Infantile Hemangiomas Lose Vascular Endothelial Cadherin During Involution: Potential Role in Cell Death?

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Background: Infantile hemangiomas (IHs) are benign endothelial cell (EC) tumors that undergo a predictable natural history, with rapid proliferation, stabilization, and involution. However, mechanisms regulating these transitions are not well understood. We have observed loss of vascular endothelial cadherin (VECAD) in involuting/involuted IHs. VECAD plays a critical role in angiogenesis, cell cycle progression, and EC survival. We hypothesize that loss of VECAD is associated with apoptosis occurring during IH involution.

Methods: Resected IH samples were clinically categorized as proliferating (n = 4), stable (n = 4), or involuting/involuted (n = 5). Neonatal dermal tissues were used as controls (n = 5). Immunohistochemistry was conducted on sectioned specimens using antibodies against EC markers VECAD and CD31. Apoptosis was assessed with terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay.

Results: CD31 signal intensity in proliferating, stable, and involuting/involuted IH ECs was unchanged relative to each other and to control ECs. VECAD signal significantly and progressively diminished as IHs progressed from proliferation to involution. Involuting/involuted IHs had significantly reduced VECAD expression compared with control ECs ($P < 0.0001$), proliferating IHs ($P < 0.0001$), and stable IHs ($P < 0.001$). As expected, the number of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling-positive ECs was significantly higher in involuting/involuted IHs ($P < 0.05$) relative to control ECs and proliferating IHs.

Conclusions: Loss of VECAD expression in IH endothelium corresponded to IH involution and increased apoptosis. It is unclear whether loss of VECAD is causative of IH involution; further studies are needed to elucidate the role of VECAD function in EC survival. (*Plast Reconstr Surg Glob Open 2024; 12:e5832; doi: 10.1097/GOX.0000000000005832; Published online 17 May 2024.*)

INTRODUCTION

Infantile hemangiomas (IHs) are the most common vascular tumor occurring in children and arise in the first few days to weeks of life.[1,2] IHs consist of benign hyperplasia of endothelial cells (ECs) and demonstrate a characteristic natural history.[3,4] The initial phase in the progression of an IH is rapid proliferation occurring between birth and around 9 months of age. In cutaneous IHs, the lesion evolves from a premonitory mark into a bright red papule or plaque.[5] This is followed by a period of stabilization, typically around age 9–10 months, as the growth of the hemangioma plateaus.[6] Finally, at about 1 year of age, IHs will undergo a slow involution in which blood vessels regress and may be replaced with a fibrofatty residuum. This regression is gradual, with roughly 90% of IH involution occurring by 4 years of age.[7,8] Although involution occurs spontaneously, normal skin is not always restored, with at least 20% of children experiencing residual skin changes such as pallor, anetoderma, or telangiectasias.[9]

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Although the causative pathophysiology behind hemangioma formation is not completely understood, IHs are thought to arise due to aberrant regulation in vasculogenesis.[10] Despite the fact that hemangioma endothelial cells (HemECs) form a vascular framework and have endothelial origins, HemECs have distinctive features from normal ECs. HemECs have been shown to be hyperproliferative and exhibit a progenitor phenotype when compared with normal ECs.[11] Furthermore, unlike most normal ECs, HemECs express *SLC2A1*, which encodes glucose transporter 1 (GLUT1).[12] Studies have further shown that the expression of various intra- and extracellular markers in HemECs can change as hemangiomas progress through different clinical phases.[13] Ultimately, however, the mechanisms underlying the initiation of IH development and the regulation of their phase progression are incompletely understood. It is not known whether these markers contribute to the abnormal regulation of vasculogenesis observed in IHs and if they play a role in regulating the transition of IH phases.

One such marker which may modulate IH phase progression is vascular endothelial cadherin (VECAD), a cell-cell adhesion molecule specific to the adherens junction complex between ECs.[14] Along with its role in maintaining endothelial integrity, VECAD is implicated in intracellular signaling pathways that directly and indirectly influence cell dynamics.[15] VECAD has been shown to exhibit various regulatory functions, including regulating cellular proliferation, modulating vascular endothelial growth factor (VEGF) receptors, and inhibiting apoptosis.[16] On a preliminary screen, our laboratory found that the endothelium of involuting/involuted IHs had a loss of VECAD expression. The purpose of this study is to systematically examine the expression of VECAD in hemangiomas at the three different phases of their progression, and to quantify the degree of apoptosis in each phase. We hypothesize that diminished expression of VECAD coincides with cell death, and that loss of VECAD may play a role in the eventual regression and involution of IH endothelium.

