Published in final edited form as:

Matrix Biol. 2009 March ; 28(2): 63–64. doi:10.1016/j.matbio.2009.03.001.

From the Editor's Desk Insights into endothelial mechanisms through the lens of vascular anomalies

Dear readers of Matrix Biology,

I have frequently reminded graduate students and postdoctoral fellows in my laboratory that science is more like a marathon than a sprint in that hard, steady work on important problems over a long period of time is the best way to prepare for success.

In the case of most projects such periods fall well within the lifetime of a grant or a fellowship, allowing the work to be published before the support ends. However, in a few cases, progress in the study of hard problems can be painfully slow and stress both financial and intellectual resources. These are the studies that truly test the commitment of talented students and postdoctoral fellows and benefit from stable, long-term funding mechanisms. Two recent publications that describe endothelial pathogenetic mechanisms associated with two different human vascular anomalies are examples of such studies.

One of these papers, by Limaye et al. (2009) provides compelling evidence for a combination of germline and somatic mutations in the endothelial receptor tyrosine kinase TIE2 in familial cases of venous malformations and for combinations of somatic mutations in the same receptor in sporadic cases of the disorder. The work builds on studies initiated more than a decade ago in my laboratory when M. Vikkula et al. (1996) demonstrated that familial cases of venous malformations are associated with heterozygous activating missense mutations in TIE2. Since then, additional studies have confirmed this finding and several different missense mutations in this tyrosine kinase receptor are now known to cause familial venous malformations (Limaye et al., 2009).

Venous malformations are multifocal (the rare familial form) or single (the most common sporadic form) cutaneous or mucosal lesions consisting of tortuous small veins with incomplete covering of the endothelium by smooth muscle cells. The localized nature of the lesions suggests that the formation of the defects in the familial form requires a localized triggering event in addition to the germline mutations in TIE2. The nature of this event and what causes the common sporadic lesions have remained a mystery – until now.

By studying cDNA isolated from the lesion of a member of a family with the “original” (Vikkula et al., 1996) heterozygous R849W missense mutation, Limaye et al. (2009) found an in-frame somatic deletion in the allele that did not carry the R849W mutation. The deletion causes loss of 43 amino acid residues from the ligand-binding domain of the receptor, loss of cell surface expression and loss of activation (phosphorylation) in the presence of ligand. Therefore, the somatic lesion-restricted mutation turns the affected TIE2 allele into a loss-of-function allele so that only the mutant R849W TIE 2 protein is functioning in the lesion. This finding led to the speculation that similar somatic changes in TIE2 may be

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associated with sporadic venous malformations. By sequencing blood-derived DNA and lesion-derived DNA (both genomic and cDNA) from a number of individuals, the authors discovered both single and double somatic mutations in sporadic venous malformations. In general, the mutations are activating mutations and in cases where two mutations have occurred (in the same allele!) the mutations have added effects on receptor activation. The study firmly establishes that abnormal activation of TIE2 signaling is the underlying cause of both the rare familial and the common sporadic forms of venous malformations and provides strong support for the idea that therapies aimed at inhibiting TIE2 dependent processes may be effective in both inherited and sporadic forms.

The Limaye et al. (2009) paper also provides an example of (or a contrast to, see below) genetic mechanisms that may be responsible for the discrete lesions seen in other vascular anomalies. One such disorder is the most common tumor of infancy called infantile hemangioma. This sporadic endothelial tumor, occurring in up to 10% of infants of European descent, has fascinated investigators and clinicians for a long time, for several reasons. Hemangiomas have a unique life cycle with rapid growth (proliferating phase) during infancy, followed by a longer period (months to years) of involution, resulting in complete regression. The tumors occur more frequently in females than in males, in premature babies and in cases where the pregnancy has been associated with placental abnormalities.

