Eur J Cancer Clin Oncol, Vol. 24, No. 12, pp. 1885–1889, 1988.
Printed in Great Britain

0277-5379/88$3.00 + 0.00

Estrogen Receptor Distribution in the Peripheral, Intermediate and Central Regions of Breast Cancers

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Abstract—*The central, intermediate and peripheral regions of 25 breast tumors were analyzed for estrogen receptors (ER) using a combined biochemical (BC)/immunohistochemical (IHC) micromethod. To optimize the regional comparison, the percentage carcinoma per sample (PCS) was evaluated and incorporated into the quantification of the ER.*

Correction of the measured ER for the PCS eliminated differences in receptor levels between the central and peripheral regions but not the intermediate region. Although the corrected BC-ER level in this region was found to be about 20% higher, the IHC method did not detect such a difference. Determination of the ER status at the actual growth front of a tumor, rather than in the intermediate region where ER levels appear to be highest, may be of greater clinical relevance. Intra-regional ER heterogeneity appears, however, to be particularly pronounced in this area of a tumor.

INTRODUCTION

WITHIN solid breast tumors a focal growth pattern should theoretically result in an expanding globular tumor mass that radiates from a central point. Although there are many restrictions to such a growth pattern *in vivo*, e.g. blood supply, bone proximity, etc., it may be possible to identify concentric regions such as: a peripheral region, where rapid cell multiplication takes place; an intermediate region, where the rate of cell multiplication has decreased due to crowding; and a central region, where cell maturation and necrosis may be present. Since the estrogen receptor (ER) is an important growth regulatory protein in some breast cancers, it is possible that these various regions may be associated with specific receptor levels and distribution patterns. In a preceding publication [1] we demonstrated the occurrence of intratumor ER heterogeneity by sampling at random from multiple unspecified regions of a breast tumor. In this study, sampling procedures were modified in an attempt to correlate ER levels and distribution patterns with specific regions of a tumor. There are at least three pertinent questions: (1) which region has the highest receptor level? (2) are the receptors all in the same form within the different regions? (3) can heterogeneity be detected within individual regions?

A number of studies examining differences between receptor levels in the peripheral and central regions of breast tumors have produced conflicting results. ER levels have been reported to be highest in the peripheral region [2] and in the central region [3]. Due to large variations in the actual tumor cell content between regions, particularly the peripheral and central regions, it is extremely difficult to make meaningful comparisons unless corrections for such differences in cellularity are incorporated into the quantification of the ER.

We have minimized this problem using a biochemical (BC)/immunohistochemical (IHC) microsample technique [1, 4, 5] that corrects the ER levels for differences in the viable tumor cell content between regions.

In this study multiple samples were obtained from each of 25 breast tumors in cross section (peripheral, intermediate, central, intermediate and

Accepted 14 July 1988.
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Supported by a grant from the Medical Research Council of Canada.

peripheral). Each sample was analyzed for estrogen receptor level and distribution pattern by the BC/IHC method.

MATERIALS AND METHODS

Surgical breast cancer specimens were obtained immediately after excision and stored at −70°C. Some tumors were removed intact and five microsamples from a cross section could be obtained as shown in Fig. 1. In other instances, where biopsies of the tumor passed through the center, representative samples from all five regions were not available. With small tumors strict adherence to the size of microsamples (13 × 1.5 × 1.5 mm) was not possible and samples of different dimensions, but of comparable volume, were taken. While frozen, all microsamples were divided longitudinally into 'sisterhalves'. As described previously, one half was biochemically analyzed for ER using the micromethod [1, 6]; the other half was analyzed for the percentage carcinoma per sample (PCS), and also for IHC determination of ER. In instances where the PCS was ≤5% the samples were considered unsuitable for analysis and were excluded.

