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FAL1 regulates endothelial cell proliferation in diabetic arteriosclerosis through PTEN/AKT pathway

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Abstract. – OBJECTIVE: This study aims to investigate the role of FAL1 in the occurrence and progression of diabetic arteriosclerosis and its underlying mechanism.

PATIENTS AND METHODS: FAL1 expression in coronary artery disease (CAD) tissues, normal artery tissues, and tumor necrosis factor-α (TNF-α)-induced endothelial cells was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The regulatory effects of FAL1 on cell proliferation, migration, and cell cycle were examined by cell counting kit-8 (CCK-8) assay, transwell assay, and flow cytometry, respectively. Western blot was used to detect protein expressions of proliferation-related gene PCNA (proliferating cell nuclear antigen), cell cycle-related genes cyclin D1, PTEN (phosphatase and tensin homolog deleted on chromosome ten) and AKT (protein kinase B) in HUVECs. Subsequently, rescue experiments were performed to assess whether PTEN/AKT signaling pathway is activated during the process of FAL1-regulated proliferation and migration of HUVECs.

RESULTS: FAL1 was highly expressed in CAD tissues and TNF-α-induced endothelial cells compared with that of controls. Overexpression of FAL1 in HUVECs promoted cell cycle, proliferation, and migration. FAL1 activated PTEN/AKT pathway in HUVECs, which was partially reversed by PTEN overexpression.

CONCLUSIONS: Highly expressed FAL1 can promote proliferation and migration of endothelial cells through activating PTEN/AKT signaling pathway.

Key Words:
FAL1, Diabetic atherosclerosis, Proliferation, Migration.

Introduction

Diabetes mellitus (DM) is the most common and serious disease that endangers human health. As a glycometabolic disorder, DM is considered to be a risk factor for coronary atherosclerotic heart disease[1]. Even under strict control of other risk factors for cardiovascular diseases, the mortality of myocardial infarction in DM patients is still 2-3 times higher than that of non-diabetic patients[2,3]. Long-term chronic inflammation is the most common pathogenesis of DM and cardiovascular disease[4,5]. Pathological lesions of coronary artery in DM patients are mainly characterized by involvement of multiple blood vessels, severe coronary artery stenosis, and diffuse lesions. It is believed that atherosclerosis is mainly caused by abnormal blood glucose metabolism. In-depth researches further demonstrated that diabetic atherosclerosis is the result of multiple factors[6].

Vascular endothelial cells (VECs) are significant components of the vascular wall. Selective protection of VECs, inflammatory responses in VECs, and targeted treatment of VECs have become the three major focuses that are well studied[7,8]. The vascular endothelial injury is caused by a variety of factors, such as oxidative stress, inflammation, immune response, and hypertension. Dysfunctional actin in peripheral cells and enlargement of the intercellular spaces are observed in impaired VECs, thereafter increasing the vascular permeability[9]. As one of the inflammatory cells, tumor necrosis factor-α (TNF-α) is a vital risk factor for atherosclerosis. TNF-α could regulate inflammation and apoptosis of VECs[10,11].

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Recent studies have shown that human genome can be transcribed into non-coding RNAs (ncRNAs) with varying length. NcRNAs have limited or no protein-coding function. Long non-coding RNAs (lncRNAs) are a type of ncRNAs, which participate in the development of various diseases, including tumors. For example, lncRNA p21 regulates atherosclerosis development by promoting the proliferation and apoptosis of vascular smooth muscle cells through increasing p53 activity[12]. LncRNA MALAT1 is involved in the regulation of cell proliferation, viability and inflammatory response of VECs[13]. LncRNA H19 participates in the glucose regulation by interacting with target microRNA, which serves as a risk factor for coronary diseases[14]. LncRNA FAL1 has been shown to promote cell proliferation and migration in many diseases[15-17]. The specific role of FAL1 in diabetic atherosclerosis, however, still remains to be studied.

Patients and Methods

Patients

15 coronary artery disease (CAD) tissues and 15 normal arterial tissues were surgically resected. Samples were washed with DEPC (diethyl pyrocarbonate) water (Beyotime, Shanghai, China) and preserved in liquid nitrogen. Clinical data of enrolled subjects were collected from Pathology and Medical Records Department. This study was approved by the Ethics Committee of Life Science Ethics Review Committee of Zhengzhou University. All the subjects signed the informed consent.

Cell Culture and Transfection

Human HUVECs were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium, Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco, Rockville, MD, USA). Cells were maintained in a 5% CO_2 incubator at 37°C. After cell confluence was up to 50-60%, cell transfection was performed following the manufacturer's instructions of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The corresponding lentiviruses used in the study were constructed by GenePharma (Shanghai, China).

