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Expression and prognostic significance of ELL-associated factor 2 in human prostate cancer

Yachen Zang¹, Yun Dong², Dongrong Yang¹, Boxin Xue¹, Feng Li³, Peng Gu¹, Haifeng Zhao³, Shaoxiong Wang¹, Songlin Zhou¹, Rong Ying⁴, Zhou Wang^{5,6,7}, and Yuxi Shan¹

Yuxi Shan: shanyx1002@yeah.net

¹Department of Urology, The Second Affiliated Hospital of Soochow University, 1055 Sanxiang Road, Suzhou 215004, China

²Department of Pathology, Wuxi Hospital of Traditional Chinese Medicine, Wuxi, China

³Department of Pathology, The Second Affiliated Hospital of Soochow University, Suzhou, China

⁴Department of Urology, Nanjing Medical University Affiliated Suzhou Hospital, Suzhou, China

⁵Department of Urology, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

⁶Department of Molecular Pharmacology and Chemical Biology, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

⁷Department of Pathology, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Abstract

Purpose—ELL-associated factor 2 (EAF2) is an androgen-regulated tumor suppressor in the prostate. The purpose of this study was to investigate the expression of EAF2 protein in human prostate cancer specimens along with BPH specimens as a control, and to evaluate potential association of EAF2 expression with clinical characteristics and overall survival of the prostate cancer patients.

Methods—The expression of EAF2 was evaluated in 44 prostate cancer and 23 BPH tissue specimens using immunohistochemistry. The relationships of EAF2 expression with clinical characteristics and overall survival rates were analyzed by Chi-square test and Kaplan–Meier method.

Results—The immunostaining intensity of EAF2 in BPH specimens was significantly higher than that in prostate cancer ($p < 0.05$). EAF2 expression decreased significantly in high-grade and advanced-stage human prostate tumors and inversely correlated with PSA level, Gleason scores, bone metastasis and tumor stage. Importantly, loss of EAF2 expression was associated with a significant decrease in patient survival.

Correspondence to: Yuxi Shan, shanyx1002@yeah.net.

Compliance with ethical standards

Conflict of interest None.

Conclusion—Expression of EAF2 is decreased in prostate carcinogenesis, and EAF2 loss is associated with high-risk patients and poor survival.

Keywords

ELL-associated factor 2 (EAF2); Prostate cancer; Overall survival; Prognosis

Introduction

EAF2 is a tumor suppressor initially identified as a novel androgen-responsive gene in the prostate [1, 2]. EAF2 interacts with the RNA polymerase II (pol II) elongation factor eleven-nineteen lysine-rich in leukemia (ELL) [3]. EAF2 expression is downregulated in human prostate cancer specimens and cell lines. Overexpression of EAF2 can induce apoptosis in transfected prostate cancer cells and inhibit xenograft tumor growth [4]. Furthermore, EAF2 knockout mice developed lung adenocarcinoma, hepatocellular carcinoma, B cell lymphoma and high-grade murine prostatic intraepithelial neoplasia (mPIN) [5].

EAF2 can modulate hypoxia-induced factor 1 α (HIF-1 α) and angiogenesis, via direct binding and stabilization of pVHL [6, 7]. Chen et al. have demonstrated that HIF-1 α can induce EAF2 under hypoxia. They also found that EAF2 bound to and suppressed HIF-1 α transcriptional activity [8]. Moreover, EAF2 has also recently been shown to regulate the potent antiangiogenic factor thrombospondin 1 (TSP-1) through interaction with the tumor suppressor p53 [9]. TSP-1 inhibits the proliferation and migration of endothelial cells and increases apoptosis [10]. And decreased expression of TSP-1 has been reported in prostate cancer [11]. Together, these studies indicate that EAF2 as a tumor suppressor is upregulated by androgens in the prostate.

