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Human chorionic gonadotropin in gastric carcinoma

An immunohistochemical study suggesting independent regulation of subunits

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Summary. To investigate the production of human chorionic gonadotropin (hCG) in gastric carcinoma, 124 gastric carcinomas and a choriocarcinoma with adenocarcinoma were examined immunohistochemically, using anti-hCGα and β antibodies. In choriocarcinoma, many trophoblastic cells were synchronously positive for both subunits. In contrast, the distribution of hCG-subunits in gastric carcinoma was unbalanced with hCGα in 39 and hCGβ in 63 cases. 26 cases contained α and β positive cells, whereas synchronous cells were extremely rare in four cases. Incidences of hCG-subunit-positivities were not different between early and advanced carcinomas. HCGα-positive cells appeared endocrine-like in papillotubular carcinomas and some positive cells were argyrophilic in serial sections in 23 of 39 cases. HCGβ-positive cells were much more frequent in deranged glands, especially of microtubular-mucocellular carcinomas and most were not argyrophilic. In surrounding non-neoplastic mucosa, hCGα-positive cells were more numerous with endocrine-like configurations, but hCGβ-positive cells were rarely present in deranged glands. Although subunit-profile of hCG in gastric carcinomas was different from that of normal, the difference may be quantitative: hCG-subunits may be expressed through an independent mechanism but commonly in gastric mucosa and carcinoma. These results are also discussed in relation to trophoblastic tumours arising in non-trophoblastic tissues.

Key words: Human chorionic gonadotropin – Subunit – Gastric carcinoma – Choriocarcinoma – Immunohistochemistry

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Introduction

Human chorionic gonadotropin (hCG) is primarily a product of placental trophoblastic cells. Trophoblastic tumours are, therefore, associated with hCG-production, but hCG or its subunits are also secreted by a wide variety of non-trophoblastic neoplasms. Gastric carcinoma is one of the most common neoplasms with increased blood level of hCG (Braunstein et al. 1973; Hattori et al. 1978). However, hCG secretion of some neoplasms and neoplastic cells is unbalanced in subunit-profile (Weintraub and Rosen 1973; Tashjian et al. 1973; Rosen et al. 1980; Hattori et al. 1980; Heitz et al. 1983). HCG consists of two different subunits: the α-subunit is nearly identical in structure to other glycoprotein hormones, ie, luteinizing hormone (hLH), follicle stimulating hormone (hFSH) and thyroid stimulating hormone (hTSH), whereas the β-subunit is specific to each hormone (Pierce and Parsons 1981). In addition to neoplastic tissues, hCG- and/or hCGβ-like immunoreactivities have been demonstrated in various tissues other than the placenta (Yoshimoto et al. 1977, 1979; Braunstein et al. 1979, 1984). Immunohistochemical distribution of hCG-subunits is also unbalanced in these tissues, such as the stomach, lung and colon (Fukayama et al. 1986a, 1986b, 1987). HCGα-immunoreactive cells are identified much more often than hCGβ-positive cells and the two cells are not identical.

In the present paper, we have studied subunit-profile of hCG in gastric carcinoma immunohistochemically, which has not been studied in detail. The aims of the study are to determine (a) whether production or distribution of hCG-subunits is coordinate or not in gastric carcinomas, and (b)

whether its subunit-profile is different or not from that of normal mucosa. We will then discuss the significance of hCG-subunits in non-trophoblastic tumours, in an effort to clarify the regulatory mechanism for hCG-subunits in neoplasia of non-trophoblastic tissues.

