Distribution of Keratins in Normal Endothelial Cells and a Spectrum of Vascular Tumors: Implications in Tumor Diagnosis

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Vascular endothelial cells are specialized mesenchyme-derived epithelial-like lining cells which are the essential participants in benign and malignant vascular tumors. Although endothelia in lower animals often express keratins (K), human endothelia are generally K negative and vimentin-positive. However, K expression has been noted in some endothelia and in some epithelioid vascular tumors. In this study, we systematically examined normal human vascular endothelia and a spectrum of human vascular tumors (n = minimum of 137 tumors with each marker) for simple epithelial keratin polypeptides of the Moll catalogue (K7, K8, K18, and K19). Selected vascular tumors were also evaluated with antibodies to K14 and the monoclonal antibody 34βE12 that recognizes several keratins of stratified epithelia. Endothelia of normal veins, venules, and lymphatics commonly exhibited focal positivity for K7 and K18, whereas K8, K14, and K19 were not seen in non-neoplastic endothelia with the antibodies used. Lymphangiomas (6 of 7) and venous hemangiomas (6 of 13) often showed K7-positive endothelial cells; K18 was detected less commonly, whereas K8 and K19 were not detected. Epithelioid hemangioendotheliomas (EHEs) showed K7 and K18 expression in the majority of cases (50% and 100%, respectively), while K8 was seen in 10% cases and K14 and K19 in none. In contrast, epithelioid angiosarcomas (EAs) were often positive for K8 and K18 (approximately 50%), whereas they less commonly showed K7 and only occasionally K19; all tumors were negative for K14 and with the antibody 34βE12. Nonepithelioid angiosarcomas (AS) less commonly showed keratin expression with K7, K8, and K18 being positive in 20% of cases, and K14 and K19 in none of the cases. Epithelial membrane antigen (EMA) was occasionally detectable in EHE (2/19) but was present in 4 of 16 (25%) EAs and 17 of 48 (35%) nonepithelioid AS. These findings document the common presence of focal reactivity for K7 and K18 in subsets of normal endothelia and also the frequent presence of simple epithelial keratins in malignant vascular tumors, while such expression is uncommon in nonepithelioid angiosarcomas. K- and EMA-positivity in neoplastic endothelia needs to be considered in the evaluation of human tumors. K antibodies such as those specific to K19 or AE1 that do not react with K8 and K18 should be used in the differential diagnosis of epithelioid vascular tumors and carcinomas. HUM PATHOL 31:1062-1067. Copyright © 2000 by W.B. Saunders Company

Key words: **angiosarcoma, differential diagnosis, endothelial cells, immunohistochemistry, keratin 7, keratin 8, keratin 18, EMA.**

Abbreviations: **K, keratin; EMA, epithelial membrane antigen; ABC, avidin-biotin peroxidase; EA, epithelioid angiosarcomas; EHE, epithelioid hemangioendothelioma.**

Human vascular endothelial cells form an epithelial-like lining in the vascular channels. The intermediate filament cytoskeleton of these cells is typically composed of vimentin,[1] but vascular endothelia in lower species, such as the trout and Xenopus frog, have been found to extensively coexpress vimentin and epithelial (cyto)keratins (K).[2-5] Among the keratins in the Moll catalog (numbered 1 to 20), the lower molecular weight keratins K7, K8, K18, and K19 are typically expressed in simple, nonstratified epithelia, and therefore, they are commonly referred to as simple epithelial keratins. Higher molecular weight keratins K1 to K6 and K9 to K17 are variably expressed in complex (stratified glandular, squamous, and transitional cell) epithelia.[4-7]

Systematic studies on human tissues with antibodies to simple epithelial keratins have shown K18-expression in some human vascular endothelial cells of soft tissues.[2] Also, one study reported K19 in pulmonary microvascular capillaries,[8] and we recently described keratin 7 (K7) immunoreactivity in subsets of normal vascular endothelial cells.[9] Human malignant epithelioid vascular tumors have been reported to be K-positive with polyclonal and monoclonal antibodies based on small numbers of cases,[10-14] but there is no systematic data on the expression of specific K polypeptides in different types of vascular tumors. Although vimentin is consistently present in sarcomas, such as angiosarcomas, it is commonly expressed in poorly differentiated carcinomas, limiting the differential diagnostic value of this marker.[7,15]

