Original Article
CircATP5SL promotes infantile haemangiomas progression via IGF1R regulation by targeting miR-873-5p

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Abstract: Infantile haemangiomas (IH) are the most common soft-tissue tumours in infants. Several studies have demonstrated the importance of circular RNA (circRNA) for the regulation of various cancer cells. The present study aims to evaluate the functions and molecular mechanisms of circATP5SL in IH progression. In this study, we found that circATP5SL is significantly dysregulated in IH. We conducted Transwell, MTT, and flow cytometry analysis to evaluate the role of circATP5SL in IH cell proliferation, invasion, migration, and apoptosis. Meanwhile, by using subcellular distribution detection, as well as dual-luciferase reporter test and RIP analysis, it has been confirmed that miR-873-5p directly binds to the 3'UTR of IGF1R mRNA, thereby inhibiting the expression of IGF1R. Besides, circATP5SL promoted IGF1R expression by directly adsorbing miR-873-5p, an IGF1R inhibitor, thereby promoting cellular invasion, proliferation, and migration as well as inhibition of apoptosis. In summary, our study suggests that circATP5SL promotes IH progression by regulating IGF1R expression through adsorption of miR-873-5p, elucidating circATP5SL as a promising therapeutic target for the prognostication and treatment of IH.

Keywords: Infantile haemangiomas, circRNA, circATP5SL, IGF1R, miR-873-5p

Introduction

Infantile haemangiomas (IHs) are one of the most benign endothelial tumors in infants and young children [1, 2]. Most IHs regress spontaneously and do not require therapy, but about 10-15% of clinical cases show rapid growth with complications such as disfigurement, ulceration, or obstruction and require treatment [3, 4]. A better understanding of the molecular mechanisms of tumors will be beneficial for effective diagnosis and novel treatment strategies. Despite a large amount of research has been focused on this disease, the pathogenesis has not been fully elucidated. The current theory has supported that hypoxic stress is the triggering signal that could also cause the elevated expression of angiogenic factors like vascular endothelial growth factors (VEGF), through hypoxia-inducible factor alpha (HIFα) [5, 6]. Therefore, the detailed study of molecular mechanisms involved in the regulation of IH progression could be of increased clinical importance to develop novel and effective cancer treatments.

Circular RNAs (circRNAs) are closed circular structures made up of linear RNA molecules, in which the 5' and 3' ends are attached by covalent bonds and these are characteristically stable, conserved, and space-time specific [7]. In the past few years, many circRNAs are extensively associated with multiple biological processes including cell cycle, proliferation, invasion, migration, and apoptosis [8-10]. Moreover, microRNAs (miRNAs) have been identified as endogenous small RNA molecules lacking coding capabilities, which are potentially associated with the regulation of protein-coding genes in terms of their expression levels [11]. Previously, several studies have demonstrated the significant role of miRNAs in metastasis, development, and tumorigenesis of various cancer types [12-14]. Recently, circRNAs have been

appeared to act as "miRNA sponges" as circR-NAs contain binding sites for miRNA [15, 16]. Also, circRNAs separate miRNAs to stop the miRNAs induced regulation of target genes [17]. The microarray analysis identified 374 downregulated and 234 upregulated circRNAs during the event of haemangioma. Gene ontology and pathway analysis showed that these differentially expressed circRNAs are involved in angiogenesis and cell development. RNA sequencing of haemangioma also showed that 249 circRNAs were differentially expressed in comparison with normal skin tissue [18]. These findings are indicative of the role of circRNA in the development of haemangiomas and its function and potential molecular mechanism warrant further study.

In this study, through data collation and analysis of the hemangioma gene chip, several circR-NAs abnormally expressed in hemangioma were screened. Through verification and validation studies, it was found that circATP5SL had the most significant difference in IH tissues. The human umbilical vein endothelial cells (HUVECs) and also haemangioma-derived endothelial cells (HemECs) were examined by knockdown and overexpression of circATP5SL and thereby the relative effects of circATP5SL on the cell migration, apoptosis, and proliferation were evaluated. Importantly, we found that circATP5SL and miR-873-5p competitively regulate the downstream protein IGF1R and thus participate in the development of haemangiomas. Our findings provide new insights into the pathogenesis and treatment of haemangiomas.

Materials and methods

Human tissue specimens

We collect samples from IH surgery performed from 2015 to 2019 at Ningbo Women and Children's Hospital. The resected samples were soaked in RNAstore (CWBIO, China) at 4°C overnight and stored at -80°C.

Cell culture

The human umbilical vein endothelial cells (HUVECs) and hemangioma-derived endothelial cells (HemECs) were obtained from the American Type Culture Collection (USA). All selected cell lines were grown on RPMI 1640 medium (Hycolon, USA) added with 1% penicil-

lin-streptomycin (Sigma-Aldrich, USA) and 10% fetal bovine serum (FBS).

Total RNA extraction and reverse transcription

In order to extract the total RNA from the grown culture of cells or tissues, TRIzol reagent (Invitrogen, CA) was utilized following the manufacturer's instructions. Then, the extracted RNA was reverse transcribed into cDNA by following the ReverTra Ace qPCR RT Kit's instructions (TOYOBO, Japan).

RNA localisation experiments

RNA was directly reverse transcribed into cDNAs after being treated with 3 U/mg RNase R (Epicenter, USA) for 15 minutes at 37°C. However, a PARIS Kit (Invitrogen, USA) was employed to perform the nuclear and cytoplasmic RNA isolations following the manufacturer's protocol and was also reverse transcribed into cDNA.

Quantitative real-time polymerase chain reaction

Light Cycler 480 SYBR Green I Master (Roche, Switzerland) was employed to carry out Quantitative real-time polymerase chain reaction (qRT-PCR).

The primer sequences are listed in Supplementary Materials. The qRT-PCR analysis was conducted on the Light Cycler 480 (Roche, Switzerland) and data were evaluated by considering the comparative cycle threshold (CT) method ($2^{-\Delta\Delta CT}$).

Plasmids construction

To construct IGF1R overexpression plasmid, IFG1R cDNA synthesis was accomplished and the resultant product was then cloned into pcDNA3.1(+) vector. To construct circATP5SL overexpression plasmid, circATP5SL cDNA synthesis was performed and the resultant product was cloned into pcD5-ciR vector (Geenseed Biotech Co). The pGPU6/Neo vector was used to construct circATP5SL shRNA.

siRNAs, miRNA mimic, plasmid, shRNA, and transfection experiments

GenePharma (China) provided us with circATP5SL siRNAs, the vector of circATP5SL shRNA, and the mimic of miR-873-5p along with non-

targeting negative control. The plasmid of circATP5SL was synthesized at Geneseed (China). Lipofectamine 2000 (Invitrogen, Germany) was used for transfection. Thirty-six hours post-transfection, the cells were used for RNA harvesting and then detected by RT-qPCR. Three circATP5SL siRNAs were used for knockdown of circATP5SL (Supplementary Tables 1, 2 and Supplementary Figure 1).

MTT assay

Thiazolyl Blue (MCE, USA) was used for cell proliferation assay. HemECs and HUVECs were seeded into 96-well plates. Then, 10 μL MTT (3.5 mg/mL in PBS) was introduced into the medium. Subsequently, the media plates were incubated at 37°C for a period of 4 h. Through aspiration, the supernatant was removed carefully, and then every single well was added with 100 μL DMSO. Finally, the absorbance was measured at 490 nm.

