International Journal of Gynecological Pathology
28:19–22, Lippincott Williams & Wilkins, Baltimore

Original Article

Gonadotropin-releasing Hormone Receptor Expression in Endometrial Cancer

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Summary: Several studies have previously reported the expression of the gonadotropin-releasing hormone receptor (GnRHr) in cases of endometrial cancer. However, the relationship between GnRHr expression and a variety of clinicopathologic parameters remains unclear. This study was conducted with 141 endometrial cancer patients, all of whom had undergone operations between 1993 and 2002. Paraffin-embedded tissue blocks were sectioned and immunostained with monoclonal anti-GnRHr antibody. Clinicopathologic variables were also evaluated, with 10% cutoff values for GnRHr positivity. Seventy specimens (49.6%) stained as GnRHr-positive. Mean parity was higher in the patients with GnRHr-positive tumors than those with GnRHr-negative tumors (2.50 ± 1.92 versus 1.82 ± 1.37, $P = 0.016$). Body mass indices were also higher in the patients with GnRHr-positive tumors (26.6 ± 4.6 versus 24.7 ± 4.2, $P = 0.010$). However, GnRHr positivity was not determined to be statistically significantly associated with any other clinicopathologic characteristics, including age, menopausal status, histotype, disease stage, tumor differentiation, lymph node metastasis, and myometrial invasion. The results of this study, although they may require further investigation, suggested that obese and multiparous women with endometrial cancer might be greatly influenced by endogenous gonadotropin-releasing hormone and/or exogenous gonadotropin-releasing hormone analogs. **Key Words:** Endometrium—Carcinoma—Gonadotropin-releasing hormone receptor.

Endometrial cancer is the most common malignancy of the female genital tract in developed countries. Although this malignancy frequently presents as an early stage disease, advanced and recurrent cases of this disease represent a persistent problem, due to the refractory nature of this condition to cytotoxic chemotherapy or radiotherapy. On the basis of hormonal dependency of endometrial cancer, progestin has been employed for treatment, and the gonadotropin-releasing hormone (GnRH) and its receptor have recently moved into the limelight as a new potential target for the treatment of endometrial cancer.

The hypothalamic decapeptide, GnRH, performs an important role in the control of mammalian reproduction, and binds specifically to the gonadotropin-releasing hormone receptor (GnRHr) (1). Previously, the identification of GnRHr expression in human reproductive tissues and human gynecologic cancers perpetuated a number of investigations of the possible use of GnRH agonists and antagonists in endometrial cancer patients (2). However, although the results of several previous studies reported

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This work was supported by Cancer Research Institute, Seoul National University College of Medicine (CRI-06-05).

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DOI: 10.1097/PGP.0b013e318178e54f

approximately 80% of GnRHr expression in cases of endometrial cancer, these results were the product of studies involving a very small series of cancer tissues (3,4). Moreover, it is currently uncertain as to what is the most salient issue to be considered during the selection of possible candidates for GnRH agonist or antagonist therapy among diverse patients with this malignancy. Thus, we evaluated GnRHr expression in a large series of endometrial cancer patients, and analyzed the association of GnRHr expression with several clinical characteristics.

MATERIALS AND METHODS

In this study, we used archived paraffin-embedded tissue blocks obtained from 141 patients with endometrial cancer. The patients in this study were the same as those previously described (5), with the exception of 3 stage I patients and a stage IV patient whose tissue blocks evidenced no remaining cancer tissue. The mean patient age was 51.0 years (range: 26–76 yr). Staging was assigned according to the classifications established by the 1988 International Federation of Gynecology and Obstetrics (FIGO) surgical staging for endometrial cancer: stage I, n = 103 (73.0%); stage II, n = 13 (9.2%); stage III, n = 17 (12.1%); and stage IV, n = 8 (5.7%). Tumor grading was performed by a gynecologic pathologist (So Yeon Park) using FIGO grading system proposed in 1989.

