啓明醫大論文集 第 9 卷 第 3 號
The Keimyung Univ Med J
Vol 9, No.3, September, 1990

Detection of Estrogen Receptors with Monoclonal Antibodies in Formalin-Fixed, Paraffin-embedded Breast cancer tissue* (Comparison of immunohistochemical method with ER - D5 antibody and ER-ICA)

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=國文秒錄=

파라핀 포매된 유방암 조직에서 단클론항체에 의한 에스트로겐 수용체 검색 (ER-D5 항체로 하는 면역조직화학적 방법과 ER-ICA 방법과의 비교)

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포르말린 고정 파라핀 포매된 유방암 조직으로 면역조직화학적 기법에 의해서 에스트로겐 수용체(ER)를 검색하는 방법을 기술하였다. 엄선된 원발성 유방암 72예(1988년 7월~1990년 4월)를 단클론항체 ER-D5로 Biotin-StreptAvidin 방법으로 염색하여 58%의 환자의 유방암이 ER 양성을 나타내었다. 이 중 동결절편-ER-ICA 방법도 동시에 시행한 30예와는 80%의 일치율을 보였고, 또 DCC방법에 의한 생화학분석을 시행한 21예와는 76%의 일치율을 나타내었다. 이 중 10예에서는 3가지 방법을 다 시행하였고, 나머지 30예는 소급하여 파라핀 포매조직에서만 시행하였다.

파라핀 포매조직에서 양성으로 염색되는 암세포의 비율은 0에서부터 거의 전 암세포에 (100%) 염색되는 다양한 양상을 보였고 그 염색강도는 1+에서부터 3+로 역시 다양하였으며 드물게는 ±로 아주 희미하게 염색되기도 하였다. 파라핀 절편의 염색이 다른 방법보다 더 예민하여 ER 양성율이 많았다.

본 논문의 목적은 통상적으로 포르말린에 고정한 유방암조직에서 에스트로겐 수용체를 면역조직화학적 분석으로 쉽고 신속히 할 수 있는 방법의 확립과 또 이 방법의 다른 방법과의 일치성을 확인하는데 있으며 위와 같이 확인되었다. 본 연구에서 시행한 기법은 다른 기법보다 시행비용이 저렴하고 또 작은 조직에서도 가능하며 조직진단을 하고 난 후에도 파라핀 불록에서 간단히 할 수 있고, 조직학적 형태를 동시에 볼 수 있으며 영구히 표본을 보존할 수 있는 등의 장점이 있다.

Key Words: Estrogen receptor, Breast cancer tissue, Immunohistochemical method, Fomalin-fixed, Paraffin-embedded,

Introduction

The presence of an estrogen receptor(ER) in breast cancer is an important parameter in selection of breast cancer patients for hormonal therapy[1,2], because it is a general agreement that ER-rich tu-

* The summary of preliminary results of this study has been posted at the Spring Meeting of the Korean Society of Pathologists 1990.

* This study was supported by a special research grant of Keimyung University Dong San Medical Center, 1989.

mors are more likely to respond to endocrine therapy than to chemotherapy[3] and ER positive breast cancer is positively correlated with a prolonged disease-free interval[4,5,6].

The development of monoclonal anti-ER antibodies allows the direct identification of the actual ER protein[7,8]. The estrogen-receptor immunocytochemical assay (ER-ICA) developed in 1982[7] and 1985 has been used with reproducible results and a good correlation with conventional ER assays.[9,10]. But the ER-ICA has only been used on fresh or frozen tissue specimens. The techniques have disadvantages as they require fresh tissue and can take no direct account of either the proportion of carcinoma in the sample or the heterogeneity of estrogen receptor expression within the tissue submitted for analysis. From the surgical pathologist's perspective, the ideal histochemical ER assay should be reliably performed in routinely processed, formalin-fixed tissue.

The aims of this study were to establish an easy and rapid technique for ER immunohistochemical assay in routinely processed formalin-fixed, paraffin-embedded histological specimens and to determine the correlation between immunohistochemical method and immunocytochemical and biochemical assays. The authors describe a simple and reproducible method with an ER-D5 monoclonal antibody, using an amplified Biotin-StreptAvidin method to determie ER content in paraffin sections. Our observations indicate that this can be accomplished in a reliable and reproducible manner through the use of selected antibodies on tissues that have been embedded routinely in paraffin after fixation in formalin.

