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17β-hydroxysteroid dehydrogenase type 11 (Pan1b) expression in human prostate cancer

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Androgens are reported to be actively produced *in situ* in human prostate cancer. These locally produced androgens are also demonstrated to play important role in the pathogenesis and development of human prostate cancer. The status of locally produced androgen inactivation and metabolism, however, remains unclear. Therefore, it is important to examine the status of this *in situ* androgen metabolism and inactivation in order to improve clinical response to endocrine therapy in the patients diagnosed with prostate cancer. 17β-hydroxysteroid dehydrogenase type 11 (Pan1b) was demonstrated to display greatest activity with 5α-androstan-3α, 17β-diol (3α-diol) as substrate in several human androgen metabolizing tissues, suggesting that this enzyme may play important role in androgen metabolism. However, its details including the expression level of Pan1b have not been studied in human prostate cancer. In this study, we evaluated immunolocalization of Pan1b in human prostate cancer specimens obtained from surgery (n=40), and correlated the findings with clinicopathological features of the patients in order to study its clinical significance. Pan1b immunoreactivity was detected in 19 cases (48%) and was significantly associated with cancer of seminal vesicle invasion (P<0.05). These data suggest that Pan1b expression could be connected wth advanced prostate cancer.

Key words: 17ß-hydroxysteroid dehydrogenase type XI, prostate cancer, immunochemistry

Androgens play important role in the pathogenesis of human prostate cancer [1]. *In situ* production of androgens has been also suggested to play a pivotal role in the pathogenesis and/ or development of human prostate cancer [1, 2]. Suppression of androgen secretion and/or a blockade of their actions represent the basis for many forms of effective hormonal treatment of the patients diagnosed with prostate cancer [3]. However, the status of further metabolism or inactivation of these locally produced androgens still remains unclear. Therefore, it becomes very important to examine the levels of expression of androgens metabolizing and inactivating enzymes in the prostate cancer tissue in order to obtain a better understanding of the possible roles of *in situ* androgen metabolizing.

Human 17β-hydroxysteroid dehydrogenase type 11 (Pan1b) is known to display greatest activity with 5α-androstan-3α, 17β-diol (3α-diol) as substrate and to convert it to androsterone in several human androgen metabolizing tissues, suggesting its possible roles in human androgen metabolism [4, 5]. 3α-diol is known to be capable of stimulating cell proliferation in androgen-sensitive prostate cancer cell line (LNCaP) [6]. Laplante *et al.* demonstrated that Pan1b mRNA level was higher than that of 17β-hydroxysteroid dehydrogenase type 5 (AKR1C3), one of androgen-producing enzymes in LNCaP cells [6]. However, the status of this Pan1b in human prostate cancer has not been studied at all. Therefore, in this study, we examined Pan1b immunoreactivity in human prostate cancer, and correlated the findings with the status of androgen receptor (AR), estrogen receptor beta (ERβ), and other relevant clinicopathological findings in order to explore the possible biological significance of this androgen-matabolizing enzyme in human prostate cancer.

Materials and Methods

Patients and tissues. Forty surgical specimens of prostate carcinoma were obtained from the patients who underwent prostatectomy from 1998–2003 at the Department of Urology, Tohoku University Hospital (Sendai, Japan). The mean age of the patients was 65.9 y (range: 54–77 y). All patients examined in this study did not receive radiation, chemotherapy, or hormone therapy before surgery. Clinical data, including patient age, serum prostate specific antigen (PSA) concen-

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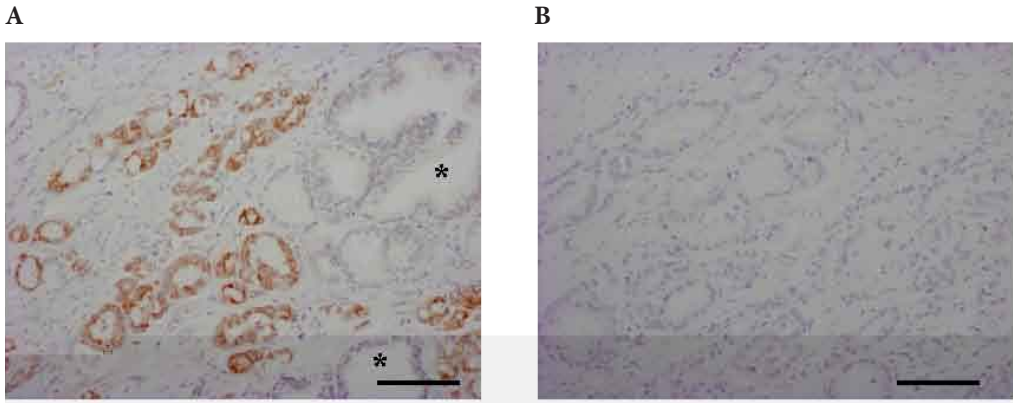

