Transcription factor Foxp1 stimulates angiogenesis in adult rat after myocardial infarction

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Abstract

Background: Coronary heart disease is the leading cause of death and major socioeconomic burden worldwide. The ischemia and hypoxia caused by the sudden occlusion of the main coronary arteries lead to myocardial infarction, leading to hypertrophic growth of cardiomyocytes and left ventricular dilatation, which eventually develops into heart failure. Timely and effective revascularization is extremely important for saving the infarcted myocardium and improving heart function.

Purpose: To explore the effect of transcription factor Foxp1 on angiogenesis after myocardial infarction from two levels of in vivo and in vitro experiments.

Methods: The myocardial infarction model was established in male SD rats to detect FoxP1 expression; lentivirus carrying FoxP1 interfering RNA was injected by multi-point method for 4 weeks, and tissue immunofluorescence and immunohistochemical experiments were performed to observe myocardial endothelial cell proliferation and collagen deposition. Human umbilical vein endothelial cells (HUVEC) were cultured in a hypoxic cell incubator to detect the expression changes of FoxP1 transcription level and protein level at different time points; Lentiviruses carrying specific FoxP1 gene interfering RNAs were used to transfect HUVECs, and cells were used for transfection. Immunofluorescence and flow cytometry were used to detect the proliferation ability of HUVEC in different groups; meanwhile, cell scratch assay was used to detect the migration ability of HUVEC of different groups and tubule formation experiment to observe the tube formation ability.

Results: The myocardial infarction rat model was successfully established. Compared with the sham-operated group, immunofluorescence experiments showed that the expression of FoxP1 was significantly increased. After the lentivirus of FoxP1 interfering RNA was injected into myocardial infarction rats, the expression of FoxP1 decreased, endothelial cell proliferation decreased, and collagen deposition increased. Angiogenesis is reduced. When HUVECs were treated in a 5% hypoxia incubator, it was found that the expression of FoxP1 in endothelial cells increased within 24 hours. After transfection of HUVECs with FoxP1 interfering RNA lentivirus, their proliferation, migration and tube formation abilities decreased significantly.

Conclusions: Hypoxia can promote the expression level of endothelial cells FoxP1 to a certain extent; foxp1 may promote the formation of new blood vessels in the infarct area by promoting endothelial cell proliferation and inhibiting endothelial cell apoptosis after myocardial infarction. FoxP1 may provide a promising therapeutic target by promoting effective angiogenesis in the treatment of myocardial infarction.

Background

Coronary heart disease (CHD) is reported as the one of the dominating causes of death and main socioeconomic burden worldwide(1,2). According to reports, ischemic heart disease (IHD) causes over 370,000 deaths each year in the United States (3). Ischemia and hypoxia caused by sudden occlusion of

the main coronary artery leads to myocardial infarction (MI), which causes hypertrophic growth of cardiomyocytes and dilatation of the left ventricle (LV) and eventually developed into heart failure (1,4). As we all know, although current treatment of CHD is becoming more mature and most cases can get better curative effects by revascularized through emergency PCI and use of dual antiplatelet therapy (5), the long-term mortality remains high. In addition, the sequelae of PCI surgery such as stent thrombosis or stent restenosis and gastrointestinal bleeding and oral bleeding caused by long-term use of antiplatelet drugs hinders the prognosis of coronary heart disease. These facts urge people to find new treatment methods to improve the heart function after acute myocardial infarction. Resent years myocardial revascularization is a hot research topic in the treatment of coronary heart disease and with the rapid development of molecular biology technology, remedial angiogenesis has become a promising new method for patients with advanced CHD (6,7). Researchers believe that wound healing after MI relates to a strong angiogenic response, which starts in the marginal zone and extends to the necrotic infarct core(8). The fibroblast growth factor (FGF) family and vascular endothelial growth factor (VEGF) family have been used in therapeutic angiogenesis in animal experiments and clinical studies (9,10). In a study of heart tissue regeneration and protection, insulin-like growth factor-1 (IGF-1) could inhibit the apoptosis of cardiomyocytes, recruit endogenous stem cells, and promote angiogenesis (11).

The fork head box (Fox) family has 17 subfamilies, with more than 100 members, and its DNA binding region has a special wing-like spiral structure(12). The proteins encoded by the Fox family have diverse functions not only involved in embryonic development and cell cycle regulation and other biological processes, but also related to glucose and lipid metabolism, species aging, and immune regulation processes(13). FoxP1 is a member of the P subfamily of the Fox transcription factor superfamily, and is mainly expressed in an overlapping pattern in lung, nerve, lymph, and heart tissues (14,15). Studies have found that FoxP1 expression is observed in the entire heart, including the myocardium, endocardium and endocardial cushion tissue at E11.5, and FoxP1 is observed in aortic and pulmonary artery endothelial cells at E12.5d and E14.5d (16). In the apoe KO hyperlipidemia mouse model, the loss of endothelial cell FoxP1 increases atherosclerosis, and endothelial FoxP1 affects the adhesion, migration and penetration of monocytes into the blood vessel wall in the pathogenesis and progression of atherosclerosis. It is very important in regulation (17). Studies have observed that FoxP1 gene knockout mice die at the embryonic stage at day E14.5 due to perivascular hemorrhage and edema (18), confirming that FoxP1 may play an extremely important role in the growth, differentiation and angiogenesis of the mouse heart. In an angiogenesis study, after induction of ischemia in the hind limbs of mice, the expression of FoxP1 was found to be up-regulated at the site of neovascularization(19). At present, the research on the transcription factor FoxP1 in cardiac function mostly focuses on myocardial cell proliferation and atherosclerosis, and may inhibit myocardial fibrosis and cardiac remodeling. Its relationship with angiogenesis after myocardial infarction has not been reported yet. Our data indicate FoxP1 plays a pro-angiogenic role on endothelial cell after myocardial infarction, and we also intend to clarify the mechanism during this process.

