Upregulation of miR-192-5p inhibits the ELAVL1/PI3Kδ axis and attenuates microvascular endothelial cell proliferation, migration and angiogenesis in diabetic retinopathy

Xiao-Lin fu
Hainan West Central Hospital
Fu-Tao He (futaohehe1234@163.com)
Hainan West Central Hospital
Mo-Han Li
Hainan West Central Hospital
Chun-Yan Fu
Hainan West Central Hospital
Jian-Zhi Chen
Hainan West Central Hospital

Research Article

Keywords: diabetic retinopathy, ELAVL1, miR-192-5p, PI3Kδ, microvascular endothelial cell

Posted Date: July 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1871717/v1

Abstract

Background: Diabetic retinopathy (DR) is a common complication of diabetes and a sight threaten among adults. MicroRNAs (miRNAs) serve a key role in DR progression. However, the role and mechanism of miR-192-5p in DR remain unclear. We aim to investigate the effect of miR-192-5p on cell proliferation, migration and angiogenesis in DR.

Methods: The expressions of miR-192-5p, ELAVL1 and PI3Kδ in DR samples and human retinal microvascular endothelial cells (HRMECs) were assessed using RT-qPCR. ELAVL1 and PI3Kδ protein levels were evaluated by Western blot. RIP and dual luciferase reporter assays were performed to confirm the miR-192-5p/ELAVL1/PI3Kδ regulatory network. Cell proliferation, migration and angiogenesis were evaluated by CCK8, transwell and tube formation assays.

Results: MiR-192-5p was decreased in DR patients and high glucose (HG)-treated HRMECs. In function, overexpressed miR-192-5p suppressed cell proliferation, migration and angiogenesis in HG-treated HRMECs. Mechanically, miR-192-5p directly target ELAVL1 and decreased its expression. We further verified that ELAVL1 bound to PI3Kδ and maintained PI3Kδ mRNA stability. Rescue assays demonstrated that the inhibitive effects of HG-treated HRMECs caused by miR-192-5p upregulation were overturned by overexpressed ELAVL1 or PI3Kδ.

Conclusion: MiR-192-5p alleviated DR progression via targeting ELAVL1 and reducing PI3Kδ expression, indicating a biomarker for therapy in DR.

Introduction

Diabetic retinopathy (DR) is one of the most serious clinical microvascular complications of diabetes, manifesting as vision loss and even blindness [1]. Clinical studies have shown that DR was found in 90% of patients with type 1 diabetes and 55% of patients with type 2 diabetes [2]. DR is a disease with multifactorial etiologies, including oxidative and hypoxic stress, retinal inflammation, which can cause proliferation and migration of retinal microvascular endothelial cells and the formation of capillary tube [3]. Clinical treatment of DR is mainly divided into surgical treatment, retinal laser treatment, intravitreal injection treatment and systemic treatment [4]. However, the curative effect is not good, and symptoms often show progressive aggravation, which seriously affects the quality of life [5]. Therefore, more molecular mechanisms and new biological diagnostic markers are urgently needed for the treatment of DR.

MicroRNAs (miRNAs) are a class of single-stranded non-coding RNAs of approximately 22 nucleotides and are associated with various cellular processes, such as proliferation, apoptosis and angiogenesis [6]. Importantly, miRNAs are involved in DR [7]. For example, miR-126 was decreased in retina of DR patients [8]. Dysregulation of miR-374a regulated HRMECs proliferation and migration [9]. Notably, miR-192-5p expression was decreased in diabetes, and its overexpression could reduce blood glucose levels in HepG2

cells [10]. Besides, miR-192-5p was decreased in diabetic retina [11]. Nevertheless, the precise mechanisms of miR-192-5p in DR remain poorly understood.

ELAV-like RNA binding protein 1 (ELAVL1) is a RNA-binding protein which binds to a variety of proteins and maintains mRNA stability [12]. Literatures have found that ELAVL1 was up-regulated in the retina and HRECs of diabetic rats, and combined with VEGF mRNA to promote the expression of VEGF and angiogenesis [13, 14]. However, the mechanism needs to be further explored. We recently found that miR-192-5p could bind to ELAVL1 by starbase prediction. Herein, we speculated that decreased miR-192-5p could promote ELAVL1 expression and contribute to DR development.

Phosphatidylinositol 3-kinase delta (PI3Kδ) is a PI3K class IA catalytic isoform encoded by the PIK3CD gene (p110δ) and is an important member of the lipid kinase family [15]. Currently, it has been reported that the expression of PI3Kδ was increased in high glucose-induced human retinal microvascular endothelial cells (HRMECs). Furthermore, PI3Kδ regulated the activation of Akt signaling pathway in vascular endothelial cells to promote cell proliferation, migration and angiogenesis, while the inactivation of PI3Kδ attenuated pathological retinal angiogenesis [16]. Nevertheless, the specific contribution of PI3Kδ to DR is largely unknown. According to the prediction of starbase, ELAVL1 had a binding site with PIK3CD. Herein, we hypothesized that ELAVL1 might play a role in DR by binding to PIK3CD.

Herein, we aimed to investigate the role and mechanism of miR-192-5p in modulating the pathogenesis of DR. We hypothesize that in DR, up-regulation of miR-192-5p can suppress ELAVL1/PI3Kδ axis and thereby attenuate microvascular endothelial cell proliferation, migration and angiogenesis.

Materials And Methods

1. Clinical samples

Samples of retinal fibrovascular membrane (FVM) were obtained from twenty DR patients who were admitted to Hainan West Central Hospital from September of 2020 to September of 2021. Epiretinal membranes (EM) from twenty patients who underwent vitrectomy for rhegmatogenous retinal detachment without proliferative vitreoretinopathy were assigned to the control group. All specimens were stored in 80˚C until being used. Informed consent was provided by all patients. Approval was obtained from the Ethics Committee of Hainan West Central Hospital.

2. Cell culture and DR cell model

Human retinal microvascular endothelial cells (HRMECs) were provided by Procell (Wuhan, China), which were later cultivated within endothelial cell medium (ECM, Gibco, Grand Island, NY, USA) based on the concentration of 10% fetal bovine serum (FBS, Gibco) under the conditions of 5% CO2 and 37°C. To construct DR cell model, HRMECs were exposed to different glucose concentrations, namely, normal-glucose (5.5 M, Normal), high-glucose (HG, 30 mM) or osmotic control group (Mannitol group, 5.5 mM HG supplemented with 24.5 mM mannitol).

