In situ androgen producing enzymes in human prostate cancer

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Abstract

Androgens have been proposed to be actively produced *in situ* in human prostate cancer. These locally produced androgens have also been considered to play important roles in the pathogenesis and development of prostate cancer. Therefore, it is important to examine the status of this *in situ* androgen metabolism and/or synthesis in detail in order to improve the clinical response to hormonal therapy in patients diagnosed with prostate cancer. Several studies have previously demonstrated the expression of androgen-producing enzymes such as 5 α -reductase types 1 and 2, and 17 β -hydroxysteroid dehydrogenase type 5 (17 β -HSD5), in human prostate carcinoma cells. However, their biological significance has remained largely unknown. In this study, we evaluated the immunoreactivities of these steroidogenic enzymes in human prostate cancer obtained from surgery ($n=70$), and correlated the findings with clinicopathological features of the patients. 17 β -HSD5 immunoreactivity was detected in 54 cases (77%), 5 α -reductase type 1 in 51 cases (73%) and 5 α -reductase type 2 in 39 cases (56%). 5 α -reductase type 2 immunoreactivity was significantly correlated with that of androgen receptor (AR), and 17 β -HSD5 positive cases were significantly associated with clinical stage (TNM stage pT3 vs pT2). These data all suggest that androgen-producing enzymes, such as 5 α -reductase type 1 and type 2, and 17 β -HSD5 are expressed in a majority of prostate cancers, and are involved in the local production and actions of androgens in prostate cancers.

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Introduction

Androgens play important roles in the pathogenesis of prostate cancer (Lopez-Otin & Diamandis 1998). In addition, the *in situ* production of androgens has been proposed to play a critical role in the pathogenesis and/or development of human prostate cancer (Lopez-Otin & Diamandis 1998). Moreover, suppression of androgen secretion and/or a blockade of their actions represent the basis for many forms of effective hormonal treatment of patients diagnosed with prostate cancer (Negri-Cesi *et al.* 1998). Therefore, it is very important to examine the status of expression of androgen metabolizing and/or producing enzymes in prostate cancer patients in order to obtain a better understanding of the possible roles of *in situ* androgen production and its action.

Testosterone, the main circulating androgen, is converted in prostate cancer tissues by 5 α -reductases into the bioactive and potent androgen, 5 α -dihydrotestosterone (DHT) (Russell & Wilson 1994). Therefore, 5 α -reductases are considered to play important roles as local regulators of androgen and other sex steroid actions in prostate cancer tissues. The type 1 isozyme of 5 α -reductase is located on the distal short arm of chromosome 5, and is mainly expressed in the liver and skin (Thigpen *et al.* 1993, Russell & Wilson 1994). The type 2 isozyme of 5 α -reductase is located in band p23 of chromosome 2, and is, in addition to its strong expression in the liver, also markedly expressed in the prostate, seminal vesicle, and epididymis (Thigpen *et al.* 1992, Thigpen *et al.* 1993). In a recent study by Dufort and co-workers, 17 β -HSD5, which specifically catalyzes the reduction

of androstenedione to testosterone, was cloned (Dufort *et al.* 1999). Several studies have previously demonstrated the presence of 5 α -reductase isozyme mRNAs and enzyme activities in human prostate carcinoma cells (Delos *et al.* 1998, Negri-Cesi *et al.* 1998, Soderstrom *et al.* 2002, Thomas *et al.* 2003). The mRNA for 17 β -HSD5 was also reported to be expressed in prostate cancer tissues (Koh *et al.* 2002). These findings indicate that *in situ* production of 5 α -dihydrotestosterone (DHT) from inactive androstenedione by 17 β -HSD5 (reduction of androstenedione to testosterone), and via 5 α -reductase (metabolism of testosterone to DHT) in human prostate tissue may be occurring. However, the status of expression for these enzymes and their relation to clinicopathological findings has not been examined in human prostate cancer. Therefore, in this study, we first examined the immunolocalization of 5 α -reductase isozymes, and 17 β -HSD5 in human prostate carcinoma tissue specimens obtained from surgery. We then correlated these findings with various clinicopathological parameters of patients including the status of the androgen receptor (AR) in 70 cases of human prostate carcinoma in order to evaluate the possible roles of these androgen metabolizing/synthesizing enzymes in human prostate cancer.

