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The Clinical Relevance of Fragile Histidine Triad Protein (FHIT) in Patients with Bladder Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: The present study aimed to investigate the clinical relevance of fragile histidine triad protein (FHIT) in patients with bladder cancer (BC).

Material/Methods: Three independent BC microarray studies were collected and reanalyzed. The expression of FHIT was evaluated between BC samples and normal bladder tissues. The correlation between the expression of FHIT and clinicopathological features was analyzed using the chi-square test. Log-rank based survival analysis was conducted to detect the survival significance of FHIT in patients with BC. Gene set enrichment analysis (GSEA) was performed to identify the mechanisms.

Results: FHIT was significantly downregulated in BC cells (p=0.0044). BC patients in the FHIT high expression group had better clinical characteristics (including invasiveness, tumor grade, disease progression, and T staging) than those in the FHIT low expression group (p<0.0001, p<0.0001, p=0.031, p<0.0001, and p=0.056, respectively). Patients in the FHIT high expression group had better cancer-specific survival (p<0.0001) and overall survival (p=0.0008) than those in the FHIT low expression. GSEA results indicated that BC samples in the FHIT low expression group were enriched in interferon alpha response, apoptosis, androgen response, interferon gamma response, heme metabolism, and transforming growth factor (TGF) beta signaling.

Conclusions: FHIT predicts better clinical relevance for patients with BC, which may be a promising therapeutic target.

MeSH Keywords: Gene Expression • Prognosis • Urinary Bladder Calculi

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Background

Bladder cancer (BC), the second most common urologic malignancy, has been the fourth and the eighth most commonly diagnosed cancer in men and in women, respectively [1,2]. With the aging of the world population, the incidence of BC is rising, and this disease will be a public health challenge in the future [3]. Managements for patients with invasive BC and non-invasive BC are different. For patients with non-invasive BC, transurethral resection of the bladder tumor (TURBT) is recommended as the standard surgical option, and the five-year overall survival for these patients reaches 90%, while about 40% to 80% of these patients will develop disease relapse or progression [4]. For patients with invasive BC, neoadjuvant cisplatin-based combination chemotherapy has become a standard of care; once it becomes metastatic cancer, the five-year overall survival for patients with invasive BC is a dismal 6% [5]. Molecular diagnosis is now a part of clinical management for many types of cancers; however, for BC there has been no significant progress with the widespread use of molecular diagnosis in clinical practice [6]. Previous studies have demonstrated that fragile histidine triad protein (FHIT) is involved in the tumorigenesis of a variety of human malignancies, including lung carcinoma, breast cancer, pancreatic cancer, renal carcinoma, etc. [7,8]. Nevertheless, its prognostic value in patients with BC remains to be elucidated.

In the present study, we attempted to make clear the relationship between the expression of FHIT and the clinicopathological features of patients with BC by reanalyzing three independent BC microarray studies.

Material and Methods

Data source

BC gene expression profiles GSE3167 [9], GSE13507 [10,11], and GSE31189 [12] were obtained from Gene Expression Omnibus (GEO). Total RNA was extracted from normal bladder tissues or BC cells of these three studies. The gene expression data of GSE3167, GSE13507, and GSE31189 were normalized using RMA algorithm, quantile normalization and log2 transformation, and MAS5 algorithm, respectively. GSE3167, annotated with Affymetrix Human Genome U133A Array and comprised of 50 BC samples and nine normal bladder tissues, was applied to evaluate the expression of FHIT between normal bladder tissues and BC cells. GSE13507, annotated with Illumina Human-6 v2.0 Expression BeadChip and comprised of 165 primary bladder cancer samples, 23 recurrent non-muscle invasive tumor tissues, 58 normal looking bladder mucosae surrounding cancer and 10 normal bladder mucosae, was applied to investigate the relationship between the expression of FHIT and clinicopathological features. GSE31189, annotated with Affymetrix Human Genome U133 Plus 2.0 Array, which included 52 BC samples, was applied to perform gene set enrichment analysis (GSEA) [13,14] to identify potential mechanisms regarding the impact of FHIT on the proliferation of BC cells.