Previous study demonstrated that EAF2 protein down-regulation, allelic loss, promoter hypermethylation and possibly homozygous deletion was identified in more than 80 % of advanced prostate cancer specimens examined (Gleason >7) [4]. However, there were no study reported the relationship of EAF2 expression with clinical characteristics and prognostic significance in prostate cancer patients. Therefore, the aim of this study is to investigate the expression of EAF2 in prostate cancer along with benign prostatic hyperplasia (BPH) specimens as control, and to detect the association of EAF2 expression with clinical characteristics and overall survival of the prostate cancer patients.

Methods

Patient samples

Archived specimens of prostate cancer and BPH were collected from a total of 67 patients who underwent prostatectomy and transurethral resection of the prostate (TURP) in the second affiliated hospital of Soochow University. The study cohort consisted of 44 prostate cancer and 23 BPH tissue specimens. The information of prostate-specific antigen (PSA), tumor stages, Gleason score and patient's age were obtained (Table 1). The study was reviewed and approved by institutional ethics committee.

Immunohistochemistry

Immunohistochemical stains were performed on 4- μ m sections of paraffin blocks. Briefly, the sections were deparaffinized, rehydrated and subjected to antigen retrieval in citrate buffer (10 mM, pH 6.0) for 30 min. After blocking with peroxide for 10 min at room temperature, the slides were then incubated overnight at 4 °C with rabbit anti-EAF2 (1:200 dilution, abcam, Cambridge, UK) and followed by incubation with a secondary anti-rabbit antibody (Dako, Carpinteria, CA) for 30 min at room temperature. After a complete wash in PBS, the slides were developed in freshly prepared diaminobenzidine solution (DAB) then counterstained with hematoxylin, dehydrated, mounted and analyzed using an Olympus BX51 Microscope (Olympus, Tokyo, Japan). Omitting the primary antibody was used as negative control.

Assessment of EAF2 expression

Slides were reviewed independently by two observers (Dong Y and Li F). The staining for EAF2 was mainly confined to the nuclear and presented as brown granular materials. In scoring expression of EAF2 protein, both the extent and intensity of immunopositivity were evaluated.

The extent of positivity was scored according to the percentage of cells showing positive staining: 0 (0–1 %), 1 (2–24 %), 2 (25–49 %), 3 (50–74 %) and 4 (75–100 %). The intensity of the positivity was scored as follows: 0, negative; 1, weak; 2, moderate; 3, strong. The final score was determined by multiplying the intensity of positivity and the extent of positivity scores, yielding a range from 0 to 3. The expression for EAF2 was considered negative when the scores were less than 3 and positive when the scores were three or more [12].

Statistical analyses

The relationship between EAF2 expression and clinical characteristics was analyzed with Chi-square test. Overall survival rates were performed by the Kaplan–Meier method and log-rank test. Overall survival time was defined from the day of surgery to the day of death or last follow-up. For all tests, p value <0.05 was considered significant.

Results

EAF2 expression is decreased in prostate cancer

In total, 67 tissue specimens of prostate cancer ($n = 44$) and BPH ($n = 23$) were examined for EAF2 expression using immunohistochemistry (Table 1). The staining of EAF2 was detected in nuclei in most of the BPH specimens, while in most prostate cancer, the staining was weak (Fig. 1). This pattern is consistent with the previously published data [4]. Each of the specimens was scored as EAF2 positive or EAF2 negative according to the aforementioned criteria. As shown in Table 1, the expression of EAF2 in BPH specimens (30.4 %, 7/23) is significant higher than in prostate cancer (59.1 %, 26/44) ($p < 0.05$). The result shows that EAF2 expression is decreased in prostate cancer.

The association of EAF2 expression with clinical characteristics of the prostate cancer patients

The expression level of EAF2 in 44 prostate cancer patients with several clinical characteristics is detailed in Table 2. The statistical results demonstrated that the EAF2 expression were dramatically lower associated with higher PSA level, Gleason scores and tumor stage ($p < 0.05$). Furthermore, it was significant that there were less EAF2-negative cases among tumors with bone metastasis compared to non-bone metastasis ($p < 0.05$). No statistical association between EAF2 expression and the age of patients was found. All the results above show that lost EAF2 expression was found to be associated strongly with the high risk and progression of prostate cancer.

