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Immunohistological Localization of β-HCG in Breast Carcinomas*

RENATE WACHNER,† CHRISTIAN WITTEKIND‡ and SABINE VON KLEIST†§

†*Institute of Immunobiology, University of Freiburg, Stefan-Meier-Str. 8, 7800 Freiburg, F.R.G. and* ‡*Institute of Pathology, University Freiburg, Albertstr. 19, 7800 Freiburg, F.R.G.*

Abstract—*As reports about HCG positivity in serum or tissue of patients with breast cancer show divergent results, the present investigation was undertaken to study the frequency of positive staining for beta-HCG in breast cancer tissue and to correlate the staining pattern with the histology of the tumor and stage of disease. With the immunoperoxidase technique we studied 129 cases of breast carcinomas. We obtained a positive staining for beta-HCG in 12%. However, in most of the tumors only very few cells contained beta-HCG. Morphologically the positive cells could not be distinguished from β-HCG-negative cells. We conclude from our data that until now the β-HCG staining provides no immediate useful information for the management of patients.*

INTRODUCTION

HUMAN chorionic gonadotropin (HCG) is normally produced by the placenta. It is also demonstrable in patients with gestational and non-gestational trophoblastic tumors [1]. In more recent years HCG has been reported to be detectable in the serum of patients with non-trophoblastic tumors [2–4], including mammary carcinomas.

In trophoblastic tumors the HCG-serum level generally correlates with disease activity in so far as a decrease of the serum level marks a response to therapy and an increase is often seen prior to relapse. By immunohistochemistry the site of production of the HCG has been revealed to be the choriocarcinomatous elements or syncytiotrophoblastic-like giant cells (STLGC) in the trophoblastic tumors [5]. With respect to HCG tissue positivity in breast carcinomas, some discrepancies are apparent concerning the antibodies used and the frequency of positively stained cases [6–8].

The present investigation was therefore undertaken to study whether ectopic β-HCG-positivity was demonstrable in breast cancer tissue and, if so, to attempt a correlation of the staining pattern with the histological type of tumor. Furthermore, the results were analyzed with regard to tumor burden, i.e. to the frequency of involved axillary lymph nodes.

MATERIALS AND METHODS

β-HCG staining was performed with the indirect immunoperoxidase technique on tissue samples from 129 breast carcinomas and 39 benign breast lesions. The mean age of patients with breast carcinoma was 60 yr, ranging from 28 to 90 yr, 21 patients being premenopausal (16%). The mean age of patients with benign breast lesions was 48 yr (29–79 yr).

The tissues were obtained from the Institute of Pathology of the University of Freiburg. They were formalin-fixed, embedded in paraffin wax and cut into 5-μm sections. After being deparaffinized, the endogenous peroxidase was blocked with 1% hydrogen peroxide in methanol. Then the sections were incubated with 5% ovalbumin in order to reduce background staining. Between each step of incubation the sections were washed three times with TBS (Tris-buffered saline) at pH 7.4. The HCG-antiserum, raised in rabbits against the β-chain of HCG (Dakopatts, Denmark), was tried in several dilutions in TBS containing 25% normal swine serum (1:20, 1:30, 1:50), and 1:20 was chosen because of the incubation time being the most convenient.

Control sections incubated with a beta-HCG antiserum absorbed against purified β-HCG (Boehringer Mannheim, F.R.G.) showed negative

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§To whom requests for reprints should be addressed.

staining results. The incubation with the first antiserum was performed for 2 hr at 37°C, with the second antiserum (in dilution 1:20) for 30 min at room temperature. Then the sections were stained with 3,3'-diaminobenzidintetrahydrochloride and 0.012% hydrogen peroxide. Finally the sections were counterstained in Mayer's hemalaun, dehydrated and mounted in Entellan.

RESULTS

The immunohistological staining of β-HCG gave positive results in only a small percentage of the studied cases.

β-HCG was detected in 16 carcinomas out of 129 (12%) and in 2 benign breast lesions out of 39 (5%). With respect to histology, no characteristic pattern could be revealed since 8/76 invasive ductal carcinomas, 2/10 ductal carcinomas with a predominant intraductal component, 2/4 mucinous and 2/15 tubular carcinomas, 2/6 lobular carcinomas, 0/15 undifferentiated, 0/2 intraductal carcinomas and 0/1 medullary carcinoma were positive (Table 1). These positive sections also remained positive when stained with the β-HCG-antiserum diluted 1:50. The normal breast and normal lactating breast tissues studied were both negative for β-HCG.