3. Cell transfection

The shRNAs specifically targeting ELAVL1 (sh-ELAVL1) and negative control were provided by GenePharma (Shanghai, China). ELAVL1-overexpressing plasmid (oe-ELAVL1) and PI3Kδ-overexpressing plasmid (oe-PI3Kδ) were constructed by GenePharma (Shanghai, China). The miR-192-5p mimic, miR-192-5p inhibitor and negative controls were bought from GenePharma (Shanghai, China). Cells were seeded in 6-well plates. At 70% confluence, cell transfection was carried out by utilizing Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instruments. 48 h later, cells were harvested for following experiments.

4. Western blotting assay

This work utilized RIPA buffer (Beyotime, Shanghai, China) for extracting total cellular proteins. BCA method (Keygen Biotech, Nanjing, China) was applied in determining protein content. After separating protein aliquots through SDS-PAGE, this study transferred on PVDF membranes (Millipore, MA, USA). Later, 5% defatted milk was later utilized to block membranes for a 1-h period. Blots were cultivated with anti ELAVL1 antibody (#12582, 1:1000, Cell Signaling Technology, Danvers, MA, USA) as well as PI3Kδ antibody (ab1678, 1:1000, Abcam, Cambridge, MA, USA) overnight under the temperature of 4˚C. Subsequently, this study adopted secondary antibody (Cell Signaling Technology) for additional 1-h incubation under ambient temperature. Signals were detected by enhanced chemiluminescence (Millipore). β-actin was used as internal reference.

5. Real-time quantitative PCR (RT-qPCR)

In order to perform the extraction of total cellular and tissue RNAs, TRIzol reagent (Invitrogen) was used. Isolated mRNA was reversed with PrimeScript RT reagent kit (Takara, Dalian, China). Apart from that, the Mir-XTM miRNA First Strand Synthesis Kit (Takara) was applied in miRNA transcription. SYBR Green detection system (Takara) was used to quantify gene expression. The designed primers used in this study were: miR-192-5p F: 5'-GGCGCCTGACCTATGAATTG-3', R: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGCTGT-3'; ELAVL1 F: 5'-CGCAGAGATTCAGGTTCTCC-3', R: 5'- CCAAACCCTTTGCACTTGTT-3'; PIK3CD F: 5'-TAAGTTTGAGGGCAGCGAGG-3', R: 5'-ACCTGCAGCGTGTAGTCTTC-3';. U6 and GAPDH were employed as the internal control. Results were analyzed with the standard $2^{-\Delta\Delta Ct}$ method.

6. Cell viability assay

The detection of HRMECs cell viability was made by applying Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan). After transfection, this work inoculated cells in the 96-well plates (5000 cells/well). Twenty-four hours after seeding, the supplementation of each well was made with 10 μL CCK-8 solution. At 2-h post-incubation, we evaluated cell viability by measuring the absorbance (OD) (Bio Rad Laboratories, Inc., Hercules, CA, USA) at 450 nm.

7. Transwell assay

3×10^3 cells/well were added into top Transwell chambers (8 μm, Millipore) using serum-free medium, whereas medium that contained 10% FBS was added to bottom chamber. At 24-h post-cultivation, 4% paraformaldehyde (PFA) was utilized to fix migrating cells, followed by staining using the 0.1% crystal violet. Olympus microscope (Tokyo, Japan) was adopted for taking cell images. At the same time, cell number was calculated from 5 random fields.

8. Tube formation assay

Matrigel (50 μL/well, BD Biosciences, Franklin Lakes, NJ) was added to 96-well plates and allowed to polymerize for 60 min at 37°C before seeding HRMECs on Matrigel-coated wells (7×10^3/well). Eight hours after seeding, capillary-like structures were captured using microscopy (Olympus). Tube formation was quantified by selecting five different fields randomly using ImageJ software (NIH, USA).

9. Dual-luciferase reporter assay

This work cloned mutant (MUT) or wild-type (WT) 3'UTR in ELAVL1 to pmirGLO vector (Promega, Madison, WI, USA). Then, HRMECs were subjected to co-transfection using NC mimic or miR-192-5p mimic and vectors carrying ELAVL1-WT or ELAVL1-MUT with Lipofectamine 3000 (Invitrogen). 2 days later, the dual-luciferase reporter assay system (Promega) was used for making the measurement of luciferase activities.

10. RNA immunoprecipitation (RIP)

EZ-Magna RIP kit (Millipore) was utilized for RIP assay. Transfected cells were lysed using RIP lysis buffer. The extract was added to magnetic beads and exposed to ELAVL1 antibody (Santa Cruz, USA), Ago2 antibody (Abcam) or anti-IgG (Sigma) at 4°C overnight. Finally, the relative enrichment of genes was calculated by RT-qPCR.

11. Statistical analysis

Results were represented by mean ± SD and explored with Graphpad Prism 8 (San Diego, CA, USA). Each experiment was conducted in 3 replicates. We used the Student's t-test for making the comparison between 2 groups, while One-way ANOVA among several groups. In this study, $p < 0.05$ stood for statistical significance.

Results

1. MiR-192-5p was down-regulated in DR clinical samples and DR cell model

Firstly, we detected miR-192-5p levels in samples from patients with DR or non-diabetic individuals by RT-qPCR and demonstrated that miR-192-5p expression was decreased dramatically in DR patients

compared to the control (Fig. 1A). Next, HRMECs were used to construct DR cell model and treated with normal glucose (5.5 mM), high glucose (HG, 30 mM) or mannitol (5.5 mM HG supplemented with 24.5 mM mannitol). According to RT-qPCR analysis, miR-192-5p was greatly low in HRMECs with HG, compared with normal glucose group or mannitol group (Fig. 1B). Therefore, miR-192-5p was reduced in DR patients and by HG treatment.

2. MiR-192-5p upregulation suppressed microvascular endothelial cell proliferation, migration and angiogenesis in DR cell model

To further explore the function of miR-192-5p in DR, HG-induced HRMECs were transfected with miR-192-5p mimic. RT-qPCR showed that miR-192-5p was overexpressed in HG-induced HRMECs (Fig. 2A). Next, CCK8 experiment revealed that HRMECs viability increased after HG treatment, but cell viability decreased in HG cells with miR-192-5p upregulation (Fig. 2B). Meanwhile, HG induced cell migration. Nevertheless, cell migration diminished by overexpressed miR-192-5p (Fig. 2C). Moreover, tube formation ability of HRMECs was induced after HG stimulation, but was impaired in HG cells with miR-192-5p upregulation (Fig. 2D). Thus, these results suggest that cell proliferation, migration and angiogenesis in HG-treated HRMECs were suppressed by miR-192-5p overexpression.