Materials and Methods

The material for this study came from 72 consecutive biopsies from patients with primary breast cancer. All tumors were removed surgically at Keimyung University Dongsan Medical Center between from July 1988 to April 1990. The breast cancer tissue was formalin fixed and paraffin embedded blocks have been stored for up 2 years at room temperature.

Monoclonal antibody: The ER-D5 monoclonal antibody(Amersham) raised against affinity-purified cytosol estradiol receptor was utilized. ER-D5 recognizes a 29kd non-hormone binding protein associated with the estrogen receptor which has a cytoplasmic location rather than the nuclear locus of the estradiol binding unit.

ER immunohistochemical staining was performed using an amplified Biotin-StreptAvidin (B-SA) system (BioGenex Laboratories, CA. U. S. A.)

Preparation and staining of paraffin sections:

Tumors were fixed in neutral buffered formalin at room temperature for 12 to 24 hours and processed for routine paraffin embedding. The original hematoxylin and eosin stained sections were reviewed and a representative section of each case was selected. Sections four to six microns thick were cut from the corresponding formalin fixed, paraffin embedded blocks of tumor tissue and placed on gelatin-coated slides (DIFCO's Bacto gelatin), heat-fixed at 37°C for 4 to 8 hours, deparaffinized in xylene, and hydrated through graded alcohols. The sections were then placed in PBS, pH 7.6, incubated with a blocking reagent (3% hydrogen peroxide) for 10, 20, 30 minutes, rinsed for 5 minutes with two changes, and covered with a blocking reagent (normal goat serum) for 20 minutes and then incubated in a moist chamber with 1~2 drops of primary antibody (ER-D5 Amersham, 1:50 dilution) at room temperature. After rinsing well in PBS for 5 minutes with two changes again then the link antibody (biotinylated goat anti-mouse immunoglobulin) was used for 20 minutes and then rinsed well with PBS for 5 minutes. Then a labelling reagent (peroxidase labelled avidin) was applied and again rinsed well with PBS and followed by an application of substrate solution (peroxidase as labelling enzyme) and then rinsed well with deionized water, and finally counterstained with Mayer's hematoxylin, and then rinsed with tap water. The slides were immersed in ammonia water, rinsed gently with running tap water, dehydrated, cleared and mounted.

Interpretation:

Results of staining in paraffin sections were initially read independently by two observers and then a final score was agreed upon after simultaneous

viewing of slides by both observess on a dual-head microscope. Stains were rated on the basis of visually estimated percentages of neoplastic cells with positive cytoplasmic staining by counting 200 morphologically malignant cells in multiple random fields using x40 objectives and the predominant staining intensity among the positive cells (0 for negative, ± for faintly positive, +1 for weakly positive, 2+ for moderately positive, +3 for strong positive).

A tumor was considered "positive for ER" if greater than 10 percent of neoplastic cells showed any intensity of cytoplasmic staining and "negative for ER" if none of the neoplastic cells revealed any intensity of cytoplasmic staining and/ or lesser than 10 percent of positive ER of tumor cells.

Results

The results of histochemical staining are shown in table 1.

