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Review

Evolution of hemangioma endothelium

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ABSTRACT

Infantile hemangioma is a benign vascular tumor, characterized by a unique life cycle consisting of rapid growth and spontaneous regression. Three distinct phases (proliferating, involuting, and involuted) take place over the course of approximately 5–8 years, with specific cell types defining each separate phase. The origin of the cells comprising hemangiomas has been deliberated over since the late 1800s. We have recently provided experimental evidence that hemangiomas arise from multipotent stem cells. These hemangioma stem cells that give rise to the endothelial cells are also the essential source of adipocytes during hemangioma involution. The molecular mechanisms that regulate the differentiation of the hemangioma stem cells remain unclear. Although recent studies have elucidated a number of signaling pathways underlying hemangioma pathogenesis, many unanswered questions remain. Herein, we review the unique cellular composition of infantile hemangioma, as well as some of the signaling pathways active within hemangioma-genesis. Understanding the mechanisms behind changes in cellular fate throughout the hemangioma growth pattern will not only provide insight into the stem cell population that resides within the tumor, but will help to establish more effective eradicating therapies.

Contents

Introduction

Infantile hemangioma

Infantile hemangioma, a benign tumor of vascular endothelial cells (ECs), is the most common type of childhood tumor (Bruckner and Frieden, 2006; Mulliken and Glowacki, 1982). These vascular lesions appear in 1 out of every 100 newborns (Krol and MacArthur, 2005; North et al., 2001), predominantly affecting infants of Caucasian heritage (Bruckner and Frieden, 2003; Krol and MacArthur, 2005; Ritter et al., 2007). While the reasons remain obscure, infantile hemangiomas develop three times more often in females than in males (Mulliken, 1992; Ritter et al., 2007). It has also been noted that preterm infants are at significantly higher risk of developing hemangiomas (Amir et al., 1986). Interestingly, hemangiomas exhibit a strong predilection for the head and neck regions, which account for at least 60% of all hemangiomas (Chiller et al., 2002;

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Waner et al., 2003). Recent studies suggest that hemangiomas are not randomly distributed, but occur in areas of developmental boundaries where fusion of embryonic cranium takes place (Haggstrom et al., 2006b; Waner et al., 2003). These findings suggest a plausible connection between embryonic development and hemangioma pathogenesis.

Clinical features and classification of hemangiomas

Uniquely, hemangiomas are characterized by distinct phases of proliferation and regression (Mulliken and Glowacki, 1982; Ritter et al., 2007). While these vascular lesions may differ in the age of inception and possibly few clinical characteristics, on average, the patient exhibits no symptoms at birth. Subsequently, a red patch arises within the first few weeks of life (Bruckner and Frieden, 2003). Following the formation of the initial minute lesion, the tumor progresses rapidly into the first phase, the proliferating period. Typically, proliferation perseveres for a period of 1 year during which masses of ECs are observed, but defined vascular architecture is not prominent (Mulliken and Glowacki, 1982; Ritter et al., 2007). Approximately at the age of 1 year, the tumor enters the involuting phase, which may last between 3 and 5 years. During this stage, changes are observed solely at the cellular level where EC hyperplasia is reduced and mature vessels become distinct. The final stage of this tumor is known as the involuted phase. This stage is reached by the age of 5–8, and is characterized by the replacement of blood vessels by fibrofatty tissue. Although the tumor regresses spontaneously over time, the child may be left with a discolored area of thin epidermis situated where the lesion had initially formed (Enjolras and Mulliken, 1993; Ritter et al., 2007).

Clinically, hemangiomas are classified based on morphological traits and anatomical location. Slightly elevated lesions situated on the epidermis that appear red or purple in color are classified as superficial hemangiomas (Holmdahl, 1955). Lesions located beneath the skin and within internal organs have often been referred to as deep hemangiomas (Holmdahl, 1955; Ritter et al., 2007). Some have also proposed categorizations of the vascular tumors as localized, segmental, indeterminate and multifocal lesions (Chiller et al., 2002). Further vocabulary used to describe morphological features of hemangiomas include strawberry, capillary, port-wine, cavernous, capillary-cavernous, and lymphangiohemangioma (Cohen, 2007; Mulliken, 1993; Mulliken and Glowacki, 1982). Unfortunately, much of the widely used nomenclature is obsolete and obscure, resulting in erroneous classification of vascular tumors as vascular malformations and vice versa (Cohen, 2007). Subsequently, designation of hemangiomas should be based on the work consummated by Mulliken and Glowacki (Mulliken and Glowacki, 1982), which established proper classification based on the cellular and the histological features. Most notably, the work demonstrated that hemangiomas grow by hyperplasia, whereas, vascular malformations are developmental defects and exhibit a quiescent endothelium (Mulliken and Glowacki, 1982).

Proper categorization of hemangiomas can be achieved most reliably by the examination of three determining factors; ability of the tumor to enlarge as the child grows, when the tumor first becomes apparent, and the process of evolution (and regression) that the tumor undergoes. While the size of vascular malformations are commensurate with the patient's age, hemangiomas do not grow with the same proportionality (Smolinski and Yan, 2005). Hemangiomas typically are not apparent at birth and emanate at 1 week to 1 month after birth (Cohen, 2007). However, vascular malformations are discernible immediately post-birth as they are developmental defects. Lastly, hemangiomas undergo inimitable evolution during which the tumor proliferates and spontaneously regresses over time (Beck and Gosain, 2009; Bruckner and Frieden, 2003; Mulliken and Glowacki, 1982). This is peculiar to hemangiomas, as no other vascular tumors have demonstrated this type of life-cycle pattern.

To date, the most accurate diagnostic confirmation of hemangiomas can be achieved by staining for Glucose transporter-1 (Glut-1) (Bruckner and Frieden, 2003; Khan et al., 2008). Glut-1 is a member of a large transporter protein family, which is most commonly associated with erythrocytes (Gould and Holman, 1993; North et al., 2000). This protein has been noted to be highly expressed in ECs at the blood–brain-barrier, in the placenta, as well as in the eyes and nerves (Froehner et al., 1988; Gould and Holman, 1993; Jansson et al., 1993; North et al., 2000). A large study conducted by North et al. (2001) established the unique immunoreactivity of endothelial Glut-1 in hemangioma specimens, which was not observed in normal skin or other vascular anomalies (including malformations). The similar pattern of Glut-1 expression observed throughout various stages of hemangiomas and placenta insinuates the possibility of a connection between the two, especially when it comes to the origin of the tumor (North et al., 2000).

Present therapies for the treatment of hemangiomas

It is important to determine the mechanisms behind hemangioma formation in order to establish an effective eradicating therapy. While most hemangiomas are left untreated, as they pose no risk to the patient due to the spontaneous involuting nature, some must be treated (Beck and Gosain, 2009; Frieden et al., 1997). It has been noted that up to 20% of hemangioma cases can arise in areas that may pose life-threatening complications (MacArthur et al., 1995; Weber et al., 1990). For example, vascular lesions surrounding vital organs like the eyes or the nasal region can result in significant vision and airway obstruction in the infant (Enjolras et al., 1990). Internal bleeding and ulceration (Haggstrom et al., 2006a), and congestive heart failure (Cooper and Bolande, 1965) have also been shown to be complications of hemangiomas obstructing internal organs.

