Neuroendocrine differentiation in prostate cancer


Military Medical Academy, *Institute of Pathology, Belgrade; Clinical Hospital Center "Dragiša Mišović", †Department of Urology, Belgrade; Clinical Center of Serbia, ‡Institute of Urology and Nephrology, Belgrade

Background. In numerous recent studies attention has been focused to neuroendocrine differentiation (NED) in prostate cancer (PC). Focal NED is present in almost all PCs, but it is prominent in only 5–10% of the carcinomas. The prognostic significance of focal NED in PC is controversial, but current evidence suggests its influence on the onset and/or conversion of hormone resistant tumor phenotype. The aim of this study was to evaluate the relationship between NED status, based only on immunohistochemical use of neuroendocrine (NE) markers, with PC grade and stage, and preoperative serum levels of prostate-specific antigen (PSA). Methods. The study included the biopsy material of 73 untreated PC patients (pts.) obtained by transurethral resection (TUR) (37 pts.), and radical retropubic prostatectomy (RRP) (36 pts.). Two representative tissue samples (typically the block containing the largest amount of neoplasm) were selected for immunohistochemical (IHH) staining. NE cells were identified using a panel of IMM markers: chromogranin A, neuron-specific enolase, and serotonin. The level of PC exocrine differentiation was detected by monoclonal antibodies against PSA. Results. Significant expression of NE cells was demonstrated in 26 (70.2%) pts. with PC after TUR. In this group, serum preoperative PSA values ranged from 0.1 to 9.6 ng/ml. The majority of pts. with NED had low differentiated PC with Gleason grade score (GGS) >7, and normal PSA values below 4 ng/ml (77%), in clinical stage D (54%). Statistically significant correlation (p<0.01) of positive NED with higher stage and grade and low PSA values was established. Among the pts. with localized PC in whom RRP was performed (n=36), significant expression of NE cells was found in 13 pts. (41.7%), 8 (33.3%) in pT2 stage, and 7 (46.7%) in pT3 stage. Significant correlation between NED with preoperative PSA values and stage of PC in pts. with RRP was not found. Conclusion. We demonstrated the significant NED in poorly differentiated PC in patients in the advanced stage of the disease. The expression of NED in organ-confined PC did not correlate with tumor stage, but it correlated with tumor grade (GGS≥7).

Key words: carcinoma, neuroendocrine; prostatic neoplasms; tumor markers, biological; prostate-specific antigen.

Introduction

Prostate cancer (PC), the most common noncutaneous human malignancy in the Western world, was estimated to have an incidence of 220 900 cases in 2003, accounting for approximately one third of new cancer diagnoses in men. Prostate cancer alone accounts for approximately 33% (230 110) of incident cases in men. In 2003, 28 900 prostate cancer-related deaths were expected, a mortality burden surpassed only by that of lung cancer (1). The two main issues for clinicians and pathologists concerning PC-based work are the early detection of cancer and the identification of prognostic factors that predict the outcome in individual patients. Tumor grade, clinical stage, and serum prostate specific antigen (PSA) levels have become the cornerstone variables in the determination of treatment options for patients with PC. Much research effort has been set forth regarding the PC prognostic factors, and they were the topics of two recent international consensus conferences. In the first one of the consensus conferences, organized by the
College of American Pathologists, a multidisciplinary group of clinicians, pathologists, and statisticians analyzed the existing predictive factors, and stratified them into categories reflecting the significance of published evidence and taking into account the opinions of the prostate working group members of the College of American Pathologists. Factors were ranked as: Category I – the ones proved to be of prognostic significance and useful in clinical patient management; Category II – those that have been extensively studied biologically and clinically, but whose importance remained to be validated in statistically robust studies, and Category III – all other factors not sufficiently studied to show their prognostic value. Factors in category III included neuroendocrine differentiation, and factors such as oncogenes, tumor suppressor genes, apoptosis genes, etc (2, 3).

