Pattern of Hormone Receptor Status of Secondary Contralateral Breast Cancers in Patients Receiving Adjuvant Tamoxifen

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ABSTRACT

In breast cancer patients receiving adjuvant tamoxifen after unilateral treatment, contralateral breast cancer (CBC) is extremely rare. As a result, only limited data are available on the hormone receptor status of CBCs evolving in tamoxifen-treated patients. The aim of our investigation was to evaluate the pattern of hormone receptor status of CBCs in patients treated with adjuvant tamoxifen at our institution. Material was collected from 35 patients. We have found that 27 of the 35 patients included into our investigation developed an estrogen receptor (ER)-positive CBC despite adjuvant tamoxifen. Seven ER-positive CBCs occurred after tamoxifen had been discontinued, and 20 patients developed an ER-positive CBC while receiving tamoxifen. Notably, 80% of these CBCs displayed moderate-to-strong levels of ER. In our opinion, the selection of ER-negative CBCs, which has previously been implicated to be the pivotal mechanism of tumor escape of CBCs evolving in tamoxifen-treated patients, is only one mechanism of tumor escape in patients receiving antiestrogen treatment. The emergence of ER-positive CBCs in the majority of tamoxifen-treated patients suggests that alternative escape mechanisms may be equally relevant. These include the emergence of ER-positive CBCs that display tamoxifen-dependent growth properties, the selection of CBCs that are tamoxifen resistant because of ER mutations with altered ER function, and, finally, the selection of ER-positive CBCs that overexpress c-erbB2.

INTRODUCTION

The rising incidence of breast cancer, coupled with improved survival after effective treatment, has placed an increasing number of women at risk for the development of a cancer in the contralateral breast (1). CBC3 was first described in 1921 by Kilgore (2) and has been the subject of an increasing number of reports over the last decade (3–7). Although CBC is uncommon compared with the overall incidence of breast cancer, the relative risk of a breast cancer patient developing a contralateral tumor is ~2–6-fold higher than the risk of primary cancer in the general female population (6, 8, 9). As a consequence, both the prevention and the treatment of contralateral disease are important issues to be considered in patients with a prior history of breast cancer.

Tamoxifen is currently the endocrine treatment of choice for all stages of ER-positive breast cancer in postmenopausal women and has also shown promise in the treatment of premenopausal women with ER-positive disease (10–15). In addition, there is substantial evidence that tamoxifen also reduces the incidence of CBC (10–15). The 1998 Oxford overview analysis (12) demonstrated a highly significant reduction of 30% in the incidence of CBC in patients treated with tamoxifen as compared with patients never treated with the drug. Rutqvist et al. (16) found a 40% decrease in CBC in patients receiving adjuvant tamoxifen, and a randomized, double-blinded and placebo-controlled trial performed by Fisher et al. (17) showed similar results. In addition, investigators in the National Surgical Adjuvant Breast and Bowel Project P-1 (NSABP) study B-14, in which patients with ER-positive tumors were treated, found a statistically significant 37% reduction in CBC in the tamoxifen arm of their study (18).

Acting as an antiestrogen on the breast, tamoxifen inhibits the proliferation of ER-positive breast cancer cells (19–23). It is well known that breast cancer is heterogeneous and may contain both ER-positive and ER-negative tumor cell clones. As a consequence, when breast cancer is treated with tamoxifen, ER-positive tumor cells are thought to respond by decreasing proliferation whereas the ER-negative cells may continue to grow because of selective pressure (22). Ultimately, this could allow the emergence of an ER-negative tumor that displays tamoxifen-independent growth properties (24). Hypothetically, tamoxifen may, thus, have crucial implications for the ER status of CBCs evolving in patients who receive adjuvant tamoxifen after unilateral treatment. Because the ER status on its part is known to have crucial implications for the efficacy of subsequent endocrine treatment of the secondary cancer, the hormone receptor status of CBCs evolving in patients who receive adjuvant tamoxifen is of high clinical interest. However, because

1 The abbreviations used are: CBC, contralateral breast cancer; ER, estrogen receptor; PgR, progesterone receptor.
CBC is extremely rare in the era of widespread clinical use of tamoxifen, only limited data are available on the ER status of CBCs that occur in patients receiving adjuvant tamoxifen. Therefore, we have screened our database and investigated the pattern of hormone receptor status in secondary CBCs in patients treated with adjuvant tamoxifen at our institution. We were able to identify 35 patients for whom all of the relevant data (ER status and PgR status of both the primary tumors and CBCs) were available.

