FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 49, No. 4, 2011
pp. 631–635

ORIGINAL STUDY

VIA MEDICA

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 immunoexpression 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-

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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 Bialystok and the Department of Gynecology District Hospital in Bialystok 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 Bialystok. 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.

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

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

	No. of cases	P53	BCL-2
Type I no. (%)	76	18 (23.7)	44 (57.9)
Type II no. (%)	22	12 (54.5)	8 (36.4)
p		0.006	0.041

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

	No. of cases	P53 no. (%)	BCL-2 no. (%)
Type I			
Stage			
I	43 (56.6)	7 (16.3)	38 (88.4)
II	24 (31.6)	6 (25.0)	5 (20.8)
III	9 (11.8)	4 (44.4)	1 (11.1)
p		0.17	< 0.001
Type II			
Stage			
I	5 (22.7)	2 (40.0)	5 (100)
II	10 (45.5)	8 (80.0)	3 (42.9)
III	7 (31.8)	2 (28.6)	0
p		0.084	0.002

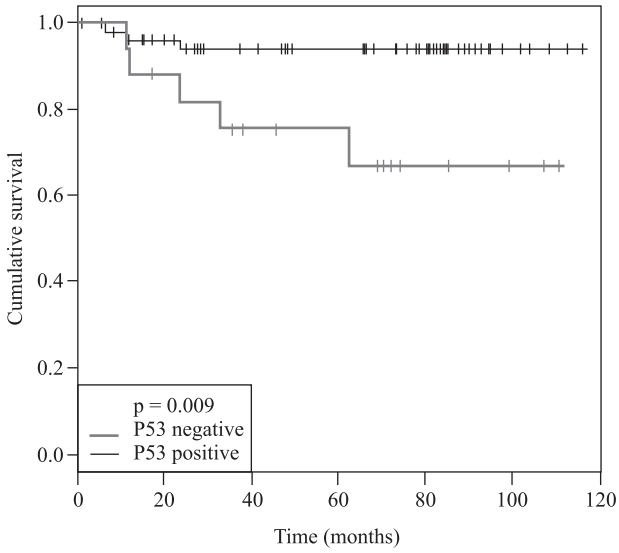

Figure 1. Kaplan–Meier survival analysis concerning P53 immunoexpression and cumulative survival in type I endometrial cancer

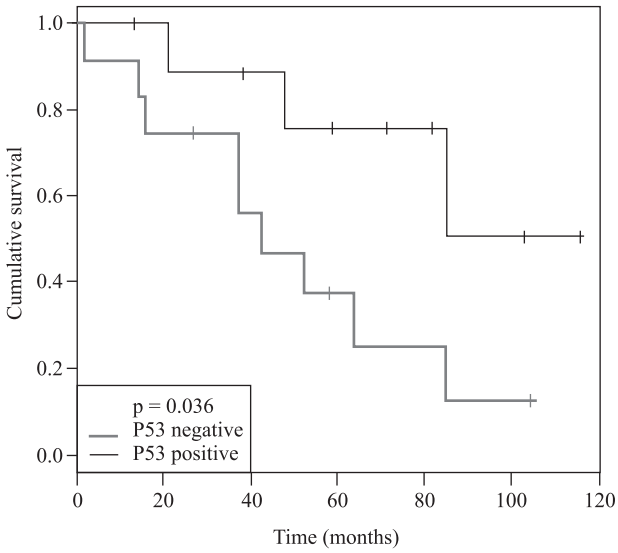

Figure 2. Kaplan–Meier survival analysis concerning P53 immunoexpression and cumulative survival in type II endometrial cancer

