Expression of the epidermal growth factor system in endometrial cancer after adjuvant tamoxifen treatment for breast cancer

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Summary

The aim of our study was to describe the expression of cerbB-1, cerbB-2, cerbB-3 and cerbB-4 in endometrial cancer tissue and its correlation with clinicopathologic features and prognosis of endometrial cancer patients diagnosed during or after tamoxifen treatment for breast cancer.

Thirteen tamoxifen-related endometrial cancers were identified from the archives of the Department of Obstetrics and Gynecology of the University of Patras, Medical School. Tissue specimens from endometrial lesions were immunostained for cerbB-1, cerbB-2, cerbB-3 and cerbB-4.

For cerbB-1, five cases were positive and eight were negative. For cerbB-2, ten cases were positive and three were negative. For cerbB-3, nine cases were positive and four were negative. For cerbB-4, eight cases were positive and five were negative.

However, a limitation of our study is that the number of cases was small, and further investigations are necessary to allow a more focused evaluation of cerbB-1, cerbB-2, cerbB-3 and cerbB-4 status, as a prognostic factor for endometrial cancer after tamoxifen treatment.

Key words: Endometrial cancer; Tamoxifen; Breast cancer; Epidermal growth factor system; Cerb-B receptors.

Introduction

Tamoxifen (TAM) is a non-steroidal, selective estrogen receptor modulator (SERM) that has potent anti-estrogenic activity in the breast while displaying weak estrogen activity in the endometrium. It has been the antihormonal treatment of choice for postmenopausal breast cancer patients with positive estrogen receptors over the past two decades and its use has been convincingly shown to improve the disease-free survival as well as overall survival [1].

One of the most significant and deleterious side-effects of TAM treatment in postmenopausal women with breast cancer appears to be its proliferative effect on the endometrium. Overall endometrial pathologies, including hyperplasia, polyps, carcinoma and sarcoma have been identified in up to 36% of postmenopausal breast cancer TAM-treated patients [2]. The pathogenetic mechanism for the development of TAM-associated malignant endometrial tumors has not yet been yet clearly defined.

During the last decade efforts have focused on attempting to identify cytokinetic or molecular events that correlate with the malignant potential of endometrial cancers. Several laboratories have evaluated the expression of oncogenes and tumor suppressor genes.

The epidermal growth factor (EGF) system is a type I growth factor family consisting of four receptors: epidermal growth factor receptor (EGFR) (also called cerbB-1, HER-1), cerbB-2 (also called HER-2), cerbB-3 (also called HER-3), cerbB-4 (also called HER-4). The receptors are transmembrane glycoproteins with an extracellular ligand-binding domain, a transmembrane region and an intracellular domain. The intracellular domains of cerbB-1, cerbB-2 and cerbB-4 display tyrosine kinase activity. Activation of the receptors induces dimerization. CerbB-1 and cerbB-4 form either homo- or heterodimers, whereas cerbB-2 functions as a cofactor for the other receptors, and cerbB-3 needs heterodimerization because of its lack of tyrosine kinase activity. There are at least 11 known ligands for the EGF system.

The EGF system is ubiquitous in human organs and plays a fundamental role in diverse processes such as embryogenesis, development, proliferation, differentiation, cell motility and survival [3, 4].

Only a limited number of studies concerning the EGF system and the endometrium have been published [10, 12]. In these studies one or few members of the EGF system have been investigated. Most of these studies are based on immunohistochemistry of single biopsies from women undergoing hysterectomy on benign indications.

The aim of our study was to describe the expression of cerbB-1, cerbB-2, cerbB-3 and cerbB-4 in endometrial cancer tissue and its correlation with the clinicopathologic features and prognosis of endometrial cancer patients diagnosed during or after tamoxifen therapy for breast cancer.

