in gene transcription of molecules associated with the tight junction ZO-1 signaling pathway (ZO-1, rock, rhoGEF, cingulin, and MLC-2) in different dietary Se-treated groups. Our findings revealed that gene transcription of each molecule increased with increasing Se concentration in the vascular tissue. Specifically, the L group exhibited reduced expression compared to the C group, while the H group showed higher expression than the C group (Fig. 4A). Western blot analysis confirmed consistent results between protein and mRNA expression of ZO-1 signaling pathway (Fig. 4B). It

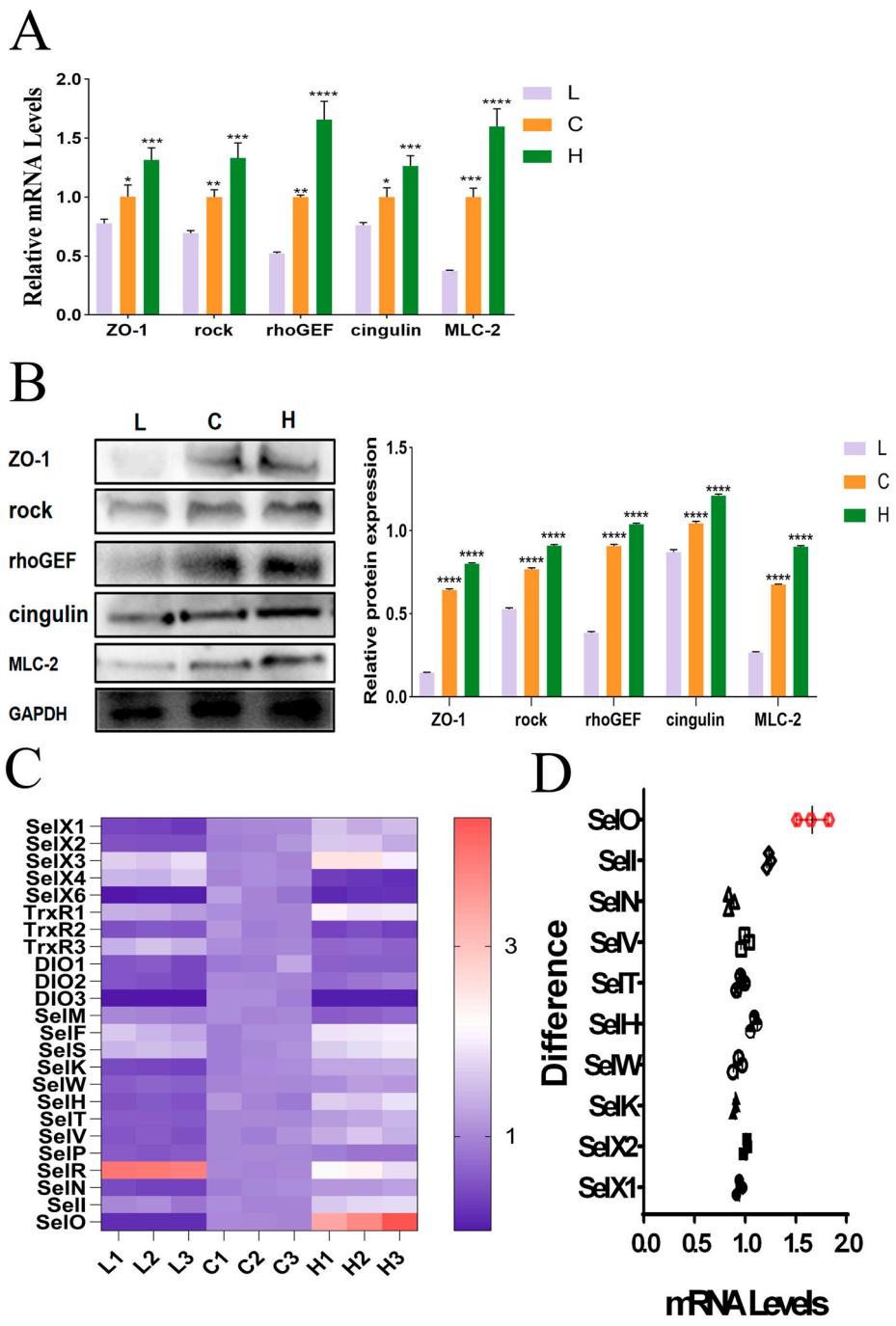

Fig. 4. Effect of Se on the ZO-1 signaling pathway in the vasculature. (A) The mRNA levels of ZO-1 signaling pathway (ZO-1, rock, rhoGEF, cingulin, and MLC-2) in the vascular (N = 3). (B) The protein levels of ZO-1 signaling pathway (ZO-1, rock, rhoGEF, cingulin, and MLC-2) in the vascular. (C)–(D) Heatmap (C) and volcano plot (D) of Sel mRNA sequencing results of differential expression in vascular (N = 3). (E) Analysis of the genetic correlation between SelO and vascular endothelial permeability. Results are presented as mean ± SEM. Statistical significance was obtained by one-way ANOVA. *P < 0.05, significant difference; **P < 0.01, more significant difference; ***P < 0.001, very significant difference; ****P < 0.0001, very significant difference.

indicated a correlation between Se and the ZO-1 tight junction signaling pathway.

3.5. SelO promotes the expression of vascular adhesive plaque proteins

Se, an essential trace element in the human body, exerts its functions primarily through Sel incorporation. In this study, we investigated 24 Sel in mice and identified those with the most significant alterations in expression within vascular tissues under varying Se concentrations (Fig. 4C). Screening for Sel, which met expectations, revealed a strong association between SelO and vascular tissue, with SelO expression increasing with increasing selenium levels and the trend being most pronounced (Fig. 4D). Furthermore, utilizing IBM SPSS Statistics 25 analysis, we discovered a significant association between SelO and vascular endothelial adhesive plaques protein (vinculin, talin and paxillin), intercellular junctional effector molecules (connexin, JAM-A, claudin-1, and cadherin), as well as tight junction ZO-1 pathway (ZO-1, rock, rhoGEF, cingulin and MLC -2) were significantly correlated with each other (Table 6). Subsequently, we evaluated both mRNA and protein expressions of SelO in HUVECs treated with different Se content

Table 6
SPSS analysis of the correlation between Sel O and factors associated with vascular endothelial permeability.

