REVIEW

Neuroendocrine transdifferentiation of prostate carcinoma cells and its prognostic significance

MĂDĂLINA MARCU, E. RADU, MARIA SAJIN

"Carol Davila" University of Medicine and Pharmacy, Bucharest

Abstract

Neuroendocrine (NE) cells are a distinct epithelial cell compartment of the normal human prostate gland. Their phenotype and range of endocrine secretion products are similar, but not identical to those of NE-like cells from prostate carcinoma. Neuroendocrine differentiation (NED) is a feature commonly seen in prostate carcinoma and a number of studies pointed out that its extent is associated to hormone therapy refractory and aggressive disease. However, less information is available on the significance of NED in organ-confined prostate cancer, although identification of early predictors of aggressive disease would obviously allow for more adequate therapy. We review here recent advances in understanding the differentiation pathways of normal and malignant neuroendocrine cells as well as current information regarding the prognostic and therapeutic implication of NED assessment.

***Keywords*:** neuroendocrine cells, prostate, prostate cancer, cell differentiation, prognosis.

Introduction

Prostate cancer is the most frequent malignancy in men with an estimated age-standardized incidence of 106.2 new cases per 100 000 people for EU25 countries in 2006, almost a quarter of overall cancer incidence; in Romania, the estimated incidence in the same year was 32.2 [1]. Other statistical data [2] show that in Romania malignant neoplasm of prostate is the hospital discharge diagnostic for 64.80 patients in 100 000 inhabitants and is responsible for a standardized death rate of 15.30/100 000 inhabitants. Although deaths caused by prostate cancer rank third among cancer deaths in both sexes [1], most cases are unlethal.

Currently, prognostics tools based on statistical models with the ability to predict various risks for individual cases are used to guide clinical decisions and provide counseling for patients (reviewed in [3]). These tools include nomograms, probability tables (such as the "Partin staging tables"), classification and regression tree analyses and artificial neural networks. Of all these algorithms, nomograms are the most accurate and discriminating tools for predicting outcomes in patients with prostate cancer.

Prostate biopsy is indicated in patients up to 75-year-old with elevated serum PSA levels and/or suspicious digital rectal examination findings. Clinical features (preoperative serum PSA levels, clinical stage) and histopathological biopsy findings (primary and secondary Gleason grades, number of positive and negative biopsy cores) are shown to be quite accurate predictors of the tumor pathology. Patients are stratified according to disease extension: organ-confined cancer (pT1, T2) benefits of definitive therapy (radical prostatectomy, radiotherapy, neoadjuvant therapy), while extraprostatic tumor extension or metastasis are an indication for hormone therapy. However, T1–T2 prostatic carcinoma is a heterogeneous tumor regarding outcome after radical prostatectomy; recurrence, metastasis and/or biochemical relapse can occur sometimes rapidly in a small number of patients.

Although pre-operative prognostics tools are validated estimators of disease pathology, their ability to predict biochemical relapse, prostate cancer-related metastasis or death is limited. Nomograms currently in use and are also inadequate for the identification of patients that require minimal or no treatment (the so-called "insignificant cancers", less than 0.5 cm^3 in volume, regardless of stage). Current therapeutic strategies (prostatectomy, hormone and adjuvant therapy) may be over-treating a number of the patients, while being insufficient for those with aggressive forms of disease. Due to high prevalence of the disease, the treatment side effects, the increasing age of the population; it would be of tremendous benefit to be able to recognize aggressive forms of prostatic cancer.

Hormone therapy aimed at androngen deprivation of malignant cells is well established for the treatment of metastatic disease, recurrence after radical prostatectomy or radiotherapy and as neoadjuvant therapy. However, 18 to 36 month after an initial response to anti-androgen therapy, most of the prostate carcinomas switch to a hormone resistant phenotype, entering into a more aggressive and ultimately fatal stage of disease.

Neuroendocrine differentiation (NED) is seen in virtually all cases of prostatic carcinoma, mostly in a focal pattern; extensive NED is associated to hormone therapy refractory, aggressive disease. Yet neuroendocrine differentiation is included by the *College of American Pathologists Consensus Statement 1999* in Category III of prognostic and predictive factors

(not sufficiently studied to demonstrate prognostic value) [4]. This review discusses recent advances in the study of neuroendocrine differentiation mechanisms, prognostic value and implication for therapy.

