Short Communication

Cyclooxygenase-2 and p53 Expressions in Endometrial Cancer

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Abstract

Cyclooxygenase-2 (COX-2) has been known to be related with various types of carcinoma, but we have insufficient knowledge about the association between COX-2 and endometrial cancer. Many have reported a close relationship between p53 expression and a poor prognosis in endometrial cancer, but it is unclear whether p53 is an independent prognostic factor. To clarify these uncertainties, we examined the expressions of COX-2 and p53 in endometrial cancer tissues. The study was carried on 152 endometrial cancer patients who had operation at Seoul National University Hospital. Paraffin-embedded tissue blocks were sectioned and immunostained using monoclonal anti-COX-2 and anti-p53 antibodies. Twenty-seven (17.8%) specimens stained as COX-2 positive. COX-2 positivity was more frequently observed in postmenopausal patients than in premenopausal patients (8.8% versus 25.0%; P = 0.009). However, COX-2 positivity did not show a statistically significant association with any other clinicopathologic characteristic (parity, body mass index, histotype, International Federation of Gynecology and Obstetrics stage, grade, lymph node metastasis, deep myometrial invasion, or p53 overexpression). Thirty-one (20.4%) specimens showed p53 overexpression and this was significantly correlated with an advanced stage (P = 0.001), poor differentiation (P < 0.001), lymph node metastasis (P = 0.012), and deep myometrial invasion (P < 0.001). Multivariate Cox regression analysis showed that advanced stage was an independent prognostic factor of survival, but p53 overexpression was not. COX-2 may be associated with endometrial cancer carcinogenesis during the postmenopausal period but not with tumor aggressiveness and p53 overexpression. The p53 overexpression was found to be strongly associated with endometrial cancer aggressiveness. (Cancer Epidemiol Biomarkers Prev 2004;13(9):1538–42)

Introduction

Endometrial cancer is the most common malignancy of the female genital tract in developed countries and is expected to account for 6% of new cancer cases among women in the United States in 2003 (1). Korea has experienced a remarkable increase in endometrial cancer incidence, that is, 132 patients in 1991 and 581 patients in 2001 according to the tumor registry (2).

Recently, much attention has been focused on cyclooxygenase (COX), which is a rate-limiting enzyme in the biosynthesis of prostaglandins from arachidonic acid. Two isoforms of COX are known, COX-1 and COX-2, both of which participate in the formation of a variety of eicosanoids, including prostaglandins D2, E2, I2, and F2α, and thromboxane A.

COX-2 is an inducible immediate-early gene, which is up-regulated by various stimuli, including mitogens, cytokines, growth factors, and tumor promoters (3). Several studies have shown that COX-2 expression is aberrantly increased in various human epithelial cancers, including those of colorectal, esophageal, gastric, lung, and bladder origin (4–9). These findings suggest that the cellular up-regulation of COX-2 may be a common mechanism in epithelial carcinogenesis. In addition, many studies have shown that COX-2 expression reduces apoptosis, increases the invasiveness of malignant cells, and promotes angiogenesis (10–15). To our knowledge, two reports are available on the expression of COX-2 and its possible clinical significance in patients with endometrial cancer (16, 17), but they have shown quite different results each other.

p53 is often called the cellular gatekeeper or as the guardian of the genome, and its importance has been emphasized by the discovery of mutations in p53 in >50% of all human tumors. The nature of these genetic changes in cancer cells is most commonly a missense mutation in one allele, producing a faulty protein that is observed at high concentrations in these cells followed by a reduction to homozygosity (18).
In endometrial cancer, mutations of the p53 tumor suppressor gene have been found in ~10% to 20% and more frequently in serous papillary and clear cell histologic types (19). Although the use of the immunohistochemical expression of p53 as an indication of the level of p53 mutation presents problems in terms of false positivity and negativity (20), many clinical correlated studies of p53 immunostaining have reported. The abnormal expression of p53 has been associated with an advanced stage and poor survival by several studies, although some have reported that the overexpression of p53 is not an independent prognostic factor in endometrial cancer (21-31). In addition, several reports suggested that p53 and COX-2 might have close relationship (32-34).

To clarify these uncertainties, in the present study, we examined the expressions of COX-2 and p53 in endometrial cancer and their relationships with clinicopathologic characteristics.

Materials and Methods

This study included 152 endometrial cancer patients with archived paraffin-embedded tissue blocks. All patients were admitted, treated, and followed up at the Department of Obstetrics and Gynecology, Seoul National University Hospital. Mean patients’ age was 50.9 years (range 26–76 years), and no patient had history of regular drug ingestion such as nonsteroidal anti-inflammatory drugs or COX-2 inhibitors. Staging was done according to the 1988 International Federation of Gynecology and Obstetrics classification: stage I, n = 112 (73.7%); stage II, n = 13 (8.6%); stage III, n = 18 (11.8%); and stage IV, n = 9 (5.9%). Two to 3 weeks after staging laparotomy, all the patients, except stage Ia with grade 1 or 2, received a radiation therapy. Patients with stage IV disease had six or more cycles of cisplatin-based chemotherapy after radiation. As of August 2003, the median follow-up period was 33.5 months (range 1–124 months). During the follow-up period, nine died of the disease and eight experienced disease recurrence. Of those that experienced recurrence, four died of the disease and four were alive with disease.

