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NEUROENDOCRINE CELLS IN THE PROSTATIC CARCINOMAS AFTER NEOADJUVANT HORMONAL THERAPY

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INDEXING WORDS

prostate cancer ; neuroendocrine cell ; immunohistochemistry

SYNOPSIS

Prostate cancer is a malignant tumor that is likely to increase in incidence in Japan. The most troublesome facet of this tumor is its conversion from a hormone-sensitive status to an insensitive one. Neuroendocrine differentiation may help explain this phenomenon. We studied the prevalence of neuroendocrine cells in prostate carcinoma after androgen ablation therapy. Radical prostatectomy specimens from 28 patients who had undergone neoadjuvant endocrine therapy with luteinizing hormone-releasing hormone analogue were retrospectively studied. Immunohistochemical staining was used to assess the localization of neuroendocrine cells. Neuroendocrine differentiation index, defined as the sum of the immunoreactivity scores of the specimens against anti-chromogranin A and anti-neuron specific enolase antibodies, was developed. Overall, 46.4% and 53.6% of the prostate carcinomas contained chromogranin A and neuron-specific enolase positive cells, respectively . Localization of these neuoendocrine cells in tumors was patchy . Two specific types of neuroendocrine cells were identified: a closed type and an open type based on the location of the neuroendocrine cells. The neuroendocrine differentiation index correlated with tumor grade, but not with tumor stage . The prevalence of neuroendocrine cells in higher grade prostatic carcinoma may help to explain the insensitivity of these cancers to hormonal therapy.

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INTRODUCTION

In the United States, prostate cancer has become one of the top two causes of mortality in males over 50 years of age for the last 10 years .[15] It has also become a major cause of male death in Japan .[13] It is estimated that approximately 50 % of males in the US over the age of 70 years may develop prostate carcinoma .[13] There are several therapeutic options in the management of prostate carcinomas including surgical resection ,[17,12] hormonal treatment with or without anti-cancer agents ,[11,16] and external or internal radiation therapy .[3] Sensitivity to hormonal treatment is a significance of the prognostic factor in patients with prostate cancer. Hormone insensitivity has been studied extensively during the past decade, and several mechanisms, including neuroendocrine differentiation of prostatic carcinoma, have been postulated to have prognostic relevance .[6,4]

There are few reports describing neuroendocrine differentiation of prostate carcinoma following hormonal therapy .[1] In the present paper, we describe the neuroendocrine nature of prostate carcinoma after endocrine treatment using immunohistochemical techniques.

MATERIALS AND METHODS

Patients

A total of 28 consecutive patients (aged 48 to 78 years, median : 66 years) with prostate cancer who had undergone radical prostatectomy using Walsh's nerve-sparing technique [17] at Kobe University Hospital or at Miki Municipal Hospital between 1992 and 1996 were studied. Preoperative staging was performed using transrectal ultrasonography (US), pelvic magnetic resonance imaging (MRI), pelvic computed axillary tomography scan (CT), bone scintigraphy, and chest CT scan. Preoperative pathologic diagnosis was made on evaluation of either US guided sextant prostate core biopsies [9] or on transurethral resection of the prostate that were initially diagnosed as benign prostatic hypertrophy. Prior to radical surgery, all patients received neoadjuvant therapy using a luteinizing hormone-releasing hormone analogue . Following radical prostatectomy, patients were staged according to standards set forth by the Japanese Urological Association. Eighteen patients were classified with stage B disease and 10 with stage C. Two pathologists independently graded resected specimens. Gleason scores of 2-3, 4-7, and 8-10 were considered well differentiated, moderately differentiated, and poorly differentiated, respectively. Overall , 8 patients had well-differentiated adenocarcinomas, 8 had moderately-differentiated adenocarcinomas, and 12 had poorly-differentiated tumors.

Immunohistochemical staining

Surgically resected prostate specimens were fixed in 10% buffered formalin , embedded in paraffin, and ten 4-μm thick sections of each block containing the primary tumor