Table 1. Histological classification and Estrogen Receptor status

Case No.	Age	LM. Dx.	ER-PAP(B-SA)	ER-ICA	ER-DCC**
1	50	Ductal carcinoma, infiltrating	++(*diff* 100%)	+(40%)	+(5.8fmol/mg)
2	40	Ductal carcinoma, infiltrating	+(*diff* 100%)	+(20%)	+(14.99fmol/mg)
3	60	Lobular carcnoma, infiltrating	+(80%)	+(70%)	+(74.91fmol/mg)
4	42	Ductal carcinoma, infiltrating	+>−(*diff* 85%)	+(75%)	+(24.1fmol/mg)
5	50	Ductal carcinoma, infiltrating	±(*diff* 100%)	+(20%)	−(4.47fmol/mg)
6	48	Ductal carcinoma, infiltrating	+<−(*focal* 20%)	−	+(18.3fmol/mg)
7	47	Intraductal carcinoma	±(*diff* 100%)	−	−(1.47fmol/mg)
8	51	Ductal carcinoma, infiltrating	−(*occ* 5%)	+(27%)	−(0fmol/mg)
9	40	Medullary carcinoma, infiltrating	−	−	+(9.40fmol/mg)
10	40	Cystosarcoma phyloides, malignant	−	−	−(0fmol/mg)
11	43	Ductal carcinoma, infiltrating	++(*diff* 100%)	+(51%)	·
12	39	Ductal carcinoma, infiltrating	++(85%)	+(32%)	·
13	48	Intraductal carcinoma	++(*diff* 100%)	+(28%)	·
14	50	Ductal carcinoma, infiltrating	+~++(*diff* 100%)	+(65%)	·
15	41	Ductal carcinoma, infiltrating	+(80%)	+(65%)	·
16	30	Ductal carcinoma, infiltrating	+(*focal* 25%)	−	·
17	35	Lobular carcinoma, infiltrating	++(20%)	−	·
18	51	Ductal carcinoma, infiltrating	+	−	·
19	32	Ductal carcinoma, infiltrating	−	+(56%)	·
20	39	Mucinous carcinoma	−	−	·
21	54	Ductal carcinoma, infiltrating	−	−	·
22	49	Ductal carcinoma, infiltrating	−	−	·
23	45	Papillary carcinoma, infiltrating	−	−	·
24	45	Ductal carcinoma, infiltrating	−	−	·
25	30	Ductal carcinoma, infiltrating	−	−	·
26	35	Medullary carcinoma	−	−	·
27	41	Ductal carcinoma, infiltrating	−	−	·
28	46	Ductal carcinoma, infiltrating	−	−	·
29	53	Ductal carcinoma, infiltrating	−	−	·
30	46	Ductal carcinoma, infiltrating	−	−	·
31	69	Ductal carcinoma, infiltrating	+(*diff* 100%)	·	+(126.75fmol/mg)
32	32	Ductal carcinoma, infiltrating	+++(*diff* 100%)	·	+(26.31fmol/mg)

33	48	Ductal carcinoma, infiltrating	+(*diff* 80%)	·	+(14.3fmol/mg)
34	33	Ductal carcinoma, infiltrating	+>−(75%)	·	+(43.25fmol/mg)
35	49	Intraductal carcinoma	++(75%)	·	−(2.23fmol/mg)
36	63	Ductal carcinoma, infiltrating	++(*diff* 100%)	·	−(0fmol/mg)
37	41	Ductal carcinoma, infiltrating	+,±(30%)	·	−(0.6fmol/mg)
38	40	Ductal carcinoma, infiltrating	±>+(*focal* 35%)	·	−(0fmol/mg)
39	60	Ductal carcinoma, infiltrating	±>+(*focal* 30%)	·	−(0fmol/mg)
40	53	Medullary carcinoma	−	·	−(0fmol/mg)
41	29	Intraductal carcinoma, comedo type	−	·	−(0fmol/mg)
42	38	Ductal carcinoma, infiltrating	+++(*diff* 100%)	·	·
43	43	Lobular carcinoma, infiltrating	+++(*diff* 100%)	·	·
44	41	Intraductal carcinoma. Paget's ds.	++(*diff* 100%)	·	·
45	50	Mucinous carcinoma	+,++(*partial diff* 80%)	·	·
46	37	Medullary carcinoma	+,++,+++(*focal* 85%)	·	·
47	60	Ductal carcinoma, infiltrating	++(*diff* 100%)	·	·
48	38	Ductal carcinoma, infiltrating	+~++(*diff* 85%)	·	·
49	37	Ductal carcinoma, infiltrating	+<++(*diff* 100%)	·	·
50	48	Ductal carcinoma, infiltrating	+>++(*diff* 90%)	·	·
51	64	Ductal carcinoma, infiltrating	+>++(*diff* 90%)	·	·
52	40	Ductal carcinoma, infiltrating	+(*infilt**20%)	·	·
53	63	Ductal carcinoma, infiltrating	+(85%)	·	·
54	47	Ductal carcinoma, infiltrating	+(70%)	·	·
55	31	Ductal carcinoma, infiltrating	+(*partial diff* 80%)	·	·
56	47	Ductal carcinoma, infiltrating	±~+(*focal* 35%)	·	·
57	50	Ductal carcinoma, infiltrating	+,±(*diff* 80%)	·	·
58	64	Ductal carcinoma, infiltrating	±>+(*focal* 20%)	·	·
59	47	Mucinous carcinoma	±>+(*diffl* 100%)	·	·
60	37	Ductal carcinoma, infiltrating	±(40%)	·	·
61	53	Tubular carcinoma	±(25%)	·	·
62	50	Medullary carcinoma, infiltrating	−	·	·
63	55	Medullary carcinoma	−	·	·
64	53	Ductal carcinoma, infiltrating	−	·	·
65	59	Ductal carcinoma, infiltrating	−	·	·
66	39	Ductal carcinoma, infiltrating	−	·	·
67	49	Ductal carcinoma, infiltrating	−	·	·
68	49	Ductal carcinoma, infiltrating	−	·	·
69	44	Ductal carcinoma, infiltrating	−	·	·
70	43	Secretory carcinoma	−	·	·
71	31	Ductal carcinoma, infiltrating	−	·	·
72	49	Lobular carcinoma, infiltrating	−	·	·