The finding of β-HCG could not be correlated either with tumor size or with lymph nodal stage: small tumors of less than 2 cm as well as big ones of over 5 cm diameter were positive; also, those having metastasized into the regional lymph nodes were just as frequently positive as those still being localized. In all cases examined there was a heterogeneity in both the percentage of positive tumor cells and the intensity of the staining of cells of a given tumor (Fig. 1). In one tumor all carcinoma cells were stainable for β-HCG, while all other tumors had only some positive cells or single groups of positive cells (Fig. 2). Morphologically, the positive cells could not be distinguished from β-HCG-negative cells. In benign lesions β-HCG could be seen in proliferating areas of one intraductal papilloma and one ductal hyperplasia. Both patients were postmenopausal (49 and 62 yr).

DISCUSSION

It has been known for some time that gestational choriocarcinomas or germinomatous tumors are accompanied by elevated β-HCG-serum levels [4, 5]. However, in some tumors of the lung, liver, gastrointestinal tract, adrenal cortex and urogenital tract, raised HCG-serum levels have also been observed. In all these tumors choriocarcinomatous elements or STLGC have been demonstrated [9]. There is only one case of infiltrating ductal breast carcinoma associated with choriocarcinomatous elements staining positive for β-HCG [10].

Few authors reported tissue-HCG-positivity in human tumors without choriocarcinomatous areas and without STLGC; they examined tumors of the lung, gastrointestinal tract, ovary, oral cavity and breast [11, 12]. The highest incidence of HCG-positivity in breast cancer tissue was reported by Horne *et al.* [8], who detected it in 60% of 50 breast carcinomas. It is known that most of these antisera do not discriminate between LH, FSH, TSH and HCG because of the similarity of their alpha-chains. There is one study that dealt with the tissue localization of α-HCG in breast cancer [13], and this author described a positivity of 23% (12/53 cases). This result is similar to the

Table 1. Characteristics of β-HCG-positive breast cancer tissues

Case No.	Age (yr)	TNM-stage	Histological classification (WHO)
1	59	$T_1N_0M_0$	tubular carcinoma
2	42	$T_1N_0M_0$	mucinous carcinoma
3	52	$T_1N_{1b}M_0$	invasive ductal carcinoma
4	63	$T_xN_0M_x$	invasive ductal carcinoma
5	55	$T_xN_{1b}M_0$	invasive ductal carcinoma
6	45	$T_2N_{1b}M_x$	invasive ductal carcinoma with a predominant intraductal component
7	76	$T_2N_0M_0$	invasive ductal carcinoma
8	69	$T_2N_0M_0$	invasive ductal carcinoma
9	74	$T_2N_{1b}M_0$	mucinous carcinoma
10	65	$T_2N_{1b}M_0$	lobular carcinoma
11	61	$T_2N_{1b}M_0$	invasive ductal carcinoma
12	48	$T_2N_2M_0$	invasive ductal carcinoma
13	70	$T_2N_2M_{oss}$	tubular carcinoma
14	89	$T_3N_xM_x$	lobular carcinoma
15	55	$T_3N_0M_x$	invasive ductal carcinoma with a predominant intraductal component
16	62	$T_3N_3M_{oss}$	invasive ductal carcinoma

