


# Inhibiting Interleukin-6/Signal Transducers and Activators of Transduction-3/Hypoxia-Inducible Factor-1 $\alpha$ Signaling Pathway Suppressed the Growth of Infantile Hemangioma

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## Abstract

**Objective** This study aims to evaluate the expression of interleukin 6 (IL-6) in patients with infantile hemangioma (IH) and investigate the role of the IL-6/signal transducers and activators of transduction-3 (STAT3)/hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) pathways in the progression of IH.

**Methods** Serum samples were obtained from the patients with IH and normal infants to measure IL-6 expression. Hemangioma-derived stem cells (HemSCs) were transfected with small interfering RNA (siRNA) targeting IL-6, HIF-1 $\alpha$ , or STAT3. Then, cell viability and wound healing assays were conducted. After that, the HemSC tumor mouse model was established. The in vivo anticancer effect of the IL-6 inhibitor was investigated.

**Results** The patients with IH had much higher IL-6 levels compared with the healthy controls ( $p = 0.005$ ). HemSCs transfected with IL-6 siRNA had significantly lower viability and migration rates than normal HemSCs. HemSCs transfected with STAT3 siRNA or HIF-1 $\alpha$  siRNA had similar tendencies. On tumor-bearing mice, the IL-6 inhibitor treatment significantly delayed tumor growth. Compared with the control group, caspase-3 was significantly increased in the IL-6 inhibitor group ( $p < 0.05$ ), whereas Ki-67 was decreased in the IL-6 inhibitor group ( $p < 0.05$ ). In the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, the IL-6 inhibitor group had much higher apoptosis rates than the controls ( $p < 0.05$ ).

**Conclusion** Our findings indicate that inhibiting the IL-6/STAT3/HIF-1 $\alpha$  signaling pathways could suppress IH growth.

## Keywords

- ▶ infantile hemangioma
- ▶ signal transducers and activators of transduction-3
- ▶ hypoxia inducible factor-1 $\alpha$
- ▶ apoptosis
- ▶ interleukin 6

\* These authors have contributed equally to this study and retain the first authorship.

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## Introduction

Infantile hemangioma (IH) is a common vascular tumor during infancy caused by endothelial cell (EC) proliferation.<sup>1</sup> It often appears on the head or face at approximately 2 weeks of age.<sup>2</sup> The tumor rapidly proliferates within 6 to 10 months and slowly regresses over several years.<sup>1</sup> Although IH is a benign tumor, it can cause facial deformation and vision obstruction and be life-threatening.<sup>3</sup> Thus, studying the mechanisms of IH and finding the appropriate treatment strategies are necessary.

It has been reported that many cytokines were elevated in patients with IH, including interleukin 6 (IL-6).<sup>2,4</sup> IL-6 is a pleiotropic cytokine secreted by various cell types, such as immune and tumor cells, and plays an important role in tumor growth.<sup>5</sup> The IL-6-related pathways were reported to be associated with epithelial-mesenchymal transition<sup>6</sup> and immune reaction in tumor microenvironments.<sup>7</sup> Furthermore, the role of the IL-6 signaling pathway on angiogenesis has also been previously studied. Zegeye et al found that IL-6 could regulate the sprouting angiogenic function of endothelial cells (ECs).<sup>8</sup> Hegde et al demonstrated that IL-6 induced the expression of vascular endothelial growth factor receptor 2 in ECs, leading to angiogenesis in breast cancer.<sup>9</sup> Signal transducers and activators of transduction-3 (STAT3) are important downstream molecules of IL-6. After being phosphorylated by IL-6, STAT3 binds to the promoter of the gene encoding the vascular endothelial growth factor, stimulating the formation of tumor blood vessels.<sup>10</sup> Thus, activating the IL-6/STAT3 pathways was found to facilitate angiogenesis,<sup>11</sup> while inhibiting these pathways was found to reduce angiogenesis and cancer cell progression.<sup>12</sup>

The initiation of IH was found to be related to the dysfunction of the balance between pro- and antiangiogenic signals.<sup>13</sup> As mentioned above, the IL-6-related signaling pathway plays a vital role in tumor angiogenesis. Thus, IL-6 may also participate in the formation and progression of IH. A study conducted by Fu et al found that IL-6 was significantly increased in hemangioma cells compared with normal ECs.<sup>13</sup> And inhibiting IL-6 could suppress the invasion and proliferation of hemangioma cells.<sup>13</sup> However, the definite role of IL-6 in the progression of IH has not been determined.

In this study, we aim to evaluate the expression of IL-6 in patients with IH and investigate the role of the IL-6/STAT3/hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) pathways in the progression of IH.

## Materials and Methods

### Patients

Serum samples were obtained from 15 patients with IH. Control serum samples were obtained from 15 sex- and age-matched normal infants. Of the 15 patients with IH, the mean (standard deviation [SD]) age was 50.2 (9.8) days; 60% (9/15) were females. The IHs were located on the head and/or neck ( $n = 10$ , 66.7%) or at other body sites ( $n = 5$ , 33.3%). The IHs were localized, segmental, or indeterminate in 11 (73.3%), 3 (20.0%), and 1 (6.7%) patients, respectively. No patient re-

ceived treatment before the serum samples were collected. Of the 15 normal infants, the mean (SD) age was 49.5 (10.2) days; 60% (9/15) were females. All collected serum samples were immediately stored in liquid nitrogen. The human samples were obtained with written informed consent from the guardians of the children, according to the Declaration of Helsinki. The Ethics Committee of Children's Hospital of Xinjiang Uygur Autonomous Region approved this study.

### Cell Culture

Hemangioma-derived stem cells (HemSCs) were obtained from Procell Life Science&Technology Co., Ltd. (Wuhan, China) and maintained in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco, Grand Island, New York, United States), accompanied by 10% fetal bovine serum and 1% antibiotic-antimycotic penicillin-streptomycin solution (Sangon Biotech, Shanghai, China) at 37°C and 5% CO<sub>2</sub>.