Materials and methods

Patients and tissues

Seventy surgical pathology specimens of prostate carcinoma were obtained from patients who underwent prostatectomy from 1998–2003 at the Department of Urology, Tohoku University Hospital (Sendai, Japan). The mean age of the patients was 65.7 years (range: 48–77). All patients examined in this study did not receive radiation, chemotherapy, nor hormone therapy before surgery. Clinical data, including patient age, serum prostate specific antigen (PSA) concentration, clinical stage according to the International Union Against Cancer TNM classification (1987), lymph node status, and Gleason's score (Veltri *et al.* 1996) were retrieved from detailed patient charts describing individual patient histories. The histological grade of each tumor was evaluated by three of the authors (Y N, T S and H 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

5 α -reductase type 1 and type 2 antibodies used in this study were rabbit polyclonal antibodies against synthesized peptides corresponding to amino acids 232–256 for 5 α -reductase type 1, and amino acids 227–251 for 5 α -reductase type 2, respectively (Thigpen *et al.* 1993). These antibodies were kindly provided by Dr D W Russell (University of Texas Southwestern Medical Center, Dallas, TX). The polyclonal antibody for 17 β -hydroxysteroid dehydrogenase type 5 (17 β -HSD5) was raised in a rabbit against a synthetic peptide corresponding to amino acids 297–320 for 17 β -HSD5 and was kindly provided by Dr V Luu (Laval University Hospital Center, Québec, Canada) (Pelletier *et al.* 1999). Characterization of these three antibodies was confirmed by immunoblotting, and the use of antibodies for 5 α -reductase type 2 and 17 β -HSD5 on immunohistochemistry has been previously reported (Thigpen *et al.* 1993, Silver *et al.* 1994, Pelletier *et al.* 1999). mAb against AR was purchased from DAKO Corporation (Carpinteria, CA).

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 (Suzuki *et al.* 1994). For immunostaining of AR, the slides were heated in an autoclave at 120 °C for 5 min in citric acid buffer (2 mmol/l citric acid and 9 mmol/l trisodium citrate dehydrate, pH 6.0) after deparaffinization for antigen retrieval. The dilutions of primary antibodies used in our study were as follows: 5 α -reductase type 1, 1 : 1000; 5 α -reductase type 2, 1 : 1000; AR, 1 : 100; and 17 β -HSD5, 1 : 1000. 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% H₂O₂] and counterstained with hematoxylin. Tissue sections of liver and prostate were used as positive controls for 5 α -reductase types 1 and 2, respectively, and non-neoplastic breast tissue was used as a positive control for 17 β -HSD5 (Thigpen *et al.* 1993, Pelletier *et al.* 1999). As a negative control, normal rabbit or mouse IgG was used instead of the primary antibodies, and no specific immunoreactivity was detected in these reported tissue sections.

Scoring of immunoreactivity

Scoring of immunoreactivity was performed based on previous reports with some modifications (Soslow *et al.*

2000, Suzuki *et al.* 2001). For statistical analyses of 5 α -reductase type 1 and type 2, and 17 β -HSD5 immunoreactivity, the carcinoma cases were classified into the following two groups: +, positive, more than 10% positive cells; and –, no immunoreactivity, less than 10% positive cells according to Soslow and colleagues (Soslow *et al.* 2000). The evaluation (+, positive carcinoma cells; and –, no immunoreactivity) was performed by three of the authors (Y N, T S and H S), independently. Cases with discordant results among these investigators above were re-evaluated. Scoring of AR in carcinoma cells was performed on high power fields ($\times 400$) using standard light microscopy. In each case, more than 500 carcinoma cells were counted independently by the three authors above, and the percentage of immunoreactivity, i.e. labeling index (LI), was determined (Suzuki *et al.* 2001). In the present study, interobserver differences were less than 5%, and the mean of these three values was obtained. In addition, as a control, immunoreactivity for 5 α -reductase type 1 and 2, 17 β -HSD5, and AR was examined in the peripheral zone of non-neoplastic glands in each case.

