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Case report

Epithelioid angiosarcoma of the intestinal tract with endothelin-1-like immunoreactivity

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Abstract. Two rare cases of intestinal epithelioid angiosarcoma arising in the sigmoid colon and small intestine are reported. The small intestinal tumours were located in the duodenum and upper jejunum. All tumours showed reddish-black protuberant masses with comparatively clear margins. Histology reveals solid and epithelioid growth of large polygonal cells, mimicking undifferentiated carcinoma. Vascular differentiation such as lumina containing red cells and intracytoplasmic vacuolization is noticed. Intense immunoreactivity to *Ulex europaeus* agglutinin I and JC70 (CD31), and sporadic positive reaction to factor VIII related antigen were detected in one case but not in the other. However, both tumours have cells which show intense endothelin-1 immunoreactivity. Positive immunostaining occurred with low molecular weight keratins suggesting epithelial differentiation and suggested epithelioid angiosarcoma as a diagnosis, a specific entity within angiosarcoma. Because of their histological features, epithelioid angiosarcomas may be confused with undifferentiated carcinomas. Endothelin-1-like immunoreactivity seems to be a good marker for this type of angiosarcoma, even when the tumour cells lack usual marker substances for endothelial cells.

Key words: Angiosarcoma – Intestinal tract – JC70 – Endothelin – Epithelioid angiosarcoma

Introduction

Although angiosarcomas are not infrequently encountered in the skin, breast, bone and retroperitoneum, the same tumour is uncommon in the gastrointestinal tract and only a few cases have been reported (Ordonez et al. 1983; Saito et al. 1987; Smith et al. 1990; Taxy and Battifora 1988) although a number have been reviewed (Barclay and Schapira 1983; Grain et al. 1976; Ormos and Sin 1959; Ostermiller et al. 1966; Steiner and Palmer 1949; Waterhouse et al. 1981; Wilson et al. 1974). Their histological features are extremely variable and range from a well-differentiated type associated with rich vessel-like structures to undifferentiated types composed of sheet-like proliferation of anaplastic epithelioid cells. When the undifferentiated type of angiosarcoma arises in the gastrointestinal tract, it may be confused with undifferentiated carcinoma or poorly differentiated adenocarcinoma. The very low reported incidence of angiosarcoma of gastrointestinal tract might be due to this confusion. We report this neoplasm arising in the sigmoid colon and small intestine.

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Case reports

Case 1. A 70-year-old woman was admitted to the hospital because of a large amount of sudden anal bleeding. A solitary haemorrhagic protuberant tumour was found by endoscopic examination. On the day of admission, sigmoid colectomy was performed. At operation, there were no regional lymph nodal or visceral metastases. The post-operative course has been uneventful for 2 years.

Case 2. A 64-year-old man was admitted to hospital with a complaint of persistent gastrointestinal bleeding. Radiographic examination revealed two discrete tumours in the duodenum and upper jejunum. Partial duodeno-jejunectomy was carried out. He died of pulmonary metastasis a year after operation. Permission for autopsy was not granted.

Materials and methods

Excised specimens obtained at surgery were fixed in neutral formalin, and embedded in paraffin wax. The sections were cut at 3 μm and stained with haematoxylin-eosin, periodic-acid Schiff (PAS), alcian blue, and silver impregnation for reticulin.

The immunohistochemical studies using biotin-streptavidin-peroxidase complex method were carried out on paraffin-embedded sections. The primary antibodies or reagents applied and their working dilutions were: epithelial antigen (Dakopatts, 1:50), carcinoembryonic antigen (CEA, Dakopatts, 1:50), epithelial mem-

brane antigen (EMA, BioGenex, 1:1), muscle actin (HHF35, Enzo Diagnostics, 1:8000), desmin (BioGenex, 1:1), vimentin (BioGenex, 1:1), S–100 protein (Dakopatts, 1:500), factor-VIII related antigen (FVIII, Dakopatts, 1:5000), *Ulex europaeus* agglutinin I (UEA–I, Dakopatts, 1:50; anti-UEA–I, 1:200), JC70 (CD31, Dakopatts, 1:200), keratins: AE1, AE3 (Chemical Credential, 1:200), 34BE12 (Dakopatts, 1:50), NCL–5D3 (Novocastra, 1:200), NCL–LL002 (Novocastra, 1:50).

