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Small cell carcinoma of the stomach: An immunohistochemical and electron microscopic study

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Summary. A 4 x 6 cm ulcerative mass in the antrum was found to consist of papillary adenocarcinoma in the surrounding wall and the small round cell neoplasm at its base. Immunohistochemical staining revealed that elements of the papillary adenocarcinoma were positive for carcinoembryonic antigen, epithelial membrane antigen, keratin, endocrine granule constituent, and CA19-9, while components of the small cell carcinoma were weakly positive only for neuron-specific enolase. In one portion of the small cell carcinoma, particularly large cells with pleomorphic nuclei which were intensely positive for desmin were detected. Electron microscopic examination revealed dense-cored granules and intercellular junctions in the small neoplastic cells and bundles of intermediate filaments in the desmin-positive large cells. These findings suggest that ultrastructural examination is vital in diagnosis of small cell carcinoma and they reveal the capability of this carcinoma toward multidirectional differentiation.

Key words: Small cell carcinoma, Electron microscopy, Dense-cored granule, Desmin, Stomach

Introduction

Extrapulmonary small cell carcinoma is reported to have been detected in various organs (Ibrahim et al., 1984). However, small cell carcinoma of the stomach appears to be extremely rare. Matsusaka et al. (1976) reported only two in their review of about 2000 cases of gastric carcinoma, and only seven other cases have been reported in the literature (Chejfec and Gould, 1977; Eimoto and Hayakawa, 1980; Fer et al., 1981; Ibrahim et al., 1984; Fukuda et al., 1988; Hussein et al., 1990). We herein describe a case of gastric small cell carcinoma with papillary glandular differentiation and review cases reported in the literature.

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Materials and methods

Case report

A 68-year-old woman was admitted to hospital because of upper abdominal pain. She had not noticed weight loss or fever. The laboratory test data were within normal limits, except for slight anaemia. A barium enema of the upper alimentary tract and gastroscopic examination indicated gastric cancer. Biopsy specimens from the tumor confirmed well-differentiated tubular adenocarcinoma. A total gastrectomy with dissection of regional lymph nodes was performed. Despite extensive examination, including chest X-ray and CT scan, no other potential primary lesion was found.

Light microscopy

For microscopic studies, tissues were fixed in 10% formalin and embedded in paraffin. Four μm sections were prepared for staining with haematoxylin-eosin, periodic acid-Schiff (PAS), Grimelius, and Fontana-Masson stains, as well as for immunohistochemical examination.

Immunohistochemical staining was carried out by the peroxidase-antiperoxidase (PAP) method with a PAP kit (DAKO JAPAN, Kyoto, Japan). Preparations were treated with 0.3% hydrogen peroxide in absolute methanol for 30 min to block endogenous peroxidase. After treatment with normal rabbit or swine serum, the preparations were incubated with the primary antibodies for 30 min, and then incubated sequentially with the second antibodies for 30 min, and finally with the PAP complex for 30 min. The peroxidase reaction was developed with 0.06% 3,3'-diaminobenzidine tetrahydrochloride in 0.05M Tris buffer (pH 7.7) containing 0.03% hydrogen peroxide. Methyl green was used for counterstaining. Sources and dilutions of the primary antibodies were as follows: antisera to carcinoembryonic antigen (CEA, 1/150, Kyowa

