Published in final edited form as:

Ann Plast Surg. 2018 April ; 80(4 Suppl 4): S211–S213. doi:10.1097/SAP.0000000000001438.

Analysis of Follicle-Stimulating Hormone Receptor in Infantile Hemangioma

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Abstract

Introduction—The life-cycle of infantile hemangioma (IH) and secretion of follicle-stimulating hormone (FSH) are identical. We previously have shown that IH contains the receptor for follicle-stimulating hormone (FSHR). The purpose of this study was to identify which cell type(s) in IH expresses FSHR.

Methods—Human proliferating IH tissues obtained during a clinically-indicated surgical procedure were used. Paraffin sections and isolated cell populations (endothelial, pericyte, stem cell) were subjected to immunofluorescence for FSHR. Tissues were co-stained with DAPI, anti- α smooth muscle actin, or biotinylated ULEX Europaeus Agglutinin I to identify nuclei, pericytes, and endothelial cells, respectively. Whole tissue and purified single cell populations underwent PCR for FSHR. Positive control specimens (ovary, sertoli cells) and negative control tissues (skin/subcutis, hepatic cells) were included.

Results—Immunofluorescence of 9 IHs demonstrated that FSHR was enriched in pericytes compared to endothelial cells. FSHR was expressed in 6 of 6 whole tissue IHs along with the positive control via PCR. FSHR was not present in the negative control samples. Four of 5 sets of pericytes expressed FSHR by PCR. Neither IH endothelial cells, IH stem cells, or negative control cells exhibited FSHR by PCR.

Conclusions—Because the secretion of FSH correlates with the growth pattern of IH, FSH might be involved in the disease process. FSHR is enriched in the pericytes of IH suggesting that this cell type may be involved in the pathogenesis of the tumor.

Keywords

tumor; hemangioma; follicle-stimulating hormone; receptor; vascular

INTRODUCTION

Infantile hemangioma (IH) is the most common neoplasm of infancy, affecting 5% of Caucasians.¹ At approximately two weeks of age the tumor appears and then enlarges

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The authors have no disclosures

(proliferating phase); 80% of its growth is completed by 5 months of age.² The tumor begins to regress at approximately 12 months which is completed by 3.5 years of age (involuting phase).³

The mechanism for the growth and involution of IH is unknown. We hypothesized that follicle-stimulating hormone (FSH) might influence the pathophysiology of this tumor because it surges after birth when IH rapidly enlarges.⁴ As the proliferation of the lesion slows, FSH levels decrease. IH stops proliferating at the same time that an infant ceases production of FSH.⁴ Girls are 3 times as likely to develop this tumor, and infant FSH levels are 5 to 7 times higher in females.⁵ Children that are low birth-weight have a higher frequency of developing IH and have increased secretion of FSH.^{6,7} We previously have shown that IH tissue has the receptor for FSH (FSHR) by immunohistochemistry.⁸ The purpose of this study was to determine which type(s) of IH cells contain FSHR.

METHODS

Patient Specimens

After approval from the Committee on Clinical Investigation at Boston Children's Hospital, proliferating infantile hemangioma specimens were collected during a clinically-indicated procedure. Positive controls (ovary and testis) and negative controls (normal skin/subcutis and liver) were acquired from the Departments of Pathology at Boston Children's Hospital.

Immunofluorescence

Immunofluorescence was used to determine the presence and location of FSHR. FFPE tissue sections were deparaffinized and immersed in a heated citric acid based antigen unmasking solution (Vector Labs; Burlingame, CA; H-3300). Sections were blocked using a blocking reagent (Perkin Elmer; Waltham, MA; FP1020). Sections were stained overnight at 4°C with anti-human FSHR antibody (GeneTex; Irvine, CA; GTX71309) alone in a 1:200 dilution or along with one of the following antibodies: anti-alpha smooth muscle actin (Abcam; Cambridge, MA; ab7817) in a 1:200 dilution, anti-CD31 (Dako; Glostrup Denmark; JC70A) in a 1:50 dilution, or anti-Calponin (Abcam; Cambridge, MA; ab700) in a 1:50 dilution. The following day slides were incubated in biotinylated anti-rabbit, anti-mouse, or Ulex Europaeus Agglutinin I secondary antibodies (Vector Labs; Burlingame, CA; BA-1100, BA-2000, B-1065) in a 1:200 dilution followed by Alexa Fluor 488-conjugated or 594-conjugated Streptavidin (Jackson ImmunoResearch; West Grove, PA; 016-540-084, 016-580-084) in a 1:150 dilution. Slides were stained with DAPI (Southern Biotech; Birmingham, AL; 0100-20) as a nuclear marker. Images were obtained using either a SPOT RT3 2Mp Slider camera (Diagnostic Instruments Inc.; Sterling Heights, MI) mounted to a Nikon Eclipse 80i microscope or a Zeiss LSM 880 with Airyscan (Zeiss; Oberkochen, Germany) confocal microscope.

