

## Evaluation of Estrogen Dependency of Human Breast Cancers

### I. Cytochemical study of estrogen receptor in human breast cancer tissue\*

Masayuki NISHIKI, Motoi YAMANE, Kuniki AMANO,  
Katsuki YASUDA, Tsuneo OKUMICHI and Haruo EZAKI

*The Second Department of Surgery, Hiroshima University School of Medicine, 1-2-3,  
Kasumi, Minami-ku, Hiroshima 734, Japan*

(Received March 5, 1984)

*Key words: Estrogen receptor, Cytochemical study, Breast cancer, Fluorescent estradiol conjugate*

### ABSTRACT

We have had an opportunity to determine estrogen receptor (ER) in cytoplasmic fractions of primary breast cancer specimens taken from 79 patients under a fluorescent microscope using fluorescent estradiol conjugate as a tracer. In this study, an estrogen receptor is reported as positive when not less than 10% of carcinoma cells incorporate fluorescent estradiol conjugate into cytoplasm.

The ER positive rate was 61% in the total cases, varying by age: 70% in 40s and younger, 43% in 50s, and 60% in 60s and older patients.

Sixty-eight % of premenopausal breast cancers compared to 54% of postmenopausal ones were ER positive.

Broken down by the histological type, the rates were 70% in papillo-tubular carcinoma, 59% in medullary tubular carcinoma, and 57% in scirrhous type.

The rates by tumor size according to the clinical TNM classification were 70% in T<sub>1</sub>, 59% in T<sub>2</sub>, 91% in T<sub>3</sub>, and 34% in T<sub>4</sub>.

The rates by stage defined by the pathological TNM classification were 65% in Stage I, 50% in Stage II, 87% in Stage III, and 45% in Stage IV.

We discuss our findings and review others, as well.

### INTRODUCTION

The efficacy of the endocrinotherapy, which mostly depended on extirpation and destruction of endocrine organs before, in both advanced and recurrent breast cancers has been known for many years<sup>2)</sup>. Further studies on the hormone dependency of the breast cancer defined indications and efficacy of individual hormonal manipulations, and consequently reduced the use of extirpation of important organs and administration of drugs with strong side effects. As more importance has been attached to a role of ER in the response to hormonal manipulation, the cytoplasmic receptor protein has been

assayed more often. An assay method most widely used at present is the dextran-coated charcoal assay (DCC assay)<sup>12)</sup>.

On the other hand, many studies including ours were made to invent a technique of determining cytoplasmic receptors at the tissue level. We have been studying by Dr. Lee's method since 1979<sup>5,8,9)</sup>, and revealed the good correlation between ER positive rate by DCC assay and our data<sup>14)</sup>.

The ER positive rate generally reported is 50-70%<sup>4,12)</sup>. Judging from the ER positive rate, about 60% of breast cancers seemed to respond to hormonal therapy, although it had been reported to be about 30%<sup>6,11)</sup>. ER determina-

\*<sup>1)</sup> 西亀正之, 山根 基, 天野国幹, 安田克樹, 奥道恒夫, 江崎治夫: ヒト乳癌におけるエストロゲン依存性の検討 I. ヒト乳癌組織におけるエストロゲンレセプターの細胞化学的研究

tion has been very useful to evaluate and predict the response and prognosis. In this paper, we show our cytochemical study.

### MATERIALS AND METHOD

We determined the presence of ER in 79 cases of primary breast cancer consisting of 78 females and 1 male.

#### *Cytochemical Staining of Estrogen Receptors in Tissue Sections*

Breast cancer tissues were obtained at the time of their surgical procedure. They were frozen with liquid nitrogen and preserved in a deep-freezer at  $-80^{\circ}\text{C}$ . Each tissue specimen was cut into about 4-micron thick frozen sections in a cryostat, and a series of 3 to 4 preparations was made. The slides were dried for 15 to 20 min at  $4^{\circ}\text{C}$ , and fixed by 95% alcohol at  $-20^{\circ}\text{C}$  for 30 min.

