Significance of Aromatase Activity in Human Breast Cancer

W. R. Miller, R. A. Hawkins, and A. P. M. Forrest

University Department of Clinical Surgery, Medical School, Teviot Place, Edinburgh EH9 9AG, Scotland

Abstract

The significance of in vitro aromatization of \([7\alpha-^3H]\)testosterone to estradiol by human breast cancer has been investigated by correlation with (a) estrogen receptor activity and (b) clinical response to endocrine therapy. Evidence for estradiol synthesis was obtained in 66 and estrogen receptor activity in 71 of 110 tumors. Whereas most estrogen receptor-positive tumors synthesized estradiol, the majority of receptor-negative tumors did not. This tendency for aromatization to be associated with estrogen receptor-positive tumors was statistically significant \((p < 0.005)\). Mean level of receptor was also significantly higher in tumors with aromatization than in tumors without estradiol synthesis \((p < 0.001)\). Forty patients with advanced breast cancer that had been treated by endocrine therapy. There was a significant trend for tumors with aromatization to be associated with response to treatment \((p < 0.05)\), but the correlation was not absolute and may simply reflect the association between aromatase activity and estrogen receptors. Within the small subgroup of patients treated with aminoglutethimide or adrenalectomy, tumors with high aromatization activity responded whereas those without aromatization did not. Tumor estrogen biosynthesis may therefore be of clinical significance in selecting patients for treatments which remove sources of precursor for aromatization or inhibit aromatase activity itself.

Introduction

Many groups \((4, 7, 14, 24)\) have now demonstrated that some breast cancers synthesize estrogen from androgen precursors in vitro. It is not known whether this potential for aromatization is merely an example of tumor differentiation or whether it is of significance to the need of the tumor for estrogens. In the latter event, correlations between tumor aromatase and hormonal sensitivity would be expected.

In this study, we have related tumor aromatase activity to estrogen receptors and, in a small group of patients with advanced breast cancer, to response to endocrine treatment.

Materials and Methods

Tumors. Breast cancer tissue was obtained from 110 patients. Of these, 24 were premenopausal, 4 were menopausal (less than 5 years since the last menstrual period), and 82 were postmenopausal.

Tumor was obtained at mastectomy or biopsy and immediately placed on ice. Following removal of tissue for histopathological diagnosis, the remainder was assayed for estradiol synthesis (aromatase activity) and estrogen receptor activity.

Estrogen Synthesis. A portion of each tumor \((0.5 \text{~g})\) was finely minced and incubated for 2 hr at \(37^\circ\text{C}\) in Krebs-Ringer phosphate buffer, pH 7.4, containing an NADPH-generating system and 22.5 \(\mu\text{Ci} [7\alpha-^3H]\)testosterone \((8.9 \text{ Ci/mmol})\). The reaction was stopped by the addition of methanol, and 500 \(\mu\text{g}\) of nonradioactive estradiol were added to monitor losses during purification and characterization. Estradiol was extracted and purified by thin-layer chromatography \((14, 15)\). Characterization was by chemical derivative formation \((14)\) and was based on chromatographic behavior of parent and derivatized estradiol being identical with that of authentic steroids and by the maintenance of consistent specific radioactivity throughout the procedures. Synthesis was determined by measuring radioactivity in the purified estradiol fraction. Conversions in excess of 0.02% of the original precursor are detectable.

Estrogen Receptors. Concentration of estrogen receptor was determined by saturation analysis \((5)\). Tumor cytosol was incubated overnight at \(4^\circ\text{C}\) with \(17\beta-[2,4,6,7-^3H]\)estradiol and varying amounts of nonradioactive \(17\beta\)-estradiol. Separation of free and bound steroid was by addition of dextran-coated charcoal; the bound fraction was measured by liquid scintillation counting. Concentration of receptors was determined by Scatchard analysis \((23)\). Activities in excess of 5 fmol/mg cytosol protein were designated receptor positive \((8)\).

Clinical Response. Forty patients with advanced breast cancer were treated with endocrine procedures including oophorectomy, adrenalectomy, hypophysectomy, tamoxifen, diethylstilbestrol, and aminoglutethimide.

For the purpose of this study, objective response to treatment was graded independently by 2 members of the Department of Clinical Surgery who did not know the results of the biochemical studies. Patients were classified as having a response if they showed evidence of tumor regression during therapy.

Results

Evidence for estradiol synthesis from testosterone was obtained in 66 of the human breast cancers examined \((60\%)\). Level of conversion is shown in Chart 1 and ranged from 0.02 to 0.5%.

Estrogen receptor activity was detected in 81 tumors \((74\%)\), and the relationship between the presence of receptors and potential for aromatization is shown in Table 1. Most estrogen receptor-positive tumors \((68\%)\) synthesized estradiol, whereas only the minority of receptor-negative tumors \((38\%)\) did so. This tendency for aromatization to be associated with estrogen receptor-positive tumors was statistically significant \((p < 0.005)\).

