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Glomeruloid Structures as Vascular Reaction in Human Gastrointestinal Carcinoma

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Malignant tumors induce angiogenesis and modulation of microvasculature. Based on histologic and immunohistochemical analysis of human surgical material, we describe here the occurrence of glomeruloid structures in gastrointestinal carcinomas, and compare them with the microvasculature in inflammatory granulation tissue. The glomeruloid structures were composed of clusters of mutually fused capillaries with prominent swelling of endothelial cells and pericytes. They were thought to be specific for glioblastoma of the brain. The glomeruloid structures were observed juxtaposed to carcinoma nests in one-third of gastric carcinoma of intestinal type and colorectal carcinoma in the area of invasive growth beyond the muscularis mucosae. They were not observed in gastric carcinoma of diffuse type, intramucosal carcinoma, or inflammatory granulation tissue. The glomeruloid structures can be regarded as an extreme example of endothelial hyperplastic changes observed in cancer stroma. Our results suggested that glomeruloid structures can occur in carcinomas as vascular reaction, a mechanism different from that in inflammatory granulation tissues.

Key words: Carcinoma — Stromal reaction — Angiogenesis — Endothelial proliferation — Immunohistochemistry

Malignant tumors and inflammation are two major pathologic conditions associated with angiogenesis, a process of blood vessel proliferation from preexisting vessels. Angiogenesis is a key phenomenon for active proliferation of solid tumors.[1] Since all human tissues except cartilage and the cornea are vascularized, angiogenesis inevitably induces modulation of microvasculature. Swelling of endothelial cells is a feature of microvessels in the stroma of human tissues.[2,3] Microvasculature in cancer stroma is heterogenous in expression patterns of markers for endothelial cells and pericytes.[4] The glomeruloid structures are composed of a cluster of capillaries with prominent endothelial proliferation; they are known in glioblastoma multiforme and thought to be specific for this tumor. Indeed, occurrence of the glomeruloid structures is one of the histologic criteria of malignancy of glioma.[5] The question then arises; do these structures occur in other malignant tumors or not? In most carcinomas, desmoplasia takes place as another stromal reaction, and this makes the confirmation of microvascular changes on routine histologic slides quite difficult. Therefore, exact and reliable methods are needed to identify microvasculature in human surgical materials.

The present authors have already clarified that gastrointestinal carcinomas have totally different microvascular patterns from that in the normal tissue.[6–8] Occurrence of immature capillaries with marked endothelial hyperplastic changes was frequent. von Willebrand factor (vWF; formerly factor VIII related antigen) was abundantly labeled in vessels in cancer stroma when frozen sections were used. However, this immunoreactivity decreased significantly on routinely processed paraffin blocks. Laminin is a major component of the basal lamina, and its immunohistochemical detection in vascular endothelial cells and pericytes has been well documented.[9,10] We found that double digestion of specimens before immunostaining was effective for laminin staining using routinely processed, paraffin blocks, especially for immature vessels in cancer stroma. We examined muscle actin because of its usefulness for the identification of pericytes and smooth muscle cells of the blood vessels.[4] We also stained CD31, a recently-introduced endothelial cell marker.[11] Here, we describe for the first time the occurrence of glomeruloid structures in cancer stroma and we discuss the heterogeneity of microvascular patterns in different histologic types of cancers.

MATERIALS AND METHODS

Tissues Seventy-one successive cases of carcinoma of the stomach and large intestine were selected from the lists of surgical specimens of the Department of Pathology, Tohoku University School of Medicine and Tohoku Rosai Hospital (Table I). One representative block was used for each case. Invasive gastric carcinomas were classified into intestinal and diffuse types.[12,13] The intestinal type corresponded to tubular, papillary and well-differentiated mucinous adenocarcinomas, and the diffuse type to signet ring cell and poorly differentiated mucinous adenocarcinomas according to the WHO's classification.[13] Intestinal-type gastric carcinoma was basically

similar to colon carcinoma. Therefore, the two types are described together in the same group. The diffuse type was frequently characterized by diffuse proliferation of isolated carcinoma cells with pronounced deposition of interstitial collagen fibers (scirrhous stroma). In the present study, no mucinous carcinomas are included. Colon carcinomas were classified according to the WHO's classification.[14] Adenomas with severe dysplasia were regarded as carcinomas confined to the lamina propria. Inflammatory bowel disease and granulation tissue in gastric ulcers were used as controls for angiogenesis (18 cases). As another control, a case of glioblastoma multiforme was selected, which contained well-developed glomeruloid structures. Ten to twenty serial sections, 3 μm in thickness, were cut from each block.