Neuroendocrine (NE) cells in normal prostate

Normal prostate epithelium comprises three cell types: luminal (secretory), basal and neuroendocrine cells (reviewed in [5]). Prostatic glandular component arises during organ development from epithelial stem cells under the inductory influence of urogenital sinus mesenchyme; small epithelial buds branch and develop into mature secretory acini.

A small population of stem cells persists in the adult organ, most probably confined within the basal cell compartment. Several observations support this hypothesis: some of the basal cells (as defined by their histological characteristics) show the typical infrequent proliferation associated to adult stem cells, yet have high self-renewal capacity; they are able to reconstitute the gland after experimental androgen ablation in animal models through differentiation into secretory and neuroendocrine cells. Terminal differentiation, at least into secretory cells, is preceded by a stage of transit-amplifying epithelial cells characterized by poor self-renewal capacity but high mitotic activity.

According to this model [6], neuroendocrine and secretory cells arise both from a common progenitor – adult prostate stem cells (PSC). Several experiments support this view: androgen administration in castrated rats leads to regeneration of the prostate in a pattern similar to normal development [7, 8]. Blastocyst complementation studies in p63-/- mice show that transplanted p63+ embryonic stem cells differentiate into both basal and luminal secretory cells [9]. However, Kurita T *et al.* concluded from UGS transplantation studies that p63 is essential for differentiation of basal cells, but p63 and thus basal cells are not required for differentiation of prostatic neuroendocrine and luminal epithelial cells [10]. Recent studies also point to a common origin for NE and epithelial cells in the prostate: in a mouse transplantation model, Leong KG *et al.* showed that an entire prostate gland, including neuroendocrine cells, can be generated starting from an individual adult prostate stem cell with the phenotype Sca-1+CD133+CD44+CD117+ [11]. It has also been demonstrated that CD133, a marker expressed by a number of somatic stem cells, is present on stem-like prostate cells that have high clonogenic potential and a basal location [12, 13], and are able to differentiate *in vitro* into both secretory and neuroendocrine cells [14, 15]. Moreover, expression of oct4, a "stemness" signature gene was recently demonstrated in human basal p63+ prostatic cells [16].

It should be mentioned that a 1999 study by Aumüller G *et al.* suggests a different origin for neuroendocrine cells. The authors found that in 10-week-old human embryos UGS epithelium was free of neuroendocrine cells that were present in the surrounding mesenchyme and paraganglia. Later on, after week 12, chromogranin A positive cells were present within the epithelial buds that ultimately develop into prostate glandular components. These findings were interpreted by the authors as circumstantial evidence that neuroendocrine cells have neurogenic origin, distinct from the UGS-derived basal and luminal cells [17].

Neuroendocrine cells are distributed throughout the normal prostate, with higher frequency in ducts than in acinar tissue. Two types of prostatic neuroendocrine cells have been described, according to their morphology: the "open" cell type with long processes reaching the lumen and the "closed" type without luminal extensions. Both cell types show irregular, dendritic processes that extend between adjacent cells ([18] cited by Abrahamsson PA [19]). NE cells have argentaffin and argyrophilic characteristics and their vast majority express/secrete a number of neuroendocrine markers: chromogranin A, neuron specific enolase, serotonin, calcitonin, bombesin, somatostatin and a number of other neuropeptides and proteins (reviewed in [20]). Neuroendocrine cells express synaptophysin, PGP 9.5, CD56, do not proliferate (Ki67-), and do not express p63 protein or PSA [21]. Another phenotype characteristic of NE cells that is considered relevant in pathologic circumstances is their lack of androgen receptor expression [22]. The neuroendocrine component of prostatic carcinoma will survive androgen deprivation therapy, which renders the tumors castration-resistant and incurable.

