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Demonstration of Estrogen Receptor by Immunohistochemical Staining in Paraffin Sections of Breast Carcinoma

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Paraffin embedded sections of 64 breast carcinomas were stained immunohistochemically using a commercially available monoclonal antibody to estrogen receptor. To improve the sensitivity of the staining, the authors used a Pronase® enzyme pretreatment, biotinylated antibody to rat IgG as secondary antibody, streptavidin-alkaline phosphatase as tertiary reagent and fast red as chromogen. When compared to the results of estrogen receptor enzyme immunoassay, this method yielded an 85.9% concordance rate, 86.2% specificity and 85.7% sensitivity. When compared to estrogen receptor immunocytochemistry(ER-ICA) in frozen section and considering the inherent advantages of immunohistochemical staining over biochemical assay, the major advantages of this method are good morphology, suitability for retrospective study and reduced cost of staining due to dilution of expensive primary antibody. Thus, this method offers an alternative to ER assay using fresh tissue and should provide additional valuable information about estrogen receptor.

Key Words : Estrogen receptor, immunohistochemical staining, paraffin sections, breast carcinoma

The prognosis of breast cancer influenced by a variety of clinical and pathologic factors. Among these, the axillary lymph node status and the tumor size have been found to be the best prognostic indicators (Fisher *et al.* 1984). A recent important development in the evaluation and management of breast cancer is the realization that the presence of estrogen receptor(ER) correlates with the response to hormone therapy and prognosis(Jensen *et al.* 1971; Rubens and Hayward 1980; Knight *et al.* 1983; Lippmann and Allegra 1980; Osborne *et al.* 1980; Fisher *et al.* 1983; Howat *et al.* 1985). Although the significance of ER analysis in the treatment of breast cancer remains somewhat problematic, there is general agreement that receptor-rich tumors are more likely respond to endocrine therapy(Jensen *et al.* 1976; DeSombre and Jensen 1980; Jensen 1981; Jensen *et al.* 1982; Howat *et al.* 1985). The ER can be measured in vitro by various techniques such as sucrose density gradient(Jensen *et al.* 1971), dextran-coated charcoal assay(Korenman and Dukes 1970), gel electrophoresis(Wagner and Jungblut 1976) and gel filtration assay(Godefroi and Brooks 1973). The development of highly specific monoclonal antibodies to ER protein by Greene *et al.* (1980) has permitted the development of ER-radioimmunoassay(ER-RIA), ER-enzyme immunoassay(ER-EIA) and ER-immunocytochemistry(ER-ICA), based on direct antigen recognition(Greene and Jensen 1982; king *et al.* 1985). Several reports indicate that the results of these methods bear a semiquantitative relationship to those of biochemical assay(King *et al.* 1985; Goussard *et al.* 1986; Remmele *et al.* 1986; Thorpe 1987; Van Netten *et al.* 1987). Although ER-ICA using frozen sections showed encouraging and reproducible results, the use of frozen sections has several disadvantages, such as suboptimal histology, the need for fresh tissue and the lack of suitability for retrospective studies. So attempts have been made to develop a reli-

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able ER-ICA technique which can be performed on formalin-fixed and paraffin-embedded tissue sections with encouraging results(Andersen *et al.* 1986; Shintaku and Said 1987; Cheng *et al.* 1988).

In this study we performed ER-ICA on paraffin-embedded tissue sections of breast cancer using commercially available kits. We compared the results with those of ER-EIA to determine the value of ER-ICA in paraffin-embedded tissue sections.

MATERIALS AND METHODS

Materials

The materials for this study came from 64 cases of breast carcinoma examined in the Department of Pathology of Severance Hospital from July 1988 to January 1990, for which ER analysis had been performed by ER-EIA. The histologic types of breast carcinoma included 59 cases of infiltrating ductal carcinoma-not otherwise specified, 2 cases of infiltrating lobular carcinoma, 2 cases of mucinous carcinoma and a case of medullary carcinoma. The ages of the patients ranged from 27 to 74 years (mean, 48.3 years). The patients were all women.