Transwell migration and matrigel invasion assays

We used a transwell setup for migration assay and a matrigel pre-coated transwell insert for invasion assay by following the manufacturer's instruction (Corning, USA). The upper chambers were added with homogeneous single-cell suspensions (1×10^5 cells per well for invasion assay and 4×10^4 cells per well for migration assay), prepared in 200 μL serum-free medium. Then, we added 750 μL medium with 10% FBS in the lower chamber as a chemoattractant and incubated the plates for 24 h. In order to quantify the rates of cell migration and invasion, the number of cells was counted in the lower chamber in at least three random fields.

Flow cytometry

Followed by transfection, apoptosis was detected by flow cytometry using the Apoptosis Detection Kit (MultiSciences, China), and stained cells were analyzed on a CytoFLEX S (Beckman, USA).

Luciferase assay

Dual-luciferase assay was performed by following the instructions from the manufacturer (Promega, USA). Luciferase reporter vector pGL3 (Promega, USA) was used for the amplification and cloning of mutant circATP5SL, IGF1R, and wildtype. 293T cells were simultaneously transfected with miR-873-5p mimics and luciferase plasmids. The firefly luciferase activities were stabilized to that of Renilla luciferase after 48 h.

RNA immunoprecipitation assay

Utilizing a Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, USA), the RNA immunoprecipitation (RIP) assay was conducted following the instructions from the manufacturer. The enrichment of miR-873-5p and circATP5SL was identified using qRT-PCR. The anti-Ago2 (ab57113) and IgG used for RIP were purchased from Abcam (UK).

Mice

A total of twenty-four 5 weeks old male BALB/c-nu mice were randomized into four groups.

HemECs stably expressing sh-circATP5SL and circATP5SL as well as the empty vector were subcutaneously injected into the nude mice by targeting their flank regions. Four weeks after injection, we sacrificed the mice to measure the growth of subcutaneous tumors. The clinical Research Ethics Committees of our hospital approved all the experiments conducted on animals.

Western blot

With the help of 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the cell protein lysates and then were moved to 0.22 μm nitrocellulose membranes (Sigma, USA). In the next step, membranes were incubated with rabbit anti-IGF1R (Cell Signaling Technology, USA) primary antibodies overnight at 4°C and subsequently with secondary antibody for one hour at room temperature. The enhanced chemiluminescence chromogenic substrate was quantified by densitometry using ImageJ (NIH, USA).

Statistical analysis

In order to compare mean values, two-tailed t-tests were performed with the help of GraphPad Prism (GraphPad Software, USA). $P < 0.05$ was established as statistically significant.
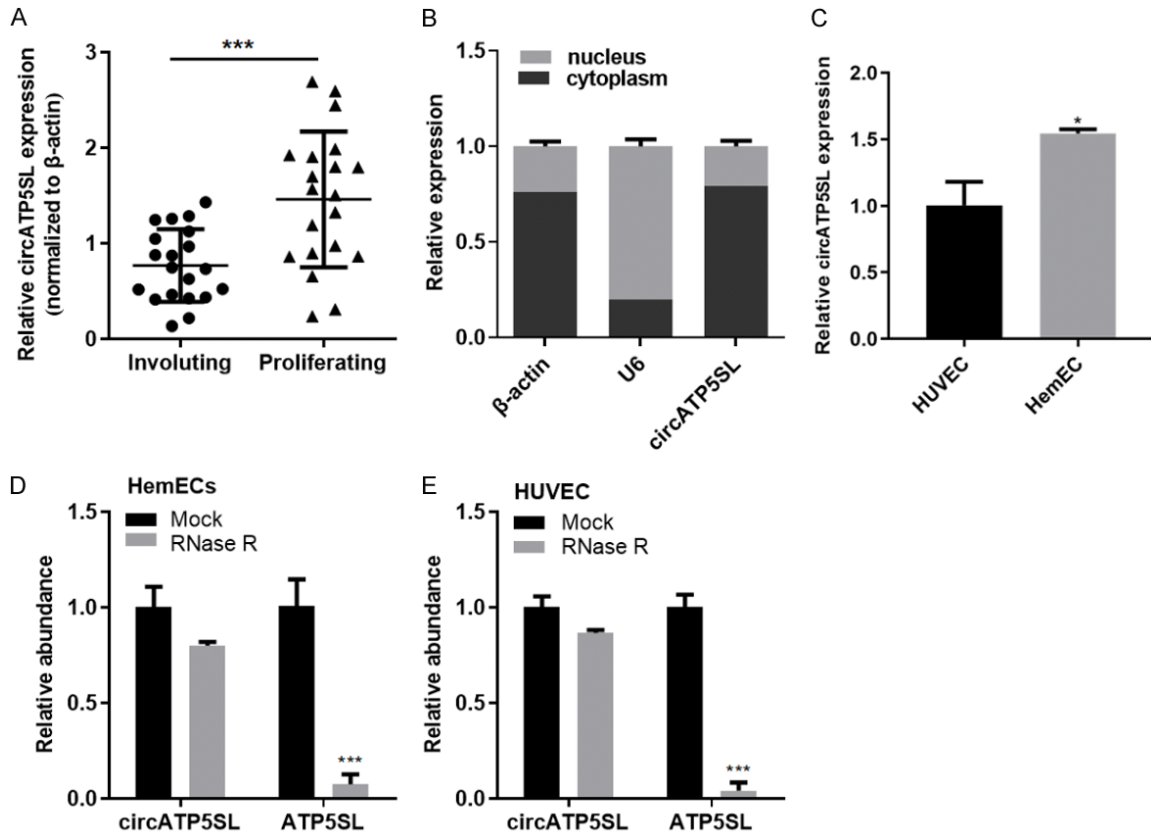
Figure 1. circATP5SL was overexpressed in haemangioma tissue and mainly distributed in the cytoplasm. A. High expression of circATP5SL in 20 sets of haemangioma tissue. B. qRT-PCR analysis of circATP5SL, U6, and β-actin in the cytoplasm and nucleus in HemECs. C. qRT-PCR assay of circATP5SL and ATP5SL expression in HemECs and HUVECs. D, E. qRT-PCR assay of circATP5SL and ATP5SL expression in HemECs and HUVECs after treatment with RNase R. Data represent the mean ± SD of three independent experiments. *$P<0.05$, **$P<0.01$, ***$P<0.001$. All experiments were performed three times in triplicate.

Results

circATP5SL was highly expressed in haemangioma tissue

Several circRNA molecules have been reported to be abnormally expressed in haemangiomas, but the function of circRNA in haemangiomas has not been verified. Based on haemangioma chip data [2], we selected circATP5SL, which show significant differences, as a candidate research molecule.

