SPANDIDOS PUBLICATIONS

Is neuroendocrine cell differentiation detected using chromogranin A from patients with bone metastatic prostate cancer a prognostic factor for outcome?

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Abstract. We evaluated the usefulness of overexpression of neuroendrocrine (NE) cell differentiation determined by immunohistochemical staining for chromogranin A (Cg A) in diagnostic needle biopsy specimens of bone metastatic prostate cancers. A total of 50 patients diagnosed as having bone metastatic prostate cancer were studied. The period of observation was between 6.9 and 79.4 months (median 48.7 months). Cg A was detected by immunostaining using the labeled streptavidin biotin method. Cg A-positivity was defined as the presence of immunostained cells in 10% or more of the tumor. All statistical analyses were carried out using the Statistical Package for Social Sciences Software, version 10.0 for Windows. Eleven patients (22%) were classified into the Cg A-positive group. There were no significant differences in clinical data between the Cg A-positive and Cg A-negative groups. The 5-year cause-specific survival rate was 34.1% for the Cg A-positive group and 55.2% for the Cg A-negative group (p=0.3763). The 3-year non-recurrence rate was 9.1% for the Cg A-positive group and 35.9% for the Cg A-negative group, and this difference was significant (p=0.0253). The 3-year cause-specific survival rates after recurrence were 38.4% and 42.3% respectively (p=0.8125). We consider that NE cell differentiation of the primary tumor in cases of bone metastatic prostate cancer is not a prognostic factor for outcome.

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Introduction

The involvement of neuroendocrine (NE) cell differentiation has been identified in recent years as a factor in the acquisition of resistance to endocrine therapy for prostate cancer (1). NE cells do not generally express androgen receptors, and do not undergo proliferation in response to androgen (2). They are accordingly believed to exhibit resistance to endocrine therapy with androgen ablation. With respect to the neuroendocrine substance secreted by NE cells, the existence of a mechanism that stimulates proliferation of neighboring tumor cells via a paracrine mechanism has been suggested (2). Autopsy investigations of patients who developed resistance to endocrine therapy and died of cancer revealed cases in which NE cells had proliferated and invaded at a high frequency (3). Chromogranin A (Cg A) is most widly expressed in NE cell differentiation (4,5). Many reports of previous research have stated that in immunohistochemical (IHC) investigations (4,6-8), serum concentration measurements of Cg A (9-11) are significantly correlated with outcome following endocrine therapy. All of these reports, however, included cases at a variety of clinical stages and undergoing different therapies. There have been no reports of studies on patients receiving the same therapy and whose outcome could be predicted by staining of diagnostic needle biopsy specimens sampled at the time of initial diagnosis of bone metastatic prostate cancer.

In this study, we examined diagnostic needle biopsy specimens from patients with bone metastatic prostate cancer for overexpression of NE cell differentiation by immunostaining for Cg A, and evaluated its usefulness as a prognostic factor for outcome.

Subjects and methods

Subjects. We studied 50 patients who had been examined at Aichi Medical University Hospital between January 1998 and December 2001, and who had been diagnosed with bone metastatic prostate cancer. The period of observation was between 6.9 and 79.4 months (median 48.7 months). The

Table I. Patient characteristics stratified by chromogranin A immunohistochemical staining results.

Characteristics	Cg A positive group	Cg A negative group	Total
Patient	11 (22.0%)	39 (78.0%)	50
Age (years)	63-82	61-91	61-91
Average age	73.9±6.1	73.3±7.8	73.4±7.4
Median age	76	72	72
Serum PSA (ng/ml)	50.0-3780.0	34.0-10060.0	34.0-10060.0
Average PSA	1016.0±1217.5	1037.8±2035.1	1033.0±1874.7
Median PSA	440	270	336.3
Gleason score			
7	0	4	4
8	5	14	18
9	4	20	25
10	2	1	3
EOD grade			
1	2	12	14
2	9	22	31
3	0	3	3
4	0	2	2
T stage			
T1c	1	1	2
T2a	3	3	6
T2b	3	9	12
T3a	1	0	1
T3b	0	4	4
T4	3	21	24
Tx	0	1	1
N stage			
N0	5	26	31
N1	6	12	18
N2	0	1	1

Cg A, chromogranin A; PSA, prostate-specific antigen; EOD, extent of disease. No differences were statistically significant.

treatment method was maximal androgen blockade in all cases, with subjects receiving anti-androgen agents with LH-RH agonists in 47 patients and anti-androgen agents in addition to bilateral orchiectomy in three patients. In all cases, PSA levels fell below 4.0 ng/ml following MAB therapy. Table I shows the patient characteristics.

