Improved Measurement of Androgen Receptors in Human Breast Cancer

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ABSTRACT

Molybdate-stabilized androgen receptors have been quantitated in cytosols derived from 1026 malignant breast tumors including all new cases of primary breast cancer reported in the western region of Norway during the 3-year period 1985–1987. A simple single point saturation assay using the synthetic labeled ligand methyltrienolone was evaluated for this purpose. This approach also allowed the simultaneous determination of estrogen and progesterone receptor from the same cytosol preparation. The cytosol content of albumin was also recorded in order to control for dilution by extracellular proteins.

Androgen receptor was the sex hormone receptor most frequently found both in primary and secondary breast cancer. In primary tumors 84.9% (723 of 852) showed a cytosol concentration higher than 10 fmol/mg protein compared to 71.2 and 67.1% for estrogen and progesterone receptors, respectively. This incidence is about 2 times higher than previously reported for androgen receptors in the literature and may be due to the stabilizing effects of molybdate and a serine protease inhibitor on the recovery of active binding sites in cytosol. Cytosol concentration of androgen receptor is generally lower than that of the other sex hormone receptors; the average level was 65.5 fmol/mg cytosol protein compared to 86.8 and 84.7 for estrogen and progesterone receptors, respectively.

Both incidence and cytosol concentrations were lower for all sex hormone receptors in soft tissue metastasis than in the primary tumor. This decrease is not likely to be due to differences in tumor cellularity since metastatic tumors appear to be more cellular as judged from a lower cytosol content of extracellular proteins (albumin). No significant differences were observed in any parameter investigated between different metastatic sites (skin, lymph nodes). Androgen receptor levels were strongly correlated to estrogen and progesterone receptor concentration in both primary and secondary cancers.

Cytosol androgen receptor concentration increases with age. This increase is more significant in metastatic than in primary tumors. Evidently, tumor cellularity is a confounding factor in primary tumors since tumor cytosols from younger patients showed a higher content of extracellular proteins. Receptor levels in lymph node metastasis did not exhibit age dependence. This may suggest that locally produced factors rather than circulating levels of sex steroids modulate tumor receptor expression.

In metastatic tissues androgen receptors are present with twice the frequency of progesterone receptors and one in four of these tumors express androgen receptor as their sole sex hormone receptor. This supports the view that some of the beneficial effects of high dose progestin treatment of advanced breast cancer are mediated through the androgen receptor. Knowledge of the tumor androgen receptor status should thus aid in the selection of optimal endocrine therapies in recurrent disease.

INTRODUCTION

Analysis of estrogen and progestin receptors in breast cancer tissue has gained widespread acceptance as prognostic parameters useful in the management of this disease (1). The presence of estrogen receptor suggests a well differentiated tumor amenable to hormonal manipulation. The concomitant presence of progestin receptor, thought to be the product of an estrogen receptor-mediated estrogenic stimulus, strengthens the likelihood that the tumor may respond to endocrine therapy. In breast cancer patients 60–80% (2, 3) of primary tumors contain measurable levels of estrogen receptor (i.e., >10 fmol/mg cytosol protein) and 40–60% show the simultaneous presence of both estrogen receptor and progesterone receptor. Endogenous estrogens stimulate breast tumor growth and most endocrine therapies are aimed at eliminating or antagonizing this influence. Considerably less is known about breast tumor sensitivity towards androgens and the occurrence and prognostic value of androgen receptors. Pharmacological doses of androgens induce regression in rats bearing 7,12-dimethylbenz(a)-anthracene-induced mammary carcinomas (4) whereas growth stimulation by physiological concentration of androgens has been encountered in Shionogi 115 cells from a murine mammary cancer (5). In this cell line the growth-stimulatory effect of high doses of estradiol seems to be mediated through the androgen receptor (6). A direct growth-inhibitory effect of androgens on hormone-dependent human breast cancer cell lines has been demonstrated (7) whereas a growth-stimulatory effect was noted in tissue culture using supraphysiological concentrations (8). In clinical trials (9) synthetic androgens gave an objective remission in about 25% of advanced breast cancer patients. Recently, reports of high response rates using high doses of progestins (10), which are partial androgen agonists, have renewed interest in the role of androgens in breast cancer treatment.