Because comprehensive data on the expression of the individual K polypeptides in human endothelia and vascular tumors is not currently available and has the potential to be diagnostically useful, we systematically evaluated normal and neoplastic human endothelia with a broad panel of antikeratin antibodies directed against simple epithelial keratins K7, K8, K18, and K19 and the complex epithelial keratin K14. The antibody 34βE12 which recognizes a constellation of keratins K1, K5, K10, and K14 was included in the evaluation. Epithelial membrane antigen (EMA) expression was also tested, because this marker is commonly used in the histopathologic evaluation, and it has not been extensively studied in vascular tumors.

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MATERIALS AND METHODS

Selected normal tissues and 185 vascular tumors were obtained from the Soft Tissue Registry of the Armed Forces Institute of Pathology, Washington, DC. Immunohistochemical studies for K polypeptides was performed using the avidin-biotin peroxidase (ABC)-complex amplification and detection system. The primary antibodies to keratins included monoclonal antibodies specific to individual keratins K7, K8, K14, K18, and K19 (numbered according to the Moll system,[1] and also the monoclonal antibody 34βE12 that reacts with keratins 5, 10, and 14.[16] Additionally, a commonly used monoclonal antibody E29 for EMA was evaluated in selected (mostly malignant) vascular tumors, because these can be confused with malignant epithelial tumors. A minimum of 137 tumors was studied with each keratin antibody.

The primary antibodies, their sources and dilutions, and enzymatic or microwave-based epitope retrieval procedures that were used before the incubation with the primary antibody are listed in Table 1. The primary antibody was followed by biotinylated horse antimouse immunoglobulin antiserum (1:200), followed by avidin combined in vitro with biotinylated peroxidase just before use (each 1:500). The color was developed with diaminobenizidine containing hydrogen peroxide. Negative and positive controls were also run using sections from a multitissue block to verify the appropriate reactions. Findings that supported high detection sensitivity in the control tissues were K8 and K18 positivity in a subset of reticulum cells in lymph nodes.[17]

RESULTS

Nonneoplastic Endothelia in Soft Tissues and Different Organs

K7-positive venules, lymphatics, and capillaries were seen in the skin, subcutaneous soft tissues, skeletal muscle, and mucosal sites including the respiratory, gastrointestinal, and genital tracts. For example, many K7-positive capillaries were seen in the esophageal wall beneath the epithelium (Fig 1A). Also, the slit-like vessels with attenuated endothelia and flaccid contours, consistent with lymphatics, were often positive in the gastrointestinal mucosa.

K18 positivity was seen in similar locations but in a smaller number of vessels. There was no reactivity for K8, K14, or K19, or for the antibody 34βE12. Vascular smooth-muscle cells in the placenta were positive for K8 and K18. Normal endothelia were negative for EMA.

VASCULAR TUMORS

Simple epithelial keratins K7 and K18 were detected in most lymphangiomas and occasional hemangiomas. They were more commonly seen in the epithelioid hemangioendotheliomas and angiosarcomas, which also expressed K8 and rarely K19. K14 was not detected in any of the vascular tumors, and none reacted with the monoclonal antibody 34βE12. The K-immunoreactivity results for the different groups of vascular tumors have been summarized in Table 1 and are presented in detail later.

Hemangiomas

Cavernous and capillary hemangiomas only exceptionally contained lesional cells with immunoreactivity for simple epithelial keratins, whereas the surrounding nonlesional venules were commonly K7-, and less commonly, K18-positive (Fig 1B). K7- and K18-positive endothelia were seen focally in 2 capillary-type intramuscular hemangiomas, and 1 cavernous-type intramuscular hemangioma contained focal K7-positive endothelial cells. One juvenile capillary hemangioma showed scattered K7-positive myopericytic cells, but the endothelial cells were negative.

Nearly half of the venous hemangiomas showed focal K7 immunoreactivity in the endothelia, and sometimes also vascular smooth-muscle of the large lesional veins were positive (Fig 1C); 3 of 13 cases showed K18-positive endothelial cells in a similar pattern. K8 and K19 positive lesional endothelia were not detected.

