DHCR24 predicts poor clinicopathological features of patients with bladder cancer
A STROBE-compliant study

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Abstract

To investigate the prognostic value of DHCR24 for patients with bladder cancer (BC). We used public bladder cancer microarray studies to evaluate the expression of DHCR24 between normal bladder tissues and BC cells, to investigate the relationship between the expression of DHCR24 and the clinical features of BC patients. Survival analysis was performed to investigate the correlation between DHCR24 expression and the survivals of BC patients. Gene set enrichment analysis was conducted to identify relevant mechanisms. The results showed that DHCR24 was up-regulated in BC cells compared with that in normal bladder tissues ($P = .0389$). Results of chi-square test suggested that BC patients in DHCR24 low expression group were proved to have better clinical characteristics (including tumor grade, disease progression, T staging, and N staging) as compared with those in DHCR24 low expression group ($P < .0001$, $P = .002$, $P = .005$, and $P = .002$, respectively). BC patients in DHCR24 low expression group were associated with better cancer-specific survival and overall survival ($P < .0001$ and $P = .0008$, respectively). DHCR24 might promote the proliferation of BC cells through several oncogenesis-associated biological processes (estrogen response, heme metabolism, P53 pathway, cholesterol homeostasis, mTORC1 signaling, peroxisome, xenobiotic metabolism, glycolysis, and protein secretion). Thus, DHCR24 might be a therapeutic target for patients with BC.

Abbreviations: BC = bladder cancer, DHCR24: 24-dehydrocholesterol reductase, GEO = gene expression omnibus, GSEA = gene set enrichment analysis, TURBT = transurethral resection of the bladder tumor.

Keywords: bladder cancer, DHCR24, prognostic relevance

1. Introduction

Bladder cancer (BC) represents the second most common cancer of the urinary system and the 6th most common cancer in men and the 11th most common in women, respectively.[1,2] Urothelial carcinoma represents the major type of BC—non-muscle invasive BC (NMIBC)—which represents approximately 70% of newly diagnosed BCs; 70% of NMBICs are pTa BCs, which is confined to bladder epithelium, 20% of NMIBCs are pT1 BCs which invade lamina propria, and 10% of NMIBCs are carcinoma in situ. For the treatment of NMIBC, transurethral

resection of the bladder tumor (TURBT) is recommended as the standard surgical option, and 45% to 80% of patients with NMIBC will experience disease recurrence and progression. Meanwhile, muscle invasive bladder cancer (MIBC) represents the remaining newly diagnosed BC. For the treatment of MIBC, radical cystectomy has become a standard of care, and once it becomes metastatic cancer, the 5-year overall survival for patients with invasive BC is dismal.[2–6] Thus, the introduction of novel markers that are associated with the clinical characteristics and predict the prognosis of BC might be of great significance.

DHCR24 is involved in multiple cellular functions, such as oxidative stress, cell differentiation, antiapoptotic function, and anti-inflammatory activity.[7,8] In addition, DHCR24 is dysregulated in various tumors including adrenal, prostate, ovarian, and testicular germ cell tumors. In this study, the relationship between the expression of DHCR24 and the clinical characteristics of BC patients was analyzed to investigate the prognostic value of DHCR24.

2. Methods

2.1. BC microarray studies

The study was approved by the local Ethics Committee (Zhongnan Hospital, Wuhan University). BC gene expression study GSE13507[9,10] and E-MTAB-1803[11] were downloaded from GEO (gene expression omnibus) database (https://www.ncbi.nlm.nih.gov/geo/) and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/). GSE13507 is a gene expression profile of 165 primary BC samples, 23 recurrent nonmuscle invasive tumor tissues, 58 normal looking bladder mucosae surrounding cancer,

and 10 normal bladder mucosae, which was annotated with Illumina human-6 v2.0 expression beadchip. The other BC microarray study E-MTAB-1803 was a gene expression profile of 85 primary BCs, which was annotated with Affymetrix Human Genome U133 Plus 2.0 Array. GSE13507 was used to investigate the expression of DHCR24 between normal bladder tissues and BC cells, to characterize the relationship between the expression of DHCR24 and the clinicopathological features of patients with BC, and to evaluate the prognostic relevance of DHCR24 for patients with BC. E-MTAB-1803 (n = 85) was used to perform gene set enrichment analysis (GSEA)[12,13] to identify potentially relevant mechanisms.

