Mosaic Tumor Vessels: Cellular Basis and Ultrastructure of Focal Regions Lacking Endothelial Cell Markers

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Abstract

Endothelial cells of blood vessels in tumors may be thin, fragile, and defective in barrier function. We found previously that the endothelium of vessels in human colon carcinoma xenografts in mice is a mosaic structure. Approximately 85% of tumor vessels have uniform CD31 and/or CD105 immunoreactivity, but the remainder have focal regions that lack these common endothelial markers. The present study assessed the ultrastructure of the vessel lining and the integrity of the basement membrane in these regions. Using immunolabeling and confocal microscopy, we identified blood vessels that lacked CD31 and CD105 immunoreactivity and then analyzed the ultrastructure of these vessels by transmission electron microscopy. Eleven percent of vessels in orthotopic tumors and 24% of vessels in ectopic tumors had defects in CD31 and CD105 staining measuring on average 10.8 μm (range, 1–41.2 μm). Ultrastructural studies identified endothelial cells at 92% of CD31- and CD105-negative sites in orthotopic tumors and 70% of the sites in ectopic tumors. Thus, most regions of tumor vessels that lack CD31 and CD105 immunoreactivity represent attenuated endothelial cells with abnormal expression of endothelial cell markers, but some are gaps between endothelial cells. More than 80% of the defects lacked immunoreactivity for multiple basement membrane proteins.

Introduction

Angiogenesis in tumors produces poorly formed blood vessels having multiple abnormalities. Morphologic and immunohistochemical studies of tumors have identified changes in gene expression, defective endothelial cell barrier function, loose attachment of pericytes (mural cells and perivascular cells), and redundant layers of vascular basement membrane (1–7). In a previous study, we assessed the cellular composition of blood vessels in human adenocarcinomas implanted in mice using green fluorescent protein (GFP)-expressing cancer cells and fluorescent antibodies to label endothelial cells (8). We found that 15% of the vessels examined, representing ~4% of the vascular surface area, had focal regions that lacked immunoreactivity to the common endothelial cell markers CD31 (platelet/endothelial cell adhesion molecule-1, PECAM-1) and CD105 (Endoglin). These vessels, termed “mosaic vessels”, had several potential explanations. First, endothelial migration during rapid vessel growth could occur without sufficient endothelial proliferation to complete the endothelial lining, leaving cancer cells exposed to the lumen. Second, endothelial cells could be shed from the vessel lining, leaving similar regions of exposed underlying tumor cells. Third, migrating tumor cells could invade the vessel wall, displacing endothelial cells from the lining. And fourth, these regions could be lined by endothelial cells that do not express common endothelial markers, and were consequently not detected.

These scenarios have implications for vessel physiology and therapeutic targeting (8, 9). Holes in the endothelium due to missing endothelial cells might be responsible for the leakiness of tumor vessels, which can actually hinder uniform drug delivery (10, 11). Alternatively, the presence of tumor cells in the vessel wall could be an indication of tumor aggressiveness or metastasis. Absence of detectable CD31 and CD105 might be accompanied by other alterations in protein expression in tumor endothelial cells, possibly induced by the hostile tumor microenvironment. This could also influence endothelial functions including barrier formation and angiogenesis.

The present study sought to determine the cellular basis of the mosaicism in CD31/CD105 staining found in tumor vessels. To address this issue, we developed a four-step approach that enabled us to examine the ultrastructure of regions of endothelium that lack expression of CD31 and CD105 identified by confocal microscopy. First, endothelial cells of tumor vessels were labeled in vivo by injection of antibodies to CD31 and CD105. Second, tumors were harvested and embedded in a resin compatible with both confocal and transmission electron microscopy (TEM). Third, adjacent 2-μm and 80-nm sections were cut, and fourth, blood vessels that had focal defects in CD31/CD105 immunoreactivity identified by confocal microscopy were examined by electron microscopy in adjacent sections. The approach made it possible to examine endothelial cell ultrastructure in regions lacking endothelial markers.

We found that most mosaic vessels in mouse models of colon carcinoma had a continuous, thin endothelial lining which lacked CD31 and CD105 immunoreactivity, rather than absence of endothelial cells or presence of tumor cells in the vessel wall. Focal openings in the endothelium were present in some tumor vessels, particularly in ectopic xenografts, which had a rapid growth rate.

