high levels of ROS. This could be further potentiated by combination with drugs that modulate specific signal transduction pathways. Future work should concentrate on identifying appropriate oxidative biomarkers as well as ROS-modulating drugs to address this possibility clinically.

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Suppressing NFAT Increases VEGF Signaling in Hemangiomas

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Infantile hemangiomas represent the most common tumor of endothelial cell (EC) origin, yet the mechanisms regulating hemangioma EC behavior are poorly understood. A new study by Jinnin et al. demonstrates that enhanced VEGFR2 signaling in hemangioma ECs is caused by suppression of NFAT (nuclear factor of activated T cells)-dependent VEGFR1 expression.

Infantile hemangiomas are benign localized lesions, comprised primarily of aberrant endothelial cells (ECs), that appear within the first weeks of life. These lesions go through a proliferation phase during the first 6–10 months and slowly regress over the following 5–10 years. During the involution phase, the vasculature undergoes apoptosis and is replaced by fibrotic fatty tissue. Depending on their location, hemangiomas can cause deformity or life-threatening complications (Barnes et al., 2007). Previously, Olsen and colleagues isolated hemangioma ECs (hemECs) from actively proliferating angiomas of nine unrelated infants and showed that these lines were clonal, arising from a single progenitor cell (Boye et al., 2001). These cells are thought to originate from placental ECs or to be differentiated toward the placental microvascular phenotype (Barnes et al., 2007). Although their origin is uncertain, hemECs have a characteristic expression pattern that is stably maintained in cultured cells and differs from those of normal ECs. They have increased rates of proliferation and enhanced VEGF-mediated migration (Boye et al., 2001). However, until now, the molecular mechanisms driving the aberrant behavior of hemECs remained unknown.

In a recent study, Jinnin et al. (2008) showed that VEGFR2 signaling is constitutively active in cultured hemECs due to decreased VEGFR1 expression. Since both receptors bind VEGF-A, VEGFR1 is thought to negatively regulate VEGFR2 signaling by acting as a decoy receptor for VEGF-A (Olsson et al., 2006). Therefore, constitutive VEGFR2 activity due to suppression of VEGFR1 could explain the increased proliferation and migration of hemECs.

With this in mind, the authors delved further into how VEGFR1 is regulated in hemECs as compared to normal ECs. Because both VEGFR1 (*FLT1*) transcript and protein levels were minimal in hemECs, Jinnin et al. sequenced part of the *FLT1* promoter from all nine hemEC lines. They demonstrated that a region of the *FLT1* promoter contains a binding site for the transcription factor NFAT, providing the first evidence that *FLT1* represents an NFAT target gene. Functionally, this finding was critical to the characterization of hemECs because the authors also demonstrated that NFAT transcriptional activity is lower in hemECs than in normal ECs. Hence, suppression of NFAT-dependent *FLT1* transcription could help drive the enhanced VEGF signaling of hemECs. Looking upstream of NFAT to a cell-surface receptor, Jinnin et al. also found that reduced NFAT activation in hemECs was associated with decreased β1 integrin activity and decreased adhesion to the β1 integrin substrates type I collagen and fibronectin, despite equivalent surface expression of β1 integrin between both cell types.

Previous studies suggested a genetic link between hemangioma growth and

either somatic or germline mutations. Examining the coding sequences of 24 candidate genes (chosen for their roles in controlling EC adhesion, proliferation, etc.), Jinnin et al. found heterozygosity for nucleotide changes resulting in amino acid substitutions in three of the nine cultured hemEC cell lines. One of these mutations was in the integrin-like protein tumor endothelial marker 8 (TEM8). While this mutation was not found in DNA samples from other individuals with a history of hemangioma, its identification suggested a role for this protein in regulation of NFAT activation and VEGFR1 expression. The authors showed that wild-type TEM8 increased VEGFR1 expression and reduced VEGFR2 signaling in hemECs. In two hemEC lines, they also found a germline mutation of VEGFR2 (C482R) that had a population frequency of 10%. This specific mutation was crucial for VEGFR2 regulation of VEGFR1 expression but did not affect VEGFR2 expression or its activity.