Immunohistochemical staining was done as described previously (14, 15) using the following primary antibodies: COX-2 (mouse monoclonal, clone 33, 1:50 dilution, Transduction Laboratories, Lexington, KY), and p53 (mouse monoclonal, clone DO-7, 1:50 dilution, DAKO A/S, Copenhagen, Denmark).

A specialized pathologist blind to the patients’ clinical features reviewed slides and quantified the immunohistochemical data. The percentage of cells expressing COX-2 was estimated by dividing the number of positively stained tumor cells by the total number of tumor cells. For COX-2 expression, if 5% (median value) or more tumor cells were stained, the sample was defined as positive. For p53 overexpression, when there were at least 10% (median value) and stage IV, n = 9 (5.9%). Two to 3 weeks after staging laparotomy, all the patients, except stage Ia with grade 1 or 2, received a radiation therapy. Patients with stage IV disease had six or more cycles of cisplatin-based chemotherapy after radiation. As of August 2003, the median follow-up period was 33.5 months (range 1–124 months). During the follow-up period, nine died of the disease and eight experienced disease recurrence. Of those that experienced recurrence, four died of the disease and four were alive with disease.

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Statistical analyses were carried out using SPSS for Windows version 10.0.5 (SPSS Inc., Chicago, IL). The association of variables was evaluated by the \( \chi^2 \) test or the \( t \) test if applicable. Log rank test and multivariate analysis with Cox regression were used for survival analysis. \( P < 0.05 \) was considered statistically significant.

Results

COX-2 immunoreactivity of normal endometrial gland and stroma was minimal irrespective of menstrual phase (proliferative or secretory). In contrast, tumor cells showed significant immunoreactivity for COX-2. Of the 152 specimens, 27 (17.8%) were COX-2 positive. Patients showing COX-2 positivity were older and more frequently postmenopausal than those showing COX-2 negativity (\( P = 0.009 \)). However, COX-2 positivity was not found to show a statistically significant association with any other clinicopathologic characteristic or p53 overexpression (Table 1).

Thirty-one (20.4%) specimens showed p53 overexpression, and this was significantly associated with advanced stage (stage III or IV; \( P = 0.001 \)), poor differentiation (grade 2 or 3; \( P < 0.001 \)), lymph node metastasis (\( P = 0.012 \)), and deep myometrial invasion (\( P < 0.001 \); Table 2).

Log rank test showed that old age (≥65 years; \( P = 0.02 \)), advanced stage (\( P < 0.001 \)), poor differentiation (grade 2 or 3; \( P < 0.001 \)), lymph node metastasis (\( P < 0.001 \)), deep myometrial invasion (\( P < 0.001 \)), and p53 overexpression (\( P < 0.001 \)) were related to patient survival. All the covariates mentioned above and COX-2 expression were considered to be independent predictors of survival.

Table 1. Clinicopathologic characteristics according to COX-2 expression

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>COX-2 expression</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Patients [n (%)]</td>
<td>125 (82.2)</td>
<td>27 (17.8)</td>
</tr>
<tr>
<td>Age, y (mean ± SD)</td>
<td>49.8 ± 11.0</td>
<td>55.9 ± 10.0</td>
</tr>
<tr>
<td>Parity (mean ± SD)</td>
<td>2.1 ± 1.6</td>
<td>2.5 ± 2.0</td>
</tr>
<tr>
<td>Body mass index, kg/m² (mean ± SD)</td>
<td>25.5 ± 4.5</td>
<td>26.3 ± 3.7</td>
</tr>
<tr>
<td>Menopausal status [n (%)]</td>
<td>62 (91.2)</td>
<td>6 (8.8)</td>
</tr>
<tr>
<td>Prenomenopausal</td>
<td>63 (75.0)</td>
<td>21 (25.0)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histotype [n (%)]</td>
<td>121 (82.3)</td>
<td>26 (17.7)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>12 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Others*</td>
<td>4 (80.0)</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>International Federation of Gynecology and Obstetrics stage [n (%)]</td>
<td>103 (82.4)</td>
<td>22 (17.6)</td>
</tr>
<tr>
<td>I, II</td>
<td>61 (61.5)</td>
<td>27 (26.5)</td>
</tr>
<tr>
<td>III, IV</td>
<td>31 (31.0)</td>
<td>18 (18.0)</td>
</tr>
<tr>
<td>Grade [n (%)]</td>
<td>1</td>
<td>74 (84.1)</td>
</tr>
<tr>
<td>2 or 3</td>
<td>14 (15.9)</td>
<td>13 (13.0)</td>
</tr>
<tr>
<td>Lymph node metastasis [n (%)]</td>
<td>92 (83.6)</td>
<td>18 (16.4)</td>
</tr>
<tr>
<td>Negative</td>
<td>10 (66.7)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myometrial invasion [n (%)]</td>
<td>97 (84.3)</td>
<td>18 (15.7)</td>
</tr>
<tr>
<td>Less than half</td>
<td>28 (75.7)</td>
<td>9 (24.3)</td>
</tr>
<tr>
<td>More than half</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53 overexpression [n (%)]</td>
<td>100 (80.0)</td>
<td>25 (20.0)</td>
</tr>
<tr>
<td>No</td>
<td>21 (77.8)</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*By the \( t \) test or the \( \chi^2 \) test.

