Biomarkers: Important Clues to the Pathogenesis of Infantile Haemangioma and their Clinical Significance

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Infantile haemangioma is the most common tumour of infancy, yet the pathogensis of this lesion remains unknown and the predictable life cycle is poorly understood. Though much new information on infantile haemangioma has emerged over the past decade, researchers continue to debate the fundamental features; including cells of origin, nonrandom distribution, and mechanisms regulating the sometimes explosive growth and slow involution. The development of biomarkers has shed light on the pathogenesis and management of infantile haemangioma. Several useful biomarkers and their suggestions as to the aetiology of infantile haemangioma are reviewed. In addition, the application in clinical diagnosis and choice of treatment methods of infantile haemangioma is summarised.

Key words: *angiogenesis, CD133, GLUT-1, immune response, infantile haemangioma, vasculogenesis*

Infantile haemangioma (IH) is differentiated from other vascular anomalies by a predictable natural history. The lesion is preponderant in females compared with males at rates of 3:1 to 5:1. It usually appears within weeks after birth, proliferates rapidly for a period of up to 12 months, and then involutes over a period of 5 to 7 years. Histologically, proliferating haemangioma is mainly composed of masses of compact capillaries lined by plump endothelial cells with high mitotic rates. In addition to endothelial cells, haemangioma includes stromal components, such as fibroblasts, pericytes, and mast cells. As the lesion involutes, mitosis gradually decreases, with increased apoptosis of endothelial cells and gradual replacement of vascular tissues by fibrofatty tissues.

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Although IH is a common vascular lesion, the cellular and molecular mechanisms that cause the abnormal endothelial cell proliferation and spontaneous regression are still poorly understood. Many theories have been proposed for the pathogenesis of IH. In addition, there are various treatment modalities for IH depending on the stage and location of the lesion. Recently, the development of useful biomarkers has shed light on the pathogenesis and management of IH.

Biomarkers for placental origin

In 2001, North et al[1] showed that several microvascular markers, including glucose transporter 1 (GLUT-1), merosin, Lewis Y antigen and FcgRII (CD32), were shared by IH and placenta. None of these markers were found in other vascular anomalies, on the normal vasculature of skin and subcutis, or on tumour vasculature. These shared patterns of expression raise the hypothesis that IH is of placental origin. Ritter et al[2] further detected increased expression of indoleamine 2,3-dioxygenase (IDO) both in placenta and in IH. Additional support of the placental theory comes from large-scale genomic analysis and other data[3].

GLUT-1 is highly expressed early in embryonic development, but foetal endothelial GLUT-1 expression rapidly disappears except in the endothelium of tissues

with barrier function, such as placenta, and blood-retina and blood-brain barriers. The expression of GLUT-1 can be upregulated by several factors, including hypoxia, glucose and oestrogen. Moreover, hypoxia and oestrogen are demonstrated to be important stimulators for the growth of IH[4,5]. GLUT-1 is a high-affinity glucose transporter protein with clear potential to provide a cellular growth advantage. Marked endothelial expression of GLUT1 in IH may be an important permissive factor in the proliferative phase of IH, rather than the suggestion of placental origin. Although debate still exists, GLUT-1 has been used as a diagnostic marker to distinguish IH from vascular malformation (especially when IH is in late involuting and/or involuted phase), rapidly involuting congenital haemangioma (RICH) and noninvoluting congenital haemangioma (NICH). With this biomarker, the nomenclature of 'facial nerve haemangiomas' is demonstrated to be classified as 'venous vascular malformations of the facial nerve' and therapeutic interventions should be delivered in the early stage of the lesion[6]. The expression of GLUT-1 in most subglottic haemangiomas reveals that this clinical entity is IH rather than congenital haemangioma[7].

Biomarkers for vasculogenesis

Vasculogenesis involves the development of vessels that differentiate from precursor cells *in situ*. The aforementioned markers are also expressed by hematopoietic cells[5]. It has been demonstrated that IH endothelial cells coexpress myeloid markers, including CD83, CD32, CD14 and CD15[8]. The present authors' data further showed that cultured monocytes were capable of differentiating into haemangioma-like endothelial cells under the angiogenic stimulation from both vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), with coexpession of CD14, GLUT-1 and normal vascular markers[9]. These shared patterns of expression serve to support the supposition that the endothelial cells of IH are arrested in a specialised, intermediate state of vascular/haematopoietic differentiation.

