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Intratumoral Variation of Cytoplasmic and Nuclear Estrogen Receptor Concentrations in Human Mammary Carcinoma*

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Abstract—*This study demonstrates that the cellular content of the cytoplasmic specific estradiol receptor varies in mammary carcinomas in a reproducible way; the central parts of the tumor contained the lowest and the peripheral parts the highest level of the receptor. These intratumoral differences in receptor content, which varied between 3- and 20-fold, were found in tumors from both pre- and postmenopausal women. In contrast to the cytoplasmic receptor the intranuclear receptor level was highest in the central parts of mammary carcinomas. The finding of large intratumoral variations in receptor content strongly suggest that tumor samples for receptor analysis should be collected in a standardized way.*

INTRODUCTION

PATIENTS whose mammary carcinomas contain estrogen receptor (ER) are likely to respond to endocrine therapy [1, 2]. The total amount of receptor may however vary within three orders of magnitude and there is an incomplete correlation between absolute receptor content and likelihood of response [2, 3]. Moreover, there are several well documented cases of patients who have responded to endocrine therapy in spite of the fact that their tumors have been 'receptor-negative' [1]. The finding of responders in the receptor-negative group has so far only been explained for women with high levels of serum estradiol. In this case it is assumed that endogenous estradiol blocks the receptor *in vivo* making it undetectable by the techniques routinely used [4]. This explanation is obviously not sufficient for most postmenopausal women. An alternative explanation which has been considered by some authors is that the cellular receptor content may vary in different parts of the tumor. However, so far only relatively minor intratumoral variations were detected [5, 6].

The aim of the present study was to investigate the cytoplasmic and nuclear estradiol receptor content in different parts of human mammary carcinomas. We report that the receptor content varies widely in different parts of a tumor. Consequently we recommend a standardized tissue sampling technique to reduce this major source of error in estradiol receptor measurements.

MATERIALS AND METHODS

Patients and tumors

Twenty-one tumors were obtained as mastectomy specimens. No preoperative radiation, chemotherapy or endocrine therapy had been given. For technical reasons only roughly spheroidal tumors with a diameter of more than 1.5 cm were used. Both pre- and postmenopausal women were included in the study. The histological classification was previously described]7]. All but one of the tumors were ductal carcinomas of different degrees of differentiation. The one remaining tumor was a pure colloid (mucoid) carcinoma.

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Measurements of specific estradiol receptor

After mastectomy, the tissue was transported on crushed ice to the pathology department. All tumors were excised and prepared for analysis within 0.5 hr after operation. An 0.5 cm thick disc-formed slice was obtained from the tumor by two parallel cuts close to 'equator' of the tumor. The slice was frozen on dry ice and prepared in either of two ways (Fig. 1); (a) the slice was divided into four identical quadrants. One quadrant was further subdivided into three parts by cuts perpendicular to the radius of the quadrant; (b) consecutive concentric ringformed sections were manually punched out using a cork-borer. The frozen samples were either analyzed directly or stored at −70°C for up to 3 weeks before analysis. Pilot experiments had shown that freezing on dry ice and storage at −70°C did not change the estradiol binding. The specific estradiol receptor content was determined as previously described using a ^{3}H-estradiol concentration of 5 nM [8]. All receptor values are expressed as fmole/μg DNA. DNA was determined by the method of Burton [9]. The intranuclear receptor content was measured as described by Garola and McGuire [10].

Chemicals

^{3}H-estradiol was obtained from New England Nuclear, Polyacrylamide gel plates were purchased from LKB, Sweden.

RESULTS

Cytoplasmic estradiol receptor values in replicate samples

As detailed in Methods, a rounded disc was cut out from 12 tumors. These discs were further divided into four quadrants (Fig. 1). Three of the four quadrants were without further subdivisions subjected to parallel ER determinations. The results depicted in Table 1 demonstrate that the maximal difference between quadrants from the same tumor was 3-fold (case 10) and that this maximal difference was approximately 2-fold for several other tumors (cases 1, 4, 7, 8, 11 and 12).

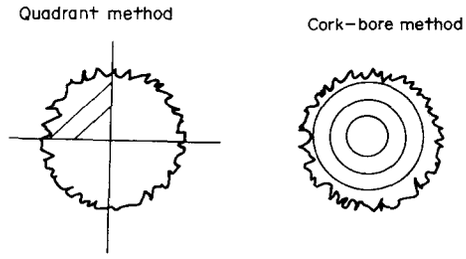

Fig. 1. A schematic illustration of the two methods used for subdividing tumor slices.