Material and Methods

Between May 1991 and December 2005, about 128 women with histologically confirmed endometrial cancer were referred to the Department of Gynecologic Oncology of the University

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g. 1

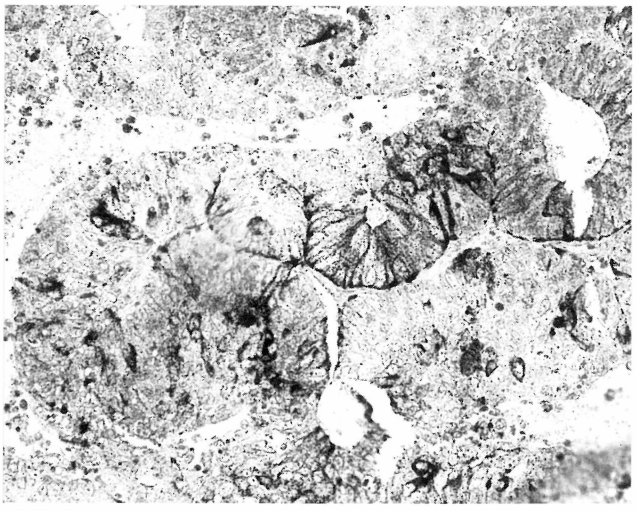
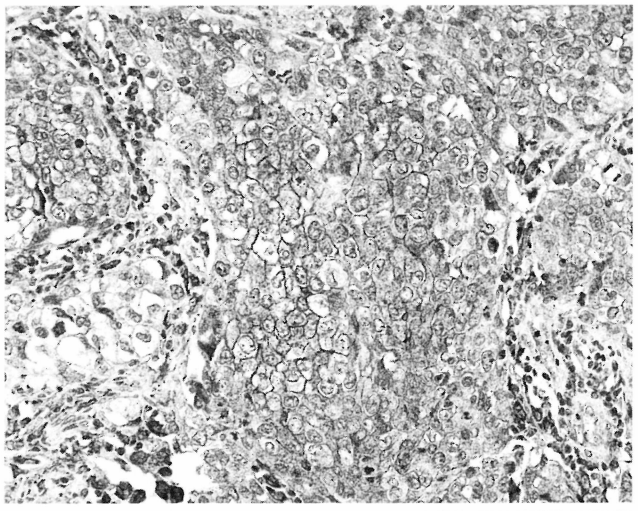