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

We used TRIzol (Invitrogen, Carlsbad, CA, USA) to extract total RNA for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). RNA concentration was determined by a spectrophotometer (Hitachi, Kyoto, Japan). The expression level of the target gene was calculated using the $2^{-\Delta\Delta CT}$ method. Primers used in the study were as follows: FAL1: F: 5'-GCAAGCGGAGACTTGTCTTT-3', R: 5'-TTGAACTCCTGACCTCGTGA-3'; PCNA: F: 5'-CCTGCTGGGATATTAGCTCCA-3', R: 5'-CAGCGGTAGGTGTCGAAGC-3'; cyclin D1: F: 5'-GCTGCGAAGTGGAAACCATC-3', R: 5'-CCTCCTTCTGCACACATTTGAA-3'; PTEN (phosphatase and tensin homolog deleted on chromosome ten): F: 5'-TGGATTCGACTTAGACTTGACCT-3', R: 5'-GGTGGGTTATGGTCTTCAAAAGG -3'; AKT (protein kinase B): F: 5'-GCTGCGAAGTGGAAACCATC-3', R: 5'-CCTCCTTCTGCACACATTTGAA-3'; GAPDH (glyceraldehyde 3-phosphate dehydrogenase): F: 5'-AGCCACATCGCTCAGACAC-3', R: 5'-GCCCAATACGACCAAATCC-3'.

Cell Counting Kit-8 (CCK-8) Assay

Transfected HUVECs were seeded in the 96-well plates at a density of 1×10^6/mL. 10 μL of CCK-8 reagent (Dojindo Laboratories, Kumamoto, Japan) was added in each well. After incubation for 2 h, the OD (optical density) value of each well was measured at the wavelength of 450 nm by a microplate reader (Bio-Rad, Hercules, CA, USA).

Western Blot

Transfected HUVECs were lysed using a cell lysis buffer, shaken on ice for 30 min, and centrifuged at 4°C, 14,000 g/min for 15 min. Total protein concentration was calculated by bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL, USA). The extracted proteins were separated on a 10% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and subsequently transferred to a PVDF (polyvinylidene difluoride) membrane (Millipore, Billerica, MA, USA). Western blot analysis was performed according to standard procedures. Protein bands were exposed by enhanced chemiluminescence (ECL).

Cell Cycle Detection

HUVECs were centrifuged at 800 rpm/min for 5 min and the precipitant was resuspended in pre-cooled 70% ethanol. After phosphate-buffered saline (PBS) wash, cells were stained with propidium iodide (PI) (Mskbio, Wuhan, China) for 25 min in the dark. The stained cells were detected by flow cytometry (FACSCalibur; BD Biosciences, Detroit, MI, USA).

Transwell Assay

HUVECs were resuspended in serum-free DMEM and seeded in the upper transwell chamber. DMEM containing 10% FBS was added in the lower chamber. HUVECs were cultured for 24 h and fixed with 0.1% crystal violet for 15 min. Penetrating HUVECs were observed and captured for cell counting.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) and GraphPad (La Jolla, CA, USA) were used for data analyses. Data were expressed as mean ± standard deviation. The continuous variables were analyzed by the *t*-test. $p < 0.05$ was considered statistically significant.

Results

High-Dose Glucose Treatment Promoted FAL1 Expression

FAL1 was highly expressed in 15 CAD tissues compared with that of 15 normal arterial tissues (Figure 1A). To explore the biological role of FAL1 in endothelial cells, HUVECs were treated in 5 mM (low-dose) or 25 mM (high-dose) glucose for different time points, respectively. The CCK-8 assay showed that the proliferative capacity of HUVECs was increased after high-dose glucose treatment (25 mM) in a time-dependent manner (Figure 1B). Moreover, the high-dose glucose treatment remarkably upregulated the FAL1 expression in HUVECs (Figure 1C). To further explore the regulatory role of FAL1 in diabetic atherosclerosis, HUVECs proliferation was detected after 50 ng/ml TNF-α induction for different time points. QRT-PCR data showed