Immunohistochemical staining was performed by using the ABC method using formalin-fixed, paraffin-embedded tissue sections as previously described (5–7). Tissue sections (4-μm) were mounted on silanized slides and dried at 37°C overnight. The sections were deparaffinized in xylene and sequentially washed using graded ethanol and phosphated-buffered saline (pH 7.4). After pretreatment of 10 mmol/L sodium citrate (pH 7.0) in a microwave for 15 minutes, endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 minutes and the samples were preincubated with protein blocking solution for 10 minutes. All slides were incubated with primary anti-human GnRHr monoclonal antibody (unconjugated, clone 0.N.313, United States Biological, Swampscott, Massachusetts, dilution, 1:100) for 60 minutes at room temperature in a humid chamber. Incubation with biotinylated link antibody (DAKO A/S, Copenhagen, Denmark), reaction with avidin/biotinylated horseradish peroxidase solution, and counterstaining with Mayer's hematoxylin were followed.

A specialized gynecologic pathologist (So Yeon Park), who was blinded to the clinical features of the patients, reviewed the slides and quantified the immunohistochemical data. All the tumor cells in the slides were reviewed and judged as positively stained or not. The percentages of GnRHr-expressing cells were estimated by dividing the number of positively stained tumor cells by the total number of tumor cells. We used an arbitrarily determined cutoff value, 10%, for the determination of the GnRHr-positive specimens.

Statistical analyses were conducted using SPSS for Windows software, version 11.0 (SPSS Inc, Chicago, Illinois). The association between variables was evaluated via the χ^2 test or the t-test where applicable. $P < 0.05$ was considered to be statistically significant.

RESULTS

Seventy (49.6%) among 141 specimens were stained as GnRHr-positive (Table 1). There were 71

TABLE 1. *Clinicopathologic characteristics according to GnRH receptor expression*

	GnRH receptor expression		
Characteristics	Negative	Positive	*P*
Patients (n [%])	71 (50.4)	70 (49.6)	
Age, yr (mean ± SD)	49.9 ± 11.9	52.1 ± 10.6	NS
Parity (mean ± SD)	1.82 ± 1.37	2.50 ± 1.92	0.016
BMI, kg/m^2 (mean ± SD)	24.7 ± 4.2	26.6 ± 4.6	0.010
Menopausal status (n [%])			NS
Premenopausal	36 (55.4)	29 (44.6)	
Postmenopausal	35 (46.1)	41 (53.9)	
Histotype (n [%])			NS
Endometrioid	70 (51.1)	67 (48.9)	
Others*	1 (25.0)	3 (75.0)	
FIGO stage (n [%])			NS
I, IIa	56 (51.4)	53 (48.6)	
IIb, III, IV	15 (46.9)	17 (53.1)	
Grade (n [%])			NS
1	42 (51.9)	39 (48.1)	
2	15 (44.1)	19 (55.9)	
3	14 (53.8)	12 (46.2)	
Lymph node metastasis†(n [%])			NS
Negative	47 (47.0)	53 (53.0)	
Positive	9 (60.0)	6 (40.0)	
Myometrial invasion (n [%])			NS
Less than half	51 (49.0)	53 (51.0)	
More than half	20 (54.1)	17 (45.9)	

*Papillary serous or clear cell type.
†Lymph node dissection was not performed in 26 cases.
*,†Small subgroup rendered low statistical power less than 20% (retrospective calculation).
BMI indicates body mass index; FIGO, International Federation of Gynecology and Obstetrics; GnRH, gonadotropin-releasing hormone; NS, not significant.

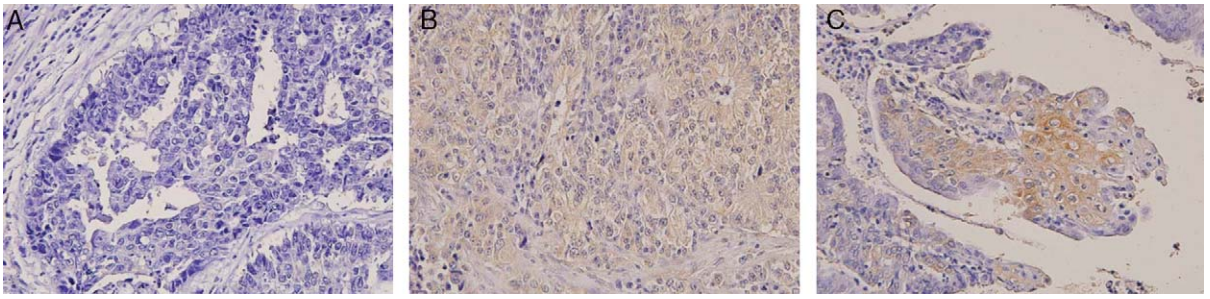

FIG. 1. A, Endometrial cancer with no expression of gonadotropin-releasing hormone receptor (GnRHr). B, Poorly differentiated tumor cells with diffuse cytoplasmic immunostain for GnRHr. C, Focal intense expression of GnRHr in well-differentiated carcinoma (hematoxylin and eosin, original magnification: 400 ×).