Androgen responsiveness should theoretically be reflected through the presence of androgen receptors within the tumor or by the presence of a recognized product of an androgenic action. Proteins secreted in gross cystic disease fluid are likely to be such a product (11, 12) and one of them, the progesterone-binding cyst protein, is present in about 60% of cytosols prepared from primary breast tumors (13). This would imply that androgen-responsive tumors occur in about two thirds of the patients. This is, however, close to twice the incidence previously reported for androgen receptors in primary tumors (14–18). This discrepancy is likely to reside in methodological shortcomings. One major problem is the inherent thermolability of the receptor itself which results in the loss of binding affinity by either spontaneous transformation or through the action of cellular proteases (19). The natural ligands testosterone and 5α-dihydrotestosterone are metabolized by intracellular enzymes and are also bound with high affinity to the SHBG present in extracellular space which will interfere in conventional binding assays. The use of the synthetic steroid ligand methyltrienolone is reported (20) to circumvent some of these problems. More recently, the stability of the androgen receptor has been shown to be greatly enhanced by molybdate ions. Inclusion of molybdate in the homogenization medium has a dramatic effect on the recovery of active binding sites (21) and would seem to be mandatory for reliable estimates of androgen receptor levels. Although previous investigations of androgen...
receptors in larger patient materials suffer from this omission, androgen receptor determination still seems to be predictive of survival and response to endocrine therapy (22).

The present investigation evaluates a simple single point saturation assay for molybdate-stabilized androgen receptor that can be used in conjunction with routine ligand-binding analysis for estrogen and progesterone receptor. We further wanted to establish more accurate and updated data on the prevalence of androgen receptors in metastatic and primary breast tumors in a large and representative patient material and to seek correlation between androgen receptor and extra- and intracellular parameters such as albumin and other sex hormone receptors.

MATERIALS AND METHODS

Patient Material

Breast tumors collected from patients in the western region of Norway during a 3-year period starting in December 1984 were analyzed as part of a routine screening procedure for sex hormone receptors. Among the 895 histologically confirmed malignant breast tumors investigated results from 26 tumors were deleted due to inadequate amounts of tissue supplied (cytosol protein less than 1 mg/ml) and 17 were excluded for other reasons (preoperative radiation, technical errors). The remaining 852 primary cancers from 837 patients represented 105% of expected new breast cancer cases as estimated from the average cancer incidence in this region in a previous 10-year period (1970–1979).

Analyses of metastatic tumors, mainly soft tissue metastasis from loco-regional recurrences and metastatic axillary lymph nodes, were restricted to patients who had not previously received any hormonal or antihormonal treatment. Metastatic tumors were grouped according to site as: lymph nodes, predominantly axillary; cutaneous, locoregional skin metastasis; and scar, including tumors in close proximity to the operation scar. When information on menopausal status was unavailable patients have been assumed to be postmenopausal after 55 years of age.

Tumors were either frozen immediately after removal in liquid nitrogen and shipped on dry ice or, alternatively, chilled on ice, brought to the laboratory within 1 h and then placed at −70°C until further processing (never exceeding 2 weeks).

Reagents

- R-1881 (specific activity, 87 Ci/mmol), R-5020 (specific activity, 82 Ci/mmol), and [2,4,6,7,16,17-3H]Estradiol (specific activity, 150 Ci/mmol) were obtained from New England Nuclear, Boston, MA.
- Protein was analyzed by a Coomassie Brilliant Blue dye-binding method (23) using bovine albumin (γ-globulin; 1:1) as reference material. Albumin was determined by rocket immunoelectrophoresis according to the method of Laurell (24) using monospecific rabbit antiserum.
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Analytical Procedure

Buffer. Tris-HCl, 10 mM, pH 7.4, containing 10 mM sodium molybdate, 10% (v/v) glycerol, 1 mM thiglycol and 1 mM phenylmethyl-sulfonyl fluoride. Thiglycol and phenylmethylsulfonyl fluoride were added shortly before use.