3. MiR-192-5p directly bound to ELAVL1 and inhibited its expression

Further, the relationship between miR-192-5p and ELAVL1 was investigated. Luciferase reporter assay revealed that fluorescence value of ELAVL1 wild-type group decreased in HRMECs with miR-192-5p mimics, while there was no significant change in ELAVL1 mutant group (Fig. 3A). Next, the results of RIP assay validated that both miR-192-5p and ELAVL1 were enriched in the Ago2 antibody complex (Fig. 3B). Furthermore, we detected decreased miR-192-5p level in HG-induced HRMECs with miR-192-5p inhibitor compared to control cells (Fig. 3C). Afterwards, ELAVL1 expression was elevated in HG-treated HRMECs, and overexpressed miR-192-5p reduced ELAVL1 expression, whereas miR-192-5p inhibitor elevated ELAVL1 level (Fig. 3D-3E). All in all, miR-192-5p could target ELAVL1 and suppress ELAVL1 expression.

4. The reduction in microvascular endothelial cell proliferation, migration and angiogenesis caused by miR-192-5p upregulation was reversed by ELAVL1 overexpression

We overexpressed ELAVL1 in HG-induced HRMECs and discovered that both ELAVL1 gene and protein expression were increased (Fig. 4A). The proliferation and migration of HG cells were suppressed by miR-192-5p upregulation. However, reductions of HG cell proliferation and migration generated by miR-192-5p overexpression could be restored by ELAVL1 overexpression (Fig. 4B and 4C). In addition, the inhibition of tube formation with miR-192-5p mimics in HG-treated cells could also be overturned by co-transfection of oe-ELAVL1 (Fig. 4D). Taken together, miR-192-5p modulated the cell proliferation, migration and angiogenesis in HG-treated HRMECs through ELAVL1.

5. ELAVL1 maintained PI3Kδ mRNA stability

We next delineate the interaction between ELAVL1 and PI3Kδ, RIP assay verified the binding of ELAVL1 to PI3Kδ in HRMECs (Fig. 5A). Notably, the abundance of PI3Kδ mRNA in ELAVL1 immunoprecipitates was decreased with overexpressed miR-192-5p, and PI3Kδ mRNA abundance in ELAVL1 immunoprecipitates was increased after knockdown of miR-192-5p (Fig. 5B). Additionally, ELAVL1 expression was diminished in HG-treated HRMECs transfected with sh-ELAVL1 (Fig. 5C). Meanwhile, the expression of PI3Kδ was significantly down-regulated after ELAVL1 knockdown, and the expression of PI3Kδ was up-regulated after ELAVL1 overexpression (Fig. 5D). In the presence of actinomycin D, knockdown of ELAVL1 accelerated the decay of PI3Kδ mRNA, whereas overexpression of ELAVL1 slowed down the decay of PI3Kδ mRNA (Fig. 5E). Altogether, the above data indicated that ELAVL1 could maintain PI3Kδ mRNA stability.

6. Overexpressed PI3Kδ overturned inhibitory effects of miR-192-5p upregulation on microvascular endothelial cell proliferation, migration and angiogenesis

Finally, we evaluated the function of PI3Kδ in miR-192-5p-regulated DR. Our results revealed increased PI3Kδ expression in HG-induced HRMECs transfected with oe-PI3Kδ compared to the control groups (Fig. 6A). Next, we discovered that miR-192-5p greatly reduced cell proliferation, migration in HG-treated cells, which was obviously overturned by the co-transfection with oe-PI3Kδ (Fig. 6B and 6C). Moreover, tube formation ability of HG cells was effectively suppressed by miR-192-5p upregulation compared to HG groups, while overexpression of PI3Kδ could reverse this effect (Fig. 6D).

Discussion

Diabetes mellitus is a metabolic disease, which can easily cause damage to tissues and organs, resulting in pathological changes [17]. DR is a common complication of type 1 and type 2 diabetes, and it is also one of the main reasons for irreversible vision loss [18]. Incidence of DR in diabetic patients is about 25% [19]. The formation of new blood vessels in DR progression contributes to the irreversible damage of vision in DR patients [20]. Therefore, reversing retinal microvascular dysfunction is of great importance for DR therapy. Here, we demonstrated that miR-192-5p upregulation inhibited cell proliferation, migration and angiogenesis in HG-treated HRMECs, which were mediated by negatively regulation of ELAVL1 and PI3Kδ expression.

Various miRNAs are reported to be involved in DR progression. For instance, miR-148a-3p led to increased cell viability and decreased apoptosis in HG-triggered DR through targeting TGFB2 and FGF2 [21]. MiR-29b-3p suppressed HRMECs proliferation and angiogenesis via binding to VEGFA and PDGFB [22]. Recently, dysregulation of miR-192-5p is observed to be related to hyperglycemia and DR. MiR-192-5p inhibition greatly elevated blood glucose content in HepG2 cells [10]. Additionally, methane exerted a protective role in DR through up-regulating retinal miR-192-5p expression [11]. In our project, miR-192-5p was greatly diminished in clinical samples and HRMECs induced by HG. Furthermore, we also found that under HG condition, miR-192-5p overexpression led to inhibition of microvascular endothelial cell

proliferation, migration and angiogenesis. These findings imply that miR-192-5p is crucial for suppressing DR development.

MiR-192-5p is reported to bind to targets in various diseases. For instance, miR-192-5p could target TRPM7, thereby inhibiting cervical cancer proliferation and invasion [23]. Overexpressed mir-192-5p rendered gastric cancer cells more sensitive to cisplatin [24]. In our project, miR-192-5p could target ELAVL1 and negatively regulate its expression. Indeed, literatures indicated that ELAVL1 played a role part in diabetes. For example, ELAVL1 depletion induced osteogensis in a mouse model of diabetes mellitus [25]. In addition, ELAVL1 was involved in renal tubular epithelial pyroptosis in diabetic nephropathy [26]. We further demonstrated that after overexpressing ELAVL1, effects of miR-192-5p on HG-regulated endothelial cell proliferation, migration and angiogenesis were weakened. Altogether, we proved that miR-192-5p reduced DR progression through binding with ELAVL1.

