ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 4/2023
785–790
https://doi.org/10.18388/abp.2020_6295

Regular paper

MicroRNA-221-3p promotes post-burn HUVEC proliferation, migration, and angiogenesis by regulating CDKN1B

Kun Miao, Fei Xie and JinGui Lin

Department of Hand and Foot Microsurgery, Fuzhou Second Hospital Affiliated to Xiamen University, Fuzhou City, Fujian Province, 350000, China

Background and objective: Previous studies have shown that miR-221-3p plays an important role in vascular remodeling, but it is unclear whether it contributes to angiogenesis after burn injury. The purpose of this study was to investigate the effect of miR-221-3p on angiogenesis in HUVECs after burn injury and to reveal its underlying molecular mechanism. **Methods:** The burn HUVECs model was established by heat treatment. Plasmid or oligonucleotide transfection altered the expression of miR-221-3p and CDKN1B in HUVECs. MTT, colony formation, Transwell, flow cytometry, and tube formation experiments were applied to assess the proliferation, migration, apoptosis, cell cycle, and tube formation capacity of HUVECs. miR-221-3p, CDKN1B, Ki-67, and PCNA expression was assessed by RT-qPCR or Western blot. The dual-luciferase reporter assay verified the targeting relationship between miR-221-3p and CDKN1B. **Results:** miR-221-3p was lowly expressed and CDKN1B was highly expressed in burn HUVECs. Overexpression of miR-221-3p promoted the proliferation, migration, and tube formation ability of burn HUVECs and inhibited apoptosis and the proportion of cells in the G0/G1 phase, whereas overexpression of CDKN1B had the opposite effect. Knockdown of miR-221-3p further inhibited the angiogenic capacity of burn HUVECs, but this effect was reversed by knockdown of CDKN1B. Mechanistically, miR-221-3p targeted CDKN1B. **Conclusion:** miR-221-3p improves the angiogenesis of burn HUVECs by targeting CDKN1B expression, and the miR-221-3p/CDKN1B axis may serve as a potential molecular target for future burn therapy.

Keywords: microRNA-221-3p, CDKN1B, Burn; Human umbilical vein endothelial cells, Angiogenesis

Received: 28 March, 222; revised: 11 July, 2023; accepted: 23 September, 2023; available on-line: 22 November, 2023

e-mail: jingui17441@outlook.com

Abbreviations: BCA, Bicinchoninic acid; CDKN1B, Cyclin-dependent kinase inhibitor 1B; cDNA, Complementary DNA; DMEM, Dulbecco's Modified Eagle's Medium; ECL, Enhanced chemiluminiscent; EDTA, Ethylene diamine tetra acetic acid; FITC, Fluorescein isothiocyanate; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HUVECs, human umbilical vein endothelial cells; JAK-STAT, Janus kinase-signal transducer and activator of transcription; miRNA, MicroRNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MUT, Mutant type; OD, Optical density; PBS, Phosphate buffered saline; PCNA, Proliferating cell nuclear antigen; PI, Propidium iodide; PI3KR, Phosphatidylinositol 3-kinase, regulatory subunit 1; PVDF, Polyvinylidene difluoride; RIPA, Radio-Immunoprecipitation Assay; RT-qPCR, Reverse transcription quantitative polymerase chain reaction; S.D., Standard deviation; siRNA, Small interfering RNA; 3′ UTR, 3′ untranslated region; vWF, Von Willebrand factor; WT, Wild type

INTRODUCTION

Burn is one of the top ten causes of death in the world, killing more than 200 000 patients each year. In addition to a significant economic burden, burn injuries have a higher risk of developing mental illness (Haagsma *et al.,* 2016). Severe burn injuries can affect various organs and systems of the body, and their severity is related to the surface area and depth of burn throughout the body, but even relatively minor burn injury can still be life-threatening and life-altering (Kanitakis *et al.,* 2011). The core problem of burn injury is the loss of vascular integrity, the destruction of physiological barriers, and the edema caused by increased interstitial pressure (Edgar *et al.,* 2011). One of the key points of burn injury treatment is to maintain the blood volume, supply blood to the organs, and accelerate the exchange of skin and important nutrients, such as oxygen (Gelfand, Donelan, and Burke 1983). Vascular damage caused by burn injury can easily cause tissue edema around the wound or even systemic (van Baar *et al.,* 2006). Therefore, post-burn angiogenesis is an important factor for a good outcome of the disease.

MicroRNA (miRNA) is a class of conservative noncoding microRNA molecules consisting of about 22 nucleotides, which can regulate gene expression (Johnson *et al.,* 2019). With the development of next-generation sequencing technology, the research on the function and mechanism of miRNA has become increasingly in-depth, and there are also more studies on miRNA targeting mRNA in the treatment of vascular-related diseases. For example, miR-342-3p/-5p has significant anti-inflammatory and pro-angiogenic effects when targeting pannexin-2 down-regulation (Ray *et al.,* 2020); miR-210 can inhibit the apoptosis of arteriosclerotic occlusive vascular endothelial cells through JAK-STAT (Yue *et al.,* 2019) and miR-125b can limit the formation of the vascular lumen through translation inhibition of VE-cadherin (Muramatsu *et al.,* 2013). This study focused on a miRNA with an important role in vascular remodeling, named miR-221-3p. miR-221-3p has also been studied to mediate vascular remodeling in perivascular adipose tissue-derived extracellular vesicles (Li *et al.,* 2019) and regulate the dysfunction of diabetic retinal microangiopathy (Wang *et al.,* 2020). However, it is unclear whether miR-221-3p plays a role in vascular remodeling after burn injury.

