Immunohistochemical Localization of Brain-Gut Hormones in Gastric Carcinoma with Relation to Argyrophil Cells

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YAMADA, S., SASANO, N. and NAKAMURA, T. *Immunohistochemical Localization of Brain-Gut Hormones in Gastric Carcinoma with Relation to Argyrophil Cells.* Tohoku J. exp. Med., 1984, **143** (1), 1-15 — A total of 87 surgical cases of gastric carcinoma including 3 carcinoid tumors were investigated with the methods of silver reaction and immunoperoxidase stain for 8 different brain-gut hormones. Argyrophil (AP) cells were demonstrated in 38 cases (44%), argentaffin (AF) cells in 18 (21%) and endocrine cells in 13 (14%). The occurrence of endocrine cells had no relation with histological types. Glicentin cells were demonstrated in 10 cases, somatostatin in 7, motilin in 3, β -endorphin in 2 and gastrin in one. Endocrine cells appeared generally in small numbers except one carcinoid tumor which had numerous somatostatin cells. No single cell positive for more than two kinds of hormones could be demonstrated. Two undifferentiated carcinomas looking like carcinoid tumors had argyrophil cells and endocrine cells of either somatostatin or β -endorphin. These results suggest that carcinoid-like carcinoma or endocrine cell carcinoma may lie on the intermediate state between carcinoma and carcinoid tumor. ——— gastric carcinoma; carcinoid tumor; argyrophil cell; endocrine cell; brain-gut hormone

Argyrophil (AP) and argentaffin (AF) cells in gastric carcinoma have repeatedly been documented by several investigators. (Hamperl 1927; Azzopardi and Pollock 1963; Soga et al. 1971; Kubo and Watanabe 1971; Watanabe 1972; Tahara et al. 1975). If these cells are identical to AP and AF cells in the normal gastrointestinal mucosa, they may produce amines and/or polypeptide hormones.

Immunohistochemical demonstration of brain-gut hormones in gastric carcinoma was successful first for gastrin (Tahara et al. 1975, 1980; Nagata 1977; Prade et al. 1982). Furthermore, Tahara et al. (1982) demonstrated somatostatin in argyrophil cells in early gastric carcinoma and somatostatin and glucagon in scirrhous argyrophil cell carcinoma of the stomach. Endocrine cells containing

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polypeptide hormones have been demonstrated in gastric carcinoid tumors (Wilander et al. 1979; Dayal et al. 1980).

The present paper deals with the immunohistochemical localization of several kinds of brain-gut hormones in carcinoma and carcinoid tumors of the stomach in relation to AP and AF cells. Discussion will be focused on the findings of endocrine cells of neoplastic and/or non-neoplastic origin appeared in gastric carcinoma.

MATERIALS AND METHODS

Materials were 87 surgical cases of gastric carcinoma, consisting of 13 early carcinomas confined to the submucosal layer, 71 advanced carcinomas involving up to the proper muscle layer and deeper and 3 carcinoid tumors. They were randomly collected from the files in the period from 1978 to 1981 of Department of Pathology, Tohoku University School of Medicine; the First Department of Surgery, Gunma University School of Medicine; and Departments of Surgery, National Sendai Hospital and Sendai City Hospital and Tsurugaya Open Hospital in Sendai.

Specimens were fixed in 10% formalin solution, Zamboni's fixative, Bouin's fixative or 4% paraformaldehyde, and were dehydrated and embedded in paraffin. One to several representative paraffin blocks including tumor tissues in each case were prepared for histological and immunohistochemical examinations.

Blocks were cut into 2.5 μ m-thick serial sections for histological and histochemical examinations.

Histology. Specimens were stained with hematoxylin and eosin, the Grimelius method for AP cells (Grimelius et al. 1976), Fontana-Masson for AF cells and PAS-alcian blue (pH 2.5).

Histological classification of gastric carcinoma and the definition of early gastric carcinoma were made according to the General Rules for the Gastric Cancer Study in Surgery and Pathology in Japan (Jap. Res. Soc. for Gastric Cancer 1981).