Lymphangioma

Six of 7 lymphangiomas showed K7-immunoreactivity in 30% to 50% of the lining endothelia (Fig 1D). Focal K18-positivity was seen in 2 of 7 cases, but K8, K14, and K19 were not detected.

TABLE 1. Monoclonal Antibodies, Their Source and Dilution and the Pretreatment Modality Used in This Study

Polypeptide	Clone	Pretreatment	Antibody dilution	Source
Keratin 7	OV-TL 12/30	PrVIII*	1:400	Dako, Carpinteria, CA
Keratin 8	Cam5.2	Pepsin†	1:40	Beckton-Dickinson, Mt. View, CA
Keratin 14	LL002	MW‡	1:40	Novocastra New Castle, UK
Keratins 1,5,10,14	34βH12	PrVIII*	1:40	Dako
Keratin 18	DC-10	MW‡	1:40	Novocastra
Keratin 19	RCK 108	MW‡	1:50	Dako
EMA	E29	MW§	1:50	Dako
CD31	JC/70	PrVIII*	1:100	Dako
von Willebrand factor	Polyclonal	PrVIII*	1:1600	Dako

* PrVIII = 0.05% Sigma protease type VIII, 3 minutes in 0.1 Mol/L phosphate buffer, pH 7.8 at 37°C
† Pepsin = crude pepsin (0.05% in HCl, pH 2.0) for 30 min. at 37°C
‡ MW = microwave heating adjusted to near to boiling in citrate-buffer for 20 min at pH 6.0, followed by a 20 min. cooling period
§ Parallel experiments were done with and without the above described microwave epitope retrieval on angiosarcomas