Preliminary experiments found that miR-221-3p was abnormally low expressed in the serum of burn injury patients. It was speculated that miR-221-3p may have a similar role in the process of vascular remodeling after burn injury. Therefore, this work focused on exploring the effects of miR-221-3p on the proliferation, migration, tube formation, apoptosis, and cell cycle of HU-

VECs in the burn environment, and revealed its potential downstream molecular mechanisms.

MATERIALS AND METHODS

Serum from burn injury patients

Ten burn injury patients (burns area ≥50%) in Fuzhou Second Hospital Affiliated to Xiamen University were enrolled, including 6 males and 4 females, aged 18–55 years. Venous blood (10 mL) was collected within 24 h before regular anti-shock treatment and centrifuged at 4°C to obtain the supernatant which was then stored at –80°C. Normal serum from 10 healthy volunteers was recruited as a control group. This study was approved by the Ethics Committee of Fuzhou Second Hospital Affiliated to Xiamen University (ethical number: 2015061129s), and written informed consent was obtained from all participants.

Heat treatment

HUVECs (BeNA, Beijing, China) were identified by STR typing. HUVECs were seeded in Petri dishes for 48 h, harvested into 15 ml centrifuge tubes, and immersed in a circulating water bath at 52°C for 3 min. The cells in the control group were placed in a 37°C water bath for 3 min. Heat-treated cells were re-seeded in Petri dishes and further incubated at 37°C. Cells were then harvested after 6 h of heat treatment.

Cell culture and transfection

Heat-treated HUVECs were grown to 80% confluence in an endothelial cell culture medium (Sciencell, USA) with 5% fetal bovine serum, 0.05% penicillin-streptomycin (Thermo Fisher Scientific, USA). Then, HUVECs were detached with 0.25% trypsin, added with a culture medium to terminate the detachment, and prepared for a cell suspension (1×10^5 cells/mL). Flow cytometry confirmed the positive expression of CD31 and VWF in the purchased HUVECs (Supplementary Fig. 1 at https://ojs.ptbioch.edu.pl/index.php/abp/).

HUVECs with a cell density of 1×10^5 cell/mL were inoculated into 96-well plates (100 μL/well), and 10% fetal bovine serum (Thermo Fisher) was added and incubated for 24 h. miR-221-3p-mimic, miR-221-3p-inhibitor, mimic/inhibitor-negative control (mimic/inhibitor-NC), small interfering RNA (siRNA) targeting CDKN1B (si-CDKN1B), si-NC, PCDNA-CDKN1B, and pcDNA 3.1 were purchased from Shanghai GenePharma. The reagent was transfected into HUVECs instantaneously according to the manufacturer's instructions for Lipofectamine 2000 (Thermo Fisher). After incubation for 48 h, the transfection efficiency was detected by RT-qPCR and western blot.

MTT method

After transfection, every 4×10^4 HUVECs in each well on the 96-well plates were combined with 20 μL of MTT solution (Sigma, USA) at 0 h, 24 h, 48 h, and 96 h. Then, the samples were further cultured for 3 h, centrifuged at 4°C for 15 min, and dissolved by adding 150 μL of dimethyl sulfoxide solution (Sigma). Finally, optical density $(OD)_{490}$ nm was measured (Zhang *et al.,* 2022).

Colony formation method

HUVECs were cultured at 700 cells/well on the 6-well plates with the culture medium changed every 3 days. The culture was terminated when macroscopic clonal clusters appeared. Then, colonies were fixed with 4% paraformaldehyde (Leagene, Beijing, China) at 1 mL/well for 40 min, stained with crystal violet solution (Leagene) at 1 mL/well for 20 min, and counted (Zhang *et al.,* 2021).

Transwell experiment

A total of 2×10^4 HUVECs suspended in a serum-free DMEM (Thermo Fisher Scientific) were added to the upper chamber of the transwell plate, and 800 μL of endothelial cell culture medium and 10% fetal bovine serum were added to the lower chamber. HUVECs after 48-h culture were dyed with crystal violet solution for 20 min and counted under a microscope (Wang *et al.,* 2022).

Flow cytometry detection

Apoptosis was assessed according to Annexin V-FITC Apoptosis Detection Kit (ThermoFisher, USA). The log-phase growing HUVECs were digested with EDTA-free trypsin, washed once with pre-cooled PBS, centrifuged at low speed for 15 min at 4°C, and centrifuged once again. After discarding the supernatant, 5 μL of Annexin V-FITC and PI were added for 10 min, and finally, 500 μL of Annexin V binding buffer was supplemented to detect cell apoptosis by flow cytometry (He *et al.,* 2021).

Cell cycle was assessed by the Propidium Iodide Flow Cytometry Kit (Abcam, USA). HUVECs were first digested with trypsin to obtain a single cell suspension, fixed with 66% ethanol for 2 h at 4°C, and centrifuged at 500×*g* for 5 min. The cell pellet was resuspended in prepared 1×PI+RNase Staining solution for 20 min at 37°C in the dark and loaded into a flow cytometer.