Materials And Methods

Experimental animals

Sprague-Dawley male rats randomly divided into MI group and control group were obtained from the experimental animal center of Chongqing Medical University. All animals in this study received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals”. All animal experiments were approved by the Institutional Animal Care and Use Committee of Chongqing Medical University.

MI model

30 male rats aged 6-8 weeks were randomly divided into sham operation group and MI group and then anesthetized intraperitoneally with pentobarbital sodium (30~50mg/kg). A catheter was intubated into the trachea and a small animal ventilator provided positive pressure ventilation at 1~2 ml/cycle and a respiratory rate of about 60 breaths per minute. After the thoracic cavity at the level of the fourth rib and along the left sternal border was opened, the left anterior descending coronary artery (LAD) was ligated with a 6-0 silk suture 3 mm from the tip of the left auricle. The chest wall was closed with a continuous 5-0 prolene suture, followed by a 4-0 polyester suture in order to close the skin. The procedure of sham operation was the same as the above-mentioned MI induction but without LAD ligation. After LAD ligation in rats, ST segment elevation in the chest leads checked by an electrocardiogram (ECG) was the sign of a successful MI surgery and the success rate was approximately 70%.

Cell culture

Human Umbilical Vein Endothelial Cells (HUVEC) were purchased from the company and cultured in RPMI-medium 1640 supplemented with penicillin, streptomycin and 10% FBS, and used to the 10th generation. The stable-growing HUVEC was seeded in 6 six-well plates at a density of 2×10^4 cells/well, and placed in a normoxic cell incubator overnight. These cells were taken out next day and placed in a hypoxic cell incubator (5% O2) for 1 hour, 2 hours, 4 hours, 12 hours, 24 hours, 48 and 72 hours.

Lentiviral transfection

The pre-experiment result showed that the most suitable MOI was10, and the formal experiment of lentiviral transfection is carried out according to the instructions. Inoculate HUVEC in advance with a 6-well plate at a concentration of $3\sim5\times10^4$/well. According to the control group, vehicle group and Foxp1 RNAi group, complete medium, 1×10^8 TU/ml negative control virus and 1×10^8 TU/ml Foxp1 interfering RNA lentivirus were added to the inoculated HUVEC respectively. The infection system was 2 ml. The optimal conditions are used for infection conditions. Change the fluid one day after infection. Three days after infection, the infection efficiency of lentiviral and cell status was observed with a fluorescence microscope.

Scratch test

Before the experiment, use a marker and a ruler to draw 6 horizontal lines on the back of the 6-hole plate, with an interval of about 0.5 to 1 cm, to make the horizontal lines evenly pass through the holes. Add

about 5×105 HUVEC cells to the six-well plate and place them in the incubator for overnight culture; the next day, use a 200uL pipette tip and a ruler perpendicular to the horizontal line on the back of the orifice to make a scratch on the cells in the orifice; wash the cells 3 times with sterile PBS buffer, and add 2 mL of serum-free simple medium to each well; Enter the 37°C 5% CO2 incubator to continue the cultivation. Observe and take pictures under an inverted phase-contrast microscope at 0, 6, 12, and 24 hours after the scratch.

Tubule formation experiment

Spread the pre-chilled Matrigel polycarbonate glue on a 96-well plate, 50μl per well, put it in a 5% CO 2 incubator at 37°C, let it stand for 60 min, and combine to solidify; Add each group of HUVEC with a density of 1.5×105/mL, add 100uL cell suspension to each well, and continue to culture; After 2 hours, the number of cells in each group was observed under an inverted phase-contrast microscope. Obvious tubular structures were visible in 2.5 hours; 6 fields of view were randomly selected for shooting, and then image J software was used for analysis and processing.

Flow Cytometry

The three groups cells were respectively seeded in a six-well plate at a density of $5*10^4$ and cultured overnight; the floating cells were collected in a centrifuge tube, and then the cells were digested into single cells with 0.25% pancreatin; The cells were collected in a centrifuge tube, added with an appropriate amount of PBS buffer, centrifuged, and the supernatant was discarded. After two times centrifugation, resuspend the cells in 100uL PBS buffer, and slowly add 900uL pre-cooled 75% ethanol. Or collect the cells in each well in several centrifuge tubes, add an appropriate amount of PBS buffer, centrifuge at 1000 r/min for 5 minutes, and discard the supernatant. Centrifuge twice in the same way; finally 1×106 cells were resuspended in PBS (PH=7.2) 500uL buffer, placed in a 1.5mL EP tube, and then sent to the School of Life Sciences, Chongqing Medical University for cell cycle and cell apoptosis detection.

Western blot analysis

For western blot analysis of FoxP1 expression, cultured cells were lysed in RIPA buffer on ice and protein concentration was quantified using the Bradford method. The equal amount of total protein (15μg) was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% milk in Tris-buffered saline Tween (TBST) (50 mM Tris, 150 mM NaCl, 0.5 mM Tween-20, pH=7.5), and then were incubated over night with antibodies directed against human FoxP1. Beta-actin was used as loading controls.

Haematoxylin-Eosin Staining

The rat hearts from each group were fixed in 4% PFA and then stained with haematoxylin-eosin (n=3 for each group at every time point). Pictures were taken using microscope at different magnification. And

then after dehydration with 70%, 80%, 90%, 100% gradient alcohol and transparent xylene, the whole embryo was embedded with paraffin and sliced with a thickness of 10um. Pictures were taken using microscope at different magnification.

Immunofluorescence

The cells, which were grown on cover slips without coated, were fixed with 4% paraformaldehyde at room temperature for 15 min, and the heart tissue sections followed by permeabilization with 0.25% Triton X-100 in PBS (0.01 mol/l) at 37°Cfor 10 min. Cells or sections were blocked with 10% goat serum (Boster Biological Technology, Pleasanton, CA, USA) at 37˚C for 10 min, and then incubated with the following primary antibodies at 4˚C overnight. The cells were then incubated with cyanine 3-conjugated goat anti-rabbit immunoglobulin G (CWBIO, Beijing, China) at 37˚C for 45 min, followed by DAPI at room temperature for 10 min. Subsequently, the cells were subjected to confocal microscopy imaging (original magnification, x400). Imaging conditions for each antibody were kept consistent across all samples.

