Estrogen Receptor and Endocrine Responsiveness in Japanese versus American Breast Cancer Patients

Y. Nomura, S. Kobayashi, O. Takatani, H. Sugano, K. Matsumoto, and W. L. McGuire

Department of Breast Surgery, National Kyushu Cancer Center Hospital, Fukuoka, Japan; Department of Surgery, Nagoya City University Medical School, Nagoya, Japan; Department of Medicine, National Cancer Center Hospital (S. K.); Department of Medicine, National Cancer Center Hospital (O. T.) and Cancer Institute, Japanese Foundation for Cancer Research (H. J.); Institute for Cancer Research, Osaka University Medical School, Osaka, Japan (K. M.); and University of Texas Health Science Center, San Antonio, Texas (W. L. M.)

SUMMARY

We have compared the incidence of estrogen receptor (ER) in breast tumors and its clinical correlation with responses to endocrine therapies in Japanese and American patients. There was no correlation between tumor histopathology and the presence of ER, and the ER values in primary and metastatic lesions from the same patients were similar in most Japanese cases. Japanese patients with low and high plasma estradiol levels had identical incidences of ER-positive tumors.

The correlation between tumor ER and response to endocrine therapy is similar between Japanese and American patients. The incidence of ER-positive tumors is higher in postmenopausal American patients in both primary and metastatic lesions. It is possible that the reported increase in tumor lymphocyte infiltration in Japanese patients may explain this difference. The reported 5-year survival advantage of Japanese breast cancer patients cannot be explained by differences between the two populations in the response to endocrine therapy for advanced disease.

INTRODUCTION

In Japan, the incidence of breast cancer is remarkably lower than in Western countries (5). When Japanese women migrate to Western countries, their risk of breast cancer increases (3). When women in Japan do develop breast cancer, they have a more favorable survival rate than American breast cancer patients, which is not explained in histopathology, tumor size, axillary node status, or the type of primary therapy (19, 24, 28).

One might speculate that the better survival rate in Japanese women might be correlated with better response to therapies for advanced disease. However, a recent report suggests that the response to endocrine ablative therapy is quite similar for Japanese and American women (2). This latter study used the same criterion of objective tumor response for both Japanese and American patients. Nevertheless, tumor ER values were not included to show that the groups were truly comparable, although it is now generally appreciated that human breast tumors that contain ER are likely to regress following endocrine therapy, whereas those tumors lacking ER usually fail to respond (16). This concept was originally proposed by Jensen et al. (9) and has been supported by data from their laboratory (10) as well as from other Western countries (17).

It is therefore the purpose of this report to compare the incidence of ER in breast tumors and its clinical correlation with responses to endocrine therapy in Japanese and American patients.

MATERIALS AND METHODS

ER. Specimens from Japanese patients were processed as follows.

Tumors were excised, trimmed of fat and normal tissue, frozen in liquid nitrogen, and used immediately or stored at −70-80°C until assay. Tissues were powdered in the frozen state and homogenized in 2 to 4 ml of buffer per g of tissue. The buffer was 0.01 M Tris-HCl at pH 7.4 (most cases) or 8.0, 0.001 M EDTA, usually with 0.5 mM dithiothreitol. In most cases, the homogenate was centrifuged at 150,000 × g for 60 min to obtain the supernatant cytosol fraction. In some samples of one investigator (40 cases), the homogenate was centrifuged at 2,000 × g for 20 min to obtain the supernatant for the dextran-coated charcoal assay. Comparison of 105,000 and 2,000 × g supernatants from a few tumors was done by this investigator, and similar values of dissociation constant and numbers of binding sites per g tissue were shown. Protein was quantitated by the method of Lowry et al. (13).

Five to 20% sucrose gradients were prepared in the Tris buffer. Samples for gradient analysis were prepared by incubating 0.25 ml of cytosol (2 to 10 mg of protein per ml) with 1 to 2 pmoles of 17β-[3H]estradiol, 90 Ci/mmole, for 3 hr at 0-4°C. Control cytosols were preincubated with 100 to 200 pmoles of nonradioactive 17β-estradiol 20 min prior to adding the [3H]estradiol. The treated samples (0.2 ml) were applied to the sucrose gradient and the tubes were centrifuged overnight at 56,000 rpm. The gradient was collected and the radioactivity was counted.

