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Original Article

Diagnostic utility of WT-1 cytoplasmic stain in variety of vascular lesions

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Abstract: Vascular lesions are commonly encountered in routine pathologic practice and often pose diagnostic challenges owing to their morphologic diversity. Although WT-1 expression was reported in some vascular tumors, little is known about its staining patterns in a spectrum of vascular lesions from various locations. We examined WT-1 immunostain in 95 cases of vascular lesions including angiosarcomas (AS, 19 cases), hemangioendotheliomas (HE, 5), Kaposi's sarcomas (KS, 4), cavernous hemangiomas (CVH, 12), capillary hemangiomas (CPH, 7), pyogenic granulomas (PG, 4), lymphangiomas (LA, 4), hemangiopericytomas (HP, 5), glomus tumors (GT, 8), vascular malformation (VM, 13) and granulation tissue (GRT, 14). Strong WT-1 cytoplasmic stain was invariably observed in all cases of malignant and borderline vascular tumors including AS (19/19), KS (4/4) and HE (5/5). WT-1 was also consistently expressed in CPH (7/7), PG (4/4), and GRT (14/14), while it became weaker in VM (10/13) and often negative in CVH (2/12) and LA (0/4). WT1 stain was not demonstrated in HP (0/5) and rarely in GT (2/8). We conclude that consistent and diffuse WT-1 cytoplasmic stain in AS, HE and KS can be useful in distinguishing these tumors from poorly differentiated tumors with mimicking features. On the other hand, reliable WT-1 stain in CPH, PG and GRT may help in differential diagnosis with non-endothelial vascular tumors such as GT and HP. Recognizing the WT-1 cytoplasmic stain in a broad spectrum of benign and neoplastic tissues is critical in formulating appropriate immunohistochemical panels and avoiding misinterpretation of results.

Keywords: WT-1, vascular tumor, endothelial proliferation, angiogenesis, immunohistochemistry

Introduction

Wilms' tumor 1 (WT-1) was originally described as a tumor suppressor gene based on its mutational inactivation in a subset of Wilms' tumor [1]. Recently, expression of WT1 was also identified in tumors from different origins, including breast [2], colon [3], soft tissue [4] and brain [5]. WT-1 is not normally expressed in these organs and no mutations in the gene have been identified in associated tumors, suggesting its potential oncogenic role. Recent studies implicated that WT-1 also plays an important role in angiogenesis mainly by regulating vascular endothelial growth factor (VEGF) [6, 7], V-ets erythroblastosis virus E26 oncogene homolog 1 (ETS1 transcription factor) [8], angioproteins [9], nestin [10] and proliferation of vascular smooth muscle cells [11].

Vascular lesions are commonly encountered in routine pathology practice and often pose diagnostic challenges owing to their morphologic diversity and morphologic similarity with other non-vascular lesions. An adequate and effective clinical management is heavily weighted on accurate histologic classification of these lesions. Immunohistochemistry with endothelial-specific markers (such as CD31) has been successfully used in histologic evaluation of vascular lesions with relatively high specificity and sensitivity. Lately, expression of WT-1 was reported in a few types of vascular tumors of skin [12-14] and soft tissue [15, 16]. The findings lead some to speculate that the WT-1 may serves as a marker for neoplastic endothelial cells to facilitate the differentiation between benign and neoplastic vascular lesions [14, 18, 19].

Despite of the observations, little is known about the WT-1 expression in a spectrum of vascular tumors from various locations, nor in non-neoplastic vascular lesions. The current

Table 1. Patient demographics and locations of lesions

Lesions	Age (years)	Sex (M/F)	Locations									
			Brain	Heart	Head/ neck	Lung/ pleura	Liver	GI tract	Skin	Soft tissue	Other	Total
AS	48.5 (20-79)	13/6		11	2	1	2			1	1 bone 1 breast	19
HE	41.6 (33-55)	2/3					3		2			5
KS	36.3 (25-42)	4/0							2	1	1 lymph node	4
CPH	48.8 (19-75)	3/4			5				2			7
CVH	54.3 (45-75)	5/7		1	7		1			2	1 spleen	12
PG	31.5 (2-49)	2/2			4							4
LA	42 (25-53)	0/4			2					2		4
HP	50.8 (35-59)	2/3	3		2							5
GLT	50.6 (31-78)	3/5						1	7			8
VM	58.1 (36-76)	3/10	5		3			2	1	1	1 spinal cord	13
GRT	51.9 (19-73)	12/2			3	2		3	1	4	1 bladder	14
Total		49/46	8	12	28	3	6	6	15	11	6	95