Cell Isolation

Single cell suspensions were prepared from IH specimens by digesting with collagenase (Roche, Indianapolis, IN) and isolated using magnetic microbeads for: (1) VE-cadherin+ endothelial cells, (2) PDGFR β + pericytes, and (3) CD133+ stem cells. Cells were seeded on

non-coated tissue culture dishes in DMEM/10% fetal bovine serum and analyzed for either endothelial, pericyte/smooth muscle, or stem cell markers.^{9,10}

Polymerase Chain Reaction

Purified cell lines were cultured, RNA was extracted using RNeasy Mini Kit (Qiagen; Hilden, Germany; 74104), and 1 μ g was used to synthesize cDNA (New England Biolabs; Ipswich, MA; E650S). cDNA from cell lines and controls were processed using a Taq RED PCR Master Mix (Genesee Scientific; San Diego, CA; 42-138) with the following primer sets for FSHR and beta-actin (ACTB): FSHR Forward (5'-TCC CGA GGA ATG CCA TTG AA-3'), FSHR Reverse (5'-GGA AGG CCT CAG GGT TGA TG-3'), ACTB Forward (5'-GCC GCC AGC TCA CCA T-3'), and ACTB Reverse (5'-TCG ATG GGG TAC TTC AGG GT-3'). PCR products were run on a 1% agarose gel alongside positive control (human testis cDNA) and negative control (human liver cDNA) (BioChain; Newark, CA; C201119 and C1234149 respectively).

RESULTS

Nine proliferating IH specimens were examined (Table 1). Immunofluorescence illustrated that FSHR was present primarily in pericytes compared to endothelial cells (Figure 1). PCR demonstrated expression of FSHR in all IH whole tissue samples. Isolated cell population PCR experiments showed FSHR in 4 of 5 pericyte specimens. Neither endothelial (n=3) nor stem cell (n=3) samples exhibited FSHR by PCR (Figure 2).

DISCUSSION

We previously observed that FSHR is expressed in whole tissue of IH by immunohistochemistry.⁸ Our current study shows that the receptor is enriched in IH pericytes, further supporting the hypothesis that the ligand for this receptor might influence the tumor's life-cycle. The primary function of FSH is to promote follicular growth and spermatogenesis in the ovary and testis.^{5,11,12} FSH might affect IH by direct hormone interaction or indirectly through a paracrine pathway. One possibility is that FSH stimulates neovascularization in this tumor. FSH has been implicated in the pathogenesis of cancer, which is dependent on angiogenesis and/or vasculogenesis.¹³ FSH promotes VEGF signaling and hypoxia-inducible factor 1 α in certain tissues.¹³⁻¹⁵

Blood vessel formation requires endothelial cells to line the vessel wall and pericytes to surround them. Pericytes regulate lumen size and vessel stability by controlling endothelial cell proliferation and survival, and assembly of the basement membrane.¹⁶⁻¹⁸ The endothelial-pericyte interaction is influenced by several molecules including angiopoietins, platelet-derived growth factor B (PDGFR- β), and vascular endothelial growth factor (VEGF)-A.¹⁹ Proliferating IH pericytes play a significant role in the tumor's growth; when IH stem cells are blocked from differentiating into pericytes in a murine model, blood vessels do not form.²⁰ IH pericytes also have decreased contractility and low levels of angiopoietin 1, suggesting they cannot fully stabilize blood vessels.¹⁰ IH pericytes continually secrete VEGF-A, which may further effect blood vessel stimulation. Because FSH has been shown to promote VEGF signaling,¹³⁻¹⁵ it may accentuate the proliferating

effect and production of VEGF-A in IH pericytes. Interestingly, propranolol, a drug used to treat IH, significantly decreases FSH secretion²¹ and has been shown to affect the contractility of IH pericytes.²²

The observation that IH pericytes express the receptor for FSH, and that the secretion of this hormone coincides with its growth, is only a correlation. Further in-vitro and in-vivo experiments are required to test if FSH affects the life-cycle of the tumor. If a FSH pericyte interaction does influence proliferation of this tumor, targeted drug therapy for these lesions might be developed. A topical, intralesional, and/or systemic FSH antagonist could potentially prevent the growth of IH which would significantly minimize its morbidity.

Acknowledgments

The authors thank Joyce Bischoff PhD and Lan Huang PhD for providing the infantile hemangioma isolated cell populations used in this studied. This work was funded by the National Institutes of Health 1R21HD081004-02 (AKG), 1K23 HD086296-01A1(RAM) and the Plastic Surgery Foundation (AKG, RAM).

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Figure 1. Follicle-stimulating hormone receptor (FSHR) is enriched in proliferating infantile hemangioma pericytes. FSHR (green) is co-localized with pericytes (red). (20x magnification)

Figure 2.

PCR demonstrated FSHR in infantile hemangioma tissue and pericytes. (A.) Infantile hemangioma samples (lanes 1–6) exhibit FSHR expression along with the positive control ovary (lane 8). FSHR is not present in the internal control H₂O (lane 7) or normal tissues (lanes 9–11). (L = ladder); (B.) Agarose gel showing the presence of FSHR mRNA in infantile hemangioma pericytes (PC) and testis (positive control), but not in infantile hemangioma endothelial cells (EC), stem cells (SC), liver (negative control), or H₂O (template control).

Table 1

Patient Characteristics of Cell Types Isolated from Infantile Hemangioma

Sample	Cell Type(s)	Age	Sex	Location
1	Pericytes Endothelial cells	12 months	F	Chest
2	Stem cells	10 months	F	Nose
3	Stem cells	4 months	F	Abdomen
4	Stem cells	9 months	F	Chest
5	Pericytes	2 months	F	Eyelid
6	Endothelial cells	3 months	M	Scalp
7	Pericytes	3 months	F	Eyelid
8	Pericytes	6 months	F	Neck
9	Pericytes Endothelial cells	4 months	F	Scalp