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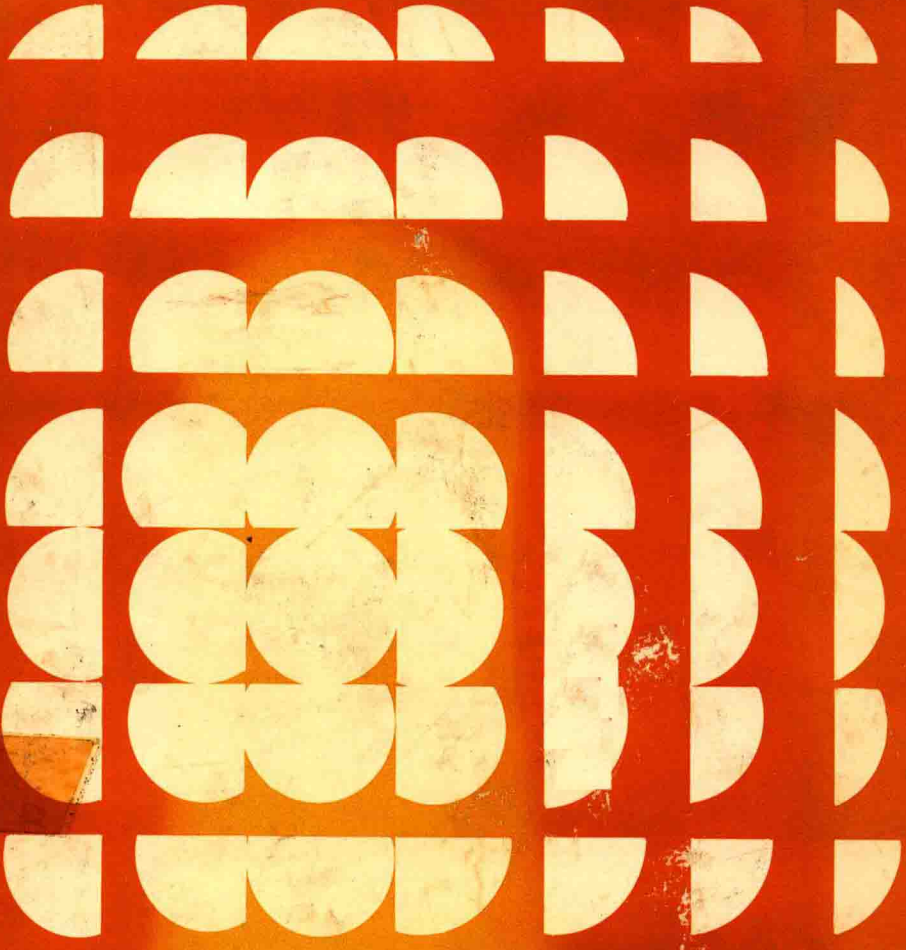
Hormones, Receptors, and Breast Cancer

Edited by
William L. McGuire



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Hormones, Receptors, and Breast Cancer

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Contents

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Estrophilin and Endocrine Responsiveness of Breast Cancer

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INTRODUCTION

It is now 12 years since we obtained a surgical sample of human breast cancer in our first attempt to determine whether an in vitro assay using tritiated estradiol could be used to differentiate hormone-dependent and hormone independent breast cancers. Even in 1966 it had long been recognized that some human breast cancers appeared to be hormone-dependent in that their growth was influenced by changes in the endocrine status of the patient. In 1896 Beatson reported the regression of metastatic breast cancer in several premenopausal women following oophorectomy (1). The modern era of endocrine therapy, resulting from the classic report in 1952 by Huggins and Bergenstal (10) on the use of adrenalectomy and in 1958 by the reports by Luft et al. (26) and by Pearson and Ray (29) on hypophysectomy for successful treatment of metastatic breast cancer in the postmenopausal woman, had clearly established that certain breast cancer patients obtained striking remissions from endocrine ablation. However with increased application of endocrine ablation to the treatment of metastatic breast cancer it became apparent that fewer than half of the patients treated actually received benefit. Thus by the 1960's there was a need for a simple method to predict a priori which breast cancer patients were most likely to respond to endocrine ablation.

Based on the early animal studies with tritiated estradiol (19) and hexestrol (8), Folca, Glascock and Irvine administered tritiated hexestrol to several patients about to undergo adrenalectomy for advanced breast cancer. They reported (7) that the lesions of the patients who later responded to this endocrine ablation took up more of the administered estrogen than did the lesions of the patients who did not respond. Although technically limited, this procedure provided the first evidence in human breast cancer that the interaction of estrogen with the tumor could be of diagnostic utility. Other laboratories, including those of King (24), Mobbs (27) and ourselves (23) used the carcinogen induced-rat mammary tumor animal model to demonstrate that hormone-dependent mammary tumors show a specific uptake of estrogen in vivo, similar to that seen in normal estrogen target tissues. Recognizing the importance of an in vitro assay, we showed (18) that by incubating slices of rat mammary tumors with tritiated estradiol in vitro one could also observe

the specific uptake of the tritiated estradiol by the hormone-dependent mammary tumors. Although this early in vitro method for differentiating hormone-dependent and autonomous human breast cancer seems quite crude by today's standards, it was able to make meaningful predictions of patient response to endocrine therapy (11).