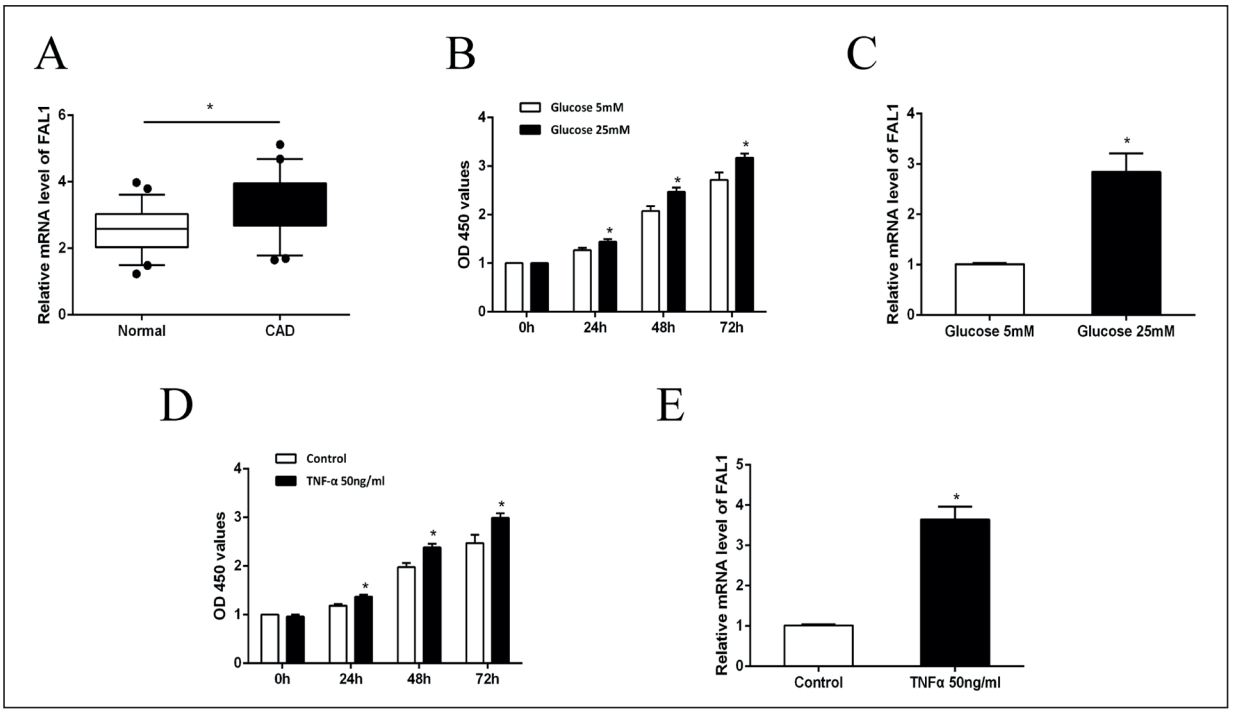

Figure 1. High-dose glucose treatment stimulated FAL1 expression. ***A,*** FAL1 was highly expressed in CAD tissues than that of normal arterial tissues. ***B,*** HUVECs proliferation was pronounced after 25 mM glucose treatment than those treated with 5 mM glucose. ***C,*** High-dose glucose treatment upregulated FAL1 expression in HUVECs. ***D,*** 50 ng/mL TNF-α induction promoted HUVECs proliferation. ***E,*** 50 ng/mL TNF-α induction upregulated FAL1 expression in HUVECs.

that TNF-α induction remarkably promoted cell proliferation and FAL1 expression in HUVECs (Figure 1D and 1E).

FAL1 Overexpression Promoted Cell Proliferation and Migration

We first verified the transfection efficacy of LV-FAL1 in HUVECs (Figure 2A). CCK-8 results illustrated that overexpression of FAL1 remarkably promoted HUVECs proliferation in a time-dependent manner (Figure 2A and 2B). In addition, both mRNA and protein levels of PCNA were also upregulated by FAL1 overexpression (Figure 2C and 2D). The effect of FAL1 on cell cycle of HUVECs was detected by flow cytometry. The data indicated that overexpressed FAL1 promoted cell cycle development (Figure 2E). Both mRNA and protein levels of cyclin D1 were upregulated after LV-FAL1 transfection in HUVECs (Figure 2F and 2G). Transwell assay demonstrated that FAL1 overexpression promoted cell migration of HUVECs as well (Figure 2H).

FAL1 Overexpression Activated PTEN/AKT Pathway in HUVECs

FAL1 overexpression downregulated PTEN and upregulated AKT in HUVECs (Figure 3A-3C). Subsequently, transfection efficacy of LV-

