Published in final edited form as:

Pediatr Dermatol. 2015 ; 32(4): 539–543. doi:10.1111/pde.12530.

Growth Hormone Induces Recurrence of Infantile Hemangiomas After Apparent Involution: Evidence of Growth Hormone Receptors in Infantile Hemangioma

Naikhoba C. O. Munabi, B.A.[*], **Qian Kun Tan, H.S.**[*], **Maria C. Garzon, M.D.**[†], **Gerald G. Behr, M.D.**[‡], **Carrie J. Shawber, Ph.D.**[§,¶], and **June K. Wu, M.D.**[*]

[*]Division of Plastic Surgery, Columbia University Medical Center, New York-Presbyterian Hospital, New York

[†]Division of Pediatric Dermatology, Columbia University Medical Center, New York-Presbyterian Hospital, New York

[‡]Division of Pediatric Radiology, Columbia University Medical Center, New York-Presbyterian Hospital, New York

[§]Department of Obstetrics and Gynecology, Columbia University Medical Center, New York-Presbyterian Hospital, New York

[¶]Department of Surgery, Columbia University Medical Center, New York-Presbyterian Hospital, New York

Abstract

Infantile hemangiomas (IHs) are the most common benign tumor of infancy, characterized by a natural history of early proliferation in the first months of life to eventual involution during childhood, often with residual fibrofatty tissue. Once involution has been achieved, IHs do not typically recur. We present two cases of exogenous growth hormone therapy resulting in the recurrence of IHs in late childhood, supported by radiological, immunohistochemical, in vitro, and in vivo evidence.

CASE REPORTS

Patient 1

A 9-year-old girl presented with a nuchal soft tissue mass. She had a history of a nuchal IH during infancy, which involuted before age 1 year without residuum after a single steroid injection. She was then diagnosed with congenital growth hormone (GH) deficiency, for which she had been prescribed GH therapy approximately 10 months before presentation to the senior surgeon. Her parents noticed an enlarging mass at the site of the previously involuted IH after commencement of GH therapy, which steadily increased in size during

Address correspondence to June K. Wu, M.D., Columbia University Medical Center, 161 Fort Washington Avenue, Suite 511, New York, NY 10032, or e-mail: jw92@columbia.edu.

Presented as a poster at the 20th International Workshop of the International Society for the Study of Vascular Anomalies, Melbourne, Australia, April 2014.

this time. Physical examination revealed a 5.7-cm × 2.2-cm nontender nuchal mass with a pebbly consistency without discoloration or thrill. MRI revealed a mass within the superficial soft tissue with heterogeneous enhancement consistent with a vascular mass (Fig. 1). Because of the unusual presentation, excisional biopsy was recommended to confirm the diagnosis of recurring IH. Intraoperative resection found the lesion to be lobular, vascular, and deep purple in color (Fig. 1). Pathology confirmed the diagnosis of IH using glucose transporter 1 (GLUT1) staining. Her postoperative course was uneventful without recurrence.

Patient 2

A 7-year-old girl with PHACE syndrome and multiple cardiac, gastrointestinal, and neurologic complications presented with several enlarging soft tissue masses of the right frontal region. She had a history of a right-sided, segmental facial IH that experienced minimal proliferation during infancy. The IH demonstrated focal areas of continued growth and de novo tumor formation at age 4 years, which was managed with resection several years before presentation. Histologic and immunohistochemical (IHC) studies were consistent with common IH. Because of growth insufficiency, the patient had been initiated on GH therapy at age 5 years. In the ensuing 3 months there was enlargement of new soft tissue masses on the right forehead and frontal scalp, as previously described (1,2). Physical examination revealed multiple reddish-purple soft tissue nodules <2cm in diameter, suggestive in appearance of recurrent IHs. Significant bradyarrhythmia at the time of presentation precluded the initiation of propranolol treatment. GH therapy was discontinued, after which the new soft tissue masses continued to enlarge. Masses were in the superficial soft tissue and enhanced with contrast on MRI. Because of pain and irritation with brushing of the hair and concerns about the visible location, two lesions were surgically resected at the time of pacemaker insertion for bradyarrhythmia. Gross clinical appearance was similar to IHs: lobular, vascular, and deep purple in color (Fig. 1). Pathology confirmed a diagnosis of IH using GLUT1 staining.

