Neuroendocrine differentiation in androgen-dependent and -independent prostate cancer

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The objective of this study was to characterize, immunohistochemically, neuroendocrine differentiation in androgen-dependent and -independent prostate cancer. A chart review of 17 consecutive patients with metastatic prostate cancer being treated in an ambulatory center, generated ten androgen-dependent and seven androgen-independent cases. The formalin-fixed, paraffin-embedded prostate cancer specimens were stained for Chromogranin A and Neuron-Specific Enolase with the Strep-Avidin Biotin Immunoperoxidase method. These were examined by a single blinded investigator. The mean age and grade of the prostatic carcinoma of the two groups did not differ significantly. Of the total number of specimens, 29.4% did not stain with neuroendocrine markers. This value was similar for both the androgen-dependent (30%) and androgen-independent (28.6%) cases. The major difference noted between the two groups was the presence of cell clusters (n=4) in the androgen-dependent group only. Neuroendocrine differentiation has been associated with a poor prognosis and poor response to hormonal therapy. If the incidence or pattern of neuroendocrine differentiation could predict a poor response to hormonal therapy other treatment options could be implemented with the possibility of improved clinical outcomes. A larger prospective study is required to elucidate the predictive importance of the incidence and pattern of neuroendocrine differentiation in metastatic prostatic carcinoma.

Neuroendocrine (NE) differentiation has been demonstrated repeatedly in both normal(1,2) and malignant prostatic tissue(1,3-8). The function of these cells is unclear. It has been suggested that they may play a role in growth and differentiation and in homeostatic regulation of secretion in the mature prostate gland(9). The identification of these cells can be achieved by several means. However, the most sensitive and specific method appears to be immunohistochemistry with Chromogranin A (ChrA) and Neuron-specific Enolase (NSE)(2,4,6,7,10-12).

NE differentiation is reported to be a poor prognostic factor (7,12-14). There is a significant correlation between survival and the absence of NE differentiation in prostatic carcinoma ($p<0.001$)(14). NE differentiation has been shown to be an independent prognostic factor. This has been reinforced by demonstrating that this prognostic factor was superior to the Gleason Grading System(12).

Others have noted that prostatic carcinomas with NE differentiation have a poor response to hormonal therapy(10-13). It is suggested that this is a selective response to an androgen-free environment so that NE cells, which are not androgen-sensitive, will proliferate(10,13). For this reason alternative therapies for metastatic prostate cancer, such as radiation and chemotherapy, have been proposed for these cases (7,10,14,15).

At the present time, anti-androgen therapy is the primary treatment for metastatic prostate cancer. However, many of these cases relapse and show androgen resistance. The recognition of NE differentiation early in the course of the disease may have implications for alternative therapies resulting in increased survival rates and improved quality of life for patients affected by metastatic prostate cancer.

The objective of this study is to charac-

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terize neuroendocrine differentiation in androgen-dependent and -independent prostate cancer.

DESIGN

A sample of 10 androgen-dependent and 7 androgen-independent cases was selected from a chart review of consecutive patients with metastatic prostatic adenocarcinoma from an ambulatory setting. Androgen-dependence was defined as a positive response to hormonal therapy such as a decrease in serum prostate specific antigen (PSA) or an improvement in symptoms or bone scan. Conversely, androgen- independence was characterized by an increasing PSA, worsening bone scan, or progression of symptoms despite hormonal manipulation. Hormonal therapies included: bilateral orchiectomy, luteinizing hormone releasing hormone (LHRH) analogs, and anti-androgens. All but 3 of the specimens (3/17) were obtained prior to the initiation of hormonal therapy. These latter three cases all belonged to the androgen-independent group. No cases of carcinoid or small cell carcinoma were used.

METHODS

Formalin-fixed, paraffin-embedded specimens were obtained from surgical pathology archives. The Strep-Avidin Biotin Immunoperoxidase method was used. Both the monoclonal ChrA and NSE Immunostains were performed on each tissue specimen(2,4,6,7,10-12). The negative control was established by the substitution of buffer for the primary antibody. Positive controls were pancreatic islet cell tumor and pheochromocytoma.

A single blinded investigator (DLM) examined all specimens in a standardized fashion. Specimens were considered positive for NE differentiation if they stained for either ChrA or NSE. The degree of NE differentiation was defined as: 0 equals no NE differentiation, + 1 equals less than 2% of the sample, +2 equals less than 25% of the sample, and +3 equals greater than 50% of the specimen. Samples were also examined for the pattern of NE differentiation including cell clustering (a group of 3 or more adjacent cells) and scattered cell patterns. A selected subgroup of the cases were reviewed independently by a blinded pathologist (BAW) to assess the reproducibility of the scoring system. Statistical analysis was performed using a two-tailed t test.

