Expression of Wilms Tumor 1 Gene Distinguishes Vascular Malformations From Proliferative Endothelial Lesions

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Background: Vascular malformations and hemangiomas, which are endothelial lesions of childhood, may result in considerable morbidity because they can cause discomfort and functional impairment and have a negative affect on the patient’s appearance. Although vascular malformations may initially appear very similar to hemangiomas, they have distinct clinical courses. Infantile hemangiomas progress through 3 stages: proliferative, involuting, and involuted. The proliferative phase is characterized by clinical growth. Once hemangiomas reach their maximum size, they begin to regress or involute. Histologically, this stage is characterized by endothelial apoptosis. Finally, the involuted stage of the hemangioma occurs when the original lesion is replaced by a connective tissue remnant. In contrast to hemangiomas, vascular malformations do not involute but continue to enlarge as the patient grows.

Observations: The biochemical differences between hemangiomas, which involute, and vascular malformations, which do not involute, are not well understood. We found that the transcription factor encoded by the Wilms tumor 1 (WT1) gene is expressed in the endothelium of hemangiomas but not in vascular malformations.

Conclusions: Defects in WT1 signaling may underlie the inability of malformation endothelial cells to undergo physiologic apoptosis and remodeling. The availability of WT1 staining in hospital laboratories may allow the clinician to distinguish hemangiomas from vascular malformations and thus to give appropriate therapy to the patient.

Arch Dermatol. 2005;141:1297-1300
RESULTS

Hemangiomas revealed endothelial cytoplasmic immunopositivity for WT1 in 89% (8) of 9 samples (Table). Some of the slides that were positive for WT1 in tumor cells also exhibited background blood vessel staining (capillaries, venules, or arterioles). Only 1 hemangioma sample (11%) did not stain for WT1 at all. Other vascular tumors that showed positive staining for WT1 included pyogenic granulomas (100%), angiosarcomas (100%), an epithelioid hemangioendothelioma (100%), and a hobnail hemangioendothelioma (100%). The malignant hemangioendothelioma was negative for WT1. Of note, additional samples of hemangiomas revealed staining of normal background blood vessels (capillaries, venules, or arterioles). Also, the pyogenic granulomas, 1 angiosarcoma, the epithelioid hemangioendothelioma, and the hobnail hemangioendothelioma exhibited normal background blood vessel staining.

The vascular malformations in our study did not show any positive staining of endothelium (Figure 1). Two port-wine stains (100%), 10 venous malformations (100%), and 8 lymphatic malformations (100%) were completely negative for WT1. As with the vascular tumors, there were some samples that displayed normal background blood vessel staining with WT1, including venous malformations and lymphatic malformations.

The positive controls (mesothelioma sections) revealed positive nuclear staining in the endothelium. Mesotheliomas also showed staining of background blood vessels (capillaries, venules, or arterioles). Antigen retrieval was performed using heat-induced antigen retrieval and primary antibody adsorption with antigen-specific antibody adsorbed with antigen.

STATISTICS

The total number of lesions with positive endothelial staining was divided by the total number of positive- and negative-staining lesions with the same diagnosis.
angiomas, vascular malformations do not involute, nor present at birth or develop later in life. In contrast to hemangiomas, rapid involution of the hemangioma.

Hemangiomas most commonly appear at birth or shortly afterward and are characterized by a rapid growth phase, called the proliferative phase, which is distinguished by endothelial proliferation, and activation of the tie-2 receptor.

The tie-2 receptor serves as the receptor for angiopoietins 1 and 2, which are involved in endothelial remodeling. Also, the levels of interferons alpha and beta are reduced in the epidermis overlying hemangiomas, which may provide a permissive environment for hemangioma growth. Finally, gene array has identified insulin growth factor 2 to be highly expressed in proliferative hemangiomas and may serve as an endothelial growth factor. Hemangiomas involute, and this process is accompanied by endothelial apoptosis and induction of interferon-regulated genes. Then, the hemangioma is replaced by a fibrofatty scar. The life cycle of a hemangioma thus demonstrates the ability of the hemangioma’s endothelial cells to undergo remodeling. Administration of high-dose glucocorticoids or interferon alfa results in more rapid involution of the hemangioma.