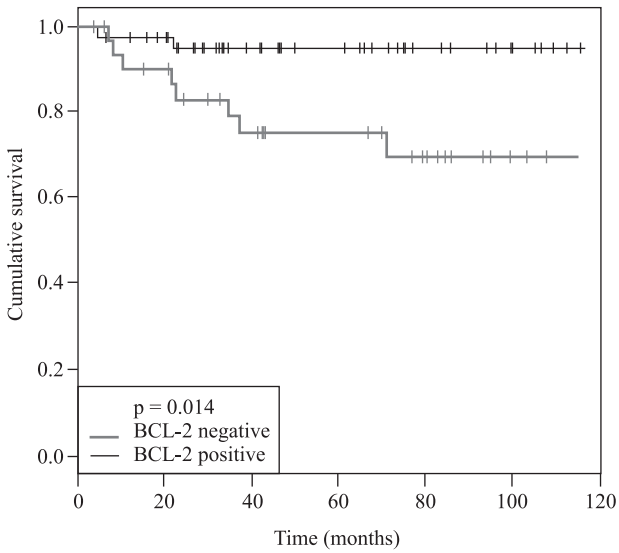

Figure 3. Kaplan–Meier survival analysis concerning BCL-2 immunoexpression and cumulative survival in type I endometrial cancer

the clinical stage of type I and II tumors ($p < 0.001$ and $p = 0.002$, respectively) (Table 2).

Among patients with positive P53 immunoexpression 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 immunoexpression 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 immunoexpression, 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 immunoexpression 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 immunoexpression, 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 immunoexpression (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 immunoexpression in type II endometrial cancer, the Kaplan–

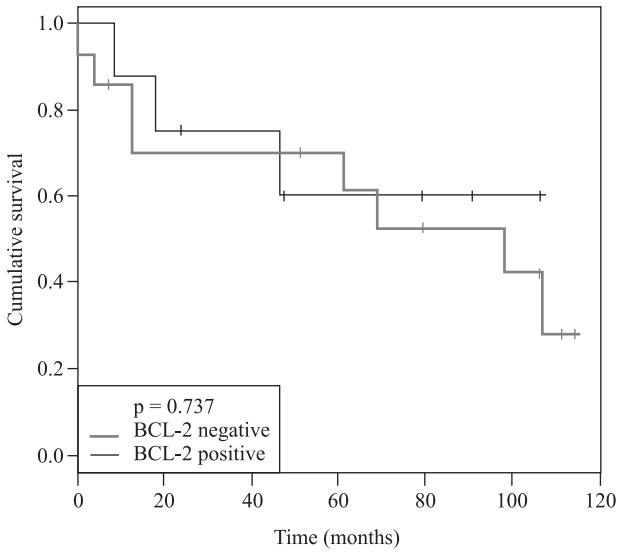

Figure 4. Kaplan–Meier survival analysis concerning BCL-2 immunoexpression and cumulative survival in type II endometrial cancer

–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 nonfunctional 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 immunoexpression in type II tumors but P53 immunoexpression was not found to be statistically different when surgical staging was analyzed. Studies have correlated P53 immunoexpression 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 immunoexpression 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 immunoexpression and tumor type, stage and patient survival. Several studies have shown increased BCL-2 immunoexpression 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 immunoexpression 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

