Prognostic Usefulness of Estrogen Receptor Immunocytochemical Assays for Human Breast Cancer

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Abstract

Breast cancers of postmenopausal patients at high risk for recurrence participating in an adjuvant therapy protocol were independently assayed for estrogen receptor by conventional dextran-coated charcoal steroid binding assays and by immunocytochemistry (ER-ICA) to compare the two assays and to assess the prognostic usefulness of ER-ICA. The ER-ICA was based on a monoclonal antibody to the estrogen receptor and was applied to lightly fixed, frozen sections of the cancers. Excellent agreement was found between the two estrogen receptor methods. It was found that a combination of the distribution of ER-ICA stained cells and the overall staining intensity gave a statistically significant correlation with the quantitative estrogen receptor dextran-coated charcoal steroid binding assay value. In addition, the overall appraisal of the lesion as ER-ICA positive or negative as well as the ER-ICA staining intensity and proportion of ER-ICA stained cancer cells related to patient disease-free interval and survival, independent of patient lymph node involvement. This relationship of ER-ICA status to prognosis appeared not to relate only to responses to adjuvant tamoxifen treatment since it also was observed with patients who did not receive the antiestrogen.

Introduction

Results from many laboratories over the last 15 years have clearly established that the tumor content of estrogen receptor determined by steroid binding assays can help identify breast cancer patients who are likely to respond to endocrine therapy (1-4). Over the last 5 to 10 years it has also become evident that there is a direct relationship between the proportion of patients who obtain benefit from endocrine therapy and the quantity of estrogen receptor present in their cancers (5-9); furthermore, when the cancer contains significant quantities of both ER and PR, there is an increased likelihood of response to endocrine therapy (4, 9, 10).

Despite the overwhelming evidence for the usefulness of steroid binding assays for steroid receptors, there are still some generally recognized limitations to their successful application. Even though essentially all the breast cancers of patients who benefit from endocrine therapy contain significant amounts of estrogen receptor, not all patients with ER, or for that matter ER and PR, in their cancers respond to such therapies. Because they are carried out on extracts obtained by homogenizing the tissue, steroid binding assays of breast cancers cannot indicate how much of the receptor may come from normal or nonmalignant epithelial cells and how much from actual invasive cancer. If any significant proportion of the receptor were derived from noninvasive elements of the tissue, the steroid binding assay result could be misleading with regard to the behavior of the metastatic lesion. Since the stroma associated with breast cancers is generally found to be receptor negative, its presence in samples subjected to homogenization and steroid binding assays for ER would not cause an ER negative cancer to be designated ER positive; however, in the endometrium, where the normal stroma as well as the epithelium has been found to be ER positive (11), inclusion of normal tissue in the sample used for the steroid binding assay could give rise to a misleading receptor positive result not necessarily conveying the ER status of the cancer. Even when all the ER of the sample is derived from the cancer, a low to moderate quantity of ER could reflect either a low proportion of cells containing significant amounts of estrogen receptor or a high proportion of ER containing cells with a low concentration of estrogen receptor. These two different types of cancers could have significantly different clinical behavior, including different sensitivity to hormones.

Thus there are a number of important reasons why one would like to be able to use reliable histochemical or immunocytochemical methods to evaluate the content and distribution of steroid receptors in cancer tissues. Unfortunately, the first attempts to meet this need were based on procedures which, while often showing a moderately good correlation with quantitative estrogen receptor assays by steroid binding methods, clearly are now understood to measure not the receptor but rather some other, generally weaker affinity, estrogen binding components in the cells (4, 12); however, following the development and definitive characterization of monoclonal antibodies to the estrogen receptor of human breast cancer cells (13, 14), a method has been devised to localize the estrogen receptor in the cells of frozen sections of tissues using these monoclonal antibodies (15, 16). A recent study in our laboratory (17) using this method compared the ER-ICA and steroid binding assay results (from sedimentation analysis for ER) for estrogen receptor in human breast cancers and showed an excellent correlation between the two methods; however, since that initial study consisted simply of sequential accessions of breast cancers, the prognostic or treatment utility of the ER-ICA results could not be readily appraised.