Most commonly, corticosteroids administered locally or systemically are used as the first line of defense for low and high risk hemangiomas of varying sizes (Beck and Gosain, 2009). While generally the use of small doses of corticosteroids is safe, patient response to the treatment is variable, with only 30% exhibiting accelerated regression and involution (Enjolras et al., 1990; Fost and Esterly, 1968). Nonetheless, corticosteroid therapy has been noted to cause severe adverse effects in children administered high doses for long periods of time (Greinwald et al., 1999). Some of the adverse effects include growth retardation, immunosuppression and edema (Greinwald et al., 1999). Another treatment used for hemangioma management is interferon alpha-2a. Interferon α-2a is an antiviral agent that has recently gained acceptance for use in hemangioma patients with life-threatening vascular lesions (Beck and Gosain, 2009). This type of therapy appears to be effective when administered in the early proliferative stage of the tumor (Greinwald et al., 1999). Common side effects such as fever, headache, irritation and malaise are seen in patients undergoing this treatment (Nguyen and Fay, 2009). However, upon cessation of the therapy, the side effects disappear (Enjolras et al., 1990). Nevertheless, a very severe neuromuscular complication, spastic diplegia, has been observed in 20% of infants administered interferon alpha-2a (Barlow et al., 1998; Worle et al., 1999). Thus, continuous neurological testing must be performed on patients undergoing the treatment to ensure effective detection of neurotoxicity and timely cessation of the therapy (Nguyen and Fay, 2009).

The most recently proposed therapy for hemangiomas is propranolol, a nonselective beta-blocker (Nguyen and Fay, 2009). A study conducted by Leaute-Labreze et al. (2008) demonstrated propranolol's ability to suppress the growth of large severe hemangiomas. While it is yet to be established how the treatment produces its effects, it is believed to act on the pericytes surrounding capillaries that cause vasoconstriction (Nguyen and Fay, 2009). Although the current therapies available offer effective elimination of the tumor in some cases, the plausible side effects from their use cause great concerns

for the health and wellbeing of the children affected. Thus, further understanding of the cellular and molecular basis of hemangiomas is required in order to prevent complications and properly treat the vascular tumor.

Alarming hemangiomas not responding to medical therapy can also be surgically resected to ensure that the complications do not worsen. Some surgical excisions are also performed to minimize psychosocial effects from the skin deformities that result from lesion formation on the face.

Current theories of hemangioma origin

Several hypotheses exist on the origin of hemangiomas. Two plausible mechanisms have gained acceptance; the intrinsic defect hypothesis, and the extrinsic defect hypothesis. The intrinsic hypothesis entails that a somatic mutation in one or more genes controlling EC proliferation is responsible for the tumor formation. This hypothesis suggests that the ECs in hemangiomas originate from a single stem/progenitor cell and therefore implies that the cells are clonal in nature. A recent study conducted by Boye et al. (2001) examined methylation patterns in the ECs from hemangioma lesions. They observed that all hemangioma-derived endothelial cells (hemECs) demonstrated a similar X-chromosome inactivation pattern, supporting the notion that the cells arise from same parent progenitor cell. Although patches of cells with similar X-chromosome inactivation are found in various tissues, these findings are quite compelling. Other studies (Jinnin et al., 2008; Walter et al., 2002) provided further evidence to support the presence of somatic mutations by identifying a missense mutation in vascular endothelial growth factor receptor-2 (VEGFR2) (Walter et al., 2002) within hemangioma lesions but not normal adjacent tissue.

In contrast to the intrinsic theory, the extrinsic hypothesis suggests that the tumor microenvironment regulates hemangioma-genesis. For example, Bielenberg et al. (1999) identified hyperplasia and increased angiogenesis in the epidermis overlaying proliferating hemangioma. However, tissue adjacent to involuted hemangioma did not exhibit the same characteristics. This finding suggests a possible signal imbalance in the epidermis adjacent to proliferating hemangioma, which may be contributing to the progression of the tumor. However, the location of this signal imbalance (epidermis vs the hemangioma tumor itself) is not fully clear. It is possible that rapid expansion of the tumor during the proliferating phase underlies an imbalance in the growth factors and cytokines and ultimately leads to epidermal hyperplasia.

Recently, an extrinsic hypothesis with an intrinsic component has been brought forward to explain hemangioma-genesis. Disturbances in the microenvironment, such as transcervical chorionic villus sampling (CVS), a method of prenatal diagnosis of cytogenetic abnormalities (Rhoads et al., 1989), has been suggested to increase the incidence of infantile hemangioma (Burton et al., 1995; Holmes, 2009). It is believed that the invasive procedure causes intravascular dislodgment of placental cells, which enter the circulation and embolize to the developing fetus (Bree et al., 2001; Kaplan et al., 1990; North et al., 2002). The extrinsic component consists of the mechanical disruption and the permissive environment for homing. While the intrinsic component consists of the progenitor nature of the cells that are dislodged.

Evolution of hemangioma endothelium

The origin of the cells comprising hemangiomas has been deliberated over since the late 1800s (Fig. 1). It was in 1863 that Virchow (Virchow, 1863) first proposed a possible relation between hemangiomas and embryonic development, suggesting the possibility of remnant embryonic mesoderm giving rise to the vascular tumor. The theory was further perpetuated by Pack and Miller (1950), and Malan (1974) who advocated that activation and growth of dormant angioblastic cells during fetal development were responsible for hemangioma formation. While the theories presented a vision for hemangioma-genesis, it was not until histological examinations that the cellular components of the vascular tumor began to be unraveled.

Currently, immunohistochemistry offers the most reliable way of studying intact specimens at the cellular level. The cellular components of hemangiomas consist largely of ECs and perivascular cells (Hopfel-Kreiner, 1980; Lo et al., 2009). It was the initial histochemical work of Mulliken and Glowacki (1982), examining the EC morphology, that shed some light on the cellular components of hemangiomas. Typically, infantile hemangiomas undergo three distinct phases that can be characterized by the developmental state of the ECs. Plump endothelium comprising convoluted capillaries characterizes the early phase of proliferation in hemangiomas (Mulliken and Young, 1988). As the tumor continues to further develop, the ECs flatten and the vessels dilate, creating a mature vascular appearance (Mulliken and Young, 1988). As the last stage of the hemangioma approaches, fibroadipose tissue surrounds collapsed blood vessels and EC debris (Mulliken and Young, 1988). As the hemangioma research field continued to expand, unusual characteristics of the tumor were revealed. It was the study by North et al., (2000, 2001) that discovered the unusually high expression of Glut-1 in ECs in all stages of hemangiomas. Although Glut-1 positive vessels are seen in all three phases of the hemangioma lifecycle, a fair number of the vessels exhibit a 'mosaic' phenotype (unpublished observations). In other words, the ECs in these vessels are a mixture of Glut-1 positive and Glut-1 negative. It is plausible that the ECs in these vessels are derived from different sources or processes.