METHODS

Resected tissues were obtained under approval of the Columbia University Institutional Review Board and analyzed. Resected tissues were formalin fixed and paraffin embedded as described previously (3). IHC and immunofluorescence staining were performed on paraffin sections of resected tissues for GLUT1, CD31, and the GH receptor (GHR). Hemangioma stem cells (HemSCs), believed to be the origin of IHs, were isolated from resected tissues using CD133 (stem cell marker) antibody-coated magnetic beads (3,4). HemSCs were characterized to assess multipotency using flow-assisted cell sorting (FACS) analysis with antibodies against CD90 (stem cell marker) and CD31 (endothelial cell marker).

HemSC cytotoxicity to propranolol was determined using a fluorescence-based cell viability assay (5). HemSCs were seeded overnight on a 96-well plate coated with fibronectin (BD Biosciences, San Jose, CA) in EGM-2 (Lonza, Allendale, NJ) with 20% fetal bovine serum (Life Technologies, Grand Island, NY) at a concentration of 4×10^3 cells/well. Cells were then treated with increasing concentrations of propranolol for 24 hours before cell viability

was assessed as previously described (5). All concentrations were performed in sextuplet, and solutions were diluted from a stock solution of propranolol hydrochloride (Sigma Aldrich, St. Louis, MO) dissolved in sterile water at pH 3.0.

Finally, the ability of HemSCs isolated from recurrent IHs to recapitulate IH in an in vivo mouse model was determined. A total of 3×10^6 HemSCs were resuspended in Matrigel (BD Biosciences) and injected subcutaneously into the dorsum of nude mice, as described previously (4). After 3 weeks, implants were harvested, formalin fixed, and paraffin embedded. Implants were assessed for the presence of vascular channels on hematoxylin and eosin (H&E) staining and for GLUT1-positive endothelium on IHC staining. Tissues and HemSCs from individuals with IHs who were not exposed to GH therapy served as controls.

RESULTS

H&E staining of resected tissues from both patients demonstrated a dense network of dilated capillaries surrounded by fibrous stroma. GLUT1-positive staining of CD31-positive endothelium confirmed the diagnosis of IH (Fig. 2A). In patient 1, hemorrhagic areas within the mass were consistent with the heterogeneous enhancement pattern observed on MRI. IHC staining showed GHR expression on GLUT1-positive endothelium, as well as in another distinct GLUT1-negative cell population, likely HemSCs. Furthermore, GHR expression was observed in both patients' tissues and in control IH tissues from patients unexposed to GH (Fig. 2B).

HemSCs from our GH-treated patients had similar biologic profiles and behavior to HemSCs from untreated patients. FACS analysis demonstrated low CD31 and high CD90 expression consistent with control samples and suggestive of true stem cell characteristics (Fig. 3A). HemSCs from our cases were as sensitive to the cytotoxic effects of propranolol as control HemSCs, with an 50% lethal concentration between 90 and 130 μM at 24 hours (Fig. 3B). Finally, in a murine model of IH, these HemSCs formed vascular channels with GLUT1-positive endothelium as seen on H&E and IHC staining, confirming the ability of HemSCs from these resected samples to develop IH in vivo (Fig. 3C).

DISCUSSION

To the best of our knowledge, there is only one prior case report of GH therapy leading to IH recurrence; one of the reported patients was patient 2 of our series (1). We have been able to extend this observation of GH being linked to IH recurrence by adding an additional patient and performing analysis on the resected clinical specimens and isolated cells. The clinical presentation and histopathologic analyses of resected tissues suggest that the soft tissue masses observed in both cases represent true recurrences of IHs after exposure to GH and despite an extended period of apparent involution. In addition, the FACS profiles of HemSCs isolated from these patients, the observed cytotoxicity to propranolol in vitro, and the ability to recapitulate GLUT1-positive vascular endothelium in vivo suggests that these recurrent IHs had similar behavior and responsiveness to treatment as in typical IH pathology.