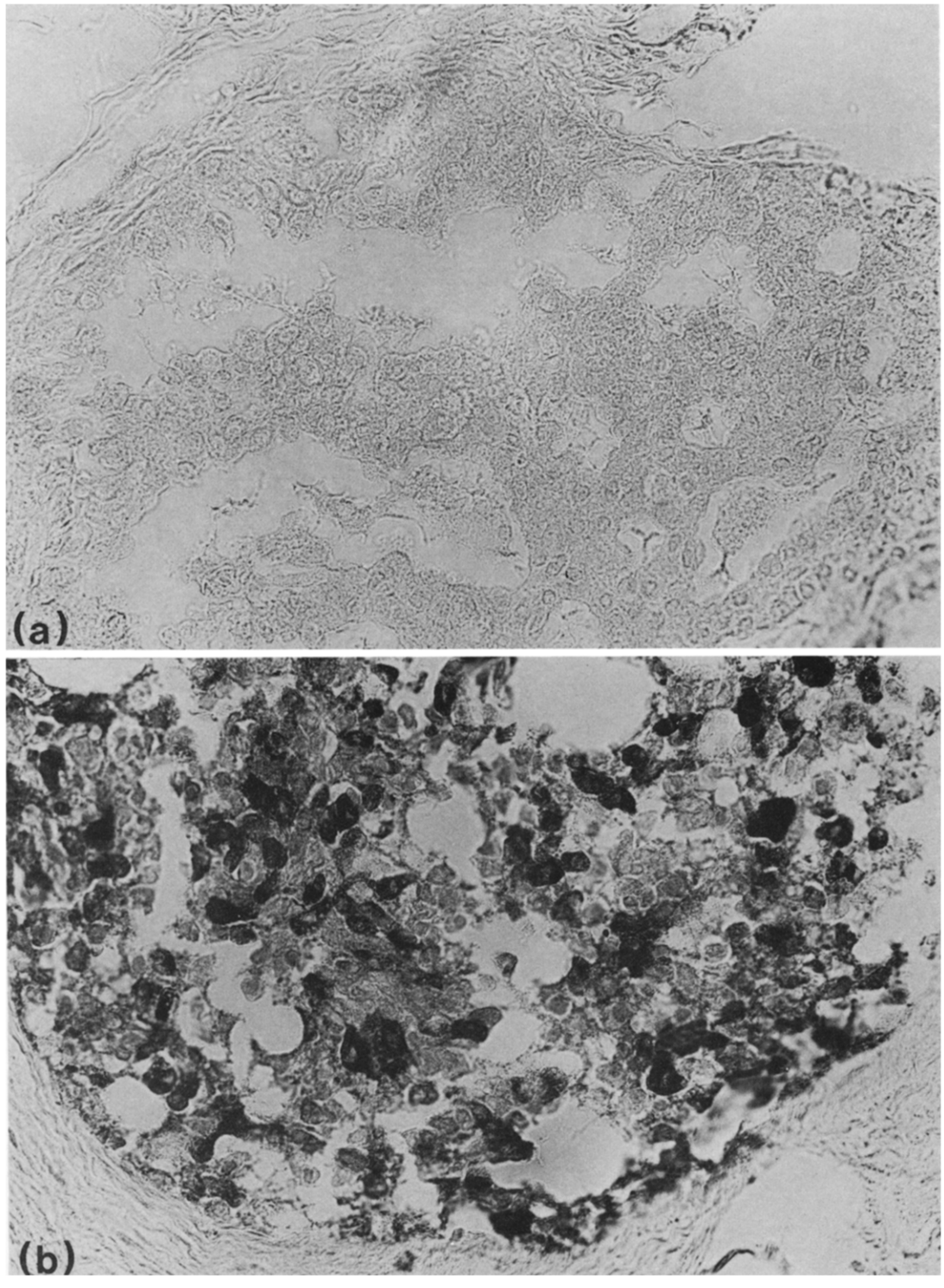

Fig. 1. Invasive ductal carcinoma: all cells staining positive for β-HCG with various intensity (no counterstaining, ×340).