METHODS

ER-ICA of paraffin sections

Routine formalin-fixed and paraffin-embedded tissue blocks were cut at four microns, mounted on glass slides without tissue adhesives and dried in an oven at 60°C overnight. Slides were then deparaffinized for 15 minutes in xylene, hydrated through graded alcohols for 5 minutes and rinsed in 0.05M TRIS buffer (pH 7.5 at 25°C). Sections were then treated with 0.03 gm/ml of Pronase® enzyme (protease, Streptomyces griseus 50,000 units, CALBIOCHEM Corporation) in TRIS buffer for 10 minutes at 37°C. After TRIS buffer wash for 10 minutes, sections were incubated overnight at room temperature with primary antibody (ER-ICA Monoclonal, Abbott Laboratories) at 1 : 10 dilution. Slides were rinsed with TRIS buffer for 10 minutes,

Table 1. List of methods used for ER-ICA

Pretreat -ment Enzyme	Dilution of Primary Ab	Incubation Time of Primary Ab	Type of Secondary Reagent	Type of Tertiary Reagent	Name of Chromogen
Pronase®	1:10	Overnight	Biotinylated anti-rat Ig	Alk-P conjugated Strept Avidin	Fast red
Pronase®	1:10	One Hour	Biotinylated anti-rat Ig	Alk-P conjugated Strept Avidin	Fast red
Pronase®	1:10	Overnight	Biotinylated anti-mouse Ig	Alk-P conjugated Strept Avidin	Fast red
Pronase®	1:10	Overnight	Biotinylated anti-rat Ig	ABC	AEC
Pronase®	1:10	Overnight	Biotinylated anti-mouse Ig	ABC	AEC
Pronase®	1:10	Overnight	Goat anti-rat Ig	PAP complex	AEC
Trypsin	1:10	Overnight	Biotinylated anti-rat Ig	Alk-P conjugated Stept Avidin	Fast red
Trypsin	1:10	Overnight	Biotinylated anti-rat Ig	ABC	AEC

Ig ; Immunoglobulin, Alk-P ; Alkaline Phosphatase,
ABC ; Avidin Biotin Complex, AEC ; Aminoethylcarbazole,
PAP ; Peroxidase Anti-Peroxidase Complex

and biotinylated antibody to rat immunoglobin G (VECTOR Laboratories) was added for 30 minutes followed by TRIS buffer washing for 10 minutes. Streptavidin-alkaline phosphatase (BioGenex Laboratories) was added for 30 minutes. After final wash, sections were then incubated in chromogen substrate solution (Fast red, BioGenex Laboratories) for 15 minutes. Sections were then washed in water, lightly counterstained with Mayer's hematoxylin and coverslipped with glycerin-jelly. A negative control was run during each procedure on some of the paraffin sections with the negative control antibody (normal rat serum) supplied in the Abbott Kit. Sections of stomach carcinoma were also stained as negative controls. After interpreting the results of staining by this method, we attempted several other methods using different combinations of reagents. The methods tried were as follows(Table 1).

ER-EIA

Fresh samples of breast tumor were sent to the department of clinical pathology at the time of surgery for estrogen receptor analysis using the ER-EIA monoclonal kit(Abbott Laboratories). Values of less than 20 femtomoles/mg cytosol protein were considered to be negative, while values of more than 20 femtomoles/mg cytosol protein were interpreted as positive.

Evaluation of ER-ICA

The results of staining were interpreted without knowing the results of ER-EIA. Stains were rated on the basis of visually estimated proportions of positive tumor cells as "+++" when more than two-thirds of tumor cell nuclei showed positive staining; "++" when approximately one-third to two-thirds of tumor cell nuclei showed positive staining; "+" when less than one-third of tumor cell nuclei were positive; and "0" when there was no tumor nucleus staining. For statistical analysis of the results, we regarded the results of staining as "positive" if any portion of the tumor cell nuclei showed positive staining. The staining intensity of tumor cell was interpreted as "++" when the tumor cell showed predominantly intense, opaque, red staining of nuclei and "+" when the tumor cell showed predominantly speckled red staining of the nuclei.

RESULTS

ER-ICA in paraffin section

The results of ER-ICA using paraffin-embedded sections are shown in Table 2. The quality of immunostaining was good, and it was easy to interpret the results of staining because of a clean background and good contrast of fast red dye even in tumors with weak staining. The positive reaction was confined to the nuclei of breast cancer cells (Fig. 1) and normal ductal epithelial cells (Fig. 2). No specific cytoplasmic staining reaction was observed and no nuclear staining occurred in negative controls. Heterogeneity of the staining reaction was conspicuous, and the percentage of positively stained neoplastic cells ranged from 0 to 100 percent. Among the 34 cases with positive ER-ICA staining, 3 cases showed "+" positivity, 7 cases showed "++" positivity and 24 cases showed "+++" positivity. The distribution of positive nuclei varied considerably even within the same section, and there was a tendency toward focal clustering of positive tumor cells. Positive tumor cells also showed considerable heterogeneity in the intensity of the reaction, and there was a tendency for tumors with high percentage of positive tumor cells to show intense staining of the nuclei. Non-neoplastic ductal epithelial cells also occasionally showed nuclear staining in both ER-positive and ER-negative tumors, and their staining intensity and the number of positive cells varied from case to case. Thirty-one of the 59 cases of infiltrating ductal carcinoma-NOS showed a positive reaction, both cases of mucinous carcinoma showed diffuse positive staining(Fig. 3), one of two cases of infiltrating lobular carcinoma showed positive staining and one case of medullary carcinoma showed a negative reaction. It was, however, difficult to correlate positivity with histologic type due to the small numbers of types other than infiltrating ductal carcinoma-NOS.