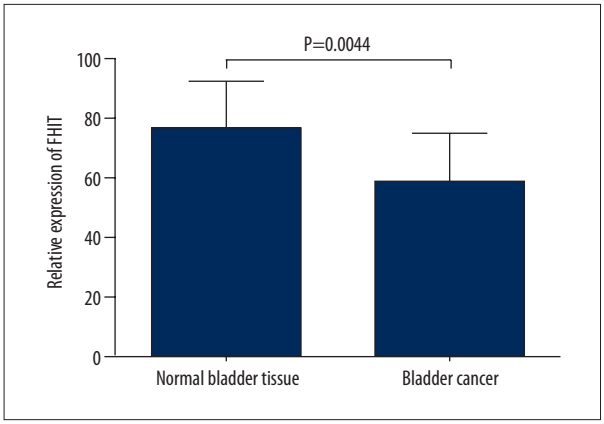

Figure 1. FHIT was downregulated in bladder cancer.

Statistical analysis

The expressions of FHIT (the probe ID is 206492 in GSE3167) in BC samples and normal bladder tissues were reported as mean ± standard deviation (SD). BC samples were classified into a FHIT low expression group and a FHIT high expression group according to the median of FHIT expression in GSE13507 (the corresponding probe ID is ILMN_1766123). Chi-square analysis was conducted to determine the correlation between the expression of FHIT and the clinical characteristics (including age, gender, invasiveness, grade, recurrence, progression, and TNM staging). We performed log-rank based survival analysis to investigate the relationships between the expression of FHIT and the cancer-specific survival and overall survival of BC patients. Hazard ratio and its 95% confidence interval were calculated using the Mantel Haenszel approach. Cancer-specific survival and overall survival were defined as described by Kim et al. [10,11]. A p value less than 0.05 was regarded as statistical significant for the chi-square test and survival analysis. GSEA was performed to investigate the relevant mechanisms involved in the regulation of FHIT on BC cells. In addition, h.all.v5.2.symbols.gmt was used as a reference for GSEA. Differences were considered statistically significant at nominal p value <0.05 and false discovery rate <25%.

Results

FHIT was downregulated in BC cells

As shown in Figure 1, the expression of FHIT in normal bladder tissues was significantly increased compared to BC cells (76.78±16.029 versus 58.66±16.541, p=0.0044).

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Table 1. The clinical characteristics of bladder patients in FHIT low expression and FHIT high expression group.

Characteristics	FHIT expression		Chi-square	P value
	Low expression (n=83)	High expression (n=82)		
Age (year)				
<60	18 (21.7)	24 (29.3)	1.25	0.264
≥60	65 (78.3)	58 (70.7)		
Gender No. (%)				
Male	20 (24.1)	10 (12.2)	3.927	0.048
Female	63 (75.9)	72 (87.8)		
Invasiveness No. (%)				
Non-muscle invasive	35 (42.2)	68 (82.9)	29.213	<0.0001
Muscle invasive	48 (57.8)	14 (17.1)		
Grade No. (%)				
Low	36 (43.4)	69 (84.1)	29.633	<0.0001
High	47 (56.6)	13 (15.9)		
Recurrence No. (%)				
No	23 (27.7)	44 (53.7)	0.01	0.919
Yes	12 (14.5)	24 (29.3)		
Progression No. (%)				
No	62 (74.7)	72 (87.8)	4.644	0.031
Yes	21 (25.3)	10 (12.2)		
T staging No. (%)				
Ta–T1	36 (43.4)	68 (82.9)	27.694	<0.0001
T2–T4	47 (56.6)	14 (17.1)		
N staging No. (%)				
N0	69 (83.1)	80 (97.6)	8.879	0.003
N1–N3	13 (15.7)	2 (2.4)		
M Staging No. (%)				
M0	77 (92.8)	81 (98.8)	3.667	0.056
M1	6 (7.2)	1 (1.2)		

The relationship between FHIT expression and the baseline characteristics of BC patients

Next, we analyzed the distribution of the clinical characteristics (age, gender, invasiveness, grade, recurrence, progression, and TNM staging) of BC patients in the FHIT low expression group (n=83) and the FHIT high expression group (n=82). As shown in Table 1, there was no significant difference between the FHIT low expression group and the FHIT high expression group in terms of age distribution of BC patients. BC patients in the FHIT high expression group were proven to have better clinical characteristics (including invasiveness, tumor grade, disease progression, and T staging) compared to those in the FHIT low expression group (p<0.0001, p<0.0001, p=0.031, p<0.0001, and p=0.056, respectively). Meanwhile, although the differences did not reach statistical significance, more BC patients in the FHIT low expression group developed distant metastases compared with those in the FHIT high expression group (p=0.056).

