Estrogen and Prolactin Receptor Concentrations in Rat Mammary Tumors and Response to Endocrine Ablation

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SUMMARY

Estrogen and prolactin receptor concentrations were measured in 24 carcinogen-induced rat mammary tumors and correlated with the tumor response to host ovariectomy or hypophysectomy. It was found that essentially all of the tumors contained some specific estrogen receptor, and all but three contained prolactin receptor. The values for each receptor comprised a continuum from very low to relatively high concentrations, suggesting that previous considerations of hormone dependence on the basis of presence or absence of hormone receptors may be oversimplified. The concentration of each receptor tended to be lower in the hormone-independent than in the hormone-dependent tumors, but there were a number of hormone-independent tumors with higher receptor levels than some of the hormone-dependent tumors had. A better correlation of tumor response to endocrine ablation resulted from a combination of the 2 receptor levels than from either receptor concentration alone. These results suggest that there is a complex relationship between mammary tumor response to endocrine ablation and levels of estrogen and prolactin receptors and that some tumors may be dependent upon 1 or both of these hormones for growth.

INTRODUCTION

A significant number of human breast cancers respond to endocrine ablations such as oophorectomy, adrenalectomy, and hypophysectomy, and, therefore, these cancers are considered to be hormone dependent. Since the correlation of breast cancer remission to endocrine ablation with tumor estrogen receptor content permits prediction of the response of many patients to such therapy, it is likely that estrogen is involved in growth regulation of these cancers. However, there are suggestions that prolactin (10, 26) and possibly other hormones (7, 22) also may be important in breast cancer. In the DMBA3-induced mammary cancer of the rat, most of the tumors regress following host castration or treatment with drugs that inhibit prolactin release and, hence, are deemed hormone dependent (6, 9, 11, 23). In this model system, there is considerable disagreement as to whether estrogen or prolactin alone, or both hormones in concert are responsible for tumor growth regulation. Since there is a growing body of evidence that both steroid and peptide hormones act through the mediation of their specific receptors (27), we have approached this problem by comparing the rat mammary tumor response to host ovariectomy or hypophysectomy with the tumor content of both estrogen and prolactin receptors.

MATERIALS AND METHODS

Mammary tumors were induced in 50-day-old female Sprague-Dawley rats by a single i.g. feeding (12) of 20 mg of DMBA in 2 ml of sesame oil. Transplant tumors were obtained after s.c. transplant of about 100 mg of DMBA in 2 ml of sesame oil. Transplant tumors were obtained after s.c. transplant of about 100 mg of tumor either as a small piece or as a homogeneous mince in 0.9% NaCl solution into 40- to 55-day-old intact female rats. Since the Sprague-Dawley rat is not highly inbred, tumor growth after transplant is seen in only 10 to 30% of the animals. Each transplant tumor used had established a consistent growth pattern before the host was subjected to ovariectomy. Although such tumors show a definite tendency to lose their ability to regress following ovariectomy with subsequent transplant generations, we have observed retention of this ability to regress for up to 4 transplant generations in the Sprague-Dawley rat. Although some histological changes often occur on transplantation of the tumors, the transplant tumors used were carcinomas as were the primary tumors studied.

Tumor volumes, used to assess the tumor response to endocrine ablation, were calculated from weekly tumor measurements by caliper (6). Hormone-dependent tumors are defined as those tumors that regressed to a volume less than one-half presurgical size. This group of tumors was excised while the animal was under light ether anesthesia generally 1 to 3 weeks following endocrine ablation. The hormone-independent tumors used were those which, at time of tumor excision, generally from 3 to 5 weeks after ovariectomy, had a calculated volume greater than the presurgical size.

For the receptor determinations, the tumors were excised from animals under ether anesthesia, the tumors were minced, weighed, frozen in liquid nitrogen, and pulverized at liquid nitrogen temperature with a Thermovac autopulver-

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2 Recipient of Research Career Development Award HD 46249 from the National Institute of Child Health and Human Development. To whom requests for reprints should be addressed, at the Ben May Laboratory for Cancer Research, University of Chicago, 950 E. 59th St., Chicago, Ill.
3 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene, i.g., intragastric.

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izer (Thermovac Industries Corp., Copiague, N. Y.). The tumor powder was homogenized in 4 volumes of cold 10 mm Tris buffer, pH 7.4, containing 1 mm dithiothreitol, using a Polytron PT-10 homogenizer (Brinkmann Instruments, Inc., Westbury, N. Y.). A high-speed cytosol, used for the estrogen receptor determination, was obtained by centrifugation of the tumor homogenate for 30 min at 250,000 x g and 2°. The total particulate fraction, obtained as the sediment in this centrifugation, was resuspended in the homogenization buffer, frozen in liquid nitrogen, and stored at liquid nitrogen or dry ice temperatures until used to prepare the membrane fraction for prolactin receptor assay.