**PATIENTS AND METHODS**

**Patient Selection.** A retrospective analysis was carried out in 1129 breast cancer patients who had the initial diagnosis made between 1980 and 1998 and received 5 years of tamoxifen (20 mg daily) as a single adjuvant treatment. Secondary CBC was defined as a tumor of the contralateral breast that occurred after a minimum interval of 6 months after the diagnosis of the first breast cancer. Patients were included in the study on the condition that they had developed a secondary CBC and that paraffin-embedded tissue samples could be obtained from both tumors. Material was collected from 35 patients.

**Patient Characteristics.** Median age of the patients at the diagnosis of the first breast tumor was 65 years (range, 39–86 years). It was 69 years at the diagnosis of the contralateral tumor (range, 40–87 years). The median time interval between the diagnosis of the first breast cancer and the contralateral tumor was 34 months (range, 8–144 months).

**Tumor Characteristics.** Staging of the tumors was done according to the TNM classification of the Union Internationale Contre le Cancer. Tumor diameter was determined on the gross specimen. All of the specimens were embedded in paraffin, stained with H&E, and graded according to the methods described by Bloom and Richardson (25).

**Immunohistochemical Assay.** As described previously (26, 27), ERs and PgRs were determined immunohistochemically on paraffin sections using the ER-ICA and PgR-ICA kit (Abbott Laboratories, North Chicago, IL). Tumors that showed at least 10% receptor-positive nuclei were scored positive. Staining intensity for ER and PgR was scored as 0, negative; +, slight; +++, moderate; and +++, strong. In case of heterogeneous staining intensity within the tumor, the most predominant intensity was scored. All of the specimens were re-examined by the same experienced pathologist (M. Rudas) to confirm the histological type, staging, nuclear grading, and hormone receptor status of the respective tumors.

**RESULTS**

**Tumor Characteristics of Primary Cancers and CBCs.** Table 1 illustrates the tumor characteristics (tumor size, tumor histology and histological grading) of the primary breast cancers and the CBCs in 35 patients included in our investigation. Fig. 1 shows the ER and PgR status of the primary tumors and CBCs as well as the time interval between the occurrence of both tumors.
Hormone Receptor Status of Primary Breast Cancers. First, we looked at the hormone receptor status of the primary breast cancers in our study population: 25 (71%) of 35 primary breast cancers were ER-positive. Of these tumors, 10 (40%) also scored positive for PgR, whereas 15 (60%) displayed a PgR-negative phenotype. 10 (29%) of 35 primary breast cancers were ER-negative. Of these, 8 (80%) also scored negative for PgR, whereas 2 (20%) displayed a PgR-positive phenotype.

Hormone Receptor Status of CBCs Occurring During or After Treatment with Adjuvant Tamoxifen. Next, we addressed the hormone receptor status of CBCs occurring during or after adjuvant tamoxifen: Of 35 CBCs, 27 (77%) were ER-positive and 8 (23%) were ER-negative; 26 CBCs (74%) occurred during treatment with adjuvant tamoxifen, whereas 9 (26%) occurred after tamoxifen had been discontinued. Of the 26 CBCs that occurred during adjuvant tamoxifen, 20 (77%) were ER-positive, whereas 6 (23%) displayed an ER-negative phenotype. Fifteen (75%) of 20 ER-positive CBCs also scored positive for PgR, whereas 5 (25%) of 20 tumors were PgR-negative. All of the ER-negative CBCs that occurred during adjuvant tamoxifen treatment also scored negative for PgR. Of the nine CBCs that occurred after adjuvant tamoxifen treatment, 7 (78%) were ER-positive, whereas 2 (22%) displayed an ER-negative phenotype. Five (71%) of the seven ER-positive tumors also scored positive for PgR, whereas two tumors (29%) were PgR-negative. Both of the ER-negative tumors occurring after adjuvant tamoxifen treatment also scored negative for PgR.

