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Heterogeneity of Soluble and Nuclear Oestrogen Receptor Status of Involved Nodes in Relation to Primary Breast Cancer

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Abstract—*Both soluble and nuclear oestrogen receptors were measured in at least two different portions of primary breast cancer and in concurrent metastatic tissue from axillary nodes. Oestrogen receptor (ER) status of involved nodes was found highly consistent with that of primary tumours. Of the 67 patients studied, 30 had metastatic nodes which contained both soluble and nuclear ER. Of these, 27 were associated with a primary cancer which also had both soluble and nuclear ER, determined in at least two separate parts of the primary cancer. Conversely, none of the completely negative primaries gave rise to fully receptor positive metastatic tissue.*

Surprisingly, 17 out of 20 heterogeneous primary tumours, i.e. those containing both receptor positive and negative components, generated receptor negative metastatic nodes. Moreover, in 7 of the 8 patients with N-2 stage nodal involvement, the metastatic disease had arisen from primaries which were either completely receptor negative or with a heterogeneous ER status. It is suggested that macroscopic heterogeneity of ER status in primary breast cancer is associated with poor prognosis.

INTRODUCTION

BREAST cancers can be divided into those which are clearly aggressive and those which grow more slowly and result in significantly longer survival [1]. Two indices which are used to discriminate these two types of disease are (a) nodal involvement, and (b) steroid receptor status. However, neither discriminant, on is own, has been completely reliable. In the latter case, the presence of soluble oestrogen receptor (ER) in a biopsy of the primary disease was thought to be an index of good prognosis for prolonged survival [2, 3]. However, the survival benefit of soluble ER is not confirmed by all groups [4, 5]. When a measure of physiological action of the receptor is included, such as the presence of either nuclear receptor [6] or progesterone receptor [7], the difference in disease-free interval or total survival time is greater, but again not all studies confirm this [8]. The following study was undertaken to try to improve our ability to recognise aggressive disease and, perhaps, explain some of the discrepancies among previous studies.

It is well recognised [9] that metastatic spread of disease to regional lymph nodes is more common in patients with ER negative primaries than in those with ER positive primaries. Assays of ER status in histologically involved nodes might, therefore, be considered more useful than those in primary disease, particularly when the amount of biopsy tissue from the primary may be limiting. Further, the cellularity of a nodal biopsy is usually higher than that in a primary [10].

Nevertheless, if adequate tissue for receptor assays can be obtained from both primary and nodal sites, then the results have the potential to indicate which tumours might escape hormonal growth control. This assumes that, in a primary tumour containing both receptor positive and negative cells, the receptor negative cells are the more aggressive and so more likely to metastasise to the axillary nodes. Thus a change in receptor status from positive to negative between the primary and nodal sites would be expected to influence the prognosis of the patients and, possibly, the selection of therapy.

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Little work has been published comparing the receptor status of the primary disease with that of the lymph nodes in the same patient. In one paper by Corradini *et al.* [11], receptor negative primaries were always associated with receptor negative metastatic nodes, whereas about a quarter of receptor positive primaries gave rise to receptor negative nodes. In this study Corradini *et al.* measured only the receptor status of the soluble fraction from a single biopsy of the primary disease. However, it is now recognised that tumour heterogeneity can lead to changes in receptor status between different parts of a single primary [12–14]. For this reason, the present study determined the ER status in at least two different portions of the primary and in the nodes. We also measured both soluble and nuclear receptor in each sample, since we have shown that the combination is a better index of hormone dependence than is soluble receptor alone [6]. The main objective of our study was to determine whether tumours of variable receptor status, i.e. those containing both receptor positive and negative components, always generate receptor negative metastatic nodes, as would be expected if receptor negative cells are, indeed, the more aggressive. This class of tumours, showing variable receptor status across the primary (as determined by biochemical assay), constitutes 25–30% of all breast and endometrial cancers [12, 15].

PATIENTS AND METHODS

Patients (67 in all) attended the breast cancer clinic of M. Ascoli Cancer Institute and Department of Surgery, Palermo University—Policlinico. Patients were selected because, at the time of presentation, they had involved axillary nodes but no other overt metastases. Both N-1 ($n = 59$) and N-2 ($n = 8$) patients were included. The age range of the patient was 29–71 years. Of these, 28 were premenopausal and 39 postmenopausal. All patients underwent modified radical mastectomy coupled with node sampling. The mean number of nodes sampled was 12.4 and mean number of involved nodes was 6.7. The proportion of nodes investigated that were found to contain malignant cells was similar in all three categories of Hormone Sensitivity [(++) = 54%, (+−) = 56% and (−−) = 63%]. These three categories are defined later. Of the eight patients with N-2 nodes, four had (−−) primaries, three (+−) and only one a (++) primary.

