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ORIGINAL ARTICLE

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miR-9 promotes canine endothelial-like cell migration by targeting *COL15A1*

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Abstract

Background: Endothelial cell migration is the initial stage of angiogenesis. In previous studies, miR-9 has been found to regulate angiogenesis and cell migration in human medicine.

Objectives: This study aimed to reveal the regulatory effect of miR-9 on canine endothelial cell migration.

Methods: Embryonic canine ventricle myocardium tissues were collected and induced to differentiate into endothelial-like cells (ELCs). A transwell and invasion assay were used to evaluate the impact of miR-9 on the migration capacity of ELCs, after which a luciferase reporter assay, western blotting, RNA sequencing and reverse transcription-polymerase chain reaction were conducted to explore the regulatory mechanism.

Results: Our results showed that we successfully induced the primary cells derived from canine cardiac embryo tissues into ELCs. MiR-9 also promoted the migration and invasion of canine ELCs, and inhibited the expression of collagen XV, an angiogenic inhibitor, at the translational level by targeting the 3′ untranslated region of *COL15A1* gene. Furthermore, RNA sequencing showed that overexpression of miR-9 impacted several signalling pathways and eight genes involved in angiogenesis and cell migration in canine ELCs.

Conclusions: These findings suggest that miR-9 enhances the migration of canine ELCs and may serve as a potential diagnostic and therapeutic target for canine diseases involved in endothelial cells migration and angiogenesis, but more further studies are needed.

KEYWORDS
canine endothelial-like cell, cell migration, COL15A1, collagen XV, miR-9

1 | INTRODUCTION

Angiogenesis is vital in both physiological and pathological processes. A key element of angiogenesis is endothelial cell migration (Lee et al., 2022). The molecular mechanisms driving endothelial cell migration are firmly modulated by proangiogenic and antiangiogenic factors (Li et al., 2005). As venous endothelial cells in the adult stage are resting and lose their migratory ability, there must be a vibrant angiogenic process to restore their migration ability (Lee et al., 2022). However, the molecular mechanisms involved in this are not well understood.

MicroRNAs (miRNAs) are a type of non-coding regulatory RNA, and mature miRNAs are single-stranded RNA molecules of 21–23 nt in length (Ding et al., 2020). Current research has shown that miRNAs can modulate angiogenesis during normal physiological processes and

different stages of tumour progression (Orso et al., 2020). By inhibiting angiogenesis inhibitors, miRNAs increase the expression level of angiogenic growth factors (Ding et al., 2020). A study has indicated that miR-9 can enhance angiogenesis by upregulating the expression of a gene that controls vascular endothelial growth factor (VEGF) (Ma et al., 2010). Zhuang et al (2012) found that miR-9 secreted by tumour cells significantly promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway. However, the effect of miR-9 on canine endothelial cells still remains unknown.

Therefore, our objective was to determine the regulatory effect of miR-9 on the migration of canine endothelial cells, and provide a theoretical basis for miR-9 as a potential diagnostic and therapeutic target for canine diseases involving endothelial cell migration and angiogenesis.

2 | MATERIALS AND METHODS

2.1 | Cell isolation, culture and differentiation

Embryonic canine ventricle myocardium tissues from a dog with a terminated pregnancy were used for deriving primary cells. The myocardium tissue was sterilely surgically excised from the embryos, rinsed in cold sterile phosphate-buffered saline (PBS) and placed on ice. Then, the tissue was cut into small pieces removing blood, fat and fibrous connective tissues. Small pieces (~1 mm^3) were cultured with canine endothelial cell growth medium (CECGM) (Lonza Bioscience) at 37°C with 5% CO_2. When the cells reached 90% confluence, they were dissociated with TrypLE Express Enzyme (Thermo Fisher Scientific) and pipetted onto plates precoated with 2% gelatin. Then, they were cultured using CECGM with additional 1% endothelial cell growth supplement (ScienCell Research Laboratories) to induce their differentiation into endothelial cells (Liu et al., 2015; Oosterhoff et al., 2016), called endothelial-like cells (ELCs).

2.2 | Tube formation assay

The tube formation assay is typically used to demonstrate the angiogenic activity of vascular endothelial cells in vitro (Cheng et al., 2021; Smith & Andreadis, 2022). Matrigel (Corning Life Sciences) was precooled overnight at 4°C and diluted with CECGM to a concentration of 50%. The diluent was added to wells of 96-well plates and solidified by incubating for 30 min at 37°C, after which 2.0×10^4 cells were added into wells and cultured at 37°C with 5% CO_2. The formed tubes were observed under a microscope every 2 h.

2.3 | Reverse transcription-polymerase chain reaction

To understand the endothelial cell properties of the primary cells, total RNA was extracted from the cells at passage 3 using TRIzol (Tiangen). Total RNA (1 μg) was reverse transcribed using a PrimeScript RT reagent kit with gDNA (genomic DNA) Eraser (Takara Biotechnology). The following genes (Table S1) were detected to identify the molecular properties of the cultured cells: *CD31* (marker of endothelial cells), *α-SMA* (alpha-smooth muscle actin, marker of the myofibroblastic phenotype) and *Vimentin* (marker of mesenchymal origin cells) (Hinz et al., 2012). GAPDH and RPS19 were used as housekeeping genes. Reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed using TB Green Premix Ex Taq II (Tli RNase H Plus) (Takara Biotechnology). The 2-ΔΔ CT method was utilized to analyse the relative expressions of these genes.