PI3Kδ is an essential component of the downstream signaling mechanisms of multiple immune receptors, such as T cell receptor and B cell receptor [27]. Meanwhile, PI3Kδ is responsible for the production of PIP3 and the activation of AKT [28]. Recently, a study reported that PI3Kδ promoted Akt activation, cell proliferation, migration, and tube formation in vascular endothelial cells of DR [16]. Moreover, PI3Kδ induced vascular contractility in type 1 diabetes mice [29]. In this study, we confirmed that ELAVL1 could bind to PI3Kδ mRNA and maintain its stability. Additionally, suppressive effect of miR-192-5p upregulation on microvascular endothelial cell proliferation, migration and angiogenesis could be attenuated by overexpressed PI3Kδ. Therefore miR-192-5p could modulate angiogenesis in DR through PI3Kδ.

In summary, the research revealed that miR-192-5p was markedly decreased in DR. Overexpressed miR-192-5p could inhibit microvascular endothelial cell proliferation, migration and angiogenesis. Moreover, miR-192-5p exerted protective effects by negatively regulating the ELAVL1/PI3Kδ pathway. Our findings indicate miR-192-5p might act as one novel therapeutic target to inhibit DR progression. Potential anti-angiogenic effect of miR-192-5p in DR needs to be further investigated using animal models.

Abbreviations

diabetic retinopathy	DR
ELAV-like RNA binding protein 1	ELAVL1
fibrovascular membrane	FVM
high glucose	HG
human retinal microvascular endothelial cells	HRMECs
microRNAs	miRNAs

phosphatidylinositol 3-kinase delta	PI3Kδ

Declarations

Ethics approval and consent to participate

Samples of retinal fibrovascular membrane (FVM) were obtained from twenty DR patients who were admitted to Hainan West Central Hospital from September of 2020 to September of 2021. Informed consent was provided by all patients. Approval was obtained from the Ethics Committee of Hainan West Central Hospital.

Consent for publication

The informed consent was obtained from study participants.

Availability of data and materials

All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This work was supported by Hainan Health Industry Scientific Research Project (21A200347).

Authors' contributions

Xiao-Lin fu: Conceptualization; Methodology; Validation;

Fu-Tao He: Formal analysis; Investigation; Resources; Data Curation;

Mo-Han Li: Writing - Original Draft;

Chun-Yan Fu: Visualization; Supervision;

Jian-Zhi Chen: Writing - Review & Editing; Project administration; Funding acquisition

References

1. Simó-Servat, O., C. Hernández, and R. Simó, *Diabetic Retinopathy in the Context of Patients with Diabetes*. Ophthalmic Res, 2019. **62**(4): p. 211–217.

2. Kashim, R.M., P. Newton, and O. Ojo, *Diabetic Retinopathy Screening: A Systematic Review on Patients' Non-Attendance*. Int J Environ Res Public Health, 2018. **15**(1).
3. Rodríguez, M.L., et al., *Oxidative Stress and Microvascular Alterations in Diabetic Retinopathy: Future Therapies.* Oxid Med Cell Longev, 2019. **2019**: p. 4940825.
4. Wang, W. and A.C.Y. Lo, *Diabetic Retinopathy: Pathophysiology and Treatments*. Int J Mol Sci, 2018. **19**(6).
5. Jampol, L.M., A.R. Glassman, and J. Sun, *Evaluation and Care of Patients with Diabetic Retinopathy*. N Engl J Med, 2020. **382**(17): p. 1629–1637.
6. Lu, T.X. and M.E. Rothenberg, *MicroRNA.* J Allergy Clin Immunol, 2018. **141**(4): p. 1202–1207.
7. Li, X., et al., *MicroRNAs: Potential Targets in Diabetic Retinopathy*. Horm Metab Res, 2020. **52**(3): p. 142–148.
8. Qin, L.L., et al., *MicroRNA-126: a promising novel biomarker in peripheral blood for diabetic retinopathy*. Int J Ophthalmol, 2017. **10**(4): p. 530–534.
9. Wang, Z., et al., *Dysregulation of miR-374a is involved in the progression of diabetic retinopathy and regulates the proliferation and migration of retinal microvascular endothelial cells*. Clin Exp Optom, 2021: p. 1–6.
10. Cai, H., et al., *Circular RNA HIPK3 contributes to hyperglycemia and insulin homeostasis by sponging miR-192-5p and upregulating transcription factor forkhead box O1*. Endocr J, 2020. **67**(4): p. 397–408.
11. Wu, J., et al., *Protective effects of methane-rich saline on diabetic retinopathy via anti-inflammation in a streptozotocin-induced diabetic rat model*. Biochem Biophys Res Commun, 2015. **466**(2): p. 155–61.
12. Schultz, C.W., et al., *Understanding and targeting the disease-related RNA binding protein human antigen R (HuR)*. Wiley Interdiscip Rev RNA, 2020. **11**(3): p. e1581.
13. Platania, C.B.M., et al., *Novel indole derivatives targeting HuR-mRNA complex to counteract high glucose damage in retinal endothelial cells*. Biochem Pharmacol, 2020. **175**: p. 113908.
14. Amadio, M., et al., *The PKCbeta/HuR/VEGF pathway in diabetic retinopathy*. Biochem Pharmacol, 2010. **80**(8): p. 1230–7.
15. Bier, J. and E.K. Deenick, *The role of dysregulated PI3Kdelta signaling in human autoimmunity*. Immunol Rev, 2022.
16. Wu, W., et al., *PI3Kδ as a Novel Therapeutic Target in Pathological Angiogenesis*. Diabetes, 2020. **69**(4): p. 736–748.
17. Cloete, L., *Diabetes mellitus: an overview of the types, symptoms, complications and management*. Nurs Stand, 2022. **37**(1): p. 61–66.
18. Crabtree, G.S. and J.S. Chang, *Management of Complications and Vision Loss from Proliferative Diabetic Retinopathy*. Curr Diab Rep, 2021. **21**(9): p. 33.