Materials and methods

Gastric carcinomas were surgically resected (67 males and 48 females, 32–90 years old). The tumours consisted of 50 early carcinomas (invasion depth is confined within the mucosa or submucosa), and 74 advanced carcinomas (carcinoma invasion extended to or beyond the muscular layer). A case of choriocarcinoma with adenocarcinoma was also examined which developed at the oesophago-gastric anastomosis and was considered to arise from Barrett's type oesophagus (Aonuma et al. 1986). All stomachs were opened along the greater curvature as soon as possible after resection. They were fixed in 17% formalin. One or two blocks were taken: In 109 carcinomas less than 10.0 cm in length, one tissue-block contained the entire longitudinal cut surface along the lesser curvature in 50 early and 24 advanced carcinomas, or contained at least one peripheral and the area of central deepest invasion in 35 advanced carcinomas. In 15 carcinomas larger than 10.0 cm, two seperate blocks were sampled corresponding to one peripheral and one central portion. Tissues were processed routinely and embedded in paraffin. Serial sections were cut at 3 microns thick and stained with haematoxylin and eosin (HE) and alcian blue, periodic acid-Schiff double stain (ABPAS, pH 2.5) for diagnostic examination. Serial sections were then immunostained with anti-hCGα and β antibodies. When any immunoreactive cell was identified, another adjacent serial section was stained by Grimelius silver technique. In gastric carcinomas containing more than 10 hCGα-immunoreactive cells, the sections were also immunostained with hLHβ, hFSHβ, and hTSHβ, each parallel to the sections stained with anti-hCGα-antibody.

For classification of the histological types of gastric carcinomas, the criteria of the Japanese Research Society for Gastric Cancer (1979) were applied. However, because frequent transition among histological types was observed in each carcinoma, the classification was simplified to two categories: papillotubular and microtubular-mucocellular groups. The papillotubular group included papillary and well and moderately differentiated tubular carcinomas. The microtubular-mucocellular group covered poorly differentiated and signet ring cell carcinomas. In this simplification, papillotubular or microtubular-mucocellular groups corresponded to intestinal or diffuse type in Lauren's classification (1965) respectively. The site of origin of the carcinomas of less than 10.0 cm in maximum length was determined by the roentogenologic and macroscopic findings and was described as cardia, body and antrum. The surrounding non-neoplastic mucosa of the early carcinomas was also evaluated as an intrinsic control. These were classified by the nature of glands; pyloric and fundic with or without intestinalized glands.

For immunohistochemistry, the peroxidase antiperoxidase (PAP) method of Sternberger (1979) was applied to the formalin fixed and paraffin embedded sections. Purification of each hCG-subunit and generation of rabbit antiserum for each (provided by H. Okumura, Teikoku Hormone Manufacturing Company, Kawasaki, Japan) have been reported elsewhere (Okumura et al. 1976, 1978). Antisera for hLHβ, hFSHβ and hTSHβ were provided by National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK, USA). The optimal dilutions of rabbit antisera were 1:1000 for hCGα, 1:500 for hCGβ, 1:20000 for hLHβ, 1:5000 for hFSHβ and 1:50000 for hTSHβ. Prior to the application of rabbit antisera, sections were pretreated with 0.1% pronase (Type VII, Sigma, St. Louis, USA) for 3 min. Incubation of primary antibodies was carried out overnight at 4 C.

Control studies have been reported (Fukayama et al. 1986a, 1986b, 1987). There was no immunostaining without antisera or with nonimmune sera. Immunoreactivity for each hCG-subunit in the placenta was abolished by absorption with each corresponding antigen, but it was not affected with counterpart antigen. Different fixatives (formalin, Bouin's solution or periodate-lysine-paraformaldehyde) had no effect on the immunostaining of both subunits. Immunoreactivity for hCGα crossreacted with other α-subunits of glycoprotein hormones (Okumura et al. 1978). However, because single common gene codes α-subunit of glycoprotein hormones (Fiddes and Talmage 1984), immunoreactivity with anti-hCGα-antibody was called simply as hCGα-immunoreactivity. Immunoreactivity for hCGα was not affected with absorption of a variety of gastrointestinal peptides or amine (gastrin 1–17 and 1–34, serotonin, gastrin releasing peptide, calcitonin, adrenocorticotropic hormone 1–39, β-endorphin, γ-melanocyte stimulating hormone, insulin, glucagon, glucagon like peptide, somatostatin, pancreatic polypeptide, and polypeptide YY). In the present study, both antisera were further absorbed with serine proteinases to eliminate unexpected cross reaction (Jagiello and Mesa-Tejada 1979). Cationic trypsin, chymotrypsin B, and elastase I were kindly provided by M. Ogawa (Second Department of Surgery, Osaka University, School of Medicine, Japan) (Fujimoto et al. 1980; Iwaki et al. 1983a, 1983b; Murata et al. 1983). Diluted antisera for hCGα and β were absorbed with each proteinase (20 microgram/ml) and then applied to the control sections (placenta, non-neoplastic antral mucosa and gastric carcinomas containing hCGβ-positive cells). Immunostaining in control sections was the same with or without absorption.

