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*Original Article*

# Role of endothelial-mesenchymal transition in the tumorigenesis of infantile hemangioma

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**Abstract:** The aim of this study was to investigate the relationship between endothelial-mesenchymal transition (EndoMT) and the progression of infantile hemangioma (IH) and to provide references for clinical. Sixty-five patients were enrolled in this study. Tissues obtained from these patients were analyzed by tissue microarray (TMA). Serial sections from TMA blocks underwent immunohistochemistry with the primary antibodies for EndoMT markers (Twist, Zeb1, Smad, N-cadherin, Vimentin, and α-SMA). Intensity and extent points were counted to quantitate the markers expression. All sixty-five patients were diagnosed as IH, which distributes all over the body from head to planta pedis. Progressive phases could be distinguished with H&E staining. The expressionof Twist, Zeb1, Smad, α-SMA, Vimentin and N-cadherin in the abnormal endothelial cells were significantly increased compared with normal controls (***P < 0.01). Average expression points (intensity + extent) in proliferating and involuting phases were 7.69 and 7.80 for Twist; 8 and 7.90 for Zeb1; 4.43 and 3.80 for N-cadherin; 6.72 and 6.85 for Smad; 7.31 and 6.87 for α-SMA, and 6.42 and 7.00 for Vimentin. EndoMT involves in the tumorigenesis of IH. The endothelial cells have the capacity to transdifferentiate into mesenchymal cells when IH proliferates and these mesenchymal cells may further transdifferentiate into adipocytes or fibroblasts in involuting phase.

**Keywords:** Endothelial-mesenchymal transition, infantile hemangioma, immunohistochemistry, tumorigenesis, proliferation

## Introduction

Infantile hemangioma (IH) is one of the most common benign tumors of infancy that occurs in 5% to 10% Caucasian infants. Most of the patients exhibit significant clinical symptoms withintwo years, with the female-to-male ratio-of 3:1 approximately [1]. IH arises from areas close to head and neck skin and grows rapidly within two years after birth with characteristic capillary flame or "stretch-like" plaques [2, 3]. The tumorigenesis of IH can be divided into two consistent stages: proliferating and involuting phase. Although IH is self-limited, the minority of the lesion will grow largeand lead to serious complications such as ulceration, bleeding, and infection [4, 5].

Clinical strategies for patients with IH are limited. Surgical resection always causes bleeding and postoperative disfiguration [6]. β-blocker propranolol serves as the preferred drug for IH with obvious side effects, including convulsions, decreased heart rate, and asthma [7, 8].

The molecular mechanisms of IH tumorigenesis are still under debate, in spite of recent advances in diagnosis. The characteristic immunophenotype features that distinguish IH from other vascular tumors include thepositivity for GLUT1,