Procedure. Frozen tumor tissue, 0.2–0.5 g, was finely sliced with a scalpel and homogenized in 3 ml buffer using two 20-s bursts from a Polytron homogenizer (Kinematica GmbH, Lucerne, Switzerland) at a setting of 8. The homogenate was centrifuged for 1 h at 105,000 × g using a Beckman T 70 rotor. The resulting supernatant, cytosol, was used for single point saturation assays. Cytosol was incubated with saturating concentrations of labeled ligand in the presence and absence of a 100-fold excess of cold steroid to correct for nonspecific (unsatuated) binding. Specifically, androgen receptor was determined in aliquots of 200 μl cytosol by incubating overnight (16–20 h) at 0–4°C with 10 nM [3H]-labeled methyltrienolone (R-1881). Parallel tubes received in addition 1 μM cold methyltrienolone. In order to block binding of labeled steroid to corticosteroid and progesterone receptors cold triamcinolone acetonide was added to all tubes to a final concentration of 5 μM (25). Steroids were maintained in and added from alcoholic stock solutions up to a final cytosolic alcohol concentration of 2%. Excess (unbound) steroid was then removed by adding 500 μl of a charcoal suspension (0.25% Norit A plus 0.025% dextran) in buffer. Following 30 min of incubation charcoal was removed by centrifugation at 3000 × g for 15 min. Aliquots of 500 μl were then analyzed for bound radioactivity by liquid scintillation counting.

Calculation of Cellular Protein. Extracellular blood-derived proteins present in cytosol will dilute cellular proteins to a varying degree depending on the cellularity of the tumor. Albumin (Alb.) measurements have been shown previously to adequately reflect extracellular protein contribution (26) and can be used to compute cellular protein concentration using the formula

\[
\text{Rec. fmol} = \frac{\text{Rec. fmol} \times 100}{\text{mg cell protein} - \text{% Alb.} \times 2.0}
\]

The factor 2.0 refers to the average ratio of total protein to albumin found in serum in this age group using the methods listed above. Due to the increasing inaccuracy in this estimate when albumin concentration approaches 50%, this transformation has been limited to cytosols containing less than 40% albumin.

RESULTS

Androgen receptor assay precision was evaluated on a pool of cytosol with a close to average content of androgen receptor. Within assay the coefficient of variation was 8.2% (N = 10). Repeated analysis of this pool during a 3-month interval showed an interassay coefficient of variation of 14.1% (N = 8). There was no significant decrease in androgen receptor content with length of storage at −70°C (P = 0.21) for periods up to 3 months. Repeated freezing and thawing (up to three times) showed no effect on recovery. The accuracy of the single point saturation assay was compared with a 6-point Scatchard analysis and against sucrose density gradient centrifugation analysis. The results were all within 10% of each other. Scatchard plot analysis revealed a single class of binding sites with an apparent association constant of 2.19 ± 0.1 M⁻¹ (not shown). Sucrose density gradient centrifugation analysis showed a predominant binding component sedimenting in the 8S region. However, significant saturable binding activity was also present in the 4–5S region (not shown).

Competition studies using various representative steroids displaced labeled R-1881 in the following order of effectiveness, R-1881 > 5α-dihydrotestosterone > R-5020 > progesterone > estradiol > dexamethasone, which is consistent with the specificity reported for the human androgen receptor (27).