Quite clearly studies of the hormone dependence of human breast cancer are more sophisticated today. Determination of estrogen receptor, or estrophilin as it has come to be called, is more quantitative and is almost universally accepted as a diagnostic tool (28). We are no longer satisfied with only separating the patients into two categories, those likely to respond to endocrine therapies from those unlikely to respond, but now hope to predict accurately the response of each patient. It is anticipated that such a goal might be reached not only by recognizing the importance of the presence of estrophilin in the breast lesion but also by evaluating a second parameter which might be indicative of a functioning estrogen-estrophilin interaction pathway. Nonetheless, it is also important to realize just how effective the predictive test based only on estrophilin content, as assayed by sedimentation methods, can be. This chapter, therefore, will be devoted largely to an updating of our studies of estrophilin levels in human breast cancer, first as regards the recent clinical correlations with response to endocrine therapy; second, as relates to the use of estrophilin assay of the primary tumor for prediction of subsequent response to endocrine therapy at the time of recurrence; and finally, as regards recent studies with antibody to estrophilin.

EXPERIMENTAL PROCEDURES

With the exception of the first ten patients whose tumor tissue was assayed by in vitro uptake of tritiated estradiol (11), all tumors have been analyzed for estrophilin by sedimentation analysis (4). For this purpose the tumor specimen is immersed in liquid nitrogen and shattered in a Thermovac autopulverizer. The resulting tissue powder is homogenized with cooling in 10mM Tris buffer, pH 7.4, containing 0.5mM dithiothreitol, usually 4 ml per g tissue, and a particulate-free cytosol is prepared by centrifugation of the homogenate for 30 min., 2°C, at 210,000 x g. For assay of cytosol content of estrophilin two 150 µl-portions of cytosol are used, one is incubated with buffer, the other, the specificity control, is incubated with 50µl of 0.25µM unlabelled diethystilbestrol in the recent assays and 50µl of 1 µM Parke-Davis CI628 in the earlier studies. After 30 minutes at 2°C, 50µl of 2.5nM tritiated estradiol is added to both portions and, following a second incubation for one hour in the cold, 200µl-aliquots of the incubation mixtures are layered on separate, preformed 10-30% sucrose gradients in 10mM Tris, pH 7.4 buffer containing 10mM KCl and 1mM EDTA. Following centrifugation for 16 hr at 250,000 x g the gradients are fractionated and analyzed for tritium (6). While we recognize that this method underestimates the estrophilin content, especially for those cytosols containing large concentrations of estrogen receptor (5), it has been found to be sensitive for detecting low to moderate amounts of 8S estrophilin.

Antibody to calf uterine nuclear estrophilin (9) was obtained from a

New Zealand white rabbit immunized by the intradermal injection procedure of Vaitukaitis et al. (30) using a primary immunization with approximately 20 g of purified estrophilin with complete Freund's adjuvant and additional tubercle bacilli and booster injections with up to 50μg of purified estrophilin in Freund's incomplete adjuvant. The highly purified calf uterine nuclear estrophilin was obtained from crude calf uterine nuclear sediment incubated for 60 min. at 25° with calf uterine cytosol containing 10nM tritiated estradiol. The nuclear estrophilin-estradiol complex was extracted in 10mM Tris, pH 8.5 buffer containing 400mM KCl and purified by a sequence of salt precipitation, gel filtration through Sephadex G-200 and electrophoresis in polyacrylamide gel as described elsewhere (3). Different preparations were found to contain from 10 to 50% of the tritiated estradiol expected for the pure receptor complex. Blood was collected via the marginal ear vein and a crude immunoglobulin fraction was prepared from the serum by precipitation with 33% saturated ammonium sulfate in 50mM phosphate buffer, pH 7.4.

MCF-7 cell extracts were provided by Dr. Christopher Nolan, Abbott Laboratories, North Chicago, Illinois. To obtain nuclear MCF-7 H^3-estradiol-estrophilin complex a 10mM Tris, pH 7.4 homogenate of MCF-7 cells was incubated with 10nM H^3 estradiol at 25°C for 60 minutes, or, alternatively, cells were grown for 70 hours in the presence of 10nM H^3 estradiol at 37°C prior to homogenization. In each case crude nuclei were isolated by centrifugation at 1000 x g and the nuclear estrophilin complex was extracted with 400mM KCl in 10mM Tris, pH 8.5 buffer. Uncomplexed nuclear estrophilin was obtained from MCF-7 cells not exposed to estradiol but extracted as above and incubated with H^3 estradiol. Cytosol estrophilin was obtained from a 210,000 x g supernatant of MCF-7 cells homogenized in 10mM Tris, pH 7.4 buffer. Cytosol estrophilin complex was obtained after incubation of MCF-7 cytosol with 2.7nM H^3 estradiol at 2°C followed by treatment with dextran-coated charcoal to remove excess steroid.