First, we verified the expression of circATP5SL in haemangioma tissue. We employed qRT-PCR to determine the expression of circATP5SL in 20 sets of haemangioma tissues and found there was a significantly higher expression of circATP5SL compared with normal tissues (**Figure 1A**). Different spatially distributed circRNAs play different functions and mecha-

nisms [19]. Therefore, we further analyzed the release of circATP5SL in HemECs cells using nuclear-plasma separation experiments. Using U6 and actin as controls, we found that circATP5SL was mainly distributed in the cytoplasm (**Figure 1B**). At the same time, we also identified various expression levels of circATP5SL in HemECs and HUVECs cells (**Figure 1C**). To test whether circATP5SL has higher stability than linear RNA molecules, we treated RNA with RNase R and found that circATP5SL tolerated the digestive capacity of RNase R while linear ATP5SL was digested by RNase R (**Figure 1D** and **1E**).

circATP5SL silencing inhibited haemangioma cell proliferation, invasion, and migration and promoted apoptosis

We designed the interference sequence of circATP5SL, si-circATP5SL, which spanned the

splice site of circATP5SL. After the transfection of si-circATP5SL and negative control sequences, we observed that the expression of circAT-P5SL was significantly reduced by si-circAT-P5SL (**Figure 2A**). MTT cell proliferation experiments indicated that after circATP5SL was silenced, the proliferation ability of HemECs and HUVECs was potentially lower than that of the control group (**Figure 2C**). Transwell cell invasion and migration experiments revealed that interferences of circATP5SL expression reduce the invasion and migration capabilities of HemECs and HUVECs (**Figure 2E**). By flow cytometry, it has been shown that circATP5SL knockdown significantly promoted apoptosis in HemECs and HUVECs (**Figure 2G**). Also, the elevated expressions of circATP5SL considerably promoted the migration, invasion, and proliferation of haemangioma cells while inhibited cellular apoptosis.

Moreover, to further explore the effects of circATP5SL associated with proliferation, invasion, migration, and apoptosis of haemangioma cells, we constructed an overexpression plasmid of circATP5SL. After transfection, the overexpression level of circATP5SL was detected by qRT-PCR (**Figure 2B**). MTT experiments verified that the proliferation of HemECs and HUVECs was significantly increased after inducing overexpression of circATP5SL (**Figure 2D**). Furthermore, we found that apoptosis of HemECs and HUVECs were decreased while invasion and migration ability was significantly enhanced (**Figure 2F** and **2H**).

circATP5SL targeted miR-873-5p

The above cell function experiments show that circATP5SL may affect the progression of haemangioma by promoting proliferation, invasion, and migration and reducing apoptosis, thus we aimed to elucidate the underlying molecular mechanisms. Many studies have shown that circRNA are potent sponges for miRNA, leading to loss of function that affects the expression of downstream target genes [20, 21]. We found that circATP5SL contained multiple miRNA binding sites through analysis using Circinteractome (https://circinteractome.nia.nih.gov/) and miRanda 2010 (http://www.microrna.org/microrna/getDownloads.do). By intersecting these two databases (**Figure 3A**), we found that circATP5SL was able to bind to

four miRNAs, namely miR-210, miR-198, miR-873-5p, and miR-330-5p. By overexpressing circATP5SL in HemECs and HUVECs, only miR-873-5p was significantly downregulated with no effects on the expression levels of other miRNAs (**Figure 3B**).

To further confirm the existing association between circATP5SL and miR-873-5p, we designed wildtype and mutant plasmids of circATP5SL and performed a double luciferase gene reporting experiment (**Figure 3C**). The results of the study revealed that the co-transfection of miR-873-5p mimics and circATP5SL wildtype significantly reduced the luciferase activity while no significant change in luciferase activity was observed after being co-transfected with circATP5SL mutant plasmids (**Figure 3D**), indicating that miR-873-5p and circAT-P5SL could interact. We also performed RIP experiments and observed that Ago2 was able to enrich miR-873-5p and combine with circAT-P5SL (**Figure 3E**), further indicating that there was an interaction between the two. On the contrary, the knockdown of circATP5SL verified that miR-873-5p was found with a significantly higher expression level as shown (**Figure 3F**). miR-873-5p expression was significantly downregulated in 20 sets of fresh haemangioma tissue and matching adjacent tissues (**Figure 3G**). Moreover, the statistical analysis identified a completely negative correlation between circATP5SL and miR-382-5p in these samples (**Figure 3H**).