Two hundred µl of cytosol or 2,000 × g supernatant (1 to 3 mg of protein per ml) prepared in the Tris buffer were incubated with increasing quantities of 17β-[3H]estradiol, 43 to 90 Ci/mmole (0.006 to 0.50 pmole), for 16 hr at 0-4°C.
Control cytosols were preincubated with 100 pmoles of nonradioactive 17β-estradiol 20 min prior to adding the 17β-[3H]estradiol. One-half ml of dextran-coated charcoal suspension (Norit A, 0.25 g/100 ml, and dextran, 0.0025 g/100 ml, in 0.01 M Tris-HCl, pH 8.0) was then added at 4° and mixed vigorously for 30 min at 4°. The mixture was then centrifuged for 15 min at 2,000 x g and the supernatant radioactivity was quantitated. The results were analyzed according to Scatchard (23) or Baulieu and Raynaud (1). A tumor is considered ER+ if it contains more than 2 fmoles of ER per mg of cytosol protein (Japanese) or more than 3 fmoles ER per mg cytosol protein (American).

The methods for collection and assay of ER for the American patients are practically identical to the method described above and have been previously described in detail (18).

Assay of Serum 17β-Estradiol. Serum 17β-estradiol levels were determined by radioimmunoassay (15). The antiserum was produced by the immunization of rabbits by 17β-estradiol-6-(0-carboxymethyl)oxime bovine serum albumin. Two ml of serum samples to which 1000 dpm of 17β-[3H]estradiol (40 Ci/mmol) (Radiochemical Centre, Amersham, England) were added to correct for procedural losses were extracted with ether, and the dried extracts were applied to microcolumns of Sephadex LH-20. The columns were washed and eluted with benzene:methanol (85:15). The dried eluates, after being added with 10,000 dpm of 17β-[3H]estradiol, were incubated at room temperature for 30 min with the antiserum in a 1:50,000 dilution of the borate buffer (pH 8.0) containing 0.06% of bovine serum albumin and 0.05% of bovine γ-globulin. The free and bound steroids were separated by saturated (NH₄)₂SO₄, and the radioactivities in the supernatant were counted. Recovery after extraction and chromatography was 74.8 ± 4.7% (SD). The water blank per sample was 6.7 ± 1.8 pg. The interassay coefficient of variation in normal females (follicular phase) obtained from 10 assays was 8.5%.

Criteria for Objective Remissions. The published criteria of the Cooperative Breast Cancer group were used (4).

Specifically, tumor responses were classified as objective remissions only when: (a) at least 50% of all directly measurable lesions decreased 50% in size, or (b) osseous lesions recalcified, and (c) no new lesions appeared.

RESULTS AND DISCUSSION

Tumor ER Levels. Charts 1 and 2 show the values for ER in more than 700 Japanese and American breast tumor specimens. In both countries, the values range from zero to more than 1000 fmoles/mg cytosol protein. The wide range of values may be due to a combination of factors. First, since tumors commonly exhibit cellular heterogeneity, the ER content might vary directly with the proportion of cell types that contain cytoplasmic ER. The first studies from western countries showed no obvious correlation between the histology of a tumor and its ability to bind estrogen (16), and this is supported by the present data from Japan (Table 1). More recently, however, a strong association between ER and invasive lobular carcinoma has been described, while a low frequency of ER is seen in tumors with a prominent local lymphocyte reaction (21). Second, one might suppose that contamination of a tumor specimen by normal breast cells would give variable assay results if those cells contained ER. However, this is not the case, since ER cannot be readily detected in nonlactating human (6, 7, 11) or animal (8, 20, 25, 27) mammary cells. Finally, endogenous estrogen secreted by the patient must be considered, since high levels of endogenous estrogen would occupy ER sites and make them unavailable for assay by conventional techniques, thus leading to variability in results. Maass et al. (14) and Sakai and Saenz (22) have recently reported that most breast tumor ER values are indeed underestimated for this reason, but they point out that the error is probably insufficient to explain the higher values for free cytoplasmic ER in postmenopausal patients. This is supported by the present data in which blood estrogen levels as well as breast tumor ER were examined in Japanese patients (Chart...
Estrogen receptor and histology of breast cancers in Japanese patients