Materials and Methods

Green fluorescent protein construct. To provide an endogenous marker for cancer cells, a LS174T colon carcinoma cell line was transfected with a GFP construct driven by a constitutive promoter, EF1α. Selection was

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carried out by exposing the transfected cell lines to puromycin (1 μg/mL). Stable transfectants were obtained using fluorescence-activated cell sorting gated on the GFP-positive population (8).

**Tumor models.** Fluorescent LS174T cells, designated LSEFG, were implanted in female severe combined immunodeficiency mice anesthetized with ketamine/xylazine (100/10 mg/kg, i.m.). All GFP-expressing tumor cells were implanted at the same passage number. To assess the effect of host microenvironment on tumor vasculature, tumors were grown in orthotopic (cecum) or ectopic (ovarian pedicle) sites. For the orthotopic tumor model, a midline incision was made in the abdominal wall to expose the cecum. A thin cut was made in the serosa of the cecum, and 0.05 to 0.1 mL of LSEFG tumor slurry was injected into a pocket in the cecal wall. Tumors in this site grew slowly, reaching a diameter of 6 to 10 mm over a period of 7 to 10 weeks. Alternatively, a small piece (0.125 mm³) of tumor tissue was implanted in the cecal wall. In this case, tumors reached a diameter of 6 to 10 mm in 3 to 4 weeks. In both models, the tumor blood supply formed from cecal blood vessels. Because the size and overall wall structure of tumor vessels formed after implantation of a slurry of cells or piece of tumor were similar, the data were analyzed together.

In the ectopic model, 0.1 mL of tumor slurry was injected into the ovarian pedicle after ovarioctomy. Tumors were isolated from surrounding tissue by enclosure in a bag of Parafilm (American National Can Company, Menasha, WI). In this model, the tumor blood supply originates from branches from the main artery and vein in the ovarian fat pad (12). Ectopic tumors grew to a diameter of 6 to 10 mm within 10 to 14 days (13). A total of nine orthotopic tumors and six ectopic tumors were studied.

**Antibody infusion.** For confocal microscopy, endothelial cells of tumor vessels were labeled in situ by i.v. injection of a mixture of two fluorescent antibodies: rat anti-mouse CD31 (PECAM-1, clone MEC 133, Pharmingen, San Diego, CA) and rat anti-mouse CD105 (Endoglin, Pharmingen). Anti-CD31 and anti-CD105 antibodies label most endothelial cells in mice uniformly, but anti-CD105 labeling is particularly strong in angiogenic tumor vessels (14–16). Before injection, both antibodies were labeled with Cy5 fluorophore according to the manufacturer’s protocol (FluoroLink kit, Amersham Pharmacia Biotech, Piscataway, NJ). After anesthesia with ketamine/xylazine (100/10 mg/kg, i.m.), mice were infused with a mixture of the two antibodies (total antibody protein, 50–60 μg in 200 μL) via tail vein over a 30-minute period. The antibodies were allowed to circulate for 1 hour before the tumors were fixed by vascular perfusion (see below). No signal was detected when Cy5-labeled rat IgG was injected according to the same protocol as a negative control. Labeling tumor vessels with systemically injected antibodies ensured that the analyzed vessels were functional and perfused with blood at the time of fixation. Few vessels were unperfused or completely devoid of CD31 and CD105 expression, as judged by comparing the electron microscopy and confocal microscopy vessel maps of the same tissue.

**Sequential confocal microscopy and electron microscopy analyses.** For correlation of confocal and TEM images, tumors were fixed by vascular perfusion of fixative consisting of 3% paraformaldehyde and 0.1% glutaraldehyde in 0.9% PBS (pH 7.4; ref. 17). The thorax was opened, a perfusion of fixative was performed by exposing the transfected cell lines to puromycin (1 μg/mL). Stable transfectants were obtained using fluorescence-activated cell sorting gated on the GFP-positive population (8).