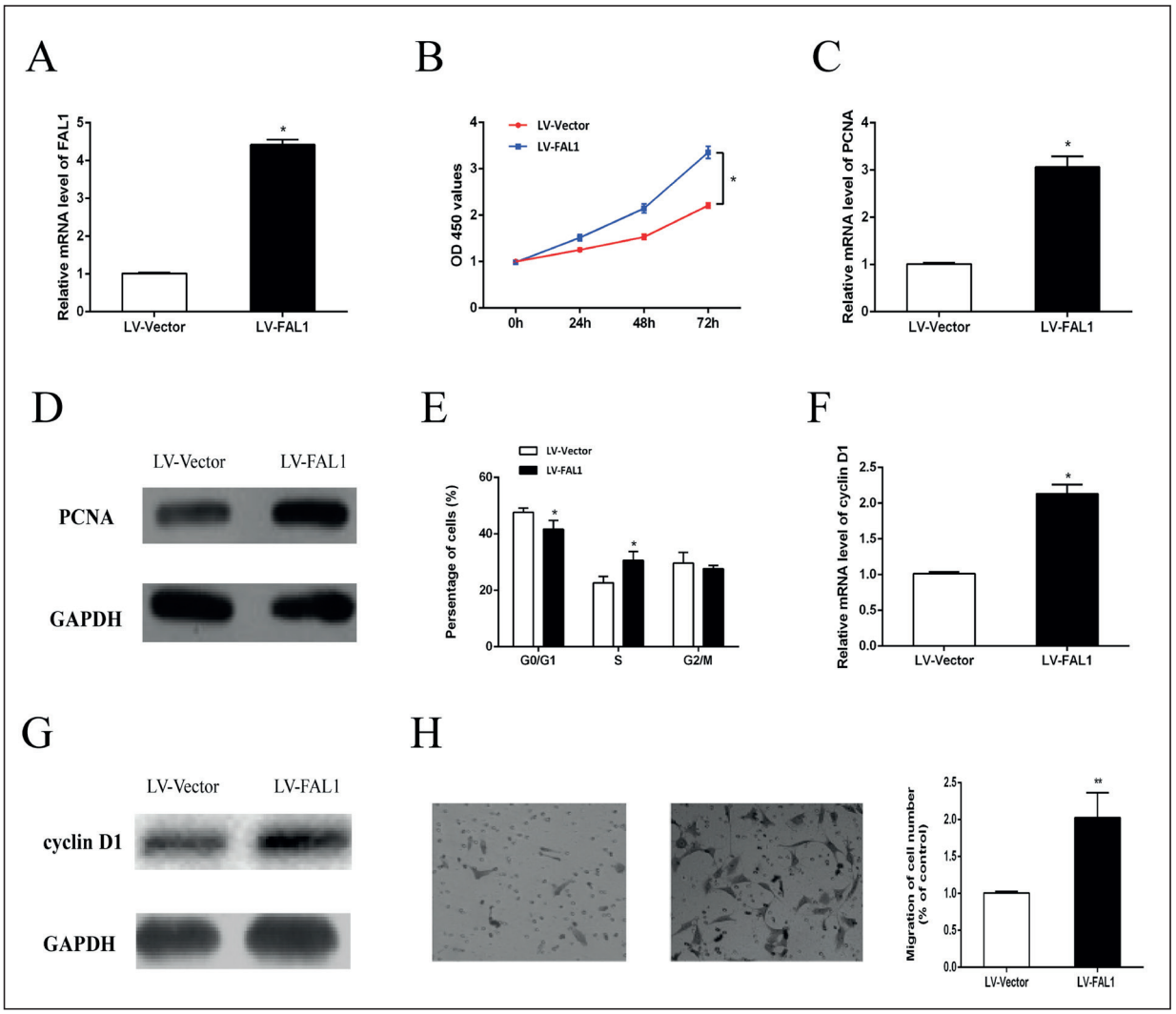

Figure 2. FAL1 overexpression promoted proliferation and migration of HUVECs. ***A,*** LV-FAL1 transfection upregulated FAL1 expression in HUVECs. ***B,*** FAL1 overexpression promoted HUVECs proliferation. ***C,*** FAL1 overexpression promoted mRNA level of PCNA. ***D,*** FAL1 overexpression promoted protein level of PCNA. ***E,*** FAL1 overexpression promoted cell cycle of HUVECs. ***F,*** FAL1 overexpression promoted mRNA level of cyclin D1. ***G,*** FAL1 overexpression promoted the protein level of cyclin D1. ***H,*** FAL1 overexpression promoted HUVECs migration.