NE-like prostate cancer cells

Neuropeptides released by NE cells may facilitate the development of androgen independence, acting as autocrine and paracrine growth factors for malignant cells. In prostate cancer cells, neuropeptides have been shown to promote cell growth, migration and protease expression [21]. Bombesin/gastrin-releasing peptide (GRP) transmit their signals through G protein-coupled receptors, which are often overexpressed in prostate cancer, and aberrantly activate androgen receptor (AR) in the absence of androgen [23].

However, the origin of NE cells and the molecular mechanism of NE cell enrichment during prostatic carcinoma progression are not fully understood. Recent data suggest that adenocarcinoma cells undergo a trans-differentiation process to become NE-like cells, which acquire the NE phenotype and express NE markers and could thus be termed 'NE-like PCa cells' [24]. Underlying this naming convention are the demonstrated differences between normal and cancer NE cells: the former express cytokeratin 5, a basal cell marker [25], while the latter have characteristics of luminal cells such as cytokeratin 18 and prostatic acid phosphatase expression [21, 26]. Also, as opposed to normal prostate NE cells, NE-like malignant cells express anti-apoptotic protein bcl-2 [27] and α-methylacyl-CoA racemase (AMACR) [26].

Furthermore, a number of studies in animal models, cell lines and primary tumor cells suggest some of the molecular mechanisms leading to prostate carcinoma cells transdifferentiation into NE-like cells (also reviewed

in [24]): androgen depletion (through RPTPα-, PTPB-1- or protocadherin-PC-mediated signaling pathways), increase of intracellular cAMP concentration and IL-6 signaling through associated cell receptors.

A different model of prostate tumorigenesis, proposed by Bonkhoff H and Remberger K [6], that has recently received attention is the cancer stem cell (CSC) model. It states that tumors contain a pool of self-renewing cells that maintain the heterogeneous malignant cell population. Cancer stem' cells are resistant to therapy and can survive and repopulate the tumor. Origin of CSC is still uncertain, with evidence suggesting that either normal prostate stem cells, progenitor or even differentiated secretory cells could acquire epigenetic modifications and genetic mutations that would give rise to CSC [28]. The most frequently identified markers of cancer stem cells include CD44 [29], CD133 [13, 15], and integrins [30]. Some of the properties of identified prostate cancer stem cells are common to a number of other organs CSC, including high colony-forming capacity, prolonged survival in cell cultures, growth *in vitro* as spheres of aggregated cells, and the ability to give rise to tumors in immunocompromised animals. Noteworthy, Palapattu GS *et al*. have recently shown [31] that neuroendocrine tumor cells of prostate cancer selectively express CD44 *in vivo* and *in vitro*, leading them to conclude that such cells are significant for therapy resistance and tumor recurrence. Furthermore, the number of cells coexpressing oct4, a stemness marker, and chromogranin A or synaptophysin increases in prostate cancer compared to benign prostate and these cells represent NE-like prostate cancer cells [16].

Prognostic value of NED in prostatic carcinoma

All of the above characteristics of normal and cancer-associated neuroendocrine cells have led to a number of studies that analyzed the value of neuroendocrine differentiation assessment for prostate carcinoma prognostics and therapy. Although there is no accepted definition of NED in PCa, it is often identified by scattered clusters of differentiated NE cells among a predominant population of adenocarinoma cells, except for rare cases of small cell carcinoma or carcinoid [32]. Two of the most frequently used methods to study neuroendocrine differentiation are the immunodetection of NE tissue and/or serum markers such as chromogranin A. Results obtained are conflicting possibly due to differences in lot size and selection criteria, in analysis end-points definition and in methods used. It is also conceivable that increasing NE differentiation is a phenomenon associated to worse tumor stage and/or grade, rather than being an independent prognostic factor. Thus, in advanced, non-organ confined prostate cancer correlation of NE differentiation with a poor prognosis might be coincidental, not causative.