GH is known to be mitogenic for a variety of vascular cell types because of expression of the GH receptor; upregulation of the GHR in vascular tumor endothelium can serve as a marker

of current or impending malignancy (6). Although IH is a benign tumor, our clinical and in vitro findings suggest that GHR is expressed in IH endothelium and other cell types. These findings suggest that residual GLUT1-positive endothelial cells that express GHR persist subclinically after resection or involution and can respond to exogenous GH, leading to clinical recurrence.

Our findings raise a number of questions. First, if GHR is present in IH tissues, it is surprising that there have not been reported instances of IH recurrence in healthy children with physiologic GH levels, especially given that IH is the most common benign pediatric tumor. We speculate that in children with normal GH levels, their IH tissues may be conditioned to GH stimulation and thus achieve homeostasis as far as their proliferative response. In children with GH deficiencies, the lack of GH exposure may cause GHR on IH tissues to be more sensitive to GH stimulation. When these children are then given exogenous GH, their IH tissues are primed to respond and proliferate. Further investigation will be needed to confirm this hypothesis.

Patient 2 in our series is an even more unusual case because her IH continued to enlarge after discontinuing GH therapy. This observation raises a second question of whether her IH pathology was even further altered or responsive to additional stimuli. Histopathologic analysis of the IHs isolated from patient 2 was no different from that of patient 1 or control specimens, although we speculate that patient 2 may possess a large and active HemSC population that is responsive to multiple stimuli including but not limited to GH, or the pathologic environment surrounding IH tissues may be particularly supportive of HemSC activation in this patient.

In conclusion, the presence of GHR in IH tissue suggests that GH therapy may result in recurrence of previously involuted IHs. It is possible that some patients may have greater responsiveness of IH to GH than others, but this variability has not been elucidated. The potential role of GH in IH recurrence is especially relevant for patients with PHACE syndrome, which has been associated with forms of pituitary dysfunction, and who may be offered GH therapy during peripubescence (7,8). Pediatric endocrinologists contemplating GH therapy should obtain a careful history of IH in their patients and counsel them regarding this possibility. The association between GH therapy and IH recurrence may also become significant to dermatologists or plastic surgeons when patients present with emerging vascular tumors beyond of the normal time frame for IH proliferation (<1 yr of age). In such instances, clinicians should probe for a history of endocrine dysfunction or GH therapy. If the clinical diagnosis is in doubt, preoperative imaging studies are recommended, although imaging may be atypical because of factors such as internal hemorrhage, as shown in patient 1. Thus incisional or excisional biopsy may be required to confirm a diagnosis of IH.

Acknowledgments

We would like to acknowledge grants 1R21EB 016515-01 (CJS) and 1K08HL 102068-01 (JKW), which supported the completion of this research.

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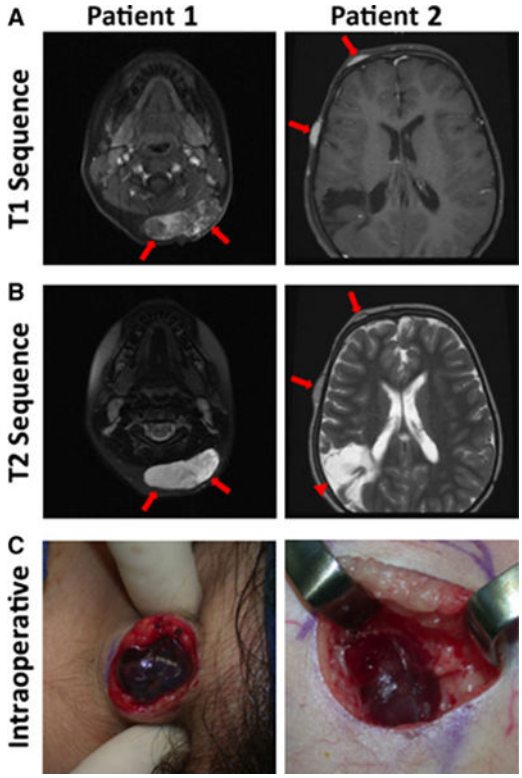

Figure 1.

