

HER-2/neu Overexpression and Hormone Dependency in Endometrial Cancer: Analysis of Cohort and Review of Literature*

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Abstract. *Background:* Overexpression of HER-2/neu (HER) is associated with unfavorable prognoses in both endometrial and breast cancer. *Materials and Methods:* To determine whether an association exists between HER expression and markers of hormone dependency in endometrial cancers, we subjected hysterectomy specimens from 125 patients to immunohistochemical staining for HER. HER was visually interpreted as negative/weakly positive (HER-) versus strongly positive (HER+). Estrogen receptor (ER) and progesterone receptor (PR) levels were quantitated on fresh tissue using a dextran-coated charcoal assay. *Results:* HER+ was observed in 12% of endometrioid tumors and 22% of nonendometrioid tumors ($p=0.07$). Mean ER and PR levels were 255 fmol/mg and 457 fmol/mg in endometrioid tumors, compared with 74 and 104 in nonendometrioid tumors ($p<0.01$). Hyperplasia associated with the tumor was related to high levels of both ER and PR ($p<0.05$), but not with HER expression. Age was significantly related to high levels of ER ($p=0.007$). Both recurrence and death rates were significantly associated with low levels of ER and PR ($p<0.01$). Mean ER and PR levels were 270 and 466 fmol/mg, respectively, in HER- tumors, compared with 95 ($p=0.14$) and 138 fmol/mg ($p=0.02$) in HER+ tumors. *Conclusion:* HER overexpression may be an important step in hormone-independent growth and proliferation in a subgroup of endometrial cancers.

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Endometrial cancer is the most common malignancy of the female reproductive tract in the United States and is exceeded annually in overall frequency only by cancers of the breast, colon and lung. It has been estimated that during the calendar year 2005, 40,880 new cases of endometrial cancer will be diagnosed and 7,310 deaths will occur (1).

Unfavorable prognoses are associated with HER-2/neu overexpression in both breast and endometrial cancer (2, 3). In breast cancer, HER-2/neu overexpression has been inversely correlated with levels of estrogen receptor (ER) and progesterone receptor (PR) (4). HER-2/neu overexpression appears to confer a growth advantage to neoplastic cells in a nonestrogenic environment (5) and a poor response to antiestrogenic treatment (4). The aim of this study was to determine whether an association exists between HER-2/neu expression and markers of hormone dependency in endometrial cancers.

Materials and Methods

Between 1984 and 1993, 815 surgically treated patients with endometrial cancer were accrued to the database at Mayo Clinic (Rochester, Minn, USA). Before the study, Institutional Review Board approval was obtained. We selected for review 612 cases with epithelial endometrial cancer who satisfied the following inclusion criteria: i) patients had been surgically managed with hysterectomy and removal of remaining adnexal structures and ii) they had not been diagnosed with another malignancy within 5 years before or after the diagnosis of endometrial cancer (with the exception of patients with carcinoma *in situ* or those with skin cancer other than melanoma). During the 10-year period, 299 of the above 612 patients had both initial preoperative endometrial sampling and definitive surgery at our institution. Sixteen of the 299 patients were removed from the study because clinicopathological data or histological blocks were not available. A cohort of 283 patients remained, from which 125 women were selected by using a case-cohort design, including all 49 women experiencing a recurrence and 76 randomly chosen progression-free patients. This was not a random sample of the whole cohort, but the study was done in accord with the design of Prentice (6).

We made the assumption that the randomly selected subgroup of 76 nonrecurrent cases was representative of the whole population of the 234 recurrence-free patients.

Statistically, every patient who did not have a recurrence had a higher weight than patients who had a recurrence. Thus, we extrapolated the results observed in the population of 125 patients for the overall population of 283. We had previously used this statistical design (with recurrence as outcome variable) for prediction of recurrence and survival in patients with endometrial cancer, based on immunohistochemical results reported on the preoperative endometrial biopsy (7). The current study used the same population as the previous analysis, but looked at a different outcome. As a consequence of the statistical design, most of the following results are reported as estimated percentages for the overall population, and not as actual numbers.