**Table 1.** Clinical, biological characteristics and laboratory examination of patients with IH

| Case | Sex | Age (Year/Month/Day) | Tumor size (cm) | WBC ($10^9$/L) | NR (%) | LYMR (%) | RBC ($10^{12}$/L) | PLT ($10^9$/L) | Hb (g/L) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 8M15d | 2×1×1 | 5.04 | 26.84 | 59.14 | 3.99 | 291 | 100 |
| 2 | M | 8M14D | 2×1.3×0.7 | 11.12 | 40.34 | 49.64 | 4.26 | 310 | 98 |
| 3 | F | 2M20D | 1.2×1×0.8 | 8.45 | 20 | 68 | 3.27 | 444 | 99 |
| 4 | F | 2M23D | 3.5×23×1.5&2×1.2 | 1.9 | 28.8 | 62.1 | 3.87 | 438 | 107 |
| 5 | F | 2M12D | 2.2×1.5×1&1.3×1.2×0.5 | 8.23 | 16.8 | 72.1 | 3.445 | 364 | 83 |
| 6 | F | 1M22D | 1.4×1.2×0.6 | 10.48 | 22.4 | 71.6 | 3.39 | 547 | 104 |
| 7 | F | 3M | 1.2×0.8×0.4 | 7.58 | 16.1 | 74.3 | 4.31 | 314 | 123 |
| 8 | F | 8M9D | 1.3×1.2×1.5 | 9.28 | 37.8 | 51.1 | 4.8 | 460 | 119 |
| 9 | M | 7M10D | 1.8×1.2×0.7 | 11.4 | 22.2 | 68.4 | 4.82 | 465 | 128 |
| 10 | F | 1Y | 3.3×2×1.2 | 12.58 | 36.3 | 56.3 | 4.44 | 422 | 121 |
| 11 | F | 2M12D | 2.2×1.6×0.6 | 5.16 | 23.6 | 64 | 3.33 | 372 | 97 |
| 12 | F | 2M13D | 2.1×1.6×1 | 7.48 | 33.2 | 59.1 | 3.32 | 723 | 95 |
| 13 | M | 8M18D | 2.6×2.2×1.3 | 15.51 | 15.3 | 81.9 | 4.45 | 355 | 112 |
| 14 | F | 10M9D | 3.5×2.3×1.2 | 11.24 | 22.5 | 68.6 | 3.9 | 474 | 103 |
| 15 | F | 1Y1M | 2.1×1.5×0.7 | 8.37 | 33.74 | 57.74 | 4.1 | 247 | 111 |
| 16 | F | 1Y4M | 3×2×1.5 | 14.04 | 25.9 | 64.8 | 4.37 | 239 | 118 |
| 17 | F | 8M25D | 2.7×2.3×1.2 | 11.88 | 31.4 | 56.1 | 4.33 | 384 | 113 |
| 18 | F | 9M13D | 5×3×1.2 | 9.07 | 40.1 | 49.4 | 4.36 | 329 | 110 |
| 19 | F | 1Y4M | 4×3.3×2.4&2.5×2.2 | 6.46 | 30.5 | 57.7 | 4.36 | 338 | 108 |
| 20 | M | 9M | 4×3×2&2.5×2 | 8.48 | 16 | 68.3 | 4.53 | 399 | 98 |
| 21 | F | 1Y9M | 3×2×1.5&1.2×1.2×1.5 | 10.53 | 31.4 | 62.6 | 4.2 | 340 | 116 |
| 22 | F | 8M19D | 2.5×2×1.5 | 8.11 | 25.6 | 65.1 | 4.51 | 275 | 112 |
| 23 | F | 7Y | 2×1.2×1 | 8.03 | 48.4 | 34.2 | 4.47 | 302 | 121 |
| 24 | F | 5M27D | 1.7×1.5×1 | 9.96 | 17.9 | 69.1 | 4.38 | 333 | 117 |
| 25 | M | 8M18D | 3.4×1.5×1.2 | 7.61 | 30 | 51.2 | 4.25 | 281 | 105 |
| 26 | F | 1Y | 2.3×1.8×0.8 | 14.03 | 24.5 | 62 | 4.39 | 441 | 114 |
| 27 | F | 1Y1M | 2×1×1&2×1.5×0.5 | 4.42 | 28.6 | 53.8 | 4.34 | 237 | 111 |
| 28 | M | 8M26D | 2×1.5×0.8 | 12.01 | 21.7 | 65.4 | 5.3 | 511 | 97 |
| 29 | M | 1Y4M | 3.8×3.7×1.5 | 10.95 | 20.7 | 64.8 | 5.02 | 291 | 129 |
| 30 | M | 10M26D | 5.5×4×1.3 | 8.28 | 24.2 | 63 | 4.35 | 361 | 109 |
| 31 | F | 11M2D | 4×3×2 | 9.27 | 52.6 | 34.4 | 4.34 | 249 | 108 |
