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Original Article

Clinical significance of Nrf2 expression in benign prostatic hyperplasia and prostate cancer tissues

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Abstract: Objective: To investigate the clinical significance of nuclear factor erythroid 2-related factor 2 (Nrf2) expression in benign prostatic hyperplasia and prostate cancer tissues. Methods: We collected specimens from 50 patients with benign prostatic hyperplasia and 42 patients with prostate cancer who underwent surgical treatment at our hospital between June 2007 and March 2013. The expression of Nrf2 protein was compared between benign prostatic hyperplasia and prostate cancer samples using immunohistochemical staining, and we determined the clinical significance of Nrf2 expression. Results: The rate of positive Nrf2 expression was 58% in prostate cancer tissues, which was significantly higher than that in benign prostatic hyperplasia (14.3%) ($P < 0.05$). The number of cases with positive Nrf2 expression was unrelated to patient age or T classification. By contrast, positive Nrf2 expression was related to patient Gleason grade, lymph node metastasis and distant metastasis ($P < 0.05$). Furthermore, the five-year survival rate was significantly lower in patients with positive Nrf2 expression compared with patients with negative expression ($P = 0.04$). Conclusion: Nrf2 is an important factor in the development and progression of prostate cancer, and methods for targeting the expression of Nrf2 may have important clinical implications for the treatment of prostate cancer.

Keywords: Benign prostatic hyperplasia, prostate cancer, Nrf2

Introduction

Prostate cancer is the third most common malignant tumor in Chinese males, and the incidence of this disease is rising along with the aging Chinese population and continuous improvements in the standard of living [1]. A deeper understanding of the pathogenesis of prostate cancer should have significant implications for the treatment of this disease. Nuclear factor erythroid 2-related factor 2 (Nrf2), a nuclear transcription factor, is a member of the cap'n'collar (CNC) family of leucine zipper transcriptional activators. Many previous studies have shown that Nrf2 plays an important role in the development, progression and invasion of several types of tumors, including prostate cancer [2]; however, to date, there are few studies addressing how Nrf2 expression is linked to the clinical and pathological characteristics or prognosis of prostate cancer patients. In this study, we compared Nrf2 expression between benign prostatic hyperplasia and prostate cancer tissues and we analyzed the relationship between Nrf2 expression and various clinical and pathological characteristics to provide a reference for the diagnosis, treatment and prognosis of prostate cancer.

Materials and methods

General information

Fifty patients (65 ± 8.5 years old) who underwent surgical treatment at our hospital and were diagnosed with prostate cancer by post-operative pathological examination between September 1998 and July 2006 were included in this study. In addition, 42 patients (66 ± 7.7 years old) with benign prostatic hyperplasia were also included in this study. This study was approved by the Ethics Committee of our hospital, and all patients signed an informed consent form prior to surgery. Information concerning general patient data was collected, including name, age, lab results before and after surgery,

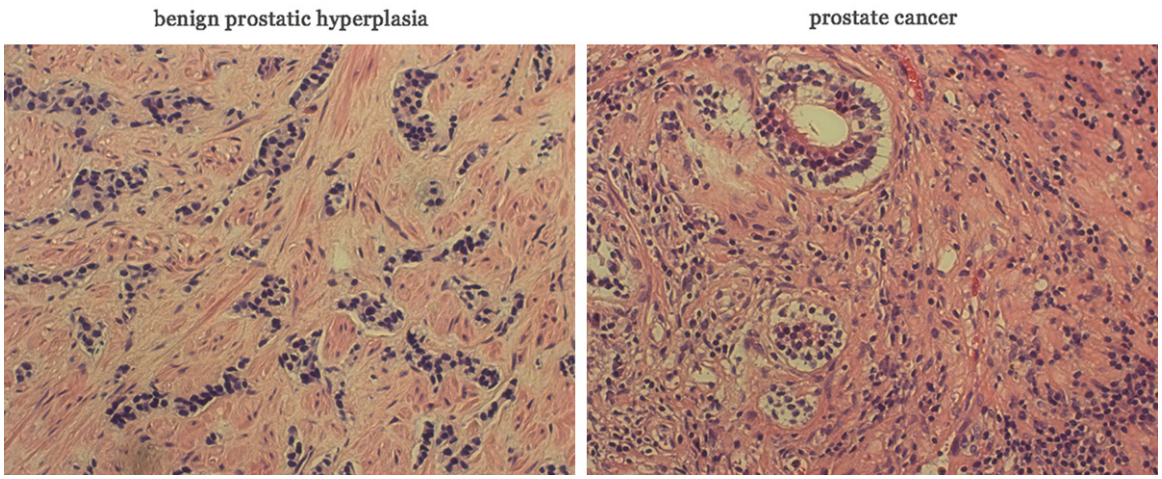

Figure 1. HE staining of benign prostatic hyperplasia and prostate cancer tissues.