Vascular malformations, on the other hand, may be present at birth or develop later in life. In contrast to hemangiomas, vascular malformations do not involute, nor do they respond to glucocorticoid or interferon therapy. Distinguishing large hemangiomas from vascular malformations is clinically important because interferon therapy is potentially toxic and should not be administered to patients who are unlikely to respond.

We have shown that the transcription factor WT1 is present in vascular tumors but not in vascular malformations. A significant portion of the hemangiomas, pyogenic granulomas, angiosarcomas, and hemangiendotheliomas that stained for WT1 revealed positive staining of the proliferative endothelial cells. The background staining of normal blood vessels seen in large number of the vascular tumors as well as in some of the vascular malformations serves as an internal positive control for WT1 staining. This staining of normal blood vessels was also seen in the positive control, mesothelioma, in addition to proliferative endothelial staining of that tumor. Whereas the mesothelioma shows WT1 in a nuclear location, the vascular tumors that stain positive for WT1 reveal a cytoplasmic location of the transcription factor. This finding may indicate a cytoplasmic function for the WT1 protein.

Recently, a cytoplasmic role for WT1 has been described as a major component of polysomes as a translational regulator. Cytoplasmic WT1 has been previously described in other tumors, including rhabdomyosarcoma, breast cancer, and colon cancer. The cytoplasmic-nuclear WT1 protein ratios of cell types differ. To confirm that the cytoplasmic WT1 staining we observed was not an artifact, we performed RT-PCR analysis of cultured endothelial cells in the presence of angiogenic factors, including vascular endothelial growth factor and angiopoietins 1 and 2, and found that WT1 messenger RNA was highly expressed in these endothelial cells (Figure 2).

Of interest, there is a prior report, involving an experimental model of myocardial infarction, on the localization of WT1 in endothelial cells. Experimental infarction of the rat myocardium led to a high level of expression of WT1 in remodeling and hypoxic endothelial cells in the wound. Wilms tumor 1 is involved in embryonic mesenchymal migration, and mice deficient in WT1 have lethal defects in the epicardium as a result of defective migration. Loss of WT1 could potentially lead to a vascular malformation phenotype through the following mechanisms: WT1 has been shown to stimulate the production of platelet-derived growth factor family members, and loss of WT1 may account for defective investment of WT1-deficient endothelial cells by smooth muscle.

Clinically, vascular malformations are characterized by a failure to remodel to appropriate physiologic stimuli. Also, many vascular malformations are characterized by abnormally large lumina with deficient smooth muscle or pericyte investment. Loss of WT1 may account in part for some of these defects. Staining for WT1 may guide the clinician in difficult cases, as positive results would suggest a proliferative vascular lesion and appropriate therapy (eg, systemic steroids and interferon), while negative results might point to a vascular malformation and thus avoid the need for systemic therapy.

Accepted for Publication: March 30, 2005.

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Author Contributions: Study concept and design: Arbiser. Acquisition of data: Cohen. Analysis and interpretation of data: Lawley, Cerimele, and Weiss. Drafting of the manuscript: Cerimele and Arbiser. Critical revision of the manuscript for important intellectual content: North, Koza-kewich, Mulliken, and Arbiser. Statistical analysis: Cohen. Obtained funding: Arbiser. Financial Disclosure: None.

Funding/Support: Dr Arbiser was funded by grant RO1 AR47901 from the National Institutes of Health, Bethesda, Md, and the Sturge-Weber Foundation, Mt Freedom, NJ.

Figure 2. Wilms tumor 1 messenger RNA is present in human dermal microvascular endothelial cells. Lane 1 represents RNA from human dermal microvascular endothelial cells treated with vascular endothelial growth factor (VEGF) alone; lane 2, RNA from endothelial cells treated with VEGF and angiopoietin 1; and lane 3, RNA from endothelial cells treated with VEGF and angiopoietin 2.
REFERENCES