RNA extraction and quantitative real-time PCR (RT-qPCR)

Cells were collected and lysed with TRIzol (Takara Bio Inc., Otsu, Japan) to extract total RNA, which was reverse-transcribed to complementary DNA using a Prime Script Reverse Transcriptase reagent kit (cat. no. RR047A; Takara Bio Inc.) according to the manufacturer's protocols. β-actin was used as an internal reference for each gene. The relative gene expression levels were calculated using the 2-ΔΔCq method and normalized to β-actin.

Statistical analysis

All experiments were repeated three times. Values are expressed as the mean ± standard deviation.The data were analyzed using SPSS (version 20.0; IBM Corp., Armonk, NY, USA), and one-way analysis of variance followed by a Tukey's test was applied for comparison between groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

The expression of FoxP1 protein increased in the heart after myocardial infarction in rats

To explore the role of Foxp1 in cardiovascular angiogenesis in adult rat, we generated MI model as described previously. After the operation, the ECG of the limb lead monitoring showed obvious ST-segment elevation (Fig. 1A,B). Subsequently, we took heart specimens from rats in the sham operation group, 14days, and 21 days after MI and we observed that the expression of foxp1 increased significantly after myocardial infarction in rats using immunofluorescence experiments (Fig. 1C). Endothelial marker CD 31 (green) and FoxP1 (red) and nuclear staining (DAPI, blue) immunofluorescence double-staining localization showed FoxP1 is expressed in capillary endothelial cells and FoxP1 is upregulated at sites of neovascularization(Fig. 1D). Additionally, the double-stained confocal fluorescence detection of CD31 specifically expressed by endothelial cells and the proliferation index Ki67 showed that the myocardial

infarction group had more endothelial cell proliferation than the sham operation group (Fig. 1E). That indicated that endothelial cell proliferation, the most important part of angiogenesis, may be regulated by the transcription factor FoxP1.

Foxp1 knockdown affects angiogenesis and cardiac function in MI rats

In order to further ascertain whether foxp1 is involved in the regulation of angiogenesis after myocardial ischemia, a heart-specific foxp1 KO model was constructed by using the dot matrix injection method to inject Foxp1 interfering RNA lentivirus into the heart of MI rats. After 4 weeks of coronary artery ligation, double immunofluorescence staining was used to detect the proliferation of rat cardiac endothelial cells, and the angiogenesis of myocardial infarction mice was evaluated. As shown in Figure 2A, the number of endothelial cells proliferating in the MI+FOXP1 knockdown group was significantly lower than that in the MI group. These observations suggest that foxp1 knockdown affects angiogenesis in rats with myocardial infarction 4 weeks later. And echocardiography showed that the left ventricular ejection fraction of the MI + foxp1 knockdown group was lower than that of the myocardial infarction group (left ventricular ejection fraction: 85.33±2.1vs 74.67±2.1; $P<0.01$) , though there were also significant differences in short-axis shortening rates, all groups were within the normal range(fig.2B,C). Rats in three groups were all sacrificed 4 weeks after the operation to obtain specimens, which were fixed in paraformaldehyde and then cut into sections and embedded in paraffin. Results showed that the MI group had significantly more collagen deposition than the sham-operated group, and the myocardial tissue was disordered. In contrast, MI rats treated with foxp1 knockdown had more severe collagen deposition and more obvious fibrosis (fig.2C). The results suggested that foxp1 knockdown reduces cardiac function and worsens fibrosis in rats with myocardial infarction.

Hypoxic culture of endothelial cells induces increased foxp1 expression

For the purpose of narrowing down the relationship between angiogenesis and the transcription factor foxp1, a short-term hypoxia group and a long-term hypoxia group for the well-growing HUVECs were set up, as HUVECs are one of the most commonly cells used for the study of angiogenesis. The short-term hypoxia group is set to 0h, 1h, 2h, 4h, 12, and 24h, and the long-term hypoxia group is set to 0h, 12h, 24h, 48h and 72h. The RT-PCR results displayed that within 24 hours of short-term hypoxia, the expression of FoxP1 was significantly increased compared with the normoxia group ($p<0.05$) (fig.3A). However, after 24 hours, the expression of foxp1 dropped significantly, and the expression of FoxP1 even was lower than that of the normoxic group (fig.3B). And the results of western blot showed a similar phenomenon that foxp1 protein increased significantly within 24 hours of hypoxic culture, and gradually decreased during 48 and 72 hours (fig.3C-F). These data indicates that the hypoxic microenvironment affects the expression of foxp1, and proper hypoxic culture of endothelial cells increases the expression of foxp1 protein.

FoxP1 knockdown affects endothelial cell proliferation

Similarly, the interfering RNA lentivirus of foxp1 was transfected into human umbilical vein endothelial cells according to the instructions(Fig.S1), and cultured in a normal oxygen incubator and a hypoxia incubator for 24 hours respectively. Subsequent immunofluorescence staining of ki67 and PH3 showed that the proliferation of FoxP1RNAi group was significantly reduced (Fig. 4A, B). The results of flow cytometry also showed that under the condition that the total amount of cells was basically the same, the cells in the FoxP1RNAi group with double to quadruple DNA, that is, the G1 phase-S phase and the S phase-G2 phase were significantly less than those in the control group (Fig. 4C,E). As expected, compared with the control group, the apoptotic cell detection results still showed that the number of apoptotic cells in the Foxp1 knockdown group was significantly increased (Fig. 4D,F). We reasoned that it was because of the knockdown of foxp1 that endothelial cell proliferation was attenuated, indicating that foxp1 has an indispensable role in endothelial cell proliferation.