Medex, Tokyo, Japan); alpha-1-fetoprotein (1/5000, DAKO JAPAN); human chorionic gonadotropin (1/500, DAKO JAPAN); prostate-specific antigen (1/500, DAKO JAPAN); epithelial membrane antigen (EMA, 1/100, DAKO JAPAN); keratin (KL-1, 1/100, Immunotech, Marseille, France); cytokeratin (1/500, DAKO JAPAN); cytokeratin associated with nonsquamous epithelium (1/100, Lipshaw, Detroit, MI, USA); cytokeratin associated with squamous epithelium (1/100, Lipshaw); vimentin (1/5, DAKO JAPAN); S-100 (1/500, DAKO JAPAN); desmin (1/100, DAKO JAPAN); myoglobin (1/1000, DAKO JAPAN); factor-VIII (1/200, DAKO JAPAN); CA19-9 (1/10, Centocor, Malvern, PA, USA); CA125 (no dilution, Centocor); neuron-specific enolase (NSE, 1/300, DAKO JAPAN); Leu 7 (1/30, Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA); leukocyte common antigen (1/200, DAKO JAPAN); MT-1 (1/5, Bio-science, Emmenbrucke, Switzerland), MB-1 (1/5, Bio-science); Leu M1 (1/100, Becton Dickinson Immunocytometry Systems); 4KB5 (1/25, DAKO JAPAN), UCHL-1 (1/50, DAKO JAPAN); MAC 387 (1/100, DAKO JAPAN); Ki-1 (1/20, DAKO JAPAN); L26 (1/100, DAKO JAPAN); secretory component (1/80, DAKO JAPAN); endocrine granule constituent (EGC, no dilution, MILAB, Malmo, Sweden); alpha-1-antichymotrypsin (1/2000, DAKO JAPAN); alpha-1-antitrypsin (1/2000, DAKO JAPAN); growth hormone (no dilution, Bio Genex); thyroid stimulating hormone (no dilution, Bio Genex); antidiuretic hormone (no dilution, Bio Genex); follicle-stimulating hormone (no dilution, Bio Genex); luteinizing hormone (no dilution, Bio Genex); parathyroid hormone (1/300, MILAB); human placental lactogen (1/500, DAKO); placental alkaline phosphate (1/100, DAKO JAPAN); pancreatic polypeptide (1/500, MILAB); triiodothyronine (1/100, Cambridge Medical Diagnostics, Billerica, MA, USA); thyroxine (1/100, Cambridge Medical Diagnostics); thyroglobulin (no dilution, Nichirei, Tokyo, Japan); calcitonin (no dilution, Lipshaw); serotonin (no dilution, Lipshaw); vasoactive intestinal polypeptide (1/100, Immuno Biological Laboratories, Fujioka, Gunma, Japan); somatostatin (no dilution, Nichirei); insulin (1/5, Nichirei); gastrin (1/100, Medical and Biological Laboratories, Nagoya, Aichi, Japan); and glucagon (1/500, Linco Research, St. Louis, MO, USA).

Electron microscopy

For electron microscopic studies, specimens fixed in formalin were cut into 1 mm cubes and fixed in 2% glutaraldehyde in a phosphate buffer. The specimens were then washed with the buffer solution, postfixed in 2% OsO_4 in phosphate buffer, dehydrated, and embedded in Quetol 812. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEM-1200EX electron microscope.

Results

Gross examination revealed the tumor to be located slightly posterior to the lesser curvature of the antrum of the stomach; it was considered to be a Borrmann III type carcinoma, and measured 6x4x2 cm. The cut surface was soft and greyish, and the tumor expanded into the subserosa.

Light microscopic examination revealed well-differentiated papillary adenocarcinoma in the peripheral area combined with undifferentiated carcinoma consisting of small round cells in the central area (Fig. 1). Well-differentiated papillary adenocarcinoma was limited to the submucosa with vascular invasion (Fig. 1). In contrast, the undifferentiated small cell carcinoma infiltrated into the subserosa and had invaded vessels. Most neoplastic cells in the undifferentiated area were uniform, small, round or oval, and had intensely hyperchromatic nuclei and scarce cytoplasm (Fig. 2). The nucleoli were not prominent, and numerous mitotic figures were seen. There were also a few large multinuclear cells with scarce cytoplasm in the undifferentiated area (Fig. 2). Gradual transition from small cell carcinoma to well-differentiated papillary adenocarcinoma was seen, and these areas were closely related to each other. No lymph node metastasis was found.

Fig. 1. Well-differentiated papillary adenocarcinoma and area of transition to neoplastic small cells resembling pulmonary small cell carcinoma are seen. Haematoxylin-eosin. x 50

Argyrophilia upon Grimelius staining was very weak in the small cell carcinoma and absent in well-differentiated adenocarcinoma. Both areas were negative for silver impregnation by Fontana-Masson method and PAS staining.

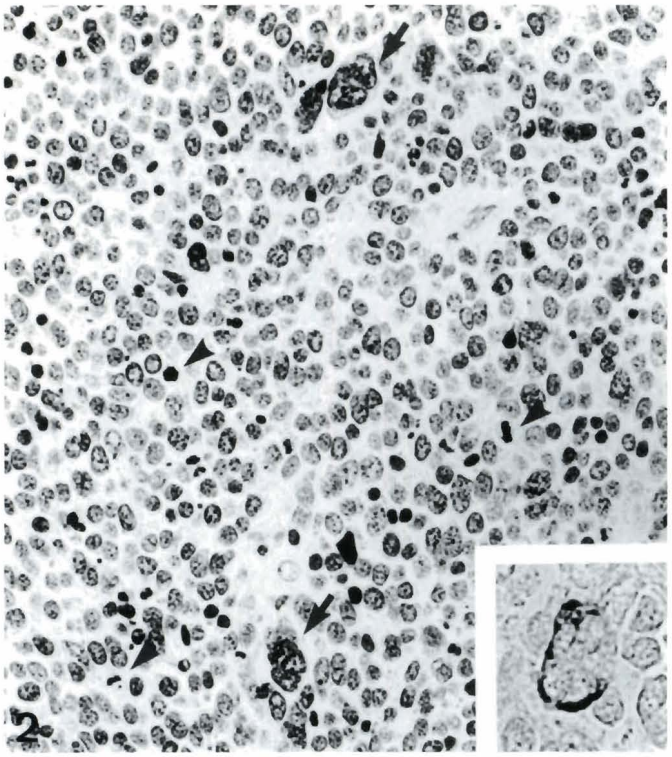
The well-differentiating papillary adenocarcinoma was strongly positive for immunohistochemical stains with CEA, EMA, keratin (KL-1), EGC, and CA19-9 (Fig. 3). Neoplastic small cells were very weakly positive for NSE and negative for all others. However, within the small cell carcinoma, a few large neoplastic cells were strongly positive for desmin (Fig. 2).