Statistical analysis

Values for patient age, serum PSA levels, and LI for AR were presented as the mean $\pm$ 95% confidence interval (95% CI), and associations between 5 α -reductase immunoreactivity and the parameters described above were evaluated using the Bonferroni test. Statistical differences between immunoreactivity for 5 α -reductases and stage, lymph node status, histological grade, and immunoreactivity for 17 β -HSD5, were evaluated in a cross-table using the χ^2 -test. $P < 0.05$ was considered significant.

Results

Immunohistochemistry

Immunoreactivity for 5 α -reductase type 1 and type 2, and 17 β -HSD5 was detected in the cytoplasm of carcinoma cells, whereas AR immunoreactivity was detected in the nuclei of carcinoma cells (Fig. 1A–D). In the non-neoplastic peripheral zone, immunoreactivity for 5 α -reductase type 2, 17 β -HSD5, and AR was detected in the glandular epithelium, but

Figure 1 Immunohistochemistry of 5 α -reductase type 1 (A) and type 2 (B), 17 β -HSD5 (C), and AR (D) in human prostate carcinoma, and immunohistochemistry of 5 α -reductase type 1 (E) and type 2 (F), 17 β -HSD5 (G), and AR (H) in non-neoplastic glands of the peripheral zone of human prostate. Immunoreactivity of 5 α -reductase type 1 (A) and type 2 (B), 17 β -HSD5 (C) was detected in the cytoplasm of carcinoma cells, whereas that of AR (D) was detected in the nucleus of carcinoma cells. In addition, immunoreactivity of 5 α -reductase type 2 (F), and 17 β -HSD5 (G) was detected in the cytoplasm of non-neoplastic glands of the peripheral zone, whereas that of AR (H) was detected in the nucleus of non-neoplastic glands of the peripheral zone. However, immunoreactivity of 5 α -reductase type 1 (E) was not detected. Original magnification, $\times 400$.

Table 1 Correlation among 5 α -reductases immunoreactivity and 17 β -HSD type 5 immunoreactivity in human prostate cancer

	<i>P</i> value
5 α -reductase type 1 and 5 α -reductase type 2	0.0004*
5 α -reductase type 1 and 17 β -HSD type 5	0.0993
5 α -reductase type 2 and 17 β -HSD type 5	0.0049*

**P*<0.05.

5 α -reductase type 1 immunoreactivity was negative in this study (Fig. 1E–H). The number of positive cases and their corresponding percentages were as follows: 51/70 (73%) for 5 α -reductase type 1, 39/70 cases (56%) for 5 α -reductase type 2, and 54/70 cases (77%) for 17 β -HSD5.

Correlation among 5 α -reductases and 17 β -HSD5 immunoreactivity

A statistically significant positive correlation was detected between 5 α -reductase type 1 and type 2 immunoreactivity (*P*=0.0004), and between 5 α -reductase type 2 and 17 β -HSD5 immunoreactivity (*P*=0.0049) (Table 1). A similar trend was also detected between 5 α -reductase type 1 and 17 β -HSD5 immunoreactivity, but the correlation did not reach statistical significance (*P*=0.0993) (Table 1).

Correlation between immunoreactivities and clinicopathological parameters

Immunoreactivity for 5 α -reductase type 1 was not significantly correlated with clinicopathological parameters including patient age, concentration of serum

Table 2 Correlation between 5 α -reductase type 1 immunoreactivity and clinicopathological parameters in human prostate cancer

