Virchows Arch [Pathol Anat] (1986) 409:365–373

Virchows Archiv A

Human chorionic gonadotropin in primary liver carcinoma in adults

An immunohistochemical study

Yasuni Nakanuma[1], Masashi Unoura[2], Hiroshi Noto[2], and Goroku Ohta[1]

[1] Second Department of Pathology, and [2] First Department of Internal Medicine, School of Medicine, Kanazawa University, Kanazawa 920, Japan

Summary. Production of human chorionic gonadotropin (hCG) by extragonadal tumours is not a rare phenomenon. In the liver, similar results have been reported in hepatoblastomas. The present study was attempted to survey hCG level in serum and hCG-immunoreactivity in primary liver carcinoma in adults. Although hCG was elevated in serum in 2 (22.2%) of 9 autopsied cases with hepatocellular carcinoma (HCC), the hCG-reactivity of carcinoma cells was found in 2 (2.1%) of 95 HCC cases. Carcinoma cells positive for immunoreactive hCG was found in 2 (15.4%) of 13 cases with cholangiocarcinoma (CC). The patients with hCG-immunoreactivity in carcinoma and/or elevated serum level of hCG failed to reveal distinct clinical and endocrinological disturbance due to excess hCG. The hCG-positive cells were focal within the carcinoma and showed poor histological differentiation in both HCC and CC, and there were no trophoblastic cells. It is suggested that hCG is one of the hormones produced by primary liver carcinoma in adults and can be localised immunohistochemically in a small number of poorly differentiated carcinoma cells.

Key words: Hepatocellular carcinoma – Cholangiocarcinoma – Human chorionic gonadotropin – Immunohistochemistry

Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein, normally secreted by syncytiotrophoblast cells of the placenta (Midgley and Pierce 1962; Dreskin et al. 1970). Germ cell tumors frequently produce and secrete hCG which is now a useful marker in such tumours (Taylor et al. 1978; Miyake et al. 1981). Recently, the ectopic presence of hCG-like substance has been demonstrated by a specific radioimmunoassay (RIA) technique in various

Offprint requests to: Y. Nakanuma at the above address

normal tissues including the colon and liver (Yoshimoto et al. 1977). Production of hCG by extragonadal tumours, especially those of the stomach and lung, is well known, and there have been several reports on immunohistochemical localization of hCG in tumour cells and their morphology (Dreskin et al. 1970; McManus et al. 1976; Okano 1979; Ito and Tahara 1983). It is well known that some of the extragonadal tumours with hCG production are composed of trophoblastic cells (choriocarcinoma), some of mixed histology of trophoblastic and nontrophoblastic elements, and the remaining of nontrophoblastic elements (Fine et al. 1962; Kuwajima et al. 1981; Tahara et al. 1982; Watanabe et al. 1982).

In the liver there have been several reports on hepatoblastoma producing hCG and presenting with sexual precocity (Watanabe et al. 1982; Beach et al. 1984) where hCG is identified in tissue resembling syncytiotrophoblast (Watanabe et al. 1982). However, there have been few reports on hCG and primary liver carcinoma in adults to date (Braunstein et al. 1973A and 1973B; Yoshimoto et al. 1980). We have recently seen hepatocellular carcinoma (HCC) from an adult who disclosed elevated serum level of hCG and immunoreactivity of hCG in carcinoma cells. This experience prompted us to survey hCG in serum and carcinoma tissue in primary liver carcinoma in adults.

Materials and methods

1. Serum level of hCG

Serum level of hCG was measured in 9 autopsied cases with HCC by double-antibody RIA using tracer quantities of hCG iodinated with ^{125}I and rabbit antiserum against β-subunit of hCG (hCG RIA kit, CIS). Sera were frozen at $-80°$ C until used. In these 9 cases the immunohistochemical study for hCG was also carried out in the carcinoma tissue, as described below.

2. Morphologic examination

A total of 108 cases (98 autopsied and 10 surgically resected liver specimens) of primary liver carcinoma were investigated. These were composed of 95 cases of HCC and 13 cases of cholangiocarcinoma (CC). They were all over 30 years of age and 80 were in males. Choriocarcinoma, hepatoblastoma, yolk sac tumour and carcinoid of the liver were not included. The material was obtained from the files of the Second Department of Pathology, Kanazawa University School of Medicine (1979–1984), and the Department of Pathology of the Fukui Prefectural Hospital (1980–1982) and of the Ishikawa Prefectural Hospital (1980–1981). All specimens were fixed in 10% neutral formalin. More than three blocks including carcinoma tissue were obtained from each case and embedded in paraffin.