RT-qPCR detection

Total RNA was extracted with Trizol reagent (Thermo Fisher Scientific), reverse-transcribed into cDNA with the reverse transcription kit (Thermo Fisher Scientific). All primers were synthesized by Sangon (Shanghai, China). Taking U6 and GAPDH as the internal references, miR-221-3p and CDKN1B expression was calculated by the $2^{-\Delta\Delta CT}$ method (Miao *et al.,* 2020).

Western blot

Protein lysates were harvested by adding RIPA (Merck, Germany) and the concentration was examined by a BCA kit (Enzyme-Linked Biotechnology, Shanghai, China). Proteins were separated by protein electrophoresis on sodium dodecyl sulfate-polyacrylamide gel (Thermo Fisher Scientific) and transferred to PVDF membranes for reaction with CDKN1B (1:1000, sc-1641), Ki67 (1:1000, ab92742), PCNA (1:1000, sc-56) and GAPDH (1:1000, ab8245), together with goat anti-rabbit secondary antibody (1:5000, 7074). ECL solution (GlpBio, USA)-developed bands were tested to analyze the gray value (Lu & Huang, 2021).

Luciferase reporter gene assay

Prediction from the website https://starbase.sysu.edu.cn shows that miR-221-3p has a binding site for CDKN1B. Wild-type and mutant CDKN1B sequences containing the miR-221-3p binding site were cloned into the PGL4 luciferase reporter vector (Promega). The above luciferase reporter vector and miR-221-3p mimic and mimic-NC were then co-transfected into HUVECs using Lipofectamine 2000 (Invitrogen). The cells were collected 48 h after trans-

fection, and the luciferase activity was detected according to the instructions of the luciferase activity detection kit (Promega). Renilla luciferase activity was considered as a reference for signal intensity (Dong *et al.,* 2021).

In vitro HUVECs tube formation model

Matrigel (Merck, Germany) was added to the center of the μ-slide well plate and left for 15 min. Cell suspension (50 μL, 3×10^4/mL) was centrifuged, washed twice with serum-free DMEM, and centrifuged again. Cells were resuspended in 50 μL endothelial cell culture medium (containing 10% burns serum or 10% control serum) and added to μ-slide well plates. Images were taken in 5 fields of view 6 h later and analyzed by Image-pro Plus 6.0 software (Stefanini *et al.,* 2009).

Statistical analysis

All data were analyzed using SPSS 21.0. Data were presented as mean ± standard deviation (S.D.). Two groups were compared by Student's *t*-test while multiple groups were compared by One-way ANOVA. All functional experiments were run in triplicate. Results were plotted using GraphPad Prism 7.0 software. $P<0.05$ indicated statistical significance.

RESULTS

Abnormally low expression of miR-221-3p in burn environment

miR-221-3p Expression was decreased in the serum of burn injury patients (Fig. 1A). Subsequently, miR-221-3p expression was found to be reduced in heat-treated HUVECs (Fig. 1B).

Overexpression of miR-221-3p restores the angiogenic capacity of burn-injured HUVECs

miR-221-3p-mimic Was transfected into burn-injured HUVECs. miR-221-3p-mimic promoted miR-221-3p expression in burn-injured HUVECs (Fig. 2A). MTT assay and colony formation assays showed that heat treatment inhibited the proliferation and clonogenic ability of HUVECs, while overexpression of miR-221-3p alleviated this phenomenon (Fig. 2B, C). Transwell assay indicated that heat treatment inhibited the migration ability of HUVECs, whereas the migration ability of HUVECs was increased after overexpression of miR-221-3p (Fig. 2D). Western blot reported that heat treatment decreased the expression of the proliferation proteins Ki-67 and PCNA in HUVECs, whereas overexpression of miR-221-3p prevented this change (Fig. 2E, F). Flow cytometry demonstrated that heat treatment increased the apoptotic rate of HUVECs and arrested cells in G0/G1 phase, while overexpression of miR-221-3p alleviated this phenomenon (Fig. 2G, H). Tube formation experiments manifested that heat treatment reduced the angiogenic capacity of HUVECs, but overexpression of miR-221-3p increased tube formation in HUVECs (Fig. 2I).

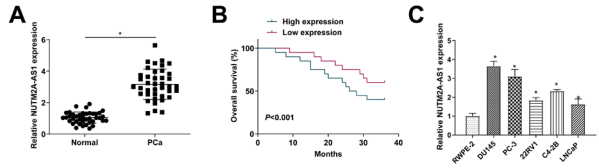

Figure 1. miR-221-3p and CDKN1B signatures in HUVECs
(**A**) RT-qPCR to detect miR-221-3p in the serum of healthy subjects and burns patients; (**B**) RT-qPCR to detect miR-221-3p in the normal and heat-treated HUVECs. Data are presented as mean ± S.D. *$P<0.05$.