Multiple Comparisons							
Dependent Variable:			Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
(I) VAR00001						Lower Bound	Upper Bound
LSD	Sel O	vinculin	-1.18000*	0.10457	0.000	-1.3949	-0.9651
		talin	1.67333*	0.10457	0.000	1.4584	1.8883
		paxillin	.76333*	0.10457	0.000	0.5484	0.9783
		connexin	.89667*	0.10457	0.000	0.6817	1.1116
		JAM-A	.88333*	0.10457	0.000	0.6684	1.0983
		claudin-1	-5.92333*	0.10457	0.000	-6.1383	-5.7084
		cadherin	.37333*	0.10457	0.001	0.1584	0.5883
		ZO-1	-1.32333*	0.10457	0.000	-1.5383	-1.1084
		rock	1.68000*	0.10457	0.000	1.4651	1.8949
		rhoGEF	2.06333*	0.10457	0.000	1.8484	2.2783
		cingulin	-7.12667*	0.10457	0.000	-7.3416	-6.9117
		MLC-2	1.09333*	0.10457	0.000	0.8784	1.3083

and observed significantly reduced SelO mRNA and protein expression in group L compared to group C; conversely, it was significantly higher in group H (Fig. 5A and B). Collectively, these findings suggest that SelO plays a crucial role in mediating vascular endothelial permeability injury induced by Se deficiency.

To further investigate the role of SelO in vascular endothelial cell injury, we silenced SelO expression in HUVECs by cell transfection and subsequently examined its expression in vascular endothelial cells. Our findings revealed that HUVEC cells with SelO interference exhibited normal expression of the SelO gene in the siRNA-NC group, while mRNA expression of SelO was significantly reduced in the siRNA-L, siRNA-M, and siRNA-H groups (Fig. 5C). Western blot analyses were consistent with mRNA results (Fig. 5D), confirming successful establishment of the SelO interference model. We also assessed mRNA expressions of vascular adhesive plaque proteins (vinculin, talin, and paxillin) in HUVECs treated with different concentrations of Se. The data demonstrated an increase in vinculin, talin, and paxillin expression with increasing Se content (Fig. 5E). Western blot analyses mirrored these findings (Fig. 5F). Furthermore, we evaluated the impact of interfering with SelO on vascular adhesive plaque protein expression using HUVECs and observed significantly reduced mRNA expressions for vinculin, talin, and paxillin genes in the siRNA-L, siRNA-M, and siRNA-H groups compared to the siRNA-NC group without significant differences among these three experimental groups (Fig. 5G). Validation by Western blot analysis showed that the protein expression of vinculin, talin and paxillin was significantly decreased in the siRNA-L, siRNA-M and siRNA-H groups compared to the siRNA-NC group (Fig. 5H). This suggests that SelO promotes the expression of vascular junction effector molecules.

3.6. SelO promotes the upregulation of vascular junction effector molecules

The mRNA expressions of vascular intercellular junctional effector molecules, including connexin, JAM-A, cadherin, and claudin-1 were detected in HUVECs. The results demonstrated that the expression of connexin, claudin-1, and cadherin increased with increasing Se content, while the expression of JAM-A decreased. Western blot analyses were consistent with mRNA findings (Fig. 6A). However, protein expression of connexin, claudin-1 and E-cadherin increased with increasing Se content while JAM-A and N-cadherin decreased (Fig. 6B). We further investigated the expression of vascular endothelial intercellular junctional effector molecules after transfection with siSelO for 24 h. It was observed that in SelO-interfered HUVEC cells, the siRNA-L group showed significantly reduced expression of connexin, claudin-1 and cadherins genes compared to the siRNA-NC group; whereas the siRNA-M and siRNA-H groups exhibited similar trends but to a lesser extent. Conversely, the expression of JAM-A gene was significantly higher in all three interference groups compared to the siRNA-NC group (Fig. 6C). Western blot analyses confirmed these findings by demonstrating significantly reduced protein expressions for connexin, claudin, and E-cadherin while higher expressions for JAM-A and N-cadherin proteins in all three interference groups compared to siRNA-NC group (Fig. 6D). These results suggest that SelO promotes the upregulation of vascular junction effector molecules.

3.7. SelO has a positive effect on the expression of the ZO-1 signaling pathway

The mRNA expressions of ZO-1 signaling pathway components, including ZO-1, rock, rhoGEF, cingulin and MLC-2, were detected in HUVECs. The results demonstrated a significant increase in the expression of ZO-1, rock, rhoGEF, cingulin and MLC-2 with increasing Se content (Fig. 7A). Consistent protein expression patterns were observed (Fig. 7B). We investigated the impact of SelO interference on the expression of ZO-1 signaling pathway components within vascular endothelial cells. In HUVEC cells subjected to SelO interference (siRNA-L, siRNA-M and siRNA-H groups), there was a significant reduction in gene expression of ZO-1, rock, rhoGEF, cingulin and MLC-2 compared to the siRNA-NC group (Fig. 7C). As shown in Fig. 7D, Western blot results revealed that the expression of ZO-1, rock, rhoGEF, cingulin and MLC-2 proteins was significantly decreased in the siRNA-L, siRNA-M and siRNA-H groups compared to the siRNA-NC group (Fig. 7D). The above results suggest that SelO has a positive effect on the expression of the ZO-1 signaling pathway.

4. Discussion

Se deficiency can lead to a variety of diseases in humans and animals. The previous researches had reported Se deficiency was an important factor in the occurrence of disease, such as Chronic Liver Disease (CLD), central nervous system disorder, Cartilage Injury and et al. [44–46]. The immune properties of selenium allow supplementation to prevent H1N1 flu infection [47]. Among them, the role of selenium deficiency in the occurrence of cardiovascular diseases has received the most extensive attention [48]. Se deficiency has been identified as a common cause of vascular disease, with significantly reduced blood Se content observed in patients with chronic vascular diseases compared to healthy individuals [49].The reduced production of GSH-PX and SOD mediated by Se deficiency, as well as a significant decrease in enzyme activity, leads to intense lipid peroxidation, which impairs cell membrane fluidity and repair and mediates vascular endothelial cell damage [50]. However, dietary intake is the only way for the body to absorb Se, so Se content can regulate Sel expression in the body [51]. SelS has demonstrated its ability to preventing atherosclerosis-induced vasculature formation [52]. However, as Se exposure decreases, vascular endothelial adhesion molecules increase and Sel expression decreases, exacerbating the

