Significance of α -subunit HCG demonstrated in breast carcinomas by the immunoperoxidase technique

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SUMMARY Fifty-three breast carcinomas were examined by an indirect immunoperoxidase technique for the presence of the α -subunit of human chorionic gonadotrophin. Positive staining occurred in 12 (22.6%). There was no correlation between α -subunit production and specific histological features, but production was related to the presence of lymph node metastases and hence a poorer prognosis. The α -subunit could be used as a prognostic indicator in the assessment of breast carcinoma.

The secretion of human chorionic gonadotrophin (HCG) by non-trophoblastic tumours is now well recognised (Weintraub and Rosen, 1971; Braunstein *et al.*, 1973). Braunstein *et al.* used a radioimmunoassay method to detect HCG in a variety of tumours, of which carcinoma of the breast was one, and there have been similar reports based on the use of an antisera which measured whole molecule HCG and the β -subunit of HCG (Goldstein *et al.*, 1974). Secretion of the α -subunit of HCG, which is very similar in structure to the α -subunit of thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and luteinising hormone (LH), has been described in a small number of carcinomas, including breast (Franchimont *et al.*, 1972), and in HeLa cell lines (Lieblich *et al.*, 1976).

Immunohistochemical methods have given similar results. Using an immunoperoxidase technique Horne *et al.* (1976) detected HCG in 60% of breast carcinomas. Naughton *et al.* (1975) using a similar technique examined human tumour cells, including infiltrating duct breast carcinomas, and detected β -subunit HCG. Horne *et al.* found that the patients whose breast carcinomas secreted pregnancy specific proteins (of which HCG is one) had a shorter survival time, and they thought that this could be related to a local immunosuppressive effect by these substances. Naughton *et al.* also commented that the β -subunit of HCG could have an immunological role.

Since, therefore, there is good evidence for the production of HCG by breast carcinomas a series

of such neoplasms was examined retrospectively for the presence of the α -subunit of HCG. The morphology of the neoplasms was studied in detail, and in cases in which the lymph nodes had been examined the absence or presence of metastases was noted.

Materials and methods

Fifty-three carcinomas removed from 52 patients were studied, one patient having bilateral tumours. Out of the 53, 40 were received from the Queen Elizabeth Hospital, Birmingham, between February 1976 and February 1977, and 13 from Selly Oak Hospital, Birmingham, between November 1976 and February 1977. The tissues were fixed in 4% formaldehyde in 0.15 M sodium chloride solution, dehydrated, cleared, and embedded in paraffin. Sections for morphological examination were stained with haematoxylin and eosin. Specific antiserum was raised in a rabbit against purified α -subunit HCG. At 50% binding the antiserum showed complete cross-reactivity with the α -subunits of TSH, FSH, and LH; 5% cross-reactivity with HCG, and <0.3% with β -subunit HCG; 11.6% cross-reactivity with LH and less than 0.3% with β -subunit LH; 16.3% cross-reactivity with FSH and 5.2% with β -subunit FSH. Cross-reactivity with human growth hormone and human placental lactogen was less than 0.3%.

IMMUNOPEROXIDASE TECHNIQUE

The method was essentially that described by Curran and Jones (1977). Sections were treated with 0.1% trypsin (Difco 1:250) pH 7.8 at 37°C for 10, 20, and 30 minutes to 'unmask' the antigen (Curren and

Fig. 1 Part of poorly differentiated breast carcinoma showing clustering of positive cells with negative areas between them. (Immunoperoxidase $\times 140$)

Fig. 2 High-power view of small number of positive cells showing diffuse staining of cytoplasm with focal intensity. (Immunoperoxidase $\times 250$)

Gregory, 1977). Endogenous peroxidase was blocked using 0.2% HCl in methanol. Non-specific background staining was reduced by using normal swine serum at a dilution of 1 in 5 for 10 minutes. The sections were then treated with antisera against α -subunit HCG. A dilution of 1 in 100 was found

to be optimal for staining with negligible background staining. Swine anti-rabbit gamma-globulin serum (Dakopatt) was then applied, followed by peroxidase antiperoxidase complex (Dakopatt). The diaminobenzidine reaction was used to localise the site of peroxidase. Control sections were treated with

normal rabbit serum and with specific antiserum after absorption with purified α -subunit HCG in place of the specific antiserum. Sections from term placenta which had been processed the same as the carcinomas were treated with specific antiserum.

MORPHOLOGY

The carcinomas were classified as follows: (1) Non-infiltrative—intraduct, intralobular. (2) Infiltrative—infiltrating duct, infiltrating lobular, infiltrating (no in-situ seen), medullary, mucinous, and papillary. They were graded according to the criteria of Bloom and Richardson (1957), with 40 random high power fields being examined. The extent of the scirrhous reaction was assessed and graded as absent, mild, moderate, or marked. The lymphocyte infiltration was similarly assessed and divided into two groups depending on whether the infiltrate was throughout the tumour or confined to the periphery. Circumscription of the carcinoma was determined microscopically and noted if present. The absence or presence of metastases in lymph nodes, when these were available, was determined in the same way.