Numerous studies have confirmed the initial reports (18, 19) of the prognostic utility of the presence of estrogen receptor in tumors as determined by steroid binding assays. It was of some interest therefore to assess the possible prognostic utility and clinical meaning of the results of ER-ICA on the primary breast cancers of postmenopausal patients at high risk for recurrence, some of whom would receive adjuvant hormonal treatment with the antiestrogen, tamoxifen, in a randomized prospective clinical trial. The results, presented herein, indicate that certain features of the ER-ICA determinations on the cancers correlate...
well with the patient’s prognosis for recurrence and length of
survival.

Materials and Methods

Patients

Biopsies from 126 patients with breast cancer registered by the
DBCG were studied. The organization, design, and follow-up of the
DBCG program have been described in detail elsewhere (20). The
patients were postmenopausal women between 45 and 80 years of age
and at high risk for recurrent disease. One hundred and three of them
were entered into DBCG adjuvant endocrine protocols 77-lc or 82-lc.
A woman was defined as being postmenopausal when menostasia had
persisted for at least 5 years. Patients were designated as being high
risk if positive lymph nodes were found, if the tumor was >5 cm
diameter, or if the tumor invaded the skin or fascia. To enter the study,
patients must have had no evidence of advanced disease as estimated
by physical examination, blood tests, X-rays of the chest and bone, or
bone scintigraphy; furthermore, they must not have had any previous
or concomitant other malignant disease, and they must have given their
verbal informed consent. The major reasons for exclusion from the
protocol were metastasis at the time of the primary diagnosis, other
concurrent cancers, medical contraindications to the therapy, or an
operation which deviated from the protocol. The primary surgical
treatment was total mastectomy and partial axillary dissection. Two to
4 weeks postoperatively, patients in the 77-lc protocol received radio-
therapy to the mastectomy area and to the supraclavicular and axillary
lymph nodes at a dose equivalent to 1335 rads equivalent therapeutic.
Eligible patients were allocated at random to receive either no drug
therapy (radiotherapy) or tamoxifen (radiotherapy plus TAM), 10 mg
3 times daily for 48 weeks. In the 82-lc protocol patients were allocated
at random to receive radiotherapy plus TAM, TAM, or TAM plus
cytoxan, methotrexate, and 5-fluorouracil.

Of the 103 patients 5 had a receptor analysis performed on the
metastasis rather than the primary tumor tissue: the ER-DCC assays
failed for 2, while the ER-ICA failed for 3 patients. These patients are,
therefore, not included in the clinical analysis of the data; thus, 93
patients on protocol had both ER-DCC and ER-ICA assays performed
on the primary tumor tissue. Although comparisons of ER-ICA vari-
ables with ER-DCC were conducted with all available data, time to
recurrence and survival analyses were carried out only with the data
from patients on the protocols and with receptor analyses performed
on the primary tumor. Seventy-two patients were entered in the 77-lc
protocol and 21 were entered in the 82-lc protocol.

Follow-up studies and registration of patient data were conducted as
previously described (20). Patients included for this paper were entered
between September 1981 and June 1983. All data were evaluated as of
July 5, 1984 at which time the median observation period was 22
months.

Estrogen Receptor Analysis

DCC. Estrogen receptors were measured using the DCC method and
Scatchard analysis of the data in accord with the recommendations of
the European Organization for Research on Treatment of Cancer (21).
Continuous quality control studies of the ER assay are performed in
collaboration with other European laboratories in the European Orga-
nization for Research on Treatment of Cancer receptor group. Only
patients with verified malignant tissue in the biopsy specimen sent for
receptor analysis were included in this paper. Tumors containing at
least 10 fmol ER/mg cytosol protein were considered positive for ER.

