Expression of the epidermal growth factor system in endometrial cancer

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Summary

The aim of our study was to describe the expression of c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4 in endometrial cancer tissue and its correlation with clinicopathologic features and prognosis of the patients. One hundred and six cases of endometrial cancer were identified from the archives of the Department of Obstetrics and Gynecology of the University of Patras. Tissue specimens from endometrial lesions were immunostained for c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4. Statistical analyses were performed using the chi square test, Kaplan-Meier method and Cox analysis. We found a significant association between c-erbB-1 expression and patient survival. A reverse correlation was found between tumor grade and c-erbB-1 expression. Tumor grade was not significantly correlated with the expression of the remaining three receptors. Stage of the tumor showed no relationship with the expression of these receptors. 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 to gynecologic oncologists for definitive surgical treatment.

Key words: Endometrial cancer; EGF system; c-erbB receptors; Prognosis.

Introduction

Endometrial cancer is the most common malignancy of the female genital tract. Overall, about 2% to 3% of women develop endometrial cancer during their lifetime [1]. Endometrial cancer is a malignancy that occurs primarily in postmenopausal women. It most often occurs in the sixth and seventh decades of life, at an average age of 60 years. Several risk factors have been identified (nulliparity, late menopause, obesity, unopposed estrogen therapy, atypical endometrial hyperplasia, diabetes mellitus and tamoxifen therapy). Most of these risk factors are related to prolonged, unopposed estrogen stimulation of the endometrium [2].

The overall survival rate for endometrial cancer is 84%; this reflects its early clinical declaration [1].

A wide variety of prognostic factors (age, stage, tumor grade, histologic type and depth of myometrial invasion) have been described and evaluated in detail.

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 c-erbB-1, HER-1), c-erbB-2 (also called HER-2), c-erbB-3 (also called HER-3), c-erbB-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 c-erbB-1, c-erbB-2 and c-erbB-4 display tyrosine kinase activity. Activation of the receptors induces dimerization. C-erbB-1 and c-erbB-4 form either homo- or heterodimers, whereas c-erbB-2 functions as a cofactor for the other receptors, and c-erbB-3 needs heterodimerization because of its lack of tyrosine kinase activity. There are at least 11 known ligands for the EGF system [3].

The EGF system is ubiquitous in human organs and plays fundamental roles in diverse processes such as embryogenesis, development, proliferation, differentiation, cell motility and survival [4, 5].

The EGF system signaling network induces a wide variety of biologic responses and is involved in many physiologic and pathologic conditions. The biologic outcome of signaling through the EGF system depends on the cellular and environmental context. Dysregulation of the EGF signaling network is implicated in multiple human pathologies, of which the role of EGF in cancer is the best characterized, particularly for c-erbB-1 and c-erbB-2 [5].

Only a limited number of studies concerning the EGF system and the endometrium have been published. In these studies one or few members of the EGF system have been investigated [6-9]. Most of these studies are based on immunohistochemistry of single biopsies from women undergoing hysterectomy for benign indications.

The aim of our study was to describe the expression of c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4 in endometrial cancer tissue and its correlation with clinicopathologic features and prognosis of the patients.

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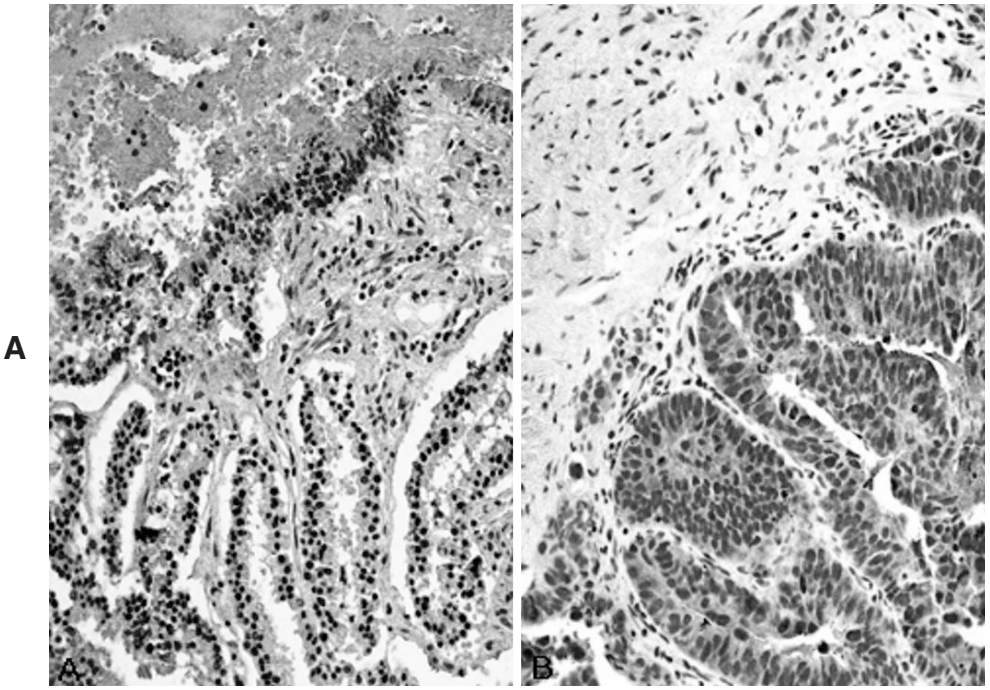

