The prognostic significance of the immunohistochemical expression of P53 and BCL-2 in endometrial cancer

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Abstract: The objective of this study was to verify the frequency of P53 and BCL-2 immunohistochemical expression in 98 patients with endometrial carcinoma, and to correlate it with clinical stage and patient survival. A significant difference was found regarding the frequency of P53 expression when comparing type I and II tumors (23.7% and 54.5%, respectively; p = 0.006). A positive correlation was observed between P53 immunexpression and patient survival in type I and II tumors (p = 0.009 and p = 0.036, respectively). BCL-2 expression was significantly more frequent in early clinical stages in both types of endometrial cancer (p < 0.001 and 0.002) and correlated with a decrease in overall survival in type I endometrial cancer (p = 0.014). Thus, the prognostic value of these biomarkers in endometrial cancer needs to be further investigated. (Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 4, pp. 631–635)

Key words: endometrial cancer, P53, BCL-2, prognosis

Introduction

Endometrial carcinoma is one of the commonest gynecological malignancies. The age-adjusted incidence rate in Poland is 13.7 per 100,000 women per year [1]. Acknowledged clinicopathological prognostic markers used to determine the course of endometrial carcinomas include histological type, tumor grade, depth of myometrial invasion, lymph-node involvement and clinical stage [2]. In the majority of cases, the neoplasm is histologically diagnosed as an endometrial carcinoma of endometrioid type (type I) and its stage at the time of diagnosis as type I according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). The second major group of endometrial cancer is histologically classified as serous carcinoma (type II) [2].

In the past few years, several studies have assessed the clinical relevance of different biological factors evaluated in tissue samples from patients with endometrial cancer in order to detect markers capable of predicting the outcome [3–8]. Several publications have regarded the TP53 and the BCL-2 genes as possible markers of endometrial cancer aggressiveness [9–11]. Some 20–50% of endometrial carcinoma cases show abnormal P53 expression. The expression of P53, as seen in immunohistochemical analysis, seems to be related to undifferentiated, aggressive tumors, with a poor prognosis [6]. BCL-2 expression is rela-
tively high in the normal endometrium, decreases in endometrial cancer and has been related to well-differentiated endometrial tumors, early-stage disease and diseases with a good prognosis [9, 10].

The aim of the present study was to estimate the frequency of P53 and BCL-2 immunohistochemical expression in patients who have been surgically treated for endometrial carcinoma, and to correlate this expression with clinical stage and survival.

Material and methods

Patients and clinical samples. A total of 98 patients with endometrial cancers (aged 49–72, median value — 61.7 years) treated at the Department of Gynecology Medical University of Białystok and the Department of Gynecology District Hospital in Białystok between 1999 and 2003, were included in this study. None of the patients had received chemotherapy, hormonal therapy or radiation therapy prior to surgery. All patients had primary cancers and were receiving first treatment. Cases selected in the present study showed the same stage, both clinically and surgically. All tumors were staged according to the FIGO criteria. All patients underwent abdominal hysterectomy and bilateral oophorectomy. Pelvic and paraaortic lymphadenectomy was done at stage II (34 cases) and stage III (16 cases). Adjuvant chemotherapy was added to the treatment for stage III patients.

Clinicopathological information was obtained from medical charts. Histopathological examination was performed according to the WHO classification. Representative samples of hysterectomy specimens were stained with H&E for light microscopic study and evaluated to confirm a tumor stage and histological type. Patients were informed and gave their consent for the study. The protocol was previously approved by the Bioethical Committee of the Medical University of Białystok. Follow-up data was completed until January 2010.

Immunohistochemical analysis. The following primary antibodies were used: monoclonal mouse anti-human P53 protein, Clone: Do-7; Code M7001 and monoclonal mouse anti-human BCL-2 oncoprotein, Clone: 124; Code M0887 (Dako, Glostrup, Denmark). All reactions were performed using positive controls (breast cancer for P53 expression and tonsils for BCL-2 expression).

