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Peroxidase Activity in Human Breast Carcinomas: Correlation with Histological and Biochemical Parameters*

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Abstract—*Peroxidase activity was found in 68% of 56 human breast carcinomas. Median activity was higher in ductal carcinomas than in tumors with lobular components. Activity did not correlate with tumor grade or stage, or the presence or absence of axillary node involvement. Activity, however, correlated with tumor cellularity, lymphocyte infiltration levels, and total protein and alkaline phosphatase levels. It is concluded that variation in peroxidase activity in human breast tumors is at least in part due to lymphocyte infiltration. Furthermore, this contamination of breast tumors with lymphocytes may explain the lack of correlation between estradiol receptor levels and peroxidase activity.*

INTRODUCTION

THE ABILITY to differentiate between hormone-dependent and independent breast carcinomas is a prerequisite for rational ablative and additive hormone therapy. This can now be done reasonably well with the combined estradiol (ER) and progesterone receptor (PR) assays in breast tumor cytosols [1]. Thus approximately 70–80% of tumors containing both ER and PR respond to hormonal therapy, while tumors lacking both receptors rarely respond to such treatment. The rationale for using the PR in addition to the ER is probably based on the belief that PR is induced by estradiol and is thus a marker for a functional ER.

We [2] and others [3, 4] have shown that the enzyme peroxidase is also induced by estradiol at least in the immature rat uterus. If a similar induction occurred in human breast tumors, peroxidase could also act as a marker for functional ER in these tissues and thus possibly provide a more reliable index of hormonal responsive tumors.

Peroxidase activity has now been detected in dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumors [4], transplanted mice mammary tumors [5, 6] and human breast carcinomas [3, 7, 8]. Moreover, in the animal tumors peroxidase activity was higher in the hormone-dependent group than in the independent group [5, 6]. Also following estradiol administration to rats containing DMBA-induced tumors, Jellinck *et al.* found a moderate correlation between nuclear ER and peroxidase activity [4]. However, in human breast carcinomas, no correlation has been found between basal levels of cytoplasmic ER and peroxidase activity [7, 8]. The reason or reasons for this lack of correlation are unknown; it does not appear to be due to either endogenous progesterone inhibiting estradiol induction of the enzyme or the presence of nuclear ER in the absence of cytoplasmic ER [7]. In this investigation we have therefore attempted to correlate peroxidase activity with a variety of histological and biochemical parameters in order to establish further what factors contribute to peroxidase variations in human mammary carcinomas.

MATERIALS AND METHODS

Breast carcinomas were stored, homogenized and assayed for peroxidase activity as previously described [7]. CEA was measured by

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radioimmunoassay using a commercial kit supplied by CIS. Before assay, tumor homogenates which had been centrifuged at 800 *g* for 10 min were re-centrifuged at 15,000 *g* for 15 min and then diluted appropriately with CEA-free serum. Alkaline phosphatase activity and protein in the 800 *g* supernatant were determined using kits supplied by Boehringer and Bio-Rad respectively.

Histology grade was based on mitotic frequency (total number of mitoses per 10 high-power fields) and was based on a scale of 1 (low) to 4 (high). Lymphocyte infiltration was estimated semi-quantitatively on a scale of 1 (few or no lymphocytes) to 4 (very dense infiltration). Epithelial cellularity was estimated as the percentage area of tissue in available sections that was occupied by tumor cells and expressed in arbitrary units of 10 from less than 10% to 90% or more. Histology types were classified as either ductal or tumors with lobular components, i.e. pure lobular or mixed lobular and ductal. Tumor stage was based on the Manchester classification. For the majority of tumors the pathological and biochemical investigations were carried out on matched portions of tissues.

Correlation coefficients were calculated using Kendall's Tau equation. The results were computed using the 'Statistical Package for the Social Sciences'. The statistical explanation was taken from the SPSS Statistical Algorithms, Release 8 © 1978, SPSS Inc.

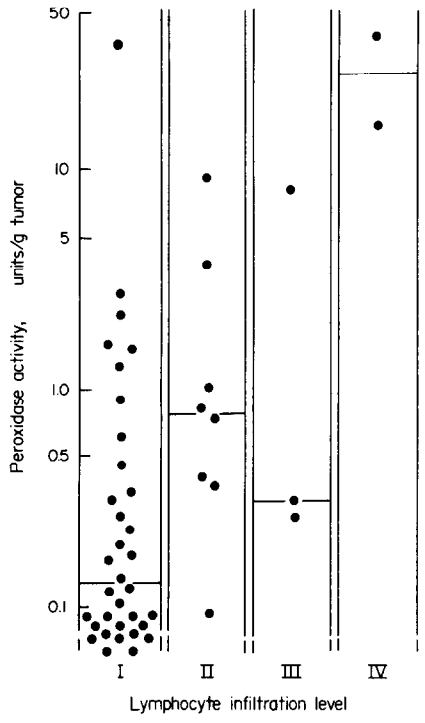

Fig. 1. Distribution of peroxidase activity in human breast carcinomas with different levels of lymphocyte infiltration. Procedures as described in section on methodology.

RESULTS

Correlation of peroxidase activity with histological parameters

Peroxidase activity (>0.1 U/g wet weight) was found in 38/56 primary breast carcinomas and varied from undetectable levels to 38.8 U/g wet weight of tissue. Median activity was statistically higher in ductal tumors than in carcinomas with lobular components (0.8 vs 0.06 U/g, $P = 0.003$). However, activity did not correlate with either tumor stage, grade, size, or the presence or absence of axillary node involvement. On the other hand, activity correlated significantly with cellularity ($r = 0.23$, $P = 0.003$, $n = 37$) and levels of lymphocyte infiltration ($r = 0.37$, $P = 0.001$, $n = 44$; see Fig. 1).

Correlation of peroxidase activity with biochemical parameters

Peroxidase activity showed no correlation with concentrations of the onco-fetal gene product CEA but did correlate with alkaline phosphatase activity levels ($r = 0.42$, $P = 0.001$, $n = 50$) and total proteins levels ($r = 0.29$, $P = 0.002$, $n = 47$). Alkaline phosphatase levels, however, also correlated with lymphocyte infiltration ($r = 0.37$, $P = 0.003$, $n = 38$).

DISCUSSION

Previous work [2–4] has shown that peroxidase activity was an excellent marker for estrogen action in the immature rat uterus. In contrast to other estrogen-inducible proteins such as the PR and 'induced protein' (IP), the assay of peroxidase is simple and cheap. Moreover, peroxidase is a relatively stable protein. Thus, if peroxidase activity was induced specifically by estrogens in human breast carcinomas, its assay could have major advantages over the PR assay as an adjunct to the ER determination. However, results to date show no correlation between basal levels of peroxidase activity and ER concentrations in human mammary carcinomas [7, 8]. These were unexpected findings and require further investigations.

We show here that many of the breast tumours with high levels of peroxidase activity also had high levels of lymphocyte infiltration. Previously, an inverse relationship was found between ER levels and lymphocyte infiltration

[9]. Thus the presence of high peroxidase activity in some tumours with low concentrattons of ER may be explained by lymphocyte infiltration. We conclude, therefore, that the lack of correlation between ER and peroxidase activity in human breast carcinomas is due at least in part to contamination of the latter with lymphocytes.

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