Endocrine-paracrine cells of the prostate (also known as APUD or neuroendocrine cells) constitute, in addition to basal and exocrine secretory cells, one third of population of highly specialized epithelial cells in the prostate gland. These cells contain, and most likely secrete, serotonin and calcitonin, as well as a variety of other peptides (4). Little is known of the functional role of these cells, but they probably subserve a paracrine or local regulatory role (5). Prostatic neuroendocrine (NE) cells, both benign and malignant, do not express the androgen receptor, in contrast to non-neuroendocrine prostate cancer cells, which usually express the androgen receptor (4). NE cells were also found in primary prostatic malignancies and in metastatic carcinomas and had been suggested to be a part of the malignancy, rather than having been trapped by the tumor (6). Normal NE cells are believed to be terminal differentiated, postmitotic cells. By increasing the intracellular levels of cAMP, PC cells can be induced to become postmitotic NE differentiated cells, morphologically similar to normal NE cells (4).

Both eutopic and ectopic hormone content/production have been associated with PC. Immunohistochemical (IMM) detection of eutopic hormones includes serotonin, thyroid-stimulating hormone, somatostatin, calcitonin, calcitonin gene-related protein, etc. Ectopic hormones detected by IMM include adrenocorticotropic hormone, beta-endorphin, human chorionic gonadotrophin, parathormone, leu-enkephalin, and glucagon. Paraneoplastic syndromes reported include Cushing’s syndrome, inappropriate secretion of antidiuretic hormone, and hypercalcemia (5, 6).

Focal neuroendocrine differentiation (NED) is present in virtually all prostate cancers but is prominent in only 5–10% of them. Prognostic significance of focal NED in PC is controversial, but current evidence suggests the influence on prognosis related to hormone resistant tumors and/or a role in the conversion to a hormonal resistant phenotype (4–8). The most important criteria in evaluating the prognostic significance of focal NED in PC are the selection of cases based on tumor Gleason grade score (GGS), and concomitant or previous hormone therapies. Moreover, the type, duration, and intermittency of hormone therapy may be equally relevant (9). Comprehension of neuroendocrine biology will lead to better treatment options of PC metastases (10). Several reports have shown an increased number of NE cells in advanced tumor stages, high-grade versus low-grade tumors, especially after androgen suppression therapy during tumor progression (7, 9, 11, 12). Studies of the prognostic value of NED in biopsy samples obtained after radical prostatectomy have yielded conflicting results (13, 14). Many explanations could be offered concerning the number of studies which demonstrated the prognostic significance of NED. The most important explanation is that different cohorts of patients were studied. Other explanations include methodological differences in determining the presence of NE cells, or interpretation differences (5, 6, 12).

The aim of this study was to evaluate the relationship between NED status, exclusively based on IMM detection of NE markers, with PC grade and stage, and preoperative PSA serum levels.

**Methods**

The study utilized the biopsy material of 73 untreated PC patients (pts.) obtained by transurethral resection (TUR) (37 pts.), and radical retropubic prostatectomy (RRP) (36 pts.). The analysis of serum PSA was performed by Hybri-tech method of monoclonal immunoassay. Normal serum PSA levels were ≤ 4.0 ng/ml, and intermediate PSA levels were 4.1–10 ng/ml. PC staging was classified by Whitmore-Jewet and TNM system. Clinical staging combined digital rectal examination, serum PSA level, transrectal ultrasonography, bone scan, and abdominal CT. The prostate, seminal vesicles, and lymph nodes were examined histologically in a standard fashion to determine extraprostatic extension status, surgical margin status, seminal vesicle involvement, and lymph node status. All the specimens were fixed in 10% phosphate-buffered formalin, processed into wax paraffin, and stained with hematoxylin-eosin for diagnostic purposes and histological grading. A GGS was based on the primary and secondary Gleason pattern. Two representative tissue samples (typically the block containing the largest amount of neoplasm) were selected for immunohistochemical staining. The level of PC exocrine differentiation by monoclonal antibodies against PSA was detected through IMM-PAP analysis. NED was scored as NE-positive (>10/10HPF). Microwave antigen retrieval procedure was performed in 0.5 mol/kg citrate buffer pH 6.0. Monoclonal mouse anti-human neuron-specific enolase (NSE) (DAKO Code No M0873, dilution 1:50), chromogranin A(CgA) (DAKO Code No M0869, dilution 1:100), and serotonin (SER) (DAKO Code No M0758, dilution 1:50), were used as primary antibodies, LSAB+ as visualization systems, and DAKO AEC+ as a chromogen. As a negative control, nonimmunized mouse serum was used instead of the NE antibody. Statistical analysis was done by Statistica for Windows v5.0 StatSoft Inc. software. P-values less than 0.05 were considered significant.
Results

