A CYTOPLASMATIC ESTROGEN RECEPTOR RELATED PROTEIN (ER-D5 Ag) IN BREAST CANCER

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Summary: A monoclonal antibody against an Estrogen Receptor Related Protein (ER-D5 AG by Amersham) was analyzed in evaluating hormone dependence in 188 breast cancers, in addition to current index of steroid receptors. The Authors observed that the concentration of this new Antigen is not related with PgR but with ER concentration. In fact, increasing the ER values, increases the concentration of ER-D5 Ag, showing a good correlation between these two tumoral markers. In regard to Progestosterone Receptor ranges, the ER-D5+ves were equally distributed between PgR−ves and PgR+ves. Our experience suggests the application of ER-D5 Ag as R−ves tumor screening marker and emphasizes the importance of a second level in determining therapy and prognosis in breast cancer.

Key words: breast cancer; monoclonal antibody; estrogen receptor.

In breast cancer, Estrogen Receptor (ER) assay gives the best predictive correlation of hormonal therapy response. However, there are frequent situations in which few ER− patients benefit from hormonal therapy because around 60% ER+ patients obtain objective remission following endocrine treatment. Nevertheless, a better response rate is obtained with the ER+/PgR+ phenotype than ER+/ PgR−, and patients with ER+/PgR+ tumors show a longer survival than other groups. Recently, Coffer and King (1, 2) identified a protein associated with estrogen receptor (ER-D5 Ag) and preliminary evidence suggests ER-D5+ breast tumors have a high response rate, while ER-D5− tumors a high failure rate to endocrine treatment.

ER-D5 Ag could be an opportunity for a new approach at identifying those ER+ and ER− patients who will respond.

MATERIAL AND METHODS

Human tissue processing: Breast cancer tissues obtained from 188 mastectomy were placed immediately into liquid nitrogen and stored at −80 °C until steroid receptor status and ER-D5 Ag was determined.

Receptor assay: The lower dry weight of the tissues was 0.5 gms and the protein concentration for cytoplasmatic assay was standardized at 2.0 mg/ml for PgR and 1.0 mg/ml for ER, using the estimate of the nucleic acid which guided the dilution (3).

We used the single saturation analysis when we could utilize 1.2 ml of cytosol in triplicate (DES 8 × 10−10 M, 3H-E2 8 × 10−12 M, DHT + F 1 × 10−6 M, Cold-R 5020 5 × 10−7 M, 3H-R 5020 20 × 10−10 M). ER and PgR values equal to 0 fmol/mg of proteins were considered negative undetectable, and values below 3 fmol/mg as negative detectable, 3-10 fmol/mg as borderline range, 10-100 fmol/mg as positive with a prognostic value and more than 100 fmol/mg as positive with predictive value.

ER-D5 Ag-RIA: This procedure provides a reliable assay using a monoclonal antibody which recognizes an Estrogen Receptor Associated Protein, so-called D5 Ag. 25% in triplicate, of cytosol (1-2 mg/ml of protein concentration) and Std (1.48-360 U.I./ml) were incubated in darkroom for 90 min in preseleced tubes for enhanced binding of ER-D5 Ag present in the unknown sample or reference standard supplied by Amersham. After washing with deionized water, 125I labelled monoclonal antibody to ER-D5 Ag was incubated (37 °C/2 hrs) in the tubes and became bound of the ER-D5 Ag on the walls of the tubes. Washing for unbound tracer removal and measurement of the amount of 125I labelled in Cristal-MDGS-Packard was done for 1 min.

The results were expressed in I.U./ml and the cut-off in our experience is 35 I.U./mg of cytosol protein.

Statistical analysis: Data were stored and analyzed using the IBM PC XT 10MK, the U-Test, the Wilcoxon Rank-Sum Test and finally, the t-Test (4).
RESULTS

In our experience, 60.6% (114/188) of the cases were tumors ER+, 53.7% (101/188) were ER-D5+, while 39.9% (75/188) were PgR+ (table 1).

Table 1. – Positive and negative cases of Estrogen, Progesterone Receptors determined by ligand binding and ER-D5 Ag assayed by IRMA-Amersham-Kit.

<table>
<thead>
<tr>
<th>ER</th>
<th>PgR</th>
<th>ER-D5 Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>74 (39.3%)</td>
<td>113 (60.1%)</td>
</tr>
<tr>
<td>+</td>
<td>114 (60.6%)</td>
<td>75 (39.9%)</td>
</tr>
</tbody>
</table>

The 89% of ER+ tumors were ER-D5 Ag+, too. In regard to estrogen receptor range (tab. 2) we observed that when ER was negative undetectable (0 fmol/mg) the ER—D5 Ag mean value was 36.3 U.I./mg reflects the fixed cutoff of 35 U.I./mg of cytosolic protein. The ER-D5 Ag mean value in the ER+ range was clearly higher (117 U.I./mg of cytosolic protein) (Tab. 2).