Histological characteristics were abstracted from the original pathology reports. The hematoxylin and eosin-stained slides of the hysterectomy specimens of the 125 selected cases were reviewed by 2 of us (TJS, GLK), who confirmed the histological diagnosis, assessed the tumor grade and subtype, and verified the presence of associated hyperplasia.

Staging was defined according to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system (8). In patients operated on before 1988, stage was retrospectively determined on the basis of postsurgical pathology reports. Histological classification was performed according to the World Health Organization classification (9). Architectural grading was based on the degree of glandular differentiation, in accordance with the FIGO guidelines (8). Hyperplasia associated with tumor was diagnosed when an area of hyperplasia was found adjacent to the tumor in the same sample.

Follow-up of patients was performed on the basis of information reported in the clinical histories. When information about survival and recurrence was not sufficiently detailed in the histories, death certificates were obtained and letters to patients and family physicians or telephone calls were used to obtain follow-up information. When death from disease was the end-point, we considered as censored all patients who were alive (with or without disease) at the time of follow-up or who had died of a cause not related to the disease, and we considered as uncensored patients who died of the disease. When recurrence of the disease was the end-point, censoring was at the date of last contact or death in case of no relapse, and we considered as uncensored all patients who had recurrence of the disease.

Analysis of molecular parameters. Hematoxylin and eosin-stained sections of tumor-containing paraffin blocks were reviewed by one of us (TJS) to identify the most representative block to cut.

The immunohistochemical staining technique used a modification of the avidin-biotin method reported by Hsu *et al.* (10). The paraffin blocks were cut in 5- μ m sections and mounted on silanized glass slides. The slides were dewaxed in 2 changes of xylene and rehydrated through graded solutions of ethanol. Endogenous peroxidase activity was blocked by treatment with 0.6% peroxide/methanol for 20 minutes at room temperature, followed by a rinse in tap water. All sections were subjected to heat-induced epitope retrieval in 1 mM EDTA, pH 8.0, for 20 minutes, using a steamer, followed by a 10-minute cooldown at room temperature.

Nonspecific binding sites were then blocked by incubation in 5% goat serum for 10 minutes at room temperature. Automated

immunohistochemical staining was performed on a Techmate 500. All detection reagents were obtained from Ventana Medical Systems and used according to the manufacturer's instructions. Staining was performed with primary antibodies against HER-2/neu (A0485, Dako Corp., diluted 1:1,600). Sections were incubated with primary antibodies for 60 minutes at room temperature. The slides were then rinsed and treated with biotinylated goat secondary antibodies for 20 minutes at room temperature. After another rinsing, the slides were treated with peroxidase-labelled avidin-biotin complex for 20 minutes at room temperature. After rinsing, the slides were exposed to diaminobenzidine/H₂O₂ for 15 minutes, rinsed again, counterstained in a diluted hematoxylin solution for 1 minute, dehydrated and mounted with a coverslip.

Grading of the slides for the cytoplasmic marker HER-2/neu included glandular staining intensity relative to the corresponding negative control slide (*i.e.*, endometrial tissue that did not stain for HER-2/neu). Scoring of HER-2/neu followed the guidelines developed for the Dako test method (11): 0, no staining was observed or membrane staining was seen in less than 10% of tumor cells; 1, faint, barely perceptible membrane staining was seen in no more than 10% of the tumor cells, with the cells being stained in only part of their membrane; 2, weak to moderate complete membrane staining was observed in more than 10% of the tumor cells; 3, moderate to strong complete membrane staining was observed in more than 10% of the tumor cells.

During quantitation of the cytoplasmic marker, the slides were subjectively assessed for their adequacy and preservation by one of us (TJS). At the time of the quantitation of the tissues, the reviewer was blinded regarding the other results of the analysis. Cases were eliminated from the analysis if the tissue was poorly preserved and hence the staining was uninterpretable, or if no tumoral tissue was present on the slide.

Analysis of hormone receptor (ER, PR) status. ER and PR levels, retrospectively abstracted from the histological reports, were available in 77 and 62 patients, respectively. ER and PR ligand binding levels had been assessed on fresh tissue with a dextran-coated charcoal assay, as previously described (12). The tissue that was immediately adjacent to the area where ER and PR were measured had been studied by hematoxylin and eosin histological examination in paraffin-embedded sections to confirm the presence of cancer. ER and PR levels were abstracted retrospectively from the pathology reports.