Representative magnetic resonance imaging from both patients is shown in axial cross-sections of T1 contrast-enhanced and T2 sequences, as well as intraoperative findings. **(A)** In patient 1, T1 fast spoiled gradient echo postcontrast scans display a mass in the subcutaneous soft tissue with marked heterogeneity and increased enhancement peripherally (red arrows), which is atypical for infantile hemangioma (IH) but could reflect hemorrhagic components seen on pathology. In patient 2, T1 fat-saturated postgadolinium images reveal subcutaneous masses with homogeneous enhancement, a moretypical finding in IH. **(B)** Relatively homogeneous signal and well-defined masses were visible in both patients on T2 sequence. Patient 2 exhibits hyperintense T2 signal in the right posterior parietal cortex due to encephalomalacia from a known prior cerebrovascular accident (red arrowhead). **(C)** Intraoperatively, masses appeared dark red, vascular, and well encapsulated.

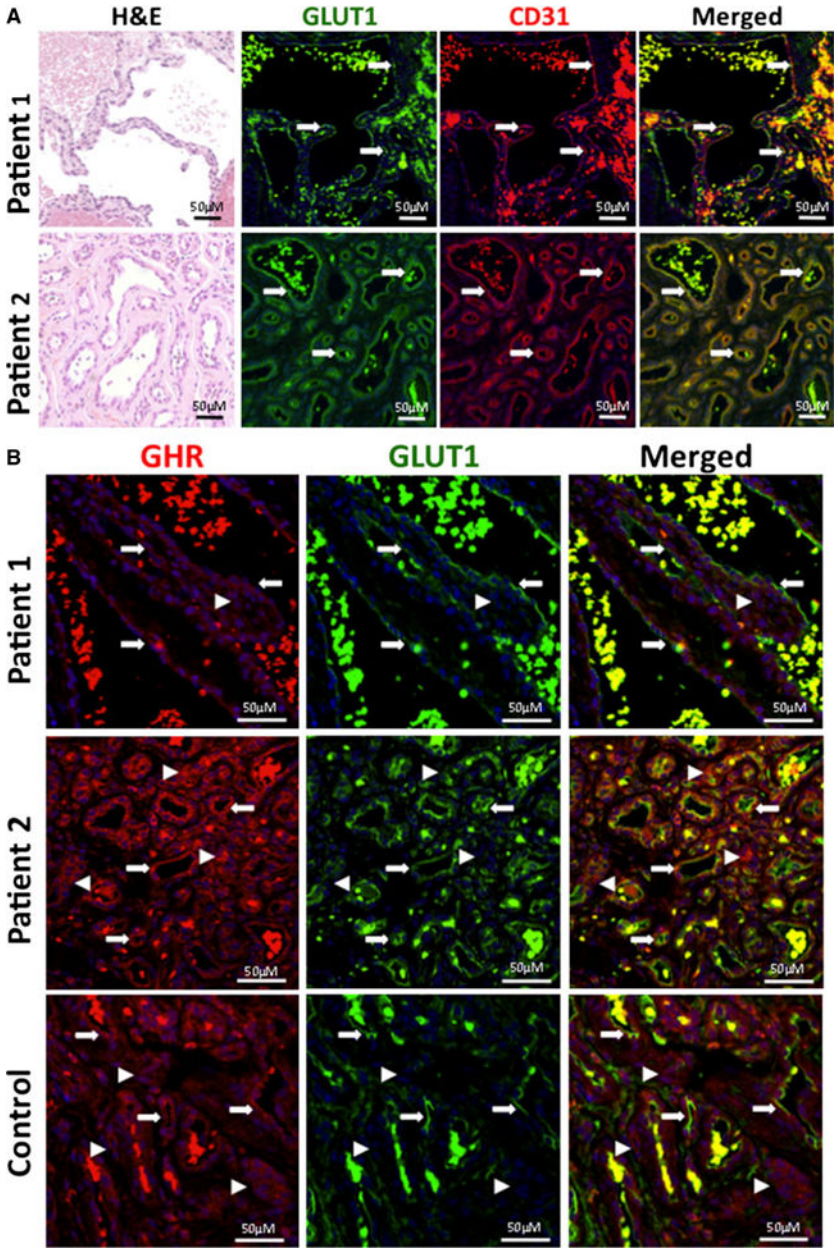

Figure 2.