Organ-confined disease

Only a few studies addressed the prognostic significance of NE differentiation in localized prostate cancer, and data collected on prostatic biopsies is even scarcer. Weinstein MH *et al.* [33] studied 104 patients with clinically organ-confined CaP treated only by radical prostatectomy, with the end-point of biochemical disease progression. Results showed that histological grade and NE differentiation seen in prostatectomy samples predicted progression in multivariate analysis. Moreover, extent of NE differentiation (more than 70 chromogranin A positive cells per representative section, as revealed by immunohistochemistry) separated patients with tumors of Gleason sum less than or equal to six into groups with high and low risks for progression, independent of Gleason sum. The latter observation could provide a basis for stratifying the estimated 85% of newly diagnosed prostate cancers that are organ-confined [34].

Theodorescu D *et al.* [35] studied 71 patients with localized disease treated by radical prostatectomy, using immunohistochemistry to identify expression of chromogranin A and cathepsin D. The authors concluded that the level of Chr A immunoreactivity is a strong predictor of disease specific survival and is superior to standard pathologic prognostic factors (such as Gleason score, capsular penetration, seminal vesicle invasion, and percentage of tumor in the specimen).

Recently, a multivariate analysis of NE differentiation and malignant cell proliferation (Ki-67 labeling index) on radical prostatectomy specimens found NED to be the second most significant predictor of biochemical progression, after Gleason score [36]. The authors also observed a significantly higher Ki-67 reactivity in the chromogranin A positive group in comparison with chromogranin A negative specimens, and concluded that NE differentiation could serve as additional prognostic marker in radical prostatectomy samples. Another study by Gunia S *et al.* [37] also demonstrated that Ki67 index and NED significantly aggravate established adverse prognostic parameters such as nodal status, tumor stage, pretherapeutic PSA-level, and Gleason score.

However, other studies lead to diverging conclusions: Ahlegren G *et al.* [38] found that NE differentiation in localized prostate cancer is not *per se* a prognostic factor of failure after radical prostatectomy. More recently, Revelos K *et al.* [39] studied 130 patients with clinically localized prostate cancer, treated with radical prostatectomy. Although a positive association was detected between NED and pathological features of the tumors, the extent of neuroendocrine differentiation was not found to be an independent prognostic factor for biochemical failure of therapy in multivariate analysis. Similar conclusions were drawn by Autorino R *et al.* [40], after noting that in univariate analysis NE expression did not predict biochemical recurrence free survival, whereas it was associated with clinical recurrence.

In prostate cancer extending beyond the organ limits, most of the studies pointed out a strong association between the extent of NE differentiation and aggressive disease, but here also there are conflicting conclusions regarding the value of NED as an independent prognostic factor. Increased NE differentiation was found to be associated with aggressive disease [41, 42], Gleason

score [43, 44], anti-androgen therapy failure [41] and survival [44, 45]. However, McWilliam LJ *et al.* concluded that detection of neuroendocrine differentiation in conventional prostatic adenocarcinoma is not an independent indicator of prognosis [44].

Hormone-refractory carcinoma

Prostate cancer has been recognized as an androgen-sensitive disease since the work of Huggins and Hodges in 1941. Androgen ablation, involving antiandrogens and luteinizing hormone-releasing hormone analogues, remains the main therapeutically approach of metastatic cancer, recurrence after definitive treatment and as neoadjuvant therapy concurrent with radiotherapy [46]. Anti-androgen therapy prolongs overall survival, reduces bone and soft tissue metastases mass and suppresses PSA levels in most of the patients with metastatic prostate cancer. However, disease progresses despite therapy at a median of 2–3 years, with a subsequent expected survival of 16–18 months from the time of progression [47]. Duration of response to therapy is variable, with 5–10% of patients remaining alive 10 years after initiating ADT [48].

The efficacy of different drugs or drug combinations to induce androgen suppression is estimated in relation to the goal of achieving serum levels of total testosterone below 50 ng/dL. When progression occurs despite maintenance of target serum androgen levels, the disease is considered to be 'androgen-independent' or 'hormone-refractory'.

There are several theories addressing the mechanism of hormone-refractory PCa [47–49]. These theories propose either continued AR signaling *via* alternative pathways, or truly AR-independent mechanisms. Continued signaling through the AR is dependent on increased AR sensitivity through AR amplification or mutation. These alterations increase AR sensitivity to DHT, nonandrogenic steroid molecules or anti-androgens.

