EXPRESSION OF p53 AND Bcl–2 PROTEINS IN EPITHELIAL OVARIAN CARCINOMA WITH DIFFERENT GRADE OF DIFFERENTIATION

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ЭКСПРЕССИЯ БЕЛКОВ p53 И Bcl–2 В ЭПИТЕЛИАЛЬНЫХ ОПУХОЛЯХ ЯИЧНИКА РАЗЛИЧНОЙ СТЕПЕНИ ДИФФЕРЕНЦИРОВКИ

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Expression of p53 and Bcl-2 as well as nuclear antigen of proliferating cells (NAPC) detected with the IPO-38 MoAb was studied in serous epithelial ovarian tumors of different grade of differentiation (32 patients in total). Overexpression of the mutant p53 and NAPC was shown to be typical of poorly differentiated malignant ovarian tumors with high aggressiveness and vast dissemination (FIGO stage IIIc and IV). Well-differentiated tumors, according to our data, were characterized by the absence of the mutant p53 expression, the low proliferation index and the high Bcl-2 levels. These data suggest that the detection of p53 and Bcl-2 expression as well as expression of the NAPC in ovarian tumors could be useful for prognosis and therapy correction.

Key Words: **malignant ovarian carcinoma, grade of differentiation, p53, bcl-2, nuclear antigen of proliferating cells.**

У 32 больных с серозными эпителиальными опухолями яичников различной степени дифференцировки определена экспрессия белков — регуляторов апоптоза p53, Bcl-2 и антигена, ассоциированного с пролиферацией клеток (АПК). Установлено, что избыточная экспрессия мутантного белка p53 и АПК характерна для низкодифференцированных злокачественных опухолей яичников, отличающихся крайней степенью злокачественности, агрессивным течением заболевания и обширной распространенностью процесса по классификации FIGO (IIIc и IV). Опухоли с высокой степенью дифференцировки, по нашим данным, отличаются отсутствием экспрессии мутантного белка p53, низким индексом пролиферации опухолевых клеток и повышенной экспрессией белка Bcl-2. Определение экспрессии белков p53, Bcl-2 и АПК в ткани опухолей яичника на основе полученных данных может быть использовано для прогноза заболевания и коррекции терапии.

Ключевые слова: **злокачественные эпителиальные опухоли яичников, степень дифференцировки, p53, Bcl-2, антиген пролиферирующих клеток.**

Malignant ovarian carcinoma is one of the leading cause of death of patients with gynecological malignancy [1, 2]. Tumor progression occurs mainly by dissemination through peritoneum resulting in relatively low–symptomatic disease. 80% of such cancers are diagnosed only at the late stages when effective treatment could not be achieved [2]. Histological type of ovarian cancer is one of the major prognostic factors determining clinical outcome. Poorly differentiated tumors are characterized by high metastatic rate and aggressiveness that influence the treatment outcome [1].

Over the last few years there has been increasing evidence that expression of certain genes, such as *p53* and *bcl–2*, may affect the cellular response to an apoptotic stimulus and therefore modulate the sensitivity of cells to anticancer means [3–7]. At the same time, mutations in genes controlling proliferation, differentiation and apoptosis could be one of the causative factors in the origin of malignant neoplasm [4–9].

Wild–type p53 is recognized as positive regulator of apoptotic cell death. A high incidence of *p53* gene point mutations is found in various types of human ma–

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Abbreviations used: NAPC — nuclear antigen of proliferating cells.

lignancies, such as colorectal, lung, breast, endometrial cancers and ovarian neoplasms [4, 8, 9–15]. Pathological expression of Bcl–2 has been described in a wide variety of human cancers [4, 8, 10–14]. Bcl–2 expression in certain types of tumors such as neuroblastomas and prostate carcinoma is considered as a poor prognostic marker [11, 15]. At the same time, lung and breast cancer patients with Bcl–2 positive tumors have better survival [16, 17]. The relationship between Bcl–2 and p53 is of particular interest. Although an inverse relationship between Bcl–2 and p53 expression has been reported in breast cancer [18], their roles in apoptosis have not yet been extensively investigated.

The role of wild–type and mutated *p53* gene as well as Bcl–2 in the pathogenesis of ovarian cancer is intriguing. Positive p53 immunostaining is common in the serous histologic type of ovarian cancer and is associated with poor outcome, poor histologic grade, and S–phase fraction size greater than the median sized, as determined by DNA flow cytometry [19]. On the other hand, there are contradictory results concerning the Bcl–2 as a prognostic factor in patients with ovarian carcinoma. While some authors have observed that Bcl–2 expression was related to more favorable prognosis [20], other authors have shown increased Bcl–2 expression in patients with shortened overall and disease–free survival [21, 22]. Because the clinical significance of p53 and Bcl–2 expression has not yet been established in ovarian carcinoma, we aimed to detect the expression of p53, Bcl–2 and nuclear antigen of proliferating cells (NAPC) in serous epithelial ovarian carcinoma in association with both of clinicopathologic factors and grade of tumor differentiation.

MATERIALS AND METHODS

Tumor specimens of 32 patients (the median age 48 years) with ovarian cancer have been studied. Clinical staging was made according to the International Federation of Gynecology and Obstetrics (FIGO) staging system. Of 32 patients in the study, 9 were staged in stage Ic, 11 — stage IIIc, 4 — stage IIIb, 8 — stage IV. The fragments from different sites of the tumors were fixed in 10% neutral formalin solution, dehydrated in alcohols of ascending concentration, and embedded in paraffin blocks. The sections were stained with hematoxylin and eosin and investigated by light microscopy.