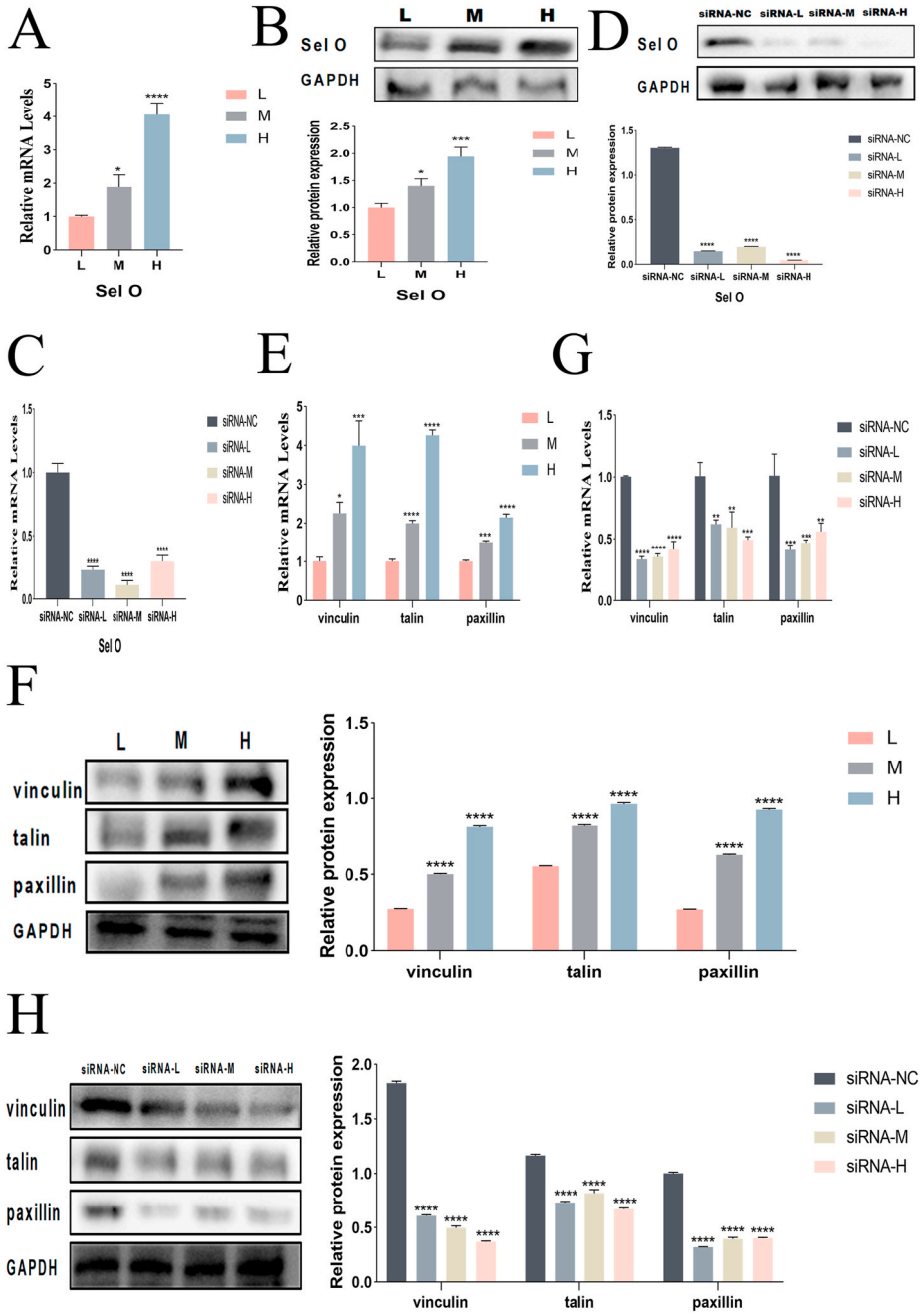

Fig. 5. SelO promotes the expression of vascular adhesive plaque proteins. (A) The mRNA levels of SelO in HUVECs (L, M, H were HUVECs cultured with 0 ng/mL, 5 ng/ml, and 10 ng/ml selenocysteine, N = 3). (B) The protein levels of SelO in HUVECs (N = 3). (C) After transfection with siSelO for 24 h, the mRNA expression of SelO in HUVECs (siRNA-NC was HUVECs transfected with siRNA-SelO negative control were cultured using normal medium. siRNA-L, siRNA-M, and siRNA-H were HUVECs transfected with siRNA-SelO were cultured with a selenocysteine content of 0 ng/mL, 5 ng/ml, and 10 ng/ml, N = 3). (D) After transfection with siSelO for 24 h, the protein expression of SelS in HUVECs (N = 3). (E) The mRNA levels of adhesive plaque protein (vinculin, talin, and paxillin) in HUVECs (N = 3). (F) The protein levels of adhesive plaque protein (vinculin, talin, and paxillin) in HUVECs (N = 3). (G) After transfection with siSelO for 24 h, the mRNA levels of adhesive plaque protein (vinculin, talin, and paxillin) in HUVECs (N = 3). (H) After transfection with siSelO for 24 h, the mRNA levels of adhesive plaque protein (vinculin, talin, and paxillin) in HUVECs (N = 3). Results are presented as mean ± SEM. Statistical significance was obtained by one-way ANOVA. *P < 0.05, significant difference; **P < 0.01, more significant difference; ***P < 0.001, very significant difference; ****P < 0.0001, very significant difference.

inflammatory response and vascular endothelial dysfunction [53]. The results of the present study showed that Sel expression was differentially affected by the different concentrations Se diets with in vascular tissues. The expression of Sel O was most obvious with the changes in Se levels. The present study found that there may be potential associations between SelO and factors related to the formation of adhesion plaque proteins, the structure of vascular endothelial cell junctions, and the ZO-1 pathway of tight junction.

Cell adhesion is an important component of functions such as cell transfer, which is based on the synergistic action of the cell with the extracellular matrix, requiring the cooperative action of the transmembrane glycoprotein receptor family, such as the integrin family, the actin cytoskeleton and a number of cytoplasmic proteins [54]. However, low expression of Sel resulted in reduced expression of adhesive plaque proteins and impaired vascular resistance to injury and intercellular transfer integration. For example, GPX1 mediates metastasis of