IGF1R was a direct target of miR-873-5p

It is evident from several studies that IGF1R plays a key role in promoting the migration and proliferation of hemangioma [22, 23]. MiRNAs can act on the 3' untranslated region (UTR) of mRNA to regulate gene expression. We explored the target genes downstream of miR-873-5p through database analysis. We established that IGF1R was a potential target gene of miR-873-5p and through miRanda 2010 analysis, we determined that miR-873-5p had a binding site for IGF1R (**Figure 4A**). Based on its binding site, we designed a wildtype and mutant plasmid of IGF1R 3' UTR, which was combined with miR-873-5p mimics and co-transfected into HemECs. Double luciferase gene reporter showed that wildtype IGF1R 3' UTR reduced luciferase activity while mutant plasmids had
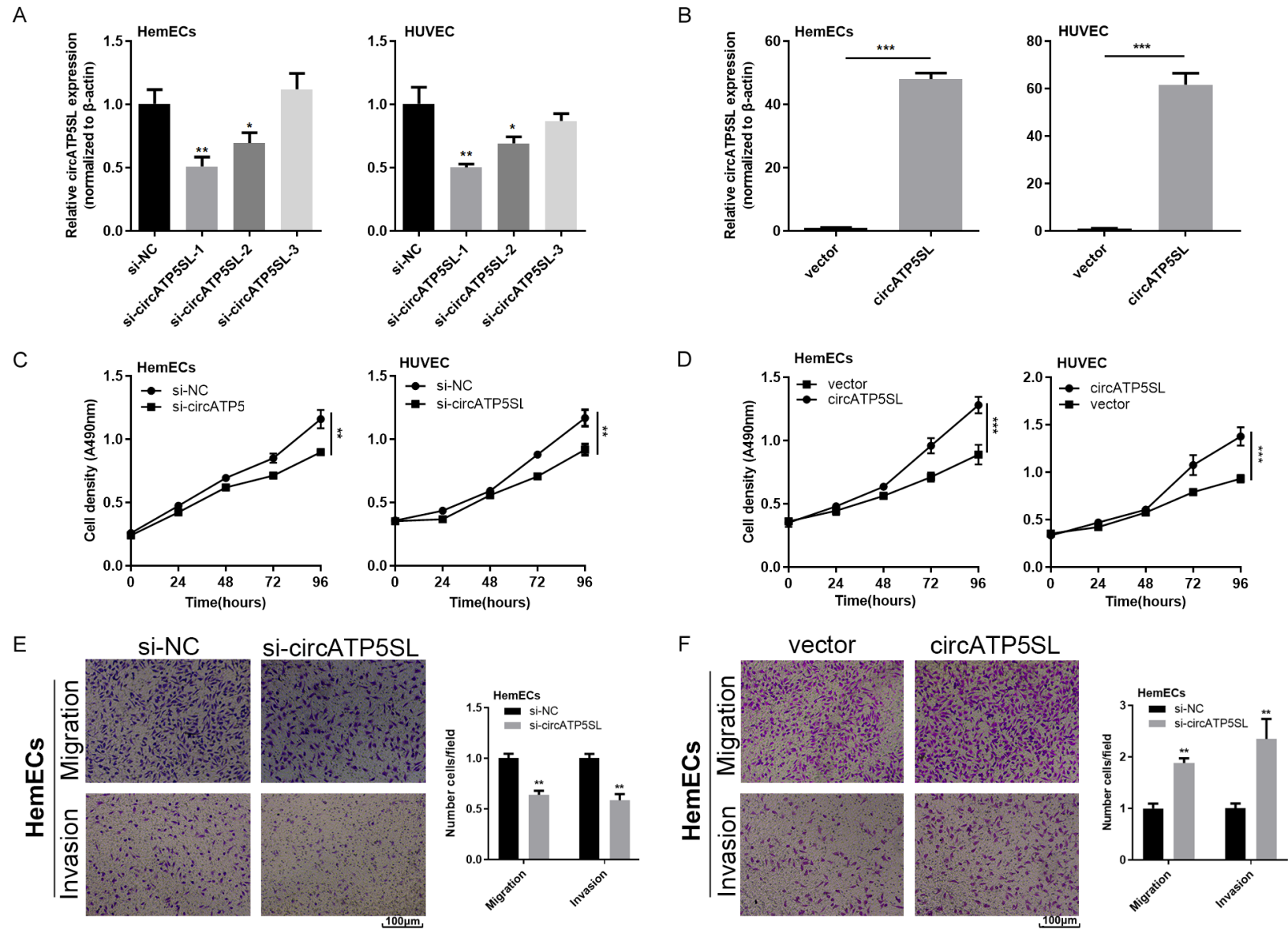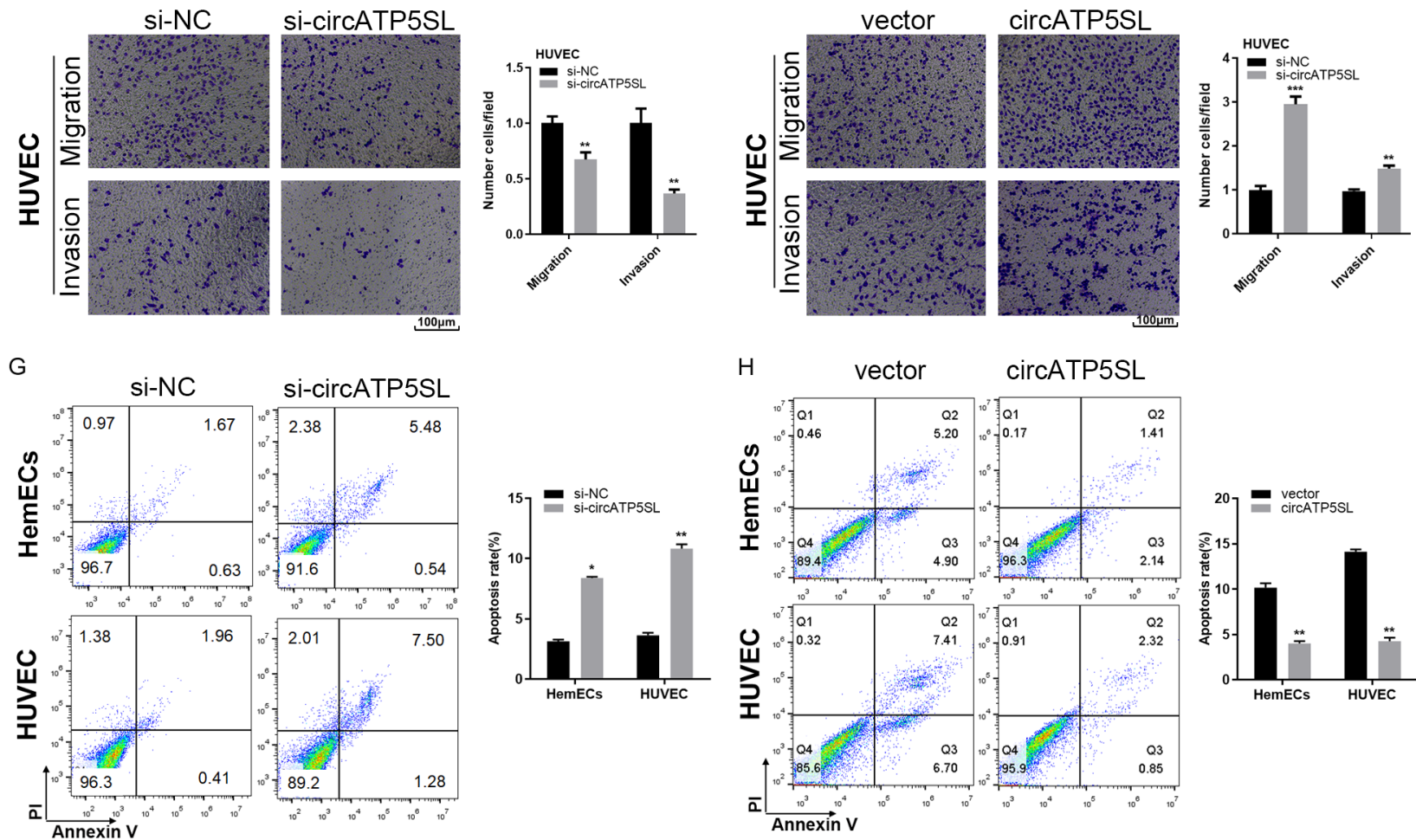
Figure 2. circATP5SL promoted the proliferation, invasion, and migration of haemangioma cells and inhibited apoptosis. A. qRT-PCR determination of circATP5SL expression in HemECs and HUVECs transfected with gene-specific siRNAs. B. qRT-PCR assay of circATP5SL expression in HemECs and HUVECs transfected with circATP5SL plasmid. C, D. The MTT analyses were performed to evaluate the viability of haemangioma cells treated with si-circATP5SL or circATP5SL plasmid in HemECs and HUVECs cells. E, F. The invasion and migration ability of HemECs and HUVECs cells treated with si-circATP5SL or circATP5SL plasmid were evaluated by the transwell assays. G, H. The apoptotic ability of HemECs and HUVECs cells treated with si-circATP5SL or circATP5SL plasmid were evaluated by the flow cytometry assays. *$P<0.05$, **$P<0.01$, ***$P<0.001$.
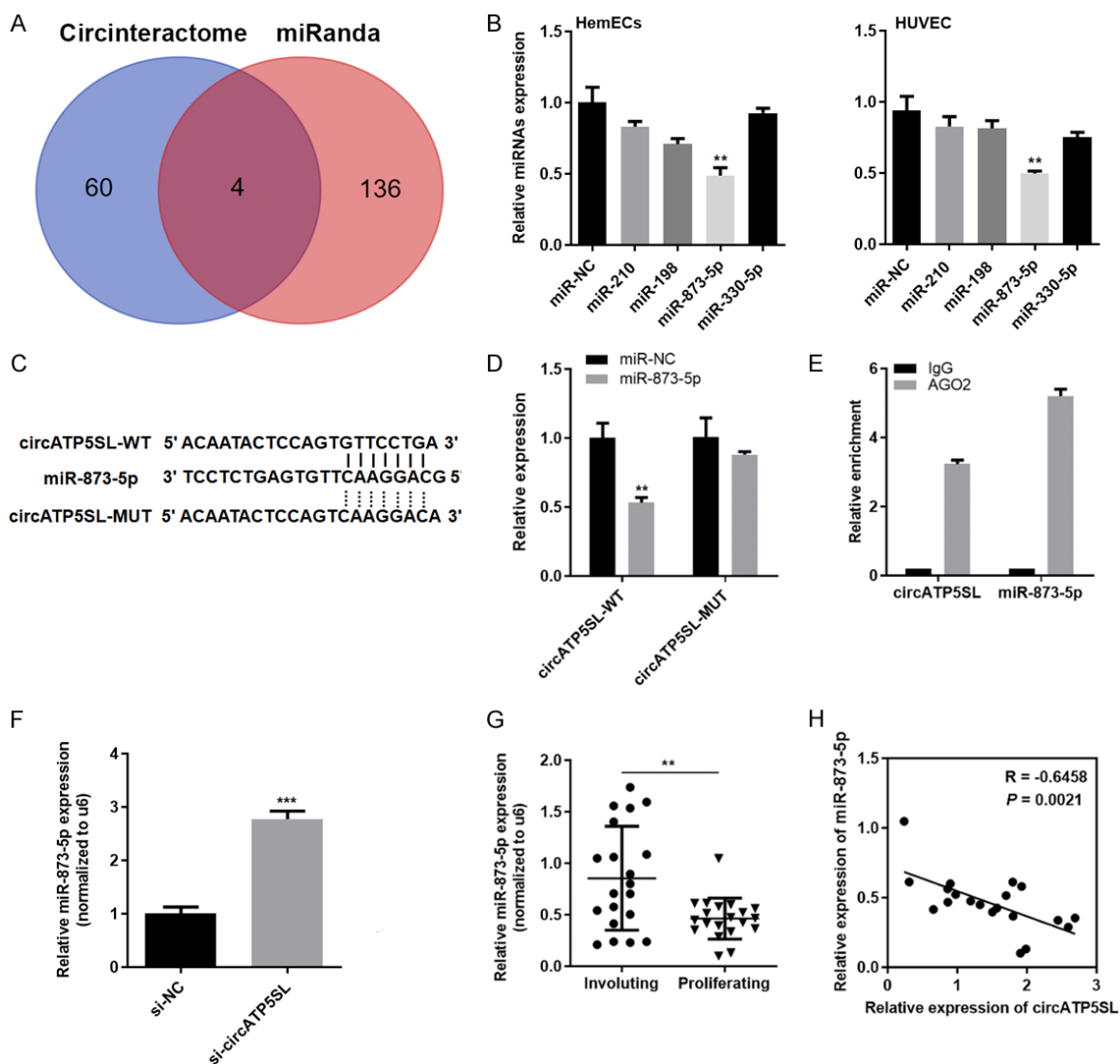
Figure 3. circATP5SL targeted miR-873-5p. A. Venn diagram showing overlapping of the target miRNAs of circATP5SL predicted by Circinteractome and miRanda. B. The relative level of 4 miRNA candidates and miR-NC in HemECs and HUVECs detected by qRT-PCR. C. A schematic of wildtype and mutant circATP5SL luciferase reporter vectors. D. Luciferase activity of wildtype or mutant circATP5SL after transfection with miR-873-5p mimics in 293T cells. E. Expression of circATP5SL and miR-873-5p in HemECs cells after the pull-down of Ago2 by RIP assay. F. circATP5SL knockdown promoted miR-873-5p expression. G. Increased expression of miR-873-5p in haemangioma tissue. H. Correlation between circATP5SL and miR-873-5p expression. All experiments were conducted three times in triplicate. **P<0.01, ***P<0.001. miR-NC, miRNA mimics as a negative control.

