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Estrogens, Estradiol Receptors and Peroxidase Activity in Human Mammary Carcinomas*

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Abstract—*Estrogens, cytoplasmic estradiol receptors, nuclear estradiol receptors and peroxidase activity were measured in human breast carcinomas. No correlation was found between either estrogens, cytoplasmic estradiol receptor or nuclear estradiol receptors and peroxidase activity. The lack of correlation between cytoplasmic estradiol receptors and peroxidase activity is not likely to be due to inhibition of estrogen-inducible peroxidase by endogenous progesterone or the presence of nuclear estradiol receptors in the absence of cytoplasmic receptors. It is concluded that peroxidase activity is not a specific marker for a functional cytoplasmic estradiol receptor in human mammary carcinomas.*

INTRODUCTION

IT IS now well established that approximately 50–55% of patients with metastatic breast carcinoma respond to hormone manipulative therapy if their primary tumor contains estrogen receptors (ER). On the other hand, less than 10% of ER-negative tumors respond [1]. Thus the greatest use of the ER assay is in predicting metastatic tumors unlikely to respond to endocrine therapy. One possible reason why some ER-positive tumors fail to respond is that there may be a defect in estradiol action after binding to the cytoplasmic receptor [2]. Therefore, by combining measurement of a specific protein induced by estradiol with the ER, assay of a functional ER might be possible and hence be a more accurate prediction of hormone responsive tumors. It has been known for some time that administration of estrogen to rats increases uterine peroxidase activity [3]. More recent evidence suggested that the estrogen-induced peroxidase only occurred in those rat tissues whose growth was affected by the steroid such as the uterus, vagina and carcinogen-induced mammary tumors [4]. No induction was found in non-target organs of estrogens or in target tissues such as the pituitary or hypothalmus where the steroid effect is mostly functional [4]. From these findings, De Sombre and co-workers [4] suggested that peroxidase activity might be a useful marker for estrogen-stimulated growth responses.

Peroxidase activity has now been detected in dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumors [5], transplanted mice mammary tumors [6] and human breast carcinomas [4, 7]. Moreover in the animal tumors, peroxidase activity was higher in the hormone-dependent group than in the independent group [6, 8]. The purpose of this study was to investigate the relationship between estrogens, ER and peroxidase activity in human mammary carcinomas.

MATERIALS AND METHODS

Primary breast carcinomas were frozen in liquid nitrogen as soon as possible after operation and then stored at −70°C. Cytoplasmic ER (CER) was assayed using a single saturating concentration of 5 nM [^{3}H]estradiol as previously described [9]. Total nuclear ER (NER) were assayed using hydroxylapatite as described by Garola and McGuire [10]. However, Tween was not added to the washing buffer. The cut-off points were 50 fmol/g wet weight for CER and 25 fmol/g wet weight for NER. The CER concentrations varied from 0 to 5468 fmol/g in the NER-containing

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tumors and from 0 to 1249 fmol/g in tumors lacking NER. The NER levels varied from 0 to 792 fmol/g in the CER-positive tumors and from 0 to 604 fmol/g in the CER-negative tumors. The extraction and assay of peroxidase was a modification of the method described by Lyttle and DeSombre [4], Aliquots of the supernatants removed after centrifuging at 800 *g* were re-centrifuged at 15,000 *g* for 15 min at 0–4°C. The sediments were homogenized in 10 mM Tris–HCl buffer (pH 7.4) containing 0.5 M $CaCl_2$. The extracts were again centrifuged at 15,000 *g* for 15 min and the supernatants were assayed for peroxidase activity. The reaction mixture, in a final volume of 3 ml, consisted of 13 mM guaiacol and 0.3 mM H_2O_2 in 10 mM Tris–HCl buffer (pH 7.4). The reaction was started by the addition of tumor extract and the initial rate of activity was determined by following the increase in absorbancy at 470 nm. An enzyme unit was defined as the amount of enzyme required to produce an increase of one absorbancy unit/min. Tumors containing greater than 0.1 units/g wet weight were considered as peroxidase-positive. Total estrogens and progesterone were measured in the supernatant from the first centrifugation at 15,000 *g* described above [11, 12]. The antisera against both steroids were supplied by Abraham. The antibody used in the estrogen assay was produced against estriol-3, 16α, 17β-trihemisuccinate-HSA. The cross reactivity for both estradiol and estrone was 140% (taking estriol as 100%). Total estrogens, rather than estradiol, were measured, as estrone is quantitatively the most important estrogen in postmenopausal females and most of the tumors in the present investigation were from postmenopausal patients. The antiserum for the progesterone assay was obtained against 11-desoxycortisol-21-monohemisuccinate-HSA. The cross reactivity for both 17-hydroxyprogesterone and 11-desoxycortisol was 90% (taking progesterone as 100%).

RESULTS

Table 1 shows the percentage of peroxidase-positive tumors and the median peroxidase activity in CER-positive–NER-positive, CER-positive–NER-negative, CER-negative–NER-positive and CER-negative–NER-negative carcinomas. There was no significant difference in median activities between the four groups. Also, there was no correlation between either CER or NER and peroxidase activity. Furthermore, no correlation was found between either cytoplasmic total estrogens or progesterone and peroxidase activity.

