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Original Article

Up-regulation of *SULF2* is associated with a poor prognosis of patients with prostate cancer

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Abstract: Background: The heparan sulfate 6-O-endosulfatase 2 (*SULF2*) promotes growth and metastasis of solid tumors and is linked with the prognosis of various tumors. In the present study, we aimed to detect the expression of *SULFs* and estimate its prognostic relevance in prostate cancer (PCa) patients. Methods: The relative expression of *SULF2* in 128 PCa tissues and 53 healthy tissues at mRNA and protein level were evaluated by qRT-PCR and western blot, respectively. The association between *SULF2* expression and clinical factors as well as overall survival was severally assessed using chi-square test and Kaplan-Meier analysis. Meanwhile, cox regression analysis was applied to evaluate the independent prognostic significance of *SULF2*. Results: The relative expression levels of *SULF2* was significantly higher in tumor tissues compared with healthy tissues both at mRNA and protein level ($P<0.05$). And the high expression of *SULF2* was correlated with PSA, metastasis, and tumor stage. Kaplan-Meier analysis indicated that patients with high *SULF2* expression had a poor overall survival than those with low expression (log rank test, $P=0.000$). Moreover, multivariate analysis showed that increased expression of *SULF2* was an independent predictor of the prognosis of PCa. Conclusion: Our data indicated that *SULF2* was up-regulated and might be a novel prognostic indicator in PCa.

Keywords: *SULF2*, prostate cancer, prognosis

Introduction

Prostate cancer (PCa), is the most common non-skin neoplasm and the third leading cause of cancer-related death after lung and colorectal cancers among men in Western countries which is recognized to be a major public health problem [1, 2]. Recently, the incidence and mortality rates of PCa have obviously increased in Asia [3-5]. Despite the fact that multiple therapeutic choices, such as surgery, hormonal therapy, and radiotherapy, are available for the treatment of PCa patients, there are still lack efficient strategies for the treatment of castrate-resistant and metastatic PCa which lead to a poor prognosis [6]. Therefore, it is imperative to identify novel and efficient prognostic markers for human PCa.

Heparan sulfate 6-O-endosulfatase 2 (*SULF2*) is a member of the sulfatase family and located on chromosome 20q13 [7, 8]. Multiple studies have demonstrated that the increased expression of *SULF2* was found in various cancers such as breast cancer, brain cancer, gastric cancer, esophageal cancer, and hepatocellular carcinoma [9-14]. In addition, it was reported to be over-expression in PCa and related to the tumorigenicity of cancer cells in previous study [15]. However, the prognostic value of *SULF2* still remains unknown.

In the current study, we detected the *SULF2* expression in PCa and investigated its relationship with clinicopathological features of patients with PCa. What's more, the prognostic value of *SULF2* was explored.

Methods and materials

Patients and tissue samples

The study was approved by the Research Ethics Committee of Affiliated Hospital of Shandong

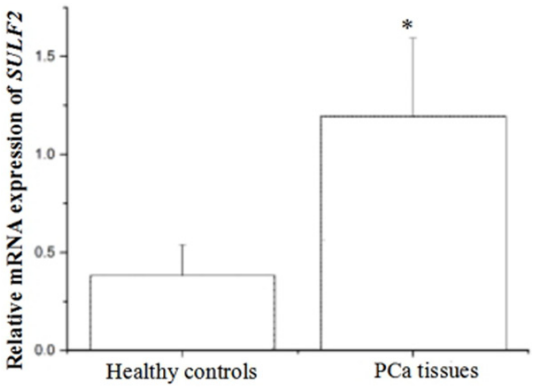

Figure 1. The relative mRNA expression of *SULF2* in PCa tissues and normal tissues. The *SULF2* mRNA expression was significantly higher in PCa tissues compared with that in healthy tissues ($P<0.05$).