Fig. 2



g. 3

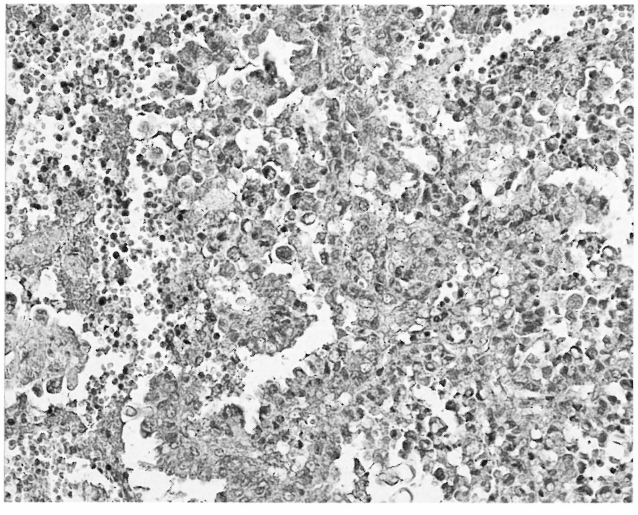


Fig. 4

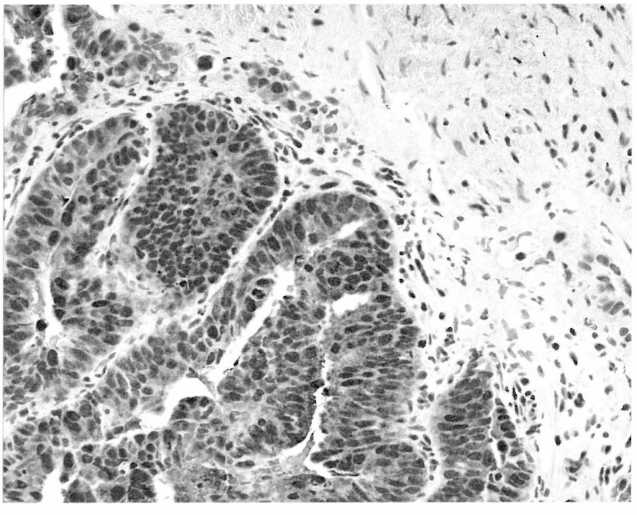


Figure 1. — CerbB-1 positive immunostaining in endometrial carcinoma after TAM treatment.
Figure 2. — CerbB-2 positive immunostaining in endometrial carcinoma after TAM treatment.
Figure 3. — CerbB-3 positive immunostaining in endometrial carcinoma after TAM treatment.
Figure 4. — CerbB-4 positive immunostaining in endometrial carcinoma after TAM treatment.

of Patras Medical School. Among them, 13 cases of endometrial cancers were found to be associated with TAM-treatment in breast cancer patients.

The patients were diagnosed with endometrial cancer, at least six months after cessation of TAM treatment for breast cancer.

All 13 patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. Lymph node sampling and cytologic tests of the peritoneal fluid were performed in all patients. All staging procedures were performed by a gynecologic oncologist.

All tissue specimens were stained with hematoxylin-eosin. Staging was determined using the surgical staging system for endometrial cancer established by the International Federation of Obstetrics and Gynecology (FIGO). Tumor histologic classification was performed using the criteria of the World Health Organization (WHO).

Formalin-fixed paraffin-embedded tissue sections, representative of the tumor in each case, were immunostained using the biotin-streptavidin peroxidase method. The following antibodies against cerbB-1, cerbB-2, cerbB-3 and cerbB-4 were used: a) anti–EGFR mouse monoclonal antibody (Santa Cruz Biothechnology Inc., UK) in a dilution 1:20; b) anti-HER-2 mouse polyclonal antibody (Dako Cytomation, Denmark), in a dilution 1:300; c) anti-HER-3 mouse polyclonal antibody (Santa Cruz Biothechnology Inc., UK) in a dilution 1:100 and d) anti-HER-4 mouse polyclonal antibody (Santa Cruz Biothechnology Inc., UK) in a dilution 1:200.

Tumors were scored by the proportion of tumor cells stained. Immunostaining of 5% of the tumor cells was considered as an optimized cut-off for tumor positivity. The staining for cerbB-1, cerbB-3 and cerbB-4 was mainly cytoplasmic, though nuclear staining was also detected in some of the cases. The staining for cerbB-2 was membrane, and though cytoplasmic staining was also detected in some of the cases, only the membrane immunostaining was considered as positive.

Statistical analyses were performed using the SPSS-13 for Windows. The association between cerbB-1, cerbB-2, cerbB-3 and cerbB-4 expression, as well as between the molecule expression and other clinicopathological markers, was analyzed using the chi square test. Life tables were calculated according to the Kaplan-Meier method. The survival time was calculated from the date of initial surgery for the endometrial cancer.

Results

The median age at diagnosis of endometrial cancer was 66.8 years (range 49-82 years) and the median interval between diagnoses of two cancers was 95.5 months (range 6-251 months).

All patients had been treated with 20 mg of TAM daily. The median duration of TAM use for the cases was 95.5 months (range 6-251 months), with three patients having taken TAM for less than 24 months, one patient between 24 and 59 months and nine patients for more than 60 months.

None of the patients had been on estrogen replacement therapy.

Among the 13 patients, we had nine (69.2%) at Stage I, two (15.4%) at Stage II, one (7.7%) at Stage III and one (7.7%) at Stage IV, according to the FIGO classification.