AR-independent mechanisms of androgen-independent PCa could at least in part be due to the presence of NE differentiation. Malignant NE cells do not express AR, are more resistant to apoptosis and also express and secrete a number of molecules that can act as antiapoptotic and growth factors on adenocarcinoma cells [21].

Neuroendocrine differentiation (NED) and hormone-refractory disease seem to be associated phenomena: extensive NED of a tumor renders it androgen-independent, and androgen blockade induces NED. Moreover, the extent of neuroendocrine component of a prostatic tumor is related to Gleason score, thus advanced prostatic cancer, which is the main indication for hormone therapy, already has, in most instances, a significant neuroendocrine component.

Evidence for the impact of anti-androgen therapy on NED comes from a number of studies shown that chromogranin A expression in either prostate tissue or serum increases in continuous androgen deprivation therapy, pointing out that NED progresses more rapidly if androgen deprivation is carried out more intensively [50–53].

Therapeutical implications

Alternatives to standard androgen deprivation therapy, such as intermittent androgen suppression and estrogen therapy, hold the potential to reduce the switch towards androgen-independent growth of CaP while maintaining clinical benefit (reviewed in [48]). However, once the hormone-refractory stage of prostate cancer has been reached, the only non-experimental therapeutic option is chemotherapy, which is usually also of limited benefit to patients.

Pharmaceutical agents able to block the tumor-promoting action of neuroendocrine cancer cells products are under investigation. Serotonin and bombesin antagonists under study show promising experimental results. Also IL-6 could be considered as a new therapeutic approach, since *in vitro* and *in vivo* experiments have demonstrated that IL-6 induced NE transdifferentiation of prostate cancer cells has a significant inhibitory effect on tumor growth [21], [32]. Somatostatin inhibits NE activity and, through specific receptors, is able to reduce angiogenesis, proliferation and to promote apoptosis of cancer cells. Somatostatin analog used in combination with other agents lead to PSA and symptomatic responses in several studies [32].

Neuroendocrine transdifferentiation of prostate carcinoma cells is a constant phenomenon, with possible prognostic significance and consequences on therapy response. In organ-confined disease, assessment of NED could identify a group of patients that would benefit from more aggressive therapy, while extended NED in HRPC could be a trigger for a particular anti-androgen regimen.

References

[1] FERLAY J, AUTIER P, BONIOL M, HEANUE M, COLOMBET M, BOYLE P, *Estimates of the cancer incidence and mortality in Europe in 2006*, Ann Oncol, 2007, 18(3):581–592.

[2] ***, *The Statistical Office of the European Communities (EUROSTAT) Dissemination Database*, http://ec.europa.eu/eurostat, accessed in January 2009.

[3] SHARIAT SF, KARAKIEWICZ PI, MARGULIS V, KATTAN MW, *Inventory of prostate cancer predictive tools*, Curr Opin Urol, 2008, 18(3):279.

[4] BOSTWICK DG, GRIGNON DJ, HAMMOND ME, AMIN MB, COHEN M, CRAWFORD D, GOSPADAROWICZ M, KAPLAN RS, MILLER DS, MONTIRONI R, PAJAK TF, POLLACK A, SRIGLEY JR, YARBRO JW, *Prognostic factors in prostate cancer. College of American Pathologists Consensus Statement 1999*, Arch Pathol Lab Med, 2000, 124(7):995–1000.

[5] ISAACS JT, *Prostate stem cells and benign prostatic hyperplasia*, Prostate, 2008, 68(9):1025–1034.

[6] BONKHOFF H, REMBERGER K, *Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model*, Prostate, 1996, 28(2):98–106.

[7] LESSER B, BRUCHOVSKY N, *Effect of duration of the period after castration on the response of the rat ventral prostate to androgens*, Biochem J, 1974, 142(2):429–431.

[8] EVANS GS, CHANDLER JA, *Cell proliferation studies in the rat prostate: II. The effects of castration and androgen-induced regeneration upon basal and secretory cell proliferation*, Prostate, 1987, 11(4):339–351.

