Int J Hum Genet, 22(2): 48-56 (2022)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
DOI: 10.31901/24566330.2022/22.02.801

Long Noncoding RNA FENDRR Facilitates Progressions of Hemangioma Endothelial Cells via Sponging MicroRNA-424

Qiang Liu[1], Hongzhi Jiang[2], Qilong Zhang[3], Haizhen Ju[4] and Fei Lu[5*]

[1,2,3,4] The Affiliated Weihai Second Municipal Hospital of Qingdao University, Pediatric Surgery, 51 Guangming Road, Weihai City, Shandong Province, 264200, China
[5]Deputy Chief Physician, Yuyao People Hospital, Zhejiang, 315400
E-mail: [1]<rosgurqojcwo@163.com>, [2]<zdyauvtonxgo@163.com>, [3]<plqbwmfpomci@163.com>, [4]<qlrbichqccdg@163.com>, [5]<uy788231@126.com>

KEYWORDS Angiogenesis. Cell Viability. Hemangioma. Human Hemangioma Cells. Non-coding RNA

ABSTRACT The present study investigated effects of FENDRR and miR-424 on modulating HemECs progressions. Using RT-qPCR, FENDRR was detected to be upregulated in HemECs. The knockdown of FENDRR inhibited HemECs viability, migratory and invasive abilities but accelerated the cell apoptosis. Additionally, MMP-9 and VEGFA were also suppressed. Luciferase reporter test then verified that miR-424 in HemECs was sponged by FENDRR and downregulated in HemECs. Furthermore, overexpressed FENDRR restrained miR-424 mimics-induced high miR-424 expression. Beyond that, suppressed HemECs viability, invasiveness and migratory ability and increased apoptosis caused by miR-424 mimics were also reversed by FENDRR overexpression. Moreover, miR-424-induced low MMP-9 and VEGFA were restored by overexpressed FENDRR.

INTRODUCTION

Infantile hemangioma (IH), a benign lesion, occurs in infant period and the incidence rate is about 4.5 percent (Léauté-Labrèze et al. 2017). This soft-tissue tumor appears in the first few weeks of life and most of IHs can resolve spontaneously without treatment (Acevedo and Cheresh 2008). Unfortunately, complications of IHs result in permanent scars or fat accumulation and even disfigurement (Yang et al. 2017). Moreover, airway obstruction, visual disturbance and cardiac failure are more severe complications caused by IHs (Tiemann and Hein 2020). IHs happen mainly in females (about 3 times higher than males), premature infants and babies with placental anomalies (Tiemann and Hein 2020). As for IHs treatment, propranolol is the priority drug for systemic treatment while surgery and laser treatment are efficient methods to alleviate damages of skins (Krowchuk et al. 2019). Though hemangioma has been reported to be formatted by the excessive proliferation of vascular endothelial cells (Ye et al. 2019), mechanisms about the progression of hemangioma was still obscured.

Noncoding RNAs have been deemed as junk genes, which take about 98 percent in genomic transcripts (Adams et al. 2017). Nevertheless, recent research has reported that those noncoding RNAs played critical roles in many kinds of cancers (Wang et al. 2019). Long noncoding RNAs (lncRNAs) are over 200 nucleotides in length and have no capacity to encode protein. Roles of lncRNAs are multifarious in cancers, including hemangioma. LncRNA FOXD2-AS1 was upregulated in hemangioma cells and facilitated proliferation, invasiveness and migration by upregulating PDRG1 through sponging miR-324-3p (Zhao et al. 2020). LncRNA MALAT1 accelerated proliferation, tube formation, invasiveness and migration of IH cells by stimulating MEKK3-induced IKK/NF-κB signaling pathway through competitively sponging miR-424 (Li et al. 2019). LncRNA MEG3 was downregulated in hemangioma cells and inhibited proliferation and cell cycle arrest via regulating PTEN/PI3K/AKT signaling pathway by sponging miR-494 (Dai et al. 2018). LncRNA FENDRR, also named FOXF1-AS1, has a positively correlation with FOXF1 in lung adenocarcinoma samples (Herrera-Merchan et al. 2020). FENDRR has been also found to be upregulated in samples of IH by microarray analysis (Liu et al. 2016). Howev-

*Address for correspondence:
Fei Lu
Deputy Chief Physician,
Yuyao People Hospital,
Zhejiang, 315400. China.
Phone: 86-18067201743,
E-mail: uy788231@126.com

er, roles of FENDRR in IH were unknown. Therefore, this study would analyze mechanisms of FENDRR in regulating cell progression.

MicroRNAs (miRNA) are small noncoding RNAs (19-22nt) that are endogenously expressed in cells (Chen et al. 2021). MiR-501 was upregulated in cells of hemangioma and facilitated proliferation, cell cycle and other progressions via targeting homeobox D10 (Zeng et al. 2019). MiR-139-5p was verified to restrain invasiveness of hemangioma cells, which was detected to be negatively interplayed with linc00152 in hemangioma. Moreover, miR-424 has been downregulated in senile hemangioma, which has the capacity to hamper the angiogenesis via targeting MEK or cyclin E1 (Nakashima et al. 2010). Moreover, mIR-424 was reported to be sponged by FENDRR in colorectal cancer but their interaction in hemangioma were never mentioned (Nakashima et al. 2010). Hence, researchers hypothesized that FENDRR might also regulate progressions of hemangioma cells via sponging miR-424.

