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Urologic Oncology: Seminars and Original Investigations 37 (2019) 299.e7–299.e18

UROLOGIC ONCOLOGY

Original article

High expression of Chitinase 3-like-1 is an unfavorable prognostic factor in urothelial carcinoma of upper urinary tract and urinary bladder

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Received 28 November 2018; received in revised form 27 December 2018; accepted 1 January 2019

Abstract

Background: Metabolic adaptation in cancer cells is important for cancer cell survival. Alternation in cellular metabolism getting more energy to support cell proliferation played a critical role in disease progression. We initially analyzed the public transcriptome of urothelial carcinoma in Gene Expression Omnibus database (GSE31684) with particular focus on genes associated with carbohydrate metabolism, and found that *Chitinase 3-like-1* (*CHI3L1*) was a significantly up-regulated gene associated with advanced disease status. This study was aimed to evaluate the expression and prognostic significance of CHI3L1 in upper urinary tract urothelial carcinoma (UTUC) and urinary bladder urothelial carcinoma (UBUC).

Materials and Methods: We performed immunohistochemical study to evaluate CHI3L1 expression in 2 well-defined cohorts of urothelial carcinoma, including UTUC ($n = 340$) and UBUC ($n = 295$). CHI3L1 expression level was determined by *H*-score method. The associations between CHI3L1 expression and clinicopathological features, disease-specific survival (DSS) and metastasis-free survival (MFS) were analyzed.

Results: High expression of CHI3L1 was significantly associated with adverse clinicopathological features in UTUC or UBUC, including advanced tumor status (pT), nodal metastasis, high histological grade, vascular invasion, perineural invasion, and high mitotic activity (all $P < 0.05$). Kaplan-Meier survival analysis revealed that patients with high CHI3L1 expression had shorter DSS and MFS in both UTUC and UBUC (all $P < 0.05$). In multivariate survival analyses, high expression of CHI3L1 acted as an independent prognostic factor for worse DSS ($P < 0.001$ in UTUC and $P = 0.036$ in UBUC) and MFS ($P = 0.002$ in UTUC and $P = 0.003$ in UBUC) in both UTUC and UBUC groups.

Conclusions: High expression of CHI3L1 was significantly associated with aggressive clinicopathological features and acted as an independent prognostic factor for worse outcome in urothelial carcinoma.

Keywords: CHI3L1; Urothelial carcinoma; Prognosis

1. Introduction

Urothelial carcinoma (UC) is the most common malignancy involving the urinary system in Taiwan and worldwide. Various chemical carcinogens, such as cigarette smoke [1], arsenic exposure [2,3], consumption of aristolochic acid

This study was supported by grants from Chang Gung Hospital (CMRPG8G0581) and An Nan Hospital (ANHRF103-10).

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https://doi.org/10.1016/j.urolonc.2019.01.001
1078-1439/

contained in Chinese herbs [4], and occupational exposure to aromatic amines [5,6] have been identified to be associated with the occurrence of UC. Moreover, with the advances in genome-wide expression analyses and sequencing techniques, numerous genetic alternations have been found to play an important role in the initiation and progression of UC. These genetic changes can help us to understand disease pathogenesis and molecular subtyping of tumors. Thus, searching for key molecular regulators involved in the progression of UC and identifying potential therapeutic targets are of great importance.

Cancer is a disease condition characterized by uncontrolled cell proliferation. Cancer cells need more energy than normal cells to support rapid cell growth and to prolong cell survival. Changes in cellular metabolism play a critical role in cancer progression, including increased glucose uptake, fatty acid synthesis, and glutaminolysis [7–9]. Hence, we tried to identify which genes associated with carbohydrate metabolism have influences on cancer progression in UC. Through analysis of the public transcriptome of UC of urinary bladder in Gene Expression Omnibus database (Accession number: GSE31684) with particular focus on genes associated with carbohydrate metabolic process, we found that *Chitinase 3-like-1 (CHI3L1)* was a significantly up-regulated gene correlated with advanced disease status. *CHI3L1* is a protein-coding gene and located on chromosome 1q32.1. CHI3L1, also known as YKL-40, is a glycoprotein member of the glycosyl hydrolase 18 family [10]. The protein does not have chitinase activity and is secreted by chondrocytes, synovial cells, endothelial cells, neutrophils, and activated macrophages. It has been found to play an important role in inflammation, fibrosis, and tissue remodeling [11]. Overexpression of CHI3L1 is also found in several types of cancer, including breast cancer [12], gastric cancer [13], lung cancer [14], prostate cancer [15], ovarian cancer [16], pancreatic cancer [17], renal cell carcinoma [18], papillary thyroid carcinoma [19], and glioblastoma [20]. In most of these cancer types, the expression of CHI3L1 is of prognostic significance, and some have biologic roles that regulate cell growth, migration, and invasion.