Figure 1. — A) C-erbB-1 positive immunostaining. B) C-erbB-2 positive immunostaining.

Materials and Methods

Case selection

Between May 1991 and December 2004, about 106 women with histologically confirmed endometrial cancer were referred to the Department of Gynecologic Oncology of the University of Patras Medical School.

Among them, 12 patients denied surgical intervention and they underwent radiotherapy.

Ninety-four 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.

Histopathology and immunohistochemistry

Three pathologists reviewed all hematoxylin-eosin stained sections. 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. Sections were quenched with H_2O_2 (0.6%) in 100% methanol for 20 min to inhibit endogenous peroxidase activity. Microwave pretreatment was used to unmask epitopes. Non specific binding was blocked by incubating the sections in TBS solution containing 3% BSA.The following antibodies against c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4 were used: a) anti-EGFR mouse monoclonal antibody (Santa Cruz Biothechnology Inc, UK) in a dilution 1:20, b) anti-HER-2 mouse monoclonal antibody (Biogenex), in a dilution 1:100, 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.

Labeling was detected using the streptavidine-biotin complex method, while 3, 3' diaminovenzidine (DAB) was used as a chromogen. Sections were counterstained with hematoxylin. Negative staining controls were included in which no primary antibody had been added.

Evaluation section-immunopositivity was done by three pathologists through a Zeiss light microscope. Immunostaining of 5% of the tumor cells was considered as an optimized cut-off for tumor positivity (we chose this evaluation system because there is no acceptable scoring system for endometrial cancer as there is for breast cancer). The staining for c-erbB-1, c-erbB-3 and c-erbB-4 was mainly cytoplasmic, though nuclear staining was also detected in some of the cases (Figures 1A, 2A, and 2B). The staining for c-erbB-2 was membrane, although cytoplasmic staining was also detected in some cases, only the membrane immunostaining was considered as positive (Figure 1B).

Statistical analysis

Statistical analyses were performed using the SPSS-13 for Windows. The association between c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4 expression, as well as between 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 the initial diagnosis. Multivariate survival analyses were performed with the Cox proportional hazards model, entering the following covariates: a) c-erbB-1 expression (negative vs positive), b) c-erbB-2 expression (negative vs positive), c) c-erbB-3 expression (negative vs positive), d) c-erbB-4 expression (negative vs positive), e) tumor stage (I, II, III and IV), and f) tumor grade (I, II and III). In Cox regression analysis a p of 0.05 was adopted as the limit for inclusion of a covariate. All statistical tests were two-sided. For each method every $p < 0.05$ was considered as statistically significant.