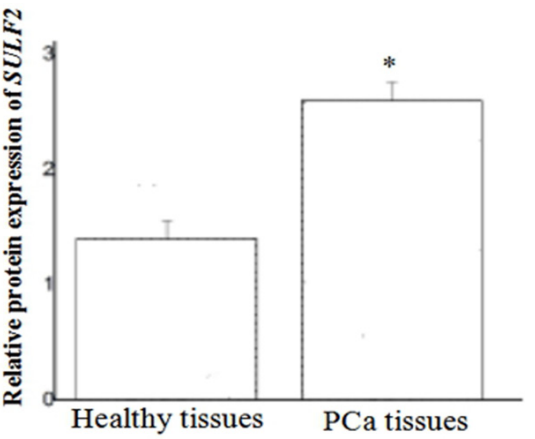

Figure 2. The relative protein expression of SULF2 in PCa tissues and normal tissues. It was increased in PCa tissues compared to that in healthy controls ($P<0.05$).

Academy of Medical Sciences. Patients who were diagnosed with PCa were obtained from Affiliated Hospital of Shandong Academy of Medical Sciences and none of them had received any radiotherapy or chemotherapy before sampling. Besides, 53 healthy volunteers were taken as healthy controls. Informed consent was obtained from each participant in advance.

Tumor tissues and healthy tissues were collected from patients with PCa and healthy controls. Then the tissues samples were frozen by liquid nitrogen immediately and stored at -80°C for use. All specimens were handled and made anonymous according to the ethical and legal standards. Clinicopathologic characteristics including age, Gleason score, PSA, metastasis, and tumor stage were recorded in a database. A 5 years' follow-up was conducted and patients who were died from unexpected events or other diseases were excluded from our study.

RNA isolation qRT-PCR analysis

The isolation of total RNA from frozen tissues samples was carried out using RecoverAlleTM Total Nucleic Acid Isolation Kit (Ambion, Life Technologies, CA, and USA) following the providers' specifications. Total RNA (100 ng) with a 260/280 nm absorbance ratio of 1.5-2 was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies, CA, USA) according to the manufacturer's indications. The RT-PCR reaction was performed in ABI 7500-fast thermocycler and *GAPDH* was used as internal controls. Each sample was in triplicate. The relative mRNA expression of *SULF2* was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Total protein was isolated from all tissues samples and separated on 12.5% SDS-PAGE gels, respectively. Then the brands were transferred onto PVDF membranes (Millipore). The membrane was blocked in TBS-T buffer contain 5% skim milk at room temperature for 2 h. The primary antibody anti-*SULF2* antibody (1:2,500) was added and incubated with membranes for overnight at 4°C. After washing with TBS-T, the membrane was incubated with TBS-antibody (1:5,000) for 60 min at room temperature. Proteins of interest were detected and visualized by autoradiography after various exposure times. The β-actin was used as an internal loading control.

Statistical analyses

All statistical analyses were performed using Origin Pro 9.0 software (Microcal, USA). Data were presented as mean ± SD. The differences between two groups were analyzed by students' t test. Chi-square test was used to analyze the association between *SULF2* expression and clinicopathologic features of patients with PCa. Kaplan-Meier methods with the log-rank test were used to estimate differences in overall survival among PCa patients. Multivariable analysis adjusted for clinical factors

Table 1. Relationship between *SULF2* expression and clinicopathologic parameters of patients with PCa

Characteristics	Cases (n=128)	*SULF2* expression level		*P* values
		Low (n=53)	High (n=75)	
Age (years)				0.816
≤66	86	35	51	
>66	42	18	24	
Gleason score				0.266
>8	53	25	28	
≤8	75	28	47	
PSA				0.015
Negative	68	34	34	
Positive	60	19	41	
Metastasis				0.016
No	81	40	41	
Yes	47	13	34	
Tumor stage				0.006
T1/2	92	45	47	
T3/4	36	8	28	

PSA: prostate-specific antigen.

was used to evaluate the prognostic value of *SULF2* using cox regression analysis. The difference was considered to be significant when the *P* value was less than 0.05.