The prognostic value and molecular mechanism of CHI3L1 in UC is still unknown. In this study, we evaluated CHI3L1 expression in 2 large cohorts of UC, including upper urinary tract urothelial carcinoma (UTUC) and urinary bladder urothelial carcinoma (UBUC). The relationships between CHI3L1 expression and key clinicopathological parameters were analyzed. More importantly, we also investigated the prognostic significance of CHI3L1 in patients with UC.

2. Materials and methods

2.1. Analysis of public transcriptome of urinary bladder UC

The public transcriptome dataset with the accession number of GSE31684 was obtained from Gene Expression Omnibus database. For data analysis, the raw CEL files available in the Affymetrix HUMAN Genome U133 Plus 2.0 microarray platform were imported into Nexus Expression 3 software (BioDiscovery, EI Segundo, CA). All probes in the analysis were included without preselection or filtering. We performed supervised comparative analyses and focused on genes associated with carbohydrate metabolic process (GO:0005975) to identify significantly and differentially expressed genes between tumors with high-stage (pT2-pT4) and low-stage (pTa-pT1). Those genes with $P < 0.01$ and log2-transformed expression fold change $>\pm 0.1$ were selected.

2.2. Patients and tumor samples

This study was approved by the Institutional Review Board of the Chi Mei Medical Center (IRB10501-005). There were 635 cases who were diagnosed as UC between 1996 and 2004 enrolled in this study, including 340 UTUCs and 295 UBUCs. Those with synchronous UCs in the upper tract and urinary bladder were excluded. Paraffin-embedded tissue samples were obtained from the archives of the department of Pathology in Chi-Mei Medical Center. Patients with UTUC received biopsies initially in the Chi-Mei Medical Center, followed by nephroureterectomy and regional lymph node dissection. Patients with superficial UC in urinary bladder (pTa and pT1) underwent a transurethral resection of the bladder tumor with or without intravesical application of the Bacillus Calmette-Guerin therapy. A salvage cystectomy was performed for those with tumor recurrence. Radical cystectomy with bilateral pelvic iliac lymph node dissection was performed for tumors with invasion of muscle layer. Those UBUC patients with either pT3-4 stage or lymph node metastasis received cisplatin-based chemotherapy. No patients received neoadjuvant chemotherapy in our study. The tumor staging was determined by 8th American Joint of Cancer Committee system. The mean follow-up durations for patients with UTUC and UBUC were 44.7 and 30.8 months, respectively.

2.3. Immunohistochemical study and scoring of CHI3L1 expression

Tissue sections of 3-μm thickness were cut from paraffin-embedded tissue blocks, and routinely underwent deparaffinization, rehydration, and antigen retrieval. The sections were incubated with a primary antibody targeting CHI3L1 (Cat No. MABC196, Clone mAY, 1:500; Merck millipore) for 1 hour. The scoring of CHI3L1 immunostaining was based on the *H*-score method, calculated using the following equation: $H\text{-score} = \Sigma \text{Pi}(i + 1)$, where i is the intensity of stained tumor cells (0–3+), and Pi is the percentage of stained tumor cells for each intensity ranging from 0% to 100%. This formula produced a score range from 100 to 400. The median of *H*-score acted as a cut-off value to sub-classify all cases into 2 subgroups, high, and low expression of CHI3L1. High expression of CHI3L1

was defined as the *H*-score greater than or equal to the median while low expression of CHI3L1 was defined as the *H*-score less than the median.

