Exp Oncol 2009
31, 4, 246–249

Experimental ONCOLOGY

CORRELATION BETWEEN HISTOLOGICAL TYPE AND IMMUNOHISTOCHEMICAL PROFILE OF PROSTATE CANCER AND GLEASON SCALE GRADATION

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***Aim*: To evaluate the characteristics of prostate cancer (PC) morphogenesis, taking into consideration the role of proliferation and apoptosis in tumor cells. *Methods*: p53, $p16^{INK4a}$, Bcl-2 and Ki-67 proteins expression was analyzed by immunohistochemistry in paraffin embedded sections of biopsy specimens from PC patients. The level of tissue immunoreactivity was evaluated by semi-quantitative method with estimation of 100% colored cells content over 1000 cells in one specimen. Patients were divided into three groups in accordance to Gleason scale gradation: group 1 — with Gleason scale < 5 (n = 13); group 2 — with Gleason scale > 5 and < 8 (n = 8); group 3 — with the highest Gleason scale > 8 (n = 6). *Results:* Upon histological examination of prostate biopsy specimens, it was found that in the first group in 6 out of 13 (46%) cases small acinic cell PC developed on the background of chronic prostatitis with PIA (proliferative inflammatory atrophy) locus, frequently in combination with prostatic intraepithelial neoplasia (PIN) locus. Hyperchromic epithelial cells in PIA locus were characterized by nuclear expression of p53 and Ki-67 proteins, and cytoplasmic expression of Bcl-2. The precancerous foci in the PIN and PIA in the biopsy specimens of the second group of PC patients were found in 2 out of 8 (25%) cases of large and small acinic cell adenocarcinoma observations. The expression level of p53, p16INK4a, Bcl-2 proteins and especially Ki-67 protein adequately increased in tumors of group 2 in comparison with group 1. Group 3 comprised of patients with Gleason scale > 8, predominantly solid structures or scirrhus of PC, which were characterized by the highest nuclear expression of p53, $p16^{INK4a}$ and Ki-67, and also by overexpression of cytoplasmic Bcl-2. *Conclusions*: Obtained results showed the direct correlation between patients' Gleason scale, and the expression level of p53, $p16^{INK4a}$, Bcl-2 proteins and, particularly, Ki-67 marker of proliferating cells in PC tumor cells.**

***Key Words*: prostate cancer, Bcl-2, p53 and Ki-67 proteins, PIN and PIA locus, Gleason scale, biopsy specimens.**

PC is the most common cancer in men in Europe that was ranked third after lung and colorectal cancers in 2006 [1]. In Ukraine in 2007 the annual age-standardized incidence rate of PC was 19.3/100.000 ranking fourth after lung, skin and stomach cancers [2].

The PC morbidity growth changed during the last 20 years from 12.0 in 1989 year to 28.1 in 2007. This fact allows to assume the role of the negative impact of ecological environmental factors, including the consequences of the Chernobyl accident [3].

Precancerous changes of prostate gland can be also present in young men, are more natural for men aged 50 years, though the PC manifestations usually diagnosed in patients aged 60–70 years [4]. In 2001, for men aged 60 years the PC risk was 30%, the possibility of symptomatic manifestations was only 8–9% with PC lethal outcome of 2.9% [4–6]. It is well-known that PC could be defined by means of autopsy in 30–40% of men aged 50–70 years. At the same time, only in 10% of cases cancer has clinical manifestations, and in 4% the disease leads to lethal outcome [7].

To optimize the early PC detection and for prognosis of the disease outcome the complex study should be conducted. It should cover the individual histological and immunohistochemical tumor characteristics, which influence its growth, level of differentiation and metastatic activity.

Molecular biology studies elucidated some mechanisms of control of cell division, cell death, signal transduction from the receptors to the nucleus. It is well-known that *p53* gene mutations can promote the development of genetic instability. The *p53* gene functional disorders are general molecular features of many human neoplasms. These disorders can occur in the normal cells, thus, increasing the possibility of neoplastic clone formation, or they take place in malignant cells, which promote tumor progression [8, 9].

It is also well-known that protein encoded by *Bcl-2* pro-oncogene can arrest the apoptosis of prostate cells induced by p53 protein or other stimuli, including cytostatic drugs. In case of Bcl-2 protein overexpression it acts as oncogene and participates in the formation of androgen-resistant phenotype and tumor resistance to chemotherapeutic compounds [10, 11].