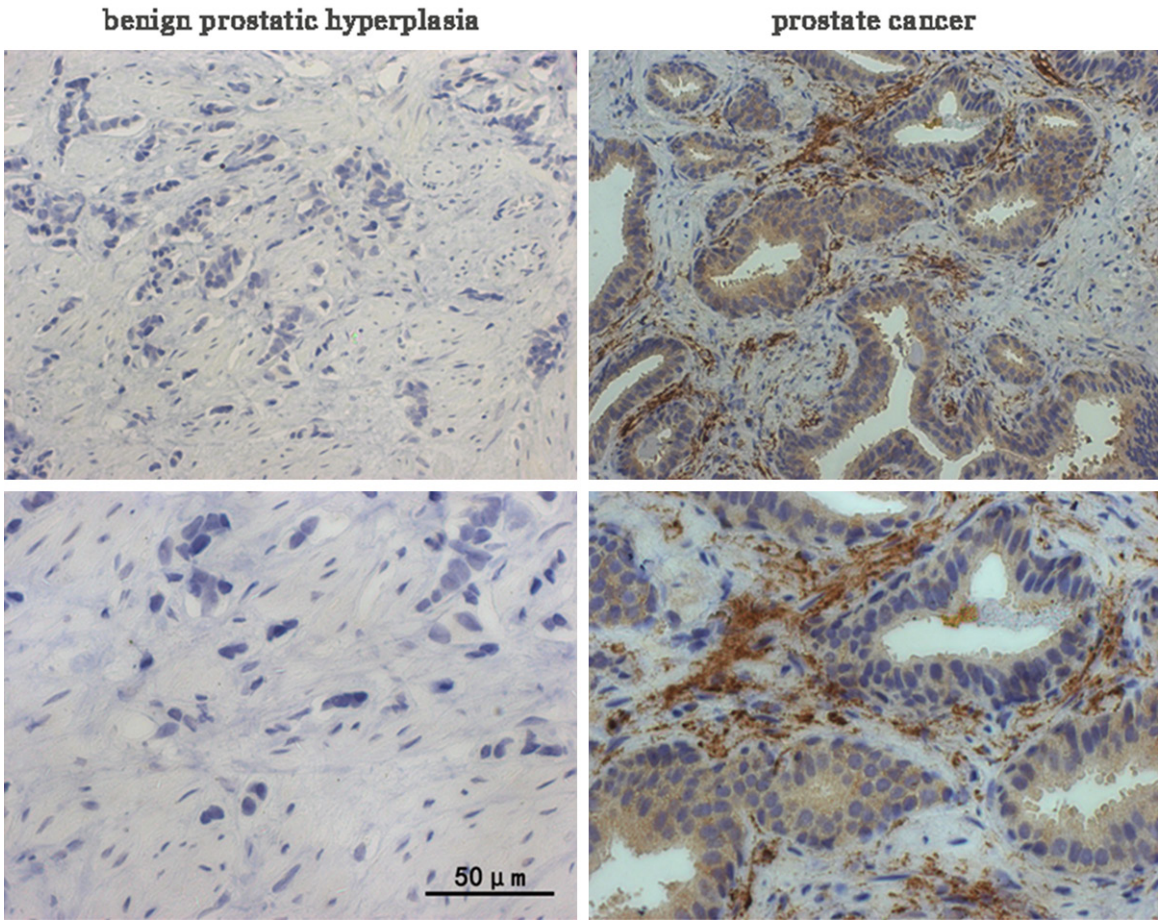

Figure 2. Typical examples of Nrf2 expression in benign prostatic hyperplasia and prostate cancer tissues (representative images). Nrf-2 expression was low in benign prostatic hyperplasia tissues, whereas it was significantly higher in the cytoplasm and nuclei of prostate cancer tissues.

tumor, node and metastases (TNM) stage, Gleason grade, and treatment. Patients who had received radiotherapy and chemotherapy before surgery were excluded from this study.