Anti-endothelin 1 (Immunobiological Laboratory, Japan) was raised in rabbit by subcutaneous injections of synthetic peptide coupled with thyroglobulin (Sigma). Antiserum obtained was fractionated to IgG through protein A AvideGel (Bio Probe International) and specifically purified through antigen-bound, activated thiol AvideGel affinity chromatography (Bio Probe). The specificity of the antibody was checked by the enzyme linked immunosorbent assay (ELISA) method in which the primary and endothelin-related antigens (50 ng/well) including vasoactive intestinal contractor (Peptide Inst., Japan) was immobilized in ELISA plate. After incubation with the antibody, anti-rabbit IgG coupled with horseradish peroxidase was applied to the plate and the peroxidase reaction product was visualized by orthophenyline diamine (OPD) substrate (Sigma). Optical density was detected by BioRad immunoreader.

Pretreatment in 0.1% trypsin (Difco) for 15 min at 37° C was carried out before the application of the primary antibodies except for muscle actin, S–100 protein, vimentin and anti-endothelin-1. The reaction products were visualized by immersion of slides into Graham-Karnovsky solution (Graham and Karnovsky 1966). Finally, the nuclei were counterstained with haematoxylin or methyl green.

The primary endothelin-1 antibody pre-absorbed with 5 µg of the homologous antigen at 4° C overnight was used to confirm the specificity of immunohistochemistry.

