Original Paper

Correlation between histopathological form and the degree of neuroendocrine differentiations in prostate cancer

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Abstract
Prostate cancer (PCa) is the most frequent neoplastic condition in males, but only 64–65% of the cases are sensitive to hormone therapy. The aim of this study was to investigate the neuroendocrine component of the prostatic carcinoma, in relation to the histopathological form and the degree of differentiation. Biopsies were obtained through transurethral resection, from 82 patients with prostate cancer. In order to assess the histopathological form and the Gleason score, one section from each case was stained with Hematoxylin–Eosin. Additional sections were stained with chromogranin A. We considered neuroendocrine cell hyperplasia to have a higher value than that observed in benign prostatic hyperplasia (BPH) and normal prostate (over three neuroendocrine cells/gland). The quantification of neuroendocrine differentiation (NED) has been significant; the reaction was considered to be weak (2–10% neuroendocrine cells), moderate (10–20%) and intense (over 50%). Cells positive for chromogranin A have been identified in all the cases, but a larger number than that registered in normal tissue has been noted in 59 patients (71.95%). In most of the cases, the neuroendocrine cells have been distributed in small groups among the neoplastic cells, and rarely isolated. In two cases of small cell carcinoma most of the tumoral cells have been positive for chromogranin A. In conclusion, the study of neuroendocrine differentiation in patients with prostatic carcinoma revealed hyperplasia of positive chromogranin A cells, in 71.95% of cases. Neuroendocrine prostatic differentiation is correlated with the advanced stage of evolution and possibly with the resistance to hormonal treatment.

Keywords: chromogranin A, neuroendocrine differentiation, prostatic adenocarcinoma, small cell carcinoma.

Introduction
Prostate tumors with neuroendocrine differentiation represent a heterogeneous group of entities. There is no accepted definition of NED in PCa. NED is often identified by scattered clusters of differentiated NE cells, among a predominant population of adenocarcinoma cells, except for rare cases of small cell carcinoma or carcinoid [1]. The prostatic small cell carcinoma is a tumor that exhibits a very aggressive behavior. Most of the patients exhibit metastasis at the time of diagnostic, with low chances of survival. The decrease is registered in less than two years after setting the diagnostic. Adenocarcinoma with neuroendocrine differentiation expresses neuroendocrine markers, prostate-specific antigen (PSA) and specific acid phosphatase (PSAP). Establishing the differential diagnostic helps detect malignant prostatic lesions. Neuroendocrine differentiation can be unapparent on the morphological Hematoxylin–Eosin staining, being detectable only in immunohistochemistry. Palapattu GS et al. have recently shown [2] that neuroendocrine tumor cells of prostate cancer selectively express CD44 in vivo and in vitro, leading them to conclude that the presence of such cells is significant for therapy resistance and tumor recurrence. Furthermore, the number of cells co-expressing Oct4, a stemness marker, and chromogranin A or synaptophysin is increased in prostate cancer compared to benign prostate, and these cells represent NE-like prostate cancer cells [3]. AR-independent mechanisms of androgen-independent PCa could be, at least in part, 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 anti-apoptotic and growth factors on adenocarcinoma cells [4]. Neuro-endocrine differentiation (NED) and hormone refractory disease seem to be associated phenomena: extensive NED of a tumor renders it androgen-independence, and androgen blockade induces NED. Moreover, the extent of neuroendocrine component in 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. The aim of this study was to investigate the neuroendocrine component in prostate cancer, correlated with the histopathological form and the degree of differentiation.

Materials and Methods
We have investigated 82 cases of prostate tumors obtained through biopsies. Most of the patients were
aged between 70–80 years; only five cases were aged between 40–50 years. The PSA level was elevated in 39 cases (more than 10 ng/mL), associated with the clinical symptomatology. After 48 hours fixation in 10% buffered formalin, and paraffin embedding, 3 µm sections were performed for each case. To establish histopathological diagnosis and the Gleason score, one slide, from each case, was stained using the standard method Hematoxylin–Eosin. To determine neuroendocrine differentiation, immunohistochemical staining with chromogranin A (CH A) was performed for each case. The dew axed and rehydrated sections were heated in a microwave oven, in pH 6 citrated buffer, for 10 minutes, for antigen retrieval. Endogenous peroxidase was inhibited using 3% oxygenated water for 5 minutes. The slides were incubated with primary antibody (polyclonal rabbit anti-human chromogranin A), 1:400 dilution for 30 minutes. We used EnVision as working system (DAKO Denmark), and the final reaction product was visualized, in brown, with 3,3'-diaminobenzidine. The nuclear staining was performed with Lillie’s watery Hematoxylin. We used as positive control the neuroendocrine cells from normal prostate glands and negative control the basal and secretory cells from normal tissue. In normal tissue, we identified 1–2 neuroendocrine cells per gland. The results were assessed with Nikon Eclipse E600 microscope and images (taken in JPEG format) were captured and processed using Lucia G software system.