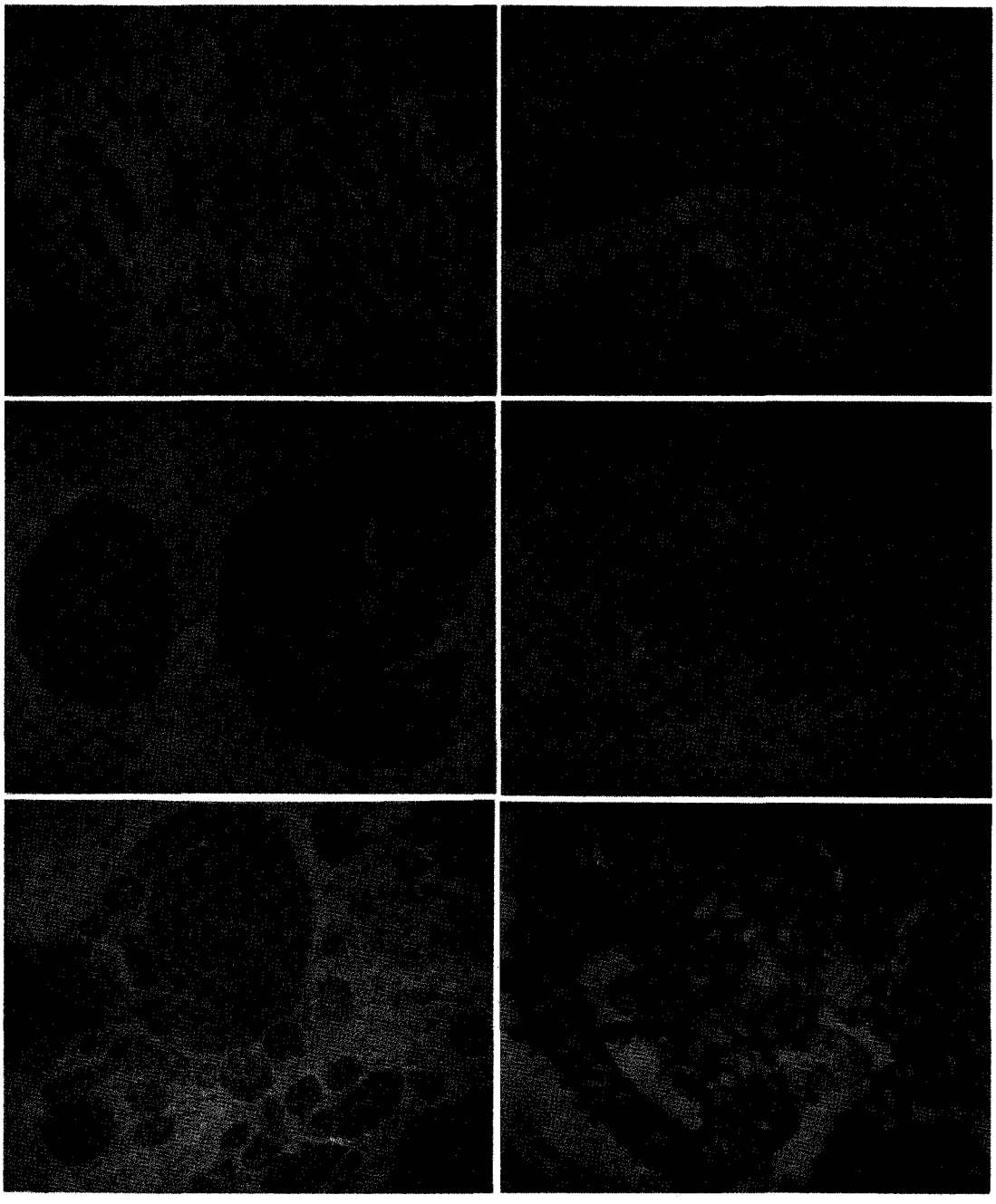
diff: diffuse
* Only infiltrating tumor cells
**>5fmol/mg: Positive