Objectives

This present study aimed to analyze functions of FENDRR in regulating cell viability, migratory ability, invasiveness, apoptosis and proteins related to angiogenesis in HemECs and its interaction with miR-424.

MATERIAL AND METHODS

Main Reagents

DMEM medium (Gibco, USA), 10 percent FBS (Gibco), 1 percent penicillin-streptomycin (10000U/ml, Gibco), Lipofectamine 3000 (Invitrogen, USA), TRI reagent (Sigma-Aldrich, USA), PrimeScript RT Reagent Kit (Takara, Japan), TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA), ProFlex™ PCR System (Applied Biosystems), SYBR Green PCR Master Mix (Applied Biosystems), Cell Counting Kit-8 (Beyotime, Shanghai, China), Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich), 0.1% crystal violet (Beyotime), RIPA Buffer (Sigma-Aldrich), QuantiPro BCA Assay Kit (Sigma-Aldrich), SDS-PAGE (Beyotime), TBSTw (Beyotime), anti-MMP-9 (ab76003, Abcam, UK), anti-VEGFA (ab52917) and anti-GAPDH (ab9485), Goat Anti-Rabbit IgG H&L (HRP) (ab6721), BeyoECL Plus (Beyotime).

Cell Culture

Human Umbilical Vein Endothelial Cells (HUVECs) and human hemangioma cells (HemECs) were obtained from BLUEBIO (Shanghai, China). Cells were both cultivated in DMEM medium (high glucose, Gibco, USA) supplemented with 10 percent FBS (Gibco) and 1 percent penicillin-streptomycin (10000U/ml, Gibco) containing 5 percent CO_2 at 37^0.

Cell Transfection

To explore effects of FENDRR and miR-424 on HemECs, cell transfection was performed. GenePharma (Shanghai, China) provided siFENDRR-1/-2/-3, oeFENDRR and miR-424 mimics and their negative controls (siNC, oeNC and NC mimics). Before transfection, HemECs were incubated after cell confluence reached 85 percent and Lipofectamine 3000 (Invitrogen, USA) was used to transfect siNC, siFENDRR, oeNC, oeFENDRR, NC mimics, miR-424 mimics and miR-424 mimics with oeFENDRR into HemECs, respectively. Cells then were cultivated for 48 hours, and RT-qPCR was to examine relative expressions of FENDRR and miR-424.

RT-qPCR

Total RNA was segregated from cells using TRI reagent (Sigma-Aldrich, USA) to detect RNA expressions. Afterward, cDNAs of FENDRR were synthesized using PrimeScript RT Reagent Kit (Takara, Japan) while cDNAs of miR-424 were compounded using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems). Then, ProFlex™ PCR System (Applied Biosystems) and SYBR Green PCR Master Mix (Applied Biosystems) was used for PCR. Sequences about primers were shown: FENDRR: F: 5'-CCACATGGATGGTTG CCACT CTC-3', R: 5'-GCTGGTACTCGGCCTTCTAATTGG-3'; (Lu et al. 2019) miR-424: F: 5'-GCGGCGGCAGCAGCAATTCATG-3', R: 5'-ATCCAGTGCAGGGTCCGAGG-3'; (Yu et al. 2020) GAPDH: F: 5'-GGAGCGAGATCCCTCCAAAAT-3', R: 5'-GGCTGTTGTCATACTTCT-

CATGG-3' (Lu et al. 2019) and U6: F: 5'- GGAACGATACAGAGAAGATTAGCA-3', R: 5'- GTGCAGGGTCCGAGGT-3' (Yu et al. 2020). GAPDH and U6 were internal controls of FENDRR and miR-424, respectively. Relative expressions were calculated by $2^{-\Delta\Delta Ct}$ method. The experiment was run in a triplicate.

Cell Viability

To examine cell viability of HemECs after FENDRR or miR-424 transfection, Cell Counting Kit-8 (Beyotime, Shanghai, China) was applied. HemECs were first seeded onto 96-well plates ($5x10^3$ cells/well) and cultivated at 37^0, 5 percent CO_2. After transfection, 10μl CCK-8 was added at 24 hours, 48 hours and 72 hours and cells were cultured for another 1 hour. Thereafter, cell viability of HemECs was detected at 450nm wavelength using Cytation Hybrid Multi-Mode Reader (BioTek, USA). The experiment was run in a triplicate.

Cell Apoptosis

To analyzed apoptosis rate of HemECs after the transfection of FENDRR or miR-424, flow cytometry was conducted. Annexin V-FITC Apoptosis Detection Kit from Sigma-Aldrich (USA) was for evaluating the percentage of apoptosis. Transfected HemECs were washed using PBS and then cells were adjusted in Binding Buffer at a concentration of 110^6 cells/ml. Next, 500μl of the suspension was added into 1275mm test tubes and each tube was added with 5μl Annexin V-FITC and 10μl of Propidium Iodide (Ge et al. 2021). Then, tubes were cultured for 15 min at 25^0 and protected from light. The apoptosis rate of HemECs was validated using BD Accuri C6 Plus Flow Cytometer (BD Biosciences, USA). This experiment was done in triplicate.