Table 1. Cytoplasmic ER-values in different quadrants from square slices of breast cancer

Case No.	ER-value in fmole/μg DNA Quadrant No. 1	2	3
1	0.98	1.17	0.68
2	0.41	0.49	0.34
3	0.92	0.94	0.70
4	2.61	3.66	1.84
5	0	0.02	0.01
6	0	0.01	0.03
7	4.24	2.87	6.48
8	1.61	1.19	3.34
9	3.05	1.50	2.57
10	1.80	0.57	0.84
11	1.81	1.95	3.01
12	1.37	1.39	1.38

Case No. 7 = colloid carcinoma, all others = ductal carcinomas. Case Nos. 2 and 5 = premenopausal, all others = postmenopausal.

Cytoplasmic estradiol receptor values in central and peripheral parts of the tumor

One of the quadrants from the tumor disc described above was further subdivided so that a central portion, an intermediate portion and a peripheral portion of the tumor was obtained (Fig. 1). Estrogen receptor assay on these three portions indicated a decreased value in the central part of the tumor in seven out of ten receptor-containing tumors (Table 2). Furthermore, the maximal difference between the central and peripheral parts of the tumors was much wider (e.g., case 1) than the difference between the replicate parts described above. We therefore decided to perform a more detailed study of the intratumoral variation of the nuclear and cytoplasmic ER-content.

Cytoplasmic and nuclear receptor levels in different parts of tumors from postmenopausal women

Cork-borers were used to obtain consecutive ringformed sections from six tumors as described in Methods (Fig. 1). Figures 2 and 3 show the cytoplasmic estradiol receptor values from the centre to the periphery. The centre contained only low or unmeasurable receptor values. This value then increased towards the periphery and was maximal in

Table 2. Cytoplasmic ER-values in central, intermediate and peripheral zones of quadrants from square slices of breast cancer

Case No.	ER-value in fmole/μg DNA		
	Zone		
	Central	Intermediate	Peripheral
1	0	0.45	0.90
2	0.10	0.16	0.20
3	0.08	0.33	0.22
4	1.17	1.94	1.47
5	0.03	0.01	0.03
6	0	0	0.01
7	4.28	3.26	6.30
8	0.43	1.55	0.63
9	1.03	2.28	2.23
10	1.00	0.29	1.29
11	0.50	2.79	2.57
12	2.63	2.66	1.08

Case No. 7 = colloid carcinoma, all others = ductal carcinomas. Case Nos. 2 and 5 = premenopausal, all others = postmenopausal.

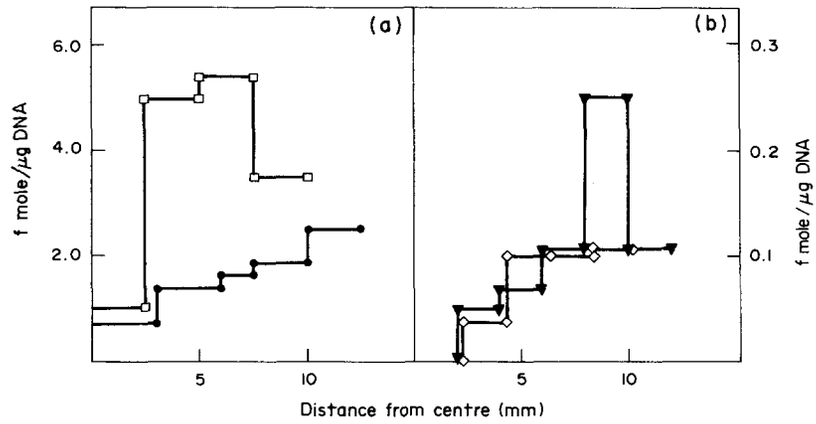

Fig. 2. The cytoplasmic estradiol receptor level in mammary carcinomas from postmenopausal women. The cellular content of cytoplasmic receptor (expressed as fmole/μg *DNA) was determined in different parts of tumors with a high (Fig. 2a) and low (Fig. 2b) total receptor content, respectively. Concentric tumor specimens were prepared as described in Materials and Methods. The distance from the tumor centre (orig.) is given in mm. The size of each segment is given by the horizontal bars which represent the distance between the inner and outer radius of the respective segment.*

one of the most peripheral parts. It is noteworthy that four tumours which had no, or extremely low levels of receptor in the centre all had values of more than 0.1 fmole/μg DNA in specimens taken from the periphery. It is thus clear from these experiments that the amount of ER in the tumor sometimes may depend on where the specimens are taken.