Bcl–2 expression was detected in frozen sections using anti–Bcl–2 monoclonal antibodies (MoAb) (Dakopatts, Denmark) by immunohistochemical peroxidase–antiperoxidase (PAP) method. NAPC was assessed with IPO–38 MoAb (R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, Kyiv, Ukraine) [23]. This antigene is absent in nonproliferating cells and becomes to be detectable in G_1 phase of the cell cycle, differing in some respects from PCNA and Ki–67.

The pieces of tumor tissue were frozen in liquid nitrogen and placed into cryostat. 4–6–μm–thick sections were further mounted on poly–L–lysine coated slides, dried in air and fixed in acetone for 5 min at room temperature. The protein expression was assayed at the same day, otherwise the specimens were wrapped into aluminum foil and stored at –20°C. Immediately before immunohistochemical assay the frozen specimens were brought up to the room temperature for 20 min. To reduce nonspecific background staining the sections were covered with normal rabbit serum. Upon incubation with optimal dilutions of MoAbs the specimens were washed 3 times for 5 min in phosphate–buffered saline (PBS), covered with excess of rabbit serum against mouse immunoglobulins and further incubated for 1 h. Then the specimens were washed in PBS again and incubated with PAP–complex for 45 min. Upon washing out non–bound PAP–complex, peroxidase activity was assayed histochemically. After detection of peroxidase activity the sections were additionally stained with hematoxylin for 2–3 min, embedded in balm and studied with a light microscope. p53 expression was also detected by immunohistochemical PAP method in paraffin sections with anti–p53 MoAb (clone BP53–12, reacting with a C–therminal epitope of the p53 both of wild and mutant type was kindly provided by Dr. JoHilgers, The Netherlands). 4–6–μm–thick paraffin sections were left for a day at 37°C. Prior to immunohistochemical assay the specimens were placed in a thermostat at 60°C for 10 min, and were deparaffined. For the optimal immunoperoxidase staining, antigens were disclosed by treatment of sections 10 min with 0.06% trypsin solution at 37°C.

RESULTS AND DISCUSSION

The first group included 12 patients with poorly differentiated serous ovarian carcinoma forming mainly solid structures, where papillae were practically absent, though separate glands were revealed sometimes. Tumor cells were different in size and highly polymorphic, with the features of dystrophy. Mitotic cells were abundant with pronounced pathology of mitotic patterns. Marked necrotic fields, lymphoid infiltration, and deep invasion into stroma were also evident.

The second group included 8 patients with moderately differentiated serous ovarian tumors with cells forming glandular papilla structures, paved predominantly by multilayer polymorphic epithelium. Glandular formations were observed with moderate cell atypism, areas of solid growth with cell polymorphism and sharply atypical cells. In some areas invasion in stroma and perifocal lymphoid infiltration were observed.

The third group included 12 patients with well differentiated serous ovarian tumors. In these tumors parenchyma prevailed over the edematic stroma with a small content of cells. In the majority of such tumors numerous papillae and cysts with hyperchromic multilayer epithelium could be observable. In several tumors the sparse layers of desquamated epithelium were evident. The mitotic cells were rather scarce.

High expression of both mutant p53 and NAPC was found in poortly differentiated tumors (Table). The highest p53 expression was observed in ovarian tumors of large size, with massive necrotic areas and nuclear polymorphism. High malignancy of such tumors was also confirmed by a high expression of NAPC detected with IPO–38 MoAb. At the same time, Bcl–2 expression was found only in single cells along the layers of low differentiated tissue, whereas in the most solid structures with active cell proliferation Bcl–2 expression has not been detected (Table).

Table. Expression of p53, Bcl–2 and nuclear antigen of proliferating cells (NAPC) in epithelial ovarian cancer with different grade of differentiation

Grade of differentiation	mp53	Bcl–2	NAPC
Poorly differentiated	+++	–	+++
Moderately differentiated	+/–	+/–	+/–
Well differentiated	–	+++	+

mp53 – mutant p53.

20–30% of moderately differentiated ovarian tumors expressed mutant p53 and NAPC (Table) with the traces of Bcl–2–specific immunostaining in cytoplasm. Only in 3 (10%) of 12 tumors low expression of mutant p53 as well as NAPC was found, while in the remaining tumors expression of these proteins was rather abundant. The expression of Bcl–2 was found in the majority of the cells of low–grade tumors. More pronounced expression of Bcl–2 was observed in patients staging as Ia (Table).

Therefore, overexpression of the mutant p53 and NAPC detected with the IPO–38 MoAb was shown to be typical of poorly differentiated malignant ovarian tumors (IIIc and IV FIGO staging). It was shown that the mutations of *p53* gene occur at the late stages of ovarian tumor development and can serve as a relevant prognostic factor of tumor aggressiveness [5, 7, 13, 14]. It is also known, that high proliferative capacity of tumor cells is a marker of poor prognosis [3, 12].

Our present study has shown that poorly differentiated epithelial ovarian tumors are characterized by overexpression of p53 and a high cell proliferation index that could serve as a marker of unfavourable prognosis. Well differerntiated tumors, according to our data, are characterized by the absence of mutant p53 expression, the low cell proliferation index, and high level of Bcl–2 protein. This observation is in agreement with results of other authors having demonstrated similar expression patterns of the proteins under study in several other tumors [15–20].

In conclusion, our results indicate that p53, Bcl–2 and NAPC are differently expressed in epithelial ovarian carcinoma varying by histological grade as well as biological features which could be useful for both prognosis and therapy correction.

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