19. Hammes, H.P., *Diabetic retinopathy: hyperglycaemia, oxidative stress and beyond*. Diabetologia, 2018. **61**(1): p. 29–38.
20. Whitehead, M., et al., *Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies*. Expert Opin Biol Ther, 2018. **18**(12): p. 1257–1270.
21. Wang, J., et al., *MicroRNA-148a-3p alleviates high glucose-induced diabetic retinopathy by targeting TGFB2 and FGF2*. Acta Diabetol, 2020. **57**(12): p. 1435–1443.
22. Tang, W., et al., *MicroRNA-29b-3p inhibits cell proliferation and angiogenesis by targeting VEGFA and PDGFB in retinal microvascular endothelial cells*. Mol Vis, 2020. **26**: p. 64–75.
23. Dong, R.F., et al., *Tumor suppressor miR-192-5p targets TRPM7 and inhibits proliferation and invasion in cervical cancer*. Kaohsiung J Med Sci, 2021. **37**(8): p. 699–708.
24. Xie, X., et al., *MiR-192-5p reverses cisplatin resistance by targeting ERCC3 and ERCC4 in SGC7901/DDP cells*. J Cancer, 2019. **10**(4): p. 1039–1051.
25. Ren, Y., et al., *ELAV-like RNA binding protein 1 regulates osteogenesis in diabetic osteoporosis: Involvement of divalent metal transporter 1*. Mol Cell Endocrinol, 2022. **546**: p. 111559.
26. Li, X., et al., *Long noncoding RNA MALAT1 regulates renal tubular epithelial pyroptosis by modulated miR-23c targeting of ELAVL1 in diabetic nephropathy*. Exp Cell Res, 2017. **350**(2): p. 327–335.
27. Sogkas, G., et al., *Homeostatic and pathogenic roles of PI3Kδ in the human immune system*. Adv Immunol, 2020. **146**: p. 109–137.
28. Wu, M., et al., *WSX1 act as a tumor suppressor in hepatocellular carcinoma by downregulating neoplastic PD-L1 expression*. Nat Commun, 2021. **12**(1): p. 3500.
29. Pinho, J.F., et al., *Phosphatidylinositol 3-kinase-δ up-regulates L-type Ca2 + currents and increases vascular contractility in a mouse model of type 1 diabetes*. Br J Pharmacol, 2010. **161**(7): p. 1458–71.

Figures

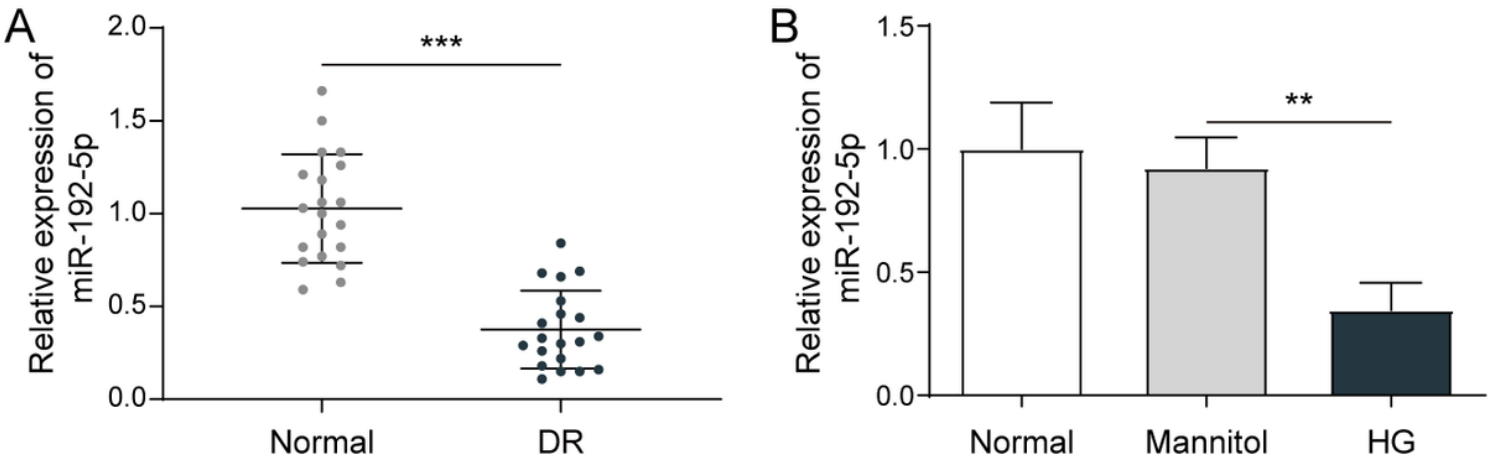

Figure 1

MiR-192-5p was down-regulated in DR clinical samples and DR cell model. (A) RT-qPCR detected miR-192-5p expression in samples from DR patients (n=20) or non-diabetic individuals (n=20). (B) MiR-192-5p level in normal group, high glucose group and mannitol group was detected by RT-qPCR. Results are presented as mean ± SD from 3 replicate experiments. *P<0.05.