(A) Tissues from both patients showed dense networks of dilated capillaries on hematoxylin and eosin staining. Samples were glucose transporter 1-positive (GLUT1; green) and CD31-positive (red), with colocalization of expression patterns (yellow), confirming a diagnosis of infantile hemangioma (IH) (white arrows). **(B)** Growth hormone receptor (GHR; red) expression was detected in both patients and control IH samples that were not exposed to growth hormone. GHR was expressed on GLUT1-positive (green) endothelium (arrows) as well as in another distinct cell population (arrow heads), most likely hematopoietic stem cells. Magnification 20×.

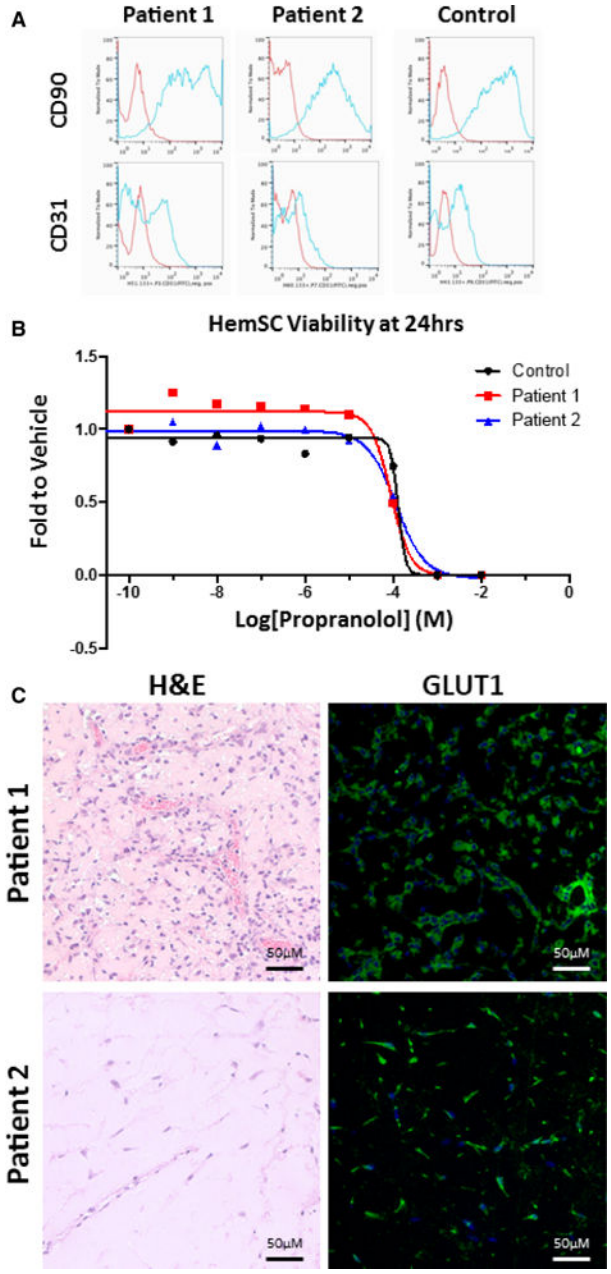

Figure 3.

(A) Flow-assisted cell sorting analysis of hematopoietic stem cells (HemSCs) isolated from patient-resected tissues (left and middle) were strongly CD90 and weakly CD31 positive, consistent with characteristics observed in control HemSCs (right). Histograms in red denote negative controls. **(B)** Cells from resected samples exhibited cytotoxic effects in response to propranolol in the same dose range (10^5–10^4 M) as control HemSCs at 2 hours. Data are presented as the viability of propranolol-treated cells as a proportion of the viability of cells in vehicle solution. **(C)** When suspended in Matrigel and injected subcutaneously into mice, cells isolated from both patients developed blood vessels, as seen with hematoxylin and

eosin staining (left) and glucose transporter 1–positive endothelium (right), confirming a diagnosis of IH.