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of cancers</th>
<th>ER+ (%)</th>
<th>ER− (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillotubular</td>
<td>73</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>Medullary</td>
<td>28</td>
<td>57</td>
<td>43</td>
</tr>
<tr>
<td>Scirrhous</td>
<td>47</td>
<td>57</td>
<td>43</td>
</tr>
<tr>
<td>Special type</td>
<td>5</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>

Estrogen receptors in breast tumors according to menopausal status

<table>
<thead>
<tr>
<th>Menopausal Status</th>
<th>Japanese</th>
<th>American</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ (%)</td>
<td>ER− (%)</td>
<td>ER+ (%)</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>57</td>
<td>43  (153)*</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>55</td>
<td>45  (166)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of patients.

ER in Primary and Metastatic Tumors. A frequent question is "How do ER values in metastatic specimens compare to ER values in the primary specimen?" There is a paucity of data in the literature on this point. Table 3 shows that, in both countries, the overall frequency of ER in metastatic tumors from American patients more frequently contain ER than tumors from Japanese patients, although the difference is confined to the postmenopausal patients (Table 2). This result is surprising, since if ER in tumors is related to endocrine responsiveness and hence prolonged survival, one would have expected the frequency of ER in tumors to be higher in Japanese patients. It seems unlikely that the discrepancy is the result of differences in assay procedures, since this would have resulted in an increased frequency of tumor ER in the premenopausal American patients as well. Nor would differences in blood estrogen levels between Japanese and American postmenopausal patients be likely to explain these differences in tumor ER incidence, although in view of dietary differences, high levels of phytoestrogens in Japanese postmenopausal women should be considered. In other American series where the overall incidence of ER is close to the Japanese experience, the preponderance of tumor ER in postmenopausal patients still exists (26). A possible explanation is that Japanese breast tumors have been reported to have considerably more lymphocyte invasion than American breast tumors (19) and such tumors have a decreased incidence of ER (21). Information on lymphocyte invasion is not available in the present study.

3). In those patients with blood estrogen levels below 50 pg/ml, 52% of tumors contained ER, while in patients with blood levels above 50 pg/ml, 53% of tumors contained ER. We conclude that endogenous estrogen levels may contribute to the variation of ER values but would rarely affect the classification of a tumor.

When tumors from both groups are compared, tumors

---

Chart 2. ER values in metastatic breast tumor specimens listed according to patient age.

Chart 3. A comparison of breast tumor ER values and serum estradiol values at the time of biopsy.
Estrogen receptors in primary and metastatic breast cancer tissue

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Japanese</th>
<th>American</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>55</td>
<td>73</td>
</tr>
<tr>
<td>Metastatic</td>
<td>43</td>
<td>63</td>
</tr>
</tbody>
</table>

Numbers in parentheses, number of patients.

Objective breast tumor regressions according to ER assay and type of therapy

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Japanese</th>
<th></th>
<th>American</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER+ (%)</td>
<td>ER- (%)</td>
<td>ER+ (%)</td>
<td>ER- (%)</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>1/3</td>
<td>1/8</td>
<td>5/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>0/3</td>
<td></td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomy and ovariectomy</td>
<td>11/16</td>
<td>0/11</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>0/1</td>
<td></td>
<td>7/10</td>
<td>0/4</td>
</tr>
<tr>
<td>Androgen</td>
<td>0/1</td>
<td></td>
<td>3/5</td>
<td>0/4</td>
</tr>
<tr>
<td>Estrogen</td>
<td>2/2</td>
<td></td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Antiestrogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14/22</td>
<td>1/24</td>
<td>15/25</td>
<td>0/20</td>
</tr>
<tr>
<td>% response</td>
<td>64%</td>
<td>4%</td>
<td>60%</td>
<td>0%</td>
</tr>
</tbody>
</table>

ER levels and tumor response to endocrine therapy

<table>
<thead>
<tr>
<th>ER value (fmoles/mg cytosol protein)</th>
<th>Japanese</th>
<th></th>
<th>American</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of regressions/no. assayed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>1/24 (4)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-10</td>
<td>6/9 (67)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;11</td>
<td>8/13 (62)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>American</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>0/20 (0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-10</td>
<td>3/6 (50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;11</td>
<td>12/19 (63)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses, percentages.