were prepared. One section was stained with hematoxylin-eosin (H&E), and those remaining were used for immunohistochemical staining. Immunohistochemical staining was performed using avidin-biotin-peroxidase complex (ABC) technique .[10] Briefly, sections were deparaffinized, hydrated through xylene and a graded ethanol series, and then rinsed for 5 min in distilled water. Tissue sections were then incubated in 0.3% methanolic hydrogen peroxide to block endogenous peroxidase activity and in 3% normal horse serum to reduce nonspecific background staining. Primary antibodies (Table) (Biomeda, Foster City, CA, USA), were diluted with 3% normal horse serum, applied to tissue sections, and incubated for 2 h at 4℃ in a moist chamber. After being washed with phosphate-buffered saline (PBS, pH 7.2), secondary antibody (biotin-labeled horse anti-mouse immunoglobulin or biotin-labeled horse anti-rabbit immunoglobulin, Vectastain *elite* ABC kit; Vector Laboratories, Burlingame, CA, USA) was applied to tissue sections and incubated for 1 h at room temperature. Slides were then washed with PBS. Avidin and biotin-labeled horseradish peroxidase complex (Vectastain *elite* ABC kit) was applied to the slides and incubated for 30 min at room temperature. After washes with PBS, tissue sections were incubated in peroxidase substrate solution (0.01% hydrogen peroxide and 0.05% diaminobenzidine tetrahydrochloride in 0.05 M tris buffer, pH 7.2) for 2 to 5min. Sections were then counterstained with hematoxylin, dehydrated, and mounted with Permount. Negative controls included substituting of the primary antibody with non-cross reacting antibody derived from the same species at an identical dilution or incubating with blocking serum alone. Fibrous tissue and transitional epithelium served as internal negative controls.

Evalution of immunoreactivity

Immunoreactivity was evaluated by a single investigator (C.X.) who was blinded as to the source of the specimen. The results were scored as: -, no staining; +, occasional staining in isolated cells (less than 2%); ++, staining in numerous cells (less than 25%); and +++, staining in numerous cells (more than 25%). A neuroendocrine differentiation index (NEDI) was developed based on the prevalence of anti-chromogranin A antibody immunoreactive cells (A, scored from 0 to 3+) and anti-NSE antibody reactive cells (B, scored from 0 to 3+). The NEDI was calculated by summing the A and B scores. All specimens were assigned NEDI scores between 0 and 6 in NEDI.

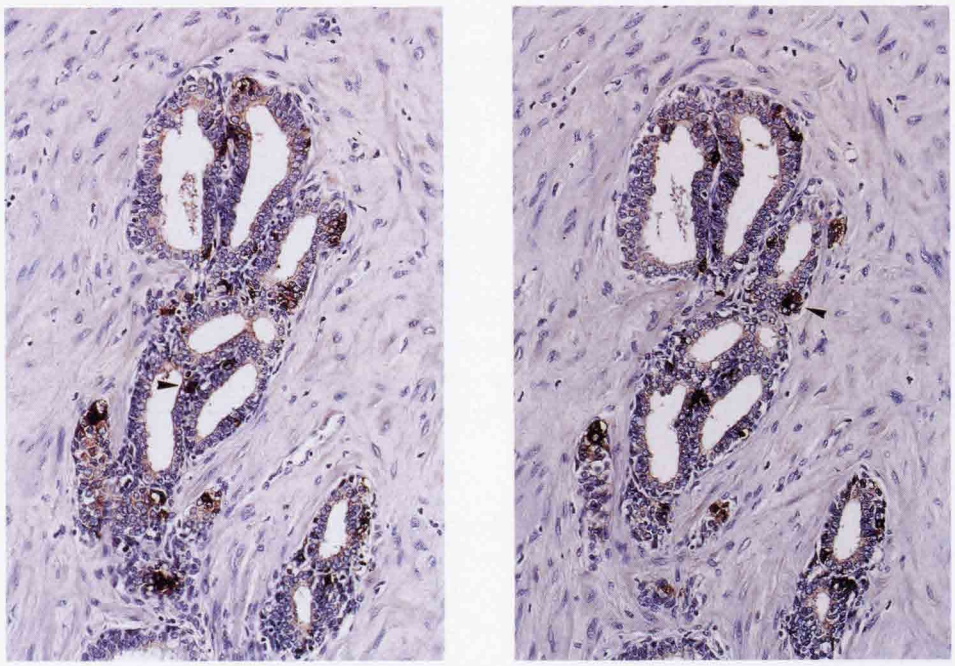
Statistical analysis

Correlative analyses between clinicopathologic variables were performed using the Mann-Whitney U test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Antibody staining patterns of prostate cancer cells

All specimens showed variable immunoreactivity with anti-EMA and anti-PSA antibodies (data not shown). Chromogranin A positive cells were found in 13 of 28 (46.4%) prostate cancers, and 15 of 28 (53.6%) contained NSE positive cells. All the specimens that contained chromogranin A positive cells showed cells immunoreactive to NSE. On the other hand, 3 specimens with tumor cells that expressed NSE did not express chromogranin A. The prevalence of neuroendocrine cells in the tumor samples was scored using the NEDI as described above. Figure 1 shows a respresentative section stained with anti-chromogranin A and



a . b .

Fig. 1 . Representative immunohistohemical staining of neuroendocrine cells in the prostatic cancinoma .
a . neuroendocrine cells stained with anti-chromogranin A antibody and immunoreactivity was scored as 2+ .
b . neuroendocrine cells stained with anti-neuron spcific-enolase (NSE) antibody and immunoreactivity was scored as 2+ .
NEDI, neuroendocrone differentication index. was scored as 4 in this spcimem.
(Original magnification, X200)

anti-NSE antibodies. This specimen was scored as 2+ for chromogranin A and 2+ for NSE, with a NEDI calculated as 4.