Transwell

Transwell was applied for measuring migratory ability and invasiveness of HemECs. Matrigel (Corning, USA) was coated or not in invasion and migration, respectively. The upper chamber (Corning, USA) then was added with serum-free medium (Gibco, USA) and $1x10^5$ cells were seeded. Meanwhile, DMEM medium (Gibco) supplemented with 10 percent FBS (Gibco) was added into the lower chamber (Corning, USA). After incubation for 24h, cells in the lower chamber were stained using 0.1 percent crystal violet (Beyotime, Shanghai, China). Using a microscope, numbers of cells were counted. The average was calculated from 6 visual fields.

Western Blot

To validate angiogenesis in HemECs, proteins related to angiogenesis were detected using western blot assay. Total protein was first isolated by RIPA Buffer (Sigma-Aldrich, USA) and then quantified by QuantiPro BCA Assay Kit (Sigma-Aldrich). Afterward, protein was segregated by SDS-PAGE (Beyotime, Shanghai, China) and shifted onto PVD Western Blotting Membranes (Sigma-Aldrich, USA). Membranes were then blocked by TBSTw (Beyotime, Shanghai, China) and incubated with primary antibodies: anti-MMP-9 (1:1000, ab76003, Abcam, UK), anti-VEGFA (1:1000, ab52917) and anti-GAPDH (1:2000, ab9485) at 4^0 overnight. Next, Goat Anti-Rabbit IgG H&L (HRP) (1:800, ab6721) then was applied to culture with membranes for 2 hours at room temperature. BeyoECL Plus (Beyotime, Shanghai, China) then was used for developing and Image J software (NIH, USA) was used to evaluate grey values of proteins.

Statistical Analysis

Data were all shown as mean±SD and then analyzed using GraphPad Prism 7.0 (GraphPad, USA). Student's t-test was used for comparison between two groups while one-way ANOVA was used for comparison in three groups and two-way ANOVA was used for comparisons in cell viability detections. P value <0.05 was considered to have significance in statistics (Song et al. 2021).

RESULTS

FENDRR was Upregulated in HemECs and Regulated Cell Viability and Cell Apoptosis

The researchers first evaluated FENDRR expressions using RT-qPCR, indicating that it was promoted in HemECs compared with HUVECs

(Fig. 1A). Furthermore, its expression was downregulated in HemECs by suppressed transfection of FENDRR (Fig. 1B). Then, functional tests were used to verify roles of FENDRR. Results of CCK-8 indicated that the cell viability of HemECs was hampered by the loss of FENDRR while flow cytometry manifested that the absence of FENDRR facilitated the apoptosis rate of HemECs (Fig. 1C, D). These results indicated that FENDRR might be an oncogenic long noncoding RNA in hemangioma.

Knockdown of FENDRR Decreased Invasiveness, Migration and Angiogenesis in HemECs

Thereafter, functions of FENDRR in modulating invasiveness and migration of HemECs were detected, showing that migratory and invasive abilities were reduced by FENDRR inhibition (Fig. 2A, B). Additionally, biomarkers related to angiogenesis were examined and results of western blot indicated that both MMP-9 and VEGFA protein expressions were downregulated after FENDRR suppression (Fig. 2C). Based on these results, FENDRR had the ability to accelerate invasiveness, migration and angiogenesis of HemECs.

MiR-424 was the Target Gene of FENDRR

To analyze underlying mechanism of FENDRR, ENCORI (http://starbase.sysu.edu.cn) has provided binding sites between FENDRR and miR-424 (Fig. 3A). Moreover, luciferase reporter test showed that the fluorescence was the low-