Statistical analysis. For statistical analysis, HER-2/neu was quantitated as a categorical variable, and patients with strong (3+) staining for HER-2/neu (HER+) were compared with all the other cases (*i.e.*, no staining, 1+, and 2+) (HER-). This was done according to our previous studies in which we compared HER-2/neu expression with prognosis (2, 7, 13). ER and PR were analyzed as continuous variables.

With regard to traditional histological variables, patients with histological grade 1 and 2 tumors were compared with those with grade 3 tumors. Furthermore, patients with endometrioid tumors were compared with patients who had all other subtypes. Wilcoxon rank-sum tests and Chi-square analysis were used for weighted statistics. Differences between groups were considered statistically significant at $p < 0.05$. SAS version 6.12 and S-Plus version 3.4 statistical software packages were used.

Table I. Associations between HER-2/neu expression, ER, and PR and other clinicopathological characteristics in patients with endometrial cancer.

Variables		Mean ER \pm SD* (n=77)	<i>p</i>	Mean PR \pm SD* (n=62)	<i>p</i>	% HER+	% HER-	<i>p</i>
Grade	1-2	291 \pm 343	0.002	552 \pm 835	<0.001	11	89	0.08
	3	165 \pm 322		204 \pm 251		19	81	
Histological subtype	Endometrioid	255 \pm 330	0.002	457 \pm 729	<0.001	12	88	0.07
	Nonendometrioid	74 \pm 98		104 \pm 167		22	78	
Associated hyperplasia	Yes	317 \pm 414	0.02	629 \pm 1,010	0.007	16	84	0.32
	No	149 \pm 237		274 \pm 367		10	90	
Age	\leq 55 yr	99 \pm 163	0.007	278 \pm 382	0.26	13	87	0.96
	>55 yr	293 \pm 358		478 \pm 733		13	87	
BMI	<30 kg/m ²	248 \pm 324	0.65	402 \pm 622	0.93	12	88	0.79
	\geq 30 kg/m ²	265 \pm 378		485 \pm 756		14	86	
HER-2/neu	0, 1+, 2+	270 \pm 361	0.14	466 \pm 734	0.02			
	3+	95 \pm 103		138 \pm 150				

*Measured in fmol/mg.

BMI, body mass index; ER, estrogen receptor; HER, HER-2/neu; PR, progesterone receptor; SD, standard deviation.

Results

The mean age (\pm standard deviation [SD]) of the 125 patients was 63.2 \pm 10.0 years (range, 26-88 years), and the mean body mass index (BMI) (\pm SD) was 29.9 \pm 8.7 kg/m² (range, 17.6-60.0 kg/m²). All patients were managed with hysterectomy and bilateral salpingo-oophorectomy. On the basis of the assessment of traditional pathological prognostic factors (including intraoperative frozen-section analysis), 82 patients were treated by lymphadenectomy; 58 patients received adjuvant radiation, 1 adjuvant cytotoxic chemo-therapy, and 8 adjuvant hormonal therapy.

During review of the immunohistochemically-stained slides, 6 cases were eliminated because of technical inadequacies (see Methods). ER binding levels were available in 77 of the 125 specimens and PR binding levels in 62. Overall, 74 hysterectomy specimens had information on both HER-2/neu staining and ER levels, and 59 had information on both HER-2/neu expression and PR levels.

We estimated that, in the overall population of 283 patients, 26% had histological grade 3, 19% nonendometrioid histological subtype, 44% associated hyperplasia, and 13% HER+. Twenty-one percent of patients were \leq 55 years of age, and 55% had a BMI <30 kg/m². Mean ER and PR levels were 254 fmol/mg (range, 0-883 fmol/mg) and 434 fmol/mg (range, 0-2,260 fmol/mg), respectively.

HER+ was associated (borderline significance) with grade 3 and nonendometrioid tumors. Although low levels of ER appeared to trend ($p=0.14$) with HER+, HER+ tumors were significantly ($p=0.02$) associated with low PR levels (Table I). ER and PR levels were inversely associated with grade 3 and nonendometrioid tumors ($p<0.01$), but both were increased in tumors associated with hyperplasia

($p<0.05$). Additionally, ER levels were higher in patients >55 years of age ($p=0.007$) (Table I).