The results in paraffin sections were compared with biochemical assays with the dextran-coated charcoal technique in 21 cases or with immunocytochemical assay (ER-ICA, Abbott) on frozen sections in 30 cases. Ten of 72 cases were analysed simultaneously by three different techniques and 30 of 72 cases were studied retrospectively. There was a significant correlation between the results obtained with three the different methods; concordance of 76% with biochemical assay and 80% with ER-ICA. Their concordance are shown in tables 2 and 3, and figures 7~8. In paraffin sections, positive staining for ERs was restricted to the cytoplasm of carcinoma cells and normal/hyperplastic duct epithelial cells, such stained ER positive non-neoplastic epithelial cells served as an inbuilt control when present (Fig 1). The immunoreactivity of ERs was maintained well in paraffin blocks stored up to 2 years. The number of positively

stained neoplastic cells in the cases studied ranged from 0% (Fig 1) to nearly 100% (Fig 6), revealing focal (Fig 2,3), and/or partially diffuse (Fig 4,5), and diffuse staining in tumorous areas (Fig 6).

In paraffin sections that contained a relatively small percentage (10~20%) of stained ER-positive cells, the positive cells were often focally clustered in one area of the section, even though the tumor appeared histologically homogenous.

The ER-positive cells show considerable heterogeneity with respect to the intensity of cytoplasmic staining in individual cells within the same section, vary-