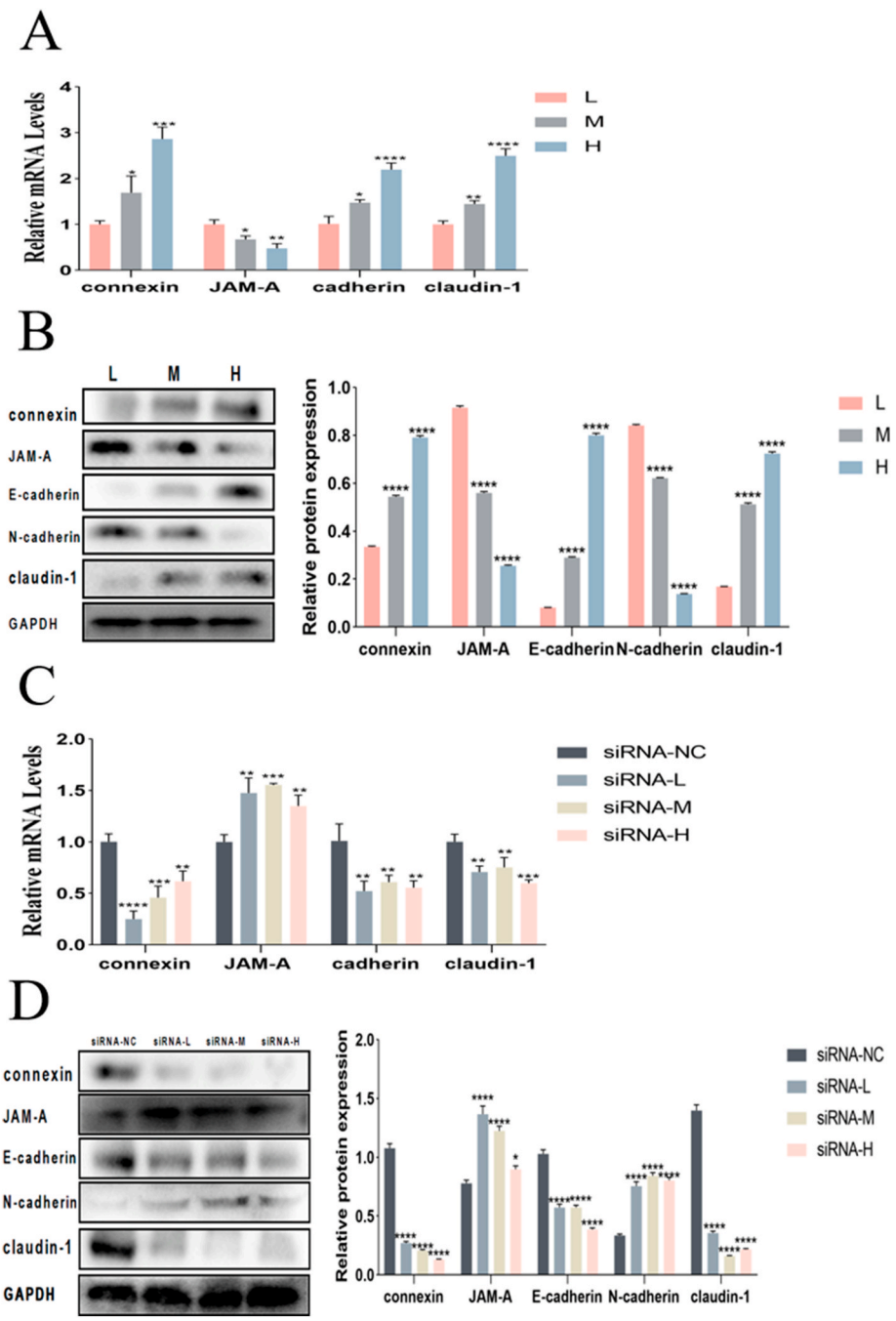

Fig. 6. SelO promotes the upregulation of vascular junction effector molecules. (A) The mRNA levels of intercellular junctions (connexin, JAM-A, cadherin, and claudin-1) in HUVECs (N = 3). (B) The protein levels of intercellular junctions (connexin, JAM-A, cadherin, and claudin-1) in HUVECs (N = 3). (C) After transfection with siSelO for 24 h, the mRNA levels of intercellular junctions (connexin, JAM-A, cadherin, and claudin-1) in HUVECs (N = 3). (D) After transfection with siSelO for 24 h, the protein levels of intercellular junctions (connexin, JAM-A, cadherin, and claudin-1) in HUVECs (N = 3). Results are presented as mean ± SEM. Statistical significance was obtained by one-way ANOVA. *P < 0.05, significant difference; **P < 0.01, more significant difference; ***P < 0.001, very significant difference; ****P < 0.0001, very significant difference.

triple-negative breast cancer cells by regulating cell adhesion when its expression is reduced [55]. Interference with Sep15 was found to shift focal adhesion to the periphery of the cellular substrate, reducing the ability of cells to migrate and invade, leading to inhibition of cell proliferation, while cell growth was restored upon removal of the interference inducer [56]. In contrast, SelS protected vascular endothelial cells from tumor necrosis factor and nitric oxide damage by regulating intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, thereby attenuating some of the vascular diseases triggered and promoted by endothelial dysfunction [57]. In this experiment, we found that the expression of the adhesion plaque proteins talin, paxillin and vinculin were increased with increasing Se content, confirming the regulatory effect of Se on the expression of adhesion plaque proteins. Se deficiency reduced the expression of adhesion plaque proteins and impaired vascular resistance to injury and intercellular transfer integration. With SelO expression decreasing, the expression of adhesion plaque protein was significantly reduced, suggesting a regulatory role of selenium on adhesion plaque protein via SelO.

Endothelial tight junctions can form a selective barrier separating the vascular lumen from extravascular organs and tissues and play an