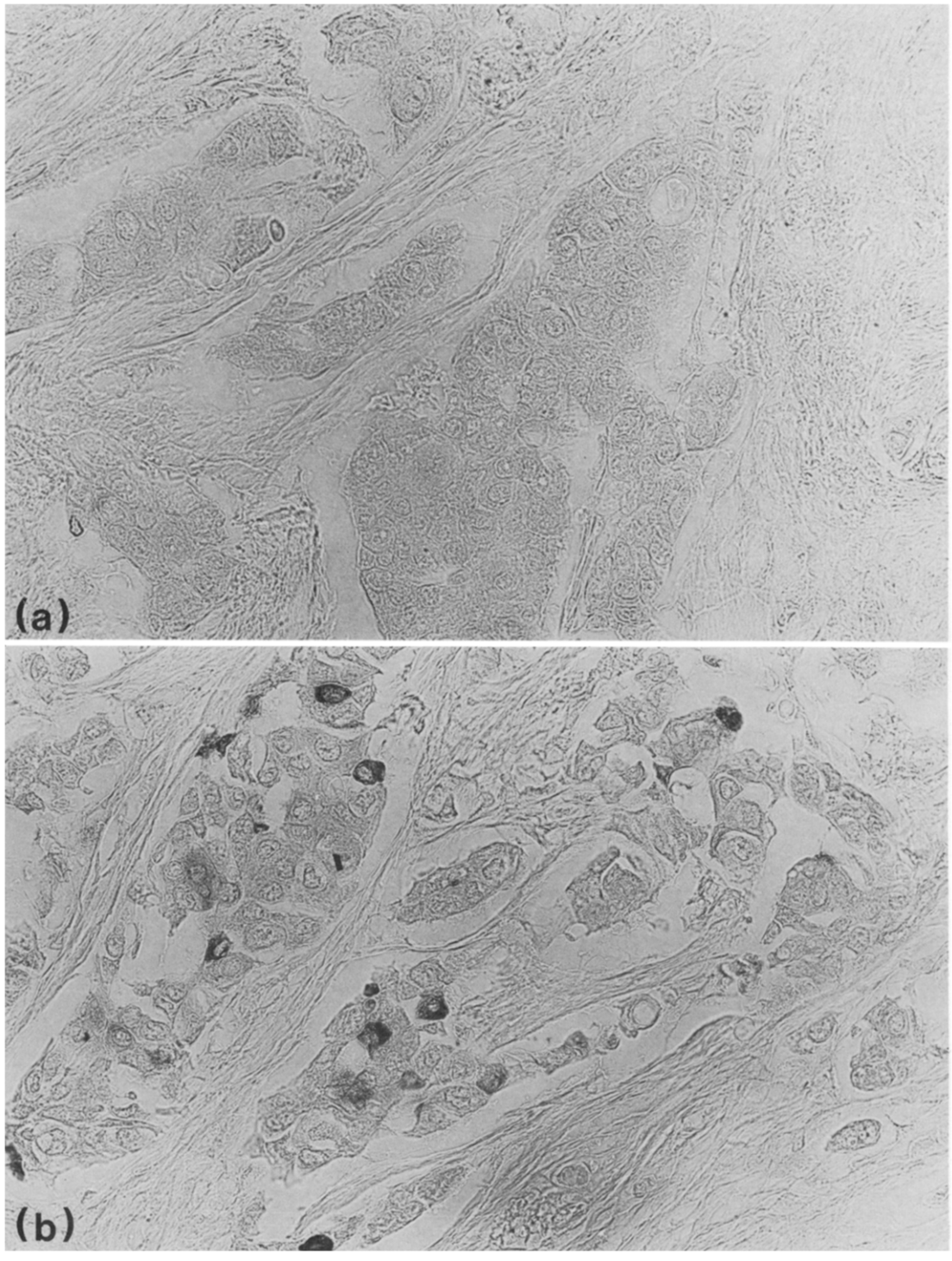

Fig. 2. Invasive ductal carcinoma with single β-HCG-positive cells (no counterstaining, ×340).

report of Dosogne-Guérin *et al.* [3], who demonstrated elevated α-HCG-serum in 30% of 116 breast patients.

In our study β-HCG was detected in the tissues of 16 out of 129 patients (15%). Our results are in good accordance with the report of Braunstein *et al.* [2], who found β-HCG in 12% (4/33) in the sera of breast cancer patients, and with the results of Sheth *et al.* [14], who reported raised β-HCG levels in 14% of their patients ($n = 64$).

Bellet *et al.* [7], however, detected a clear-out staining in only two cases (4%) in the tissues of his 53 breast cancer patients, one of them with a raised serum level (<2 ng/ml). Interestingly, 6 patients had raised β-HCG serum values in spite of the fact that the authors could not demonstrate any tissue-positivity. However, they stained only one section of every tumor, and supposedly the tumors of the patients with raised serum levels were positive in another area. Indeed, Bellet *et al.* described in one of their cases small islets of positive tumor cells.

Wahren *et al.* [15] reported a positive β-HCG immunofluorescence-staining in needle-biopsy material in only 4 of 50 breast cancer patients (8%). Yet they did not find a positive reaction in either lysates of these tumor cells or in the sera of these patients.

Cove *et al.* [16] described β-HCG in one of 30 tumor cytosols investigated, and in one out of 94 serum samples.

In our series, which is the largest yet studied, beta-HCG tissue positivity is higher than in reports with smaller case numbers. It is especially important to prepare slides with great areas of tumor because sometimes the β-HCG can be seen in very few cells of the tumor (less than 1%). Thus the cytology smearing technique is supposed not to provide reliable results.

Opinions differ on whether the immunoreactive molecule detected by an anti-β-HCG antiserum is produced *in situ* by the tumor cells or has to be considered as a plasmatic contamination. Since only few cells within a carcinoma stain for β-HCG, it seems reasonable to suppose that the molecule is indeed produced *in situ*. Also HCG-positivity is clearly located in tumor cells and not in macrophages. Furthermore, it has been shown by Tashijan *et al.* [17] that clonal strains derived from bronchiogenic carcinomas are able to produce β-HCG in cell culture.

Rutanen and Seppälä [18] reported elevated HCG-serum levels more often in older patients, regardless of the nature of their disease. The mean age of the 16 patients with HCG-positive tissue areas in our study was 62 yr; if this was compared with the mean age of all 129 patients studied (60 yr), no discrepancy was observed; hence we cannot confirm an age factor being involved in HCG production.

We have demonstrated by immunological means β-HCG and we have reason to believe that it is the placental HCG, since normal breast tissue controls did not give any positive reaction.

We conclude from our data that, for the time being, the β-HCG staining provides no immediate useful information for the clinical management of patients. Positive or negative tumors showed axillary node involvement in the same frequency. The observation of Horne *et al.* [8] that patients with HCG-positive tumors have a worse prognosis nevertheless deserves further proof.

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