2.2. Statistical analysis

Statistical analyses were conducted from October 10 to November 1. Firstly, we evaluated the expression levels of DHCR24 in normal bladder tissues and BC cells. The expression of DHCR24 (the corresponding probe ID is ILMN_1725510 in GSE13507) was presented as mean ± standard deviation (SD), and unpaired t test was conducted to evaluate the difference between normal bladder tissue and BC cells. Secondly, the primary BC samples in GSE13507 (n = 165) were classified into DHCR24 low expression group (n = 83) and DHCR24 high expression group (n = 82) based on the median of DHCR24 expression levels in these primary BC samples, and then chi-square test was conducted to investigate the relationship between the expression of DHCR24 and clinical characteristics (including age, sex, tumor grade, recurrence, progression, and TNM staging) of patients with BC. Meanwhile, we conducted logistical regression analysis to analyze the relationship between the expression of DHCR24 and the above clinical characteristics of BC patients. Finally, log-rank based survival analysis and Cox proportional-hazards regression model were conducted to investigate the prognostic relevance of DHCR24 for BC patients in GSE13507 (BC patients were categorized into DHCR24 low expression group and DHCR24 high expression using the same methods as mentioned above). Cancer-specific survival and overall survival were defined as Kim et al introduced.[9,10] P value less than .05 was regarded as statistical significance for chi-square test and survival analysis. Finally, GSEA was performed to investigate the relevant mechanisms involved in the regulation of DHCR24 on BC cells using E-MTAB-1803. BC samples in E-MTAB-1803 were also divided into DHCR24 low expression group and DGCR24 high expression group based on the median of DHCR24 expression in E-MTAB-1803. The gene set 'h.all. v5.2.symbols.gmt' was used as a reference. Differences were considered statistically significant at nominal P value <.05 and false discovery rate <25%.

3. Results

Our results showed that DHCR24 was increased in BC cells compared with that in normal bladder tissues. BC patients in DHCR24 low expression group were associated with better clinical characteristics (including tumor grade, disease progression, T staging, and N staging) than those in DHCR24 low expression group. BC patients in DHCR24 low expression group were associated with better cancer-specific survival and overall survival. The results of GSEA suggested that DHCR24 might promote the proliferation of BC cells through several oncogenesis-associated biological processes.
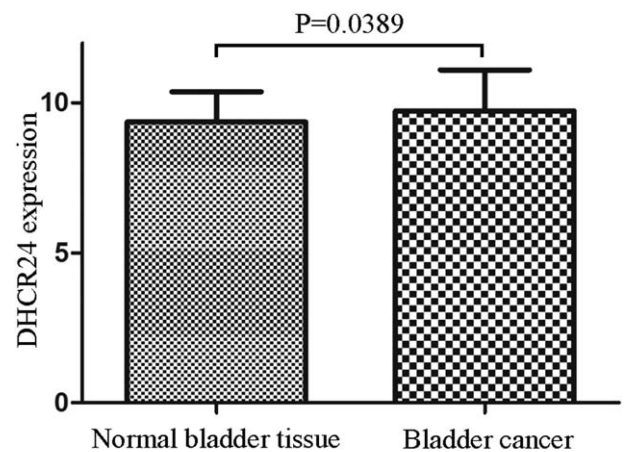
Figure 1. The expression of DHCR24 in normal bladder tissue and bladder cancer.

3.1. DHCR24 was up-regulated in BC cells

GSE13507 was used to evaluate the expression of DHCR24 between normal bladder tissues and BC cells. As shown in Fig. 1, DHCR24 (the corresponding probe ID is ILMN_1725510) was significantly up-regulated in BC cells compared with that in normal bladder tissues (9.364 ± 1.007 vs 9.74 ± 1.367; P = .0389).

3.2. The relationship between the expression of DHCR24 and the clinical characteristics of BC

The primary BC samples in GSE13507 were categorized into DHCR24 high expression group and DHCR24 low expression group according to the median of DHCR24 expression. As shown in Table 1, BC patients in DHCR24 low expression group were associated with better clinical characteristics (including sex, age, tumor grade, disease progression, T staging, and N staging) as compared with those in DHCR24 high expression group (P = .014, P = .009, P < .0001, P = .002, P = .005, and P = .002, respectively). Moreover, the results of logistic regression analysis suggested that the expression of DHCR24 was associated with the sex (P = .026), grade (P = .033), and progression (P = .048) of BC patients.