### Cell Transfection

Three small interfering RNAs (siRNA) targeting IL-6 (IL-6 siRNA1, IL-6 siRNA2, and IL-6 siRNA3), three siRNAs targeting STAT3 (STAT3 siRNA1, STAT3 siRNA2, and STAT3 siRNA3), three siRNAs targeting HIF-1 $\alpha$  (HIF-1 $\alpha$  siRNA1, HIF-1 $\alpha$  siRNA2, and HIF-1 $\alpha$  siRNA3), and the nontargeting control siRNAs (si-NC) were obtained from GenePharma (Shanghai, China). The HemSCs were seeded in 24-well plates. The aforementioned plasmids were transfected into HemSCs using Lipofectamine 2000 (Invitrogen, Carlsbad, California, United States) according to the manufacturer's instructions.

### Cell Viability Assay

Cell viability was assessed by a Cell Counting Kit-8 (CCK-8) assay. The HemSCs were seeded at a density of  $5 \times 10^4$  cells/mL and cultured for 24 hours. Then, the HemSCs were transfected with si-NC, IL-6 siRNA, STAT3 siRNA, and HIF-1 $\alpha$  siRNA. After being cultured for 24, 48, and 72 hours, the cells in each well were incubated with a CCK-8 solution at 37°C for 1 hour. Then, the absorbance of each well was detected by a microplate reader (BioTek, Winooski, Vermont, United States) at 450 nm.

### Wound Healing Assay

After transfection with si-NC, IL-6 siRNA, STAT3 siRNA, and HIF-1 $\alpha$  siRNA, the HemSCs were seeded in six-well plates at  $10^7$  cells/well and incubated overnight. Wounds of 0.5 to 1.0 cm were produced using sterile 200  $\mu$ L plastic pipette tips. Cells were further cultured with medium for 48 hours. A microscope was used to acquire images (Olympus, Japan) at  $4 \times 40$  magnification.

### Quantitative Real-Time Polymerase Chain Reaction

Total RNAs were extracted from the patients' serum samples and cells using TRIzol reagent (Invitrogen). RNA was reverse-transcribed using the cDNA Synthesis Kit (TaKaRa Bio, San Jose, CA, United States). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed with an ABI 7500 real-time PCR System (Applied Biosystems, USA). The relative gene expression was calculated using the 2<sup>- $\Delta\Delta$ Ct</sup> method. The

primers used are as follows: HIF-1 $\alpha$ , 5 $\epsilon$ -AAGTTCACCT-GAGCCTAAT-3 $\epsilon$  (forward primer) and 5 $\epsilon$ -ATGGGTCTTTG-TTCTGT-3 $\epsilon$  (reverse primer); IL-6, 5 $\epsilon$ - TTCGGTCCAG-TTGCTTCT-3 $\epsilon$  (forward primer) and 5 $\epsilon$ - GTGCCTCTTT-GCTGCTTC-3 $\epsilon$  (reverse primer); STAT3, 5 $\epsilon$ - TTTTGTGAGC-GATGGAGTA-3 $\epsilon$  (forward primer) and 5 $\epsilon$ - TTGTTG-ACGGTCTGAAGT-3 $\epsilon$  (reverse primer); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5 $\epsilon$ -AGA-AGGCTGGGCTCATTTG-3 $\epsilon$  (forward primer) and 5 $\epsilon$ -AGGGGCCATCCACAGTCTTC-3 $\epsilon$  (reverse primer).

### Western Blotting

The proteins extracted from the cells were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred onto polyvinylidene fluoride membranes (Millipore, Massachusetts, United States). After being blocked for 1 hour, the blots were incubated with primary antibodies overnight at 4°C. Antibodies against HIF-1 $\alpha$  (1:1,000, cell-signaling technology, Danvers, Massachusetts, United States), IL-6 (1:1,000, cell-signaling technology), STAT3 (1:1,000, cell-signaling technology), and GAPDH (1:3,000, cell-signaling technology) were incubated. Then, the membranes were incubated with antirabbit or antimouse immunoglobulin G (IgG)-Horseradish Peroxidase (HRP)-conjugated secondary antibodies (1:2,000, cell-signaling technology) for 1 hour at room temperature. The ECL Chemiluminescence Detection System (Thermo Fisher Scientific, Rochester, New York, United States) was applied to visualize protein bands.

### Tumor Growth Assay

The IL-6 inhibitor, corylifol A<sup>14</sup> (purity >98%), was purchased from Shanghai Winherb Medical Technology Co., Ltd (Shanghai, China).

A 4-week-old male nu/nu athymic nude mice (Cavens Biotechnology, Changzhou, China) were raised under specific pathogen-free conditions. A total of  $5 \times 10^5$  HemSCs were injected into the left armpit of the nude mice. The tumor sizes were measured by calipers (length and width) every 3 days. The tumor volumes were calculated with the formula  $V = a \times b^2 / 2$ , where  $a$  is the larger and  $b$  is the perpendicular shorter tumor axis. When the tumor volume reached 100 mm<sup>3</sup>, the mice were divided into two groups: (1) the IL-6 inhibitor group ( $n = 20$ ), and (2) the control group ( $n = 20$ ). Each mouse was treated with either 200  $\mu$ L of an IL-6 inhibitor (corylifol-A, 20.8 mg/mL) or the same volume of normal saline (control group) through intratumor injection on day 1. On days 7, 14, 21, and 27, four mice in each group were randomly chosen for tumor tissue collection. The Animal Care and Use Committee of Children's Hospital of Xinjiang Uygur Autonomous Region granted a project license to perform the animal experiments (grant no.: 2020101967).

### Immunohistochemistry

Formalin-fixed paraffin-embedded sections (5  $\mu$ M) from the tissues at different time points were incubated with rabbit antimouse primary antibodies directed against caspase-3 and K<sub>i</sub>-67 (cell-signaling technology) overnight at 4 $\epsilon$ C. Bio-

tinylated goat antirabbit anti-IgG (Wuhan Boster Biological Technology) was used as the secondary antibody. The proteins were visualized under a light microscope.

### Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling Staining

The cell apoptosis in each group was detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. The right amounts of reagent 1 (TdT) and reagent 2 (dUTP) in the TUNEL kit (Beyotime, Shanghai, China) were mixed at a ratio of 1:9 and added to cover the slices. After TUNEL staining, the cells were counterstained with 4–6-diamidino-2-phenylindole as a fluorescent tracer to detect the nuclei. TUNEL positive cell numbers were counted automatically using ImageJ software.

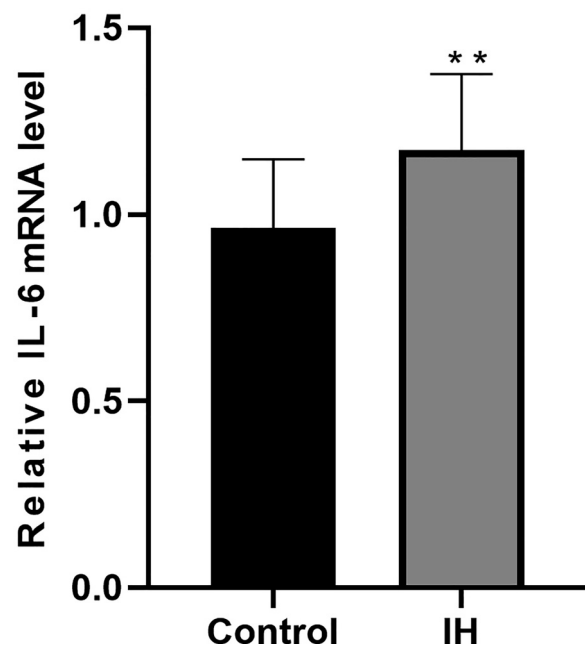
### Statistical Analysis

Data analyses were performed using the SPSS statistics 22.0 software (IBM Corp., Armonk, New York, United States). The continuous variables were expressed as mean  $\pm$  SD. The significance of the differences between groups was determined by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) or student's  $t$ -tests. A  $p$ -value of less than 0.05 was considered statistically significant.

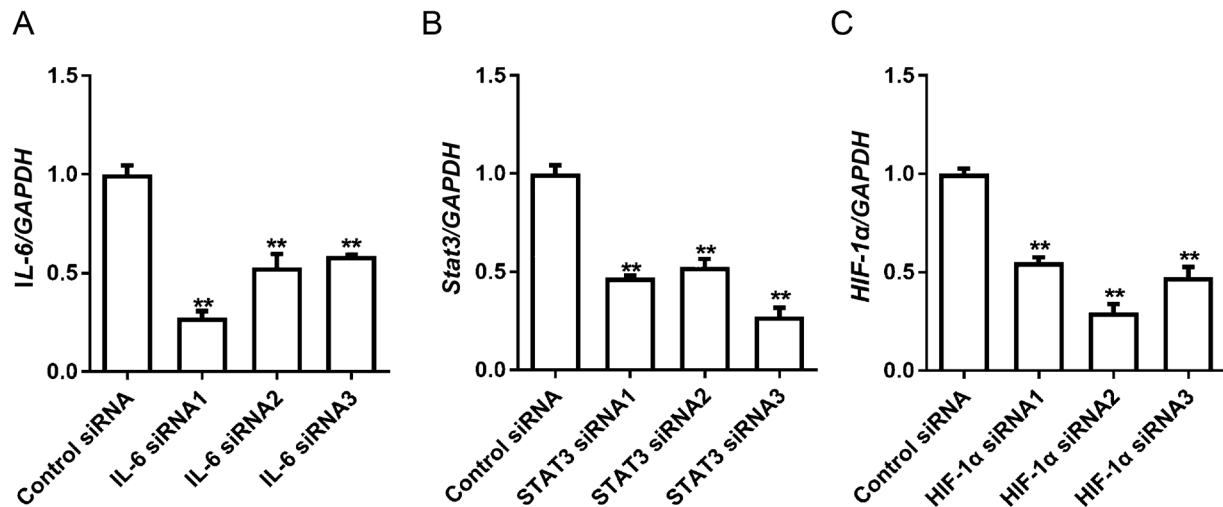
## Results

### The Expression of Interleukin-6 was Elevated in Patients with Infantile Hemangioma

The expressions of IL-6 in patients with IH and sex- and age-matched normal infants were detected by qRT-PCR. As shown in **Fig. 1**, patients with IH had much higher IL-6



**Fig. 1** IH patients had much higher IL-6 levels as comparing to the healthy controls. IH, infantile hemangioma. \*\* $p < 0.05$  versus control group. IH, infantile hemangioma; IL, interleukin; mRNA, messenger RNA.



**Fig. 2** HemSCs were transfected with siRNAs targeting IL-6 (A), STAT3 (B), or HIF-1 $\alpha$  (C), and the levels of IL-6, STAT3, or HIF-1 $\alpha$  were detected by qRT-PCR. \*\* $p < 0.05$  versus control group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HemSC, hemangioma-derived stem cell; HIF, hypoxia-inducible factor; IL, interleukin; qRT-PCR, quantitative real-time polymerase chain reaction; siRNAs, small interfering RNA; STAT3, signal transducers and activators of transduction-3.

levels compared with the healthy controls ( $1.17 \pm 0.20$  vs.  $0.96 \pm 0.18$ ,  $p = 0.005$ ).