Ultrastructural examination of the small neoplastic cells disclosed a small number of dense-cored granules of the neuroendocrine type and intercellular junctions, although ultrastructural preservation was poor due to inappropriate fixation. The granules were membrane-bound, and the dense cores ranged from 100 to 200 μm in diameter (Fig. 4). Bundles of intermediate filaments were observed in the cytoplasm of some large neoplastic cells which were probably identical to the multinuclear, desmin-positive cells (Fig. 5).

Discussion

Small cell carcinoma, initially established as a definitive type of lung neoplasm (Azzopardi, 1959), accounts for about 25% of all lung carcinomas (Cohen and Matthews, 1978). As described in a review by Ibrahim et al. (1984), extrapulmonary small cell carcinoma has been increasingly reported in various organs, including the larynx, hypopharynx, thymus, esophagus, salivary gland, nasal cavity, skin, small and large intestines, pancreas, uterine cervix, endometrium, urinary bladder, prostate, and breast. In the stomach, however, small cell carcinoma is an unusual neoplasm, with only nine cases reported in the literature (Matsusaka et al., 1976; Chejfec and Gould, 1977; Eimoto and Hayakawa, 1980; Fer et al., 1981; Ibrahim et al., 1984; Fukuda et al., 1988; Hussein et al., 1990) (Table 1).

The possible histogenesis of small cell carcinoma arising in gastrointestinal tract is controversial. It has been suggested that small cell carcinoma of the stomach can arise from Kultschitsky or APUD cells, which are widely distributed throughout the gastrointestinal tract (Matsusaka et al., 1976; Chejfec and Gould, 1977;



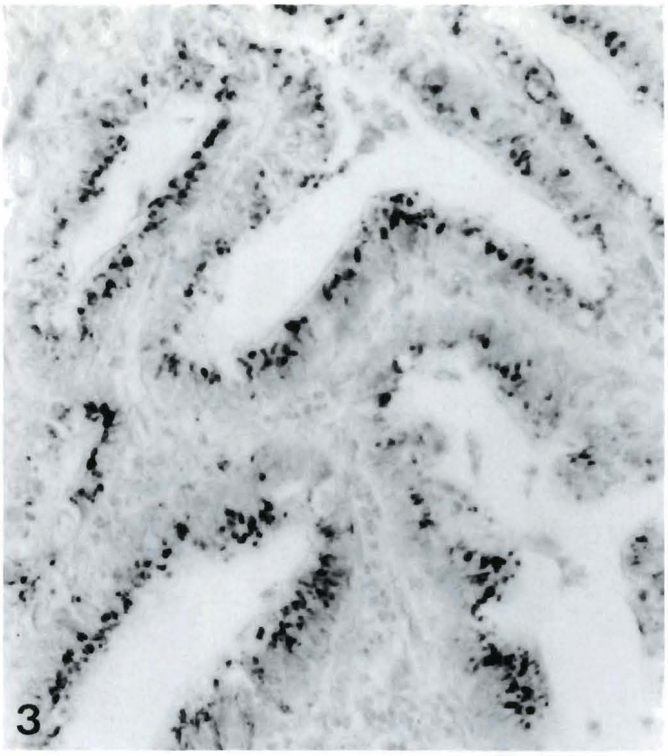


Fig. 2. Cytological details of neoplastic small cells. Nuclei are hyperchromatic with indistinct nucleoli. Note large neoplastic cells with multiple nuclei, scarce cytoplasm (arrow) and numerous mitotic figures (arrow head). Haematoxylin-eosin staining. (x 400). Inset: Large neoplastic cells intermingled with small cell carcinoma show cytoplasmic immunoperoxidase reactivity for desmin. Immunoperoxidase stain, nuclear counterstain with methyl green. x 450

