Nuclear Receptor DAX1 in Human Prostate Cancer: A Novel Independent Biological Modulator

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Abstract. The orphan nuclear receptor DAX1 (dosage-sensitive sex reversal-AHC critical region on the X chromosome gene 1; NR0B1) has been known for its various roles in human development, specifically sex determination and steroidogenesis. Its expression has been reported in endocrine and sex steroid-dependent neoplasms such as human adrenocortical, pituitary, endometrial, and ovarian tumors. Prostate cancer is also sex steroid-dependent tumor in which androgens play important roles in the pathogenesis and development via androgen receptor (AR). DAX1 is also reported to repress AR activity in human prostate cancer cell line (LNCaP) but its biological roles have remained unclear in the human prostate cancer. The aim of this study is to examine the expression of DAX1 in human prostate cancer using immunohistochemistry in order to evaluate its possible biological and/or clinical significance. In this study, we examined the DAX1 immunoreactivity in human prostate cancer obtained from surgery (n = 40), and correlated the findings with clinicopathological features of the patients. Twenty-one cases were defined as positive cases for DAX1 immunoreactivity (53%). Immunoreactivity for DAX1 was inversely and significantly correlated with Gleason score (P<0.05). However, DAX1 immunoreactivity was not significantly correlated with the status of sex steroid receptors we examined. DAX1 immunoreactivity is considered a new biological modulator of human prostate cancer, but independent to the status of sex steroid receptors in human prostate cancer tissues.

Key words: DAX1, Prostate, Cancer, Immunohistochemistry

PROSTATE cancer is known as sex steroid-dependent tumor in which androgens play important roles in the pathogenesis and development via androgen receptor (AR) [1, 2]. In addition, estrogen receptor beta (ERβ) and progesterone receptor (PR) is also expressed in prostate cancer and is known to modify its biological significance [3–5]. Therefore, it becomes interesting to examine the regulatory mechanisms of expression of these steroid receptors in human prostate cancer.

The orphan nuclear receptor DAX1 (dosage-sensitive sex reversal-AHC critical region on the X chromosome gene 1; NR0B1) is a recently characterized member of the orphan nuclear receptor family [6, 7]. DAX1 is widely distributed in the reproductive and endocrine systems [8, 9]. In addition, DAX1 is known to act as a negative regulator of steroid production [10–13]. DAX1 has also been demonstrated to repress Ad4BP/SF-1-mediated transactivation of other steroidogenic genes, and to act as a corepressor for AR, PR, and ERβ [14–20]. These interactions could play significant roles by influencing sex-steroid signaling pathways. However, the expression of DAX1 has not been examined in detail in human prostate cancer. Therefore, in this study, we examined the status and relative abundance of DAX1 in human prostate cancer, and correlated the findings with the status of AR, ERβ, PR, and other clinicopathological findings in order to examine the possible biological significance of this unique transcription factor.
Materials and Methods

Patients and tissues

Forty surgical pathology 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 specimens were retrieved from surgical pathology files of Tohoku University Hospital. The mean age of the patients was 65.9 y (range: 54–77 y). All the patients examined in this study did not receive radiation, chemotherapy, or hormone therapy prior to surgery. Clinical data, including patient age, serum prostate specific antigen (PSA) concentration, lymph node status, and clinical stage according to the International Union Against Cancer TNM classification (1987), and Gleason score were retrieved from the patient charts describing individual patient histories. The histological grades of each tumor were evaluated by two of the authors (Y.N. and T.S.). All the 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 (2003-146).

Antibodies

Rabbit polyclonal antibody for DAX1 was obtained from Santa Cruz Biotechnology, (Santa Cruz, CA, USA). Antibodies against AR and Ki-67 were purchased from DAKO Corporation (Carpinteria, CA) and Immunotech (Marseilles, France), respectively. Antibodies for ERβ and PR were also commercially obtained from Gene Tex, Inc., (San Antonio, TX) and NeoMarkers Co. Ltd. (Fremont, CA), respectively.

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, 4]. For immunostaining, 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: DAX1, 1 : 500; AR, 1 : 100; ERβ, 1 : 1,500; PR, 1 : 200; 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% H2O2] and counterstained with hematoxylin. Tissue sections of the normal adrenal gland were used as positive controls for DAX1, an invasive ductal carcinoma of the breast were used as positive controls for ERβ and PR, and normal prostate tissue was used as a positive control for AR. As for negative controls, immunohistochemical preabsorption tests were performed for DAX1, and normal rabbit IgG was also used instead of the primary antibody. No specific immunoreactivity was detected in these tissue sections (data not shown).