Using other combinations of reagents, pretreament enzyme and incubation time of primary antibody, we could not obtain results as satisfactory as with the original method.

Comparison of ER-ICA of paraffin sections with ER-EIA

The results of ER-ICA using paraffin sections were compared with content of ER measured by ER-EIA. As shown in Table 3., 30 of the 35 tumors with ER content greater than 20 fmol/mg cytosol protein displayed positive ER-ICA. Of the 29 tumor samples negative for ER(below 20 fmol/mg cytosol protein) by ER-EIA, 25 showed no immunoreactivity. Thus the results of ER-ICA agreed with those of ER-EIA in 85.9%, and the specificity and sensitivity using the results of ER-EIA as "true" values were 86.2% and

Table 2. Summary of the results of ER-ICA and ER-EIA

Surgical Number	Age	ER-EIA	ER-ICA Positivity	ER-ICA Intensity	Type	Surgical Number	Age	ER-EIA	ER-ICA Positivity	ER-ICA Intensity	Type
88- 9977	74	1.11	-	-	MEDU	89- 7334	53	466.02	+++	++	ILC
88-10587	50	6.90	-	-	IDC	89- 7844	67	17.30	-	-	IDC
88-11668	59	2.76	-	-	IDC	89- 8046	41	24.60	+++	+	IDC
88-11707	44	31.03	-	-	IDC	89- 8712	43	74.80	+++	+	IDC
88-12168	44	6.94	-	-	IDC	89- 8937	60	212.90	+++	++	IDC
88-12460	57	63.70	++	+	IDC	89- 9301	52	1.70	-	-	IDC
88-13306	60	314.50	+++	++	IDC	89- 9595	57	40.50	+	+	IDC
88-15192	57	0.20	-	-	IDC	89-10286	51	6.00	-	-	IDC
88-15472	37	14.00	+++	+	IDC	89-10710	52	0.00	-	-	IDC
89- 234	69	38.90	-	-	IDC	89-10846	47	33.40	++	+	IDC
89- 731	51	717.00	+++	++	IDC	89-11382	0	0.00	-	-	IDC
89- 886	45	593.90	++	-	IDC	89-11415	71	22.50	-	-	IDC
89- 1290	31	6.80	+++	++	IDC	89-11430	52	0.00	-	-	IDC
89- 1317	27	1.89	-	-	IDC	89-11988	56	0.00	-	-	IDC
89- 1441	43	21.40	++	+	IDC	89-11992	56	153.80	+++	++	IDC
89- 1463	45	370.00	+++	++	MUCI	89-12102	46	0.00	-	-	IDC
89- 1465	31	3.25	+++	++	IDC	89-12185	31	0.00	-	-	IDC
89- 1509	45	117.90	+++	+	IDC	89-12261	46	153.20	-	-	IDC
89- 1612	41	8.96	-	-	IDC	89-12718	34	0.39	-	-	IDC
89- 1971	50	7.51	++	+	IDC	89-13148	32	0.18	-	-	IDC
89- 2351	44	66.18	+++	++	IDC	89-13460	45	10.53	-	-	IDC
89- 2780	49	10.12	-	-	IDC	89-13918	49	3.59	-	-	IDC
89- 3388	39	59.87	+++	+	IDC	89-14107	56	625.00	-	-	IDC
89- 3607	27	20.10	+	+	IDC	89-14247	56	40.94	+++	+	IDC
89- 4205	52	113.64	+++	++	IDC	89-14248	53	147.10	+++	++	IDC
89- 5137	46	142.30	+++	+	MUCI	89-14317	63	57.77	+++	+	IDC
89- 5629	43	17.24	-	-	IDC	89-14481	44	26.81	+	+	IDC
89- 5903	53	17.78	-	-	IDC	89-15096	35	75.26	+++	++	IDC
89- 5982	42	15.84	-	-	ILC	89-15166	49	72.72	+++	+	IDC
89- 6229	57	204.08	+++	++	IDC	89-15167	57	101.90	+++	++	IDC
89- 6591	48	42.27	+++	+	IDC	89-15444	46	48.47	++	+	IDC
89- 7293	64	3.69	-	-	IDC	90- 309	43	37.51	++	+	IDC

