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VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2 (VEGFR2) AS A MARKER FOR MALIGNANT VASCULAR TUMORS AND MESOTHELIOMA – IMMUNOHISTOCHEMICAL STUDY OF 262 VASCULAR ENDOTHELIAL AND 1640 NONVASCULAR TUMORS

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Abstract

Vascular endothelial growth factor receptor 2 (VEGFR2) is a primary responder to vascular endothelial growth factor signal, and thereby regulates endothelial migration and proliferation. This receptor is expressed in endothelial cells and some vascular tumors, but many reports also detail its expression in carcinomas and lymphomas. VEGFR2 is a potential cell type marker, and data on VEGFR2 expression may also have therapeutic significance in view of recent availability of VEGFR2 inhibitors. In this study we immunohistochemically examined 262 vascular endothelial and 1640 non-vascular tumors and selected non-neoplastic tissues with a VEGFR2-specific rabbit monoclonal antibody 55B11. In early human embryo, VEGFR2 was expressed in endothelia of developing capillaries, thoracic duct, great vessels, hepatic sinusoids, epidermis, and mesothelia. In late first trimester fetus peripheral soft tissues, VEGFR2 was restricted to capillary endothelia, chondrocytes, and superficial portion of the epidermis. In normal adult tissues, it was restricted to endothelia and mesothelia. VEGFR2 was consistently expressed in angiosarcomas, Kaposi sarcomas, and retiform hemangioendotheliomas. It was detected only in half of epithelioid hemangioendotheliomas (15/27), usually focally. VEGFR2 was strongly expressed in most capillary hemangiomas and weakly or focally in cavernous, venous, and spindle cell hemangiomas, and lymphangiomas. Malignant epithelial mesothelioma was found to be a unique epithelial neoplasm with a strong and nearly consistent VEGFR2 expression, including membrane staining (35/38). Approximately 10% of squamous cell carcinomas and 23% of pulmonary adenocarcinomas contained focal positivity. The only non-endothelial mesenchymal tumors found VEGFR2-positive were biphasic synovial sarcoma (focal epithelial expression), and chordoma. All melanomas and lymphomas were negative. VEGFR2 is a promising marker for malignant vascular tumors and malignant epithelioid mesothelioma. Expression in biphasic synovial sarcoma epithelium, chordoma, and some carcinomas has to be considered in differential diagnosis. Information on VEGFR2 tissue expression may be useful in development of targeted oncologic therapy via VEGFR2-specific tyrosine kinase inhibitors.

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Keywords

angiosarcoma; Kaposi sarcoma; malignant mesothelioma; VEGFR2; endothelial cell; mesothelial cell; fetal tissue; adult tissue

INTRODUCTION

Vascular endothelial growth factor receptor 2 (VEGFR2) is a type V receptor tyrosine kinase mainly known to be expressed in vascular endothelial cells and encoded by the *KDR* gene. This receptor responds to the signal of vascular endothelial growth factor (VEGF) binding, which initiates a phosphorylation cascade that ultimately involves nuclear regulatory targets resulting in enhancement of endothelial proliferation and migration.³² VEGFR2 protein is also known as KDR (kinase insert domain receptor), Flk-1 (fetal liver kinase 1), and CD309 in the cluster of differentiation terminology of hematopoietic antigens.

VEGFR2 is expressed in many but not all vascular endothelial cells from early fetal development, and it is more highly expressed in neovascular tumor endothelia than in normal endothelia.^{5,33} VEGFR2 expression has been reported in hemangiomas and angiosarcomas. Activated VEGFR2-signaling¹⁵, sometimes by *KDR* gene mutations found in juvenile capillary hemangiomas and angiosarcomas, is believed to be pathogenetically important.^{2,35} However, no systematic information exists on VEGFR2 expression in vascular tumors.