Scoring of immunoreactivity

Evaluation of DAX1, AR, ERβ, PR, and Ki-67 immunoreactivity was performed in high-power fields (>400) using a standard light microscope. These immunohistochemical expression levels were independently reviewed by two of the authors (Y.N. and T.S.). In all cases examined, a total of more than 500 tumor cells from three different representative fields were counted independently by the two aforementioned authors, and the percentage immunoreactivity (i.e., labeling index [LI]), was determined. After completely reviewing the immunostained sections of each lesion, all cases were divided into the following two groups: + (more than 10% positive cells); – (fewer than 10% positive cells) for DAX1 immunoreactivity.

Statistical analysis

Values for patient age, serum PSA levels, and LI for AR, ERβ, PR, and Ki-67 were presented as the mean ± 95% confidence interval (95% CI), and associations between DAX1 immunoreactivity described above were evaluated using the unpaired t-test. Statistical differences between immunoreactivity for DAX1 and stage, lymph node status, histological grade, and Gleason score were evaluated in a cross-table using the χ2-test. P<0.05 was considered significant.

Results

DAX1 immunoreactive protein was detected in the...
nuclei of prostate carcinoma cells, but not in the normal prostate (Fig. 1). Twenty-one cases were defined as positive cases for DAX1 immunoreactivity (53%). There was a significant inverse or negative correlation between DAX1 immunoreactivity and Gleason score of carcinoma (Table 1) \((P<0.05)\). Immunoreactivity for DAX1 was not significantly correlated with other clinicopathological parameters including patients’ age, concentration of serum PSA levels, pT stage, lymph node status, or LI of Ki-67 (Table 1). There were no significant correlations between DAX1 immunoreactivity and AR, ER\(\beta\), or PR, immunoreactivity (Table 2).

### Discussion

In this study, we first demonstrated that immunoreactivity for DAX1 was detected in human prostate cancer in approximately 50% of the cases we examined. In addition, immunoreactivity for DAX1 was inversely and significantly correlated with Gleason score.

To the best of our knowledge, this study is the first report to examine the relative abundance of DAX1 protein in human prostate cancer tissues. DAX1 was previously reported in human testis, ovarian follicle, corpus luteum, and adrenal, and has been in general postulated to be associated with development and steroidogenesis of these tissues \([21–25]\). The presence of

### Table 1. Correlation between DAX1 immunoreactivity and clinicopathological parameters in human prostate cancer tissues (\(*P<0.05\))

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Positive ((n = 21))</th>
<th>Negative ((n = 19))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.1 ± 1.1</td>
<td>65.7 ± 1.2</td>
<td>0.802</td>
</tr>
<tr>
<td>PSA (ng/ML)</td>
<td>12.0 ± 2.3</td>
<td>16.7 ± 3.6</td>
<td>0.270</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–6</td>
<td>7 (17.5%)</td>
<td>1 (2.5%)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10 (25.0%)</td>
<td>6 (15.0%)</td>
<td></td>
</tr>
<tr>
<td>8–10</td>
<td>4 (10.0%)</td>
<td>12 (30.0%)</td>
<td>0.009*</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>11 (27.5%)</td>
<td>7 (17.5%)</td>
<td></td>
</tr>
<tr>
<td>pT3</td>
<td>10 (25.0%)</td>
<td>12 (30.0%)</td>
<td>0.324</td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2 (5.0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>19 (47.5%)</td>
<td>19 (47.5%)</td>
<td>0.168</td>
</tr>
<tr>
<td>Ki-67 LI (%)</td>
<td>7.6 ± 1.1</td>
<td>7.8 ± 1.5</td>
<td>0.903</td>
</tr>
</tbody>
</table>

### Table 2. Correlation between DAX1 immunoreactivity and the expression level of sex steroid receptors in human prostate cancer tissues

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Positive ((n = 21))</th>
<th>Negative ((n = 19))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR LI (%)</td>
<td>76.3 ± 5.2</td>
<td>72.0 ± 4.4</td>
<td>0.531</td>
</tr>
<tr>
<td>ER(\beta) LI (%)</td>
<td>44.5 ± 6.4</td>
<td>39.9 ± 6.6</td>
<td>0.624</td>
</tr>
<tr>
<td>PR LI (%)</td>
<td>10.1 ± 2.6</td>
<td>5.7 ± 2.2</td>
<td>0.214</td>
</tr>
</tbody>
</table>

![Fig. 1. Immunoreactivity of DAX1 in human prostate cancer tissues. DAX1 immunoreactive protein was detected in the nuclei of prostate carcinoma cells (A), but it was negligible in the epithelial cells of non-neoplastic prostate glands (B). Bar = 10 \(\mu\)m.](image)
DAX1 was also reported in adrenal, endometrial, and ovarian tumor [19, 26–28]. DAX1 mRNA was absent in human normal prostate, while it was expressed in androgen-dependent prostate cancer cell lines, i.e. LNCaP cells [20, 29]. Therefore, DAX1 may play an important role in development of human prostate cancer but not in normal prostate.