Figure 1. Immunoreactivity of Pan1b in human prostate cancer. A: Pan1b immunoreactive protein was detected in the cytoplasm of prostate carcinoma cells, but it was negligible in the epithelial cells of non-neoplastic prostate glands (*). B: No specific immunoreactivity was observed in the negative control section. Bar = 100 μm, respectively.

tration, clinical stage, and lymph node status according to the International Union Against Cancer TNM classification (1987), and Gleason score were retrieved from detailed patient charts describing individual patient histories. The histological grade of each tumor was evaluated by two of the authors (Y.N. and T.S.). All specimens were fixed with 10% formalin and embedded in paraffin wax at the Department of Pathology, Tohoku University Hospital. The Ethic's Committee at Tohoku University School of Medicine approved the research protocol for this study.

Antibodies. Rabbit polyclonal antibody for HUP1 (Pan1b) was kindly provided by Dr. Krozowski (Baker Heart Research Institute, Central Melbourne, Australia) [4, 5]. Antibodies against AR and Ki-67 were purchased from DAKO Corporation (Carpinteria, CA) and Immunotech (Marseilles, France), respectively. Antibodies for ERβ were also commercially obtained (Gene Tex, San Antonio, TX)

Immunohistochemistry Immunohistochemical analysis was performed employing the streptavidin-biotin amplification method using a Histofine Kit (Nichirei, Tokyo, Japan) and has been previously described in detail [2]. The dilutions of primary antibodies used in our study were as follows: Pan1b, 1: 200; AR, 1: 100; ERβ, 1: 1,500; and Ki-67, 1: 50. The antigen–antibody complex was visualized with 3,3'-diaminobenzidine (DAB) solution [1 mmol/l 3,3'-DAB, 50 mmol/l Tris–HCl buffer (pH 7.6), and 0.006% H_2O_2] and counterstained with hematoxylin. Tissue sections of normal adrenal glands were used as positive controls for Pan1b, an invasive ductal carcinoma of the breast were used as positive controls for ERβ, and normal prostate tissue was used as a positive control for AR. As for negative controls, immunohistochemical preabsorption tests were performed for Pan1b, and normal rabbit IgG used instead of the primary antibody. No specific immunoreactivity was detected in these sections (data not shown).

Scoring of immunoreactivity. Scoring of immunoreactivity was performed based on previous reports [2, 7, 8]. For statistical analyses of Pan1b immunoreactivity, the carcinoma cases were tentatively classified into the following two groups: +, positive, more than 10% positive cells; and –, no immunoreactivity, less than 10% positive cells [2]. The evaluation (+, positive carcinoma cells; and – no immunoreactivity) was performed by two of the authors (Y. N. and T. S.). Scoring of AR, ERβ, and Ki-67 in carcinoma cells was performed on high power fields (X400) using standard light microscopy. In each case, more than 500 carcinoma cells were counted independently by two of the authors above, and the percentage of immunoreactivity, i.e. labeling index (LI), was determined [2, 7, 8]. We evaluated all the slides containing carcinoma cells in each individual case.

Statistical analysis. Values for patient age, serum PSA levels, and LI for AR, ERβ, and Ki-67 were presented as the mean±95% confidence interval (95% CI), and associations between Pan1b immunoreactivity and the parameters described above were evaluated using the unpaired-t test. Statistical differences between Pan1b immunoreactivity and other clinicopathological factors were evaluated in a cross-table using the χ^2-test. $P<0.05$ was considered significant.

Results

Pan1b immunoreactive protein was detected in the cytoplasm of prostate carcinoma cells (Figure 1). Its immunoreactivity was, however, not detected or very weakly detectable in human normal prostate (Figure 1). Nineteen cases were defined as positive for Pan1b immunoreactivity (48%). There was a statistically significant positive correlation between Pan1b immunoreactivity and the status of seminal vesicle invasion ($P<0.05$) (Table 1). However, Pan1b immunoreacitivity was not significantly correlated with other clinicopathological parameters including patient age, concentration of serum PSA levels, Gleason score of carcinoma, extracapsular extension, or lymph node status (Table 1). There were no significant cor-

relations between Pan1b immunoreactivity and AR, ERβ, or Ki-67 immunoreactivity (Table 2).

Discussion

In this study, we demonstrated Pan1b immunoreactivity which was detected in human prostate cancer in approximately 50% of examined cases. Values of this immunoreactivity were significantly correlated with the status of seminal vesicle invasion.

We previously reported that androgen-producing enzymes were co-expressed in human prostate cancer, and were involved in the local production of testosterone and 5α-dihydro-testosterone (DHT), which may play important roles in biological behaviors of prostate carcinoma cells [2]. These locally produced androgens are also possibly metabolized and inactivated in human prostate cancer tissue. For example, aromatase was also reported to be expressed and in human prostate cancer tissue, possibly metabolizing testosterone into estrone (E1) [9]. In addition, AKR1C2, one of the human members of the aldo-keto reductase (AKR) 1C gene family, was reported to be expressed and convert DHT to 3α-diol in both human prostate cancer cell line and tissue [10, 11]. Therefore, results of our present study suggest that Pan1b may contribute to conversion of 3α-diol to androsterone in human prostate cancer tissue.