Numerous studies have reported VEGFR2 expression in carcinoma cells, especially in mammary^{12,28}, colorectal^{1,10,31}, lung non-small cell^{9,30}, and urothelial³⁶ carcinomas. Tumor cell expression has also been reported in malignant melanoma^{18,27} and diffuse large B-cell lymphoma.¹³ However, a recent report on common carcinomas failed to detect VEGFR2 in tumor cells and found it limited to tumor endothelia only.³³

Recent arrival of tyrosine kinase inhibitors with specificity toward VEGFR2, such as sunitinib, sorafenib, and cabozantinib, and VEGFR2 blocking by a VEGF-neutralizing antibody bevacizumab (analogous to herceptin in breast cancer), have generated expectations that these inhibitors could be effective against VEGFR2-positive tumors as targeted oncologic therapy, while the antiangiogenic effect in any tumor (whether expressing VEGFR2 or not) could also have additional or independent therapeutic effect.^{2,6,17,37}

In this study, we systematically examined vascular endothelial, non-endothelial epithelial, mesenchymal, neuroectodermal and hematolymphoid tumors for VEGFR2 to examine its endothelial specificity and the potential of VEGFR2 as a tumor type marker, and also to discover potential targets for VEGFR2 inhibitor-based targeted therapy. We used a monoclonal antibody 55B11 shown specific to this receptor.³³

Based on our results, VEGFR2 is a strong marker for angiosarcoma and Kaposi sarcoma and has very limited expression in non-endothelial mesenchymal and neuroectodermal tumors. Among epithelial tumors, its consistent and unique expression in malignant mesothelioma is of interest for both tissue diagnosis and targeted therapy with VEGFR2 inhibitors.

MATERIALS AND METHODS

Tissues

Selected normal developing and adult tissues and 262 vascular endothelial tumors were evaluated. Also studied for comparison were 793 non-endothelial mesenchymal tumors, and 847 epithelial neoplasms (almost all malignant). Most of these tissues were arranged in tightly packed multi-tissue blocks containing 25–50 cases. Thus the tissue volume was in average far greater than that in tumor microarrays based on 0.6–1 mm punch biopsies, so that such multi-tissue blocks are sometimes referred to as “macroarrays”. All tissues were obtained from surgical specimens and used in an anonymized manner. All angiosarcomas were verified as CD31-positive and Kaposi sarcomas as HHV8-positive. Other tumors were extensively verified by multiple differentiation markers. Especially, all “outliers”, unexpectedly positive or negative cases, were further investigated and verified to confirm the diagnostic category.

Immunohistochemistry

A rabbit monoclonal VEGFR2-specific antibody 55B11 from Cell Signaling Technology (Danvers, MA) was used in a dilution of 1:500. Immunostaining was performed using a Leica Bond-Max automated immunostainer. Heat-induced epitope retrieval was performed for 30 min using a Bond-Max high pH epitope retrieval buffer. Primary antibody was applied for 30 minutes, followed by Bond-Max polymer for 15 min. Diaminobenzidine was used as the chromogen, followed by a hematoxylin counterstain.

The percentage of positive tumor cells was estimated. The presence of internal control (positive capillary endothelia) was required for valid immunostain for tumors, but this was not required in normal tissues; however external control was used to verify technical adequacy for each slide. Parallel data on vascular tumors were available on other endothelial markers from previous studies: Claudin-5, ERG, Prox1, podoplanin, and CD31.^{21–23}

RESULTS

The results on VEGFR2-immunoreactivity in 262 vascular endothelial cell tumors, 793 non-endothelial mesenchymal and lymphohematopoietic tumors, and 847 epithelial neoplasms (mostly malignant), are summarized in Tables 1–3.

Developing human tissues

In an early 1st trimester embryo (estimated age, 7 weeks), VEGFR2-immunoreactivity was present in the epidermis, vascular endothelial (Fig. 1A, B), and mesothelial cells. Small capillaries with positive endothelia were detected in primitive placenta, truncal, intestinal, and pulmonary mesenchyme. Of the large truncal vessels, prominent positivity was detected in the thoracic duct and structures consistent with the orifices of the great vessels at the heart. Within the heart, endothelial staining was more prominent in the atria and peripheral portions of the ventricles, and in the orifices of great vessels. In the liver, sinusoids were positive (Fig. 1C). Portal tracts were not identifiable. Mesothelial cells were variably positive. All parenchymal epithelial, non-endothelial mesenchymal, and primitive neural elements were negative.

In a late 1st trimester fetus limbs, VEGFR2-positivity was detected in capillary endothelia, upper portion of epidermis, and cartilage.