Antibodies The first antibodies used are listed in Table II. All these antibodies were used for the identification of vascular structures in cancer stroma.

Immunohistochemistry After deparaffinization, specimens were immersed in methanol containing 0.3% hydrogen peroxide for 15 min. The specimens were treated in 0.1% trypsin (Sigma, type III)/Tris HCl buffer containing 0.1% $CaCl_2$ for 30 min. For laminin staining, the specimens were further treated in 0.4% pepsin (Sigma)/0.01 *N* HCl for 30 min at 37°C. After immersion in non-immunized goat serum, the specimens were incubated with the first antibodies (Table II). The specimens were washed in phosphate-buffered saline (PBS) three times after each incubation. Then, the biotin-streptavidin system (Histofine Kit, Nichirei, Tokyo) was used. The horseradish peroxidase reaction was carried out in 30 mg/100 ml diaminobenzidine containing 0.006% hydrogen peroxide and 0.065% sodium azide. Nuclear counterstaining was done with 2% methyl green. vWF was stained in all cases. Laminin and CD31 were stained in 66 and 12 cases, respectively.

Table I. Summary of the Results as Indicated by Number of Cases with Glomeruloid Structures/Total Number of Cases Examined by Serial Sections

Colon carcinoma		
depth of invasion		
mucosa (adenoma with severe atypia)	0/12	
submucosa	2/11	
muscularis propria or subserosa (adventitia)	3/10	
Stomach carcinoma		
depth of invasion	intestinal	diffuse
mucosa	0/10	0/4
submucosa	3/7	0/4
muscularis propria or subserosa	2/7	0/6
Granulation tissue		
gastric ulcer	0/6	
non-specific intestinal ulcer	0/2	
ulcerative colitis	0/9	
inflammatory fibroid polyp	0/1	

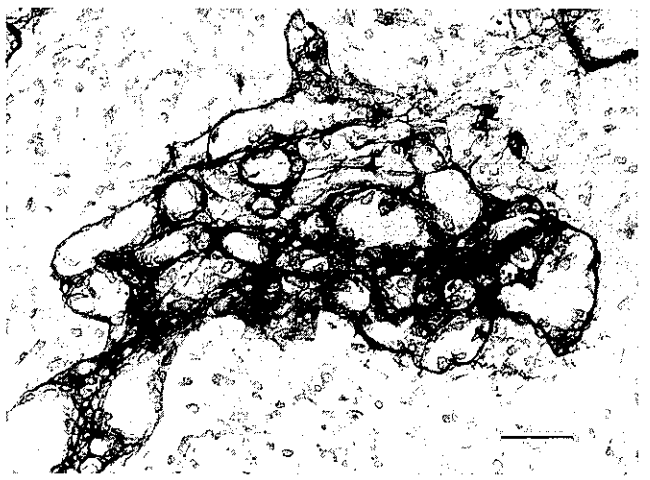
RESULTS

Identification of glomeruloid structures The glomeruloid structures were identified as clusters of capillaries, which were mutually fused with cellular proliferation. These vascular structures were essentially the same as those observed in glioblastoma multiforme (Fig. 1). In order to identify them, hematoxylin and eosin (H-E)-stained histologic sections were carefully searched for clusters of mesenchymal cells (Figs. 2A and 2B, arrow). When such clusters were found, immunoreactivity of the vascular structure was examined (vWF, laminin, muscle actin and CD31). Next, several serial sections were checked to