Table 1. Analysis of positive Nrf2 expression in benign prostatic hyperplasia and prostate cancer tissues

Group	Nrf2 expression		P
	Positive expression (n, %)	Negative expression (n, %)	
Benign prostatic hyperplasia	6 (14.3)	36 (85.7)	< 0.001
Prostate cancer	29 (58)	21 (42)	

Table 2. The relationship between Nrf2 expression, clinical and pathological characteristics, and prostate cancer stage

Group		n	Nrf2 expression		P
			Positive	Negative	
Age (years)	< 60	6	2	4	0.19
	≥ 60	44	27	17	
Gleason grade	< 7	15	2	13	< 0.001
	= 7	24	16	8	
	> 7	11	11	0	
T classification	T1-T2	21	9	12	0.06
	T3-T4	29	20	9	
Lymph node metastasis	Yes	27	24	3	< 0.001
	No	23	5	18	
Distant metastasis	Yes	26	22	4	< 0.001
	No	24	7	17	

Immunohistochemistry and hematoxylin and eosin (HE) staining

The resected specimens were fixed in 4% neutral formalin for two days, embedded in paraffin, cut into 6-μm paraffin sections, deparaffinized with xylene, and rehydrated with a graded ethanol series. The slides were placed in antigen retrieval solution and then blocked with rabbit immune serum. Next, an antibody against Nrf2 (1:50, Abcam plc, USA) was added, and the slides were placed in an incubator at 37°C for two hours. After thorough washing in phosphate buffer solution (PBS), the slides were incubated with the secondary antibody for 1 hour. After washing with PBS, horseradish peroxidase-labeled streptavidin was added for 15 minutes, and then, the slides were washed with PBS, stained with diaminobenzidine (DAB), restained with hematoxylin, and observed by microscopy. PBS was used in place of the primary antibody for the negative control samples. Routine HE staining was performed according to standard procedures.

The immunochemical staining results were interpreted as follows: two experienced pathologists independently interpreted the results in a blinded manner. They each counted 15 fields-of-view per slide at high magnification (×400). The positive cell rate was calculated as the percentage of cells with Nrf2 expression per every 100 cells. The scoring of positive immunohistochemical results (brown particles in the cytoplasm) was based on previous studies and was as follows: 0 points for negative staining intensity; 1 point for pale yellow; 2 points for yellow; and 3 points for brown. Final results were interpreted as negative between 0-2 points and positive between 3-7 points. The percentage of positive cells was then classified as negative expression (0-5%), weakly positive (6-25%), moderately positive (26-75%), or strongly positive (> 76%). Weakly positive, moderately positive and strongly positive results were scored as positive results [3].

Statistical analysis

SPSS 13.0 software was used for statistical analyses. A χ^2 test was performed to analyze count data, and the Kaplan-Meier method was used for survival analysis. $P < 0.05$ was considered statistically significant.

Results

HE staining and Nrf2 protein expression in prostate cancer and benign prostatic hyperplasia tissues

In this experiment, all specimens were confirmed by pathological diagnosis, and typical examples of benign prostatic hyperplasia and prostate cancer samples are shown in **Figure 1**. The expression levels of Nrf2 were low in the benign prostatic hyperplasia samples, whereas staining was often observed in the cytoplasm and nuclei of prostate cancer tissues (**Figure 2**). Statistical analysis revealed that the positive Nrf2 expression rate in the nuclei of prostate cancer tissues was 58%, compared with only 14.3% in benign prostatic hyperplasia tissues, which was significantly different (**Table 1**).

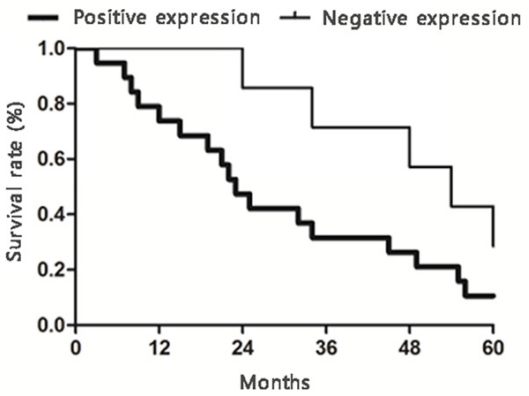

Figure 3. The relationship between Nrf2 expression and patient five-year survival rate.

