Isolation and characterization of endothelial cells from human intramuscular hemangiomas
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Introduction: Intramuscular hemangiomas are the most common form of hemangioma of deep soft tissue with a propensity for local recurrence. Hemangioma is a benign vascular tumor characterized by proliferation of endothelial cells (1). Endothelial cells (ECs) isolated from hemangiomas can serve as a useful model for studying the biology of hemangiomas. ECs from hemangiomas of liver, brain and skin have been isolated and cultured (2, 3). However there have been no reports on the isolation and culture of EC from intramuscular hemangiomas. The aims of this study were to isolate and culture human intramuscular hemangioma endothelial cells (IHECs) and to characterize their phenotypic and functional characteristics compared with normal human vessels.

Materials and Methods: (1) Isolation and culture of IHECs
Two cases of intramuscular hemangioma were obtained as surgical specimens. IHECs were isolated using a previously described method (4). Briefly, the hemangioma tissue was cut into pieces and digested enzymatically. The tissue was filtered through a mesh-sieve and centrifuged. The pellet was suspended twice in culture medium and the cells were seeded in gelatin-precoated flasks. After about 1 week in culture, the purification was carried out using fluorescence-activated cell sorter with monoclonal FITC-conjugated anti-CD31 antibody as previously described (4). The purity of human IHECs was quantified with flowcytometry by staining the cells with monoclonal antibody to CD31, a typical marker of EC.

(2) Characterization of IHECs
The morphological characteristics of IHECs were observed by phase-contrast microscopy before and after the purification process. The purified IHECs were identified by immunocytochemistry for typical EC markers such as von Willebrand factor (vWF), CD 31 and CD34. Polymerase chain reaction assays were performed to study the expression of angiogenesis-related molecules, such as vascular endothelial growth factor (VEGF), VEGF receptors (Flt, KDR), angiopoietin 1 (Ang1) and Tie2. The isolated IHECs were seeded onto the Matrigel-coated wells and examined for tube formation under an inverted phase contrast microscope. Normal endothelial cells obtained from normal vessels during surgery were used as controls.

Results: Immunocytochemical staining indicated that the cells expressed endothelial markers of CD31 (Fig. 1A, 1B), vWF and CD34. On light microscopy, the cells from donors grew with cobblestone morphology typical of endothelial cells (Fig. 1C). Purified IHECs from two samples showed 96% and 97% staining for CD31 by flow-cytometric analysis. The expression of VEGF, VEGF receptors, angiopoietin 1 and Tie2 was increased in IHECs compared with normal ECs (Fig. 2). The IHECs developed capillary-like tube structure similar to the one created by normal ECs when cultured on Matrigel (Fig. 3).

Discussion: We have successfully isolated ECs from human intramuscular hemangiomas. The phenotypic and functional characteristics of IHECs differed from normal ECs. These findings may be useful in understanding the pathophysiology of intramuscular hemangioma.