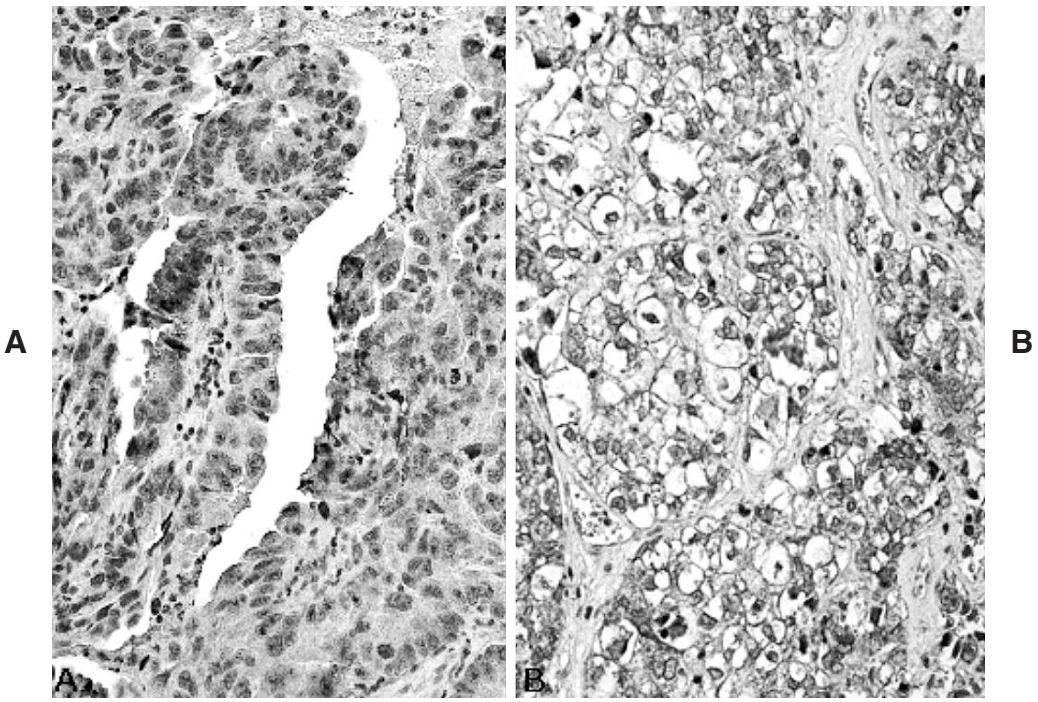

Figure 2. — A) C-erbB-3 positive immunostaining. B) C-erbB-4 positive immunostaining.

Results

Clinical findings

Median age at diagnosis of endometrial cancer was 64.2 years (range 39-89 years). Median weight was 74.5 kg (range 65-89 kg). Time of follow-up ranged from 11 to 168 months (mean 76 months). None of the patients had received hormonal therapy.

Histopathologic findings

Our study included 106 cases of endometrial adenocarcinomas who had been diagnosed by endometrial curettage. Among the 106 cases, we had 31 grade 1 (29.2%), 51 grade 2 (48.1%), and 24 grade 3 carcinomas (22.6%).

In the majority of cases (94 from 106) the diagnosis was followed by total abdominal hysterectomy with bilateral salpingo-oophorectomy. Among the 94 patients, we had 64 (60.4%) in Stage I, 15 (14.2%) in Stage II, ten (9.4%) in Stage III and five (4.7%) in Stage IV, according to the FIGO classification.

The histopathologic findings of 106 endometrial cancers are summarized in Table 1.

Immunohistochemical findings

For c-erbB-1, 66 cases (62.3%) were positive and 40 (37.7%) were negative. For c-erbB-2, 69 cases (65.1%) were positive and 37 (34.9%) were negative. For c-erbB-3, 72 cases (67.9%) were positive and 34 (32.1%) were negative. For c-erbB-4, 78 cases (73.6%) were positive and 28 (26.4%) were negative.

Table 1. — *Histopathologic findings.*

		Case numbers	Percentage (%)
Age	≤ 60 years	38	35.8
	> 60 years	68	64.2
Histologic type	Endometrioid	92	86.8
	Papillary serous	8	7.5
	Adenosquamous	4	3.8
	Clear cell	2	1.9
	Undifferentiated	0	0
Stage	I	64	60.4
	II	15	14.2
	III	10	9.4
	IV	5	4.7
Grade	G I	31	29.2
	G II	51	48.1
	G III	24	22.6
Myometrial invasion	None	7	7.4
	≤ 1/2	37	39.4
	> 1/2	50	53.2
Cervical invasion	None	70	74.5
	Superficial	10	10.6
	Deep	14	14.9
Ovarian metastasis	Presence	6	6.4
	Absent	88	93.6
Lymph node metastases	Presence	8	8.5
	Absent	86	91.5
Tumor invades bladder/bowel	Presence	4	4.2
	Absent	90	95.7
Ascites cytology	Presence	11	11.7
	Absent	83	88.3

Statistical analysis

A significant association was found between c-erbB-1 and c-erbB2 expression (Pearson correlation 0.369, $p < 0.001$), as well as between c-erbB-1 expression and the expression of c-erbB-3 and c-erbB-4 proteins (Pearson correlation 0.341 and 0.505, respectively and $p < 0.001$ in both cases). A significant correlation also emerged between the expression of c-erbB-2 and c-erbB-3 receptors (Pearson correlation 0.557, $p < 0.001$). Moreover, the expression of c-erbB-2 and c-erbB-4 was strongly correlated with one another (Pearson correlation 0.235, $p = 0.015$). Finally, c-erbB-3 and c-erbB-4 positivity also had a strong association with each other (Pearson correlation 0.368, $p < 0.001$). Finally, the correlation between the expression of c-erbB-2 and c-erbB-4 approached borderline ($p = 0.055$).

There were no significant differences in EGF system expression between endometrioid and non-endometrioid histological subtypes. Possibly, this was due to the small number of cases of non-endometrioid subtypes in our study.