After the fixation, one section was stained by hematoxylin and eosin, and other sections of the series were overlaid with 0.1 ml of fluorescent estradiol conjugate ( $17\beta$ -estradiol-6-carboxymethyl-oxime-bovine serumalbumin-fluorescent, Zeus Scientific, Inc., USA), and incubated in a humid chamber at a room temperature for 30 min. An excessive unresponded conjugates were drained and rinsed gently with staining buffer (pH 7.1-7.2, 0.01 M PBS) on a rotator three times for 5, 10, and 15 min each.

The slides mounted with glycerin were observed using an optiphot fluorescent microscope (Nikon). They were compared with hematoxylin and eosin stained preparations in terms of the presence of carcinoma cells, and the histological pattern. The following criteria were used for evaluation of ER.

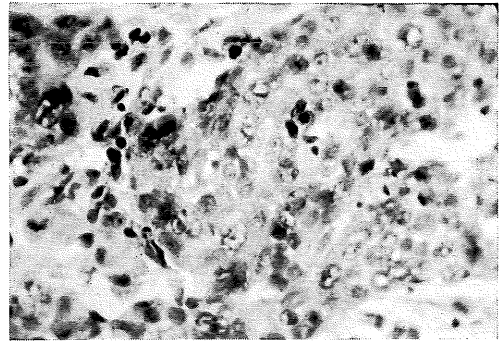
When fluorescent uptake cells account for 10-90%, a patient is ER positive.

When they account for almost 100%, she (or he) is ER strong positive.

When they account for not more than 10% including 0, she (or he) is ER negative (Figs. 1-5).

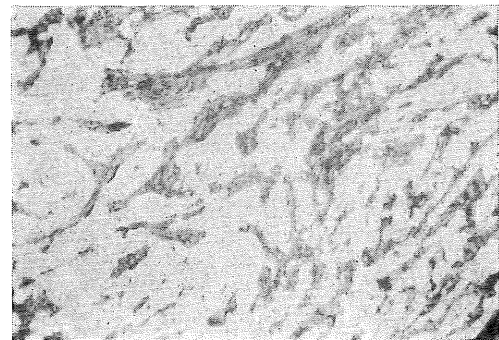
### RESULTS

Of the 79 patients with primary breast cancer, 61% (48) were positive for ER. The ER rates in subgroups classified by their histological type of the carcinoma are as follows: in common type, 70% in papillotubular carcinoma, 59% in

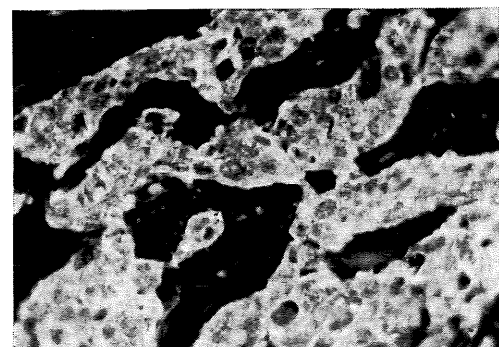


**Fig. 1.** Papillotubular Carcinoma

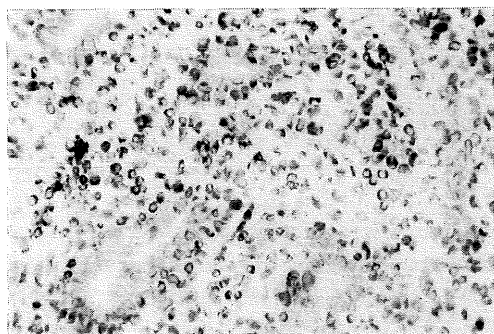
The photomicrograph shows proliferation, forming substantial or gland-like structures of cells with relatively large nuclei. Vacuolation is also noted in some cells. (H-E stain,  $\times 400$ )