**Knockdown of Interleukin-6, Hypoxia-Inducible Factor-1 $\alpha$ , or Signal Transducers and Activators of Transduction-3 Inhibited Hemangioma-Derived Stem Cells' Viability and Migration**

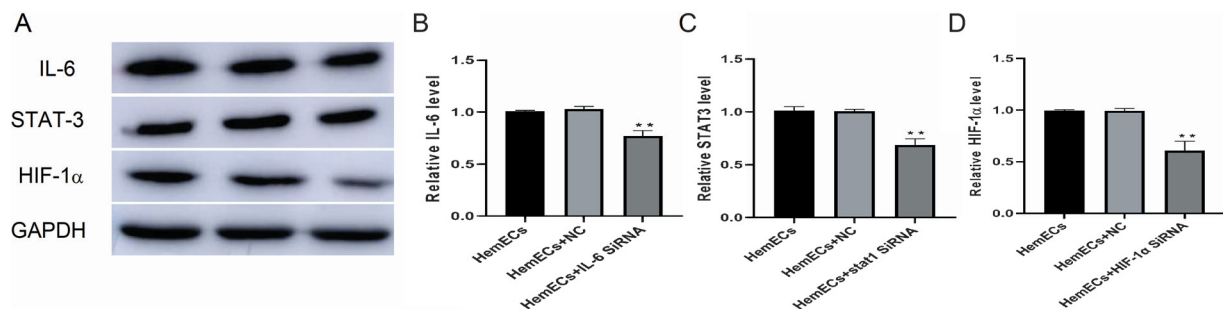
HemSCs were transfected with siRNAs targeting IL-6, HIF-1 $\alpha$ , or STAT3, and the levels were detected by qRT-PCR. As shown in **Fig. 2A**, compared with the control group, the levels of IL-6 significantly decreased in the IL-6 siRNA1, IL-6 siRNA2, and IL-6 siRNA3 groups ( $p < 0.05$ ). The IL-6 siRNA1 group had the lowest IL-6 level. The levels of HIF-1 $\alpha$  or STAT3 also decreased in the three HIF-1 $\alpha$  siRNA or STAT3 siRNA groups, respectively (**Fig. 2B and C**). And the STAT3 siRNA3 group had the lowest STAT3 level. The HIF-1 $\alpha$  siRNA2 group had the lowest HIF-1 $\alpha$  level. Thus, IL-6 siRNA1, STAT3 siRNA3, and HIF-1 $\alpha$  siRNA2 were chosen for further studies. Western blot analysis further confirmed that HemSCs transfected with IL-6 siRNA, STAT3 siRNA, or HIF-1 $\alpha$  siRNA had lower IL-6, STAT3, or HIF-1 $\alpha$  levels than normal HemSCs, respectively ( $p < 0.05$ , **Fig. 3**).

In the cell-viability assay, the HemSCs transfected with IL-6 siRNA had significantly lower viability than normal HemSCs after being cultured for 24, 48, and 72 hours ( $p < 0.05$ , **Fig. 4**). The HemSCs transfected with STAT3 siRNA or HIF-1 $\alpha$  siRNA had similar tendencies (**Fig. 4**).

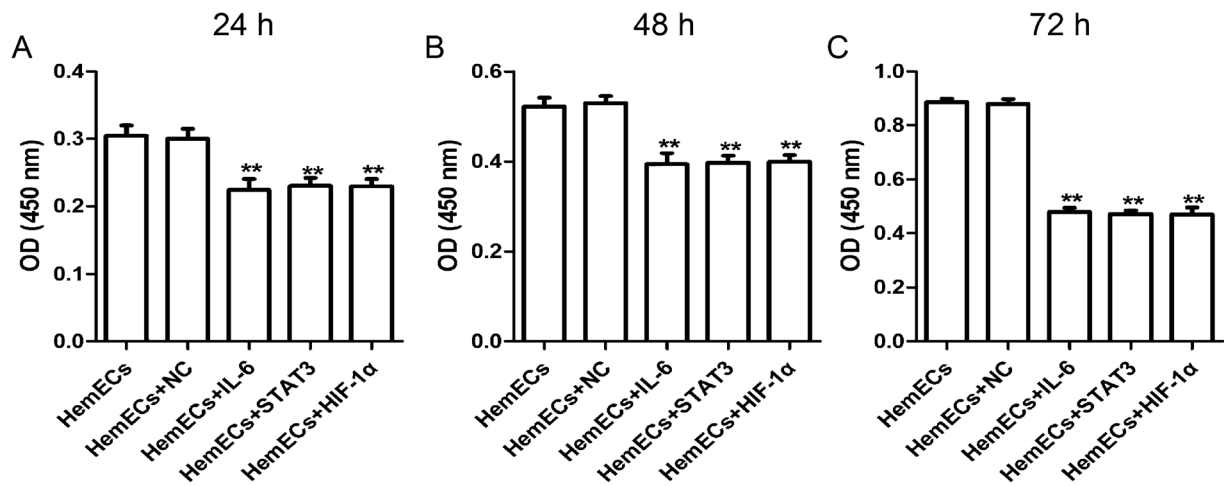
In the wound healing assay, the migration rate for the normal HemSCs was 100% after being cultured for 48 hours. But the migration rate for the HemSCs transfected with IL-6 siRNA ( $49.07 \pm 3.54$  vs.  $100.00$ ,  $p < 0.001$ ), STAT3 siRNA ( $44.98 \pm 3.91$  vs.  $100.00$ ,  $p < 0.001$ ), or HIF-1 $\alpha$  siRNA ( $43.15 \pm 1.02$  vs.  $100.00$ ,  $p < 0.001$ ) was significantly decreased compared with the normal HemSCs (**Fig. 5**).