MEDU: Medullary carcinoma, IDC: Infiltrating ductal carcinoma-not otherwise specified,
MUCI: Mucinous carcinoma, ILC: Infiltrating lobular carcinoma

85.7% respectively. Five cases showed false negative results(ER-EIA positive, ER-ICA negative), and the values of ER-EIA were 22.5, 31.03, 38.90, 153.20 and 625.00 fmol/mg cytoplasmic protein. In one case with this false negative result(ER-EIA; 625.00 fmol/mg cytoplasmic protein), ER-ICA revealed intense positive nuclear staining of adjacent normal ductal epithelium while the nuclei of the tumor cells were totally negative(Fig. 4). On the other hand, four cases with negative ER-EIA(3.25, 6.80, 7.51 and 14.00 fmol/cytoplasmic protein) showed a positive ER-ICA.

The quantitative relationship between the semiquantitative values of ER-ICA(0, +, ++, +++) and the values abtained by ER-EIA is shown in Fig. 5. Statistical analysis using simple linear regression analysis showed the semiquantitative results of ER-ICA and the values of ER-EIA to be correlated(r: 0.35 $p < 0.001$).

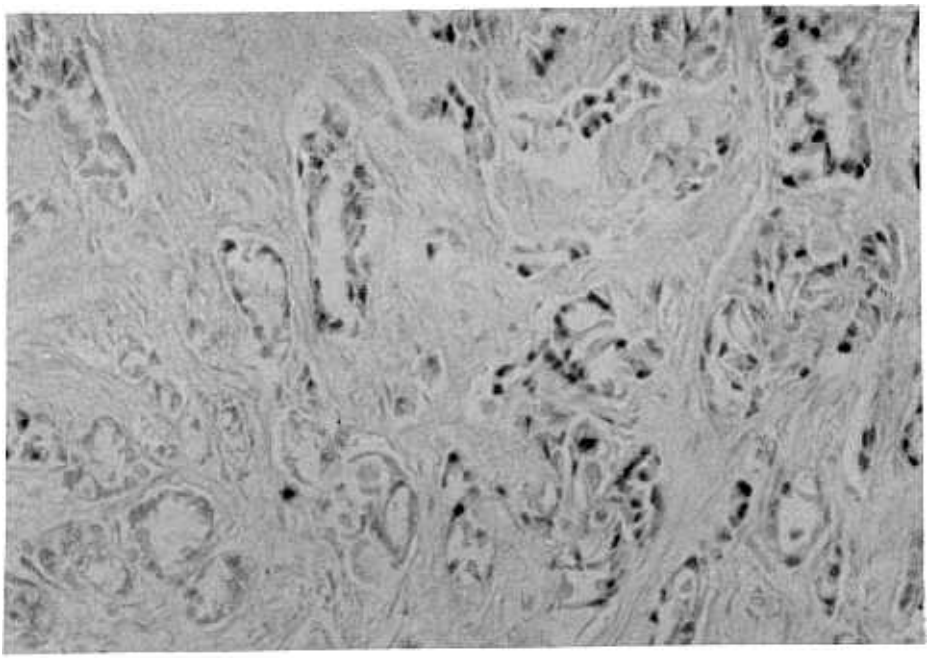

Fig. 1. Infiltrating ductal carcinoma showing intense nuclear staining by ER-ICA using paraffin section (×200).