Survival analysis

To investigate the relationships between cancer-specific survival and overall survival of BC patients in the FHIT low expression group and the FHIT high expression group, we conducted log-rank based survival analysis of BC patients in the two groups. As shown in Figure 2A, the cancer-specific survival favored patients in the FHIT high expression group over

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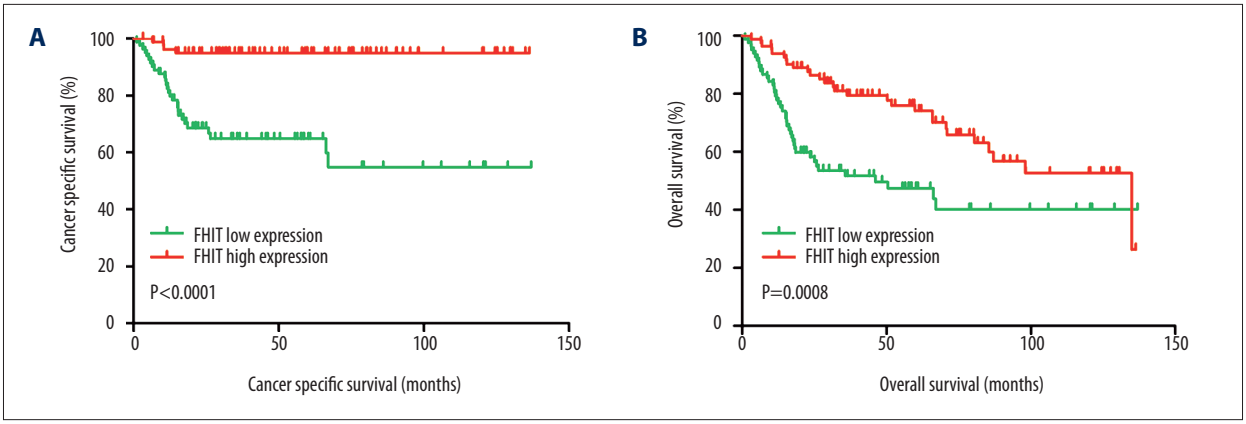

Figure 2. The cancer-specific survival (**A**) and overall survival (**B**) favored patients with higher expression of FHIT over patients with lower expression of FHIT.

Table 2. Gene set enriched in breast cancer samples with FHIT low expression.

Name	ES	NES	NOM p-val	FDR q-val
Interferon alpha response	0.6775	1.690164	0.02008	0.114189
Apoptosis	0.508901	1.636727	0.016293	0.141598
Androgen response	0.503997	1.564777	0.022177	0.17703
Interferon gamma response	0.624001	1.709146	0.02045	0.181087
Heme metabolism	0.404229	1.570244	0.008065	0.20992
TGF beta signaling	0.522607	1.509911	0.039761	0.239196

ES – enrichment score; NES – normalized enrichment score; NOM p-val – nominal p value; FDR q-val – false discovery rate.

patients in the FHIT low expression group (HR=6.018, 95% CI: 2.979–12.16, p<0.0001). Meanwhile, the overall survival favored patients in the FHIT high expression group over patients in the FHIT low expression group (HR=2.299, 95% CI: 1.412–3.743, p=0.0008, Figure 2B).

GSEA

To characterize the potentially relevant mechanisms that FHIT might inhibit the proliferation of BC cells, BC samples in GSE31189 were classified into a FHIT (the corresponding probe ID was 206492_at) high expression group and a FHIT low expression group according to the median of FHIT expression, and then GESA was conducted based on the group of FHIT expression. As shown in Table 2, BC samples in the FHIT low expression group were enriched in interferon alpha response, apoptosis, androgen response, interferon gamma response, heme metabolism, and transforming growth factor (TGF) beta signaling. These results suggested that FHIT might impact the proliferation of BC cells through the aforementioned biological processes.