Relationship between Nrf2 expression, clinical and pathological characteristics, and prostate cancer stage

Once we had confirmed that Nrf2 expression was higher in the prostate cancer samples, we then examined how this correlated with certain clinical and pathological characteristics in the patients. We found that positive Nrf2 expression was unrelated to patient age and T classification ($P > 0.05$). By contrast, Nrf2 expression was positively correlated with Gleason grade, lymph node metastasis and distant metastasis ($P < 0.05$), as the number of patients with positive Nrf2 expression increased with Gleason grade, lymph node metastasis and distant metastasis (**Table 2**).

Relationship between Nrf2 expression and clinical prognosis

The five-year survival rate was significantly higher in patients with negative Nrf2 expression than in patients with high Nrf2 expression ($P < 0.05$, **Figure 3**).

Discussion

Nrf2 (66 kDa) was originally discovered by Mori et al. in 1994, and its function and activity are mainly regulated by the cytosolic protein Kelch-like ECH-associated protein 1 (Keap1). Under basal conditions, Nrf2 is mainly present in the cytoplasm, where it binds to the cytoskeleton-associated Keap1 [4]. Oxidative stress causes the uncoupling of Nrf2 and Keap1, and unbound Nrf2 protein is then transferred from the cytoplasm to the nucleus, where it forms heterodimers with macrophage activating factor (Maf), which can then bind to antioxidant response elements (AREs) to initiate the transcription and expression of specific target genes [5]. Previous studies have shown that Keap1 mutations in tumor cells can result in the loss of Nrf2 inhibition. For example, compared with wild-type mice, lung cancer cells grown in Nrf2-knockout nude mice were more likely to metastasize, whereas the incidence of lung cancer metastasis was significantly reduced in mice lacking Keap1, suggesting that Keap1 plays a key regulatory role in the transfer and accumulation of Nrf2 in the nucleus [6]. Furthermore, a growing number of detailed studies have demonstrated that Nrf2 plays an important role in tumorigenesis and invasion. Compared with normal epithelial cells, Nrf2 is over-expressed in 91.5% of head and neck squamous cell carcinoma cases [7], and positive Nrf2 expression was observed in 26-30% of non-small cell lung cancer, breast cancer and prostate cancer samples [8], similar to what was observed for prostate cancer, suggesting that the excessive accumulation of Nrf2 in the nucleus may play an important role in the development, progression and proliferation of prostate cancer.

Previous studies have shown that Nrf2 plays a dual role in the development and progression of cancer. During early stages, Nrf2 activates the ARE signaling pathway and regulates the expression of downstream genes, thereby blocking the carcinogenic effects of harmful factors in the cell. Loss of Nrf2 function promotes the development of cancer, and Nrf2-knockout mice are more likely to develop cancer than wild-type mice [9]. However, once tumors have developed, the activated Nrf2 signaling pathway protects tumor cells from the effects of radiotherapy and chemotherapy, mainly by enhancing the ability of tumor cells to resist oxidative stress [9]. Therefore, many researchers currently believe that a lack of Nrf2 expression induces cancer, whereas Nrf2 overexpression greatly reduces the sensitivity of tumor cells to radiotherapy and chemotherapy [10], and as such, the inhibition of Nrf2 expression should have important implications for the treatment of prostate cancer. In other words, Nrf2 expression is closely related to the development and progression of prostate cancer, and the down-regulation of Nrf2 expression in the nucleus may significantly inhibit the growth and proliferation of prostate cancer cells while improving the sensitivity of these cells to chemotherapeutic drugs [11-13]. We found that Nrf2 accumulation was closely related to clinical stage, lymph

node metastasis and bone metastasis in prostate cancer patients, suggesting that Nrf2 plays an important role in the proliferation and metastasis of prostate cancer. Therefore, early detection of Nrf2 expression may provide a valuable reference for assessing prostate cancer prognosis. In addition, recent studies have confirmed that high Nrf2 expression in non-small cell lung cancer and pancreatic cancer tissues was correlated with markedly poorer prognoses [14, 15], whereas low expression was correlated with better prognoses. This study also showed that the five-year survival rate was significantly lower in patients with positive Nrf2 expression than in those with negative Nrf2 expression, which likely occurred because Nrf2 enhances the ability of tumor cells to resist oxidative stress, thereby reducing the necrosis and apoptosis of tumor cells.

In summary, the expression level of Nrf2 was significantly higher in prostate cancer tissues than in benign prostatic hyperplasia tissues, and Nrf2 expression was closely correlated with prostate cancer metastasis and progression. In addition, Nrf2 expression was closely correlated with prostate cancer prognosis. These results suggest that treatments targeting Nrf2 may be useful for the clinical treatment of prostate cancer.

Disclosure of conflict of interest

None.

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