| 32 | F | 1Y11M | 5×2.5×1.2 | 6.67 | 35.4 | 57.4 | 4.47 | 214 | 123 |
| 33 | F | 10M11D | 2.5×1.3×0.7&2×1.3×0.8 | 10.09 | 27 | 59.6 | 4.63 | 252 | 113 |
| 34 | F | 11M13D | 3×2×1.4 | 8.93 | 41.4 | 49 | 4.8 | 223 | 116 |
| 35 | F | 1Y | 3×3×1.5 | 8.39 | 25.6 | 60.4 | 4.51 | 377 | 119 |
| 36 | F | 11M12D | 1.3×1.2×1&3×2×1.5 | 10.66 | 42.6 | 41.8 | 4.28 | 361 | 109 |
| 37 | F | 1Y10M | 2.3×1.8×0.8 | 7.92 | 39.1 | 46.6 | 4.11 | 327 | 115 |
| 38 | F | 9M22D | 3.2×1×3 | 12.63 | 14.7 | 75.3 | 5 | 141 | 125 |
| 39 | M | 9M11D | 3.5×2×0.9 | 8.22 | 37.9 | 51.6 | 4.9 | 239 | 116 |
| 40 | M | 1Y | 1×0.8×0.5 | 7.06 | 24.3 | 59.1 | 4.6 | 298 | 100 |
| 41 | M | 1Y4M | 2.2×1.8×0.8 | 10.03 | 15.5 | 73.5 | 4.38 | 432 | 108 |
| 42 | F | 1Y6M | 3.5×2.5×1.5 | 10.49 | 31.9 | 62.1 | 4.26 | 362 | 121 |
| 43 | F | 9M6D | 4×3×1.5&2.5×2 | 9.54 | 14 | 79.2 | 4.25 | 487 | 119 |
| 44 | M | 1M | 3×2.1×1.5 | 12.42 | 33.7 | 59.4 | 4.4 | 328 | 115 |
| 45 | F | 1Y | 2..3×1.5×1.2 | 9.66 | 22.24 | 69.04 | 4.7 | 366 | 115 |
| 46 | F | 1Y8M | 2.5×1.5×1 | 5.56 | 28.9 | 56.1 | 4.38 | 125 | 113 |
| 47 | F | 8M24D | 0.9×0.6×0.7&2.5×2.1×0.5 | 12.25 | 32.5 | 49.6 | 4.47 | 495 | 109 |
| 48 | F | 1Y | 2.4×1.5×1.4&1.7×1×1&1.5×1×0.8 | 10.79 | 28.7 | 60.2 | 4.78 | 303 | 119 |
| 49 | M | 7M14D | 2×1.5×1.3 | 11.45 | 20.2 | 71.6 | 4.47 | 408 | 106 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 50 | M | 1Y | 1.8×1.2×0.5 | 11.28 | 29.14 | 61.74 | 4.74 | 326 | 109 |
| 51 | M | 1Y5M | 2.6×1.7×1 | 6.84 | 45.9 | 43.4 | 4.51 | 264 | 115 |
| 52 | M | 1Y6M | 2.2×1.9×1 | 9.15 | 43.3 | 43.9 | 4.91 | 428 | 131 |
| 53 | M | 10M17D | 1.4×0.7×1.2 | 7.18 | 38.9 | 41.2 | 4.24 | 255 | 109 |
| 54 | F | 8M26D | 1.8×1×0.9 | 6.51 | 23.7 | 66.1 | 4.41 | 144 | 110 |
| 55 | F | 4M24D | 2×1.2×1 | 9.52 | 26.8 | 64.3 | 3.91 | 374 | 96 |
| 56 | M | 6M8D | 1.7×1×0.5 | 11.57 | 18 | 68 | 3.9 | 319 | 99 |
| 57 | F | 6M13D | 2.8×1.5×0.9 | 15.78 | 21.2 | 57.2 | 5.08 | 547 | 120 |
| 58 | M | 1M20D | 2.1×1×0.8&2×1×0.5&1.2×0.8×0.5 | 5.56 | 18.9 | 66.2 | 3.02 | 354 | 88 |
| 59 | F | 7M24D | 2.3×1.5×1 | 15.53 | 19.9 | 72.1 | 4.59 | 370 | 111 |
| 60 | M | 4Y1M | 1.2×0.8×0.5 | 6.9 | 48.4 | 34.5 | 4.36 | 237 | 121 |
| 61 | M | 1Y8M | 1.7×1.3×0.5 | 9.04 | 21.9 | 71.6 | 4.29 | 278 | 116 |
| 62 | M | 2M24D | 3.3×2×1 | 4.71 | 14.9 | 57.3 | 3.13 | 346 | 85 |
| 63 | F | 1M26D | 3.5×2.5×1.5 | 8.95 | 17.42 | 65.81 | 3.72 | 285 | 115 |
| 64 | F | 4M12D | 3×2×1 | 68.47 | 23.7 | 62 | 3.93 | 407 | 103 |
| 65 | F | 2M8D | 3×2.2×0.4&1.7×1.9×0.3 | 9.16 | 25.54 | 59.34 | 3.39 | 296 | 106 |