Results

Two types of neuroendocrine cells were identified in the normal zone: the closed type and the open type (Figure 1). In neuroendocrine cell hyperplasia (Figure 2), we found up to three times more neuroendocrine cells per gland than in the normal prostate (one or two cells per gland) and benign prostatic hyperplasia. In cases with prostatic adenocarcinoma the positive cells were assessed according to the number of positive cells per gland. In cases with NED, the neuroendocrine cells were organized in clusters in the malignant glands. We have identified 82 cases of prostatic adenocarcinoma two of them being small cell carcinoma using the standard method Hematoxylin–Eosin. The tumors have been identified in the peripheral zone of the prostate. For the 82 cases of adenocarcinoma, the Gleason score varied between 3 and 10; most of the tumors had a lower score (Gleason less than 8), in four cases, Gleason score was 8 or 9, and for the two small cell carcinoma cases the Gleason score was 10. The neuroendocrine differentiation was weak (2–10%) with Gleason score 3–5 (Figure 3) and moderate (10–20%) with Gleason score 5–8 for adenocarcinoma cases, and intense (over 50%) with Gleason score 8–10 (Figure 4) for undifferentiated adenocarcinoma and small cell carcinoma. We have identified positive CH A cells in all cases. A larger number than that registered in normal tissue was identified in 59 patients, representing 71.95%. In the two cases with prostatic small cell carcinoma, the neuroendocrine differentiation was almost complete, most of the tumoral cells being positive for CH A. The two patients with small cell carcinoma underwent cytostatic and hormone treatment, but they died at 8, respectively 11 months from the diagnostic. In the prostatic adenocarcinoma cases, we observed the presence of isolated neuroendocrine cells, moderate (Figure 5) and reduced neuroendocrine differentiation (Figure 6), and also the presence of intratumoral positive CH A cells. Oppted to the small cell carcinoma, where the neuroendocrine differentiation was organized in large, compact groups (Figure 7), in adenocarcinoma neuroendocrine differentiation appears in small groups (Figure 8). Patients’ survival was determined by Gleason score; the survival of patients with Gleason score 3–5 was good – 11 of 17 are still alive, four of them being dead of other causes than prostatic carcinoma; with Gleason score 5–8, 44 of 59 are alive, and form those with Gleason 8–10, only one is still live (Table 1).

Table 1 – Neuroendocrine differentiation (NED) and Gleason score

<table>
<thead>
<tr>
<th>NED</th>
<th>Gleason score</th>
<th>No. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–10%</td>
<td>3–5</td>
<td>17</td>
<td>20.73</td>
</tr>
<tr>
<td>10–20%</td>
<td>5–8</td>
<td>59</td>
<td>71.95</td>
</tr>
<tr>
<td>&gt;50%</td>
<td>8–10</td>
<td>6</td>
<td>7.31</td>
</tr>
</tbody>
</table>

Figure 1 – Close-type, open-type neuroendocrine cells, CH A positive, 100×.

Figure 2 – Neuroendocrine cell hyperplasia positive CH A, 100×.
Correlation between histopathological form and the degree of neuroendocrine differentiations in prostate cancer

Discussion

The normal neuroendocrine cells and the paracrine-endocrine cells are part of the diffuse neuroendocrine system. In human prostate, these cells have been identified on morphological basis and based on secreted neuroendocrine factors. 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 transdifferentiation process to become NE-like cells. These cells acquire the NE phenotype and express NE markers, and could be termed ‘NE-like PCa cells’ [5].

Neuroendocrine differentiation is focally present in all cases of PCa. The number of detected cells in each case varies with fixation, tissue sectioning, antibody detection method, and number of the examined sections. Understanding the role played by NED in prostate cancer is essential because most of the phenotypes are associated with a reserved prognostic and the progression towards independent androgen tumors [6, 7].

Weinstein MH et al. [8] studied 104 patients with clinically organ-confined PCa 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, the 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 risk 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 [9]. In our study, neuroendocrine cells identified with chromogranin A were positive in 59 of 82 cases (71.95%).

Neuroendocrine cells have been observed in prostate cancer, their number increasing in accordance with the tumoral stage and degree and particularly with the androgenic deprivation. This aspect is associated with the results obtained in the present study, in areas of normal prostate and those of benign prostatic hyperplasia where we identified neuroendocrine cell hyperplasia (more than three cells per gland). At the level of all prostatic adenocarcinoma studied by us, we have observed positive cells for chromogranin A. The study performed by Shimizu S et al. in 2007 [10] uses CH A for the identification and quantification of tumoral neuro-endocrine cells (NETC); the identified positive cells presented characteristics specific to tumoral cells. In our study we quantified the positive reaction of neuro-endocrine as follows: weak (2–10%), moderate (10–20%), and intense (over 50%). Abrahamsson PA et al. [11] could not find a correlation between the presence of immunohistochemically positive NE cells and long-term survival. In our study, survival was quantified only in the cases of small cell carcinoma when decease occurred at eight respectively 11 months after the administration of hormone and cytostatic treatment. In these two cases, neuroendocrine differentiation has been quantified as intense (over 50%) associated with a Gleason score of 8–10. The highly aggressive pattern of this tumoral type has been described in a series of studies performed by di Sant’Agnese PA [6]; these tumors are very rare, only 1–2% of the total amount of prostatic tumors being described as part of the conventional adenocarcinoma. The correlation between the Gleason score and the neuroendocrine differentiation was identified in a study performed by Berruti A et al. in 2005 [12]. In this case, the relation between the neuroendocrine differentiation and the undifferentiated tumors has been determined. This aspect has also been demonstrated in our study, in the areas of intense neuroendocrine differentiation (over 50%), the Gleason score being over 8.

Conclusions

In the present study, we have immunohistochemically identified the neuroendocrine differentiation of prostate cancer with chromogranin A. In prostatic adenocarcinoma, two degrees of neuroendocrine differentiation were established by correlation with the Gleason score: weak (3–5) and moderate (5–8). In small cell carcinoma, the Gleason score was 8–10 and the neuroendocrine differentiation was intense. We demonstrated that neuroendocrine differentiation, detected with chromogranin A, is associated with Gleason score, aggressive disease and low rate of survival. CH A immunoreactivity is a prognostic marker of disease and is superior to standard pathologic diagnosis.

References


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