Non-neoplastic adult tissues

VEGFR2 immunoreactivity was essentially restricted to endothelia and mesothelia (Fig. 1D), and no parenchymal epithelia, neural, lymphoid or muscular tissues were positive. Small positive capillaries were noted in the soft tissue, nasal, gastrointestinal tract and urinary bladder mucosae, lymphoid and subepithelial tissues of the tonsil, pancreas and myocardium. In the kidney, glomerular endothelia were strongly and interstitial capillaries weakly positive. In normal adult liver, positivity was restricted to portal vessels and sinusoids were negative. The spleen contained positive capillaries in the red pulp, whereas sinusoidal and muscular venous and arterial endothelia were negative. In addition, muscular walls of some venules were weakly positive. No positive capillaries were detected in the brain, prostate and very few in the muscular layers of the gastrointestinal tract. Peritoneal mesothelia showed regionally variable positivity. Submesothelial (keratin-positive) cells in regenerative processes adjacent to peritoneal surface were also variably positive.

Benign angiomas

The results are summarized in Table 1 and illustrated in Fig. 2. Hemangiomas varied in their VEGFR2 immunoreactivity. Typically strongly positive were juvenile capillary hemangiomas, lobular capillary hemangiomas (Fig. 2A), and other types of capillary hemangiomas. Papillary endothelial hyperplasia components in various hemangiomas were also strongly positive. All 4 epithelioid hemangiomas showed VEGFR2 immunoreactivity in the majority of epithelioid endothelial cells.

Cavernous and venous hemangiomas typically showed weak, delicate staining (Fig. 2B) observed only in a varying numbers of lining cells (median, 25%). Hepatic cavernous hemangiomas often showed stronger staining than the peripheral examples. Spindle cell hemangioma endothelia were heterogeneous and in most cases, only minority of lining cells were positive (median, 20%, Fig. 2C), whereas the spindle cells were negative. Lymphangiomas also typically showed limited, delicate VEGFR2-immunoreactivity usually in a minority of vascular endothelia (median, 30% of endothelia, Fig. 2D). In 1 lymphangioendothelioma, 80% of lining cells were VEGFR2-positive.

Hemangioendotheliomas

Kaposiform hemangioendothelioma (Table 1) showed a VEGFR2-positive endothelial component, whereas the pericytes were negative (Fig. 3A). Dabska hemangioendothelioma components in a lymphangioma were strongly positive. All but one of 12 retiform hemangioendotheliomas showed strong positivity in the majority of tumor cells (Fig. 3B). However, only half of epithelioid hemangioendotheliomas were positive. In those tumors, the positivity was focal (median, 20% of tumor cells), often membrane-associated (Fig. 3C). One epithelioid sarcoma-like hemangioendothelioma was negative.

Kaposi sarcoma and angiosarcomas

All Kaposi sarcomas (Table 1) were positive with moderate to strong intensity of apparently cytoplasmic staining (Fig. 3D). Most angiosarcomas irrespective of site of origin or subtype were strongly positive for VEGFR2 (85/89, 96%) with variable membranous and cytoplasmic staining (Fig. 4 A–D). In addition, some angiosarcomas also showed perinuclear Golgi zone-like positivity. Only 3 cases showed positivity in 10% or less of tumor cells, and 8 of 9 epithelioid angiosarcomas were positive. The 4 VEGFR2-negative angiosarcomas were poorly differentiated examples one each from the buttock, stomach, pericardium, and brain. However, solid non-vasoformative angiosarcomas were often positive. Such a tumor involving small intestine was highlighted as a sole VEGFR2-positive

case in a block containing large cell lymphomas. This tumor was also positive for CD31, ERG, claudin 5, and Prox1 (Fig. 5) and negative for CD20 and Pax5.

Malignant epithelial neoplasms

The results are summarized in Table 2. The most striking and consistent tumor cell-specific VEGFR2-positivity was detected in malignant epithelial mesothelioma, of which 35/38 cases (92%) were positive, typically with extensive, predominantly membrane staining with some cytoplasmic positivity (Fig 6A). Tubulopapillary examples showed stronger staining, whereas solid epithelial proliferations were variably, often only focally positive. Three purely solid epithelial mesotheliomas and all 6 sarcomatoid spindle cell mesotheliomas were negative.

Focal diffuse cytoplasmic positivity without distinct membrane staining was detected in 12/53 (23%) of pulmonary adenocarcinomas (Fig. 6B). Approximately 10% of cutaneous, laryngeal, esophageal, and pulmonary squamous cell carcinomas contained focal cytoplasmic positivity (Fig 6C). All other carcinomas and testicular seminomas were VEGFR2-negative (Table 2). Nearly half of testicular embryonal carcinomas (6/15) contained clusters of positive epithelial cells with both membranous and cytoplasmic staining (Fig 6D), whereas all seminomas were negative.