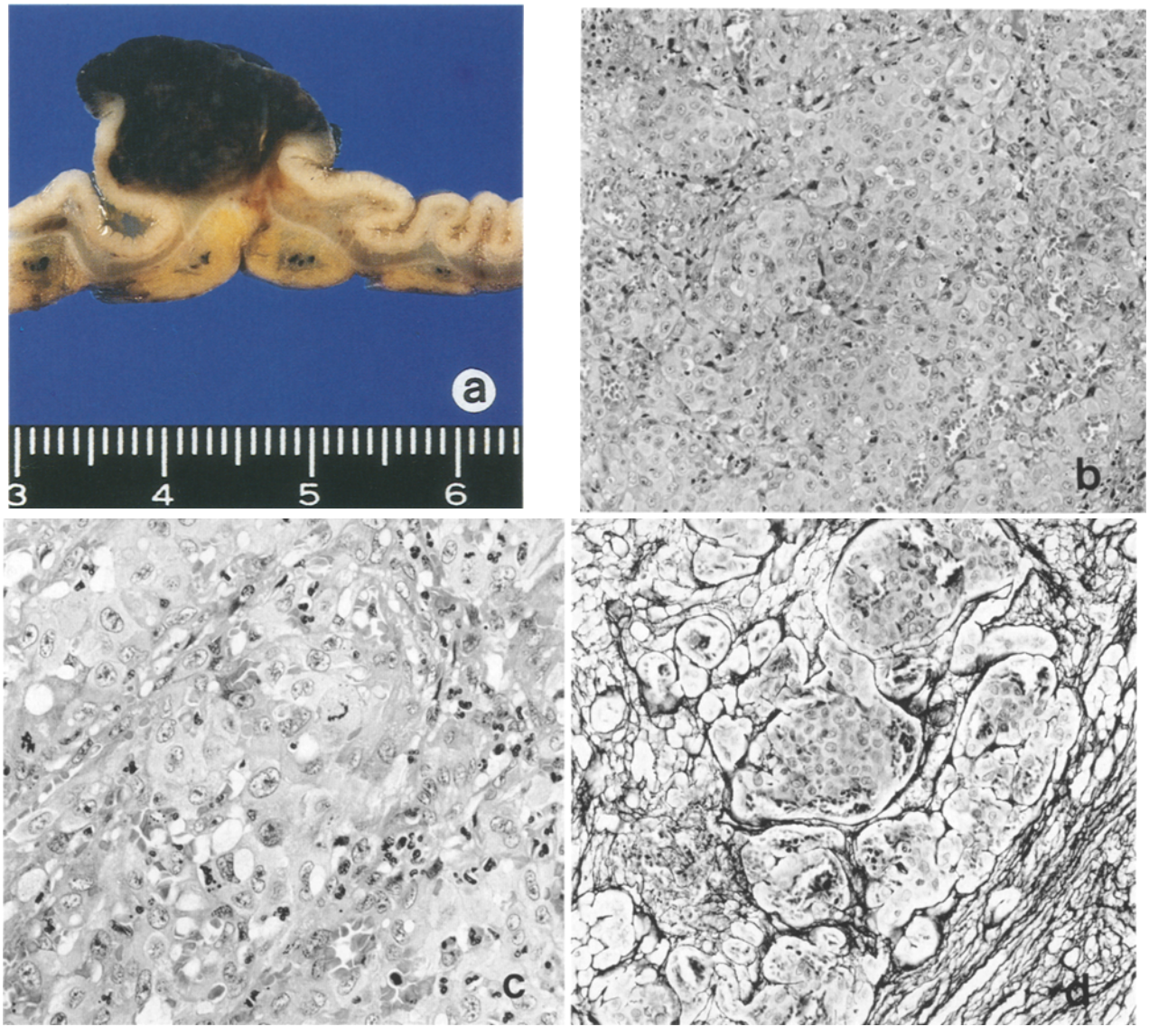

Fig. 1. Longitudinal section of the tumour in case 1, showing reddish–black protuberant mass with comparatively clear margin (**a**). Solid growth of plump epithelioid cells arranged in vague alveolar structures H.E. × 100 (**b**). High power view of the same tumour. Small lumina and intracytoplasmic vacuolization are seen. Numerous polymorphous leukocytes infiltrate in the stroma H.E. × 400 (**c**). Reticulin staining demonstrates alveolar structures with irregular vessel-like lumina × 150 (**d**)

Results

The tumour in case 1, mesured 17 mm in maximum diameter, protruded into the colonic lumen and showed dark reddish to black colour. The margins of the tumour were comparatively clear but not encapsulated and extended to proper muscle (Fig. 1a). The surface was ulcerated. Histologically the tumour was composed of polygonal or short spindle cells arranged in solid sheet and irregular alveolar structures (Fig. 1b). The cells had eosinophilic, plump cytoplasm and oval nuclei. Large intracytoplasmic vacuoles contained red cells or fibrin clots and erythrophagocytosis was not uncommon (Fig. 1c). Numerous mitotic figures were seen. Tumour cells were not stained with both PAS or alcian blue stains. Reticulin preparation revealed both minute vessel-like channels and conspicuous epithelioid structures, some of which had central lumens filled with blood cells (Fig. 1d). In the stroma, marked haemorrhage associated with inflammatory cell infiltration was noted (Fig. 1b, c). Immunohistochemically tumour cells were intensely positive for UEA–I (Fig. 2a), vimentin (Fig. 2b), JC70 and were also stained with anti-endothelin-1 (Fig. 2c). Only a few cells were labelled by FVIII.

The two tumours in case 2 measured about 30 mm and 40 mm in maximum diameter, showed pedunculated protuberant masses with ulceration and were dark reddish to black in colour, similar to those in case 1. Histologically they consisted of a solid growth of large pleomorphic and polygonal cells with ample cytoplasm. These cells were chiefly arranged in vague epithelioid nests. In some parts, alveolar structures resembling blood channels were prominent (Fig. 3a). Erythrophagocytosis and intracytoplasmic vacuolization, indicative of primitive blood vessels, were noted. PAS and alcian blue staining did not show positivity in tumour cells. Silver impregnation demonstrated small epithelioid reticulin networks associated with central vessel-like lumina (Fig. 3b). Immunohistochemically tumour cells were diffusely and intensely stained for vimentin (Fig. 3c) and anti-endothelin-1 (Fig. 3d).

In the anti-keratin antibody series, tumours of both cases showed strong positivity for AE1 (Fig. 2d) and NCL–5D3, both of which were expected to react with