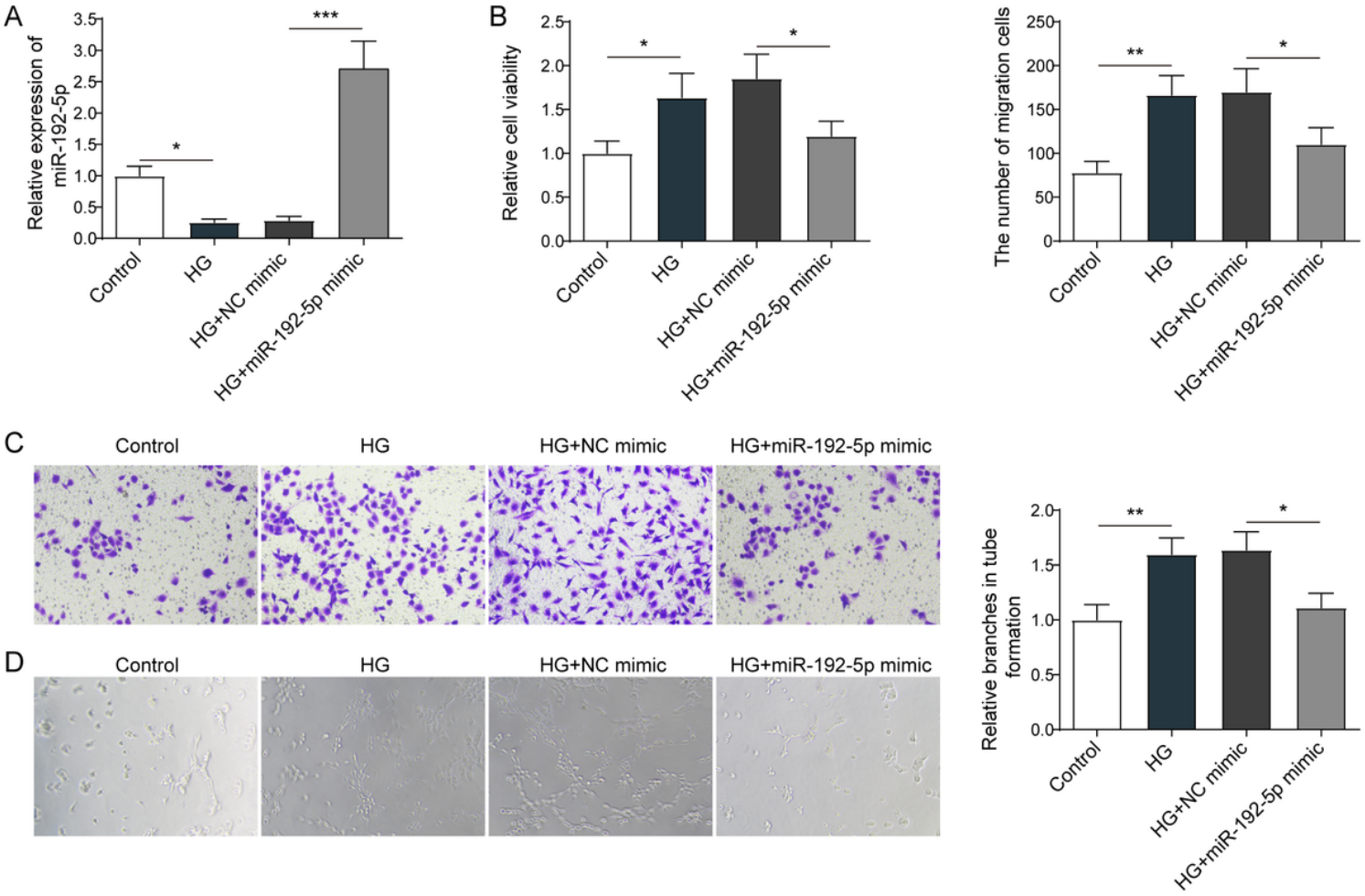

Figure 2

MiR-192-5p upregulation suppressed microvascular endothelial cell proliferation, migration and angiogenesis in DR cell model. (A) HG-induced HRMECs were transfected with miR-192-5p mimic or NC mimic. Transfection efficiency was verified by RT-qPCR. (B) CCK8 detected the cell viability. (C) Transwell assay for analysis of cell migration ability. (D) Tube formation experiment tested angiogenesis ability of HRMECs. Error bars stand for mean ± SD. Experiments were conducted in triplicate. *P<0.05.

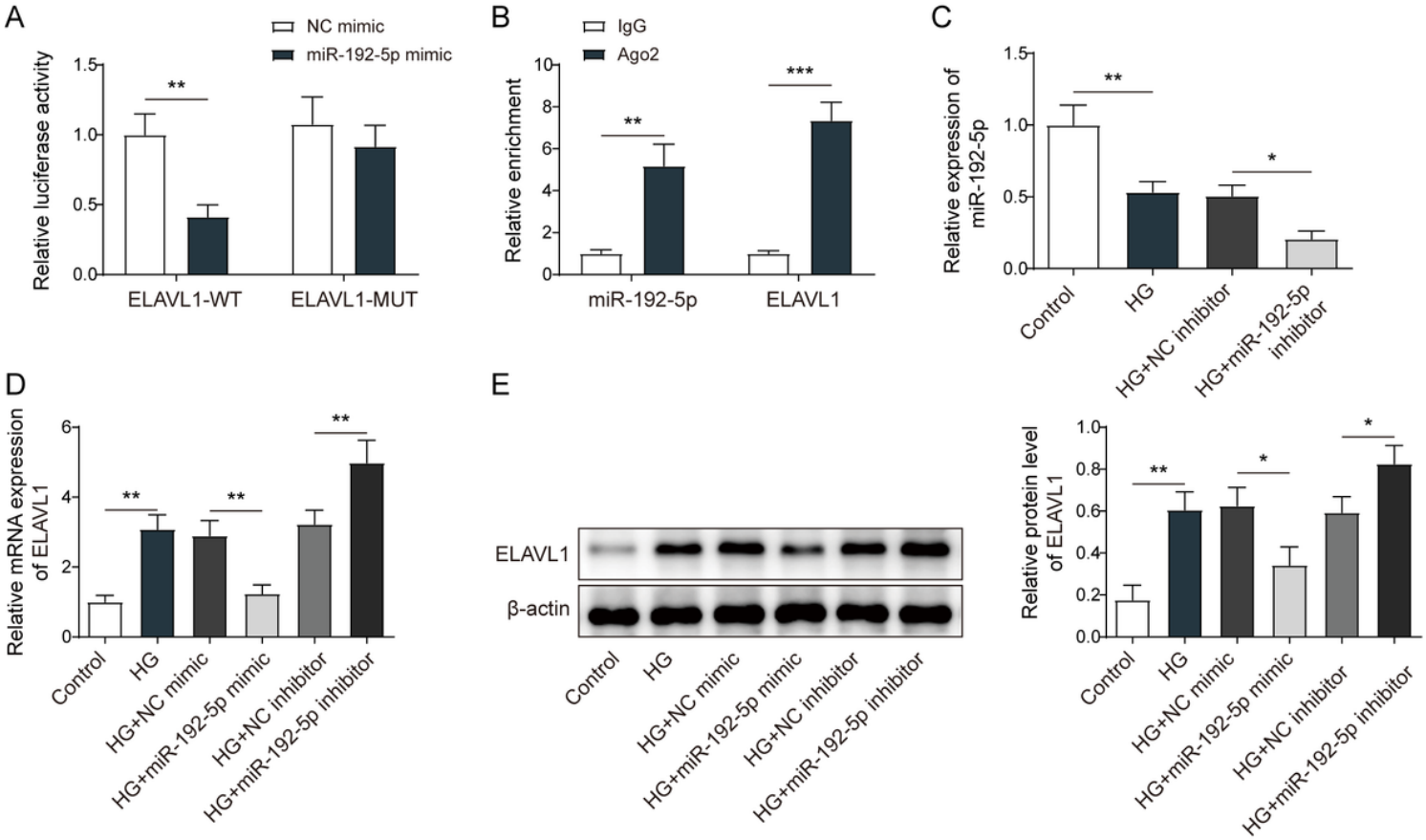

Figure 3

MiR-192-5p directly bound to ELAVL1 and inhibited its expression. (A) Luciferase activity of the wild-type or the mutant of ELAVL1 in HRMECs with miR-192-5p mimics. (B) RIP revealed binding of miR-192-5p to ELAVL1. (C) RT-qPCR analysis of miR-192-5p expression in cells transfected with miR-192-5p inhibitor or NC inhibitor. (D, E) Expression of ELAVL1 was evaluated using RT-qPCR and Western blot. All data are represented as the mean ± SD of three independent experiments. *P<0.05.