In addition to the above described tumor cell immunoreactivity, VEGFR2 was detected in tumor neovascular endothelia, especially prominently in renal cell and pulmonary small cell carcinomas and also malignant mesotheliomas.

Non-endothelial mesenchymal and hematopoietic tumors (Table 3)

VEGFR2-immunoreactivity was limited in mesenchymal non-endothelial tumors. Biphasic synovial sarcoma (7/8) contained glandular epithelial cells with cytoplasmic VEGFR2-positivity (Fig. 7). Half of the chordomas (5/10) contained cytoplasmic positivity. All other mesenchymal and neuroectodermal tumors, including melanomas, granulosa cell and Leydig cell tumors, and small and large B-cell lymphomas and T-cell lymphomas studied were negative.

DISCUSSION

In this study, we immunohistochemically evaluated expression of VEGFR2, a vascular endothelial growth factor receptor that regulates endothelial proliferation and migration. We used a monoclonal rabbit antibody 55B11 previously found specific to this receptor tyrosine kinase.³³ The results showed expression in normal endothelia and mesothelia, and widespread expression of VEGFR2 in benign and malignant vascular tumors. In addition, VEGFR2 was detected in a small fraction of epithelial neoplasms, most notably in malignant mesothelioma and testicular embryonal carcinoma, and to a lesser degree, in pulmonary adenocarcinoma and squamous cell carcinomas. Although VEGFR2 is a membrane protein and thus membrane immunoreactivity is expected, some tumors also showed diffuse cytoplasmic or Golgi zone-like paranuclear positivity. This distribution may reflect aberrant processing of the VEGFR2 protein in tumors.

VEGFR2 is strongly expressed in most angiosarcomas regardless of subtype, site, and differentiation, but was absent in non-endothelial malignant mesenchymal tumors, with very rare exceptions (biphasic synovial sarcoma, chordoma). VEGFR2 was also detected in all Kaposi sarcomas indicating that this receptor can be a useful supplemental marker for malignant vascular endothelial tumors. Ability of VEGFR2 to identify poorly differentiated angiosarcomas randomly present in multitumor blocks containing different tumor entities further illustrates its diagnostic usefulness. Similar to other endothelial markers, VEGFR2

does not discriminate between benign and malignant endothelial neoplasms, so that this distinction must be made on histological grounds. Previous studies have demonstrated VEGFR2-specific mRNA in angiosarcoma and Kaposi sarcoma³, and a small number of angiosarcomas were shown to be immunohistochemically VEGFR2-positive.²

Because of the strong VEGFR2 expression in vascular tumors and presence of *VEGFR2*-mutations in some angiosarcomas (10% in one study)², tyrosine kinase inhibitors with specificity toward VEGFR2 are being investigated as targeted oncologic therapy for malignant vascular tumors.^{2,26} Our results showing strong VEGFR2 expression in angiosarcomas regardless of differentiation level support the basis of VEGFR2 inhibitors as targeted therapy against malignant endothelial tumors.

Compared with other endothelial markers, VEGFR2 is nearly equally specific and sensitive for angiosarcoma as CD31 and ERG, each marker having different additional specificities: mesothelia/mesothelioma for VEGFR2, histiocytes and plasma cells for CD31^{19,20}, and prostate cancer and cartilage for ERG.²¹ VEGFR2 is more specific than claudin-5, which also labels a high number of various carcinomas²², and more sensitive and specific than podoplanin (D2-40) and Prox1, each of which only label a subsets of angiosarcoma (especially the cutaneous ones), and additionally many types of carcinomas and other tumors.^{16,23}

VEGFR2 expression in hemangiomas is more variable, with some variants, such as juvenile capillary and lobular capillary hemangiomas showing strong positivity, whereas other capillary and many cavernous hemangiomas showed more subtle positivity probably reflecting weaker expression. As noted in previous studies, VEGFR2 showed stronger expression in neovascular endothelia or carcinomas.³³

In hemangioendotheliomas, VEGFR2 expression is variable. Whereas the more differentiated and vasoformative types, such as kaposiform and retiform hemangioendotheliomas are strongly positive, epithelioid hemangioendotheliomas with primitive vasoformation are only focally if at all positive indicating that other vascular endothelial markers such as CD31, ERG, claudin-5 are more suitable for its detection.^{7,21,22}

VEGFR2 expression in non-endothelial mesenchymal tissues and tumors is rare. However, this receptor is expressed at least in fetal cartilage, as also reported by others⁴, and therefore might also be expected in cartilaginous tumors. Based on our observation, chordoma also seems to commonly contain VEGFR2-positive tumor cells, perhaps as reflection of its distant relationship with cartilage.