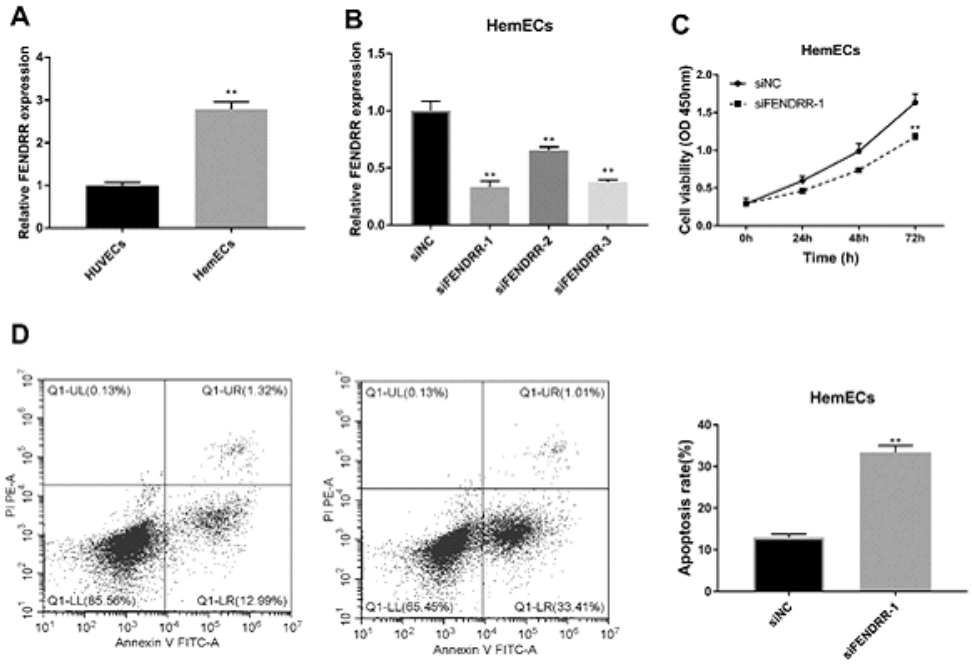

Fig. 1. LncRNA FENDRR was downregulated in HemECs and modulated cell viability and apoptosis
A: RT-qPCR was performed to analyze RNA expressions of FENDRR in HUVECs and HemECs, $^{**}P<0.05$ versus HUVECs
B: RNA expressions of FENDRR were assessed using RT-qPCR after the transfection with siNC, siFENDRR-1, siFENDRR-2 and siFENDRR-3, $^{**}P<0.05$ in comparison with the siNC
C: CCK-8 validated the cell viability of HemECs after the transfection of siFENDRR-1. D: The apoptosis rate was evaluated through flow cytometry

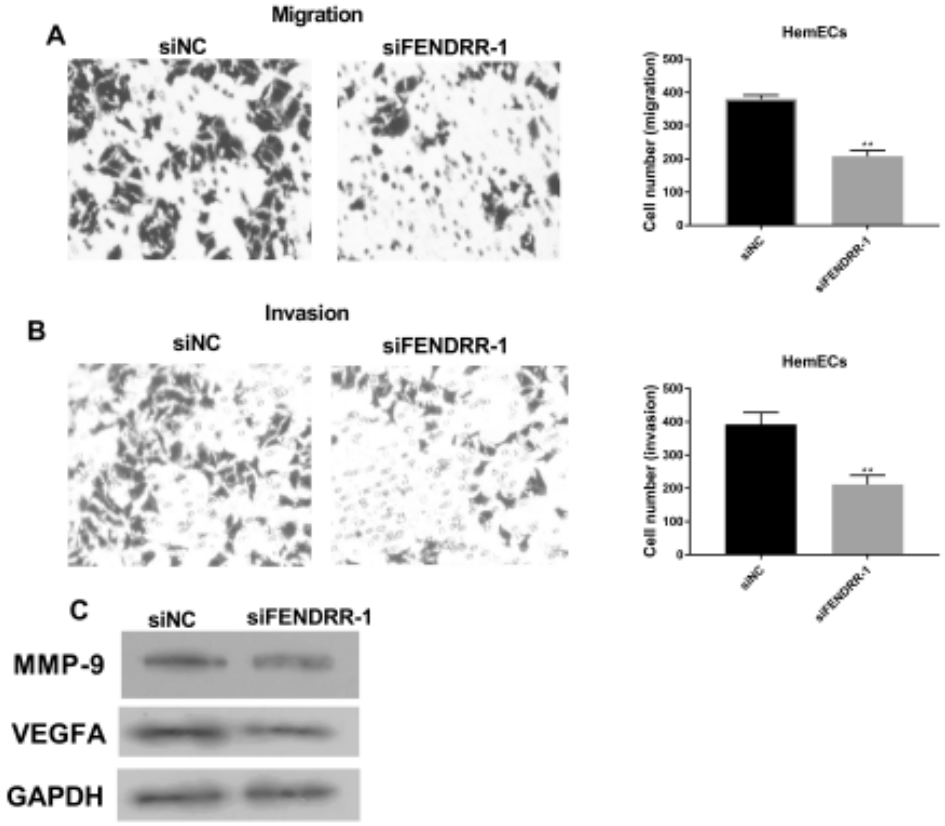

Fig. 2. Knockdown of FENDRR inhibited migration, invasiveness and angiogenesis of HemECs
A, B: Transwell assay was applied to validate migratory ability and invasiveness of HemECs after the transfection of siFENDRR-1, **P<0.05 in comparison with the siNC
C: Protein expressions of factors related to angiogenesis, MMP-9 and VEGFA, were examined using western blot, **P<0.05 versus the siNC

est in FENDRR-wt with miR-424 mimics group (Fig. 3B). Furthermore, low miR-424 expression in HemECs was confirmed by RT-qPCR. Beyond that, upregulated expression of miR-424 caused by miR-424 mimics was hindered by FENDRR overexpression (Fig. 3C, D). Therefore, miR-424 was sponged and suppressed by FENDRR.

FENDRR Regulated Cell Viability, Apoptosis, Invasiveness, Migration and Angiogenesis of HemECs by Sponging miR-424

To further examining interactions between FENDRR and miR-424, functional tests were performed. According to results of CCK-8 and flow cytometry, overexpression of FENDRR hindered the decrease of cell viability and restrained the high apoptosis level in HemECs with miR-424 mimics (Fig. 4A, B). Moreover, shown as Fig. 4C, D, migration and invasiveness of HemECs were retarded by miR-424 overexpression but restored to be high by the FENDRR upregulation. Additionally, downregulated MMP-9 and VEGFA caused by miR-424 mimics were reversed and upregulated by FENDRR upregulation (Fig. 4E). According to these detections, effects of FENDRR on HemECs were performed via sponging miR-424.