Hemangiomas are also unique in terms of cellular heterogeneity. Cellular diversity of hemangiomas was examined by Gonzalez-Crussi and Reyes-Mugica (1991) and was further strengthened by Smoller and Apfelberg (1993). The latter study examined immunoreactivity of CD34, an EC marker that is also shared by some stem cells, in the cellular components of hemangiomas. They discovered CD34 positivity in the ECs and in the interstitial cells surrounding the vasculature (Smoller and Apfelberg, 1993). The discovery led to the presumption of CD34+ cells giving rise to both ECs and pericytes. Again, suggesting the possibility that a progenitor cell gives rise to several cellular lineages in hemangiomas. Later on, Ritter et al. (2006) provided further evidence for abnormal endothelium by observing co-localization of CD31, an EC marker, with CD32, a myeloid cell marker in a portion of the hemangioma vessels. Other observations included co-localization of the von Willebrand factor (vWF) with CD83, a dendritic cell marker (Ritter et al., 2006). While the significance behind the co-expression of myeloid and EC markers is unclear, it may be indicative of the origin of hemangioma cells. The possibility of hemangiomas arising from a hematopoietic precursor cell may explain the co-expression of EC and myeloid cell markers. Alternatively, the presence of multiple cellular markers on what is perceived to be a mature EC may indicate an immature state of the ECs in hemangiomas.

It is perplexing that hemangioma endothelium expresses all markers that are associated with a fully functional endothelial phenotype (Fig. 2). Namely, hemangioma ECs (in the proliferating as well as the involuting phases) are nicely decorated with CD31, vascular endothelial (VE)-cadherin, von Willebrand Factor (vWF), CD34, and CD146. Only vascular endothelial growth factor receptor 2 (VEGFR2) displays positivity in the ECs as well as the interstitial (and perivascular) cells. What makes hemangiomas unique is this atypical endothelium which expresses mature EC markers and markers of other cellular lineages (for example myeloid markers). It is easy to appreciate the immature nature of hemangioma ECs by examining for stem/progenitor cell markers. Hemangioma specimens are immunoreactive to known human pluripotency markers including SOX2, OCT4 and Nanog, as well as multipotent stem cell marker CD133. This positivity is seen in the endothelium and interstitial cells, while CD133 positivity is primarily localized to the endothelium (Fig. 3). Could this immunophenotype

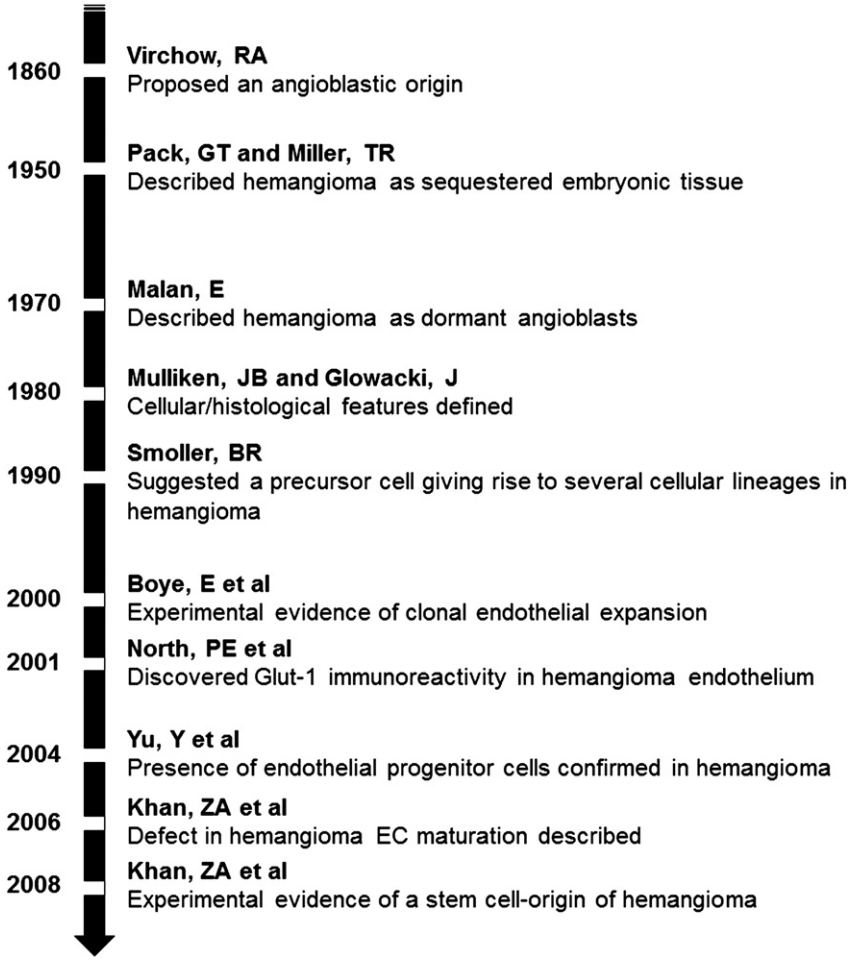

Fig. 1. Major advances in the understanding of hemangioma biology. A schematic diagram illustrating the major discoveries and advances in the knowledge of hemangioma formation and progression.

suggest a possibly delayed or disrupted differentiation pathway to endothelial cells? Dosanjh et al. (2000) isolated hemECs and compared them to fetal and neonatal ECs. The study illustrated remarkable similarities in cellular morphology and protein expression between hemangioma and fetal ECs, but not with neonatal cells. When grown in culture, hemECs and fetal cells exhibit similar spindle shape morphology and growth characteristics. The neonatal cells predominantly displayed an epitheloid shape, typically exhibited by functionally mature ECs. Expression of vWF, CD31, type IV and type I collagens were also similar in hemECs and fetal ECs. These findings clearly showed that hemangioma

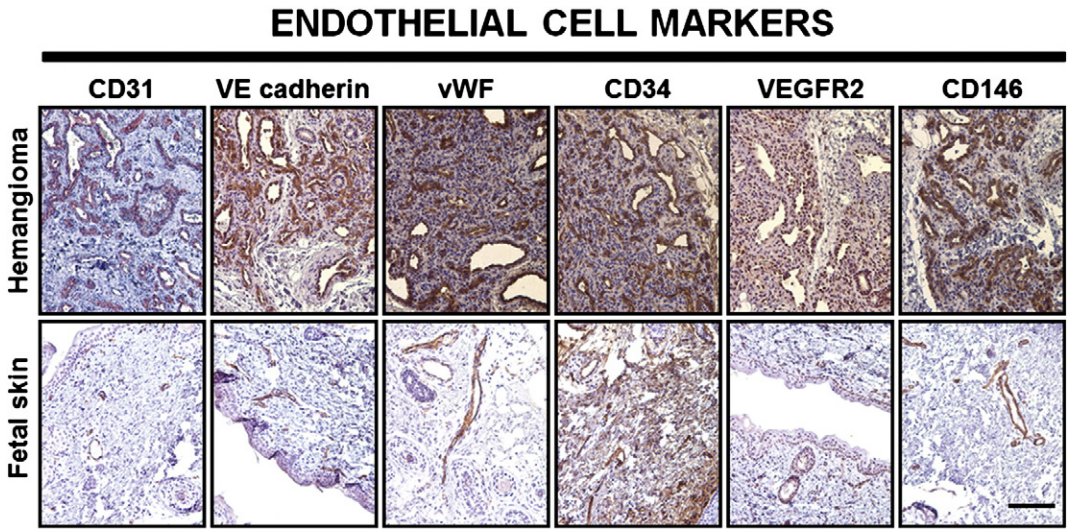

Fig. 2. Endothelial cell marker staining in proliferating hemangioma specimens. Proliferating hemangioma specimens were used following approval by the Ethics Board at the University of Western Ontario. Staining demonstrates positive immunoreactivity of all EC markers on hemangioma endothelial cells. [Fetal skin is used as positive control (Abcam); all images taken at 20× magnification; scale bar measurement represents 200 μm.]