specimens stained less than 10% of tumor cells and they were categorized as GnRHr-negative. Among GnRHr-positive specimens, 39 had 10% to 50% of stained tumor cells and 31 had 50% to 90%, respectively. Tumor cells evidenced diffuse cytoplasmic immunoreactivity for GnRHr (Fig. 1). Mean parity was higher in patients with GnRHr-positive tumors than those with GnRHr-negative tumors (2.50 ± 1.92 versus 1.82 ± 1.37, $P = 0.016$). Body mass indices (BMIs) were also found to be higher in patients with GnRHr-positive tumors (26.6 ± 4.6 versus 24.7 ± 4.2, $P = 0.010$). However, GnRHr positivity was determined not to be statistically significantly associated with any other clinicopathologic characteristics, including age, menopausal status, histotype, disease stage, tumor differentiation, lymph node metastasis, and myometrial invasion.

DISCUSSION

GnRH binds to its receptor in the anterior pituitary and regulates the production and release of luteinizing hormone and follicular-stimulating hormone and, consequently, steroid hormone secretion from the gonads. Continuous GnRH agonist therapy suppresses the pituitary gonadal axis via the downregulation and desensitization of its own receptors and this was the basic rationale for the utilization of GnRH agonists in the treatment of hormone-dependent tumors including prostate, breast, endometrial, and ovarian cancers (8). The detection of GnRHr in other tissues, including the breast, ovary, endometrium, placenta, and prostate suggested that GnRH agonists and antagonists might also exert direct actions on peripheral targets (9).

However, as was described in the previous section, reports regarding GnRHr expression in endometrial cancer were conducted using only a very small series of cancer specimens (3,4). A recent report by Yue et al. (10) also involved only 30 endometrial cancer tissue specimens. By way of contrast, the present study employed a large series of patients (141 specimens), and included additional analysis for clinicopathologic characteristics, although the only significant results obtained were the parity and BMI results. Regarding histotype and lymph node metastasis, however, we should cautiously interpret the statistical result because there is so small subgroup to reach appropriate statistical power.

As the majority of patients with endometrial cancer underwent oophorectomies during surgical staging, the direct actions of GnRH analogs on peripheral tissues seem to be critical to the intended treatments, and many authors have reported that the antitumor effects of GnRH analogs seem to be exerted directly on GnRHr in tumors (2). According to our results, approximately 50% of patients with endometrial cancer may potentially benefit from the GnRH analog therapy. In addition, high parity and BMI may constitute useful criteria in the selection of candidates for the therapy among a variety of endometrial cancer patients, although the exact relationship between GnRHr and parity and BMI currently remains to be determined.

Approximately 70% of endometrial cancer cases are detected in stage I of the disease, thus resulting in the relatively favorable prognosis of this malignancy as compared with that of other gynecologic malignancies, such as ovarian cancer (11). However, some carcinomas manifest aggressive behavior even in stage I of the disease, and recurrent and advanced diseases often prove incurable, because this tumor is generally refractory to chemotherapeutic agents and ionizing radiation. Progestins are frequently used in endometrial cancer patients, because atypical endometrial hyperplasias and endometrial carcinomas, especially those of the well-differentiated

endometrioid type, often express progesterone receptors (PRs), and their growth is suppressed by progestins (11). According to our analyses of compiled data, coupled with the results of our previous study on steroid receptor expressions in endometrial cancer cases (5), 88.7% (125/141) of our patients exhibited PR and/or GnRHr expression. Correlation between estrogen receptor/PR expression and GnRHr expression was not observed. This indicates that therapy with progestin and/or GnRH analogs may prove helpful to the majority of endometrial cancer patients and, in addition, GnRH analog therapy may be applicable in patients with advanced disease because no significant differences in GnRHr expression were detected according to pathologic type, stage, grade, lymph node metastasis, and depth of myometrial invasion in our study.

In summary, the result of this study indicated that GnRHr expression in endometrial cancer might be lower than that reported in previous studies, but also suggested that GnRHr targeted therapy might benefit patients with aggressive disease, and this benefit may be more common in obese and multiparous women.

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