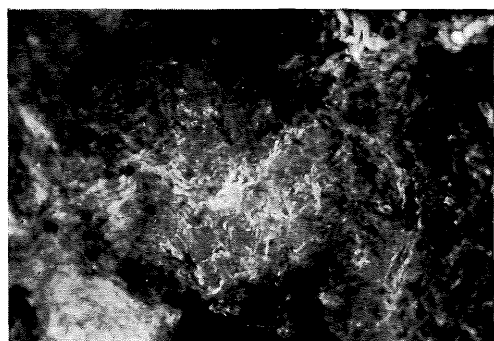
**Fig. 2.** Fluorescent photomicrograph of carcinoma cell from the same patient as Fig. 1. Stain with fluorescent estradiol conjugate shows conjugate localization in carcinoma cells with papillotubular proliferation, but not in zone of nuclei giving a perforated pattern. ( $\times 200$ )



**Fig. 3.** Fluorescent photomicrograph of carcinoma cell from the same patient as Fig. 1. Circular zones without fluorescent localization indicate no fluorescent uptake by nuclei. In cytoplasm, sand grain-like diffused fluorescent localization is noted. This case is regarded 100% positive for ER. ( $\times 400$ )



**Fig. 4.** Scirrhous Carcinoma  
The photomicrograph indicates a scirrhous type carcinoma where cells with irregularly sized, atypical nuclei show infiltrative proliferation. (H-E stain,  $\times 200$ )



**Fig. 5.** Fluorescent photomicrograph of specimen from the same case as Fig. 4. It shows only nonspecific linear fluorescence localization in zone of connective tissue, indicating no uptake of fluorescence by carcinoma cells. This case is judged negative for ER. ( $\times 200$ )

medullary tubular carcinoma, and 57% in scirrhous carcinoma. In only a few numbers of patients with special types of carcinoma, one patient (100%) with lobular carcinoma and 1 out of 2 (50%) with mucous carcinoma were positive, while one with Paget's carcinoma and one with signet ring cell carcinoma were negative (Table 1).

The ER positive rates by age groups were: 70% in patients of 49 years and downward, 43% in 50s, and 60% in 60s and up (Table 2).

As for relation to menstrual status, 68% of premenopausal patients compared with 54% of postmenopausal ones were ER positive (Table 3).

The rate in each tumor size group was: 70% in T<sub>1</sub>, 59% in T<sub>2</sub>, 91% in T<sub>3</sub>, and 34% in T<sub>4</sub>

(Table 4).

The rates related to the stage were: 65% in

**Table 1.** Relationship of Histological Type of Carcinoma and Results of ER Analysis of Tumor Tissue

	No. of Cases	ER Positive		ER Negative	
		No.	%	No.	%
<i>Common Type</i>					
Papillotubular	23	16	70	7	30
Medullary Tubular	37	22	59	15	41
Scirrhous	14	8	57	6	43
<i>Special Type</i>					
Lobular	1	1	100	—	0
Paget's	1	—	0	1	100
Mucous	2	1	50	1	50
Signet Ring Cell	1	—	0	1	100
Total	79	48	61	31	39

**Table 2.** Relationship of Age of Patients and Results of ER Analysis of Tumor Tissue

Age	No. of Cases	ER Positive		ER Negative	
		No.	%	No.	%
$\leq 49$	43	30	30	13	30
50—59	21	9	43	12	57
$\geq 60$	15	9	60	6	40

**Table 3.** Relationship of Menstrual Status and Results of ER Analysis of Tumor Tissue

Menstrual Status	No. of Cases	ER Positive		ER Negative	
		No.	%	No.	%
Premenopausal	41	28	68	13	32
Postmenopausal	37	20	54	17	46

**Table 4.** Relationship of Tumor Size and Results of ER Analysis of Tumor Tissue

Tumor Size*	No. of Cases	ER Positive		ER Negative	
		No.	%	No.	%
T <sub>is</sub>	3	2	67	1	33
T <sub>0</sub>	2	0	0	2	100
T <sub>1</sub>	10	7	70	3	30
T <sub>2</sub>	44	26	59	18	41
T <sub>3</sub>	11	10	91	1	9
T <sub>4</sub>	9	3	34	6	66

Tumor Size\*: Clinical TNM classification by UICC

**Table 5.** Relationship of Stage of Tumor and Results of ER Analysis of Tumor Tissue

Stage*	No. of Cases	ER Positive		ER Negative	
		No.	%	No.	%
I	40	26	65	14	35
II	20	10	50	10	50
III	8	7	87	1	13
IV	11	5	45	6	11

Stage\*: Pathological TNM classification by UICC

Stage I, 50% in Stage II, 87% in Stage III, and 45% in Stage IV (Table 5).