2.4 | Western blotting analysis

To further characterize the primary cells, total proteins were obtained from the adherent cells at passage 3 by adding ice-cold RIPA (radioimmunoprecipitation assay) lysis buffer (Servicebio). The equivalent protein (25 μg) was loaded to an SDS-PAGE gel and then imprinted onto a polyvinylidene fluoride membrane. The membrane was blocked with 5% skim milk for 1.5 h at room temperature and incubated with a specific primary antibody and an appropriate secondary antibody at −4°C overnight. Antibodies used included the following: anti-CD31 (bs-0195R, Bioss Antibodies, 1:500), anti-vimentin (V6389, Sigma-Aldrich, 1:2000), anti-α-SMA (A2547, Sigma-Aldrich, 1:1500), anti-collagen XV (bs-0547R, Bioss Antibodies, 1:1500) and anti-β-actin (bs-0061r, Bioss Antibodies, 1:5000). The bands on the membrane were developed via a Super ECL (enhanced chemiluminescence) Plus kit (Boster Biological Technology) and recorded via Image Lab software (4600SF, Tanon Science & Technology).

2.5 | Immunofluorescence staining

To localize the markers of endothelial cells in the primary cells, we performed immunofluorescence staining. A cell suspension with 1.0×10^3 cells was added to Biologix cell culture slides precoated with 2% gelatin and incubated for 24 h. The slides were then fixed with acetone for 10 min at −20°C, blocked with 10% goat serum/0.1% Tween 20/PBS and incubated at room temperature for 1 h. Subsequently, they were incubated with primary antibodies against CD31 (1:100), vimentin (1:1600) and α-SMA (1:400) at 4°C overnight, and then with 100 μL of goat anti-rabbit IgG (Alexa Fluor 568, A11011, Invitrogen, 1:500) or goat anti-mouse IgG (Alexa Fluor 488, A10667, Invitrogen, 1:500) secondary antibodies for 1 h at room temperature away from light. Finally, the cells were stained with one drop of Vectashield antifade mounting medium with DAPI (H-1200, Vector Laboratories) and observed under a fluorescence microscope.

2.6 | Transfection of miR-9 mimic

To explore the effect of miR-9 on canine ELCs, the cells at passage 3 were seeded onto six-well plates before transfection. When the cell density reached 50%, cells were transfected with 20 pmol/mL

miRNA mimics or a negative control (NC) using RFect siRNA/miRNA transfection reagent (Baidai Biotechnology). miR-9-5p mimics were obtained from Sangon Biotech. The miRNA sense sequence was 5′-UCUUUGGUUAUCUAGCUGUAUGA-3′.

Then, the transfected cells were incubated for 48 h before harvesting. To determine whether the cells were successfully transfected with miRNA mimics, they were observed under a fluorescence microscope, and the expression of miR-9 was detected by a Hairpin-it real-time PCR kit (Genepharma) as described in the manufacturer's manual. The U6 gene was used as an internal control.

2.7 | Migration and invasion assay

To investigate the influence of miR-9 on canine ELCs migration and invasion, 100 μL of dulbecco's modified eagle medium (DMEM) with 1.0×10^4 cells was seeded to each upper chamber of a Transwell plate (Biosharp Life Sciences). The bottom chamber was filled with CECGM. For the migration assay, after 24 h of incubation, uninvaded cells on the upper layer were erased using a swab, and the invaded cells, which had shifted to the bottom of the inserted membrane were fixed with paraformaldehyde for 10 min and stained for 15 min with 1% crystal violet. For the invasion assay, Matrigel was diluted with DMEM to a concentration of 3%. A 50-μL mixture was coated in the upper chamber. After 24 h of incubation, excess media were removed, after which the same procedure was conducted as for the migration assay.

2.8 | RNA sequencing analysis

To further determine the regulatory mechanisms by which miR-9 promotes canine ELCs migration, RNA sequencing (RNA-seq) (Novogene Bioinformatics Technology) was utilized. Briefly, the integrity of the RNA extracted from the cells transfected with mimic or NC was assessed using the Bioanalyzer 2100 system (Agilent Technologies). mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. PCR products were purified (AMPure XP system) and the quality of the library was evaluated. Then, 150-bp paired-end reads were sequenced on an Illumina NovaSeq 6000 (Illumina). The data were deposited in Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra/), and the accession number was PRJNA973936. The genes were considered as differentially expressed with an adjusted p value of <0.05 by DESeq. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment and Gene Ontology (GO) were performed using DAVID62 (https://david.ncifcrf.gov/), and the results were visualized by drawing chords on the Bioinformatics online website (http://www.bioinformatics.com.cn/). The selected different genes were verified by RT-PCR as mentioned before. The primers used are shown in Table S1.

2.9 | Luciferase reporter assay

TargetScan and miRanda databases were used to predict the binding sites between miR-9 and *COL15A1*. The recombinant pmirGLO-*COL15A1*-MUT (mutant) and pmirGLO-*COL15A1*-WT (wild-type) plasmids were purchased from Jinkairui Bioengineering. Cells were co-transfected with 50 pmol/mL miR-9-5p mimic or NC and 4 ng/μL pmirGLO-*COL15A1*-MUT or pmirGLO-*COL15A1*-WT using Lipofectamine 2000 (Invitrogen). The fluorescence intensity was detected using the Dual-Luciferase reporter assay system (Promega) after 48 h.

2.10 | Statistical analysis

Data were analysed and visualized using GraphPad Prism 5.0 (GraphPad Software). Each experiment was carried out at least in triplicate, and all results are expressed as the means ± SD. A one-way ANOVA was used to assess statistical significance.

3 | RESULTS

3.1 | Culture and identification of canine ELCs

We collected canine embryonic cardiac muscle tissue and dissociated the cells by culturing the small tissue pieces. After 1 day of seeding, the cells began to grow with an adherent pattern. After two to three passages, they were cobblestone-shaped and evenly distributed (Figure 1A). The cells could form tubules observed at 2 h and started to disintegrate at 4 h in the tube formation assay (Figure 1B).

To further explore whether the primary cells were successfully induced into endothelial cells, we detected the molecular marker expression of differentiated cells. The RT-PCR results showed the primary cells expressed *CD31*, *α-SMA* and *Vimentin* genes (Figure 2A). Additionally, these cells expressed Vimentin and CD31 protein, but barely expressed α-SMA protein (Figure 2B). Furthermore, immunofluorescence staining showed that they expressed CD31 protein on the membrane and Vimentin protein in the cytoplasm, but without α-SMA expression (Figure 2C). Together, these results indicated that we successfully obtained canine ELCs.