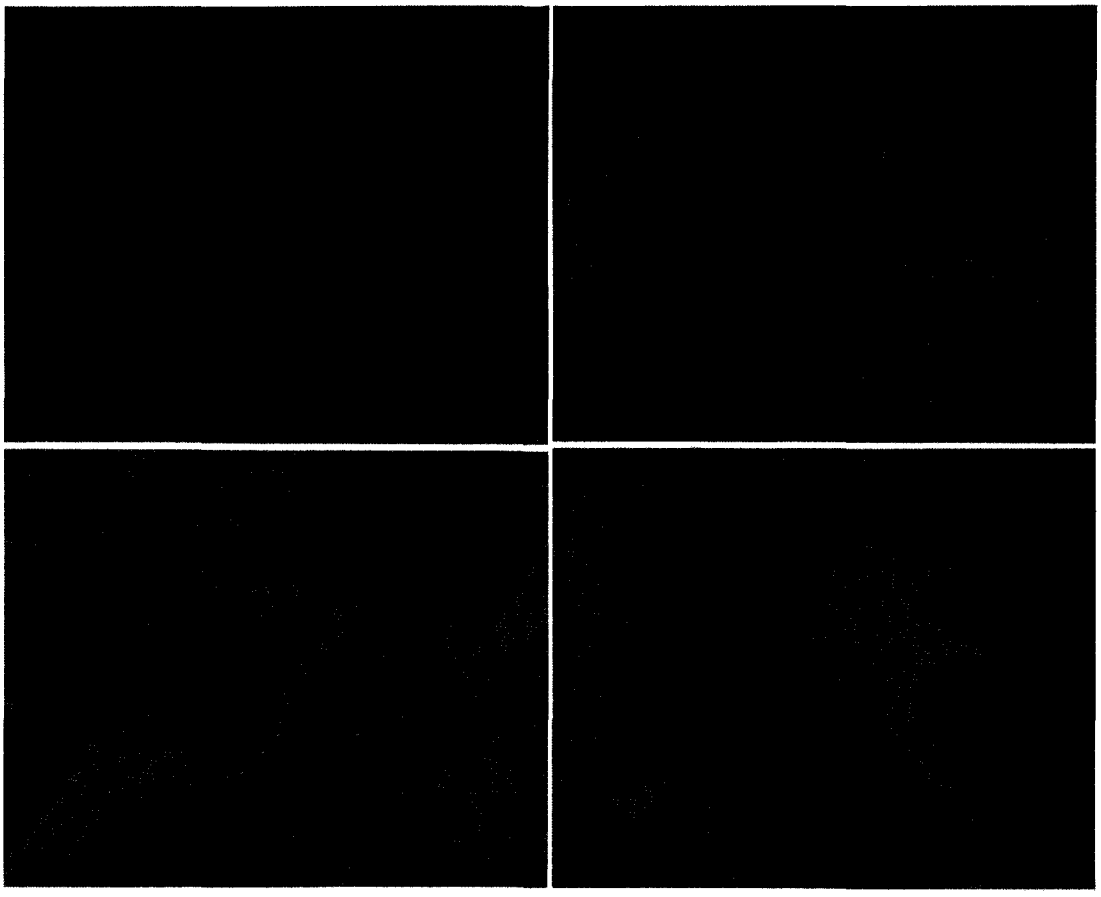


Fig 1. Infiltrating ductal carcinoma in 32-year-old woman (case 19). ER immunostaining on paraffin section shows ER-negative cancer cells but ER-positive nonneoplastic ductal epithelial cells serve as a control. Formalin-fixed paraffin section, hematoxylin counterstain. (x250)

Fig 2. Infiltrating ductal carcinoma in 39-year-old woman (case 12). ER immunostaining on paraffin section shows variable cytoplasmic staining of cancer cells ranging from 1+ to 2+ in different cancer cells. Formalin-fixed paraffin section, hematoxylin counterstain. (x250)

Fig 3. Mucinous carcinoma in 50-year-old woman (case 45). ER immunostaining on paraffin section shows variable cytoplasmic staining ranging from 1+ to 2+ in different cancer cells. Formalin-fixed paraffin section, hematoxylin counterstain. (x400)

Fig 4. Medullary carcinoma in 37-year-old woman (case 46). ER immunostaining on paraffin section shows moderate cytoplasmic staining of cancer cells. lymphocytic infiltration at the periphery. Formalin-fixed paraffin section, hematoxylin counterstain. (x250)

Fig 5. Infiltrating lobular carcinoma in 35-year-old woman (case 17). ER immunostaining on paraffin section shows 1+ to 2+ cytoplasmic staining. Formalin-fixed paraffin section, hematoxylin counterstain. (x63)

Fig 6. Infiltrating ductal carcinoma in 38-year-old woman (case 48). ER immunostaining on paraffin section shows strong positive staining masking of the cytoplasm with reddish brown coloration. Formalin-fixed paraffin section, hematoxylin counterstain. (x400)

Fig 7. Infiltrating ductal carcinoma in 43-year-old woman (case 11). (a) ER immunostaining cytochemical stanining on frozen section (ER-ICA) shows nuclear ER-positive staining in infiltrating cancer cells (x400). (b) ER immunostaining on paraffin section shows ER-positive cytoplasmic staining. Formalin-fixed paraffin section, hematoxylin counterstain. (x400)

Fig 8. Infiltrating carcinoma in 48-year-old woman (case 13). (a) ER immunostaining cytochemical on frozen section (ER-ICA) shows nuclear ER-positive staining. (x400) (b) ER immunostaining on paraffin section shows diffuse and moderate ER staining in cytoplasm. Formalin-fixed paraffin section, hematoxylin counterstain. (x63)

Table 2. Immunohistochemical ER Detection versus ER-ICA

	No. of Tumor	% of Total
ER_{P+} ER_{F+}	10/30	33.3 %
ER_{P-} ER_{F-}	14/30	46.6 %
Concordant	24/30	80.0 %
ER_{P+} ER_{F-}	4/30	13.3 %
ER_{P-} ER_{F+}	2/30	6.6 %
Disconcordant	6/30	20.0 %

Table 3. Immunohistochemical ER Detection versus ER-DCC

	No. of Tumor	% of Total
ER_{P+} ER_{C+}	9/21	42.8 %
ER_{P-} ER_{C-}	7/21	33.3 %
Concordant	16/21	76.1 %
ER_{P+} ER_{C-}	4/21	19.0 %
ER_{P-} ER_{C+}	1/21	4.7 %
Disconcordant	5/21	23.8 %

※ ER_P: ER-PAP(B-SA), ER_F: ER-ICA, ER_C: ER-DCC

ing from a faint(±) speckling of brown reaction product to a strongly positive (+3) (Fig 2~5) masking of the cytoplasm with reddish brown coloration (Fig 6).

One case (case 52) showed ER-positive infiltrating tumor cells but ER-negative in the primary main tumor cells. The false negative observed in two cases (cases 9 and 19) of paraffin sectios most likely reflect a loss of immunoreactivity resulting from tissue handling or fixation. The prevalence of ER positivity in histologic type of breast carcinoma is shown in table 4.