2.4. Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences software version 14. Chi-square test was used to determine whether there was a significant association between CHI3L1 expression and various clinicopathological parameters, including primary tumor status (pT), primary nodal status (pN), histological grade, vascular invasion, perineural invasion, and mitotic rate. The endpoints were disease-specific survival and metastatic-free survival, calculated from the time of diagnosis with UC to the date of an event (e.g., death or metastasis) occurred. These 2 survival endpoints were calculated by the Kaplan-Meier method and analyzed by the log-rank test. The relationships between survival endpoints and clinicopathological parameters were determined by using univariate and multivariate analyses. The Cox proportional hazards model was applied for multivariate analysis. All tests are 2-sided, and those with P values less than 0.05 were considered statistically significant.

3. Results

3.1. CHI3L1 was a significant up-regulated gene associated with advanced disease status in UBUC

From data mining of public transcriptome of UBUC (GSE31684) which includes 78 high-stage (T2-T4) and 15 low-stage (Ta-T1) cases, *CHI3L1*, *SLC2A3*, *PPP1R2*, *ALDH2*, *COTL1*, *AKR1B1*, *SLC2A3*, *ST3GAL5*, *GPD1L*, *IDUA*, *CAPN3*, *SMA4*, *ST3GAL5* and *HAS3* were identified as significantly and differentially expressed genes associated with carbohydrate metabolism between patients with high-stage (T2-T4) and low-stage (Ta-T1) UBUCs (Table 1 and Fig. 1). Of these, *CHI3L1* was the most significantly up-regulated gene detected by 2 probes with a log2 ratio of 2.8175- and 2.2409-fold change, accompanied with a P value of <0.0001 and 0.0001, respectively. Hence, CHI3L1 was selected for further study and analysis.

3.2. Patient characteristics and associations between CHI3L1 expression and clinicopathological parameters in UTUC

As shown in Table 2, there were 340 patients with UTUC, including 158 males and 182 females in this study. Most patients with UTUC at diagnosis were older than 65 years ($n = 202$, 59.4%). Forty-nine patients (14.4%) had synchronous tumors both in the ureter and renal pelvis, and 62 patients (18.2%) had tumor multifocality. Most tumors have high histological grade ($n = 284$, 83.5%). About half of the cases were presented with advanced pT stage (pT2-4) ($n = 159$, 46.8%), and nodal metastasis was observed in 28 patients (8.2%). One hundred and six cases (31.2%) had tumors with vascular invasion, 19 cases (5.9%) with perineural invasion, and 167 cases (49.1%) with high mitotic activity. The relationships between CHI3L1 expression and clinicopathological parameters were statistically analyzed. High expression of CHI3L1 significantly correlated with advanced tumor status ($P < 0.001$), advanced nodal status ($P < 0.001$), high histological grade ($P = 0.003$), increased vascular invasion ($P < 0.001$), increased perineural invasion ($P < 0.001$), and high mitotic activity ($P = 0.023$). The expression of CHI3L1 was not associated with gender, age, tumor location, or multifocality.

3.3. Patient characteristics and associations between CHI3L1 expression and clinicopathological parameters in UBUC

This cohort, listed in Table 2, consisted of 295 cases with UBUC and had male predominance ($n = 216$, 73.2%). There were 239 (81.0%) cases having high histologic grade, 123 (41.7%) cases at a muscle-invasive stage (pT2-T4), and 29 (9.8%) cases having lymph node metastasis. Vascular invasion, perineural invasion and high mitotic activity were found in 49 (16.6%), 20 (6.8%), and 156 (52.9%) patients, respectively. Of note, high expression of CHI3L1 (Fig. 2) was significantly associated with advanced tumor status ($P < 0.001$), advanced nodal status ($P = 0.033$), high histological grade ($P = 0.035$), and increased vascular invasion ($P = 0.020$). There was no significant association between CHI3L1 expression status and age, gender, perineural invasion, or mitotic activity.

3.4. Survival analysis and prognostic significance of CHI3L1 expression in patients with UTUC

We performed univariate and multivariate analyses with the Cox proportional hazards model to identify key prognostic factors for disease-specific survival and metastasis-free survival. In univariate analysis, tumor location ($P = 0.0079$), multifocality ($P = 0.0026$), advanced primary tumor status ($P < 0.0001$), nodal metastasis ($P < 0.0001$), high histologic grade ($P = 0.0215$), vascular invasion ($P < 0.0001$), perineural invasion ($P < 0.0001$), and high CHI3L1 expression ($P < 0.0001$) were significantly associated with worse disease-specific survival (Table 3 and Fig. 3). Therefore, tumor location, multifocality, primary tumor status, nodal metastasis, histologic grade, vascular invasion, perineural invasion, and CHI3L1 expression were selected for multivariate analysis. In multivariate analysis, high expression of CHI3L1 ($P < 0.001$) together with nodal metastasis ($P < 0.001$), high histological grade ($P = 0.008$), and perineural invasion ($P = 0.002$) acted as independent prognostic factors for shorter disease-specific survival. In terms of metastasis-free survival, the univariate analysis disclosed that the following parameters significantly