Immunohistochemistry for brain-gut hormones. Immunohistochemical stains for brain-gut hormones were performed by the peroxidase anti-peroxidase (PAP) method (Sternberger et al. 1970; Taylor 1978) and an indirect method (Taylor 1978). The indirect method was used for only gastrin. The first (specific) and the second antibodies and their dilution used were as follows: Guinea-pig anti-gastrin (GP-1305) 1:150, rabbit anti-glicentin C'-terminal (R-4804) 1:100, rabbit anti-motilin (R-1105) 1:100, rabbit anti-VIP (vasoactive intestinal polypeptide) (R-501) 1:100, rabbit anti- α -endorphin (R-3404) 1:40 and rabbit anti- β -endorphin (R-5803) 1:40, all of which were generously offered by Prof. N. Yanaihara; rabbit anti-somatostatin (Lot. 1039) 1:300 and rabbit anti-ADH (Lot. 701) 1:300 given from Dr. K. Mouri; and rabbit anti-somatostatin (JG-1) 1:150 available from the Japan Immunoresearch Laboratories Co., Ltd., Tokyo. The biochemical specificities of antibodies were confirmed by Yanaihara, N. et al. (1979, 1980, 1981), Yanaihara, C. et al. (1980a, b), Ibata et al. (1980), Mouri and Maruyama (1980) and Nishino et al. (1981). The second antisera of swine anti-rabbit immunoglobulins (Z196) 1:40, rabbit anti-guinea-pig immunoglobulins/HRP (P141) 1:40 and PAP, rabbit (Z113) 1:80 were obtained from Dakopatts, Denmark.

The following tissues were used as positive controls: normal tissue of the pancreas for the reactions of glicentin and somatostatin; normal antral mucosa of the stomach for gastrin; tumor tissue of VIP-producing pheochromocytoma for VIP; normal tissue of the pituitary for ADH and α - and β -endorphins.

The negative control for the specificity of the tissue reaction was tested with non-immune rabbit or guinea-pig serum as the first layer. After reaction the cells with brownish

cytoplasm and without staining of nuclei were adopted as positive.

Ultrastructural study. Ultrastructural studies were carried out on 2 cases of carcinoid tumor and a case of carcinoid-like carcinoma. Specimens were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde at pH 7.4 and embedded in Epon 812 by routine techniques. Thin sections of 600–800 Å were stained with uranyl acetate and lead citrate and examined with a JEOL 100B electron microscope.

RESULTS

Argyrophil and argentaffin cells in gastric carcinoma

Histological types of gastric carcinoma positive with AP and AF reactions and immunoperoxidase stains for brain-gut hormones are shown in Table 1. AP cells were demonstrated in 38 (44%) out of 87 gastric carcinomas including 3 carcinoid tumors. AF cells appeared in 18 (21%) and endocrine cells in 13 (14%). AP cells were contained in all histological types, while AF cells were absent in papillary adenocarcinoma and undifferentiated carcinoma.

Endocrine cells in gastric carcinoma

The results of the immunoperoxidase stain for brain-gut hormones and silver stains are listed in Table 2. The incidence of carcinoma with endocrine cells was 6 (37%) out of 18 cases containing both AP and AF cells and 7 (36%) out of 20 cases with AP cells alone. All cases with endocrine cells had AP cells. All with negative silver reaction had no endocrine cells. The clinicopathological details of 13 cases with endocrine cells are shown in Table 3. The patients were 40 to 75

TABLE 1. *Histological type of gastric carcinoma with positive argyrophil, argentaffin and immunostain reactions*

Histological types	Number of cases	Positive with		
		AP	AF	IM
Papillary adenocarcinoma (pap)	8	2	0	1
Tubular adenocarcinoma				
Well differentiated (tub ¹)	15	5	3	2
Moderately differentiated (tub ²)	10	4	2	3
Poorly diff. adenocarcinoma				
Medullary type (por, med)	10	4	1	1
Scirrhou type (por, sci)	27	12	5	1
Mucinous adenocarcinoma (muc)	7	4	3	2
Signet ring cell carcinoma (sig)	5	2	2	0
Undiff. carcinoma (ud)	2	2	0	2
Carcinoid tumor (cd)	3	3	2	1
Total	87	38	18	13
(%)		(43.7)	(20.7)	(14.3)

AP, argyrophil reaction (Grimelius); AF, argentaffin reaction (Fontana-Masson); IM, immunostain.

TABLE 2. *Comparison of argyrophil and argentaffin cells to immunostain for brain-gut hormones*

Argyrophil	Argentaffin	Total number of cases	Immunostain	
			Positive (%)	Negative (%)
+	+	18	6 (33.3)	12 (66.7)
+	—	20	7 (35.0)	13 (65.0)
—	+	0	0	0
—	—	49	0 (0)	49 (100.0)
Total		87	13 (14.9)	

years of age and composed of 10 males and 3 females. Eleven cases were of advanced carcinoma and 2 were early. There was no relationship of the appearance and localization of endocrine cells to the histological type or the depth of invasion. The results of immunoperoxidase stains for gastrin, motilin, glicentin, somatostatin, β -endorphin, α -endorphin, VIP and ADH in relation to AP and AF cells are shown in Table 4. Glicentin was positive in 10 cases, somatostatin in 7, motilin in 3, β -endorphin in 2 and gastrin in one. The mode of immunoreactivity in the individual cases are shown in Table 5. Positive stain for only glicentin was found in 4 cases, and for both glicentin and somatostatin in 3. Six other types of positive stain were observed in one case each. No single cell simultaneously positive for two or more brain-gut hormones was found in serial sections. Evidence of positive argyrophil reaction in endocrine cells was also observed.

