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Comparison of the Immunocytochemical Assay (ER-ICA) and the Biochemical Assay for Estrogen Receptor in Human Breast Cancer Cell Lines

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Abstract—*Estrogen receptor content of 8 cell lines from non-malignant human breast tissues was determined by an immunocytochemical assay using the Abbott ER-ICA monoclonal kit. The results were in accordance with those obtained by the conventional radiochemical (DCC) assay. Primary cultures of breast tissues are suggested as an important field for* in situ *application of the ER-ICA.*

INTRODUCTION

A LARGE number of studies on estrogen receptors (ER) in human breast cancer tissue based on a biochemical assay have been published in recent years (for review see [1]). An important limitation of the biochemical estrogen receptor assay is that it does not allow assessment of the possible heterogeneity of ER in a tissue [2] and the application of cytochemical methods have been suggested [3]. However, low sensitivity and specificity have limited the use of cytochemical methods based on immunofluorescence or radiolabelling of estrogens [4]. With the introduction on monoclonal antibodies to the estrogen receptor protein [5], an immunocytochemical assay (ER-ICA) was developed for the detection of ER in frozen sections of tissues [6]. Excellent agreement was found between this assay and the biochemical assay [7].

This work presents an application of the ER-ICA on a number of tissue culture cell lines from non-malignant and malignant human breast tissue. By a semiquantitative evaluation of the ER-ICA we have compared the results with the conventional biochemical DCC assay.

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MATERIAL AND METHODS

Breast cancer tissues

Frozen sections of 20 breast cancer tissues were studied using the ER-ICA method as described by others [6] in order to ensure that the method was working in our hands. In parallel, the biochemical (DCC) method [8] for ER determination was applied to all tissues.

Tissue culture cell lines

Eight cell lines from non-malignant and malignant human breast tissues were included in this study. MCF-7 cells [9], BT-20 cells [10], ZR-75-1 cells [11], T-47D cells [12], Cama-1 cells [13] and the tamoxifen-resistant subline of MCF-7, the AL-1 line [14] are derived from breast cancer tissues; HMT-3522 [15] is an epithelial cell line from fibrocystic disease of the breast, and HBL-100 [16] is derived from cells of human milk. BT-20, ZR-75-1, and T-47D were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.); MCF-7 and HBL-100 were kindly supplied by the Breast Cancer Task Force Cell Culture Bank, Mason Research Institute (Worcester, MA, U.S.A.); Cama-1 was obtained from J. Fogh, the Sloan-Kettering Institute (New York, MA, U.S.A.); AL-1 and HMT-3522 are developed in our laboratory. The following growth media were used: MEM + non-essential amino acids + 15% fetal bovine serum (FBS) (Cama-1) or 10% FBS (BT-20) or 5% FBS + 6 ng/ml insulin (MCF-7); RPMI 1640 + 10% FBS (ZR-75-1); RPMI 1640 + 2% FBS + 6 ng/ml insulin (T47D); McCoy'5a medium + 10% FBS

(HBL-100); DME/F12 + insulin 6 ng/ml + 0.5% FBS (AL-1); DME/F12 + insulin 250 ng/ml + transferrin 10 μg/ml + epidermal growth factor 10 ng/ml + Na-selenite 10^{-8} M + estradiol 10^{-10} M + hydrocortisone 0.5 μg/ml + prolactin 5 μg/ml without serum (HMT-3522). All media were supplemented with glutamine 2×10^{-3} M.

Preparation of cells

Cells from a confluent monolayer washed twice with phosphate-buffered saline (PBS) were scraped from the plastic by the use of a rubber policeman, resuspended in PBS and centrifuged at 280 ***g*** for 10 min. Smears were made from the cell pellet and fixed in formaldehyde 3.7% for 10 min, washed in PBS for 5 min, and further fixed in cold methanol (−20° C) for 3–5 min and cold acetone (− 20° C) for 3 min and washed twice in PBS for 5 min.

Some of the cell lines were also assayed *in situ* on the monolayer without removing the cells. However, the staining was weaker and more variable than on smears of cells. Therefore, we have only included results on cell smears.