For correlation of confocal and TEM images, tumors were fixed by vascular perfusion as above except that the fixative consisted of 3% glutaraldehyde in cacodylate buffer (20). Tumors were removed, and pieces measuring ~1 to 3 mm were cut, rinsed with 100 mmol/L cacodylate buffer, fixed with 1% OsO₄ in 100 mmol/L cacodylate buffer at 4°C for 2 hours, rinsed with water, stained en bloc with 2% aqueous uranyl acetate for 48 hours at 38°C, dehydrated with acetone, and embedded in epoxy resin (20). Sections 0.5 μm in thickness were stained with toluidine blue for light microscopy, and sections 60 to 80 μm in thickness were stained with lead citrate and examined with a Zeiss EM-10C or Philips TEM 300 electron microscope. All vessels visible by electron microscopy in sections of orthotopic tumors from three mice were photographed sequentially until images of 100 vessels were obtained. In addition, 38 vessels with a potentially abnormal wall structure were identified in toluidine blue–stained sections of ectopic tumors from two mice, and then 80 mm sections of the same vessels were cut, stained with uranyl magnesium acetate, counterstained with lead citrate, and photographed by electron microscopy. Endothelium was identified by the following criteria: (a) the cells formed a continuous polarized surface layer adluminal to basement membrane, (b) all cells had an intact plasmalemma membrane, (c) junctions (overlapping or abutting) were evident between adjacent cells, and (d) in nucleated regions, extensive rough and smooth endoplasmic reticulum, some vesicles and an occasional Golgi complex were evident.

Peeling of the endothelium due to sectioning was very rarely observed. However, in some vessels, the endothelial lining was extremely fragile and the 80 mm Hg perfusion was sufficient to detach it. This does not occur when the same procedure is used for normal tissue. On the rare occasion when plasmalemma membrane disruption was visible, the vessels were excluded from the study.
Results

Comparison of orthotopic and ectopic xenografts. The two xenograft models of LS174T colon carcinoma had different patterns of growth. Tumors implanted orthotopically in the cecum grew slowly, invaded the gut wall, and formed multiple small tumor nodules surrounded by host tissue (Fig. 1A). By comparison, ectopic tumors grew rapidly, supported by a blood supply from branches of ovarian arteries and veins, but the Parafilm bag prevented the tumors from invading neighboring tissue (Fig. 1B; ref. 12). Blood vessels within orthotopic and ectopic tumors were approximately the same size (6-16 μm in diameter, Fig. 1C and D).

Frequency of regions lacking CD31 and CD105. A quantitative analysis of tumor vessels examined by confocal microscopy after staining for CD31 and CD105 immunoreactivity revealed focal regions without detectable CD31 or CD105 immunoreactivity in 11% (226 vessel profiles) and 24% (353 vessels profiles) of vessels in the orthotopic and ectopic tumors, respectively. The remaining vessels had uniform CD31 and/or CD105 (CD31/CD105) immunoreactivity (Fig. 2A and B). This feature applied both to orthotopic and to ectopic tumors. Most blood vessels of both types of tumor were surrounded by multiple, irregular layers of basement membrane that extended well beyond the endothelium into the stroma (Fig. 2C). Electron microscopic studies revealed that endothelial cells of most tumor vessels were thin in regions other than the nucleus (Fig. 2D and E). Tumor cells were located close to the wall in most vessels (Fig. 2F).

Transmission electron microscopy analysis of regions lacking CD31 and CD105. A total of 46 tumor vessels with focal regions lacking detectable CD31/CD105 immunoreactivity (26 in six orthotopic tumors and 20 in three ectopic tumors) were examined sequentially by confocal microscopy and electron microscopy. Thin endothelial cells, some focally detached from the matrix, were found in 24 of the 26 vessels (92%) lacking detectable CD31/CD105 staining in orthotopic tumors. In only two vessels, representing 0.8% of vessel profiles examined in orthotopic tumors, did focal lack of CD31/CD105 immunoreactivity coincide with an ultrastructurally confirmed opening in the endothelium. The gaps ranged from 1.9 to 4.1 μm.

Among the 20 vessels in ectopic tumors with focal lack of detectable CD31/CD105 immunoreactivity, 14 were confirmed by TEM to have a luminal cell layer in these locations. In most instances, these cells were extremely thin endothelial cells. (Fig. 3A-F). In rare cases, the subcellular structure of the cell layer and limited resolution prevented classification of the cell (Fig. 3G-I).

The remaining six vessels from ectopic tumors (30% of the vessels with CD31/CD105-negative regions) had focal openings in the endothelium or regions where endothelial cells had separated from one another or were missing (Fig. 4).