Table II. List of the First Antibodies Used

Name	Source	Working dilution incubation time	Pretreatment	Specificity
Anti-laminin (rabbit)	E-Y Lab. (San Mateo, CA)	1:300, 1 h	Trypsin+Pepsin[a)]	Basal lamina
Anti-von Willebrand factor (rabbit)	DAKO (Glostrup, Denmark)	1:300, overnight	Trypsin	Endothelial cells[b)]
Anti-muscle actin (mouse monoclonal)	Enzo (New York, NY)	1:3000, overnight	Trypsin	Pericytes, smooth muscle cells, myofibroblasts
Anti-CD31 (mouse monoclonal)	DAKO	1:40, overnight	Trypsin	Endothelial cells, some lymphocytes and macrophages

a) For details, see "Materials and Methods."
b) In cancer stroma, pericytes are also positive because of antigen deposition around them.[6]



exclude the possibility that they were tangentially sectioned vessels. vWF stained primarily endothelial cells (Figs. 2C and 2D). In cancer stroma, however, vWF can also be positive in pericytes because of deposition of the antigen around pericytes.[6] Laminin staining depicted the basal lamina of endothelial and pericytes (Fig. 3). CD31 stained the luminal surface of endothelial cells, but its reactivity was weak in hypertrophied endothelial cells, so it was not effective for this purpose (data not shown).

Fig. 1. A typical, large glomeruloid structure in a case of glioblastoma multiforme as revealed by laminin immunohistochemistry. ×180. Scale bar=50 μm.