ICA. At the time of analysis for ER by the conventional DCC assay
method, a central portion of the tumor tissue was removed, placed in a
cryotube with a screw stopper, and kept in a Revco freezer at −70°C
until time of the ER-ICA analysis. Immunocytochemical staining of
coded biopsies was performed as described previously (17) using an
indirect peroxidase antiperoxidase sandwich technique, except that
tissue sections were fixed for 5 min at room temperature with picric
acid-paraformaldehyde (22) rather than ethanol. All immunological
reagents were diluted in 10% normal goat serum in phosphate-buffered
saline to reduce nonspecific staining. Every section evaluated for the
presence of ER was compared to a negative control consisting of an
adjacent section for which normal rat IgG was substituted for monoclono-
nal anti-ER (H226). The estimation of the values of semiquantitative
variables of ER-ICA staining was also performed according to previ-
ously published protocols (17). In addition, the number of stained cells
per mm² of cross-section was determined with the aid of an ocular grid.
The entire tissue section was scanned for this evaluation; furthermore,
at least three randomly chosen low power (×100) fields of each tissue
section were assayed by tallying the frequency with which an ER-ICA
stained cell or group of cells coincided with any of 99 intersection
points on a 1-μm² ocular grid. The mean value for the assayed fields
was expressed as the positive cell index for that tumor. Evaluation of
immunocytochemical staining was performed with no knowledge of the
results of steroid binding assays.

As previously described for breast cancers (15, 17) as well as normal
tissues of the reproductive tract (11, 15, 16), immunohistochemical
staining of estrogen receptor rich tissues with the monoclonal anti-
estrogen receptor antibodies H222 or H226 shows mainly nuclear stain-
ing. This staining is specific since substituting normal rat IgG for the
specific rat monoclonal antibody yields no nuclear staining; further-
more, prior incubation of the antibody with ER containing but not with
ER depleted cytosol eliminates the specific nuclear staining (15).

Statistics

Data processing and statistical analyses were done on IBM 3081 and
DEC-20 computers at the University of Chicago Computation Center,
using programs of MINITAB (copyright, Pennsylvania State Univer-
sity) and SPSS (Version M, Release 9.1, SPSS, Inc.), and Social
Statistics

Results

Relation of ER-ICA to Conventional ER Assays. Although we
were most interested in discovering the possible prognostic
usefulness of the ER-ICA procedure with frozen sections of
breast cancer tissue, it was also of interest to see whether the
semiquantitative features of the ER-ICA staining related in a
significant way to the quantity of ER determined by steroid
binding assays (ER-DCC) of the same breast cancers. During
the evaluation of the ER-ICA staining of the breast cancers we
recorded a subjective overall evaluation of each tumor as ER-
ICA positive or negative, simply reflecting an educated feeling
about the relative predominance of the staining distribution
and intensity. Twelve cancers were designated plus-minus (bor-
derline) in the initial evaluation. Closer examination of the
unambiguously ER-ICA positive and negative specimens
revealed that they could be distinguished by the number of ER-
ICA positive cells per mm² (ER-ICA positive were ≥100; ER-
ICA negative, <100). The 12 tumors in question were then
classified according to these criteria. The comparison of this
subjective evaluation with the independent, steroid binding
assay for ER is shown in Fig. 1. As expected, almost all of the
tumors with the higher levels of ER were judged to be ER-ICA
positive. In the range of 15 to around 50 fmol/mg cytosol
protein there was some overlap but tumors with low ER,
between 4 and 12 fmol/mg, were all judged ER-ICA negative.

Fig. 2 illustrates the nature of the differences in staining
intensity that were observed among the ER-ICA stained sec-
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Estrogen Receptor Immunocytochemistry

Fig. 1. Danish Adjuvant Breast Cancer Study findings on ER-ICA status as related to quantitative ER found by steroid binding assay (ER-DCC).

ER-ICA features, in particular staining intensity and the proportion of positively stained epithelial cells, relate fairly directly to the quantity of cytosol estrogen receptor found by conventional biochemical assays. We therefore carried out a linear regression analysis of the best fit of various semiquantitative ER-ICA variables to the quantity of ER [as the log (ER + 1) since the ER-DCC results demonstrated a lognormal distribution]. We found that the 2 features which contribute most to the correlation of ER-ICA results with the independent quantitative biochemical assay results are the staining intensity and proportion of positively stained cells in the cancer. Fig. 6 presents the correlation of the predicted ER values obtained from the best fit equation with the actual ER-DCC values. The correlation coefficient for the regression equation including ER-ICA intensity and stained cell distribution is $R = 0.82$. While addition of data on tumor cellularity increased $R$ to 0.825, the difference was not statistically significant. The $R^2$ value thus shows that most but not all of the variability in the ER assessed by steroid binding assay can be accounted for these 2 ER-ICA variables; hence, the data indicate that the semiquantitative ER-ICA features, in particular staining intensity and the proportion of ER-ICA stained epithelial cells, relate fairly directly to the quantity of cytosol estrogen receptor found by conventional steroid binding assays.