Biochemical ER analysis

The BC-ER method has been described elsewhere in detail [1]. Briefly, each sample to be analyzed for ER was minced and homogenized in 500 µl of TEDM buffer [tris(hydroxymethyl)-methylamine]1,4-dithiothreitol, ethylene-diaminetetraacetate, sodium molybdate (10/0.5/1.0/10 mmol/l, pH 7.5). The resulting homogenate was centrifuged at 50,000 ***g*** for 0.5h at 4°C. Two 50 µl aliquots of each sample supernatant were incubated for 0.5 h with tritiated estradiol (3×10^{-10} M). One tube was warmed to 56°C for 30 min prior to incubation to serve as a control. Each aliquot was then electrophoresed using the CAGE method [6]. One hundred microliters of each supernatant was used for protein determination according to the method of Lowry *et al.* [7].

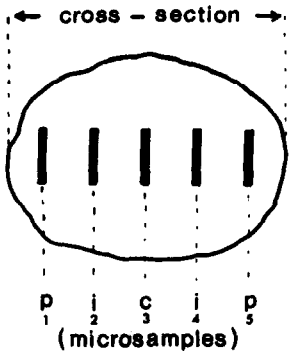

Fig. 1. Diagrammatic illustration of sampling procedure from peripheral (p), intermediate (i) and central (c) regions of breast tumors.

The correction of measured ER values on the basis of PCS was performed according to the equation:

$$\text{Corrected ER} = \text{Measured ER} \times 100/\text{PCS}.$$

The PCS was estimated by four observers (including a patholgoist) working independently. When disagreement occurred between observers, slides were reevaluated and PCS values were assigned by mutual agreement.

As in a previous publication [1], the BC-ER cutoff point for moderate vs. high receptor level was arbitrarily set a 100 fmol/mg of tumor protein. Microsamples with measured ER levels <3 were not used in this study.

Immunohistochemical analysis of ER

The frozen 'sisterhalf' of each biochemical portion was cut on a cryostat into 6 µm sections for the estimation of the PCS and for the IHC-ER determination. The IHC-ER procedure used was as described in the Abbott ER-ICA monoclonal antibody kit (Abbott Laboratories International, North Chicago, IL 60065).

The IHC-ER staining was evaluated by three observers working independently using a double grading system in which the percentage of carcinoma cells as well as the intensity of specific staining for ER was assigned a value. For the percentage of staining 1 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, 10 = 91–100%. For the intensity of staining the numbers 0, 1, 2, 3 were used to define absent, low, moderate and high staining, respectively. For each microsample, the value assigned for staining intensity was added to the range value assigned for the percentage of specific staining to generate a 'Histoscore' [1]. A histoscore from 1 to 8 was considered moderate, and ≥9 was considered high for ER. Microsamples with <5% specific staining were not used in this study.

RESULTS

Of the 25 breast cancers assayed, 22 were classified as infiltrating ductal carcinomas, two as infiltrating lobular carcinomas (AM, RK, Fig. 2), and one as a medullary carcinoma (GS, Fig. 2). For the BC analysis, a total of 107 sisterhalves were obtained from these tumors: 23 were taken from the central region; 42 from both the peripheral and intermediate regions. For the IHC analysis, a total of 103 sisterhalves were obtained from these tumors: 21 were taken from the central region; 41 from both the peripheral and intermediate regions. Figure 2 shows that in all but two cases (CM, AM) the methods correlated with respect to the presence or absence of receptors. The overall quantitative

