Am J Transl Res 2022;14(9):6596-6603
www.ajtr.org /ISSN:1943-8141/AJTR0134793

Original Article

CTRP9 mitigates vascular endothelial cell injury in patients with hypertensive heart disease by inhibiting PI3K/Akt/mTOR axis

Lingyun Pan[1], Xiaocui Sun[1], Haixia Che[1], Mingzhe Li[2]

[1]*Department of Emergency Medicine, The Second People's Hospital of Liaocheng, The Second Hospital of Liaocheng Affiliated to Shandong First Medical University, Liaocheng, Shandong, China;* [2]*Shandong ENT Hospital Affiliated to Shandong University, Jinan, Shandong, China*

Received April 29, 2021; Accepted March 26, 2022; Epub September 15, 2022; Published September 30, 2022

Abstract: Objective: To investigate the mechanism of factor-alpha-related protein 9 (CTRP9) in mitigating the vascular endothelial cell (VEC) injury in patients with hypertensive heart disease (HHD) by the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) axis. Methods: 43 patients with HHD admitted to our hospital from February 2018 to February 2019 were included in the study group, and another 39 healthy controls from the same period were the reference group. The total protein of transfected VECs was detected by western blotting, and the proliferation rate of the VECs was determined by Cell Counting Kit-8 (CCK-8). The levels of CTRP9, high sensitivity C-reactive protein (hs-CRP), thrombomodulin (TM), and von Willebrand factor (vWF) were detected by ELISA. The mechanism of CTRP9 in alleviating VEC injury in HHD patients by inhibiting the PI3K/Akt/mTOR axis was analyzed. Results: The two groups did not differ in terms of their general data (P>0.05). The CTRP9 level in the study group was higher than in the reference group (P<0.001). Study group had higher levels of endothelin-1 (ET-1), hs-CRP, TM, vWF (P<0.001), and markedly lower phospho-PI3K (p-PI3K) and phospho-protein kinase B (p-AKT) protein levels (P<0.05). Compared to the reference group, the proliferation capacity of trophoblast cells in the study group was sharply decreased (P<0.05). The study group had lower phosphorylation levels of PI3K, Akt, and mTOR proteins than the reference group (P<0.05). Phosphorylation of Akt occurred at 15 min and reached its peak at 30 min. A drastically reduced invasion capacity of VECs was observed in the study group compared to the reference group (P<0.05). Conclusions: CTRP9 mitigates VEC injury in patients with HHD by inhibiting the PI3K/Akt/mTOR axis.

Keywords: CTRP9, PI3K/Akt/mTOR axis, stimulation, hypertensive heart disease, vascular endothelial cell injury

Introduction

Hypertensive heart disease (HHD) impairs the structure and function of the heart. It is characterized by dyspnea and fatigue after exercise, and is usually triggered by hypertension. To date, HHD can be controlled and alleviated only by drugs, as a radical treatment has not yet been found [1, 2]. Mutations in some components of the PI3K/Akt/mTOR signaling pathway can induce cell transformation, proliferation, and migration. Previous studies have shown that the expression of effector molecules in the PI3K/Akt/mTOR axis is higher during episodes than that in remission in patients with HHD, suggesting a key role of the PI3K/Akt/mTOR axis in the occurrence and progression of HHD [3]. C1q/TNF-related protein 9 (CTRP9), an endogenous cardiovascular protective factor, is essential to immune, blood, energy metabolism, and cardiovascular protection systems. Clinical studies have found that CTRP9 exerts multiple protective effects on systemic circulation arteries, including the ability to promote nitric oxide (NO) production, as well as anti-atherosclerosis and anti-inflammatory effects [4]. Moreover, CTRP9 plays a role in inhibiting vascular inflammation and leukocyte adhesion induced by perivascular cytokines [5-7]. The above effects of CTRP9 suggest its promising application prospect in the treatment of cardiovascular and cerebrovascular diseases. However, the mechanism of the inhibitory effect of CTRP9 on the PI3K/Akt/mTOR axis to mitigate

vascular endothelial cell (VEC) injury in HHD patients has hitherto not been reported. Accordingly, the present study analyzes the effect of CTRP9 on VEC injury in patients with HHD by inhibiting the PI3K/Akt/mTOR axis, aiming to further provide a clinical basis for future treatment. The innovation of this research lies in the inhibition of signal pathways to achieve damage repair, which provides a basis for future targeted therapy or immunotherapy.

