Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Expression of neuroendocrine differentiation markers in lethal metastatic castration-resistant prostate cancer

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ARTICLE INFO

Keywords:
Neuroendocrine differentiation
Immunohistochemistry
Castration-resistant prostate cancer
Androgen receptor
Chromogranin A
Synaptophysin

ABSTRACT

Neuroendocrine differentiation (NED) is a common phenomenon in prostate cancer, and it has been associated with poor prognosis in some studies of primary prostate cancer. Incidence and patterns of NED in metastatic prostate cancer sites have not been examined widely. In this study, we studied expression of three commonly used markers of NED (chromogranin A, neuron specific enolase and synaptophysin) in 89 metastases from 31 men that died of castration-resistant prostate cancer and underwent rapid autopsy, and in 89 hormone-naïve primary tumors removed by radical prostatectomy. In addition, we examined NED association with androgen receptor, ERG and Ki-67 expression in metastatic tumor sites. Morphologically, 1 of 31 cases was classified as small cell carcinoma, and the remaining 30 were classified as usual prostate adenocarcinoma using a recently proposed classification of prostate cancers with NED. Metastases showed more expression of neuron specific enolase and synaptophysin compared to prostatectomies (6.3% of cells vs. 1.0%, $p < 0.001$ and 4.0% vs. 0.4%, $p < 0.001$, respectively). At least focal expression of one of the markers was seen in 78% of metastases. Strong expression was relatively uncommon, seen in 3/89 (chromogranin A), 8/89 (neuron specific enolase), and 5/89 (synaptophysin) metastases. Expression of chromogranin A and synaptophysin correlated with each other ($r = 0.64$, $p < 0.001$), but expression of neuron specific enolase did not correlate with the two other markers. Extent of NED varied significantly between different metastatic sites in individual patients. Absent androgen receptor expression was associated with strong expression of chromogranin A ($p = .02$) and neuron specific enolase ($p = .02$), but not with focal expression of any marker. No clear association was found between expression of NE markers and ERG or Ki-67. In conclusion, NED is a common and heterogeneous phenomenon in metastatic, castration-resistant prostate cancer. NED is more often present in castration-resistant prostate cancer compared to hormone-naïve disease, and it is associated with androgen receptor negativity. More research is needed to understand significance of NED in the progression of prostate cancer.

1. Introduction

Prostate cancer (PCa) is a highly prevalent cause of cancer death among males worldwide [1]. The majority of PCa deaths are caused by metastatic disease that has progressed to castration resistant prostate cancer (CRPC) after patients have received androgen deprivation treatment. It has been suggested that the presence of neuroendocrine differentiation (NED) in prostate cancer cells could have therapeutic implications for men with CRPC [2].

PCa cells with NED differ from neuroendocrine (NE) cells in normal prostate: PCa cells with NED have proliferative activity, and their protein expression is often different from normal NE cells. The origin of PCa cells expressing neuroendocrine markers is unclear. They might either originate from the same stem cells as normal NE cells, or be differentiated from cancerous prostate epithelia [3].

Cancer cells with NED can be found in 10–100% of all PCas [4]. This broad reported range is likely due to variable tissue imaging and staining techniques, and variable criteria used to evaluate NED in different studies [4]. Traditionally, PCas with NED have been categorized into conventional prostate adenocarcinomas with focal NED, carcinoid tumors and small cell NE carcinomas. However, Epstein et al. have recently proposed a new working classification consisting of usual prostate adenocarcinoma with NED, adenocarcinoma with Paneth cell −like NED, carcinoid tumor, small cell carcinoma, large cell NE

Abbreviations: PCa, prostate cancer; CRPC, castration-resistant prostate cancer; NED, neuroendocrine differentiation; NE, neuroendocrine; CgA, chromogranin A; NSE, neuron specific enolase; TMA, tissue microarray; DNPC, double-negative prostate cancer; AR, androgen receptor; ERG, ETS-related gene

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https://doi.org/10.1016/j.prp.2018.04.015
Received 6 January 2018; Received in revised form 9 April 2018; Accepted 20 April 2018

carcinoma and mixed tumor with NE carcinoma and adenocarcinoma features [5].