The tissue samples were graded as well, moderately, or poorly differentiated. The distribution of tumour grades is outlined in Tables 1 and 2, together with the score and pattern of NE differentiation for each tissue sample.

RESULTS

The mean age of the patients was 75 $\pm$ 2 (standard error of the mean, SEM) years. There was no significant difference between the mean age of the androgen-dependent (74 $\pm$ 3 years) and androgen-independent groups (76 $\pm$ 2 years) (p=0.5). Initially, three patients underwent a radical prostatectomy and seven were treated with external beam radiation with or without gold seed implantation. All 17 patients were treated with hormonal therapy. For androgen-dependent patients, the mean number of months between the initiation of hormonal therapy and most recent follow-up appointment was 12.6 $\pm$ 4 months. The same value for the androgen-independent group was 25.6 $\pm$ 6 months (p=0.08).

It was noted that the prostatic needle core biopsies (n=8) provided little tissue and were thus difficult to interpret. Of all the samples studied 5/17 (29.4%) did not demonstrate NE differentiation, 3/10 (30%) in the androgen-dependent group and 2/7 (28.6%) in the androgen-independent group. Of the remaining samples which were positive for NE differentiation: 10/12 (83.3%) were quantified as +1; 2/12 (16.7%) were quantified as +2; none (0%) were quantified as +3. See Tables 1 and 2.

Table 1: Androgen-dependent group characteristics (n=10)

Patient No.	NE Diff. Score	NE Diff. Pattern	Grade of prostatic Ca
1	0	-	moderate
2	0	-	moderate
3	+1	cell clusters	moderate
4	+2	cell clusters	moderate
5	+1	scattered cells	moderate
6	+1	cell clusters	moderate
7	0	-	well
8	+2	cell clusters	moderate
9	+1	scattered cells	moderat
10	+1	scattered cells	moderate

Table 2: Androgen-independent group characteristics (n=7)

Patient No.	NE Diff. Score	NE Diff. Pattern	Grade of prostatic ca
1	0	-	moderate
2	+1	scattered cells	poor
3	+1	scattered cells	moderate to poor
4	+1	scattered cells	moderate
5	+1	scattered cells	moderate
6	0	-	moderate
7	+1	scattered cells	poor

In those specimens with NE differentiation, four demonstrated clustering of NE cells. All cell clustering was noted in the androgen-dependent group. The remaining eight had focal scattered NE cells. See Figure 1.

DISCUSSION

The mean age of the two groups did not differ sig-

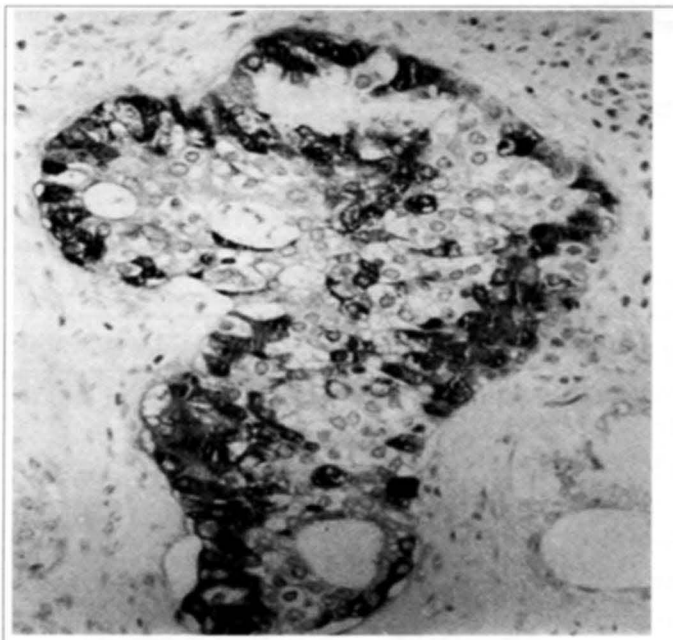

Figure 1a) shows prostatic adenocarcinoma at 20X stained with Chromogranin A demonstrating cell clusters