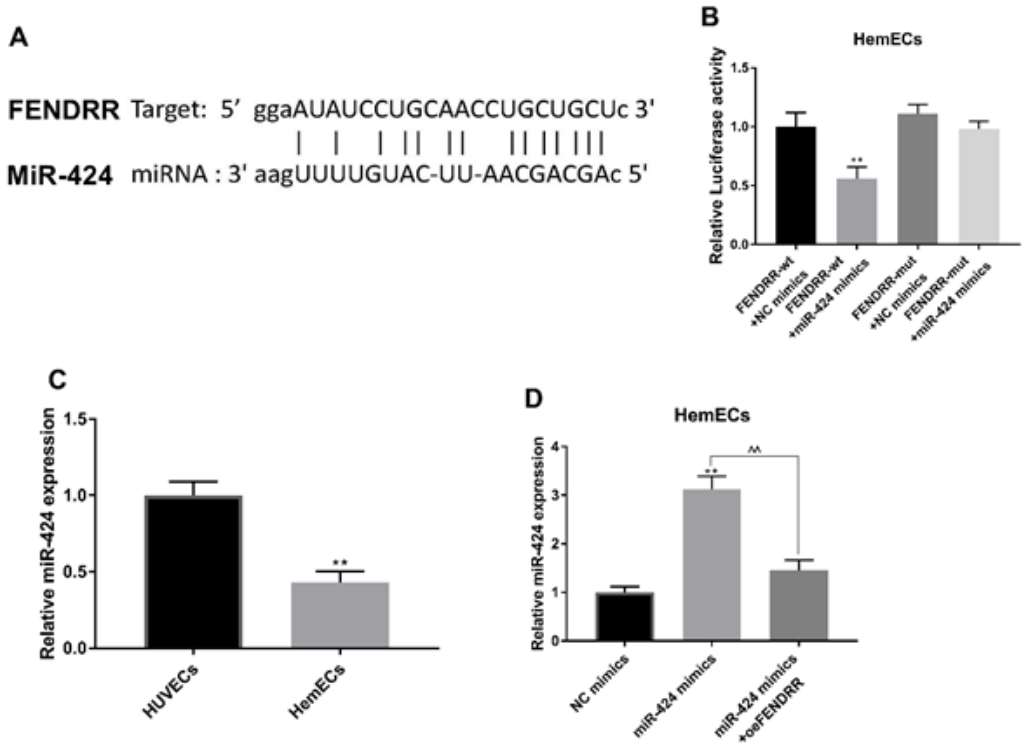

Fig. 3 MiR-424, the target of FENDRR, was low expressed in HemECs
A: Binding sites of miR-424 in FENDRR was provided by ENCORI (http://starbase.sysu.edu.cn)
B: Luciferase reporter assay was applied to detect fluorescence in FENDRR-wt/mut with mimics of NC/miR-424, **P<0.05 versus the FENDRR-wt with NC mimics, FENDRR-mut with NC mimics and FENDRR-mut with miR-424 mimics
C: RNA levels of miR-424 in HUVECs and HemECs were assessed by RT-qPCR, **P<0.05 compared to HUVECs
D: RNA expressions of miR-424 after transfected with miR-424 mimics and miR-424 mimics with oeFENDRR were analyzed using RT-qPCR, **P<0.05 versus the NC mimics and ^^P<0,05 versus the miR-424 mimics

DISCUSSION

According to previous studies, dysregulation of angiogenesis can lead to occurrence of IH (Léauté-Labrèze et al. 2017). Though β blockers are commonly used for treating IH, side effects of these medicines limit their efficacies (Ji et al. 2014). Therefore, the key point at present stage is to figure out deep mechanism of IH and to prevent its development in early stage. In this research, FENDRR has been discovered to be upregulated, which positively regulated progression of HemECs by targeting miR-424 in HemECs.

Cheng et al. have determined that FENDRR was downregulated in colorectal cancer tissues and its overexpression suppressed the cell viability, invasion and migration by targeting miR-424-5p directly (Cheng et al. 2020). FENDRR also restrained cell viability, migration, epithelial-mesenchymal transition and invasiveness but accelerated apoptosis of cervical cancer cells via sponging miR-15a/b-5p and promoting tubulin alpha 1a (Zhu et al. 2020). LncRNA FENDRR overexpression promoted proliferation, migration and vascular endothelial growth factor expression in human retinal endothelial cells after high glucose induction while knockdown of FENDRR caused reversed results (Shi et al. 2019). Although most studies have examined suppressed functions of FENDRR, it could acceler-