Light microscopy. 5 μm sections of these blocks were stained with H & E, Gomori's reticulin, periodic acid-Schiff (PAS), Shikata's orcein for intracytoplasmic HBsAg, Grimelius technique for argyrophil reaction and Masson-Fontana for argentaffin reaction (Shikata et al. 1974; Sano 1976).

Immunohistochemistry was carried out on 5 μm thick deparaffinized sections using the avidin-biotin-peroxidase complex method (Hsu et al. 1981). Briefly, sections were pretreated with methanolic-H_2O_2 and then normal goat serum (diluted in 1:5 in phosphate buffer solution (PBS)). Primary rabbit antisera to whole-hCG (Dako Co. Denmark) were diluted in 1:500 in PBS, and applied for 2 h at room temperature. The sections were then treated with biotinylated goat anti-rabbit IgG (Vector Lab. USA) at 1:400 dilution for 30 min. During this time

the avidin-biotin-peroxidase complex was prepared (25 µl of avidin DH + 25 µl of biotinylated peroxidase + 5 ml of PBS (Vector Lab. USA)), and it was applied for 30 min. The histochemical reaction for peroxidase was carried out using a H_2O_2-3,3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Co. USA) solution.

Several staining and specificity controls were performed. Normal placental tissue from the 15th week of gestation was always used at the same time as a positive control. No positive stain was obtained when H_2O_2 without DAB or DAB without H_2O_2 was applied. Positive stain was abolished when PBS or nonimmune serum was used as the first layer as well as after preincubation of diluted primary antiserum with 50 µg/ml of hCG (Sigma Chemical Co., USA).

Results

1. Serum level of hCG (Table 1)

Two (22.2%) of the 9 patients with HCC revealed elevated serum level of hCG, and one of the two also disclosed hCG-immunoreactivity in carcinoma tissue. The remaining 8 cases failed to reveal such hCG positive cells.

2. Incidence of hCG-positive carcinoma cells in primary liver carcinoma

These cells were found in 2 (2.1%) of the 95 cases of HCC and 2 (15.4%) of the 13 cases of CC. Non-neoplastic hepatic tissue failed to reveal immuno-

Table 1. Human chorionic gonadotropin in serum and carcinoma cells of 9 cases with hepatocellular carcinoma

Case	Serum level* (IU/ml)	hCG-immunoreactive carcinoma cell**	Case	Serum level* (IU/ml)	hCG-immunoreactive carcinoma cell**
1	54.3	+	6	1.4	–
2	6.4	–	7	13.8	–
3	10.0	–	8	3.8	–
4	3.2	–	9	5.5	–
5	6.5	–			

* = normal: ≤ 10 IU/ml, ** = immunohistochemical method, + = positive, – = negative

Table 2. Main features of 4 autopsied cases of primary liver cancer with hCG-immunoreactivity

Case	Age (years) Sex	Main tumor histology	Histology of hCG-positive carcinoma cell	Nonneoplastic liver
A	57, male	Trabecular HCC	Pleomorphoic and giant cells with desmoplasia	Alcoholic liver disease
B	65, male	Trabecular HCC	Pleomorphic and giant cells	Chronic active hepatitis
C	75, female	Tubular adenocarcinoma	Poorly differentiated adenocarcinoma	Mild liver fibrosis
D	55, male	Tubular adenocarcinoma	Poorly differentiated adenocarcinoma	Mild liver fibrosis