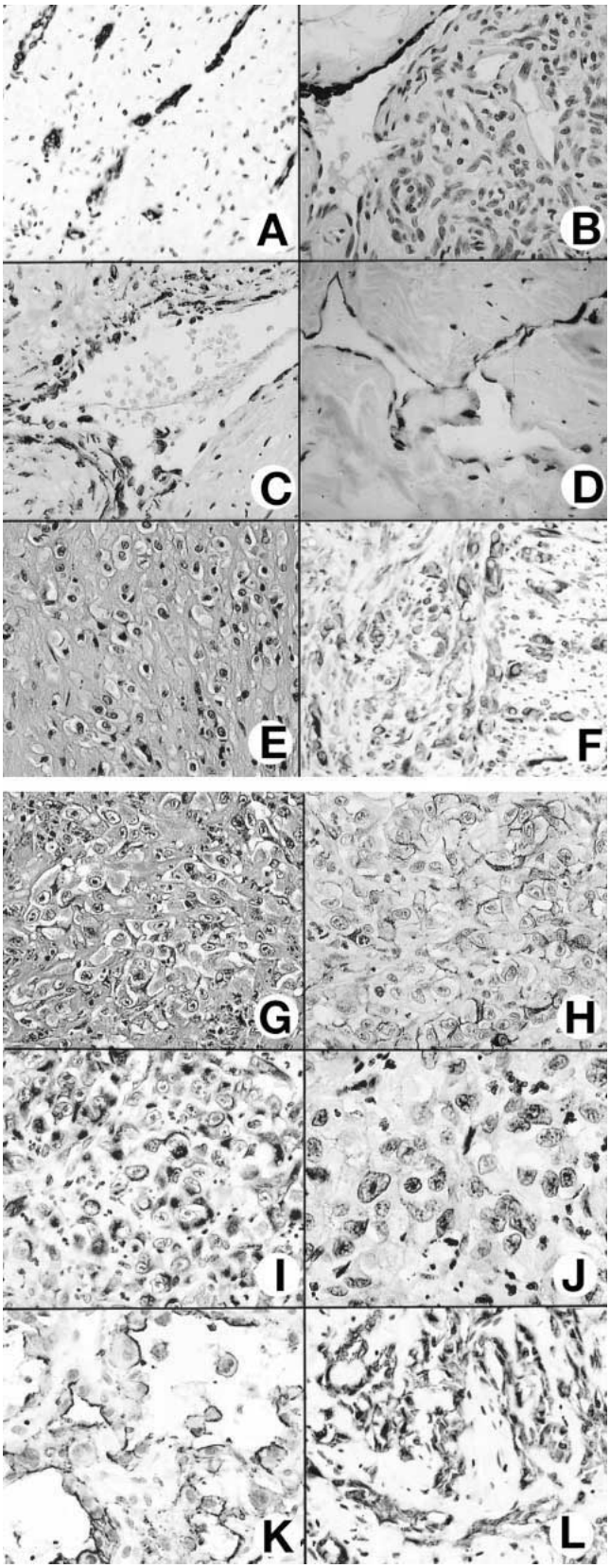

FIGURE 1. (A) Endothelial cells of esophageal subepithelial capillaries are positive for K7. (B) This cellular (juvenile) capillary hemangioma is negative for K7, but a venule next to the tumor has K7-positive endothelial cells. (C) A venous hemangioma with immunoreactivity to K7 in both the vascular smooth muscle and some endothelial cells. (D) The attenuated endothelial cells of lymphangioma vessels are K7-positive. (E) Epithelioid hemangioendothelioma is composed of cords and nests of primitive epithelioid endothelial cells embedded in a myxocollagenous matrix. (F) Epithelioid hemangioendotheliomas typically contain lesional cells positive for K18, as seen in this tumor that invades the wall of a vein. (G) Epithelioid angiosarcoma shows sheets of poorly differentiated epithelioid cells with large nuclei and prominent nucleoli. Note hemosiderin deposition in the tumor. (H) The cell membranes of epithelioid angiosarcoma is positive for CD31. (I) Epithelioid angiosarcoma cells with immunoreactivity for K8. (J) Epithelioid angiosarcoma with focal, linear EMA-positivity on the cell membranes. (K, L) Two angiosarcomas, an epithelioid (K) and a nonepithelioid example (L), show prominent EMA-positivity lining the vascular lumina. Both tumors were also positive for CD31.

Spindle Cell Hemangioma (Hemangioendothelioma)

All 10 of these tumors presented in the distal extremities as small nodules. K18 was the only simple epithelial K detected. This was usually expressed focally and was detected in 6 of the cases. The apical cytoplasm of the vascular endothelia was often lined by a delicate EMA-positive zone.

Epithelioid Hemangioendothelioma

These tumors were from a variety of soft-tissue sites in adult patients. Histologically, they were composed of cords, clusters, and nests of mildly to moderately atypical epithelioid endothelial cells that were often embedded in a collagenous or myxocollagenous matrix and frequently invaded the walls of venous channels. The tumor cell nuclei often had an open chromatin pattern with peripheral chromatin clumping and an occasional nucleolus. The cytoplasm was pale to deeply eosinophilic, and sometimes contained vacuoles (intracellular lumina). Mitotic activity was low (Fig 1E). All of the EHEs were at least focally positive either for CD31 with distinctive membrane staining or for von Willebrand factor (factor VIII-related antigen) with a cytoplasmic staining.

Among the simple epithelial keratins, K18 was detected in all EHEs that were tested (17 cases), 8 of which showed positivity in more than 50% of tumor cells (Fig 1F). K7 was seen in 10 of 20 cases, and 5 tumors showed extensive reactivity in 50% or more of the lesional cells. K8 reactivity was rare and was seen only focally in 2 cases. K14 and K19 were not detected, and there was no reactivity with the 34βE12. Two tumors showed focal EMA-positivity lining the cytoplasmic vacuoles (primitive lumina) of the neoplastic cells.

Epithelioid Angiosarcoma

Of the 18 epithelioid angiosarcomas (EA) that were analyzed, 9 were from the deep soft tissues, 3 were from the retroperitoneum or pelvis, 2 were from the scalp, and 1 each was from the spleen, nasal cavity, prostate, and lung (metastatic). These tumors were

composed of large epithelioid cells with ample variably eosinophilic or amphophilic cytoplasm. The tumor cells had large atypical nuclei with prominent "melanoma-like" nucleoli. All examples were high grade (Fig 1G). The diagnosis was based on vasoformative architecture, cytoplasmic reactivity for von Willebrand factor and membrane staining for CD31 (Fig 1H).