TABLE 3. *Gastric carcinoma with positive*

Case	Age	Sex	Location*	Size (cm)	Histological type	Depth of Invasion
1	56	M	A	3.5 × 3.0	ud	sm
2	63	M	A	5.2 × 2.5	tub ²	sm
3	40	F	M	4.0 × 4.0	tub ²	s
4	53	M	A	4.5 × 4.0	cd	s
5	65	M	A	4.0 × 2.0	ud	pm
6	67	M	AM	7.0 × 7.6	pap	s
7	69	M	A	6.8 × 3.7	tub ¹	pm
8	75	M	A	N.D.	tub ¹	s
9	73	M	A	9.0 × 8.0	muc	s
10	40	F	C	10.2 × 8.0	por, sci	s
11	53	M	MA	6.0 × 6.0	por, med	s
12	69	F	MA	7.0 × 10.0	muc	s
13	69	M	A	9.0 × 10.0	tub ²	s

AP, argyrophil reaction ; AF, argentaffin reaction ; GLI, glicentin ; SOMT, soma LIM, localization of immunostain positive cells.

* The stomach is separated into the upper (C), middle (M) and lower (A) portions curvature.

TABLE 4. *Total number of positive immunostain cases in gastric carcinoma in relation to argyrophil and argentaftin cells*

	<i>Argyrophil</i>	+	+	Total
	<i>Argentaftin</i>	+	—	
Number of cases		6	7	13
Immunostain for				
Positive	Gastrin	1	0	1
	Motilin	2	1	3
	Glicentin	5	5	10
	Somatostatin	2	5	7
	β -Endorphin	0	2	2
	α -Endorphin	0	0	0
	VIP	0	0	0
	ADH	0	0	0

The frequency of these cells varied with individual cases. Endocrine cells were relatively small in number in most cases, but somatostatin cells in a carcinoid tumor were exceptional and distributed diffusely and densely.

Localization of endocrine cells and tumor histology

Endocrine cells within the tumor were variable in appearance and number.

Tubular adenocarcinoma. Endocrine cells were demonstrated in 5 out of 25 cases. In case No. 7, carcinoma cells positive for glicentin, somatostatin, gastrin immunostain for brain-gut hormones

AP	AF	Positive immunostain	LIM	BS (mg/100 ml)
+	—	SOMT	sm	75
+	+	GLI+SOMT	sm	82
+	—	GLI+SOMT	sm	71
+	—	GLI+SOMT+ β -END	pm	88
+	—	β -END	pm	83
+	—	GLI+SOMT	pm	88
+	+	GLI+SOMT+MOT+GAS	pm	101
+	+	GLI	s	160
+	—	GLI+SOMT+MOT	pm	N.D.
+	+	GLI	sm	91
+	+	MOT	m, sm	107
+	+	GLI	m, sm	109
+	—	GLI	sm	117

tostatin; MOT, motilin; GAS, gastrin; β -END, β -endorphin; BS, blood sugar;

by drawing lines between the corresponding trisectional points on the greater and lesser

TABLE 5. *Positive immunostain for brain-gut hormones in the individual cases*

Immunostain	Number of cases
GLI only	4
GLI+SOMT	3
GLI+SOMT+MOT	1
GLI+SOMT+ β -END	1
GLI+SOMT+MOT+GAS	1
SOMT only	1
MOT only	1
β -END only	1
Total	13

GLI, glicentin ; SOMT, samatostatin ; MOT, motilin ; GAS, gastrin ; β -END, β -endorphin.

and motilin were demonstrated (Figs. 1, 2 and 3). Both glicentin and gastrin cells were confirmed to be argyrophil by a serial slides of hematoxylin and eosin, Grimelius and immunoperoxidase stains. Somatostatin cells were weakly argyrophil and motilin cells were negative for argyrophil nor argentaffin reactions.