Androgen receptor blank values estimated from the spread of duplicate samples (variation coefficient, 7.8%) pooled from the low range of receptor values indicate a detection limit of 4–5 fmol/mg cytosol protein for the average cytosol. Androgen receptor blank values may also be affected by interference by blood-derived proteins, such as SHBG. To assess possible interference by SHBG blanks were prepared using 17 sera of widely differing SHBG content at a dilution of 1:20 (total protein, 4 mg/ml). These "cytosols" revealed an "androgen receptor" value which was highly significantly correlated (r = 0.94) to SHBG concentration. The equation of the linear regres-
sion line was

Androgen receptor blank value (fmol/ml cytosol)

\[ = 11.6 + 3.9 \times \text{SHBG (nm)} \]

In 49 tumor cytosols analyzed for SHBG by immunoradiometric assay the average SHBG concentration was 1.2 nm with a range of 0.2 to 6.1 nm. SHBG may thus contribute an average of 4 fmol/mg cytosol protein to the recorded androgen receptor values with an upward range of about 9 fmol/mg protein.

Receptor values and incidence (i.e., >10 fmol/mg protein) obtained for estrogen receptor and progesterone receptor analysis were generally in good agreement with previously published values (26, 28, 29) for primary breast cancer obtained using a homogenization buffer lacking molybdate and protease inhibitor, although values for the progesterone receptor tended to be higher. There was also excellent correlation between estrogen receptor and progesterone receptor determined by the present dextran-coated charcoal method and a double-site immunoenzymatic assay (Abbott Laboratories); correlation coefficients were 0.98 and 0.95 for estrogen receptor and progesterone receptor, respectively, in 28 cytosols analyzed.

None of the parameters recorded in our tumor material passed tests for being normally distributed (Smirnow-Kolmogorov test) and hence nonparametrical statistical tests have been used throughout in analyzing the results.

The distribution of androgen receptor values found in primary cancer (Fig. 1) seem to suggest the presence of two tumor populations: one with low or zero receptor content; and one with log normally distributed values. A natural cutoff point between the two populations appears to be approximately 20 fmol/mg cytosol protein. The range of androgen receptor values was definitely more narrow than those of the other receptors and the mean value of 65.5 fmol/mg protein was clearly lower than that of the mean estrogen receptor and progesterone receptor (Table 1). In metastatic tumors the average androgen receptor value was higher than progesterone receptor but still lower than estrogen receptor (Table 2). Compared with primary tumors there was a highly significant \( P < 0.001 \) drop in both values (Mann-Whitney \( U \) test) and incidence \(<10 \) fmol/mg protein, \( \chi^2 \) test) for all three receptors (Tables 1 and 2). In both primary (Fig. 2) and metastatic tumors all three receptors were highly intercorrelated \( P < 0.0001, \) Spearman rank correlation analysis; Table 3). Albumin content in various cytosols was measured in order to correct for extracellular protein components (Tables 1 and 2). In agreement with earlier studies (26) albumin and blood-derived extracellular proteins make a significant contribution to what is generally termed “cytosol” protein and also show a great variation between tumor groups and with patient age. As could well be predicted all receptors showed a negative and highly significant correlation to the albumin content in primary tumors (Table 3). In metastatic tumors this correlation was absent. In order to compensate for dilution of cellular protein by extracellular proteins a conversion was made as outlined above to calculate receptor concentration per mg cell protein. Results are included in Tables 1 and 2. Androgen and estrogen receptors both showed a significant increase with patient age in both metastatic and primary tumors. Progesterone receptor levels were remarkable independent of age especially in primary tumors. Since tumor albumin content also is a function of age in primary tumors (Table 3) part of the age dependence of androgen receptor concentration could be explained by differences in tumor cellularity. When the correlation analysis is repeated using receptor values transformed into fmol/mg cell protein using the formula above, the significant correlation between age and androgen receptor is lost (Table 3). Similar results were obtained when tumors were grouped according to the menopausal status of the patients (Table 1). Tumor cellularity thus seems to be a confounding factor in the age dependence of receptor levels in primary tumors. Tumor albumin content is not a function of age in metastatic tumors. Compared to primary tumors metastatic tumors showed a higher cellularity as judged by a significantly lower albumin content \( P < 0.001, \) Mann-Whitney \( U \) test). The highly significant correlation between androgen receptor, estrogen receptor, and age was thus not altered by transforming these receptor data into fmol/mg cell protein. Interestingly, receptor content in lymph node metastasis showed no age dependence (Spearman rank analysis, \( P > 0.58 \)) and no difference between pre- and postmenopausal patients (Mann-Whitney \( U \) test, \( P > 0.3 \)). Otherwise, receptor content was about the same in different metastatic sites, none of the apparent differences reaching significance (Table 2). If tumors are classified as “positive” and “negative” using a conventional cutoff point of 10 fmol/mg protein, androgen receptor emerges as the most prevalent sex hormone receptor in both primary and metastatic cancer (Tables 1 and 2). Differences in incidence between post- and premenopausal patients were evident only for estrogen receptor in primary cancer (Table 1). Furthermore, there were no differences in incidence of any receptor among the various sites of soft tissue metastasis \( P > 0.3, \chi^2 \) test).