Morphologically, two types of prostatic neuroendocrine cells could be recognized: a closed type separated from the lumen by other cells (Fig. 2), and an open type that extended to the glandular lumen (Fig. 3). The distribution of neuroendocrine cells was patchy both in the acinus and the prostatic urothelium.

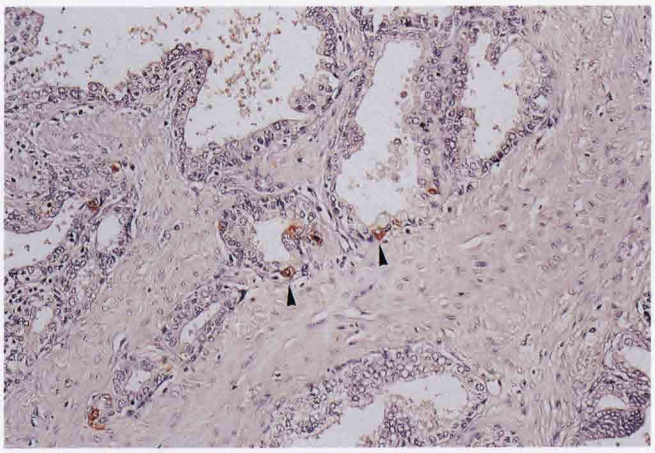


Fig.2 . Immunohistochemical staining of neuroendocrine cells with anti-chromogranin A antibody. Closed type neuroendocrine cells (arrow heads) are stained brown . (Original magnification, X200) .

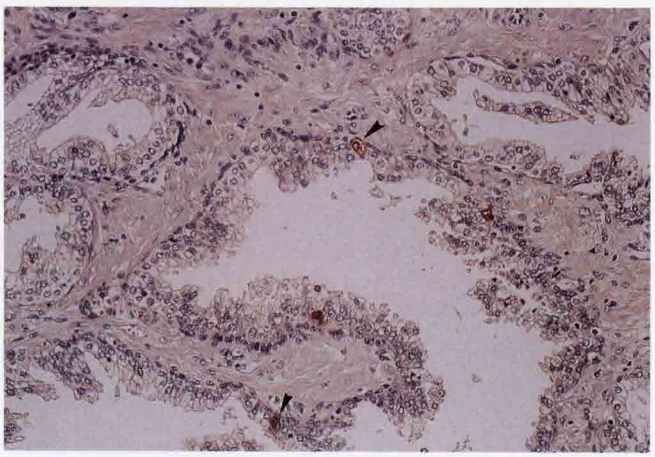


Fig.3 . Immunohistochemical staining of neuroendocrine cells with anti-chromogranin A antibody. Open type neuroendocrine cells (with arrows) are stained brown . (Original magnification, X200)

Staging and the neuroendocrine differentiation index

Although more than half of the patients with stage B disease had tumors without neuroendocrine differentiation, tumors of patients with stage C disease contained various numbers of cells with neuroendocrine differentiation (Fig. 4). There was, however, no statistically significant difference between these two groups.

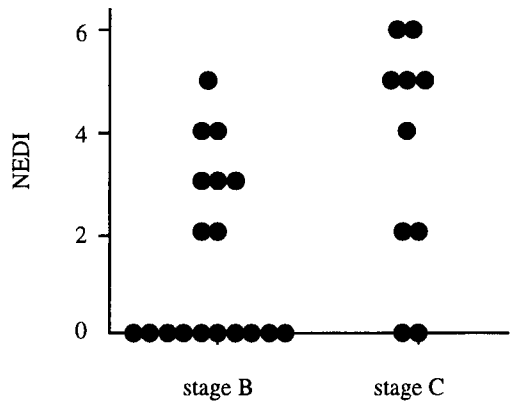


Fig.4 . Distribution of NEDI among patients with Stage B and C prostate cancer. No statistical significant difference was demonstrated between these groups.

Grading and the neuroendocrine differentiation index

The median NEDI of well-differentiated, moderately-differentiated, and poorly-differentiated adenocarcinomas of the prostate were 0, 2.5, and 4.5, respectively (Fig.5). The moderately- and poorly-differentiated proststic adenocarcinomas had significantly higher NEDI than the well-differentiated tumors ($p<0.01$).