3.2 | miR-9 promotes migration and invasion of ELCs

To investigate the influence of miR-9 on ELCs, we transfected them with miR-9-5p mimics. RT-PCR results confirmed that transfected canine ELCs overexpressed miR-9 (Figure S1). Additionally, we found that miR-9 overexpression markedly increased the migration and invasion ability of these cells (Figure 3). Thus, these results demonstrated that miR-9 promoted the migration and invasion of canine ELCs.

3.3 | miR-9 targets *COL15A1* gene and inhibits collagen XV expression

To further determine the regulatory mechanism by which miR-9 promoted canine ELCs migration, TargetScan and miRDB databases were used in an overlap analysis to identify the potential target genes of

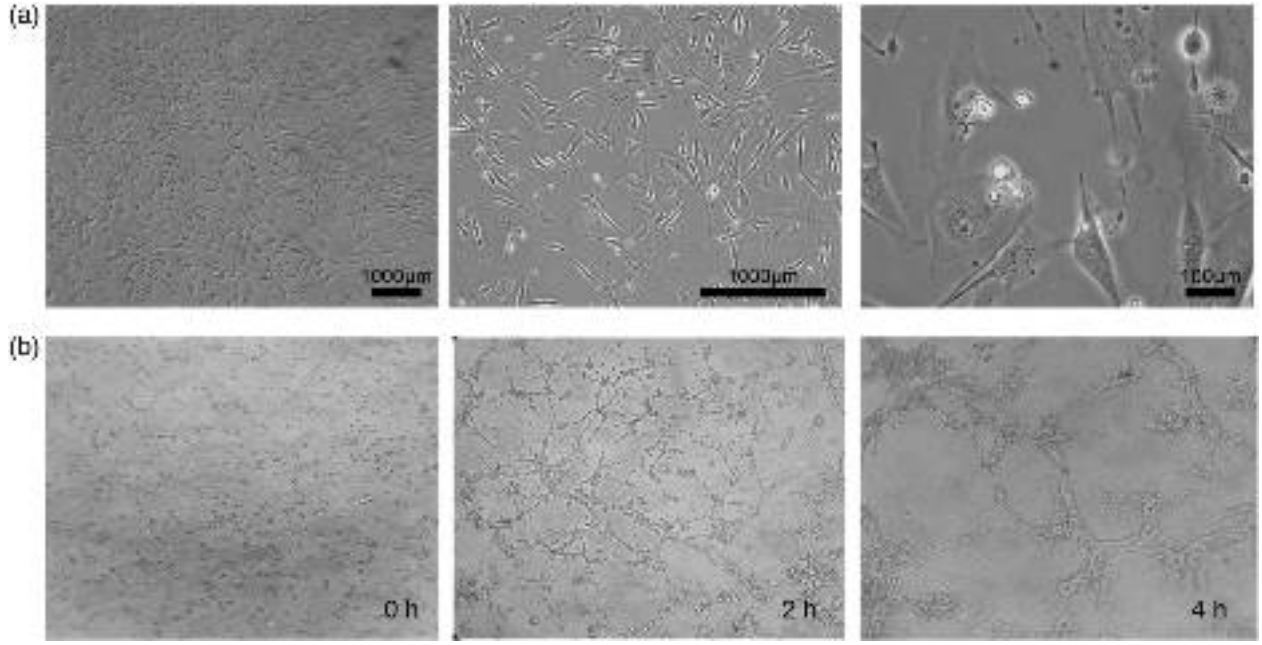


FIGURE 1 Morphology and tube forming capacity of canine ELCs. (A) Most cells extracted from embryonic canine ventricle myocardium tissues exhibit a cobblestone morphology at passage 3, and the cell morphology was observed under 40×, 100× and 400× magnification. (B) The tube formation at 0, 2 and 4 h of canine ELCs. After 2 h, the cells show a dense tubular structure. At 4 h, the tubular structure starts to collapse. These experiments were conducted in triplicate.

miR-9. The results showed that 408 genes may be possible target genes in canines. Among them, *COL15A1* was selected from five genes related to angiogenesis and cell migration because of the strongest potential to bind miR-9 (Figure 4A). In the Dual-Luciferase assay, the luciferase activity of cells in the miR-9 + pmirGLO-*COL15A1*-WT treatment group was noticeably stronger than that in the NC + pmirGLO-*COL15A1*-WT treatment group, whereas in the miR-9 + pmirGLO-*COL15A1*-MUT treatment group, there was no difference in luciferase activity compared with that in NC + pmirGLO-*COL15A1*-MUT treatment group (Figure 4B,C). We further found there was no difference in the expression of the *COL15A1* gene between the miR-9 group and the NC group (Figure 4D). But the expression of collagen XV protein in ELCs transfected with miR-9-5p mimics was significantly lower than that in the NC treatment group (Figure 4E). Together, we speculated that miR-9 could bind to *COL15A1* gene and then inhibit the expression of collagen XV.

3.4 | miR-9 participates in translational regulation

To further investigate the mechanism of miR-9 on promoting the migration and invasion of ELCs, we analysed the RNA-seq data. The expression of 185 genes in the miR-9 group was significantly different from that in the NC group (Figure 5A and Table S2). KEGG enrichment analysis revealed that the most enriched signalling pathways of upregulated and downregulated genes in the miR-9 group were the TGF-β signalling pathway and regulation of the actin cytoskeleton, respectively (Figure 5B,C). GO enrichment analysis found that many genes involved in regulating the protein translation process were downexpressed (Figure 6). Together, we thought that miR-9 might inhibit the expression of related proteins by regulating the translation process.