Table 1
Summary of differentially expressed genes associated with carbohydrate metabolic process (GO:0005975) and showed positive associations to cancer invasiveness in the transcriptome of urothelial carcinoma of urinary bladder (GSE31684).

	Comparing T2-4 to Ta-T1				
Probe	Log ratio	*P* value	Gene symbol	Biological process	Molecular function
209395_at	2.8175	<0.0001	*CHI3L1*	Carbohydrate metabolic process, chitin catabolic process	Catalytic activity, cation binding, chitinase activity, extracellular matrix structural constituent, hydrolase activity; hydrolyzing O-glycosyl compounds, sugar binding
209396_s_at	2.2409	0.0001	*CHI3L1*	Carbohydrate metabolic process, chitin catabolic process	Catalytic activity, cation binding, chitinase activity, extracellular matrix structural constituent, hydrolase activity; hydrolyzing O-glycosyl compounds, sugar binding
202499_s_at	1.4254	0.0003	*SLC2A3*	Carbohydrate metabolic process, carbohydrate transport, glucose transport, transport	Glucose transmembrane transporter activity, sugar: hydrogen ion symporter activity, transporter activity
202166_s_at	1.2134	0.0001	*PPP1R2*	Carbohydrate metabolic process, generation of precursor metabolites and energy, glycogen metabolic process, regulation of phosphoprotein phosphatase activity, regulation of signal transduction	Protein binding, protein phosphatase inhibitor activity, type 1 serine/threonine specific protein phosphatase inhibitor activity
201425_at	1.1369	0.001	*ALDH2*	Alcohol metabolic process, carbohydrate metabolic process, metabolic process	Aldehyde dehydrogenase (NAD) activity, aldehyde dehydrogenase [NAD(P)+] activity, electron carrier activity, oxidoreductase activity
224583_at	1.1211	0.0046	*COTL1*		Actin binding, enzyme binding, protein binding
201272_at	1.0651	0.0043	*AKR1B1*	Carbohydrate metabolic process, response to stress	Aldehyde reductase activity, aldo-keto reductase activity, electron carrier activity, oxidoreductase activity, protein binding
202497_x_at	1.0569	0.0002	*SLC2A3*	Carbohydrate metabolic process, carbohydrate transport, glucose transport, transport	Glucose transmembrane transporter activity, sugar: hydrogen ion symporter activity, transporter activity
239755_at	−1.026	0.003	*ST3GAL5*	Carbohydrate metabolic process, ganglioside biosynthetic process, glycosphingolipid biosynthetic process, protein amino acid glycosylation	Lactosylceramide alpha-2;3-sialyltransferase activity, neolactotetraosylceramide alpha-2;3-sialyltransferase activity, sialyltransferase activity, transferase activity, transferase activity; transferring glycosyl groups
212510_at	−1.1329	0.0091	*GPD1L*	Carbohydrate metabolic process, glycerol-3-phosphate catabolic process, glycerol-3-phosphate metabolic process	NAD binding, coenzyme binding, glycerol-3-phosphate dehydrogenase (NAD+) activity, oxidoreductase activity, oxidoreductase activity; acting on CH-OH group of donors, oxidoreductase activity; acting on the CH-OH group of donors; NAD or NADP as acceptor
205059_s_at	−1.204	<0.0001	*IDUA*	Carbohydrate metabolic process, disaccharide metabolic process, glycosaminoglycan metabolic process, metabolic process	L-iduronidase activity, catalytic activity, cation binding, hydrolase activity, hydrolase activity; acting on glycosyl bonds, hydrolase activity; hydrolyzing O-glycosyl compounds
210944_s_at	−1.4018	<0.0001	*CAPN3*	Carbohydrate metabolic process, metabolic process, muscle development, proteolysis	Alpha-glucosidase activity, calcium ion binding, calcium-dependent cysteine-type endopeptidase activity, cysteine-type endopeptidase activity, cysteine-type peptidase activity, hydrolase activity, hydrolase activity; acting on glycosyl bonds, hydrolase activity; hydrolyzing O-glycosyl compounds, peptidase activity, signal transducer activity
214850_at	−1.5083	<0.0001	*SMA4*	Carbohydrate metabolic process, nervous system development, skeletal development	Catalytic activity, cation binding, hydrolase activity; hydrolyzing O-glycosyl compounds
203217_s_at	−1.627	0.0003	*ST3GAL5*	Carbohydrate metabolic process, ganglioside biosynthetic process, glycosphingolipid biosynthetic process, protein amino acid glycosylation	Lactosylceramide alpha-2;3-sialyltransferase activity, neolactotetraosylceramide alpha-2;3-sialyltransferase activity, sialyltransferase activity, transferase activity, transferase activity; transferring glycosyl groups
223541_at	−1.6319	0.0024	*HAS3*	Carbohydrate metabolic process	Hyaluronan synthase activity, transferase activity, transferase activity; transferring glycosyl groups