**Table 2.** Summary of primary antibodies used for immunohistochemistry

| Antibody | Clone | Supplier | Dilution |
|---|---|---|---|
| Twist | Twist2C1a | Abcam | 1:50 |
| Zeb1 | 2A8A6 | Santa Cruz | 1:50 |
| Smad | H-2 | Santa Cruz | 1:50 |
| N-cadherin | 6G11 | Dako | 1:100 |
| Vimentin | V9 | Dako | 1:100 |
| SMA | 1A4 | Dako | 1:100 |

Lewis Y antigen, FcγRII, CD15, CCR6, indoleamine 2,3-deoxygenase, and IGF2 [9-14].

Endothelial-Mesenchymal Transition (EndoMT) contributes to the carcinogenesis of several vascular tumors. EndoMT and Epithelial-Mesenchymal Transition (EMT) exhibit similarities [15]. The cells undergoing EndoMT lose endothelial characteristics and acquire, at least in part, mesenchymal cell traits [16]. Previous studies have shown the close relationship between vascular support cells (pericytes or smooth muscle cells) and endothelial cells, indicating the involvement of EndoMT [17]. EndoMT-transforming cells are usually capable of enhanced motility and exhibit spindle-shaped morphology.

A set of biomarkers allow investigators to gain more insights into whether EndoMT contributes to the tumorigenesis of IH. High levels of transcription factors Twist and Zeb1 lead to the loss of cell-cell contacts and cell scattering, which are accompanied with decreased junction proteins such as E-cadherin and β-catenin [18]. Vimentin and α-SMA are mesenchymal cell markers with expressionofa decline of E-cadherin. Downregulation of E-cadherin is often accompanied with increased N-cadherin, referred as “cadherin switch”. Furthermore, transforming growth factor beta (TGF-β) can induce EndoMT through Smad pathway [19].

Although the results of several studies indicate that EndoMT may play an important role in the tumorigenesis of IH, the molecular characterization of IH is still suffered from the lack of direct evidence. In this study, we established tissue microarray tissue (TMA) from patients with different stage of IH. Immunohistochemistry (IHC) staining was used to investigate the relationship between EndoMT and the progression of IH. Our data provided preliminary evidence that EndoMT can promote the proliferation and transformation of abnormal endothelial cells.

## Materials and methods

### *Patients and clinical data*

Sixty-five IH patients (23 males and 42 females) were enrolled in this study. Age ranged from 1 month to 7 years old. The diagnosis was established based on the combination of macro features, H&E, IHC (GLUT1-positive cases) and color Doppler ultrasonography. Thirty normal skin samples from patient with lipoma were served as normal control. The clinical data were summarized in **Table 1**. As for ethical concerns,