METHODS

Tissue Samples

Human hemangioma tissues were obtained immediately after surgical excision with IRB approval from Columbia University Vagelos College of Physicians & Surgeons (IRB # AAAA9976). The resected IH tissues were collected as representative stages of the IH life cycle and were categorized as proliferating ($n = 4$), stable ($n = 4$), or involuting/involuted ($n = 5$) based on patient age, clinical appearance, and histologic characteristics. No patients involved in the study received systematic treatment. Normal dermal vasculature obtained from neonatal foreskin tissues were used as controls ($n = 5$). Tissues were either fixed and frozen or embedded in paraffin for sectioning (5 µm).

Immunohistochemistry

Immunofluorescence staining was performed for the EC markers VECAD and CD31. Paraffin-embedded IH

Takeaways

Question: Mechanisms governing the natural history of proliferation, stabilization, and involution of infantile hemangiomas (IHs) are poorly understood. Do changes in expression of the endothelial cell junctional protein vascular endothelial cadherin (VECAD) play a role in the progression of IHs?

Findings: Involuting IHs lose VECAD expression and exhibit increased apoptosis relative to proliferating hemangiomas. VECAD expression progressively diminished as IHs progressed from proliferation to stabilization to involution.

Meaning: Loss of VECAD expression may play a role in infantile hemangioma involution.

specimens along with control tissues were stained as previously described.[17] The primary antibodies used were VECAD (1:100; R&D Systems; Minneapolis, USA) and CD31 (1:50; Dako; Glostrup, Denmark). Secondary antibodies with immunofluorescent dye Alexa Fluor 488 (green) and Alexa Fluor 594 (red) were used for detection (Invitrogen; Carlsbad, Calif.). To visualize the nuclei, 4',6-diamidino-2-pheylindole (DAPI) (Thermo Fisher Scientific; Waltham, Mass.) was used.

Apoptosis Assay

IH tissues from specimens used in immunohistochemical analysis were also used for analysis of cell death. Fixed-frozen sections were immunostained with CD31 as previously described to visualize ECs. Apoptosis of HemECs and control ECs was assessed with terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay used according to manufacturer's protocol (Roche; Mannheim, Germany). DAPI (Thermo Fisher Scientific; Waltham, Mass.) was used to visualize nuclei.

Quantification and Statistical Analysis

Quantitative analysis was conducted using ImageJ software (ImageJ 1.51, U.S. National Institutes of Health, Bethesda, Md.). The fluorescence intensity for endothelial VECAD and CD31 was measured across three separate fields for each tissue sample. The signal intensity of VECAD and CD31 was normalized by the area measured. Data are presented as mean signal intensity over vessel length (nm). The numbers of apoptotic ECs and HemECs were measured as the ratio of TUNEL-positive nuclei to DAPI-stained nuclei. Statistical analysis with one-way ANOVA and Student t test was performed with GraphPad software (Prism 9.0.0; San Diego, Calif.), and a P value less than 0.05 was considered statistically significant.