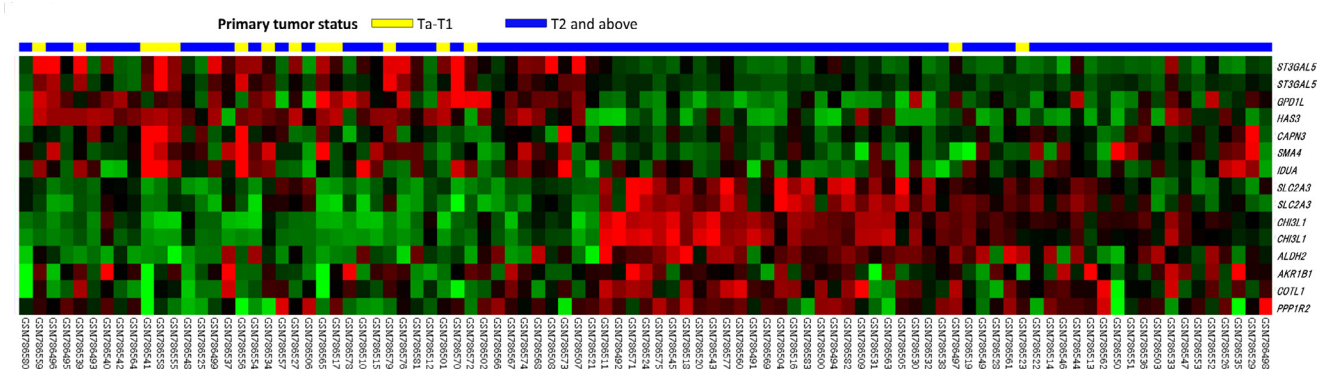

Fig. 1. Analysis of publicly available transcriptome of urinary bladder urothelial carcinoma (GSE31684) with special focus on genes associated with carbohydrate metabolism (GO:0005975) found that *CHI3L1* was the most significantly up-regulated gene correlated with advanced primary tumor (pT) status. The pT status of each sample showed on the top of the heat map, illustrated by yellow color for Ta-T1 stage and blue color for T2-T4 stage, respectively. The gene expression levels are demonstrated as a spectrum of red and green for those with up-regulation and down-regulation, respectively (Color version of figure is available online.).

correlated with a poor outcome: high CHI3L1 expression ($P < 0.0001$), multifocality ($P = 0.0127$), advanced primary tumor status ($P < 0.0001$), nodal metastasis ($P < 0.0001$), high histologic grade ($P = 0.0027$), vascular invasion ($P < 0.0001$), and perineural invasion ($P < 0.0001$). Furthermore, CHI3L1 expression, multifocality, primary tumor status, nodal metastasis, histologic grade, vascular invasion, and perineural invasion were selected for multivariate analysis. In multivariate analysis, high expression of CHI3L1 ($P = 0.002$) together with multifocality ($P = 0.001$), nodal metastasis ($P = 0.005$), high histological grade ($P = 0.040$), vascular invasion ($P = 0.007$), and perineural invasion ($P = 0.032$) were significantly associated with worse metastasis-free survival.