The median time to recurrence for the uncensored subgroup was 7 months (range, 0-103 months), whereas the median follow-up of censored patients was 91 months (range, 4-151 months). One patient was lost to follow-up at 8 months after the operation; she was excluded from the analysis of survival. We estimated that of the 283 patients, 17% had recurrence, and 12% died of the disease. Age, BMI, and associated hyperplasia did not significantly influence survival. Recurrence and compromised survival were correlated ($p<0.01$) with grade 3 histology and nonendometrioid subtypes (Table II) and with low ER and PR levels (Table III). Moreover, HER+ was significantly associated with an adverse recurrence rate (Table II).

Discussion

Hormone-dependent breast cancer represents a prototype for investigating the role of hormones in cancer development. Estrogens are physiological mitogens for the mammary cells; they can stimulate cell growth by modulating the expression of oncogenic growth factor receptors (14). About one-third of invasive breast carcinomas are responsive to antiestrogenic therapy (15). However, invasive cancers can become estrogen independent, presumably through the acquisition of genetic changes (16). In fact, during the first steps of malignant transformation, epithelial cells undergo numerous mitoses, with subsequent progressive accumulation of genetic alterations such as HER-2/neu amplification and p53 deletion (14).

Like breast tumors, endometrial cancer is generally a hormone-responsive neoplasm (17). However, there are 2

Table II. Recurrence and survival frequencies according to single variables in patients with endometrial cancer.

Variables		% with recurrence	p	% DOD	p
Grade	1-2	12	0.001	7	0.001
	3	29		26	
Histological subtype	Endometrioid	12	0.001	7	0.001
	Nonendometrioid	33		31	
Associated hyperplasia	Yes	15	0.26	15	0.35
	No	22		10	
Age	≤55 yr	15	0.67	9	0.39
	>55 yr	18		13	
BMI	<30 kg/m ²	18	0.58	12	0.88
	≥30 kg/m ²	16		13	
HER-2/neu	0, 1+, 2+	14	0.03	10	0.10
	3+	29		20	

BMI, body mass index; DOD, dead of disease.

categories of endometrial cancer. Tumors of the most common type (type 1) develop in an estrogenic environment, are well-differentiated, have an endometrioid histological subtype, are associated with high levels of hormone receptors, and usually respond to hormonal therapy with progestins. Such tumors generally arise in a background of endometrial hyperplasia. Tumors of the second type (type 2) are poorly-differentiated, are generally of a nonendometrioid histological subtype, frequently lack steroid receptors, are more likely to recur after treatment, and are not responsive to antiestrogenic therapy. These tumors often arise within atrophic endometrium. HER-2/neu overexpression is more common in the second type of endometrial cancer (18).

Steroid hormone receptors are intracellular proteins that are necessary for the function of steroids within the cell (19). In endometrial cancer, ER and PR are inversely correlated with grade, stage, and histological tumor type (20-22). The prognostic value of hormone receptor status in endometrial cancer has previously been demonstrated in a number of studies (21, 22). The presence of PR in tumor cells is a stronger prognostic factor than ER (21), because its expression is related both to more favorable differentiation (22) and to hormone responsiveness (22, 23).

The HER-2 oncogene is one of the most frequently altered genes in human cancer. It encodes a 1,255-amino acid, 185-kDa receptor tyrosine kinase (p185neu). The protein is a transmembrane receptor, whose overexpression is associated with malignant transformation (24). The reported percentage of endometrial cancers that overexpress HER-2/neu protein varies considerably (2, 12, 25-28). HER-2/neu overexpression is predictive of

Table III. Recurrence and survival frequencies according to estrogen receptor (ER) and progesterone receptor (PR) levels.

	ER, mean, fmol/mg	p	PR, mean, fmol/mg	p
Recurrence				
Yes	126		163	
No	288	< 0.001	508	< 0.001
Dead of disease				
Yes	130		131	
No	278	< 0.01	499	< 0.001

unfavorable outcomes in some studies (2, 12, 25, 27, 28), but not in others (25). A possible explanation for the lack of concordance in the literature with respect to HER-2/neu protein expression includes differences in populations, techniques, antibodies used and interpretation of results (28). For our analysis, we used an antibody that has been approved by the Food and Drug Administration for determining a patient's HER-2/neu status (29).