3.5 | miR-9 affects angiogenesis-related genes

Based on previous studies, we screened the signalling pathways related to cell migration and angiogenesis in those KEGG signalling pathways, and found 13 differentially expressed genes. RT-PCR results confirmed that eight genes were actually up-expressed in the miR-9 group compared with that in the NC group, including diaphanous-related formin 2 (DIAPH2), DNA methyltransferase 3 alpha (DNMT3A), frizzled class receptor 5 (Fzd5), hepatic growth factor (HGF), platelet-derived growth factor D (PDGFD), peroxisome proliferator-activated receptor γ coactivator 1β (PPARGC1B), sodium voltage-gated channel α subunit 5 (*SCN5*) and protein tyrosine phosphatase receptor type F (PTPRF) (Figure 7A). These genes were involved in multiple signalling pathways related to cell migration and angiogenesis showed as in the KEGG enrichment chord graph (Figure 7B). These findings imply that miR-9 may have the capacity to control cell migration via modulating several genes and signalling pathways linked to cell migration and angiogenesis.

4 | DISCUSSION

In 1973, endothelial cells derived from human umbilical vein were first successfully cultured, leading to the development of modern vascular biology (Jaffe et al., 1973). Since then, researchers have

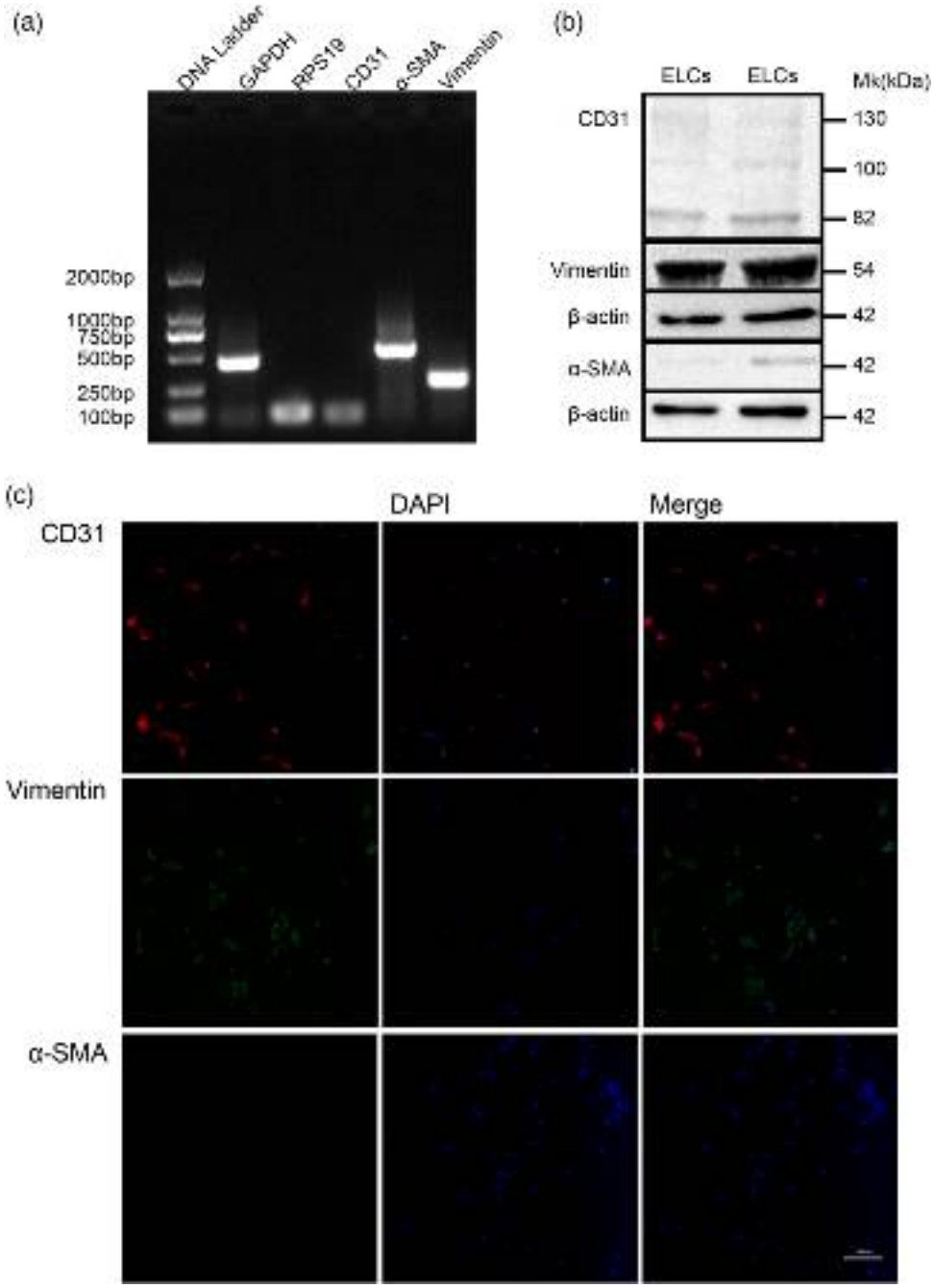


FIGURE 2 The primary cells extracted from embryonic canine ventricle myocardium tissues were characterized using CD31 (marker of endothelial cells), α-SMA (marker of myofibroblastic cells) and Vimentin (marker of mesenchymal origin cells). (A) Cells were characterized by RT-PCR. *CD31*, *α-SMA* and *Vimentin* were expressed by the cells. *GAPDH* and *RPS19* were used as a housekeeping gene. (B) Cells were characterized by western blotting. Those cells express Vimentin and CD31 protein, but they barely express α-SMA protein. β-Actin was used as an internal control. (C) The cells were characterized by immunofluorescence staining. Cells are positive for CD31 (red) and vimentin (green), but negative for α-SMA. DAPI (blue) stained cell nuclei. Scale bar = 100 μm.