Histologically, the tumors were mainly infiltrating ductal carcinoma (50/72), of these 32(64 %) were ER-positive immunohistochemically, and the uncommon histologic type, mucinous carcinoma, infiltrating lobular carinoma, intraductal carcinoma and tubular carcinoma were characterized with a high prevalence for ER. In contrast, papillary carcinoma, secretory carcinoma, malignant cystosarcoma phylloides and medullary carcinoma were negative or rarely ER-positive(table 4). Poorly differentiated carcinomas were more likely receptor-negative than their well differentiated counter part.

Discussion

The immunohistochemical method for detection of estrogen receptors has long been sought and the recent introduction of a number of different methods for the detection of estrogen receptors in the paraffin embedded sections[11] using several different monoclonal antibodies such as D547Spγ, D5883μ, D75P3γ, H222Spγ and H226Spγ[12] have been studied. The frequency of receptor positivity in the literature va-

Table 4. Prevalance of Estrogen Receptor Positivity in Histologic Types of Breast Carcinoma

Histologic type of carcinoma	No. of Positive/Total ER-PAP(B-SA)	ER-ICA	ER-DCC
Ductal carcinoma, infiltrating	32/50(64%)	10/21(47.6%)	8/14(57.1%)
Lobular carcinoma, infiltrating	3/4	1/1	1/1
Mucinous carcinoma	2/3	0/1	
Intraductal carcinoma	3/5	1/2	0/3
Medullary carcinoma, infiltrating	1/6	0/2	1/2
Tubular carcinoma	1/1		
Cystosarcoma phylloides	0/1	0/1	0/1
Secretory carcinoma	0/1		
Papillary carcinoma	0/1		
Total	42/72(58.3%)	12/30(40.0%)	10/21(47.6%)

ries from 50% to 80%[13,14]. In our study the frequency of ER-positive tumors was 58% by paraffin section, 40% by frozen section and 47% by DCC assay respectively. These results are a little lower than the reported results because the population of our study were women predominantly in 3rd to 5th decades with a rather low mean age (45.7 years) than the postmenopausal group(50~79 years) in previously published report[14].

DeRosa et al[15] applied the D75 antibody and an avidin-biotin technique to paraffin-embedded tissue fixed in Bouin's solution or buffered formalin, each at various temperatures and for a varying length of time. Using these findings as a guide, we employed formalin fixation as usual. The reproducible technique was achieved with a Biotin-StreptAvidin system with a primary monoclonal antibody (ER-D5) and was performed on tissues fixed in neutral-buffered formalin and paraffin embedded. Thirty cases were simultaneously done by a paraffin section and frozen section methods (ER-ICA), one of which (3.3%) was ER-negative in the paraffin method and five of the 30 cases (16.6%) were negative in the frozen section while positive in the paraffin method. One of the 21 cases (4.7%) simultaneously done with the DCC method was negative in the paraffin section, and seven of the 21 cases (34.1%) were ER negative in DCC assay while positive in the paraffin method.

Our findings of ER positivity in paraffin sections appeared to be more sensitive than in other methods, revealing ER counts of 16.6% and 34.1% greater than in frozen section and DCC assay respectively. The overall concordance of the immunohistochemical method with the biochemical assay ranges from 65~93%[16-22] and 97%[11] and with the cytochemical method 94%[11], and 95% in published values[23]. In a recent study by Paterson et al[24] the result showed that a complete agreement between by DCC biochemical assay and paraffin section method treated with DNAse before applying a PAP method using ER-ICA monoclonal antibodies was seen in 85%. In another recent study by Cudahy et al[25], comparison of the biochemical assay with a polyclonal anti-E_2 assay (immunohistochemical technique using formalin-fixed tissue) showed a concordance 63%, and with an ER-ICA on frozen section showed a concordance 86%. The fomer value (63%) is lower than the 79% agreement rate achieved by Pascal et al[26].

Our data in this study indicate a significant correlation between the immunohistochemical method and the quantitative biochemical assay, a concordance of 76%, and an 80% concordance between the immunohistochemical method and ER-ICA applying an avidin-biotin peroxidase system. It appears that both nuclear and cytoplasmic ER are relevant to a tumor cell's hormonal receptor status[23]. However, elegant immunocytochemical specificity studies performed by King and Greene[12] indicate that the staining seen with ER-ICA is specific for type I ER and is not due to cross-reactivity with other cellular components.

