<table>
<thead>
<tr>
<th>Title</th>
<th>The prognostic value of the HNK-1 (Leu-7) antigen in prostatic cancer--an immunohistochemical study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Liu, Xiu-heng; Yoshiki, Tatsuhiro; Kokuho, Masanori; Okada, Yusaku; Tomoyoshi, Tadao; Higuchi, Kayoko</td>
</tr>
<tr>
<td>Citation</td>
<td>泌尿器科紀要 (1993), 39(5): 439-444</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1993-05</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/2433/117845">http://hdl.handle.net/2433/117845</a></td>
</tr>
<tr>
<td>Type</td>
<td>Departmental Bulletin Paper</td>
</tr>
<tr>
<td>Textversion</td>
<td>publisher</td>
</tr>
</tbody>
</table>
THE PROGNOSTIC VALUE OF THE HNK-1 (Leu-7) ANTIGEN IN PROSTATIC CANCER
—AN IMMUNOHISTOCHEMICAL STUDY—

Xiu-heng Liu, Tatsuhiro Yoshiki, Masanori Kokuho, Yusaku Okada and Tadao Tomoyoshi

From the Department of Urology, Shiga University of Medical Science

Kayoko Higuchi

From the Department of Surgical Pathology, Kyoto National Hospital

The anti-HNK-1 (Leu-7) monoclonal antibody (MAb) was revealed to be reactive with non-cancerous and cancerous prostatic epithelial cells, although this antibody was originally found to be reactive against natural killer cells. However, the prognostic significance of HNK-1 antigen in prostatic cancer patients remains unknown. The expression of HNK-1 antigen on prostatic cancer was investigated immunohistochemically using the avidin-biotin-peroxidase complex (ABC) method with the anti-HNK-1 monoclonal antibody. Of the 52 patients with prostatic cancer, 49 patients (94%) showed reactivity to anti-HNK-1 MAb and the immunoreaction was associated with the histological differentiation of prostatic cancer. Well differentiated cancer showed the highest percentage of positively stained cancer cells and poorly differentiated cancer showed the lowest percentage. No statistically significant differences existed between groups classified by stage, although the more advanced cancers tended to have weaker reactions. The five-year survival rate and interval free of progression were then studied using the Kaplan-Meier method on 33 patients with stage D1 disease who had received endocrine therapy. The findings indicated that a high survival rate and a longer interval free of progression were associated with a higher fraction of positively stained cancer cells. In conclusion, the expression of HNK-1 antigen on prostatic cancer may be a useful prognostic factor in patients with prostatic cancer.


Key words: HNK-1, Prostatic cancer, Immunohistochemistry, Prognostic factor

INTRODUCTION

HNK-1 (Leu-7) antigen was initially found to be expressed on natural killer (NK) cells3). Subsequent studies have shown that anti-HNK-1 monoclonal antibody (MAb) was also reactive with myelinated nerves, pancreatic islet cells, chief cells of the stomach, adrenal medullary cells and other normal tissues and tumors derived from the neuroectoderm and the amine precursor uptake and decarboxylation systems2). In 1985, anti-HNK-1 was first reported to detect an antigen on normal, benign and malignant prostatic tissues3,4). However, to date, no reports have assessed the relationship between the anti-HNK-1 immunohistochemical reactivity and the biological behavior of prostatic cancer. In this report, we investigated the extent (fraction of positively stained cancer cells) of immunostaining in 52 cases of prostatic adenocarcinoma using the avidin-biotin-peroxidase complex (ABC) method and attempt to reveal the relationships between anti-HNK-1 MAb reactivity and histological differentiation, survival rate and the interval free of progression.

MATERIALS AND METHODS

Fifty-two patients with prostatic cancer were diagnosed at the Department of Urology, Shiga University of Medical Science, from June 1982 to September 1991. Their average age at diagnosis was 70.3 years (from 53 to 87 years). Three patients were in stage A, 2 in stage B, 14 in stage C and 33 in stage D. The average follow-up period was 39.4 months (from 2 to 118 months).
Immunohistochemical staining of tissues was performed according to the procedure described by Hsu et al. Briefly, tissue sections were deparaffinized and rehydrated through a xylene and graded alcohol series. Endogenous peroxidase activity was blocked by immersion for 30 minutes in 0.1% hydrogen peroxide (H₂O₂) and non-specific binding of antibodies was blocked by incubation for 30 minutes with Block Ace solution (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). The tissue sections were then incubated overnight with anti-HNK-1 MAb (Becton Dickinson Immunocytometry System, CA, U.S.A.) at a dilution of 1:100 and subsequently incubated with biotinylated antimouse IgM (Vector Laboratories, Inc., CA, U.S.A.) at a dilution of 1:200 for 60 minutes. Subsequently the sections were incubated with ABC complex at a dilution of 1:200 for 60 minutes and then immersed in the diaminobenzidine substrate.