For determination of the estrogen receptor content, 200-μl portions of the high-speed tumor cytosols were incubated for 30 min at 0° with 50 μl of homogenization buffer or 50 μl of 10-6 m diethylstilbestrol in homogenization buffer, followed by addition of 50 μl of 12 nm 17β-[2,4,6,7-3H]estradiol (specific activity, 109 Ci/m mole; New England Nuclear Corp., Boston, Mass.) in the same buffer. After incubation for an additional 60 min in ice, a 200-μl portion of each mixture was layered on 3.4 ml of a 10 to 30% sucrose gradient containing 10 mM KCl and 1 mM EDTA in 10 mM Tris buffer, pH 7.4, and centrifuged at 2° for 16 hr at 250,000 x g. Successive 100-μl fractions were collected from the bottom of the centrifuge tube by displacement with paraffin oil. Radioactivity was determined in a toluene-Triton X-100 scintillation fluid at 28 to 33% efficiency. The estrogen receptor content was calculated from the diethylstilbestrol-inhibited dpm in both 8 S and 4 S regions of the gradient, and is expressed as fmoles of specific estradiol binding per g of fresh tumor tissue.

For determination of prolactin receptor content, the high-speed tumor sediment was thawed, homogenized in 0.3 M sucrose, and centrifuged for 20 min at 14,500 x g. The supernatant fraction was subjected to a 2nd centrifugation at 105,000 x g at 2° to isolate the membrane-containing pellet used for the prolactin receptor determinations. After colorimetric determination of protein content (19), replicate portions of the tumor membrane fractions, suspended in 0.025 M Tris buffer containing 10 mM CaCl2, pH 7.8, were incubated for 48 hr at 4° with 67,000 ±5%, cpm of [125I]iodinated prolactin. The prolactin (NIH-S10; National Pituitary Agency, NIH) was iodinated by a lactoperoxidase method (8) and had a specific activity of about 40 μCi/μg. Specific prolactin binding refers to the amount of radioactive iodinated prolactin that could be displaced by excess unlabeled prolactin, and is expressed as cpm/200 μg of membrane fraction protein. The reproducibility of the assay using 3 to 4 replicate samples is generally ±2.2%. Nonspecific binding refers to the nondisplaceable cpm and generally represents 3 to 5% of the total counts. This assay has previously been shown to demonstrate high-affinity binding of prolactin to mammary tumor membrane fraction (16).

RESULTS

In this preliminary study, 24 rat mammary tumors were followed for response to host ovariectomy or hypophysectomy, and the tumor levels of estrogen and prolactin receptors were determined (Table 1). It can be seen from the results that, although the hormone-independent tumors as a group consist of the lower end of the spectrum of estrogen receptor levels, there is overlap of the higher values of the hormone-independent tumors and the lower values of the hormone-dependent tumors. Likewise, there is considerable overlap in the values for specific prolactin binding by hormone-dependent and hormone-independent tumors, although 2 of the 3 tumors showing no specific prolactin binding were hormone-independent tumors. Chart 1 shows the relation between the 2 receptor levels and tumor response to endocrine ablation. It is quite clear from this correlation that all of the tumors with high levels of both prolactin and estrogen receptor regressed following endocrine ablation, whereas only 1 of the 5 tumors with low values for both receptors regressed. Lines were drawn to obtain the best correlation of receptor levels with response (450 fmoles/g for estrogen receptor and 2200 cpm/200 μg of protein for prolactin receptor). In the group with low levels of one receptor and higher levels of the other receptor, there are both hormone-dependent and hormone-independent tumors.

The Table 1

<table>
<thead>
<tr>
<th>Tumor*</th>
<th>Estrogen receptor (fmoles/g tumor)</th>
<th>Prolactin binding (cpm/200 μg membrane protein)</th>
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<tr>
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</tr>
<tr>
<td>8</td>
<td>602</td>
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* Tumors 4, 5, 6, and 21 were transplant tumors; all of the rest were primary carcinogen-induced tumors. Based on tumor response after host hypophysectomy (Tumors 10 and 18) or ovariectomy.
cut-off line in Chart 1 (Tumors 12 and 13 of Table 1) appeared to grow slightly during the 1st week before undergoing rapid regression.

In an attempt to correlate further the tumor responses to endocrine ablation with tumor content of estrogen and prolactin receptor, different combinations of the 2 receptor levels were tested for their ability to correlate with response to ablation. It was found, as presented in Chart 2, that when 10% of the prolactin receptor value was added to the estrogen receptor value for the tumors, a better correlation with response was obtained. In this chart, the lines dividing positive and negative values for receptor levels were drawn so as to retain all of the hormone-independent tumors within the range of negative receptor levels. By this procedure, the combination of receptor values plot gives only 2 false predictions, whereas the correlation with either estrogen receptor alone (left) or prolactin receptor alone (right) includes in each case 7 hormone-dependent tumors in the receptor negative area.