The regulation of cell proliferation is controlled by gradual activation of cyclins and corresponding cyclin-dependant kinases. The cyclin kinases activity is determined by the expression level of relevant cyclins and the activity of specific inhibitors. There are several families of cyclin kinases inhibitors' genes. One of the most studied is $p16^{INK4a}$ gene, which suppresses the activity of cyclin-dependent D kinases, and by this obstructs advancing of the cell cycle G1 phase. The $p16^{INK4a}$ gene functional disorder or its inactivation can lead to the loss of control on cell mitosis [12].

Ki-67 protein is a cell proliferation marker that belongs to the regulatory proteins. It assists during the cell mitosis and disappears when the cell passes to the resting phase or at the time of DNA repair [13]. Antibodies against Ki-67 proteins are often used for evaluation of proliferating activity of cancer cells, including PC cells. Ki-67 index is an independent and

Received: October 30, 2009.
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Abbreviations used: PC – prostate cancer; PIA – proliferating post-inflammatory atrophy; PIN – prostatic intraepithelial neoplasia.

widely spread marker for prognosis of PC recurrence and patients' survival [12].

Therefore, genes and their products related to the tumor progression are considered to be tumor markers. They are widely used for early diagnostics (screening), latent metastasis or disease recurrences detection, and also for chemotherapy efficiency monitoring and estimation of metastatic tumour potential.

The aim of our study was to evaluate the characteristics of PC morphogenesis, taking into consideration the role of proliferation and apoptosis in tumor cells.

MATERIALS AND METHODS

The biopsy specimens were received from PC patients, who underwent observation in Institute of Urology of Ukrainian Academy of Medical Sciences for the period of 2004–2006. All studies were performed according to the Institute's Ethical Committee regulations. The patients' age was from 58 to 82 years, an average age was 70 ± 2.02. All of them are Kiev citizens, where density of polluted soil by radioactive (Cs^{137}, Cs^{134}) comprises 0.5–5 Curie\1 km^2.

Biopsy specimens were immediately preserved in buffered formalin (phosphate buffer, pH 7.4) with subsequent preparation of paraffin blocks. Besides the ordinary histological examination, the expression of tumor suppressor proteins p53 and $p16^{INK4a}$, apoptosis inhibitor Bcl-2, marker of proliferating cells Ki-67 was examined by immunihistochemistry in 4 microns paraffin embedded sections. Immunohistochemical staining was conducted by conventional method using avidin-biotin-peroxidase complex (ABC) and mouse monoclonal antibodies against p53 (DO-7 clone), anti-$p16^{INK4a}$, anti-Bcl-2 and Ki-67 mAbs (DAKO, Denmark). Histological verification of tumor was conducted in accordance with the latest WHO International classification (2004), defining the total gradation of PC according to the Gleason scale.

To evaluate the level of tissue immunoreactivity the semi-quantitative method with estimation of colored cells percent among 1000 cells in each specimen was applied. Not stained cells has got "0"; < 10% corresponded to "1"; more than 10% but < 50% — "2"; > 50% — "3" .

All analyzed tumors were divided into three groups in accordance to Gleason scale gradation of PC. The first group interconnected 13 tumors with the Gleason scale: group 1 (n = 13) — < 5; group 2 (n = 8) — more than 5 but less than 8 by the Gleason scale; group 3 (n = 6) — the highest Gleason scale > 8.

The statistical analysis of the research results was carried out by χ^2 Pearson criteria. The discrepancy between the data was considered to be statistically relevant with accuracy not less than 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Upon histological examination of prostate biopsy specimens from group 1, the 46% of cases (6 out of 13) were predominantly small acinic cell PC, which developed on the chronic prostatitis background with proliferative inflammatory atrophy (PIA) foci, frequently in the combination with PIN zones, which are considered as precancerous conditions in prostate cancerogenesis (Fig. 1, *a, b*). Moreover, the nuclear expression of p53 and Ki-67 proteins and especially high cytoplasmic expression of Bcl-2 (> 50% of colored cells) was found in PIA focuses of small (less than 10% of cells) and expanded (> 10% but < 50%) groups of frequently hyperchromic epithelial cells, laying atrophied ectatic glands. (Fig. 1, *c, d*). The expression of $p16^{INK4a}$ protein was rather low or absent.

The precancerous foci (PIN and PIA) were found in 2 out of 8 cases (25%) in group 2, which consisted of large and small acinic cell prostate adenocarcinoma with generation of cribriform, rarely solid structures. In neoplasms of this group the expression level of p53, $p16^{INK4a}$, Bcl-2 proteins and especially of Ki-67 protein significantly increased ($p < 0.05$), in comparison with tumors of group 1 (Fig. 2, *a*, *b*).