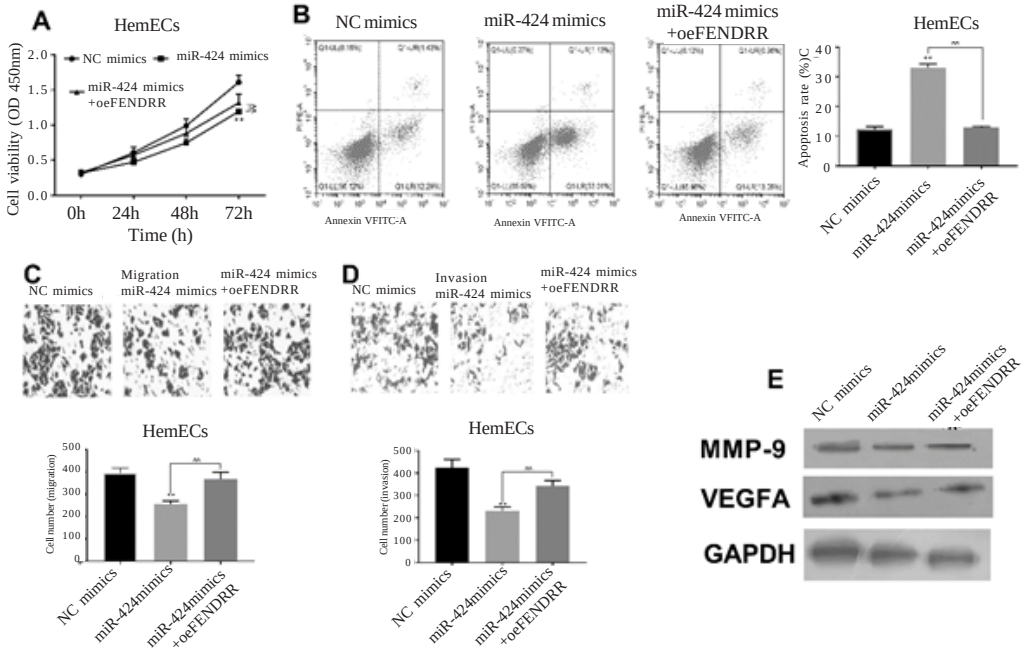

Fig. 4. Overexpression of FENDRR accelerated progressions of HemECs by downregulating miR-424
A: The cell viability of HemECs with miR-424 mimics and miR-424 mimics with oeFENDRR was evaluated using CCK-8, **P<0.05 versus the NC mimics and ^^P<0,05 versus the miR-424 mimics
B: Apoptosis rates of HemECs were analyzed by flow cytometry, **P<0.05 versus the NC mimics and ^^P<0,05 versus the miR-424 mimics.
C, D: Migration and invasiveness of HemECs after transfected with miR-424 mimics or miR-424 mimics with oeFENDRR were measured using transwell, **P<0.05 versus the NC mimics and ^^P<0,05 versus the miR-424 mimics
E: MMP-9 and VEGFA protein expressions in HemECS were detected by western blot, **P<0.05 versus the NC mimics and ^^P<0,05 versus the miR-424 mimics

Figure 1	
RT-qPCR for FENDRR	**P<0.0001
RT-qPCR for FENDRR	**P<0.0001
CCK-8 for cell viability of HemECs with siFENDRR	**P<0.0001
Flow cytometry for apoptosis of HemECs with siFENDRR	**P<0.0001
Figure 2	
Transwell for migration with siFENDRR	**P<0.0001
Transwell for invasion with siFENDRR	**P=0.0023
Figure 3	
Luciferase reporter test	**P=0.0004
RT-qPCR for miR-424	**P=0.0011
RT-qPCR for miR-424 with miR-424 mimics or miR-424 mimics with oeFENDRR	**P=0.0002; ^^P=0.0011
Figure 4	
CCK-8 for cell viability of HemECs	**P<0.0001; ^^P=0.0058
Flow cytometry for apoptosis rate of HemECs	**P<0.0001; ^^P<0.0001
Transwell for migration	**P=0.0007; ^^P=0.0047
Transwell for invasion	**P=0.0012; ^^P=0.0025

ate migratory and invasive abilities of osteosarcoma cells by activating Forkhead box protein F1/MMP-2/-9 signaling pathway (Kun-Peng et al. 2017). In the study of Liu et al. fold change of FENDRR was the highest compared with other lncRNAs (Liu et al. 2016). In this study, roles of

this lncRNA were examined in HemECs and FENDRR was significantly upregulated in HemECs. Furthermore, the knockdown of FENDRR caused low cell viability, invasiveness and migration but the apoptosis was promoted. Hence, FENDRR had the ability to accelerate progression of HemECs, which suggested that FENDRR might be a biomarker for diagnosing IH.

In this study, ENCORI provided binding sites between miR-424 and FENDRR and FENDRR directly sponged miR-424 in HemECs by further experiment. MicroRNAs have been well known as key regulators in malignancies. MiR-125b was upregulated by propranolol facilitated the suppression of cell viability and the promotion of apoptosis in HemECs (Huang et al. 2019). MiR-187-3p was downregulated in HemECs and it enhanced sensitivity of HemECs to propranolol by targeting NIPBL (Shi et al. 2019). Low expressed miR-424 in IH tissues blocked bFGF/FGFR1 signaling pathway resulting in suppression of proliferation, migration and tube formation (Yang et al. 2017). Moreover, miR-424 was sponged by lncRNAMALAT1, causing upregulation of MEKK3 and activation of IKK/NF-κB signaling pathway, which resulted in accelerated progression of IH (Li et al. 2019). Present study indicated that the expression of miR-424 was inhibited by FENDRR overexpression. Beyond that, decreased cell viability, migration and invasiveness caused by miR-424 mimics were also hampered by FENDRR overexpression. Compared to former research, this one has analyzed interactions of miR-424 with FENDRR in HemECs, which might provide a new way to inhibit functions of FENDRR.