Materials and methods

General data

Forty-three patients with HHD admitted to our hospital from January 2019 to January 2020 were included as a study group, and 39 healthy controls from the medical examination center during the same period were selected as the reference group. Inclusion criteria: (1). The classification of hypertension was levels 1 and 2. (2). Doppler echocardiography showed left ventricular hypertrophy with hypodiastolic left ventricular function (restricted left ventricular filling and decreased E/A ratio). Exclusion criteria: Patients who had cardiac insufficiency such as cardiomyopathy and coronary heart disease. This study was reviewed and approved by the internal Medical Ethics Committee (No. 2018-09-12), and all the participants signed an informed consent.

Methods

ELISA: Fasting elbow venous blood (3 mL) was collected from both groups in the early morning. Then the blood samples were added to 2% and 3.2% 2 mL of sodium citrate respectively and oscillated evenly. After centrifugation, the supernatant was collected and placed in the refrigerator at -20°C for later analysis. The levels of CTRP9, high sensitivity C-reactive protein (hs-CRP), thrombomodulin (TM), and von Willebrand factor (vWF) were detected by ELISA with the ELISA kits (Shanghai Renjie Biotechnology Co., Ltd., China) and DR-3508 microplate reader (Wuxi Hiwell-Diatek Instruments Co., Ltd, China). All operations were in strict accordance with the instructions.

Western blotting: Western blotting was used to detect the total proteins extracted from the VECs and transfected cells. BCA protein quantitative kit (R21250, Shanghai yuanye Bio-Technology Co., Ltd, China) was adopted to quantify the total protein. First, the proteins were added to 4X protein loading buffer in proportion, denatured at 95°C for 5 min, and stored in a refrigerator at -20°C. Then, the polyvinylidene fluoride (PVDF) membrane was rinsed with 3% tris buffered saline tween (TBST) 3 times, 5 min each time, and sealed with the BSA blocking buffer for 2 hours of incubation at room temperature. After the PVDF was rinsed, primary and second antibodies were dropwise added in sequence for incubation [8, 9]. The freshly prepared electrochemiluminescence (ECL) solution (Shanghai yuanye Bio-Technology Co., Ltd, R21240) was added to the surface of the PVDF membrane for image collection and analysis. For protein quantification, the relative optical density (OD) value was used to represent the protein expression, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference. This experiment was repeated at least 3 times. Western blotting was used to test the expression of proteins related to the p-PI3K/p-Akt pathway.

The phosphorylation levels of apoptosis-related proteins PI3K/Akt were analyzed by Western blotting. After rinsing, the cells were thoroughly lysed with a cell lysate containing protease inhibitors. After centrifugation, the supernatant was collected and heated to boiling for 10 min. Then, an appropriate amount was processed for gel electrophoresis and subsequently transferred to the PVDF membrane. Then, rabbit anti-Bax, B cell CLL/lymphoma-2 (Bcl-2), phosphorylated Akt, total Akt, and mouse anti-GAPDH antibodies were added for overnight incubation at 4°C. The dilution rate of the primary antibodies was all 1:1000, and of the secondary antibody was 1:2000 (Shanghai Beyotime Biotechnology Co., Ltd., China). The next day, it was rinsed 3 times with 0.05% TBST, and HRP labeled goat anti-rabbit or goat anti-mouse antibodies were then added. Finally, the ECL substrate was used for development to present the observation image.

CCK-8 assay: The proliferation rate of VECs was measured by a CCK-8 assay. Cells in the logarithmic growth phase were seeded into a 96-well plate at a specification of 4×10/well after being processed with 0.25% trypsin. Each well was then cultured with high-glucose Dul-

Table 1. Comparison of general data in the two groups

Category	Study group (n=43)	Reference group (n=39)	χ^2/t	P
Gender			0.003	0.957
Male	24 (55.81)	22 (56.41)		
Female	19 (44.19)	17 (43.59)		
Mean age (year)	53.95±4.52	54.24±4.39	0.294	0.764
Mean BMI (kg/m^2)	21.17±2.56	21.23±2.62	0.105	0.917
Average height (cm)	168.27±9.26	168.38±9.04	0.054	0.957
Education				
College	11 (25.58%)	13 (33.33%)	0.594	0.441
High School	18 (41.86%)	15 (38.46%)	0.098	0.754
Primary School	14 (32.56%)	11 (28.21%)	0.183	0.669
Residence			0.000	0.991
Urban	21 (53.85%)	19 (48.72%)		
Rural Area	22 (56.41%)	20 (51.28%)		