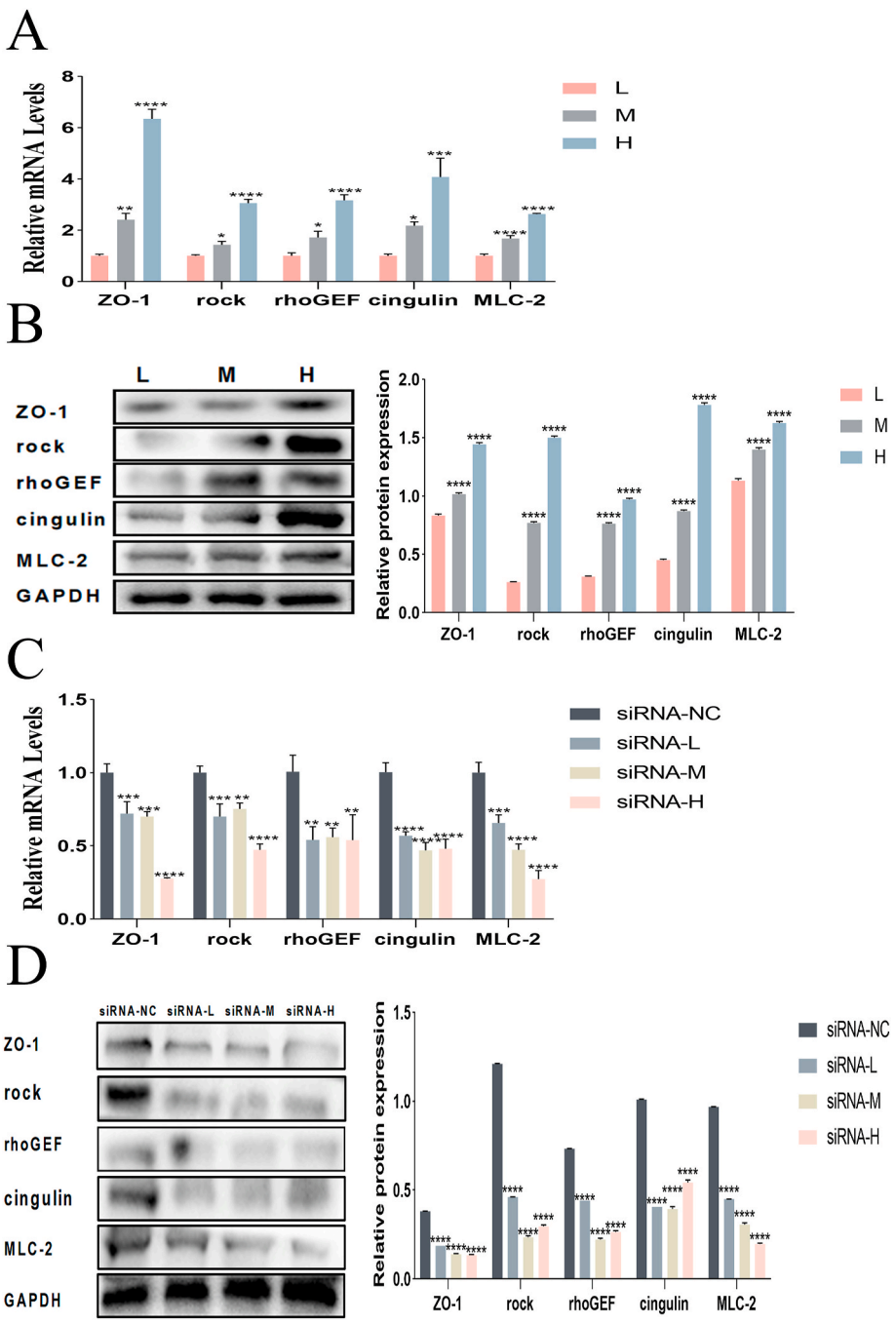

Fig. 7. SelO has a positive effect on the expression of the ZO-1 signaling pathway. (A) The mRNA levels of ZO-1 signaling pathway (ZO-1, rock, rhoGEF, cingulin, and MLC-2) in HUVECs (N = 3). (B) The protein levels of ZO-1 signaling pathway (ZO-1, rock, rhoGEF, cingulin, and MLC-2) in HUVECs (N = 3). (C) After transfection with siSelO for 24 h, the mRNA levels of ZO-1 signaling pathway (ZO-1, rock, rhoGEF, cingulin, and MLC-2) in HUVECs (N = 3). (C) After transfection with siSelO for 24 h, the protein levels of ZO-1 signaling pathway (ZO-1, rock, rhoGEF, cingulin, and MLC-2) in HUVECs (N = 3). Results are presented as mean ± SEM. Statistical significance was obtained by one-way ANOVA. *P < 0.05, significant difference; **P < 0.01, more significant difference; ***P < 0.001, very significant difference; ****P < 0.0001, very significant difference.

important role in maintaining the homeostasis of the systemic vasculature [58]. JAM-A is a member of the immunoglobulin superfamily of transmembrane adhesion molecules that maintain the integrity of endothelial cell tight junctions through affinity. It has been shown that under inflammatory stimulation, activation of endothelial cells causes JAM-A to migrate back to the surface of endothelial cells and promotes monocyte and T-cell adhesion and transcellular migration, indicating that JAM-A plays an important role in the process of pathological vascular injury such as atherosclerosis [59]. It has been shown that SelU regulates tight junctions through claudin-3 and that disruption of SelU in chicken testis supporting cells significantly reduces the expression of claudin-3, compromising the integrity of its junction-associated molecules [60]. In the present study, qPCR and Western blot results confirmed that the tight junction function of endothelial tissues and cells was impaired in selenium deficiency, and the related genes were abnormally expressed. The present study was consistent with the previous findings that the expression of tight junction-related factor claudin-1 was decreased and JAM-A expression was increased when SelO was interfered with, confirming the regulation of selenium on vascular endothelial tight junction molecules via SelO.

The protein composition of the linker on the gap junction membrane is connexin, and its abnormal protein expression directly affects the function of gap junction communication, which will affect cell growth, differentiation, proliferation, metabolism and apoptosis, as well as disrupting cellular structural homeostasis [61]. Some researchers have found that when colorectal cancer cells and vascular endothelial cells adhere to each other, the expression of connexin factors is reduced, gap junctions and the establishment of intercellular communication between the two is reduced, and the metastatic ability of cancer cells is increased [62]. In this experiment, it was found that as selenium levels in the diet increased, connexin expression gradually increased and gap junction function returned to normal. In addition, the expression of the gap junction protein connexin was significantly reduced in vascular endothelial cells transfected with siRNA-Sel O in this experiment.

Adhesion junctions between endothelial cells are essential for neovascularisation and the maintenance of vascular integrity and stability. This junction is mainly mediated by a variety of adhesion junction proteins, including vascular endothelial calreticulin, neuronal calreticulin and epithelial-type calreticulin [63]. When E-cadherin is expressed on the surface of tumor cells, tumors complete metastasis by collective migration, whereas ectopic expression of N-cadherin in breast cancer cells promotes cancer metastasis [64]. In this experiment, we examined the expression of adhesion junction effector molecules at elevated selenium concentration gradients and found that N-cadherin expression was increased and E-cadherin expression was decreased in selenium deficiency. Endothelial cells transfected with siRNA-SelO showed abnormal expression of adherens junction effector molecules, i.e. increased N-cadherin expression and decreased E-cadherin expression, confirming the regulation of selenium on adhesion junction effector molecules by SelO.

Tight junctions are an important structural basis for maintaining the blood-brain barrier and ZO-1 is an important tight junction protein. Once the distribution and expression of ZO-1 protein is altered, the structure and function of tight junctions will be directly affected [65]. Se deficiency reduced the expression of ZO-1 and E-cadherin by promoting the expression of LncRNA-MORC3 and inflammatory factors, which can lead to the disruption of tight junctions resulting in tissue damage in the small intestine of piglets [66]. rock and rhoGEF, as important intracellular signaling pathway, are closely related to cell contraction and negatively regulate cell adhesion, skeletal rearrangement and cell migration. Se deficiency reduced SelK production, which impairs the ability of cells to migrate via the rock and rhoGEF pathways [67]. MLC is an important component of myosin, and many cytokines, such as intercellular signal-regulated protein kinases and inflammatory mediators, mediate cell contraction through MLC phosphorylation, leading to widening of the cell gap, resulting in increased vascular endothelial permeability and the formation of vasculitic lesions [68]. Se deficiency has been found to reduce uterine smooth muscle contractility by abnormally decreasing rock and MLC expression in mouse uterine smooth muscle [69]. In the mouse model of differential selenium concentration established in this study, the expression of factors rock, rhoGEF, cingulin and MLC-2 of the ZO-1 pathway was found to increase gradually with the increase in selenium. As for the HUVEC model transfected with siRNA-SelO, the expression was significantly lower than that of the siRNA-NC group, confirming the regulatory effect of selenium on the ZO-1 pathway through SelO. These results are consistent with the results of previous studies.