Table 2. Antibody Characterization, Dilution, and Cellular Localization

Antigen	Species (clone)	Dilution	Staining pattern	Source
CD31	Mouse IgG1/kappa (JC70A)	1:50	Membrane/cytoplasm	DAKO, Carpinteria, CA
CD34	Mouse IgG1/kappa (My10, 8g12)	1:25	Membrane/cytoplasm	BD Biosciences, San Jose, CA
D2-40	Mouse IgG1 (D2-40)	1:75	Membrane/cytoplasm	Signet Laboratories, Dedham, MA
FLI-1	Rabbit polyclonal IgG (FLI-1)	1:100	Nucleus	Santa Cruz Biotechnology, Santa Cruz, CA
HHV-8	Mouse IgG1 (13B10)	1:100	Nucleus	Novo-Castra, Newcastle, UK
WT-1	Mouse IgG1/kappa (6F-H2)	1:100	Nucleus or cytoplasm	DAKO, Carpinteria, CA
SMA	Mouse IgG (1A4)	Pre-diluted	Cytoplasm	Ventana Medical Sys, Tucson, AZ

study was aimed to elucidate the staining patterns of WT-1 in the vasculatures from reactive angiogenesis and a wide variety of benign and malignant vascular tumors. The potential applications of WT-1 immunostain in histopathologic evaluation of vascular lesions are discussed.

Material and methods

From 2009 to 2011, a total of 95 cases of vascular lesions were identified from the Surgical Pathology database in the Department of Pathology and Genomic Medicine in our institution. Since cardiac angiosarcomas are rare, the search included cases from 2000 to 2011. The vascular lesions include angiosarcomas (AS, 19), hemangioendotheliomas (HE, 5), Kaposi's sarcomas (KS, 4), capillary hemangiomas (CPH, 6), cavernous hemangiomas (CVH, 12), pyogenic granulomas (PG, 4), lymphangiomas (LA, 4), hemangiopericytomas (HP, 5), glomus tumors (GT, 8), and vascular malformations (VM, 13). Samples of granulation tissue (GRT, 14) from various locations were included for comparison. The patient's demographic data and location of the lesions are listed in **Table 1**. The study was conducted with institutional IRB approval (IRB0608-0112).

Pathologic evaluation and tissue microarray

The cases were reviewed by two pathologists (SG, YG) independently and the original diagnoses were agreed upon. Tissue microarrays were assembled from representative portions of formalin-fixed and paraffin-embedded tissue blocks.

Immunohistochemistry and interpretation

Tissue microarray sections were stained immunohistochemically for CD31, CD34, FLI-1 and WT1. Additional immunostains for D2-40 and HHV8 were performed on sections of LAs and KSs, respectively. Sections of HPs and GTs were also stained for smooth muscle actin (SMA). The sources, characterizations, concen-

Table 3. Results of Immunohistochemical stains on Various Vascular Lesions

Lesions	Cases (n)	Intensity scores of immunohistochemical stain (number of cases)						
		WT-1	CD31	CD34	FLI-1	SMA	D2-40	HHV8
AS	19	1+ (2) 2+ (2) 3+ (15)	2+ (2) 3+ (17)	2+ (3) 3+ (16)	3+ (19)	NP	NP	NP
HE	5	2+ (2) 3+ (3)	2+ (1) 3+ (4)	3+ (5)	3+ (5)	NP	NP	NP
KS	4	2+ (3) 3+ (1)	3+ (4)	3+ (4)	3+ (4)	NP	NP	3+ (4)
CPH	7	2+ (3) 3+ (4)	2+ (1) 3+ (6)	3+ (7)	3+ (7)	NP	NP	NP
CVH	12	- (10) 1+ (1) 2+ (1)	2+ (4) 3+ (8)	3+ (12)	2+ (2) 3+ (10)	NP	NP	NP
PG	4	2+ (1) 3+ (3)	3+ (4)	3+ (4)	3+ (4)	NP	NP	NP
LA	4	- (4)	1+ (2) 2+ (2)	- (1) 1+ (2) 2+ (1)	3+ (4)	NP	3+ (4)	NP
HP	5	- (5)	- (5)	- (1) 3+ (4)	2+ (3) 3+ (2)	3+ (5)	NP	NP
GT	8	- (6) 1+ (2)	- (8)	- (1) 2+ (1) 3+ (6)	- (1) 1+ (4) 2+ (3)	3+ (8)	NP	NP
VM	13	- (3) 1+ (6) 2+ (4)	2+ (5) 3+ (8)	2+ (3) 3+ (10)	3+ (13)	NP	NP	NP
GRT	14	1+ (1) 2+ (5) 3+ (8)	2+ (2) 3+ (12)	3+ (14)	3+ (14)	NP	NP	NP

