Cytohistochemical Studies on Estrogen Receptors of Breast Cancer Tissue Using an Immunoperoxidase Technique

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ABSTRACT: The estrogen receptors (ER) in breast cancer tissues were investigated in 122 patients using an immunoperoxidase method. ER (+) were evident in 77 of 122 patients (63.1 per cent). If classified according to pre- and postmenopausal subjects. ER (+) was seen in 61.4 per cent and ER (−) in 32.9 per cent before menopause, and ER (+) in 65.4 per cent and ER (−) in 30.8 per cent after menopause with no marked difference between the two. If classified according to histological type, ER (+) was seen in 73.2 per cent of those with papillotubular carcinoma and in 62.0 per cent of those with scirrhous carcinoma, whereas ER (−) was seen in 44.9 per cent of those with medullary tubular carcinoma. ER (+) was seen in carcinoma with apocrine metaplasia, lobular carcinoma and Paget's carcinoma. Concerning the relationship between primary tumors and metastatic lymph nodes, ER (+) for both was seen in 20 of 41 patients (48.8 per cent) whereas ER (−) for both was found in 9 of 41 patients (22.0 per cent). Four patients with local recurrences had a positive ER (+) at the beginning of treatment, but the ER became negative after hormonal treatment and chemotherapy.

KEYWORDS: estrogen receptor, breast cancer, immunoperoxidase technique

INTRODUCTION

Endocrine therapy is one of the most effective treatments for advanced breast cancer, albeit the response rate being only 50-60 per cent. The estrogen receptor is one index used to determine the indications of this therapy. In attempts to obtain more effective responses of endocrine therapy for breast cancer, we studied estrogen receptors cytohistochemically and tried to establish criteria for selecting patients responding to such therapy. Morphological studies of estrogen receptors in breast cancer tissues in terms of histogenesis of breast cancer as well as tumor effects of endocrine therapy were also done. Estrogen receptors in breast cancer tissues were histocytologically investigated by the immunoperoxidase method.

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MATERIALS AND MMETHODS

The breast cancer tissues used were those obtained after biopsy or surgery at the First Department of Surgery, Kinki University

School of Medicine. The tissues were fixed in 10 per cent formaldehyde solution or by the freeze-substitution method[1] just after collection, and 6 μ paraffin sections were prepared.

One hundred and twenty-two women with primary breast cancer were examined for ER with respect to the menopausal status, histological type and stage classification of the cancer, primary and metastatic tumors, and recurrent breast cancer. A comparison of histological ER and biological ER was made.

Staining technique for estrogen receptors

Estrogen receptors were stained by the immunoperoxidase technique. The paraffin sections were deparaffinized and covered with normal porcine sera dissolved in 0.5 M Tris buffer solution, pH 7.6, followed by incubation for 30 minutes at room temperature. The porcine sera were absorbed by filter paper. As the primary serum, rabbit anti-17β-estradiol-6 bovine serum albumin (Miles-Yeda, U.S.A.), diluted five fold with Tris buffer solution, was placed in a moist chamber for 24 hours at 4°C. For the following two hours, the temperature was gradually increased to room temperature. The sections were washed three times in Tris buffer solution for three minutes. Then, as the secondary serum, porcine anti-rabbit Ig G (Dako, Denmark) was diluted 100 fold with Tris buffer solution, followed by a 30 minute incubation at room temperature. The sections were washed three times in Tris buffer solution for three minutes. Then, rabbit peroxidase anti-peroxidase complex (Dako) was placed in a moist chamber for 30 minutes at room temperature and 3, 3′-diaminobenzidine tetrahydrochloride solution with 0.005 per cent hydrogen peroxide added was used for preparation of the Karnovsky solution. The DAB solution was subjected to a color reaction for five minutes and immediately the solution was washed well in Tris buffer solution and finally in distilled water. Nuclei were stained with hematoxylin and then, dehydrated, cleared and mounted. Those from which the primary or secondary sera were removed were used as controls. The criteria of ER level were based on the percentage of ER positive cells in the cancer cell population. If ER positive cancer tissues accounted for 50 per cent or more of the total number of cancer cells, the subject was assessed as ER positive; 50-10 per cent, ER (±); and 10 per cent or less, ER negative.

Biochemical ER measurement method

The breast cancer tissues were immediately cut and sections measuring 1 cm were prepared, followed by preservation in a deep freezer. ER were measured in accordance with the dextran-coated charcoal method (DCC method). The material was classified as ER positive when the ER levels exceeded 10 fmol/mg protein and when they were less than 10 fmol/mg protein, as ER negative.

Results

Intracellular localization of ER in breast cancer could be classified into three types: that localized in the cytoplasm, that in the nucleus and that in both the cytoplasm and nucleus. ER, localized only in the nucleus, seemed to be a nuclear estrogen receptor. In breast cancer tissues, ER positive cancerous cells and ER negative cancerous cells were mixed and formed a so-called mosaic pattern.