Statistical analysis was carried out by using Chi-square test. Binominal analysis (z-test) was also applied to the incidence of carcinomas containing both subunits, comparing with multiple incidences of isolated subunit-positivity.

Results

HCG-subunits in 'ectopic' choriocarcinoma with adenocarcinoma

Syncytiotrophoblastic cells usually showed both positivities for hCG-subunits. Many cytotrophoblastic cells were also synchronously positive for both subunits, but in the typical nests of choriocarcinoma hCGβ-positive cytotrophoblastic cells appeared to be slightly more numerous than hCGα-positive ones (Fig. 1a and b). In transition to adenocarcinoma, there were several cells positive for either isolated subunit, and several hCGα-positive cells were identified in tubular adenocarcinoma (Fig. 1c). In comparison with serial sections stained with Grimelius silver technique, the cells positive for both hCG-subunits were not argyrophilic.

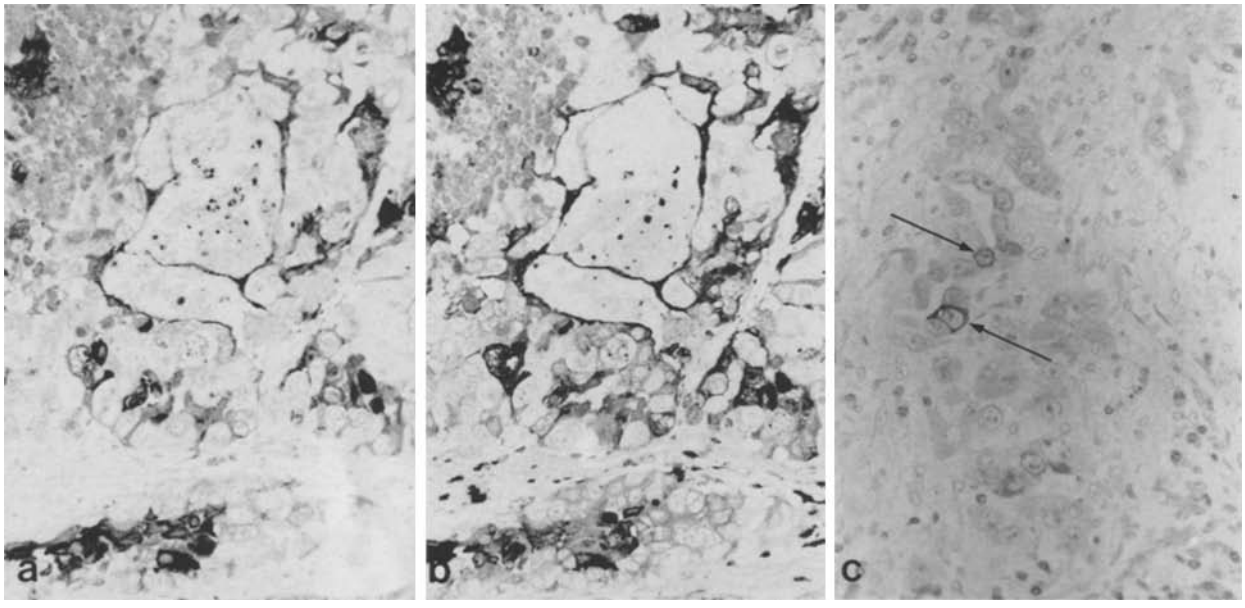

Fig. 1. Serial sections of a choriocarcinoma with andenocarcinoma (45 years old, male) were stained with anti-hCGα (**a** and **c**) and β (**b**) antibodies. Many trophoblastic cells are synchronously positive for both subunits (**a** and **b**). A few immunoreactive cells for isolated hCGα are present in a portion of tubular carcinoma (c, *arrows*). PAP-immunostaining. **a** and **b**, ×150, and **c**, ×300