The histopathologic findings of 13 TAM-related endometrial cancers are summarized in Table 1.

Table 1. — *Histopathologic findings.*

		Case numbers	Percentage (%)
Age	≤ 60 years	5	38.5
	> 60 years	8	61.5
Histologic type			
	Endometrioid	10	76.9
	Papillary serous	1	7.7
	Adenosquamous	2	15.4
	Clear cell	0	0
	Undifferentiated	0	0
Stage	I	9	69.2
	II	2	15.4
	III	1	7.7
	IV	1	7.7
Grade			
	G I	3	23.1
	G II	6	46.1
	G III	4	30.8
Myometrial invasion			
	None	2	15.4
	≤ 1/2	6	46.1
	> 1/2	5	38.5
Cervical invasion			
	None	10	76.9
	Superficial	2	15.4
	Deep	1	7.7
Ovarian metastasis	Present	1	7.7
	Absent	12	92.3
Lymph node metastases	Present	1	7.7
	Absent	12	92.3
Tumor invading bladder/bowel	Present	1	7.7
	Absent	12	92.3
Ascites cytology	Present	2	15.4
	Absent	11	84.6

For cerbB-1, five cases (38.5%) were positive and eight (61.5%) were negative. For cerbB-2, ten cases (76.9%) were positive and three (23.1%) were negative. For cerbB-3, 9 cases (69.2%) were positive and four (30.8%) were negative. For cerbB-4, eight cases (61.5%) were positive and five (38.5%) were negative.

The median follow-up was 55.7 months (range 14-120 months).

The 5-year cumulative endometrial carcinoma-specific survival was 68.2%.

The 5-year cumulative endometrial carcinoma-specific survivals for patients < 60 years and ≥ 60 years of age at diagnosis of endometrial cancer were 100% and 58.8%, respectively.

The 5-year cumulative endometrial carcinoma-specific survival for TAM treatment ≥ 60 months was 83.3%.

Discussion

The pathogenetic mechanism for the development of TAM-associated malignant endometrial tumors has not yet been clearly defined. TAM may act as an initiator of carcinogenesis via estrogen agonistic activity in the endometrium. Perhaps TAM uses pathogenetic pathways similar to sporadic cancer [5]. TAM may increase the proliferation of a subset of cells, thereby increasing the likelihood of mutations. Alternatively, it may promote the growth of cells that have already sustained mutations. As a result of either possibility, TAM exposure could lead to the production of a spectrum of mutations similar to that of sporadic endometrial cancer [5]. Another study showed that the carcinogenetic effect of TAM maybe due to genotoxic DNA damage [6].

The Stockholm trial showed a continued divergence of the cumulative incidence curves of endometrial cancer for the TAM-treated and control groups, even several years after cessation of TAM-treatment [7]. The relative risk (RR) for endometrial cancer, as compared to non TAM-treated patients with gradual increase in duration of TAM-treatment, increases up to 60 consecutive months. Stage III and IV endometrial cancers occurred more frequently in long-term (≥ 60 months) TAM-users than in non-users [8]. In our study one Stage III patient received TAM for 53 months and another Stage IV patient received TAM for 61 months, respectively. The median duration of TAM use was 104.2 months (range 12-251 months), with eight patients having taken TAM for more than 60 months. We could not find any significant correlation between the duration of TAM-use and expression of cerbB-1, cerbB-2, cerbB-3 and cerbB-4, but the number of cases was very small.

Endometrial pathologies are associated with high cumulative doses of TAM administered to postmenopausal breast cancer patients. Women who received 20 mg of TAM daily developed endometrial pathologies after longer periods of treatment compared to those who were treated with 40 mg of TAM daily [9]. In our study all women received 20 mg of TAM daily.