Methods. A prostate-specific antigen assay was conducted using a Tandem-R assay kit. (Hybritech, San Diego, CA, USA). Systematic sextant biopsy of prostate was carried out using an Aloka (Tokyo, Japan) SSB-650CL with an 18-gauge biopsy needle (Biopty, C. R. Bard, Covington, GA, USA) under transrectal ultrasound guidance. The clinical stage and extent of disease (EOD) grade were determined by computed tomography, magnetic resonance imaging, and bone scans. The difference between groups was tested for significance by using the Mann-Whitney U-test and the χ^2 test, with a value of $p<0.05$ considered significant. Survival and non-recurrence periods were calculated by using the Kaplan-Meier method, and the log rank test was used to determine the significance of difference. All statistical analyses were carried out using the Statistical Package for Social Sciences Software (SPSS, Chicago, IL, USA), version 10.0 for Windows. The day on which the clinical stage was determined was regarded as day 0 of observation. Recurrence was defined as the presence of biological failure and three consecutive rises in PSA levels, with the first rise counted as the date of recurrence. The final date of observation was December 31, 2004. The Gleason classification (12) was used for histopathological classification, and the Union Against Cancer Classification system (13) was used to evaluate the primary tumor and lymph node metastasis.

Immunohistochemical staining procedure and evaluation. Cg A was detected by immunostaining using the labeled streptavidin biotin method. Paraffin sections were prepared from the biopsy specimen: after deparaffinization and quenching the endogenous peroxidase activity with 0.3% hydrogen peroxide in methanol, the specimen was washed in phosphate-buffered

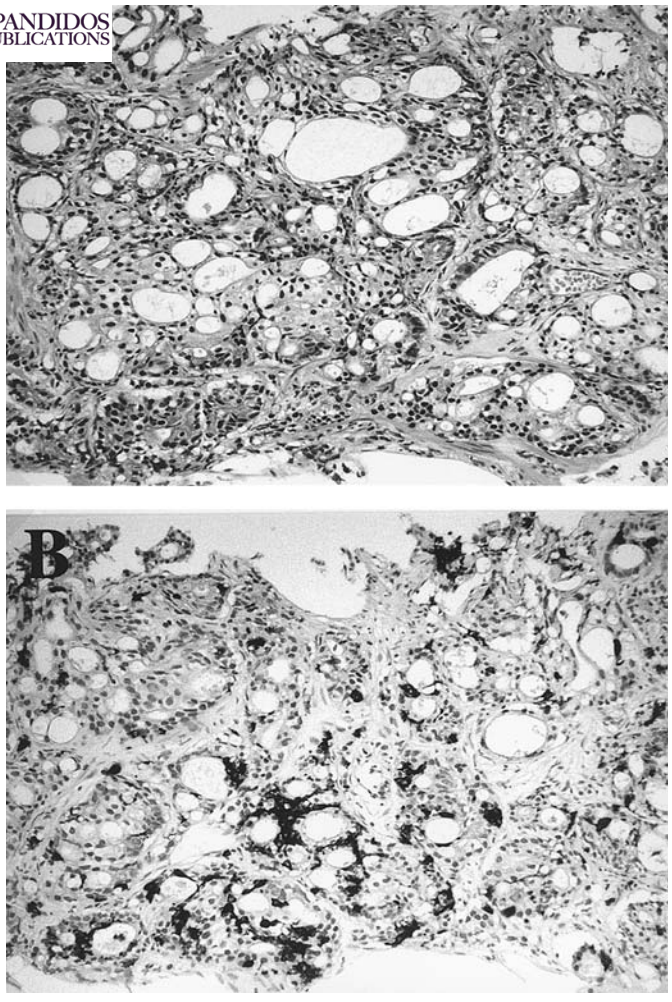

Figure 1. A, Hematoxylin and eosin staining of prostate specimen with Gleason score 4+4. B, Chromogranin A immunochemical stain showing granular staining in the cytoplasm.

saline (PBS, pH 7.4). Non-specific reactions were suppressed with 5% normal goat serum, and rabbit anti-human Cg A (Dako Cytomation, Glostrup, Denmark) diluted at 1:400 was applied as the primary antibody and allowed to react at 37˚C for 30 min. After washing in PBS, biotin-labeled mouse anti-rabbit IgG antibody was allowed to react at 37˚C for 30 min, after which the specimen was washed in PBS, reacted with horseradish peroxidase-labeled streptavidin at 37˚C for 30 min, and washed again. It was subsequently developed in diamino-benzidine and counterstained with hematoxylin for nuclear staining. The specimen were examined by two experienced pathologists and considered positive if the positively stained cells comprised 10% or more of the tumor area (Fig. 1).