Table 1. Incidence of hCG-subunit-immunoreactivity in gastric carcinoma

Histological group	Number of cases	Number of positive cases		
		hCGα	hCGβ	(both)
Papillotubular	57	21	23*	(15)***
Early	30	11	8**	(7)***
Advanced	27	10	15	(8)
Microtubular-mucocellular	67	18	40*	(11)
Early	20	7	15**	(5)
Advanced	47	11	25	(6)
Total	124	39	63	(26)

Significant difference was detectable in incidences of hCGβ-positivities between papillotubular and microtubular-mucocellular groups (* $p<0.05$), which may be due to differences in early carcinomas (** $p<0.005$, Chi-square test). Incidence of carcinomas bearing both subunits seemed to be higher in papillotubular carcinomas than that deduced from multiple incidences of both isolated positivities of the corresponding groups (*** $p<0.05$, z-test)

HCG-subunits in gastric carcinoma

Immunoreactive cells for hCG-subunits were identified in 76 of 124 gastric carcinomas (Table 1) in variable numbers with hCGα in 39 (31.5%) and hCGβ in 63 cases (50.8%). The number of positive cells was usually small: the number of hCGα- or β-positive cells were less than ten in 23 of 39, or 20 of 63 cases respectively. HCGα-immunoreactivity was present in 36.0% of early carcinoma and 28.4% of advanced carcinomas. HCGβ-positive cells were demonstrated in 46.0% of early and 54.1% of advanced carcinomas. 26 carcinomas contained cells positive for both units (12 early and 14 advanced). The incidences of hCG-subunit-positivities were not significantly different between early and advanced carcinomas ($p<0.05$).

In the papillotubular group (Intestinal type) 29 of 57 cases exhibited hCGα or β-positive cells (21 and 23 cases respectively). HCGα-immunoreactive cells were usually solitary within the neoplastic glands. They were like intraglandular endocrine cells: triangular or ovoid in shape (Fig. 2a). However, hCGβ-positive cells were found in deranged glands or in solitary and clustered in the interstitium (Fig. 3). The shape of hCGβ-immunoreactive cells was variable; they might be slightly spindle shaped, flat, or large and irregular.

15 cases contained cells positive for both units. The incidence of this finding was significantly higher than that deduced from incidences of isolated subunit-positivities ($p<0.05$) if it was assumed that they were independent. However, immunoreactive cells for both hCG-subunits were present in different parts in 11 carcinomas. A few or several synchronous cells were identified in only four carcinomas, in which positive cells for isolated hCG-subunits were also present in close proximity (Fig. 4a, b).