Many hypotheses have been proposed for the cellular and molecular mechanisms responsible for the appearance and rapid growth of the hemangioma lesion in the early postnatal period, but based on the available evidence it has not been possible to identify one specific pathogenetic mechanism that explains all the cellular and molecular properties of hemangiomas. With a recent paper from my own laboratory, this situation has now changed. Our study (Jinnin et al., 2008) builds on a previous investigation of X-chromosome inactivation patterns in sporadic hemangioma lesions. In collaboration with the laboratory of Joyce Bischoff, we found (Boye et al., 2001) that endothelial cells from the lesions of female patients in all cases tested, showed complete skewing of X-chromosome inactivation, clearly consistent with clonality; in contrast, non-endothelial cells from the lesions were just as clearly not clonal. During the past 6 years we have therefore examined the biochemical properties of hemangioma-derived endothelial cells with the aim of identifying molecular properties that are unique to hemangioma endothelial cells and distinguish these cells from several different types of control endothelial cells.

The paper by Jinnin et al. (2008) provides, I believe, justification for saying that we were quite successful in reaching this aim. We found that transcript and protein levels of the decoy receptor VEGFR1 are abnormally low in hemangioma endothelial cells compared to control endothelial cells (several different kinds), resulting in constitutive, VEGF-dependent activation of VEGFR2 receptor and its downstream targets. Jinnin et al. (2008) further demonstrated that low levels of VEGFR1 expression in hemangioma is a consequence of repressed activation of the transcription factor NFATc2 in hemangioma endothelial cells. Consistent with this, we found that the expression of other known NFAT target genes is also low in the cells. The repressed activation of NFAT could be traced back to abnormalities in an endothelial surface protein complex containing of VEGFR2, β 1 integrin and the “integrin-like” transmembrane protein known as TEM8 or Anthrax toxin receptor 1. Sequencing of several candidate genes with DNA from nine different hemangioma patients resulted in identification of heterozygous missense mutations in TEM8 and VEGFR2 in three patients. These mutations result in abnormal VEGFR2/ β 1 integrin/TEM8 complex formation, suppression of β 1 integrin activation and loss of the ability of the complex to induce NFAT-dependent VEGFR1 expression when cells are treated with VEGF or β 1 integrin stimulatory antibodies.

Interestingly, we found that abnormal VEGFR2/ β 1 integrin /TEM8 complex formation is a characteristic feature of all hemangioma endothelial cells, even those derived from patients where mutations in TEM8 or VEGFR2 have not been identified. We believe, therefore, that this complex may contain additional components and that some of these components may be candidates for hemangioma-causing mutations as well.

Since the mutations already identified in TEM8 and VEGFR2 are heterozygous germline mutations, they cannot explain the clonality of endothelial cells within the lesions of these patients. Like the heterozygous germline TIE2 mutations in familial venous malformations, they also do not explain the localized nature of the lesions. Whether hemangioma lesions in patients with germ-line mutations in TEM8 or VEGFR2 are initiated because of somatic mutations similar to what happens in familial venous malformations is not known. Since venous malformations do not regress like hemangiomas, we have argued (Jinnin et al., 2008) that the initiating event in hemangioma formation may be a reversible pathophysiological event rather than a somatic mutation, but a somatic mutation cannot be ruled out. However, we speculate that most sporadic hemangiomas, like venous malformations, may be caused by somatic mutations in genes, such as TEM8 and VEGFR2, that have already been found to carry a germline mutation in some cases. With access to large a number of DNA and lesional tissue samples collected from hemangioma patients, these possibilities can hopefully be addressed faster than the time we have spent so far to open the door to the pathogenetic “mystery” of proliferating hemangiomas. However, we are also preparing ourselves for another “marathon run” in that we plan studies of the mechanisms by which hemangiomas undergo the slow process of involution leading to regression. While the current studies suggest that anti-VEGF therapy may be effective in treating clinically problematic hemangiomas in the proliferating phase, only a complete understanding of the involution process may set the stage for attempts to accelerate the process in patients with already established tumors. Understanding the involution mechanisms may even help in developing better strategies for inducing vascular regression in angiogenesis-dependent diseases other than hemangiomas.

So, I keep reminding my students and postdoctoral fellows that science is not a sprint...”

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