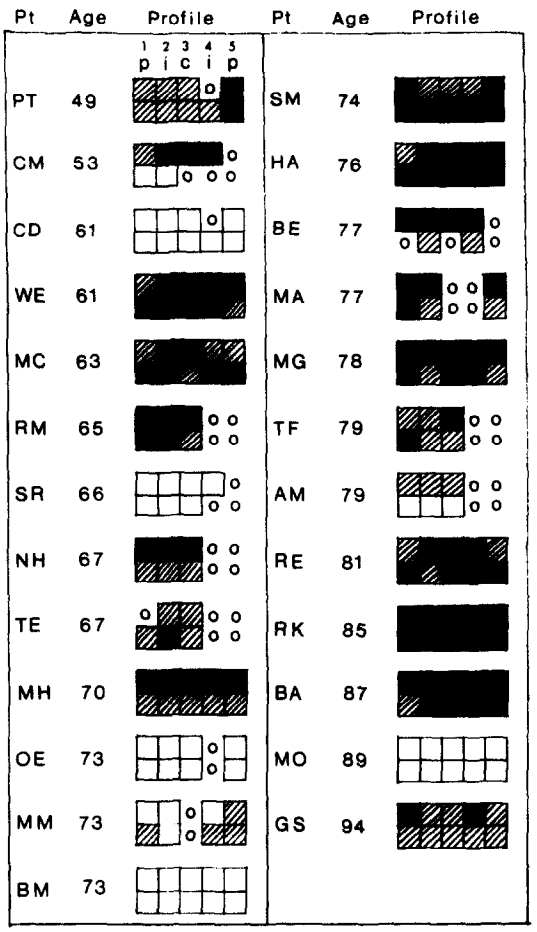

Fig. 2. Diagrammatic representation of ER levels in the peripheral (p_1), intermediate (i_2), central (c_3), intermediate (i_4) and peripheral (p_5) regions of 25 breast tumors. For each tumor the profile illustrates regional variation in ER level and distribution by the BC (top row) and IHC (bottom row) methods using sisterhalves of microsamples. ■ High level of receptors; shaded: intermediate level of receptors; □ absence of receptors; ○ sample was not analyzed.

correlation coefficient (r) between the two methods was 0.46.

Table 1 indicates that the lowest measured BC-ER was observed in the peripheral region. Correction of the measured BC-ER for percentage carcinoma eliminated differences in receptor levels between the peripheral and central regions but not from the intermediate region where it was found to be approx. 20% higher. On the other hand, the average 'Histoscore' varied by less than 10% between all regions.

Table 2. Number of breast tumor microsamples categorized as having a 'high' ER level as measured by HC and IHC methods

Region	BC*	IHC†
Central	11	7
Intermediate	22	16
Peripheral	18	18

*≥100 fmol/mg protein.
†Histoscore values ≥9.

Using the three categories of receptor level previously established [1]—absent, moderate, high—Fig. 2 illustrates that ER assessment by both methods placed sisterhalves in the same category in more than 50% of the cases. Table 2 indicates that more microsamples were categorized as having 'high' ER content by the BC compared to the IHC method. Such differences were observed in the central and intermediate regions, but not in the periphery where the methods were in agreement.

The intra-regional ER heterogeneity was evaluated by calculating the ratio of percentage ER positive cells in corresponding peripheral and intermediate regions (i.e. p_1/p_5 and i_2/i_4, Fig. 1). Table 3 shows that of the 13 tumors assessed, values >1.50 were observed in the peripheral regions of six tumors and in the intermediate region of only one tumor.

Table 4 indicates that the mean corrected ER level in large tumors is about 40% higher than that of smaller tumors, irrespective of region. For small as well as large tumors the intermediate regions show the highest mean corrected ER levels.

DISCUSSION

Agreement between the BC and IHC methods, with respect to the presence or absence of ER, occured in 23 of the 25 cases. Using the corrected ER level and the 'Histoscore' the quantitative correlation (r) was 0.46, which is considerably lower than the correlation coefficient noted in a previous publication ($r = 0.75$) [1]. Other discrepancies between current and previous studies were also observed. In a previous study, the cut-off points between the moderate and high ER ranges were arbitrarily selected so that an equal number of tumors would be categorized in the high range by

Table 1. Mean carcinoma content and receptor level (fmoles/mg) *in the central, intermediate and peripheral regions of breast tumors using a BC/IHC method. Numbers in parentheses are number of microsamples*