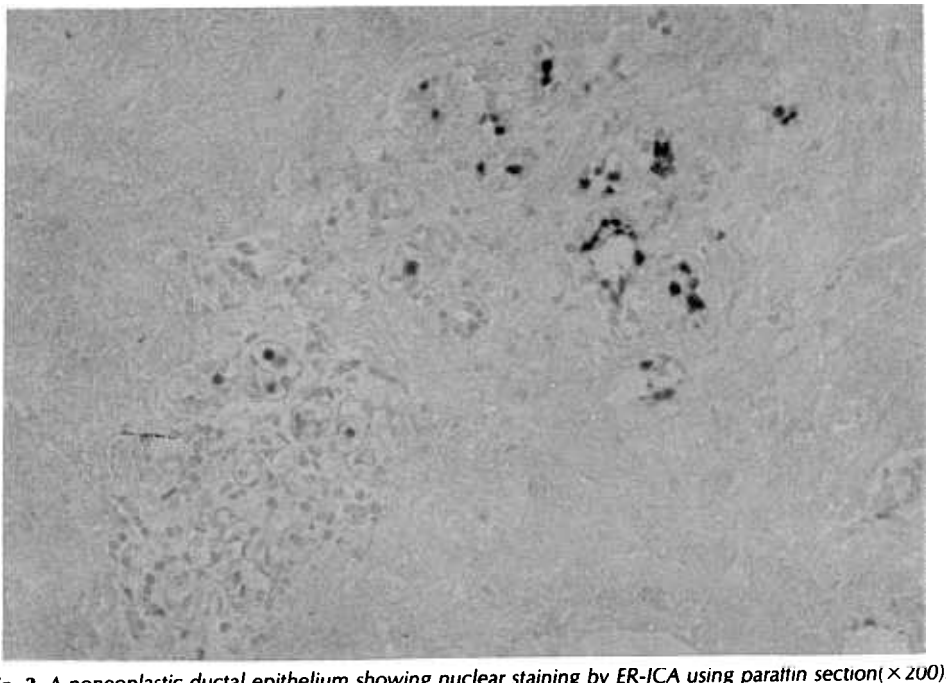

Fig. 2. A noneoplastic ductal epithelium showing nuclear staining by ER-ICA using paraffin section(×200).

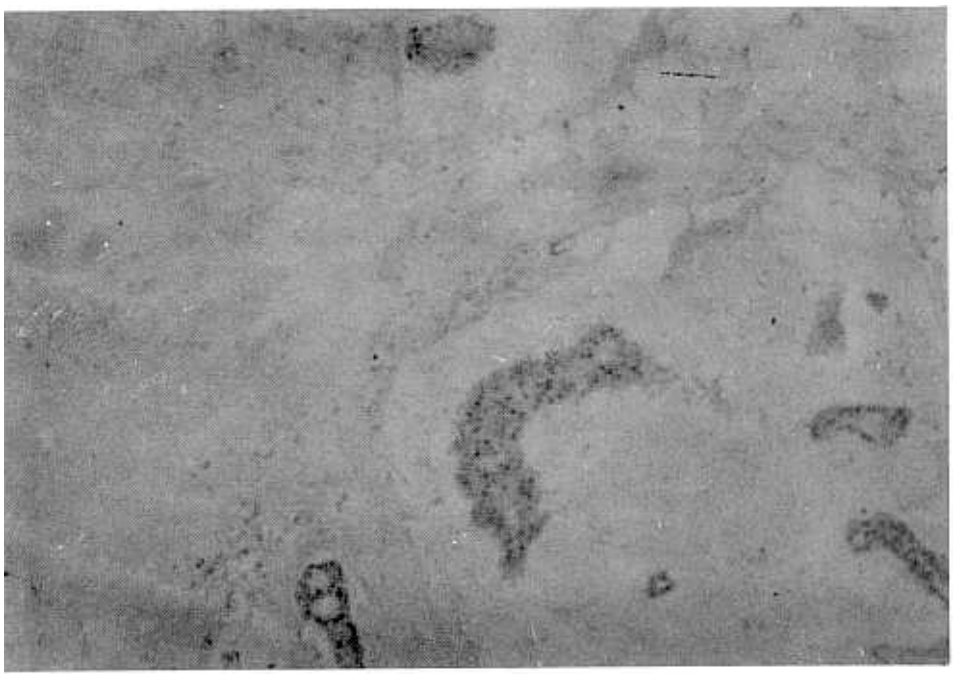

Fig. 3. *Mucinous carcinoma showing nuclear staining by ER-ICA using paraffin section(×100).*

Table 3. Comparison of ER-ICA of paraffin section with ER-EIA

ER-EIA	Number of cases	ER-ICA Negative	Positive
Negative	29	25	
Positive	35	5	30

Concordance rate=55/64, Specificty=25.29 Sensitivity=30/35

DISCUSSION

The use of monoclonal antibody against estrogen receptor protein has permitted direct measurement of the amount of estrogen receptor protein by immunologic assay such as enzyme immunoassay and radioimmunoassay(Green *et al.* 1980; Jensen *et al.* 1982; Greene and Jensen 1982). Because these methods, like the previously used biochemical methods, require fresh tissue homogenates, they provide little information about the heterogeneity of ER distribution in tumor tissue. Also the bothersome and time-consuming procedures hinder wide clinical applications. The development of ER-ICA using frozen tissue sections makes it possible to correlate the histopathologic characteristics of a tumor with its ER status, even with very small amounts of tissue (King *et al.* 1985). Several studies with ER-ICA report that the results of immunostaining are significantly correlated with the concentrations of ER as measured by biochemical assay, so ER measurement by ER-ICA appears to be useful in predicting the response to hormonal therapy(King *et al.* 1985; McClleland *et al.* 1986; Pertschuk *et al.* 1985). Because this method works on fresh frozen tissue, it has suboptimal morphologic features and it is impossible to perform a retrospective study. Recent developments in the field of immunohistochemistry have led to technical modifications with increased sensitivity. Among these, the avidin-biotin methods are popular because of their increased sensitivity and versatility(Hsu *et al.* 1981). Due to the markedly increased sensitivity of these immunostaining methods, several antigens previously detected only in freash tissue can be demonstrated in routinely fixed and paraffin-embedded tissue, and several