explored new resources for endothelial cell culture, and proved that endothelial cells can be differentiated from pluripotent stem cells, monocytes and embryonic stem cells (Cheng et al., 2021; Ferreira et al., 2007; Smith & Andreadis, 2022). In canines, yolk sac cells can also be transduced to change their morphology and show ELCs (Fratini et al., 2016). In this study, we successfully cultured and differentiated canine cardiac embryonic cells into ELCs which possessed the key characteristics of endothelial cells, including expressing CD31 protein (Figure 2) and exhibiting tubular formation properties (Figure 1). The mRNA expression of interstitial cell markers (α-SMA, Figure 2) in cell cultures suggests the presence of interstitial cells contamination and/or endothelial–mesenchymal transition which are frequent issues in primary cultures when using enzymatic digestion (Bischoff & Aikawa, 2011; Butcher & Nerem, 2007). However, our

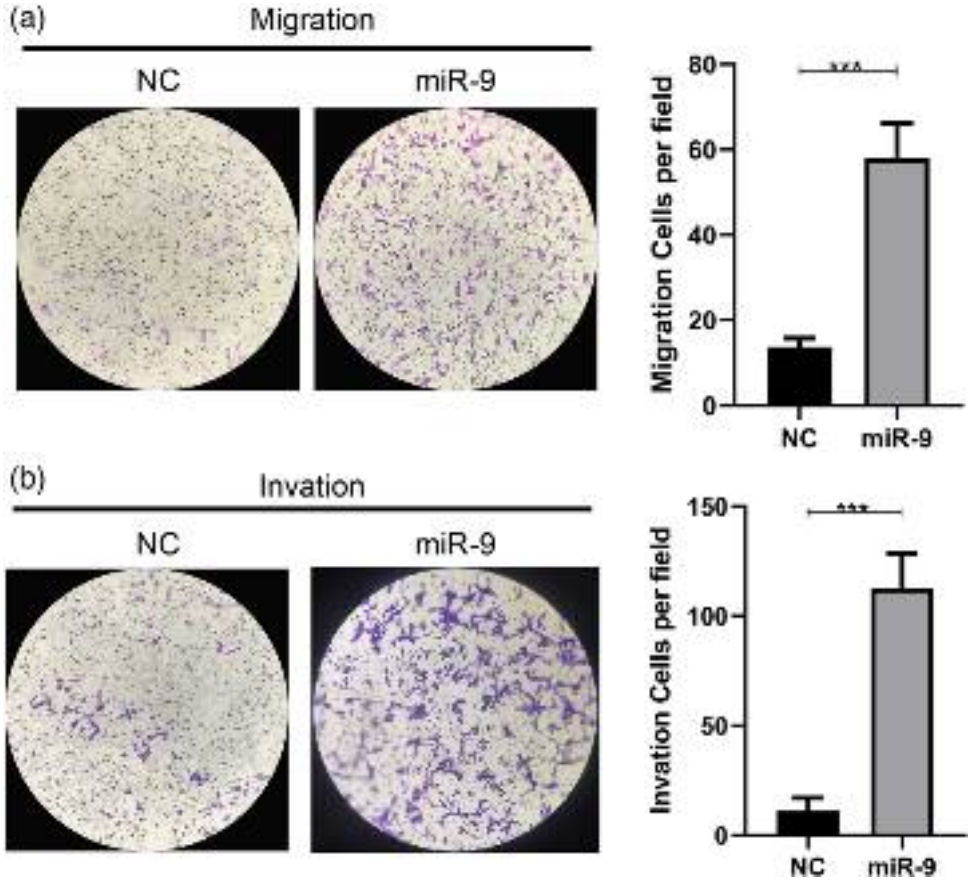


FIGURE 3 Overexpression of miR-9 promotes the migration and invasion of ELCs. The number of cells on the lower chamber migrating (A) or invading (B) across the membrane were counted. Data are presented as the mean ± SD of three independent experiments. ***$p<0.001$. Original magnification, ×100.

results showed that α-SMA protein barely detected in spite of obvious expression of its mRNA in ELCs, which meant the primary cells were highly purified. Therefore, the canine ELCs obtained in this study can be used for future studies of canine endothelial cells. However, a problem is that the culture of ELCs requires a special culture environment and cannot be indefinitely passaged, which needs to be solved urgently.

In human medicine, many studies have explored the effect of miRNAs on pathological processes and their potential clinical applications (Saliminejad et al., 2019). Several studies indicate that miR-9 can enhance endothelial cell migration and angiogenesis (Ma et al., 2010; Zhuang et al., 2012). In the current study, we also found that miR-9 could promote the migration of canine ELCs (Figure 3). In veterinary medicine, other researchers have also found that miR-9 can promote the migration and invasion of canine tumour cells (Fenger et al., 2016, 2014). High expression of miR-9 can be detected in exosomes, which are couriers for cell–cell communication and promising in liquid biopsy (Valencia & Montuenga, 2021), derived from canine mammary tumour cells (Fish et al., 2018). Therefore, in veterinary medicine, miR-9 may also participate in canine diseases, and will hopefully become a promising target for diagnosis, treatment and prognostication of canine diseases.

miRNAs are post-transcriptional modulators and bind complementary sequences typically in the 3′UTR of one or more target mRNAs to translationally inhibit or degrade target genes (Sun et al., 2018). We found that the possible target gene of miR-9 was *COL15A1* gene, which is a 45-kb gene coding for one polypeptide chain of 1388 aa named collagen XV (Bretaud et al., 2020). In humans, collagen XV is expressed by various cell types, including connective tissue cells, epithelial cells and endothelial cells (Amenta et al., 2005). It is a non-fibrillar collagen localized to basement membrane zones to connect large fibrillar collagens and enhance tissue structural integrity to prevent cell migration through the basement membrane (Amenta et al., 2005; Clementz & Harris, 2013; Rasi et al., 2010). A previous study shows that *COL15A1* down-expression increases the migration of cultured human aortic smooth muscle cells (SMCs) (Durgin et al., 2017). Additionally, collagen XV has an anti-angiogenic domain region in its non-collagenous C-terminal called restin, which can inhibit the migration of endothelial cells (Ramchandran et al., 1999). Our experiments verified that miR-9 could target the 3′UTR of the *COL15A1* gene and decrease the expression of collagen XV protein (Figure 4). Transcriptome sequencing also showed that translation-related genes of ELCs transfected with the miR-9-5p mimic were down-expressed (Figure 6). These results indicate that miR-9 may inhibit the translation of targeted mRNA into