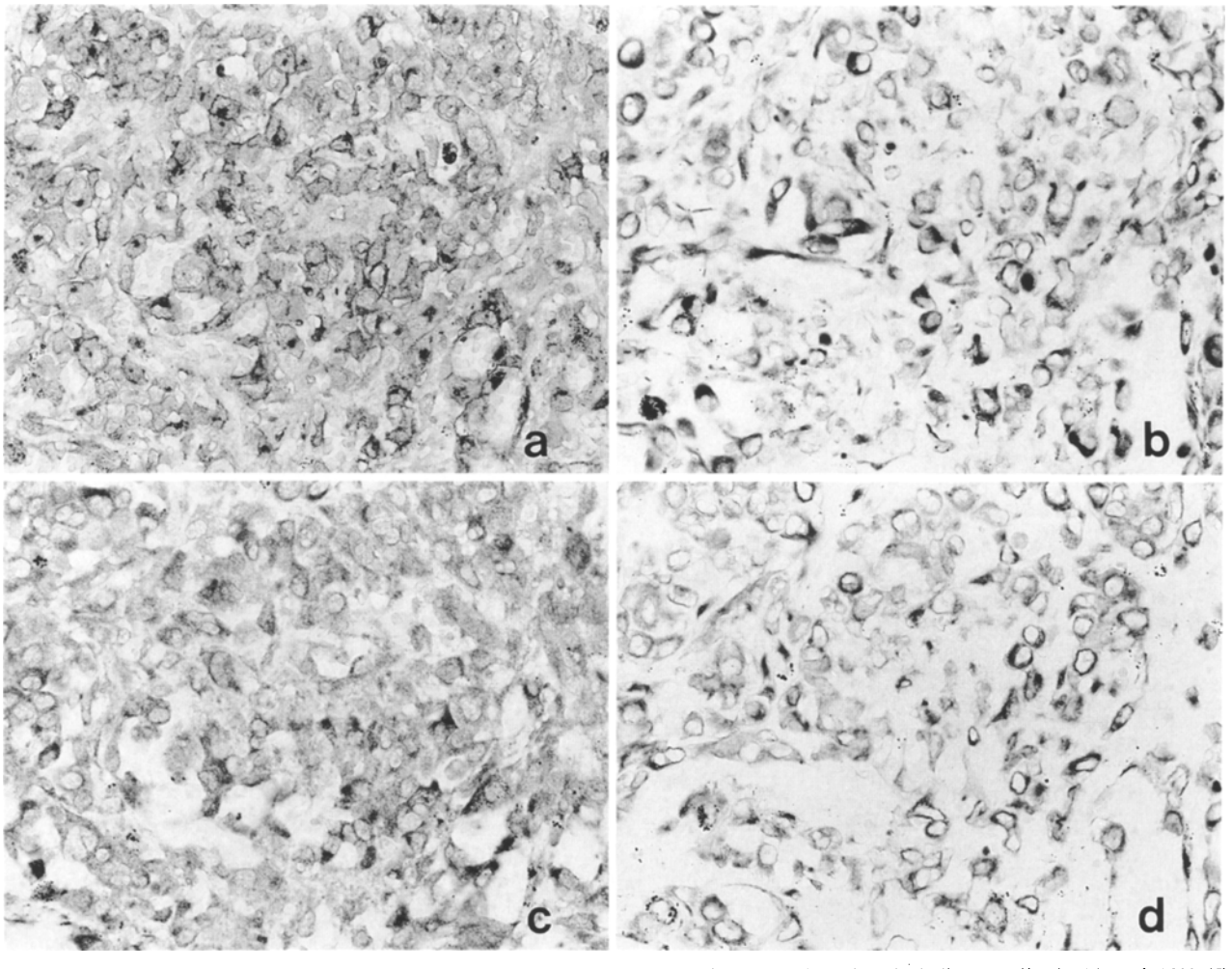

Fig. 2. Intense positive staining of tumour cells of case 1 for UEA–I (**a**), vimentin (**b**) anti-endothelin-1 antibody (**c**) and AE1 (**d**). ×300. UEA–I mainly reacts with cellular membrane and the other antibodies immunostained the cellular cytoplasm (biotin-streptavidin-peroxidase complex method)