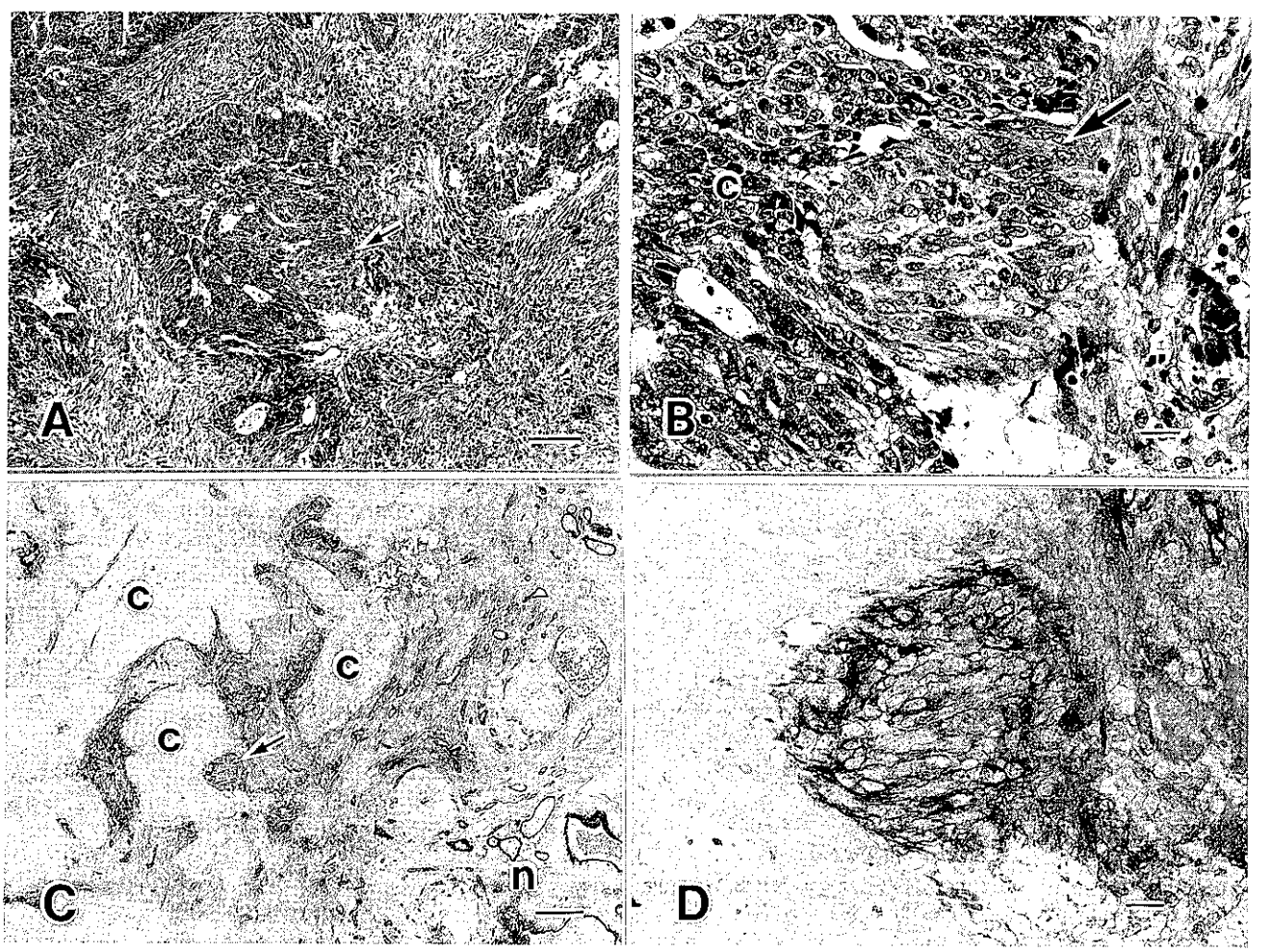


Fig. 2. Glomeruloid structures (indicated by arrows) in colon carcinoma. A: Low magnification. ×70. Scale bar=100 μm. B: Higher magnification of the glomeruloid structures in Fig. 2A. ×270. Scale bar=20 μm. C: vWF staining in a serial section. vWF is irregularly positive around carcinoma cells (c). Note well organized pattern of vWF in neighboring normal tissue (n). ×30. Scale bar=200 μm. D: Higher magnification of Fig. 2C, corresponding to Fig. 2B. ×270. Scale bar=20 μm.

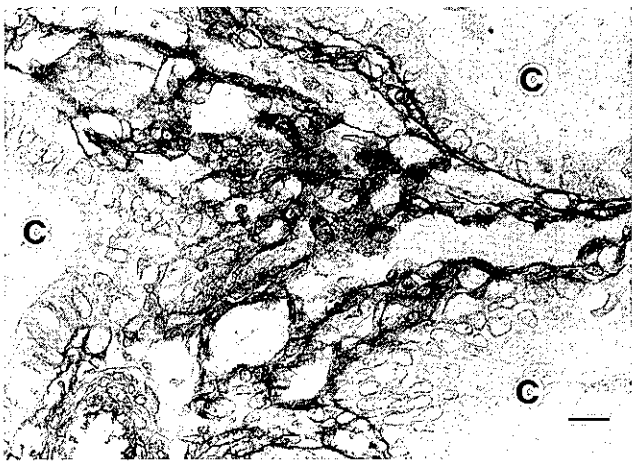


Fig. 3. Glomeruloid structures in intestinal-type gastric carcinoma as revealed by laminin immunohistochemistry. This vascular structure is surrounded by cancer cells (c). ×280. Scale bar=20 μm.

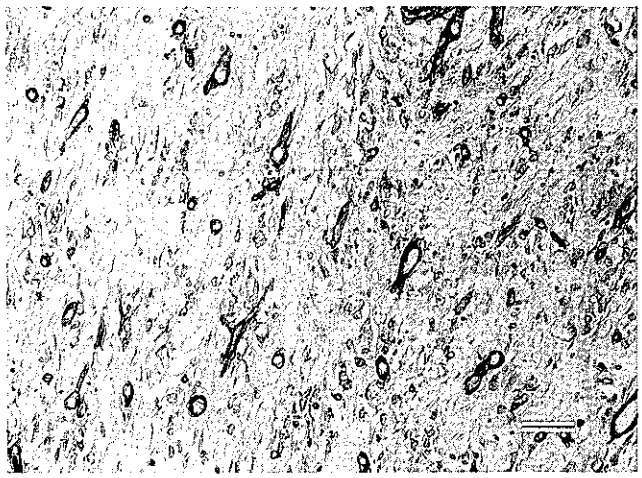


Fig. 5. Laminin staining in diffuse-type gastric carcinoma. Capillaries are homogeneously distributed without glomeruloid structures. ×135. Scale bar=50 μm.