Tumors can be divided into 8 groups based on the various combinations of receptor “negative” or “positive” tumors (Fig. 3). The most frequent constellation found in both primary and...
metastatic disease is the one that is “positive” for all three receptors. Tumors containing androgen receptor as their sole receptor occur in about 10% of primary cancers but increase to become the second largest group in metastatic disease where 1 in 4 tumors belongs to this group.

**DISCUSSION**

The recommendation of R-1881 as a suitable ligand in the assay for androgen receptor has in part been based on its low affinity for SHBG (20). The present study shows that SHBG may significantly influence the blank value and detection limit in androgen receptor determination although in most tumors this contribution will be low. Still androgen receptor values between 10 and 20 fmol/mg protein should be interpreted with caution in the presence of a high cytosol content of extracellular (albumin) protein. The negative correlation between receptor values and extracellular (albumin) protein concentration also suggests that the SHBG contribution to the measured androgen receptor values is not of major significance.

Another potential drawback using R-1881 as ligand relates to the high affinity for progesterone receptor. Theoretically this binding should be blocked by the addition of excess unlabeled triamcinolone. Only 42 (4.1%) of 1026 tumors analyzed contained (>10 fmol/mg protein) progesterone receptor without the simultaneous presence of androgen receptor. This small number may still be considered high enough to rule out any methodological bias due to binding of R-1881 to the progesterone receptor. Overestimation of progesterone receptor due to binding of R-5020 to androgen receptor (30) can most likely be ignored since as many as 254 (25%) cytosols registered as progesterone receptor negative in the presence of significant amounts of androgen receptor.

As judged by incidence only (greater than 10 fmol/mg protein) androgen receptor is by far the most common receptor present in both primary and metastatic tumors. The average value in fmol/mg protein is, however, lower (Tables 1 and 2) than the other receptors and the distribution of values definitely more narrow. This may suggest that androgen receptor levels are more stringently controlled than are other sex hormone receptors.

In agreement with previously published results (14–17) all three receptors are highly intercorrelated (Fig. 2; Table 3), an observation that may reflect the stage of tumor differentiation.