ECs are similar to fetal ECs but not to fully mature ECs. Soon after, an aberrant functional feature of hemECs was also identified. Boye and colleagues showed that hemECs had an abnormal response to endostatin, an angiogenesis inhibitor. Unlike the control human dermal microvascular endothelial cells (HDMECs) that were inhibited by endostatin, hemECs showed increased migration in the presence of the anti-angiogenic peptide. The significance of this finding, however, was not fully clear.

A significant number of endothelial progenitor cells (EPCs) in proliferating phase hemangiomas have recently been reported (Yu et al., 2004). The study conducted by Yu et al. examined expression of CD133 mRNA in hemangiomas. Using RT-PCR, they were able to detect significant levels of CD133 mRNA in proliferating hemangiomas. The involution of hemangiomas was also associated with a significant drop in the CD133 transcript level. To validate the novel finding, anti-CD133 and anti-VEGFR2 antibody-labeled cells were pulled from a proliferating hemangioma and examined by flow cytometry. The study established that approximately 2% of the cell population was positive for both VEGFR2 and CD133 markers, indicating an endothelial progenitor nature of the cells. To further study the behavior of hemECs, we have isolated $CD133^+$ and $CD133^-$ hemangioma cells (Khan et al., 2006) (Fig. 5). The cells were then differentiated into ECs in culture. Using these isolated cells, we assessed the cellular activity in response to endostatin. We showed that hemEPCs (designated as CD133+ derived ECs) and hemECs (CD133-derived) increased adhesion, migration and proliferation in response to endostatin. Interestingly, this aberrant behavior was also observed in normal cord blood endothelial progenitor cells (cbEPCs) but not in the mature HDMECs (Khan et al., 2006). This suggested that the counterintuitive response to endostatin is a unique property of endothelial precursors. We then wanted to know if this 'precursor' response would dampen as the cells matured in culture. To our surprise, we found that hemangioma EPCs and ECs retained this precursor response for far longer periods than normal cord blood-derived EPCs (Khan et al., 2006). The continued abnormal stimulation of hemEPCs and hemECs by endostatin suggested a possible defect in the maturation process in the cells.

Most recently, we isolated hemangioma CD133+ stem cells and implanted them into immunodeficient mice to analyze their potential to form hemangiomas (Khan et al., 2008). We showed for the first time that these hemangioma stem cells (hemSCs) are able to produce blood vessels in vivo. Intriguingly, the blood vessels were Glut-1 positive, indicating that the isolated cells in fact had formed hemangiomas. This study was the first to provide experimental evidence that a stem cell is the hemangioma-initiating cell. This was a major advancement in the field, as we now know the cellular target for therapy. In light of these findings, a novel drug candidate for hemangiomas has come into play recently. Rapamycin, an mTOR (mammalian target of Rapamycin) inhibitor, has been shown to suppress the self-renewal of hemSCs and induce their differentiation into a perivascular phenotype (Greenberger et al., 2011). In addition, it has been shown to inhibit the vasculogenic capacity of these cells in vivo (Greenberger et al., 2011), implying its potential use for the treatment of proliferative hemangiomas. However, limited experience with this drug indicates that further studies must be conducted in order to assess its associated adverse effects.

Other cellular components of hemangiomas

Hemangiomas have an abundant level of mast cells surrounding the vasculature during the proliferative phase (Mulliken and Glowacki, 1982). Quantitative immunohistochemical analysis of hemangiomas revealed a 30–40 fold increase in mast cells surrounding proliferating vessels as compared to the vessels in the involuted phase (Mulliken and Young, 1988). Similar observations were also reported by another research group (Pasyk et al., 1984), that showed increased mast cells in both proliferating and involuting hemangiomas. While the role of mast cells in hemangiomas remains obscure, Pasyk et al. (1984) have suggested that mast cells may act as precursors/initiators for the involution phase of hemangiomas. Other investigations have demonstrated that mast cells are more prominent in hemangiomas when the lesions undergo involution (Tan et al., 2004). The observed presence of mast cells in different phases of hemangiomas has been attributed to their proangiogenic and antiangiogenic roles (Sun et al., 2007). Presence of mast cells during proliferation may also be indicative of an immunological response. Mast cells during involution may be representative of apoptosis and stimulation by chemokine signaling, which may account for reduced vessels and increased fibrofatty tissue formation.

Much attention has been allotted to the examination of the heterogenous cellular components of hemangiomas. Pericytes, also known as Rouget cells (Dore-Duffy and Cleary, 2011), are the elongated cells which surround the ECs (Yamagishi and Imaizumi, 2005). These cells of mesodermal origin (Yamagishi and Imaizumi, 2005) have been shown to be imperative in the maintenance of microvascular homeostasis (Yamagishi et al., 1993a, 1993b). However, their role in hemangiomas is not well studied. Immunohistological analyses reveal that perivascular cells in hemangiomas are immunoreactive to α-smooth muscle actin (α-SMA) (Li et al., 2003). The cells also express NG2, Calponin and Caldesmon in hemangiomas (Fig. 4). Interestingly, platelet-derived

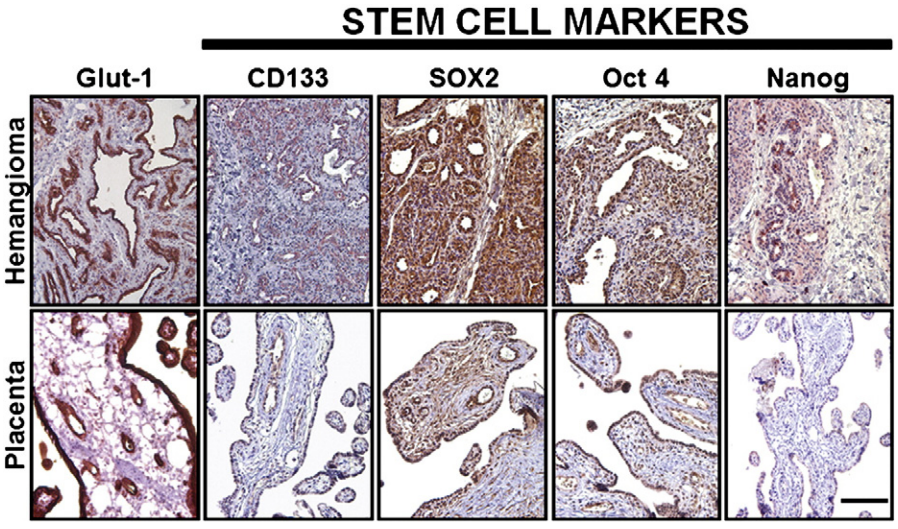

Fig. 3. Stem cell marker staining in hemangioma specimens. Positive CD133 and Glut-1 immunoreactivity is seen in endothelial cells. SOX2, Oct4 and Nanog also showed immunoreactivity in the interstitial cells. [Placenta is used as positive control; all images taken at 20× magnification; scale bar measurement represents 200 μm].