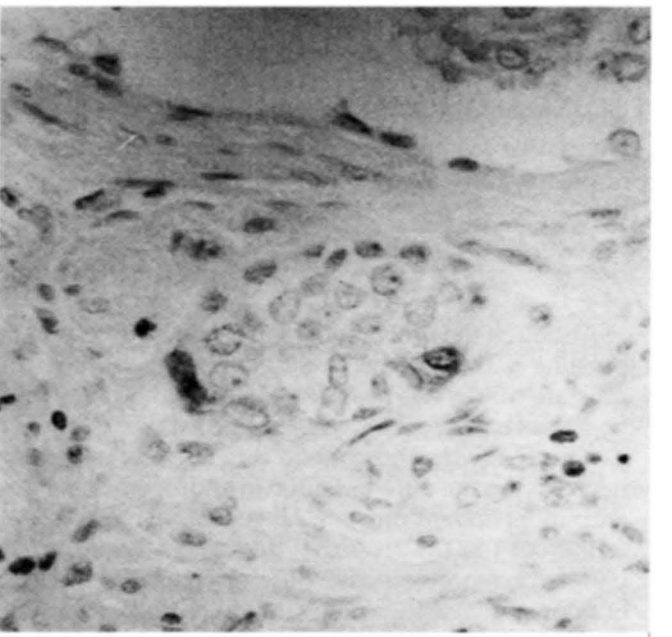

Figure 1b) shows prostatic adenocarcinoma at 40X stained with Neuron-Specific Enolase demonstrating scattered cell pattern.

Figure 1: Demonstration of the patterns of neuroendocrine differentiation: *see captions.*

nificantly (p=0.5). The grade of prostatic carcinoma was similar in the androgen-dependent and -independent groups (see Tables 1 and 2). As well, the mean number of months from the onset of hormonal therapy to the last date of follow-up did not differ significantly between the androgen-dependent (12.6 $\pm$ 4 months) and independent (25.6 $\pm$ 6 months) groups. However, this is a small sample size and there is a relative difference in these values. It must be considered that with longer follow-up some of the androgen-dependent group may progress to become non-responsive to hormonal therapy and thus become members of the androgen-independent group.

Of the total sample studied 29.4% (5/17) did not stain positively for NE differentiation. It was noted that similar values were found in the androgen-dependent (30% or 3/10) and androgen-independent (28.6% or 2/7) groups. Previous studies have shown varying results for the recognition of NE differentiation in prostate cancer from 10%(3) to 100%(1) presumably because of improvements in staining techniques. The discrepancy between our 70% identification and the latter study may be the result of differences in specimen collection and study methods. The study with 100% positive staining used 40 fresh specimens preserved in Bouin's Solution and 80 paraffin-embedded specimens and three separate immunohistochemical techniques(1). Our study utilized 17 formalin-fixed, paraffin-embedded specimens and two antibodies of neuroendocrine differentiation.

Neuroendocrine differentiation has been shown to be an independent prognostic factor(14). A retrospective study (n=52 with a minimum six year follow-up) showed that patients with specimens positive for NE differentiation had 0% survival at 4 years compared to those with negative staining which had 80% survival at 7 years ($p<0.0001$)(14). Another retrospective investigation (n=110) with a minimum four year follow-up found that clinical stage and NE differentiation were better predictors of prognosis than histological grade(12). Other studies have also demonstrated that the presence of NE differentiation is associated with a poor prognosis(1,13).

Prostate cancer which is refractory to hormonal treatment may be responsive to other therapies such as chemotherapy and radiation(7,10,14,15). Some authors have noted that prostate cancers with neuroendocrine differentiation have a poor response to hormonal therapy and that this is a selective response to an androgen-free environment rather than an adaptive response(10,13).

In the present study of metastatic prostatic carcinoma there was no difference in the prevalence of NE differentiation in the two groups studied. However, cell clusters were noted only in the androgen dependent group; this has not been noted previously.

Thus, if certain pathological characteristics such as the presence or patterns of NE differentiation are able to identify which prostate cancers will be refractory to hormonal treatment alternative therapy could be implemented. The ability to detect and predict which prostate cancers will respond to androgen therapy is important clinically. The accepted present day treatment

of metastatic prostate cancer is anti-androgen therapy. However, some of these cases go on to develop resistance to hormonal therapy and progress rapidly. If one were able to predict these cases at the time of diagnosis alternative therapies could be applied earlier with the hopes of improving survival time and quality of life.

CONCLUSION

Although the function of neuroendocrine cells in the prostate is unclear, neuroendocrine differentiation in prostate cancer has been associated with a poor prognosis and a poor response to hormonal therapy.

Our study demonstrates a difference in patterns of neuroendocrine differentiation in androgen-dependent and -independent prostate cancers. Cell clusters were found only in the androgen-dependent group. If the pattern of neuroendocrine differentiation is a predictor of poor response to hormonal therapy then alternative therapies such as chemotherapy might be indicated earlier in the disease process and may increase survival and quality of life in these cases. A large, prospective study examining the incidence and patterns of neuroendocrine differentiation and response to hormonal therapy in metastatic prostate cancer is indicated.

ACKNOWLEDGMENT

We thank Sandra MacDougall for her assistance with this project.

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