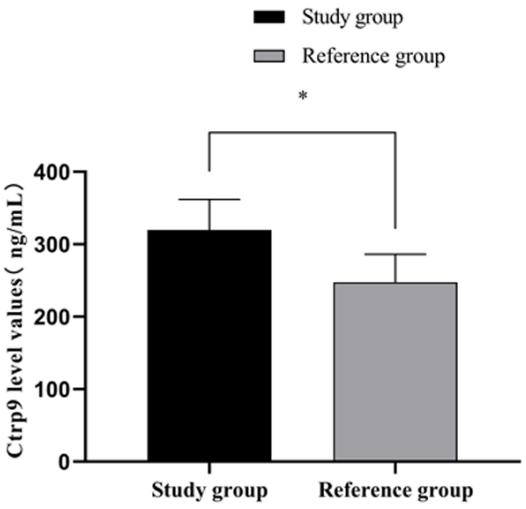

Figure 1. Comparison of CTRP9 level between two groups (x±sd). Note: CTRP9 level in the study group was (319.52±42.33) ng/mL, and that of the reference group was (247.46±38.56) ng/mL; A Significant difference in CTRP9 level between two groups (*P<0.05).

becco's modified eagle medium (DMEM/HG, 200 μL) with 10% fetal bovine serum (FBS), and the original culture medium was discarded when the cell fusion reached 70-80%. When incubated in a constant temperature incubator (37°C) with 5% CO_2 and saturated humidity for 12, 24, 36, and 48 hours, CCK8 reagent (10 μL) was added to each well. The culture plate was then shaken slightly and placed in the cell incubator for 2 hours. The 96-well plate was oscillated for 2-5 min. The OD value was detected at 490 nm with a microplate reader. According to the formula (reference group OD-study group OD)/reference group OD, the cell proliferation rate of each group was calculated and analyzed. Each group was set with 5 replicate wells, and the experiment was repeated 3 times independently.

Transwell cell invasion assay: Transwell cell invasion assay: the matrigel was dissolved at 4°C, diluted in the proportion of 1:5 in DMEM/HG medium without FBS, and then placed in the incubator for 4 hours until the matrigel solidified. Cells at the logarithmic phase were collected and starved in DMEM/HG containing 1% FBS for 24 hours. Then the cells were digested by 0.25% trypsin and suspended in DEME/HG without FBS to a density of 4×10/mL. The lower chamber was added 500 μL DMEM/HG containing 10% FBS. Each group was set in triplicate and placed in a constant temperature incubator of 37°C with 5% CO_2 for 48 hours. Then the upper chamber was withdrawn and rinsed with PBS twice, and wet cotton swabs were used to wipe out cells that failed to infiltrate the matrigel in the upper chamber. Subsequently, the cells were fixed with absolute alcohol for 15 min and then dried at room temperature [10, 11], followed by 30 min of staining with 0.1% crystal violet at room temperature. After 2 times of PBS washing, the number of transmembrane cells was observed under an inverted microscope, and 5 fields were randomly selected from each group for counting.

Statistical analysis

The experimental data were statistically analyzed by SPSS 20.0 and visualized by GraphPad

Table 2. Comparison of vascular endothelial cell injury-related markers between the two groups (x±s)

Group	n	hs-CRP (mg/L)	TM (μ/L)	vWF (IU/dL)
Study group	43	21.38±1.36	5.62±0.48	92.73±7.92
Reference group	39	8.41±1.48	2.31±0.35	67.43±6.72
t		42.314	36.537	17.973
P		0.001	0.002	0.001

Prism 7 (GraphPad Software, San Diego, USA). The count data, analyzed by the χ^2 test, were represented by [n (%)]. The measurement data were expressed as (x±sd), analyzed by a t-test. The difference was considered significant when a *p*-value was less than 0.05.

Results

Comparison between general data

There were no significant differences in gender ratio, average age, mean BMI, average height, education, and residence between the two groups (P>0.05), which were comparable (**Table 1**).

Comparison of CTRP9

The CTRP9 level in the study group was (319.52±42.33) ng/mL, and that of the reference group was (247.46±38.56) ng/mL (P< 0.001; **Figure 1**).

Comparison of VEC injury

Significantly higher ET-1, hs-CRP, TM, and vWF levels were determined in the study group as compared to the reference group (P<0.05; **Table 2**).

