Published in final edited form as:

J Craniofac Surg. 2009 March ; 20(Suppl 1): 695–697. doi:10.1097/SCS.0b013e318193d6ac.

Progenitor cells in infantile hemangioma

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Abstract

Infantile hemangioma is a vascular tumor that occurs in 5–10% of infants of European descent. A hallmark of infantile hemangioma is its lifecycle, which is divided into three stages. The proliferating phase spans the first year of postnatal life, and is characterized by cellular masses without a defined vascular architecture as well as nascent blood vessels with red blood cells evident within the luminal space. The involuting phase begins around one year of age and continues for 3–5 years. Proliferation slows or stops in this phase, and histology shows that the blood vessel architecture becomes more obvious and vessel size is enlarged. The involuted phase is reached by 5–8 years of age, at which point blood vessels are replaced with a fibrofatty residuum and capillary-sized channels. The growth and involution life-cycle of infantile hemangioma is very different from other vascular tumors and vascular malformations, which do not regress and can occur at any time during childhood or adult life. Many laboratories have reported on the endothelial characteristics of the cellular masses that are prominent in the proliferating phase of infantile hemangioma, as well as their immature appearance. These studies, along with isolation and characterization of hemangioma-derived cell populations with progenitor cell properties have lead to an emerging hypothesis that hemangioma is caused by an abnormal or delayed differentiation of mesodermal progenitor cells into the disorganized mass of blood vessels. Here we discuss the literature that support this emerging hypothesis.

Introduction

Infantile hemangioma, referred to herein as hemangioma, is a common childhood tumor composed of disorganized blood vessels and immature endothelial cells (1,2). Hemangioma usually follows a benign course, however in some children the tumors can threaten tissue and organ function and even become life-threatening. There are many examples of tissue and organ damage that can ensue: deprivation amblyopia from eyelid hemangioma, airway compromise from subglottic hemangioma, ulceration and bleeding that can require transfusion and/or surgery, and in large hemangiomas, high-output congestive heart failure(3). Children with endangering or life-threatening hemangiomas are treated with corticosteroids, surgery or interferon- α . However, approximately 30% of hemangiomas do not respond to steroids and irreversible neurologic toxicity has been associated with interferon- α administration to infants with hemangioma (4–7). Interferon- α is now rarely used; vincristine, a chemotherapeutic agent, has become an option for IHs that do not respond to steroids. A recent report showed the beta-adrenergic receptor antagonist, propranolol, as an effective treatment for children with severe hemangioma (8) indicating that new safe and fast-acting treatments are being actively sought. Understanding the precise cellular mechanisms that drive growth and involution of hemangioma will provide a basis for targeted therapies to either stop the proliferating phase

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or accelerate the involuting phase in order to minimize tissue destruction and morbidity associated with hemangioma.

Cellular Origin of Infantile Hemangioma

An understanding the cellular origin of hemangioma and the molecular regulation of the proliferating, involuting and involuted phases has been pursued by several clinical and basic science investigative teams. An important place to start has been to examine the cellular components of the proliferating phase at the morphological level in hemangioma tissue sections. A second important effort has been to dissect the lesions into purified cell types that can be studied, tested and compared in the laboratory. Here in we will discuss several papers that have made important inroads in understanding the cellular complexity of hemangioma.

Interstitial cells

Although hemangioma is most often noted for its vascularity and angiogenic profile (9), others have focused on the cellular elements that appear to be primitive or immature cells in the interstitial regions between the vascular channels(10). Smoller and Apfelberg focused intensively on these “interstitial tumor cells”, using histology and immunostaining to identify highly cellular areas composed of cuboidal and spindle-shaped cells. The interstitial cells did not react with anti-factor VIII-related antigen (also known as von Willebrand factor, vWF), a marker of differentiated endothelial cells. Instead most of the interstitial cells were positive for factor XIIIa, a marker of dermal dendrocytes, some positive for CD34, a stem cell and endothelial marker, and even fewer positive for α -smooth muscle actin, a pericyte/smooth muscle cell/myofibroblast marker. Anti- α -SMA also stained the perivascular cells surrounding the vascular lumens, as expected. Importantly, most of the proliferating cells were seen in the interstitial, non-vascular areas. In this 1993 publication, the authors put forth the concept that the proliferating phase hemangioma is composed of a primitive progenitor cell capable of differentiating into endothelial cells and pericytes(10). This concept was extended, again by Smoller and colleagues, by quantifying the increased proliferative index in interstitial cells compared to the endothelial cells(11). Increased expression of the anti-apoptotic gene *bcl-2* in the interstitial cells was also shown, suggesting these cells might have a survival advantage. Intriguingly, their quantitative analyses suggested that proliferation and *bcl-2* expression was prolonged in lesions with markedly more vascular channels (11), which the authors interpreted as an indication that more highly vascularized hemangiomas undergo longer periods of growth before the onset of the involuting phase. A more in-depth understanding of this observation might provide a means to predict which hemangiomas will grow to an endangering size. Two studies have investigated expression of intracellular adhesion molecule-2 (ICAM-3) in hemangioma, and shown that it serves as a marker of immature vascular elements and nascent vessels in hemangioma (12,13).