NED is commonly focal in hormone-naïve primary PCa, usually constituting < 1% of all tumor cells. Androgen deprivation therapy often leads to increased NED [6,7]. Increased NED has also been associated with poorly differentiated PCa (Gleason Score > 6) [8]. NED prognostic significance in hormone-naïve and well-differentiated prostate cancer is controversial, although it has been researched in several studies [2,3,9]. However, NED appears to predict poorer prognosis in patients with high grade PCa both prior and after androgen deprivation therapy: Berruti et al. showed that NED predicts shorter time to PSA progression and shorter survival in patients treated with androgen deprivation therapy but not in patients that have "hormone-naïve" (no exposure to androgen deprivation) disease [10], and Krauss et al. found that primary PCas (Gleason Score ≥7) with > 1% NE cell populations treated with radiation therapy only are more likely to develop distant metastases, whereas PCas (Gleason Score ≥7) with < 1% NE cells have outcomes similar to those where NED is absent [11].

The most commonly used immunohistochemical markers for detecting NE phenotype cells are chromogranin A (CgA), neuron specific enolase (NSE) and synaptophysin [12]. Despite being widely used, it has recently been speculated that NSE might not be a sufficiently specific marker for NED [5]. Of these three markers, CgA appears to be the most specific in detecting NED and predicting the prognosis of PCa [12].

The incidence of NED in primary prostate tumors and its effect on clinical outcome has been examined in several previous studies [2,10,11]. However, although NED in primary PCa is associated with higher incidence of metastatic disease, few articles have focused on NED in metastatic tumor sites.

Mucci et al. described CgA and synaptophysin expression in 2.8% and 2.7% of metastatic CRPC autopsy samples, respectively, using tissue microarray (TMA) technique. However, both CgA and synaptophysin expression was detected five times more often in whole tissue sections of the same samples [13]. Shah et al. and Roudier et al. reported in their autopsy studies of patients that had died of metastatic CRPC, that expression of CgA or synaptophysin was mostly focal, and was not associated with survival of patients [14,15]. In a recent autopsy study, Bluemn et al. evaluated 10% of metastatic CRPCs as neuroendocrine PCa. In the same study, they observed a significant increase (5% to 21%) in proportion of a "double-negative prostate cancer" (AR negative, NED marker negative, DNPC) in men who died of metastatic CRPC in 1997–2011 (pre-approval of abiraterone and enzalutamide) vs 2012–2016 (post approval of these drugs). In the same study, the proportion of neuroendocrine PCa did not differ among the two groups [16]. In other related work, Aprikian et al. and Quek et al. studied NED in hormone-naïve PCa lymph and bone metastases [17,18]. CgA was expressed in 46% [16] and 12% [18] of lymph node metastases, and in 52% of bone metastases [17]. Quek et al. reported that any CgA expression in lymph metastases predicted worse prognosis [18].

In total, NED in metastatic CRPC tissue has previously been studied in only 140 patients in four different studies [13–16]; Hormone-naïve metastatic tissue has been studied in 202 patients, 54 of whom had received hormonal treatment but reportedly had not developed castration resistance at the time of obtaining study specimens [17,18]. Supplementary Information contains a more detailed review of these six studies focused on NED in metastatic CRPC.

To extend understanding of the role of NED in lethal metastatic PCa, we examined NED in multiple metastatic sites in 31 men. We used three different NE markers, CgA, NSE and synaptophysin, and examined NED association with AR expression, ERG expression, and proliferation marker Ki-67.

2. Materials and methods

The use of clinical material was approved by the ethical committee of the Tampere University Hospital (TAUH) and the National Authority for Medicolegal Affairs and the Johns Hopkins Medicine Institutional Review Board (autopsy samples).