The authors also discovered a complex between β1 integrin, TEM8, and VEGFR2 in both EC lines that was significantly more abundant in hemECs. Identified mutants of TEM8 and VEGFR2 also enhanced complex formation. These findings suggested that TEM8 and VEGFR2 negatively regulate β1 integrin activation and in turn suppress NFAT transcriptional activity. Jinnin et al. then showed that decreased VEGFR1 expression, controlled by NFAT, is linked to enhanced VEGFR2 signaling in hemECs and appears to be negatively regulated by increased complex formation between TEM8, VEGFR2, and β1 integrin (Figure 1).

While the authors have made substantial inroads into the mechanistic differences between normal ECs and hemECs and put forth an interesting model for hemEC dysfunction, some unanswered questions remain. For example, integrin binding to the extracellular matrix requires both alpha and beta integrin subunits. β1 integrin can interact with various alpha subunits; therefore, it would be interesting to determine whether a specific alpha subunit is also responsible for regulation of NFAT. In normal ECs, VEGFR2 crosstalks with other integrins, such as αvβ3 or αvβ5, to affect downstream signaling (Hood et al., 2003; Mahabeleshwar et al., 2007). Could the interaction between VEGFR2 and αvβ3 or αvβ5 integrin also be dysregulated in hemECs? Although only one mutation each of TEM8 and VEGFR2 was identified in this study, could other mutations within the same regions also be responsible for upregulated VEGFR2 signaling in hemECs? An important caveat to this study is that while these experiments were performed with cultured cells derived from hemangiomas, it remains unclear whether this same pathway is active in hemangiomas in vivo. Unfortunately, there are no physiologically relevant in vivo models for infantile hemangioma to date, making it difficult to address this issue (Ritter et al., 2007).

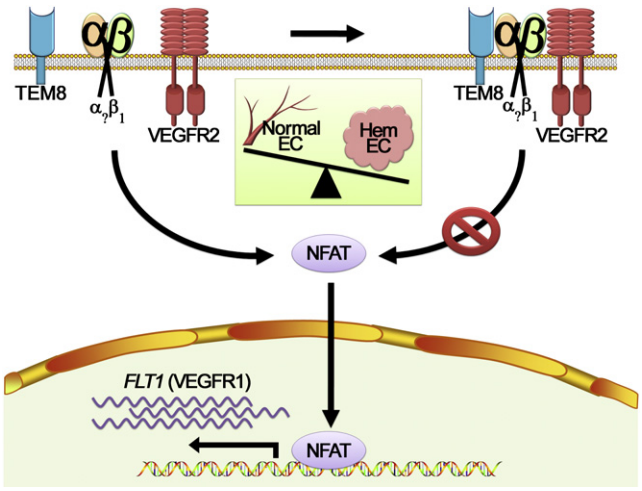

Figure 1. TEM8 and VEGFR2 Can Negatively Regulate β1 Integrin-Mediated NFAT-Dependent VEGFR1 Expression through Complex Formation

In normal endothelial cells (ECs), β1 integrin activation leads to NFAT-dependent transcription of *FLT1* (VEGFR1), the VEGF decoy receptor. However, this pathway can be dampened by complex formation between β1 integrin, VEGFR2, and TEM8. In hemangioma ECs (hemECs), constitutive VEGF signaling may be due to enhanced complex formation in hemECs versus normal ECs, which then leads to decreased *FLT1* transcription.

In conclusion, Jinnin et al. have identified a molecular pathway that may elucidate the altered proliferation and migratory behavior of hemECs relative to normal ECs. Currently, corticosteroids are used to treat infantile hemangiomas. However, their mechanism of action is poorly understood, and they can have undesired side effects such as growth retardation and increased risk of infection (Ritter et al., 2007). This new model suggests that treatment with anti-VEGF, VEGFR2 kinase inhibitors, or other agents affecting β1 integrin, TEM8, and NFAT could be viable therapies to ameliorate this condition.

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