Malignant epithelial mesothelioma is a notable exception as a strongly VEGFR2 positive epithelial neoplasm. VEGFR2 expression of mesothelioma parallels its expression in normal adult (and fetal) mesothelial cells. VEGFR2 is therefore a new supplemental positive marker for malignant mesothelioma, along with calretinin, keratins 5, and WT1. However, additional mesothelial/epithelial and vascular markers may be needed to separate mesothelioma from angiosarcoma, another tumor with strong VEGFR2 expression. Because sarcomatoid mesotheliomas lack VEGFR2-expression, this marker is not suitable for their detection. Similarly, some solid epithelial mesotheliomas also show limited, or in some cases, no expression.

Based on strong VEGFR2 expression in mesothelioma cells, this tumor would be a particularly strong candidate for targeted therapy with VEGFR2 tyrosine kinase inhibitors. Indeed, in vitro studies have demonstrated that tyrosine kinase inhibitors sunitinib and vandetanib, which mainly inhibit VEGFR2, inhibited growth of three mesothelioma cell lines.²⁵ Also, all 17 mesothelioma tissue sections were immunohistochemically positive

with the 5B11 antibody; this result was confirmed in the present study. Similarly, VEGFR2-inhibitors might also be of interest in VEGFR2-positive chordomas, 50% subset of these tumors, based on our findings.

Embryonal carcinoma of testis was another epithelial tumor with distinct membranous VEGFR2-positivity. Such positive cells were seen in clusters as a minority of tumor cells. It is difficult to ascertain their nature, but early vascular or mesothelial progenitor-type of differentiation is possible. It is of interest to note that angiosarcoma is the second most common somatic evolution into a sarcoma in germ cell tumors, after rhabdomyosarcoma.⁸

VEGFR2 was otherwise rarely expressed in common carcinomas, in general agreement with a recent study with the same 5B11 antibody.³² In our study, no carcinomas of breast, colon, endometrium, ovary, pancreas, prostate, or stomach were positive. However, 23% of pulmonary adenocarcinomas contained positive cells. Their focal cytoplasmic positivity, however, was in contrast with membrane staining observed in most mesotheliomas, so that the pattern of staining (membrane vs. cytoplasmic) can be useful in separation of mesothelioma and pulmonary adenocarcinoma.

Our results and those of Smith et al.³³ finding either rare or no VEGFR2 expression in common carcinomas, are in contrast with numerous older studies, in which 40% or more of carcinomas, such as pulmonary non-small cell carcinomas^{9,30}, mammary^{12,28}, colorectal^{1,10,31}, gastric³⁴, and transitional cell carcinomas of the bladder³⁶ were found to have VEGFR2 expression in tumor cells, and this difference is most likely attributable to using of specific (less staining) vs. non-specific antibodies (more staining). We also found no VEGFR2 expression in hepatocellular carcinoma, in contrast with a previous study reporting this receptor in 61% of hepatomas with authors' own antibody.¹⁴ Because many of these studies report tumor cell VEGFR2 expression as an adverse prognostic factor, these findings may have to re-interpreted.

VEGFR2-expression was also detected in approximately 10% of squamous cell carcinomas. This finding might correspond with the fetal squamous epithelial phenotype with VEGFR2-positivity. Thus, VEGFR2-positivity in some squamous cell carcinomas might represent oncofetal antigen expression – reversion of tumor expression pattern into that of fetal tissue. A previous study using another VEGFR antibody showed a higher frequency of VEGFR2 in head and neck squamous carcinomas, with nearly all cases (17/18) being focally positive.²⁴ However, another report stated VEGFR2-negativity of head and neck squamous cell carcinoma cells, observing positive stromal cells instead.²⁹

We were also not able to replicate previous findings showing common (>50%) cytoplasmic VEGFR2 expression in diffuse large B-cell lymphomas¹³, malignant melanomas^{18,27}, and ovarian granulosa cell tumors¹¹. Those studies with VEGFR2-positive findings were also based on VEGFR2-antibodies determined unspecific by a recent investigation³³, or the antibody source was not stated. The less frequent true VEGFR2 expression carcinomas and absence of this receptor in lymphoma and melanoma cells may lead to modification of oncologic strategies related to the use of VEGFR2-specific inhibitors, unless these inhibitors are used merely against tumor neovascularization.

