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Localization of the β Subunit of Human Chorionic Gonadotrophin on Various Tumors*

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Abstract—*Using an immunohistochemical technique, the authors made a screening of various tumors to detect the presence of a molecule immunoreacting with an anti β-subunit of the chorionic gonadotrophin hormone (β-HCG) antibody. Only 7 out of the 93 studied cases were positive (2/53 breast, 5/25 testis, 0/8 ovarian, 0/8 uterine, 0/2 pancreatic tumors). So, the presence of this immunoreactive molecule can be considered as rare. However, a more thorough study of testis tumors comparing immunohistochemistry, HCG and α-foetoprotein blood rates, clinical evolution strongly suggest that the presence of rare cells labelled by this technique could be a factor of bad prognosis. In this type of tumor, this technique seems to be of special interest as, alone, it may be able to point out, before any detectable blood HCG, what could be a chorial participation. This hypothesis has to be corroborated by the study of a larger number of cases.*

INTRODUCTION

THE β-SUBUNIT of human chorionic gonadotrophin (β-HCG) is frequently found at elevated concentrations in the serum of patients bearing trophoblastic tumors [1] and at lower level in the blood of some patients with non-trophoblastic tumors [2]. The site of synthesis (or of storage) of this subunit has been pinpointed by an immunohistochemical technique, which showed it to be localized in syncytial trophoblastic cells [3].

Other studies have demonstrated the ectopic presence of HCG and its β subunit, or, at least, of an immunologically cross-reactive molecule, in the serum of patients with various types of non-trophoblastic tumors [4, 5]. The site of production of this subunit has been revealed by immunohistochemistry to be the tumor cells themselves [6–8].

Some discrepancies are apparent in the studies carried out in this field. It therefore seemed to us that it would be useful, first, to verify whether ectopic β-HCG production really does occur; and if so, to examine the frequency of occurrence of such an ectopic synthesis in various cases of non-trophoblastic tumors.

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MATERIALS AND METHODS

Materials

We studied histological sections from 53 cases of mammary adenocarcinomas. In 6 cases the plasma β-HCG level has previously been found, by radioimmunoassay, to be elevated (over 2 ng/ml). In addition, we studied 2 cases of mastosis and one of fibroadenoma, for a total of 3 cases of benign and 53 cases of malignant breast tumors.

Likewise we studied sections from other tumors, which can be histologically classified as follows: 5 cases of uterine adenocarcinoma; 2 cases of pancreatic adenocarcinoma; 8 cases of ovarian mixed tumors (4 seminomas, 1 teratoma, 1 carcinoid, 1 epithelioma and 1 cystadenoma); 21 cases of testis seminoma and 4 cases of embryonal carcinoma. Finally, we studied one case of testis choriocarcinoma (Table 2)

Immunohistochemical technique

The tumors were fixed in Bouin's solution, embedded in paraffin, and sectioned at 4 μm. Three sections were treated by an indirect labelling method employing peroxidase.

In order to demonstrate the presence of a molecule reactive against the anti-β-HCG antibody on tumor cells, the sections are

treated first with rabbit anti-β-HCG antibody, and then with peroxidase-labelled sheep antibody anti rabbit gamma globulin (peroxidase-labelled sheep antibody and Fab fragments, directed against rabbit immunoglobulins, were obtained from Institut Pasteur Production).

Afterwards, the color reaction is developed using the enzymatic properties of the peroxidase: decomposition of hydrogen peroxide by the enzyme, followed by oxidation of the diaminobenzidine to yield a brown color [9].

The antiserum was obtained by i.d. injection of HCG β-subunit to the rabbit (β-HCG batch CR 119-3, kindly provided by Dr. Bialy, NIH, Bethesda) and the presence of anti β-HCG antibodies was checked over using the Ouchterlony immunodiffusion technique.

The cross-reactivity of the antiserum AL56 to LH was measured by RIA at the Institut Pasteur. The cross reaction, as calculated by Abraham's method, was 7%.

The immune serum was used to prepare an absorbed immune serum for that, 2 mg of highly purified β-HCG (batch CR 119-3) were fixed to cyanogen bromide activated sepharose under gentle agitation. This mixture was poured into a glass column; 2 ml of the rabbit immune serum were layered onto this column.

The first elution at pH 8 discards unfixed antibodies. The presence of proteins in this effluent is spectrophotometrically followed at 280 nm until O.D. returns to baseline level. This elution product is radioimmunologically assayed to make sure of the absence of anti β-HCG antibody and therefore, will be called "absorbed immune serum" and used as a control serum.