Comparison of p-PI3K and p-Akt expression levels

Relative protein expression levels of p-PI3K and p-AkT in the study group were (0.43±0.09) and (0.47±0.08), respectively; while those in the reference group were (1.64±0.28) and (1.46±0.24), respectively. The differences in p-PI3K and p-Akt between the two groups were significant (P<0.05; **Figure 2**).

Effect of CTRP9 on the proliferation of transfected VECs

The amount (time point) of the proliferation of VECs in the study group was (0.21±0.08) (12 h), (0.31±0.06) (24 h), (0.45±0.12) (26 h), (0.53±0.06) (48 h), while that in the reference group was (0.23±0.05) (12 h), (0.34±0.07) (24 h), (0.53±0.11) (26 h), (0.79±0.05) (48 h). Results of the CCK-8 assay demonstrated that compared to the reference group, the trophoblast proliferation ability in the study group was decreased (P<0.05), which indicated a significant inhibitory effect of CTRP9 on the proliferation of VECs (**Figure 3**).

Effect of CTRP9 on the PI3K/Akt/mTOR axis in patients with HHD

Protein relative expression levels of PI3K, AkT, and mTOR in the study group were 1.53±0.06, 1.50±0.04, and 2.06±0.54, respectively; while those in the reference group were 0.64±0.28, 0.46±0.24, and 1.07±0.44, respectively. The differences in relative protein expression of PI3K, Akt, and mTOR between two groups were significant (P<0.05). According to the results of western blotting, the study group yielded lower phosphorylation levels of PI3K, Akt, and mTOR proteins in comparison to those in the reference group (P<0.05), indicating that CTRP9 inhibitors could block the PI3K/Akt/mTOR signaling pathway (**Figure 4**).

Effect of CTRP9 on the invasion of VECs

Average cell number of the study group (102.63±5.36) was lower than (200.63±7.36) respectively; the difference in VEC invasion between groups was significant (P<0.05). The results of Transwell assay suggested that the invasion of VEC in the study group was inferior to that in the reference group (P<0.05; **Figure 5A-C**).

Discussion

Clinical studies have confirmed that CTRP9 is crucial in the anti-inflammatory response, myocardial protection, inhibition of vascular remodeling after intimal injury, and protection of vascular endothelium [12, 13]. Prior studies [14] have pointed out the effect of CTRP9 on increasing the stability of carotid atherosclerotic plaque and reducing the expression of intercellular adhesion molecule (ICAM)-1, thus inhibiting cytokine-induced leukocyte adhesion and vascular inflammation. PI3K is a lipase that can phosphorylate the third hydroxyl group of phos-

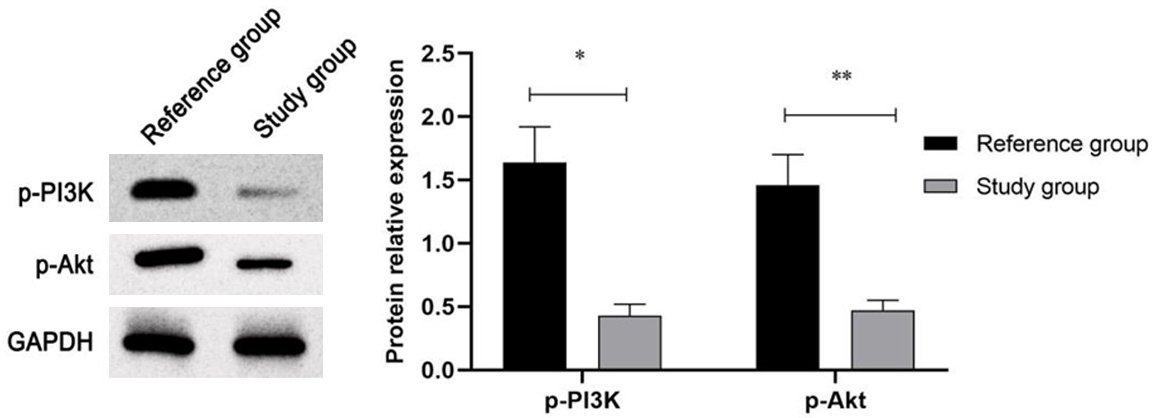

Figure 2. Comparison of p-PI3K and p-Akt expression levels between two groups (x±s). Note: Relative protein expression levels of p-PI3K, and p-AkT in the study group were 0.43±0.09 and 0.47±0.08, respectively; while those in the reference group were 1.64±0.28 and 1.46±0.24, respectively. A difference was found in p-PI3K between the two groups (t=5.818, *P=0.028), as well as in p-Akt (t=5.534, **P<0.01).