Discussion

BC is one of the most common malignancies worldwide. Although the risks for metastasis and death of patients with non-invasive BC remain relatively low, 40% to 80% of non-invasive BC cases can develop into invasive BC [1–3]. BC tumors that progress to invasive disease have a dismal 6% five-year overall survival rate [3].

During the past decades, significant advances have been achieved in the diagnosis and therapeutics of BC patients. Voided urine cytology, detection of urine nuclear matrix proteins (NMPs), and the UroVysion multicolor FISH test have been widely used in the screening or diagnosis of BC [15]. Meanwhile, several targeted therapies have been introduced in BC management including anti PD-L1 monoclonal antibodies (atezolizumab, durvalumab, avelumab), anti-PD-1 monoclonal antibodies (pembrolizumab, nivolumab), CTLA4 receptor inhibitor (ipilimumab), which have significantly improved the response rates of BC patients [16]. Thus, identification of novel molecules that were associated with clinical outcomes of patients with BC is meaningful [17].

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In the present study, we re-analyzed three independent BC microarray studies, and found that FHIT was downregulated in BC cells. Moreover, patients in the FHIT high expression group displayed better clinicopathological features and survival rates; thus, FHIT might inhibit the proliferation of BC cells through several oncogenesis-associated biological processes.

FHIT, located at the FRA3B site of chromosome 3p14.2, is one of the histidine triad gene family members, which has been reported to be correlated with multiple human cancers [6,7]. The loss of FHIT may alter multiple biological functions in human malignancies including decreased apoptosis, increased epithelial-mesenchymal transition (EMT), increased resistance to genotoxic agents, altered production of reactive oxygen species, and ongoing genome instability [18,19]. Malak et al. demonstrated that FHIT was downregulated in acute lymphoblastic leukemia and could be used to monitor the minimal residual disease [20]. Su et al. indicated that the frequency of FHIT hypermethylation was significantly increased in breast cancer compared with benign breast disease [21]. Wu et al. proved that FHIT loss conferred cisplatin resistance in lung cancer via the AKT/NF-κB/Slug-mediated PUMA reduction [22]. Kapitanović et al. supported that reduced FHIT expression was associated with tumor progression in sporadic colon adenocarcinoma [23]. A literature review suggested that FHIT acted as a tumor suppressor in variety of human malignancies, which was similar to our result that FHIT was downregulated in BC cells. Thus, FHIT might participate in the oncogenesis of BC.

Cystoscopy is regarded as the gold standard for the initial diagnosis and staging of BC. Histological tumor grade of BC is based on the degree of aggressiveness of tumor cells. Low-grade BC is made up of less aggressive cells, which grow slower, look quite normal, and act similar to normal cells. High-grade BC is characterized by fast-growing cells, which look and act in a disordered way, and are more likely to progress into the muscle layer of the bladder [21]. In our study, we observed that more BC patients in the FHIT high expression group were diagnosed with low-grade BC compared with those in the FHIT low expression group, indicating that FHIT might inhibit the progression of BC cells.

T staging refers to the degree to which the cancer has grown in the adjacent layers of tissue, from the connective tissue just beneath the urothelium to the tissue structures outside the bladder. The invasiveness of BC is determined according to T stages of BC; tumors with stages Ta–T1 are categorized as non-invasive BC, and tumors with stages ≥T2 are categorized as invasive BC. Ta–T1 tumors may progress to invasive BC. N staging and M staging represent the lymph node and distant metastasis, respectively [24,25]. Our results suggested that more BC patients in the FHIT high expression group underwent non-muscle invasive, Ta–T1, N0, and M0 BC compared to those in the FHIT low expression group, suggesting that FHIT might be associated with the tumor staging of BC.

The results of survival analysis showed that patients in the FHIT high expression group had better cancer-specific survival and overall survival, indicating that FHIT predicted better survival of patients with BC.

Conclusions

Our results demonstrated that FHIT predicted better clinical prognosis in BC patients, which might be a promising therapeutic target for BC patients.

Conflicts of interest

None.

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