Patient Characteristics and Prognosis. The variables related to conventional assays for ER and PR as well as ER-ICA were tested in a proportional hazards model with regard to prediction of time to recurrence and duration of survival. The results, Table 1, showed that most of the tumor steroid receptor variables as well as the patient’s lymph node status were found to be statistically significantly related to estrogen prognosis. The $\chi^2$ test and associated Ps indicate that the best fit of the model for disease-free interval was found for ER-ICA or ER-DCC status;
Fig. 2. Variation in ER-ICA staining intensity in breast cancers. Shown are a weakly staining lesion (A) contrasted with one showing intense staining (B). × 250.

Fig. 4. Variation in ER-ICA distribution in breast cancers. Examples of breast cancers showing low (A), moderate or focal (B), and high proportion (C) of ER-ICA stained epithelial cells. × 100.

Fig. 3. Comparison of ER-ICA staining intensity and ER-DCC according to a Danish Breast Cancer Study.

Fig. 5. Relation between percentage of ER-ICA positive epithelium and ER-DCC.
but similar highly significant correlations were also seen for the quantity of ER and PR (by SBA) as well as for the percentage of epithelium staining positively in ER-ICA; also highly significant but with smaller $\chi^2$ values were the number of positive lymph nodes, a group in general at lower risk for recurrence, one sees different rates of recurrence depending on whether the cancer is ER-ICA positive or negative. Even in the patients with fewer than 4 positive lymph nodes, a group in general at lower risk for recurrence, the ER-ICA status of the cancers. Even in the patients with fewer than 4 positive lymph nodes, a group in general at lower risk for recurrence, one sees different rates of recurrence depending on whether the cancer is ER-ICA positive or negative. Furthermore, there is a statistically significant difference in recurrence rates in both lymph node groups depending upon the ER-ICA status of the cancers. Even in the patients with fewer than 4 positive lymph nodes, a group in general at lower risk for recurrence, one sees different rates of recurrence depending on whether the cancer is ER-ICA positive or negative. This suggests the correlation of the information in the ER-ICA status variable with information collectively contained in lymph node status and PR concentration. It is of interest to see that categorizing the lymph node variable appeared to make its information so much more relevant to the model.

When the forward stepwise procedure was applied to relating the independent variables to duration of survival, no other variable, even lymph node status, provided enough information to be added to the model after ER-ICA status; however, when the backward stepwise process was tried, ER-ICA status was discarded as not competing with a combination of other variables, which in turn were discarded to leave only the percentage of ER-ICA positive epithelium in the model. This is demonstrated most clearly by the percentage of ER-ICA positive epithelium in the model. One may infer from these phenomena that collectively the variables are redundant and that ER-ICA status by its nature contains information equivalent to that of several other variables; however, it must be kept in mind that though definitely related to disease-free interval and to survival duration, these variables cannot predict either outcome specifically because of the variability inherent in the data.

ER-ICA and Disease-free Interval. We next looked at the actual relationship between ER-ICA and ER-DCC status and the patients' disease-free intervals. Fig. 7. Both of these estrogen receptor related variables clearly differentiate 2 populations with different disease-free experience. These differences are highly significant ($P < 0.0001$) and indicate that ER-ICA and ER-DCC statuses show similar power to differentiate patients with significantly different time to recurrence. Since lymph node status is such a significant prognostic factor in breast cancer it was important to consider the ER-ICA status with regard to patient lymph node status as well when looking at the disease-free intervals of the patients. As can be seen in Fig. 8, ER-ICA status is a prognostic factor independent of the lymph node status. The earliest recurrences are seen in patients with 4 or more positive nodes whose cancers are ER-ICA negative; furthermore, there is a statistically significant difference in recurrence rates in both lymph node groups depending upon the ER-ICA status of the cancers. Even in the patients with fewer than 4 positive lymph nodes, a group in general at lower risk for recurrence, one sees different rates of recurrence depending on whether the cancer is ER-ICA positive or negative.