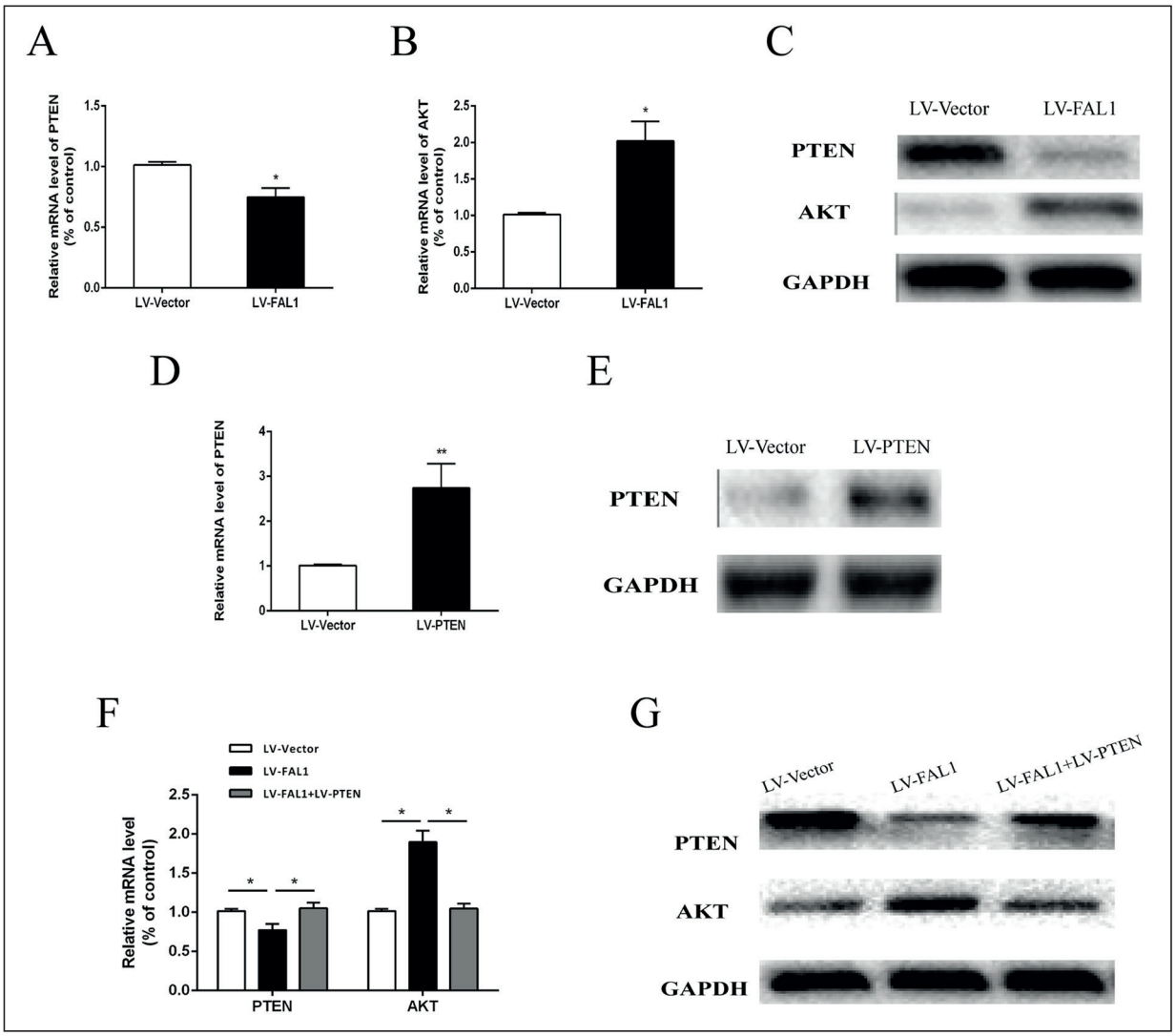

Figure 3. FAL1 overexpression activated PTEN/AKT pathway in HUVECs. ***A,*** FAL1 overexpression downregulated mRNA level of PTEN. ***B,*** FAL1 overexpression promoted mRNA level of AKT. ***C,*** FAL1 overexpression upregulated AKT and downregulated PTEN. ***D,*** LV-PTEN transfection promoted mRNA level of PTEN in HUVECs. ***E,*** LV-PTEN transfection promoted protein level of PTEN in HUVECs. ***F,*** The mRNA levels of PTEN and AKT were reversed by PTEN overexpression. ***G,*** The protein levels of PTEN and AKT were reversed by PTEN overexpression.

PTEN was verified by qRT-PCR and Western blot (Figure 3D and 3E). PTEN downregulation and AKT upregulation induced by FAL1 overexpression were reversed after HUVECs were transfected with LV-PTEN (Figure 3F and 3G), indicating that FAL1 exerts its biological function in HUVECs *via* PTEN/AKT pathway.

Proliferative Role of FAL1 Was Inhibited by PTEN/AKT Pathway Suppression

Increased proliferation in HUVECs induced by FAL1 overexpression was partially reversed by PTEN overexpression (Figure 4A). Protein expressions of PCNA and cyclin D1 in HUVECs were also reversed by PTEN overexpression (Figure 4B and 4C). Similarly, cell migration was reversed after LV-PTEN transfection in HUVECs (Figure 4D).