After deparaffinization and rehydration, antigen retrieval, inactivation of endogenous peroxidase and blocking of non-specific reactions, the sections were incubated for 2 h at ambient temperature with a diluted solution of primary antibodies (1:50 for P53 and 1:100 for BCL-2). Primary antibody binding was revealed by streptavidin-biotin peroxidase complex and diaminobenzidine tetrahydrochloride (LSAB, Dako, Glostrup, Denmark).

Tumors were considered positive for P53 expression if 10% or more of the nuclei of tumor cells stained brown, and for BCL-2 expression if 10% or more of the cytoplasm of tumor cells stained brown.

Statistical analysis. Statistical analysis was performed using Statistica software version 9.0PL (StatSoft, Inc., StatSoft Polska Sp. z o.o., Poland). A chi-square test was used to evaluate the relationship between categorical variables. Fisher’s exact test was used to determine significance between the two groups. A p-value of < 0.05 was considered as statistically significant. In addition, survival time was calculated from the date of surgery to the date of death and survival analysis was performed using the Kaplan–Meier method.

Results

The tumors were classified as follows: 76 cases were type I (endometrioid endometrial carcinomas) and 22 cases were type II (19 cases of serous and three of clear-cell carcinomas). Among patients with type I endometrial cancer, 43 had tumors classified as stage I, 24 had tumors classified as stage II, and nine had tumors classified as stage III.

The type II tumors were classified as follows: five cases were in stage I, ten cases were in stage II, and seven cases were in stage III. All patients were followed up. At a median follow-up of 60 (range 1–126) months, 39 (39.8%) patients had died as a consequence of cancer progression.

In type I tumors, P53 immunoexpression was positive in 18 cases (23.7%), while BCL-2 immunoexpression was positive in 44 cases (57.9%). In type II tumors, P53 and BCL-2 immunoexpression was detected in 12 cases (54.5%) and eight cases (36.4%), respectively. The correlations of P53 and BCL-2 immunoexpression between these two types of tumors are summarized in Table 1.

Table 1. Comparison of P53 and BCL-2 immunoexpression between patients with type I and II endometrial cancer

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>P53</th>
<th>BCL-2</th>
</tr>
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<tbody>
<tr>
<td>Type I no. (%)</td>
<td>76</td>
<td>18 (23.7)</td>
<td>44 (57.9)</td>
</tr>
<tr>
<td>Type II no. (%)</td>
<td>22</td>
<td>12 (54.5)</td>
<td>8 (36.4)</td>
</tr>
<tr>
<td>p</td>
<td>0.006</td>
<td>0.041</td>
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No correlation was found between the frequency of P53 immunoexpression in both types of tumors and stages (p = 0.17 and p = 0.084). P53 immunoexpression was more frequent in type II tumors (p = 0.006). A statistically significant correlation was found between the frequency of BCL-2 immunoexpression and
the clinical stage of type I and II tumors (p < 0.001 and p = 0.002, respectively) (Table 2).

Among patients with positive P53 immunopropheresion in type I endometrial cancer, the Kaplan–Meier survival estimates of the 1-year, 5-year, and 10-year survival rates were 97.4%, 92.1%, and 90.8%, respectively, while the respective rates among patients with negative P53 immunopropheresion were 97.4%, 93.4%, and 93.4%. A statistically significant difference was observed between survival rates over time (p = 0.009) (Figure 1).

In the group with type II endometrial cancer with positive P53 immunopropheresion, the Kaplan–Meier survival estimates of the 1-year, 5-year, and 10-year survival rates were 86.4%, 77.3%, and 72.7%, respectively, while the respective rates among endometrial cancer patients with negative P53 immunopropheresion were 100%, 86.4%, and 84.8%. A statistically significant difference was observed between survival rates over time (p = 0.036) (Figure 2).

In type I endometrial cancer patients with positive BCL-2 immunopropheresion, the 1-year, 5-year, and 10-year survival rates were 96.1%, 94.7%, and 94.7%, compared to those with negative BCL-2 immunopropheresion (93.4%, 90.8%, and 89.5%, respectively), and the differences in survival were significant (p = 0.014) (Figure 3).