Sedimentation analysis studies of the interaction of estrophilin antibody with breast cancer estrophilin complexes were conducted by incubating the breast cancer extract with the immunoglobulin fraction (200-400μg protein) of the immunized or normal rabbit serum at 2°C for 1 to 2 hours. Aliquots of the incubation mixtures were then layered on preformed sucrose gradients which were centrifuged at 2°C for 16 hrs at 253,000 x g, fractionated and assayed for H^3 by scintillation counting. The sedimentation markers were analyzed in a separate gradient tube at the same time.

RESULTS AND DISCUSSION

While the early assay methods for evaluating estrogen receptor in human breast cancer, mainly using the slice uptake procedure developed in our laboratory (18), had a number of distinct limitations, the early clinical correlations were very promising. Our first report of 26 evaluable patients treated by endocrine therapies, all but one patient having been subjected to ablative procedures, showed 5 of the 7 patients whose lesions were classified as positive experienced remissions while only one of the 19 patients with borderline or negative type lesions had

an objective response. In retrospect it appears that part of the early prediction success may indeed have resulted from the relative insensitivity of the methodology used, although it is also likely that the uptake method may have at least partially detected nuclear estrophilin (by exchange occurring at the 37°C incubation temperature) as well as cytosol estrophilin. Because of the relative insensitivity of the methods our early reports (6,11-14,20) did not take sufficient cognizance of the quantitative significance of the results.

With the increased sensitivity of sedimentation analysis for detecting small amounts of cytosol estrophilin along with the availability of tritiated estradiol containing 4 tritium atoms, thus with a higher specific activity, it became apparent that one could detect extremely small amounts of estrophilin. It became important therefore to consider whether there was any predictive relevance to the quantitative levels of estrophilin in the breast cancers. This question took an added importance since some investigators at the 1974 Workshop on Estrogen Receptor in Breast Cancer (28), especially those using non-sedimentation analytical methods, were reporting that most of the breast cancers assayed contained estrogen receptor while it is clear that only 25 to 30 percent of the patients treated by endocrine therapy will have objective remissions. At that meeting we reported that there appeared to be a group of patients whose lesions had a low but detectable cytosol estrophilin content but who did not respond to endocrine therapy (21). It therefore was suggested that there may be a critical level of cytosol estrophilin below which patients did not respond to endocrine therapy. This concept of the critical level of estrophilin has withstood the almost doubling of the clinical results since 1974 (4,5,15-17,22). Our most recent data is shown in figure 1. It can be seen that indeed the estrophilin content of human breast cancer represents a continuum from zero to over 2500 fmoles per gram. Furthermore, in the premenopausal patients the distribution of estrophilin content is skewed toward the lower levels with essentially only two samples having a content significantly over 1000 fmoles/gram. In the 110 postmenopausal women, with only 4 exceptions, no patient whose tumor had less than 750 fmole estrophilin per gram experienced an objective remission. Similarly, the data for the 50 premenopausal women showed that no remissions were found in patients whose tumors contained less than 300 fmoles estrophilin per gram. Despite the increased number of cases the definition of the apparent critical level of estrophilin required for response has remained the same (22). However it is still evident that while essentially all responses occur in patients whose cancers have more than this critical level of estrophilin, not all patients whose cancers have at least the critical level of estrophilin respond. In figure 2 the response rate is related to tumor cytosol estrophilin content for the postmenopausal patients. The critical level phenomenon is again evident when the data are presented this way since the response rates are about 5% in the 2 lowest groups which have the majority of the patients. Furthermore, there appears to be a tendency toward increasing response rate with increasing estrophilin content between 750 and 2000 femtomoles/gram. More clinical correlations with patients whose tumors are estrophilin-rich (i.e. have more than the critical level of estrophilin) will be needed to confirm this apparent trend.