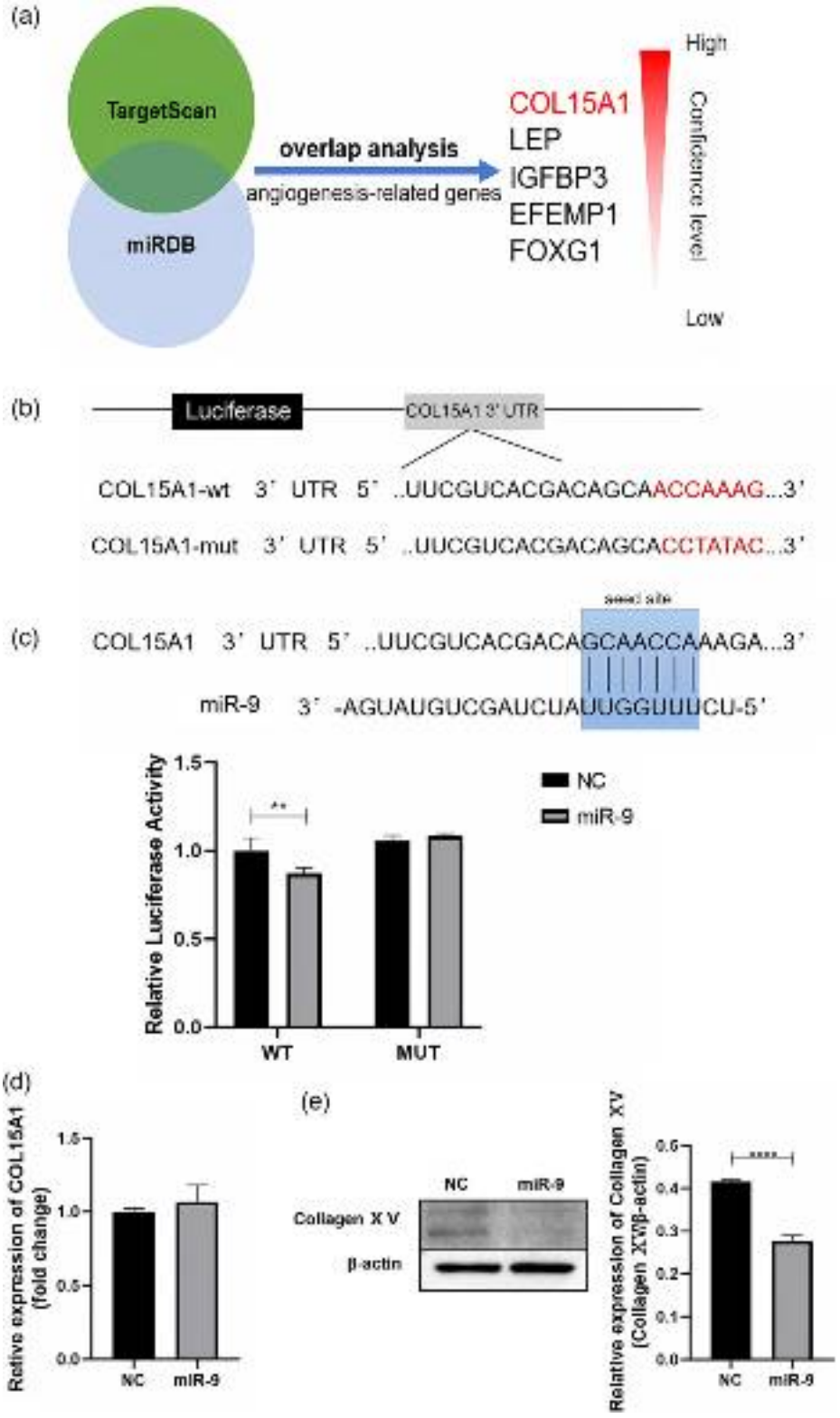


FIGURE 4 miR-9 targets *COL15A1* gene and inhibits the expression of collagen XV protein. (A) TargetScan and miRDB database overlap analysis was used to predict the potential target genes of miR-9. *COL15A1* gene was selected from five angiogenesis-related genes because of the strongest potential to bind miR-9. (B) Sequences of the *COL15A1* 3′UTR reporter plasmid that were wild-type (wt) or mutant (mut). (C) Schematic illustration of miR-9 and *COL15A1* 3′UTR binding sites accompanied with luciferase reporter assay in cells following co-transfection of *COL15A1* wild-type (WT) or mutant (MUT) and miR-9-5p mimics or NC, respectively. $^{**}p<0.01$. (D) There is no difference in the expression level of *COL15A1* mRNA between the miR-9 group and the NC group. (E) The protein level of collagen XV is inhibited in canine ELCs after transfection with miR-9-5p mimics. $^{****}p<0.0001$.