EAF2 expression is increased in aggressive prostate cancer

We compared the expression of EAF2 with Gleason grade and stage group of the prostate cancer. Weak/moderate positive EAF2 expression was observed in prostate cancer tissue of Gleason score <7 , while in Gleason score >7 , the staining was very weak or negative (Fig. 1e, f). When the EAF2 staining was stratified according to Gleason score <7 , 7 or >7 , significantly decreased EAF2 expression was observed in Gleason score >7 group compared with Gleason score <7 , 7 and BPH (Fig. 2a). With regards to EAF2 expression in every stage of prostate cancer, we have shown the results in Fig. 2b. The patients were divided into stages from I (the least advanced) to IV (the most advanced), and dramatically decreased EAF2 expression was detected in high stage (III and IV) when compared to lower stage (I and II). The results indicate that EAF2 expression decreases significantly with higher tumor grade and advanced tumor stage in human prostate cancer.

The correlation between EAF2 expression and overall survival

Survival status information was available for all the 44 prostate cancer patients. The average duration of follow-up was 46 months (range 15–93 months), and the Kaplan–Meier survival curve for prostate cancer patients with EAF2 positive and negative expression is shown in Fig. 3. We found a significant decrease in survival in patients with EAF2 protein negative compared to EAF2 positive, it means that patients with EAF2 downregulation had poorer survival than EAF2 positive prostate patients.

Discussion

This is the first study investigating the expression associated with clinical characteristics and prognostic relevance of EAF2 in human prostate cancer. In the present study, we provide evidence that expression of EAF2 is decreased in the human prostate cancer as compared with BPH and that EAF2 downregulation is associated with more aggressive prostate cancer and poor survival. This finding is consistent with EAF2 playing an important tumor-suppressive role and being a potential biomarker in prostate cancer.

The Gleason grading system is one of the most powerful prognostic factors in prostate carcinoma and is central to stratifying patients into risk groups and in determining the management of patients with prostate cancer [13]. We found that loss of EAF2 expression correlated significantly with increasing Gleason score when the EAF2 staining was stratified

according to Gleason score <7 , 7 or >7 , with loss of EAF2 expression most frequently in the cohort with Gleason score >7 . The percentage of negative EAF2 expression is about 92 % (11/12) in the group of Gleason score >7 , while it is 43.7 % (14/32) in the other groups. (Gleason score <7 , $=7$). In a previous study, EAF2 downregulation was observed in one out of nine (1/9) prostate cancer specimens with Gleason score of 7 or lower. In contrast, EAF2 downregulation was detected in seven out of eleven (7/11) in tumors with Gleason score of 8–9. The findings were consistent with ours and revealed that EAF2 loss is associated with aggressive prostate cancer [14]. In another study by Xiao and colleagues, they revealed that EAF2 protein is downregulated in human prostate cancer specimens. In their experiment, significant EAF2 downregulation was observed in 19 of 23 radical prostatectomy specimens with Gleason scores of 7–10 [4]. However, their study did not contain specimens of Gleason score <7 , which may be one reason for the higher percentage of EAF2 downregulation in Xiao's result as compared to this study.

One difference between this study and the previous study is that the patients in the present study are all Chinese in China, whereas the previous study used specimens from US prostate cancer patients, presumably most of them being Caucasians. However, EAF2 downregulation was frequent in both patient populations with advanced prostate cancer. This suggests that EAF2 is likely to play important roles in prostate cancer in both Chinese and American men, and it can act as a tumor suppressor regardless of the ethnic background and environmental differences.