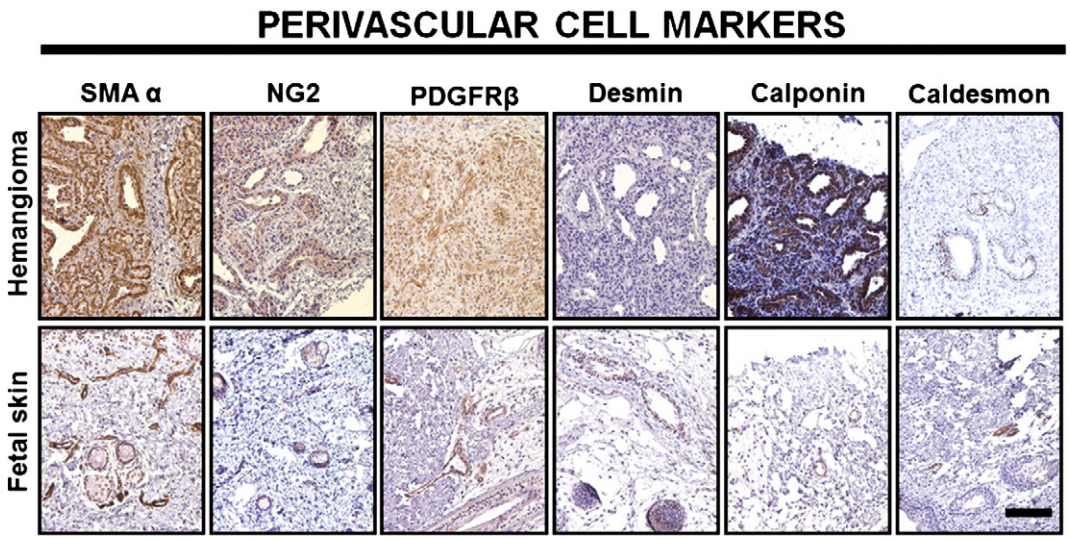

Fig. 4. Perivascular cell marker staining in hemangioma specimens. Perivascular marker staining demonstrated positive immunoreactivity in cells surrounding the capillaries in hemangioma specimens. Only PDGFRβ displayed a diffused pattern of expression throughout the entire tissue. [Fetal skin used as positive control; all images taken at 20× magnification; scale bar measurement represents 200 μm.]

growth factor receptor-β (PDGFRβ), a marker suggested to be expressed by pericytes and some stem/precursor cells, is not restricted to the vessels in hemangiomas, but rather is dispersed throughout most of the cells in the tissue. Caldesmon, a protein found in mature smooth muscle cells (Huber, 1997), is also positive in the mature enlarged hemangioma vessels but not the nascent capillaries. In fetal skin, caldesmon is positive in the small capillaries (Fig. 4). This immunohistological pattern may demonstrate that hemangioma capillaries are fully functional vessels, but may display a variation in perivascular cell marker expression depending on the maturity level.

Molecular and biochemical alterations in hemangiomas

While the exact aberrant EC signaling remains elusive in hemangioma-genesis, several signaling pathways have been proposed to play an important role. As hemangiomas exhibit disorganized EC

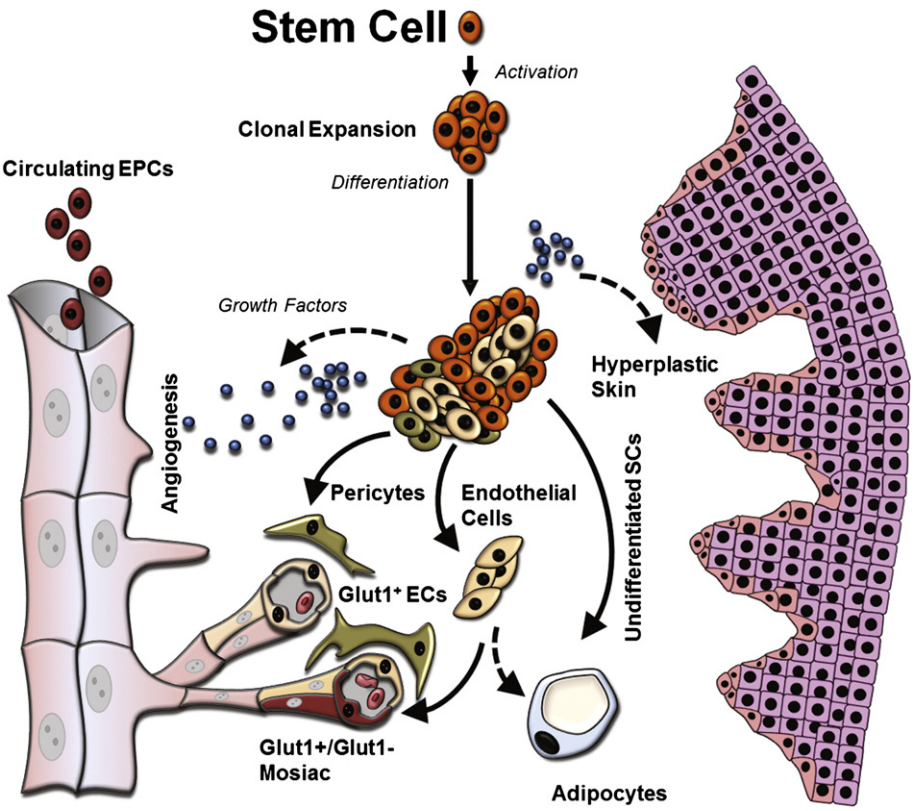

Fig. 5. Schematic of our working hypothesis. Hemangioma SCs undergo atypical activation and expansion. These CD133+ cells give rise to the Glut-1+ microvascular ECs and also pericytes during the proliferating phase. Increased growth factor expression may also cause adjacent tissue to exhibit hyperplasia and angiogenesis. Circulating endothelial progenitor cells may also incorporate into developing vessels producing mosaic Glut-1+ and Glut-1− vessels. As hemangiomas progress, immune cells may regulate disappearance of hemangioma vessels, thus triggering the involution and hemangioma stem cell differentiation into adipocytes.

proliferation, much attention has been turned to VEGF expression. VEGF plays a role in angiogenesis and vasculogenesis through its ligand-receptor interaction (Berse et al., 1992; Millauer et al., 1993; Shweiki et al., 1992). Jinnin et al. (2008) showed that a decrease in the availability of VEGF receptor-1 (VEGFR1) created an increased concentration of readily available VEGF in hemECs. Accessible VEGF ultimately activates the VEGFR2 and triggers EC proliferation. Absence of VEGFR1 has proven to be lethal in mice, causing vascular obstructions due to uncontrollable proliferation of the ECs (Kearney et al., 2002). VEGFR1 functionality is eminently important for binding VEGF and ultimately preventing unduly interaction with VEGFR2 (Ferrara, 2005; Roberts et al., 2004). Jinnin et al. (2008) also identified protein complexes carrying mutated forms of VEGFR2 and other proteins like tumor endothelial marker 8 (TEM8), in hemangioma ECs. The research group proposed that the protein complexes play an important role in down-regulating the activity of nuclear factor of activated T cells (NFAT), a transcription factor required for VEGFR1 activity regulation (Jinnin et al., 2008). Thus, with decreased availability of VEGFR1, more VEGF is readily available to bind and activate VEGFR2. This finding perpetuated the notion that it is the imbalance in VEGFR1 and VEGFR2 expression that causes increased VEGFR2 activity and ultimately aberrant EC proliferation. An outstanding question was whether hemangioma SCs also exhibit this aberrant VEGF signaling or is this property acquired once the SCs differentiate into ECs. A study conducted by the Bischoff lab has also implicated VEGFR-1 as the key mediator of EC differentiation in hemangiomas (Boscolo et al., 2011a). They concluded that the differentiation may occur through VEGF-A or VEGF-B induced ERK 1/2 phosphorylation in the hemSCs (Boscolo et al., 2011a). These studies were supported with in vivo experimentation, which demonstrated decreased vasculogenesis and ERK 1/2 phosphorylation in response to short-hairpin (sh)-RNA-mediated silencing of VEGFR1.