Among patients with positive BCL-2 immunopropheresion in type II endometrial cancer, the Kaplan–
Meier survival estimates of the 1-year, 5-year, and 10-year survival rates were 95.5%, 86.4%, and 86.4%, respectively, while the respective rates among patients with negative BCL-2 immunoexpression were 90.9%, 81.8%, and 63.6%. No statistically significant difference was observed between survival rates over time (p = 0.737) (Figure 4). The cumulative disease-specific survival curves of patients grouped according to P53 and BCL-2 immunoexpression are shown in Figures 1–4.

Discussion

A recent model of endometrial carcinogenesis revealed that there are two different types of tumor. Type I tumors develop slowly and progressively as a result of endometrial hyperplasia in unopposed estrogen activity. These tumors are characterized by sustained BCL-2 expression and late mutations to the TP53 gene, as confirmed by the low expression of P53 in stages I and II in our study. On the other hand, type II tumors develop in an atrophic epithelium without estrogen stimulation. They are aggressive and occur in a distinct biological environment. Gene mutation and P53 protein accumulation are often found in type II lesions [10, 12, 13].

The presence of P53 mutations in endometrial cancer has been extensively studied in literature. Approximately one in three endometrial cancers have abnormalities of P53 by immunohistochemistry [7, 14–17]. A number of studies have shown that both P53 overexpression and mutation are associated with poor prognosis [9, 10, 14, 15, 17]. It is worth noting that earlier grade 3 endometrioid carcinomas were classified as type II tumors. Molecular data now exists suggesting that most grade 2, and some grade 3, tumors have molecular profiles similar to grade 1 tumors [12].

In type II endometrial cancer, approximately 80% of carcinomas have mutations in P53, implying a role for its inactivation early in the development of this aggressive tumor type. This is in contrast to type I, in which P53 mutations are relatively uncommon and are largely confined to grade 3 tumors. The exact mechanism for the cause of this mutation is still unclear. After DNA damage, nuclear P53 accumulates and causes cell cycle arrest by inhibiting cyclin-D1 phosphorylation of the Rb gene and therefore promoting apoptosis. Thus, mutated P53 results in a non-functional protein that accumulates in the cell and acts as a double negative inhibitor of the wild-type P53, leading to propagation of aberrant cells [18].

A much lower P53 mutational rate in type I tumors makes P53 a useful molecular marker that distinguishes type I from type II endometrial cancers [19]. Overexpression of tumor P53 protein has been studied by immunohistochemistry; its prevalence in endometrial cancer reportedly ranged from 30% to 80%. Several studies have concluded that the examination of tumor P53 provides independent prognostic information [14, 15], while others have failed to show such a relationship [7, 16, 17, 20].

In our study, we found that 54.5% of type II tumors and 23.7% of type I tumors expressed P53 and this difference was statistically significant. In previous publications, the difference in P53 expression between these two types of tumors has proved to be more distinct [5, 14, 17, 21]. We noted an increased P53 immunexpression in type II tumors but P53 immunexpression was not found to be statistically different when surgical staging was analyzed. Studies have correlated P53 immunexpression with a decrease in overall survival in patients with endometrial tumors [22, 23]. In our study, P53 positivity was also associated with the risk of death.

BCL-2 immunexpression was observed in 57.9% in type I tumors and 36.4% of type II that we analyzed. The literature describes rates of 30–85% [9, 16, 24], depending on the selection of patients and type of lesion. Our study demonstrated a correlation between BCL-2 immunexpression and tumor type, stage and patient survival. Several studies have shown increased BCL-2 immunexpression to be associated with some favorable prognostic factors in type I tumors in initial stages [9, 10, 24, 25]. In our study, a statistically significant correlation between BCL-2 and disease free survival was found in type I tumors.

In conclusion, P53 immunexpression was significantly more frequent in type II tumors and correlat-
ed with a decrease in overall survival in both types of endometrial cancer. A positive correlation was observed between decreased BCL-2 immunoexpression and patient survival in type I tumors. Thus, the prognostic value of these biomarkers in endometrial cancer needs to be further investigated.

References