[9] SIGNORETTI S, PIRES MM, LINDAUER M, HORNER JW, GRISANZIO C, DHAR S, MAJUMDER P, MCKEON F, KANTOFF PW, SELLERS WR, LODA M, *p63 regulates commitment to the prostate cell lineage*, Proc Natl Acad Sci U S A, 2005, 102(32):11355–11360.

[10] KURITA T, MEDINA RT, MILLS AA, CUNHA GR, *Role of p63 and basal cells in the prostate*, Development, 2004, 131(20):4955–4964.
[11] LEONG KG, WANG BE, JOHNSON L, GAO WQ, *Generation of a prostate from a single adult stem cell*, Nature, 2008, 456(7223):804–808.
[12] COLLINS AT, HABIB FK, MAITLAND NJ, NEAL DE, *Identification and isolation of human prostate epithelial stem cells based on alpha(2)beta(1)-integrin expression*, J Cell Sci, 2001, 114(Pt 21):3865–3872.
[13] RICHARDSON GD, ROBSON CN, LANG SH, NEAL DE, MAITLAND NJ, COLLINS AT, *CD133, a novel marker for human prostatic epithelial stem cells*, J Cell Sci, 2004, 117(Pt 16):3539–3545.
[14] LITVINOV IV, VANDER GRIEND DJ, XU Y, ANTONY L, DALRYMPLE SL, ISAACS JT, *Low-calcium serum-free defined medium selects for growth of normal prostatic epithelial stem cells*, Cancer Res, 2006, 66(17):8598–8607.
[15] VANDER GRIEND DJ, KARTHAUS WL, DALRYMPLE S, MEEKER A, DEMARZO AM, ISAACS JT, *The role of CD133 in normal human prostate stem cells and malignant cancer-initiating cells*, Cancer Res, 2008, 68(23):9703–9711.
[16] SOTOMAYOR P, GODOY A, SMITH GJ, HUSS WJ, *Oct4A is expressed by a subpopulation of prostate neuroendocrine cells*, Prostate, 2009, 69(4):401–410.
[17] AUMÜLLER G, LEONHARDT M, JANSSEN M, KONRAD L, BJARTELL A, ABRAHAMSSON PA, *Neurogenic origin of human prostate endocrine cells*, Urology, 1999, 53(5):1041–1048.
[18] DI SANT'AGNESE PA, DE MESY JENSEN KL, *Endocrine-paracrine cells of the prostate and prostatic urethra: an ultrastructural study*, Hum Pathol, 1984, 15(11):1034–1041.
[19] ABRAHAMSSON PA, *Neuroendocrine cells in tumour growth of the prostate*, Endocr Relat Cancer, 1999, 6(4):503–519.
[20] ABRAHAMSSON PA, *Neuroendocrine differentiation in prostatic carcinoma*, Prostate, 1999, 39(2):135–148.
[21] VASHCHENKO N, ABRAHAMSSON PA, *Neuroendocrine differentiation in prostate cancer: implications for new treatment modalities*, Eur Urol, 2005, 47(2):147–155.
[22] KRIJNEN JL, JANSSEN PJ, RUIZEVELD DE WINTER JA, VAN KRIMPEN H, SCHRÖDER FH, VAN DER KWAST TH, *Do neuroendocrine cells in human prostate cancer express androgen receptor?*, Histochemistry, 1993, 100(5):393–398.
[23] YANG JC, OK JH, BUSBY JE, BOROWSKY AD, KUNG HJ, EVANS CP, *Aberrant activation of androgen receptor in a new neuropeptide-autocrine model of androgen-insensitive prostate cancer*, Cancer Res, 2009, 69(1):151–160.
[24] YUAN TC, VEERAMANI S, LIN MF, *Neuroendocrine-like prostate cancer cells: neuroendocrine transdifferentiation of prostate adenocarcinoma cells*, Endocr Relat Cancer, 2007, 14(3):531–547.
[25] SCHALKEN JA, VAN LEENDERS G, *Cellular and molecular biology of the prostate: stem cell biology*, Urology, 2003, 62(5 Suppl 1):11–20.
[26] HUANG J, YAO JL, DI SANT'AGNESE PA, YANG Q, BOURNE PA, NA Y, *Immunohistochemical characterization of neuroendocrine cells in prostate cancer*, Prostate, 2006, 66(13):1399–1406.
[27] XUE Y, VERHOFSTAD A, LANGE W, SMEDTS F, DEBRUYNE F, DE LA ROSETTE J, SCHALKEN J, *Prostatic neuroendocrine cells have a unique keratin expression pattern and do not express Bcl-2: cell kinetic features of neuroendocrine cells in the human prostate*, Am J Pathol, 1997, 151(6):1759–1765.
[28] KASPER S, *Stem cells: The root of prostate cancer?*, J Cell Physiol, 2008, 216(2):332–336.
[29] HURT EM, KAWASAKI BT, KLARMANN GJ, THOMAS SB, FARRAR WL, *CD44+ CD24(-) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis*, Br J Cancer, 2008, 98(4):756–765.
[30] LAWSON DA, XIN L, LUKACS RU, CHENG D, WITTE ON, *Isolation and functional characterization of murine prostate stem cells*, Proc Natl Acad Sci U S A, 2007, 104(1):181–186.