In our study, the ER-D5 monoclonal antibody (antibody subclass IgG_1) which raised against an affinity-purified cytosolic estradiol receptor from human myometrium gives only cytoplasmic staining[27] of human breast tumors[28,29] as compared to a nuclear locus of an estradiol binding unit[12].

ERs have been subdivided into high-affinity, type I, and lower-affinity (type II and III). It is now a generally known fact that there are multiple classes of steroid binding sites, which are supported by recent studies[30-32]. Many workers believe that the new procedure were probably identify the lower affinity/type II estrogen binding proteins rater than the high-affinity/ type I customerily measured biochemically [33].

Heterogeneity of staining features was seen in different parts of the tumor within the section, its causes have been presumably explained in that a tumor may harbor different clones each with characteristic ER content, and expression of the ER protein may depend on the stage of the cell such as a different ER expression throught the cell cycle in MCF-7 human breast cancer cells[34]. Molecular heterogeneity of an ER with respect to size and surface ionic charge has been demonstrated in cytosol from human breast carcinomas[35]. Jakesz et al[36] found an inverse relation between ER levels and the proliferative rate of MCF-

7 cells. Proliferating cells slowly accumulated higher concentrations of ER due to increased synthesis or reduced degradation, or both. High proliferative activity as defined by a high proliferative index are related to ER negativity[37], in contrast well differentiated carcinomas were more frequently receptor positive than poorly differentiated tumors[38].

Ours is the first study to compare the accuracy between immunohistochemical assay with ER-D5 monoclonal antibody in paraffin sections and immunocytochemical assay performed on frozen section (ER-ICA) and/or biochemical assays with the dextran-coated charcoal technique to my knowledge.

The immunohistochemical method is relatively rapid and utilizes formalin-fixed paraffin sections of the same tissue that is used for standard histologic diagnosis. The excellent morphology achieved permits an assessment of the staining of individual cells in relation to the usual histologic criteria employed in diagnosis of breast tumor and it stands in contrast to the DCC method that takes no account of variations in receptor expression by tumor cells.

This immunohistochemical method opens up a range of possibilities such as the use of the wealth of samples stored in the pathology department for retrospective studies, determination of the ER content in tumors on which the DCC assay or the ER-ICA was not performed, the correlation of the ER content with histopathological features, the ER analysis on very small tissue samples[39] and it has the additional advantage of costing considerably less than any other method.

In conclusion, ER analysis is now an integral part of the assessment of breast cancer. The results in our study on conventionally formalin-fixed, paraffin embedded breast cancer tissue seem comparable to those achieved by assay using frozen tissue sections (ER-ICA), and are highly correlated with those obtained by the conventional biochemical DCC assay.

Summary

An immunohistochemical technique for demonstrating estrogen receptor (ER) protein in paraffin-embedded sections of formalin-fixed breast tumor is described. The purpose of our study was to establish an easy and rapid technique for ER immunostaining that can be carried out in any surgical pathology laboratory. Suitable histologic materials of seventy two cases of breast cancer were stained with an ER-D5 monoclonal antibody, using an amplified Biotin-StreptAvidin method, and 58% of cases were ER-positive tumors.

ER content in paraffin sections were partly compared simultaneously with those on frogen section (ER-ICA method) in 30 cases and with those on cytalolic values determined by a dextran-coated charcoal method in 21 cases.

The percentage of positively stained cancer cells in paraffin sections ranged from 0 to nearly 100 percent with staining intensities ranging from 1+ to 3+, rarely±. The estrogen receptor immunostaining on paraffin sections was found to be in concordance with that on frozen section (ER-ICA) and with the biochemical assay (dextran-coated charcoal) in 80% and 76% of cases respectively. The staining on paraffin sections showed more ER positive tumors than either frozen section staining or cytosolic assay. In this study 31 of 72 cases were studied retrospectively. ER detection from the paraffin embedded breast cancer provides certain advantages over the conventional ER-ICA method or DCC method, especially for determination of ER status in patient in which only formalin fixed, paraffin embedded tissue is available.

Acknowledgements

The authors wish to thank to Professor Tae Ho Chung, head of the Institute of Basic Medical Science, School of Medicine, Kyungpook National University, for providing the biochemical cytosol assay, Mr. Sung Gene Choi for skillful technical assistance and Miss Mi Ae Kim for typing the manuscript.

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