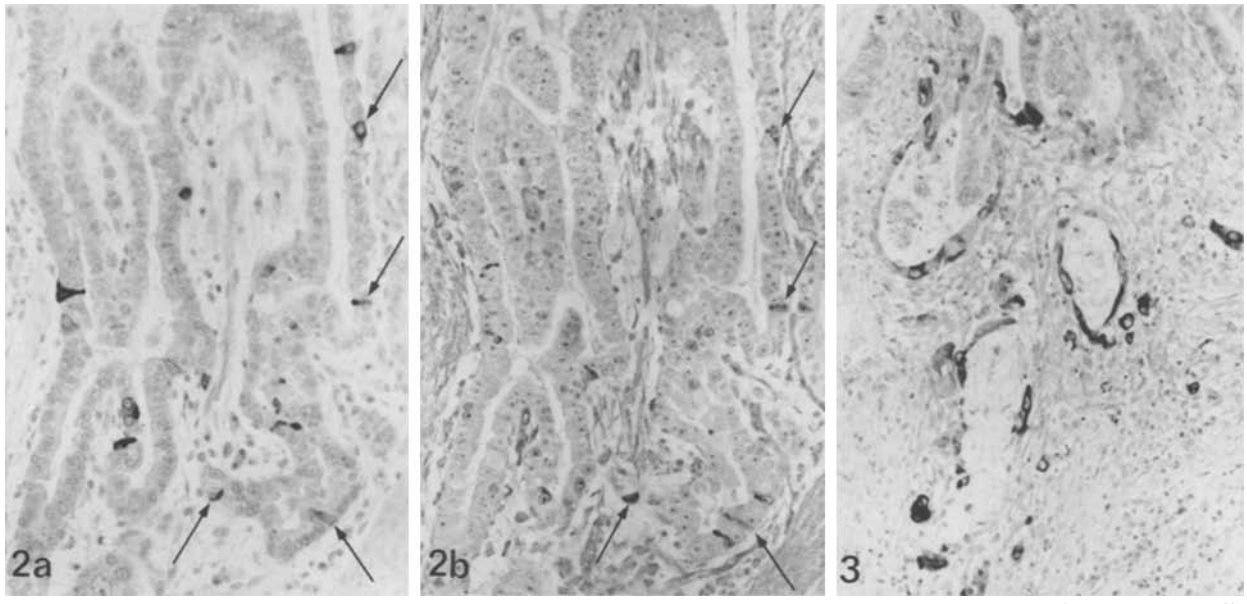

Fig. 2. Serial sections of an advanced papillotubular carcinoma (48 years old, male) were immunostained with anti-hCGα antibody (**a**) and stained by Grimelius silver technique (**b**). Some hCGα-positive cells are argyrophilic (*arrows*). In another serial section, no hCGβ-immunoreactive cell was identified. ×200

Fig. 3. Many hCGβ-positive cells in an advanced papillotubular carcinoma (77 years old, male). PAP-immunostaining for hCGβ, ×150

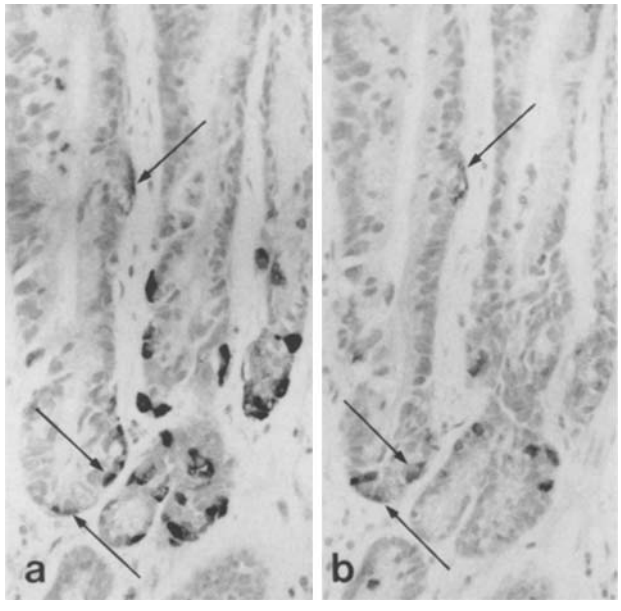

Fig. 4. Serial sections of an early papillotubular carcinoma (79 years old, male) were immunostained with anti-hCGα (**a**) and β (**b**) antibodies. A few cells are synchronously positive for both subunits (*arrows*). In another adjacent sections, these positive cells were argyrophilic. PAP-immunostaining, ×240

In comparison with argyrophilic cells in serial sections, some of hCGα-positive cells were argyrophilic in 13 of 21 cases (Fig. 2a and b), whereas such a relation was extremely rare between hCGβ-positive cells and argyrophilic cells – in three of 23 cases ($p<0.005$). These three cases also contained hCGα-positive argyrophilic cells.

In the microtubular-mucocellular group (Diffuse type) 47 of 67 carcinomas showed hCGα- or β-positivity (18 and 40 cases respectively). In early carcinomas, hCGβ-positivity was found in 15 of 20 cases, and the incidence was significantly higher than its counterpart in the papillotubular group ($p<0.005$).