[31] PALAPATTU GS, WU C, SILVERS CR, MARTIN HB, WILLIAMS K, SALAMONE L, BUSHNELL T, HUANG LS, YANG Q, HUANG J, *Selective expression of CD44, a putative prostate cancer stem cell marker, in neuroendocrine tumor cells of human prostate cancer*, Prostate, 2009, 69(7):787–798.
[32] KOMIYA A, SUZUKI H, IMAMOTO T, KAMIYA N, NIHEI N, NAYA Y, ICHIKAWA T, FUSE H, *Neuroendocrine differentiation in the progression of prostate cancer*, Int J Urol, 2009, 16(1):37–44.
[33] WEINSTEIN MH, PARTIN AW, VELTRI RW, EPSTEIN JI, *Neuroendocrine differentiation in prostate cancer: enhanced prediction of progression after radical prostatectomy*, Hum Pathol, 1996, 27(7):683–687.
[34] COOPERBERG MR, BROERING JM, LITWIN MS, LUBECK DP, MEHTA SS, HENNING JM, CARROLL PR; CAPSURE INVESTIGATORS, *The contemporary management of prostate cancer in the United States: lessons from the cancer of the prostate strategic urologic research endeavor (CapSURE), a national disease registry*, J Urol, 2004, 171(4):1393–1401.
[35] THEODORESCU D, BRODER SR, BOYD JC, MILLS SE, FRIERSON HF JR, *Cathepsin D and chromogranin A as predictors of long term disease specific survival after radical prostatectomy for localized carcinoma of the prostate*, Cancer, 1997, 80(11):2109–2119.
[36] MAY M, SIEGSMUND M, HAMMERMANN F, LOY V, GUNIA S, *Prognostic significance of proliferation activity and neuroendocrine differentiation to predict treatment failure after radical prostatectomy*, Scand J Urol Nephrol, 2007, 41(5):375–381.
[37] GUNIA S, ALBRECHT K, KOCH S, HERRMANN T, ECKE T, LOY V, LINKE J, SIEGSMUND M, MAY M, *Ki67 staining index and neuroendocrine differentiation aggravate adverse prognostic parameters in prostate cancer and are characterized by negligible inter-observer variability*, World J Urol, 2008, 26(3):243–250.
[38] AHLEGREN G, PEDERSEN K, LUNDBERG S, AUS G, HUGOSSON J, ABRAHAMSSON PA, *Neuroendocrine differentiation is not prognostic of failure after radical prostatectomy but correlates with tumor volume*, Urology, 2000, 56(6):1011–1015.
[39] REVELOS K, PETRAKI C, SCORILAS A, STEFANAKIS S, MALOVROUVAS D, ALEVIZOPOULOS N, KANELLIS G, HALAPAS A, KOUTSILIERIS M, *Correlation of androgen receptor status, neuroendocrine differentiation and angiogenesis with time-to-biochemical failure after radical prostatectomy in clinically localized prostate cancer*, Anticancer Res, 2007, 27(5B):3651–3660.
[40] AUTORINO R, LAMENDOLA MG, DE LUCA G, DE SIO M, GIULIANO F, D'ARMIENTO M, DE PLACIDO S, CONTI P, DI LORENZO G, *Neuroendocrine immunophenotype as predictor of clinical recurrence in 110 patients with prostate cancer*, Int J Immunopathol Pharmacol, 2007, 20(4):765–770.
[41] TAPLIN ME, GEORGE DJ, HALABI S, SANFORD B, FEBBO PG, HENNESSY KT, MIHOS CG, VOGELZANG NJ, SMALL EJ, KANTOFF PW, *Prognostic significance of plasma chromogranin A levels in patients with hormone-refractory prostate cancer treated in Cancer and Leukemia Group B 9480 study*, Urology, 2005, 66(2):386–391.
[42] GROBHOLZ R, GRIEBE M, SAUER CG, MICHEL MS, TROJAN L, BLEYL U, *Influence of neuroendocrine tumor cells on proliferation in prostatic carcinoma*, Hum Pathol, 2005, 36(5):562–570.
[43] BERRUTI A, MOSCA A, TUCCI M, TERRONE C, TORTA M, TARABUZZI R, RUSSO L, CRACCO C, BOLLITO E, SCARPA RM, ANGELI A, DOGLIOTTI L, *Independent prognostic role of circulating chromogranin A in prostate cancer patients with hormone-refractory disease*, Endocr Relat Cancer, 2005, 12(1):109–117.
[44] MCWILLIAM LJ, MANSON C, GEORGE NJ, *Neuroendocrine differentiation and prognosis in prostatic adenocarcinoma*, Br J Urol, 1997, 80(2):287–290.
[45] KAMIYA N, SUZUKI H, KAWAMURA K, IMAMOTO T, NAYA Y, TOCHIGI N, KAKUTA Y, YAMAGUCHI K, ISHIKURA H, ICHIKAWA T, *Neuroendocrine differentiation in stage D2 prostate cancers*, Int J Urol, 2008, 15(5):423–428.
[46] SHARIFF AH, ATHER MH, *Neuroendocrine differentiation in prostate cancer*, Urology, 2006, 68(1):2–8.
[47] PIENTA KJ, BRADLEY D, *Mechanisms underlying the development of androgen-independent prostate cancer*, Clin Cancer Res, 2006, 12(6):1665–1671.