In conclusion, the present study supports a regulatory role for selenium in modulating vascular endothelial adhesion plaque proteins, vascular endothelial intercellular junctional structures and the ZO-1 signaling pathway by affecting SelO expression. Our study reveals the biological mechanism of selenium deficiency leading to impaired permeability of vascular endothelial cells, provides a theoretical basis for selenium to regulate vascular endothelial adhesion plaque production, vascular endothelial intercellular junctional structure and tight junction ZO-1 signaling pathway through SelO, and provides abundant experimental data for the study of selenium deficiency leading to vascular injury and indicates the direction of further research.

CRediT authorship contribution statement

Jiawei Wu: Writing – original draft, Data curation, Conceptualization. **Yanhe Zhang:** Methodology, Investigation. **Tianjing Liu:** Visualization. **Jie Yang:** Software. **Xiaoran Sun:** Project administration. **Xuejiao Gao:** Supervision.

Declaration of competing interest

The authors declare that they have to known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by funding from the National Natural Science Foundation of China (31802262).

References

[1] V. Kravchenko, T. Zakharchenko, Thyroid hormones and minerals in immunocorrection of disorders in autoimmune thyroid diseases, Front. Endocrinol. 14 (2023) 1225494.
[2] Q. Sun, E. Oltra, D. Dijck-Brouwer, T. Chillon, P. Seemann, S. Asaad, K. Demircan, J. Espejo-Oltra, T. Sánchez-Fito, E. Martín-Martínez, W. Minich, F. Muskiet, L. Schomburg, Autoantibodies to selenoprotein P in chronic fatigue syndrome suggest selenium transport impairment and acquired resistance to thyroid hormone, Redox Biol. 65 (2023) 102796.
[3] S. Xu, Z. Kang, K. Li, X. Li, Y. Zhang, X. Gao, Selenium deficiency causes iron death and inflammatory injury through oxidative stress in the mice gastric mucosa, Biol. Trace Elem. Res. (2023), https://doi.org/10.1007/s12011-023-03754-5.
[4] R. Xu, J. Cao, T. Xu, T. Liu, M. Zhu, M. Guo, Selenium deficiency induced inflammation and apoptosis via NF-κB and MAPKs pathways in muscle of common carp (Cyprinus carpio L.), Fish Shellfish Immunol. 138 (2023) 108847.
[5] S. Carr, B. Crites, H. Shinde, P. Bridges, Transcriptomic changes in response to form of selenium on the interferon-tau signaling mechanism in the caruncular tissue of beef heifers at maternal recognition of pregnancy, Int. J. Mol. Sci. 24 (24) (2023).
[6] J. Xiao, H. Cao, S. Guo, S. Xiao, N. Li, M. Li, Y. Wu, H. Liu, Long-term administration of low-dose selenium nanoparticles with different sizes aggravated atherosclerotic lesions and exhibited toxicity in apolipoprotein E-deficient mice, Chem. Biol. Interact. 347 (2021) 109601.
[7] S. Zheng, J. Zhao, H. Xing, S. Xu, Oxidative stress, inflammation, and glycometabolism disorder-induced erythrocyte hemolysis in selenium-deficient exudative diathesis broilers, J. Cell. Physiol. 234 (9) (2019) 16328–16337.
[8] Z. Wei, M. Yao, Y. Li, X. He, Z. Yang, Dietary selenium deficiency exacerbates lipopolysaccharide-induced inflammatory response in mouse mastitis models, Inflammation 37 (6) (2014) 1925–1931.
[9] D. Luo, X. Tang, Y. Wang, S. Ying, Y. He, H. Lin, P. Khoso, S. Li, Selenium deficiency exacerbated Bisphenol A-induced intestinal toxicity in chickens: apoptosis and cell cycle arrest mediated by ROS/P53, Sci. Total Environ. 913 (2024) 169730.
[10] R. Nishi, K. Sagiyama, K. Hamada, T. Fukumoto, R. Kato, T. Yamamoto, Y. Fuku, H. Amitani, A. Asakawa, Macrocytic anemia induced by selenium deficiency in the course of anorexia nervosa: a case report, Medicine 102 (51) (2023) e36740.
[11] M. Wacker, A. Ball, H. Beer, I. Schmitz, K. Borucki, F. Azizzadeh, M. Scherner, G. Awad, J. Wippermann, P. Veluswamy, Immunophenotyping of monocyte migration markers and therapeutic effects of selenium on IL-6 and IL-1β cytokine axes of blood mononuclear cells in preoperative and postoperative coronary artery disease patients, Int. J. Mol. Sci. 24 (8) (2023).
[12] K. Poudel-Tandukar, E. Bertone-Johnson, K. Poudel, Serum selenium and inflammation in individuals with human immunodeficiency virus infection, AIDS Res. Hum. Retrovir. 39 (12) (2023) 671–676.
[13] O. Abozaid, M. Sallam, S. El-Sonbaty, S. Aziza, B. Emad, E. Ahmed, Resveratrol-selenium nanoparticles alleviate neuroinflammation and neurotoxicity in a rat model of alzheimer's disease by regulating Sirt1/miRNA-134/GSK3β expression, Biol. Trace Elem. Res. 200 (12) (2022) 5104–5114.
[14] Z. Qing, Z. Kaixin, H. Yanfei, Z. Yiming, X. Hua, Z. Ling, S. Guangliang, L. Shu, MicroRNA-223 triggers inflammation in porcine aorta by activating NLRP3 inflammasome under selenium deficiency, J. Cell. Physiol. 236 (6) (2021) 4555–4564.