3.5. Survival analysis and prognostic significance of CHI3L1 expression in patients with UBUC

As shown in Table 4, the univariate analysis revealed that advanced primary tumor status ($P < 0.0001$), nodal metastasis ($P = 0.0002$), high histologic grade ($P = 0.0013$), vascular invasion ($P = 0.0024$), perineural invasion

Table 2
Correlations between CHI3L1 expression and other important clinicopathological parameters in urothelial carcinomas

		Upper urinary tract urothelial carcinoma				Urinary bladder urothelial carcinoma			
			CHI3L1 expression				CHI3L1 expression		
Parameter	Category	Case no. *n* (%)	Low *n* (%)	High *n* (%)	*P* value	Case no. *n* (%)	Low *n* (%)	High *n* (%)	*P* value
Gender	Male	158 (46.5)	74 (21.8)	84 (24.7)	0.277	216 (73.2)	110 (37.3)	106 (35.9)	0.534
	Female	182 (53.5)	96 (28.2)	86 (25.3)		79 (26.8)	37 (12.5)	42 (14.2)	
Age (y)	<65	138 (40.6)	73 (21.5)	65 (19.1)	0.377	121 (41.0)	57 (19.3)	64 (21.7)	0.435
	≥65	202 (59.4)	97 (28.5)	105 (30.9)		174 (59.0)	90 (30.5)	84 (28.5)	
Tumor location	Renal pelvis	141 (41.5)	61 (17.9)	80 (23.5)	0.180	–	–	–	–
	Ureter	150 (44.1)	83 (24.4)	67 (19.7)		–	–	–	–
	Renal pelvis and ureter	49 (14.4)	26 (7.6)	23 (6.8)		–	–	–	–
Multifocality	Single	278 (81.8)	138 (40.6)	140 (41.2)	0.779	–	–	–	–
	Multifocal	62 (18.2)	32 (9.4)	30 (8.8)		–	–	–	–
Primary tumor (T)	Ta	89 (26.2)	64 (18.8)	25 (7.4)	**<0.001**[a]	84 (28.5)	59 (20.0)	25 (8.5)	**<0.001**[a]
	T1	92 (27.1)	56 (16.5)	36 (10.6)		88 (29.8)	45 (15.3)	43 (14.6)	
	T2-T4	159 (46.8)	50 (14.7)	109 (32.1)		123 (41.7)	43 (14.6)	80 (27.1)	
Nodal metastasis	Negative (N0)	312 (91.8)	165 (48.5)	147 (43.2)	**<0.001**[a]	266 (90.2)	138 (46.8)	128 (43.4)	**0.033**[a]
	Positive (N1-N2)	28 (8.2)	5 (1.5)	23 (6.8)		29 (9.8)	9 (3.1)	20 (6.8)	
Histological grade	Low grade	56 (16.5)	38 (11.2)	18 (5.3)	**0.003**[a]	56 (19.0)	35 (11.9)	21 (7.1)	**0.035**[a]
	High grade	284 (83.5)	132 (38.8)	152 (44.7)		239 (81.0)	112 (38.0)	127 (43.1)	
Vascular invasion	Absent	234 (68.8)	143 (42.1)	91 (26.8)	**<0.001**[a]	246 (83.4)	130 (44.1)	116 (39.3)	**0.020**[a]
	Present	106 (31.2)	27 (7.9)	79 (23.2)		49 (16.6)	17 (5.8)	32 (10.8)	
Perineural invasion	Absent	321 (94.4)	168 (49.4)	153 (45.0)	**<0.001**[a]	275 (93.2)	139 (47.1)	136 (46.1)	0.362
	Present	19 (5.6)	2 (0.6)	17 (5.0)		20 (6.8)	8 (2.7)	12 (4.1)	
Mitotic rate (per 10 high power fields)	<10	173 (50.9)	97 (28.5)	76 (22.4)	**0.023**[a]	139 (47.1)	72 (24.4)	67 (22.7)	0.523
	>=10	167 (49.1)	73 (21.5)	94 (27.6)		156 (52.9)	75 (25.4)	81 (27.5)	

[a] Statistically significant.