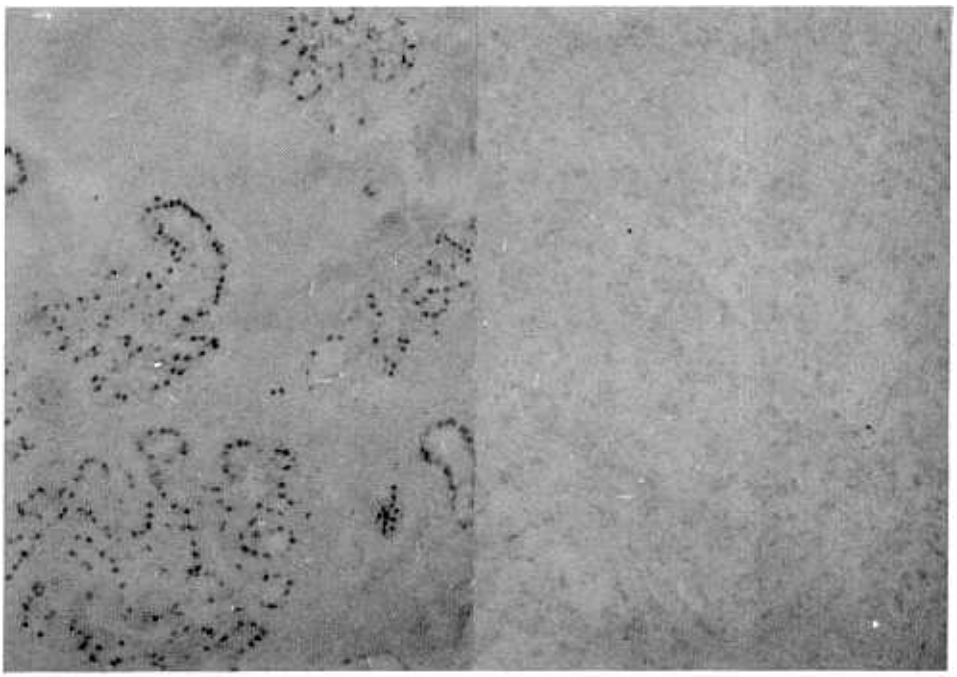

Fig. 4. False negative case showing intense nuclear staining of noneoplastic ductal epithelium(left) with negatiye nuclear staining of tumor cells(right) (×200).

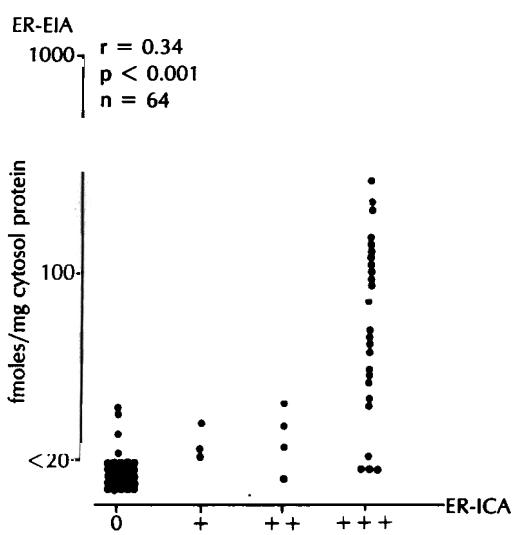

Fig. 5. Relationship between semiquantitative ER-ICA values and ER-EIA.

techniques were reported recently for the detection of estrogen receptor in paraffin sections using different combinations of reagents(Andersen *et al.* 1986; Shintaku and Said 1987; Cheng *et al.* 1988). In this study we used the enzyme-labelled avidin method, the most sensitive method yet known (Giorno 1984), and Pronase® enzyme pretreatment to demonstrate estrogen receptor in formalin-fixed tissue using monoclonal antibody directed against estrogen receptor.