A second elution of the column will occur at pH 2.8 (glycerine–HCl buffer 0.2 M) in order to unhook the β-HCG specific antibodies. As above, spectrophotometry at 280 nm and RIA are used to check the quality of this second elution product.

For each tumor studied, we examine 3 sections: one treated with normal rabbit serum; the second, by absorbed immune serum (control); and the third one by specific rabbit immune serum (specific anti β-HCG antibody). Each section is de-paraffinized (cleared) in xylene (RP Prolabo), then, after rinsing with ether, washed in ordinary tap-water for 15 min. The section is then placed in a 7.5% H_2O_2 bath for 5 min in order to eliminate any blood which may be present. After washing in ordinary water, it is placed in a 2.28% periodic acid bath (Merck) in order to block the endogenous peroxidase activity which might lead to false positive results. After another washing in water, each slide is dipped in an 0.02% potassium borohydride bath (Merck Shuchardt), after which it is once again washed in water and put in PBS buffer, pH 7.4. Each slide is then placed in a humidified chamber and covered with a non-specific sheep serum diluted 1 : 10 in serum albumin (5 min). After washing in PBS, the first section is covered with normal rabbit serum diluted 1 : 50, the second with the absorbed immune serum diluted 1 : 50 and the third with the specific immune serum diluted 1 : 50. The 3 sections are then placed in a humidified chamber for 15 min.

After washing, the sections are covered with a peroxidase labelled anti-rabbit globulin diluted 1 : 50 (Institut Pasteur Production), washed again and developed for immune complex bound peroxidase by H_2O_2 and diaminobenzidine (Prolabo).

The course and the quality of the coloured reaction can be monitored under microscope.

Finally, the plates are counter-coloured with Mayer's hemalum.

For each examined tumor, the slide treated with normal rabbit serum serves as a negative control; however, the "best control" of specificity is provided by the slide treated with the absorbed immune serum.

In addition, as a test of the quality of the staining procedure for each tested tumor, as testicular choriocarcinoma (3 slides) is treated under conditions which are rigorously identical. It is well-established that this trophoblastic tumor secretes large quantities of HCG and its β-subunit.

RESULTS

Whatever the studied tumor could be, no staining was ever observed either on slides treated with normal rabbit serum or on those treated with absorbed immune serum. On the other hand, the slides with testicular choriocarcinoma treated with specific antibody, which served as positive controls, showed a significant staining of the cytoplasm of the syncytiotrophoblastic cells and of several cytotrophoblastic cells each time the assay was run. This staining procedure causes a brown staining to appear, with a granular appearance and cytoplasmic localization.

The results obtained with the specific anti β-HCG antibody, for the various tumors studied are summarized in Table 1.

(1) No labelling was observed for the 3

Table 1. *Incidence of β-HCG in various tumors (presence of a molecule reacting with anti β-HCG antibody)*

Localization	Histology	No. of cases	Immunohistochemistry	
			β-HCG positive cases	β-HCG negative cases
Mammary	Fibroadenoma	2	0	2
	Mastosis	1	0	1
	Adenocarcinoma	47	1	46
	Adenocarcinoma with seric β-HCG level > 2ng	6	1	5
Uterine	Adenocarcinoma	5	0	5
Pancreatic	Adenocarcinoma	2	0	2
Ovarian	Seminoma	4	0	4
	Teratoma	1	0	1
	Carcinoma	1	0	1
	Epithelioma	1	0	1
	Cystadenoma	1	0	1
Testis	Seminoma	21	3	18
	Embryonal carcinoma	4	2	2

studied cases of benign breast diseases as well as for the 5 cases of uterine adenocarcinoma, the 2 cases of pancreatic adenocarcinoma, the 8 cases of ovarian tumors.

(2) Among 53 cases of malignant breast tumors, 2 cases demonstrated clear-cut staining of the cytoplasm. Although in both of the positive cases only tumor cells were labelled, in one of these tumors many malignant cells were stained, whereas, in the other, only a few showed cytoplasmic staining. In this latter case, the tumor tissue was taken from a patient whose serum showed an elevated level of β-HCG previously discovered by RIA.

(3) For the testicular tumors 5/25 were found positive. This result is of particular interest if, besides histology, seric productions of HCG and α-foetoprotein on one hand, and metastasis existence on the other hand, are considered (Table 2).