almost no effect (**Figure 4B**). Further experiments displayed that the IGF1R expression was greatly promoted with the transfection of miR-873-5p inhibitor (**Figure 4C**). We overexpress IGF1R in haemangioma cells and check its expression by Western blot (**Figure 4D**). Also, a rescue experiment was conducted with co-transfection of miR-873-5p mimics and IGF1R overexpressed plasmid to evaluate further the

effects of IGF1R and miR-873-5p mutual interaction towards the hemangioma cells proliferation. The overexpression of IGF1R reversed the influence of miR-873-5p by inhibiting proliferation in haemangioma cells (**Figure 4E**, **4F**). We then measured the expression of IGF1R in haemangioma tissue by qRT-PCR and observed that IGF1R was negatively correlated with miR-873-5p and positively correlated with circAT-
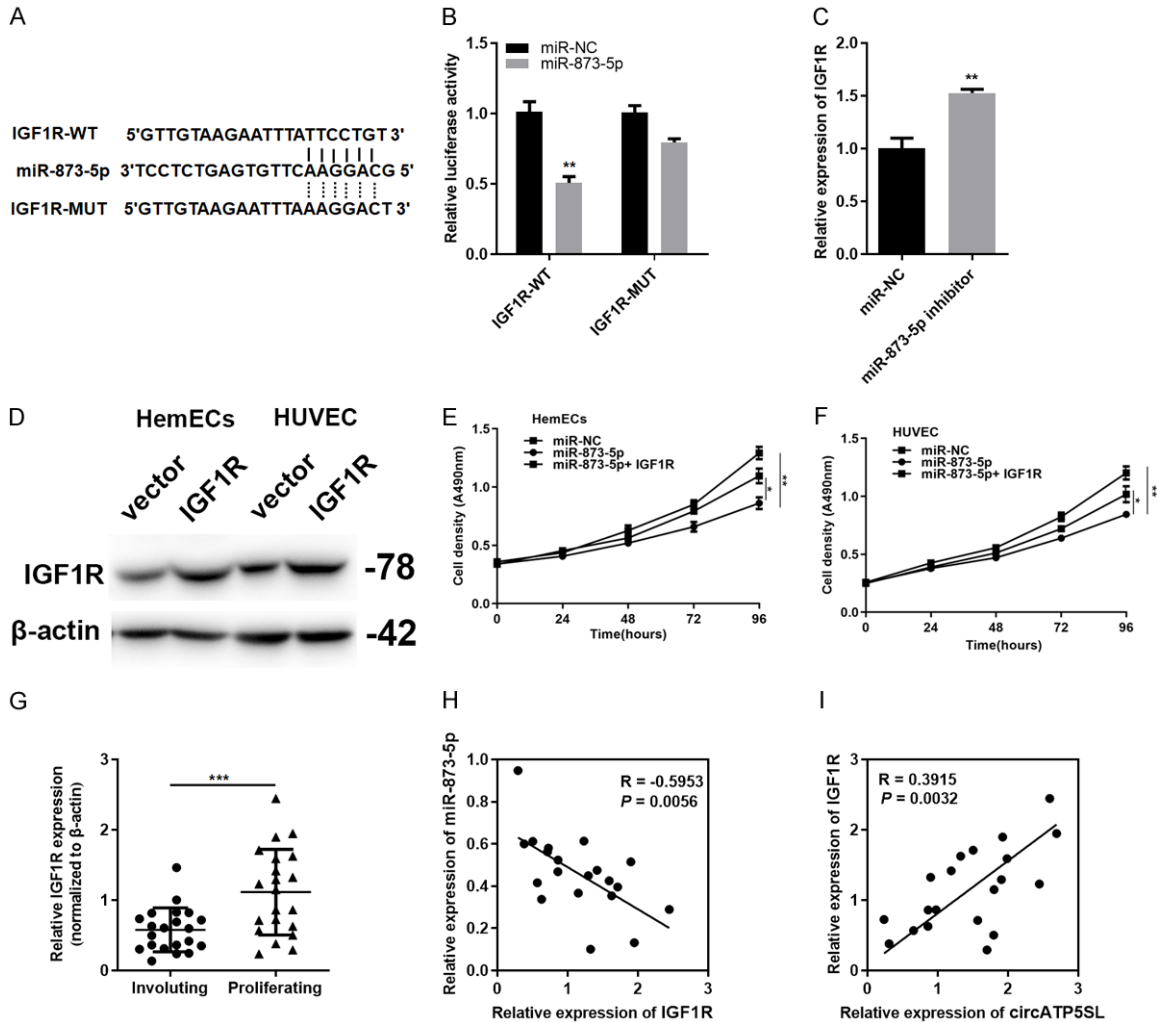
Figure 4. IGF1R was a direct target of miR-873-5p. A. A schematic of wildtype and mutant IGF1R luciferase reporter vectors. B. The luciferase activity of wildtype or mutant IGF1R after being transfected with miR-873-5p mimics in 293T cells. C. miR-873-5p knockdown promoted IGF1R expression. D. Over-expression of IGF1R in haemangioma cells significantly up-regulates the expression of IGF1R by Western blot analysis. E, F. MTT assay of cell proliferation in HemECs and HUVECs after being transfected with miR-873-5p mimics combined with or without IGF1R at the indicated time points. G. High expression of IGF1R in haemangioma tissue. H. Correlation between IGF1R and miR-873-5p expression. I. Correlation between circATP5SL and IGF1R expression. All experiments were conducted three times in triplicate. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