In summary we evaluated a large number of vascular and non-vascular endothelial tumors. Consistent expression of VEGFR2 in angiosarcoma and Kaposi sarcoma, together with limited expression in other mesenchymal and epithelial tumors, makes this marker potentially useful in the diagnosis of malignant vascular tumors. Strong VEGFR expression in malignant epithelioid mesothelioma and relatively rare expression in other epithelial tumors make this marker also worthy supplemental marker for epithelial malignant mesothelioma. Furthermore, the results strengthen the basis for application of tyrosine

kinase inhibitors with specificity to VEGFR2 in targeted oncologic treatment of malignant vascular tumors and epithelial mesothelioma.

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Fig. 1. VEGFR2 expression in normal tissues. A. In early human embryo, the epidermis and early capillaries in the primitive mesenchyme are positive. B. Placental capillaries are positive and trophoblast is negative. C. Hepatic sinusoids are positive in early embryonic liver. D. Human adult peritoneal mesothelial cells and endothelia of stromal capillaries are positive.

Fig. 2. VEGFR2 expression in benign angiomas. A Lobular capillary hemangioma endothelium is strongly positive. B. Cavernous hemangioma endothelium shows subtle positivity. C. A minority of endothelial cells in spindle cell hemangioma are positive. D. Lymphangioma endothelia and intratumoral capillaries are positive.

Fig. 3. VEGFR2 in hemangioendotheliomas and Kaposi sarcoma. A. Endothelial component in Kaposiform hemangioendothelioma is positive and pericytes negative. B. Retiform hemangioendothelioma cells are strongly VEGFR2-positive. C. Cords of epithelioid hemangioendothelioma cells contain focal membranous VEGFR2-immunoreactivity. D. Kaposi sarcoma spindle cells are VEGFR2-positive.

Fig. 4. VEGFR2-positivity in angiosarcomas. A. Well-differentiated cutaneous angiosarcoma of the scalp. B. Poorly differentiated solid angiosarcoma (Stewart-Treves syndrome). C. Hepatic angiosarcoma (negative areas are narrow columns of hepatocytes). D. Splenic pleomorphic angiosarcoma shows subtle, focal positivity.

Fig. 5.
A. Poorly differentiated solid “lymphoma-like” angiosarcoma involving small intestine, verified by three new endothelial cell markers. B. Cytoplasmic and perinuclear Golgi-like positivity for VEGFR2. C. Nuclear positivity for ERG. D. Diffuse cytoplasmic positivity for claudin-5.

Fig. 6. VEGFR2 in epithelial neoplasms. A. Tubulopapillary malignant mesothelioma shows strong membranous and some cytoplasmic immunoreactivity. B. Pulmonary adenocarcinoma contains foci of tumor cells with cytoplasmic VEGFR2-positivity. C. Cutaneous squamous cell carcinoma is focally positive. D. Epithelial cells of embryonal carcinoma of testis are focally positive.

Fig. 7.
Biphasic synovial sarcoma epithelium shows focal cytoplasmic VEGFR2-immunoreactivity.

Table 1

Expression of VEGFR2 in 262 vascular endothelial tumors.

Tumor type	Positive/total	Comment on endothelial cell labeling
Hemangioma variants	81/81	
Capillary hemangioma, juvenile	12/12	Strong, uniform
Capillary hemangioma, lobular	9/9	Strong, uniform
Capillary hemangioma, NOS	13/13	Usually strong
Cavernous hemangioma	17/17	Weak but uniform
Epithelioid hemangioma	4/4	Strong, majority of cells
Spindle cell hemangioma	13/13	Weak and variable
Venous hemangioma	13/13	Weak, focal
Papillary endothelial hyperplasia	5/5	Strong, uniform
Lymphangioma	9/9	Usually weak, focal
Lymphangioendothelioma	1/1	
Hemangioendotheliomas	32/47	
Epithelioid hemangioendothelioma	15/28	Typically focal, delicate
Epithelioid sarcoma-like hemangioendothelioma	0/1	
Papillary intralymphatic hemangioendothelioma (Dabska tumor)	1/1	
Kaposiform hemangioendothelioma	5/5	Endothelial component
Retiform hemangioendothelioma	11/12	Usually strong
Angiosarcoma	85/89	Usually strong
Scalp and face	18/18	
Other cutaneous sites (non-radiation associated)	4/4	
Deep soft tissue	7/8	
Radiation associated, mainly chest wall and breast	10/10	
Postmastectomy angiosarcoma (Stewart-Treves)	1/1	
Splenic	3/3	
Visceral/gastrointestinal	10/11	
Body cavity-associated	8/8	
Cardiac and pericardial	9/10	
Other (includes metastases)	15/16	
Kaposi sarcoma	30/30	Moderate to strong
Total for vascular tumors	243/262	