Menopausal status

The number of premenopausal patients was 70 and the postmenopausal patients, 52. After ER staining, 43 (61.4 per cent) of the 70 premenopausal patients were classified as ER positive and 34 (48.6 per cent) of the 52 postmenopausal patients as ER positive. No significant difference was observed.

Histological type

The relation between histological type and histological ER of the primary tumors is shown in Table 1.

TNM staging of breast cancer and histological ER

Seventy-three (59.8 per cent) patients were classified as stage II, 24 (19.7 per cent) as stage I, 13 (10.7 per cent) as stage III, 10 (8.2 per cent) as stage IV and 2 (1.6 per cent) as TIS. The rate of ER positive was highest (70.8 per cent) for stage I, 61.5 per cent for stage

Table 1. The Relation between Histological Type and Histological ER of the Primary Tumors

Histological Type	Histological ER			Total
	ER (+) (%)	ER (+) (%)	ER(−) (%)	
Papillotubular ca.	30 (73.2)		11 (26.8)	41
Medullary tubular ca.	25 (51.0)	2 (4.1)	22 (44.9)	49
Scirrhous ca.	13 (62.0)	4 (19.0)	4 (19.0)	21
Mucous ca.	4		1	5
Modullary ca. with lymphoid infiltration	1		1	2
Lobular ca.	2			2
Ca. with apocrine metaplasia	1			1
Paget's ca.	1			1
Total	77 (63.1)	6 (4.9)	39 (32.0)	122

III, 60.3 per cent for stage II, 60.0 per cent for stage IV and 100.0 per cent (two patients) for TIS. No difference was observed in the relationship between the growth rate of cancer and the ER positive rate.

Histological activity of ER in primary tumors and metastatic foci to the regional lymph nodes

In 41 (33.6 per cent) out of the 122 patients with primary tumors, metastatic lymph nodes were present. A comparison was made between ER activity in primary tumors and ER in metastatic lymph nodes. The ER positive rate was higher in primary tumors than in the metastatic foci. Out of the 41 patients, 20 (48.8 per cent) were ER positive in both the primary tumors and metastatic lymph nodes. Nine (22.0 per cent) were ER negative in both and one (2.4 per cent) was ER false positive in both. Thus, in 30 (73.2 per cent) patients, ER status in the primary tumors coincided with that in the metastatic lymph nodes, while in eight (19.5 per cent) patients, ER status in the primary tumors did not coincide with that in the metastatic lymph nodes.

Comparison between histological ER and biochemical ER

Among 32 patients undergoing biochemical measurement, 22 (68.8 per cent) were ER positive. Out of the same patients, 23 (71.9 per cent) were ER positive, in terms of histochemistry. No significant difference was observed between the two. The number of patients who were ER positive in both measurement methods was 18 (56.3 per cent), while five (15.6 per cent) were ER negative. Thus the ER status was confirmed in a total of 23 (71.9 per cent) patients after the two examinations.

ER staining in recurrent breast cancer

Four patients with local recurrent breast cancer were studied histologically. At the first operation, three were ER positive and the remaining one, ER (±). For the treatment of local recurrence, chemotherapy (ADM, FT 207 and 5 FU) and endocrine therapy (adrenalectomy, oophorectomy and tamoxifen administration) were performed. Thereafter, ER in those with a recurrence was negative.

Discussion

The histochemical methods for determining estrogen receptor in breast cancer tissues have long been debated.[2-6] We measured ER by staining with peroxidase which reflected binding of antibodies to estradiol-17β and E_2 with estrogen. In this method, the important point is whether estrogens bound to estrogen receptors are preferentially stained. There is the possibility that not only estrogen receptors specifically bound to estrogen but also E_2, an estrogen binding substance nonspecifically binding to estrogen, may also be stained in

this method. However, at the present stage where no monoclonal antibody of ER has been identified, this method can be considered to be adequate as an ER staining method.

In the present study, ER staining was according to the PAP method reported by Shimizu et al.[7] We used rabbit anti-17β-estradiol-6-BSA as the primary serum and porcine anti-rabbit IgG as well as antisera of peroxidase anti-peroxidase complexes as the secondary sera and for this reason, this method is different from others.

Concerning the menopausal status and ER in breast cancer, no difference was observed between the pre- and the postmenopausal patients. With the biochemical measurement method, the ER positive rate is higher in postmenopausal patients,[8] whereas in Japan, many investigators reported that there is no difference in the ER positive rate between pre- and the postmenopausal patients.[9] With respect to the histological type of the breast cancer and ER, the ER positive rate was higher in the order of papillotubular carcinoma, scirrhous carcinoma and medullary tubular carcinoma. Thus, the ER positive rate increased in those with a well differentiated carcinoma and decreased in case of a poorly differentiated carcinoma.[10]

The results of the biochemical measurement method[11] revealed that the ER positive rate was low in cases of medullary tubular carcinoma and medullary carcinoma with lymphoid infiltration but was high in cases of papillotubular carcinoma and mucous carcinoma.