Results

IMMUNOPEROXIDASE

Out of the 53 cases 12 (22.6%) gave a positive reaction with the immunoperoxidase technique. In the patient with bilateral carcinomas one was negative and the other positive. There was no staining in control sections treated with normal rabbit serum and none after absorption of the specific antiserum with purified α -subunit HCG, 0.67 μ g being required to absorb a dilution of 1 in 100. There was staining in the trophoblast of term placenta, a tissue which contains large amounts of α -subunit.

The number of cells staining was increased by treatment with trypsin, with the exposure time needed to do this depending on the fixation of the tissue. Each carcinoma varied in the number of cells that stain, but a constant feature was the clustering of positive cells with negative areas of varying sizes between them (Fig. 1). The cytoplasm stained diffusely, with a stronger focal granular reaction predominantly in the perinuclear region (Fig. 2).

MORPHOLOGY

The classification and grading of all tumours and positive cases are shown in the Table. All the positive cases were infiltrating duct or infiltrating carcinomas, and their grades correlate with the proportion of grades for all carcinomas. The small number of positive cases made assessment difficult but there was a slight excess of positive cases

Table Classification and grading of all tumours and positive cases

Classification	All carcinomas	Positive cases	Negative cases
Intraduct carcinoma	1	—	1
Infiltrating duct carcinoma	34	9	25
Infiltrating lobular carcinoma	4	—	4
Infiltrating carcinoma	11	3	8
Medullary carcinoma	1	—	1
Mucinous carcinoma	1	—	1
Papillary carcinoma	1	—	1
Grade			
I	8 (15.5%)	2 (17%)	6 (25%)
II	24 (46%)	5 (41.5%)	19 (47.5%)
III	20 (38.5%)	5 (41.5%)	15 (37.5%)

(41.6% (5 out of 12)) showing a marked scirrhous response, compared with 30.0% (12 out of 40) for negative cases. Other categories were comparable. Lymphocytic infiltrate was similar in positive and negative cases.

Overall there were eight carcinomas with microscopic circumscription. Three of them were positive cases. Twenty-two carcinomas had metastases in the lymph node, 22 did not, and in nine cases no lymph nodes were found in the specimen. Eight of the positive cases had metastasised, three had not, and in one case no lymph nodes were found. This showed an excess of metastases when compared with negative cases, in which there were 14 with metastases, 19 without, and eight cases where no lymph nodes were found. If this latter category is included in the node-negative group (although the absence of lymph nodes may be due to the nature of the mastectomy) the excess becomes more striking.

Discussion

In this study, using the very sensitive indirect immunoperoxidase technique with peroxidase-antiperoxidase complex (Burns, 1975), the α -subunit of HCG was detected in 12 out of 53 carcinomas (22.6%). Franchimont *et al.* (1972) found the α -subunit in the serum of three patients with breast carcinoma, using a radioimmunoassay method, but did not state the incidence. HCG and β -subunit HCG have been detected in breast carcinomas by radioimmunoassay methods (Braunstein *et al.*, 1973; Goldstein *et al.*, 1974) and the immunoperoxidase technique (Horne *et al.*, 1976; Naughton *et al.*, 1975), with incidences respectively of 12%, 54%, and 60%. Naughton *et al.* do not quote incidence. None of these workers mention the α -subunit of HCG, which has to be present for the formation of whole molecule HCG.

The pattern of staining is interesting, with

clustering of positive cells and variable areas of negative cells, suggesting that a clone of cells at the positive site had become derepressed. The finding of diffuse cytoplasmic staining with focal intensity in the perinuclear region differs from the findings for β -subunit HCG. Naughton *et al.* (1975) found, using the immunoperoxidase technique on breast carcinoma cells, that there was a more intense band of staining at or near the surface.

Because the production of α -subunit might be related to cellular dedifferentiation (Franchimont *et al.*, 1972) the carcinomas were examined histologically to assess differentiation, but there were no differences in grades between those producing α -subunit and those not. Although there were slight differences between positive and negative cases in the extent of scirrhous reaction these were not significant. There was no difference in circumscription.

There was, however, an excess of lymph node metastases in those carcinomas producing α -subunit. Since the presence of lymph node metastases at the time of presentation is linked with a poorer prognosis these findings tend to support the conclusion of Horne *et al.* (1976). HCG is said to have immunosuppressive properties (Contractor and Davies, 1973). Positive responses to α -subunit HCG as well as HCG and β -subunit HCG were found by lymphocytes from patients with breast carcinoma, using a macrophage electrophoretic mobility test (Wass *et al.*, 1977).

This study shows that breast carcinoma may now be included with carcinomas of the lung (Tashjian *et al.*, 1973), stomach and pancreas (Rosen and Weintraub, 1974), and HeLa cells (Lieblich *et al.*, 1976) as a tumour that can produce the α -subunit. There is no correlation between production and specific histological features. There is a relationship between production and the presence of lymph node metastasis, and hence poorer prognosis. This may be due to immunological factors related to the properties of the α -subunit.

The α -subunit of HCG could be used as a prognostic indicator in assessing breast carcinoma, but it is of little use diagnostically owing to its relatively low incidence and its presence in other carcinomas.

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