T classification and Stage mentioned above are according to the clinical TNM and pathological TNM classifications by UICC, respectively.

## DISCUSSION

It is the DCC technique, a biochemical assay, that have been used world wide to assay estrogen and progesterone receptors. However, we have been applying a method which requires neither such radioactive materials as radioisotope, nor expensive equipments including ultracentrifuge and scintillation counter. It is a cytochemical method to observe whether or not cytosol estrogen receptors incorporate fluorescent estradiol conjugate, using the fluorescent microscope.

Alouson who found only 67% of correlation between the DCC method and a fluorescent technique using antibody to receptor protein had an impression that it was inapplicable to clinical practice<sup>1)</sup>, while Pertschak demonstrated 92% of correlation with the same technique as Alouson's<sup>16,17)</sup>. Curtin obtained 83% of correlation, which was very similar to our result<sup>9)</sup>, that is, 89.2%<sup>14)</sup>. This result justifies our view that it is applicable to clinical practice.

In this paper, we discuss advantages and disadvantages of the fluorescent technique mainly based on our findings.

### *Estrogen Receptors and Histological Type of Breast Cancer*

McGuire demonstrated that estrogen receptor level tended to be low in poorly differentiated cancers but not related to histological type<sup>11)</sup>. We found the ER positive rate was relatively high (70%) in papillotubular type compared to scirrhous type (57%).

### *Estrogen Receptors and Age/Menstrual Status*

Seventy % of breast cancers developed in relatively young patients (49 yrs) were positive for ER. Sixty-eight % premenopausal compared to 54% of postmenopausal patients were ER positive. This finding was not consistent with the view generally accepted, that is, postmenopausal patients have higher estrogen receptor levels because of a decrease in endogenous estrogen secretion and carcinoma cells under estrogen-hungry conditions.

### *Estrogen Receptors and Tumor Size/Stage*

ER positive rate was high in T<sub>3</sub> (91%) and Stage III (87%) but low in T<sub>4</sub> (34%) and Stage IV (45%). It may be partly explained by the fact that patients in T<sub>3</sub> and Stage III had more accessible biopsy sites rich with cancer cells. The lower ER levels in T<sub>4</sub> and Stage IV seemed to be consistent with others' finding that more ER negative cases were seen in rapidly advancing carcinoma with poor prognosis<sup>10,13,18)</sup>. Many reports, however, demonstrated no relation between ER status and tumor size or stage.

Although our findings are slightly discrepant with others obtained through DCC technique, our method has various advantages, ie, simple, and requiring only a short time and a small amount of materials. Assay to reduce materials have been invented using Tru-cut material<sup>21)</sup>, fine needle aspiration material<sup>4,19)</sup>, etc.

Our method which determines ER at cell level allows us to assay ER even in cells from aspirate for cytodiagnosis, thoracic fluid and ascitic fluid, in nipple discharge and in cystic cellular fraction.

Since ERs may not uniformly distribute over a carcinoma tissue<sup>15,20)</sup>, it seems necessary to observe a few sites at the same time.

We are going to study this subject further with more patients.

## REFERENCES

1. **Alonso, K. and Brownlee, N.** 1981. Estrogen Receptors by Immunofluorescence: Comparison with a Dextran Coated Charcoal Technique. *Ann Clin. Lab. Sci.* 11: 132-137.
2. **Beatson, G.T.** 1896. On The Treatment of Inoperable Cases of Carcinoma of The Mamma: Suggestions for a New Method of Treatment, with Illustrative Cases. *Lancet* II: 104-107.
3. **Curtin, C. T., Pertschuk, L. P. and Mitchel, V.**