[15] M. Aviram, B. Fuhrman, LDL oxidation by arterial wall macrophages depends on the oxidative status in the lipoprotein and in the cells: role of prooxidants vs. antioxidants, Mol. Cell. Biochem. 188 (1998) 149–159.
[16] J. Heo, Selenium-stimulated exosomes enhance wound healing by modulating inflammation and angiogenesis, Int. J. Mol. Sci. 23 (19) (2022).
[17] P. Toh, J. Nicholson, A. Vetter, M. Berry, D. Torres, Selenium in bodily homeostasis: hypothalamus, hormones, and highways of communication, Int. J. Mol. Sci. 23 (23) (2022).
[18] F. Zhang, X. Li, Y. Wei, Selenium and selenoproteins in health, Biomolecules 13 (5) (2023).
[19] D. Wang, T. Wu, Y. Zheng, Y. Ma, X. Xie, Nomogram developed with selenoprotein S (SelS) genetic variation and clinical characteristics predicting risk of coronary artery disease in a Chinese population, Cardiovasc. Diagn. Ther. 10 (4) (2020) 770–777.
[20] Q. Chi, Q. Zhang, Y. Lu, Y. Zhang, S. Xu, S. Li, Roles of selenoprotein S in reactive oxygen species-dependent neutrophil extracellular trap formation induced by selenium-deficient arteritis, Redox Biol. 44 (2021) 102003.
[21] M. Shin, H. Cha, S. Park, Y. Kim, J. Kim, S. Park, Selenoprotein W deficiency does not affect oxidative stress and insulin sensitivity in the skeletal muscle of high-fat diet-fed obese mice, Am. J. Physiol. Cell Physiol. 317 (6) (2019) C1172–C1182.
[22] S. Nettleford, C. Liao, S. Short, R. Rossi, V. Singh, K. Prabhu, Selenoprotein W ameliorates experimental colitis and promotes intestinal epithelial repair, Antioxidants 12 (4) (2023).
[23] A. Cox, A. Lehtinen, J. Xu, C. Langefeld, B. Freedman, J. Carr, D. Bowden, Polymorphisms in the selenoprotein S gene and subclinical cardiovascular disease in the diabetes heart study, Acta Diabetol. 50 (3) (2013) 391–399.
[24] W. Talbi, T. Ghazouani, D. Braconi, R. Ben Abdallah, F. Raboudi, A. Santucci, S. Fattouch, Effects of selenium on oxidative damage and antioxidant enzymes of eukaryotic cells: wine Saccharomyces cerevisiae, J. Appl. Microbiol. 126 (2) (2019) 555–566.
[25] M. Mariotti, P. Ridge, Y. Zhang, A. Lobanov, T. Pringle, R. Guigo, D. Hatfield, V. Gladyshev, Composition and evolution of the vertebrate and mammalian selenoproteomes, PLoS One 7 (3) (2012) e33066.
[26] T. Yoshida, M. Watanabe, D. Engelman, R. Engelman, J. Schley, N. Maulik, Y. Ho, T. Oberley, D. Das, Transgenic mice overexpressing glutathione peroxidase are resistant to myocardial ischemia reperfusion injury, J. Mol. Cell. Cardiol. 28 (8) (1996) 1759–1767.
[27] T. Ago, J. Sadoshima, Thioredoxin and ventricular remodeling, J. Mol. Cell. Cardiol. 41 (5) (2006) 762–773.
[28] A. Rose, P. Hoffmann, Selenoproteins and cardiovascular stress, Thromb. Haemostasis 113 (3) (2015) 494–504.
[29] A. Pfenniger, M. Chanson, B. Kwak, Connexins in atherosclerosis, Biochim. Biophys. Acta 1828 (1) (2013) 157–166.
[30] C. Cencer, J. Silverman, L. Meenderink, E. Krystofiak, B. Millis, M. Tyska, Adhesion-based capture stabilizes nascent microvilli at epithelial cell junctions, Dev. Cell 58 (20) (2023), 2048-62.e7.
[31] C. Zihni, C. Mills, K. Matter, M. Balda, Tight junctions: from simple barriers to multifunctional molecular gates, Nat. Rev. Mol. Cell Biol. 17 (9) (2016) 564–580.
[32] L. Chen, Z. Chen, M. Ge, O. Tang, Y. Cheng, H. Zhou, Y. Shen, F. Qin, Monocytic cell junction proteins serve important roles in atherosclerosis via the endoglin pathway, Mol. Med. Rep. 16 (5) (2017) 6750–6756.
[33] M. Takeichi, Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling, Nat. Rev. Mol. Cell Biol. 15 (6) (2014) 397–410.
[34] L. Ding, L. Li, B. Hu, J. Wang, Y. Lu, R. Zhang, X. He, C. Shi, L. Wu, C. Wu, B. Yang, L. Zheng, B. Ping, Y. Hu, Q. Wang, TM4SF19 aggravates LPS-induced attenuation of vascular endothelial cell adherens junctions by suppressing VE-cadherin expression, Biochem. Biophys. Res. Commun. 533 (4) (2020) 1204–1211.
[35] M. Adil, S. Narayanan, P. Somanath, Cell-cell junctions: structure and regulation in physiology and pathology, Tissue Barriers 9 (1) (2021) 1848212.
[36] C. Turner, K. Pietras, D. Taylor, C. Molloy, Angiotensin II stimulation of rapid paxillin tyrosine phosphorylation correlates with the formation of focal adhesions in rat aortic smooth muscle cells, J. Cell Sci. (1995) 333–342.
[37] T. Tu, H. Liu, Z. Liu, Y. Liang, C. Tan, D. Feng, J. Zou, Amelioration of Atherosclerosis by lycopene is linked to the modulation of gut microbiota dysbiosis and related gut-heart axis activation in high-fat diet-fed ApoE mice, Nutr. Metabol. 20 (1) (2023) 53.
[38] T. Chmiel, M. Gardel, Confluence and tight junction dependence of volume regulation in epithelial tissue, Mol. Biol. Cell 33 (11) (2022) ar98.
[39] S. Holzner, S. Bromberger, J. Wenzina, K. Neumüller, T. Holper, P. Petzelbauer, W. Bauer, B. Weber, K. Schossleitner, Phosphorylated cingulin localises GEF-H1 at tight junctions to protect vascular barriers in blood endothelial cells, J. Cell Sci. 134 (17) (2021).
[40] Y. Zhang, J. Cui, K. Li, S. Xu, H. Yin, S. Li, X. Gao, Trimethyltin chloride exposure induces apoptosis and necrosis and impairs islet function through autophagic interference, Ecotoxicol. Environ. Saf. 267 (2023) 115628.
[41] R. Xu, F. Han, H. Wang, J. Wang, Z. Cai, M. Guo, Tea polyphenols alleviate TBBPA-induced inflammation, ferroptosis and apoptosis via TLR4/NF-κB pathway in carp gills, Fish Shellfish Immunol. 146 (2024) 109382.
[42] T. Liu, J. Yang, J. Wu, X. Sun, X. Gao, Polyethylene microplastics induced inflammation via the miR-21/IRAK4/NF-κB axis resulting to endoplasmic reticulum stress and apoptosis in muscle of carp, Fish Shellfish Immunol. 145 (2024) 109375.
[43] Q. Zhang, F. Wang, S. Xu, J. Cui, K. Li, X. Shiwen, M. Guo, Polystyrene microplastics induce myocardial inflammation and cell death via the TLR4/NF-κB pathway in carp, Fish Shellfish Immunol. 135 (2023) 108690.