Endothelial cell migration reduced by knocking down FoxP1

It is well known that angiogenesis is accompanied by the formation and migration of endothelial cell migration tubules in addition to the proliferation of endothelial cells. Therefore, scratch test was performed to examine the migration ability of the HUVECs under hypoxic conditions. The migratory ability of endothelial cells with knockdown of foxp1 gene was strongly decreased compared with the normal control and negative control virus groups (Fig. 5A,C). In addition, we also performed tubule formation experiments using Matrigel, as shown in Fig. 5Band 5D, after adding cells for 2.5 hours, the tubules formed in the three groups were significantly different. The number of tubules in the Foxp1 interference group was less and the size was more uneven, and most of them were disordered or damaged. Thus, foxp1 plays an important role in promoting endothelial cell migration and the formation of new blood vessels.

Discussion

Angiogenesis refers to the process in which vascular endothelial cells proliferate, migrate and form new micro-vessels in the existing vasculature under some pathological and physiological states, such as injury repair and tissue ischemia regeneration(20). Angiogenesis is also a pathological process that is critical to the development and progression of certain diseases and, in addition, may have the potential to treat and improve tissue perfusion in ischemia. Uncontrolled angiogenesis has been shown to promote tumors and retinopathy, and insufficient angiogenesis can lead to coronary artery disease. In tumors, the mRNA expression of vascular endothelial growth factor was significantly enhanced in the area around necrotic foci, suggesting a mechanism by which hypoxic microenvironment may stimulate tumor angiogenesis(21). In wounds, capillary injury creates a hypoxic environment, and experimental changes in wound oxygenation alter the recovery of angiogenic responses. Based on the molecular characterization of several angiogenic growth factors, an early landmark study showed that hypoxia can induce platelet-derived growth factor (PDGF) mRNA and vascular endothelial growth factor (VEGF) mRNA expression in tissue culture(22).

Fork head box protein P1 (Fork head box protein P1, FoxP1) is a large multi-domain transcriptional regulator of the Fox family with a winged helical DNA-binding protein that plays an important role in cardiovascular homeostasis and disease. These proteins play important roles in regulating gene transcription and controlling cell-type and tissue-specific gene expression during early development and organogenesis(23). Among Fox transcription factors, members of the Fox P subfamily (FoxP1, FoxP2 and FoxP3) have recently attracted attention. The Fox family has diverse functions in various physiological and pathophysiological processes, ranging from speech development (FoxP2)(24) to immune regulatory cells of regulatory T (FoxP3) (25) and cardio-genesis (FoxP1, FoxP4)(26). Spontaneous mutation of Fox P protein in mouse and human disease highlights its functional importance. The dysregulation of FoxP1 expression has been found in a variety of tumors, leading to speculation that FoxP1 is a tumor suppressor gene (27). FoxP1 was first mentioned in the cardiovascular field by Wang et al., describing the phenotype of hereditary FoxP1 deficiency in mouse embryonic stem cells (16): Inactivation of FoxP1 leads to severe cardiac defects, including poor separation of the ventricle and outflow tract and valve formation. Due to the diversity of expression, FoxP1 is highly regulated by alternative splicing of tissue- and cell-type-specific domains, suggesting that the same FoxP1 protein performs different functions in different cells and tissues(13, 28–31). Especially in cardiac tissue, FoxP1 is expressed in both myocardium and endocardium, and is involved in different transcriptional regulation of different types of cells(32, 33), such as cardiomyocytes (CMS), endothelial cells (ECs), and vascular smooth muscle cells (VSMCs). The widespread distribution and alternatively spliced isoforms of FoxP1 suggest that it has distinct functions in different cardiac and vascular cell and tissue types.

It is well known that angiogenesis includes two major biological processes, endothelial cell proliferation and migration. In order to study the specific effect of FoxP1 on endothelial cell angiogenesis, and FoxP1 is highly expressed in endothelial cells, we transfected Foxp1 interfering RNA lentivirus into HUVEC to explore the role of FoxP1 in HUVEC angiogenesis. According to the purpose of the experiment, we set up the control group, empty group (transfected with negative control virus) and experimental group (transfected with FoxP1 interfering RNA lentivirus). The lentivirus used in this experiment is a stable serotype virus with a long expression period and stable passage. Hence, after transfection into HUVEC, there is no need for repeated transfection and detection, and it still maintains a high expression after more than 5 generations.. Cell immunofluorescence results showed that the expression of proliferation-related antibodies Ki67 and pH3 decreased significantly after knockdown of FoxP1 gene in HUVECs. In addition, the migration ability and tube-forming ability of HUVEC showed a significant decrease compared with the control group and the empty group, and the formed tubules were more irregular and fewer in number. This result revealed that FoxP1 may have a great role in endothelial cell angiogenesis. This is consistent with the findings of Sebastian et al. (19), where FoxP1 knockdown inhibited endothelial cell proliferation, tube formation and migration. At the same time, we used SD male rats (220g-280g) to establish a myocardial infarction model by the traditional method of ligating the anterior descending branch of the main coronary artery, and using the five-point method to inject RNA interference lentivirus (the virus and the cell experimental transfection used the same species Adenovirus) to observe the effect of FoxP1 on angiogenesis after myocardial infarction in adult rats. Similar to the results of the cell

experiment, compared with the sham-operated group and the MI group, the expression of FoxP1 protein in the experimental group injected with FoxP1 interfering RNA lentivirus was significantly decreased, indicating that the lentivirus injection was successful, but immunofluorescence with CD31 and Ki67 double staining showed that, Endothelial cells in the experimental group were significantly reduced, with almost no proliferation, while in the MI group, not only the expression of Foxp1 was increased, but the number and proliferation of endothelial cells were more significant than those in the sham-operated group. Studies have demonstrated that FoxP1 is important in regulating cell proliferation, apoptosis, oxidative stress, fibrosis, angiogenesis, cardiovascular remodeling, and dysfunction. Enhanced EC-FoxP1 function is protective against pathological cardiac remodeling and improves cardiac insufficiency (34). Loss of FoxP1 results in pathological cardiac remodeling, increased atherosclerotic lesion formation (17), prolonged occlusive thrombosis, severe cardiac defects, and embryonic death. In contrast, activation of FoxP1 has broad physiological effects(35), including cell growth, hypertrophy, differentiation, angiogenesis, and cardiac development. More importantly, FoxP1 has anti-inflammatory and anti-atherosclerotic effects in the control of coronary thrombosis and myocardial infarction (33). Consequently, targeting FoxP1 signaling has emerged as an early warning biomarker and a new therapeutic approach for CVD progression, and an in-depth understanding of the role of FoxP1 signaling in the cardiovascular system will facilitate the development of effective interventions.