*Papillary serous or clear cell type.

*Lymph node dissection was not done in 27 cases.
were included in multivariate Cox regression analysis, and it showed that advanced stage was an independent prognostic factor of survival but not p53 overexpression (Table 3).

**Discussion**

In this study, high COX-2 expression was observed more frequently in postmenopausal patients than premenopausal women with endometrial cancer. Although the exact cause of this difference is unknown, it can be supposed that endometrial cancer arising from the postmenopausal endometrium may have quite a different regulatory system of COX-2 or may use the COX-2 gene more frequently than a cancer from the perimenopa-usal or normal cyclic endometrium. This postulation corresponds well with the long-standing knowledge that there are two different pathogenetic types of endometrial cancer. The most common type occurs in younger, perimenopausal women with a history of exposure to unopposed estrogen, either endogenous or exogenous. The other type of endometrial cancer occurs in women with no source of estrogen stimulation of the endome-trium (35). Therefore, this study may provide a molecular basis on the genesis of two different pathogenetic types of endometrial cancer and may be grounds for further studies on the preventive effect of nonsteroidal anti-inflammatory drugs or COX-2 inhibitors on endometrial cancer as it occurs in postmenopausal women.

As commented in Introduction, two studies on COX-2 expression and clinical significance in endometrial cancer showed quite different results each other. Fujiwaki et al. (16) analyzed 63 patients with endometrial cancer and found that COX-2 immunoreactivity did not correlate with any clinical variables (menopausal status, International Federation of Gynecology and Obstetrics stage, histologic type, tumor size, histologic grade, or myometrial invasion) or with prognosis. Ferrandina et al. (17) studied 69 patients with endometrial cancer in Italy and reported that COX-2 positivity was higher in endometrial cancer with cervical or extraterine involvement than in tumors limited to the corpus. They also found that COX-2 positivity gradually increased from grade 1 to grade 3 endometrial carcinoma, and COX-2 positive patients tended to have shorter disease-free survivals than COX-2-negative patients, although this was not statistically significant. The cause of the disagreement between these two studies is unknown, but it may have been due to the technical differences, such as different antibodies and/or quantification of staining. Another explanation for the discrepancy can be a racial or regional differences of the study population (Japanese versus Italian). In addition, although different from the Japanese study in the antibody type used, the proportion of the menopausal women, and the criteria for COX-2 positivity, the present study on Korean women shows very similar results with
that on Japanese but not with that on Italian. This might suggest the racial difference in endometrial carcinogenesis such as the role of COX-2 in carcinogenesis and progression.

Although the close relationship between the expressions of COX-2 and p53 was reported in several studies (32-34), it remains unknown that why there was no relation in this study. This may suggest the possibility of tissue-specific modulation of COX-2 gene regulation.

There is the substantial debate as to whether p53 overexpression is an independent prognostic factor in endometrial cancer. As shown by the work of Lundgren et al. (30) and Erdem et al. (31), we found that p53 expression is not an independent prognostic factor. However, Lundgren et al. (30) did not include age, lymph node metastasis, and myometrial invasion depth as prognostic variables. Such differences prevent direct comparisons of these three studies, which showed similar results by multivariate analysis in terms of identifying independent prognostic factors of endometrial cancer. In the previously mentioned studies (21-31), diverse antibodies were used against p53 and different criteria were used for p53 positivity. However, the overexpression of p53 protein was found to be associated with advanced stage, poor differentiation, lymph node metastasis, and deep myometrial invasion in the current study, and this corresponded well with the findings of other studies (24, 36, 37).

Many have reported that p53 mutation or immunostaining is significantly higher in papillary serous than in endometrioid carcinomas (38-44). In the present study, no significant association was found between p53 overexpression and nonendometrioid carcinoma. The relatively small number of nonendometrioid carcinomas (only five cases) in the study may be a cause. However, nonendometrioid carcinoma showed a trend toward more frequent p53 overexpression.

In conclusion, COX-2 may be associated with the carcinogenesis of endometrial cancer during the postmenopausal period but not with tumor aggressiveness and p53. Moreover, the COX-2 expression in endometrial cancer may be influenced by ethnicity. In addition, this study shows that p53 expression is strongly associated with the aggressiveness of endometrial cancer but not an independent prognostic factor of survival.

References
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