The results of this study indicate that ER-ICA using paraffin embedded sections is a reliable method having many advantages over other ER assay methods. The concordance rate, specificity and sensitivity of this method using the results of ER-EIA as "true" values were 85.9%, 86.2% and 85.7% respectively. Four cases with ER-EIA values below 20 fmol./mg cytosol protein showed positive nuclear staining using our method. These false positive cases demonstrated nuclear staining over at least 60 % of tumor cells, however, we believe that this may be an indication of the limitaions of ER-EIA rather than actual false positivity. Five cases with

ER-EIA values over 20 fmol/mg cytosol protein showed no nuclear staining of tumor cells. The ER-EIA values of these false negative cases ranged from 22.5 to 625.00 fmol/mg cytosol protein. The false negativity associated with low ER-EIA values may be due to loss of immunoreactivity resulting from fixation and paraffin-embedding procedures. But in view of the ER-ICA positivity in four cases with ER-EIA values below 20 fmol/mg cytosol protein, we believe other factors may be a cause of this discrepancy. In one case (ER-EIA value 625.00 fmol/mg cytosol protein), the ER-ICA showed intense positivity within the adjacent normal ductal epithelium without positive staining of tumor cells, indicating a high ER-EIA value due to the high ER content of normal epithelial cells. The sensitivity and specificity of ER-ICA using paraffin sections may therefore be higher than that indicated by the results of this study.

Other methods using different combinations of commercially available reagents did not show results as reliable and reproducible as that of the original one. Although antibody to mouse immunoglobulin is reported to cross-react to antibody to rat immunoglobulin, and some reported good results with anti-mouse immunoglobulin instead of anti-rat immunoglobulin (Wang *et al.* 1988), we could not obtain results as satisfactory as the original one. With short incubation times(1 hour), we could not obtain satisfactory results using a combination of reagents. Thus in view of the high cost of primary antibody, it is preferable to perform overnight incubations with 1:10 dilution of primary antibody. Pronase® enzyme pretreatment allows exposure of double-stranded DNA and increases the sensitivity of immunostaining with paraffin-embedded tissue. We tried trypsin enzyme pretreatment instead of Pronase®, but we failed to obtain satisfactory results. Other combinations of secondary and tertiary reagents, such as the ABC and PAP methods, did not show reliable results due to the inherent sensitivity of the methods.

We conclude that the application of Pronase® enzyme pretreatment and the alkaline-phosphatase labelled avidin biotin method to ER-ICA in paraffin-embedded sections offers an alternative to ER-ICA using frozen section, ER-EIA, ER-RIA and other biochemical assays. We feel that its major advantages, such as reduced cost of staining due to dilution of expensive primary antibody, good morphologic detail; suitability for retrospective study, technical simplicity, and abilty to correlate histologic characteristics of a tumor and status of ER even with a very small amounts of specimen will provide valuable additional information about estrogen receptors.

REFERENCES

Andersen J, Orntoft T, Skovgaard Poulsen H : Semiquantitative estrogen receptor assay in formalin-fixed paraffin sections of human breast cancer tissue using monoclonal antibodies. *Br J Cancer 53 : 691-694, 1986*

Cheng L, Binder SW, Fu YS, Lewin KJ : Demonstration of estrogen receptors by monoclonal antibody in formalin-fixed breast tumors. *Lab Invest 58:346-353, 1988*

DeSombre ER, Jensen EV: Estrophilin assays in breast cancer: Quantitative features and application to the mastectomy speciman. *Cancer 46 : 2783-2790, 1980*

Fisher B, Redmond C, Brown A, Wickerham DL, Wolmark N, Allegra J, Escher G, Lippman M, Savlov E, Wittliff J, Fisher ER : Influence of tumor estrogen and progesterone receptor levels on the response to tomoxifen and chemotherapy in primary breast cancer. *J Clin Oncol 1 : 227-241, 1983*

Fisher ER, Sass R, Fisher B: Pathologic findings from the national surgical adjuvant project for breast cancer (Protocol No. 4) x-Discrimants for tenth year treatment failure. *Cancer 53 : 712-723, 1984*

Giorno R : A comparison of two immunoperoxidase staining methods based on the avidin-biotin interaction. *Diagn Immunol 2 : 161-166, 1984*

Godefroi VC, Brooks SC : Improved gel-filtration method for analysis of estrogen receptor binding. *Anal Biochem 51 : 335-344, 1973*

Goussard J, Lechevrel C, Martin PM, Roussel G : Comparison of monoclonal antibodies and tritiated ligands for estrogen receptors assays in 241 breast cancer cytosols. *Cancer Res(Supplement) 46 : 4282s-4287s, 1986*

Greene GL, Nolan C, Engler JP, Jensen EV : Monoclonal antibodies to human breast estrogen receptor. *Proc Nat Acad Sci(U.S.A) 77 : 5115-5119, 1980*

Greene GL, Jensen EV : Monoclonal antibodies as probes for estrogen receptor detection and characterization. *J Steroid Biochem 16 : 353-359, 1982*