RESULTS

VECAD Expression

A total of 13 distinct IH tissues in different phases of progression were used in our analysis of VECAD signal intensity. Proliferating IHs ($n = 4$) showed a high cell
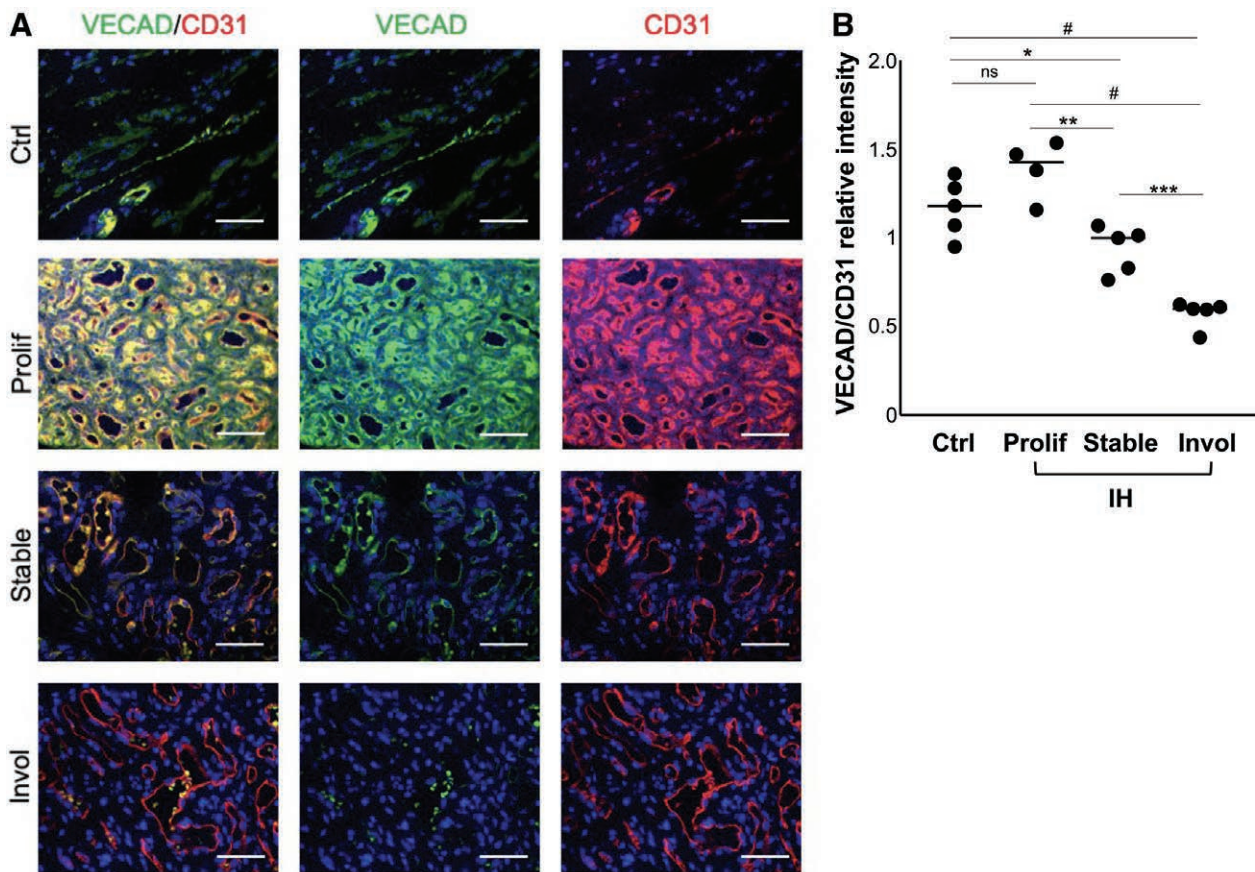
Fig. 1. IHs lose VE-cadherin expression during involution. A, Control neonatal foreskin (top row, $n = 5$), proliferating (second row, $n = 4$), stable (third row, $n = 4$), and involuting (bottom row, $n = 5$) IHs were stained for expression of VECAD (green) and CD31 (red). Scale bar, 50 μm. B, Quantification of signal intensity of VECAD/CD31. ns, not significant; #$P < 0.0001$, *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$. VECAD, VE-cadherin; Ctrl, control; Prolif, proliferating; Invol, involuting; IH, infantile hemangioma.

density with poorly defined vascular spaces with narrowed lumens relative to normal endothelium. In contrast, stable IH samples ($n = 4$) had well-defined but tightly packed vascular channels. Involuting hemangiomas ($n = 5$) showed dilated vascular channels with surrounding fatty infiltrates.

Immunohistochemical analysis demonstrated that CD31 was uniformly expressed in proliferating, stable, and involuting/involuted HemECs. The mean signal intensity of CD31 was unchanged in all three phases relative to control endothelium. Expression of VECAD significantly diminished in IHs at each progressive phase in the hemangioma life cycle (Fig. 1). Involuting/involuted IHs had significantly reduced VECAD/CD31 expression compared with control ECs ($P < 0.0001$), proliferating IHs ($P < 0.0001$), and stable IHs ($P < 0.001$). The relative intensity of VECAD in proliferating IHs was similar to that of control dermal endothelium, then showed a progressive decrease with each subsequent phase of the IH clinical course.