**Interleukin-6 Inhibitors Hindered Hemangioma-Derived Stem Cells Growth In Vivo**

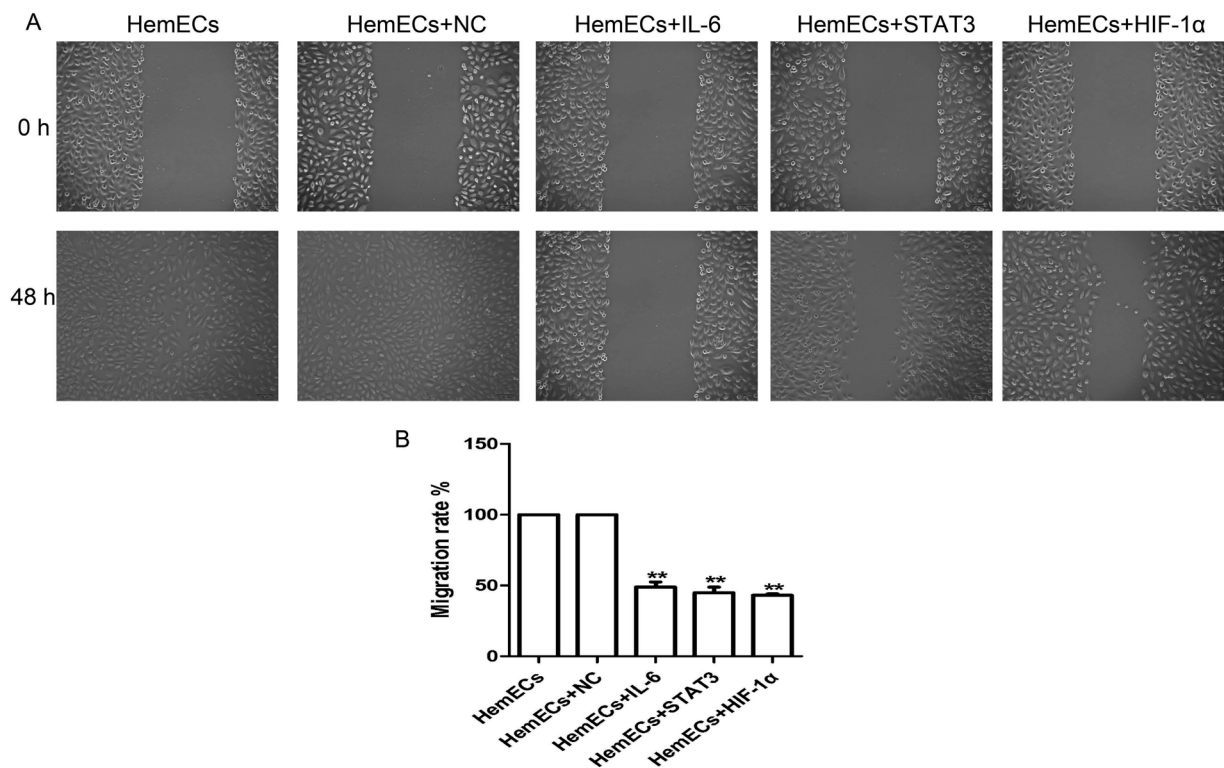
The HemSCs xenograft mouse models were created and randomly assigned to receive IL-6 inhibitors or normal saline (control). Our results indicated that the IL-6 inhibitors significantly delayed the tumor growth of the mice (**Fig. 6A and B**).



**Fig. 3** HemSCs were transfected with siRNAs targeting IL-6, STAT3, or HIF-1 $\alpha$  and the levels of IL-6, HIF-1 $\alpha$ , or STAT3 were detected by western blot. (A) The representative images; (B) the relative IL-6 expressions; (C) the relative STAT3 expressions; (D) the relative HIF-1 $\alpha$  expressions. \*\* $p < 0.05$  versus normal HemSCs group (control). HemSC, hemangioma-derived stem cell; HIF, hypoxia-inducible factor; IL, interleukin; qRT-PCR, quantitative real-time polymerase chain reaction; siRNAs, small interfering RNA; STAT3, signal transducers and activators of transduction-3.



**Fig. 4** HemSCs were transfected with si-NC, IL-6 siRNA, STAT3 siRNA and HIF-1 $\alpha$  siRNA, and the cell viability was measured at 24 hours (A), 48 hours (B), and 72 hours (C) after culture. \*\* $p < 0.05$  versus normal HemSCs group (control). HemECs, Hemangioma-derived stem cells; HIF, hypoxia-inducible factor; IL, interleukin; OD, optical density; si-NC, nontargeting control siRNA; siRNS, small interfering RNA; STAT3, signal transducers and activators of transduction-3.

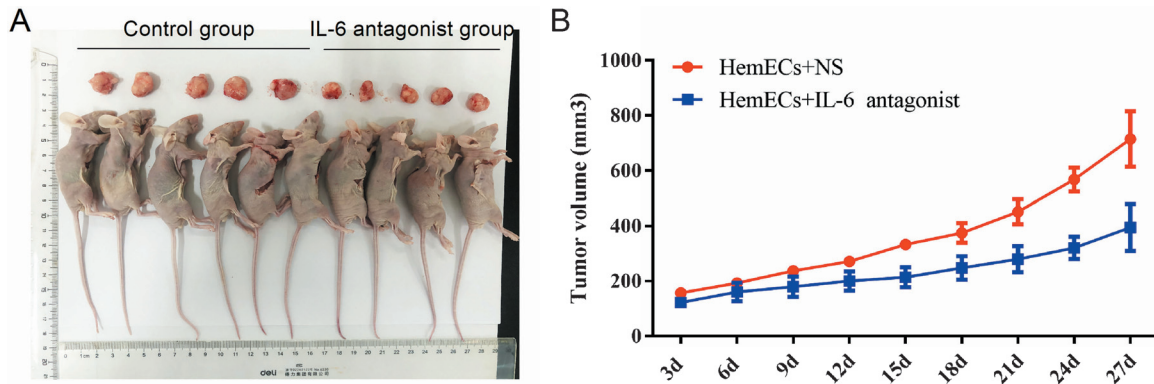


**Fig. 5** HemSCs were transfected with si-NC, IL-6 siRNA, STAT3 siRNA, and HIF-1 $\alpha$  siRNA, and the migration rate was measured at 48h after culture. (A) The representative images at 0 and 48 hours; (B) the comparisons among groups. \*\* $p < 0.05$  versus normal HemSCs group (control). HemSC, hemangioma-derived stem cell; HIF, hypoxia-inducible factor; IL, interleukin; si-NC, nontargeting control siRNA; siRNS, small interfering RNA; STAT3, signal transducers and activators of transduction-3.