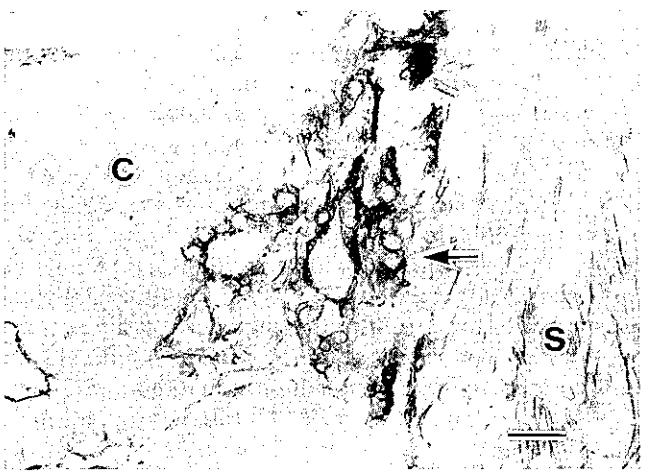


Fig. 4. Glomeruloid structures in colon carcinoma as revealed by actin immunostaining (arrow). c = cancer cells. s = stroma. ×370. Scale bar=20 μm.

Actin was positive in proliferated pericytes, but diffuse reactivity in the stromal fibroblasts (myofibroblasts) hindered detection of this reactivity in pericytes (Fig. 4). Of these markers, laminin and vWF staining were most effective. The results in each lesion are summarized in Table I. The diameter of the glomeruloid structures in gastrointestinal carcinomas was usually smaller than those in glioblastoma, being in the range of 80–160 μm.

Invasive gastrointestinal carcinoma Colonic adenocarcinoma and gastric carcinoma of intestinal-type had similar microvascular patterns in the stroma. The distribution pattern was largely influenced by carcinoma nests, and vessels were frequently more densely distributed around carcinoma nests (Fig. 2C). Swelling and increase of endothelial cells were prominent, rendering the lumen narrow (Fig. 2B). The glomeruloid structures were detected in 10 of 35 cases of this group, being sporadically distributed in cancer stroma. They were always juxtaposed (in close contact) to carcinoma nests, and were never found remote from cancer cells. There were no specific histological changes between cases with and without the glomeruloid structures. In diffuse-type gastric carcinoma, the distribution of microvessels was uniform (Fig. 5). Swelling of endothelial cells and narrowing of the lumen were not prominent. The glomeruloid structures were not observed in this group. In cancer stroma, mere proliferation of endothelial cells without formation of vascular structure has not been discovered (see our previous reports[6-8]).

Intramucosal carcinoma of the stomach and colon Neither intramucosal carcinomas nor intramucosal carcinomatous component of submucosal invasive carcinoma contained the glomeruloid structures. Patency of the capillary lumen was well preserved (Figs. 6A and 6B).

Inflammatory lesions and granulation tissues Increase of blood vessels was observed in granulation tissues of gastric and intestinal ulcers, and in active inflammatory areas in ulcerative colitis (Fig. 7). Proliferated vessels were separated from neighboring vessels and no glomeruloid structures were observed in this group.