The ER analysis

ICA. The ER-ICA is carried out as described previously [6] using the Abbott ER-ICA Monoclonal kit (Abbott Diagnostics, Wiesbaden, West Germany). A semiquantitative evaluation was attempted by scoring the staining intensity of the positive cells as weak (+), moderate (++) and high (+++) at high power light microscopy. For each preparation, 10 randomly chosen fields were evaluated corresponding to a total number of 300–600 cells.

Biochemical assay. Near confluent cultures were harvested for receptor determination. The preparation of the cytosol has been described previously [17]. The dextran–charcoal technique [8] was used for determinations of free estrogen receptors.

RESULTS

Eighteen out of 20 human breast cancer tissues gave similar results with the ER-ICA and the DCC assay, i.e. 11 were positive and 7 were negative with both methods. Two tissues with positive cells in the ER-ICA (> 1% stained cells) contained less than 10 fmol/mg protein of ER in the DCC assay.

Since the ER-ICA method seemed to work in our hands we applied it then to cell lines both on monolayer cultures *in situ* and on cell smears prepared from cultured cells. Using the fixation method as recommended in the ABBOTT ER-ICA Monoclonal kit cell smears gave a stronger and more reproducible staining. In all positive cell lines the staining was localized to the nucleus. As also observed in the tissue sections, the variation in staining ranged from negative to strong positive staining in the same cell line (Fig. 1). By a semiquantitative evaluation of the ER-ICA all positive cell lines showed the highest percentage of cells in the weak positive (+) group (Table 1). Some variation in staining within the same preparation was found as determined by the standard deviation of the percentage of positive cells in 10 fields. Four of the cell lines were ER-positive and 4 were ER-negative with both methods. When the mean percentage of positive cells of each of the 4 positive cell lines was compared to the ER content as determined by the biochemical method, a good correlation between the 2 methods was found (correlation coefficient = +0.92).

DISCUSSION

An excellent correlation between the ICA and the biochemical assay method for the determination of ER in human breast cancer tissues has been demonstrated by others [7]. A similar good correlation was found by us in 20 samples of human breast cancer tissues confirming that the method was working in our hands. The presence of ER in 4 human breast cancer cell lines as determined by the DCC assay could also be demonstrated by the ICA and 4 cell lines without demonstrable ER by the DCC were negative using the ICA. As previously described with MCF-7 cells [6], the staining was localized over the nucleus in all cell lines. This is in accordance with the recent concept that both free ('cytosolic') and bound ('nuclear') estrogen receptor are confined to the nucleus in intact cells [6, 18, 19]. It is not possible from the present study to conclude whether the ER-ICA detects bound and/or free receptors. However, by an ER enzyme immunoassay (ER-EIA) it has been observed that the same monoclonal antibody as we have used, binds to both free and nuclear-bound receptor [20].

The main advantage of the ER-ICA over the DCC assay is the demonstration of heterogeneity in the cell population. Although cell lines are expected to comprise a homogeneous cell population due to a constant selection pressure over long periods of time, the ER-ICA has shown that all of the investigated ER-positive cell lines were heterogeneous with respect to ER content. Whether this heterogeneity is due to variations in ER content through the cell cycle or differences in permeabilization of the cellular membranes by the fixative is not clear.

This study was carried out on smears of cells. However, preliminary studies have shown that the ER-ICA may be applied *in situ* to monolayer cultures although the choice of fixative seems to be more critical. The outspread monolayer cells may be more exposed to loss of free ER during the fixation procedure.