<table>
<thead>
<tr>
<th>Tumor parameter</th>
<th>All patients (N = 852)</th>
<th>Premenopausal (N = 249)</th>
<th>Postmenopausal (N = 603)</th>
<th>P difference in post/premenopausal patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (yr)</td>
<td>63.5 ± 0.5*</td>
<td>45.6 ± 0.4</td>
<td>70.9 ± 0.3</td>
<td>0.023</td>
</tr>
<tr>
<td>Cytosol protein (mg/ml)</td>
<td>4.59 ± 0.07</td>
<td>4.80 ± 0.14</td>
<td>4.49 ± 0.09</td>
<td>0.017</td>
</tr>
<tr>
<td>Albumin (% of cytosol protein)</td>
<td>20.5 ± 0.3</td>
<td>21.6 ± 0.6</td>
<td>20.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Androgen receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg cytosol protein</td>
<td>65.5 ± 2.4</td>
<td>54.6 ± 3.4</td>
<td>70.0 ± 3.1</td>
<td>0.025</td>
</tr>
<tr>
<td>fmol/mg cell protein</td>
<td>108.7 ± 3.9</td>
<td>99.0 ± 6.4</td>
<td>112.7 ± 4.8</td>
<td>0.11 (NS)*</td>
</tr>
<tr>
<td>Incidence (&gt;10)</td>
<td>84.9%</td>
<td>81.9%</td>
<td>86.2%</td>
<td>0.11 (NS)</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg cytosol protein</td>
<td>86.8 ± 4.6</td>
<td>29.0 ± 3.1</td>
<td>148.9 ± 6.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>fmol/mg cell protein</td>
<td>137.7 ± 7.0</td>
<td>47.1 ± 4.5</td>
<td>175.3 ± 9.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Incidence (&gt;10)</td>
<td>71.2%</td>
<td>60.6%</td>
<td>75.8%</td>
<td>0.0001</td>
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<tr>
<td>Progesterone receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg cytosol protein</td>
<td>84.7 ± 4.7</td>
<td>78.5 ± 7.9</td>
<td>87.2 ± 5.8</td>
<td>0.83 (NS)</td>
</tr>
<tr>
<td>fmol/mg cell protein</td>
<td>141.3 ± 7.8</td>
<td>144.0 ± 15.5</td>
<td>140.1 ± 9.0</td>
<td>0.75 (NS)</td>
</tr>
<tr>
<td>Incidence (&gt;10)</td>
<td>67.1%</td>
<td>65.9%</td>
<td>68.0%</td>
<td>0.55 (NS)</td>
</tr>
</tbody>
</table>

* Average ± SE.

**Table 2** Metastatic tumors

<table>
<thead>
<tr>
<th>Tumor parameter</th>
<th>All patients (N = 174)</th>
<th>Cutaneous (N = 56)</th>
<th>Lymph nodes (N = 46)</th>
<th>Scar (N = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (yr)</td>
<td>62.9 ± 1.1*</td>
<td>64.4 ± 1.8</td>
<td>59.0 ± 2.4</td>
<td>64.4 ± 1.6</td>
</tr>
<tr>
<td>Cytosol protein (mg/ml)</td>
<td>4.06 ± 0.18</td>
<td>3.49 ± 0.27</td>
<td>4.99 ± 0.35</td>
<td>3.92 ± 0.31</td>
</tr>
<tr>
<td>Albumin (% of cytosol protein)</td>
<td>17.5 ± 0.6</td>
<td>17.6 ± 0.9</td>
<td>15.0 ± 0.8</td>
<td>19.2 ± 1.2</td>
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<tr>
<td>Androgen receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg cytosol protein</td>
<td>48.3 ± 4.3</td>
<td>39.7 ± 4.9</td>
<td>59.2 ± 10.8</td>
<td>48.1 ± 6.6</td>
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<tr>
<td>fmol/mg cell protein</td>
<td>73.9 ± 6.2</td>
<td>63.2 ± 7.4</td>
<td>84.8 ± 14.4</td>
<td>75.2 ± 10.2</td>
</tr>
<tr>
<td>Incidence (&gt;10)</td>
<td>78.2%</td>
<td>83.9%</td>
<td>76.1%</td>
<td>72.2%</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg cytosol protein</td>
<td>69.3 ± 10.4</td>
<td>77.9 ± 22.8</td>
<td>81.3 ± 24.1</td>
<td>55.0 ± 9.1</td>
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<tr>
<td>fmol/mg cell protein</td>
<td>102.1 ± 14.6</td>
<td>109.1 ± 29.7</td>
<td>117.8 ± 35.0</td>
<td>86.5 ± 14.4</td>
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<tr>
<td>Incidence (&gt;10)</td>
<td>47.7%</td>
<td>42.9%</td>
<td>50.0%</td>
<td>50.0%</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg cytosol protein</td>
<td>44.3 ± 7.3</td>
<td>42.1 ± 9.6</td>
<td>45.7 ± 14.1</td>
<td>45.0 ± 13.3</td>
</tr>
<tr>
<td>fmol/mg cell protein</td>
<td>69.8 ± 11.5</td>
<td>71.4 ± 16.4</td>
<td>62.6 ± 18.5</td>
<td>73.1 ± 22.1</td>
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<tr>
<td>Incidence (&gt;10)</td>
<td>42.5%</td>
<td>46.4%</td>
<td>43.5%</td>
<td>38.9%</td>
</tr>
</tbody>
</table>