We analyzed the expression of NED in the material obtained from TUR specimens in 37 pts. with PC, with preoperative PSA values of less than 10 ng/ml in the advanced stages (clinical stage C and D) of the disease, and from operative specimens after RRP in 36 pts. with preoperative PSA above 10 ng/ml. The secretory product of PSA was detected immunohistochemically in all the tissue specimens. The analysis demonstrated the presence of NE cells in all normal prostate tissues and in most hyperplastic and intraepithelial neoplastic lesions (Figure 1). Significant expression of NE cells was demonstrated in 26 (70.2%) pts. with PC after TUR, using CgA, NSE, and SER. In this group of pts., serum preoperative PSA values ranged from 0.1 to 9.6 ng/ml. GGS was below 7 in 7 pts., while 27 pts. (72.9%) had GGS above 7. Clinical stage of the disease was evaluated as C in 9 pts. (24.3%), and D in 28 pts. (75.7%). The distribution of preoperative PSA values was below 4 ng/ml in 27 pts. with PC (72.9%), and intermediate 4–10 ng/ml in 10 pts. (27.1%). PSA was below 4 ng/ml in 27 pts. (72.9%) with positive NED, while intermediate PSA values 4–10 ng/ml were detected in 5 pts. (13.5%) with positive NED.

Fig. 2 – Poorly differentiated PC with extensive serotonin immunoreactive NE cells (×100).

where RRP was performed (n= 36), significant expression of NE cells was found in 15 pts. (41.7%), 8 (53.3%) having pT2 stage and 7 (46.7%) having pT3 stage. Mean PSA (x ± SD) in pts. with PC and NED were 13.9 ng/ml (13.92 ± 11.84), while the mean PSA (x ± SD) in the whole group (n = 36) was 14.7 ng/ml (14.7 ± 11.7). We diagnosed 12 PC (80%) with GGS<7, and 3 PC (20%) with GGS>7. NED was significantly associated with GGG<7 (p<0.05). Significant correlation between NED with preoperative PSA values and the stage of PC was not found.

Discussion

The concept of the diffuse neuroendocrine system, first proposed by Feyter in 1938, was further refined and termed the Amine Precursor Uptake and Decarboxylation (APUD) System by Pearse (5). Research on the prostatic NE system is still in its infancy (15). Very little is known about the specific role that these cells have in the prostate, or their relationship to the pathogenesis, growth, and prognosis of prostatic diseases. Maturation of NE cells precedes that of PSA secretory cells for about 10–16 years (16). NE cells are located in all regions of the human prostate at birth, but rapidly decrease in the peripheral prostate after birth, and reappear in puberty (5, 12). After puberty, the number of NE cells seems to increase until an apparently optimum level is reached, persisting during the period 25–54 years of age (17). In 1994, Bostwick et al. published a study on prostatic intraepithelial neoplasia (PIN) and NE differentiation (18). Additional interest in prostate NE cells relates to the possibility that NE peptide products of these cells may influence the behavior of the tumors. The evidence that NE cells tend to occur in close proximity to proliferating cells in PC suggests the possibility that NE peptides may stimulate the growth of PC cells (4, 6). Three NE tumor phenotypes can be distinguished: (I) small cell NE carcinoma, accounting
for 1–2% of prostatic malignancy; (II) carcinoid-like tumors, also rare and poorly defined, and (III) conventional prostatic adenocarcinoma with focal NED, which is very common (15).