Poorly differentiated adenocarcinoma. Endocrine cells were demonstrated in 2 out of 37 cases. In case No. 10 of the scirrhus type mixed with signet-ring cells, glicentin cells were confirmed to be argyrophil in the adjacent slide of serial

Fig. 1. Well differentiated tubular adenocarcinoma. Arrows indicate the same cell in three serial sections stained with hematoxylin and eosin (HE), Grimelius (GR) and glicentin by the PAP method (GL). Glicentin cells are argyrophilic. (Case 7. $\times 400$)

section (Fig. 4). In the medullary type of case No. 11, motilin cells were demonstrated (Fig. 5), and negative for argyrophil and argentaffin reactions.

Mucinous adenocarcinoma. Endocrine cells were demonstrated in 2 out of 7 cases. In case No. 9, carcinoma cells positive for glicentin, somatostatin and motilin were demonstrated. In case No. 12, the immunostain was successful only for glicentin.

Carcinoid-like carcinoma in undifferentiated carcinoma. In case No. 1, tumor cells were significantly pleomorphic and atypic. Carcinoma cells were mostly positive with Grimelius stain and negative with Fontana-Masson stain. Only somatostatin cells were demonstrated (Fig. 6). Electron microscopically, round secretory granules, 150–200 nm in diameter, and pleomorphic granules, 300–400 nm in diameter, were observed (Figs. 7A, 7B). In case No. 5, tumor cells were pleomorphic and arranged in an alveolar pattern focally with a rosette structure (Fig. 8). Many AP cells and a few β -endorphin cells were demonstrated. These cases were regarded to be compatible with carcinoid-like carcinoma or endocrine cell carcinoma.

Carcinoid tumor. Typical carcinoid tumors were detected in 3 cases. The majority of the tumor cells were AP in all 3 cases and AF in 2 cases. A tumor of case No. 4 located in the antrum was composed of small and uniform cells arranged in alveolar, trabecular, ribbon-like and solid structures consistent with mixed type (Soga et al. 1971) (Fig. 9). Many somatostatin cells and a few β -

Fig. 2. Well differentiated tubular adenocarcinoma. Arrows indicate the same cell in three serial sections stained with hematoxylin and eosin (HE), Grimelius (GR) and somatostatin by the PAP method (SO). Somatostatin cells are weakly argyrophilic. (Case 7. $\times 400$)

Fig. 3. Well differentiated tubular adenocarcinoma. Arrows indicate the same cell in two serial sections stained with Grimelius (GR) and gastrin by the indirect method (GA). Gastrin cells are argyrophilic (Case 7 $\times 400$)

Fig. 4. Poorly differentiated adenocarcinoma of scirrhous type with focal signet-ring cell carcinoma. Arrows indicate the same cell in three serial sections stained with hematoxylin and eosin (HE), Grimelius (GR) and glicentin by the PAP method (GL). Glicentin cells are argyrophilic. (Case 10. $\times 400$)

Fig. 5. Poorly differentiated adenocarcinoma, medullary type. Tumor cells are small in size and oval or spindle in shape with hyperchromatic nuclei. These findings are consistent with carcinoid-like carcinoma. A few carcinoma cells show positive immunoperoxidase stain for motilin (MO), but are negative for argyrophil and argentaffin stains. (Case 11. HE $\times 400$, MO $\times 400$)

Fig. 6. Carcinoid-like carcinoma. Tumor cells are pleomorphic and show marked cellular atypism. Most of the tumor cells are positive with Grimelius stain. Arrows indicate the same cell in three serial sections stained with hematoxylin and eosin (HE), Grimelius (GR) and somatostatin by the PAP method (SO). Somatostatin cells are argyrophilic. (Case 1. $\times 400$)

Fig. 7. Electron microscopically round secretory granules, 150–200 nm in diameter (A), and pleomorphic granules, 300–400 nm in diameter (B) are observed in the cytoplasm. A part of carcinoid-like carcinoma in Case 1. ($\times 10,000$)

Fig. 8. Undifferentiated carcinoma. The tumor cells show pleomorphism and alveolar patterns with focally rosette structure. (Case 5, hematoxylin and eosin $\times 200$)

Fig. 9. Carcinoid tumor. The tumor is composed of small and uniform tumor cells with alveolar (left), trabecular and ribbon-like (middle) and solid arrangement (right). (Case 4. hematoxylin and eosin $\times 400$)

Fig. 10. Carcinoid tumor. The tumor cells arranged in an alveolar pattern show diffusely positive reaction for somatostatin (SO), focally positive for glicentin (GL) and irregularly positive for β -endorphin (β -EN) by the PAP method. (Case 4. $\times 400$)

endorphin cells were demonstrated in only a part of the alveolar pattern (Fig. 10). Electron microscopically, secretory granules, 200–250 nm in diameter, were observed in the cytoplasm. Two other cases were positive with silver reaction but negative for gastrin, glicentin, somatostatin, endorphins, VIP and ADH. Electron microscopical examinations performed on one of these two cases revealed secretory granules, 100–200 nm in diameter.