1982. Histochemical Detection of Estrogen and Progesterone Binding in Fine Needle Aspirates of Breast Cancer. *Acta Cytologica* 26 : 841-846.
4. **Fernandez, D., Sauren, P., Alaghband-Zadeh, J. and B., J.I.** 1982. Variability of Estrogen and Progesterone Receptor Status Between Primary Breast Cancer and Nodal Metastases. *J. Roy. Soc. Med.* 75 : 719-722.
  5. **Hanna, W., Ryder, D. E. and Mobbs, A. B.** 1982. Cellular Localization of Estrogen Binding Sites in Human Breast Cancer. *Ann. Soc. Clin. Pathol.* 77 : 39-395.
  6. **Howanitz, J. H.** 1981. Hormone Receptors and Breast Cancer. *Human Pathol.* 12 : 1057-1059.
  7. **Johnson, R. B. and Nakamura, R. M.** 1977. Improved Estrogen Receptor Assay in Human Mammary Cancer. *Am. J. Clin. Pathol.* 67 : 444-449.
  8. **Lee, S. H.** 1978. Cytochemical Study of Estrogen Receptor in Human Mammary Cancer. *Am. J. Clin. Pathol.* 70 : 197-203.
  9. **Lee, S. H.** 1979. Cancer Cell Estrogen Receptor of Human Mammary Carcinoma. *Cancer* 44 : 1-12.
  10. **Maynard, P. V., Blamey, R. W., Elston, C. W., Haybittle, J. L. and Griffiths, K.** 1978. Estrogen Receptor Assay in Primary Breast Cancer and Early Recurrence of The Disease. *Cancer Research* 38 : 4292-4295.
  11. **McGuire, W. L.** 1975. Current Status of Estrogen Receptors in Human Breast Cancer. *Cancer* 36 : 638-644.
  12. **McGuire, W. L., Carbone, P. P., Seats, M. F. and Escher, G. C.** 1975. Estrogen Receptors in Human Breast Cancer. Raven Press : 1-7.
  13. **Neifeld, J. P., Lawrence, W., Brown P. W., Banks, W. L. and Terz, J. J.** 1982. Estrogen Receptors in Primary Breast Cancer. *Arch. Surg.* 117 : 753-757.
  14. **Nishiki, M., Amano, K. and Ezaki, H.** 1982. Estrogen Receptors by Fluorescent Estradiol Histochemical Technique. *J. J. Surg. Soc.* 83 (suppl) : 135 (in Japanese).
  15. **Paulsen, H. S., Jensen, J. and Hermansen, C.** 1981. Human Breast Cancer. *Cancer* 48 : 1791-1793.
  16. **Pertschuk, L. P., Gaetjens, E., Carter, A. C., Brigati, D. J., Kim, D. S. and Fealey, T. E.** 1979. An Improved Histochemical Method for Detection of Estrogen Receptors in Mammary Cancer. *Am. J. Clin. Pathol.* 71 : 504-508.
  17. **Pertschuk, L. P., Tobin, E. H., Brigati, D. J., Kim, D. S., Bloom, N. D., Gaetjens, E., Berman, P. J., Carter, A. C. and Degenshein, G. A.** 1978. Immunofluorescent Detection of Estrogen Receptors in Breast Cancer. *Cancer* 41 : 907-911.
  18. **Samaan, N. A., Buzdar, A. U., Aldinger, K. A., Shultz, P. N., Yang, K., Romsdahl, M. M. and Martin, R.** 1981. Estrogen Receptor: A Prognostic Factor in Breast Cancer. *Cancer* 47 : 554-560.
  19. **Silfverswärd, C. and Humla, S.** 1980. Estrogen Receptor analysis on Needle Aspirates from Mammary Carcinoma. *Acta Cytologica* 24 : 54-57.
  20. **Straus, M. J., Moran, R., Muller, R. E. and Wotiz, H. H.** 1982. Estrogen Receptor Heterogeneity and The Relationship Between Estrogen Receptor and The Tritiated Thymidine Labeling Index in Human Breast Cancer. *Oncology* 39 : 197-200.
  21. **van Netten, J. P., Algard, F. T., Coy, P., Carlyle, S. J., Brigden, M. L., Thornton, K. R. and To, M. P.** 1982. Estrogen Receptor Assay on Breast Cancer Microsamples. *Cancer* 49 : 2383-2388.