Tumors with aromatizing capacity were not only more likely to have estrogen receptor activity but, in addition, the mean level of receptor of these tumors was significantly higher than that in tumors lacking estradiol synthesis \((Chart 2)\). The converse was not so; amounts of estrogen synthesized by tumors with receptors did not differ significantly from those without receptors. In tumors with both aromatizing and estrogen receptor activity, no significant quantitative relationship was found \((Chart 3)\).

The relationship between tumor potential for aromatization and clinical response of 40 patients with advanced breast cancer treated with endocrine therapy is presented in Chart 4. There was a statistically significant trend for aromatization to be associated with response, but the correlation was not ab-
solute. This effect was largely accounted for by the association of aromatase with estrogen receptors, all tumors with aromatase activity responding to treatment also being estrogen receptor positive.

It was also of interest to examine the relationship between tumor aromatization and response to specific therapies which might be active against aromatization. The results are presented in Table 2, both for adrenalectomy, which removes the major source of C-19 steroid precursors in postmenopausal women, and for the administration of aminoglutethimide, which also inhibits tumor aromatase (2).

Although numbers are small, tumors with the greatest in vitro conversion to estradiol were those which responded while those without aromatization failed to do so.

Discussion

Our report that human breast cancer may synthesize estrogen from C-19 steroid precursors (13) has been confirmed by several groups (1, 4, 7, 24). When compared with ovarian production in premenopausal women, the levels of estrogen synthesized by a tumor may appear insignificant. However, in postmenopausal women with breast cancer, a tumor may represent a major estrogen-synthesizing organ. Using identical conditions of in vitro incubation, we have found that breast cancers may have higher aromatase activity than do adrenal cortex, liver, or fat.  

Fat is generally assumed to be the major site of peripheral conversion of androgens to estrogens (10, 21). However, direct comparisons of adipose tissue and breast cancer have always shown higher biosynthesis of estrogen in tumor (3, 22). Furthermore, using the methods of the present studies, biosynthesis of estrogens from dehydroepiandrosterone, Δ4-androstenedione, and testosterone was consistently below detectable levels in adipose tissue. The large mass of fat in the body may compensate for its low synthetic activity and make it the major source of circulating estrogen in postmenopausal women. However, “on site” tumor production of estrogen may be more important for the growth of the breast cancer.

It has been calculated that the MCF-7 cell line of human breast cancer might synthesize sufficient intracellular estrogen to stimulate estrogen-mediated events (11). In the present studies, similar calculations indicate that 0.5 to 12.5 pmol are formed during incubation. This is sufficient to half-saturate estrogen receptor sites in the majority of breast cancers. However, it should be noted that endogenous levels of testosterone in breast tumors are likely to be considerably lower than are those used in our incubations (20). On the other hand, in the breast there are high concentrations of other C-19 steroid precursors (18) which breast tumors may convert to testosterone or metabolize directly to estrogen (1, 7, 17, 19).

In addition to these theoretical considerations, it is important to relate the results from in vitro incubations to estrogen receptor status which has already been established to be of clinical value (12).

The finding of a positive correlation between aromatase and estrogen receptor activity confirms our previous report (17) but is not in agreement with others (3, 9, 24). However, in these latter studies, smaller numbers were reported. This may be important, since the relationship between aromatase and re-

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Table 1

<table>
<thead>
<tr>
<th></th>
<th>Estrogen synthesis</th>
<th>Without synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor positive</td>
<td>55</td>
<td>26</td>
</tr>
<tr>
<td>Estrogen receptor negative</td>
<td>11</td>
<td>18</td>
</tr>
</tbody>
</table>

Chart 1. Level of estradiol synthesis in tumors with aromatization.

Chart 2. Level of estrogen receptor in tumors with and without estradiol synthesis. The difference between groups is significant by Wilcoxon rank test ($p < 0.001$).
be a positive relationship between aromatase activity and clinical response, this was neither additive to, nor independent of, estrogen receptor activity. The relationship between tumor aromatase activity and endocrine responsiveness may be best shown by studying those forms of treatment which deprive the tumor of its precursor C-19 steroids or directly inhibit tumor aromatase. These include adrenalectomy and aminoglutethimide, and it is encouraging to note that tumors with highest aromatase activity appeared to be more responsive to these therapeutic methods. However, the numbers of patients studied are small, and it is now important to establish a prospective study to determine the relationships between aromatase activity, steroid receptor, and tumor sensitivity to endocrine treatment.

It is difficult to attribute a physiological role for estrogen synthesis in tumors which lack estrogen receptor activity, although it has been suggested (1) that these tumors may appear hormone independent by virtue of their de novo synthesis. Further studies are required to elucidate this.

One can conclude that estrogen-synthetic activity can be demonstrated in approximately half of all breast cancers and in many is likely to be of sufficient magnitude to induce estrogen-stimulated events. Although aromatase is correlated with estrogen receptor activity, the relationship is not absolute. However, we have some evidence that estrogen synthesis may be an important influence on the hormonal sensitivity of a tumor, especially to regimens such as adrenalectomy and aminoglutethimide.

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References


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