Phosphorylation Status of Fas-associated Death Domain Protein Is Associated With Biochemical Recurrence After Radical Prostatectomy

Tomohiro Ikeda, Nobumichi Tanaka, Keiji Shimada, Yoshiaki Matsumura, Makito Miyake, Satoshi Anai, Atsushi Tomioka, Eijiro Okajima, Akihide Hirayama, Kiyohide Fujimoto, Noboru Konishi, and Yoshihiko Hirao

OBJECTIVE To assess whether the phosphorylated Fas-associated death domain protein (FADD) at 194 serine (p-FADD) is valuable as a marker of biochemical recurrence in hormone-naive patients who had undergone radical prostatectomy.

MATERIALS AND METHODS We used radical prostatectomy specimens from 106 patients. None of the patients had received neoadjuvant or adjuvant therapy. The percentage of positive p-FADD cells (nuclear staining) was immunohistochemically evaluated. The correlation between FADD phosphorylation and the clinicopathologic parameters was assessed. The correlation between the biochemical recurrence-free rate and the p-FADD expression level was analyzed using the Kaplan-Meier method.

RESULTS Overall, 39 patients developed biochemical recurrence. We investigated the expression of p-FADD in 106 patients with prostate cancer using immunohistochemistry. We compared our findings with the clinicopathologic parameters, including the follow-up data. Patients with a greater positive p-FADD rate had a significantly lower biochemical recurrence rate than those with a lower positive p-FADD rate ($P < .001$). A significant inverse correlation was found between the positive p-FADD rate and the Gleason score.

CONCLUSION A low expression of p-FADD could be a predictor of biochemical recurrence in hormone-naive patients who have undergone radical prostatectomy. UROLOGY 81: 607–610, 2013.

Prostate cancer is a common cancer in men, and its incidence has been increasing in Japan. Although most patients with prostate cancer have a relatively good prognosis after primary treatment, some have a poor prognosis. The prognosis of patients who have undergone radical prostatectomy (RP) is uncertain, even when the Gleason score is used. Organ-confined prostate cancer can be curatively treated with surgery. However, once metastasis has occurred, the disease will eventually take a fatal course. This fact underscores the necessity of novel prognostic markers. Thus, we focused on the phosphorylation status of Fas-associated death domain protein (FADD) in prostatectomy specimens.

Financial Disclosure: *The authors declare that they have no relevant financial interests.*
From the Department of Urology, Nara Medical University, Kashihara, Nara, Japan; and Department of Pathology, Nara Medical University, Kashihara, Nara, Japan

Reprint requests: Nobumichi Tanaka, M.D., Department of Urology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan. E-mail: sendo@naramed-u.ac.jp

Submitted: June 18, 2012, accepted (with revisions): November 19, 2012

The FADD is one of the death receptor family members and is well known to be associated with the execution of Fas-mediated apoptosis in various cancer cell types.[1-3] It has previously been reported that FADD is phosphorylated at 194 serine during the G_2/M cell cycle, and phosphorylated FADD is closely associated with cell cycle regulation.[4] We have demonstrated that phosphorylated FADD at 194 serine (p-FADD) is associated with prostate cancer progression and showed a correlation between the Gleason score and a positive rate of p-FADD in prostate cancer specimens.[5] In addition, we have reported a correlation between the biochemical recurrence rate and a positive rate of p-FADD staining in patients who had undergone RP after neoadjuvant hormonal treatment. According to our data, using a p-FADD cutoff of 15%, the group with >15% had a significantly greater biochemical recurrence-free rate.[6]

In the present study, we assessed whether p-FADD is a potential marker to predict biochemical recurrence in hormone-naive patients who have undergone RP.

0090-4295/12/$36.00

http://dx.doi.org/10.1016/j.urology.2012.11.032

Table 1. Patient characteristics at diagnosis

Characteristic	Biochemical Recurrence		*P* Value
	No (n = 67)	Yes (n = 39)	
Age (y)	67.1 ± 6.4	68.7 ± 4.4	NS
PSA (ng/mL)	12.0 ± 9.1	14.1 ± 8.3	NS
Nadir PSA (ng/mL)	0.02 ± 0.04	0.14 ± 0.29	< .01
Biopsy Gleason score	6.4 ± 1.4	6.7 ± 1.7	NS
Pathologic Gleason score	6.7 ± 1.4	6.9 ± 1.4	NS

NS, not significant; PSA, prostate-specific antigen.

MATERIAL AND METHODS

Tissue Samples and Patients

In the present study, we examined 106 primary tissue specimens obtained from hormone-naive patients who had undergone RP from 1999 to 2006 at our hospital. The patient age range was 53-78 years (median 68.4). Biochemical recurrence was defined as a prostate-specific antigen (PSA) level of ≥0.2 ng/mL.