trations, and cellular localizations of the primary antibodies are detailed in **Table 2**. After overnight incubation in a 60°C oven, deparaffinization, and hydration in water, the slides were placed in a pressure cooker for 20 minutes and rinsed them in deionized water. The slides were then treated with 3% hydrogen peroxide for 8 minutes and rinsed them 3 times. The slides were loaded onto an Autostainer Universal Staining System (DAKO; Carpinteria, CA), which was preprogrammed according to the specific antibody, and Mouse EnVision (DAKO) was used as a secondary antibody. To visualize the color, we incubated the slides with DAB (3,3'-diaminobenzidine tetrahydrochloride) chromogen for 16 minutes, counterstained them with Mayer hematoxylin and coverslipped them with Permount.

The staining patterns and intensities for each of the markers were interpreted by two pathologists independently (SG, YG). The staining patterns were recorded as cytoplasmic or nuclear, or combined pattern. The staining intensity was graded based on 4-tier grading criteria: negative (no staining above background), + (focal weak staining), ++ (intermediate staining), and +++ (strong and diffuse staining). The average staining intensity of a given lesion was calculated by the following formula: total score of staining intensity (staining intensity x number of cases)/ total cases of a given lesion.

Results

The results of immunohistochemical stains are summarized in **Table 3** and **Figure 1**. The WT-1 staining was strictly in the cytoplasm of endothelial cells with granular texture. In contrast to the classic nuclear staining pattern of WT-1 in Wilms' tumor, desmoplastic small round cell tumor and ovarian tumor, there was no nuclear staining identified in any of the vascular lesions in this study.

Diffuse cytoplasmic positivity of WT-1 was invariably observed in all cases of malignant and borderline vascular tumors including AS (19/19, **Figure 2A**), KS (4/4, **Figure 2B**) and HE (5/5, **Figure 2C**). The intensity of WT-1 staining was unrelated to tumor location and stronger staining was often associated with solid and spindle cell morphology in ASs. Consistent expression of WT-1 was also observed in cer-

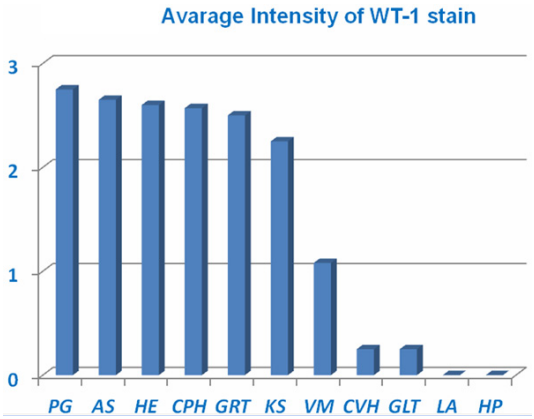

Figure 1. Average intensity scores of WT-1 stain in vascular lesions. PG: pyogenic granuloma; AS: angiosarcomas; HE: hemangioendothelioma; CPH: capillary hemangioma; GRT: granulation tissue; KS: Kaposi's sarcoma; VM: vascular malformation; CVH: cavernous.

tain types of benign vascular tumors, including CPH (7/7, **Figure 2D**) and PG (4/4, **Figure 2G**). However, the WT-1 stain in other benign vascular tumors, including CVH (2/12, **Figure 2E**) and LA (0/4, **Figure 2F**) was mostly negative with focal weak stain in rare cases (**Table 3**). The WT-1 expression level in VMs (10/13) was generally low to intermediate (**Figure 2H**) and was completely negative in 3 cases. Interestingly, a strong and diffuse WT-1 stain was seen in a case of thrombosed vascular malformation with prominent endothelial hyperplasia seen in the eyelid (**Figure 2I**). In addition, WT-1 was also expressed endothelial cells in GRT in association with benign vascular proliferation (14/14, **Figure 2J**). In tumors of non-endothelial origin, WT-1 stain was not demonstrated in HPs (0/5, **Figure 2K**), while GTs were often negative with focal weak stain in 2 cases (2/8, **Figure 2L**).