The EGF system is ubiquitous in human organs and plays a fundamental role in diverse processes such as embryogenesis, development, proliferation, differentiation, cell motility and survival [3, 4]. Dysregulation of the EGF system signaling network is implicated in multiple

human pathologies, of which the role of EGF in cancer is the best characterized, particularly for cerbB-1 and cerbB-2 [4].

The four receptors have different levels during the menstrual cycle. CerbB-1 shows the highest value in the early proliferative phase, cerbB-2 and cerbB-4 in the early secretory phase, and cerbB-3 in the late secretory phase [10].

CerbB-1 is localized to the basal part of the surface epithelial cells, only in stromal cells, or both to epithelial and stromal cells [10]. It is overexpressed in multiple human malignancies, including cancers of the breast, head, neck, lung and gliomas [11]. In our study cerbB-1 was positive in five cases. We could not find any significant correlation between cerbB-1 and stage, grade or prognosis, but the number of cases was very small.

CerbB-2 is localized basolaterally, and solely to the glands and epithelium [10, 12]. With no direct ligand identified to date, cerbB-2 functions as a preferred partner for heterodimerization with other members of the EGF system, and thus plays an important role in coordinating the EGF system signaling network that is responsible for regulating cell growth and differentiation [4]. It is overexpressed in many types of cancer and has been shown to be an indicator of more aggressive disease and poorer prognosis in patients with breast cancer [13], while it is also overexpressed in some stomach and ovarian carcinomas [14]. CerbB-2 overexpression in endometrial cancer has been correlated with unfavorable prognosis in some studies [15], but not in others [16]. Possible explanations for the lack of concordance in the prognostic value of cerbB-2 expression among the studies include differences in populations studied, techniques used, antibodies used, or interpretation of results. The conflicting results reported in the literature about its possible prognostic role, the lack of independent prediction of patient outcome, the subjectivity in its measurement, and the concerns expressed regarding its reproducibility would minimize the potential role of cerbB-2 as a marker in the preoperative evaluation of patients with endometrial cancer [12, 13, 14]. In our study cerbB-2 was positive in ten cases. It seems that there is an association between cerbB-2 and cerbB-3, cerbB-4 expression. We could not find any significant correlation between cerbB-2 and stage, grade or prognosis, but the number of cases was very small.

CerbB-3 is localized in epithelium [10]. It is expressed in some gastric and breast carcinomas [17], while little is known regarding its precise intracellular function. In our study it seems that there was an association between cerbB-2 and cerbB-3 expression. We could not find any significant correlation between cerbB-3 and stage, grade or prognosis, but the number of cases was very small.

CerbB-4 is the most recently identified member of the family, and is localized to epithelial and stromal cells [10, 18]. It is expressed in many adult and fetal tissue-lining epithelia of the skin, gastrointestinal, urinary reproductive and respiratory tracts, skeletal muscle, circulatory, endocrine and nervous systems [18], as well as in the majority of ovarian cancers [19]. In our study it seems that there was an association between cerbB-2 and cerbB-4 expression. We could not find any significant correlation between cerbB-4 and stage, grade or prognosis, but the number of cases was very small.

The main limitation of our study is that the number of cases was small, and further investigations are necessary to allow a more focused evaluation of cerbB-1, cerbB-2, cerbB-3 and cerbB-4 status, as a prognostic factor for endometrial cancer after TAM treatment.

The ability to predict increased risks of advanced disease, recurrence, and death from abnormal molecular markers detected in curettage or endometrial biopsy specimens will facilitate pretreatment referral of these patients with endometrial cancer to gynecologic oncologists for definitive surgical treatment. Prospective studies with an appropriate panel of antibodies could lead to a better definition of risk groups.

Conclusion

The ability to predict increased risks of advanced disease, recurrence, and death from abnormal molecular markers detected in curettage or endometrial biopsy specimens will facilitate pretreatment referral of these patients with endometrial cancer to gynecologic oncologists for definitive surgical treatment. Prospective studies with an appropriate panel of antibodies could lead to better definition of risk groups.

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