The third group of PC with predominantly solid structures or scirrhus, and Gleason rate higher than 8 (n = 6) was characterized by the highest nuclear expression p53, $p16^{INK4a}$ and Ki-67 ($p < 0.05$), and also by cytoplasmic hyperexpression of Bcl-2 protein. It indicated the highest level of proliferative activity of cancer cells as a result of *Bcl-2* gene alteration and overexpression of nuclear Ki-67 (Fig. 2, *c*, *d*).

Our data confirm the existing opinion on histogenetic interaction between PIN and adenocarcinoma, because they accurately manifest phenotypic similarity. Furthermore, in fact, the occurrence of PIA in PC indicates the increased aggressiveness and disposition to the invasive tumor growth [14].

In accordance to the foregoing information, Ki-67 index is considered to be the independent prognostic marker for PC patients [10]. There also exists the direct correlative dependence between the number of Ki-67 positive tumor cells, and the stage of PC [13]. There is the direct dependence between the Ki-67 proliferative index, gradation by Gleason scale, tumor infiltration of seminal vesicle, tumor size, the existence of PIN locus and the level of generic PSA in blood serum [10].

CONCLUSION

Obtained results showed the direct correlation between Gleason scale of PC and the expression level of p53, $p16^{INK4a}$, Bcl-2 proteins and, particularly, Ki-67 marker of proliferating cells. This is evidently emphasizing the fact that Gleason scale has both diagnostic and prognostic values.

According to the results of our research, chronic prostatitis, which goes with PIN and PIA locus, is proved to be favorable background for PC development. Moreover, the high expression level of protein Bcl-2 reveals itself in PIN and in a lesser degree PIA zones, so that it indicates the earlier *Bcl-2* gene alteration, inhibiting apoptosis. This phenomenon can be distinguished as one of the primary and incipient presentation of apoptosis molecular affects in PC pathogenesis. It can be used as immunohistochemical test in order to determine the early preclinical stages of PC under biopsy specimen analysis.