Size and number of endothelial gaps in tumor vessels. We selected LR White for our combined confocal/TEM study.
because it allowed analysis of the same vessels by both

techniques. To supplement our TEM data, we completed

additional studies in which tissue was embedded in epoxy resin

to optimize preservation of cellular detail. Although it was not

possible to visualize immunofluorescence using this medium

(and thus, could not confirm lack of CD31/CD105 expression),

we were able to confirm the existence of the same endothelial

abnormalities seen in the LR White embedded tissue. In a

systematic TEM study of 100 sequential vessels in three

orthotopic tumors, half of the vessel profiles had regions of

thin endothelium (mean thickness, 0.4 µm; range, 0-2 µm)
immediately adjacent to tumor cells, separated by an average
distance of only 0.2 µm (range, 0.01-1.6 µm; Fig. 5A and B).

This analysis also revealed the presence of small gaps (mean size,
0.9 μm; range, 0.1-3.3 μm) between endothelial cells in 15% of vessel profiles (Fig. 5C-E).

In a separate study, vessels of ectopic tumors embedded in epoxy resin were examined and similar ultrastructural defects were identified, first in 1 μm toluidine blue sections and then by TEM. Of the 31 vessels identified, 39% had a complete endothelial lining and underlying basement membrane, 19% had a thin but complete endothelial cell lining and attenuated basement membrane with tumor cells close to the endothelium, and 42% had an abnormally tented or discontinuous endothelium (size range, 0.5-25 μm).

**Basement membrane of tumor vessels with CD31/CD105-negative regions.** Immunohistochemical staining showed that basement membrane was present in some CD31/CD105-negative regions (Fig. 6A), but was missing in most of these regions (Fig. 6B). Ultrastructural analysis showed similar basement membrane abnormalities at endothelial gaps (Figs. 5E and 6C). In order to assess basement membrane association in CD31/CD105-negative regions, we labeled type IV collagen and laminin (major basement membrane components). In orthotopic tumors, only 10% of vessels with intact CD31/CD105 staining had regions lacking immunoreactivity for one of the basement membrane proteins, but 83% of vessels with CD31/CD105-negative regions had sites lacking a basement membrane marker (Table 1). In ectopic tumors, all but 3% of vessels with intact CD31/CD105 had staining for the basement membrane markers, but 90% of vessels with CD31/CD105-negative regions had focal defects in basement membrane.

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**Figure 3.** Ultrastructure of vessels lacking detectable CD31/CD105 immunoreactivity. A (inset), tumor vessel in a toluidine blue–stained 1-μm section of an LR White-embedded tumor viewed by light microscopy. A and B, the same vessel in an adjacent 2 μm section viewed by confocal microscopy (red, CD31/CD105 immunoreactivity of endothelium; green, GFP-expressing tumor cells). Only the red channel of this vessel is shown in (B); bar, 20 μm in (A and B). C and D, detail of the boxed region at left in (B). C, TEM image from an 80 nm section adjacent to the one shown in (A and B). D, enhanced version of (C) to show the cancer cells (pale green) and the endothelium (blue). Note the endothelial layer is complete although there is lack of immunostaining in some regions (A and B). E, detail of the boxed region in (B, right). F, enhanced version of (E) to show the cancer cells (pale green) and the endothelium (blue). Note the endothelial layer and immunostain are both complete in this region. Bar, 5 μm (C-F). G, blood vessel in an orthotopic tumor with a region of endothelium lacking CD31/CD105 staining. G (inset), vessel in a 1 μm toluidine blue–stained section. Confocal microscopic image of the same vessel (red, CD31/CD105 immunoreactivity) surrounded by GFP expressing tumor cells (green). CD31/CD105 is partially missing in the boxed region. H and I, TEM image of the same tumor vessel shows the presence of cell processes in the region lacking CD31/CD105 staining. Here, the subcellular structure of the cell layer and the limit of resolution prevented clear determination of cell phenotype. Furthermore, the unstained cell processes in this region were not continuous with the recognizable regions of adjacent endothelium, and no overlapping or abutting cell junctions could be identified. I, diagram showing enhanced version of this image. CD31/CD105-positive endothelial cells are blue, tumor cells are pale green, and CD31/CD105-negative/GFP-negative cell processes are orange. Bar, 5 μm (G-I).
staining (Table 1). Two other basement membrane components, perlecan and nidogen, gave similar values for the proportion of vessels with basement membrane defects (data not shown). These findings suggest a possible link between the loss of CD31/CD105 immunoreactivity and focal disruption of the underlying vascular basement membrane.