DAX1 protein has been reported to be localized mainly in the nuclei of various cells and to be postulated to work as a nuclear orphan receptor in the human normal and neoplastic tissues [19, 21–28]. Results of recent studies suggest that DAX1 was also detected in the cytoplasm, functioning as a potent corepressor for steroid hormone receptors in mammalian cells [30–32]. However, in our present study, DAX1 immunoreactivity was clearly detectable in the nuclei of prostate carcinoma cells but not so in their cytoplasm, which is compatible with results of most previously reported studies [19, 21–28]. Therefore, these results all suggest that DAX1 protein is predominantly present in the nuclei of prostate carcinoma cells and may play an important role as a nuclear orphan receptor in human prostate cancer tissues. However, it requires further investigations to clarify the significance of DAX expression in both the nucleus and cytoplasm in human prostate carcinoma cells.

Gleason score is the most important and established predictors of biologic behavior of human prostate carcinoma [33]. In this grading system, the five basic grade patterns are used to generate a histologic score, which ranges from 2 to 10, by adding the primary grade pattern and the secondary grade pattern. Gleason score 2–4 carcinoma is commonly regarded as well-differentiated, Gleason score 5–7 as moderately differentiated, and Gleason score 8–10 as poorly differentiated [33]. However, Gleason score 7 carcinoma is known to harbor an element of high-grade pattern carcinoma, and is intermediate in clinical aggressiveness between patterns 5–6 and 8–10, and is postulated not to be included in a moderately differentiated category [33–36]. Therefore, the 2–6 vs 7 vs 8–10 lump is considered most appropriate for low patient numbers in a research setting, as used in this study [33].

In our present study, the status of DAX1 immunoreactivity was inversely and significantly correlated with Gleason score of the cases. Saito et al. previously reported that there is a significant inverse correlation between DAX1 immunoreactivity and histological grade in endometrial carcinoma, suggesting that DAX1 may inhibit cell proliferation and the progression of endometrial carcinoma [19]. DAX1 expression level is postulated to be associated with the differentiation of mammary epithelial cells [37]. In contrast, Abd-Elaziz et al. reported that DAX1 immunoreactivity is considered to be an independent marker of poor prognosis or adverse clinical outcome in patients with epithelial ovarian carcinoma [28]. DAX1 was, however, reported not to be associated with any clinicopathological factors including histological grades in human breast cancer [31]. In our study, DAX1 immunoreactivity was detected in approximately 50% of prostate cancer tissues. The loss of expression or decreased expression of DAX1 in endometrial carcinoma was reported to result in active or increased intratumoral steroids metabolism or production in endometrial carcinoma, which subsequently result in the estrogen-dependent proliferation of carcinoma cells [19]. Similarly, the loss of expression or decreased expression of DAX1 in prostate cancer may play an important role in intratumoral steroid metabolism or production, which subsequently influences the proliferation of prostate carcinoma cells. However, further in vitro investigations are required to clarify the precise roles of DAX1 in regulating cell growth and development in human prostate carcinoma cells.

DAX1 has been reported by Zhang et al. to play important roles in the regulation of ER transactivation [30]. DAX1 has been also reported to inhibit the transcriptional activity of liganded ER by a sequential mechanism, possibly involving the recruitment of corepressors [16]. Saito et al. also reported that there was a statistically significant positive correlation between DAX1 and ERα and β expression levels, suggesting that this nuclear factor may inhibit the proliferation and progression of endometrial carcinoma through inhibition of estrogenic actions, possibly by interacting with ER present in carcinoma cells [19]. However, the status of DAX1 immunoreactivity was not significantly correlated with that of AR, ERβ, or PR in all the cases of prostate carcinoma cases which we examined. These findings suggest that DAX1 does not regulate the expression levels of these steroid hormone receptors in human prostate carcinoma cells compared to the cases of human ovarian and endometrial cancer tissues. However, DAX1 was also reported to suppress agonist-dependent activity of AR in human prostate carcinoma cell lines and PR in human breast cancer cells [20]. In our study, 21 cases were
defined as positive for DAX1 immunoreactivity (53%). Among these DAX-1 positive cases, the case with relatively high expression levels (LI more than 10%) was detected in 20 cases for AR, 20 cases for ERβ, and 8 cases for PR (data not shown), respectively. These findings also suggest the co-localization of DAX1 and these steroid hormone receptors resulting in their interaction and possible regulation by DAX-1 in the function of these steroid hormone receptors in human prostate cancer tissues. It awaits further examinations to clarify the correlation between DAX1 and these steroid hormone receptors in human prostate cancer tissues.

In summary, we demonstrated that DAX1 protein was detected in approximately 50% of prostate cancer tissues, and significant inverse or negative correlation was present between DAX1 immunoreactivity and Gleason score of carcinoma. These findings indicate that this nuclear orphan receptor is considered to be a new biological modulator of human prostate cancer.

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