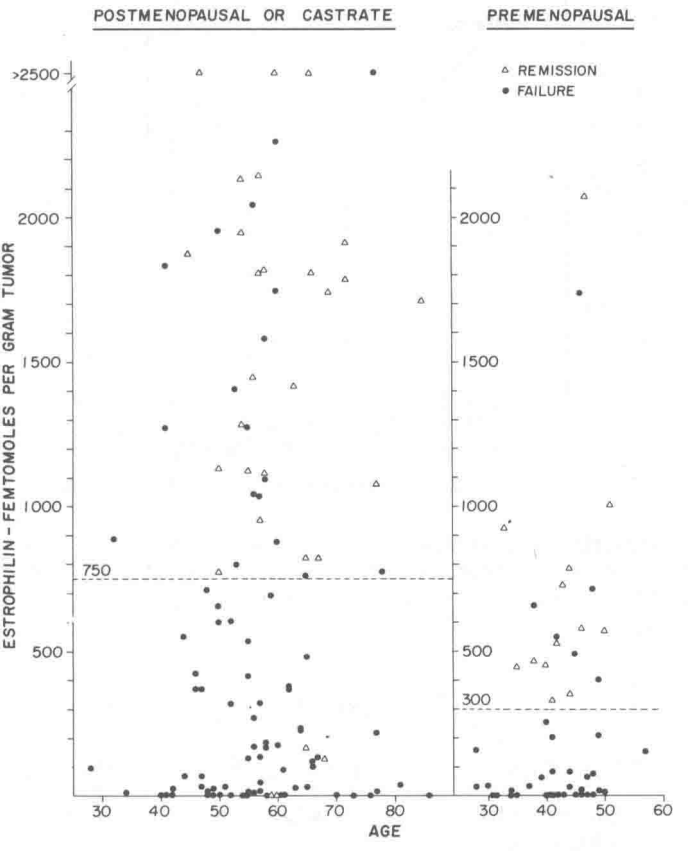

Fig. 1. Correlation of tumor cytosol estrophilin content with response to endocrine therapy for 160 patients with metastatic breast cancer.

Evaluation of the patient responses to the various endocrine treatments as correlated with tumor estrophilin for all 170 evaluated cases is shown in Table 1. One hundred seventeen patients were treated by endocrine ablation and 31 remissions were observed for an overall response rate of 26.5%. However, all but two of the remissions occurred in the estrophilin- rich group which showed a 71% response rate to ablation. The accuracy of prediction of response to endocrine ablation using the critical level of estrophilin was 88% (29+74 of 117).

Fifty-three patients were treated by hormone additive therapy. Both the overall response rate (12/53 or 22.6%) and the accuracy of prediction of the response based on estrophilin content (10+30 of 53 or 75%) were somewhat lower in this group than in the ablation group. However here also the remissions occurred primarily in the patients with estrophilin-rich tumors. Of the total of 170 patients it is clear that the patients with estrophilin-poor tumors have little chance (4%) of objective

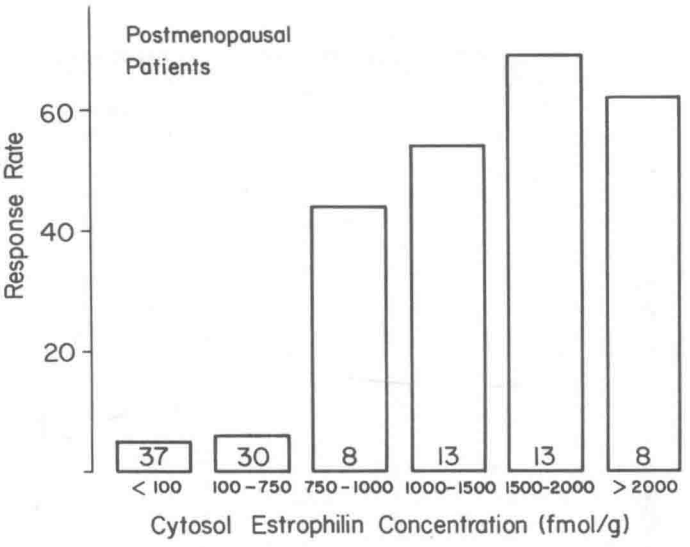

Fig. 2. Relation of response rate of postmenopausal breast cancer patients to endocrine therapy and tumor cytosol estrophilin concentration. The number inside each bar shows the number of women in that group.

TABLE 1. Remissions to Endocrine Therapy

ABLATION	ER - Rich[a]	ER - Poor[a]
Adrenalectomy	4/6[b]	0/20[b]
Adrenalectomy + oophorectomy	14/19	1/17
Hypophysectomy	2/4	0/9
Oophorectomy	9/12	1/30
Total Ablation - 117	29/41 (71%)	2/76 (3%)
HORMONE		
Androgen	0/1	0/4
Estrogen	4/5	1/9
Estrogen + Progestin	5/13	1/18
Antiestrogen	1/2	0/1
Total Hormone - 53	10/21 (48%)	2/32 (6%)
TOTAL CASES - 170	39/62 (63%)	4/108 (4%)

[a]Based on the critical estrophilin levels shown in figure 1.

[b]Objective remissions/total cases.

benefit from endocrine therapy but nearly two-thirds of the patients with estrophilin-rich tumors will obtain remissions. The overall utility of the critical levels of estrophilin for prediction of response to endocrine treatment for these 170 patients is shown by the 84% prediction accuracy based on this parameter.