Our study material consists of 136 metastases obtained in rapid autopsies from 32 men who died of metastatic CRPC between 1995 and 2005. These men consented to participate in an integrated clinical-molecular study of lethal metastatic prostate cancer (PELICAN study), and received care both in community practice and tertiary oncology centers as previously reported [19]. The average age at diagnosis was 63 years (range 40–79), and average years between diagnosis and death was 6 years (range 1–15 years). Among the 32 men, ancestry was as follows: White, Hispanic (1), African-American, Non-Hispanic (4), and White, Non-Hispanic (27). Definitive pelvic radiation therapy was received by 16 of these men, and radical prostatectomy was performed in 8 men. After metastatic disease became clinically evident, all 32 men received androgen deprivation therapy of various kinds, including orchiectomy in 10, and various drug therapies in 29. Clinical data for the autopsy cases are tabulated in Supplementary Table 1.

In addition, we studied 116 primary PCas in men undergoing radical prostatectomy in Tampere University Hospital between 1995 and 1997. These patients had not been treated with androgen deprivation or other prostate cancer therapy prior to radical prostatectomy. The average age at diagnosis was 63 years (range 51–77 years).

All tissue samples examined were formalin-fixed and paraffin-embedded. Representative areas of tissue blocks were chosen, and TMA cores combined in TMA blocks as described previously [20]. CgA, NSE and synaptophysin immunostains were performed at Fimlab laboratories (Tampere, Finland) using a Leica Bond III automated immunostain system (Leica Biosystems Newcastle Ltd). Deparaffinization and epitope retrieval were performed onboard using Leica Biosystems reagents (Bond Dewax AR9222 and Bond Epitope retrieval 2, AR9640). All antibodies were diluted in Bond antibody diluent (AR9352) and antibody incubation time and temperature for each antibody was 30 min at room temperature. The clones and dilutions for each antibody were as follows: CgA (Dako A430, Clone polyclonal) 1:2000 dilution; NSE (Dako M0873, Clone BBS/NC/VI-H14) 1:5000 dilution; and synaptophysin (Leica Novocastra PA02999, Clone 27G12) as prediluted by supplier. All immunostains were detected and visualized using Bond Polymer Refined Detection kit DS9800 (Leica Biosystems Newcastle Ltd).

Adjacent 4 μm sections from the PELICAN and Tampere TMAs were immunostained for CgA, NSE, and synaptophysin. Within each TMA spot a representative region of 100 contiguous tumor cells was visually selected, and the number of cells expressing the NE marker were counted. All cells were counted if there were under 100 tumor cells in the sample. In addition, 100 representative normal glandular cells were counted in the prostatectomy samples if found, and scored similarly. For some analyses we classified the metastases into three groups according to the fraction of cells expressing each NE marker. Over 10% of cells showing positivity to the marker was classified as strong expression, 1–10% positivity as focal expression and 0% as no expression. Fig. 1 shows representative images from the immunostained metastasis TMA slides illustrating no expression, focal expression and strong expression of each NE marker, and representative whole TMA spot images for all immunostains used in the study are shown in Supplementary Fig. 1. Examples of stains in the Tampere prostatectomy samples are shown in Fig. 2. Scoring was performed first by a bachelor of medicine, and after that verified by a surgical pathologist.

We combined CgA, NSE, and synaptophysin results with previously performed stains of the same array for androgen receptor (AR), ETS-related gene (ERG) and proliferation marker Ki-67, where antibodies used in immunohistochemistry were: 318 (Novocastra Laboratories Ltd.) against AR, EPR3864 (Epitomics Inc.) against ERG and 138G6 (Cell Signaling Technology) against Ki-67 [20]. For AR and ERG, TMA spots were scored positive/negative, and for Ki-67 the percentage of cells expressing the protein was determined as described [20]. ERG-