Targeted regulation of CDKN1B by miR-221-3p

Subsequently, the potential downstream target genes of miR-221-3p were explored. miRNAs can often bind to the 3' UTR of mRNA to regulate their expression (Yang *et al.,* 2021). Through the bioinformatics prediction website https://starbase.sysu.edu.cn, it was found that miR-221-3p had a binding site with CDKN1B (Fig. 3A). The luciferase reporter gene assay

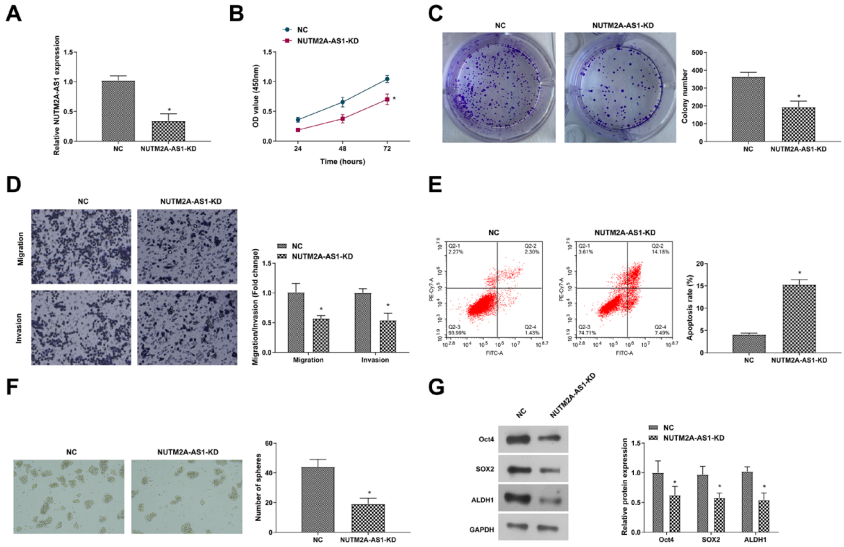

Figure 2. Overexpression of miR-221-3p restores the angiogenic capacity of burn-injured HUVECs
miR-221-3p-mimic was transfected into burn-injured HUVECs to upregulate miR-221-3p. (**A**) RT-qPCR to detect miR-221-3p in HUVECs; (**B**) MTT assay to detect the proliferation of HUVECs; (**C**) Clone formation assay to evaluate the clonogenic ability of HUVECs; (**D**) Transwell assay to detect the migration of HUVECs; (**E–F**) Western blot to measure Ki-67 and PCNA; (**G–H**) Flow cytometry to determine apoptosis and cell cycle; (**I**) Tube formation assay to assess the tube-forming ability of HUVECs; data are expressed as mean ± S.D. *$P<0.05$.

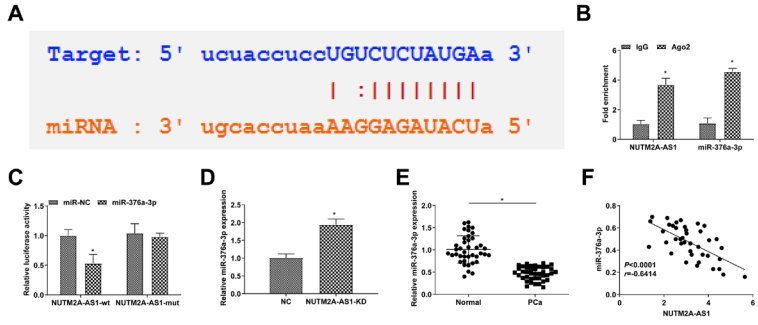

Figure 3. Targeted regulation of CDKN1B by miR-221-3p
(**A**) The binding region of miR-221-3p and CDKN1B on https://starbase.sysu.edu.cn; (**B**) Luciferase activity of cells after co-transfection; (**C**) RT-qPCR and Western blot to detect CDKN1B in burn-injured HUVECs; (**D**) RT-qPCR and Western blot to detect the effect of overexpression of miR-221-3p on CDKN1B expression; data are expressed as mean ± S.D. **P*<0.05.

results verified the binding relationship between the two. The co-transfection of WT-CDKN1B and miR-221-3p-mimic reduced luciferase activity, but that of MUT-CDKN1B and miR-221-3p-mimic had no effect on luciferase activity (Fig. 3B). In burn-injured HUVECs, an abnormal increase in CDKN1B expression (Fig. 3C) was determined. Furthermore, overexpression of miR-221-3p suppressed CDKN1B expression (Fig. 3D).

Overexpression of CDKN1B enhances the inhibitory effect of burn on HUVECs angiogenesis

Subsequently, pcDNA-CDKN1B was transfected into burn-injured HUVECs to explore the role of CDKN1B. pcDNA-CDKN1B increased CDKN1B expression in HUVECs (Fig. 4A). Functional experiments verified that overexpression of CDKN1B further inhibited the proliferation and colony ability of burn-injured HUVECs, decreased the number of migrating cells, suppressed Ki-67 and PCNA protein expression, promoted cell apoptosis, blocked cells at G0/G1 phase, and reduced the number of tube formations (Fig. 4B–I).

miR-221-3p Targets CDKN1B expression to improve angiogenesis in burn-injured HUVECs

A functional rescue experiment was implemented to probe the regulatory role of the miR-221-3p/CDKN1B axis in burn-injured HUVECs. The transfection designs were as follows: Inhibitor-NC+si-NC, miR-221-3p-inhibitor+si-NC and miR-221-3p-inhibitor + si-CDKN1B. The results presented that miR-221-3p inhibitor promoted CDKN1B expression, while si-CDKN1B reversed this effect (Fig. 5A). Functional experiments manifested that after transfection of miR-221-3p-inhibitor, cell proliferation and cloning abilities were attenuated (Fig. 5B, C), the number of migrating cells was reduced (Fig. 5D), and Ki67 and PCNA protein expressions were reduced (Fig. 5E, F), the apoptotic rate was promoted, cells in G0/G1 phase were increased (Fig. 5G, H), and tube-forming ability was impaired (Fig. 5I), and these effects were reversed by knockdown of CDKN1B.