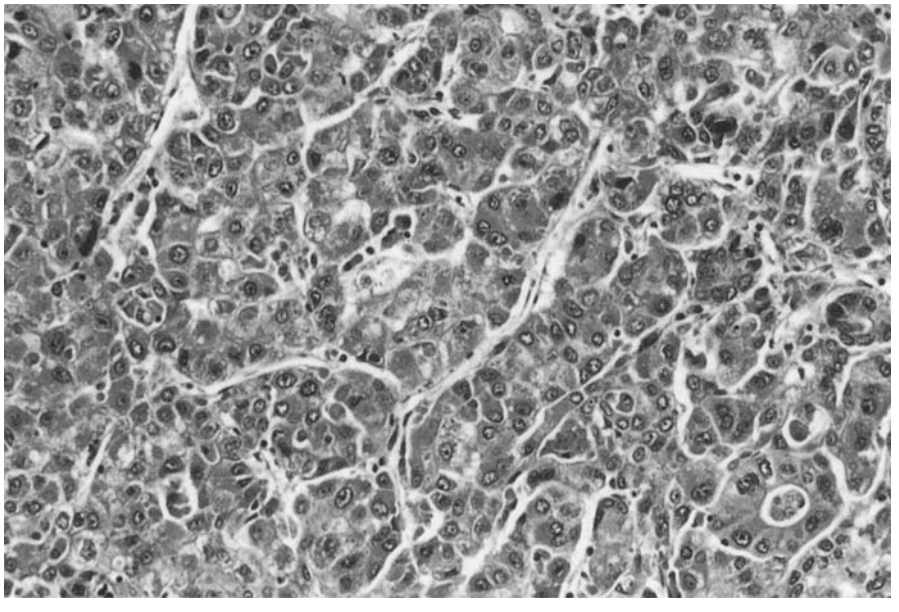

Fig. 1. Well differentiated trabecular hepatocellular carcinoma with pseudoglandular element. HE., ×180

reactivity for hCG in all cases. The main clinicopathological features of these 4 cases are shown in Table 2. There was neither gynaecomastia nor feminization in 3 males, and amenorrhoea was not found in a female patient who was post-menopausal. The two hCG-positive HCC cases showed a well-differentiated trabecular pattern with bile production (Fig. 1). There were focally desmoplastic areas where carcinoma cells showed pseudoglandular pattern or small-sized cell cluster with pleomorphic or giant cells, some of them being positive for hCG (Fig. 2) in one HCC case. In the other case there were focally pleomorphic areas including bizarre and multinucleated giant cells, some of them also being positive for hCG (Fig. 3). Non-neoplastic tissue showed no evidence of cirrhosis in these two cases, though 81% of the remaining hCG-negative HCC cases were cirrhotic. In two cases of CC showing immunoreactivity for hCG, hCG was preferentially positive in poorly differentiated areas (Fig. 4) where bizarre and giant cells were also seen, whereas as majority of the carcinoma tissue showed moderately differentiated adenocarcinoma where hCG was weakly stained or negative. The intensity of the immunostaining in the positive cells was variable. There was, however, neither distinct evidence of trophoblasts nor argyrophil or argentaffin reaction in these hCG-positive HCC and CC. There was no significant difference in the morphology of carcinoma between HCC with hCG-immunoreactivity and those without, and the same was true of CC.

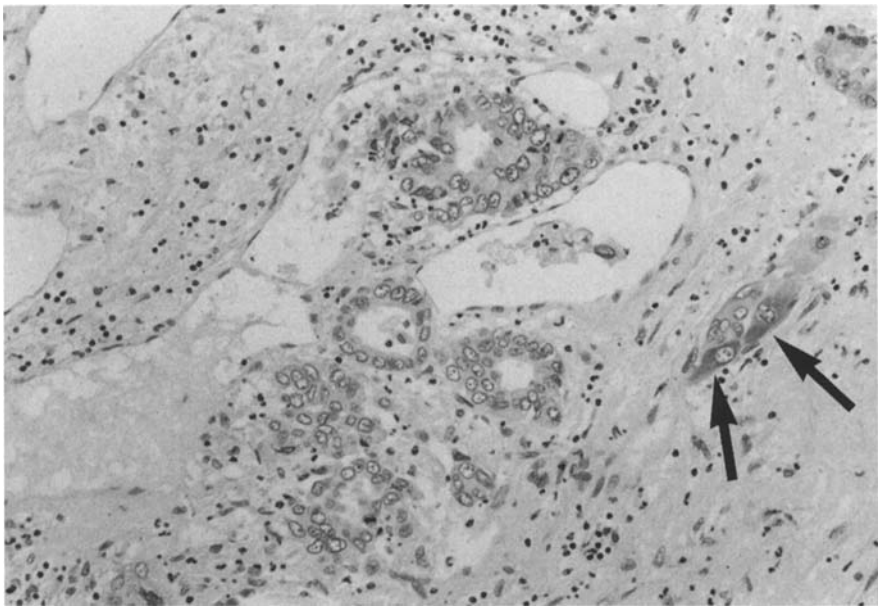

Fig. 2. Some of carcinoma cells (*arrows*) with pleomorphism show immunoreactivity for hCG. ABC method for hCG and haematoxylin. The same case of Fig. 1. ×180