Region	% carcinoma	ER measured	ER corrected	ER histoscore
Central	39 (23)	46 (23)	141 (23)	6.1 (21)
Intermediate	38 (44)	48 (42)	173 (42)	6.2 (41)
Peripheral	35 (45)	37 (42)	142 (42)	6.6 (41)

Table 3. Ratio of percentage specific staining in corresponding peripheral and intermediate regions of breast tumors

Patient	Peripheral (p_1/p_5)	Intermediate (i_2/i_4)
PT	1.16	1.02
WE	1.45	1.29
MC	1.05	1.05
MH	9.40	1.16
MM	5.30	—
SM	1.16	1.07
HA	1.08	1.01
MA	1.75	—
MG	1.55	1.35
RE	1.14	1.58
RK	1.33	1.12
BA	3.26	1.13
GS	2.54	1.25

Table 4. Mean corrected BC-ER levels (fmoles/mg) *in breast tumors categorized by size. Numbers in parentheses are number of microsamples*

Region	Tumor size ≤2 cm	>2 cm
Central	106 (10)	175 (11)
Intermediate	133 (19)	204 (19)
Peripheral	88 (17)	169 (21)

both methods [1]. In this study, using the same cut-off points, more tumors were categorized as high by the BC method as opposed to the IHC method. This inconsistency was observed in samples taken from the central and intermediate regions, but not in those taken from the peripheral regions where the methods were in agreement (Table 2). Additionally, the number of samples in the 'BC-ER-positive/IHC-ER-negative' class was increased over that observed previously. These differences may be explained, in part, by the fact that sodium molybdate (10 mM) was added to the homogenization buffer used in this, but not in previous studies.

We do not know what may be responsible for this apparent differential effect of sodium molybdate in the three regions. It has been suggested [8, 9] that the addition of sodium molybdate to breast tumor homogenates can result in an increased ER concentration by stabilizing a specific form of the receptor [8–12]. Our data may indicate that sodium molybdate stabilizes a form of the receptor which binds the ligand (estradiol) but is not readily recognized by the antibody used in the IHC-ER analysis. The possibility that not all forms of the ER system are equally well recognized by the antibodies has been proposed by Raam [13].

Silfversward *et al.* [2] found higher cytoplasmic ER levels in the peripheral area of breast tumors when compared with the central area, and suggested that differences in receptor turnover may be responsible for this observation. Davies *et al.* [3] reported a higher ER content in the central area of 22 of 24 breast tumors and suggested tumor size might partly explain this discrepancy. Our data (Table 1) indicate that correction of measured ER for variations in the PCS eliminated differences between the central and peripheral regions and this may explain these discrepancies. Irrespective of tumor size (Table 4), the highest BC-ER levels were generally found in the intermediate region. However, the assessment of ER levels in this region may not determine the receptor status of cells that are actually infiltrating into the normal tissue. Since the ER is a growth regulatory protein in some tumors, determination of receptor levels in cells at the actual growth front of a tumor may be more relevant. However, the extreme heterogeneity within this area itself poses an enigma (Fig. 2, Table 3) [14]. It is possible that variations in the ER level, ER distribution, and ER form or subunit composition could be associated with differential growth rates or stages of maturation in breast tumor cells.

Preliminary evidence from short term tissue culture of human breast cancers indicates that rapid disappearance of ER positive cells can occur when tumor cells are removed from the host environment [van Netten, unpublished observations]. Local variations in the microenvironment of the invaded normal tissues could be responsible for the observed ER heterogeneity in the peripheral region of tumors. The possibility that ER expression may be influenced by manipulation of the local environment could have important implications for the growth potential of human breast cancer.

Acknowledgements—We thank Mike MacDonald, A.R.T.; Marleen Purdy, B.Sc., R.T.; and Barbara Miyasaki, R.T. for their expert technical assistance.

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