The extent of staining was classified into four groups, according to the fraction of positively stained cancer cells: "-" denoting that no positive cells were present; "+" denoting that the number of positively stained cancer cells was less than one third of the number of total cancer cells; "++" denoting that between one-third and two-thirds of the cancer cells were stained positively and "+++" denoting that more than two-thirds of the cancer cells were positively stained.

Consecutive sections which were not incubated with anti-HNK-1 MAb were used as negative controls and BPH sections were used as positive controls. Sections stained with hematoxylin and eosine were reviewed to judge the histological grade of the prostatic cancer according to the grading system of the Japanese Urological Association.

All survival curves were estimated according to the Kaplan-Meier method. Non-progression was defined as complete or partial remission or no change following treatment according to the criteria described by Schmidt et al. The correlation between the reactivity of anti-HNK-1 MAb and the histological grade or clinical stage was evaluated by the Chi-square test.

RESULTS

Forty-nine of 52 cases (94%) of prostatic cancer and all BPH specimens were posi-

Fig. 1. The tissue sections were stained with anti-HNK-1 antibody using ABC Method A: Section of BPH tissue. Heterogeneity was obvious in the glands. The fibromuscular stroma was not stained. ×200 B: Section of well differentiated prostatic cancer. More than two-thirds of the cancer cells were stained positively. ×100 C: Section of poorly differentiated prostatic cancer. Less than one-third of the cancer cells were stained positively. ×100
tively stained. Positive staining was localized in the cytoplasm of epithelial cells of benign or malignant prostatic tissue. The fibromuscular stroma was not stained. Heterogeneity was observed in BPH tissues, as well as in the nests of the cancer cells (Fig. 1).

The extent of staining was correlated with the histological grade (p<0.05, Table 1). Well differentiated cancers had the highest percentage of positively stained cells and poorly differentiated cancers had the lowest percentage.

Prostatic cancers in early stages (stages A and B) showed higher percentages of positively stained cancer cells; more advanced cancers tended to have lower fractions of positively stained cancer cells, although no statistically significant difference existed (Table 2).

The relationship between the five-year survival rate and the probability of a progression-free course was studied in 33 patients with stage D2 cancer who had received endocrine therapy. During the follow-up period, 17 died of prostatic cancer, and 4 died of cardiovascular diseases or other

<table>
<thead>
<tr>
<th>Grade</th>
<th>Extent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wel</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Mod</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>Por</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>52</td>
</tr>
</tbody>
</table>

Table 1. Relationship between the extent of immunostaining and grade

<table>
<thead>
<tr>
<th>Stage</th>
<th>Extent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>14</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>52</td>
</tr>
</tbody>
</table>

*NS: Not significant
diseases. Fig. 2 shows the cancer-specific survival rates, according to the extent of staining. The group with more than two-thirds of their cancer cells positively stained showed a higher survival rate than the group with less than two-thirds of their cancer cells positively stained (p<0.05, at 25 months). As shown in Fig. 3, a longer interval without progression was observed in the cases with more than two-thirds of their cancer cells stained positively, compared to the cases with less than two-thirds of their cells stained positively (p<0.05, at 60 months).

DISCUSSION

In general, tumor markers may aid in diagnosis, detection of metastases, staging, prediction of therapeutic response and prognosis of cancers. Of the tumor markers for prostatic cancer, prostate-specific acid phosphatase (PAP) and prostate-specific antigen (PSA) have been widely studied and clinically accepted. Determinations of serum PAP and PSA were reported to be useful in predicting endocrine treatment response, lymph node involvement and progression of prostatic carcinoma. However, immunohistochemical studies on formalin-fixed sections stained for PAP have yielded conflicting results concerning the correlation between the immunoreaction and tumor differentiation or progression of the disease. Immunoperoxidase studies with PSA have indicated that PSA is more sensitive than PAP and that PSA staining is related to the histological differentiation of the tumor. Nevertheless, only about 76~81% of all patients with prostatic cancer showed elevated serum PSA levels. The sensitivity of PSA is still insufficient in detecting prostatic cancer. It is necessary to discover new markers that are more sensitive.