Discussion

The main pathological change underlying diabetic cardiovascular diseases is atherosclerosis. Diabetic atherosclerosis patients present earlier onset, aggravated and wider lesions, and worse

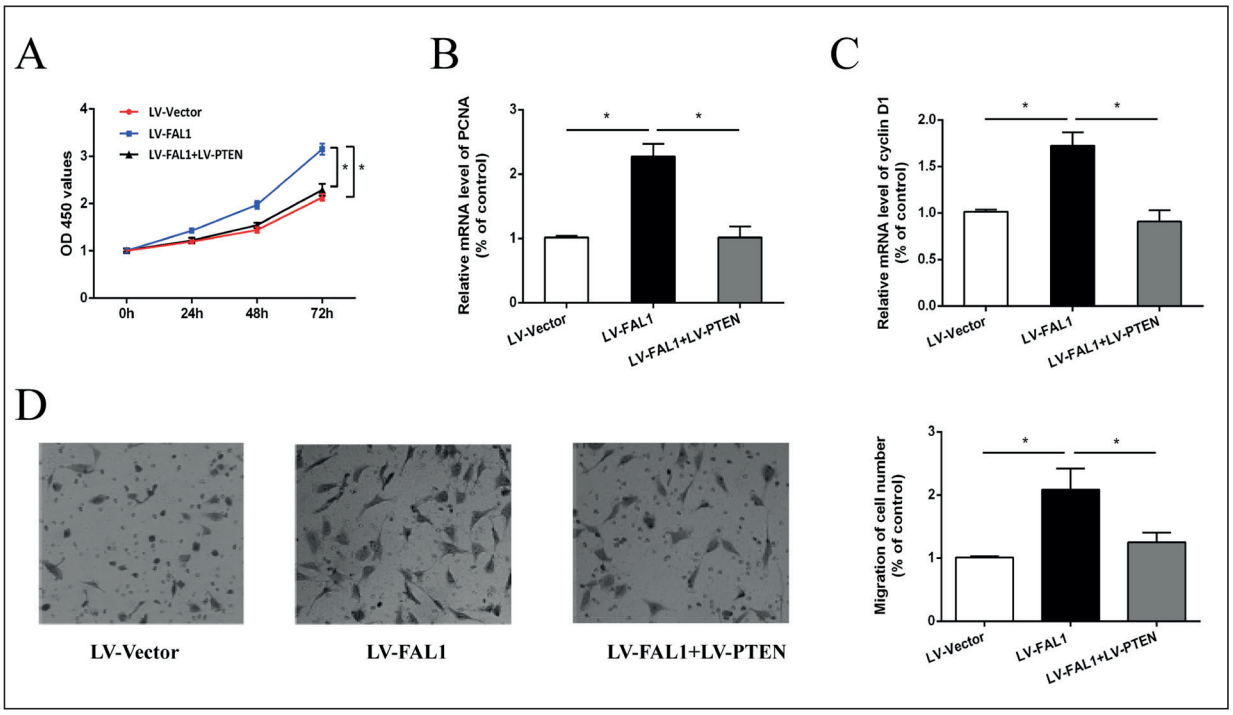

Figure 4. Proliferative role of FAL1 was inhibited by PTEN/AKT pathway suppression. ***A,*** Increased proliferation of HUVECs induced by FAL1 overexpression was reversed by PTEN overexpression. ***B,*** Upregulated PCNA in HUVECs induced by FAL1 overexpression was reversed by PTEN overexpression. ***C,*** Upregulated cyclin D1 in HUVECs induced by FAL1 overexpression was reversed by PTEN overexpression. ***D,*** Increased migration of HUVECs induced by FAL1 overexpression was reversed by PTEN overexpression.

prognosis than those atherosclerosis patients with normal blood sugar level[18,19]. Hence, we considered that DM is closely and complicatedly related to atherosclerosis. Current studies believed that lipid infiltration, thrombosis, and injury response are responsible for atherosclerosis development[20]. The potential mechanism of diabetic atherosclerosis, however, has not been fully elucidated. VECs dysfunction and atherosclerosis deterioration may aggravate coronary disease[21].

A growing number of experimental studies have demonstrated that lncRNAs are widespread in the human genome. LncRNAs exert crucial regulatory roles in biological processes, including cell differentiation, proliferation, apoptosis, and metastasis. Multiple mechanisms are involved in lncRNA regulation, such as DNA replication, chromatin modification, transcription factor recruitment and activation, regulation of RNA polymerase II activity and mRNA stability[22]. Studies have shown that lncRNA FAL1 upregulates the phosphorylated level of BMI1 by stabilizing PCR1. FAL1 also affects proliferation and metastasis of malignant tumors *via* reducing cell adhesion. In the present work, we found that FAL1 was highly expressed in CAD tissues than that of controls. Besides, FAL1 expression was higher in proliferative endothelial cells induced by TNF-α compared with those of controls. Overexpression of FAL1 promoted proliferation and migration of HUVECs.