Results

SULF2 expression in patients with PCa was up-regulated

To detect the expression of *SULF2*, qRT-PCR and western blot analyses were performed in 128 fresh human PCa tissues and 53 normal tissues. As shown in **Figure 1**, the relative mRNA expression of *SULF2* was significantly higher in PCa tissues than that in healthy controls ($P<0.05$). Meanwhile, western blot analysis revealed that relative *SULF2* protein expression was increased in PCa tissues compared with healthy tissues ($P<0.05$; **Figure 2**). These findings suggested that *SULF2* was up-regulated in PCa specimens, and might be an important carcinogenic factor in PCa.

Associations of SULF2 expression with clinicopathologic features in PCa patients

To assess the correlation of *SULF2* expression with clinicopathologic data, the patients with PCa were categorized as low or high group in relation to the mean value. Chi-square test demonstrated that the high *SULF2* expression has a tight correlation with PSA ($P=0.015$), metastasis ($P=0.016$) and tumor stage ($P=0.006$). However, there was no significant correlation between *SULF2* expression and other clinicopathologic features, such as patients' age and Gleason score ($P>0.05$, **Table 1**).

Correlation between SULF2 expression and overall survival of patients with PCa

To analyze the clinical significance of *SULF2*, we carried out a 5 years' follow-up. Based on the data of the follow-up, we analyzed the overall survival of patients with PCa by the Kaplan-Meier analysis and log-rank test. It showed that patients with high *SULF2* expression had a significantly shorter overall survival than those with low expression (log rank test, $P<0.001$, **Figure 3**). Multivariate analysis using the cox regression analysis adjusted for the clinical factors revealed that *SULF2* was an independent prognostic factor for overall survival of patients with PCa ($P=0.000$; HR=3.745, 95 % CI=1.739-8.066, **Table 2**).

Discussion

PCa is a slow-growing malignant tumor that often occurred in males [16]. The mortality accounts for about 10% of all cancer deaths [17]. Age, ethnic origin, lifestyle, environmental factors and genetic variants are major risk factors for PCa [18]. Among them, genetic variation partly contributes to the development, prognosis and progression of PCa [19]. Hence, it is of great clinical significance to identify more molecular markers for the detection, diagnosis and prognosis of PCa.

SULF2 has been discovered in mammals and its absence can results in a partially penetrant phenotypes of reduced embryonic viability, reduced postnatal weight, and adult lung abnormalities [20, 21]. The function of *SULF2* in cancer is controversial, and the enzymes are reported both as anti and as pro-tumorigenic [22]. It also confirmed to play important roles in several cancers. For instance, *SULF2* is up-regulated in NSCLC and other cancers and implicated as a driver of carcinogenesis in NSCLC, pancreatic cancer, and hepatocellular carcino-