Since in most patients sufficient amounts of the primary breast tumor are generally available at the time of mastectomy while samples of metastatic lesions often are difficult to obtain at time of recurrence, it is important to determine how well the assay for estrophilin in the primary tumor predicts the response to endocrine therapy much later, at time of recurrence. Although probably a large number of estrophilin assays have been performed on mastectomy samples from patients with early disease, there are few published reports (4,16) showing the efficacy of such assays to predict treatment responses at recurrence. Our results with 34 patients are shown in Table 2. The results are encouraging in

TABLE 2. Use of Estrophilin Assay of Mastectomy Specimen for Predicting Response after Recurrence.

ER Rich 9/13			ER Poor 1/21		
Rx[a]	Months[b]	Response	Rx[a]	Months[b]	Response
O	49	R	E	67	R
O	46	R	O	60	F
Ord	36	F	An	31	F
Ad	29	R	EP	27	F
EP	26	F	EP	18	F
E	24	F	Ord	16	F
E	24	R	Ord	15	F
O	23	R	AdO	12	F
AdO	14	R	H	12	F
E	9	F	O	11	F
AdO	5	R	An	10	F
E	1	R	AdO	8	F
			Ord	8	F
			Ord	7	F
Ablation 6/7			O	7	F
			EP	7	F
Hormone 3/6			E	4	F
			E	1	F
			EP	III IV[c]	F
			E	III IV[c]	F

[a] AdO, adrenalectomy plus oophorectomy; An, androgen; E, estrogen; EP, estrogen + progestin; H, hypophysectomy; O, oophorectomy; Ord, radiation castration.

[b] Time between mastectomy and treatment of recurrent disease.

[c] Patients had evidence of distant metastases at time of mastectomy so treatment was started immediately.

hormone-dependent cells; secondly, variable levels of tumor estrophilin may reflect the various proportions of estrophilin-containing, hormone-dependent cells and estrophilin-negative autonomous cells. If the proportion of autonomous cells is large, the response of the dependent cells to endocrine therapy could be masked by the continued growth of the autonomous cells. It is difficult to differentiate these two alternatives without the capacity to detect estrophilin at the cellular level. Our approach to this problem is to try to use estrophilin important to match other prognostic disease parameters, especially nodal status at time of mastectomy, to be able to adequately evaluate the independent contribution of estrophilin content to recurrence time or disease free interval. However, if patients with estrophilin-poor primary tumors show early recurrence, such patients would obviously be prime candidates for aggressive adjuvant chemotherapy. Thus the knowledge of tumor cytosol estrophilin content may be important for selection of certain patients for early chemotherapy as well as for choosing patients for endocrine therapy.

Although our data clearly are consistent with the requirement for moderate to substantial amounts of cytosol estrophilin for hormone response of the tumor, the fundamental reason for the critical level is less evident. Two reasonable alternatives are: first, the critical level may represent the minimum amount of cytosol receptor to maintain that the estrophilin assay of the mastectomy sample correctly predicted response to endocrine therapy in 29 of the 34 patients. Nine of the 13 patients (69%) with estrophilin-rich primary tumors responded to endocrine therapy, even though the interval time between mastectomy and treatment of recurrent disease could be quite long, up to more than 4 years. The correlation was again especially good with ablative treatment. Only one of the patients with an estrophilin-poor primary

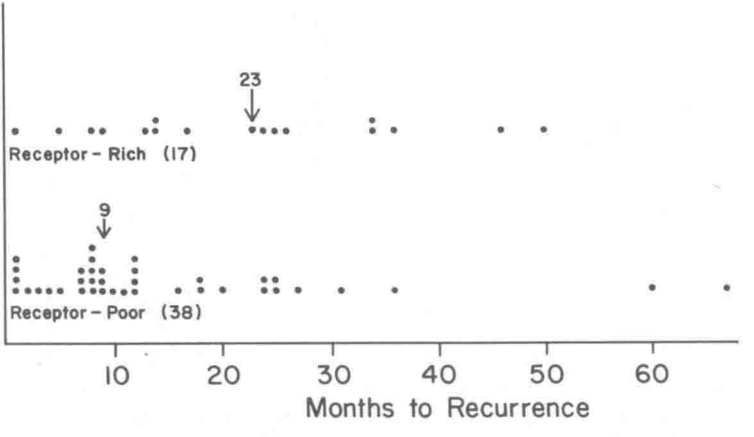

Fig. 3. Interval between mastectomy and recurrence in 17 patients with estrophilin-rich primary breast cancers and 38 patients with estrophilin-poor cancers. Median time to recurrence in each group is shown by the arrow.