HCGα-positive cells (Fig. 5a) were usually solitary cells in the micronests or infiltrating in the interstitium. HCGβ-positive cells were observed more often in clustered form, and they appeared to be relatively pleomorphic. There were 11 cases containing cells positive for both units, but the incidence of this change could be deduced when both positivities were assumed to be independent ($p<0.05$). In serial sections, both cells were apparently different. Some of hCGα-positive cells showed argyrophilia in 10 of 18 cases (Fig. 5a, b), but there were no hCGβ-positive cells, which were argyrophilic.

In 50 early and 59 advanced carcinomas less than 10.0 cm in size, the site of origin could be determined (Table 2). The location did not appear to affect the incidence of hCG-subunit-positivities ($p<0.05$).

There were 16 cases of gastric carcinoma which contained more than ten hCGα-positive cells. In serial sections, there was no immunoreactivity

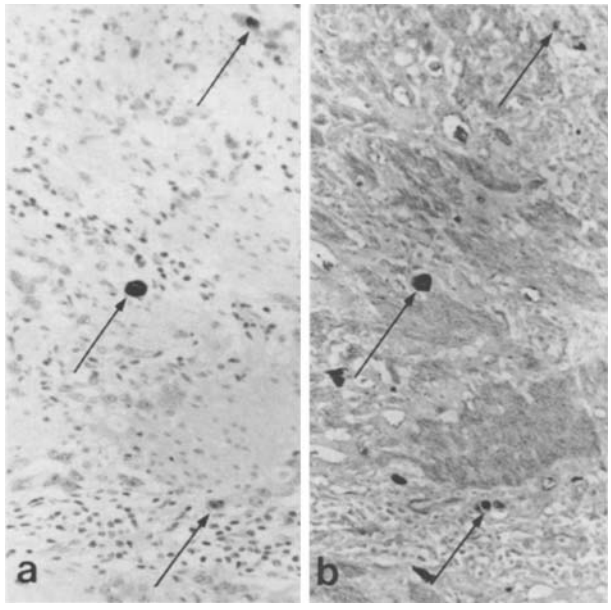

Fig. 5. Serial sections of an advanced carcinoma of microtubular-mucocellular group (35 years old, female) were immunostained with anti-hCGα antibody (**a**) and stained by Grimelius silver technique (**b**). HCGα-positive cells are argyrophilic (*arrows*). ×200

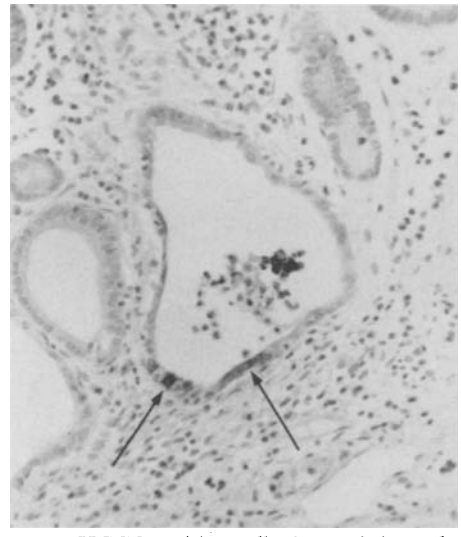

Fig. 6. HCGβ-positive cells (*arrows*) in a deranged gland of non-neoplastic antral mucosa (49 years old, female). Other glands show intestinal metaplasia. PAP-immunostaining for hCGβ, ×200

Table 2. Relation between hCG-subunit-positivity and the location of the carcinoma

Location	Number of cases	Number of positive cases		
		hCGα	hCGβ	(both)
Antrum	51	17	29	(11)
Body	40	12	16	(8)
Cardia	18	7	7	(5)
Total	109	36	52	(24)

The location did not affect the positivities of hCG-subunits (Chi-square test, $p < 0.05$)

for hLHβ, hFSHβ or hTSHβ in hCGα-positive cells.