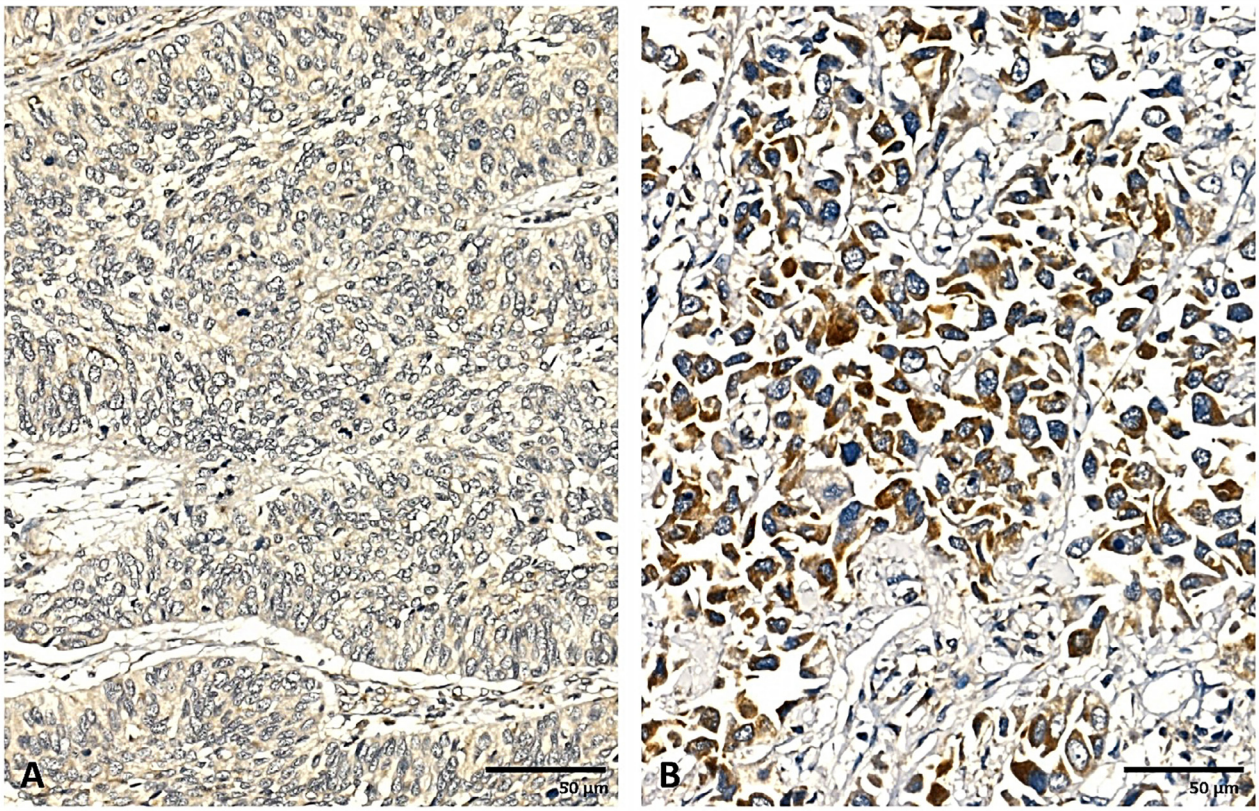

Fig. 2. Representative tissue sections of CHI3L1 immunostaining showed that the expression of CHI3L1 was low in superficially invasive urothelial carcinoma (A) and high in muscle-invasive urothelial carcinoma (B).

($P = 0.0001$), high mitotic activity ($P < 0.0001$), and high expression of CHI3L1 ($P < 0.0001$) were significantly associated with shorter disease-specific survival (Fig. 3). Hence, primary tumor status, nodal metastasis, histologic grade, vascular invasion, perineural invasion, mitotic activity, and CHI3L1 expression were selected for further multivariate analysis. In multivariate analysis, high expression of CHI3L1 ($P = 0.036$) together with advanced primary tumor status ($P < 0.001$), perineural invasion ($P = 0.016$), and high mitotic activity ($P = 0.009$) acted as independent prognostic factors for shorter disease-specific survival. With regard to metastasis-free survival, the univariate analysis disclosed that the following parameters significantly correlated with a poor survival: high CHI3L1 expression ($P < 0.0001$), advanced primary tumor status ($P < 0.0001$), nodal metastasis ($P < 0.0001$), high histologic grade ($P = 0.0007$), vascular invasion ($P = 0.0001$), perineural invasion ($P = 0.0007$), and high mitotic activity ($P < 0.0001$). Therefore, CHI3L1 expression, primary tumor status, nodal metastasis, histologic grade, vascular invasion, perineural invasion, and mitotic activity were selected for multivariate analysis. In multivariate analysis, high expression of CHI3L1 ($P = 0.003$) together with advanced primary tumor status ($P = 0.014$), perineural invasion ($P = 0.048$), and high mitotic activity ($P = 0.019$) were independent prognostic factors for worse metastasis-free survival.