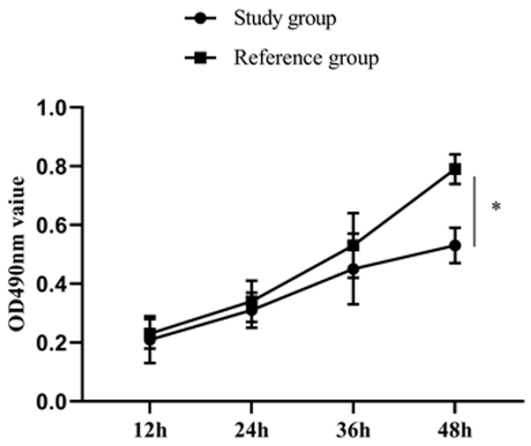

Figure 3. Effect of CTRP9 on the proliferation of transfected vascular endothelial cells (x±sd). The proliferation ability of cells in the two groups changed at 12 h, 24 h, 26 h and 48 h. Note: The amount (time point) of the proliferation of vascular endothelial cells in study group was 0.21±0.08 (12 h), 0.31±0.06 (24 h), 0.45±0.12 (26 h), 0.53±0.06 (48 h), while that in reference group was 0.23±0.05 (12 h), 0.34±0.07 (24 h), 0.53±0.11 (26 h), and 0.79±0.05 (48 h). A significant difference in the proliferation of transfected vascular endothelial cells between the two groups (t=4.708, *P=0.042).

phatidylinositol and is commonly involved in insulin signal transduction, glucose metabolism, and cell growth [15, 16]. Akt is a serine protein kinase that mainly participates in insulin-mediated glucose uptake, as well as invasion and metastasis of tumor cells. Previous studies have stated that the regulation of cell proliferation, differentiation, apoptosis, and glucose transport was supported by the PI3K/Akt pathway which is able to modulate the proliferation and apoptosis of pancreatic cancer cells [17, 18]. Excessive activation of the PI3K/Akt pathway is related to tumor pathology. The mTOR, an atypical serine kinase, can promote RNA transcription and act as an integrator of various input signals to regulate lipid synthesis, glucose metabolism, apoptosis process, and growth of tumor cells [19, 20]. Aberrant expression of the PI3K/Akt/mTOR signaling pathway was detected in cancer cells of patients with gastric cancer [21]. The immunohistochemical method also found that the expression level of p-Akt in gastric cancer tissues was higher than that in paracancerous tissues. Similar results of the expression level of mRNA in gastric cancer were detected according to RT-PCR analysis. Hence, the PI3K/Akt/mTOR signaling pathway is abnormally activated in gastric cancer tissues, through which targeted inhibitors of various molecules can exert anti-tumor effects.

The occurrence, development, invasion, and metastasis of tumor cells have been proven to be closely related to the abnormal activation of some intracellular signaling pathways. The correlation of various signaling pathways with the occurrence and development of vascular endothelial cell (VEC) injury has therefore become a focus of clinical research. The PI3K/Akt/mTOR signaling pathway is expressed in VECs of patients with HHD and participates in the regulation. The activation of this signaling pathway can improve the production of angiogenic