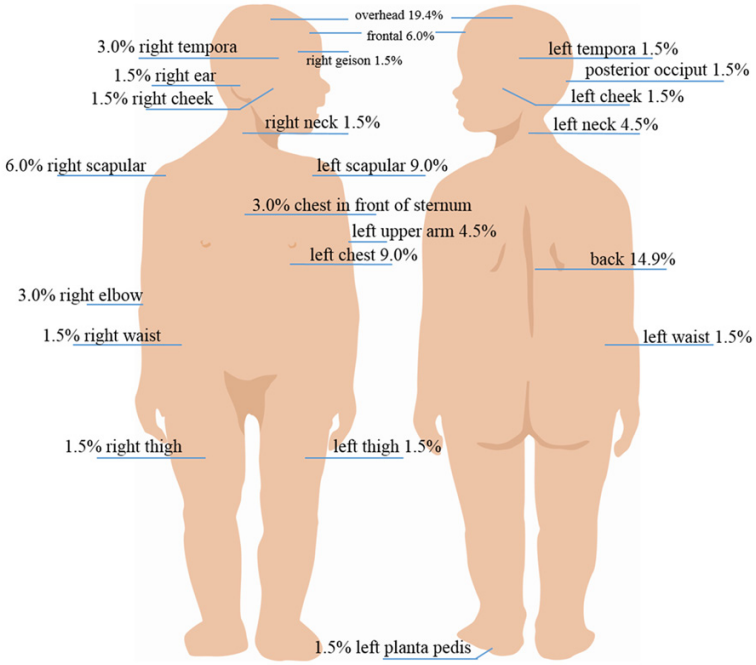


**Figure 1.** Location of IH. IH mostly occurred in head, which represented 37.4% of the total cases, then scapular (15.0%), back (14.9%), chest (12.0%) and neck (6.0%), occasionally in upper arm, thigh. More than one lesion might exist in one patient.

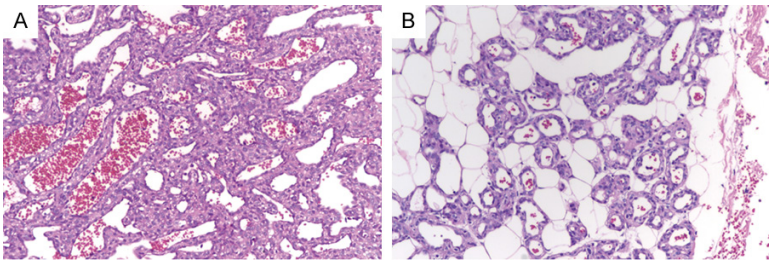


**Figure 2.** H&E staining of IH. A. Proliferating phase of IH. IH tissue was composed of cellular masses of plump endothelial cells and pericytes which form capillaries. Diameters of capillaries varied significantly (original magnification ×200). B. Involuting phase of IH. Surrounding stroma was abundant with the appearance of adipocytes (original magnification ×200).

all patients gave written informed consent to participating in the study in accordance with the Helsinki Declaration. This study was approved by the Regional Ethics Committee (Medical Ethics Committee of Jiangxi Children's Hospital and The Fourth Affiliated Hospital of Nanchang University).

*Histological examination and TMA*

Formalin-fixed, paraffin-embedded tissues were arrayed by using the manual tissue arrayer TMAjr (Pathology Devices, USA). Histopathological evaluation was performed by two independent researchers (L Zhou and Q Feng) who were blinded regarding patient details.

*IHC staining*

TMA blocks were cut into 4 μm serial sections. Antigen retrieval was performed following the dewaxing and rehydration. The slides were incubated with primary antibodies for EndoMT (**Table 2**) overnight at 4°C and secondary-antibodies for 30 minutes at room temperature. The chromogenic reaction was developed by using DAB. Slides were analyzed and scored basing on the intensity: 1: none; 2: weak; 3: medium; 4: strong, and extent: 1: ≤25%; 2: 26%-50%; 3: 51%-75%; 4: ≥ 76%.

*Statistical analysis*

Wilcoxon rank sum tests were used to compare the EndoMT marker groups with the control groups for assessing whether the differences between the markers intensity and extent are pro pronounced. Results are considered to be significant at $P< 0.05$. Statistical analyses were carried out using SPSS 20 and GraphPad Prism 6.