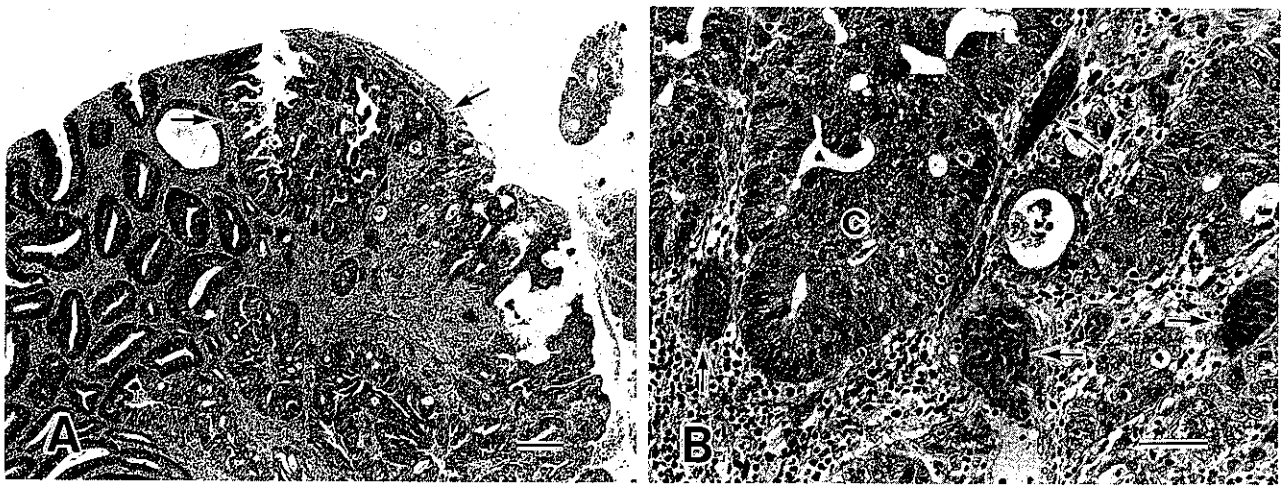


Fig. 6. A: Low magnification of colon carcinoma (the same case as in Fig. 2A). Arrows indicate carcinoma cells located in the lamina propria. ×30. Scale bar=200 μm. B: Higher magnification in the area indicated by the arrows in Fig. 6A. Most of the capillaries and venules had a wide, patent lumen and endothelial swelling was not prominent (arrows). No glomeruloid structures were present in carcinomatous lesions in the lamina propria. ×180. Scale bar=50 μm.

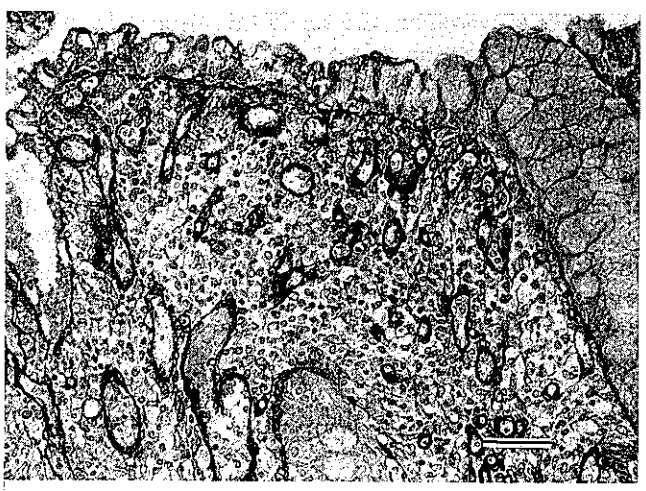


Fig. 7. Laminin staining in a case of ulcerative colitis. ×180. Scale bar=50 μm.

DISCUSSION

The present paper is the first to identify glomeruloid structures in carcinoma tissues except brain tumors. They were found in one-third of colorectal and intestinal-type gastric carcinomas when carcinoma cells invaded beyond the muscularis mucosae. They were not induced in inflammatory lesions or in diffuse-type gastric carcinomas, which had scirrhous stroma.