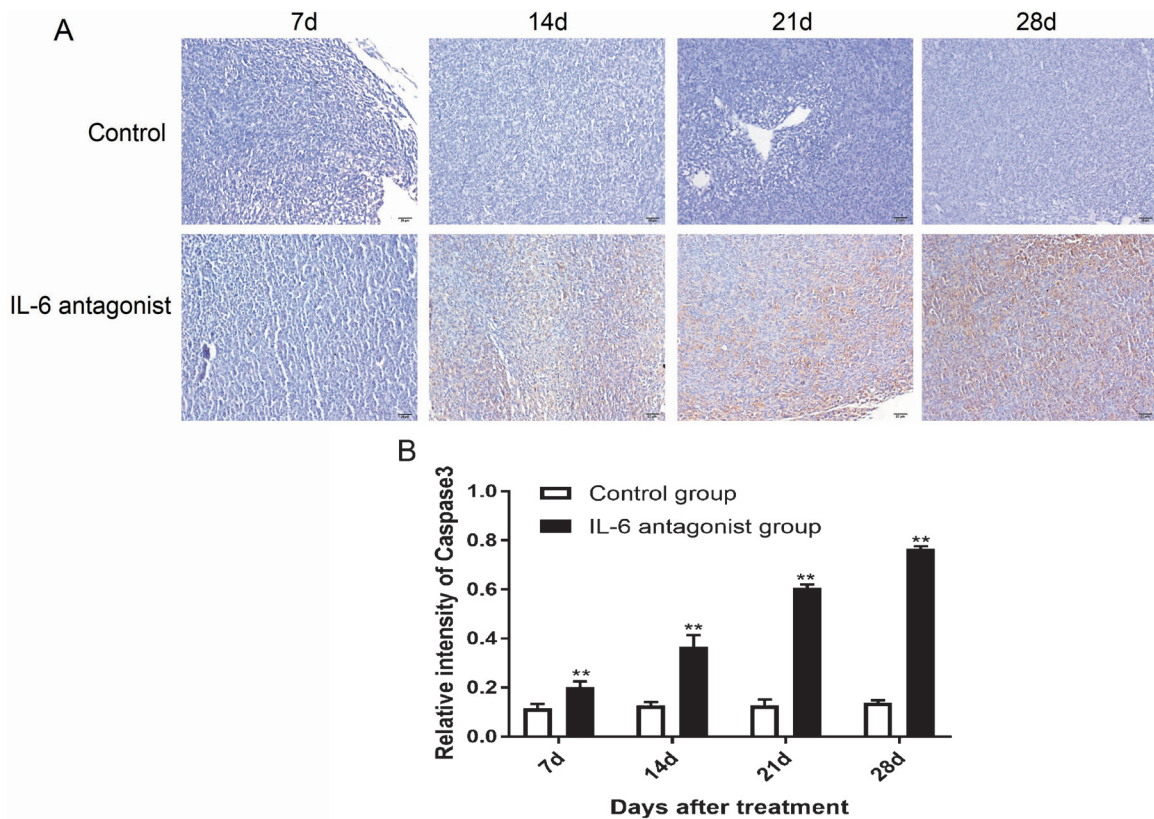
Tumor tissues from the mice in each group were obtained on days 7, 14, 21, and 27 after treatment. And immunohistochemistry (IHC) was used to assess the expression of Ki-67 and caspase-3 in the tumors. Compared with the control group, caspase-3 was significantly increased in the IL-6 inhibitor groups at each time point ( $p < 0.05$ , **Fig. 7**), whereas Ki-67

was decreased ( $p < 0.05$ , **Fig. 8**), indicating the IL-6 inhibitors could reduce HemSCs proliferation in vivo.

A TUNEL assay was used to detect cell apoptosis in each group. As shown in **Fig. 9**, the IL-6 inhibitor groups had much higher apoptosis rates than the control at days 7 ( $0.16 \pm 0.01$  vs.  $0.12 \pm 0.01$ ,  $p = 0.008$ ), 14 ( $0.21 \pm 0.04$  vs.



**Fig. 6** HemSCs xenograft mouse models were created and randomly assigned to receive IL-6 inhibitor or normal saline (control). (A) The final tumor volume on day 27; (B) tumor growth curve in each group. HemSC, hemangioma-derived stem cell; IL, interleukin.



**Fig. 7** The expression of Caspase 3 in each group. (A) The representative images ( $\times 400$ ); (B) the comparisons of Caspase 3 expression between groups at different time point. \*\* $p < 0.05$  versus control group.