The HNK-1 antigen seems promising as a tumor marker since it is expressed strongly by prostatic cancer, as well as by normal and benign prostate. In this study on formalin-fixed, paraffin-embedded sections stained using the ABC method, all BPH cases and 49 of 52 (94%) patients with prostatic cancer showed anti-HNK-1 MAb reactivity and the immunoreaction was correlated with the histological differentiation of the cancer. Well differentiated cancers showed the highest percentage of positively stained cancer cells and the more poorly differentiate cancers showed the lowest percentage. Wahab and Wright reported that HNK-1 was more sensitive than PAP and PSA in identifying prostatic cancer. In a recent study which compared anti-HNK-1 MAb with anti-PAP MAb and anti-PSA MAb which were produced in our laboratory, we obtained similar results (data not shown).

At present, it is unclear whether HNK-1 can function as a parameter for predicting the prognosis of prostatic cancer. It is widely accepted that the prognosis of prostatic cancer depends on the histological grade and clinical stage. In an effort to exclude the influence of stage on survival, we analyzed 33 patients with stage D2 cancer who had received similar anti-androgen treatments. The fraction of positively stained cancer cells significantly correlated with the survival rate and interval free of progression. Longer survival times and intervals free of progression were observed in patients showing higher HNK-1 expression; patients showing decreased HNK-1 expression had a less favorable outcome.

We also observed that more advanced prostatic cancer tended to show weaker anti-HNK-1 MAb reactions. Further study is needed to determine if the NHK-1 antigen is useful in the staging of prostatic cancer.

Although the number of patients in this study was small, the results indicate that the percentage of cancer cells stained positively with anti-HNK-1 may be useful in predicting the survival rate and interval free of progression of patients with prostatic cancer.

Further investigation will be required to determine whether the HNK-1 antigen is released into the blood in sufficient amounts to be clinically useful.
REFERENCES


(Received on October 22, 1992) (Accepted on January 19, 1993)
前立腺癌における HNK-1（Leu-7）抗原の予後因子としての検討
—免疫組織化学的検討—

滋賀医科大学泌尿器科学教室（主任：友吉唯夫）
劉 修恒，吉貴 俊寛，國保 昌紀
岡田 裕作，友吉 唯夫

国立京都病院検査部
穂 口 佳 代 子

Editorial comment

著者は ABC 法により，HNK-1（Leu-7）抗原の前立腺の良性腺上皮や高分化癌で染色性が高くなるのが分かっているため，高分化・未分化癌の区別が maken と推定していた。しかし，前立腺癌における NK 細胞の抗原性および非腫瘍性の前立腺組織にも反応することが判明した。しかし，前立腺癌における HNK-1 抗原の予後因子としての意義はまだ明らかではない。今回われわれは，前立腺癌組織における HNK-1 抗原の発現を抗 HNK-1 抗体を用いて ABC 法での免疫組織化学的検討した。その結果，HNK-1 抗原が 52例中 49例（94％）に陽性で，しかもその反応性は癌の組織分化度と相関した。高分化癌では陽性癌細胞の比率が最も高く，低分化癌でももっとも低かった。進行癌での反応性は弱くなる傾向があったが，各臨床病期に統計学的に有意な反応性の違いはなかった。内分泌療法を受けた33例の stage D2 症例について，5年生存率と非進展率を Kaplan-Meier 法で検討すると，HNK-1 抗原が3分の2以上陽性のグループが，3分の2未満のグループに比べて有意に良好な生存率と非進展率を示した。以上より HNK-1 抗原は，前立腺癌の有用な予後因子になりえると考えられる。

（泌尿誌要 39: 439-444, 1993）

前立腺癌の予後因子としては，臨床的インパクトはさほど評価しがたいと思う。組織化学の上でも，clear cutなデータは多くないが，すでにわれわれは PAPや PSAをを持っているからである。われわれが真実必要とするのは，一言物語であろうが，正常細胞ではみられず癌化によって出現する，あるいは組織の malignant potential を予知できる「物質」である（一時注目された ras腫瘍遺伝子産物，P21蛋白も結局夢に終わったようである）。

とはいえ前立腺癌，ことに stage D 症の予後因子として，HNK-1がどのような価値をもつか，現在臨床的に最良とされる Gleason スコアを越えるかどうか，さらに追試の上記の機会にまた教示していただきたい。

文献
1) 内田温士，杉江勝治：CD56とCD57. Medical Immunol 24: 491-495, 1992
2) Markey AC: HNK-1 antigen is not specific for natural killer cells. Invest Dermatol 92: 774-775, 1989