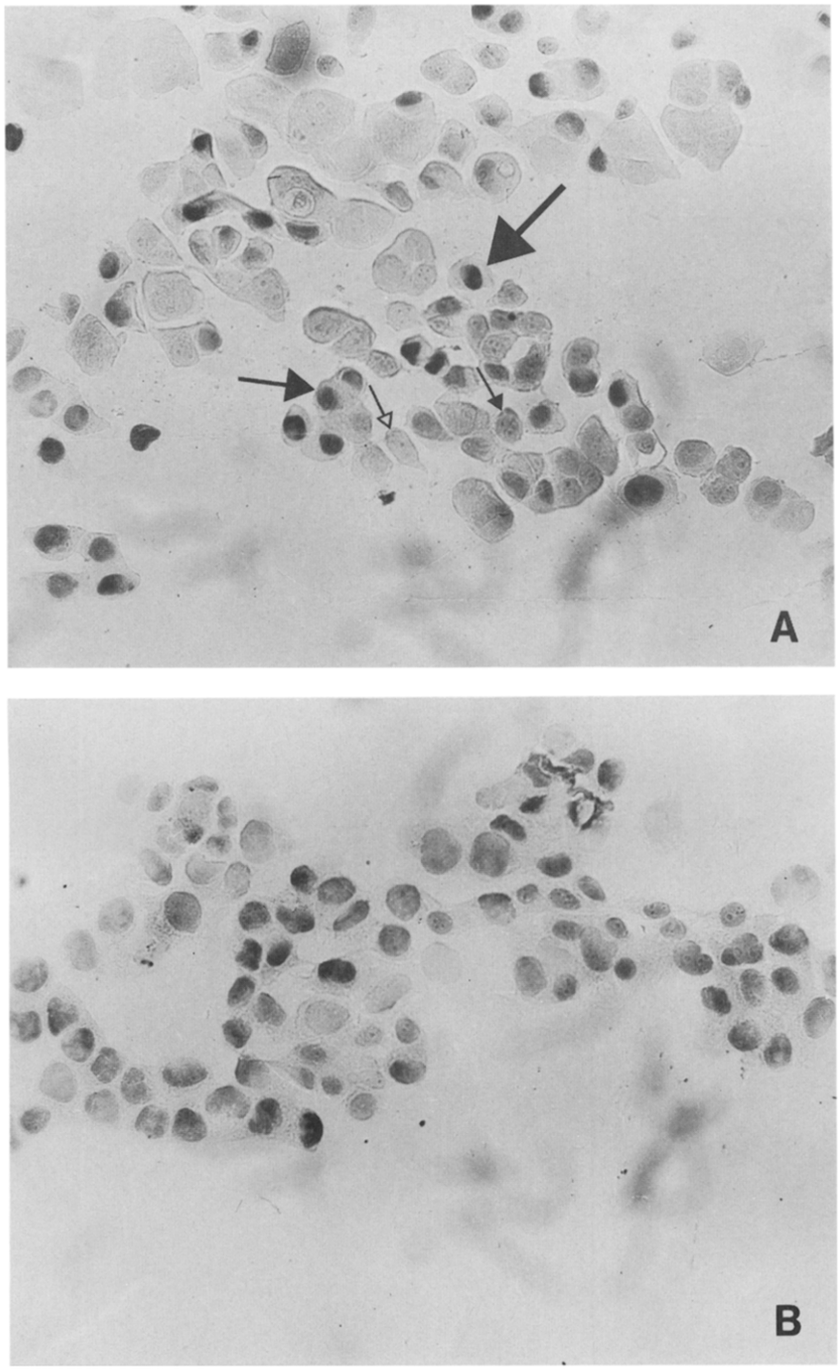

Fig. 1. *Immunocytochemical localization of estrogen receptors in a human breast cancer cell lines. A. MCF-7; B. T-47D. Staining was evaluated semiquantitatively as* +++ (*large arrow*), ++ (*medium size arrow*), + (*small black arrow*), *and negative* (*small white arrow*), *see Fig.* 1*A.*

Table 1. Estrogen receptor (ER) in 8 human breast cell lines as determined by the ER-ICA and the DCC methods

Cell line	ER-ICA					DCC (free ER)
	−	+	++	+++	%pos (S.D.)	fmol/mg protein
MCF-7	217	163	113	54	60 (12)	61
	286	168	66	19	47 (12)	
T-47D	82	211	39	2	75 (7)	143
	171	240	27	2	61 (9)	
AL-1	124	143	29	0	54 (21)	70
	125	106	32	0	52 (11)	
ZR-75-1	355	48	31	43	26 (16)	20
	342	52	33	34	26 (15)	
Cama-1					negative	< 10
BT-20					negative	< 10
HMT-3522					negative	< 10
HBL-100					negative	< 10

An important field for *in situ* application of the ER-ICA is primary cultures of breast tumors in which a variety of non-malignant and malignant cell types may be represented. Only the ER-ICA and not the biochemical assays will be suited for evaluating the ER content of the various cell types. Comparison of results of primary cultures and frozen sections of the tumor tissue will be valuable.

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