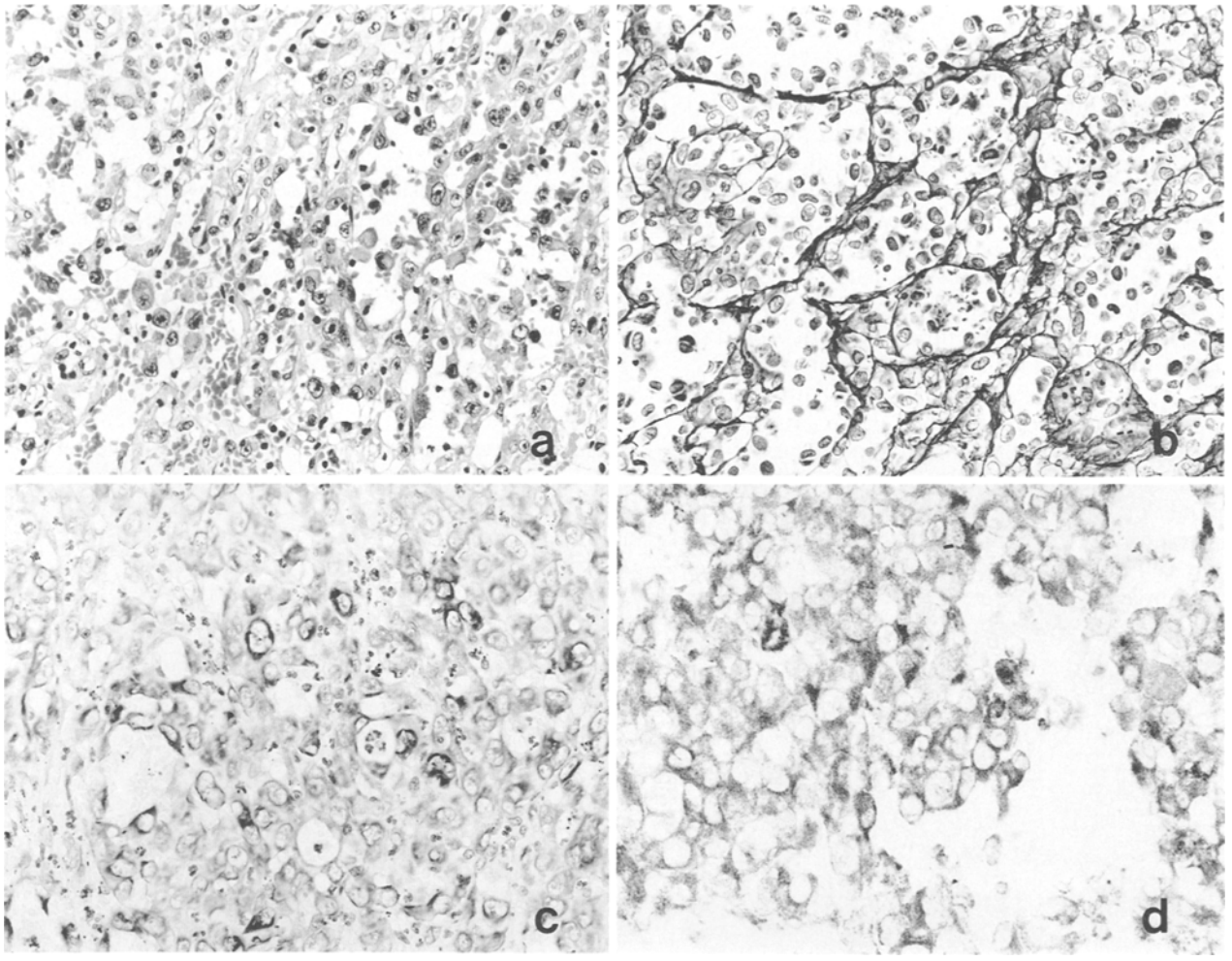

Fig. 3. Case 2. Alveolar structures loosely lined by the pleomorphic tumour cells, resembling the blood channels H.E. × 150 (**a**). Silver impregnation exhibits the characteristic reticulin networks × 150 (**b**). Immunopositive staining of tumour cells for vimentin × 200 (**c**) and anti-endothelin-1 × 300 (**d**). (**c, d:** biotin-streptavidin-peroxidase complex method)

low molecular keratins. However, AE3, 34BE12 and NCL–LL002 recognizing high molecular keratin did not show immunopositivity for tumour cells.

The other antibodies applied (epithelial antigen, CEA, EMA, muscle actin, desmin, S–100 protein) were all negative in both cells of both tumours.

Anti-endothelin-1 preabsorbed with the homologous antigen completely lost its immunoreactivity with tumour cells.

Discussion

Epithelioid angiosarcoma has characteristic morphological and immunohistochemical features and is a specific entity within the angiosarcomas (Eusebi et al. 1990; Fletcher et al. 1991). Fletcher et al. (1991) reported eight cases of epithelioid angiosarcoma arising in deep soft tissue. Their tumours were composed of eosinophilic, large epithelioid cells arranged in diffuse sheets. Intracellular vacuoles, some of which contained red blood cells were frequently noticed. Both endothelial and epithelial differentiation of tumour cells was demonstrated immunohistochemically. The same tumour, arising in the gastrointestinal tract is extremely rare and seems not to have been reported in the literature. In our cases, tumour cells having large eosinophilic cytoplasm showed sheet-like epithelioid arrangements and intracellular vacuoles with red blood cells, changes very similar to those in reported cases. Silver impregnation also revealed the vasoformative nature of the present tumours. Immunocytochemistry demonstrated many positive cells for low molecular keratins, AE1 and NCL–5D3, as observed in epithelioid angiosarcoma of soft tissues (Fletcher et al. 1991) and thyroid (Eusebi et al. 1990).