[48] HARRIS WP, MOSTAGHEL EA, NELSON PS, MONTGOMERY B; MEDSCAPE, *Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion*, Nat Clin Pract Urol, 2009, 6(2):76–85.

[49] TAPLIN ME, *Drug insight: role of the androgen receptor in the development and progression of prostate cancer*, Nat Clin Pract Oncol, 2007, 4(4):236–244.

[50] SASAKI T, KOMIYA A, SUZUKI H, SHIMBO M, UEDA T, AKAKURA K, ICHIKAWA T, *Changes in chromogranin A serum levels during endocrine therapy in metastatic prostate cancer patients*, Eur Urol, 2005, 48(2):224–229; discussion 229–230.

[51] SCIARRA A, DI SILVERIO F, *Effect of nonsteroidal anti-androgen monotherapy versus castration therapy on neuro-endocrine differentiation in prostate carcinoma*, Urology, 2004, 63(3):523–527.

[52] TARLE M, AHEL MZ, KOVACIĆ K, *Acquired neuroendocrine-positivity during maximal androgen blockade in prostate cancer patients*, Anticancer Res, 2002, 22(4):2525–2529.

[53] HIRANO D, OKADA Y, MINEI S, TAKIMOTO Y, NEMOTO N, *Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy*, Eur Urol, 2004, 45(5):586–592; discussion 592.

Corresponding author

Mădălina Marcu, MD, PhD, Pathology Department, "Carol Davila" University of Medicine and Pharmacy, "Victor Babeş" National Institute for Research and Development in Pathology and Biomedical Sciences, 99–101 Independenței Avenue, 050096 Bucharest, Romania; Phone +40744–367 136, e-mail: mad_marcu@yahoo.com

Received: November 3rd, 2009

Accepted: January 25th, 2010