Immature endothelial cells

A pioneering study by Mulliken and colleagues showed that it was possible to isolate hemangioma-derived endothelial cells (HemECs) and study their phenotype in isolation in vitro (14). Dosanjh and colleagues took this a step further and compared the in vitro characteristics of HemECs to fetal skin endothelial cells (isolated from second trimester pregnancy terminations) and neonatal skin endothelial cells (isolated from newborn foreskin following routine circumcision) (15). The HemEC were found to share many features with fetal ECs and could be distinguished from neonatal ECs. The HemEC and fetal EC exhibited a spindle-shaped morphology when grown in vitro and produced type I collagen but not type IV collagen. Conversely, neonatal ECs showed a cobblestone morphology and produced type IV collagen but not type I collagen. Most notably, HemECs and fetal ECs exhibited a diffuse intracellular pattern of (platelet endothelial adhesion molecule-1 (PECAM-1, also known as

CD31) and vWF immunostaining, which suggested these were not fully differentiated endothelial cells. The authors concluded their study with the speculation that hemangioma may represent a dysregulation of endothelial differentiation and maturation(15). This concept can be integrated with those of Smoller and colleagues by considering hemangioma as a vascular progenitor cell that can give rise to pericytes/perivascular cells and to endothelial cells, but that cells differentiating towards the endothelial lineage become delayed or disrupted due to a maturation defect.

Endothelial progenitor cells in hemangioma

In my laboratory, we isolated hemangioma-derived endothelial cells (HemECs) from proliferating hemangioma specimens and together with Boye and Olsen showed the HemECs were clonal and exhibited increased growth and migratory properties (16). Clonality was also shown in cells analyzed directly from tissue sections of proliferating hemangiomas by Marchuk and colleagues (17), which argues against the clonality being due to in vitro culture favoring a small number of cells. We subsequently isolated increasingly less differentiated cells from proliferating hemangioma tissue, including hemangioma-derived endothelial progenitor cells (HemEPCs)(18,19). Our working hypothesis was that endothelial progenitor cells in hemangioma tissue are the cellular precursors of the HemEC/endothelial cells lining the vascular channels of the tumor. To test this, we examined cellular growth, migration and adhesion of HemEPCs and HemEC, with the expectation that the cells would exhibit the same properties, in particular the HemEPCs would show the same stimulatory response to endostatin (an angiogenesis inhibitor (20)) that we found in HemECs (16). The HemEPCs were stimulated by endostatin, but unexpectedly so were normal healthy cord blood-derived EPCs(19). In summary, we found that HemEPCs, HemECs and cord blood EPCs were similar to each other in several in vitro assays, including mRNA transcriptional profiling for expression of cell-cell and cell-matrix adhesion molecules. Similar to the study of Dosanjh and colleagues(15), HemECs could be distinguished from human dermal microvascular endothelial cells. Our conclusion is that HemEPCs and HemECs are immature endothelial cells and share properties with cord blood EPCs. This raises the question of whether circulating EPCs are recruited into proliferating hemangioma lesions and if so, do they contribute to growth. In support of this, Kleinman and colleagues reported an increased number of circulating CD133+/CD34+ EPCs in children with hemangioma(21). However, the age-matched controls were somewhat older (average age 38 months) than the hemangioma patients (average age 25months). Since levels of circulating EPCs are likely to vary during infancy and early childhood, it would be important to extend these studies to a larger number of more closely matched controls.

Angiogenesis or Vasculogenesis?

Hemangioma has long been considered an angiogenic disease because of the tangled disorganized mass of blood vessels in the tumor (9,22), but the idea that hemangioma arises by a process more akin to vasculogenesis – the de novo formation of vessels from progenitor cells – was proposed nearly fifty years ago by Pack and Miller(23). Progenitor cells or “dormant angioblasts” could reside within fetal or neonatal tissue, as proposed, and/or be recruited to the lesion site from placenta or another reservoir of stem/progenitor cells. An event would stimulate vasculogenesis, leading to the onset of the proliferating phase. Once underway, angiogenesis from pre-existing vessels in the surrounding tissue should contribute by establishing connections between the nascent vessels and existing vessels. Circulating EPCs may also play a role.