Cell Death

A total of seven frozen IH sections at different phases of progression and five control specimens were evaluated for cellular apoptosis. The percentage of ECs that were apoptotic was low in normal control dermal vasculature (20%, Fig. 2). Proliferating IH endothelium also demonstrated low levels of apoptosis (14%). However, the percentage of TUNEL-positive nuclei in involuting/involuted IHs was significantly increased when compared with proliferating IHs or control dermal vasculature (70%, $P < 0.05$ for both comparisons).

DISCUSSION

The molecular mechanisms driving the characteristic natural history of IHs from proliferation to involution are not completely understood. In this study, we demonstrate that VECAD is initially expressed on the surface of proliferating HemECs at a similar signal intensity as in control dermal vessels in normal skin but is lost as IHs progress through the different phases of their natural clinical course. This loss of VECAD expression coincides with involution and increased endothelial apoptosis.

Understanding IH expression profiles may give insights into IH pathophysiology and clinical care. Expression markers such as GLUT1, which is consistently expressed in all IH phases, have been used in clinical pathology to confirm IH diagnosis.[18] However, the IH expression
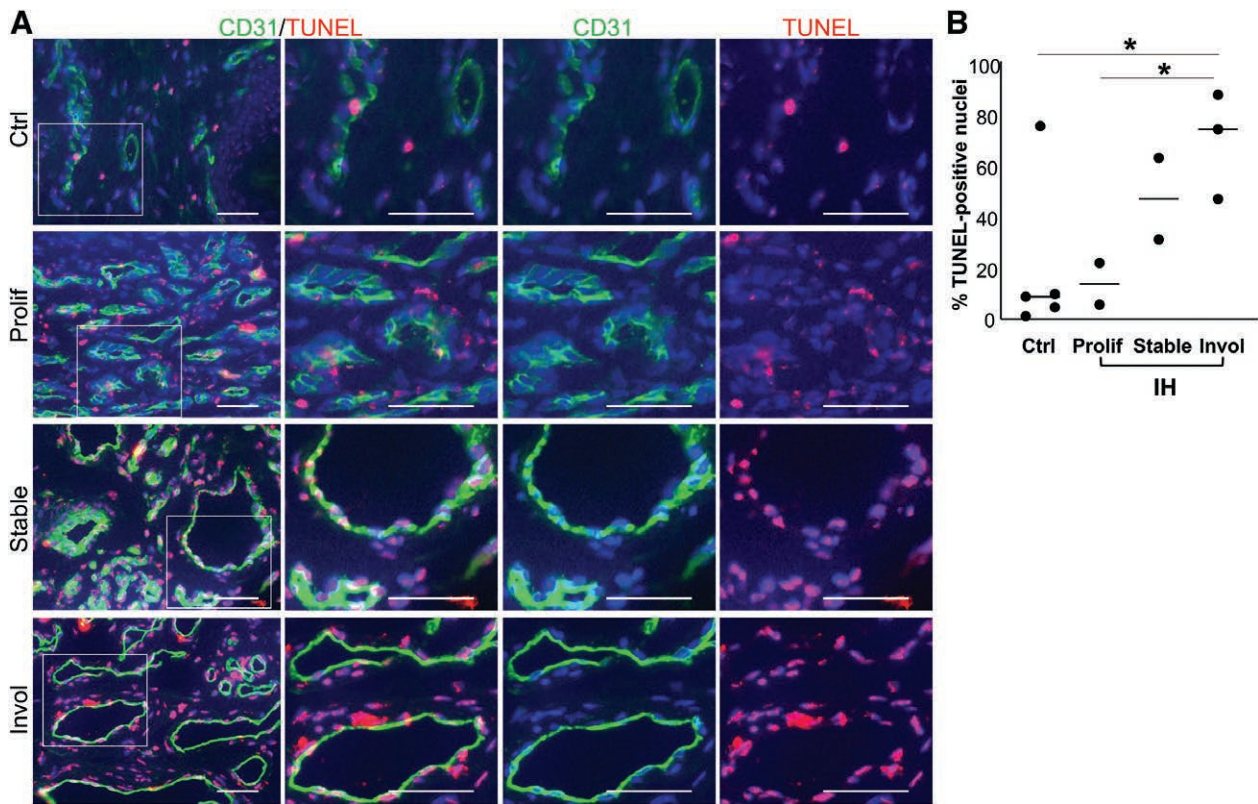
Fig. 2. Involuting hemangiomas have increased apoptosis. A, Control neonatal foreskin (top row, *n* = 5), proliferating (second row, *n* = 2), stable (third row, *n* = 2), and involuting (bottom row, *n* = 3) IHs were stained for expression of CD31 (green) and TUNEL (red). Boxed area in left column magnified. Scale bar, 50 μm. B, Quantification of TUNEL-positive ECs. *$P < 0.05$. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; Ctrl, control; Prolif, proliferating; Invol, involuting; IH, infantile hemangioma.