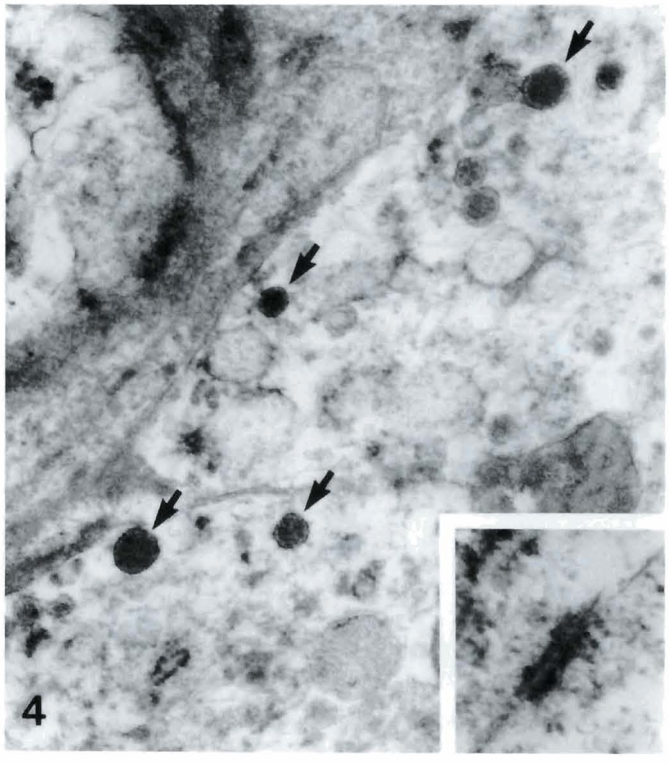
Fig. 3. Neoplastic cells, which differentiated to papillary adenocarcinoma, are positive for CA19-9. Immunoperoxidase stain, nuclear counterstain with methyl green. x 400

Eimoto and Hayakawa, 1980; Fer et al., 1981; Ibrahim et al., 1984). However, some researchers suggest that extrapulmonary small cell carcinomas, including these in the stomach (Fukuda et al., 1988; Hussein et al., 1990), derive from pluripotent stem cells (McDowell et al., 1981; Ho et al., 1984; Blomjous et al., 1988; Hagood et al., 1991). In the present case, the neoplastic cells showed neuroendocrine differentiation as well as epithelial differentiation. Moreover, some neoplastic cells were differentiated into desmin-positive cells with intermediate filaments. These findings are compatible with the concept cited above that small cell carcinoma are derived from an undifferentiated cell with the capacity for multidirectional differentiation.

Of the nine cases reported in the literature (Matsusaka et al., 1976; Chejfec and Gould, 1977; Eimoto and Hayakawa, 1980; Fer et al., 1981; Ibrahim et al., 1984; Fukuda et al., 1988; Hussein et al., 1990), eight were in males who ranged in age from 42 to 79 years (mean, 62.9 years). Histologically, five tumors were pure small cell carcinoma, and four were combined with adenocarcinoma and/or squamous cell carcinoma. Cytoplasmic argyrophilia was seen in all tumors except one, and neurosecretory granules were found in all six tumors examined ultrastructurally. Immunohistological studies were performed in only two small cell carcinomas (Fukuda et al., 1988; Hussein et al., 1990), both of which were positive for NSE.

The present tumor was a combined type. Neoplastic small cells were very weakly positive for Grimelius' silver impregnation and negative for Fontana-Masson method. Although immunohistochemical staining included those for tumor markers, cell surface antigens, enzymes, cytoskeletal components, oncofetal substances, hormones, and polypeptides, in neoplastic small cells only a very weakly positive reaction to NSE was seen. Electron microscopy showed dense-cored membrane-bound granules in the cytoplasm, even in the materials fixed once in formalin; ultrastructural examination is vital in confirming neuroendocrine differentiation in small cell carcinoma.