The possibility of bone marrow-like cells contributing to vasculogenesis in the proliferating phase was suggested by Nguyen and colleagues (24) based on detection of cells expressing HLA-DR and CD68 antigen in a close proximity to vessel structures in hemangioma tissue sections. The cells were shown to be distinct from pericytes or immune cells such as T-cells,

B-cells, NK cells or mast cells. Their morphology and immunophenotype was suggested to be most closely aligned with antigen presenting cells of the monocyte/macrophage/dendritic lineage. Others used the lymphatic endothelial hyaluronan receptor-1 (LYVE-1), CD34 and VEGF-R3 antibodies to characterize the cellular elements in proliferating and involuting hemangioma (25). In this study, the similar immunophenotype between vessels in proliferating phase hemangioma and the embryonic cardinal vein endothelium that gives rise to lymphatic and venular endothelium led the authors to propose that hemangioma vessels are “arrested” at an immature stage of vascular differentiation. Immunostaining for LYVE-1 was not observed in involuting phase hemangioma, prompting the authors to suggest LYVE-1 as a novel marker of the proliferating hemangioma. However, in the same year, Xu and colleagues reported that hemangiomas were negative for LYVE-1 immunostaining whereas vessels in angiosarcomas were positive (26). Such discrepancies underscore the importance of using multiple approaches to study gene and protein expression in hemangioma.

Hemangioma Multipotent Stem Cells

We recently reported on an immature progenitor-like cell we isolated from proliferating hemangioma specimens – we refer to the cells as hemangioma-derived stem cells (HemSCs) (27). The HemSCs exhibited robust proliferative and clonogenic capacity, and can differentiate into cells of multiple lineages, thereby fulfilling two important features of stem cells. Importantly, we showed that clonal HemSC populations expanded *in vitro* from single cells produced human GLUT-1-positive microvessels *in vivo*. HemSCs that had differentiated into endothelium *in vivo*, as determined by cell surface CD31 expression, could be retrieved and implanted into secondary recipients, where the cells formed blood vessels once more. This indicates the robust vasculogenic potential of these HemSCs. The vasculogenic potential is restricted to the HemSC because HemEC, HemEPCs, cord blood EPCs, normal human fibroblasts and bone marrow-derived mesenchymal stem cells do not form vessels *in vivo* in this model. Over time in the *in vivo* model, the human blood vessels diminished and human adipocytes became evident, reminiscent of the involuted phase. GFP-labeled HemSCs were shown to form vessels and adipocytes at 14 and 56 day time points, respectively, confirming that the vessels and adipocytes were not host-derived. These results demonstrate that HemSCs are the cellular precursors of infantile hemangioma and may in fact be the primitive cells that have been described in histological sections. In summary, our findings provide evidence for a stem cell origin of hemangioma, which is in contrast to the long-held view that hemangioma arises from ECs (22).

A limitation of our study was that we have not identified the origin of the α -SMA pericytes that prominently surround the vessels in hemangioma. These cells could also be derived from the HemSC, which can be tested directly, or may arise from normal mesenchymal stem cells recruited into the hemangioma. In a previous study, we isolated and characterized mesenchymal stem cells from hemangioma tissue following published procedures that rely on rapid adherence of the cells to plastic non-coated Petri dishes (28). A second limitation of our study is that the hemangioma lesions formed in mice do not grow appreciably, as would be expected for a proliferating hemangioma. There are several potential explanations for this. First, it might be that the proliferative potential has slowed by the time the lesion is removed from the child and the cells are purified and expanded in the laboratory. Second, systemic factors that influence post-natal vasculogenesis might be significantly different between an infant and a 6–8 week old mice. Third, our model may be missing critical cellular components; this can be addressed experimentally.

Summary

The evidence for a stem/progenitor cell as the cellular origin of hemangioma has been accumulating for many years, originating from different laboratories using a variety of

experimental approaches. Several questions remain to be explored – including the trigger for the involuting phase – yet identifying the cellular origin of IH is a critical step forward that will facilitate efforts to develop safe and fast-acting therapies for children with hemangioma. Our *in vitro* and *in vivo* models using HemSCs offer powerful tools to evaluate potential therapeutic strategies and more broadly, may lead to new insights into the role of post-natal human multipotent stem/progenitor cells in the *nov*o formation of blood vessels.

Acknowledgments

Funding: NIH P01 AR48564

The author thanks Dr Mulliken for being a mentor and collaborator in her studies on the cellular and molecular mechanisms of hemangioma.

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