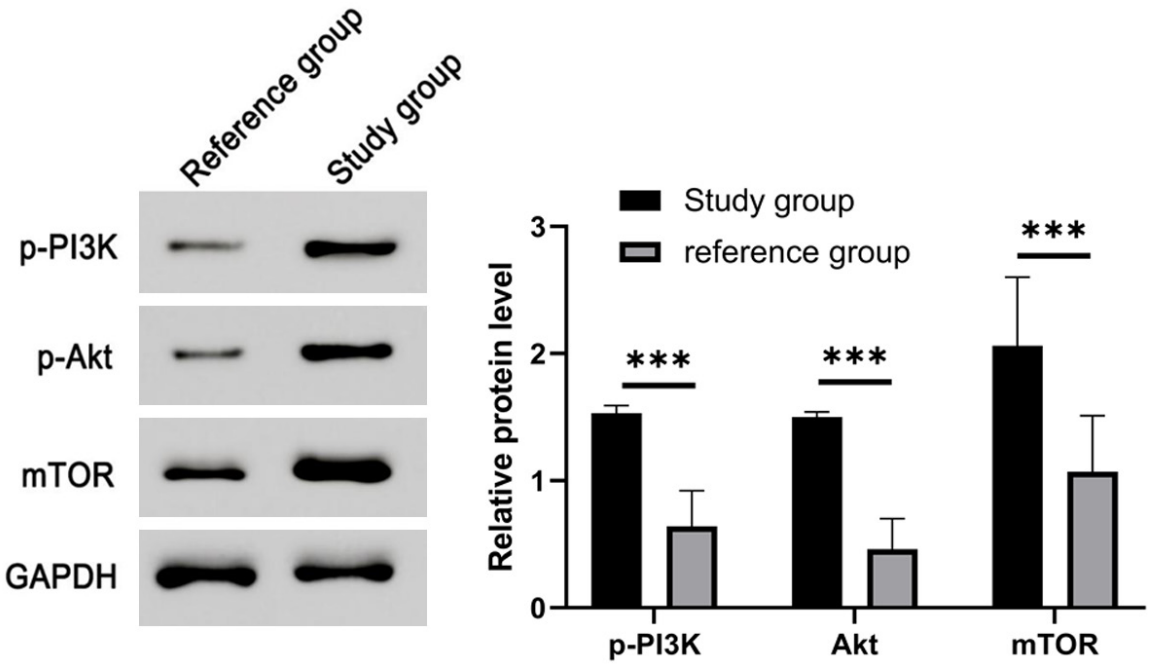

Figure 4. Effect of CTRP9 on the PI3K/Akt/mTOR signaling pathway. Comparison of the relative expression of PI3K, Akt, and mTOR proteins between the two groups. Note: Relative protein expression levels of PI3K, AkT, and mTOR in the study group were 1.53±0.06, 1.50±0.04, and 2.06±0.54, respectively; while those in the reference group were 0.64±0.28, 0.46±0.24, and 1.07±0.44, respectively.

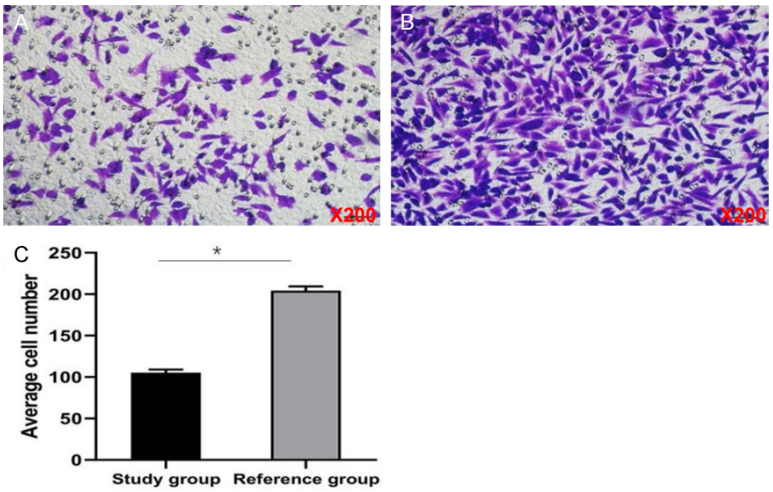

Figure 5. Effect of CTRP9 on vascular endothelial cell invasion and comparison of vascular endothelial cell invasion between the two groups (x±sd). (A) Cell invasion experiment in the study group. (B) Cell invasion experiment in the reference group. (C) Graph of cell invasion data in the two groups. Note: Magnification ratio of (A and B) is ×200. The horizontal axis of (C) represents the study group and reference group from left to right, and the vertical axis represents the average cell number. In the study group this was 102.63±5.36, which is lower than 200.63±7.36 in the reference group (t=4.786, *P=0.041).

factors such as VEGF, which can relieve the VEC injury. Therefore, the correlation between this signaling pathway and VECs is well-established [22, 23]. In recent years, with the attention of the medical community to molecular targeted therapy, targeted drugs have been developed for clinical application. mTOR signaling molecule, located in the central position of the PI3K/Akt signaling pathway, is closely related to the proliferation, differentiation, and apoptosis of tumor cells. Though this study centers on the mitigation of VEC injury in patients with HHD after the inhibition of PI3K/Akt/mTOR axis by CTRP9, the limitation lies in the absence of further exploration on drug targets. In future studies, the number of research cases will be expanded, the follow-up time will be extended, and further targeted research on drug targets will be carried out to obtain more convincing and comprehensive

data. Therefore, it is of great significance for the medical community to improve understanding of the mTOR feedback mechanism and to study the molecular mechanism of VEC injury related signal pathways in patients with HHD, so as to provide a new direction for clinical therapeutic drugs [24, 25].