Besides that, researchers also examined angiogenesis in hemangioma through detecting related protein expressions with FENDRR and miR-424. MMP-9 was upregulated by LSF, resulting in accelerated angiogenesis of HUVECs while in vivo experiments indicated that suppression of MMP-9 abolished LSF-induced angiogenesis and tumorigenesis in nude mouse models (Santhekadur et al. 2012). Besides that, angiogenesis of malignancies primarily depends on VEGFA-driven responses (Claesson-Welsh and Welsh 2013). Suppression of VEGFA caused by ACE2, leading to low angiogenesis in breast cancer (Zhang et al. 2019). Interestingly, FENDRR and VEGFA were both upregulated in vascular endothelial cells (VECs) of mice and human brain microvascular endothelial cells after thrombin treatment and VEGFA positively regulated by FENDRR accelerated the apoptosis of HBMECs by targeting miR-126 (Dong et al. 2018). Based on these studies, this research also analyzed these two proteins related to angiogenesis in HemECs and results revealed that MMP-9 and VEGFA was suppressed by knockdown of FENDRR and miR-424 mimics but upregulated by FENDRR overexpression. Hence, FENDRR might also accelerate angiogenesis in HemECs to prompt progression of HEMECs.

CONCLUSION

LncRNA FENDRR was upregulated HemECs and accelerated cell viability, invasion, migration and angiogenesis but suppressed apoptosis through sponging miR-424, suggesting that FENDRR might be a new biomarker to prevent malignant development of hemangioma. Nevertheless, further studies in vivo and clinical stages are requested to provide more information about this lncRNA.

RECOMMENDATIONS

LncRNA FENDRR was promoted in HemECs while miR-424 was inhibited. Thereafter, miR-424 was verified to be sponged by lncRNA FENDRR. Moreover, FENDRR facilitated HEMECs viability, migratory ability, invasiveness and angiogenesis but suppressed apoptosis by sponging miR-424.

ABBREVIATION LIST

FENDRR: Fetal-lethal non-coding developmental regulatory RNA;
HemECs: hemangioma endothelial cells;
HUVEC: Human Umbilical Vein Endothelial Cell;
VEGFA: Vascular Endothelial Growth Factor A;
IH: Infantile hemangioma;
MMP: matrix metalloproteinase;
DMEM: Dulbecco's Modified Eagle Medium;
PDRG1: p53 and DNA damage regulated 1;
SD: standard deviation;
LSF: Late sv40 factor.

REFERENCES

Acevedo LM, Cheresh DA 2008. Suppressing nfat increases vegf signaling in hemangiomas. *Cancer Cell*, 14(6): 429-430.

Adams BD, Parsons C, Walker L, Zhang WC, Slack FJ 2017. Targeting noncoding rnas in disease. *J Clin Invest*, 127(3): 761-771.

Chen D, Yang X, Liu M, Zhang Z, Xing E 2021. Roles of mirna dysregulation in the pathogenesis of multiple myeloma. *Cancer Gene Ther*, 28(12): 1256-1268.

Cheng C, Li H, Zheng J, Xu J, Gao P, Wang J 2020. Fendrr sponges mir-424-5p to inhibit cell proliferation, migration and invasion in colorectal cancer. *Technol Cancer Res Treat*, 19: 1533033820980102

Claesson-Welsh L, Welsh M 2013. Vegfa and tumour angiogenesis. *J Intern Med*, 273(2): 114-127.

Dai Y, Wan Y, Qiu M, Wang S, Pan C, Wang Y, Ou J 2018. Lncrna meg3 suppresses the tumorigenesis of hemangioma by sponging mir-494 and regulating pten/ pi3k/akt pathway. *Cell Physiol Biochem*, 51(6): 2872-2886.

Dong B, Zhou B, Sun Z, Huang S, Han L, Nie H, Chen G, Liu S, Zhang Y, Bao N, Yang X, Feng H 2018. Lncrna-fendrr mediates vegfa to promote the apoptosis of brain microvascular endothelial cells via regulating mir-126 in mice with hypertensive intracerebral hemorrhage. *Microcirculation*, 25(8): e12499.

Ge X, Jiang Y, Hu X, Yu X 2021. Microrna-106a-5p alleviated the resistance of cisplatin in lung cancer cells by targeting jumonji domain containing 6. *Transpl Immunol*, 69: https://doi.org/10.1016/j.trim.2021.101478.

Herrera-Merchan A, Cuadros M, Rodriguez MI, Rodriguez S, Torres R, Estecio M, Coira IF, Loidi C, Saiz M, Carmona-Saez P, Medina PP 2020. The value of lncrna fendrr and foxf1 as a prognostic factor for survival of lung adenocarcinoma. *Oncotarget*, 11(13): 1172-1185.

Huang J, Jiang D, Zhao S, Wang A 2019. Propranolol suppresses infantile hemangioma cell proliferation and promotes apoptosis by upregulating mir-125b expression. *Anticancer Drugs*, 30(5): 501-507.

Ji Y, Chen S, Li K, Li L, Xu C, Xiang B 2014. Signaling pathways in the development of infantile hemangioma. *J Hematol Oncol*, 7: 13.

Krowchuk DP, Frieden IJ, Mancini AJ, Darrow DH, Blei F, Greene AK, Annam A, Baker CN, Frommelt PC, Hodak A, Pate BM, Pelletier JL, Sandrock D, Weinberg ST, Whelan MA 2019. Clinical practice guideline for the management of infantile hemangiomas. *Pediatrics*, 143(1).