	+(n=51)	–(n=19)	<i>P</i> value
Age (years)	65.2 $\pm$ 0.9	66.6 $\pm$ 1.3	0.3851
PSA (ng/ml)	14.0 $\pm$ 1.8	17.2 $\pm$ 4.0	0.6540
Gleason score			
2–6	8 (11.4%)	5 (7.1%)	0.2612
7	22 (31.4%)	10 (14.3%)	
8–10	21 (30.0%)	4 (5.7%)	
Stage			
pT2	20 (28.6%)	9 (12.9%)	0.5380
pT3	31 (44.3%)	10 (14.3%)	
Lymph node status			
+	6 (8.6%)	2 (2.9%)	0.0849
–	45 (64.3%)	17 (24.3%)	
AR LI (%)	68.1 $\pm$ 3.2	69.1 $\pm$ 4.0	0.8703

Table 3 Correlation between 5 α -reductase type 2 immunoreactivity and clinicopathological parameters in human prostate cancer

	+(n=39)	–(n=31)	<i>P</i> value
Age (years)	65.3 $\pm$ 1.1	66.0 $\pm$ 0.9	0.6203
PSA (ng/ml)	13.5 $\pm$ 1.9	17.0 $\pm$ 3.3	0.4390
Gleason score			
2–6	6 (8.6%)	7 (10.0%)	0.7203
7	19 (27.1%)	13 (18.6%)	
8–10	14 (20.0%)	11 (15.7%)	
Stage			
pT2	14 (20.0%)	15 (21.4%)	0.2920
pT3	25 (35.7%)	16 (22.9%)	
Lymph node status			
+	3 (4.3%)	5 (7.1%)	0.2704
–	36 (51.4%)	26 (37.1%)	
AR LI (%)	73.4 $\pm$ 3.1	62.1 $\pm$ 4.0	0.0281*

*Data was considered significant.

PSA levels, Gleason's score, pT stage, lymph node states, and AR LI (Table 2). There was a statistically positive correlation between 5 α -reductase type 2 immunoreactivity and AR LI (*P*=0.0281) (Table 3), however, it was not significantly correlated with other clinicopathological parameters (Table 3).

17 β -HSD5 immunoreactivity was significantly associated with clinical stage in this study (TNM stage pT3 vs pT2) (*P*=0.0115) (Table 4).

Discussion

It is well known that androgens play important roles in the pathogenesis of prostate cancer (Lopez-Otin &

Table 4 Correlation between 17 β -HSD type 5 immunoreactivity and clinicopathological parameters in human prostate cancer

	+(n=54)	–(n=16)	<i>P</i> value
Age (years)	65.5 $\pm$ 0.9	65.8 $\pm$ 1.1	0.8716
PSA (ng/ml)	14.7 $\pm$ 1.7	13.8 $\pm$ 6.2	0.6204
Gleason score			
2–6	8 (11.4%)	5 (7.1%)	0.2895
7	25 (35.7%)	7 (10.0%)	
8–10	21 (30.0%)	4 (5.7%)	
Stage			
pT2	18 (25.7%)	11 (15.7%)	0.0115*
pT3	36 (51.4%)	5 (7.1%)	
Lymph node status			
+	7 (10.0%)	1 (1.4%)	0.4585
–	47 (67.1%)	15 (21.5%)	
AR LI (%)	69.4 $\pm$ 2.8	64.8 $\pm$ 6.1	0.4572

*Data was considered significant.

Diamandis 1998). Although plasma concentrations of testosterone have been shown to decrease more than 90% following castration, androgen levels in prostate cancer tissues decreased only 50–60%, suggesting the importance of *in situ* androgen production in prostate cancers (Labrie 1991, Mizokami *et al.* 2004). Results from our present study suggest that androgen-producing enzymes are co-expressed in human prostate cancers, and involved in the local production of DHT, which may play important roles in biological behavior of prostate carcinoma cells.

In the present study, the number of cases positive for steroidogenic enzymes and the corresponding percentages was 39/70 cases (56%) for 5 α -reductase type 2. In addition, there was a statistically significant positive correlation between 5 α -reductase type 2 immunoreactivity and AR LI. It has been reported that type 2 5 α -reductase was expressed in prostate cancer, and was very important in the process of *in situ* production of DHT (Torres *et al.* 2003). In addition, type 2 5 α -reductase has been also hypothesized to play a role in the pathogenesis and/or development of prostate cancer (Torres *et al.* 2003). Moreover, it has also been reported that men who have had some polymorphism of the type 2 5 α -reductase gene have a significantly increased risk of prostate cancer development and/or progression (Nam *et al.* 2001). Our data are consistent with these reports, and suggest that 5 α -reductase type 2 may play an important role in the *in situ* production and action of DHT in human prostate cancer tissues.