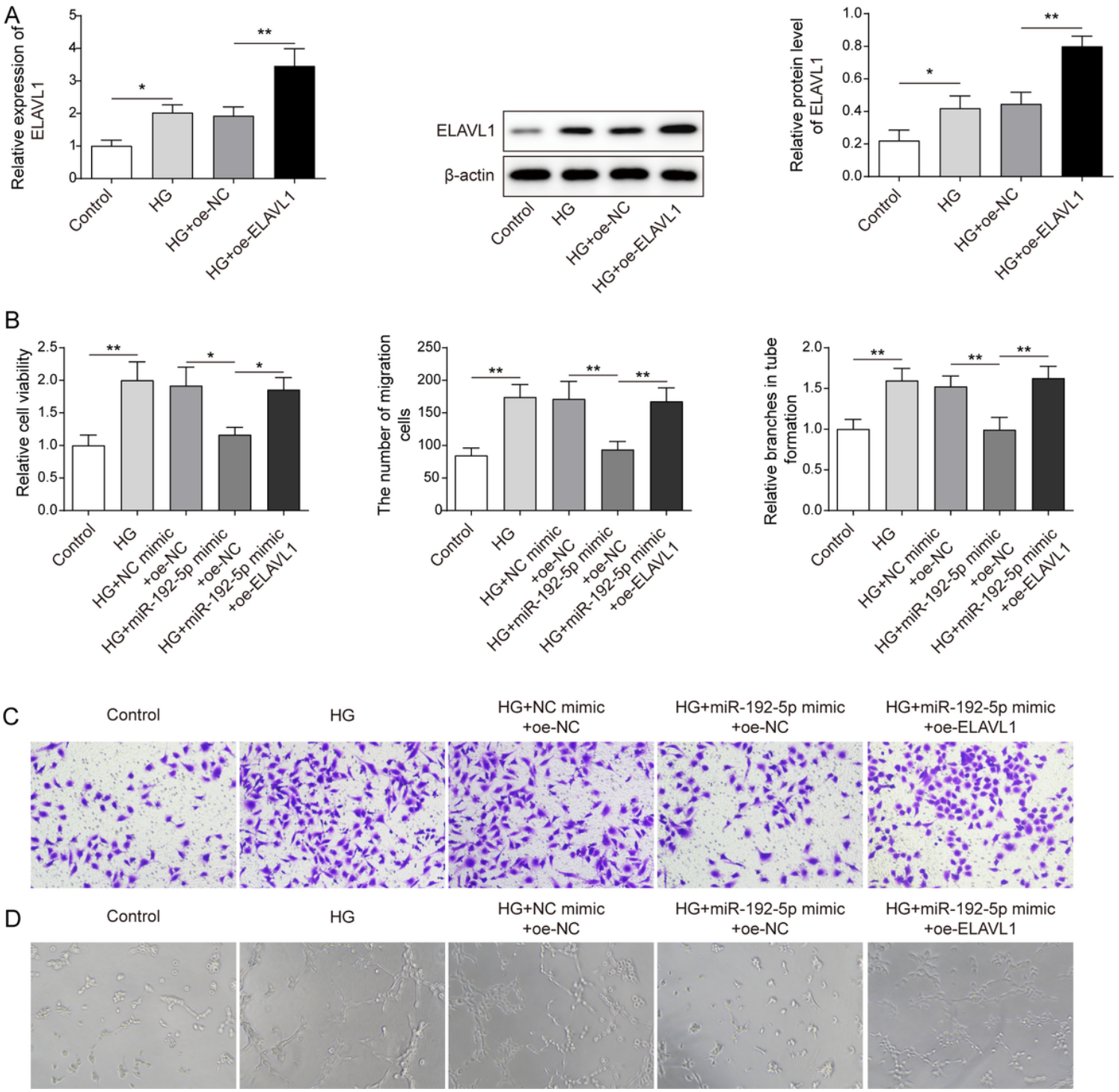

Figure 4

The reduction in microvascular endothelial cell proliferation, migration and angiogenesis caused by miR-192-5p upregulation was reversed by ELAVL1 overexpression. (A) HG-induced HRMECs were transfected with oe-ELAVL1 or oe-NC. RT-qPCR and Western blot tested ELAVL1 level. (B) CCK8 assay determined cell viability in different groups. (C) Transwell assay for analysis of cell migration in different groups. (D) Tube formation experiment assessed angiogenesis ability of HRMECs in different groups. Values were expressed as mean ± SD of three separate determinations. *P<0.05.

