Angiogenesis in congenital vascular malformations: a dynamic view on a static lesion

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Chapter 3

Angiogenesis in benign vascular lesions
– basic aspects
Angiogenesis in benign vascular lesions
It is of interest to see that the early classification of Mulliken and Glowacki in 1982, which clearly separated angioma from malformation, is “a classification based on endothelial characteristics” (as the authors state in the title of their manuscript). Endothelial cell proliferative activity was measured by means of $[^3]$H thymidine radioautography, and revealed (initial) endothelial proliferative activity in angioma, but only quiescent endothelial growth in the various types of vascular malformations. Since then, much research has been done to elucidate the mechanisms of vascular growth, and specifically whether they relate to either vasculogenesis, angiogenesis and/or (in arteriovenous malformations) arteriogenesis. Insight in mechanisms of vessel growth in the clinically different types of vascular lesions can be important for a proper understanding of the pathogenesis of disease, but may also lead to improvement of treatment strategies, for example with anti-angiogenic drugs.2,3

Vasculogenesis is the process of formation of ‘blood islands’ during embryonic development.4 Mesodermal derived precursor cells (hemangioblasts) migrate and differentiate to form new blood islands. Important players in this process are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) including their receptors, and also the Ephrins.5 VEGFR-2 seems to mediate the major growth and permeability actions of VEGF.6 Although vasculogenesis mainly takes place during embryogenesis it can also occur in postnatal life, initiated by stem/progenitor cells.

Angiogenesis refers to a process in which new vessels arise after a primary vascular plexus is formed, thus referring to growth of new blood vessels derived from pre-existing ones.7,8 There are non-sprouting (intussusception or splitting) and sprouting (budding) forms of angiogenesis. In non-sprouting angiogenesis the initial network is modified through pruning and enlargement of pre-existing vessels, to form the interconnecting branching patterns with maturation of vessels.5,9 Sprouting angiogenesis refers to a process in which a new capillary network is formed from pre-existing vessels. Angiogenesis appears to be fundamental not only for physiological vascularization, but also in pathological states such as wound healing, inflammation and neoplasias (tumor growth, invasion and metastasis).10 In many cases, tissue hypoxia/ischemia is the driving force. As a response to such a trigger, angiogenic growth factors are expressed and bind to specific receptors of endothelial cells (ECs), which lead to endothelial cell activation and proliferation.8 The pre-existing vessels become permeable and dilate. The next step involves loosening of the intercellular junctions and degradation of the extracellular matrix, followed by EC differentiation and migration of ECs. Endothelial cells form tubes, produce a new basement membrane and recruit pericytes, which leads to stabilization of the new vessels. Once the vessels have matured, their endothelial cells remain relatively quiescent.
Also in angiogenesis, VEGF is one of the key regulators. It stimulates migration, proliferation of ECs, and matrix degradation. VEGF works together with other angiogenic growth factors. Angiopoietin-1 (Ang-1) is a ligand of the Tie2 receptor located on endothelial cell surfaces and promotes EC migration and survival. Angiopoietin-2 functions as an antagonist for Ang-1. It stimulates angiogenesis in the presence of VEGF, but contributes to regression of vessels in absence of VEGF.

Proteases, and specifically the family of matrix metalloproteinases (MMPs) secreted by ECs, influence angiogenesis by degrading extracellular matrix molecules components of the tissues to be vascularized.

In contrast to vasculogenesis and angiogenesis, arteriogenesis refers to a process in which pre-existing arteriolar anastomoses enlarge by getting a more muscularized vascular wall, form collateral vessels through growth and proliferation, and then stabilize. Here, the growth factors bFGF, platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β) produced by endothelial cells have been identified as the key regulators. A hallmark for the onset arteriogenesis is that increased levels of flow forces are required.

**Hemangiomas**

Histopathology shows that infantile hemangiomas are initially extremely cellular, and apart from the proliferating microvessels, there are also proliferating “interstitial” cells present. The progenitor cell marker CD133 is expressed on a small subset of scattered cells indicating the undifferentiated status of some cells in the proliferating phase. A multipotent progenitor cell capable of de novo blood vessel formation has also been isolated from infantile hemangioma. These findings support a role for vasculogenesis, which is the de novo formation of vessels from progenitor cells. Immature ECs could arise from stem/progenitor cells that reside within the lesion, for example dormant angioblasts, or derived from a reservoir of progenitor cells such as placenta or bone marrow. After initiation of this process, angiogenesis may also contribute to vessel growth, including ingrowth of vessels form surrounding tissues; the expression of the major angiogenic growth factors bFGF, VEGF-A, Tie2 and angiopoietin-2 that are required in this proliferating stage is well documented in the literature.

