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THE EFFECTS OF THERAPY ON ESTROGEN RECEPTORS IN BREAST CANCER

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Summary—The importance of estrogen receptors (ER) in predicting the results of therapy in advanced-stage breast carcinoma is now generally accepted. It is, therefore, important to know whether therapy itself, besides other factors, could affect ER status. The aim of the authors was to investigate this problem by reviewing the data from the literature. They have taken into account the effects of hormonal and/or chemotherapy and of radiotherapy, moreover, they have considered the importance of the time elapsed since the suspension of treatment. Hormonal therapy appears to be the kind of treatment more clearly correlated with a loss of ER: the authors have reported some hypotheses about the possible mechanisms of this action. The effect of chemotherapy is much less clear; the data about radiotherapy are few, unhomogeneous and, often, insufficient. Instead, it appears quite clear that ER tend to regain their original status after the suspension of therapy. More studies, are needed before any definitive conclusion can be drawn; it will be necessary to take into account also the possible effect of the different criteria for the preselection of patients. The actual data appear, anyway, to confirm the importance of routine receptor assay on breast tumors, especially after systemic treatment and independently of the kind of therapy itself.

INTRODUCTION

It is now generally accepted that all breast cancers contain steroid receptors [1]. This paper focuses upon estrogen receptors (ER), which have been studied most thoroughly, but it is worthwhile noting, incidentally, that there are also various other receptors of equal interest and importance.

In recent years it has been demonstrated that ER assays are useful in the treatment of patients with breast cancer. Many studies have clearly shown the validity of ER levels in predicting the results of endocrine therapy, while some authors have also suggested a similar role for ER concentrations in chemotherapy [2].

Breast cancer is often a chronic disease requiring multiple therapies over a period of years: in patients with advanced breast cancer chemotherapy and/or hormonal therapy are often the choice for treatment. The hypothesis has been made that hormonal therapy could affect the hormone responsive clones while chemotherapy could control the receptor-negative cells.

On the other hand, the possible effects that the therapy itself could have on the ER concentrations have to be checked, in order to maintain the quality of the treatment.

The latter problem is the focus of this work. We reviewed data from several authors and subdivided them according to the different kinds of therapy whose effects were to be studied. We took into account also radiotherapy, some data from experimental studies, on animals or *in vitro*, and the possible effects of the suspension of therapy.

Where possible we organized the data into tables: works are reported in chronological order, distinguishing the patients' ER status prior to therapy, positive or negative, and the changes occurring after treatment, defined either as from positive to negative and vice versa, or, whenever possible and only for the originally ER + cases, as "decrease" or "increase" (arbitrarily, a change of ER values of more than 20%, higher or lower).

We have to note that the definition of ER "positivity" or "negativity" varies according to the different authors, the borderline value going from 2 to 10 fmol/mg of tissue protein. We kept to the single definitions, but we considered the borderline values as negative when they comprised a certain range of concentrations [3].

EFFECTS OF HORMONAL THERAPY

Table 1 describes the effects of hormonal therapy on a total of 194 cases, 142 ER + and 52 ER −. The kind of treatment was specified in 170 cases: 57% of these patients underwent additive therapy, mainly tamoxifen, 42% ablative and only 1% (2 cases) had both kinds of treatment. Independently of the kind of hormonal therapy, about 42% of ER + cases shifted to ER −, while only about 4% of ER − became ER +. Where changes were specified more precisely (106 cases) a "decrease" was evident in 78% and an "increase" in 7% of ER + cases.

Relative to additive therapy only, there was a change to negative in 36% of ER + cases (26 out of 72), to positive in none of 25 ER − cases, a "decrease" in 91% and an "increase" in 2% of 43 ER + cases.

Table 1. Effects of hormonal therapy

Authors	PTS	ER status prior to therapy	ER status changes[a]	ER variations[b]
Walt *et al.*[4]	12	+	5	11↓
				1↑
Leclercq and Heuson[5]	7	+	3	5↓
	2	−	0	2=
Singhakowinta *et al.*[6]	5	+	1	4↓
				1↑
Rosen *et al.*[7]	2	−	1	
Webster *et al.*[8]	2	+	1	
Allegra *et al.*[9]	8	+	4	8↓
	2	−	0	
Nomura *et al.*[10]	30	+	13	
	29	−	1	
Namer *et al.*[11]	13	+	6	13↓
	8	−	0	
Heuson and Leclercq[12]	9	+	5	8↓
	8	−	0	1↑
Waseda *et al.*[13]	11	+	5	10↓
				1=
Kiang[14]	12	+	7	10↓
	2	−	0	2↑
De Sombre and Jensen[15]	6	+	2	4↓
	1	−	0	1=
				1↑
Allegra and Lippman[16]	8	+	4	8↓
	2	−	0	
Yeu-Tsu and Lee[17]	4	+	1	2↓
				2↑
Hull *et al.*[3]	15	+	3	

[a]Number of patients who changed their ER status after therapy, from positive to negative or vice versa

[b]Only for ER+ cases in which ER values and variations were more precisely specified. If not otherwise specified by the authors, we arbitrarily considered as a "decrease" (↓) or an "increase" (↑) ER variations of more than 20% with respect to the original values.