When the forward stepwise procedure was applied to examining the relationship of independent variables to disease-free interval, the first variable selected was ER-ICA status, the "most significant" variable in Table 1. No other variable provided enough additional information to be included in the model; however, when lymph node status was redefined bimodally (either fewer than 4 or at least 4 positive nodes), the new lymph node status variable and the log (PR + 1) variable were not only added to the model but jointly caused the expulsion of the ER-ICA variable as being unnecessary. This suggests the correlation of the information in the ER-ICA status variable with information collectively contained in lymph node status and PR concentration. It is of interest to see that categorizing the lymph node variable appeared to make its information so much more relevant to the model.

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Fig. 6. Regression analysis of ER-ICA and ER-SBA. Relationship of the ER content calculated by the regression equation based on ER-ICA intensity and distribution to the actual (measured) ER-DCC. The correlation coefficient ($R$) relates to the fit of the calculated results to the actual data. Log (ER + 1) = 0.515 + 0.25 (intensity) + 0.015 (% ER-ICA positive).

Table 1 Relationship of individual variables to disease-free interval and survival in the Cox Proportional Hazards model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Disease-free interval</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-ICA</td>
<td>$\chi^2$ 19.78</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>ER-DCC (positive or negative)*</td>
<td>$\chi^2$ 17.99</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Log (PR + 1)</td>
<td>$\chi^2$ 15.40</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Log (ER + 1)</td>
<td>$\chi^2$ 14.84</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>% ERICA positive</td>
<td>$\chi^2$ 12.89</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>No. positive lymph nodes</td>
<td>$\chi^2$ 7.38</td>
<td>$P = 0.0066$</td>
</tr>
<tr>
<td>ERICA intensity</td>
<td>$\chi^2$ 6.77</td>
<td>$P = 0.0092$</td>
</tr>
<tr>
<td>Positive cell index</td>
<td>$\chi^2$ 4.08</td>
<td>$P = 0.0434$</td>
</tr>
<tr>
<td>Positive cells/mm$^2$</td>
<td>$\chi^2$ 3.18</td>
<td>$P = 0.0743$</td>
</tr>
<tr>
<td>Treatment</td>
<td>$\chi^2$ 0.44</td>
<td>$P = 0.5063$</td>
</tr>
</tbody>
</table>

* ER-DCC positive corresponds to all ER-DCC ≥ 10 fmol/mg cytosol protein.

Fig. 7. Disease-free interval and ER-ICA status (left) and ER-DCC status (right). ER-DCC plus samples were those in which the quantity of biochemically assayed estrogen receptor was ≥ 10 fmol/mg cytosol protein.
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Fig. 8. Disease-free interval, ER-ICA and lymph node statuses. Lymph node status indicates whether the patient had <4 or ≥4 positive lymph nodes.

Fig. 9. Disease-free interval, treatment, and ER-ICA status. --- --- ---, ER-ICA positive cancers; --- --- ---, ER-ICA negative cancers. Rad, radiotherapy; Tam, TAM.

It would therefore appear that the ER-ICA status of the cancer combined with data on patient lymph node status can identify the patients at very high risk for recurrence (i.e., ≥4 positive lymph nodes and ER-ICA negative) as well as indicating those with a lesser risk for recurrence (ER-ICA positive), despite their possibly unfavorable lymph node status.

When the disease-free intervals for the patients treated in the adjuvant setting with radiation therapy alone were compared with those given radiation plus tamoxifen for 48 weeks, the differences were not of statistical significance, as was indicated in Table 1. In the larger patient group studied by the BBCG (24) a significant advantage in recurrence-free survival was seen due to tamoxifen therapy in patients whose cancers contained more than 100 fmol ER/mg cytosol protein. Even though the stratification of the smaller group of patients included in our analyses makes statistical evaluation more tenuous, we looked at such differences related to ER-ICA status. As shown in Fig. 9 the patients with the longest disease-free intervals are those whose lesions were judged to be ER-ICA positive and treated with tamoxifen. With the smaller number of patients in the study groups the rather large apparent differences between patients treated with tamoxifen whose lesions were ER-ICA positive and negative do not reach the 5% probability level, i.e., P = 0.15. Although these trends will have to be tested more definitively on a larger patient sample, Fig. 9 suggests that even for patients treated with radiation therapy alone, those with ER-ICA positive lesions may have a better prognosis.