DISCUSSION

Burn injury damages the body caused by thermal exposure, radiation, and chemical or electrical contact, which

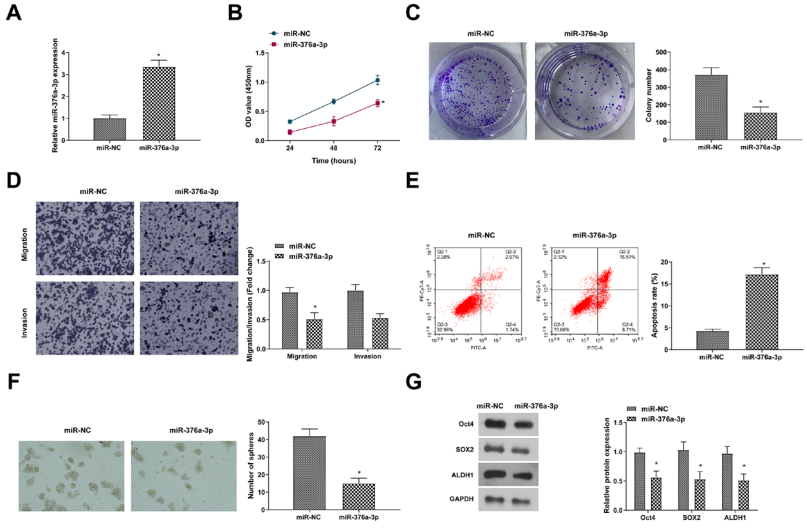

Figure 4. Overexpression of CDKN1B enhances the inhibitory effect of burn on HUVECs angiogenesis
pcDNA-CDKN1B was transfected into burn HUVECs to upregulate CDKN1B expression. (**A**) RT-qPCR and Western blot to detect CDKN1B in HUVECs; (**B**) MTT assay to detect the proliferation of HUVECs; (**C**) Clone formation assay to evaluate the clonogenic ability of HUVECs; (**D**) Transwell assay to detect the migration of HUVECs; (**E–F**) Western blot to measure Ki-67 and PCNA; (**G–H**) Flow cytometry to determine apoptosis and cell cycle; (**I**) Tube formation assay to assess the tube-forming ability of HUVECs; data are expressed as mean ± S.D. **P*<0.05.

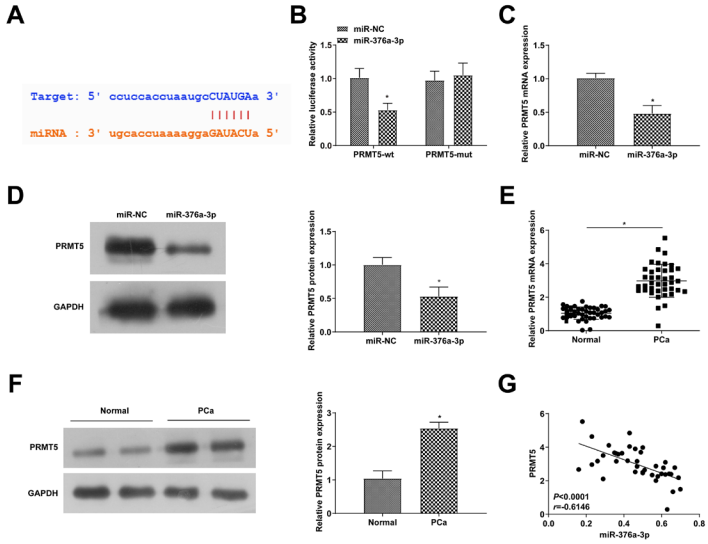

Figure 5 miR-221-3p targets CDKN1B expression to improve angiogenesis in burn-injured HUVECs
miR-221-3p-inhibitor and si-CDKN1B were co-transfected into burn-injured HUVECs (**A**) RT-qPCR and Western blot to detect CDKN1B in HUVECs; (**B**) MTT assay to detect the proliferation of HUVECs; (**C**) Clone formation assay to evaluate the clonogenic ability of HUVECs; (**D**) Transwell assay to detect the migration of HUVECs; (**E–F**) Western blot to measure Ki-67 and PCNA; (**G–H**) Flow cytometry to determine apoptosis and cell cycle; (**I**) Tube formation assay to assess the tube-forming ability of HUVECs; data are expressed as mean ± S.D. **P*<0.05.

induce activation of peripheral nerve fibers, initiating a sustained hypersensitivity response to thermal and mechanical stimuli (Laycock *et al.,* 2013). The consequences of burns are severe, with a high mortality rate and common in low- and middle-income countries (Atwell *et al.,* 2020). With the development of science and technology, there have been many studies on gene technology treatment for burn injury (Zhang *et al.,* 2021). Bioinformatics analysis has identified 43 miRNAs as potential regulators of early burn injury response among which miR-212-3p is down-regulated in dermal interstitial fluid (Foessl *et al.,* 2021). In addition to post-burns pain management, psychological counseling, scar healing, etc., post-burns angiogenesis is the basis of many burn treatment principles (Eyuboglu *et al.,* 2018). The physiological basis of tissue edema and body fluid extravasation after burn injury is the change of vascular permeability which is one of the main manifestations of vascular endothelial cell damage (Tian *et al.,* 2015).