In our analysis, we used a previously reported population of patients with endometrial cancer (7). The main advantage of using the case-cohort design was that we were able to estimate the results in a consecutive series of patients, thus eliminating the selection bias due to the inclusion in the study group of all patients who had recurrence of disease and only some of recurrence-free patients. Moreover, the estimation of results in the overall population of 283 patients permitted us to base the statistical results on an extrapolated higher number of patients. However, according to the statistical design, we needed to assume that the limited group of 76 patients who did not have recurrence and who are included in the present analysis are representative of all patients without recurrence in the overall population. Therefore, all the results are based on an estimation and are reported as percentages and not as actual numbers.

In our retrospective analysis, the levels of ER and PR were measured with a dextran-coated charcoal assay. This technique has now been replaced at our institution by immunohistochemical assay. Immunohistochemical assay permits one to make a qualitative estimation of the distribution and cellular localization of the receptors, with the possibility of a semiquantitative evaluation of receptor presence based on the intensity of immunostaining and the fraction of tissue stained by the antibodies. The immunohistochemical technique permits a more direct histological control of receptor determination than the quantitative techniques, because nonmalignant tissue components may contribute to the total amount of receptors

determined in an endometrial tumor biopsy (19). However, in our series, the tissue that was immediately adjacent to the biopsy submitted for ER and PR quantitation was usually studied by histological examination in paraffin-embedded sections to confirm the presence of cancer. This reduced the probability of measuring nonmalignant tissue. Moreover, ligand-binding assays show excellent correlation with immunohistochemical assays, with a higher sensitivity than immunohistochemistry for detecting both ER and PR (19).

In the present study, we compared ER/PR expression with various indicators of hormone dependency in endometrial cancer, including histological grade and subtype, presence of tumor-associated hyperplasia, BMI and age (Table I). High-grade tumors with nonendometrioid histological subtypes are more likely to have hormone-independent growth (30). Moreover, endometrial cancers associated with hyperplasia are usually hormone-dependent tumors and are associated with a good prognosis (31). Furthermore, younger patients with a high BMI are more likely to have hormone-dependent tumors (32), high levels of circulating estrogens (33), and a high PR/ER ratio (34). In the present study (Table I), we confirmed that low levels of ER and PR are significantly associated with high-grade and nonendometrioid tumors (21). Furthermore, the presence of tumor-associated hyperplasia was significantly related to high levels of hormone receptors. However, BMI and age were not significantly related to PR levels, but low levels of ER were observed in younger patients with endometrial cancer (Table I). The latter finding presumably reflects higher serum levels of estradiol, associated with the hormonal milieu in premenopausal women, and the corresponding lower percentage of ER-positive endometrial cancers (35). Similarly, ER levels are significantly higher in older breast cancer patients (36).

In the present study, HER-2/neu overexpression was associated (borderline significance) with high-grade tumors and nonendometrioid histological subtypes (Table I). Furthermore, a poor prognosis correlated with tumors demonstrating nonendometrioid/high-grade histology, low levels of hormone receptors, and HER-2/neu overexpression (Tables II and III). All the above findings, which are in accord with the literature (2, 18, 21, 23, 27), suggest a possible role of HER-2/neu in the carcinogenesis and growth of a subset of high-risk type-2 endometrial cancers.

The most cogent observation in this study is that HER-2/neu overexpression was significantly associated with low levels of PR (Table I), suggesting that HER-2/neu may have a functional role in hormone-independent proliferation in a subgroup of endometrial cancers. It is recognized that expression of PR is up-regulated by estradiol, both in the normal endometrium (37) and in ER-positive endometrial cancer cells (34); progesterone, through the PR, modulates the growth-stimulatory effect