* Average ± SE.

**Table 1** Primary carcinoma

**Table 2** Metastatic tumors
A natural cutoff point dividing the two populations appears to be based on the content of the three sex hormone receptors. A cutoff point between "positive" or "negative" tumors is an "negative" will divide the tumors into eight different categories. This supports the present finding that approximately twice the one of these earlier investigations (22) found that the prognosis in an additional 1 in 3 of patients with borderline androgen receptor values was equal to those who were receptor positive. This supports the present finding that approximately twice the frequency of receptor-positive cases is a more reliable estimate of the true incidence and in closer agreement with the expression of putative androgen-dependent cyst proteins in primary tumors. Classification of tumors as receptor "positive" or "negative" will divide the tumors into eight different categories based on the content of the three sex hormone receptors. A cutoff point between "positive" or "negative" tumors is an arbitrary one and is commonly chosen to be 10 fmol/mg protein. Androgen receptor distribution seems to be bimodal and a natural cutoff point dividing the two populations appears to be closer to 20 fmol/mg protein.

If, as suggested (31), response to endocrine therapy is dependent not only on the presence but also on the actual concentration of receptor, then a better estimate should be obtained through the use of cellular instead of cytosol protein. This will allow an assessment of the representativity of the analyzed tumor samples. In the present study 3.3% of the primary tumor cytosols contained more than 40% albumin and, by inference, less than 20% of the cytosol protein was derived from cellular material. Since most of these cytosols were classified as receptor negative one can assume that a maximum of about 3% of the tumor samples can be considered false negatives.

Results from many different model systems show that the cellular levels of hormone receptors are regulated by sex hormones. It is generally accepted that the expression of progestin receptor is a measure of a receptor-mediated estrogenic stimulation (32). Hence, one is forced to conclude that primary tumors are adequately or at least equally stimulated in post- and premenopausal women. The sharp increase in tumor estrogen receptor concentration with age could then be interpreted as an adaptation by up regulation of the receptor apparatus to compensate for the declining supply of circulating sex steroids (estrogens). Like estrogen receptor tumor content, androgen receptor concentration increases with age of the patients. This is in contrast to the finding of Mercer et al. (18), showing a decrease with age. This increase is less pronounced in primary tumors where part of this dependence can be explained by a lower cellularity in tumors from the younger patients as judged by cytosol albumin measurements (Table 1). This would suggest that androgen receptor concentration is rather independent on circulating levels of sex hormones when the tumor is present in its primary site but could well be under negative control of both estrogens and androgens at the metastatic site except in lymph node metastasis. The latter observation is not well understood and no evidence can be found for a response rate in lymph nodes that would be different from other soft tissue metastatic sites when subjected to endocrine therapy. It still suggests that local formation of potent hormones or other paracrine factors may be involved in the regulation of steroid receptor levels. All receptors show a significant drop in level and incidence when primary tumors are compared with metastatic tumors which is in agreement with our previous results (13) as well as with results obtained by others (16, 18). The drop in concentration is not the result of differences in cellularity since