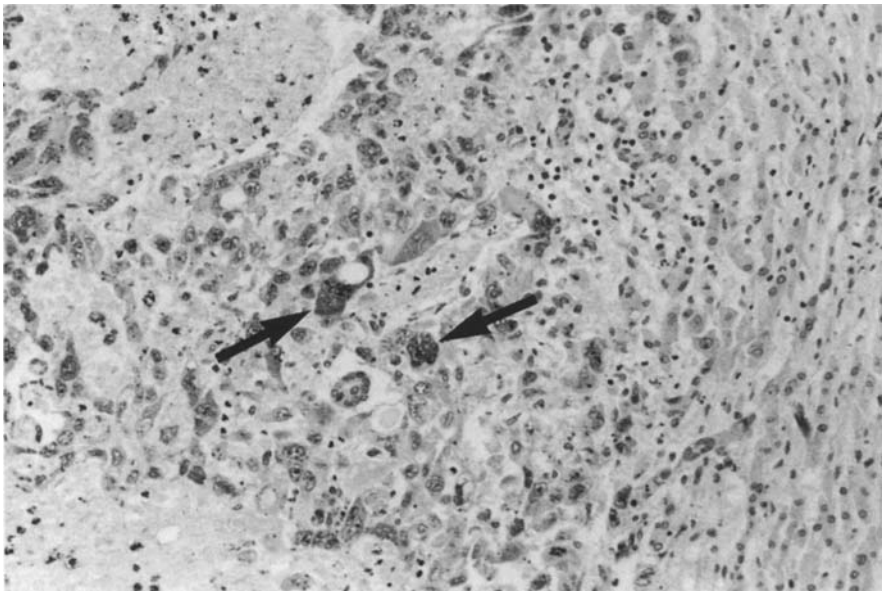

Fig. 3. Some of giant cells (*arrows*) are positive for hCG. ABC method for hCG and haematoxylin. ×180

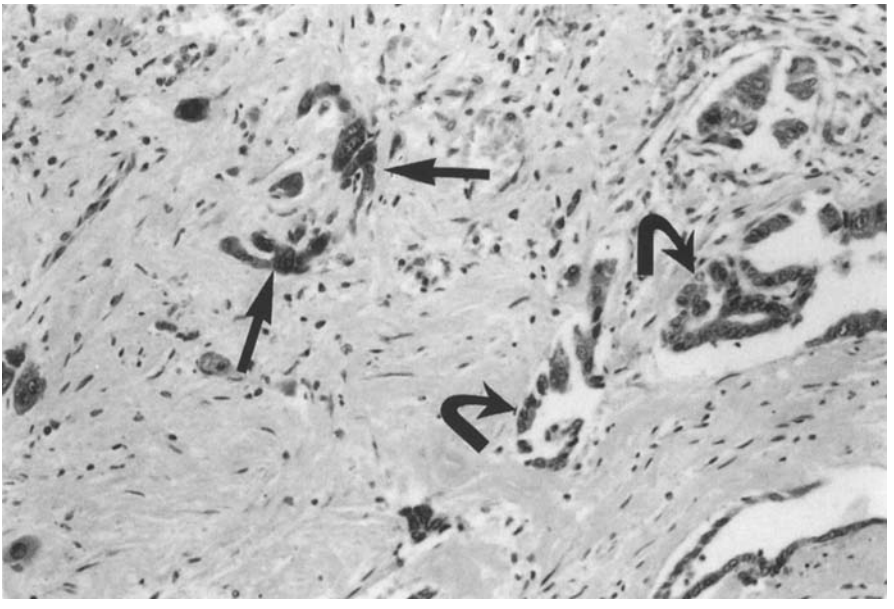

Fig. 4. Cholangiocarcinoma. Poorly differentiated adenocarcinomas (*straight arrows*) show an intensive staining for hCG and tubular elements (curved arrows) were weakly positive or negative for hCG. ABC method for hCG and haematoxylin. × 250

Discussion

Neoplasms are known to produce a number of antigenic markers, which are expressed during ontogeny and oncogenesis (Gold and Freedman 1965; Fine et al. 1967; Haur and Saxena 1974; McManus et al. 1976). Among them, hCG is expressed in the earliest part of embryonic life (Haur and Saxena 1974) and recently developed methodologies have enabled us to examine hCG levels in serum and hCG-immunoreactivity in tissue routinely. As a result, the production of hCG by a variety of extragonadal tumors is known not to be a rare phenomenon and has become one of the main concerns in oncology as well as endocrinology (McManus et al. 1976; Yoshimoto et al. 1980).