Discussion

In a previous study, we reported that ~15% of blood vessels in human colon carcinoma xenografts have focal regions that lack immunoreactivity for the endothelial cell markers CD31 and CD105 (8). The present study of the same tumors extends this previous study by assessing whether CD31/CD105-negative regions represented missing endothelial cells or were, instead, defects in endothelial cell expression of these markers. When CD31/CD105-negative regions were examined by TEM in both orthotopic and ectopic tumors, we found an extremely thin endothelium closely apposed to tumor cells; surprisingly, the endothelium was generally continuous even in regions with no observed expression of common endothelial markers. In colon carcinoma xenografts implanted orthotopically, the majority (92%) of the mosaicism was explained by undetectable CD31 and CD105 expression rather than missing endothelial cells. However, in tumors implanted ectopically in the ovarian bursa, 30% of vessel profiles with CD31/CD105-negative regions had openings in the endothelium or missing endothelial cells. Most CD31/CD105-negative regions did not have detectable immunoreactivity for the basement membrane proteins type IV collagen or laminin.

Our approach of combining immunofluorescence, confocal microscopy, and TEM analysis allowed assessment of specific immunoreactivity of cell markers and tissue ultrastructure in the same vessel. The perfusion of antibodies conjugated with Cy-5 labels the endothelium of functional vessels expressing CD31 and/or CD105, whereas TEM provides high-resolution images of the cells forming the vessel wall. These two techniques were exploited to determine the ultrastructure of the walls of tumor vessels lacking both CD31 and CD105. The specific ultrastructural changes identified in tissue embedded in LR White (thin
endothelium, gaps, missing endothelial cells, basement membrane defects) were also validated in tissue prepared for conventional TEM (which is optimally preserved by using a high glutaraldehyde concentration and secondary fixation with osmium tetroxide techniques not used for embedding tissue in LR White).

Tumor vessels lack a hierarchical arrangement and have irregular diameters, high tortuosity, random branching, and defective endothelial barrier function (21, 22). Pericytes and basement membrane of tumor vessels are also abnormal (6, 7). These abnormalities result from the bizarre microenvironment in tumors, with growth factor under- or overproduction combined with hypoxia and metabolic disturbances. The present study addressed a peculiar abnormality in tumor vessels—focal absence of endothelial expression of CD31 and CD105—that is relevant to endothelial barrier function in tumors (8). The results confirmed the frequency of this abnormality, which affected 11% of vessels in orthotopic tumors and 24% in ectopic tumors, and demonstrate that defects in CD31/CD105 expression in tumor vessels are associated with multiple structural aberrations. It is possible that the focal hyperpermeability of tumor vessels could be caused by these defects in the endothelial lining and basement membrane (23). Although relatively rare, these hyperpermeable regions could significantly dissipate the transvascular hydrostatic pressure gradient necessary for efficient delivery of therapeutics (1, 10, 11).

Abnormalities in the endothelium may reflect the dynamic nature and continuous remodeling of tumor vessels undergoing angiogenesis, which can be accompanied by degradation of the vascular basement membrane and temporary loss of pericytes (24). Basement membrane degradation and emigration of pericytes are not, however, a consistent feature of tumors (1, 6, 7). Loss of CD31/CD105 expression could also be a response to inflammatory factors such as tumor necrosis factor-α, histamine, or vascular endothelial growth factor, which cause redistribution of junctional molecules to the lateral plasma membrane (14, 25) or down-regulate their expression (26, 27). It remains to be seen whether haphazard cooption of preexisting vessels (28) or high levels of protease activity in tumors (29) contribute to basement membrane degradation and endothelial defects.

**Figure 5.** Electron micrographs showing unusual features of blood vessels in orthotopic LS174T tumors. A and B, tumor vessel with thin endothelium closely apposed to tumor cells. Region in black box in (A) is shown at higher magnification in (B). B, long arrows mark both sides of the endothelial cell. Short arrows mark the surface of the closely adjacent tumor cell. C-E, examples of endothelial gaps (short arrows) in tumor vessels with otherwise continuous endothelium (long arrows). E, endothelial gap that exposes a continuous underlying basement membrane. Bar, 1.4 μm in (A); 0.4 μm in (B); 2.2 μm in (C); 1.2 μm in (D); and 0.8 μm in (E).
An unexpected finding in this study was the higher incidence of endothelial cell defects in ectopic tumors. This observation suggests that vessel wall abnormalities can be affected by the site of tumor growth, such as differences in host microenvironment, infiltrating blood vessels, or stromal cells (30, 31). Orthotopic tumors developed within the wall of the cecum and recruited blood vessels and stromal cells from neighboring tissues. By comparison, ectopic tumors, which were partially isolated by the Parafilm enclosure, recruited vessels and stromal cells from branches of the ovarian artery and vein. In addition, LS174T tumor cells grew more slowly in the orthotopic location. Perhaps the slower growth rate enabled tumor vessels to develop a more stable wall structure.