In a similar study, Bischoff's group looked at the mechanisms behind hemSC-to-pericyte differentiation. They found that JAGGED1, a ligand of the NOTCH signaling family, is up-regulated in hemECs and is required for both the differentiation of hemSCs to pericytes, and for proper blood vessel formation in vivo (Boscolo et al., 2011b). They also concluded that direct contact with endothelial cells is a necessary component for differentiation to the mesenchymal lineage. Taken together, the Bischoff group proposes that high levels of VEGF-A induces differentiation of hemSCs to hemECs in an autocrine fashion. hemECs are then able to promote neighboring stem cells to become pericytes, allowing vascular networks to form. These are very promising studies and a natural derivation would be a comparative analysis between hemSC- derived ECs and pericytes with those isolated from actual hemangioma specimens examining their phenotypic and functional properties in controlled culture conditions.

Other signaling pathways may also play a role in hemangioma EC proliferation. For example, the angiopoietin/tie signaling system has been shown to play a critical role in normal vascular development during embryogenesis, and vascular remodeling in adult mice (Maisonpierre et al., 1997; Sato et al., 1995; Suri et al., 1996). Disruption of the tie2 signaling pathway is lethal in mice due to interruption of angiogenesis during vascular system development (Jones and Dumont, 2000). Analysis of the tie2 mRNA expression by Yu et al. (2001) identified elevated tie2 in cultured hemECs in comparison to HDMECs. In contrast, other tyrosine kinases, including, tie1, VEGFR2, VEGFR1 (also known as Flt-1), Neuropilin-1, VE-cadherin and CD31 showed similar expression in hemECs and HDMECs (Yu et al., 2001). This is in contrast to the recent studies showing imbalance between VEGFR1 and VEGFR2 in hemangioma-derived cells and mature ECs. Whether the difference is due to 'which' cell types are isolated (cell surface markers and other methods used for isolating hemECs) or cell culture conditions, remains unclear. Tie2 was the only factor that exhibited differential expression between ECs isolated from hemangioma patients and those acquired from normal fetal skin. The difference in expression promoted the speculation of a possible increased activity in the angiopoietin/tie2 pathway due to an acquired somatic mutation contributing to a defect in the ECs making up the benign tumor.

Summary

There are many questions surrounding the pathogenesis of infantile hemangioma that have yet to be answered. The existence of a specific population of hemSCs has been well received, though not much is known regarding the switch between phases of hemangioma growth. Whether hemSCs are responsible for the cellular fate at each distinct phase has yet to be determined. Though hemSCs are typically considered to retain both endothelial and mesenchymal potential, their contribution to adipogenesis has not been elucidated. Recent studies have concluded that mesenchymal stem cells reside in the perivascular region of hemangiomas, and may be responsible for fibrofatty tissue production seen within the involuted phase (Yuan et al., 2012). Are these mesenchymal stem cells the same as hemangioma SCs or are these recruited from circulation/bone marrow? We must also consider whether it is more therapeutically advantageous to stop the proliferation of ECs within the proliferative phase, or rather accelerate the differentiation to adipocytes. Perhaps treatment is case-specific, making knowledge of the mechanism of action at each stage equally important. In addition, the role of other cell types within hemangiomas must be further examined. Mast cells, myeloid cells, as well as lymphocytes are all abundant components of hemangiomas that may play significant roles in tumorigenesis and even blood vessel regression, but are difficult to study using an in vivo mouse model. Although, hemangioma studies have made remarkable headway in recent years, it is clear that there is much more to uncover. Understanding the pathogenesis will not only aid in eradicating this tumor of infancy, but will also provide links to other diseases of vascular origin.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Amir, J., Metzker, A., Krikler, R., Reisner, S.H., 1986. Strawberry hemangioma in preterm infants. Pediatric Dermatology 3, 331–332.

Barlow, C.F., Priebe, C.J., Mulliken, J.B., Barnes, P.D., Mac Donald, D., Folkman, J., Ezekowitz, R.A., 1998. Spastic diplegia as a complication of interferon Alfa-2a treatment of hemangiomas of infancy. The Journal of Pediatrics 132, 527–530.

Beck, D.O., Gosain, A.K., 2009. The presentation and management of hemangiomas. Plastic and Reconstructive Surgery 123, 181e–191e.

Berse, B., Brown, L.F., Van de Water, L., Dvorak, H.F., Senger, D.R., 1992. Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. Molecular Biology of the Cell 3, 211–220.

Bielenberg, D.R., Bucana, C.D., Sanchez, R., Mulliken, J.B., Folkman, J., Fidler, I.J., 1999. Progressive growth of infantile cutaneous hemangiomas is directly correlated with hyperplasia and angiogenesis of adjacent epidermis and inversely correlated with expression of the endogenous angiogenesis inhibitor, IFN-beta. International Journal of Oncology 14, 401–408.

Boscolo, E., Mulliken, J.B., Bischoff, J., 2011a. VEGFR-1 mediates endothelial differentiation and formation of blood vessels in a murine model of infantile hemangioma. The American Journal of Pathology 179, 2266–2277.

Boscolo, E., Stewart, C.L., Greenberger, S., Wu, J.K., Durham, J.T., Herman, I.M., Mulliken, J.B., Kitajewski, J., Bischoff, J., 2011b. JAGGED1 signaling regulates hemangioma stem cell-to-pericyte/vascular smooth muscle cell differentiation. Arteriosclerosis, Thrombosis, and Vascular Biology 31, 2181–2192.