In the liver, there have been several clinicopathological and immunohistochemical studies on hepatoblastoma producing hCG. There have been, however, few studies on hCG in primary liver carcinoma in adults, mainly concerning serum level of hCG and clinical features of the patients (Braunstein et al. 1973A and 1973B; Yoshimoto et al. 1980; Miyake et al. 1981). The reported incidence of increased hCG level, when measured in serum, is usually from 10% to 20% in HCC and from 0% to 10% in biliary tract carcinoma. Braunstein et al. (1973A), who compared clinical features of the HCC patients with measurable serum level of hCG with those without, found that the hCG-positive group was older and had a lower incidence

of association with elevation of serum α-fetoprotein level than did the hCG-negative group.

In this study we have successfully demonstrated the localization of hCG in carcinoma cells of HCC and CC using the avidin-biotin-peroxidase complex method. Tumour cells continating hCG were found in 2.1% of HCC and 15.4% of CC cases, though the distribution of the positive cells in the tumours was focal. There were, however, no trophoblastic elements in the HCC and CC. The incidence of HCC with hCG-immunoreactivity was low when compared with those of stomach, lung and pancreas (Okano 1979; Ito and Tahara 1983). The reason why the incidence of hCG in serum was high as much as 22.2% of HCC patients, while hCG-immunoreactivity was rare in HCC tissue, is only speculative. Sampling error of the examined specimens may allow this discrepancy because distribution of hCG-positive cells was focal. The same was found in gastric carcinoma reported by Kuwajima et al. (1981).

Extragonadal tumours producing hCG could be divided into those with trophoblasts and those without. Tumours with trophoblasts might originate from ectopic or hamartomatous gonadal tissue, or they might represent abnormal differentiation of carcinoma cells to acquire a primitive germ cell nature with an ability to produce hCG (Greenstein 1974; Cochrane and Williams 1976; Okano 1979; Ito and Tahara 1983). However, the mechanism of hCG production by tumours without trophoblastic cells is problematic. The present study disclosed that immunoreactivity for hCG was preferentially found in undifferentiated or poorly differentiated cells and there were no trophoblastic elements in either HCC or CC. Tahara et al. (1982) reported similar findings in gastric carcinoma without trophoblastic elements and suggested that poorly differentiated carcinoma has much more polypeptide hormones, amines and hCG than the well differentiated type. Okano (1979) suggested that a degree of histological differentiation of the tumour rather than the origin is more important for ectopic synthesis of hCG. These suggestions seem applicable to our cases. DNA coded for the ectopic synthesis of polypeptides, hCG or proteins which are retained but suppressed in adult organs, becomes derepressed during dedifferentiation or convergence of the carcinoma (Greenstein 1974; Cochrane and Williams 1976). However, a majority of the poorly differentiated carcinoma areas of the HCC or CC cases examined were negative and only limited and focal areas of a small number of cases were positive for hCG production. The mechanism of hCG production in HCC and CC without trophoblasts seems complicated and there are probably, several control factors for hCG expression at the cellular level.

Evidence of endocrinological disturbance induced by excessive hCG, such as gynaecomastia and amenorrhae, are occasionally reported in hCG-producing extragonadal tumours with trophoblast (Charles et al. 1973; Yoshimoto et al. 1980; Miyake et al. 1981; Tahara et al. 1982; Ito and Tahara 1983). Such features are, however, usually absent or not prominent in those without trophoblastic elements, as in the present four cases. The reasons for this absence of endocrinological disturbance have been thought to be

that the serum or tissue level of hCG is not high enough to bring about the clinical signs, and/or that hCG produced by extragonadal tumours without trophoblast has little or no biological activity (Kuwajima et al. 1981; Watanabe et al. 1982).

In conclusion, the data indicate that the incidence of ectopic synthesis of hCG in HCC and CC is not high, and the hCG-positive carcinoma cells in both tumours are focal in distribution when present and poor in morphological differentiation. These factor have not been described hitherto in the field of hepatology.

Acknowledgment. We are very grateful to Dr. Kenji Doishita, Fukui, and Dr. Morimoto Hayashi, Kanazawa, for permission of examination of hepatocellular carcinoma.

References

Abelev GI, Assecritova IV, Kraevsky NA, Perova SD, Perevodchikova NI (1967) Embryonal serum α-globulin in cancer patients: Diagnostic value. Intern J Cancer 2:551–558.