Biopsy samples were divided and parallel sections taken for pathological examination and oestrogen receptor assay. For all receptor assays reported here, adequate quantities of histologically malignant cells were seen in the parallel section. For the primary tumour, separate samples were taken from the growing edge of the tumour and the older, more central part. Samples were transported on ice to the receptor laboratory and stored as previously described until assay [16]. The oestrogen receptor assay for each sample measured the receptor content of both the soluble and nuclear fraction of each biopsy using an 8-point assay with [3H]-oestradiol in the range $1–10 \times 10(-10)$M. Diethylstilbestrol at 100-fold concentration was used to determine non-specific binding. The assay has been described in detail elsewhere [17] and can be carried out satisfactorily on 50 mg tissue. This standard DCC (Dextran-coated charcoal) assay was adopted for this study, in preference to either the HPLC or antibody assay, for the following reasons: (a) comparability with existing data; (b) investigation of the significance of the macroscopical heterogeneity of receptor distribution—microscopic cellular heterogeneity is seen in many tumours on histological examination and is confirmed by staining with antibodies against oestrogen receptor. However, the clinical significance of macroscopic and microscopic heterogeneity of receptor distribution may well be different.

DNA content was determined by a modification of the Burton method [18] and protein content by the standard Lowry method.

In the majority of cases, only one involved node (confirmed by histology) was assayed for ER status. However, in seven cases, receptor status was determined in at least three nodes. Receptor status of all nodes from any one patient was always consistent.

Definition of HS status

We have shown previously [6, 25] that, for a single biopsy of tumour, presence of soluble and nuclear receptor is a much better index of hormone dependence, than is soluble receptor alone. This discriminant is even better (data unpublished) if both soluble and nuclear receptor can be shown

Table 1. *Menopausal status of patients in relation to hormone sensitive status*

	HS status		
	(++)	(+−)	(−−)
PreM ($n = 28$)	10	9	9
PostM ($n = 39$)	19	11	9

HS status is defined in the Methods section and differentiated heterogeneous receptor distribution (+−) from uniformly receptor negative (−−) or receptor positive (++) primary tumours. Any biopsy is only defined as receptor positive if both soluble and nuclear receptor are present.

Table 2. Breast tumour size in relation to hormone sensitive status

	HS status (++)	(+−)	(−−)
T1–T2 (n = 40)	19	12	9
T3–T4 (n = 27)	10	8	9

Patient distribution in each tumour size was T1 = 12, T2 = 28, T3 = 11 and T4 = 16.

Table 3. Oestrogen receptor status of metastatic nodes in relation to the hormone sensitive status of the primary tumour

	HS status (++)	(+−)	(−−)
No of patients	29	20	18
ER+ Nodes	27	3	0
ER− Nodes	2	17	18

Nodes were classified as receptor positive only if both soluble and nuclear fractions contained ER.

to be present in two different parts of tumour. We have, therefore, defined three categories of Hormone-Sensitivity (HS status). Primary tumours which had both soluble and nuclear ER in both central and peripheral biopsies are defined as (++). If either soluble or nuclear receptor was missing from either biopsy, the tumour was classified as (+−) and, when no receptor was detected in any of the four fractions, given that adequate tumour cells were present, then the tumour was (−−).

RESULTS

The patients were first subdivided according to menopausal status. The results are shown in Table 1. Stage of disease in relation to HS classification of receptor content is shown in Table 2. Table 2 shows that over one-third of the patients selected for this study had a large primary disease. This figure is typical of that seen in Sicilian breast cancer clinics [15]. Receptor status of the metastatic nodes, in relation to HS classification of the receptor content of the primary tumour is shown in Table 3. Only 2 of 29 patients with (++) primary disease had receptor negative nodes, whereas 17 of 20 patients in the (+−) group had receptor negative nodes.

Mean values of receptor content for the receptor positive biopsies are shown in Table 4 for the

Table 4. Concentration of soluble and nuclear oestrogen receptors in metastatic nodes (M), and in central (C) and peripheral (P) portions of those primary tumours which were (++) HS status

Biopsy site	C (n = 28)	P (n = 28)	M (n = 23)
ERs fmol/mg pr.	70.1 ± 49.8	74.1 ± 47.3	70.4 ± 49.5
ERs fmol/μg DNA	1.75 ± 0.98	2.46 ± 1.77	1.41 ± 0.93
ERn fmol/μg DNA	1.51 ± 0.93	1.75 ± 1.12	0.78 ± 0.57
ER(s+n) fmol/μg DNA	3.26 ± 1.59	4.19 ± 2.26	2.18 ± 1.33

Receptor concentrations are shown for the soluble fraction alone (ERs) and for the combination (ER(s+n)).