Table 2

VEGFR2 expression in 847 epithelial neoplasms.

Tumor type	Positive/total	% positive
Malignant mesothelioma, epithelial [*]	35/38	92
Malignant mesothelioma, sarcomatoid	0/6	
Breast, ductal carcinoma	0/50	
Breast, lobular carcinoma	0/5	
Colon, adenocarcinoma	0/55	
Endometrium, adenocarcinoma	0/18	
Esophagus, squamous cell carcinoma	2/21	10
Intestines, sarcomatoid carcinoma	0/21	
Kidney, renal cell carcinoma	0/51	
Larynx, squamous cell carcinoma	3/32	9
Liver, hepatocellular carcinoma	0/35	
Liver, cholangiocarcinoma	0/15	
Lung, adenocarcinoma ^{**}	12/53	23
Lung, squamous cell carcinoma ^{**}	2/28	7
Lung, small cell carcinoma ^{**}	3/25	12
Ovary, serous carcinoma	0/60	
Ovary, endometrioid carcinoma	0/9	
Pancreas, ductal adenocarcinoma	0/24	
Pancreas, islet cell tumor	0/5	
Parathyroid adenoma	0/26	
Prostate, adenocarcinoma	0/43	
Stomach, glandular adenocarcinoma	0/35	
Stomach, signet ring cell carcinoma	0/10	
Skin, basal cell carcinoma	0/24	
Skin, squamous cell carcinoma ^{**}	1/9	11
Testis, seminoma	0/16	
Testis, embryonal carcinoma ^{***}	6/15	40
Thymoma	1/31	3
Thyroid, papillary carcinoma	0/16	
Urinary bladder, transitional cell carcinoma	0/46	
Uterine cervix, squamous cell carcinoma	0/25	
Total	65/847	

^{*} Extensive, membrane-based positivity^{**} Focal cytoplasmic positivity^{***} Focal membranous and cytoplasmic positivity

Table 3

VEGFR2 expression in 793 non-endothelial mesenchymal, neuroectodermal, and hematopoietic tumors.

Tumor type	Positive/total	% positive
Angiomatoid fibrous histiocytoma	0/9	
Blue nevus, cellular	0/4	
Cardiac myxoma	0/5	
Chordoma	5/10	50
Clear cell sarcoma of tendons and aponeuroses	0/16	
Desmoid fibromatosis	0/25	
Epithelioid sarcoma	0/8	
Ewing sarcoma	0/18	
Fibrothecoma	0/16	
Fibrous histiocytoma, benign, cutaneous	0/27	
Gastrointestinal stromal tumor, gastric	0/45	
Gastrointestinal stromal tumor, intestinal	0/37	
Glomus tumor	0/5	
Granulosa cell tumor of ovary	0/21	
Leiomyosarcoma	0/19	
Leydig cell tumor ovary/testis	0/3	
Lymphoma, small B-cell	0/25	
Lymphoma, large B-cell	0/39	
Lymphoma, T-lymphoblastic/ALL	0/3	
Lymphoma, peripheral T-cell	0/15	
Extramedullary myeloid tumor, blastic	0/9	
Lymphoma, anaplastic large cell	0/5	
Malignant peripheral nerve sheath tumor	0/27	
Melanoma, metastatic	0/107	
Melanoma, desmoplastic, primary	0/17	
Meningioma	0/26	
Nodular fasciitis	0/31	
Paraganglioma, retroperitoneal	0/14	
Schwannoma	0/32	
Solitary fibrous tumor/hemangiopericytoma	0/59	
Synovial sarcoma, biphasic	7/8	88
Synovial sarcoma, monophasic	0/53	
Undifferentiated pleomorphic sarcoma/malignant fibrous histiocytoma	0/55	
Total	12/793	