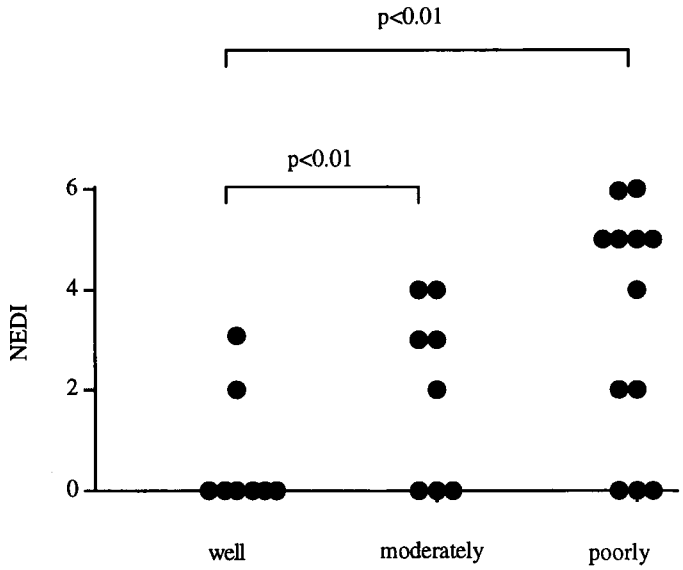


Fig.5 . Correlation of prostate cancer grade and the NEDI. The NEDI of well-differentiated adenocarcinoma of the prostate was significantly lower thnthat of moderately-or poorly-differentiated prostatic adenocarcinomas.

DISCUSSION

Neuroendocrine differentiation of tumors has received increasing attention over the past few years .[5] With the introduction of more sensitive techniques like silver staining and immunohistochemistry using a variety of antibodies directed against specific neuroendocrine peptides, the detection of neuroendocrine differentiation in prostate carcinoma has been facilitated [8] Using a combination of antibodies increases the detection rate of neuroendocrine cells. [8] In the present study , we used two distinct antibodies raised against the two neuroendocrine markers chromogranin A and neuron specific enolase. The prevalence of neuroendocrine cells demonstrated as positive staining for chromogranin A and/or NSE varied from tissue to tissue. Therefore, we developed a new scoring system, the NEDI, which is calculated by summing the immunoreactivity of these two antibodies. The distribution of neuroendocrine cells in tumor samples was patchy and focal , a finding similar to those reported in previous papers .[8,2] Since prostate core biopsies obtain only small portions of prostatic tissue, neuroendocrine cells that may be only focally present in the prostate may not be detected by this procedure (Okada,

unpublished data).

We observed two different types of neuroendocrine cells in prostatic carcinomas. Those of the closed type were separated from the lumen by other cells. The cells of the open type reached the glandular lumen. The former cells have dendritic cytoplasmic processes that interdigitate between neighboring cells. The latter cells are hypothesized to be involved in endocrine, paracrine, and neuroendocrine regulation.[14]

In the present study, neuroendocrine differentiation was detected in 46.4% of patients with stage B and in 53.6% of the patients with stage C prostate carcinoma. Deftos et al.[7] observed neuroendocrine differentiation in 9.1% (2/11) and 43% (3/7) of stage B and C prostatic carcinomas, respectively. Our data showed a higher detection rate of neuroendocrine differentiation in stage B disease. This difference can be partially explained by our use of two different markers, chromogranin A and NSE, instead of only chromogranin A. Also because all of our patients received endocrine therapy. Although there was no reports describing differences in neuroendocrine differentiation based on tumor stage, ours is the first to demonstrate no statistical difference in the rate of neuroendocrine differentiation between stage B and C disease. It had been reported that increasing neuroendocrine differentiation is associated with higher tumor grade.[14] Our results support these observations. Correlation between tumor grade and the number of neuroendocrine cells found in prostate carcinomas may well account for part or all of the reported prognostic value of neuroendocrine differentiation.

Our results were derived from patients who underwent endocrine treatment and demonstrating tumors with neuroendocrine differentiation in more than half of these patients support the hypothesis that neuroendocrine phenotypic expression is not suppressed by androgen ablation.[2] Moreover, it had been documented that tumor cell populations contain greater numbers of neuroendocrine cells following long-term anti-androgen therapy.[8] Neuroendocrine cells lack androgen receptors,[6] and this observation has been postulated to explain the rapid progression and insensitivity to hormonal therapy.[8] There is contradictory evidence relating to the prognostic implications of neuroendocrine differentiation in conventional prostatic adenocarcinoma. Some studies suggest a strong prognostic correlation[8,6] and the others cannot identify such a correlation.[14] Large-scale long-term clinical follow-up studies using multiple regression analysis will be required to clarify these issues.

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Table. Primary antibodies used.

Antibody against	Monoclonal/Polyclonal	Dilution
Chromogranin A	mouse monoclonal	X20
NSE	mouse monoclonal	X50
EMA	mouse monoclonal	X100
PSA	rabbit polyclonal	X100

NSE:Anti-Neuron Specific Enolase Antibody.

EMA:Epithelial Membrane Antigen Antibody.

PSA: Anti-Prostatic Specific Antigen Antibody.