Results from the present study also demonstrated that 5 α -reductase type 1 was frequently expressed in human prostate cancer (73%). In addition, we demonstrated that a significant positive correlation was demonstrated between 5 α -reductase type 1 and type 2 immunoreactivity. It has been suggested that individuals with greater 5 α -reductase are at an increased risk for developing prostate cancer. Furthermore, previous reports have demonstrated that type 1 5 α -reductase mRNA and activity were much greater in prostate cancers than in benign prostate tissues (Steers 2001, Iehle *et al.* 1999). These data suggest that both type 1 and type 2 5 α -reductases are likely to be important in the local production and action of DHT in human prostate cancer. It has also been reported that treatment of finasteride, a 5 α -reductase type 2 inhibitor, to patients diagnosed with metastatic prostate cancer resulted in only minor effects in this cancer (Presti *et al.* 1992), and decreased serum PSA levels by only 7% in men with advanced adenocarcinoma of the prostate (Brufsky *et al.* 1997). In contrast, however, dutasteride, which inhibits both type 1 and type 2

5 α -reductases, treatment has recently been reported to result in almost complete suppression of intraprostatic DHT, and increased regression of prostate cancer (Andriole *et al.* 2004). Results from these studies suggest that 5 α -reductase type 1, as well as the type 2 isozyme, are associated with the growth of prostate cancer. Furthermore, knowing the expression pattern and profile of 5 α -reductase isozymes may be important when considering the use of 5 α -reductase inhibitors in reducing the progression of prostate carcinoma in patients diagnosed with this aggressive cancer via the inhibition of *in situ* DHT production. However, further investigations are required to clarify the role of 5 α -reductase isozymes and related steroidogenic enzymes in the pathogenesis of prostate carcinoma.

Iehle and colleagues demonstrated that 5 α -reductase type 1 mRNA is abundantly expressed in the epithelial cells of normal prostate, whereas stromal cells of this glandular tissue demonstrated greatly reduced levels (Iehle *et al.* 1999). In our study, we could not identify significant immunoreactivity of 5 α -reductase type 1 in the non-neoplastic peripheral zone of the prostate. A previous study also demonstrated that type 1 5 α -reductase mRNA and activity were much lower in benign prostate tissues than in prostate cancers (Steers 2001). Therefore, protein expression levels of 5 α -reductase type 1 in non-neoplastic areas of the human prostate were considered below the limits of detection by immunohistochemistry.

In the present study, 17 β -HSD5 immunoreactivity was detected in carcinoma cells of 77% of prostate cancer tissues, and was positively associated with clinical stage ($P < 0.05$; TNM stage III vs TNM stage II). It is well-known that cell proliferation in the normal prostate and in prostate cancer is mainly controlled by testosterone following intracellular conversion to DHT (Ross & Henderson 1994, Imperato-McGinley *et al.* 1992). However, it has also been demonstrated that the remodeling of prostate stromal tissue via testosterone may play a role in the early stage of prostate carcinogenesis, which is generally considered to be favorable for tumor development (Li *et al.* 2001). Therefore, 17 β -HSD5 may be involved in increasing the local concentration of testosterone in prostate cancer tissues, resulting in the progression, invasion, and further development of prostate cancer.

In summary, we have demonstrated that 5 α -reductase types 1 and 2, and 17 β -HSD5 were all detected in the great majority of prostate cancer tissues, and significant positive association was detected between 5 α -reductase types 2 and AR and/or 17 β -HSD5 and clinical stage. These data also indicate the importance of these androgen metabolizing/synthesizing enzymes

in the local production and action of androgens in human prostate cancer.

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