In the surrounding mucosa of 50 early carcinomas, 42 sections showed one type of glands and 8 carcinomas were situated at the margin of pyloric and fundic glands. Thus, 31 pyloric and 27 fundic glands were evaluated.

There were hCGα-positive cells, in variable numbers, in all 31 sections of pyloric gland mucosa and in 13 of 27 fundic mucosae. As has been described (Fukayama et al. 1986a), hCGα-positive cells corresponded to endocrine-like cells both in pyloric and fundic glands.

HCGβ-positive cells were rarely observed in three sections of pyloric and one of fundic gland mucosa. In these sections, a few hCGβ-positive cells were present in deranged glands with or without neutrophilic infiltration (Fig. 6).

Discussion

It is well established that human chorionic gonadotropin (hCG) or its subunits are produced in non-trophoblastic neoplasms and neoplastic cells (Hattori et al. 1980; Rosen et al. 1980). In gastric carcinomas, increased blood level of immunoreactive hCG was observed in 10.5% to 23.5% of cases (Braunstein et al. 1973; Cailani et al. 1976; Hattori et al. 1978; Wasada et al. 1979). However, when hCG is assayed in urine (Papapetrou et al. 1980) or in tumour tissues (Hattori et al. 1978), its incidence of detection more than doubles. In the present study, hCGα- or β-positive cells were found in 61.3% (31.5% and 50.8% respectively) and the incidence of hCGβ-positivity is higher than that of other immunohistochemical studies (7% to 34%, Mori et al. 1982; Ito and Tahara 1983; Manabe et al. 1985; Wittekind et al. 1986). Because sampling methods did not seem to be different among the studies, the difference might be due to sensitivity of immunohistochemistry: different antisera may be used or staining method might be with or without pronase treatment. It is unlikely that our results suffered from false positivity, because our control studies of immunohistochemistry of hCG-subunits seemed to be comparable with

or more strict than those used in other work. Nevertheless, the incidence of hCG-subunit-positivity in this study may be found if the other sensitive methods described above were applied. Therefore, hCG-subunit production in gastric carcinomas may be fairly frequent and seems to be more frequent than that in non-small cell carcinomas of the lung (hCGα: 8/60, hCGβ: 14/60, Fukayama et al. 1986b) or in carcinomas of the rectosigmoid colon (hCGα: 1/50, hCGβ: 14/50, Fukayama et al. 1987).

In previous immunohistochemical studies, the α-subunit of hCG has not been studied. This may be due to the fact that α-subunit is nearly identical in structure to other glycoprotein hormones which is coded by a single common gene (Fiddes and Talmage 1984); the β-subunit has been assumed to limit the production of 'biologically active' combined hormone (Pierce and Parsons 1981). However, unbalanced production of hCG-subunits has been demonstrated in some neoplasms and carcinoma cells (Weintraub and Rosen 1973; Tashjian et al. 1973; Rosen et al. 1980; Hattori et al. 1980; Heitz et al. 1983). HCG-subunits are apparently regulated by a different gene (Fiddes and Talmadge 1984) and the gene-regulation is not always coordinate even in trophoblast-derived cells (Brunside et al. 1985). In the present study, many syncytio- and cytotrophoblastic cells were synchronously positive for both subunits in choriocarcinoma. Conversely, both positive cells were distinctly different populations in most of gastric carcinomas. Although expression of isolated subunit-positivities in papillotubular carcinomas did not appear to be independent in the analyses of their incidences, synchronous cells were extremely rare in four of 15 papillotubular or none of 11 microtubular-mucocellular carcinomas which contained both immunoreactive cells. Therefore, unbalanced distribution or production may be one of characteristics of hCG-production in gastric carcinoma.