Kun-Peng Z, Chun-Lin Z, Xiao-Long M 2017. Antisense lncrna foxf1-as1 promotes migration and invasion of osteosarcoma cells through the foxf1/mmp-2/-9 pathway. *Int J Biol Sci*, 13(9): 1180-1191.

Léauté-Labrèze C, Harper JI, Hoeger PH 2017. Infantile haemangioma. *Lancet*, 390(10089): 85-94.

Li MM, Dong CX, Sun B, Lei HZ, Wang YL, Gong YB, Sun LL, Sun ZW 2019. Lncrna-malat1 promotes tumorogenesis of infantile hemangioma by competitively binding mir-424 to stimulate mekk3/nf-êb pathway. *Life Sci*, 239: 116946.

Liu X, Lv R, Zhang L, Xu G, Bi J, Gao F, Zhang J, Xue F, Wang F, Wu Y, Fu C, Wang Q, Huo R 2016. Long noncoding rna expression profile of infantile. *Biol*, 37: 15977-15987.

Lu Q, Liu T, Feng H, Yang R, Zhao X, Chen W, Jiang B, Qin H, Guo X, Liu M, Li L, Guo H 2019. Circular rna circslc8a1 acts as a sponge of mir-130b/mir-494 in suppressing bladder cancer progression via regulating pten. *Mol Cancer*, 18(1): 111.

Nakashima T, Jinnin M, Etoh T, Fukushima S, Masuguchi S, Maruo K, Inoue Y, Ishihara T, Ihn H 2010. Down-regulation of mir-424 contributes to the abnormal angiogenesis via mek1 and cyclin e1 in senile hemangioma: Its implications to therapy. *PLoS One*, 5(12): e14334.

Santhekadur PK, Gredler R, Chen D, Siddiq A, Shen XN, Das SK, Emdad L, Fisher PB, Sarkar D 2012. Late sv40 factor (lsf) enhances angiogenesis by transcriptionally up-regulating matrix metalloproteinase-9 (mmp-9). *J Biol Chem*, 287(5): 3425-3432.

Shi Y, Chen C, Xu Y, Liu Y, Zhang H, Liu Y 2019. Lncrna fendrr promotes high-glucose-induced proliferation and angiogenesis of human retinal endothelial cells. *Biosci Biotechnol Biochem*, 83(5): 869-875.

Shi Y, Zhang J, Liu M, Huang Y, Yin L 2019. Smad3 inducing the transcription of styk1 to promote the emt process and improve the tolerance of ovarian carcinoma cells to paclitaxel. *J Cell Biochem*, 120(6): 10796-10811.

Song B, Wang XX, Yang HY, Kong LT, Sun HY 2021. Mir-141 attenuates sepsis-induced cardiomyopathy by targeting dapk1. *Hum Exp Toxicol*, 40(12_suppl): S137-s149

Tiemann L, Hein S 2020. Infantile hemangioma: A review of current pharmacotherapy treatment and practice pearls. *J Pediatr Pharmacol Ther*, 25(7): 586-599.

Wang J, Zhu S, Meng N, He Y, Lu R, Yan GR 2019. Ncrna-encoded peptides or proteins and cancer. *Mol Ther*, 27(10): 1718-1725.

Yang L, Dai J, Li F, Cheng H, Yan D, Ruan Q 2017. The expression and function of mir-424 in infantile skin hemangioma and its mechanism. *Sci Rep*, 7(1): 11846.

Ye C, Hu Y, Wang J, Liu D, Du J 2019. Mono (2-ethylhexyl) phthalate (mehp) triggers the proliferation of hemangioma-derived endothelial cells via yap signals. *Chem Biol Interact*, 311: 108773

Yu M, Ozaki T, Sun D, Xing H, Wei B, An J, Yang J, Gao Y, Liu S, Kong C, Zhu Y 2020. Hif-1α-dependent mir-424 induction confers cisplatin resistance on bladder cancer cells through down-regulation of pro-apoptotic unc5b and sirt4. *J Exp Clin Cancer Res*, 39(1): 108.

Zeng Z, Liu S, Cai J, Li Z, Wu H, Chen H, Huang Y 2019. Mir-501 promotes hemangioma progression by targeting hoxd10. *Am J Transl Res*, 11(4): 2439-2446.

Zhang Q, Lu S, Li T, Yu L, Zhang Y, Zeng H, Qian X, Bi J, Lin Y 2019. Ace2 inhibits breast cancer angiogenesis via suppressing the vegfa/vegfr2/erk pathway. *J Exp Clin Cancer Res*, 38(1): 173.

Zhao T, Zhang J, Ye C, Tian L, Li Y 2020. Lncrna foxd2-as1 promotes hemangioma progression through the mir-324-3p/pdrg1 pathway. *Cancer Cell Int*, 20: 189.

Zhu Y, Zhang X, Wang L, Zhu X, Xia Z, Xu L, Xu J 2020. Fendrr suppresses cervical cancer proliferation and invasion by targeting mir-15a/b-5p and regulating tuba1a expression. *Cancer Cell Int*, 20: 152.

Paper received for publication in November, 2021
Paper accepted for publication in February, 2022