In addition to IMM studies, the clinical significance of NED in PC was evaluated by measuring serum NE markers CgA, NSE, and secretoneurin. CgA is frequently elevated in patients with advanced PC (19, 20). Increased serum levels of CgA were more consistent in patients with androgen-insensitive prostate tumors and there was a significant correlation between NE serum markers and distant metastases, but not with locally progressive disease. Patients with tumors with high plasma CgA or NSE levels were suggested to have poorer prognosis, which was less recognized by tumor grading alone, than the patients not presented with this type of differentiation (15). In contrast to chromogranin A, the utility of measuring NSE in patients with advanced prostate cancer is uncertain. The incidence of high NSE levels in patients with prostate cancer was lower than in the ones with high chromogranin A levels (21). Cussenot et al. (21) found that high NSE levels were more frequent during hormonal escape, but without prognostic value, compared with CgA expression, and in poor concordance with IMM data. However, the determination of NE markers in serum may not be specific for prostate activity, and only the analysis of a very large stratified population may determine whether there is a significant serum marker of NE activity in prostate cancer (15). In the study by Kadmon et al. (22) all the patients with elevated CgA were with aggressive hormone-resistant disease, and a third was negative for PSA. They reported that the plasma CgA level was elevated in 48% of 25 patients with stage D2 prostate cancer, and suggested that this marker could be used to monitor the clinical course of these patients (21). Our study was based exclusively on IMM detection of NE markers, without comparing serum and tissue antigens in the same PC patients.

Focal NED was present in 52–81% of patients with PC who underwent transurethral resection of the prostate (TURP) (23). We demonstrated focal NED in 70.2% of TURP specimens. Serum PSA levels were not elevated in 54% of TURP patients with poorly differentiated cancer and focal NED in the disseminated stage. Speights et al. (23) compared TURP patients with high-grade/high-stage cancer with the ones with low-grade/low-stage cancer and obtained similar results: IMM detection of NED increased in high grade and stage PC. Our data processing demonstrated significantly higher (p<0.01) NED in TURP patients with poorly differentiated PC and D stage.

Available literature contains some results for low serum PSA level in advanced PC. Low serum PSA level, identified in advanced PC, may be a consequence of genetic copy. In 1996, Baffa et al. (24) showed that malignant and normal prostatic tissues expressed the same PSA genetic copy. PSA molecule changes in PC patients represented certain post-translation event (24). However, PSA molecule with masked epitope was detected in cytoplasmatic protein complexes. The loss of some PSA epitope in PC may cause persistently low serum PSA levels during the diagnosis and monitoring progression (25). Recently, Kollara et al. (26) demonstrated that the lack of PSA production in PC-3 cells was a result of their lack of androgen receptor expression.

The biological significance of NE cells in PC was not minimized by the absence of a significant correlation between NED and the disease progression (14). Many explanations could be proposed concerning the number of studies which demonstrated the prognostic significance of NED. The most important explanation was that different cohorts of patients were studied. Other explanations included methodological differences in determining the presence of malignant NE cells, or interpretation differences. In this study, two different cohorts of patients were compared according to the biopsy mode, stage, and preoperative PSA values, while the progression of the disease and cancer-specific survival were not explored. Current methods for the analysis of NED are semiquantitative, and standards need to be set using precise tissue imaging techniques with three NE parameters to obtain consistency in interpretation. Finally, rather unequal distribution of NE cells in most tumors may cause serious sampling errors if biopsy specimens or limited tissue samples, are studied (5, 6, 15).

Studies of the prognostic value of NED in biopsy samples obtained after radical prostatectomy have yielded conflicting results. Several studies, including our study results, did not find a correlation between the number of NE cells and tumor stage in RP specimens (12–14). In contrast, significant correlation (p<0.05) between the number of NE cells and tumor grade (GGS≤7) in RP was found in this study. Weinstein et al. (27) found in the series of 104 patients that NED in PC improved the prediction of progression after radical prostatectomy, but only if the analysis was restricted to the 59 cases with Gleason grade 5 and 6. In the group of pts. after RRP, we found that preoperative PSA values were lower (13.9 ng/ml) than in comparison to the mean PSA in the whole group of pts (14.7 ng/ml), although without statistical significance.