profile has been shown to change at different phases.[13,19] Tan and colleagues have found that VEGF is expressed in proliferating IHs but is not observed in involuting or involuted IHs.[13] An imbalance in VEGFR1 and VEGFR2 expression in IHs has also been reported, which could lead to the abnormal proliferation found in early IHs.[20] A large DNA microarray analysis conducted by Ritter et al. found that insulin-like growth factor 2 and integrin αVβ3 were highly expressed on the endothelium of proliferating IHs.[21] The expression pattern of VECAD in IHs at different phases, however, has not been previously reported. VECAD expression is of particular interest in IHs as it plays a significant role in EC physiology.

VECAD is a junctional protein specific to ECs and plays a key role in maintaining vascular integrity in normal endothelium through cell-cell adhesions.[22] In addition to its role in ensuring vessel structural integrity, VECAD has been implicated in various signaling pathways that can directly impact cell growth and apoptosis.[16,22] An in vivo study by Carmeliet and colleagues demonstrated that a targeted inactivation or truncation of VECAD in normal endothelium led to impaired vascular remodeling and ultimately to apoptosis,[23] highlighting the critical role of VECAD in EC survival. The loss of VECAD expression in HemECs of involuting IHs may thus lead to the increase in EC cell death that is observed during involution. The loss of VECAD-driven antiapoptotic signaling represents a

potential mechanism for the increase in cell death during the involution phase. Furthermore, Carmeliet et al demonstrated that VEGF-A signaling was unable to rescue EC survival when there is loss of VECAD.[23] This, together with decreased VEGF expression in involuting/involuted IHs,[13] may work in concert to promote apoptosis in involuting/involuted IHs.

Decreased expression of cadherins in various types of epithelial tumors has typically been associated with increased invasiveness and metastatic dissemination by detachment of cells from the primary mass.[24] Malignant vascular tumors, including angiosarcomas and hemangioendotheliomas, have also been shown to express low levels of VECAD when compared with normal endothelium.[14] The loss of these junctional proteins in malignant ECs is strongly associated with increased tumor growth and incidence of hemorrhagic events.[25] In contrast to these studies, our data show that VECAD is normally expressed in early proliferating IHs but is lost as IHs begin to involute. However, unlike malignant vascular tumors that show various levels of cellular dedifferentiation, IHs are a benign proliferation that retain their EC identity throughout their natural history, as our study demonstrated that IHs continued to express CD31 and GLUT1 while losing VECAD. Our data confirmed the endothelial identity of involuting IHs but also demonstrated critical differences in endothelial characteristics across IH phases.

The natural history and phase progression of IHs is well documented.[3,4] Staging of IHs is based on clinical examination and history; however, with the use of propranolol, rebound growth has been reported well outside of the expected period of proliferation.[26,27] In such cases where IH staging is unclear, VECAD can potentially be used as a marker to confirm involution and guide treatment.

It is unclear if the loss of VECAD in involuting/involuted IHs is a factor contributing to cell death or a result of endothelial apoptosis. While increased apoptosis has been found in VECAD-deficient ECs,[23] others have demonstrated that inducing apoptosis in ECs through the deprivation of growth factors can cause degradation of VECAD.[28] Our study shows an association of loss of VECAD with IH involution; further studies are required to elucidate causality along with the precise role of VECAD in IH physiology. Although our study demonstrated an association between loss of VECAD and IH EC death, future studies are needed to investigate the hypothesis that the loss of VECAD causes IH involution by inducing cell death.

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DISCLOSURE

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