Beach R, Betts P, Radford M, Millward-Sadler H (1984) Production of human chorionic gonadotropin by a hepablastoma resulting in precocious puberty. J Clin Path 37:734–737

Braunstein GD, Vogel CL, Vaitukaitis JL, Ross GT (1973A) Ectopic production of hCG in Ugandan patients with hepatocellular carcinoma. Cancer 32:223–226

Braunstein GD, Vaitukaitis JL, Carbone PP (1973B) Ectopic production of human chorionic gonadotropin by neoplasm. Ann Intern Med 78:39–45

Charles MA, Claypool R, Schaaf M (1973) Lung carcinoma with production of three placental proteins. Arch Intern Med 132:427–431

Cochrane M, Williams R (1976) Humoral effects of hepatocellular carcinoma. In: Hepatocellular carcinoma. K Okuda, RL Peters (eds) John Wiley & Sons, New York, pp 333–352

Dreskin RB, Spicer SS, Greene WB (1970) Ultrastructural localization of chorionic gonadotropin in human term placenta. J Histochem Cytochem 18:862–874

Fine G, Smith RW, Pathter MR (1962) Primary extragenital choriocarcinoma in the male subject. Case report and review of literature. Am J Med 32:776–794

Gibson JB, Sobin LH (1978) Histological typing of tumors of the liver, biliary tract and pancreas. World Health Organization. Geneva

Gold P, Freedman SO (1965) Specific carcinoembryonic antigens of the human digestive system. J Exp Med 122:467–481

Greenstein JP (1974) Biochemistry of Cancer. Academic Press, New York

Haur F, Saxena BB (1974) Detection of a gonadotropin in rabbit blastocytes before implantation. Science 185:444–445

Hsu M, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody [PAP] procedures. J Histochem Cytochem 29:557–580

Huang W, Blizzard RM, Migeon CJ, Camacho MD, Nyhan WL (1963) Precocious puberty in a boy with hepatoma and circulating gonadotropin. J Pediatr 63:895–903

Ito H, Tahara E (1983) Human chorionic gonadotropin in human gastric carcinoma. A retrospective immunohistochemical study. Acta Path Jap 33:287–296

Kuwajima M, Sato H, Sekiguchi S, Tomita K (1981) Gonadotropin-like substance in gastric carcinoma and pathology. Gan No Rynsho 27:289–291 (In Japanese)

McManus LM, Naughton MA, Martinez-Hernandez A (1976) Human chorionic gonadotropin in human neoplastic cells. Cancer Res 36:3476–3481

Midgley AR, Pierce GB Jr (1962) Immunohistochemical localization of human chorionic gonadotropin. J Exp Med 115:289–294

Miyake A, Hirota Y, Kobayashi Y, Hongo J, Yokoyama Y (1977) HCG producing tumors. Seijinbyo 17:38–50 (In Japanese)

Okano T (1979) Immunohistochemical and clinical study of hCG-producing tumors. Jpn J Clin Oncol 9:215–224 (In Japanese)

Sano Y (1976) Histological technics: Theoretical and applied. 5th ed. Tokyo: Nanzando Co 1976 (In Japanese)

Shikata T, Uzawa T, Yoshiwara N, Akatsuka T, Yamazaki S (1974) Staining method of Australia antigen in paraffin section – detection of cytoplasmic inclusion bodies. Jpn J Exp Med 44:25–36

Tahara E, Ito H, Nakagami K, Shimosato F, Yamamoto M, Sumi K (1982) Scirrhous argyrophil cell carcinoma of the stomach with multiple production of polypeptide hormones, amine, CEA, lysozyme, and HCG. Cancer 49:1904–1915

Taylor CR, Kurman RJ, Warner NE (1978) The potential value of immunohistologic techniques in the classification of ovarian and testicular tumors. Hum Pathol 9:417–427

Watanabe I, Morinaga S, Kojima M, Yamaguchi M, Matsumura Y (1982) hCG-producing hepatoblastomas. Shonigeka 14:453–463 (In Japanese)

Yoshimoto Y, Wolfsen AR, Odell WD (1977) Human chorionic gonadotropin-like substance in nonendocrine tissues of normal subjects. Science 197:575–577

Yoshimoto Y, Hattori M, Goto B, Matsukura S, Fujita T, Arakawa S (1980) Human chorionic gonadotropin (hCG). Rynshokagaku 16:841–848 (In Japanese)

Accepted January 29, 1986