Inhibitor in the homogenization buffer. It is noteworthy that one of these earlier investigations (22) found that the prognosis in an additional 1 in 3 of patients with borderline androgen receptor values was equal to those who were receptor positive. This supports the present finding that approximately twice the frequency of receptor-positive cases is a more reliable estimate of the true incidence and in closer agreement with the expression of putative androgen-dependent cyst proteins in primary tumors (13). Classification of tumors as receptor "positive" or "negative" will divide the tumors into eight different categories based on the content of the three sex hormone receptors. A cutoff point between "positive" or "negative" tumors is an arbitrary one and is commonly chosen to be 10 fmol/mg protein. Androgen receptor distribution seems to be bimodal and a natural cutoff point dividing the two populations appears to be closer to 20 fmol/mg protein.

If, as suggested (31), response to endocrine therapy is dependent not only on the presence but also on the actual concentration of receptor, then a better estimate should be obtained through the use of cellular instead of cytosol protein. This will allow an assessment of the representativity of the analyzed tumor samples. In the present study 3.3% of the primary tumor cytosols contained more than 40% albumin and, by inference, less than 20% of the cytosol protein was derived from cellular material. Since most of these cytosols were classified as receptor negative one can assume that a maximum of about 3% of the tumor samples can be considered false negatives.

Results from many different model systems show that the cellular levels of hormone receptors are regulated by sex hormones. It is generally accepted that the expression of progestin receptor is a measure of a receptor-mediated estrogenic stimulation (32). Hence, one is forced to conclude that primary tumors are adequately or at least equally stimulated in post- and premenopausal women. The sharp increase in tumor estrogen receptor concentration with age could then be interpreted as an adaptation by up regulation of the receptor apparatus to compensate for the declining supply of circulating sex steroids (estrogens). Like estrogen receptor tumor content, androgen receptor concentration increases with age of the patients. This is in contrast to the finding of Mercer et al. (18), showing a decrease with age. This increase is less pronounced in primary tumors where part of this dependence can be explained by a lower cellularity in tumors from the younger patients as judged by cytosol albumin measurements (Table 1). This would suggest that androgen receptor concentration is rather independent on circulating levels of sex hormones when the tumor is present in its primary site but could well be under negative control of both estrogens and androgens at the metastatic site except in lymph node metastasis. The latter observation is not well understood and no evidence can be found for a response rate in lymph nodes that would be different from other soft tissue metastatic sites when subjected to endocrine therapy. It still suggests that local formation of potent hormones or other paracrine factors may be involved in the regulation of steroid receptor levels. All receptors show a significant drop in level and incidence when primary tumors are compared with metastatic tumors which is in agreement with our previous results (13) as well as with results obtained by others (16, 18). The drop in concentration is not the result of differences in cellularity since
ANDROGEN RECEPTORS IN HUMAN BREAST CANCER

Metastatic tumors are generally more cellular as judged by their lower albumin content. Among the sex hormone receptors, androgen receptor content appears to be best preserved during the process of metastasis. In all metastatic tumors examined, androgen receptor is approximately twice as prevalent as progesterone receptor. As much as 25% of metastatic tumors, conventionally classified as receptor “negative” based on estrogen and progesterone receptor measurements, still contain significant amounts of androgen receptor. Several synthetic gestagens commonly used in breast cancer treatment also show androgenic or synandrogenic effects (33). Taken together, these observations strongly support the view (10, 34) that the beneficial effects of high dose progestin treatment, even in progestosterone receptor-“negative” tumors, are mediated through the androgen receptor. Knowledge of tumor androgen receptor content could aid in selecting optimal endocrine therapy in recurrent disease. The present tumor material will form the basis for a prospective study in order to confirm whether improved androgen receptor measurements will supplement and improve the prognostic information gained in conventional tumor receptor analysis.

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