With the cytohistological method, cancerous tissues were examined on a cell-unit basis, whereas in the biochemical method, ER of the whole tumor consisting of cancerous cells was determined.

Therefore, the tissues with a lot of connective tissue such as scirrhous cancer show apparently low ER levels in many cases. Whether or not a site for sampling[12] is predominantly ER positive or ER negative also affects the ER levels obtained. The results of the present study are in agreement with those of studies where the histological ER was compared with the biochemical ER by the fluorescent antibody technique or the immunoperoxidase method.[13-16] Concerning the stage classification of cancer and ER, no difference was observed between the individual stage groups. The results of the biochemical measurement revealed no significant differences and these results were coincident with those obtained after the present study. Accordingly, there was no relationship between the growth rate of cancer and ER status.

In a comparison of ER in primary tumors and in their metastases to regional lymph nodes, the ER positive rate was lower in the metastatic lesions than in the primary tumors. The ER state in primary tumors was not always coincident with that in metastatic lesions. The same phenomena were noted when biochemical measurements were made.[9]

The results of ER staining in the present study indicated that patients showing ER positive results in the primary tumors tended to be ER positive or ER negative in the metastatic lymph nodes and that patients showing ER negative results in the primary tumor tended to be ER negative in the metastatic lymph nodes. After chemotherapy and endocrine therapy was prescribed for the treatment of recurrent breast cancer, the ER status became negative. Therefore, ER positive cancerous cells respond to endocrine therapy and ER negative cancerous cells to chemotherapy. Thus, chemo-endocrine therapy seems to be more effective for treatment of breast cancer.

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References

1. Yasutomi M. An improved freeze-substitution method for histochemical studies. Acta Med Osaka Univ 1964; 16: 181-190. (in Japanese)
2. Dandliker WB, Levision SA, Brawn RJ. Hormone binding by cells and cell fragments as visualized by fluorescence microscopy. Res Commun Chem Pathol Pharmacol 1976; 14: 103-109.

3. Levision SA, Dandliker WB, Brawn RJ, Vanderlaan WP. Fluorescence polarization measurement of the hormone binding site interaction. Endocrinology 1976; 99: 1129-1143.
4. Neci I, Beccati MD, Piffanelli A, Lanza G. Detection and dynamic localization of estradiol receptor complexes in intact target cells by immunofluorescence technique. J Steroid Biochem 1976; 7: 505-510.
5. Pertschuk LP. Detection of estrogen binding in human mammary carcinoma by immunofluorescence; A new technique ultilizing the binding hormone in a polymerized state. Res Commun Chem Pathol Pharmacol 1976; 14: 771-774.
6. Kurzon RM, Sternbergen LA. Estrogen receptor immunocytochemistry. J Histochem Cytochem 1978; 26: 803-808.
7. Shimizu M, Wajima O, Miura M, Katayama I. PAP immunoperoxidase method demonstrating endogenous estrogen in breast carcinomas. Cancer 1983; 52: 486-492.
8. McGuire WL. Current status of estrogen receptors in human breast cancer. Cancer 1975; 36: 638-644.
9. Nomura Y, Kobayashi S, Takatani O, Sugano H, Matsumoto K, McGuire WC. Estrogen receptor and endocrine responsiveness in Japanese versus American breast cancer patients. Cancer Res 1977; 37: 106-110.
10. Parl FF, Wagner RK. The histopathological evaluation of human breast cancer 1980; 46: 362-367.
11. Silfversward C, Gustafsson J, Gustafsson SA, Humla S, Nordenskjold B, Wallgren A, Wrange O. Estrogen receptor concentrations in 269 cases of histologically classified human breast cancer. Cancer 1980; 45: 2001-2005.
12. Tilley WD, Keightley DD, Cant ELM. Intersite variation of estrogen receptors in human breast cancers. Br J Cancer 1978; 38: 544-546.
13. Lee SH. Cancer cell estrogen receptor of human mammary carcinoma. Cancer 1979; 44: 1-12.
14. Mercer WD, Lippman ME, Wahl TM, Carlson CA, Wahl DA, Lezotte D, Teague PO. The use of immunocytochemical techniques for the detection of steroid hormones in breast cancer cells. Cancer 1980; 46: 2859-2868.
15. Taylor CR, Cooper CL, Kurman RJ, Goevelsmann U, Markland FS Jr. Detection of estrogen receptor in breast and endometrial carcinoma by the immunoperoxidase technique. Cancer 1981; 47: 2634-2640.
16. Pertschuk LP, Tobin EH, Gaetjens E, Carter AC, Degenshein GA, Bloom ND, Brigati DJ. Histochemical assay of estrogen and progesterone receptors in breast cancer; Correlation with biochemical assay and patients' response to endocrine therapies. Cancer 1980; 46: 2896-2901.