Traditional endothelial marker CD31 was performed to confirm the lesions with endothelial origin. As expected, the maker was positive in all vascular lesions except for GT (0/8) and HP (0/5) (**Table 3**). FLI-1 is a newly discovered endothelial marker which showed strong nuclear staining in proliferative endothelial cells. Interestingly, FLI-1 was also positive in HPs (5/5) and majority of GTs (7/8), so did CD34 (**Table 3**). The blood vessels in normal tissue and GRT were consistently positive for the markers mentioned above. HHV8 immunostain showed diffuse positivity in KSs (4/4). Immunostain for D2-40 was positive in LA (4/4), confirming the diagnosis. GTs and HPs were also positive for SMA on immunostain.

Discussion

Although the WT-1 immunohistochemical stain was originally described in Wilms' tumor, many recent observations have found that it stains a wide variety of normal and abnormal tissues. Two WT-1 staining patterns have been recognized in the literature. The traditional nuclear staining pattern can be observed in Wilms' tumor, ovarian tumors, acute leukemia and desmoplastic small round cell tumor [20]. More commonly, a granular cytoplasmic staining pattern can be seen in tumors from gastrointestinal tract, lung, breast, uterus, prostate, urinary tract, as well as malignant melanoma and several types of sarcoma [20, 21]. In routine practice of surgical pathology, however, only the nuclear staining pattern is commonly considered as positive. How to interpret WT-1 cytoplasmic stain is confusing due to lack of consensus or guidelines. As a result, WT-1 cytoplasmic stain is often interpreted as either non-specific or negative background stain. The diagnostic utility of cytoplasmic WT-1 staining pattern has not been widely explored.

In the current study, we examined the cytoplasmic expression pattern of WT-1 in a wide spectrum of vascular lesions from a variety of organ systems. The pathogenesis of the vascular lesions varies from reactive proliferation, vascular malformation, to benign and malignant vascular neoplasms. The study demonstrated that the cytoplasmic WT-1 stain was associated with endothelial cells in many of the vascular lesions. Notably, the cytoplasmic WT-1 was invariably expressed in lesions with malignant and borderline endothelial proliferation such as AS, HE and KS. These findings are consistent with a previous study [16] that demonstrated expression of WT-1 in all 9 ASs and 2 epithelioid and hobnail HEs. Similar findings were also observed in other early reports [12, 17]. It appeared that WT-1 was a highly reliable and sensitive marker for malignant and borderline endothelial tumors independent to their locations. The positive WT-1 cytoplasmic stain may be useful, in conjunction with other endothelial markers, in distinguishing malignant and borderline vascular tumors from other poorly differentiated neoplasms with mimicking morphology. It is important to be aware of the