A reverse correlation was found between tumor grade and c-erbB-1 expression (Pearson correlation = -0.2, $p = 0.040$). Tumor grade was not significantly correlated with the expression of the rest three receptors ($p = 0.317$, $p = 0.171$, $p = 0.206$ for c-erbB-2, c-erbB-3 and c-erbB-4 respectively). Stage of the tumor showed no relationship with the expression of the c-erbB receptors.

Kaplan-Meier plots revealed a significant association between c-erbB-1 expression and patient survival ($p = 0.011$). For the other three receptors, Kaplan-Meier plots showed a tendency toward decreasing survival with protein expression, though not statistically significant.

To evaluate the potent correlation between the molecule expression and patient outcome, a Cox regression analysis was also performed. According to this, c-erbB-1 is a significant independent prognostic factor for patient death (ExpB = 4.93, $p = 0.043$), which means that patients whose carcinomas express c-erbB-1 are 4.93 times more likely to die of their disease, compared with those whose tumors do not express c-erbB-1. C-erbB-1 was also significantly correlated with patient death (Pearson correlation 0.302, $p = 0.002$). In logistic regression analysis, c-erbB-1 was found to be a predictor factor for patient death ($p = 0.016$ ExpB = 0.140, 95% CI for ExpB: 0.028-0.693). C-erbB-2 was also strongly correlated with patients death in bivariate analysis (Pearson correlation 0.209, $p = 0.036$), but no significant association was found in multivariate Cox regression analysis.

No association emerged between c-erbB-3 and c-erbB-4 expression and patient death in either bivariate (Pearson correlation 0.131, $p = 0.195$) or multivariate Cox regression analysis ($p = 0.622$ and $p = 0.161$, respectively).

Discussion

The EGF system signaling network induces a wide variety of biologic responses and is involved in many physiologic and pathologic conditions. The biologic outcome of signaling through the EGF system depends on the cellular and environmental context. Dysregulation of the EGF signaling network is implicated in multiple human pathologies, of which the role of EGF in cancer is the best characterized, particularly for c-erbB-1 and c-erbB-2 [5].

The four receptors have different levels during the menstrual cycle. C-erbB-1 shows the highest value in the early proliferative phase, c-erbB-2 and c-erbB-4 in the early secretory phase and c-erbB-3 in the late secretory phase [9]. The results that emerged from our study indicate that the four receptors are also overexpressed in endometrial adenocarcinomas of postmenopausal women.

C-erbB-1 is localized to the basal part of the surface epithelial cells [6, 9], 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 c-erbB-1 was the only molecule whose expression was proven to have a prognostic factor for patient survival even in multivariate (Cox) analysis. Moreover its expression was correlated with tumor grade, a finding that agrees with other published studies.

C-erbB-2 is localized basolaterally, and solely to the glands and epithelium [9]. With no direct ligand identified to date, c-erbB-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 [12]. Overexpression of c-erbB-2 has been found to play a role in cellular transformation, tumorigenesis, and metastasis [13]. 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 [14], while it is also overexpressed in some stomach and ovarian carcinomas [15, 16]. Opinions in the literature regarding the prognostic value of c-erbB-2 overexpression in endometrial cancer are conflicting, and the marker has been correlated with unfavorable prognosis in some studies [17], but not in others [18]. Possible explanations of the lack of concordance in the prognostic value of c-erbB-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 c-erbB-2 as a marker in the preoperative evaluation of patients with endometrial cancer. According to our findings, c-erbB-2 seemed to have a strong relationship with patient death, though this relationship was not conserved in multivariate analysis. However its role in the appearance of a more aggressive phenotype of the tumor cells should always be kept in mind.

C-erbB-3 and c-erbB-4 are receptors activated by the neuregulin family of ligands, which comprises of a number of different isoforms of NEU differantiation factor/Heregulin [19].

C-erbB-3 is localized in the epithelium [9]. It is expressed in some breast and gastric carcinomas [20, 21], while little is known regarding its precise intracellular function. In our study the expression of c-erbB-3 receptor had no association either with tumor grade and stage, or with the outcome of the disease.

C-erbB-4 is the most recently identified member of the family, and is localized to epithelial and stromal cells [7-9, 22]. It is expressed in much adult and fetal tissue - lining epithelia of skin, gastrointestinal, urinary reproductive and respiratory tracts, skeletal muscle, circulatory, endocrine and nervous systems, as well as in the majority of ovarian cancers [22]. In our study the expression of c-erbB-4 receptor had no association either with tumor grade and stage, or with the outcome of the disease.

The most interesting observation of our study was the strong association of all four receptors with each other, which implies their potent common or consecutive activation and overexpression during the malignant process, maybe with a different role in each step of cell transformation.

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.

Conclusion

The c-erbB-1 gene has the ability to predict patient survival in endometrial cancer cases. 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 to gynecologic oncologists for definitive surgical treatment.

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