lesion responded to endocrine therapy and this patient had the longest interval before recurrence. Indeed, as has been pointed out earlier (4,25), it is interesting that patients with estrophilin-poor lesions appear to have a shorter interval to recurrence than patients with estrophilin-rich lesions. Figure 3 shows the intervals to recurrence for the 55 patients studied thus far. The median time to recurrence of the estrophilin-rich breast cancer patients was 23 months while that of the estrophilin-poor cancer patients was only 9 months. It will obviously be antibodies to detect estrophilin in tissue sections and see whether the proportion of estrophilin positive cells correlates with the quantitative level of estrophilin by cytosol assay. For sometime now our laboratory has been actively engaged in the purification of both cytosol and nuclear estrophilin from calf uterus and recently we were able to demonstrate production of antibody to uterine nuclear estrophilin (9). Fortunately, this antibody to nuclear estrophilin from calf uterus is able to recognize both cytosol and nuclear estrophilin from a number of species, including man. Figure 4 shows the interaction of this rabbit antibody with cytosol from an estrophilin-rich breast cancer specimen. It can be seen that a distinct increase in the sedimentation rate, due to the association of antibody with the tritiated estradiol-estrophilin complex, is observed whether the sedimentation analysis is conducted under high salt or low salt conditions. This sedimentation change is not observed with estradiol

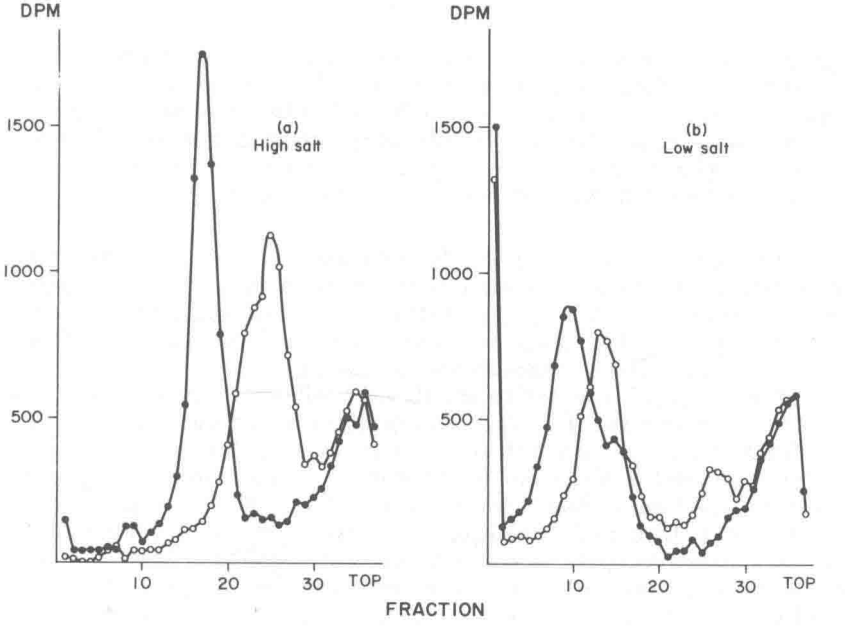

Fig. 4. Sedimentation analysis of human breast cancer cytosol estrophilin incubated with immunoglobulin of normal rabbit serum (open circles) or immuno-globulin of estrophilin-immunized rabbit serum (closed circles), as described in the experimental procedures. Reproduced from reference 9.

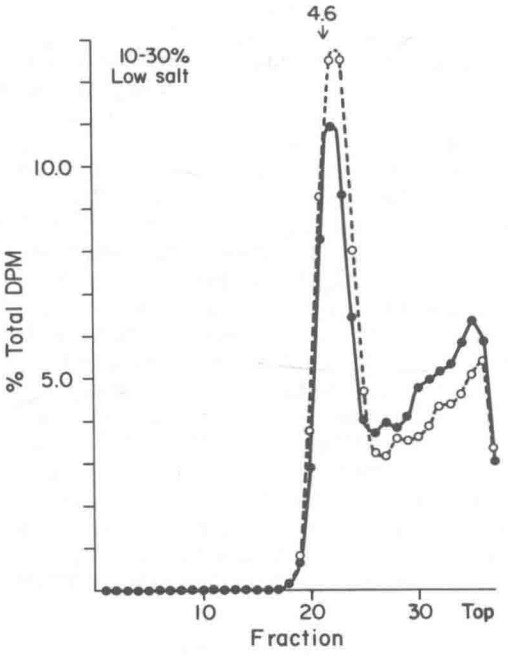

Figure 5. Lack of reactivity of human breast cancer cytosol non-specific H^3 estradiol binding components with estrophilin antibody. Estrophilin negative cytosol was incubated with diethylstilbestrol and tritiated estradiol prior to incubation with immunoglobulin of normal rabbit serum (open circles) or estrophilin-immunized rabbit serum (closed circles) and sedimentation analysis under low salt conditions.

itself (9), , or as shown in Figure 5, with the non-specific cytosol binding components. In addition, we have recently been able to demonstrate that the antiestrophilin antibody associates with estrophilin whether or not it is complexed with hormone. On the other hand this antibody does not react with androgen or progesterone receptors.