[44] Y. Nakahata, T. Hanai, T. Miwa, T. Maeda, K. Imai, A. Suetsugu, K. Takai, M. Shimizu, Effect of selenium deficiency on the development of overt hepatic encephalopathy in patients with chronic liver disease, J. Clin. Med. 12 (8) (2023).
[45] M. Pereira, L. Lima, J. Souza, N. de Souza da Costa, J. da Silva, I. Guiloski, A. Irioda, C. Oliveira, Evaluation of the neuroprotective effect of organic selenium compounds: an in vitro model of alzheimer's disease, Biol. Trace Elem. Res. (2023), https://doi.org/10.1007/s12011-023-03893-9.
[46] X. Meng, X. Meng, Z. He, Y. Yuan, Y. Fan, L. Yin, Y. Tong, Z. Hong, S. Zhu, Q. Zhang, Q. Bi, Selenium deficiency can promote the expression of VEGF and inflammatory factors in cartilage differentiation and mediates cartilage injury, Biol. Trace Elem. Res. (2023), https://doi.org/10.1007/s12011-023-04003-5.
[47] L. Yu, L. Sun, Y. Nan, L. Zhu, Protection from H1N1 influenza virus infections in mice by supplementation with selenium: a comparison with selenium-deficient mice, Biol. Trace Elem. Res. 141 (2011) 254–261.
[48] K. Lundin, Y. Qadeer, Z. Wang, S. Virani, R. Leischik, C. Lavie, M. Strauss, C. Krittanawong, Contaminant metals and cardiovascular health, Journal of cardiovascular development and disease 10 (11) (2023).
[49] I. Andrade, F. de Souza, F. Fonseca, C. Aranda, R. Sarni, Selenium-related nutritional status in patients with common variable immunodeficiency: association with oxidative stress and atherosclerosis risk, BMC Immunol. 22 (1) (2021) 31.
[50] P. Lear, J. Prohaska, Atria and ventricles of copper-deficient rats exhibit similar hypertrophy and similar altered biochemical characteristics, Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY) 215 (4) (1997) 377–385.
[51] J. Avery, P. Hoffmann, Selenium, selenoproteins, and immunity, Nutrients 10 (9) (2018).
[52] Y. Ye, F. Fu, X. Li, J. Yang, H. Liu, Selenoprotein S is highly expressed in the blood vessels and prevents vascular smooth muscle cells from apoptosis, J. Cell. Biochem. 117 (1) (2016) 106–117.
[53] E. Lopes Junior, H. Leite, T. Konstantyner, Selenium and selenoproteins: from endothelial cytoprotection to clinical outcomes, Transl. Res. : J. Lab. Clin. Med. 208 (2019) 85–104.
[54] C. Arriagada, P. Silva, V. Torres, Role of glycosylation in hypoxia-driven cell migration and invasion, Cell Adhes. Migrat. 13 (1) (2019) 13–22.
[55] E. Lee, A. Choi, Y. Jun, N. Kim, J. Yook, S. Kim, S. Lee, S. Kang, Glutathione peroxidase-1 regulates adhesion and metastasis of triple-negative breast cancer cells via FAK signaling, Redox Biol. 29 (2020) 101391.
[56] J. Bang, J. Huh, J. Na, Q. Lu, B. Carlson, R. Tobe, P. Tsuji, V. Gladyshev, D. Hatfield, B. Lee, Cell proliferation and motility are inhibited by G1 phase arrest in 15-kDa selenoprotein-deficient chang liver cells, Mol. Cell. 38 (5) (2015) 457–465.
[57] S. Cui, L. Men, Y. Li, Y. Zhong, S. Yu, F. Li, J. Du, αSelenoprotein S attenuates tumor necrosis factor–induced dysfunction in endothelial cells, Mediat. Inflamm. 2018 (2018) 1625414.
[58] N. Rahimi, Defenders and challengers of endothelial barrier function, Front. Immunol. 8 (2017) 1847.
[59] D. Hou, F. He, L. Ma, M. Cao, Z. Zhou, Z. Wei, Y. Xue, X. Sang, H. Chong, C. Tian, S. Zheng, J. Li, K. Zen, X. Chen, Z. Hong, C. Zhang, X. Jiang, The potential atheroprotective role of plant MIR156a as a repressor of monocyte recruitment on inflamed human endothelial cells, J. Nutr. Biochem. 57 (2018) 197–205.
[60] H. Sattar, J. Yang, X. Zhao, J. Cai, Q. Liu, M. Ishfaq, Z. Yang, M. Chen, Z. Zhang, S. Xu, Selenoprotein-U (SelU) knockdown triggers autophagy through PI3K-Akt-mTOR pathway inhibition in rooster Sertoli cells, Metallomics : integrated biometal science 10 (7) (2018) 929–940.
[61] M. Abbaci, M. Barberi-Heyob, J. Stines, W. Blondel, D. Dumas, F. Guillemin, J. Didelon, Gap junctional intercellular communication capacity by gap-FRAP technique: a comparative study, Biotechnol. J. 2 (1) (2007) 50–61.
[62] C. Qi, S. Acosta Gutierrez, P. Lavriha, A. Othman, D. Lopez-Pigozzi, E. Bayraktar, D. Schuster, P. Picotti, N. Zamboni, M. Bortolozzi, F. Gervasio, V. Korkhov, Structure of the connexin-43 gap junction channel in a putative closed state, Elife 12 (2023).
[63] E. Dejana, C. Giampietro, Vascular endothelial-cadherin and vascular stability, Curr. Opin. Hematol. 19 (3) (2012) 218–223.
[64] F. Rodriguez, L. Lewis-Tuffin, P. Anastasiadis, E-cadherin's dark side: possible role in tumor progression, Biochim. Biophys. Acta 1826 (1) (2012) 23–31.
[65] W. Ren, K. Wu, X. Li, M. Luo, H. Liu, S. Zhang, Y. Hu, Age-related changes in small intestinal mucosa epithelium architecture and epithelial tight junction in rat models, Aging Clin. Exp. Res. 26 (2) (2014) 183–191.
[66] Y. Xue, H. Wang, B. Tian, S. Wang, X. Gao, Selenium deficiency promotes the expression of LncRNA-MORC3, activating NLRP3-caspase-1/IL-1β signaling to induce inflammatory damage and disrupt tight junctions in piglets, Biol. Trace Elem. Res. 201 (5) (2023) 2365–2376.
[67] L. Zhang, H. Xia, K. Xia, X. Liu, X. Zhang, J. Dai, Z. Zeng, Y. Jia, Selenium regulation of the immune function of dendritic cells in mice through the ERK, akt and RhoA/ROCK pathways, Biol. Trace Elem. Res. 199 (9) (2021) 3360–3370.
[68] W. Cole, D. Welsh, Role of myosin light chain kinase and myosin light chain phosphatase in the resistance arterial myogenic response to intravascular pressure, Arch. Biochem. Biophys. 510 (2) (2011) 160–173.
[69] C. Chen, P. Xiao, Y. Chen, R. Fang, Selenium deficiency affects uterine smooth muscle contraction through regulation of the RhoA/ROCK signalling pathway in mice, Biol. Trace Elem. Res. 192 (2) (2019) 277–286.