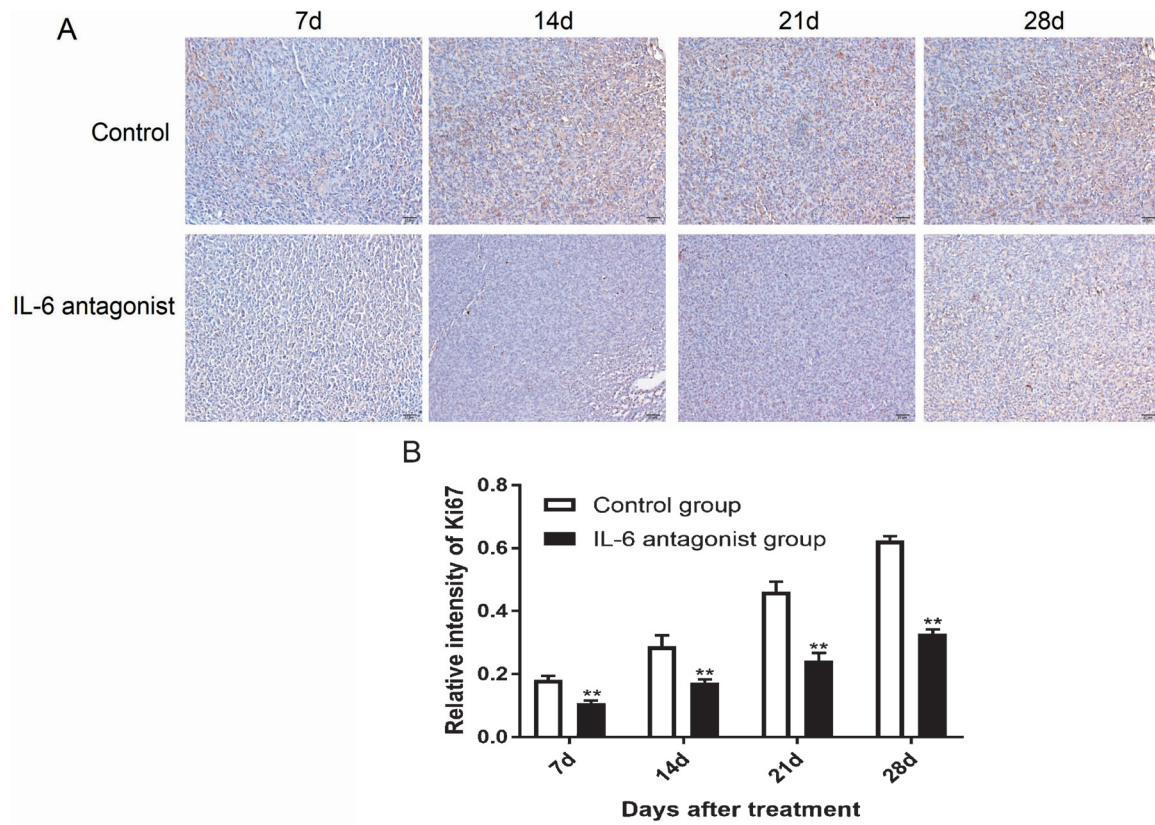
$0.10 \pm 0.01$ ,  $p = 0.010$ ), 21 ( $0.35 \pm 0.02$  vs.  $0.15 \pm 0.01$ ,  $p < 0.001$ ), and 27 ( $0.43 \pm 0.01$  vs.  $0.17 \pm 0.03$ ,  $p < 0.001$ ), respectively.

### Discussion

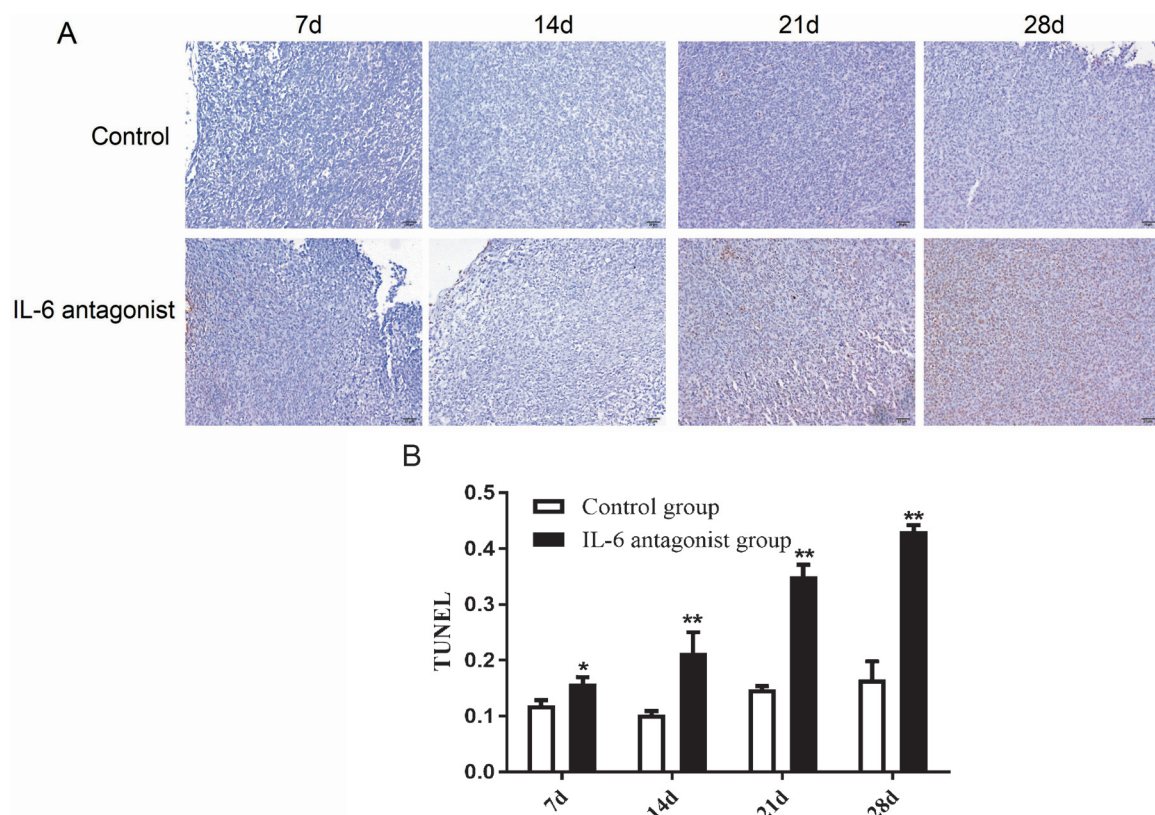
In the present study, we found that the expression of IL-6 was elevated in patients with IH. Knockdown of IL-6, HIF-1 $\alpha$ , or STAT3 could significantly inhibit the HemSCs viability and migration in vitro. Using the IL-6 inhibitors could delay tumor growth in the IH mouse models in vivo. Our results

indicated that regulating the IL-6/STAT3/HIF-1 $\alpha$  signaling pathways might inhibit IH growth.

IL-6 is an inflammatory cytokine that strongly correlates with tumor progression.<sup>15</sup> In patients with IH, the serum levels of IL-6 were increased.<sup>2,16</sup> Elevated IL-6 expression is associated with IH progression. Fu et al found that elevated IL-6 triggered the malignancy of IH via the induction of proliferation and migration of hemangioma cells.<sup>13</sup> In the present study, we also found that patients with IH had much higher IL-6 levels compared with the healthy controls. IL-6 binds to its receptor, leading to the phosphorylation of



**Fig. 8** The expression of Ki-67 in each group. (A) The representative images ( $\times 400$ ); (B) the comparisons of Ki-67 expression between groups at different time point.  $**p < 0.05$  versus control group.