Early revascularization is extremely important for patients with myocardial infarction, which can minimize myocardial cell damage, reduce myocardial tissue collagen deposition, reduce ventricular remodeling, and preserve cardiac function as much as possible(8). Although there are some shortcomings in this study such as FoxP1 overexpression experiments were not performed, and it was not validated in myocardial endothelial cell-specific FoxP1 transgenic mice. The specific mechanism of foxp1 regulating angiogenesis during myocardial ischemia has not been further explored. In this topic, we observed that the proliferation ability, migration ability and tube-forming ability of endothelial cells decreased in FoxP1 knockdown group, which proved that FoxP1 has the effect of promoting endothelial cell angiogenesis. After myocardial infarction rats were injected with FoxP1 interfering lentivirus, endothelial cells Significantly decreased collagen deposition, but increased collagen deposition. Therefore, it was proved that FoxP1 may promote angiogenesis and improve collagen deposition after myocardial infarction, thereby enhancing cardiac function. In addition, we also found that hypoxia promotes the expression of FoxP1 in myocardial infarction rats. It was explained from another perspective and provided feasibility theoretical basis and experimental evidence for the application of therapeutic angiogenesis in the repair of infarcted/ischemic myocardium.

Declarations

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions:

QS, XJ,BL,and DW designed the experiments; DW,TX,BL, JW, HY and WY performed the experiments; DW analyzed the data and wrote the paper. QS reviewed and revised the manuscript.All authors read and approved the final manuscript.

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Conflicts of interest

There is no conflicts of interest.

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Figures

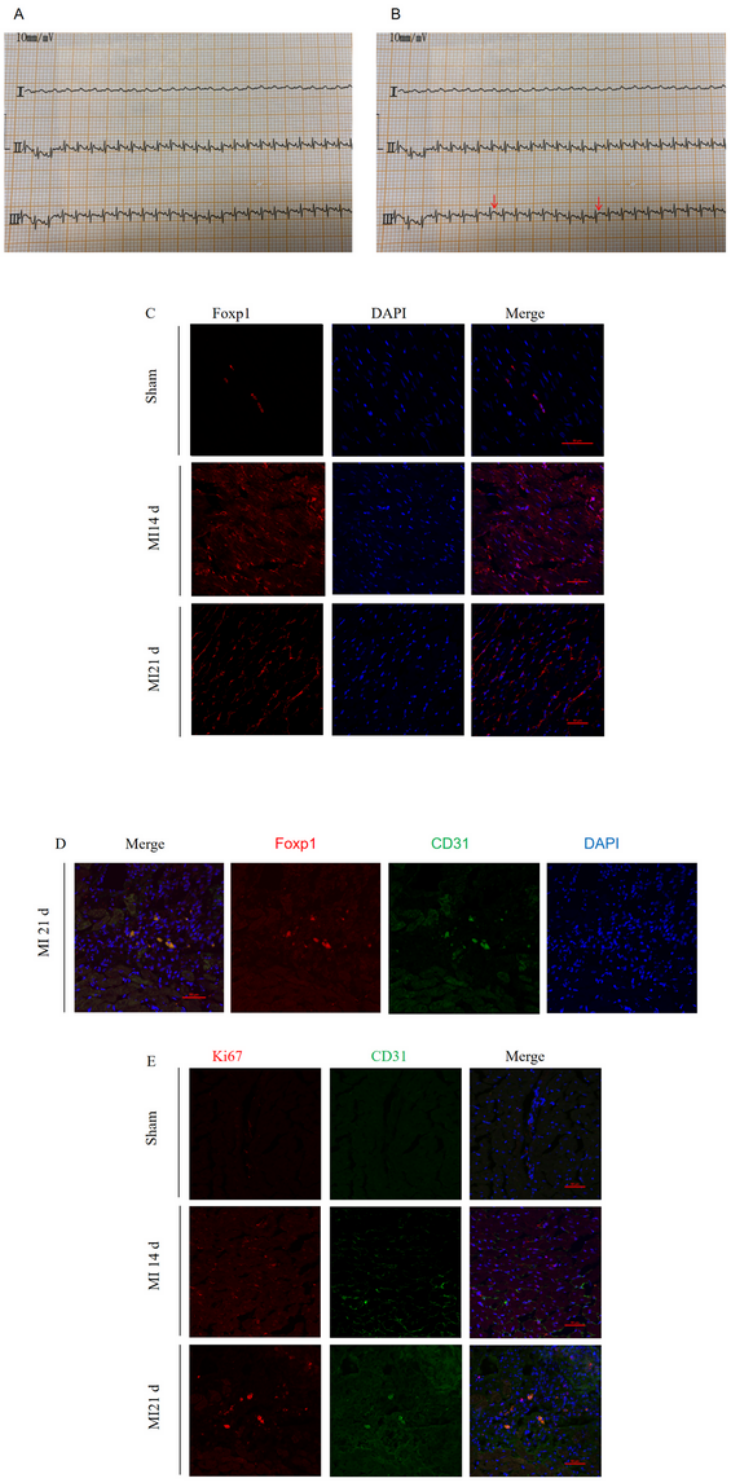

Figure 1

The expression of FoxP1 protein in the heart after myocardial infarction in rats.A-B: electrocardiogram of limb leads before and after myocardial infarction in rats.C: expression of foxp1 in heart tissue of sham-operated rats and 14 days and 21 days after myocardial infarction surgery. D: endothelial markers CD31 and FoxP1 and nuclear staining (DAPI) immunofluorescence double staining localization showed FoxP1 expression in the vascular endothelial cells of the heart after myocardial infarction.E:Endothelial cell

proliferation in myocardial infarction group and sham-operated group was detected by double-stained confocal fluorescence of endothelial cell specific marker CD31 and proliferation index Ki67.Scale bar = 50 mm in C,D and E.