Similar mutually fused vascular structures were reported in experimental carcinoma.[15] The glomeruloid structures were always juxtaposed to carcinoma nests in differentiated-type adenocarcinoma and were not observed in inflammatory lesions or granulation tissues. This suggests that glomeruloid structures are specific for the host reaction to a certain type of carcinoma. Furthermore, occurrence of the glomeruloid structures can be regarded as an extreme example of endothelial hyperplastic changes, because endothelial hyperplastic changes were prominent in gastric carcinoma of intestinal type and colon carcinoma, but not so remarkable in gastric carcinoma of diffuse type.[6-8] Ultrastructural localization of vWF in endothelial cells was also different between the two subtypes of gastric carcinoma; vWF is present in well-developed rough endoplasmic reticulum in intestinal type and in Weibel-Palade bodies in diffuse type.[8] The present and previous data indicate that histological type of carcinomas can determine the vascular pattern and suggest the heterogeneity of angiogenic stimuli between different histologic types. The two subtypes of gastric carcinomas differ not only in histologic features but also in biologic behavior. Diffuse type tends to spread via lymphatic vessels and the serosal surface, and intestinal type hematogenously to the liver or lungs.[16] Our study[8] confirmed that the difference between the two types includes the stromal changes. We had already suspected that rarity of central necrosis in diffuse-type carcinoma may be related to the uniform distribution of capillaries in their stroma.[8] Furthermore,

intramucosal carcinoma did not induce glomeruloid structures. This was best demonstrated in the intramucosal carcinomatous component of submucosal invasive carcinoma, where neither glomeruloid structures nor desmoplastic reaction was observed. This occurrence pattern is the same as that of "solid capillary buds" in cancer stroma, another vascular reaction to cancer growth.[7] This suggests that the microenvironment in the lamina propria mucosae is important to determine angiogenesis. Absence of glomeruloid structures or desmoplasia was confirmed in more than 50 cases of intramucosal carcinomas of the stomach on H-E-stained slides (unpublished observation). The present study disclosed no specific differences between invasive adenocarcinomas of differentiated type with and without the glomeruloid structures. Clarification of the clinicopathological significance of the glomeruloid structures will be a focus of future studies.

Endothelial cells of the glomeruloid structures in glioblastoma multiform express mRNAs for platelet-derived growth factor (PDGF) A and B chains and PDGF β receptor, suggesting an autocrine growth loop in endothelial cells.[17, 18] Namely, endothelial cells stimulated by tumor cells produce PDGF to upregulate their own growth. This hypothesis is attractive since a similar mechanism may be involved in prominent proliferation of endothelial cells and pericytes in our cases. In fact, PDGF-β receptor is immunohistochemically overexpressed in proliferated vessels in differentiated-type adenocarcinomas of the colon (our unpublished data).

Recently several reports have described active contributions of stromal cells in carcinoma tissue, i.e., stromelysin III in stromal cells in breast cancer,[19] urokinase-type plasminogen activator (u-PA) in fibroblastic cells in colon carcinoma,[20] uPA inhibitor in endothelial cells in colon carcinoma[21] and collagenase in stromal cells of colon carcinoma.[22] These reports clearly showed that stromal cells are involved in the degradation of extracellular matrix components, suggesting the importance of stromal cell reaction to carcinoma growth. Furthermore, c-*ets 1* proto-oncogene is expressed in endothelial cells in tumor stroma and granulation tissue,[23] in which transcription of u-PA, stromelysin, and collagenase genes is stimulated. Therefore, the biologic behavior of cancer should be assessed by considering not only carcinoma cells but also stromal cells activated by cancer cells.

In recent years, much attention has been focused on the function of the adhesion molecules in the immune reaction during inflammation. It has been shown that endothelial cells actively participate in recruiting lymphocytes, monocytes and granulocytes during inflammation by inducing cell adhesion molecules.[24, 25] We have already demonstrated the morphologic activation of endothelial cells and induction of endothelial leukocyte adhesion molecule-1 (ELAM-1; E-selectin) in inflammatory bowel disease.[26] In inflammatory lesions, endothelial cells are not simply induced to proliferate but are phenotypically activated. This change is reasonable considering the pathophysiology of the acute inflammation. However, little is known about the phenotypical changes in endothelial cells in human solid tumors except for CD34[27] or vWF.[6, 8, 28] Why do endothelial cells abundantly proliferate? Are they simply reactive to tumor growth for increased blood supply? Growth factors for mesenchymal cells, including transforming growth factor-β, PDGF, and basic and acidic fibroblast growth factors, may be involved in inducing these vessels. Further studies will be required to elucidate the function and mechanism of induction of microvasculature of cancer stroma, because control of growth will be therapeutically important.[1]

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