3.3. BC patients in DHCR24 low expression group were associated with better survivals

Next, we analyzed the prognostic significance of DHCR24 for patients with DHCR24. BC patients were classified into DHCR24 low expression group and DHCR24 high expression group as mentioned above. The mean follow-up period for these BC patients was 48 months (median 37 months; range 1–137 months). As shown in Fig. 2, both the cancer-specific survival and overall survival favored BC patients in DHCR24 low expression group over BC patients in DHCR24 high expression group (for cancer-specific survival: hazard ratio [HR] 6.018, 95% confidence interval [CI] 2.979–12.16, P < .0001; for overall survival: HR 2.299, 95% CI 1.412–3.743, P = .0008). Meanwhile, the results of multivariable Cox proportional-hazards regression analysis suggested that DHCR24 was an independent prognostic factor for patients with BC in terms of overall survival (HR 1.483, 95% CI 1.062–2.071, P = .021).
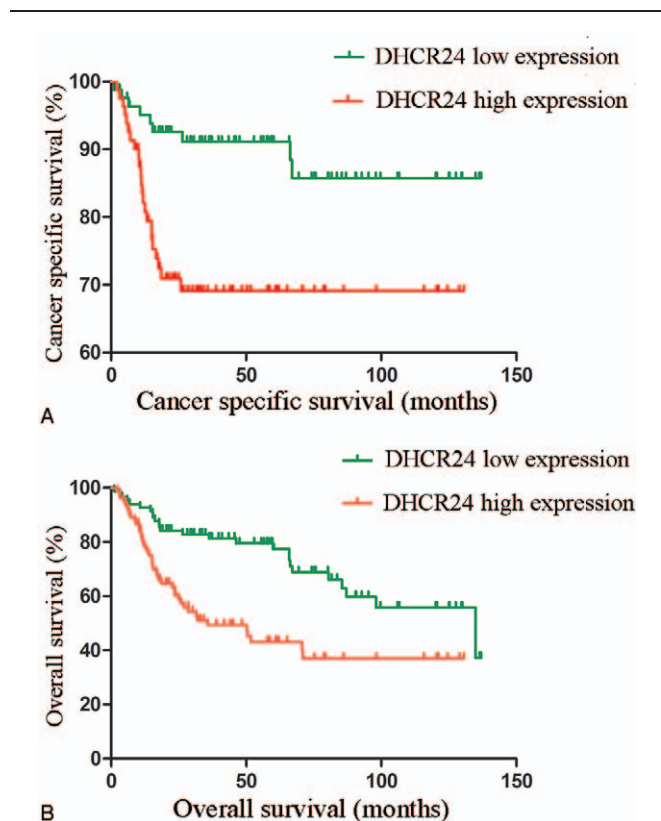
Table 1

The clinical characteristics of patients with bladder cancer in DHCR24 low expression group and DHCR24 high expression group.

Characteristics	DHCR24 expression		Chi-square	P	Logistic regression analysis			
	Low expression	High expression			OR	LCI	UCI	P
Age (y)								
<65	43	26	6.894	.009	1.028	0.997	1.061	.081
≥65	40	56						
Sex, no. (%)								
Male	74	61	6.046	.014	0.339	0.13	0.881	.026
Female	9	21						
Grade, no. (%)								
Low	67	38	21.071	<.0001	2.493	1.076	5.774	.033
High	16	44						
Progression, No. (%)								
No	75	59	9.163	.002	0.374	0.141	0.992	.048
Yes	8	23						
T staging, no. (%)								
Ta-T1	61	43	7.847	.005	1.229	0.819	1.845	.319
T2-T4	22	39						
N staging, no. (%)								
N0	81	68	9.187	.002	2.936	0.812	10.613	.1
N1-N3	2	13						
M staging, no. (%)								
M0	81	77	0.622	.43	0.447	0.042	4.737	.504
M1	2	5						

3.4. Gene set enrichment analysis

We conducted GSEA based on the expression of DHCR24. As shown in Table 2, BC samples in DHCR24 high expression group were mostly enriched gene sets like estrogen response, heme metabolism, P53 pathway, cholesterol homeostasis, mTORC1 signaling, peroxisome, xenobiotic metabolism, glycolysis, and protein secretion, indicating that DHCR24 might promote the proliferation of BC cells through these oncogenesis associated biological processes.

4. Discussion

Bladder cancer is 1 of the most common malignancies worldwide.[1] Although the risks of metastasis and death of patients with noninvasive BC remain relatively low, 40% to 80% of noninvasive BC cases can develop invasive BC.[1–3] Tumors that progress to invasive diseases result in a dismal 6% of 5-year overall survival for patients with invasive BC.[2,5] Currently, there is no highly effective standard management for both invasive and noninvasive BC. Therefore, it is of great significance to identify biomarkers that could predict the clinical prognosis of patients with BC.[14]

Figure 2. The correlation between the expression of DHCR24 and cancer-specific survival (A) and overall survival (OS) of patients with bladder cancer.

Table 2

Gene sets enriched in BC samples with higher expression of DHCR24.