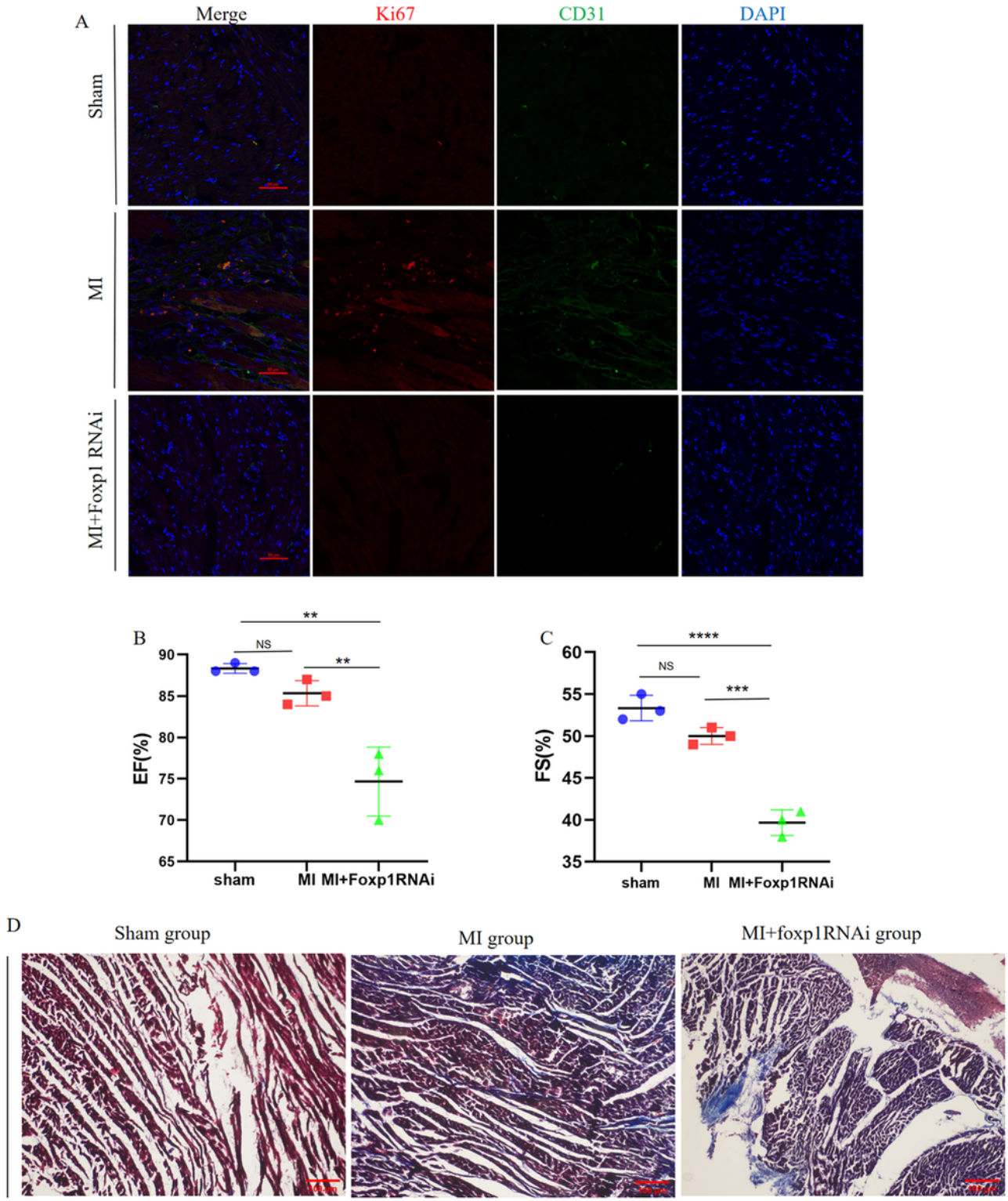

Figure 2

Foxp1 knockdown affects angiogenesis and cardiac function in MI rats. A: endothelial cells proliferated less in the MI+Foxp1 RNAi group. B-C: Cardiac function of three groups of rats. D: masson staining of rat heart tissue showed that fibrosis was aggravated after foxp1 knockdown, and blue indicated collagen deposition.**P 0.01,***P 0.001,****P 0.0001, compared with the sham group. Scale bar = 50 mm in A, scale bar = 100 mm in D.