Ablative therapy caused 48% of ER+ cases (20 out of 48) to become negative and 8% of ER− (2 out of 24) to become positive. We had more precise data for 30 ER+ patients: 80% had a "decrease" and about 17% had an "increase" in their ER concentrations.

The results from the work of Paridaens *et al.*[18] are given only as variations of mean ER values and, thus, could not be included in Table 1. They indicate an evident and reversible decrease in mean ER values from 199 down to 57 fmol/mg tissue protein after hormonal treatment, thus supporting the thesis of a depressing effect of endocrine therapy on ER concentrations.

EFFECTS OF CHEMOTHERAPY

Effects of chemotherapy on a total of 122 cases, 33 ER+ and 89 ER−, are represented in Table 2: 24% of ER+ cases changed to negative while 17% of ER− became positive. Only for 17 ER+ cases were changes more thoroughly specified: 41% (7 cases) underwent a decrease and 23% (4 cases) an increase.

Table 2. Effects of chemotherapy

Authors	PTS	ER status prior to therapy	ER status changes[a]	ER variations[b]
Rosen *et al.*[7]	2	+	2	2↓
Webster *et al.*[8]	4	+	2	
	7	−	1	
Allegra *et al.*[9]	3	+	0	
	16	−	3	
Nomura *et al.*[10]	5	+	0	
	2	−	0	
Kiang[14]	7	+	2	3↓
	35	−	4	3=
				1↑
Pouillart *et al.*[19]	2	+	0	2↑
Yeu-Tsu and Lee[17]	6	+	2	2↓
	8	−	2	3=
				1↑
Hull *et al.*[3]	4	+	0	
	21	−	3	

[a,b]See Table 1.

Pouillart *et al.*'s work [19] deserves particular attention: they reported an increase in the ER concentration in 2 out of 2 patients with advanced breast cancer, after interferon administration. In the same study, he also recorded an increase of progesterone receptors in 5 out of 6 patients after the same treatment.

Although not included in Table 2, we would like to mention the works of Mobbs[20] and Paridaens *et al.*[18]: in the first, chemotherapy brought no change in the ER status of 9 out of 11 patients (comprising both negative and positive cases); in the latter, the mean ER concentration changed from 128 to 110 fmol/mg tissue protein (14 cases), before and after treatment, respectively.

In 1977, Kiang and Kennedy[21] compared the percentages of ER+ cases before and after chemotherapy: there was no significant difference between treated and untreated patients, either in pre- or postmenopausal status (50 vs 49% in premenopausal and 67 vs 57% in postmenopausal).

Recently, Yang and Naguib[22] published the first report on the effect of methotrexate, 5 fluorouracil and vincristine on the concentration of ER in MFC-7 and MDA-MB-134 (ER+ cell lines). The results indicate that cytotoxic drugs may cause a dose-dependent reversible depletion of ER in human breast cancer, and the effect seems to be due to inhibition of the binding of estradiol to its receptors. It is interesting to note that, according to some preliminary results, the cell killing activities of these drugs, at doses of 0.5–50 μg/ml, as determined by the colony formation technique, appeared to be proportional to the degree of ER reduction caused by the corresponding doses of each drug.

EFFECTS OF HORMONOCHEMOTHERAPY

Table 3 lists 46 cases having undergone combined hormonal and chemotherapy: prior therapy, 30 were ER+ and 16 ER−. After treatment 43% of ER+ cases became negative but none of the 16 ER− changed to positive. Where more precise information was available, a "decrease" was evident in 84% (16 cases) and an "increase" in 5% (1 case) out of 19 ER+ cases.

In the work mentioned above, Paridaens *et al.*[18] also studied the effect of combining various modalities of endocrine treatment, mainly tamoxifen, with various standard chemotherapies (CMF): the mean receptor concentration was lower after treatment than before (46 vs 251 fmol/mg tissue protein, on a total of 16 patients).

EFFECTS OF RADIOTHERAPY

Great variability characterizes the few works on this particular aspect. Specimens have been examined either from primaries or from metastases, from sites previously irradiated or not, at various times after treatment, according to the different authors. As a consequence, it was not possible to evaluate completely the relative data and collect them in a comprehensive table.

The effects of radiotherapy on recurrences are taken into account in the work of Rosen *et al.*[7]. The chronological relation between irradiation and ER analysis was not homogeneous or, sometimes, even unspecified: in some cases radiotherapy immediately followed the surgical intervention on the primary, whilst other patients were irradiated prior to biopsy on the recurrences. We mention here only Rosen's patients with known primary ER characteristics. Two patients had postsurgical radiotherapy and later had metastases outside the irradiated area: both their primaries and recurrences, for both patients, were ER+. Two others had lymphnode metastases in the area of postsurgical irradiation: in both cases primaries were ER+ and recurrences ER−.

Finally, a patient had a prebioptic irradiation: her recurrence (Skin) appeared to be ER+, after an ER− primary.