The median PSA level of the prostatic biopsy was 11.8 ng/mL (range 3.9-48.0; Tandem R assay). We divided the patients into 2 groups, those with and without recurrence. The median follow-up period was 36.4 months (range 14-85).

The patient characteristics are listed in Table 1. No significant differences were found between the recurrence-free and recurrence groups, except for the nadir PSA and clinical stage.

Immunohistochemistry

Sections of the specimens were incubated for 16 hours at 4°C, and the reactions were visualized using a Histofine SAB-PO kit and diaminobenzidine as the chromogen (Nichirei, Tokyo, Japan), with hematoxylin counterstaining. The antibody used in the experiment was a polyclonal antibody. It was diluted 50 times with phosphate-buffered saline. We assessed the percentage of positive nuclear staining for p-FADD. The number of phospho-FADD-positive cells per 100 cells was designated as the percentage of positive cells in ≥1000 examined cells. The image of immunohistochemical staining of p-FADD is shown in Figure 1. We immunohistochemically examined the correlation between FADD phosphorylation in the RP specimens and the clinicopathologic parameters in patients who underwent RP and the biochemical recurrence-free rate stratified by the expression of p-FADD in RP specimens. We decided a p-FADD cutoff value of 15% for analysis of the area under the curve of the receiver operating characteristics curve. The cutoff for p-FADD expression was defined as 15%, which resulted in the greatest sum for both sensitivity (86.6%) and specificity (82.1%).

Statistical Analysis

Wilcoxon's signed rank test was used to analyze the distribution of the percentage of p-FADD-positive cells in relation to the morphology. Statistical analyses for intergroup comparisons were performed using the Mann-Whitney *U* test and Fisher's exact probability test. A survival analysis of biochemical recurrence was calculated using the Kaplan-Meier method and the log-rank test. All statistical tests were 2-sided, and statistical significance was defined as $P < .05$.

All patients provided informed consent before the specimens were collected, as appropriate. The ethics committee of Nara Medical University approved the study.

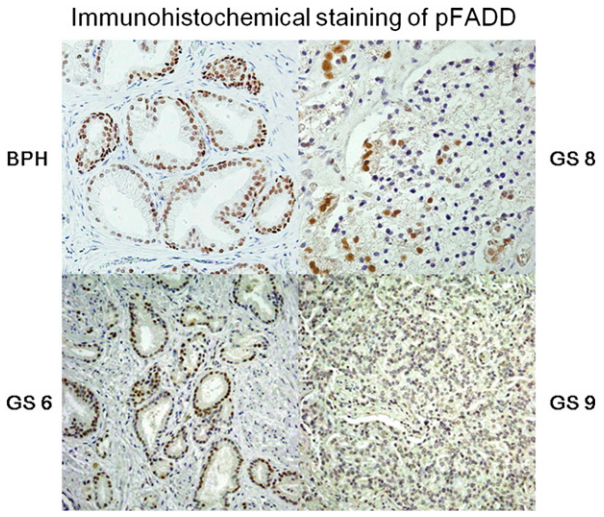

Figure 1. Image of immunohistochemical staining of phosphorylated Fas-associated death domain protein at 194 serine (p-FADD), with nuclei stained. BPH, benign prostatic hyperplasia; GS, Gleason score. (Color version available online.)

RESULTS

Expression of p-FADD in Prostatic Tissue

We investigated the phosphorylation status of FADD in normal epithelial cells and in prostate carcinoma specimens immunohistochemically (Fig. 1). The expression of p-FADD was significantly greater in the normal prostate epithelial cells, where it was predominantly located in the nucleus. However, it was lower in the cancer cells. The expression of p-FADD was also greater in the cancer cells of patients without recurrence.

Correlation Between FADD Phosphorylation in RP Specimens and Clinicopathologic Parameters

Of the 106 patients, 39 showed biochemical recurrence during the follow-up period. We compared the positive rate of FADD phosphorylation in the RP specimens with the clinicopathologic parameters.

The positive rate of p-FADD in the recurrence group was significantly lower than in the recurrence-free group. The positive p-FADD rate was significantly lower in the recurrence group (Fig. 2A). The positive p-FADD rate was significantly lower in patients with a Gleason score of 8 to 10, in both biopsy and surgical specimens, although no significant differences were found in the PSA levels at diagnosis (Fig. 2B). The patients with a greater positive p-FADD rate showed a significantly lower biochemical recurrence rate.

When the p-FADD cutoff value was defined as 15%, as in the previous study, the Kaplan-Meier analysis showed that the group with >15% had a significantly greater biochemical recurrence-free rate ($P < .001$; Fig. 3).

COMMENT

We have previously reported that p-FADD is associated with prostate cancer progression and that a correlation is