Among the simple epithelial keratins, K7, K8, and K18 were the most commonly detected; these were seen in 35%, 39%, and 64% of cases, respectively (Table 2). The expression of K8 and K18 was often concerted, with half of the positive cases exhibiting reactivity in greater than 30% of the tumor cells (Fig 1I).

Four of 16 (25%) EAs showed EMA-positivity with the immunoreactivity varying from subtle to prominent luminal staining (Figs 1J,K). Parallel staining performed without microwave epitope retrieval also gave positive results, although with a lesser intensity. K19 was only detected only in 1 case, and K14 and 34βE12-reactivity in none.

Angiosarcoma, Non-Epithelioid

This group of 52 tumors included 23 deep soft tissue angiosarcomas, 17 cutaneous angiosarcomas of the scalp or face, 4 from spleen, 3 postirradiation angiosarcomas of the chest wall, 2 postmastectomy angiosarcomas of arm, and 1 each from the breast, liver, and lung (the latter was metastatic). The neoplastic endothelial cells exhibited spindled or ovoid morphology. Distinctive vasoformation, CD31 immunoreactivity, or both were present in all cases.

Simple epithelial K immunoreactivity was less common in the nonepithelioid angiosarcomas. K18 was seen in 21% of the cases, with half of them having 50% of the tumor cells positive. K8 and K7 were present focally in 8% and 4% of the cases, respectively. None of the tumors showed K14, K19, or 34βE12-positivity.

Epithelial membrane antigen immunoreactivity was found in 17 of 48 cases (35%). In 4 of them, more than 50% of the lesional cells were positive (Fig 1L). EMA positivity was also seen without microwave epitope retrieval, although it was weaker.

Kaposi Sarcoma

All 6 Karposi sarcomas were HIV-related. The neoplastic spindle cells were positive for CD31 and CD34 and negative for simple epithelial keratins K7, K8, K18, K19, and EMA.

DISCUSSION

There is still confusion over whether endothelial cells and vascular tumors can be positive for K or epithelial membrane antigen, with reactivity for these markers often being interpreted as highly supportive, if not diagnostic, of an epithelial tumor. In this study, we have immunohistochemically analyzed normal human tissues and a large number of benign, borderline, and malignant vascular tumors for a spectrum of epithelial keratins including K7, K8, K14, K18, and K19 in the Moll catalog.[4,5]

Our evaluation of normal endothelia confirmed the previous observations that subsets of normal venules, capillaries and lymphatics in various soft-tissue, skin, and mucosal sites, display focal immunoreactivity for K7 and K18. In an extensive comparative study on the endothelial cytoskeleton of the Xenopus frog and man, Jahn et al[2] noted that human endothelial cells are immunoreactive for K8 and K18 using multiple different antibodies but failed to show K7 and K19. Our results are otherwise similar to those of Jahn et al, except that we found K7-positivity in the endothelia of some venules, capillaries, and lymphatics. Jahn et al used a different monoclonal antibody to K7, which is not applicable in paraffin-embedded tissue; the K7-antibody used by us was not available at the time of the previous study.[2] Therefore, epitope differences in the K7 antibodies or different methodology (immunofluorescence versus immunoperoxidase) are the likely explanations for the different K7 results.

In contrast to normal endothelial cells lining capillaries, venules, and lymphatics of the skin and soft tissue, endothelial cells of capillary hemangiomas are only rarely K-positive. However, lymphangiomas and venous hemangiomas often showed K7 positivity. This is consistent with the view that lymphangiomas and venous hemangiomas are, in essence, mature vascular malformations and possess endothelia that closely mirror their normal vessel counterparts. In contrast, capillary hemangiomas may possess a less differentiated endothelial phenotype or arise from capillaries that do not typically express K7.