A number of hypotheses were investigated in an attempt to explain the lack of correlation between CER and peroxidase activity. Since the CER assay carried out at 4°C measures free or unoccupied receptor, endogenous estrogens saturating the receptor might cause at least some false-negative receptor results [13]. Thus in some tumors without measurable CER but possessing peroxidase activity, receptor negativity could have been due to endogenous estrogens occupying the receptor. If this hypothesis is correct, levels of cytoplasmatic estrogens should be higher in the CER-negative–peroxidase-positive group than in the CER-negative–peroxidase-negative group.

Measurement of endogenous estrogens showed that the former group had a significantly higher concentration than the latter (Table 2). However, the median NER concentration was not significantly different in these two groups suggesting that the peroxidase activity was not induced through the ER mechanism.

Progesterone has antiestrogenic properties and has been shown to inhibit estrogen-inducible peroxidase in the immature rat uterus [5]. Could high levels of endogenous progesterone be inhibiting estrogen-inducible peroxidase activity in CER-positive tumors? Progesterone was therefore measured in cytosols from CER-positive–peroxidase-positive and CER-positive–peroxidase-negative tumors. No significant difference was found between the means of the two groups (Table 3).

Another possible explanation for the lack of correlation between CER and peroxidase could be the presence of NER in the absence of CER [10]. However, NER was found in only 6/17 tumors without CER and the median peroxidase activity in this group was not significantly different from the tumors without both CER and NER (Table 1).

DISCUSSION

Our results show no correlation between either CER or NER and peroxidase activity in human mammary carcinomas. The lack of correlation between CER and peroxidase activity is unlikely to be due to inhibition of estrogen-inducible peroxidase by endogenous progesterone or the presence of NER in the absence of CER.

Previous work using mouse and rat mammary tumors [6, 8] has shown that peroxidase

Table 1. *The distribution of peroxidase activity in breast tumors with respect to receptor status*

Receptor status	Peroxidase activity (units/g tumor)*			
	Median	Range	Mean rank	Peroxidase† (%)
CER^+ NER^+ ($n=19$)	0.171	0–7.24	26.83	74
CER^+ NER^- ($n=19$)	0.407	0–38.78	31.94	74
CER^- NER^+ ($n=6$)	0.079	0–34.99	25.5	50
CER^- NER^- ($n=11$)	0.267	0–2.791	28.04	64

*Peroxidase activity is not normally distributed—Kurtosis = 17.998; skewness = 4.100.
†A Kruskal–Wallis one-way analysis of variance was performed. No statistically significant difference was observed between the mean ranks of peroxidase activity in any of the above groups.

Table 2. *Cycloplasmic estrogen levels in CER^- tumors*

	Estrogen levels (pmol/g tumor)*		
	Median	Range	Mean rank
CER^- $Peroxidase^+$ ($n=9$)	1.69	0.29–30.38	13.43†
CER^- $Peroxidase^-$ ($n=7$)	0.75	0.52–1.79	7.36

*Estrogen levels are not normally distributed—Kurtosis = 17.728; skewness = 4.016.
†$P=0.039$ using a Mann–Whitney U Test.

Table 3. *Cytoplasmic progesterone levels in CER^+ tumors*

	Progesterone levels (pmol/g tumor)*		
	Mean	Median	Range
CER^+ $Peroxidase^+$ ($n=22$)	13.23 ± 7.43†	13.71	3.5–31.00
CER^+ $Peroxidase^-$ ($n=7$)	13.92 ± 11.25‡	8.73	3.2–34.29

*Progesterone levels are normally distributed—Kurtosis = 0.071; skewness 0.849.
†Mean ± S.D.
‡No significant difference between means. Since progesterone levels are normally distrubuted, Student's t-test was used to determine statistical significance.

activity was higher in the hormone-dependent group than in the independent group. Also Jellinck *et al.* [5] have found a moderate correlation ($r=0.45$) between NER and peroxidase activity in DMBA-induced rat mammary tumors. In human breast carcinomas we have shown in a preliminary investigation that following the administration of 1 mg of ethinyl estradiol 24 hr before biopsy or mastectomy, peroxidase activity tended to be more associated with CER-positive carcinomas than with the receptor-negative group [7]. In this investigation no steroid was administered prior to operation. It is possible that peroxidase induction in human mammary carcinoma requires super-physiological levels of estrogens. If so, this could explain the descrepancy between our previous [7] and present results. Recently Collings and Savage [14] also reported that the distribution of peroxidase-positive tumors was similar in human carcinomas with and without CER.

It would appear, therefore, that the measurement of peroxidase enzyme activity in human breast tumors cannot be used as a marker for a functional CER. Some of the peroxidase measured in these tumors may be derived from contaminating white cells or induced by other hormones. Further work is therefore necessary on peroxidase from human breast tumors to establish the origin of this enzyme and the factors which alter its activity. We are hoping to purify it and eventually to develop a radioimmunoassay for its detection. Measurement of immunoreactive breast tumor peroxidase might be a more specific marker for estrogen action in these tumors.

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