It was also of interest to see if this antibody reacted similarly with the MCF-7 receptors. This cell line, originating at the Michigan Cancer Foundation from a pleural effusion of a metastatic breast cancer patient, has been studied extensively in a number of laboratories. It has been shown to contain cytosol estrogen receptor (2) as well as nuclear receptor both free and complexed with estrogen (31). Figure 6 shows that the cytosol receptor complex in low salt consists of both 3-4S and 8S binding proteins both of which interact with the receptor antibody and thereby sediment faster. The cytosol receptor complex in high salt, mainly the 3-4S complex, also combines with receptor antibody. Figure 7 shows the similar study with the nuclear receptor complex from cells grown in the presence of tritiated estradiol. It is apparent that the antibody also completely reacts with the nuclear MCF-7 estrophilin complex. A sedimentation pattern essentially identical to that shown in Figure 7 was obtained when naked nuclear estrophilin, obtained from

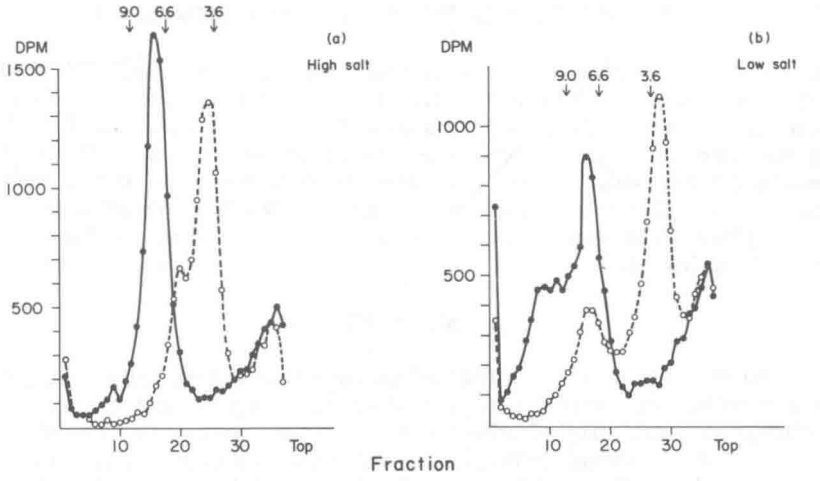

Fig. 6. Interaction of MCF-7 cytosol estrogen receptor complex with antiestrophilin antibody. MCF-7 cytosol, incubated with 2.7nM tritiated estradiol followed by treatment with dextran coated charcoal, was further incubated with immunoglobulin of normal rabbit serum (open circles) or estrophilin-immunized rabbit serum (closed circles) and analyzed by sedimentation analysis under both low salt (right) and high salt (left) conditions as described in the experimental procedures. Arrows show sedimentation positions of C^{14} labelled ovalbumin (3.6S), rabbit immunoglobulin (6.6S) and amylase (9.0S) run in a separate gradient tube.

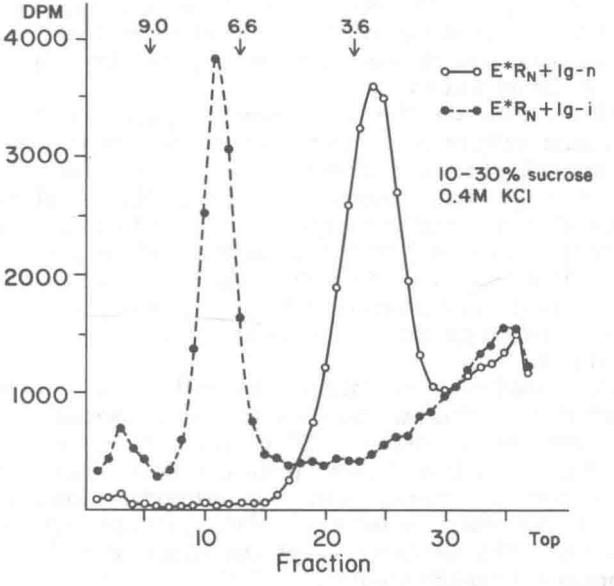

Fig. 7. Interaction of MCF-7 nuclear estrogen receptor complex with antiestrophilin antibody. Nuclear extract of MCF-7 cells grown for 70 hours in the presence of 10nM tritiated estradiol was incubated with immunoglobulin fractions and analyzed on high salt sucrose gradients as described in Fig. 6.

cells grown in estrogen deficient medium, was incubated with tritiated estradiol and estrophilin antibody. Thus the estrophilin antibody reacts with all the characterized forms of estrophilin of the MCF-7 cell line. Studies are currently underway in our laboratory to apply this recently developed antibody to the detection of estrophilin in tissue sections, using either fluorescence or peroxidase sandwich techniques, as well as to explore immunochemical methods to quantitate breast cancer estrophilin levels.