**DISCUSSION**

The observation that endocrine ablation could induce the regression of most of the carcinogen-induced tumors of the rat was reported as early as 1959 (11). Subsequent reports, in general, have suggested that at least 2 hormones, estrogen and prolactin, may be directly involved in the regulation of mammary tumor growth.

The primacy of prolactin has been proposed (30) based on experiments with triply operated rats (ovariectomized, adrenalectomized, hypophysectomized), but prolactin alone appears unable to sustain mammary tumor growth for more than a short time. Furthermore, Clemens et al. (3) and Sinha et al. (29) reported that lesions in the hypothalamic median eminence, a procedure that increases serum prolactin levels (24, 33), greatly accelerated tumor growth in rats, but subsequent ovariectomy resulted in rapid tumor regression despite high levels of prolactin. Rapid resumption of growth could be obtained by grafting ovaries into such rats, suggesting that the prolactin stimulus to mammary tumor growth is dependent on ovarian hormones.

Since there is considerable evidence that both steroid and peptide hormones act through their specific tissue receptors (27), the relation of tumor estrogen and prolactin...
receptor concentrations to tumor responses following host endocrine ablation is particularly pertinent. There are numerous reports indicating that the hormone-dependent rat mammary tumors contain significant amounts of cytoplasmic estrogen receptor (14, 17, 18, 20, 25, 28, 31) whereas the hormone-independent tumors contain little or no estrogen receptor (13, 18, 20). More recent evidence (1, 2) indicates that some apparently hormone-independent tumors do contain estrogen receptor. The estrogen receptor substances of mammary tumors show many similar characteristics to the better studied uterine estrogen receptors, in particular, sedimentation coefficients, steroid specificity and affinity, and nuclear uptake (Refs. 18 and 21; unpublished observations in our laboratory). Furthermore, recent evidence suggests that the tumor receptors are biochemically active (1).

Specific prolactin binding also has been reported in rat mammary tumors. Turkington (32) reported that DMBA-induced mammary tumors had prolactin-binding levels of 30 to 80% of lactating mammary gland, whereas the transplantable R3230AC tumor, which is prolactin responsive for milk protein synthesis but not for growth, had lower prolactin binding. McGuire et al. (4) reported that the prolactin binding of R3230AC tumors was similar in capacity and affinity to the normal mammary gland. They also reported that when both estrogen and prolactin receptor levels were compared in a hormone-dependent transplantable mammary tumor and its hormone-independent counterpart (5), both receptor levels were significantly lower in the hormone-independent tumor. An attempt to relate prolactin receptor with hormone response indicated that the amount of specific prolactin binding of rat mammary tumors correlated well with tumor growth response to prolactin administration (16).

We feel that it is of particular interest that, in the present study, essentially all of the tumors assayed had detectable amounts of specific estrogen receptor. With the increased sensitivity of present assay methods, this finding would suggest that it is no longer realistic to classify mammary tumors, either human or animal, simply as estrogen receptor present or receptor absent. Indeed, the results suggest that a more useful correlation of receptors concentration with response to endocrine approaches to therapy is to be found, it must take into account the diverse levels of receptor proteins present in the tumors. In this regard, it has been recently reported (15) that determination of a critical concentration of estrogen receptors in human breast cancer appears to improve the predictability of patient response to endocrine therapies.

What is obviously desired is some criterion or combination of criteria that can unfailingly predict response to endocrine therapies. Consideration of the estrogen receptor assay by itself shows fairly good predictability of remissions for tumors the ER level of which is greater than 450 fmoles/g (13 of 14 tumors (Chart 1)) but misses 30% of the tumors with lower ER levels that also regress. Changing the empirical criterion to less than 450 fmoles/g to include all responding tumors would also include more nonresponsive tumors. Since there are likewise some tumors that regress after endocrine ablation, but have little or no prolactin receptor, and others that fail to regress, but have relatively high prolactin receptor, it is also clear that the prolactin receptor content of tumors by itself does not fulfill the objective of an unequivocal prognostic factor. It was hoped that the knowledge of the tumor concentrations of both receptors could fulfill the above objective. Although this hope could not be entirely realized, it is clear that the use of both receptor concentrations improves the overall predictability of responses to over 90% (22 of 24 tumors (Chart 2)). It is possible, as has been suggested (7, 22), that other hormones may also be involved in the growth regulation of breast cancer, and thus, other factors must be evaluated to reach the objective of complete predictability of responses to endocrine therapies. It is also possible that the changes in serum hormone levels effected by endocrine ablations may have significantly changed the tumor receptor levels. Nonetheless, we conclude that at least in the animal model used, assay of tumor estrogen and prolactin receptor concentrations can provide highly correlative information relating to previous responses to endocrine ablation. This must now be further evaluated in a prospective manner, taking into account the assay problems due to the endogenous hormones in the intact animal.

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REFERENCES


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