It is unclear whether the greater endothelial abnormalities in ectopic tumors would influence their response to angiogenesis inhibitors (32, 33). If any, the effect may be small because the endothelial cell defects occupy such a small proportion of the total vascular surface area in these tumors. It is known that ectopic tumors respond more favorably to low-dose metronomic chemotherapy (34–36). Similar vascular abnormalities have been identified from human tumor specimens (8), but the relevance to tumor growth in patients remains to be determined.

In summary, the present study sought to determine the cellular basis of focal lack of endothelial CD31 and CD105 expression in tumor blood vessels. We developed a novel method for labeling tumor vessels in vivo and then analyzing the same vessel by immunofluorescence confocal microscopy and transmission electron microscopy. The results confirmed our earlier suggestion (8) that the tumor environment induces altered expression of PECAM-1 (CD31) and Endoglin (CD105) in endothelial cells, causing the observed mosaicism. The most common finding was loss of detectable CD31/CD105 immunoreactivity despite the presence of ultrastructurally identifiable endothelial cells. The involved endothelial cells, however, usually were thin and closely apposed to tumor cells. Other abnormalities in the endothelium

Figure 6. Basement membrane defects in regions of endothelium lacking CD31/CD105 staining. A, confocal images of blood vessel in ectopic tumor with multiple sites lacking CD31/CD105 immunoreactivity. CD31/CD105 expression (white) of tumor vessel surrounded by GFP-expressing tumor cells (green). CD31/CD105 immunoreactivity is not detectable in at least two sites (arrow and arrowheads). B, another confocal image of the same vessel showing the basement membrane protein nidogen (red), which envelopes most of the vessel including one of the CD31/CD105 defects (arrowheads) but is not detectable at another (arrow). Note that some small structures seem to have the basement membrane protein nidogen whereas no CD31/CD105 is detectable; these are likely vessels that were not carrying blood at the time of CD31 injection. C, electron micrograph of a tumor vessel with two endothelial gaps (arrows). Basement membrane is attenuated and incomplete in the region of the upper gap. Bar, 0.5 μm (C).
in CD31/CD105-negative regions included endothelial detachment, openings between endothelial cells, and missing endothelial cells. Although basement membrane was present in most of the vessels in the LS174T tumors, 80% of the vessels with regions lacking detectable CD31/CD105 staining also lacked one or more basement membrane components. Further studies are needed to elucidate the mechanisms responsible for these abnormalities and to assess their progression.

Table 1. Defects in CD31/CD105 and basement membrane staining in blood vessels of LS174T tumors

<table>
<thead>
<tr>
<th>No. of vessels</th>
<th>Complete CD31/CD105 staining? % (no. of vessels)</th>
<th>Basement membrane staining (+/-)</th>
<th>% of vessels (no. of vessels)</th>
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<tr>
<td>Orthotopic tumors*</td>
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<td></td>
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<tr>
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<td>col IV–</td>
<td>86% (12)</td>
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<td>yes: 88% (104)</td>
<td>col IV+</td>
<td>14% (2)</td>
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<td>no: 9% (10)</td>
<td>col IV–</td>
<td>16% (17)</td>
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<td>col IV+</td>
<td>84% (87)</td>
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<tr>
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<td>lam–</td>
<td>80% (8)</td>
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<tr>
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<td>yes: 76% (63)</td>
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</table>

*Data from immunohistochemically stained sections of six orthotopic tumors and three ectopic tumors analyzed by confocal microscopy. col IV, collagen IV; lam, laminin. Similar results were obtained for nidogen and perlecan staining.

References

23. Hobbs SK, Mossay WL, Yuan F; et al. Regulation of transport pathways in tumor vessels: role of tumor type...
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