TABLE 2. Summary of the Expression of Simple Epithelial Keratins 7, 8, 18, and 19 in Vascular Tumors

	K7	K8	K18	K19	EMA
Capillary hemangioma	1/22*	0/20	1/18*	0/26	
Cavernous hemangioma	1/8**	0/4	0/4	0/8	
Venous hemangioma	6/13*	0/13	3/13*	0/13	
Lymphangioma	6/7	0/7	2/7*	0/7	
Spindle-cell hemangioma	0/10	0/10	6/10*	0/10	7/7
Epithelioid hemangioendothelioma	10/20	2/20*	17/17	0/19	2/19
Epithelioid angiosarcoma	6/17	7/18	9/14	1/17	4/16
Angiosarcoma, nonepithelioid	2/48*	4/51*	10/48	0/52	17/48
Kaposi's sarcoma	0/6	0/6	0/6	0/6	0/6

* Denotes focal if any immunoreactivity.

Epithelioid hemangioendothelioma (EHE), a low-grade malignant vascular tumor, regularly expresses K18 and often has reactivity for K7, but not K8 or K19. These tumors show K expression much more often than normal endothelial cells. Although the clinicopathologic features of EHE are often quite distinctive, this tumor can histologically resemble, and therefore be mistaken for, an epithelioid sarcoma or carcinoma, and its expression of keratins compounds this problem. However, in contrast with epithelioid sarcoma, EHE typically expresses CD31 and lacks K14, K19, and 34βE12-immunoreactivity. Also, EHE commonly shows significant K7 expression, whereas this K is absent or usually only seen in sporadic cells in epithelioid sarcoma.[18] Squamous cell carcinomas also show K14, 34βE12, and K19 reactivity.

The K patterns in EA, a high-grade malignancy, differed from that seen in the other vascular tumors that we examined, and specifically differed from the K patterns seen in EHE. In contrast to EHE, it less commonly expressed K7 (EA 35% versus EHE 50%) and more commonly expressed K8 (EA 39% *v* EHE 10%). The lack of K8 immunoreactivity in normal endothelia and benign hemangiomas suggests that its expression may be a transformation-related event. Previous studies on virally transformed fibroblasts have shown selective neoexpression of K8 and K18,[19] and similar, selective K8 and K18 expression may be seen in diverse sarcomas with nonepithelial phenotypes.[7]

EMA is commonly used in the immunohistochemical differential diagnosis of epithelioid neoplasms. The usual absence of EMA in EHE helps to distinguish it from epithelioid sarcoma and carcinomas. Although EMA was previously reported negative in small numbers of EA, we found EMA-positivity in both epithelioid and nonepithelioid angiosarcomas with a nearly similar frequency (25% versus 35%, respectively). Our enhanced detection of EMA by microwave epitope retrieval is the most likely explanation for the difference from earlier, uniformly negative reports on EMA in EAs.[11-13] However, we also found EMA without epitope retrieval indicating that its detection in angiosarcomas is not an epitope retrieval artifact.

EMA-expression in EA necessitates caution in the interpretation of EMA-positivity as evidence for an epithelial tumor. EMA-immunoreactivity in angiosarcoma probably reflects the fact that some angiosarcomas attain a nearly complete epithelial immunophenotype.

The differential diagnosis between epithelioid vascular tumors and some carcinomas can be difficult considering the partially overlapping immunophenotypes. Despite the overlap, common expression of K19 and higher molecular weight keratins in carcinomas[20,21] and membrane reactivity for CD31 in vascular tumors[22-24] remain as discriminatory features. Also, keratin antibodies, such as those specific to K19 and the AE1 monoclonal antibody[5] that do not react with keratins 7, 8, and 18, are advantageous in the differential diagnosis of angiosarcoma and carcinoma.

In summary, we have systematically analyzed normal human vascular endothelia and a spectrum of human vascular tumors for the expression of simple epithelial keratins and selected stratified epithelial keratins. We showed by immunohistochemical technique that K7 and K18 are present in some normal vascular endothelia and that they are also expressed in subsets of vascular tumors, especially lymphangiomas, venous hemangiomas, and EHEs. It was also shown that angiosarcomas may acquire a nearly complete epithelial phenotype with expression of K7, K8, K18, and even EMA. However, the lack of K19 and complex epithelial keratins (high molecular weight keratins), and the expression of CD31 and von Willebrand factor, help to distinguish epithelioid vascular tumors from their mimicks, especially epithelioid sarcoma and carcinomas.

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