Tumor estrogens receptor and blood type

<table>
<thead>
<tr>
<th>Blood type</th>
<th>Japanese</th>
<th>American</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>35/68 (52)*</td>
<td>100/149 (67)</td>
</tr>
<tr>
<td>A</td>
<td>53/101 (53)</td>
<td>87/122 (71)</td>
</tr>
<tr>
<td>B + AB</td>
<td>40/79 (51)</td>
<td>37/46 (80)</td>
</tr>
</tbody>
</table>

Numbers in parentheses, percentages.

Chart 4. A comparison of breast tumor ER values from different biopsies from the same patient. Multiple values from individual patients are presented in a single vertical column (vertical lines help align the columns). •, primary breast tumor; ○, metastatic lesion to breast; □, skin recurrence; △, metastatic lymph node; ◊, metastatic liver.

Tumor ER versus Clinical Response. We now compare the usefulness of ER data in predicting the response to endocrine therapy in Japanese and American women (Table 4). Although in some of the treatment groups the number of patients is too small for definitive conclusions, the overall response rates of 64 and 60% for ER+ tumors and 4 and 0% for ER- tumors are remarkably similar. These data also compare favorably with the clinical correlation compiled from several large series in Western countries (16).

It has been previously stated that the tumor ER values which are low but definitely positive predict quite well for endocrine responsiveness (16) and this is also now supported by the present data in Japanese patients (Table 5). It is not clear why patients with low tumor ER values respond almost as well as those with higher ER values. One might speculate that remission might be prolonged in the latter patients, and this point should be clarified in future studies.

Tumor ER versus Blood Type. Finally, we turn to the question of blood types, ER, and clinical responsiveness. Lee (12) reported that of 87 patients treated by ovariectomy (with or without adrenalectomy) or by androgen administration, patients with blood group O had an 11% remission rate, compared with 23% in group A, and with 37% in groups B and AB (which were combined because of small numbers of patients). One might therefore anticipate that tumor ER values would be distributed in a similar fashion (Table 6). It can be seen that, in American patients, there is indeed a slight trend of tumor ER in accord with Lee's data (O < A < B + AB), while the Japanese patients do not show any differences between the blood groups. From the incidence of tumor ER, one might predict that the American patients should have a higher response rate than the Japanese pa-
patients. However, if Lee is correct, the opposite may be true, since a large number of American patients fall into the least favorable category (group O) whereas most Japanese patients are in the more favorable groups (A, B, and AB). More data need to be obtained relating blood groups and actual response to therapies, but the very different blood group distribution between these 2 patient populations reflects basic genetic differences and may be related to the reported survival advantage of Japanese breast cancer patients.

CONCLUSION

A comparison of ER and clinical responses between Japanese and American patients reveals a few differences and some remarkable similarities.

As in most Western cases, we do not find any obvious correlation between tumor histopathology and the presence of ER in Japanese patients. The ER values found in primary and metastatic lesions from the same patients are similar in most cases, thus supporting the notion that ER values from primary tumors probably have the same prognostic significance as a biopsy of a metastatic lesion.

Although it has been postulated that endogenous estrogens can influence the ER value in breast tumors, false negatives due to masking of receptor sites would be infrequent (14, 22). This is supported in the present study where Japanese patients with low and high plasma estradiols had virtually identical incidences of ER+ tumors.

The correlation between tumor ER and response to endocrine therapy is remarkably similar between Japanese and American breast cancer patients and is consistent with collected data from Western countries (16).

The overall incidence of ER+ tumors is higher in the American patients, particularly in the postmenopausal group. This is true for both primary and metastatic lesions. In terms of survival, however, the higher frequency of ER+ tumors in the American postmenopausal patients is perhaps offset by a greater proportion of Japanese patients falling into more favorable blood group categories. It is possible that a difference in tumor lymphocyte infiltration between the 2 groups may explain differences in the ER incidence (19, 21) or even in the therapeutic course.

In conclusion, the survival advantage of Japanese breast cancer patients cannot be explained by tumor ER incidence. Since no differences between the 2 populations in the response to endocrine therapy for advanced disease are seen, this also does not explain the higher survival rate of Japanese women. Assuming that this improved survival rate is due to improved detection and treatment, then it is possible that the improved survival rate of Japanese women is due to improved detection and treatment of distant metastases or increased immune surveillance mechanisms, thereby improving survival chances.