Table 5. Concentration of soluble and nuclear oestrogen receptors in metastatic nodes (M), and in the receptor positive central (C) and peripheral (P) portions of those primary tumours which were (+−) HS status (i.e. did not have both soluble and nuclear receptor in both central and peripheral biopsies)

Biopsy site	C (n = 10)	P (n = 10)	M (n = 3)
ERs fmol/mg pr.	40.9 ± 21.2	34.9 ± 24.1	53.8 ± 4.6
ERs fmol/μg DNA	1.45 ± 0.98	1.48 ± 1.90	1.29 ± 1.03
ERn fmol/μg DNA	1.44 ± 1.21	1.85 ± 2.07	0.62 ± 0.40
ER(s+n) fmol/μg DNA	2.89 ± 2.10	3.32 ± 3.55	1.91 ± 1.43

Receptor concentrations are shown for the soluble fraction alone (ERs) and for the combination (ER(s+n)). The receptor negative biopsies were excluded from the calculations of the mean values shown.

HS (++) group, i.e. those patients whose primary tumour had both soluble and nuclear receptor in both central and peripheral parts of the tumour. The concentration of the soluble receptor, expressed per unit protein, is very similar in primary disease and in the nodes. However, total receptor content, expressed per unit DNA, is slightly (but not significantly) lower in the nodes. It is worth noting that only two biopsies out of 29 from either the central or the peripheral portions fell below 0.5 fmol/μg DNA (cut-off 0.25 fmol/μg DNA) whereas 11 out of 27 values for the receptor positive nodes were in the range 0.25–0.5. Significantly, the DNA content of the nodal biopsies was higher than that of the primaries (232 ± 135 compared with 118 ± 55 for the peripheral and 131 ± 39 for the central primary biopsies). This

reflects, in part, the higher cellularity of the nodes, together with possible increased lymphocytic infiltration. DNA content is generally a better reference value for receptor concentration than is protein content since the receptor concentrations relative to DNA fit a Gaussian distribution much more closely [12] than do those relative to protein and so are more suitable for use in receptor comparative studies.

The receptor content for the ER positive biopsies in the (+−) group are shown in Table 5. The level of receptor measured in the receptor positive components is significantly less ($P < 0.05$), expressed by unit protein than that in the (++) group where, of course, all biopsies have both soluble and nuclear receptor. However, this difference is not so marked when the receptor content is expressed per unit DNA.

DISCUSSION

The heterogeneous nature of many breast cancers is well established [19–22]. It is recognised [23] that this heterogeneity can lead to changes in oestrogen receptor status across a single primary tumour. That this macroscopic heterogeneity of receptor status can be observed by the relatively crude DCC assay method [12] is surprising (though microscopic heterogeneity is confirmed using antibodies to ER [22]). It suggests that, in some primaries, separate colonies of the multifocal disease, some ER positive and some ER negative, have been growing for a considerable time with little coalescence [21, 23]. If it is true that receptor negative disease is more aggressive [24], then one might expect that the receptor status of involved nodes would only be positive in patients in whose primary disease the vast majority of cells were receptor positive. This is supported by the observations reported here. Of 20 patients with (+−) primary tumours, i.e. those whose primary tumour showed loss of soluble and/or nuclear receptor from one or other part of tumour, 17 (85%) had receptor negative metastatic nodes. In contrast, only 2 out of 29 (7%) with uniformly receptor-rich primary disease (++) developed receptor negative metastatic nodes. Other studies (to be published) show that, in breast cancer, biopsies of the periphery of the primary disease are less likely to contain both soluble and nuclear receptor than are biopsies of the central tumour. However, this difference in the receptor status of periphery from the central portion of the tumour is much less marked than that shown here between primary tumours and nodes.

These data suggest that the (++) group should have a much better prognosis than either the (+−) or the (−−) HS group. Consistent with the aggressive nature of the receptor negative cells is the observation that of eight patients with N-2 nodes, three had (+−) primaries and four had (−−) primaries but only one had a (++) primary. Further, the data on the small number of patients, who have reached 5-year follow-up, are also consistent with this hypothesis. Predictably, there is a poor overall survival for the (−−) group (all three dead within 48 months). Additionally, in the (+−) group two out of three patients died within 48 months, whereas the (++) HS group survive intact at 5 years. Assay for receptor content in a single, small biopsy of tumour may not differentiate the (++) group from the (+−) group. This, perhaps, explains why some groups did not find the improved survival predicted for receptor positive tumours.

The increasing availability of antibodies to ER will make the detection of heterogeneous (in terms of ER status) tumours more sensitive. However, preliminary work with antibody staining of fine needle aspirates does indicate that microscopic heterogeneity of receptor status may be observed in most, if not all, breast cancers. Therefore, until more data have accumulated, detection of macroscopic heterogeneity of receptor distribution may be of more clinical value. The evidence presented in this paper would suggest that a patient with a primary breast tumour, which has heterogeneous distribution of receptor, should be treated as having receptor negative disease if any subsequent recurrence cannot readily be biopsied and assayed directly for ER status.

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