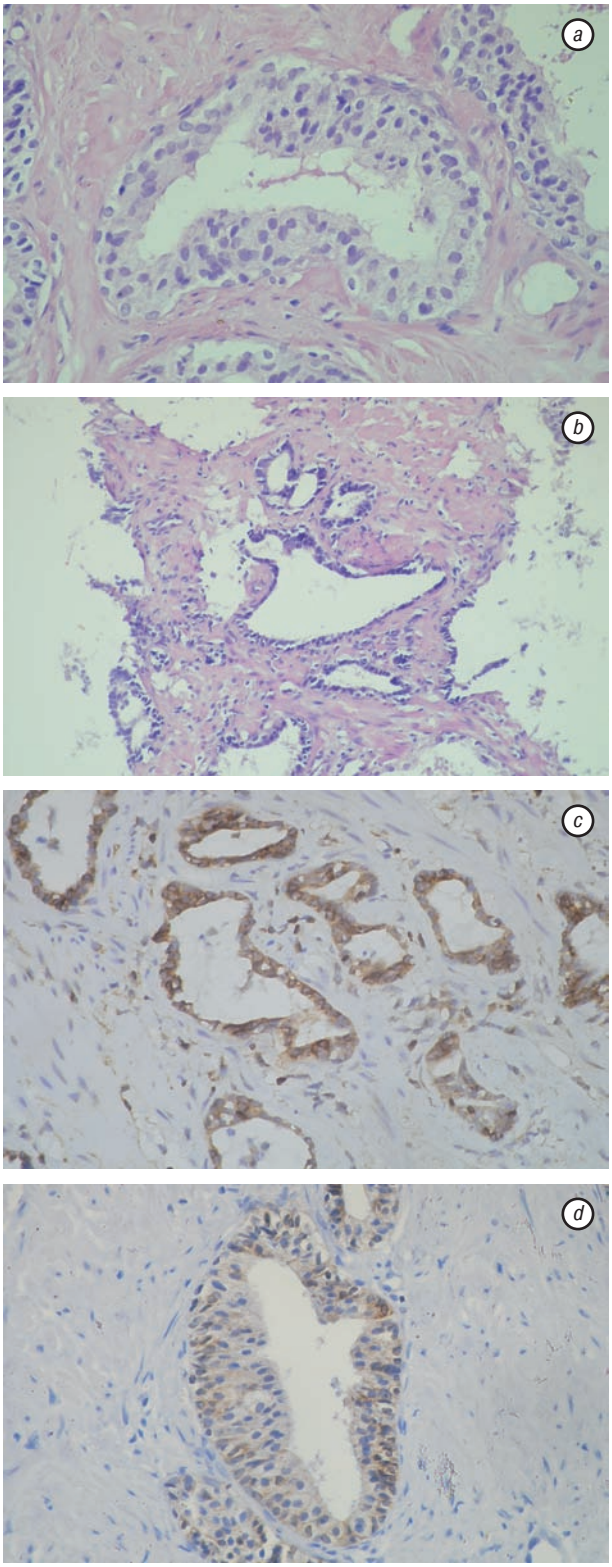

Fig. 1. Precancerous zones and Bcl-2 expression in prostate gland, 200X. *a*, PIN, hematoxylin and eosin staining; *b*, proliferating post-inflammatory atrophy, hematoxylin and eosin staining; *c*, Bcl-2 expression in PIA zone; *d*, Bcl-2 expression in PIA zone

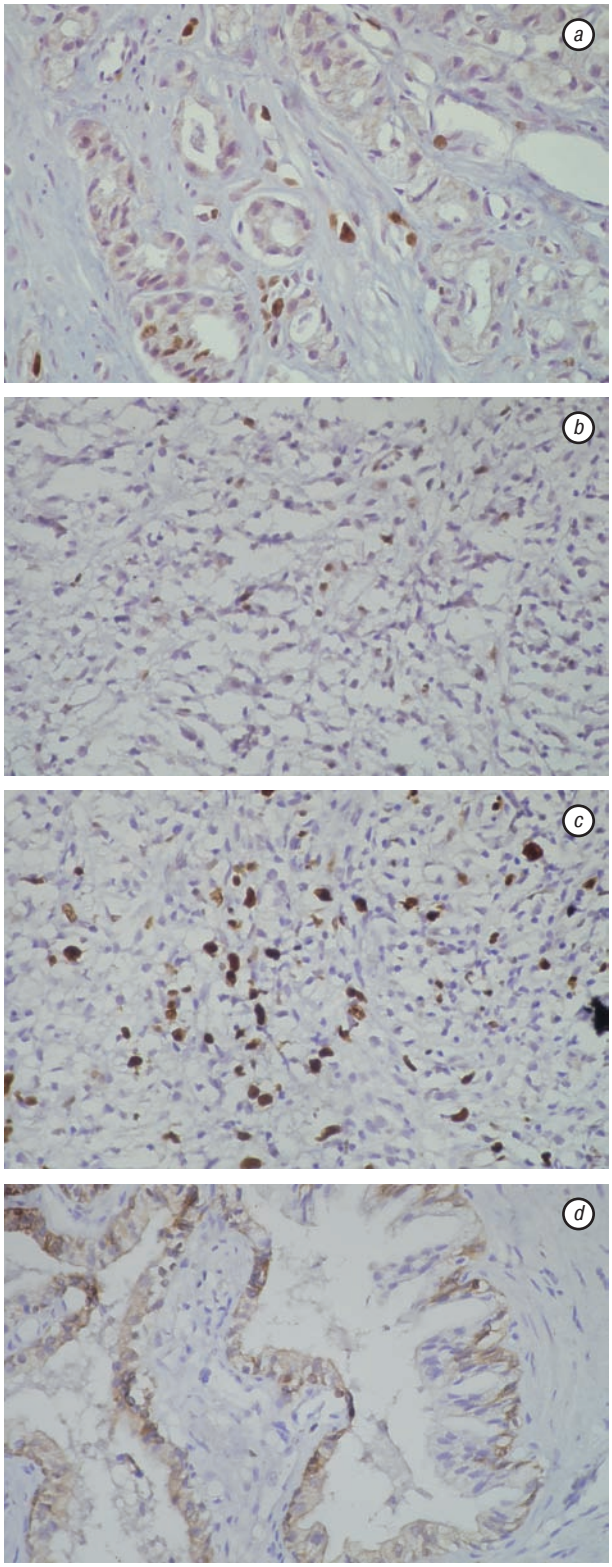

Fig. 2. Expression of Ki-67, Bcl-2 and p53 in prostate adenocarcinoma, 400X. *a*, Ki-67 expression in adenocarcinoma, Gleason stage 5; *b*, p53 expression in adenocarcinoma, Gleason stage 10; *c*, Ki-67 expression in adenocarcinoma, Gleason stage 10; *d*, Bcl-2 expression in adenocarcinoma, Gleason stage 4

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