Intensity of ER Expression of ER-positive CBCs. Fig. 2 illustrates the intensity of ER expression of ER-positive CBCs in our patient population: 16 (80%) of 20 ER-positive tumors that occurred during tamoxifen treatment displayed moderate (ER++) to strong (ER+++ ) levels of ER, whereas in 4 (20%) of the tumors, only slight (ER+) ER expression was detected. Of the seven CBCs which occurred after discontinuation of tamoxifen treatment, five tumors (71%) were classified as ER++ to ER+++ , and two tumors (29%) were classified as ER+.

Time Interval between Primary Cancers and ER-positive/ER-negative CBCs. We finally addressed the time interval between the primary cancers and CBCs and compared ER-positive with ER-negative tumors. Fig. 3 shows the median time interval and range between primary cancers and CBCs during 60 months of treatment with adjuvant tamoxifen: ER-positive tumors occurred after a median time interval of 33 months (range, 10–46 months), whereas ER-negative tumors occurred after a median time interval of 21 months (range, 8–46 months). When also taking into account the CBCs that occurred after tamoxifen had been discontinued, ER-positive tumors occurred after a median time interval of 37 months (range, 10–144 months), whereas ER-negative tumors occurred after a median interval of 27 months (range, 8–120 months).

DISCUSSION

The aim of the present study was to evaluate the pattern of hormone receptor status of secondary CBCs in patients receiving adjuvant tamoxifen. We have found that the majority of patients included in our investigation (i.e., 27 of 35) developed an ER-positive CBC, although they had been given antiestrogen treatment with tamoxifen. Of particular interest, 20 (77%) of 26 CBCs that occurred during 60 months of tamoxifen treatment were ER-positive, with 80% of the tumors displaying at least moderate levels of ER.

It is well known that tamoxifen reduces the incidence of CBC (12, 16–18). As an antiestrogen, tamoxifen selectively inhibits the growth of ER-positive breast cancer cells. Taking into account the proven effect of tamoxifen as a means of overall relapse reduction and CBC prevention, one might, therefore, expect that patients with the rare occurrence of a CBC despite adjuvant tamoxifen will show an ER-negative phenotype of the secondary cancer. Indeed, Rutqvist et al. (28) were able to demonstrate that CBCs evolving in patients receiving adjuvant tamoxifen were more frequently ER-negative than the respective primary cancers (P < 0.05) or CBCs evolving in untreated patients (P < 0.05). Likewise, Li et al. (29) found that the risk of developing an ER-negative CBC was significantly increased among women receiving adjuvant tamoxifen. Surprisingly, however, the majority of tamoxifen-treated patients from both studies, i.e., 22 (76%) of 29 patients in Rutqvist’s study and 47 (73%) of 64 patients in Li’s study developed an ER-positive CBC. Similarly, we have found that 27 (77%) of 35 patients in our study developed an ER-positive CBC despite receiving adjuvant tamoxifen. Therefore, we feel that the emergence of ER-negative CBCs is only one mechanism of tumor escape of CBCs evolving in tamoxifen-treated patients: the emergence of ER-positive CBCs in the majority (i.e., ~75%) of tamoxifen-
treated patients suggests that alternative tumor escape mechanisms may be equally relevant.

Several mechanisms may allow the occurrence of ER-positive CBCs in patients receiving tamoxifen. Firstly, ER-positive CBCs that evolve after adjuvant tamoxifen has been stopped (as in patients 5, 6, 9, 17, 18, 22, and 24 in our study; Fig. 1) may be explained by the fact that, once antiestrogen treatment has been discontinued, there is no more selective growth inhibition of ER-positive tumor cells, which allows ER-positive CBCs to eventually occur. However, 20 patients in our study developed an ER-positive CBC while receiving adjuvant tamoxifen (Fig. 1). In our opinion, mechanisms such as the following may be responsible for the emergence of ER-positive CBCs during tamoxifen treatment:

(a) Tamoxifen-stimulated growth. One possibility to explain the occurrence of ER-positive CBCs in patients treated with tamoxifen is the emergence of contralateral tumors that display tamoxifen-stimulated growth properties (30–34). Several studies in the laboratory have shown that tamoxifen-stimulated breast tumor growth may represent a highly relevant mechanism of tamoxifen resistance that may conceivably also apply to CBCs evolving in tamoxifen-treated patients (35–40). It has been speculated that the emergence of tamoxifen-stimulated breast tumor growth represents a shift from antagonist to agonist activity of tamoxifen. Conceivably, this occurs by misinterpretation of the tamoxifen signal because of alterations of the ER signal transduction pathway (30, 38). Obviously, in such cases of tamoxifen-mediated growth stimulation, tamoxifen treatment for the secondary, even though ER-positive, CBC would be contraindicated. However, alternative antihormonal agents such as pure antiestrogens may prove useful as a second-line endocrine therapy because they have been shown to retain their growth-inhibitory effects even in cases of tamoxifen-stimulated breast tumor growth (37, 38).