Results

The Cg A-positive groups consisted of 11 patients (22%) in whom 10% or more of the tumors showed positive staining. The Cg A-negative group consisted of 29 patients. Marginal cases included 8 patients with 1-5% staining and 2 with 5-9% staining. There was no statistically significant difference between the Cg A-positive group and Cg A-negative group with respect to age, serum PSA, Gleason score, EOD grade, T stage, and N stage (Table I).

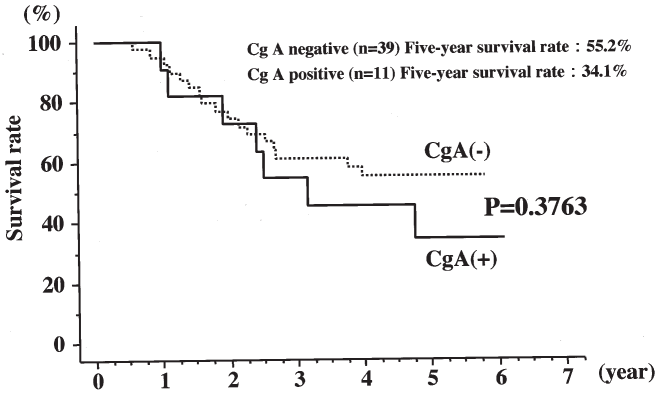

Figure 2. Cause-specific survival in Cg A-positive and Cg A-negative groups.

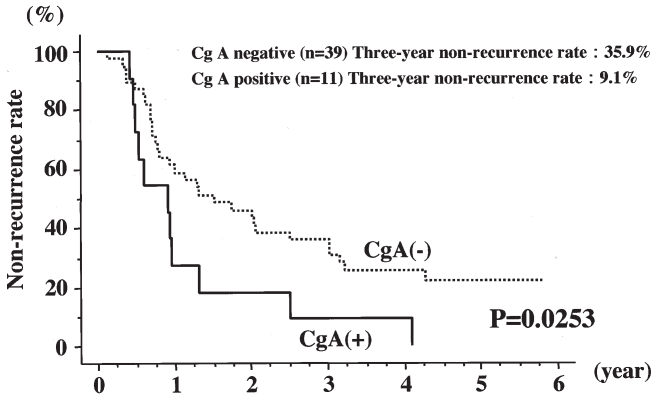

Figure 3. PSA-free recurrence in Cg A-positive and Cg A-negative groups.

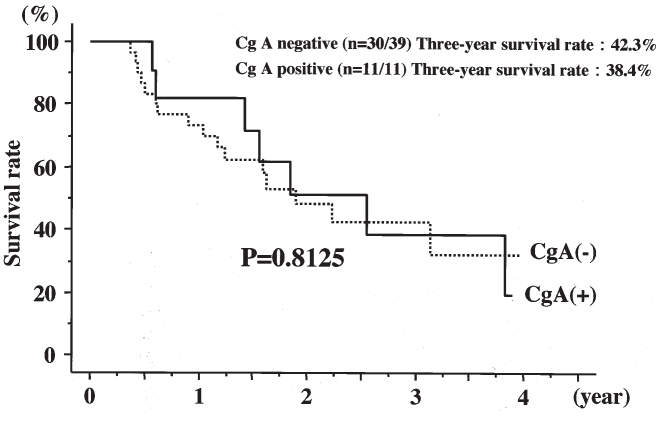

Figure 4. Cause-specific survival following recurrence in Cg A-positive and Cg A-negative groups.

The 5-year cause-specific survival rate was 34.1% for the Cg A-positive group and 55.2% for the Cg A-negative group (p=0.3763) (Fig. 2). The 3-year non-recurrence rate was 9.1% for the Cg A-positive group and 35.9% for the Cg A-negative group (p=0.0253). The cumulative 50% non-recurrence rate was reached within 0.603 year in the Cg A-positive group, but within 1.518 years in the Cg A-negative group (Fig. 3). Recurrence was observed in all 11 patients (100%) in the Cg A-positive group and 30 out of 39 patients (76.9%) in the

Cg A-negative group. The 3-year cause-specific survival rates were 38.4% and 42.3% respectively (p=0.8125) (Fig. 4).