It is very interesting that Hustin et al. (1985) observed solitary immunoreactive cells for isolated hCGα in testicular immature teratomas. These immunoreactive cells were intraglandular endocrine-like cells with close proximity to argyrophilic cells. In gastric carcinomas, hCGα-positive cells were not immunoreactive for β-subunits of glycoprotein hormones with exception of rare hCGβ-immunoreactivity. HCGα-positive cells appeared to have endocrine-like configuration in the glandular structure of papillotubular carcinoma and some hCGα-positive cells exhibited definite argyrophilia in 23 of 39 positive cases. Furthermore, we have recently demonstrated that isolated hCGα-immunoreactivity is present in endocrine cells of non-neoplastic stomach, lung and rectosigmoid colon (Fukayama et al. 1986a, 1986b, 1987). Thus, the expression of isolated hCGα may reflect endocrine cell-differentiation in gastric carcinomas, as well as non-neoplastic mucosa. Future studies using immunoelectron microscopy, however, will be necessary to elucidate the heterogeneity of argyrophilia in hCGα-positive cells in gastric carcinoma.

In contrast to the hCG-subunit profile of the non-neoplastic gastric mucosa, hCGβ-immunoreactivity was much more often seen than hCGα in gastric carcinomas. This predilection to hCGβ did not appear to be related to invading depth (no significant difference in early or advanced carcinomas) or to the original site of the carcinomas. HCGβ-positivity seemed to be more frequent in the microtubular-mucocellular group with significant differences in early carcinomas. In the rectosigmoid colon, only infiltrating carcinomas showed hCGβ-positive cells, but the incidence was unrelated to the invading depth (Fukayama et al. 1987). Because mucosal carcinomas of the stomach, especially microtubular-mucocellular group, show more deranged tubular structure than that of the colon carcinoma, some epitheliomesenchymal interaction per se may affect expression of hCGβ in stomach and colon carcinomas. In four sections of non-neoplastic mucosa, which surrounded early carcinomas, epithelial cells of deranged glands showed hCGβ-positivity. HCGβ-immunoreactive cells were also observed in disorganized alveoli of the lung (Fukayama et al. 1986b). Therefore the expression of hCGβ might not necessarily require neoplastic transformation: HCGβ may be expressed through an epithelio-mesenchymal interaction common in carcinoma and in inflammation of some non-trophoblastic tissues.

We have suggested that incidences of carcinomas containing both subunits appeared to be higher than that deduced from isolated positivities in papillotubular carcinoma. Along the line of speculation above, however, it may be due to relatively diverse tubular structures in this group: expression of hCGβ was likely to occur in deranged tubular structures. Thus, hCG-subunits in gastric carcinoma might be regulated by an independent mechanism.

In the stomach, choriocarcinomas with or without adenocarcinoma have rarely been documented (Mori et al. 1982), but they occur relatively more often there than in other intestinal tract areas (Metz et al. 1985). Although hCG-subunit-profile was unbalanced in gastric carcinoma, there were cells positive for both isolated subunits in 26 of

124 cases. This frequency appears higher than in lung carcinoma (1/60) or in the rectosigmoid colon (0/50) (Fukayama et al. 1986b, 1987). It is possible that cells expressing both isolated subunits may hybridize and develop into trophoblastic cells, although such somatic hybridization (Warner 1974) has not been observed in vivo. In the placenta, hCG-subunits are postulated to be sequentially expressed or regulated from α to β (Hoshina et al. 1985). Furthermore, a particular DNA arrangement at the hCGα-locus is suggested to be more susceptible to development of choriocarcinoma (Hoshina et al. 1985). Neoplastic endocrine cells with hCGα-production might be a likely source of trophoblastic cells following mutation in the sensitive portion of hCGα gene. The precise sequence of trophoblast-development in non-trophoblastic tissues, therefore, should be evaluated by gene modulation or hybridization of cloned carcinoma cells expressing isolated hCG-subunits.

We have demonstrated unbalanced distribution of hCG-subunits in gastric carcinomas. The subunit-profile of hCG in carcinomas was different from normal or non-neoplastic mucosa. However, the difference may be quantitative rather than qualitative: hCG-subunits may be regulated by an independent mechanism, but commonly in normal and neoplastic stomach. Future studies on this issue should also include assessment of the biological significance (Stanbridge et al. 1982) and a biochemical characterization (Fein et al. 1980; Parsons et al. 1982; Hussa et al. 1986) of both isolated subunits of hCG in the placenta and extraplacental tissues.

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