Name	ES	NES	NOM P	FDR q
Estrogen response late	0.622123	1.848372	0	0.011245
Heme metabolism	0.436732	1.724942	0	0.040279
Estrogen response early	0.556242	1.68948	0	0.051944
P53 pathway	0.57994	1.673322	.002008	0.051986
Cholesterol homeostasis	0.636135	1.888946	.004	0.016102
mTORC1 signaling	0.59105	1.742587	.004082	0.039569
Peroxisome	0.471567	1.606333	.011742	0.07405
Xenobiotic metabolism	0.459513	1.453768	.021073	0.231088
Glycolysis	0.473183	1.532974	.022222	0.138518
Reactive oxygen species pathway	0.558445	1.615541	.027197	0.075933
Protein secretion	0.426355	1.641782	.032389	0.066385

BC = bladder cancer, ES = enrichment scores, FDR = false discovery rate NES = normalized enrichment score, NOM P = nominal P.

In the present study, 3 independent BC microarray studies were analyzed, demonstrating that DHCR24 was increased in BC cells. Moreover, patients with lower expression of DHCR24 displayed better clinicopathological features, and cancer-specific survival and overall survival, and DHCR24 might promote the proliferation of BC cells through several oncogenesis-associated biological processes (estrogen response, heme metabolism, P53 pathway, cholesterol homeostasis, mTORC1 signaling, peroxisome, xenobiotic metabolism, glycolysis, and protein secretion).

DHCR24, also known as 24-dehydrocholesterol reductase, locates on 1p32.3 and comprises 8 introns and 9 exons.[8] Seladin-1, encoded by DHCR24, participates in the late steps of cholesterol biosynthesis l.[15] Moreover, several studies demonstrated that DHCR24 was involved in the tumor progression of multiple human malignancies. Dai et al demonstrated that DHCR24 was significantly increased in endometrial cancer cells, and that the DHCR24 elevation was involved in advanced clinical stage, histological grading, vascular invasion, lymphatic metastasis, and reduced overall survival of patients with endometrial cancer. Simi et al[16] demonstrated that the promoter methylation regulated the expression of DHCR24 in adrenal cancer. Battista et al[17] demonstrated that DHCR24 was downregulated in prostate cancer, and the up-regulation of DHCR24 could inhibit the proliferation of prostate cancer cells. Meanwhile, Cecchi et al[18] suggested that up-regulation of DHCR24 enhanced the resistance to Abeta toxicity of neuroblastoma cells.[18] Di Stasi et al[19] demonstrated that DHCR24 was increased in metastatic melanoma. Our results indicated that DHCR24 was up-regulated in BC cells (both MIBC and NMIBC cells), which was similar with the conclusions of literature review that DHCR24 might be associated with the carcinogenesis of BC.

Actually, Lee et al[20] suggested that DHCR24 was associated with tumor progression of patients with NMIBC by detecting mRNA expression of DGCR24 using RT-PCR and performing immunohistochemical (IHC). In their study, a total of 162 NMIBC patients were included, and the median follow-up period of these NMIBC patients was 60.0 months (range 12.1–219.9 months). During the period of follow-up, 59 (36.4%) NMIBC patients developed disease recurrence and 19 (11.7%) developed disease progression. Our results extended their conclusions to both NMIBC and MIBC patients, and proved that BC patients in DHCR24 low expression group were proved to have better clinical characteristics (including tumor grade, disease progression, T staging, and N staging) as compared with those in DHCR24 high expression group, indicating that DHCR24 was associated the progression of patients with NMIBC and MIBC. Moreover, the survival analysis of DHCR24 proved that both cancer-specific survival and overall survival favored BC patients in DHCR24 low expression group over BC patients in DHCR24 high expression group, indicating that DHCR24 was associated with poor survival of patients with BC.

The results of GSEA suggested that DHCR24 promoted the proliferation of BC cells through biological processes that had been reported to be involved in carcinogenesis, such as estrogen response,[21] heme metabolism,[22] P53 pathway,[23,24] cholesterol homeostasis,[25] mTORC1 signaling,[26] peroxisome,[27] xenobiotic metabolism,[28] glycolysis,[29] and protein secretion.

Actually, our study has limitations. Firstly, this is a retrospective study and the conclusions are not as robust as prospective studies. Second, the size of patients included in our study is not large (n = 256 in GSE13507 and n = 85 in E-MTAB-1803), which might not exclude selection bias. Thus, the conclusions of our study should be interpreted with caution and large-scale prospective studies are required to characterize the prognostic role of DHCR24 in bladder cancer.

5. Conclusions

Taken together, this study demonstrated that DHCR24 was up-regulated in BC cells, and up-regulation of DHCR24 was associated with worse clinical features for patients with BC, and BC patients in DHCR24 low expression group was proved to live longer than those in DHCR24 high expression group. DHCR24 affected the proliferation of BC cells through several biological processes involved in carcinogenesis.

Author contributions

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