Since the presence of progestin receptor has been generally found to also be of prognostic significance, we evaluated whether the ER-ICA result was giving the same type of information as did the PR content. Fig. 10 suggests that ER-ICA and progestin receptor information are independent variables although possibly conveying overlapping clinically relevant data. Clearly the best prognosis is shown by patients whose cancers have PR content greater than 100 fmol/mg, all of which are ER-ICA positive; however, for lesions with PR values <10 as well as those with PR of 10 to 100 fmol/mg, the curves for time to recurrence differ, depending on whether the lesion is ER-ICA positive or negative. This would suggest that for the intermediate and low PR cancers, knowledge of the ER-ICA status may be of value to estimate the risk of early recurrence.

ER-ICA and Patient Survival. Although the median follow-up of not quite 2 years and the more than 80% overall survival makes final conclusions on the relation of the variables to length of survival somewhat tenuous, we wanted to determine whether the relations of the variables to disease-free interval would be reflected in their relation to survival duration; indeed, ER-ICA features also appear to separate patients into statistically different survival groups. As seen in Table 1, the subjective categorization of ER-ICA positive and negative as well as ER-DCC positive and negative relates to highly significant differences in patient survival (Fig. 11). Patient survival differences are also identified by ER-ICA intensity differences (Fig. 12) and the percentage of ER-ICA positive cancer cells (not shown). In the latter case the largest survival differences occur between patients with fewer than and greater than 40% positive cells. Quantifying the number of ER-ICA positive cells per mm² identifies a poor survival group, those whose lesions have fewer than 150 positive
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Fig. 11. Patient survival, ER-ICA (left), and ER-DCC (right) statuses. Definitions are as in "Materials and Methods" and Fig. 7.

Fig. 12. Patient survival and ER-ICA intensity.

Fig. 13. Patient survival, lymph node and ER-ICA statuses.

Fig. 14. Patient survival, treatment, and ER-ICA status. Rad, radiotherapy; Tam, TAM.

cells/mm², but the relatively small survival differences between the intermediate and highest density of ER-ICA positive cells are not statistically significant (data not shown). The same result is seen when the positive cell index is correlated with survival, suggesting that these variables do not add new information to the heterogeneity shown with the percentage of ER-ICA positive epithelium parameter.

When the ER-ICA features are considered along with patient lymph node status relative to patient survival, it is again clear that the ER-ICA status appears to be a useful predictive factor. This is shown in Fig. 13. As had been seen for time to recurrence, Fig. 8, the ER-ICA status of the cancer seems to be a powerful discriminator independent of the patient’s lymph node status. Patients with ER-ICA positive cancers showed good survival probabilities, whether 3 or fewer lymph nodes were involved, or somewhat unexpectedly, even when there were 4 or more lymph nodes involved. It will be of considerable interest to verify the very dramatic difference in survival of the 2 groups with 4 or more lymph nodes involved, depending on the tumor ER-ICA status. Part of this benefit could relate to the benefit of adjuvant therapy with tamoxifen for the hormone dependent ER-ICA positive cancers as well as responses to therapy at time of metastatic disease. While there clearly are not enough patients in this initial study group to stratify according to ER-ICA status, lymph node status, and treatment, such subsequent studies will be of importance. Looking at the survival curves of the 2 major treatment groups stratified by tumor ER-ICA status, Fig. 14, suggests that the responses to adjuvant tamoxifen treatment may not be the entire explanation for this difference. The data suggest that patients with ER-ICA positive lesions had better survival duration than those with ER-ICA negative lesions, independent of treatment.

Discussion

As was concluded by the panel at the Consensus Meeting on Steroid Receptors in Breast Cancer in 1979, knowledge of the steroid receptor content of human breast cancer is of considerable value for identifying patients who are likely to respond to endocrine treatments and also to provide prognostic information of value to the physician treating the patient with breast cancer (10). Although steroid binding assays for measuring estrogen and progesterin receptor have been of considerable clinical utility, there has been a considerable effort to develop histochemical methods which allow the pathologist to assess the steroid receptor content in sections of the lesion itself (4, 12). Until the availability of monoclonal antibodies to the estrogen receptor, however, the various methods for histological assessment of receptor content did not show the necessary specificity or sensitivity to be practical and scientifically valid (12). With antireceptor antibodies, which have been rigorously shown to be specific for the estrogen receptor protein (11, 13–17), studies can now proceed to assess the clinical utility of the new types of information which can be obtained from immunocytochemical assay for ER.