## Results

*Analysis of clinical data*

Hemangiomas mostly occurred at head (37.4%, including frontal, temporals, overhead, and facial area), scapular (15.0%), back (14.9%), chest (12.0%), and neck (6.0%), occasionally atupper arm and thigh (**Figure 1**). The slow progression discouraged most patients to submit to local hospitals after the presence of clinical symptoms. The majority (91.43%) of IH exhibited abundant blood flow signal in color Doppler

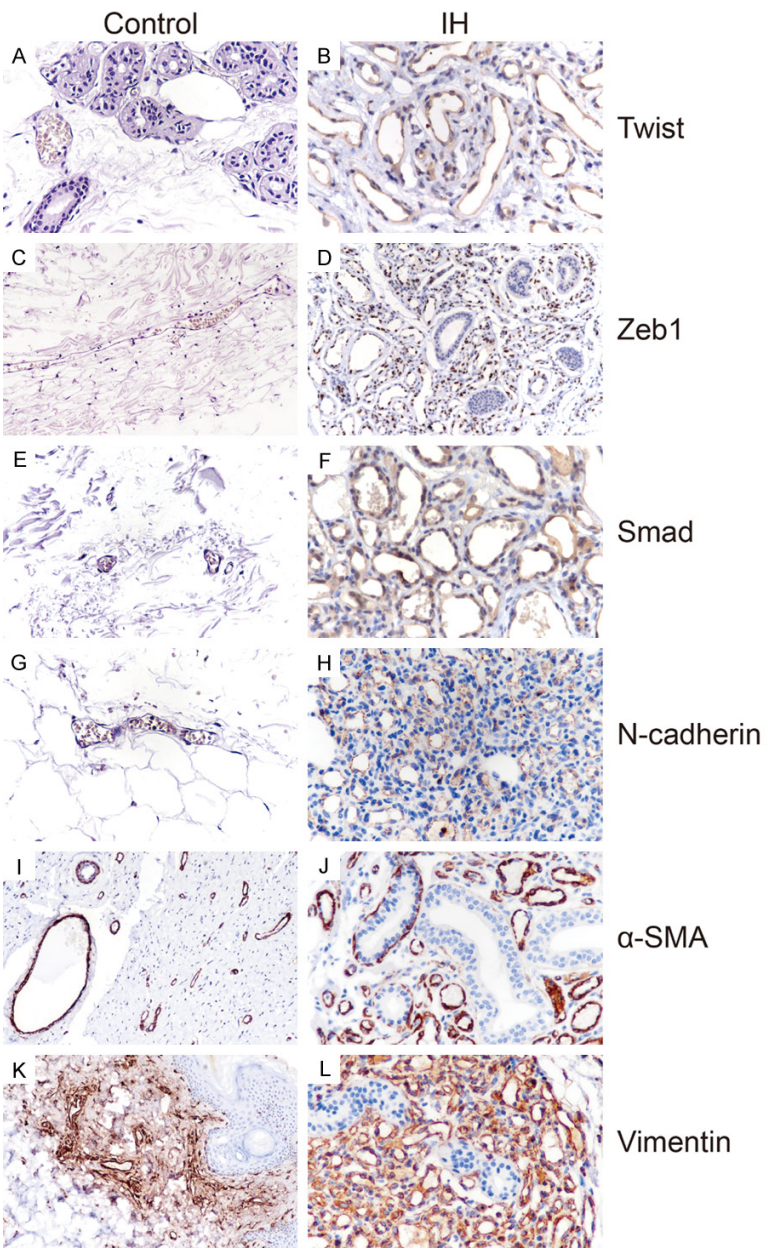


**Figure 3.** Immunohistochemical profile of IH. B, D, F, H, J, L. Disorderly endothelial cells in IH tissues exhibited positivity for all six EndoMT markers. A, C, E, G, I, K. The control groups were all negative in our cases. Primary antibodies annotations: A, B. Twist, original magnification ×400; C, D. Zeb1, original magnification ×400; E, F. Smad, original magnification ×400; G, H. N-cadherin, original magnification ×200; I, J. α-SMA, original magnification ×200; K, L. Vimentin, original magnification ×200.

ultrasonography. Laboratory studies were within normal limits.

*Histological findings*

The greatest diameters of lesions varied significantly (from 0.9 cm to 5.5 cm). Thirty-five patients were diagnosed asproliferating phase IH and 30 cases as involuting phase. In proliferating phase, tumor tissues were mainly composed of pericytes and endothelial cells. The array of endothelial cells was disorderly but well encircled. While in involuting phase, it is always difficult to pinpoint endothelial cells. However, the surrounding stroma was abundant. The appearance of adipocytes was a landmark of involuting phase (**Figure 2**).