There was more consistent evidence that NED was a prognostic factor in androgen-independent PC (6, 9). A long-acting somatostatin analog (lanreotide) was used to treat heavily pretreated prostate cancer patients with hormone refractory disease. A significant decrease in plasma chromogranin A levels was observed (without decrease in serum PSA levels), suggesting that therapy directed at malignant neoplastic NE cells in combination with other therapies might be an approach worthy of further investigation (28). Recently, serotonin has been found to show growth-promoting activity and to be functionally related to oncoproteins in PC. The design of specific antagonists for this type of receptor might be useful for the growth control of androgen-independent tumors (29).
Conclusion

Recent investigations have focused on the role of NED in the prostate and prostate cancer. We demonstrated sig-
nificant NED in poorly differentiated PC in patients in the advanced stage of the disease. The expression of NED in organ confined PC did not correlate with tumor stage, but it correlated with tumor grade (GGS£7).

LITERATURA


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A p s t r a k t


NEUROENDOKRINĂ DIFERENCIJACIJA U KARCINOMU PROSTATE

Uvod. Neuroendokrina diferencijacija (NED) u karcinomu prostate (KP), u današnje vreme, predstavlja predmet brojnih istraživanja. Fokusna NED prisutna je skoro u svim KP, ali je upadljiva samo kod 5–10% karcinoma. Iako diskutabilan, prognostički značaj fokusne NED u KP prema priključenim saznajnicima ukazuje na njen značaj u nastanku i/ili konverziji hormonski rezistentnog fenotipa tumora. Cilj analize bio je utvrđivanje korelacije između zastupljenosti NED, isključivo korišćenjem immunohistokemijskih (IMH) neuroendokrinih (NE) markera, sa graduom i stadijomom KP, kao i preoperativnim serumskim vrednostima prostata specifičnog antigena (PSA). Metode. Analizovan je biopsijski materijal 73 bolesnika sa KP, bez prethodne terapije, dobijen iz transuretralne resekcije (TUR) prostate (37 bolesnika) i radikalne retropubične prostatektomije (RRP) (36 bolesnika). Iz dva reprezentativna uzorka tkiva (sa značajnom zastupljenosti tumora) urađene su IMH analize. Korišćen je panel NE marker: homogranin A, neuron-specifična enolaza i serotonin. Monoklonalnim antitelim za PSA (IMH) određen je stepen egzokrine diferencijacije u KP. Rezultati. Značajnu NED ekspresiju utvrdili smo kod 26 (70,2%) bolesnika sa KP i urađenom TUR. Preoperativne vrednosti serumskog PSA u ovoj grupi bolesnika kretale su se od 0,1 do 9,6 ng/ml. Kod najvećeg broja bolesnika dijagnosticiran je slabo diferentovan KP sa Gleasonovim graduom (GG) iznad 7, normalne vrednosti serumskog PSA ispod 4 ng/ml (77%) i D klinički stadijum (54%). Utvrdili smo visoku statističku značajnost (p<0,01) između zastupljenosti NED, višeg stadijuma i gradusa KP i niskih vrednosti serumskog PSA. U grupi bolesnika sa učinjenom RRP zbog lokalizovana KP (n=36), značajna zastupljenost NED postojala je kod 15 (41,7%) bolesnika, od čega kod 8 (53,3%) bolesnika u pT2 stadijumu i 7 (46,7%) bolesnika u pT3 stadijumu bolesti. Nismo utvrdili značajnu razliku zastupljenosti NED od preoperativnih vrednosti serumskog PSA i stadijuma KP. Zaključak. Pokazali smo značajnu zastupljenost NED kod bolesnika sa slabo diferentovanim KP u stadijumu uznapredovale bolesti. U lokalizovanom KP postoji pozitivna korelacija NED sa GG≤7, ali ne sa stadijumom KP.

K l u č n e r e č i: karcinom, neuroendokrini; prostata, neoplazme; tumorski markeri, biološki; prostata, specifični antigen.