Belonging to miRNAs family which affects physiological processes (Amponsah *et al.,* 2017), miR-221 is located in the p11.3 region of the X chromosome and is involved in the physiological regulation of hematopoiesis and angiogenesis (Liu *et al.,* 2009). In vascular endothelial cells, miR-221 acts on CDKN1B and PI3KR1 and inhibits endothelial cell biological functions (Celic *et al.,* 2017). In human aortic endothelial cells, miR-221-3p carries the ability to block the production of peroxisome proliferator-activated receptor λ coactivator 1α, leading to mitochondrial dysfunction and apoptosis (Xue *et al.,* 2015). The present study found that miR-221-3p has a positive role in vascular remodeling after burn injury. Overexpression of miR-221-3p promoted the proliferation of HUVECs by reducing the ratio of HUVECs in the G0/G1 phase, thereby mediating HUVECs migration and tube formation, which will benefit the angiogenic capacity of HUVECs in the burn environment. Cell cycle changes are important for the proliferation of HUVECs. When cells are arrested in the G0/G1 phase, the proliferation of HUVECs is inhibited and their tube-forming ability is reduced (Cota Teixeira *et al.,* 2019; Zhang *et al.,* 2013). Several studies have demonstrated the role of miRNAs in regulating the cell cycle and proliferation of HUVECs, such as miRNAs including miR-182-5p (Su *et al.,* 2021), miR-20b (Dong *et al.,* 2020). This study speculated that the regulation of miR-221-3p on the cell cycle of HUVECs will affect the ability of vascular remodeling, and the effect of miR-221-3p on the cell cycle of HUVECs needs to be further explored in subsequent studies.

CDKN1B is located on chromosome 12p 13 and has a promoting regulatory role in tumors (Kim *et al.,* 2015), such as osteosarcoma (Gao *et al.,* 2022). As a downstream target gene of miR-221, CDKN1B can promote the proliferation of vascular smooth muscle cells in the neovascular intima (Medina *et al.,* 2008). MiR-221-3p/CDKN1B axis can regulate the proliferation and cell cycle of HUVECs after burn injury, and further influence cellular migration and tube formation ability. It is worth noting that this study only validated the role of the miR-221-3p/CDKN1B axis in angiogenesis in an *in vitro* model and could be explored in future animal burn injury models. In addition, there are few reports on the regulatory mechanism of ncRNAs in burn injury, and the regulatory mechanism of ncRNAs is crucial in vascular remodeling, skin recovery and other processes. Therefore, it is necessary to further explore the changes and mechanisms of ncRNAs in burn injury.

In conclusion, this work confirmed that miR-221-3p affects the proliferation, migration, cell cycle, apoptosis, and tube formation of burn-injured HUVECs by regulating CDKN1B. MiR-221-3p/CDKN1B axis may be of interest in future burn treatments.

REFERENCES

Amponsah PS, Fan P, Bauer N, Zhao Z, Gladkich J, Fellenberg J, Herr I (2017) microRNA-210 overexpression inhibits tumor growth and potentially reverses gemcitabine resistance in pancreatic cancer. *Cancer Lett* **388**: 107–117. https://doi.org/10.1016/j.canlet.2016.11.035

Atwell K, Bartley C, Cairns B, Charles A (2020) The epidemiologic characteristics and outcomes following intentional burn injury at a

regional burn center. *Burns* **46**: 441–446. https://doi.org/10.1016/j.burns.2019.08.002

Celic T, Metzinger-Le Meuth V, Six I, Massy ZA, Metzinger L (2017) The mir-221/222 Cluster is a key player in vascular biology via the fine-tuning of endothelial cell physiology. *Curr Vasc Pharmacol* **15**: 40–46. https://doi.org/10.2174/1570161114666160914175149

Cota Teixeira S, Silva Lopes D, Santos da Silva M, Cordero da Luz FA, Cirilo Gimenes SN, Borges BC, Alves da Silva A, Alves Martins F, Alves Dos Santos M, Teixeira TL, Oliveira RA, de Melo Rodrigues Ávila V, Barbosa Silva MJ, Elias MC, Martin R, Vieira da Silva C, Knölker HJ (2019) Pentachloropseudilin impairs angiogenesis by disrupting the actin cytoskeleton, integrin trafficking and the cell cycle. *Chembiochem* **20**: 2390–2401. https://doi.org/10.1002/cbic.201900203

Dong C, Fan B, Ren Z, Liu B, Wang Y (2021) CircSMARCA5 Facilitates the progression of prostate cancer through miR-432/PDCD10 axis. *Cancer Biother Radiopharm* **36**: 70–83. https://doi.org/10.1089/cbr.2019.3490

Dong F, Dong S, Liang Y, Wang K, Qin Y, Zhao X (2020) miR-20b inhibits the senescence of human umbilical vein endothelial cells through regulating the Wnt/β-catenin pathway *via* the TXNIP/NLRP3 axis. *Int J Mol Med* **45**: 847–857. https://doi.org/10.3892/ijmm.2020.4457

Edgar DW, Fish JS, Gomez M, Wood FM (2011) Local and systemic treatments for acute edema after burn injury: a systematic review of the literature. *J Burn Care Res* **32**: 334–347. https://doi.org/10.1097/BCR.0b013e31820ab019