*Immunophenotype of IH*

Almost all of the tumorous endothelial cells exhibited nuclear positivity for Twist, and the average expression point (intensity + extent) was 7.69 in proliferating phase and 7.80 in involuting phase. The expression of Twist was undetectable in control groups (**Figures 3A**, **3B**, **4**). A strongly and diffusely positive staining for Zeb1 was obtained in IH cases rather than normal control. The average expression point was 8 in proliferating phase and 7.90 in involuting phase (**Figures 3C**, **3D**, **4**). The expression of Smad was seen bothin nuclei and cytoplasm. The positive rate was > 95%. The average expression point was 6.72 in proliferating phase and 6.85 in involuting phase (**Figures 3E**, **3F**, **4**). The membranous positivity for N-cadherin was detected in 67.7% of IH cases, and the average expression point was 4.43 in proliferating phase and 3.80 in involuting phase, respectively (**Figures 3G**, **3H**, **4**). α-SMA and Vimentin are the markers of mesenchymal cells. Although in normal controlgroup the surrounding mesenchymal cells showed positivity for α-SMA and Vimentin, endothelial cells were negative. The positive rates of α-SMA and Vimentin were > 95%. For α-SMA, the expression point in proliferating

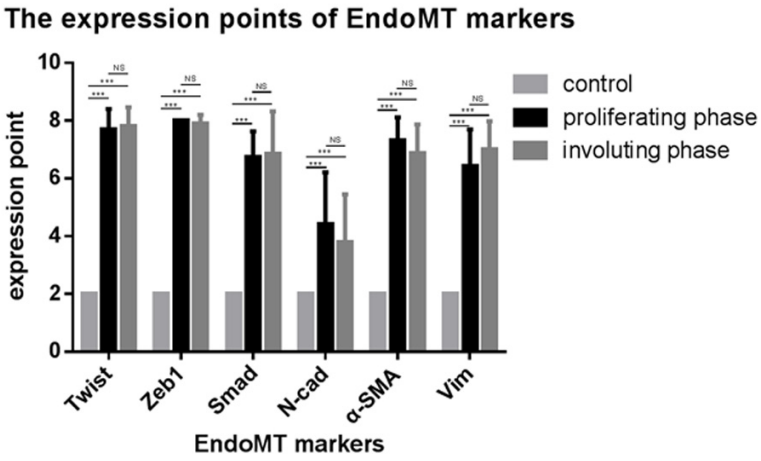


**Figure 4.** Expression points of each EndoMT markers (Twist, Zeb1, Smad, N-cadherin, α-SMA and Vimentin). All six markers were statistically significant compared to the control groups ($P<0.001$) and the expression points between proliferating and involuting phases in Twist, Zeb1, Smad, N-cadherin, α-SMA and Vimentin groups were similar ($P>0.05$).

and involuting phase was 7.31 and 6.87, respectively. While it was 6.42 and 7.00 in Vimentin (**Figures 3I-L**, **4**).

## Discussion

This is the first study focusing on the role of EndoMT in the progression of IH. IHC was employed to investigate the expression of EndoMT-related markers in endothelial cellsand surrounding cells of IH. The IHC results confirmed high levels of EndoMT markers in endothelial cells.

EndoMT occurs in endothelial cells associated with the microvasculature, which is vital for their differentiation to several lineages including fibroblasts, osteoblasts, and adipocytes [19]. IH non-destructively intermingled with adipocytes at the late stage of involuting phases, which is a feature to distinguish the phase of IH. The appearance of adipocytes indicates that IH is recovering. New studies showed that Mesenchymal Stem Cells (MSCs) resided in proliferating phase IH contributing to the adipocytes in involuting phase. However, these MSCs were not clonal, in some aspect indicating that MSCs might be formed by endothelial cells through EndoMT [20].