Boye, E., Yu, Y., Paranya, G., Mulliken, J.B., Olsen, B.R., Bischoff, J., 2001. Clonality and altered behavior of endothelial cells from hemangiomas. The Journal of Clinical Investigation 107, 745–752.
Bree, A.F., Siegfried, E., Sotelo-Avila, C., Nahass, G., 2001. Infantile hemangiomas: speculation on placental trophoblastic origin. Archives of Dermatology 137, 573–577.
Bruckner, A.L., Frieden, I.J., 2003. Hemangiomas of infancy. Journal of the American Academy of Dermatology 48, 477–493.
Bruckner, A.L., Frieden, I.J., 2006. Infantile hemangiomas. Journal of the American Academy of Dermatology 55, 671–682.
Burton, B.K., Schulz, C.J., Angle, B., Burd, L.I., 1995. An increased incidence of haemangiomas in infants born following chorionic villus sampling (CVS). Prenatal Diagnosis 15, 209–214.
Chiller, K.G., Passaro, D., Frieden, I.J., 2002. Hemangiomas of infancy: clinical characteristics, morphologic subtypes, and their relationship to race, ethnicity, and sex. Archives of Dermatology 138, 1567–1576.
Cohen Jr., M.M., 2007. Hemangiomas: their uses and abuses. American Journal of Medical Genetics. Part A 143, 235–240.
Cooper, A.G., Bolande, R.P., 1965. Multiple hemangiomas in an infant with cardiac hypertrophy. Postmortem angiographic demonstration of the arteriovenous fistulae. Pediatrics 35, 27–35.
Dore-Duffy, P., Cleary, K., 2011. Morphology and properties of pericytes. Methods in Molecular Biology 686, 49–68.
Dosanjh, A., Chang, J., Bresnick, S., Zhou, L., Reinisch, J., Longaker, M., Karasek, M., 2000. In vitro characteristics of neonatal hemangioma endothelial cells: similarities and differences between normal neonatal and fetal endothelial cells. Journal of Cutaneous Pathology 27, 441–450.
Enjolras, O., Mulliken, J.B., 1993. The current management of vascular birthmarks. Pediatric Dermatology 10, 311–313.
Enjolras, O., Riche, M.C., Merland, J.J., Escande, J.P., 1990. Management of alarming hemangiomas in infancy: a review of 25 cases. Pediatrics 85, 491–498.
Ferrara, N., 2005. The role of VEGF in the regulation of physiological and pathological angiogenesis. EXS 209–231.
Fost, N.C., Esterly, N.B., 1968. Successful treatment of juvenile hemangiomas with prednisone. The Journal of Pediatrics 72, 351–357.
Frieden, I.J., Eichenfield, L.F., Esterly, N.B., Geronemus, R., Mallory, S.B., 1997. Guidelines of care for hemangiomas of infancy. American Academy of Dermatology Guidelines/Outcomes Committee. Journal of the American Academy of Dermatology 37, 631–637.
Froehner, S.C., Davies, A., Baldwin, S.A., Lienhard, G.E., 1988. The blood–nerve barrier is rich in glucose transporter. Journal of Neurocytology 17, 173–178.
Gonzalez-Crussi, F., Reyes-Mugica, M., 1991. Cellular hemangiomas ("hemangioendotheliomas") in infants. Light microscopic, immunohistochemical, and ultrastructural observations. The American Journal of Surgical Pathology 15, 769–778.
Gould, G.W., Holman, G.D., 1993. The glucose transporter family: structure, function and tissue-specific expression. The Biochemical Journal 295 (Pt. 2), 329–341.
Greenberger, S., Yuan, S., Walsh, L.A., Boscolo, E., Kang, K.T., Matthews, B., Mulliken, J.B., Bischoff, J., 2011. Rapamycin suppresses self-renewal and vasculogenic potential of stem cells isolated from infantile hemangioma. The Journal of Investigative Dermatology 131, 2467–2476.
Greinwald Jr., J.H., Burke, D.K., Bonthius, D.J., Bauman, N.M., Smith, R.J., 1999. An update on the treatment of hemangiomas in children with interferon alfa-2a. Archives of Otolaryngology — Head & Neck Surgery 125, 21–27.
Haggstrom, A.N., Drolet, B.A., Baselga, E., Chamlin, S.L., Garzon, M.C., Horii, K.A., Lucky, A.W., Mancini, A.J., Metry, D.W., Newell, B., Nopper, A.J., Frieden, I.J., 2006a. Prospective study of infantile hemangiomas: clinical characteristics predicting complications and treatment. Pediatrics 118, 882–887.
Haggstrom, A.N., Lammer, E.J., Schneider, R.A., Marcucio, R., Frieden, I.J., 2006b. Patterns of infantile hemangiomas: new clues to hemangioma pathogenesis and embryonic facial development. Pediatrics 117, 698–703.
Holmdahl, K., 1955. Cutaneous hemangiomas in premature and mature infants. Acta Paediatrica 44, 370–379.
Holmes, L.B., 2009. Chorionic villus sampling and hemangiomas. The Journal of Craniofacial Surgery 20 (Suppl. 1), 675–677.
Hopfel-Kreiner, I., 1980. Histogenesis of hemangiomas—an ultrastructural study on capillary and cavernous hemangiomas of the skin. Pathology, Research and Practice 170, 70–90.
Huber, P.A., 1997. Caldesmon. The International Journal of Biochemistry & Cell Biology 29, 1047–1051.
Jansson, T., Wennergren, M., Illsley, N.P., 1993. Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation. The Journal of Clinical Endocrinology and Metabolism 77, 1554–1562.
Jinnin, M., Medici, D., Park, L., Limaye, N., Liu, Y., Boscolo, E., Bischoff, J., Vikkula, M., Boye, E., Olsen, B.R., 2008. Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma. Nature Medicine 14, 1236–1246.
Jones, N., Dumont, D.J., 2000. Tek/Tie2 signaling: new and old partners. Cancer Metastasis Reviews 19, 13–17.
Kaplan, P., Normandin Jr., J., Wilson, G.N., Plauchu, H., Lippman, A., Vekemans, M., 1990. Malformations and minor anomalies in children whose mothers had prenatal diagnosis: comparison between CVS and amniocentesis. American Journal of Medical Genetics 37, 366–370.
Kearney, J.B., Ambler, C.A., Monaco, K.A., Johnson, N., Rapoport, R.G., Bautch, V.L., 2002. Vascular endothelial growth factor receptor Flt-1 negatively regulates developmental blood vessel formation by modulating endothelial cell division. Blood 99, 2397–2407.
Khan, Z.A., Melero-Martin, J.M., Wu, X., Paruchuri, S., Boscolo, E., Mulliken, J.B., Bischoff, J., 2006. Endothelial progenitor cells from infantile hemangioma and umbilical cord blood display unique cellular responses to endostatin. Blood 108, 915–921.
Khan, Z.A., Boscolo, E., Picard, A., Psutka, S., Melero-Martin, J.M., Bartch, T.C., Mulliken, J.B., Bischoff, J., 2008. Multipotential stem cells recapitulate human infantile hemangioma in immunodeficient mice. The Journal of Clinical Investigation 118, 2592–2599.
Krol, A., MacArthur, C.J., 2005. Congenital hemangiomas: rapidly involuting and noninvoluting congenital hemangiomas. Archives of Facial Plastic Surgery 7, 307–311.
Leaute-Labreze, C., Dumas de la Roque, E., Hubiche, T., Boralevi, F., Thambo, J.B., Taieb, A., 2008. Propranolol for severe hemangiomas of infancy. The New England Journal of Medicine 358, 2649–2651.
Li, Q., Yu, Y., Bischoff, J., Mulliken, J.B., Olsen, B.R., 2003. Differential expression of CD146 in tissues and endothelial cells derived from infantile haemangioma and normal human skin. The Journal of Pathology 201, 296–302.
Lo, K., Mihm, M., Fay, A., 2009. Current theories on the pathogenesis of infantile hemangioma. Seminars in Ophthalmology 24, 172–177.
MacArthur, C.J., Senders, C.W., Katz, J., 1995. The use of interferon alfa-2a for life-threatening hemangiomas. Archives of Otolaryngology — Head & Neck Surgery 121, 690–693.
Maisonpierre, P.C., Suri, C., Jones, P.F., Bartunkova, S., Wiegand, S.J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T.H., Papadopoulos, N., Daly, T.J., Davis, S., Sato, T.N., Yancopoulos, G.D., 1997. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277, 55–60.
Malan, E., 1974. Vascular Anomalies (Angiodysplasias). Carlo Erba Foundation, Milan, p. 17.
Millauer, B., Wizigmann-Voos, S., Schnurch, H., Martinez, R., Moller, N.P., Risau, W., Ullrich, A., 1993. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 72, 835–846.
Mulliken, J.B., 1992. A biologic approach to cutaneous vascular anomalies. Pediatric Dermatology 9, 356–357.
Mulliken, J.B., 1993. Cutaneous vascular anomalies. Seminars in Vascular Surgery 6, 204–218.
Mulliken, J.B., Glowacki, J., 1982. Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. Plastic and Reconstructive Surgery 69, 412–422.
Mulliken, J.B., Young, A.E., 1988. Classifications of Vascular Birthmarks. Vascular Birthmarks: Hemangiomas and Malformations. W.B. Saunders, Philadelphia, pp. 24–37.
Nguyen, J., Fay, A., 2009. Pharmacologic therapy for periocular infantile hemangiomas: a review of the literature. Seminars in Ophthalmology 24, 178–184.
North, P.E., Waner, M., Mizeracki, A., Mihm Jr., M.C., 2000. GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. Human Pathology 31, 11–22.
North, P.E., Waner, M., Mizeracki, A., Mrak, R.E., Nicholas, R., Kincannon, J., Suen, J.Y., Mihm Jr., M.C., 2001. A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. Archives of Dermatology 137, 559–570.
North, P.E., Waner, M., Brodsky, M.C., 2002. Are infantile hemangiomas of placental origin? Ophthalmology 109, 633–634.
Pack, G.T., Miller, T.R., 1950. Hemangiomas; classification, diagnosis and treatment. Angiology 1, 405–426.
Pasyk, K.A., Cherry, G.W., Grabb, W.C., Sasaki, G.H., 1984. Quantitative evaluation of mast cells in cellularly dynamic and adynamic vascular malformations. Plastic and Reconstructive Surgery 73, 69–77.
Rhoads, G.G., Jackson, L.G., Schlesselman, S.E., de la Cruz, F.F., Desnick, R.J., Golbus, M.S., Ledbetter, D.H., Lubs, H.A., Mahoney, M.J., Pergament, E., et al., 1989. The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. The New England Journal of Medicine 320, 609–617.
Ritter, M.R., Reinisch, J., Friedlander, S.F., Friedlander, M., 2006. Myeloid cells in infantile hemangioma. The American Journal of Pathology 168, 621–628.
Ritter, M.R., Butschek, R.A., Friedlander, M., Friedlander, S.F., 2007. Pathogenesis of infantile haemangioma: new molecular and cellular insights. Expert Reviews in Molecular Medicine 9, 1–19.
Roberts, D.M., Kearney, J.B., Johnson, J.H., Rosenberg, M.P., Kumar, R., Bautch, V.L., 2004. The vascular endothelial growth factor (VEGF) receptor Flt-1 (VEGFR-1) modulates Flk-1 (VEGFR-2) signaling during blood vessel formation. The American Journal of Pathology 164, 1531–1535.
Sato, T.N., Tozawa, Y., Deutsch, U., Wolburg-Buchholz, K., Fujiwara, Y., Gendron-Maguire, M., Gridley, T., Wolburg, H., Risau, W., Qin, Y., 1995. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. Nature 376, 70–74.
Shweiki, D., Itin, A., Soffer, D., Keshet, E., 1992. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359, 843–845.
Smolinski, K.N., Yan, A.C., 2005. Hemangiomas of infancy: clinical and biological characteristics. Clinical Pediatrics 44, 747–766.
Smoller, B.R., Apfelberg, D.B., 1993. Infantile (juvenile) capillary hemangioma: a tumor of heterogeneous cellular elements. Journal of Cutaneous Pathology 20, 330–336.
Sun, Z.J., Zhao, Y.F., Zhao, J.H., 2007. Mast cells in hemangioma: a double-edged sword. Medical Hypotheses 68, 805–807.
Suri, C., Jones, P.F., Patan, S., Bartunkova, S., Maisonpierre, P.C., Davis, S., Sato, T.N., Yancopoulos, G.D., 1996. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87, 1171–1180.
Tan, S.T., Wallis, R.A., He, Y., Davis, P.F., 2004. Mast cells and hemangioma. Plastic and Reconstructive Surgery 113, 999–1011.