**Fig. 9** Cell apoptosis in each group were detected by TUNEL assay. (A) The representative images ( $\times 400$ ); (B) the comparisons of apoptosis rate between groups at different time point.  $**p < 0.05$  versus control group.

STAT3. IL-6/STAT3 is a well-known signaling pathway that has a profound effect on tumor initiation and growth, including the process of regulating tumor immune micro-environments, protecting tumor cells from apoptosis, driving tumor cell proliferation, and promoting tumor angiogenesis.<sup>10,17</sup> HIF-1 $\alpha$  is a transcription factor expressed in cells under hypoxia conditions.<sup>15</sup> The activation of HIF-1 $\alpha$  is associated with the growth of multiple tumors, such as lung cancer,<sup>15</sup> gastric cancer,<sup>18</sup> and colorectal cancer.<sup>19</sup> The IL-6/STAT3/HIF-1 $\alpha$  autocrine loop has been found in several tumor types.<sup>20</sup> IL-6 induces STAT3 phosphorylation, then HIF-1 $\alpha$  interacts with phosphorylated STAT3 by blocking HIF-1 $\alpha$  degradation and accelerating synthesis.<sup>15</sup> Disrupting the IL-6/STAT3/HIF-1 $\alpha$  autocrine loop was reported to reduce tumor growth significantly.<sup>21</sup>

In the present study, HemSCs were transfected with siRNAs targeting IL-6, HIF-1 $\alpha$ , or STAT3 to disrupt the IL-6/STAT3/HIF-1 $\alpha$  pathways. As expected, HemSCs transfected with IL-6 siRNA had lower viability and migration rates than normal HemSCs. And HemSCs transfected with STAT3 siRNA or HIF-1 $\alpha$  siRNA had similar tendencies. In the study by Fu et al, they used the anti-IL-6 and STAT3 inhibitors to suppress the IL-6/STAT3 pathway in hemangioma cells and found that inhibiting the IL-6/STAT3 pathway could suppress the proliferation and migration of hemangioma cells.<sup>13</sup> Consistent with the study by Fu et al, our results indicated that inhibiting the IL-6/STAT3/HIF-1 $\alpha$  pathways or reducing the expression of IL-6, HIF-1 $\alpha$ , or STAT3 could inhibit HemSCs proliferation and migration.

Our *in vivo* study further confirmed the conclusion that inhibiting the IL-6/STAT3/HIF-1 $\alpha$  signaling pathways could suppress IH growth. After IL-6 inhibitor treatment, the tumor growth of the mice was significantly delayed. Consistent with the results shown in the tumor growth measurements, the expression of Ki67 after IL-6 inhibitor treatment was significantly decreased. Ki67 is one of the most extensive cell proliferation markers which can be expressed at all stages of the cell cycle except in the G0 phase.<sup>22</sup> The IL-6 inhibitor group had lower Ki67 positive cells than the control groups, indicating that inhibiting the IL-6/STAT3/HIF-1 $\alpha$  pathways could suppress IH cell proliferation.

Apoptosis is considered one of the important mechanisms to prevent cancer development. It has been reported that the IL-6/STAT3 signaling pathway could upregulate the anti-apoptotic protein protecting tumor cells from apoptosis.<sup>10,23</sup> Inhibiting the IL-6/STAT3 signaling pathway could promote tumor cell apoptosis in ovarian cancer,<sup>24</sup> glioblastomas,<sup>25</sup> and colorectal cancer.<sup>26</sup> In the present study, the IL-6 inhibitor group had much higher apoptosis rates than the control group. Furthermore, caspase-3 was significantly increased in the IL-6 inhibitor group. Caspase-3 is a protein at the end of the caspase cascade which could be activated by both the intrinsic and extrinsic death pathways in apoptosis.<sup>27</sup> Our results indicated that inhibiting the IL-6/STAT3/HIF-1 $\alpha$  pathways could promote tumor cell apoptosis of IH. However, the underlying mechanisms of how the IL-6/STAT3/HIF-1 $\alpha$  pathways regulate IH cell apoptosis remain unclear that further studies need to explore.

Our results suggested that anti-IL-6 drugs might be effective in IH treatment. Anti-IL-6 drugs have been used to treat many diseases, such as type-1 diabetes mellitus, schizophrenia, and solid tumors.<sup>28</sup> The safety profile for anti-IL-6 medicines is manageable. The use of anti-IL-6 drugs is associated with an increased risk of severe infections. Other common adverse reactions of anti-IL-6 agents include increased transaminase blood levels and cytopenia.<sup>28</sup> Although anti-IL-6 inhibition is tolerable, we should minimize the side effects for infants in IH treatment. It is possible to apply a local IL-6 inhibition to avoid systemic side effects that needs further investigations.

## Conclusion

In conclusion, our findings revealed that the expression of IL-6 was elevated in patients with IH. Inhibiting the IL-6/STAT3/HIF-1 $\alpha$  pathways could significantly suppress the HemSCs viability and migration *in vitro* and delay tumor growth in IH mouse models *in vivo*. Our results indicated that regulating the IL-6/STAT3/HIF-1 $\alpha$  signaling pathways might inhibit IH growth, possibly opening new avenues for targeted treatments of this tumor.

### Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Children's Hospital of Xinjiang Uygur Autonomous Region. The Animal Care and Use Committee of Children's Hospital of Xinjiang Uygur Autonomous Region granted a project license to perform the animal experiments (grant no.: 2020101967).

### Data and Materials

All data generated or analyzed during this study are included in this published article.

### Authors' Contributions

A.M. and Y.A. conceived of the study, J.H. and L.Z. participated in its design, and S.-X.L. coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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### Conflicts of Interest

All of the authors had no personal, financial, commercial, or academic conflicts of interest separately.

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