P5SL (**Figure 4G-I**). These results indicate that circATP5SL affected the expression level of IGF1R by sponge adsorption miR-873-5p.

Overexpression of circATP5SL reversed miR-873-5p inhibitory effect on haemangioma cells

To further verify that circATP5SL promoted the progression of haemangioma through the regulation miR-873-5p and subsequent IGF1R expression, we performed a rescue experiment by co-transfection of miR-873-5p mimics and circATP5SL overexpression plasmid. Overexpression of circATP5SL reversed the role of

miR-873-5p on suppressing invasion, proliferation, migration, and promoting apoptosis in haemangioma cells (**Figure 5A-D**). These results indicate that circATP5SL promoted the progression of haemangiomas by controlling the axis of miR-873-5p/IGF1R.

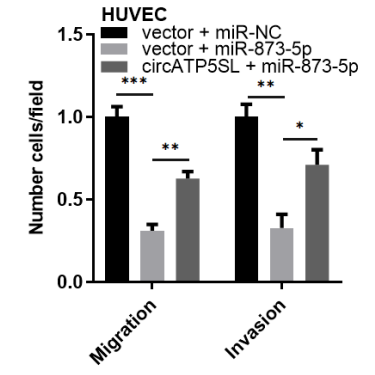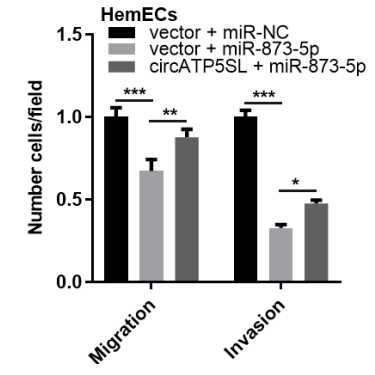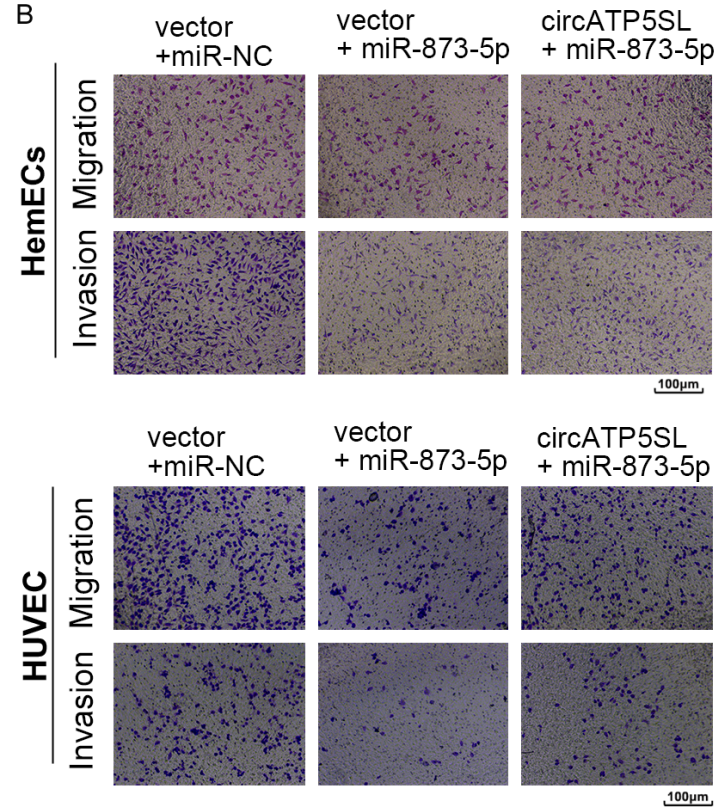*circATP5SL promoted haemangioma xenograft growth*

To provide evidence for the role of circATP5SL in regulating tumor growth *in vivo*, HemECs were transfected with circATP5SL knockdown or circATP5SL overexpression virus vector. 1 ×
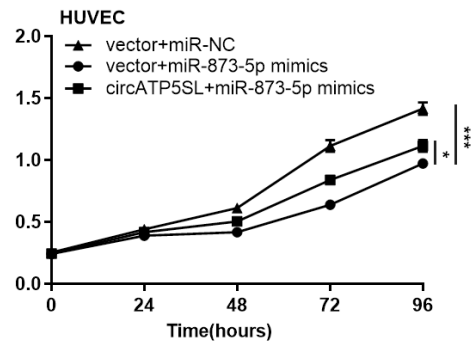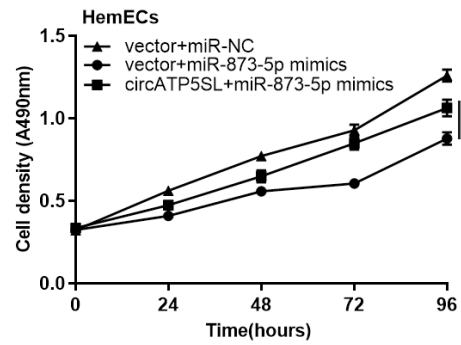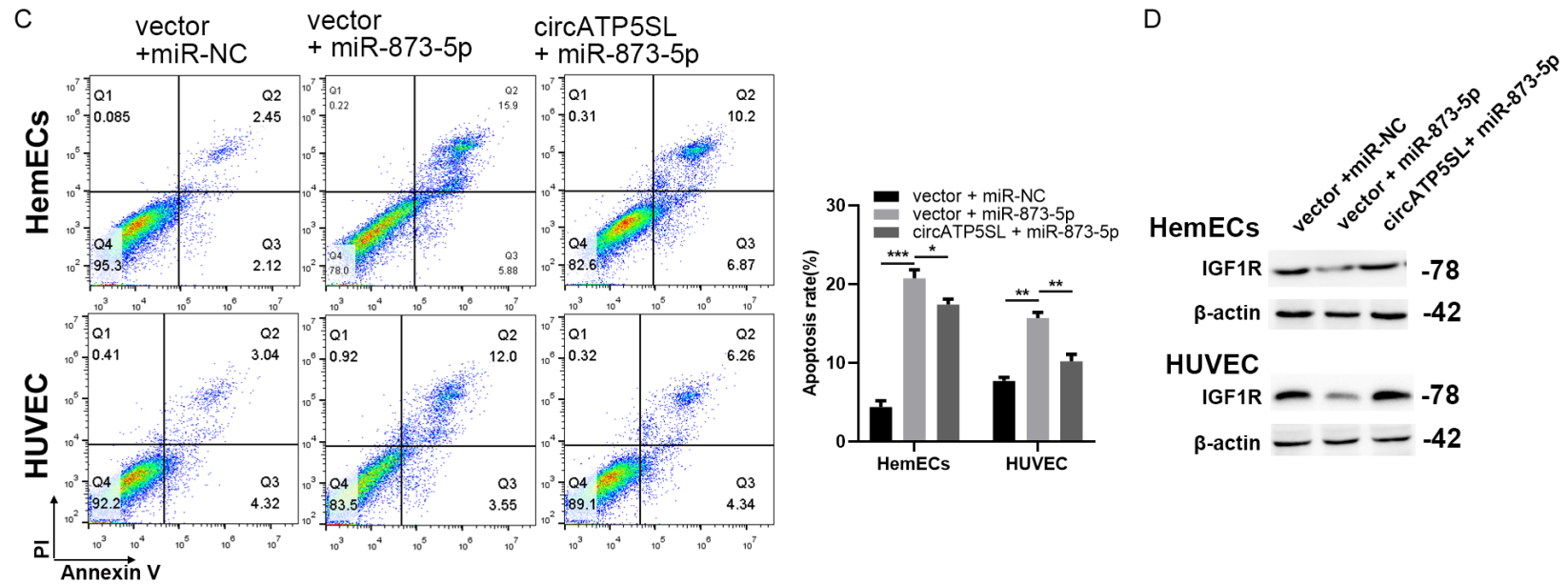
Figure 5. Overexpression of circATP5SL reversed miR-873-5p inhibition of cell proliferation, invasion, and migration and promotion of apoptosis. A. MTT assay of cell proliferation in HemECs and HUVECs after being transfected with miR-873-5p mimics combined with or without circATP5SL at the indicated time points. B. Cell migration and invasion. C. Cell apoptosis. D. Western blot. All experiments were conducted three times in triplicate. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