Just as in pulmonary small cell carcinoma, the clinical course of gastric small cell carcinoma was aggressive; all patients except one (Matsusaka et al., 1976) died within less than one year after diagnosis. Whether small cell morphological characteristics and neuroendocrine differentiation are related to the biologic aggressiveness of the tumor has not yet been determined. Dauge and Delmas have indicated that the prognosis for patients with prostate endocrine tumors is proportional to the extent of neuroendocrine differentiation (Dauge and Delmas, 1987). Conversely, some researchers have



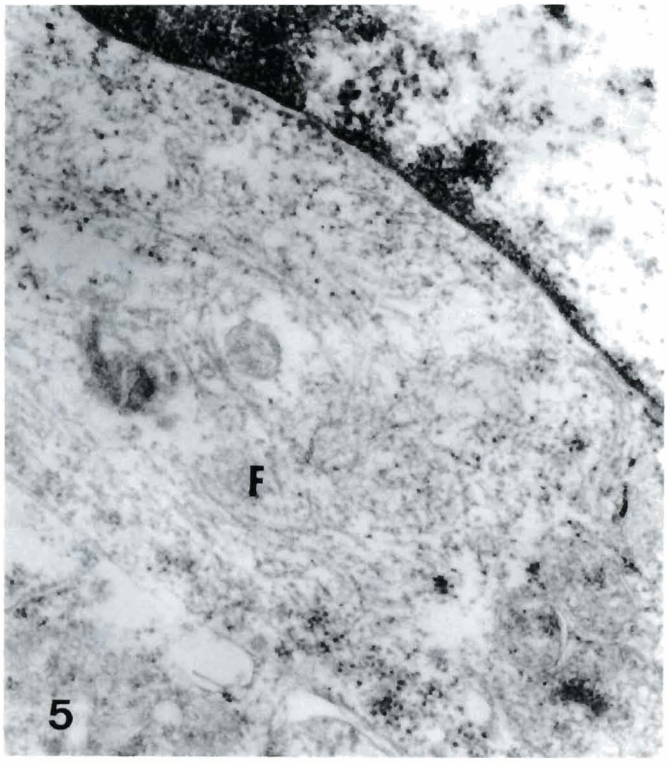


Fig. 4. Several tumor cells taken from the small cell lesion contain some dense-cored granules (arrows) and intercellular junctions (inset). Uranyl acetate and lead citrate. x 31,000; inset x 47,000

Fig. 5. A few large neoplastic cells in the small cell lesion have intermediate filaments (F). Uranyl acetate and lead citrate. x 34,000

Table 1. Clinical and histological findings in 10 cases of gastric small cell carcinoma.

Reference	Age/Sex	Histological type	Immunohistology	Neurosecretory granules
Matsusaka et al., 1976	54/M	Pure	N.D.	N.D.
	65/F	Combined	N.D.	N.D.
Chejfec and Gould, 1977	66/M	Pure	N.D.	+
	79/M	Pure	N.D.	+
Eimoto and Hayakawa, 1980	66/M	Pure	N.D.	+
Fer et al., 1981	69/M	Combined	N.D.	+
Ibrahim et al., 1984	51/M	Combined	N.D.	+
Fukuda et al., 1988	74/M	Combined	NSE, CG, Leu 7	+
Hussein et al., 1990	42/M	Pure	NSE	N.D.
Present case	68/F	Combined	NSE (very weak)	+

N.D.: not done: NSE: neuron-specific enolase; CG: chromogranin. Pure; pure small cell carcinoma; Combined: small cell carcinoma combined with adenocarcinoma or squamous cell carcinoma.

reported that small cell carcinoma of the urinary bladder is extremely aggressive in spite of slight neuroendocrine differentiation (Kim et al., 1984; Lee et al., 1986; Mills et al., 1987). Burke et al. (1991) have also described that small cell histological characteristics are the best single predictors of liver metastasis, although endocrine differentiation is highly associated with liver metastasis among colorectal carcinomas of low differentiation. In the present tumor, neoplastic cells showed obvious vascular invasion and numerous mitotic figures, although neurosecretory granules were detected in only a few neoplastic cells with electron microscopy. These findings may support Burke's suggestion in small cell carcinoma of the gastrointestinal tract.

Because of the aggressive course in small cell carcinoma, which is similar to that in pulmonary small cell carcinoma, Fukuda et al. (1988) have recommended intensive chemotherapy when it is present at any stage. Some researchers have reported effective use of chemotherapeutic regimens, similar to those administered for pulmonary small cell carcinoma, in small cell carcinoma of the gastrointestinal tract: esophagus (Kelsen et al., 1980; Fer et al., 1981; Rosenthal and Lemkin, 1983); stomach (Fer et al., 1981); colon (Redman and Pazdur, 1987; Burke et al., 1991); pancreas (Fer et al., 1981); uterine cervix (Pazdur et al., 1981); prostate (Hindson et al., 1985); and skin (George et al., 1985). However, we were unable to directly compare prognoses after surgery, chemotherapy, or radiation therapy in patients with gastric small cell carcinoma because of the small number of patients and insufficient information reported in the literature. Nevertheless, the combination of surgery, radiation and chemotherapy apparently results in the longest survival. The known aggressive course in small cell carcinoma needs prompt diagnosis.

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