When estradiol is bound to the cytoplasmic receptor the complex is translocated to the nucleus [10, 11]. Measurements of the cytoplasmic receptor will accordingly give only incomplete information about the cellular receptor content. We therefore investigated both the intranuclear and the cytoplasmic estradiol receptor in different parts of two tumors from postmenopausal women. The data in Fig. 3

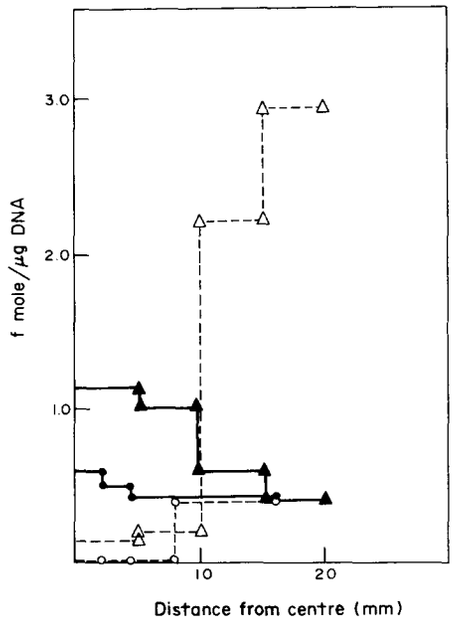

Fig. 3. The intranuclear and cytoplasmic receptor content in mammary carcinomas from postmenopausal women. The intranuclear (whole lines) and cytoplasmic (dotted lines) receptor levels were determined as outlined in Materials and Methods. The respective tumor is represented by a filled and its corresponding unfilled symbol.

demonstrate that with these two tumors the cytoplasmic and nuclear receptors did not vary in parallel. In contrast to our findings with the cytoplasmic receptor, the highest values for the intranuclear receptor were observed in the central parts of the tumors, where the values were approximately two times higher than in the periphery. It is clear from Fig. 3 that in the central parts of the tumors the amount of intranuclear receptor was several times higher than the amount of cytoplasmic receptor.

Cytoplasmic and nuclear receptor levels in different parts of tumors from premenopausal women

Figure 4 illustrates the intranuclear and cytoplasmic receptor levels observed in different parts of tumors from premenopausal women. The data show that also with these two tumors from premenopausal women higher levels of intranuclear receptor were observed in the central parts as compared to the periphery. The opposite was true for the cytoplasmic receptor of three tumors. With the two tumors analyzed for both types of receptor, there was a major difference in pattern as compared to the previously described tumors from postmenopausal women. With the tumors from the premenopausal women the total amount of receptor was much lower in the cytoplasm as compared to the nucleus indicating an efficient *in vivo* translocation of the receptor to the nucleus. One patient had no detectable cytoplasmic receptor and very low values for the intranuclear receptor.

Lack of correlation between intratumoral estrogen receptor variation and morphological parameters

With six of the tumors described above we attempted to correlate the intratumoral receptor variations to morphological parameters. Therefore, microscopic slides were prepared from parts of the sectors used for receptor analysis from 3 pre- and 3 post-

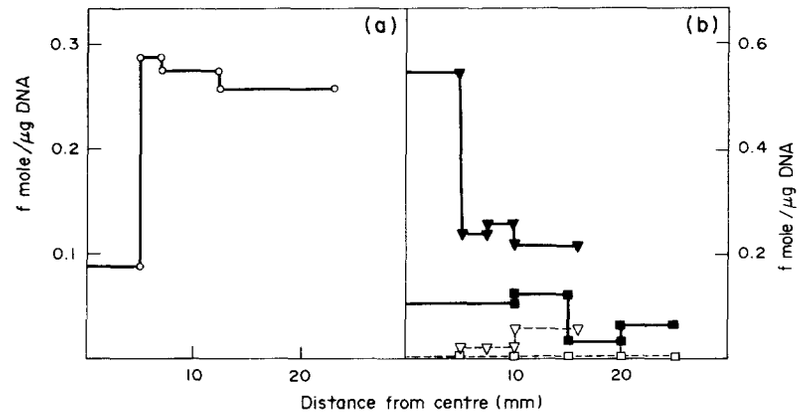

Fig. 4. The cytoplasmic and intranuclear receptor levels in mammary carcinomas from premenopausal women. The variations in cytoplasmic estradiol receptor was determined in one tumor (Fig. 4a). The intranuclear (whole lines) and cytoplasmic (dotted lines) receptor levels were measured in two tumors from premenopausal women (Fig. 4b). The respective tumor is represented by a filled and its corresponding unfilled symbol.

menopausal women respectively. With these tumors we did not detect necrotic parts but generally there was an increased sclerosis in the central parts of the tumors. None of the tumors contained significant lymphocyte infiltration. Thus our findings cannot be explained on the basis of cell death, lymphocyte infiltration or admixture of varying amounts of normal mammary tissues.

