Report

Relationship between ER-ICA and conventional steroid receptor assays in human breast cancer

G. Di Fronzo¹, C. Clemente², V. Cappelletti¹, P. Miodini¹, D. Coradini¹, E. Ronchi¹, S. Andreola² & F. Rilke³

¹Divisione di Oncologia Sperimentale C, and ²Divisione di Anatomia Patologica e Citologia, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy

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Abstract

We applied a new immunocytochemical assay for estrogen receptors (ER-ICA) to 82 human breast tumors. Results were correlated with cytosolic estrogen receptors (ERc) and nuclear ER (ERn) determined on the same sample respectively by the radioligand binding assay and by an ER enzyme immunoassay (ER-EIA). All ER-ICA-positive tumors contained more than 10 fmol/mg of protein of ERc and were therefore considered as ERc positive. In contrast, 15.4% of ERc-positive cases were ER-ICA negative. Comparison of ER-ICA results with ERn showed extensive agreement of negativity (92%), whereas 38% of ER-ICA-positive tumors were ER-EIA negative. However, the latter had ERc levels above the positivity threshold. Quantitative features of the immunocytochemical staining such as intensity and percentage of labelled cells, considered separately, did not reflect the amount of ERc or ERn. Cellularity was not significantly correlated with ER-ICA and biochemical results.

Introduction

Determination of estrogen and progesterone receptors (ER and PgR) is now a common tool for prediction of response to endocrine therapy (1, 2) and for prognostic evaluation of breast cancer patients (3–7). In fact, much data is available on the clinical role of ER, but it appears that up to 40% of women with an ER-positive breast cancer do not respond to hormone therapy (8).

On the assumption that PgR synthesis in breast cancer cells is under estrogenic control, it has been suggested that the simultaneous determination of ER and PgR should increase the accuracy of patient selection for hormone therapy (9–14). Also, the role of the debated nuclear ER (ERn) should be investigated (15). In fact, the two-step model for estrogenic action has been recently re-examined (16, 17). It was originally thought that the interaction of the hormone with cytoplasmic ER (ERc) was followed by a temperature-dependent transformation, forming a complex which translocated into the nucleus and determined the specific estrogenic effects (18). However, recent studies employing anti-ER monoclonal antibodies and cytochalasin-induced enucleation have suggested that ER always resides in the nucleus of intact cells, with release into the cytosol during homogenization (19).

The results of the evaluation of steroid receptors are critically influenced by the method employed. Until recently, steroid receptors were determined

Address for offprints: Dr. G. Di Fronzo, Istituto Nazionale Tumori, Via G. Venezian 1, 20133, Milano, Italy.
only with radioligand binding methods, which are expensive and time-consuming and have the drawback of using critical high temperature exchange assays. The development of a solid-phase enzyme immunoassay (EIA) made possible the detection of ER by immunologic methods independently of binding activity (20).

It has not yet been determined whether the ER level of a tumor biopsy reflects the heterogeneity of the cell population. A semiquantitative immuno cytotoxic assay on histologic sections for ER (ER-ICA) has been recently developed (21–24) and may clarify the behaviour of breast carcinomas with heterogeneous ER contents. It therefore seemed worthwhile to evaluate the new ER-ICA method in comparison with established radioligand assays whose clinical significance is already known.

Material and methods

Eighty-two primary breast cancer patients who underwent surgery at this institute between January and May 1985 were investigated. In the pathology laboratory samples were taken from the excised breast specimen within 15–30 min of removal. Care was taken to avoid fatty tissue and necrotic areas of the tumors and to select samples which were grossly as uniform as possible. Moreover, sections contiguous to those used for ER-ICA were examined through staining with hematoxylin and eosin and showed that the benign epithelial portion associated with the carcinoma, whenever present, was minimal. It was therefore not considered in the subsequent evaluations.

Reagents

16α-(125I)-iodoestradiol (200 Ci/mmol) in ethanol solution and 16α-ethyl-21-hydroxy-19-nor-(6,7-3H) pregn-4-ene-3,20-dione (Org 2058, 40–60 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, UK). Radioinert 17β-estradiol and Org 2058, Norit A charcoal, gelatin, and dextran T70 were purchased from Sigma Chemical Co. (St. Louis, MO). ER-ICA and ER-EIA monoclonal kits were partly provided by Abbott Laboratories Diagnostic Division (Chicago, IL). All other chemicals were analytical grade.

ERc and PgR biochemical assay

Samples for biochemical assays were immediately sent to the laboratory and stored in liquid nitrogen until assayed. ERc and PgRc were determined simultaneously by saturation analysis on the same samples by a dual label assay as previously described (25). Correction for nonspecific binding was performed on a duplicate set of tubes containing 100-fold molar excesses of radioinert 17β-estradiol and Org 2058. Incubations were carried out overnight at 0–4°C and were stopped by the addition of a suitable volume of dextran-coated charcoal (DCC) suspension (0.5% w/v Norit A, 0.05% w/v dextran T70, and 0.1% w/v gelatin in 20 mM K2HPO4, pH 7.4 at 4°C, 1 mM EDTA, and 3 mM NaN3 buffer). After 15 min the charcoal was separated by centrifugation at 2,000 × g for 10 min. The clear supernatant was counted for separate determination of bound radioactivity due to iodinated and tritiated ligands. Contribution of 125I was checked on a Multiprias 4 (United Technologies Packard, Downers Grove, IL) gamma counter whose counting efficiency (82%) was established by the formula of Eldridge and Growther (26). Thereafter, the same samples with added scintillation cocktail (Picofluor TM-15, United Technologies Packard) were recounted in a Packard 300D beta counter in the tritium region and with automatic quench compensation made on each sample to allow detection of both 3H and 125I. The appropriate 3H/125I standard efficiency curves and the 125I dpm values were used to calculate 3H dpm values. Total and nonspecific binding data were analyzed according to Scatchard (27) and expressed as femtomoles of specifically bound ligands per milligram of cytosol protein. Total protein content was measured using Coomassie blue G-250 dye (Bio-Rad, Munich, West Germany) essentially according to Bradford (28), with a mixture of human albumin and human gamma-globulin (5:3) for the protein standard curve.