In the “involuting stage”, clusters of plump immature cells are absent, and depositions of basement membranes in which also pericytes are embedded indicate formation of mature blood vessels. An interesting feature that can be noticed in the proliferative stage of hemangioma, but is absent in the involuting stage, is the presence of numerous mast cells in the interstitium of the hemangioma. Mast cells are a source for angiogenic factors which includes VEGF, angiopoietin-1, matrix metalloproteinases, tryptase, histamine and several others. This mast cell content is
not unique for infantile hemangiomas, since it can also be found in the microvascular proliferation of reactive processes like pyogenic granulomas.\textsuperscript{26} GLUT1 expression gave rise to the assumption that infantile hemangiomia could result from displacement of placental cells during fetal development. This was strengthened by the observation that the children of mothers who had a diagnostic chorion biopsy during their pregnancy showed more often infantile hemangiomas.\textsuperscript{27,28} However, molecular genetic studies have clearly determined that hemangioma-derived cells originate from the child and not from the mother.\textsuperscript{29,30}

GLUT1 could also be a permissive factor in the vasoproliferative response of hemangiomas because it is a high-affinity glucose transporter protein with clear potential to provide cellular growth advantage. GLUT1 expression is also associated with neoplastic progression in various types of carcinomas and also positivity has been noted in angiosarcomas. On the other hand in other types of angiomas, the expression of GLUT1 is notably absent, although most if not all have at least an episode of vascular growth, implying that GLUT1 expression in infantile hemangiomas is probably independent of proliferative activity and more likely an intrinsic feature of the lesion.\textsuperscript{31}

**Vascular malformations**

Vascular malformations are traditionally considered as non proliferating quiescent vascular lesions that result from embryonic errors in vascular development.\textsuperscript{9} In line with this view, a vast array of angiogenic factors that have been described in hemangiomas were not detected in vascular malformations.\textsuperscript{3,24} However, as far as angiogenic aspects are concerned, malformations appear to be the less well studied entity among vascular lesions, at least extracranially, presumably because they have been considered as static lesions since a long time.

Most of the recent insights on the vasculogenic and/or angiogenic aspects of malformations stem from cerebral lesions. There is growing evidence suggesting that in brain AVMs processes of active angiogenesis and vascular remodeling interact leading to growth, also in adult life.\textsuperscript{32} Generally, the presence of an arteriovenous shunt in the circulation results in venous hypertension downstream and arterial hypotension upstream. Such changes can trigger vascular remodeling of vessels such as dilatation, arterialization and aberrant muscularization; these structural changes can further affect local hemodynamics, and stimulate the onset of angiogenesis. Histopathological studies also presented further evidence for active angiogenesis and vascular remodeling in AVMs.\textsuperscript{32} A study on 37 cerebral AVMs and 5 controls found an approximate 7-fold increase in non-resting endothelial cells in AVMs suggesting the presence of active angiogenesis.\textsuperscript{33} This was confirmed in a study by Hatva and coworkers who found that the Ki67 index of AVM endothelial
Angiogenesis in benign vascular lesions

There are also a number of observational studies showing increased expression of VEGF in AVMs at both protein and mRNA levels. Hypoxia-inducible factor 1 (HIF-1) is a major upstream activator of VEGF. An immunohistochemical study showed that HIF-1α was expressed in a majority of (therapeutically) embolized, but also non-embolized AVM samples. The underlying mechanisms are not easy to explain. Hypoxia is the major stimulus for HIF-1α expression in tissues. But, also mechanical stretch induced by abnormally high blood flow, as has been found in studies on overloaded skeletal muscle or overactive bladder, has been identified as a trigger for HIF-1α expression.

Another interesting theory based on recent experimental work is a response-to-injury model in the pathogenesis of vascular malformations, in which various mechanisms must interact in concert to regulate angiogenic responses to injury. Initiating events could be trauma, tissue hypoxia, inflammation or irradiation. As a response, angiogenic and inflammatory pathways can either synergize with underlying congenital defects or hemodynamic injury (for example venous hypertension as an angiogenic stimulus).

Most vascular malformations occur sporadically, but some lessons can be learned from the inherited forms. In inherited forms of vascular malformations like the mucocutaneous venous malformations (VMCMs), a mutation in Tie2/Tek gene has been identified in the pathogenesis. An imbalance of Tie2 signaling will lead to vascular dysmorphogenesis. The vascular EC-specific receptor tyrosine kinase (RTK) Tie2 plays a crucial role in both vascular development and stability of vessels. In a specific familial form of malformation, the hereditary hemorrhagic teleangiectasia (HHT), mutations in the endoglin or ACVRL1 gene have been described. Both molecules play a role in TGF-β signaling, and further experimental studies have revealed the importance of endoglin in endothelial cells in the remodeling of blood vessels during angiogenesis. In experimental models, formation of AVMs resulted from a combination of vascular remodeling and an inappropriate endothelial cell proliferation response in the absence of endoglin. Sporadic forms of AVMs have been associated with local increase in soluble endoglin (which theoretically could deplete the amount of circulating ligand locally available). Endoglin is also up-regulated in endothelial cells under hypoxic conditions (for instance inflammation, hemorrhage), and here involved in a process of microvascular angiogenesis. Clearly, at this point a distinction should be made between processes of arteriogenesis and angiogenesis in AVMs.

Information on true arteriogenesis in AVMs is scant, even recent reviews report only on vasculogenesis and angiogenesis, but arteriogenesis is hardly mentioned. Obviously it is difficult to investigate whether or not new true arteries arise in the
long life of the usually slowly progressive growing arteriovenous malformations. It is likely that under conditions of high flow in the malformations, veins and collaterals will be become arterialized and develop into (malformed) arteries. Moreover such a process can be stimulated under episodic inflammatory conditions in the malformations.

References

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Angiogenesis in benign vascular lesions