Chai *et al.* previously reported high levels of Pan1b mRNA expression in the human pancreas, kidney, liver, lung, adrenal, ovary, and heart [5]. They also demonstrated that Pan1b immunoreactivity is detectable in steroidogenic cells such as syncytiotrophoblasts, sebaceous glands, Leydig cells, and granulosa cells of the dominant follicle and corpus luteum [5]. The steroidogenic Y1 mouse cell is also demonstrated to express Pan1b protein [5]. Therefore, it is reasonably postulated that Pan1b plays an important role in steroid metabolism of these tissues. The lowest expression of Pan1b has been, however, reported in the normal prostate as well as in human skeletal muscle, brain, stomach, thymus, and colon [5]. Pan1b is reported to be expressed in LNCaP cell line known as androgen-dependent prostate cancer cell line [6]. Results of our present study demonstrated that Pan1b is abundantly expressed in human prostate cancer tissue, but not in the normal prostate. All these findings suggest that Pan1b expression is possibly induced by the malignancy in human prostate cancer tissue.

In our present study, immunoreactivity for Pan1b was positively correlated with the status of seminal vesicle invasion. The prostate cancer patients with seminal vesicle invasion are considered unlikely to experience long-term biochemical (PSA) remission from cancer recurrence [12]. We previously reported that AKR1C3 immunoreactivity was positively associated with extracapsular extension in human prostate cancer tissues, which suggest that locally produced testosterone may play a role in etiology and development of human prostate cancer [2, 13]. However, androgen metabolism in advanced prostate cancer tissue may also be accelerated because of increasing testosterone production. 3α-diol is also known to be capable of stimulating cell proliferation in LNCaP cells [6]. Therefore, the elevated Pan1b expression level is considered to be associated with acceleration of androgen production and metabolism in advanced human prostate cancer tissues, and may be related to adverse clinical outcome of the patients with prostate cancer with seminal vesicle invasion. However, Pan1b immunoreactivity was not correlated with other malignant parameters including, lymph node involvement, Gleason score, or Ki-67 LI in our present study. Therefore, further studies are required to clarify the degree of involvement of the expression of Pan1b in cell proliferation and development of prostate cancer. In contrast, Pan1b immunoreactivity was not correlated with AR and ERβ LIs in our present study. Pan1b is well-known

Table 1. Correlation between Pan1b immunoreactivity and clinicopathological parameters in human prostate cancer specimens.

	Positive (n=19)	Negative (n=21)	*P* value
Age (years)	66.1±1.2	65.7±1.1	0.812
PSA (ng/ML)	15.1±3.1	13.5±2.9	0.705
Gleason score			
2–6	3 (7.5%)	5 (12.5%)	
7	8 (20.0%)	8 (20.0%)	
8–10	8 (20.0%)	8 (20.0%)	0.818
Stage			
pT2	8 (20.0%)	10 (25.0%)	
pT3	11 (27.5%)	11 (27.5%)	0.726
Extracapsular extension			
Positive	7 (17.5%)	9 (22.5%)	
Negative	12 (30.0%)	12 (30.0%)	0.698
Seminal vesicle invasion			
Positive	6 (15.0%)	1 (2.5%)	
Negative	13 (32.5%)	20 (50.0%)	0.026
Lymph node status			
Positive	2 (5.0%)	0 (0%)	
Negative	17 (47.5%)	21 (47.5%)	0.127

Table 2. Correlation between Pan1b and AR, ERβ, and Ki-67 immunoreactivity in human prostate cancer specimens.

	Positive (n=19)	Negative (n=21)	P value
AR LI (%)	70.5±5.5	77.7±4.1	0.292
ERβ LI (%)	42.8±7.0	41.9±6.1	0.921
Ki-67 LI (%)	7.2±1.2	8.1±1.3	0.583

to convert 3α-diol to androsterone as described above [4, 5]. However, to the best of our knowledge, no studies reported the association between these steroids and steroid hormone receptor expression in human prostate cancer. On the other hand, Tchédam-Ngatcha *et al.* reported that androsterone derivatives do not bind to AR or ER [14]. In addition, Yan *et al.* recently reported that 3α-diol contributed to androgen-independent prostate cancer progression [15]. Therefore, Pan1b expression may not influence AR or ERβ expression levels in human prostate cancer. However, in this study, the number of cases examined was rather limited due to tissue availability and further investigation including the analysis of more cases may be required for confirming the reliability of conclusions obtained in this study.

In summary, we demonstrated that Pan1b protein was detected in approximately 50% of prostate cancer specimens that we examined, and Pan1b expression may have an effect on the character of advanced prostate cancer.

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