Because of the characteristic epithelioid arrangement, epithelioid angiosarcoma must be differentiated from primary and metastatic carcinoma, and many sarcomas with epithelioid features. In the gastrointestinal tract, carcinoma is the most important problem in differential diagnosis but the distinction between these two tumours is possile.

Ordinary angiosarcoma of the gastrointestinal tract is also extremely rare, and only six cases in which clinical

Table 1. Summary of the cases of gastrointestinal angiosarcomas

Year of report	Author	Age	Sex	Symptoms	Site of tumour	Size	Clinical course
1983	Nelson G. et al.	80	female	Anaemia	Jejunum (two masses)	5 cm 2 cm	Died of liver metastasis
1987	Saito R. et al.	72	male	Metastasis to cervical LN	d. Colon	10 cm	Died of peritoneal dissemination
1988	Taxy JB. et al.	86	male	Epigastric pain	Stomach	7 cm	Alive for 4 months
		64	male	Bleeding	Small intestine (two masses)	–	Died of dissemination
		57	female		a. Colon	6 cm	Alive for 4 months
1990	Smith JA. et al.	16	female	Abdominal pain	s. Colon	5.5 cm	Alive for 3 years
1993	Present cases	70	female	Bleeding	s. Colon	2 cm	Alive for a year
		64	male	Bleeding	Doudenum Jejunum	3 cm 4 cm	Died of lung metastasis

d, Descending; a, ascending; s, sigmoid

and pathological details were mentioned have been reported in the literature (Ordonez et al. 1983; Saito et al. 1987; Smith et al. 1990; Taxy and Battifora 1988) (Table 1), except for some series of rather old cases (Barclay and Schapira 1983; Grain et al. 1976; Ormos and Sin 1959; Ostermiller et al. 1966; Steiner and Palmer 1949; Waterhouse et al. 1981; Wilson et al. 1974). Angiosarcoma of gastrointestinal tract including epithelioid type is thought to be a distinct and characteristic clinicopathological entity. As summarized in Table 1, these tumours mainly occur in intestine of older patients, without a sex predilection. It is noticeable that three of eight cases listed in Table 1 have multiple tumours, all of which were located in small intestine (Ordonez et al. 1983; Taxy and Battifora 1988). Clinically gastrointestinal bleeding is not infrequently observed because of mucosal ulceration and the prognosis is poor compared with ordinary gastrointestinal carcinomas, since five of eight cases died within 1 year after tumour resection, due to metastases or peritoneal dissemination. The longest survival period after operation is 3 years (alive at the time of report) (Smith et al. 1990). Silver impregnation was helpful in demonstrating vasoformative reticulin networks.

Immunohistochemical staining sometimes gives useful information on the cellular nature in malignant vascular tumours. Tumour cells in our two cases were intensely immunostained with vimentin. Although the diagnostic value of vimentin is limited because of its non-specific expression in many mesenchymal tumours and even epithelial ones, the diffuse and intense immunostaining of vimentin might be one of the characteristic features of the gastrointestinal epithelioid angiosarcoma. The FVIII antibody is used to detect endothelial cell of both blood vessels and lymphatics. FVIII is, however, thought not to be sufficiently precise in the histological diagnosis of undifferentiated malignant vascular tumours because of its rather low sensitivity and lack of reproducibility (Ordonez and Batsakis 1984; Parums et al. 1990), particularly in the poorly differentiated type (Fletcher et al. 1991). *U. europaeus* agglutinin I (UEA-1) and JC70 (CD31) are known to show higher sensitivity for vascular endothelial cells (Parums et al. 1990). In our case, these two antibodies reacted with tumour cells much more than FVIII.

Endothelin, now redesignated endothelin-1, is a polypeptide produced by vascular endothelial cells and causes hypertension through contraction of vascular smooth muscle cells (Yanagisawa et al. 1988). Immunocytochemically endothelin has been demonstrated in several organs, including normal kidney (Kitamura et al. 1989), liver and lung (Rozengurt et al. 1990). In addition, squamous cell carcinoma and adenocarcinoma are exclusively immunostained among pulmonary malignancies (Giaid et al. 1990). Tumour cells in our cases were positively labelled by anti-endothelin-1 antibody, as in the two hypertensive cases with endothelin-secreting malignant haemangioendothelioma reported by Yokokawa et al. 1991. Hypertension was absent in our two cases, but endothelin-1 immunoreactivity seems to be a good marker of this subset of angiosarcomas.

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