Discussion

Androgen receptor abnormality is one mechanism whereby tumor recurrence occurs, however, there is also another mechanism independent of androgen receptors. The first mechanism may consist of 1) androgen receptor amplification (resulting in reaction to small amounts of androgen), 2) mutations in the androgen receptor gene (binding to and reacting with sub-stances other than androgen such as anti-androgen agents, estrogen, and steroids), 3) abnormalities of the co-activator that is a factor increasing the transcriptional activity of androgen receptors, or 4) abnormalities in the production of growth factors or cytokines, resulting in androgen receptor activation. Mechanisms proposed for the androgen-receptor independent mechanism are 1) abnormalities in the group of genes related to apoptosis, resulting in the avoidance of apoptosis, and 2) the appearance and proliferation of NE-differentiated cells. However, a combination of these abnormalities may be involved rather than abnormalities occurring singly (14).

NE cells are found in prostate tissue. They extend dendrites between their neighboring epithelial cells, and their cytoplasm includes granules of accumulated peptide hormones and prohormones. The function of NE cells within the prostate is unknown, but conjecturing from the functions of NE cells in the respiratory and digestive systems and in the pancreas leads to the consideration that they are indispensable to the growth and differentiation of the prostate, as well as to the homeostatic regulation of the endocrine process (1). Cg A has been reported as being most universally expressed in prostate tissue during NE cell differentiation (15). Cg A in prostate cancer tissue has been suggested to correlate with prognosis (16).

Bostwick *et al* (16) used the number of positive cells as the criterion of positive staining. As Cg A-positive cells are observed in small numbers in normal prostate epithelium, however, in this study we defined Cg A-positive as the presence of positive cells in more than 10% of the surface area of the tumor (7). According to this classification, 11 out of 50 (22%) patients were classified as Cg-A positive, which is similar to the finding of NE cell differentiation in 25% of prostate cancers reported by Casella *et al* (15). Prueri *et al* (17) reported that the number of Cg A-positive cells was significantly greater in the group with Gleason scores of six or over. However, we found no significant differences in age, pre-biopsy serum PSA value, Gleason score, EOD grade, T stage or N stage between the Cg A-positive and Cg A-negative groups. Noordzji *et al* (18) and Isshiki *et al* (11) also found no significant differences or correlation in Gleason score or pathological stage with NE cell differentiation.

We used the Kaplan-Meier method to calculate and investigate the cumulative non-recurrence rate for the Cg A-positive and Cg A-negative groups, and found that the time to recurrence was significantly shorter for the Cg A-positive group (p=0.0253). It would normally be supposed that if the time to recurrence is short, the prognosis for life expectancy should be unfavorable. Our study, however, found no significant difference in either survival rate or survival following recurrence.

McWilliam *et al* (7) and Krijnen *et al* (8) reported the existence of NE cells as an independent prognostic factor for outcome. Their studies, however, included patients with different stages of the disease, unlike our study on patients with prostate cancer at a single stage. Aprikian *et al* (19) and Bostwick *et al* (16) studied advanced prostate cancer with lymph node metastasis, and reported no significant difference in survival rate for NE cell differentiation. In their *in vivo* research using PC-310 human prostate cancer xenograft cells, Jongsma *et al* (20) reported that androgen-dependent tumors decreased by apoptosis within a few days following castration, with 50% of the surviving cancer cells being positive for Cg A. They concluded that a proportion of tumor cells that had initially been androgen-dependent had differentiated into NE cells with no accompanying tumor proliferation following androgen ablation. Bonkhoff *et al* (2) also reported that NE cells have undergone their final differentiation and do not have the potential for proliferation. In addition, an *in vitro* study using prostate cancer cells reported that Cg A inhibits the invasion and growth of prostate cancer cells (21). This shows that in patients with bone metastatic prostate cancer with a short time to recurrence, as the cancer cells are highly biological malignant potential, it is possible that Cg A secretion increases as an attempt to suppress tumor proliferation through NE cell differentiation and to inhibit the growth of tumor cells. This may be the reason for the absence of a significant difference in survival rates, despite the significantly shorter period to recurrence.

In conclusion, NE cell differentiation determined by immunostaining for Cg A showed that the time to recurrence was significantly shorter for the Cg A-positive group than the Cg A-negative group (p<0.0253), but there were no significant differences in survival rate or survival following recurrence. We therefore consider that NE cell differentiation in the primary tumor in cases of bone metastatic prostate cancer is not a prognostic factor for outcome.

Acknowledgements

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