SUMMARY

Knowledge of the content of estrogen receptor, called estrophilin, in human breast cancer, has proved to be of significant clinical value. Although the majority of breast cancer tissues assayed can be shown to contain some cytosol estrophilin, our data indicates that only those patients whose lesions have moderate to high levels of estrophilin respond to endocrine therapy. The empirically observed critical level of estrophilin in endocrine responsive lesions appears to be about 700 fmoles per gram tissue for postmenopausal or castrate patients and 300 fmoles per gram for premenopausal patients, when sedimentation analysis with 0.5nM estradiol is used. Furthermore, in the postmenopausal patients one has the impression that there may be an increasing response rate with increasing tumor concentration of estrophilin in excess of the critical level. Of the 170 metastatic breast cancer patients evaluated for response to various endocrine therapies the overall response rate was 25%. However, almost two-thirds of the patients with estrophilin-rich lesions obtain remissions while only 4% of the patients whose lesions contained less than the critical amount of estrophilin responded. Thus, the overall accuracy of prediction of response to endocrine therapy in these 170 patients was 84%.

Further data on the correlation of estrophilin content in the primary tumor with response to endocrine treatment given subsequently, at the time of recurrent disease, are encouraging. Nine of the 13 patients whose primary lesions were estrophilin-rich showed subsequent remissions when treated from one to 49 months following mastectomy. On the other hand, only 1 of the 21 patients whose primary tumors were estrophilin-poor responded to such subsequent therapy. In addition, it appears that patients with estrophilin-poor primary lesions have a shorter interval to recurrence than the patients whose mastectomy samples are estrophilin rich.

Recent studies with antibody to calf uterine nuclear estrophilin clearly show that this antibody reacts with cytosol estrophilin from human breast cancer tissue. All reported forms of estrophilin from MCF-7 cells, a cell line derived from a breast cancer pleural effusion, have been found to interact with this antibody. However, neither free estradiol nor non-specific estrogen binding components are detected by this antibody. The antibody shows the required specificity for use in breast cancer estrophilin studies.

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REFERENCES

1. Beatson, G.T. (1896): Lancet, 2:104-107.
2. Brooks, S.C., Locke, E.R. and Soule, H.D. (1973): J. Biol. Chem., 248: 6251-6253.
3. DeSombre, E.R. and Gorell, T.A. (1975): In: Methods in Enzymology, Vol. 36A, edited by B.W. O'Malley and J.G. Hardman, pp., 349-365. Academic Press, New York.
4. DeSombre, E.R., and Jensen, E.V. (1978): In: Endocrine Surgery in Breast Cancer, edited by M. Mayer, S. Saez, and B. Stoll. Plenum Press, New York (in press).
5. DeSombre, E.R., and Jensen, E.V. (1976): In: Control Mechanisms in Cancer, edited by W. Criss, T. Ohno, and J.R. Sabine, pp. 67-82. Raven Press, New York.
6. DeSombre, E.R., Smith, S., Block, G.E., Ferguson, D.J., and Jensen, E.V. (1974): Cancer Chemother. Rep., 58: 513-519.
7. Folca, P.J. Glascock, R.F., and Irvine, W.T. (1961): Lancet, 2: 796-798.
8. Glascock, R.F. and Hoekstra, W.G. (1959): Biochem J., 72: 673-682.
9. Greene, G.L., Closs, L.E., Fleming, H., DeSombre, E.R., and Jensen, E.V. (1977) Proc. Natl. Acad. Sci. USA, 74: 3681-3685.
10. Huggins, C., and Bergenstal, D.M. (1952): Cancer Res., 12: 134-141.
11. Jensen, E.V., Block, G.E., Smith, S., Kyser, K.A. and DeSombre, E.R. (1971): Nat. Cancer Inst. Monograph, 34:55-70/
12. Jensen, E.V., Block, G.E., Smith, S., Kyser, K., and DeSombre, E.R. (1972): In: Estrogen Target Tissues and Neoplasia, edited by T.L. Dao, pp. 23-57. Univ. Chicago Press, Chicago.
13. Jensen, E.V., Block, G.E., Smith, S., and DeSombre, E.R. (1973): In: Breast Cancer: A Challenging Problem, edited by M.L. Griem, E.V. Jensen, J.E. Ultmann and R.W. Wissler, pp. 55-62. Springer-Verlag, Heidelberg.
14. Jensen, E.V., and DeSombre, E.R. (1972): In: Proceeding IV International Congress on Endocrinol., pp. 1227-1231. Excerpta Medica, Amsterdam.