DISCUSSION

The content of cytoplasmic estradiol receptor in mammary carcinomas varies over three orders of magnitude (0.01–10.00 fmole/μg DNA) [2, 3]. Tumors with a receptor value below 0.1 fmole/μg DNA are often referred to as receptor negative. Patients with receptor negative tumors seldom respond to endocrine therapy [1]. In contrast patients whose tumors are receptor positive (above 0.1 fmole/μg DNA) often benefit from endocrine therapy. It should be pointed out that there are several weaknesses in such a routine classification of tumors. First tumors from premenopausal women are often receptor negative due to the high level of endogenous estradiol which blocks the receptor and consequently makes it unmeasurable. Many such patients show, however, a well documented response to endocrine therapy [1]. Secondly, little attention has been paid to intratumoral variations in receptor content. Samples taken at random from one tumor often show a 2–3-fold variation in estradiol receptor level [5, 6]. In the present paper we want to further focus the attention on the intratumoral receptor variation and stress the importance of using a standardized sampling technique. Quadrants taken from one (and the same tumor) slice show approximately a 2-fold difference in receptor level. Such a variation appears to be of importance only in the case of tumors with a receptor value close to the limit value between the positive and negative group. Obviously in such a case the classification will be adequate only if the whole tumor is analyzed. In contrast to this moderate variation in receptor content between replicate samples there is a large difference in receptor level in samples taken from the central and peripheral parts of the same tumor. In several cases we were unable to detect a specific estradiol receptor in the central parts of the tumor while samples derived from the periphery yielded significant receptor values. Obviously if a routine sample would have been taken from the centre only, these tumors would be placed in the receptor negative group. Their eventual response to endocrine therapy would then have been unpredicted. It is possible that these findings can explain why some of the postmenopausal patients with tumors recorded as receptor negative respond to endocrine therapy. What is the explanation for these intratumoral variations in receptor content?

It is tempting to speculate that our results are related to morphological parameters such as varying amounts of tumor cells and lymphocytes in the centre and periphery of the tumor. Microscopic examination showed that the tumor centre in general was more sclerotic than the peripheral parts. Such a sclerosis will, however, not directly influence our data since the receptor values are expressed on a DNA basis. In so doing we avoid the ambiguity in normalizing the receptor values to tumor wet weight or protein content parameters which are highly dependent both on sclerosis and admixture of serum. On the other hand if the tumors were infiltrated with large amounts of lymphocytes a falsely low receptor value will be recorded when based on DNA content. A significant infiltration of lymphocytes was however not a regular finding in the tumors studied. In addition, when present, the lymphocytes were mostly localized in the periphery of the tumor, thus leading to underestimation rather than exaggeration of the differences in receptor level found between the tumor centre and the periphery. It is well known that the blood supply is poor in the centre of a tumor [12]. This results in a low metabolic activity and eventually in cell death. We found that the cytoplasmic receptor content was low while the nuclear receptor levels were high in the tumor centre. Cells in the tumor centre had thus been able to extract plasma estradiol and transfer the receptor–hormone complex to the cell nucleus. This finding excludes the possibility that with the tumors analysed the cells in the centre were metabolically inactive or even dying. One may speculate that a decreased metabolic activity in the central parts might have resulted in a slow turnover of the receptor–hormone complex, leading to liberation of only small amounts of measurable free receptor. This would then be in contrast with the peripheral parts of the tumor where the better blood supply would result in a more rapid turnover of the receptor–hormone complex and thus in a higher level of free receptor. One possibility is that the tumor cells in the centre and periphery have a different genetic make-up. This possibility is

however made unlikely by experiments of Auer *et al.* (personal communication) which show that cells from metastasis and the original tumor have the same DNA profile, suggesting a monoclonal origin of the tumor cells.

We think that our results stress the importance of using a standardized method for selecting tumor tissue for receptor analysis. Only in so doing can the receptor value be correlated to results of endocrine therapy, chemotherapy or the prognosis in a valid way.

How should tumor specimens be selected for receptor analysis? We recommend that a slice with the diameter of the tumor should be cut out and, if the tumor is small, the whole slice should be used for receptor determination. In the case of larger tumors the slice can be divided into quadrants, of which at least one should be further analyzed. In this context we would like to point out that the ER-analysis based on needle aspirates, that we recently developed, partly avoids the problem of intratumoral variation of receptor level. The aspiration procedure collects an almost pure tumor population from different parts of the tumor [13, 14].

Finally it is essential to determine the intranuclear receptor level in all tumors. This statement is based on our finding that the intranuclear and cytoplasmic receptor do not vary in parallel. In fact we have analyzed several tumors from both pre- and postmenopausal women, which have a nonmeasurable level of cytoplasmic receptor and a high content of intranuclear receptor. It is possible that the balance between these two receptor fractions can reflect the susceptibility of a tumor to endocrine therapy in a better way than measurements of only the cytoplasmic receptor.

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