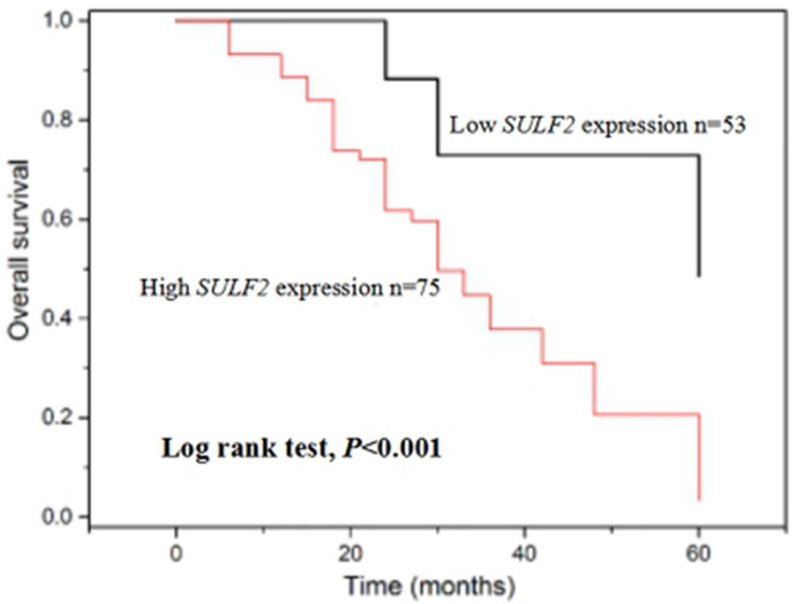

Figure 3. Kaplan-Meier analysis according to the expression of *SULF2*. Patients with high *SULF2* expression had a significantly poorer prognosis than those with low *SULF2* expression (log rank test, *P*=0.000).

Table 2. Multivariate analysis adjusted for clinicopathological factors for estimating the prognostic value of *SULF2* in patients with PCa

Features	HR	95% CI	*P* values
SULF2 expression (high vs low)	3.745	1.739-8.066	0.000

ma [22]. Keun *et al.* reported that *SULF2* was higher expressed in gastric cancer cell lines and tissues compared to normal mucosa as well as correlated with its promoter hypomethylation [23]. Lemjabbar *et al.* confirmed widespread *Sulf-2* protein expression in tumor cells of human lung squamous carcinomas [24]. Carolina *et al.* found that *SULF2* have a protumorigenic effect in prostatic cancer cell lines by transfection [15]. Ciampa *et al.*, have reported that *SULF2* chromosome locus is correlated with prostate cancer susceptibility regions [25]. However, its precise role in cancer pathogenesis and the molecular mechanisms underlying their regulation remain unclear. Moreover, the associations between *SULF2* expression and prognosis in PCa have not been reported yet.

In the present study, we detected the expression of *SULF2* in PCa. Our results showed that *SULF2* can function as a tumor oncogene as its high expression in PCa patients. This was coincident with the status of *SULF2* in other cancers. To further explore the possible roles of *SULF2* in PCa, we investigated the relationships between *SULF2* expression and different clinicopathlogical features. It revealed that the high *SULF2* expression was closely correlated with PSA, metastasis and tumor stage which suggested that *SULF2* may play crucial roles in the development and progression of PCa.

Recently, several studies have demonstrated that *SULF2* over-expression seems to be unfavorable to prognosis of the patients with different tumors. Shen *et al.* revealed that *SULF2* methylation may be a novel prognostic biomarker for gastric cancer patients treated with platinum-based chemotherapy [15]. Mathewos *et al.* reported that cytosine methylation of the *SULF2* promoter was associated with better survival of resected lung adenocarcinoma patients [26]. Caroline *et al.* demonstrated that *SULF2* gene expression was higher in skin cancer, colorectal carcinoma, testicular teratoma and liver cancer compared to their normal tissue counterpart. What's more, *SULF2* expression in primary multiple myeloma cells was associated with a poor prognosis in two independent large cohorts of patients [27]. To seek new molecular target for therapy of the PCa patients, we further investigated the correlation between *SULF2* and prognosis of the PCa patients. Patients with high *SULF2* expression showed significantly worse overall survival than those with low *SULF2* expression according to Kaplan-Meier analysis. Multivariate analysis demonstrated that the high *SULF2* expression was an independent prognostic factor for PCa patients, implying that *SULF2* may be a novel prognosis predictor and therapeutic target of the patients with PCa. These findings revealed that the aberrant expression of *SULF2* may play important roles in the tumorigenesis and aggressiveness of PCa patients for the first time.

In summary, we demonstrate for the first time that *SULF2* is up-regulated in PCa tissues. Furthermore, *SULF2* is identified as an independent marker for predicting the clinical outcome of PCa patients. These results suggest that *SULF2* may be a promising biomarker and a therapeutic target for PCa in future. However, further studies will be needed to identify mechanisms underlying its gene expression and oncogene.

Disclosure of conflict of interest

None.

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