The presence of CD133- and LYVE-1-positive endothelial cells has recently been found in proliferating IH[10,11]. CD133 (prominin-1) was the first identified member of the prominin family of pentaspan membrane proteins. It is rapidly down-regulated during cell differentiation, which makes it a unique cell surface marker for the identification and isolation of stem cells and progenitor cells. In addition to the haematopoietic system, CD133 and CD34 are also expressed on endothelial progenitor cells. The lymphatic endothelial hyaluronan receptor LYVE-1 is considered as a marker for normal and tumour-associated lymphatic vessels. However, during the formation of the vascular system, a subpopulation of venous endothelial cells have lymphatic competence. The coexpression of CD133, LYVE-1 and CD34 in endothelial cells of proliferating IH raises the hypothesis that haemangioma endothelial cells are arrested in an early developmental vascular differentiation state. In an analysis of stem/progenitor cell mobilisation in children with proliferating IH, there is a 15-fold increase in circulating endothelial progenitor cells compared with age-matched controls[12]. To clarify this mechanism, further studies demonstrate increased circulating levels of stromal cell-derived factor-1α (SDF-1α), matrix metalloproteinases-9 (MMP-9), VEGF-A, and hypoxia inducible factor-1α (HIF-1α) in children with proliferating IH[13]. This evidence suggests that IH endothelial cells are arrested in an early developmental stage of vascular differentiation, and vasculogenesis may have an important role in IH development.

Expanding evidence highlights the role of CD133 as a marker of cancer stem cells in various human tumours[14]. More recent research reveals that the CD133-selected cells isolated from IH tissue can recapitulate the development and the unique evolution of IH: the formation of blood vessels followed by involution to fatty tissue in immune-deficient mice[15]. The finding suggests that CD133-postive cells are IH-derived stem cells. With this model, it has been demonstrated that corticosteroid, the first line medicine of IH, may inhibit the vasculogenic potential of IH-derived stem cells through suppressing VEGF-A[16]. This raises the question of whether the clinical response to corticosteroid treatment is determined by the ratio of immature stem cells to mature endothelial cells in the tumour. If this were true, future pharmacotherapeutic strategies might be tailored to have anti-stem-cell (anti-vasculogenic) effects in immature lesions in which stem cells are prominent, while also having antiendothelial (anti-angiogenic) effects in tumours with high vascularity but fewer stem cells. Combination therapies targeting both stem- and endothelial-cell compartments might prove to be highly effective.

Biomarkers for angiogenesis

In addition to vasculogenesis, angiogenesis is also an important contribution to neo-vessel formation in IH. During the proliferative phase of rapid growth, many factors or markers of angiogenesis are increased in IH. These include bFGF, VEGF, MMP, proliferating cell nuclear antigen (PCNA), E-selectin, monocyte chemoattractant protein-1 (MCP-1), Tie2, insulin-like growth factor 2 (IGF-2) and type IV collagenase[17]. Furthermore, bFGF

remains elevated through early involution in both IH tissue and urine, being very low in healthy and vascular malformations in infants and children. Urinary levels of bFGF are valuable in the diagnosis and the assessment of the efficacy of treatment for IH[18,19]. During involution, IH is characterised by a five-fold increase in endothelial cell apoptosis, an increase in mast cells, and abnormal levels of angiogenic inhibitors[20]. Tissue inhibitor of metalloproteinase (TIMP), for example, is significantly increased in haemangiomas during the involuting phase[21]. Moreover, the present results (unpublished data) show that LMO2, a transcriptional regulator for angiogenesis both in normal and tumour tissues[22], is highly expressed in the proliferative phase, and gradually decreases during the involuting and involuted phases.