Howat JMT, Harris M, Swindell R, Barnes DM : The effect of estrogen and progesterone receptors on recurrence and survival in patients with carcinoma of the breast. *Br J Cancer 51 : 203-270, 1985*

Hsu SM, Raine L, Fanger H : Use of Avidin-Biotin-Peroxidase Complex(ABC) in Immunoperoxidase Technique : A Comparison between ABC and Unlabeled Antibody(PAP) Procedures. *J Histochem Cytochem 29 : 577-580, 1981*

Jensen EV, Block GE, Smith S, Kyser K,DeSombre ER : Estrogen receptors and breast cancer response to

adrenalectomy. *Natl Inst Cancer Monograph 34 : 55-70, 1971*

Jensen EV, Smith S, DeSombre ER: Hormone dependency in breast cancer. *J Steroid Biochem 7 : 911-917, 1976*

Jensen EV: Hormone dependency of breast cancer. *Cancer 47 : 2319-2326, 1981*

Jensen EV, Greene GL, Closs LE, DeSombre ER, Nadji M : Receptors reconsidered : A 20-year perspective. *Recent Prog Horm Res 38 : 1-40, 1982.*

King WJ, Desombre ER, Jensen EV, Greene GL : Comparison of immunocytochemical and steroid-binding assays for estrogen receptor in human breast tumors. *Cancer Res 45 : 293-304, 1985*

Knight WA, Livingston RB, Gregory EJ, McGuire WL : Estrogen receptors as an independent prognostic factor for early recurrence in breast cancer. *Cancer Res 37 : 4669-4671, 1977*

Korenman SG and Dukes BA: Specific estrogen binding by the cytoplasm of human breast carcinoma. *J Clin Endocrinol 30 : 639-645, 1970*

Lippmann ME, Allegra JC : Quantitaive estrogen receptor analysis : the response to endocrine and cytotoxic chemotherapy in human breast cancer and the disease free interval. *Cancer 46 : 2829-2834, 1980*

McCarty KS, Jr, Miller LS, Cox EB, Konrath J, McCarty KS, Sr : Estrogen receptor analysis. Correlation of biochemical methods using monoclonal anti-receptor antibodies. *Arch Path Lab Med 109 : 716-721, 1985*

McClelland RA, Berger U, Miller LS, Powles TJ, Coombes PC : Immunocytochemical assay for estrogen receptor in patients with breast cancer : relationship to a biochemical assay and to outcome of therapy. *J Clin Onc 4 : 1171-1176, 1986*

Osborne CK, Yochmowitz MG, Knight WA, McGuire WL : The values of estrogen and progesterone receptors in the treatment of breast cancer. *Cancer 46 : 2884-2888, 1980*

Pertschuk LP, Eisenberg KB, Carter AC, Feldman JG : Immunohistologic loclization of estrogen receptors in breast cancer with monoclonal antibodies. *Cancer 55 : 1513-1518, 1985*

Remmele W, Hilebrand U, Heinz HA, Klein PJ, Vierbuchen M, Behnken LJ, Heicke B, Scheidt E : Biochemical studies on oestrogen receptors, lectin receptors and Barr bodies in human breast cancer. *Virchows Arch(Pathol Anat) 409 : 127-147, 1986*

Rubens RD, Hayward JL : Estrogen receptors and response to endocrine therapy and cytotoxic chemotherapy in advanced breast cancer. *Cancer 46 : 2922-2924, 1980*

Shintaku P, Said JW: Detection of estrogen receptors with monoclonal antibodies in routinely processed formalin-fixed paraffin sections of breast carcinoma. *Am J Clin Pathol 87 : 161-167, 1987*

Thorpe SM : Monoclonal antibody technique for detection of estrogen receptors in human breast cancer ; greater sensitivity and more accurate classification of receptor status than dextran-coated charcol method. *Cancer Research 47 : 6572-6575, 1987* ,

Van Netten JP, Thornton IG, Caryle SJ, Brigden ML, Coy p, Goodchild NL, Gallagher S, George EJ : Multiple microsample analysis of intratumor estrogen receptor distribution in breast cancers by a combined biochemical/immunohistochemical method. *Eur J Cancer Clin Oncol 23 : 1337-1342, 1987*

Wagner RK, Jungblut PW : Oestradiol and dihydrotestosterone receptors in normal and neoplastic human mammary tissue. *Acta Endocrinol 82 : 105-120, 1976*

Wong SY, Carrol EDS, Ah-See SY, Sewell HF : Detection of estrogen receptor proteins in breast tumors using an improved APAAP immunohistochemical technique. *Cancer 62 : 2171-2175, 1988*