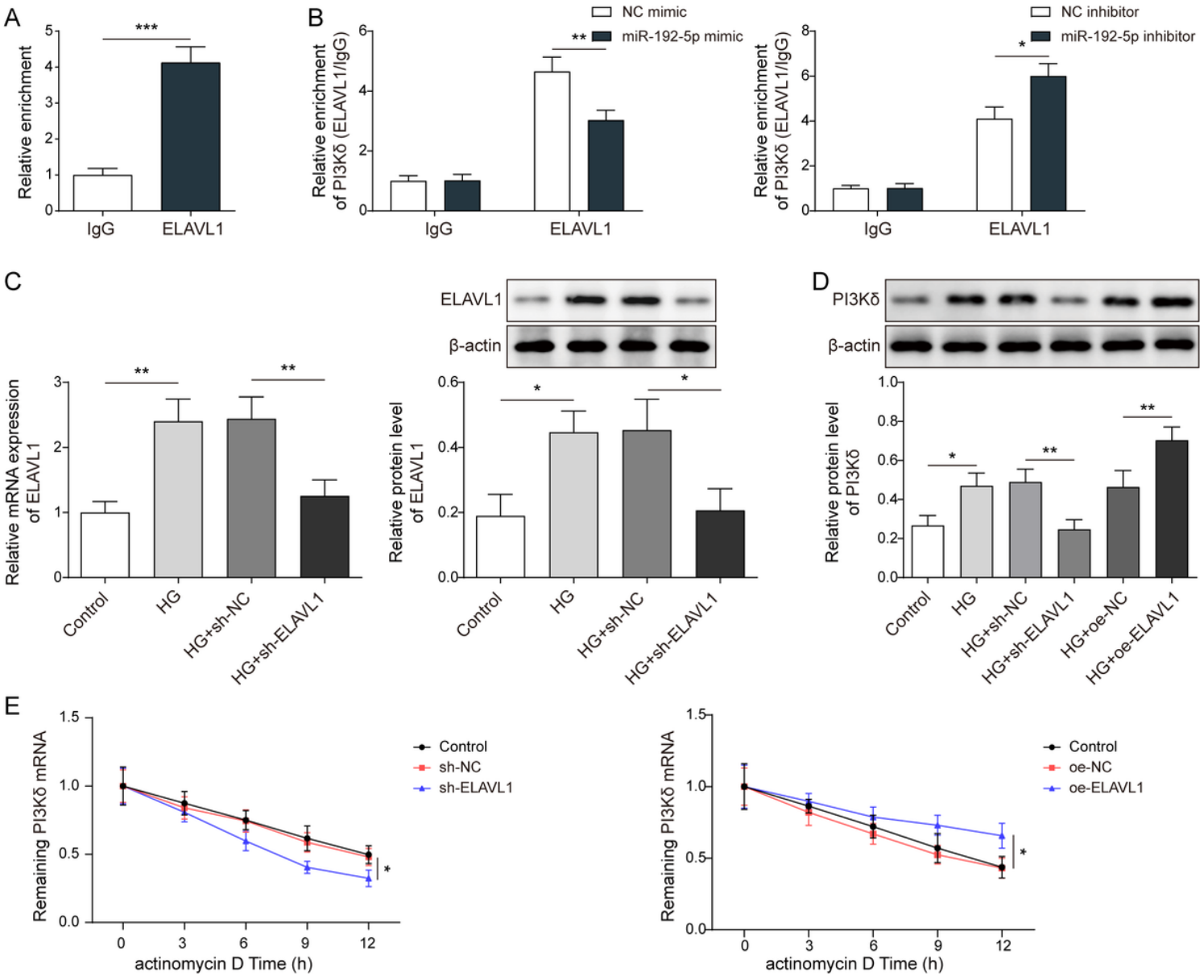

Figure 5

ELAVL1 maintained PI3Kδ mRNA stability. (A) RIP detection of the binding of ELAVL1 to PI3Kδ. (B) The abundance of PI3Kδ mRNA in ELAVL1 immunoprecipitates was detected by RIP. (C) RT-qPCR and Western blot analyzed ELAVL1 level. (D) RT-qPCR and western blot evaluated PI3Kδ expression. (E) PI3Kδ mRNA abundance treated with actinomycin D was evaluated using RT-qPCR after knockdown or overexpression of ELAVL1. Data are presented as mean ± SD of at least 3 independent experiments. *P<0.05.

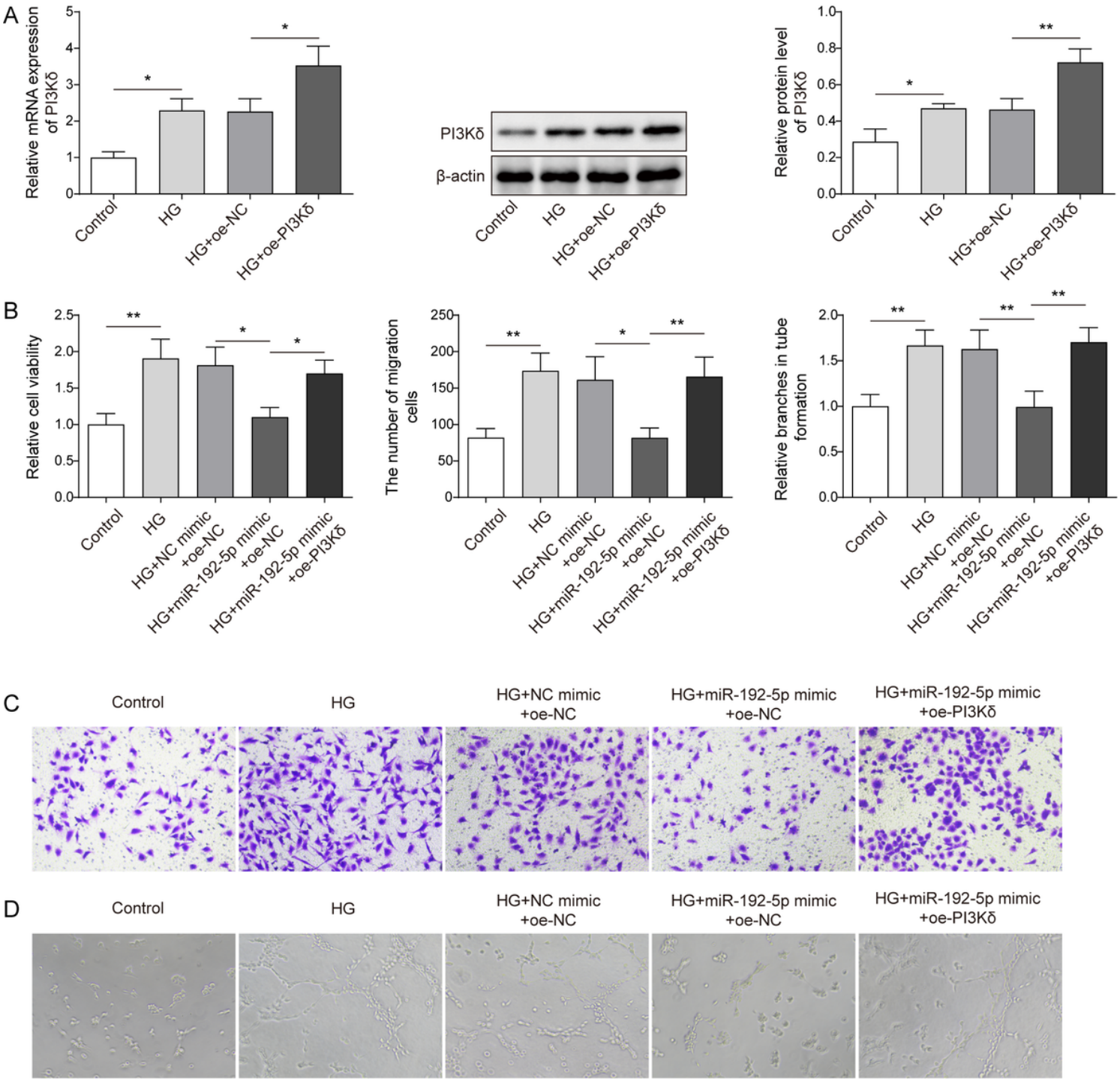

Figure 6

Overexpressed PI3Kδ overturned inhibitory effects of miR-192-5p upregulation on microvascular endothelial cell proliferation, migration and angiogenesis. (A) HG-induced HRMECs were transfected with oe-PI3Kδ or oe-NC. RT-qPCR and Western blot assessed PI3Kδ level. (B) CCK8 assay determined cell viability in different groups. (C) Transwell assay for analysis of cell migration in different groups. (D) Tube formation experiment assessed angiogenesis ability of HRMECs in different groups.. Error bars represent SD of the mean from three separate determinations. *P<0.05.