Results presented in this communication as well as an earlier
study from our laboratory (17) show that there is a very good correlation between various ER-ICA features and the estrogen receptor content of breast cancers as determined by conventional steroid binding assays. It is interesting that despite differences in tissue fixation for ER-ICA between these 2 studies, each showed a good correlation of ER-ICA results with ER by steroid binding assays. The fact that the ethanol fixation method used in the earlier study results in a somewhat less sensitive ER-ICA assay may explain the observed differences in correlation of staining intensity to the amount of ER found by conventional assay. In the earlier study the median ER content of tumors coded as intensities 2 and 3 were virtually identical, although higher than that of intensity 1. In this study there was a substantial difference in the 3 (intensity 3 > 500; intensity 2 ~ 120; intensity 1 ~ 20 fmol/mg); however, as only a subgroup of 38 of the cancers in the earlier study was assessed for quantitative staining features it was a less extensive study.

As might be expected, the two most important ER-ICA variables related to the quantity of receptor in the cancer were found to be the intensity of ER-ICA staining, which one might expect to relate to the relative quantity of receptor present in a cell, and the proportion of cells staining, indicative of the heterogeneity of the ER expression in the cancer; indeed, the results shown here indicate that there is a statistically significant correlation between these parameters and the steroid binding assay result. Since the biochemical assay is carried out on low salt extracts of the tumor, the so-called “cytosolic” receptor, whereas the immunocytochemical staining is exclusively nuclear, this high degree of correlation provides additional confirmation that the cytosolic receptor is most likely a nuclear component which is extracted from nuclei by homogenization in hypotonic media (15). This conclusion is in agreement with recent biochemical evidence obtained by enucleation experiments with GH3 cells as well (25).

Results presented herein also indicate that the ER-ICA evaluation of lightly fixed, frozen sections of breast cancers provides additional information with regard to prognosis for recurrence and length of survival of breast cancer patients. Although limited by the relatively small number of patients whose cancers were studied, nonetheless the ER-ICA status of the tumor establishes statistically significantly different patient groups with regard to disease-free interval and length of survival. While it is possible that some of this difference may relate to the improved prognosis, if not survival, of the patients treated with tamoxifen in the adjuvant setting, the trends of the data suggest that responses to tamoxifen are only a part of the basis for the differences. The analysis of the larger protocol population whose cancers were assayed for ER by SBA has pointed to a specific subgroup, namely those with estrogen receptor content greater than 100 fmol/mg cytosol protein, who appear to obtain significant benefit from the adjuvant antiestrogen (24). Other investigators have found a benefit due to treatment with tamoxifen in the adjuvant setting for patients with ER positive breast cancers (26), but there is some disagreement as to the relationship of the quantity of ER as determined by steroid binding assay and the disease-free survival (27).

It is important to note that in this study, as well as frequently in other studies concerned with testing the efficacy of new treatments in the adjuvant setting there was no uniform treatment for patients when breast cancer recurred. This obviously complicates the interpretation of survival statistics; however, we have included some significant evaluations of survival differences to indicate whether gains in disease-free interval are only temporary or if there may also be survival benefits. Even though the relatively short median follow-up time related to greater than 80% survival overall, some of the differences in survival by the ER-ICA variable are already statistically significant as shown.

A benefit related to the quantity of ER was reported for tamoxifen used in combination chemotherapy in a National Surgical Adjuvant Breast Project Trial (28). As seen in Fig. 9 the patients with the longest disease-free interval in our study group were indeed those who were treated with tamoxifen, but only if their tumor was ER-ICA positive. In this analysis as well as the evaluation of overall survival, Fig. 14, it is evident that patients with ER-ICA positive lesions appear to have better prognosis whether treated with tamoxifen or not. It will be important to verify these preliminary results on a larger patient population. Of some importance is the possibility that patients with ER-ICA negative lesions may do more poorly with adjuvant tamoxifen treatment. While the data, Fig. 14, are in this direction, the differences have not reached statistical significance. This possibility should be tested in a larger series. Certainly subsequent studies with protocols using antiestrogens in the adjuvant setting should also require steroid receptor assays so that such analyses can be performed.