None of the 16 cases of seminoma, with HCG and α-foetoprotein normal levels and metastasis negative happened to be positive. On the contrary, for 3/5 histologically seminoma showing elevated blood rates of HCG or α-foetoprotein, or metastasis positive, a labelling has been observed.

Two out of four embryonary carcinoma were positive and one of them seems of particular interest as no seric HCG was found and the labelling occurred in a ganglionic metastasis and not in the primary tumor itself.

Table 2. *Incidence of β-HCG in testis tumors*

Histological classification	Elevated HCG seric level	Elevated α-foeto seric level	Metastasis	No. of cases	Immunohistochemistry	
					β-HCG positive	β-HCG negative
Choriocarcinoma	+	−	+	1	1	0
Seminoma	+	+	+	1	1	0
	+	+	−	3	1	2
	+	−	+	1	1	0
	−	−	−	16	0	16
Embryonal carcinoma	+	+	+	1	0	1
	−	−	+	2	1	1
Embryonal carcinoma with polyembryoma	+	+	+	1	1	0

DISCUSSION

Various studies have demonstrated by immunohistochemical methods the presence of β-HCG on the surface of non-trophoblastic cancer cells.

However, immunohistochemical techniques set some problems of specificity which have to be solved before assessing the presence of a precise type of molecule. For this reason, a rigorous methodology was chosen for this study: the use of specific antibody, of an absorbed serum the loss of activity of which had been verified by radioimmúnology, and the routine use of choriocarcinoma slides which served as a "quality control" from one (day) assay to the next.

We used slides fixed with Bouin's solution after having tried successfully other types of fixation like formaldehyde. However, this type of fixation led to a more convenient use of old slides and to an easier application of routine requirements.

Several data may be pointed out: (1) We use a specific immune serum obtained from a highly purified β-HCG subunit. However, it is evident that this type of immunohistochemical technique reveals only the presence of an immunoreactive molecule against the anti-β-HCG antiserum. More, this molecule is not necessarily produced *in situ* but may be a plasmatic contamination. In this case, it becomes difficult to explain the labelling of only a few cells and it is reasonable to think that such a labelling indicates an *in situ* synthesis. (2) Some tumoral cells do bear a molecule immunoreacting with an anti-β-HCG antibody with the described technique. The presence of this molecule leads to a granular labelling of the whole cell. The cell-wall did not show any preferential affinity for this staining procedure. It is worth noting that the intensity of the labelling is similar whatever the type of the observed labelled cells. Thus, it is difficult to quantify the method and to define a sensitivity level. However, one knows that an HCG trophoblastic cell production stands at around 0.2 ng. So, it is likely that the immunohistochemical method is able to detect less than 1 ng of the immunoreactive molecule. (3) The presence of this immunoreactive molecule is uncommon in malignant non-trophoblastic cells as 7/93 tumors were positive. In these positive testicular tumors the labelling occurred in a few cells isolated in large areas of unlabelled cells (Figs. 1–3), and in the positive breast tumors, a few islets were stained. (4) In 5/7 positive cases (1/2 for breast tumors and 4/5 for testicular tumors), the presence of labelled cells is concomitant with an abnormally elevated seric rate of HCG or β-HCG, before any treatment (surgery, radiotherapy or chemotherapy) [10]. (5) Finally, the testicular tumors prove to be the most interesting localizations to study by immunohistochemistry with the antiserum anti-β-HCG.

Indeed, as none of the 16 cases of seminoma without elevated HCG or α-foetoprotein, without metastasis and of good prognosis appeared as positive, and 5/9 testicular tumors with elevated HCG or α-foetoprotein, or with multiple metastasis were positive, it may be asked whether the presence of labelled cells only in these tumors is not, itself, of bad prognosis. This concept, could lead to a revision of the treatment of this type of tumor.

One may notice that no correlation has been found between the number of labelled cells and the seric concentration of HCG. It appears that some tumors are able to store important quantities of molecule, excreting only a small part of it into the serum and, on the contrary, some other tumors immediately excrete the newly synthesised molecule. Particularly, it may happen, as seen in one case, that this type of labelling be observed while the HCG seric concentration is still normal. That is why it may be asked whether this method is not able to spot a chorial participation of a type of testicular tumor considered, so far, as non trophoblastic.