When combining the information of T, N and M categories together, along with the Gleason score and PSA level, the patients were divided into stages from I (the least advanced) to IV (the most advanced), and dramatically decreased EAF2 expression was detected in high stage (III and IV) when compared to lower stage (I and II). All the results above suggest that loss of EAF2 expression was associated with high risk and progression of prostate cancer. PSA is a pivotal downstream target gene of the androgen receptor (AR) and a serum biomarker to monitor prostate cancer progression. Also, serum PSA level is associated with high tumor stage. When the prostate cancer patients were stratified according to the PSA level, EAF2 expression was inversely correlated with the high PSA group (>20). Xiao's study [4] revealed that inactivation of androgen-dependent growth restriction pathway, via EAF2 downregulation, is common in prostate cancer progression. The downregulation of EAF2 is unlikely to be due to the lack of functional AR since prostate cancer cells are AR-positive in general. In the absence of EAF2 but in the presence of AR, androgens are likely to cause excessive proliferation in the prostate and enhance cancer progression. Furthermore, there were fewer EAF2-negative cases among tumors with bone metastasis compared to non-bone metastasis. In addition, survival curve showed that patients with EAF2 negative had poorer survival than EAF2 positive prostate patients. Thus, EAF2 expression has potential prognostic significance for prostate cancer.

The frequency of EAF2 downregulation in prostate cancer is very high and appears comparable to that of the other well-established tumor suppressors in the prostate, for example, PTEN. According to the literature, PTEN is a tumor suppressor commonly inactivated in prostate cancer. Loss of the PTEN is one of the most common somatic genetic aberrations in prostate cancer and is frequently associated with high-risk disease [15–17]. In

McMenamin's [18] study, they used 109 radical prostatectomy specimens of prostate cancer for PTEN immunostaining. Seventeen cases (15.6 %) of prostate cancer were positive, 70 cases (64.2 %) were mixed, and 22 cases (20.2 %) were negative. The loss of PTEN expression correlated with high Gleason score, particularly with Gleason score of 7 or higher advanced pathological stage. This is similar to our finding, and both studies demonstrated that detection of PTEN or EAF2 protein was correlated with the Gleason score and the pathological stage of the tumor, known prognosticators in prostate cancer. Importantly, co-downregulation of EAF2 and PTEN was detected in >50 % clinical prostate cancer specimens with Gleason scores of 8–9 [14]. Thus, EAF2 could serve as a promising tissue marker of high-risk and metastatic prostate cancers, particularly when combined with other biomarkers such as PTEN.

Conclusions

This study revealed that decreased expression of EAF2 is associated with aggressive forms of prostate cancer and demonstrated a prognostic role of EAF2 loss in prostate cancer.

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Fig. 1.

Expression of EAF2 by immunohistochemistry in prostate normal tissue and cancer tissue (a, c $\times 100$, b, d $\times 400$, e, f $\times 200$). a, b High EAF2 expression in prostate normal tissue. c, d Low EAF2 expression in prostate cancer tissue. e Weak/moderate positive EAF2 expression in prostate cancer tissue of Gleason score <7 . f Negative EAF2 expression in prostate cancer tissue of Gleason score >7

Fig. 2.

a EAF2 expression decreases dramatically in Gleason score >7 group compared with Gleason score <7, 7 and BPH. **b** EAF2 expression is significantly lower in high stage than low stage of prostate cancer and BPH

Fig. 3.

The Kaplan–Meier curve for disease-specific survival shows a significant decrease in prostate cancer-specific survival in patients with EAF2 protein negative

Table 1

The EAF2 expression in prostate normal tissues and prostate cancer tissues

Tissue type	No. of patients	EAF2 expression	
		Negative	positive
Normal tissue	23	7	16
Prostate cancer	44	26	18
Chi-squared test		$\chi^2 = 4.962$	
		$p = 0.026$	

Table 2

Clinical characteristics of the prostate cancer patients

Characteristic	No. of patients	EAF2 immunostaining		Chi-squared test
		Negative	Positive	
Age (year)				
70	17	12	5	$\chi^2= 1.515$
>70	27	14	13	$p= 0.218$
PSA (ng/ml)				
20	15	5	10	$\chi^2= 6.246$
>20	29	21	8	$p= 0.012$
Gleason score				
<7	16	8	8	$\chi^2= 8.676$
=7	16	6	10	$p= 0.013$
>7	12	11	1	
Tumor stage				
I II		6	12	$\chi^2= 8.36$
III IV	26	20	6	$p= 0.004$
Bone metastasis				
Negative	29	14	15	$\chi^2= 4.116$
Positive	15	12	3	$p= 0.042$