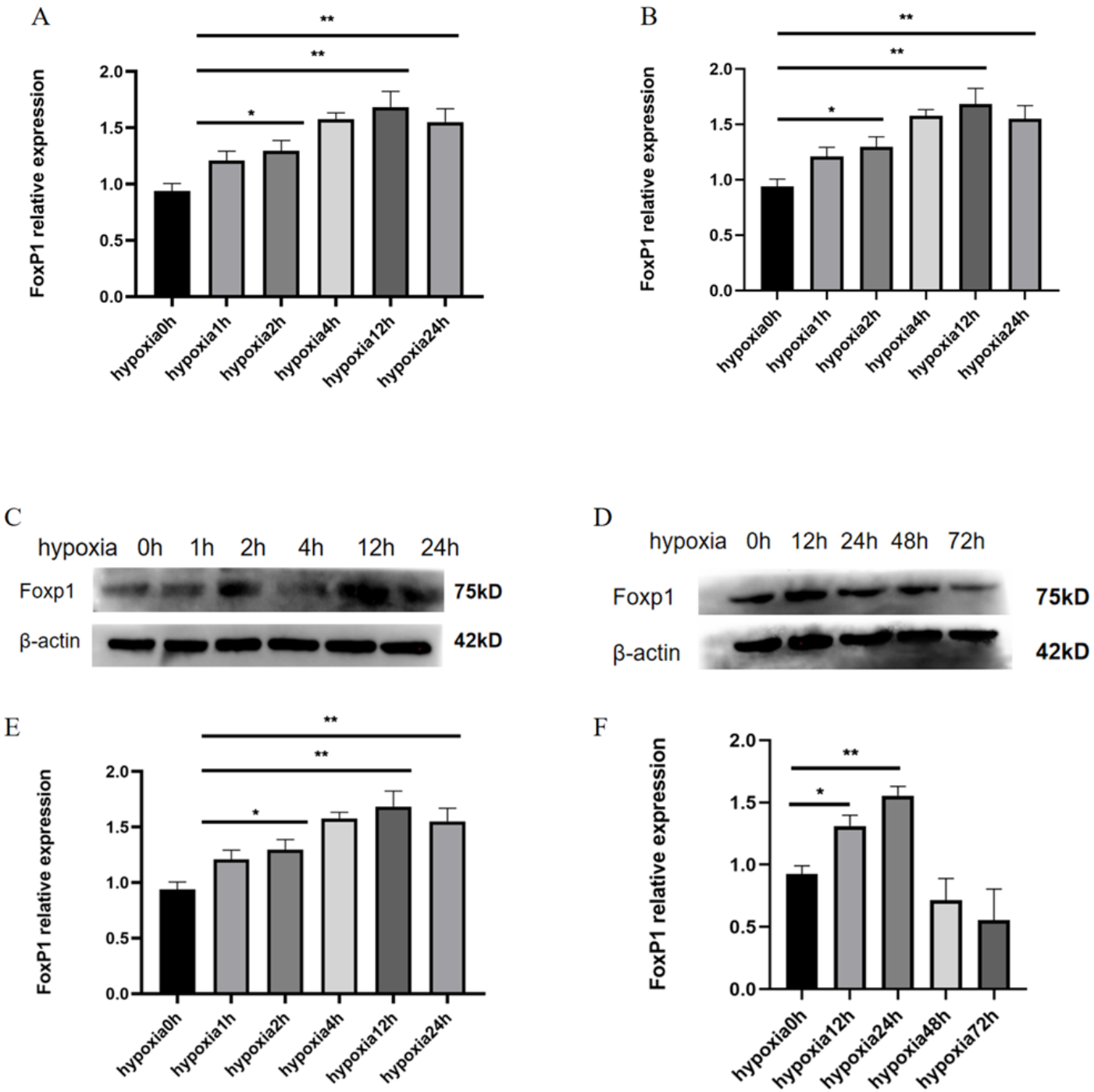

Figure 3

Expression of foxp1 in endothelial cells cultured under hypoxia. A-B: FoxP1mRNA expression level after treating HUVEC for a short time and for a long time. C-F:Changes in FoxP1 protein expression after treating HUVEC for a short time and for a long time.* P 0.05,**P 0.01

