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.

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Sections of formalin-fixed, paraffin-embedded tissue (5 µm) were stained for WT1 using a 2-step horseradish peroxidase–labeled polymer system (Envision System; Dako Corp, Carpinteria, Calif) and heat-induced antigen retrieval. The horseradish peroxidase–labeled polymer, which is conjugated with secondary antibodies, was used in combination with an automated staining system (Autostainer; Dako Corp). Hematoxylin was used as the counterstain. Negative controls were generated by substituting the primary antibody with buffer-specific antibody adsorbed with antigen.

Sections were deparaffinized in xylene and grades of alcohol and rehydrated in water. Antigen retrieval was performed by placing the sections in citrate buffer (pH 6) inside an electric pressure cooker for 3 minutes at 120°C and then cooling them for 10 minutes before immunostaining. The sections were next exposed to 3% hydrogen peroxide for 5 minutes, primary antibody for 30 minutes, horseradish peroxidase–labeled polymer for 30 minutes, diaminobenzidine as chromogen for 5 minutes, and hematoxylin as the counterstain for 15 minutes. The incubations were performed at room temperature. Between incubations, the sections were washed with Tris-buffered saline and coverslipped (Tissue-Tek SCA; Sakura Finetek USA, Inc, Torrance, Calif).

Paraffin blocks or sections for WT1 antigen staining of benign and malignant vascular tumors and vascular malformations were obtained from the pathology departments of Emory University, Atlanta, Ga; the University of Arkansas, Little Rock, and Children’s Hospital, Boston, Mass. The vascular tumors included 9 hemangiomas, 2 pyogenic granulomas, 9 angiosarcomas, 1 epithelioid hemangioendothelioma, 1 hobbain hemangioendothelioma, and 1 malignant hemangioendothelioma. The vascular malformations included 2 port-wine stains, 10 venous malformations, 8 lymphatic malformations. The slides were reviewed for diagnosis and positive staining with WT1. Mesothelioma sections were used as positive controls for WT1 staining.

HUMAN ENDOTHELIAL CELLS

Human dermal microvascular endothelial cells were obtained from the Emory Skin Disease Research Center and cultured according to the method of Swerlick et al.24 They were stimulated with angiopoietins 1 and 2 (150 ng/mL) in the presence of vascular endothelial growth factor (20 ng/mL) and harvested for reverse transcriptase polymerase chain reaction (RT-PCR) analysis. RNA was isolated from human endothelial cells, and RT-PCR analysis was performed with denaturation for 1 minute at 94°C, followed by 1 minute at 48.5°C, and then 1 minute at 72°C for 35 cycles. Primers were based on the sequence of human WT1 and were amplified using 5′-GCATCTGAAACCAGTGAGAA-3′ (sense) and 5′-TTTCCTCTGATGCATGTTG-3′ (antisense). The identity of the RT-PCR product was confirmed by sequencing.

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.

RESULTS

Hemangiomas revealed endothelial cytoplasmic immunopositivity for WT1 in 8 (89%) 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%), angiosarcoma (100%), an epithelioid hemangioendothelioma (100%), and a hobbain 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 hobbain 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 with WT1, including lymphatic malformations, which were completely negative for WT1.
vessels, as was seen in some of the vascular tumors and malformations in our study. To ensure that authentic WT1 was present in endothelial cells, we performed RT-PCR analysis on endothelial cells under conditions of growth stimulation and demonstrated authentic WT1 messenger RNA in endothelial cells (Figure 2).

Hemangiomas most commonly appear at birth or shortly afterward and are characterized by a rapid growth phase,19,20 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 alfa and beta are reduced in the epidermis overlying hemangiomas, which may provide a permissive environment for hemangiomla 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.21 Hemangiomas involute, and this process is accompanied by endothelial apoptosis and induction of interferon-regulated genes. Then, the hemangima is replaced by a fibrofatty scar. The life cycle of a hemangima thus demonstrates the ability of the hemangima'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.2,3 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.2,3

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.22,23 Recently, a cytoplasmic role for WT1 has been described as a major component of polysomes as a translational regulator.22,23 Cytoplasmic WT1 has been previously described in other tumors, including rhabdomyosarcoma, breast cancer, and colon cancer.22,23 The cytoplasmic-nuclear WT1 protein ratios of cell types differ.22,23 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.23 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.6,17

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.

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