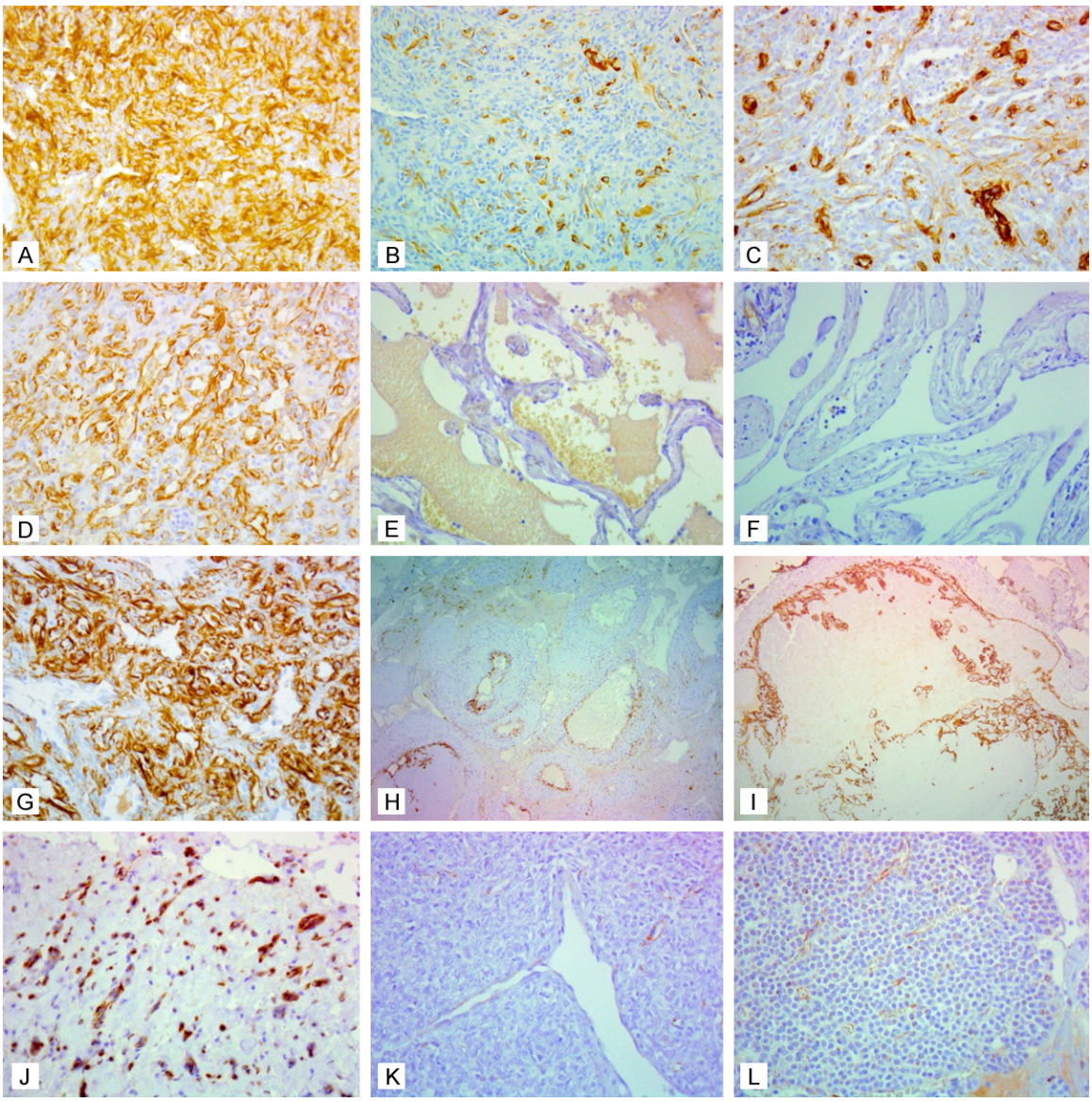

Figure 2. Immunostain of WT-1 in vascular lesions. Diffuse cytoplasmic positivity of WT-1 was observed in malignant vascular tumors including angiosarcoma (A), hemangioendothelioma (B) and Kaposi's sarcoma (C). Consistent expression of WT-1 was also observed in certain types of benign vascular lesions, including capillary hemangioma (D), pyogenic granuloma (G) and granulation tissue (J). However, WT-1 stain was mostly negative in cavernous hemangiomas (E) and lymphangiomas (F). WT-1 stain in vascular malformations was generally low or intermediate (H) but was strong in a case of endothelial hyperplasia associated with thrombosed vascular malformation (I). WT-1 stain was not demonstrated in hemangiopericytomas (K) and most of the glomus tumors (L).

strong cytoplasmic staining pattern in malignant vascular tumors helps to avoid misinterpretation when WT-1 is included in an immunostain panel for poorly differentiated tumors.

In addition to malignant vascular tumors, cytoplasmic WT-1 stain was also observed in benign vascular lesions including both reactive (GRT) and neoplastic proliferations (CPH, PG). The staining intensity and number of positive cases was similar as that seen in malignant and borderline vascular tumors. The sensitivity of WT-1 cytoplasmic stain in endothelial cells was comparable to conventional endothelial markers including CD31, CD34 and FLI1 in these lesions. Vascular malformation, however, showed lower to intermediate intensity of WT-1 cytoplasmic stain in about three quarter of the cases (10/13), more frequent than that previously reported [16-18]. The variation may

reflect the difference in staining protocols or subtypes and stages of the lesions among the study groups.

Of interest, there was no WT-1 expression detected in any cases of LAs and most of CVHs in this study. This is consistent with previous studies that demonstrated negative WT-1 staining in 17 oral LAs [12] and 8 lymphatic malformations [16]. Traditionally, LA and CVH are both considered as benign endothelial neoplasms. However, the levels of cytoplasmic WT-1 protein in these lesions may differ from that in other endothelial tumors such as CPHs or ASs.