1. Reports based on data of National Cancer Registry. The Maria Sklodowska-Curie Memorial Cancer Center, Department of Epidemiology and Cancer Prevention, *National Cancer Registry* 2004, http://epid.coi.waw.pl/krn.
2. Amant F, Moerman P, Neven P et al. Endometrial cancer. *Lancet.* 2005;366:491–505.
3. Dobrzycka B, Terlikowski SJ, Mazurek A et al. Circulating free DNA, p53 antibody and mutations of KRAS gene in endometrial cancer. *Int J Cancer*. 2010;127:612–621.
4. Dobrzycka B, Terlikowski SJ, Mazurek A et al. Mutations of the KRAS oncogene in endometrial hyperplasia and carcinoma. *Folia Histochem Cytobiol*. 2009;47:65–68.
5. Engelsen IB, Akslen LA, Salvesen HB. Biologic markers in endometrial cancer treatment. *APMIS.* 2009;117:693–707.
6. Ioachin E. Immunohistochemical tumour markers in endometrial carcinoma. *Eur J Gynaecol Oncol.* 2005;26:363–371.
7. Terlikowski S, Lenczewski A, Famulski W et al. Patterns of immunohistochemical staining for p53 expression in hyperplastic endometrium and adenocarcinoma. *Folia Histochem Cytobiol*. 2001;39(Suppl 2):195–196.
8. Terlikowski S, Lenczewski A, Famulski W et al. Proliferative activity in endometrial hyperplasia and adenocarcinoma. *Folia Histochem Cytobiol*. 2001;39:163–164.
9. Porichi O, Nikolaidou ME, Apostolaki A et al. BCL-2, BAX and P53 expression profiles in endometrial carcinoma as studied by real-time PCR and immunohistochemistry. *Anticancer Res.* 2009;29:3977–3982.
10. Appel ML, Edelweiss MI, Fleck J et al. P53 and BCL-2 as prognostic markers in endometrial carcinoma. *Pathol Oncol Res.* 2008;14:23–30.
11. Burton JL, Wells M. Recent advances in the histopathology and molecular pathology of carcinoma of the endometrium. *Histopathology*. 1998;33:297–303.
12. Lax SF. Molecular genetic changes in epithelial, stromal and mixed neoplasms of the endometrium. *Pathology.* 2007;39:46–54.
13. Abal M, Planaguma J, Gil-Moreno A et al. Molecular pathology of endometrial carcinoma: transcriptional signature in endometrioid tumors. *Histol Histopathol.* 2006;21:197–204.
14. Feng YZ, Shiozawa T, Horiuchi A et al. Intratumoral heterogeneous expression of p53 correlates with p53 mutation, Ki-67, and cyclin A expression in endometrioid-type endometrial adenocarcinomas. *Virchows Arch.* 2005;447:816–822.
15. Pijnenborg JM, van de Broek L, Dam de Veen GC et al. TP53 overexpression in recurrent endometrial carcinoma. *Gynecol Oncol.* 2006;100:397–404.
16. Erkanli S, Eren F, Pekin S et al. BCL-2 and P53 expression in endometrial carcinoma. *J Exp Clin Cancer Res.* 2004; 23:97–103.
17. Ozalp S, Yalcin OT, Tanir HM et al. p53 overexpression as a prognostic indicator in endometrial carcinoma. *Eur J Gynaecol Oncol.* 2003;24:275–278.
18. Doll A, Abal M, Rigau M et al. Novel molecular profiles of endometrial cancer - new light through old windows. *J Steroid Biochem Mol Biol.* 2008;108:221–229.
19. Lax SF, Kendall B, Tashiro H et al. The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. *Cancer.* 2000;88:814–824.
20. Alkushi A, Lim P, Coldman A et al. Interpretation of p53 immunoreactivity in endometrial carcinoma: establishing a clinically relevant cut-off level. *Int J Gynecol Pathol*. 2004;23:129–137.
21. Geisler JP, Wiemann MC, Zhou Z et al. p53 as a prognostic indicator in endometrial cancer. *Gynecol Oncol.* 1996; 61:245–248.
22. Jongen VH, Briët JM, de Jong RA et al. Aromatase, cyclooxygenase 2, HER-2/neu, and p53 as prognostic factors in endometrioid endometrial cancer. *Int J Gynecol Cancer.* 2009;19:670–676.
23. Ito K, Watanabe K, Nasim S et al. Prognostic significance of p53 overexpression in endometrial cancer. *Cancer Res.* 1994;54:4667–4670.
24. Kalogiannidis I, Bobos M, Papanikolaou A et al. Immunohistochemical bcl-2 expression, p53 overexpression, PR and ER status in endometrial carcinoma and survival outcomes. *Eur J Gynaecol Oncol.* 2008;29:19–25.
25. Coppola D, Fu L, Nicosia SV et al. Prognostic significance of p53, bcl-2, vimentin, and S100 protein-positive Langerhans cells in endometrial carcinoma. *Hum Pathol.* 1998; 29:455–462.

Submitted: 18 August, 2010
Accepted after reviews: 18 September, 2011