It also appears that the prognostic and survival value of the ER-ICA status and the proportion of ER-ICA positive tumor cells are each independent of lymph node status. As seen for both disease-free interval, Fig. 8, and survival, Fig. 13, consideration of lymph node status along with ER-ICA status of the tumor stratifies the patient population into distinct risk groups indicative of the independent contribution of each of these parameters. The proportional hazard analysis carried out suggests a degree of interaction between the tumor ER-ICA status and the combination of patient lymph node status and tumor PR content. It is of interest that despite the relatively small numbers of patients involved there are clear differences in survival of the patients with 4 or more involved lymph nodes, which most breast cancer protocol studies have shown to be a group with a poor prognosis, depending upon the ER-ICA status of the tumor. It is entirely possible that the ER-ICA status, which is related to a combination of distribution and intensity information from the immunocytochemical staining, and thus probably related to the tissue concentration as well as distribution of receptor within the cancer, may provide new and additional information of prognostic utility. As seen in Figs. 4 and 11, however, correlations of ER-ICA status and ER-DCC status to the disease-free interval and survival are quite similar. Clearly, additional studies will be needed to verify these preliminary indications. As indicated in Fig. 10 it also appears that the prognostic information obtained from ER-ICA status may provide information independent of and complementary to progestin receptor assays. While all cancers with progestin receptor levels greater than 100 fmol/mg cytosol protein were ER-ICA positive, tumors with progestin receptor content between 10 and 100 fmol/mg as well as interestingly tumors with less than PR, 10 fmol/mg, were distributed between ER-ICA positive and ER-ICA negative categories. In each case patients with tumors classified as ER-ICA positive seemed to have a better prognosis, although with the relatively small number of patients again this apparent trend did not reach statistical significance.

While it is clearly advantageous to be able to obtain the new types of information about estrogen receptor in sections of breast cancer such as the distribution of ER positive cells within the cancer, it must be recognized that at best such results can be only semiquantitative. We have not yet determined how well
independent investigators can reproduce similar semiquantitative evaluations of the same slides. Clearly as there is heterogeneity within a tumor section, there is also heterogeneity within the entire tumor. It is rather encouraging that the ER-ICA evaluation of a small piece of a cancer agrees so well with the ER-SBA on extracts of the larger tumor portion. To obtain definitive information on the tumor heterogeneity one would need to assess multiple sections from each tumor and provide multiple evaluations of each of these sections. It is not entirely evident from our studies that such exhaustive and exhausting work provides additional clinically useful information; indeed, statistically the most significant single parameter related to prognosis found in this study is simply the ER-ICA status, which is basically an educated assessment by an experienced investigator of an overall impression as to whether the section is predominantly positive or negative. This assessment obviously integrates both the intensity and heterogeneity but in an as yet not entirely defined manner; however, it did appear that there is a rough quantitative basis for the cut-off for ER-ICA positive, corresponding to more than 100 positive cells/mm² of the section, even though there may be some subjectivity in calling a cell positive. Despite the clearly relevant information obtained from the percent ER-ICA positive cells or the positive cell index, it is entirely possible that a simple impression may provide as much clinically useful information. More extensive studies on a larger patient sample with multiple readers and evaluators are obviously necessary to determine whether more detailed analysis of ER-ICA stained sections will provide additional information.

While the present study does not show a clear superiority of ER-ICA to ER-DCC results, it indicates that ER-ICA features correlate with quantitative ER-DCC assays and can provide at least data of similar clinical significance. In addition ER-ICA assessment can prove particularly valuable to confirm that the ER containing cells are indeed part of the invasive component of the lesion. It is also possible that information on the heterogeneity of the ER among the neoplastic cells of the section and the intensity of staining obtained from ER-ICA can provide new types of information that will be useful to the management of primary as well as metastatic breast cancer patients.

References

Prognostic Usefulness of Estrogen Receptor Immunocytochemical Assays for Human Breast Cancer


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