of estrogens (38). Failure of estradiol to induce PR may be a factor in the pathogenesis and/or progression of endometrial cancer (39, 40), signifying hormone-independent growth. Potentially, amplification of HER-2/neu may initiate kinase cascades that can lead to site-specific phosphorylation of hormone receptors and trigger receptor activation or inactivation of steroid-responsive genes in the absence of specific ligands (41). Because PR-positive tumors are more likely to respond to hormonal therapy compared to PR-negative endometrial cancers (22, 23), it is conceivable that HER-2/neu overexpression down-regulates the progesterone receptor ligand interaction. In fact, in breast cancer, clinical studies have shown that the response rate to tamoxifen is reduced in ER-positive tumors that overexpress HER-2/neu (42), suggesting that overexpression of HER-2/neu is a better predictor of responsiveness to endocrine therapy than is hormone receptor status itself (43). However, on the basis of our findings, we cannot demonstrate that there is a direct interaction between PR and HER-2/neu. It is possible that loss of PR in HER-2/neu-positive cases is simply a consequence of loss of differentiation in these more virulent cancers.

We did not find any significant association between HER+ tumors and ER levels in endometrial cancer (Table I). This contrasts with reports from other authors (27, 44). This observation might be explained by the differential expression of ER and PR – a more selective expression of PR as opposed to a more general expression of ER– in endometrial tumors (45). Furthermore, in a subset of ER-positive low-grade endometrial cancers, HER-2/neu overexpression may be associated with the presence of ER. Evidence suggests that HER-2/neu may be induced by estradiol and facilitate hormone-dependent cancer growth and that its expression may not be influenced by progesterone (46, 47).

The fact that overexpression of HER-2/neu may reflect escape from normal steroid hormone-related growth regulatory pathways in endometrial cancer has previously been suggested (26, 27). High HER-2/neu expression has been found to be significantly associated with absence of ER (27, 44) and PR (26, 44) by some authors, but not by others (48, 49). In particular, Bigsby *et al.* (26) noted that some endometrial tumors had heterogeneous expression of both HER-2/neu and ER/PR. The cells exhibiting HER-2/neu overexpression were PR negative. Conversely, cells that were PR positive had little or no HER-2/neu expression. However, differences in methodology do not permit a comparison among the various reported studies (Table IV).

Thus, HER-2/neu overexpression may be an important component in hormone-independent growth and proliferation in a subgroup of endometrial cancers.

Table IV. Review of the literature on the association between HER-2/neu and ER/PR.

Study	No. of patients	Inverse correlation with ER/PR	Measurement of ER/PR	Measurement of HER-2/neu
Berchuck <i>et al.</i> (27)	95	ER	DCC; f; D (≤ 7.5 vs. > 7.5 fmol/mg)	TA-1–Applied bioTechnology; f; D (0, 1+, 2+ vs. 3+)
Bigsby <i>et al.</i> (26)	49	PR ^a	Abbott; f; D (–vs. +)	TA–New England Nuclear; f; D (0, 1+, 2+, 3+ vs. 4+)
Wang <i>et al.</i> (44)	34	ER/PR ^b	Abbott; f; D (–vs. +)	Triton Diagnostics; f; D (0, 1+, 2+ vs. 3+)
Backe <i>et al.</i> (48)	222	No	Abbott; pe; D (IRS ≤ 2 vs. IRS > 2)	CB-11–BioGenex; pe; D (IRS ≤ 2 vs. IRS > 2)
Niederacher <i>et al.</i> (49)	112	No	n.a.; pe; D (–vs. +)	PCR/DNA Seq; pe; D (–vs. +)
Present study	283 ^c	PR	DCC; f; C	A0485–Dako Corp; pe; D (0, 1+, 2+ vs. 3+)

^aBoth statistical and histological correlations (*i.e.*, cells with HER overexpression were PR-negative and *vice-versa*).

^bNo topologic correlation of c-erbB-2 protein or EGFR expression with sex steroid receptor localization."

^cWe analyzed 125 patients with weighted statistics, estimating the data for the overall population of 283 patients.

C, continuous; D, dichotomous; DCC, dextran-coated charcoal; ER, estrogen receptor; f, fresh/fresh frozen; IRS, immunoreactive score (= product of staining intensity and percentage of positive cells); n.a., not available; PCR/DNA Seq, polymerase chain reaction/DNA sequencing; pe, paraffin-embedded; PR, progesterone receptor.

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