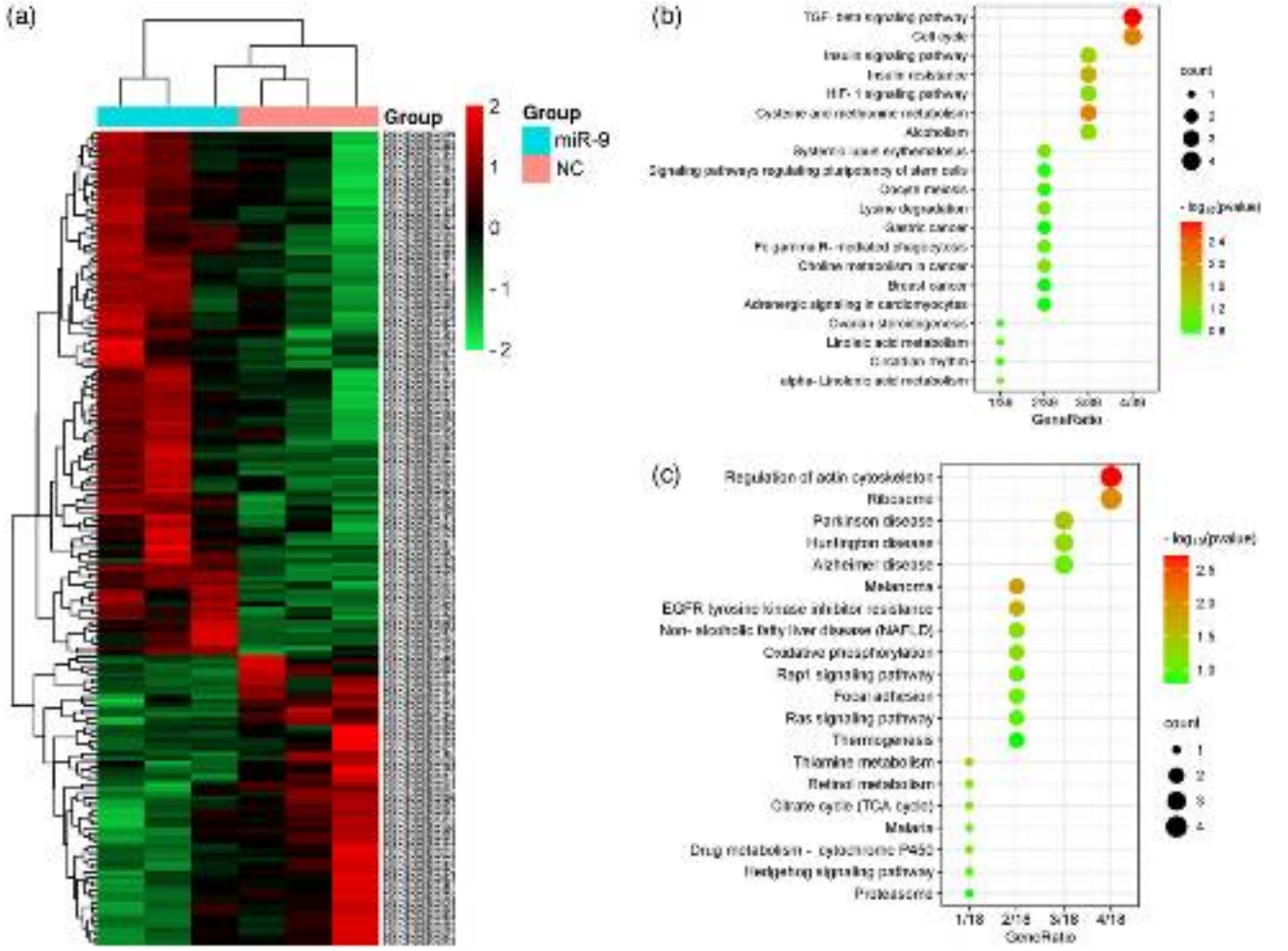
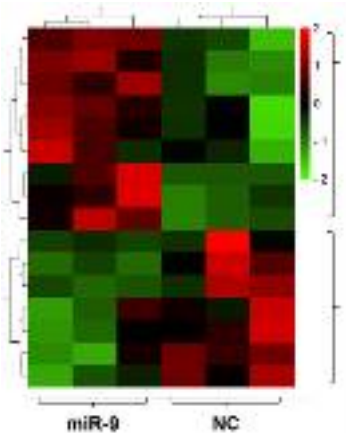


FIGURE 5 Analysis of the transcriptome expression profiles of canine ELCs transfected with miR-9-5p mimics or NC ($n = 3$). (A) Hierarchical cluster heatmap of 185 differentially expressed genes between canine ELCs transfected with miR-9-5 mimic and NC. KEGG enrichment analysis of these differentially expressed genes reveals that the most enriched signalling pathways of upregulated and downregulated genes in the miR-9 group are the TGF-β signalling pathway (B) and regulation of the actin cytoskeleton (C).



up	GO:0006457: protein folding	0.050236
	GO:0006468: protein phosphorylation	0.042118
	GO:0016310: phosphorylation	0.057333
down	GO:0006364: rRNA processing	0.158371
	GO:0016072: rRNA metabolic process	0.158371
	GO:0030684: preribosome	0.008279
	GO:0006412: translation	0.270846
	GO:0043043: peptide biosynthetic process	0.231052
	GO:0006518: peptide metabolic process	0.235447
	GO:1990904: ribonucleoprotein complex	0.24663
	GO:0005840: ribosome	0.03546
	GO:0003735: structural constituent of ribosome	0.176313
	GO:0008138: protein tyrosine/serine/threonine phosphatase activity	0.12843

FIGURE 6 Many genes are involved in inhibiting the protein translation process by GO enrichment analysis, as shown by the differential expression heatmaps on the left and the GO terms on the right.