DISCUSSION

The appearance of AP and AF cells in gastric carcinoma was first described by Hamperl (1927) and reported by several authors (Azzopardi and Pollock 1963; Tahara et al. 1975, 1982; Nagata 1977) with various incidences. With respect to the histological type of gastric carcinoma, Tahara et al. (1975) observed AP cells in 20.7% of poorly differentiated adenocarcinoma and undifferentiated carcinoma. No definite relationship between the appearance of AP cells and histological types of gastric carcinoma was, however, obtained by other investigators (Kubo and Watanabe 1971; Watanabe 1972; Nagata 1977). In the present study AP cells appeared in three histologically different carcinomas; well differentiated tubular adenocarcinoma (case No. 7), and poorly differentiated adenocarcinoma (cases No. 2 and No. 8) and undifferentiated carcinoma (cases No. 1 and No. 5) which was called as diffuse argentaffinoma (Kubo and Watanabe 1971). If these AP cells are consistent with endocrine cells, certain hormones secreted by these cells should be demonstrated. In gastric carcinoma, gastrin cells were demonstrated by Tahara et al. (1975, 1980) and Nagata (1977). Prade et al. (1982) made a detailed immunohistochemical study of a gastric adenocarcinoma with argyrophil cells. About 30 percent of the M2-positive cells stained strongly with an antigastrin serum as well as with the argyrophilic reagents.

Tahara et al. (1982) confirmed 9 cases producing polypeptides such as gastrin, somatostatin and glucagon among 16 cases of argyrophil carcinoma out of 59 gastric scirrhous carcinoma. In our study, glicentin, somatostatin, motilin, gastrin and β -endorphin cells were demonstrated (Tables 4 and 5). It is interesting that motilin cells originally specified to the small intestinal mucosa were demonstrated in 3 cases of gastric carcinoma. This may suggest a relationship between the pathogenesis of gastric carcinoma and intestinal metaplasia. Glicentin, somatostatin and gastrin cells in carcinoma tissue were argyrophil with the Grimelius stain like endocrine cells in the normal gastrointestinal mucosa (Grimelius et al. 1976; Lechago 1978; Solcia et al. 1979).

With regard to the origin of endocrine cells occurring in gastric carcinoma, Tahara et al. (1980, 1982) and Nagata (1977) explained that they originate from the tumor cells derived from the endodermal stem cells with multipotentiality. Soga et al. (1971), however, stressed that endocrine cells in carcinoma tissues are non-neoplastic because of their negligible atypism. Azzopardi and Pollock (1963) also doubted whether endocrine cells in metastatic carcinoma tissue in

lymph nodes are neoplastic or not. Concerning this our results were also nonconclusive, but it should be emphasized that some of these cells apparently showed considerable atypism. The problems of neoplastic endocrine cells will be resolved in near future.

Recently the definition of carcinoid tumors has been changed. This tumor with a definitive histological pattern is a kind of functioning tumors which secrete various peptide hormones and amines. This has been corroborated by certain investigators (Hirata et al. 1976 ; Wilander et al. 1979 ; Dayal et al. 1980 ; Marcus et al. 1980). We could immunohistochemically demonstrate the activity of somatostatin, glicentin and β -endorphin in one out of 3 gastric carcinoid tumors.

Auböck and Höfler (1983) proposed two ways in histogenesis of gastrointestinal carcinoid tumor; the one from intestinal epithelial cells and the other from disseminated extraepithelial, intraneural endocrine cells. Concerning the former way of development, a carcinoid-like carcinoma has been documented. There are two types in it. One is called composite type carcinoid (Gibbs 1963 ; Bates and Betler 1967). The other carcinoid-like carcinoma is undifferentiated carcinoma classified by Matsuzaka (1976). This type is also called endocrine cell carcinoma or oat cell carcinoma (Eimoto and Hayakawa 1980) and lies between carcinoid tumor and carcinoma. As to be asserted by Tahara et al. (1975, 1982), a spectrum from adenocarcinoma to carcinoid tumor of the stomach was demonstrated in our study. Cox and Pierce (1982) successfully demonstrated evidence for the endodermal origin of the endocrine cells in an adenocarcinoma of the colon of the rat by subcutaneous transplantation of single carcinoma cell. We have agreed to hypothesis of Soga (1982) and Tahara et al. (1982) that both carcinoma and a carcinoid tumor commonly originate from totipotent stem cells of endodermal origin in the gastric mucosa.

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