10^7 cells were injected under the skin of nude mice to establish a subcutaneous tumor model. We found that low expression of circATP5SL inhibited the growth of HemECs *in vivo* and conversely, overexpression of circATP5SL promoted its growth (**Figure 6A-I**).

Discussion

Recently, several studies have discovered that circRNAs are extensively associated with the regulation of tumors and heart disease while also playing an important role in neurodevelopment, immunity, and other processes [24-26]. In recent studies, it has been shown that there are a large number of dysregulated circRNAs molecules in IH tissues, and also bioinformatic analyses predict that they may regulate the occurrence of IH through multiple pathways [2]. However, their biological function in IH is still unknown. The present study evaluated the expression of circATP5SL in our cohort of human IH samples and the results indicated that circATP5SL was upregulated in proliferating IH compared to normal tissues, suggesting that circATP5SL may be related to the development of IH.

We further analyzed the molecular mechanism of circATP5SL. The data revealed that circATP5SL was predominantly expressed in the cytoplasm of HemECs and HUVEC, thus we speculate that circATP5SL may directly target some miRNAs in IH. Through database prediction, we identified that circATP5SL and miR-873-5p possess potential binding sites. Recently, some studies have already demonstrated miR-873-5p as a tumor suppressor in multiple cancer types. miR-873-5p is significantly suppressed in colorectal cancer cell lines and tissues and is strongly connected with poor survival [6]. In gastric cancer, knockdown of miR-873-5p profoundly promotes cell migration proliferation and colony formation [27]. In colorectal cancer, miR-873-5p directly targets TUSC3 and helps to inhibit its progression [28]. Following these findings, our study observed that miR-873-5p was also suppressed in IH and was inversely related to circATP5SL expression. Next, using double luciferase and RIP experiments, we demonstrated that circATP5SL bound to and interacted with miR-873-5p, suggesting that circATP5SL was able to sequester miR-873-5p. Therefore, we propose that circATP5SL promotes the proliferation of tumor cells through adsorption of miR-873-5p.

We also elucidated the potential mechanism of circATP5SL/miR-873-5p-mediated IH progression. Bioinformatics predicts that IGF1R is a downstream target of miR-873-5p. Studies have confirmed that IGF1R is expressed in many types of cancers such as colon cancer [29], breast cancer [30], lung cancer [31], hepatocellular carcinoma [32], gastric cancer [33], and renal cell carcinoma [34]. IGF1R intracellular signaling is mediated by IGF-1 and can activate the PI3K/AKT pathway and regulate the biological functions of tumor cells including cell cycle, proliferation, migration, invasion, and apoptosis. In IH, activation of IGF1R results in the upregulation of the protein expression level of peroxisome proliferator-activated receptor γ (PPARγ), thereby accelerating adipogenesis in haemangioma stem cells [23]. The results of our study revealed that miR-873-5p was inversely related to IGF1R expression in haemangioma samples, but also it was bound to the 3' UTR of IGF1R to inhibit IGF1R expression and function. In addition, we confirmed that IGF1R was a downstream target of the circATP5SL/miR-873-5p axis and circATP5SL promoted IGF1R expression through adsorbing miR-873-5p.

To deeply study the circRNA's regulatory mechanism *in vivo*, we used HemECs that expressed low or high levels of circATP5SL to establish a subcutaneous xenograft model in nude mice. Tumor-bearing mice in the circATP5SL overexpression group displayed more rapid tumor growth compared with mice in the control group, providing further evidence for the regulatory role of circATP5SL in haemangioma formation.

In summary, our research shows that circATP5SL competed with miR-873-5p to eliminate the inhibitory effect of miR-873-5p on IGF1R, which is leading to promote cellular invasion, proliferation, migration, and inhibition of apoptosis of haemangioma cells. Our findings provide new insights into the IH pathogenesis and new evidence for the treatment of IH.