ACKNOWLEDGMENTS

We are indebted to Dr. O. Abe, Dr. J. Kato, Dr. R. Okamoto, and Dr. H. Takikawa for cooperating with this study.

REFERENCES

17. Takikawa for cooperating with this study.
18. Takikawa for cooperating with this study.
19. Takikawa for cooperating with this study.
20. Takikawa for cooperating with this study.
21. Takikawa for cooperating with this study.
22. Takikawa for cooperating with this study.
23. Takikawa for cooperating with this study.
24. Takikawa for cooperating with this study.
25. Takikawa for cooperating with this study.
26. Takikawa for cooperating with this study.
27. Takikawa for cooperating with this study.
28. Takikawa for cooperating with this study.
29. Takikawa for cooperating with this study.
30. Takikawa for cooperating with this study.
31. Takikawa for cooperating with this study.
32. Takikawa for cooperating with this study.
33. Takikawa for cooperating with this study.
34. Takikawa for cooperating with this study.
35. Takikawa for cooperating with this study.
36. Takikawa for cooperating with this study.
37. Takikawa for cooperating with this study.
38. Takikawa for cooperating with this study.
39. Takikawa for cooperating with this study.
40. Takikawa for cooperating with this study.
41. Takikawa for cooperating with this study.
42. Takikawa for cooperating with this study.
43. Takikawa for cooperating with this study.
44. Takikawa for cooperating with this study.
45. Takikawa for cooperating with this study.
46. Takikawa for cooperating with this study.
47. Takikawa for cooperating with this study.
48. Takikawa for cooperating with this study.
49. Takikawa for cooperating with this study.
50. Takikawa for cooperating with this study.
51. Takikawa for cooperating with this study.
52. Takikawa for cooperating with this study.
53. Takikawa for cooperating with this study.
54. Takikawa for cooperating with this study.
55. Takikawa for cooperating with this study.
56. Takikawa for cooperating with this study.
57. Takikawa for cooperating with this study.
58. Takikawa for cooperating with this study.
59. Takikawa for cooperating with this study.
60. Takikawa for cooperating with this study.
61. Takikawa for cooperating with this study.
62. Takikawa for cooperating with this study.
63. Takikawa for cooperating with this study.
64. Takikawa for cooperating with this study.
65. Takikawa for cooperating with this study.
66. Takikawa for cooperating with this study.
67. Takikawa for cooperating with this study.
68. Takikawa for cooperating with this study.
69. Takikawa for cooperating with this study.
70. Takikawa for cooperating with this study.
71. Takikawa for cooperating with this study.
72. Takikawa for cooperating with this study.
73. Takikawa for cooperating with this study.
74. Takikawa for cooperating with this study.
75. Takikawa for cooperating with this study.
76. Takikawa for cooperating with this study.
77. Takikawa for cooperating with this study.
78. Takikawa for cooperating with this study.
79. Takikawa for cooperating with this study.
80. Takikawa for cooperating with this study.
81. Takikawa for cooperating with this study.
82. Takikawa for cooperating with this study.
83. Takikawa for cooperating with this study.
84. Takikawa for cooperating with this study.
85. Takikawa for cooperating with this study.
86. Takikawa for cooperating with this study.
87. Takikawa for cooperating with this study.
88. Takikawa for cooperating with this study.
89. Takikawa for cooperating with this study.
90. Takikawa for cooperating with this study.
91. Takikawa for cooperating with this study.
92. Takikawa for cooperating with this study.
93. Takikawa for cooperating with this study.
94. Takikawa for cooperating with this study.
95. Takikawa for cooperating with this study.
96. Takikawa for cooperating with this study.
97. Takikawa for cooperating with this study.
98. Takikawa for cooperating with this study.
99. Takikawa for cooperating with this study.
100. Takikawa for cooperating with this study.
Estrogen Receptor and Endocrine Responsiveness in Japanese versus American Breast Cancer Patients


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/37/1/106

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/37/1/106. Click on "Request Permissions" which will take you to the Copyright Clearance Center’s (CCC) Rightslink site.