AKT, as an effector of the PI3K/AKT pathway, is essential for tumor cell survival, proliferation, and invasion[23]. AKT is a serine-threonine protein kinase that leads to downstream interactions between the phosphorylation cascade and target proteins. It is reported[24,25] that PI3K/AKT pathway is involved in regulating a variety of biological functions, such as cell growth and survival, proliferation and apoptosis, glucose metabolism, gene transcription, neovascularization, cell migration, and cell cycle. PTEN is a tumor-suppressor gene with dual-phosphatase activity, which is a natural inhibitor of the PI3K/AKT pathway. PTEN is frequently lowly expressed in many tumors. PTEN downregulates the PI3K/AKT pathway by dephosphorylating PIP3, thereby inhibiting a series of downstream anti-apop-

totic, proliferative, and invasive signals. PTEN promotes apoptosis and inhibits proliferation and invasion *via* reducing the phosphorylation levels of several key survival kinases[26]. PTEN also inhibits mutant gene accumulation and cell transformation *via* dephosphorylation of key proteins[27,28]. We demonstrated that overexpression of FAL1 downregulated PTEN expression and upregulated AKT expression in endothelial cells. Highly expressed FAL1 promoted proliferation and migration of HUVECs by activating the PTEN/AKT pathway.

Conclusions

We demonstrated that highly expressed FAL1 can promote proliferation and migration of endothelial cells through activating PTEN/AKT signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Matsuura Y, Yamashita A, Zhao Y, Iwakiri T, Yamasaki K, Sugita C, Koshimoto C, Kitamura K, Kawai K, Tamaki N, Zhao S, Kuge Y, Asada Y. Altered glucose metabolism and hypoxic response in alloxan-induced diabetic atherosclerosis in rabbits. PLoS One 2017; 12: e175976.
2) Katakami N, Kaneto H, Shimomura I. Carotid ultrasonography: a potent tool for better clinical practice in diagnosis of atherosclerosis in diabetic patients. J Diabetes Investig 2014; 5: 3-13.
3) Tian J, Liu Y, Liu Y, Chen K, Lyu S. Cellular and molecular mechanisms of diabetic atherosclerosis: herbal medicines as a potential therapeutic approach. Oxid Med Cell Longev 2017; 2017: 9080869.
4) Guaricci AI, De Santis D, Carbone M, Muscogiuri G, Guglielmo M, Baggiano A, Serviddio G, Pontone G. Coronary atherosclerosis assessment by coronary CT angiography in asymptomatic diabetic population: a critical systematic review of the literature and future perspectives. Biomed Res Int 2018; 2018: 8927281.
5) Kurihara O, Takano M, Seino Y, Shimizu W, Mizuno K. Coronary atherosclerosis is already ongoing in pre-diabetic status: insight from intravascular imaging modalities. World J Diabetes 2015; 6: 184-191.
6) Jun JY, Ma Z, Pyla R, Segar L. Leptin treatment inhibits the progression of atherosclerosis by attenuating hypercholesterolemia in type 1 diabetic Ins2(+/Akita):apoE(-/-) mice. Atherosclerosis 2012; 225: 341-347.
7) Greene JA, Portillo JA, Lopez CY, Subauste CS. CD40-TRAF signaling upregulates CX3CL1 and TNF-alpha in human aortic endothelial cells but not in retinal endothelial cells. PLoS One 2015; 10: e144133.
8) Zhang YH, He K, Shi G. Effects of microRNA-499 on the inflammatory damage of endothelial cells during coronary artery disease via the targeting of PDCD4 through the NF-Kappabeta/TNF-alpha signaling pathway. Cell Physiol Biochem 2017; 44: 110-124.
9) Molino Y, Jabes F, Bonnet A, Gaudin N, Bernard A, Benech P, Khrestchatisky M. Gene expression comparison reveals distinct basal expression of HOX members and differential TNF-induced response between brain- and spinal cord-derived microvascular endothelial cells. J Neuroinflammation 2016; 13: 290.
10) Liang Q, Ju Y, Chen Y, Wang W, Li J, Zhang L, Xu H, Wood RW, Schwarz EM, Boyce BF, Wang Y, Xing L. Lymphatic endothelial cells efferent to inflamed joints produce iNOS and inhibit lymphatic vessel contraction and drainage in TNF-induced arthritis in mice. Arthritis Res Ther 2016; 18: 62.
11) Nallasamy P, Si H, Babu PV, Pan D, Fu Y, Brooke EA, Shah H, Zhen W, Zhu H, Liu D, Li Y, Jia Z. Sulforaphane reduces vascular inflammation in mice and prevents TNF-alpha-induced monocyte adhesion to primary endothelial cells through interfering with the NF-kappaB pathway. J Nutr Biochem 2014; 25: 824-833.
12) Wu G, Cai J, Han Y, Chen J, Huang ZP, Chen C, Cai Y, Huang H, Yang Y, Liu Y, Xu Z, He D, Zhang X, Hu X, Pinello L, Zhong D, He F, Yuan GC, Wang DZ, Zeng C. LincRNA-p21 regulates neointima formation, vascular smooth muscle cell proliferation, apoptosis, and atherosclerosis by enhancing p53 activity. Circulation 2014; 130: 1452-1465.
13) Puthanveetil P, Chen S, Feng B, Gautam A, Chakrabarti S. Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. J Cell Mol Med 2015; 19: 1418-1425.
14) Gao W, Zhu M, Wang H, Zhao S, Zhao D, Yang Y, Wang ZM, Wang F, Yang ZJ, Lu X, Wang LS. Association of polymorphisms in long non-coding RNA H19 with coronary artery disease risk in a Chinese population. Mutat Res 2015; 772: 15-22.
15) Pan C, Yao G, Liu B, Ma T, Xia Y, Wei K, Wang J, Xu J, Chen L, Chen Y. Long noncoding RNA FAL1 promotes cell proliferation, invasion and epithelial-mesenchymal transition through the PTEN/AKT signaling axis in non-small cell lung cancer. Cell Physiol Biochem 2017; 43: 339-352.
16) Jeong S, Lee J, Kim D, Seol MY, Lee WK, Jeong JJ, Nam KH, Jung SG, Shin DY, Lee EJ, Chung WY, Jo YS. Relationship of focally amplified long noncod-