Acknowledgements
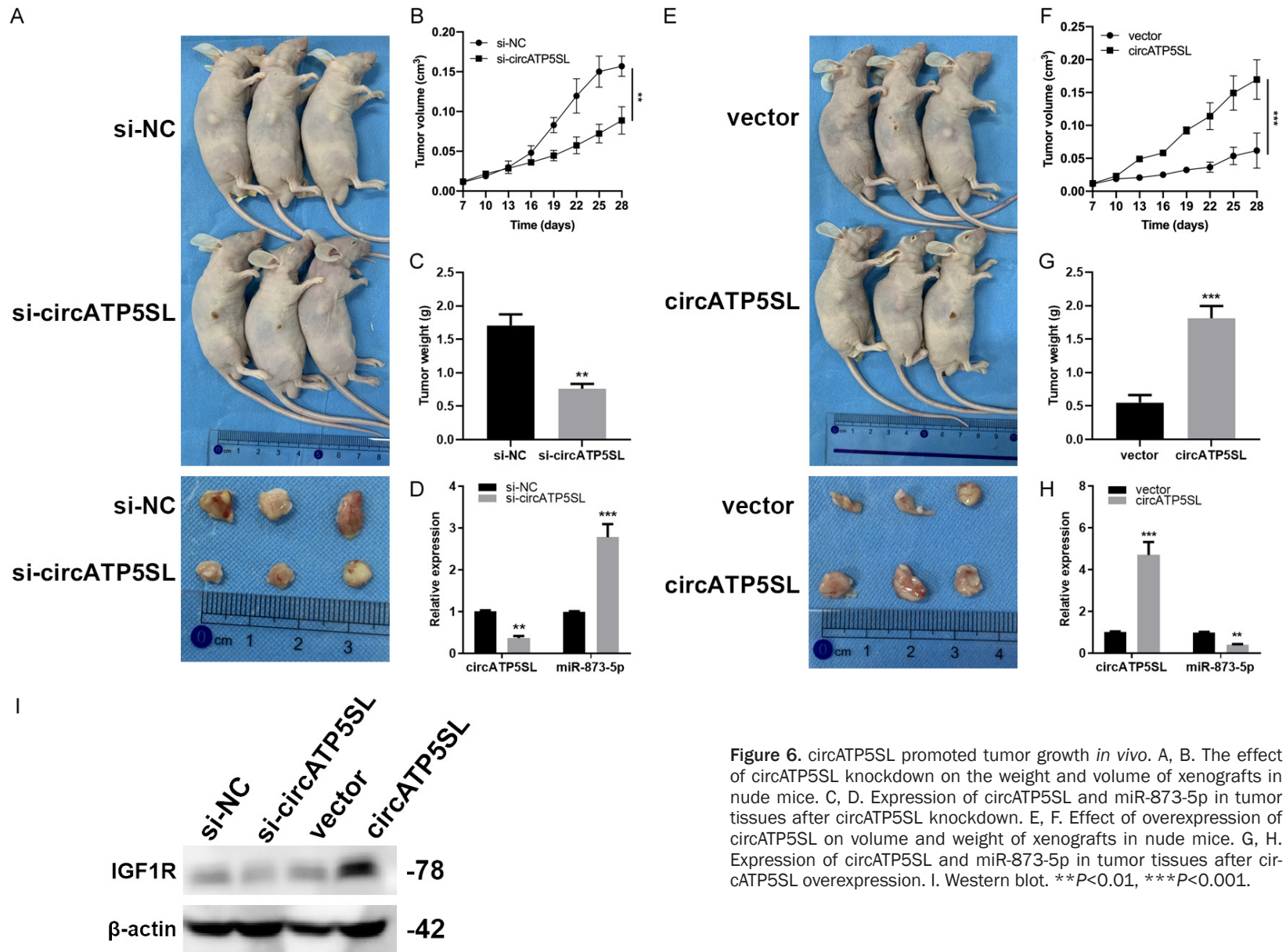
Figure 6. circATP5SL promoted tumor growth *in vivo*. A, B. The effect of circATP5SL knockdown on the weight and volume of xenografts in nude mice. C, D. Expression of circATP5SL and miR-873-5p in tumor tissues after circATP5SL knockdown. E, F. Effect of overexpression of circATP5SL on volume and weight of xenografts in nude mice. G, H. Expression of circATP5SL and miR-873-5p in tumor tissues after circATP5SL overexpression. I. Western blot. **$P<0.01$, ***$P<0.001$.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Primer sequences

| Gene	Sequence (5'→3')
circATP5SL	Sense: 5'-ACACGCTGTCCTTACACTGG-3'
	Anti-sense: 5'-CTTCTGATTGCCCTCTGGGG-3'
ATP5SL	Sense: 5'-ACACGCTGTCCTTACACTGG-3'
	Anti-sense: 5'-CTTCTGATTGCCCTCTGGGG-3'
β-actin	Sense: 5'-CACCAGCATCTTTTCCAACC-3'
	Anti-sense: 5'-AAGGCCGACTCTCCTACACA-3'
U6	Sense: 5'-CGAGCACAGAATCGCTTCA-3'
	Anti-sense: 5'-CTCGCTTCGGCAGCACATAT-3'
IGF1R	Sense: 5'-CCGACGAGTGGAGAAATC-3'
	Anti-sense: 5'-AGCAGGATGTGGAGGTAG-3'

Supplementary Table 2. RNA oligonucleotide sequences

Gene	Sequence (5'→3')
miRNA-NC	Sense: 5'-UUCUCCGAACGUGUCACGUTT-3'
	Anti-sense: 5'-ACGUGACACGUUCGGAGAATT-3'
sicircATP5SL-1	Sense: 5'-GCUGUCCUGUACCUCCUCCCUTT-3'
	Anti-sense: 5'-AGGGAGGAGGUACAGGACAGCTT-3'
sicircATP5SL-2	Sense:5'-UGUACCUCCUCCCUGCGCCUTT-3'
	Anti-sense: 5'-AGGCGCAGGGAGGAGGUACATT-3'
sicircATP5SL-3	Sense:5'-UACCUCCUCCCUGCGCCUGGUTT-3'
	Anti-sense: 5'-ACCAGGCGCAGGGAGGAGGUATT-3'
miR-873-5p mimics	Sense:5'-GCAGGAACUUGUGAGUCUCCU-3'
	Anti-sense: 5'-AGGAGACUCACAAGUUCCUGC-3'
miR-873-5p inhibitor	Sense:5'-AGGAGACUCACAAGUUCCUGC-3'
	Anti-sense: 5'-GCAGGAACUUGUGAGUCUCCU-3'

1
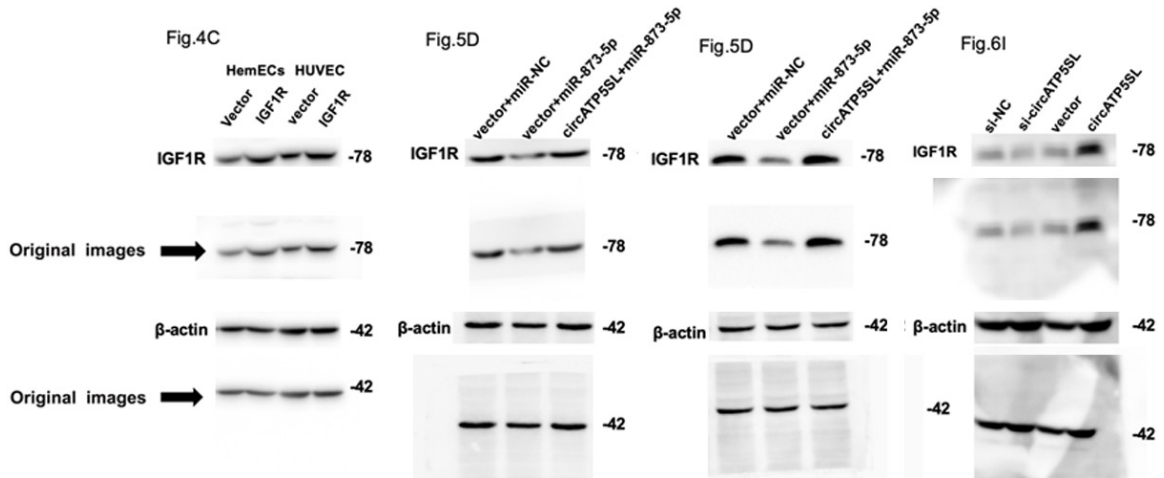
Supplementary Figure 1. Original images of Western blot.