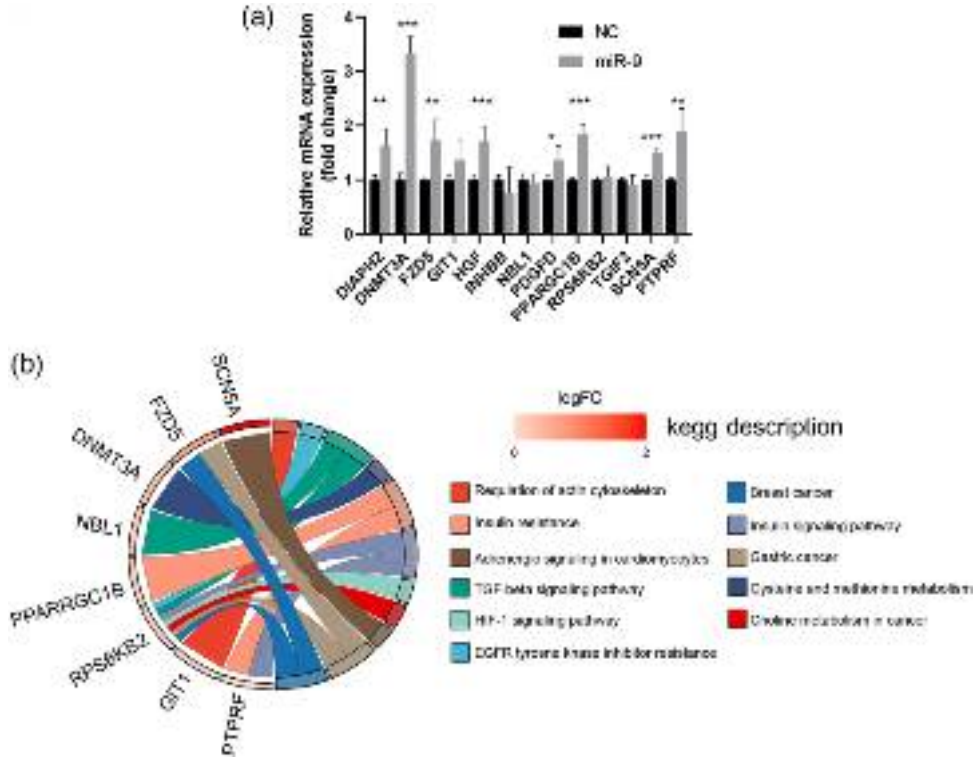


FIGURE 7 Overexpression of miR-9 increases the expression of genes related to cell migration and angiogenesis. (A) KEGG signalling pathways found 13 differentially expressed genes. Quantitative RT-PCR was used to confirm the differential expressions. Eight genes, including DIAPH2, DNMT3A, Fzd5, HGF, PDGFD, PPARGC1B, SCN5A and PTPRF, were actually increased. (B) KEGG enrichment chord graph of the eight up-regulated genes mentioned above. Fold changes (FC) of the gene expressions between the miR-9 group and the NC group.

the collagen XV protein to enhance the migration of canine ELCs, but the molecular mechanism of collagen XV on cell migration remains unclear and warrants further investigation.

Meanwhile, eight genes related to cell migration and angiogenesis were regulated by miR-9 overexpression in canine ELCs (Figure 7). Human DIAPH2 is the homolog of *Drosophila melanogaster* diaphanous, which is a member of a group of proteins that can establish cell polarity and reorganize the actin cytoskeleton in *Drosophila*. In addition, several studies have shown that DIAPH2 can regulate cell movement and adhesion (Daou et al., 2014; Lai et al., 2008; Mei et al., 2020). DNA methylation is a primary epigenetic modification of the mammalian genome including three DNA methyl transferases, including DNMT1, DNMT3A and DNMT3B (Okano et al., 1998). As a member of the DNMT3A isoform family, DNMT3Ab can strengthen gastric cancer cell migration and invasion (Cui et al., 2018). Fzd5 is a member of the Wnt/FZD family, and mediates endothelial tubule formation and cell migration in endothelial cells (Brandt et al., 2018; Peterson et al., 2017). HGF is a 92-kDa inactive single polypeptide chain precursor, and can induce the migration of vascular SMCs and human myoblasts (Catlow et al., 2008; González et al., 2017; Ma et al., 2003). PDGF was originally discovered as a component of platelets. In normal vasculature, PDGF ligands are expressed at low levels, whereas they are up-expressed, including PDGF-C and PDGF-D, in many vascular and cardiovascular diseases to promote SMCs migration (Folestad et al., 2018). Overexpression of PDGF-D also can promote macrophage migration (Xiong et al., 2021). PPARGC1B participates in the PPAR signalling network, and accelerates the migration of endothelial cells via VEGF signalling and dramatically increases muscular vessel density (Folestad et al., 2018; Rowe et al., 2011; Xiong et al., 2021). The *SCN5A* gene encodes for the alpha subunit of the main cardiac sodium channel Nav1.5, which has a critical effect on regulating cardiac electrophysiological function (Li et al., 2018). Protein tyrosine phosphatase can control cell survival, migration and invasion in coordination with tyrosine kinases (Xu & Fisher, 2012). PTPRF is one of the receptor-type PTPs (Xu & Fisher, 2012). However, the roles of SCN5A and PTPRF on cell migration are unclear.

5 | CONCLUSIONS

Our results indicate that overexpression of miR-9 increases the migration of canine ELCs. Furthermore, we found that miR-9 can bind to the 3′UTR of *COL15A1* mRNA, resulting in inhibited translation of mRNA into the collagen XV protein, and impact various pathways and genes related to cell migration and angiogenesis. Therefore, miR-9 may serve as a potential diagnostic and therapeutic target for canine diseases involving endothelial cell migration and angiogenesis; however, further research is needed.

AUTHOR CONTRIBUTIONS

Heng Jiang: Investigation; software; data curation; and writing—original draft preparation. **Mengmeng Liu**: Methodology; resources; supervision; funding acquisition. **Yao Qin**: Conceptualization; validation; formal analysis; supervision; funding acquisition. **Hong Zhang**: Conceptualization; methodology; investigation; writing—reviewing and editing; project administration; and funding acquisition.

FUNDING INFORMATION

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CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest in this work.

ETHICAL APPROVAL STATEMENT

This study was approved by the Hainan University Institutional Animal Use and Care Committee. Informed consent was obtained from the owners for the participation of their animals in this study. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

DATA AVAILABILITY STATEMENT

The RNA-seq data that support the findings of this study are openly available in Sequence Read Archive at (http://www.ncbi.nlm.nih.gov/sra/), reference number (PRJNA973936). The other data that support the findings of this study are available in the Supplementary Material of this article.

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PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.1339.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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