The study included non-endothelial vascular tumors to further access the association of WT-1 cytoplasmic stain with endothelial cell lesions. As expected, WT-1 did not stain HP and most of GT cases. This finding further suggests the important roles of WT-1 expression in the pathogenesis of endothelial proliferation. Since CD34 and FLI-1 indiscriminately stain HPs and majority of GTs, WT-1 may serve as a useful tool in differential diagnosis of endothelial versus non-endothelial vascular tumors in this context. Immunoreactivity to SMA may provide further help in differential diagnoses.

The mechanism by which the WT-1 gene is expressed differently in vascular lesions is not understood. The WT-1 gene encodes a zinc finger transcription factor and regulates the expression of several genes associated with growth, differentiation, organ development and apoptosis [22]. Recent studies implicated that WT-1 is an important regulator in angiogenesis [6-11]. The cytoplasmic positivity of WT-1 in vascular tumors has been observed previously [16], and it has been postulated that there might be a cytoplasmic function for the WT-1 protein. An explanation for the cytoplasmic presence of WT1 has been recently described as it is a major component of polysomes as a translational regulator within the cytoplasm [23, 24].

It has been implied that over expression of WT-1 may promote growth of normal vascular smooth muscle [11] and endothelial cells, but blocks differentiation that finally may give rise to angiogenic tumors [15]. On the other hand, lack of WT-1 expression was observed in a vascular malformation which is characterized by abnormally enlarged lumina, deficient smooth muscle investment and failure to remodel in responding to appropriate stimuli [16]. The findings lead some to speculate that the WT-1 expression in endothelial cells may be associated with neoplastic transformation in vascular tumors [16, 19].

However, our results disagreed with this notion based on our observation that WT-1 expression was present in a wide spectrum of vascular lesions including reactive proliferations, vascular malformations and neoplastic lesions. In addition, cytoplasmic WT-1 protein was also expressed by benign endothelial cells in normal tissues and non-neoplastic vascular proliferation in variety of solid tumors [8, 25]. Therefore, we propose that the cytoplasmic WT-1 protein may be associated with the functional status of endothelial cells, ie, the activity of angiogenesis rather than the neoplastic nature of vascular lesions. The notion is supported by the fact that high levels of cytoplasmic WT-1 protein are expressed in all lesions with active angiogenesis, including GRT, PG, CPH, KS, HE and AS. Strong and diffuse WT-1 stain associated with endothelial hyperplasia in a thrombosed vascular malformation, a lesion with otherwise low WT-1 expression, further implies the strong association of WT-1 expression with angiogenic activity. WT-1 was demonstrated in previous studies to be an important regulator in both normal and tumor-related angiogenesis by regulating VEGF transcription [7], providing further support for the notion. On the other hand, CVHs and LAs are classically featured by attenuated or flattened endothelial lining without histologic evidence of angiogenesis, suggesting an inactive functional status of endothelial cells that may correlate with absent or very low expression level of WT-1 in these lesions. The lower to intermediate expression of WT-1 in VMs as a group may also reflect the functional status of endothelial cells depending on the stage and subtypes of an individual lesion.

In summary, our study demonstrated the cytoplasmic expression of WT-1 is a sensitive and consistent endothelial marker in reactive, benign and neoplastic vascular lesions with active endothelial proliferation. Absent or reduced WT-1 expression was observed in CVHs, LAs and VMs, which may be related to lack or reduced angiogenic activity in WT1-driven angiogenesis. HPs and GTs are non-endothelial neoplasms and are largely negative

for WT-1 expression. Although not specific, WT-1 may be useful in conjunction with other markers in vascular lesions for confirmation of endothelial origin of lesional cells in appropriate morphological context. Recognizing the different patterns of WT-1 stain in a broad spectrum of reactive and neoplastic tissues, including vascular lesions is critical in formulating appropriate panels of immunohistochemical stain and avoiding misinterpretation of the results.

Our results should be interpreted with caution as we have studied relative small groups of different vascular lesions. In addition, the correlation between WT-1 immunostain and its function should be interpreted with caution because they may not correlate well. As a well-accepted concept, the immunostain intensity doesn't always reflect the level of active or functional protein. Additional studies are needed to further evaluate the correlation of cytoplasmic WT-1 expression with angiogenic activity in vascular lesions.

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Disclosure of conflict of interest

The authors do not have financial interest to claim.

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