(b) ER mutations. Alternatively, the occurrence of ER-positive CBCs during treatment with tamoxifen may be explained by the emergence of ER-positive breast cancer cells that are tamoxifen-resistant because of mutational alterations in the ER (41–44). For instance, the presence of a constitutively active ER may result in such tamoxifen resistance. An example is Tyr537Asn, which shows potent constitutive transcriptional activity (45). Cells that display this mutation grow independently of estradiol binding and are essentially unaffected by antiestrogen treatment. Tamoxifen-mediated selective growth pressure leading to the negative selection of ER-positive tumor cells that display mutational alterations in the ER may, thus, be an important mechanism that could allow the emergence of ER-positive CBCs in patients receiving antiestrogen treatment.

(c) Nonsteroidal growth-promoting mechanisms override hormonal growth-regulatory pathways. It has been shown that ER-positive tumors that overexpress the c-erbB2 oncogene are less likely to respond to endocrine therapy than those with no overexpression (46–51). Conceivably, therefore, selection of ER-positive CBCs that overexpress c-erbB2 may be another mechanism that could explain the emergence of ER-positive CBCs in patients receiving antiestrogen treatment with tamoxifen.

(d) Premenopausal patients. Finally, it has been shown that the beneficial effect of tamoxifen on the development of CBC may be dependent on menopausal status, because the reduction in the incidence of CBC is largely restricted to postmenopausal women (8, 52). Hypothetically, in premenopausal women, tamoxifen may not completely abolish the tumor growth-promoting actions of estrogen, which could eventually allow the emergence of ER-positive, estrogen-dependent CBCs.

Notably, ER-negative CBCs tended to occur earlier than ER-positive tumors in our study population (Fig. 3). Two explanations may be given for this phenomenon: Firstly, ER-negative tumors may have displayed a more aggressive phenotype that led to the earlier emergence of these tumors as compared with the more “benign” ER-positive tumors. Secondly, tamoxifen may have selectively, although transiently, inhibited the growth of ER-positive CBCs (thereby delaying their clinical emergence) whereas, at the same time, it did not have any influence on the growth of ER-negative tumors. As a result, ER-negative CBCs occurred after a shorter time interval than the ER-positive tumors.

Regardless of treatment, the following suggestions may be made on the basis of our data. Generally, in patients who develop an ER-positive CBC during adjuvant tamoxifen treatment, aromatase inhibitors or pure antiestrogens may constitute a promising alternative to tamoxifen for the treatment of the secondary cancer. However, if the ERs expressed by the CBC are nonfunctional because of mutational events, a nonhormonal treatment such as conventional chemotherapy should possibly be instituted. In patients with CBCs that overexpress c-erbB2, treatment with monoclonal antibodies against HER-2/neu may be considered. Although tamoxifen conceivably is of minimal therapeutic value in the treatment of ER-positive CBCs that occur during tamoxifen administration, tamoxifen may be considered again for CBCs that occur after tamoxifen treatment has been discontinued because such late CBCs probably constitute an independent second primary cancer in which tamoxifen may again be effective.

In conclusion, our study indicates that the majority of CBCs occurring in patients who receive adjuvant tamoxifen after unilateral treatment are ER-positive. This suggests that the selection of ER-negative CBCs is only one mechanism of tumor escape in tamoxifen-treated patients and that other tumor escape mechanisms may be equally relevant. These include the emergence of ER-positive CBCs that display tamoxifen-dependent growth properties, the selection of CBCs that are tamoxifen resistant because of ER mutations with altered ER function, and, finally, the selection of ER-positive CBCs that overexpress c-erbB2. The clinical relevance of the above-mentioned mechanisms of tamoxifen resistance in patients treated with adjuvant tamoxifen remains to be validated in future clinical trials. We hope that the present investigation will add information to better define breast tumor escape mechanisms on adjuvant tamoxifen as well as to better define possible treatment options for patients with secondary CBC after primary adjuvant tamoxifen treatment.
REFERENCES