The extracellular matrix is composed of macromolecules that influence cell functions including growth, migration and differentiation. The extracellular matrix also serves as a repository for growth factors and a modulator of cellular responses to them. The extracellular matrix of haemangiomas differs from that of normal skin and vascular malformations, with immunohistochemistry demonstrating increased vitronectin, perlecan and laminin in IH[23,24].

Since angiogenesis contributes to the growth of IH, anti-angiogenic therapy should be an effective treatment. Some angiogenesis inhibitors including batimastat (a synthetic MMP inhibitor), VEGF-toxin conjugate, angiostatin, synthetic compound TNP-470, as well as TIMP-2 have been tried in animal models of haemangioma with promising effects[25]. Corticosteroid, α-interferon (a strong FGF inhibitor) and cytotoxic sclerosing agents including sodium morrhuate, bleomycin and vincristine can destroy vascular endothelium, and consequently inhibit vascular formation[26]. Propranolol, a non-selective β-blocker, has recently been introduced as a novel modality for the treatment of problematic IH. The exact mechanism remains unclear, but vasoconstriction, down-regulation of angiogenic factors such as VEGF and bFGF and up-regulation of apoptosis of capillary endothelial cells may be partially responsible for the reduction of IH. In addition, a selective role of propranolol in inhibiting the expression of MMP-9 is a possible mechanism[27].

Biomarkers for intrinsic defect

Linkage studies have identified six families that appear to have a predisposing genetic mutation and high incidence of IH and/or vascular anomalies[28]. In these families, IH segregates as an autosomal dominant trait and a subsequent study established a linkage to chromosome 5q31-33[29], which harbours the genes for FGF receptor 4 (FGFR-4), PDGF receptor beta (PDGF-β), and FMS-related tyrosine kinase 4 (FLT-4). These genes have known roles in regulating angiogenesis. These genetic studies, while important, do not address the common sporadic form.

While investigations have presumed that IH growth is the result of uncontrolled, disorganised angiogenesis, other studies support the hypothesis that IHs result from a somatic mutation in a single endothelial (or progenitor) cell[30]. X-linked human androgen receptor gene assay (HUMARA) reveals that IH endothelial cells isolated from seven heterozygous female patients show a skewing toward a single allele[31]. A subsequent study analysed intact tissue with no selection of cells and similarly found some degree of allelic loss in 12 of 14 samples[32]. Thus, these investigations suggest that IH may arise as a consequence of clonal expansion from a single cell. These and other studies clearly indicate that IH growth does not come about by typical angiogenesis.

Biomarkers for immune response

Large numbers of haematopoietic cells of the myeloid lineage constitute a significant portion of the cells found in IH, particularly in the proliferative phase, suggesting a role for inflammation in the promotion of IH growth[8]. The present findings[33] show that intense immunoreactivity for allograft inflammatory factor-1 (AIF-1), a modulator of immune responses, is detected in 17 of 19 IH endothelial cells, at all three clinical phases. It has been found that macrophages also contribute to the promotion of IH growth. All the findings suggest a mechanism for the efficacy of corticosteroids on IH, because these agents have established anti-inflammatory effects and have been shown to induce apoptosis in myeloid cells[8].

IDO is thought to be important in preventing maternal rejection of the placenta through inhibition of T-cell function. DNA microarray analysis has identified that IDO is present in high levels in proliferating IH and decreases significantly during involution[2], thus raising the possibility that inhibition of T cells could contribute to the slow regression of IH. The conjecture is confirmed by the recruitment of a significant number of T cells, most of which are CD8-positive cytotoxic T cells, in the involuted IH[2]. This suggests that immune and immune-mediated inflammatory events may contribute to the pathogenesis of IH. The discovery of IDO expression in IH suggests that inhibiting this enzyme could lead to an increased T-cell response against IH. Further exploration of imiquimod or other compounds that modify the immune response is warranted[34].

To date, no single theory can easily explain all the characteristics of IH. IH is probably the final common expression of several pathophysiological mechanisms taking effect alone or in combination. The emergence of new biomarkers will help to unravel a general mechanism in IH. Moreover, the manufacturers favour clinically making differential diagnoses and rational treatment plans for IH.

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