Eyuboglu AA, Uysal CA, Ozgun G, Coskun E, Markal Ertas N, Haberal M (2018) The effect of adipose derived stromal vascular fraction on stasis zone in an experimental burn model. *Burns* **44**: 386–396. https://doi.org/10.1016/j.burns.2017.08.016

Foessl I, Haudum CW, Vidakovic I, Prassl R, Franz J, Mautner SI, Kainz S, Hofmann E, Obermayer-Pietsch B, Birngruber T, Kotzbeck P (2021) miRNAs as Regulators of the early local response to burn injuries. *Int J Mol Sci* **22**. https://doi.org/10.3390/ijms22179209

Gao LF, Jia S, Zhang QM, Xia YF, Li CJ, Li YH (2022) MicroRNA-802 promotes the progression of osteosarcoma through targeting p27 and activating PI3K/AKT pathway. *Clin Transl Oncol* **24**: 266–275. https://doi.org/10.1007/s12094-021-02683-w

Gelfand JA, Donelan M, Burke JF (1983) Preferential activation and depletion of the alternative complement pathway by burn injury. *Ann Surg* **198**: 58–62. https://doi.org/10.1097/00000658-198307000-00011

Haagsma JA, Graetz N, Bolliger I, Naghavi M, Higashi H, Mullany EC, Abera SF, Abraham JP, Adofo K, Alsharif U, Ameh EA, Ammar W, Antonio CA, Barrero LH, Bekele T, Bose D, Brazinova A, Catalá-López F, Dandona L, Dandona R, Dargan PI, De Leo D, Degenhardt L, Derrett S, Dharmaratne SD, Driscoll TR, Duan L, Petrovich Ermakov S, Farzadfar F, Feigin VL, Franklin RC, Gabbe B, Gosselin RA, Hafezi-Nejad N, Hamadeh RR, Hijar M, Hu G, Jayaraman SP, Jiang G, Khader YS, Khan EA, Krishnaswami S, Kulkarni C, Lecky FE, Leung R, Lunevicius R, Lyons RA, Majdan M, Mason-Jones AJ, Matzopoulos R, Meaney PA, Mekonnen W, Miller TR, Mock CN, Norman RE, Orozco R, Polinder S, Pourmalek F, Rahimi-Movaghar V, Refaat A, Rojas-Rueda D, Roy N, Schwebel DC, Shaheen A, Shahraz S, Skirbekk V, Søreide K, Soshnikov S, Stein DJ, Sykes BL, Tabb KM, Temesgen AM, Tenkorang EY, Theadom AM, Tran BX, Vasankari TJ, Vavilala MS, Vlassov VV, Woldeyohannes SM, Yip P, Yonemoto N, Younis MZ, Yu C, Murray CJ, Vos T (2016) The global burden of injury: incidence, mortality, disability-adjusted life years and time trends from the Global Burden of Disease study 2013. *Inj Prev* **22**: 3–18. https://doi.org/10.1136/injuryprev-2015-041616

He J, Chu Z, Lai W, Lan Q, Zeng Y, Lu D, Jin S, Xu H, Su P, Yin D, Chu Z, Liu L (2021) Circular RNA circHERC4 as a novel oncogenic driver to promote tumor metastasis *via* the miR-556-5p/CTBP2/E-cadherin axis in colorectal cancer. *J Hematol Oncol* **14**: 194. https://doi.org/10.1186/s13045-021-01210-2

Johnson T, Zhao L, Manuel G, Taylor H, Liu D (2019) Approaches to therapeutic angiogenesis for ischemic heart disease. *J Mol Med (Berl)* **97**: 141–151. https://doi.org/10.1007/s00109-018-1729-3

Kanitakis J, Kyamidis K, Toussinas A, Tsoïtis G (2011) Pure apocrine nevus: immunohistochemical study of a new case and literature review. *Dermatology* **222**: 97–101. https://doi.org/10.1159/000323000

Kim TH, Lee HH, Chung SH, Park J, Lee A (2015) Expression of p27 and Jun activation domain-binding protein 1 in endometriosis. *Arch Gynecol Obstet* **292**: 377–381. https://doi.org/10.1007/s00404-015-3642-0

Laycock H, Valente J, Bantel C, Nagy I (2013) Peripheral mechanisms of burn injury-associated pain. *Eur J Pharmacol* **716**: 169–178. https://doi.org/10.1016/j.ejphar.2013.01.071

Li X, Ballantyne LL, Yu Y, Funk CD (2019) Perivascular adipose tissue-derived extracellular vesicle miR-221-3p mediates vascular remodeling. *Faseb J* **33**: 12704–12722. https://doi.org/10.1096/fj.201901548R

Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C (2009) A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. *Circ Res* **104**: 476–487. https://doi.org/10.1161/circresaha.108.185363

Lu YH, Huang ZY (2021) Global identification of circular RNAs in imatinib (IM) resistance of chronic myeloid leukemia (CML) by modulating signaling pathways of circ_0080145/miR-203/ABL1 and circ 0051886/miR-637/ABL1. *Mol Med* **27**: 148. https://doi.org/10.1186/s10020-021-00395-z

Medina R, Zaidi SK, Liu CG, Stein JL, van Wijnen AJ, Croce CM, Stein GS (2008) MicroRNAs 221 and 222 bypass quiescence and compromise cell survival. *Cancer Res* **68**: 2773–2780. https://doi.org/10.1158/0008-5472.Can-07-6754