ing on chromosome 1 (FAL1) lncRNA with E2F transcription factors in thyroid cancer. Medicine (Baltimore) 2016; 95: e2592.

17) Ni N, Song H, Wang X, Xu X, Jiang Y, Sun J. Up-regulation of long noncoding RNA FALEC predicts poor prognosis and promotes melanoma cell proliferation through epigenetically silencing p21. Biomed Pharmacother 2017; 96: 1371-1379.

18) Orekhov AN, Bobryshev YV, Sobenin IA, Melnichenko AA, Chistiakov DA. Modified low density lipoprotein and lipoprotein-containing circulating immune complexes as diagnostic and prognostic biomarkers of atherosclerosis and type 1 diabetes macrovascular disease. Int J Mol Sci 2014; 15: 12807-12841.

19) Yi X, Xu L, Hiller S, Kim HS, Maeda N. Reduced alpha-lipoic acid synthase gene expression exacerbates atherosclerosis in diabetic apolipoprotein E-deficient mice. Atherosclerosis 2012; 223: 137-143.

20) Chistiakov DA, Sobenin IA, Orekhov AN, Bobryshev YV. Role of endoplasmic reticulum stress in atherosclerosis and diabetic macrovascular complications. Biomed Res Int 2014; 2014: 610140.

21) Ullevig SL, Zhao Q, Zamora D, Asmis R. Ursolic acid protects diabetic mice against monocyte dysfunction and accelerated atherosclerosis. Atherosclerosis 2011; 219: 409-416.

22) Yu Y, Yang J, Li Q, Xu B, Lian Y, Miao L. LINC00152: a pivotal oncogenic long non-coding RNA in human cancers. Cell Prolif 2017; 50:

23) Yu L, Li Z, Dong X, Xue X, Liu Y, Xu S, Zhang J, Han J, Yang Y, Wang H. Polydatin protects diabetic heart against ischemia-reperfusion injury via Notch1/Hes1-mediated activation of Pten/Akt signaling. Oxid Med Cell Longev 2018; 2018: 2750695.

24) Wang SW, Deng LX, Chen HY, Su ZQ, Ye SL, Xu WY. MiR-124 affects the apoptosis of brain vascular endothelial cells and ROS production through regulating PI3K/AKT signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 498-505.

25) Roseweir AK, Powell AG, Bennett L, Van Wyk HC, Park J, McMillan DC, Horgan PG, Edwards J. Relationship between tumour PTEN/Akt/COX-2 expression, inflammatory response and survival in patients with colorectal cancer. Oncotarget 2016; 7: 70601-70612.

26) Wang L, Dong X, Ren Y, Luo J, Liu P, Su D, Yang X. Targeting EHMT2 reverses EGFR-TKI resistance in NSCLC by epigenetically regulating the PTEN/AKT signaling pathway. Cell Death Dis 2018; 9: 129.

27) Peng F, Tang H, Liu P, Shen J, Guan X, Xie X, Gao J, Xiong L, Jia L, Chen J, Peng C. Isoliquiritigenin modulates miR-374a/PTEN/Akt axis to suppress breast cancer tumorigenesis and metastasis. Sci Rep 2017; 7: 9022.

28) Li X, Ren C, Li S, Han R, Gao J, Huang Q, Jin K, Luo Y, Ji X. Limb remote ischemic conditioning promotes myelination by upregulating PTEN/Akt/mTOR signaling activities after chronic cerebral hypoperfusion. Aging Dis 2017; 8: 392-401.