If we compare ER-D5 Ag positive rate inside the single ER range (Tab. 2), the ER-D5 Ag+ were 16.6% with undetectable EF (0.3 fmol/mg), 44% with borderline ER (3-10 fmol/mg) and 71% with positive ER (>10 fmol/mg).

Finally, we observed that 27% of ER—tumors were ER-D5 Ag+ and the ER-D5 Ag positive rate increases in a parallel way to ER concentration.

In regard to progesterone receptor ranges (Tab. 3), we observed that 53% of PgR negative tumors were ER-D5 Ag positive.

If we compare the ER-D5 Ag rate inside the single PgR range, with PgR undetectable (0 fmol/mg) the ER-D5 Ag positive tumors were 53% like to the percentage of ER-D5 Ag positive tissues (56.7%) when PgR was positive.

Table 2. – Relationship among the Estrogen Receptor ranges and the ER-D5 Ag mean value. Percentage of positivity (>35 U.I./mg of cytosolic protein).

<table>
<thead>
<tr>
<th>Estrogen Receptors</th>
<th>ER-D5 Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>fmol/mg</td>
<td>cases</td>
</tr>
<tr>
<td>Negative undetectable</td>
<td>0</td>
</tr>
<tr>
<td>Negative detectable</td>
<td>0-3</td>
</tr>
<tr>
<td>Borderline</td>
<td>3-10</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

Table 3. – Relationship among Progesterone Receptor ranges and the ER-D5 Ag mean value. Percentage of positivity (>35 U.I./mg of cytosolic protein).

<table>
<thead>
<tr>
<th>Progesterone Receptors</th>
<th>ER-D5 Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>fmol/mg</td>
<td>cases</td>
</tr>
<tr>
<td>Negative undetectable</td>
<td>0</td>
</tr>
<tr>
<td>Negative detectable</td>
<td>0-3</td>
</tr>
<tr>
<td>Borderline</td>
<td>3-10</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

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Table 4. – Comparison among the positive-negative ER-PgR tumors status and ER-D5 Ag positivity.

<table>
<thead>
<tr>
<th>ER/PgR ranges</th>
<th>ER-D5-Antigen positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>35/52 (67.3%)</td>
</tr>
<tr>
<td>+/−</td>
<td>43/59 (72.9%)</td>
</tr>
<tr>
<td>−/+</td>
<td>7/21 (33.3%)</td>
</tr>
<tr>
<td>−/−</td>
<td>14/53 (26.4%)</td>
</tr>
</tbody>
</table>

The analysis of both ER/PgR showed that when there is the estrogen action (ER+/PgR+ or ER+/PgR−) the ER-D5 Ag positivity swings between 67.3% and 72.9%; while, when the ER were negative (ER−/PgR+ or ER−/PgR−), the ER-D5 Ag positivity was very low (33.3%-26.4%) but not less interesting. This suggests that in a quarter of the case ER− (with or without PgR) we may identify the hormono-dependence of breast cancer.

DISCUSSION

Coffer (1) demonstrated that the Mr 29,000 phosphoprotein is a human specific antigen clearly related to the estrogen receptor in a specific manner. Furthermore, it was suggested that monoclonal antibodies against ER-related components recognize an epitope either on activated cytosol but not nuclear ER or an antigen that, under activating conditions, itself complexes with cytosol ER.

King (2) suggests that ER-D5 Ag recognizes a receptor-related antigen, that is different from the classical concept of the estrogen receptor.

Preliminary immunochemical data showed a quantitative relationship between P29 and soluble ER: receptor-poor cancer (<10 fmol/mg protein) showed lower D5-staining that ER-rich (>10 fmol/mg protein) tumors.

This suggests the possibility that ER-D5 Ag assay might predict the likely response to endocrine treatment in breast cancer as a new potential marker.

In our experience we may confirm this hypothesis, because we observed that in ER negative undetectable tumors, the positivity of ER-D5 Ag was lowest (16.6%); when ER were negative detectable or borderline the positivity of the ER-D5 Ag was 44-47%; when ER were positive the ER-D5 Ag was highest (71%).

No relationship exists between PgR +ve or −ve tumors and ER-D5 Ag.

The clinical relevance of these observations consists in a new approach of ER−/PgR− tumors through the ER-D5 assay in evaluating the hormonal dependency before cutting-out the endocrine treatment for good.

CONCLUSIONS

In clinical practice we know that the response to hormonal therapy in the presence of both receptors was found in 76% of the cases, while only 9% of receptor −ve tumors showed a response to endocrine treatment.

The hypothesis of McGuire is confirmed by the fact that 28% of the ER−/PgR+ patients responded like 26% of ER+/PgR− patients.

In this regard the application of a new R−ve screening marker emphasizes the importance of a second level in determining therapy and prognosis in breast cancer.

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