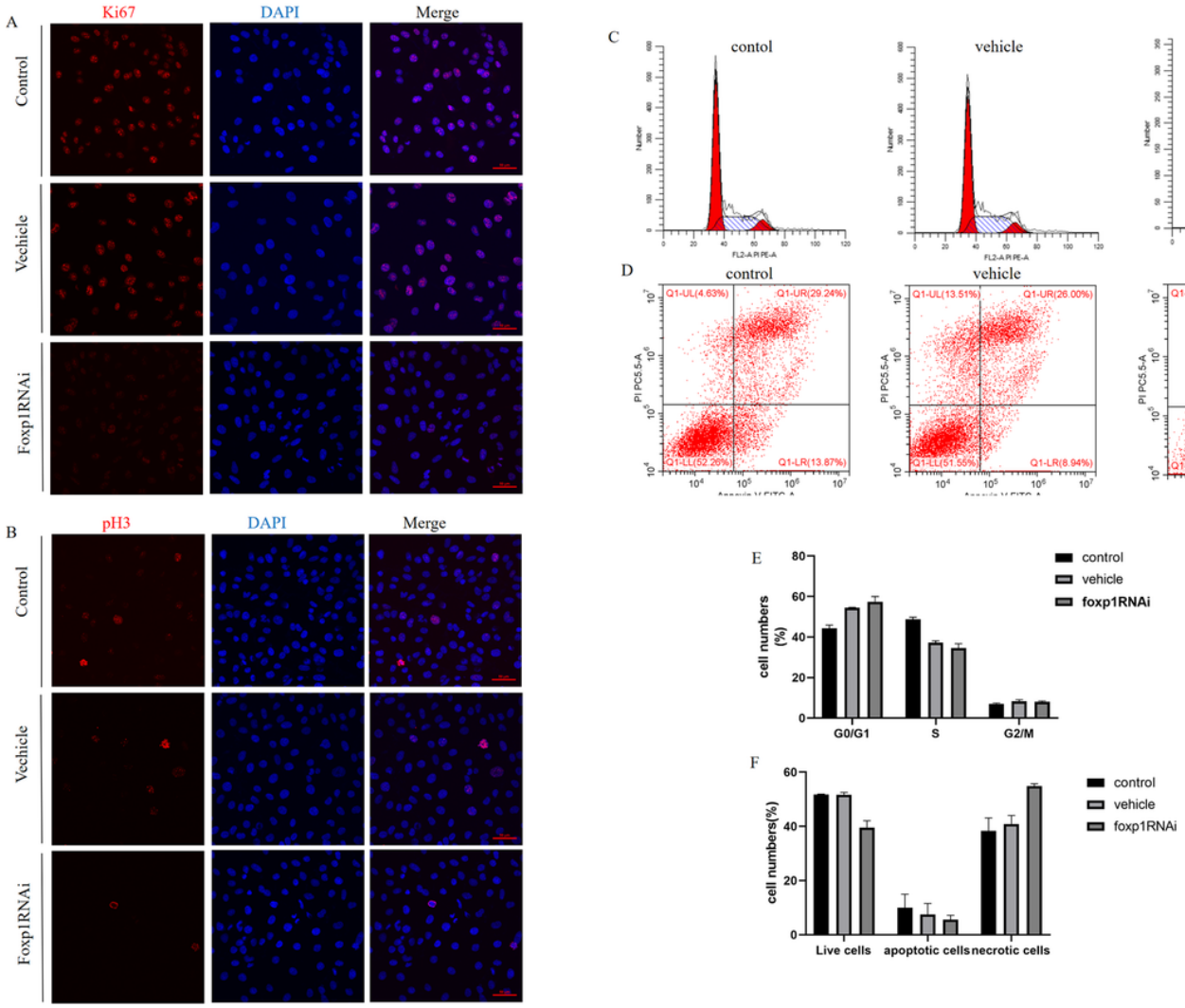

Figure 4

Cell immunofluorescence experiments and Flow cytometry showed that the proliferation of HUVECs in the experimental group (FoxP1RNAi group) were significantly reduced compared with the control group and the empty group (ie, the negative control virus group). A-B: cell proliferation index Ki67 and pH3 expression in HUVEC in the control group, vehicle group and experimental group. C,E: The proportion of cells in G1/S/G2 phase of control, vehicle and experimental group was determined by flow cytometry. D,F: Flow cytometry detection of HUVEC cell apoptosis in the control group, vehicle group and experimental group.

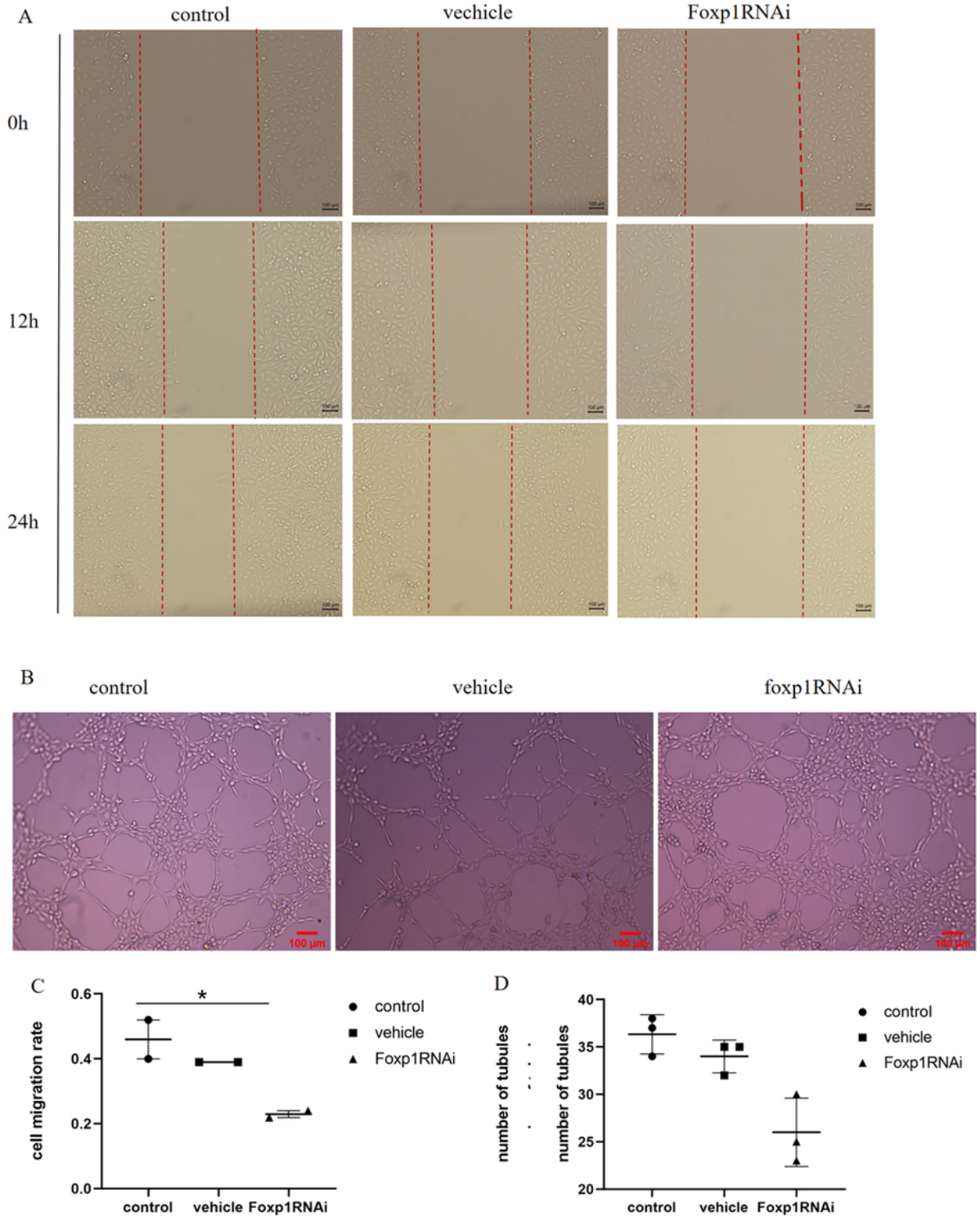

Figure 5

The migration ability of endothelial cells with knockdown of FoxP1 gene was strongly decreased compared with the normal control and negative control virus groups. A,C: Scratch test results of HUVEC cells in each group, after 24 hours of scratching, the number of cells migrated in the experimental group was significantly reduced by half compared with the control group. B,D: Three groups of HUVEC cell

tubule formation experiment results.*P 0.05, **P 0.01compared with the control group. Scale bar = 100 mm in A and B.

Supplementary Files

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- Supplementarymaterial.pdf