Miao X, Xi Z, Zhang Y, Li Z, Huang L, Xin T, Shen R, Wang T (2020) Circ-SMARCA5 suppresses colorectal cancer progression via downregulating miR-39-3p and upregulating ARID4B. *Dig Liver Dis* **52**: 1494–1502. https://doi.org/10.1016/j.dld.2020.07.019

Muramatsu F, Kidoya H, Naito H, Sakimoto S, Takakura N (2013) microRNA-125b inhibits tube formation of blood vessels through translational suppression of VE-cadherin. *Oncogene* **32**: 414–421. https://doi.org/10.1038/onc.2012.68

Ray SL, Coulson DJ, Yeoh MLY, Tamara A, Latief JS, Bakhashab S, Weaver JU (2020) The role of miR-342 in vascular health. study in subclinical cardiovascular disease in mononuclear cells, plasma, inflammatory cytokines and PANX2. *Int J Mol Sci* **21**. https://doi.org/10.3390/ijms21197217

Stefanini MO, Wu FT, Mac Gabhann F, Popel AS (2009) The presence of VEGF receptors on the luminal surface of endothelial cells affects VEGF distribution and VEGF signaling. *PLoS Comput Biol* **5**: e1000622. https://doi.org/10.1371/journal.pcbi.1000622

Su G, Sun G, Lv J, Zhang W, Liu H, Tang Y, Su H (2021) Hsa_circ_0004831 downregulation is partially responsible for atorvastatin-alleviated human umbilical vein endothelial cell injuries induced by ox-LDL through targeting the miR-182-5p/CXCL12 axis. *BMC Cardiovasc Disord* **21**: 221. https://doi.org/10.1186/s12872-021-01998-4

Tian KY, Liu XJ, Xu JD, Deng LJ, Wang G (2015) Propofol inhibits burn injury-induced hyperpermeability through an apoptotic signal pathway in microvascular endothelial cells. *Braz J Med Biol Res* **48**: 401–407. https://doi.org/10.1590/1414-431x20144107

van Baar ME, Essink-Bot ML, Oen IM, Dokter J, Boxma H, van Beeck EF (2006) Functional outcome after burns: a review. *Burns* **32**: 1–9. https://doi.org/10.1016/j.burns.2005.08.007

Wang C, Lin Y, Fu Y, Zhang D, Xin Y (2020) MiR-221-3p regulates the microvascular dysfunction in diabetic retinopathy by targeting TIMP3. *Pflugers Arch* **472**: 1607–1618. https://doi.org/10.1007/s00424-020-02432-y

Wang N, Guo Y, Song L, Tong T, Fan X (2022) Circular RNA intraflagellar transport 80 facilitates endometrial cancer progression through modulating miR-545-3p/FAM98A signaling. *J Gynecol Oncol* **33**: e2. https://doi.org/10.3802/jgo.2022.33.e2

Xue Y, Wei Z, Ding H, Wang Q, Zhou Z, Zheng S, Zhang Y, Hou D, Liu Y, Zen K, Zhang CY, Li J, Wang D, Jiang X (2015) MicroRNA-19b/221/222 induces endothelial cell dysfunction via suppression of PGC-1α in the progression of atherosclerosis. *Atherosclerosis* **241**: 671–681. https://doi.org/10.1016/j.atherosclerosis.2015.06.031

Yang L, Zhou YN, Zeng MM, Zhou N, Wang BS, Li B, Zhu XL, Guan QL, Chai C (2021) Circular RNA Circ-0002570 accelerates cancer progression by regulating VCAN *via* MiR-587 in gastric cancer. *Front Oncol* **11**: 733745. https://doi.org/10.3389/fonc.2021.733745

Yue JN, Li WM, Hong WZ, Yang J, Zhu T, Fang Y, Fu WG (2019) MiR-210 inhibits apoptosis of vascular endothelial cells *via* JAK-STAT in arteriosclerosis obliterans. *Eur Rev Med Pharmacol Sci* **23** (Suppl 3): 319–326. https://doi.org/10.26355/eurrev_201908_18663

Zhang D, Zhang Y, Zhang X, Zhai H, Sun X, Li Y (2021) Circ_0046600 promotes hepatocellular carcinoma progression via up-regulating SERBP1 through sequestering miR-1258. *Pathol Res Pract* **228**: 153681. https://doi.org/10.1016/j.prp.2021.153681

Zhang L, Zhang W, Zuo Z, Tang J, Song Y, Cao F, Yu X, Liu S, Cai X (2022) Circ_0008673 regulates breast cancer malignancy by miR-153-3p/CFL2 axis. *Arch Gynecol Obstet* **305**: 223–232. https://doi.org/10.1007/s00404-021-06149-w

Zhang P, Xu X, Hu X, Wang H, Fassett J, Huo Y, Chen Y, Bache RJ (2013) DDAH1 deficiency attenuates endothelial cell cycle progression and angiogenesis. *PLoS One* **8**: e79444. https://doi.org/10.1371/journal.pone.0079444

Zhang T, Zhang R, Xu B, Zhang M, Zhang Q, Li N, Qiu Y, Chen D, Xu K, Xiao J, Zhang N, Fang Q (2021) Spinal endomorphins attenuate burn-injury pain in male mice by inhibiting p38 MAPK signaling pathway through the mu-opioid receptor. *Eur J Pharmacol* **903**: 174139. https://doi.org/10.1016/j.ejphar.2021.174139