Conclusion

CTRP9 mitigates VEC injury in patients with HHD by inhibiting the PI3K/Akt/mTOR axis.

Disclosure of conflict of interest

None.

Address correspondence to: Mingzhe Li, Shandong ENT Hospital Affiliated to Shandong University, Room 602, Unit 4, Building 10, No. 669, Jingqi Rd, Huaiyi District, Jinan 250000, Shandong, China. Tel: +86-15168860918; E-mail: limingzhe83@163.com

References

[1] Chen JY, Lei SY, Li TT, Li J, Zuo AJ, Xu D, Song CX and Guo Y. CTRP9 induces iNOS expression through JAK2/STAT3 pathway in Raw 264.7 and peritoneal macrophages. Biochem Biophys Res Commun 2020; 523: 98-104.

[2] Huang C, Zhang P, Li T, Li J, Liu T, Zuo A, Chen J and Guo Y. Overexpression of CTRP9 attenuates the development of atherosclerosis in apolipoprotein E-deficient mice. Mol Cell Biochem 2019; 455: 99-108.

[3] Li Q, Zhu Z, Wang C, Cai L, Lu J, Wang Y, Xu J, Su Z, Zheng W and Chen X. CTRP9 ameliorates cellular senescence via PGC-1α/AMPK signaling in mesenchymal stem cells. Int J Mol Med 2018; 42: 1054-1063.

[4] Chen J, Zhang X, Liu X, Zhang C, Shang W, Xue J, Chen R, Xing Y, Song D and Xu R. Ginsenoside Rg1 promotes cerebral angiogenesis via the PI3K/Akt/mTOR signaling pathway in ischemic mice. Eur J Pharmacol 2019; 856: 172418.

[5] Huang DH, Jian J, Li S, Zhang Y and Liu LZ. TPX2 silencing exerts anti-tumor effects on hepatocellular carcinoma by regulating the PI3K/AKT signaling pathway. Int J Mol Med 2019; 44: 2113-2122.

[6] Yang Z, Zou X, Feng P, Zhan H, Xiong D and Lang J. Inhibition of the PI3K/AKT signaling pathway or overexpression of beclin1 blocks reinfection of streptococcus pneumoniae after infection of influenza A virus in severe community-acquired pneumonia. Inflammation 2019; 42: 1741-1753.

[7] Li Y, Guo Y, Fan Y, Tian H, Li K and Mei X. Melatonin enhances autophagy and reduces apoptosis to promote locomotor recovery in spinal cord injury via the PI3K/AKT/mTOR signaling pathway. Neurochem Res 2019; 44: 2007-2019.

[8] Huang S, Xu Y, Ge X, Xu B, Peng W, Jiang X, Shen L and Xia L. Long noncoding RNA NEAT1 accelerates the proliferation and fibrosis in diabetic nephropathy through activating Akt/mTOR signaling pathway. J Cell Physiol 2019; 234: 11200-11207.

[9] Chen X, Li Y, Feng M, Hu X, Zhang H, Zhang R, Dong X, Liu C, Zhang Z, Jiang S, Huang S and Chen L. Maduramicin induces cardiac muscle cell death by the ROS-dependent PTEN/Akt-Erk1/2 signaling pathway. J Cell Physiol 2019; 234: 10964-10976.

[10] Zhang Z, Wen H, Weng J, Feng L, Liu H, Hu X and Zeng F. Silencing of EPCAM suppresses hepatic fibrosis and hepatic stellate cell proliferation in mice with alcoholic hepatitis via the PI3K/Akt/mTOR signaling pathway. Cell Cycle 2019; 18: 2239-2254.

[11] Okada T, Enkhjargal B, Travis ZD, Ocak U, Tang J, Suzuki H and Zhang JH. FGF-2 attenuates neuronal apoptosis via FGFR3/PI3k/Akt signaling pathway after subarachnoid hemorrhage. Mol Neurobiol 2019; 56: 8203-8219.

[12] Zhang Y, Wang J, Hui B, Sun W, Li B, Shi F, Che S, Chai L and Song L. Pristimerin enhances the effect of cisplatin by inhibiting the miR-23a/Akt/GSK3β signaling pathway and suppressing autophagy in lung cancer cells. Int J Mol Med 2019; 43: 1382-1394.