From all these reasons, it appears that immunochemical methods operating an anti β-HCG-antiserum should be oriented toward non-trophoblastic tumor exploration as practical clinical repercussions could proceed from this type of research.

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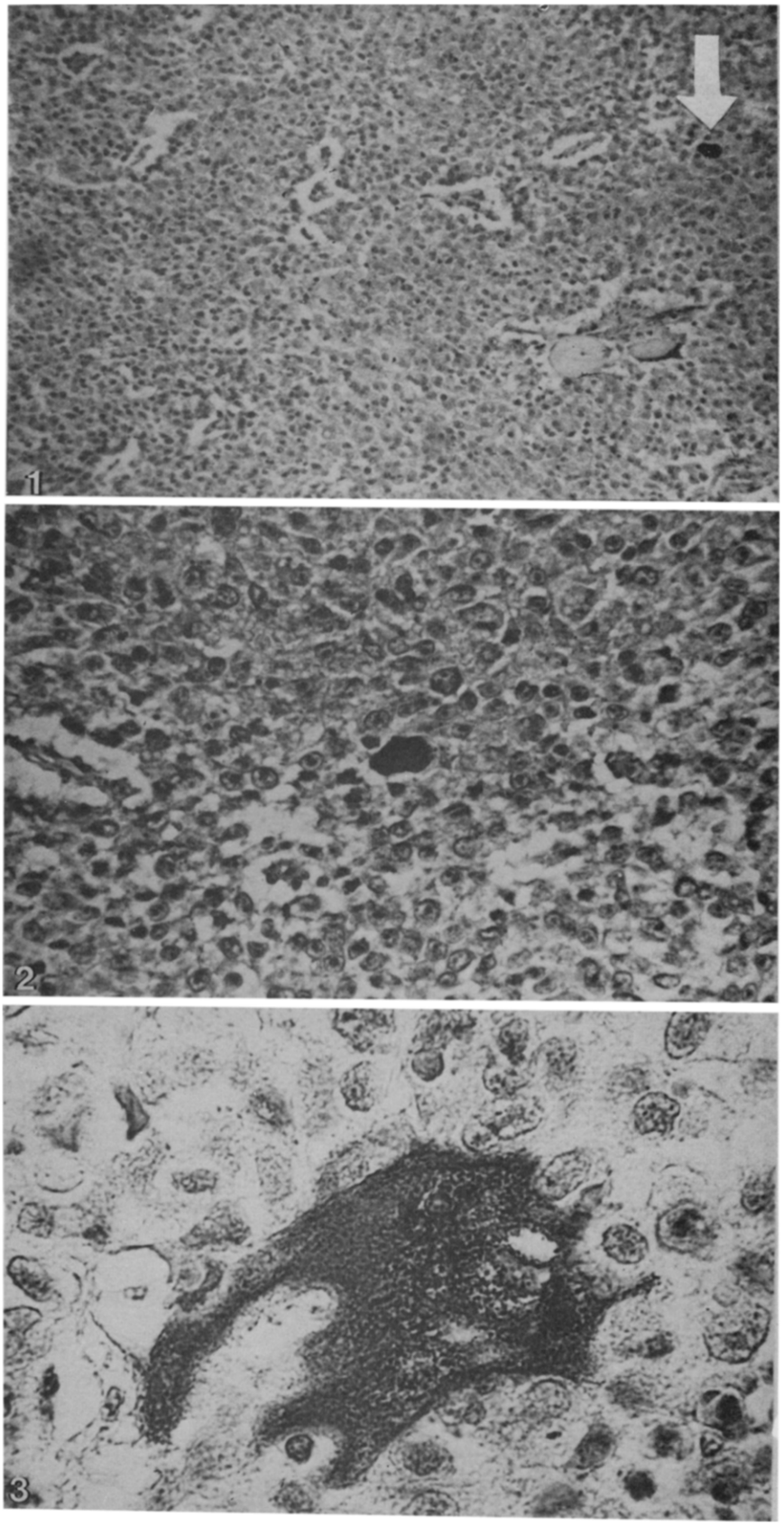

Fig. 1. *Immunoperoxydase localization of β-HCG. Isolated staining cell in seminoma tissue. Zeiss* ×40.

Fig. 2. *Immunoperoxydase localization of β-HCG. Isolated staining cell in seminoma tissue. Zeiss* ×63.

Fig. 3. *Immunoperoxydase localization of β-HCG. Syncytial aspect of isolated cell in seminoma. Zeiss* ×400.

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