Virchow, R.A., 1863. Die krankhaften geschweulste. August Hirschwald, Berlin. 306 pp.
Walter, J.W., North, P.E., Waner, M., Mizeracki, A., Blei, F., Walker, J.W., Reinisch, J.F., Marchuk, D.A., 2002. Somatic mutation of vascular endothelial growth factor receptors in juvenile hemangioma. Genes, Chromosomes & Cancer 33, 295–303.
Waner, M., North, P.E., Scherer, K.A., Frieden, I.J., Waner, A., Mihm Jr., M.C., 2003. The nonrandom distribution of facial hemangiomas. Archives of Dermatology 139, 869–875.
Weber, T.R., Connors, R.H., Tracy Jr., T.F., Bailey, P.V., 1990. Complex hemangiomas of infants and children. Individualized management in 22 cases. Archives of Surgery 125, 1017–1020.
Worle, H., Maass, E., Kohler, B., Treuner, J., 1999. Interferon alpha-2a therapy in haemangiomas of infancy: spastic diplegia as a severe complication. European Journal of Pediatrics 158, 344.
Yamagishi, S., Imaizumi, T., 2005. Pericyte biology and diseases. International Journal of Tissue Reactions 27, 125–135.
Yamagishi, S., Hsu, C.C., Kobayashi, K., Yamamoto, H., 1993a. Endothelin 1 mediates endothelial cell-dependent proliferation of vascular pericytes. Biochemical and Biophysical Research Communications 191, 840–846.
Yamagishi, S., Kobayashi, K., Yamamoto, H., 1993b. Vascular pericytes not only regulate growth, but also preserve prostacyclin-producing ability and protect against lipid peroxide-induced injury of co-cultured endothelial cells. Biochemical and Biophysical Research Communications 190, 418–425.
Yu, Y., Varughese, J., Brown, L.F., Mulliken, J.B., Bischoff, J., 2001. Increased Tie2 expression, enhanced response to angiopoietin-1, and dysregulated angiopoietin-2 expression in hemangioma-derived endothelial cells. The American Journal of Pathology 159, 2271–2280.
Yu, Y., Flint, A.F., Mulliken, J.B., Wu, J.K., Bischoff, J., 2004. Endothelial progenitor cells in infantile hemangioma. Blood 103, 1373–1375.
Yuan, S.M., Chen, R.L., Shen, W.M., Chen, H.N., Zhou, X.J., 2012. Mesenchymal stem cells in infantile hemangioma reside in the perivascular region. Pediatric and Developmental Pathology 15, 5–12.