Process of EndoMT is accompanied with some drastically changes in EndoMT-related factors-like Smad, Twist, Zeb1, α-SMA, Vimentin, and N-cadherin.

Smad is a downstream effector of TGF-β signaling [21]. TGF-β induces EndoMT of capillary endothelial cells in its tissue culture models [16]. TGF-β binds to its receptor TβRI and TβRII. Then the activated TβRII sequentially phosphorylates TβRI, Smad2, and Smad3. After combining with Smad4, Smad2/3/4 tripolymer translocates into nuclear to induce the expression of Zeb1 and Twist through interaction with specific binding motifs in gene regulatory regions [22]. Previous studies have showed that DN-Smad4 (inhibitor of Smad4) sufficiently block TGF-β-induced EndoMT in endothelial cultures, indicating the critical role of Smad in TGF-β signaling pathway [23]. In this study, the tumorous endothelial cells of both proliferating and involuting stages exhibited strong and diffuse positivity for Smad. EndoMT induced by Smad finally depends on transcription factors Zeb1 and Twist to orchestrate the cell-cell adhesion junctions. Twist expression has concerned with high-grade ductal carcinomas. Contrary to other Transcription Factors (TFs) like Zeb1, Twist has been shown to repress E-cadherin with an indirectly bound to the E-cadherin promoter [24]. More striking, Twist confers stem-like properties in epithelial cells. Nuclear positivity for Twist has been confirmed in endothelial cells of the lesion.

Zeb1 is also known to repress E-cadherin promoter. More recently, Zeb1 was described as a cofactor in Smad2/3/4 tripolymer [25]. The majority of the abnormal endothelial cells expressed high level of Zeb1. N-cadherin was first found in neuron, and then extended to other tissues. An E-cadherin to N-cadherin switch leads to disassembly of intercellular adhesion junctions thus leads EndoMT [26]. The classical membranous positivity for N-cadherin was observed in most of endothelial cells rather than surrounding mesenchymal cells.

The increased expression of mature mesenchymal markers such as α-SMA and Vimentin indi-

cates the end stage of EndoMT. However, none of abnormal endothelial cells displayed positivity for α-SMA and Vimentin. Noteworthy, the close spatial association between endothelial cells and α-SMA-positive/Vimentin-positive spindle cells raises the possibility that the mesenchymal cells may derive from endothelial cells. There are no substantial lineage-tracing data so far because of technical limitations.

Two hypotheses concerning the tumorigenesis of IH were proposed: angiogenesis and vasculogenesis. The main point of first hypothesisis the abnormal proliferation and migration of original blood vessels. Hemangioma-derived Endothelial Cells (HemECs) might be activated by gene mutation or some external factors such as hypoxia, cytokines, or chorionic villus sampling [27, 28]. Placental injury and aberrant angioblasts may increase the incidence of hemangiomas. Different from angiogenesis, vasculogenesis hypothesis focuses on Endothelial Progenitor Cell (EPC) rather than original blood vessels. The abnormally HemECs can derive from EPCs, which co-express endothelial cell marker and stem cell-progenitor cell marker [29]. However, both of the hypotheses cannot interpret the close relationship between endothelial cells and mesenchymal cells. Recently, a study reported that Hemangioma Explant-Derived Cells (HemEDCs) can differentiate into osteoblasts and adipocytes in vitro. However, the expression of predipocyte factor-1 (Pref-1), a mesenchymal differentiation inhibitor, may block the terminal stage [30]. This is supported by our IHC data that endothelial cells did not express two classical mature mesenchymal cells markers vimentin and α-SMA.

Taken together, our study provides preliminary evidence for the involvement of EndoMT in the tumorigenesis of IH. The tumor endothelial cells may transdifferentiate into mesenchymal cells through EndoMT. However, this study mainly focused on the immunophenotypes of IH. Further experiments on the subject, including in vitro and animal model studies, are required to clarify the precise relationship between endothelial cells and surrounding cells. Additionally, future studies should also consider the effect of microenvironment on the progression of IH also should be considered.

## Acknowledgements

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## Disclosure of conflict of interest

None.

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