[13] Kang M, Lee SM, Kim W, Lee KH and Kim DY. Fubp1 supports the lactate-Akt-mTOR axis through the upregulation of Hk1 and Hk2. Biochem Biophys Res Commun 2019; 512: 93-99.

[14] Jiang J, Chen S, Li K, Zhang C, Tan Y, Deng Q, Chai Y, Wang X, Chen G, Feng K, Zhang L, Xie CM and Ma K. Targeting autophagy enhances heat stress-induced apoptosis via the ATP-AMPK-mTOR axis for hepatocellular carcinoma. Int J Hyperthermia 2019; 36: 499-510.

[15] Peng T, Liu X, Wang J, Liu Y, Fu Z, Ma X, Li J, Sun G, Ji Y, Lu J, Wan W and Lu H. Long noncoding RNA HAGLROS regulates apoptosis and autophagy in Parkinson's disease via regulating miR-100/ATG10 axis and PI3K/Akt/mTOR pathway activation. Artif Cells Nanomed Biotechnol 2019; 47: 2764-2774.

[16] Liu MZ, Zhou DC, Liu Q, Xie FL, Xiang DX, Tang GY and Luo SL. Osteogenesis activity of isocoumarin a through the activation of the PI3K-Akt/Erk cascade-activated BMP/RUNX2 signaling pathway. Eur J Pharmacol 2019; 858: 172480.

[17] Mu G, Liu Q, Wu S, Xia Y and Fang Q. Long noncoding RNA HAGLROS promotes the process of mantle cell lymphoma by regulating miR-100/ATG5 axis and involving in PI3K/AKT/mTOR

signal. Artif Cells Nanomed Biotechnol 2019; 47: 3649-3656.
[18] Al-Hashem F, Al-Humayed S, Amin SN, Kamar SS, Mansy SS, Hassan S, Abdel-Salam LO, Ellatif MA, Alfaifi M, Haidara MA and Al-Ani B. Metformin inhibits mTOR-HIF-1α axis and profibrogenic and inflammatory biomarkers in thioacetamide-induced hepatic tissue alterations. J Cell Physiol 2019; 234: 9328-9337.
[19] Liu R, Chen Z, Yi X, Huang F, Hu G, Liu D, Li X, Zhou H and Liu Z. 9za plays cytotoxic and proapoptotic roles and induces cytoprotective autophagy through the PDK1/Akt/mTOR axis in non-small-cell lung cancer. J Cell Physiol 2019; 234: 20728-20741.
[20] Wu C, Ma X, Zhou Y, Liu Y, Shao Y and Wang Q. Klotho restraining Egr1/TLR4/mTOR axis to reducing the expression of fibrosis and inflammatory cytokines in high glucose cultured rat mesangial cells. Exp Clin Endocrinol Diabetes 2019; 127: 630-640.
[21] Wu Y, Zhang MH, Xue Y, Zhang T, Wu N, Guo W, Du X and Xu YL. Effect of microRNA-26a on vascular endothelial cell injury caused by lower extremity ischemia-reperfusion injury through the AMPK pathway by targeting PFKFB3. J Cell Physiol 2019; 234: 2916-2928.
[22] Yang S, Zheng Y and Hou X. Lipoxin A4 restores oxidative stress-induced vascular endothelial cell injury and thrombosis-related factor expression by its receptor-mediated activation of Nrf2-HO-1 axis. Cell Signal 2019; 60: 146-153.
[23] Wu Y, Zhang MH, Xue Y, Zhang T, Wu N, Guo W, Du X and Xu YL. Effect of microRNA-26a on vascular endothelial cell injury caused by lower extremity ischemia-reperfusion injury through the AMPK pathway by targeting PFKFB3. J Cell Physiol 2019; 234: 2916-2928.
[24] Zhang M, Wang X, Yao J and Qiu Z. Long noncoding RNA NEAT1 inhibits oxidative stress-induced vascular endothelial cell injury by activating the miR-181d-5p/CDKN3 axis. Artif Cells Nanomed Biotechnol 2019; 47: 3129-3137.
[25] Mi L, Zhang Y, Xu Y, Zheng X, Zhang X, Wang Z, Xue M and Jin X. HMGB1/RAGE pro-inflammatory axis promotes vascular endothelial cell apoptosis in limb ischemia/reperfusion injury. Biomed Pharmacother 2019; 116: 109005.