Valenstein *et al.*[23] analyzed the ER characteristics of the soft-tissue metastases (but not of the primaries) of 56 patients, from sites previously irradiated (14 cases) or not (42 cases). The percentage of ER+ biopsies was 50% in the group of nonirradiated cases and 57% in that of irradiated patients; the average ER level was slightly higher in the first group (1094 vs 930 fmol/mg tissue). The results were similar whether the patients of both groups had received prior chemotherapy or not.

The data from Janssens *et al.*[24] and Bressot *et al.*[25] can be presented together as their two works follow similar criteria. ER status was examined in primary tumors of patients who had not received

Table 3. Effects of hormonochemotherapy

Authors	PTS	ER status prior to therapy	ER status changes[a]	ER variations[b]
Walt *et al.*[4]	3	+	2	3↓
Singhakowinta *et al.*[6]	1	+	0	1↓
Rosen *et al.*[7]	1	+	0	1↑
Kiang[14]	14	+	8	12↓
	7	−	0	2=
Hull *et al.*[3]	11	+	3	
	9	−	0	

[a,b]See Table 1.

presurgical radiotherapy: each of Janssen's irradiated patients had received an amount of 20 Gy, while Bressot's received 60–70 Gy. The results from both studies show a decrease in the percentage of ER+ cases and in the average ER values of irradiated patients, when compared to nonirradiated cases (Janssens *et al.*: 54% and about 28 fmol/mg tissue protein vs 75% and 62 fmol/mg; Bressot *et al.*: 54% and about 55 fmol/mg vs 80% and 238 fmol/mg).

In another work, Janssens *et al.*[26] demonstrated an apparently dose-dependent decrease in ER values following irradiation of experimental mammary tumors (DMBA-induced tumors in Sprague–Dawley rats). However, no apparent change was demonstrated by Burke *et al.*[27] after irradiation of MCF-7 cells.

EFFECTS OF SUSPENSION OF THERAPY

It is important to know whether the above-mentioned ER variations, which follow therapy, are constant in the long term or whether there is a trend for tumors to regain their pretreatment ER status.

Valenstein *et al.*[23] demonstrated a clear tendency towards an increase of the percentage of ER+ cases, in the long term, after suspension of chemotherapy; in particular, there was an evident difference between patients who had their treatment stopped for less than 1 month and those who had it stopped for longer.

Hull *et al.*[3] provided similar results: they saw a decrease of values in 78% out of 9 patients off hormonal therapy for less than 2 months, but only in 12% out of 8 patients off treatment for more than 2 months.

King *et al.*[28] found a different proportion of changed ER phenotype after intervening radiotherapy, with or without chemotherapy: 50% when the interval between samples was from 3 to 12 months, but only 20% if the interval exceeded 12 months.

The data from a study made at the Jules Bordet Institute in Brussels were previously reported [29]. ER values were recorded before treatment and at various times after suspension of therapy. The decrease of ER concentration was more evident when the assay was carried out less than 3 months after treatment. Conversely, a general trend for tumors to regain their original ER status became apparent if ER values were analysed again later.

CONCLUSIONS

The possibility that the systemic treatment of patients with breast cancer could, to some degree, interfere with the concentration of ER is still widely discussed.

The clearest evidence appears to come from patients who have undergone hormonal therapy. The interpretation of the ER values in these patients should be done with great caution, especially if treatment has been administered within 3 months prior to the assay. The depressant effect of hormonal therapy tends, however, to vanish with time.

No conclusion can be drawn on the less clear relationship between chemotherapy and variations in ER values, in spite of the experimental work on human cell lines (MCF-7 and MDA-MB 134) reported by Yang and Naguib[22].

With regard to radiotherapy, the data are often confused; for instance, they refer to experiences not homogeneous for choice of patients and kind of treatment and insufficient, as they only seldom comprise the ER values found in single patients before treatment. Moreover, more information is needed on the possible interactions of radiotherapy with other modalities of treatment.

In the investigations of the possible mechanisms of action, several hypotheses have been made about the way hormonal treatment can affect the ER content of breast tumors. Endocrine therapy could create a population of ER-independent cells by simply killing all the ER-dependent elements or, alternatively, it could affect every single tumoral cell by interfering with its differentiation. On the other hand, the hypothesis has been made that the ER variations induced by treatment are only apparent: receptor sites could be simply masked by the hormonal drugs used [3]; this kind of interaction would be reversible and could, thus, explain the tendency of tumors to regain their original ER characteristics.

It is fairly clear that much remains to be studied before any definitive conclusion is drawn about the effects of different therapies on ER concentrations in breast tumors.

Much of the difficulty in obtaining definitive data lies in the small number of cases studied. Moreover, the observed results are influenced by the lack of the information necessary to determine the effects of some types of preselection. (For instance, the choice of treatment and the relative dosage.)

Broader studies, with clearly defined treated and control groups, matched for kind of treatment, dosage, menopausal status and primitive tumor stage, must be made to clarify the effect treatment has on this very important assay. Moreover, concurrent studies should be continued *in vitro*.

In any case, we recommend performing routine receptor assay on the primary and/or metastatic tumor, especially after systemic treatment and independent of the kind of therapy itself.

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