4. Discussion

In this study, we found that high expression of CHI3L1 was significantly associated with advanced disease status, characterized by higher tumoral and nodal stage, higher histological grade, increased vascular invasion, increased perineural invasion, and higher mitotic activity. In terms of the clinical impact of CHI3L1 expression in UC, high expression of CHI3L1 was found to be an independent prognostic factor that predicts worse disease-specific survival and metastasis-free survival. In pancreatic cancer, overexpression of CHI3L1 acted as an independent prognostic factor for both overall survival and progression-free survival [17]. In ovarian cancer, patients with high CHI3L1 expression had shorter overall survival and progression-free survival [16]. In addition to aforementioned cancer types, high expression of CHI3L1 also served as a negative prognostic for survival in papillary thyroid carcinoma, cholangiocarcinoma, clear cell renal cell carcinoma, glioma, nonsmall cell lung cancer, and breast cancer [12,18–22]. A previous study has evaluated the tissue gene expression level, serum concentration, and urinary level of CHI3L1 (also known as YKL-40) in patients with bladder cancer. Higher tissue gene expression and serum concentration of CHI3L1 were found in bladder cancer patients and were significantly associated with poor disease-specific survival in univariate

Table 3
Univariate log-rank and multivariate analyses for disease-specific and metastasis-free survivals in upper urinary tract urothelial carcinoma

			Disease-specific survival					Metastasis-free survival				
			Univariate analysis		Multivariate analysis			Univariate analysis		Multivariate analysis		
Parameter	Category	Case no. *n* (%)	No. (%) of event	*P* value	HR	95% CI	*P* value	No. (%) of event	*P* value	HR	95% CI	*P* value
Gender	Male	158 (46.5)	28 (8.2)	0.8286	–	–	–	32 (9.4)	0.7904	–	–	–
	Female	182 (53.5)	33 (9.7)		–	–	–	38 (11.2)		–	–	–
Age (y)	<65	138 (40.6)	26 (7.6)	0.9943	–	–	–	30 (8.8)	0.8470	–	–	–
	≥65	202 (59.4)	35 (10.3)		–	–	–	40 (11.8)		–	–	–
Tumor side	Right	177 (52.1)	34 (10.0)	0.7366	–	–	–	38 (11.2)	0.3074	–	–	–
	Left	154 (45.3)	26 (7.6)		–	–	–	32 (9.4)		–	–	–
	Bilateral	9 (2.6)	1 (0.3)		–	–	–	0 (0)		–	–	–
Tumor location	Renal pelvis	141 (41.5)	24 (7.1)	0.0079[a]	1	–	0.794	31 (9.1)	0.0659	–	–	–
	Ureter	150 (44.1)	22 (6.5)		0.676	0.186–2.466		25 (7.4)		–	–	–
	Renal pelvis and ureter	49 (14.4)	15 (4.4)		0.632	0.168–2.374		14 (4.1)		–	–	–
Multifocality	Single	278 (81.8)	48 (14.1)	0.0026[a]	1	–	0.198	52 (15.3)	0.0127[a]	1	–	0.001[a]
	Multifocal	62 (18.2)	18 (5.3)		2.212	0.661–7.407		18 (5.3)		2.596	1.490–4.524	
Primary tumor (T)	Ta	89 (26.2)	2 (0.6)	**<0.0001**[a]	1	–	0.127	4 (1.2)	**<0.0001**[a]	1	–	0.143
	T1	92 (27.1)	9 (2.6)		3.689	0.782–17.403		15 (4.4)		2.602	0.824–8.216	
	T2-T4	159 (46.8)	50 (14.7)		4.754	1.053–21.460		51 (15.0)		3.074	1.006–9.393	
Nodal metastasis	Negative (N0)	312 (91.8)	42 (12.4)	**<0.0001**[a]	1	–	**<0.001***	**55** (16.2)	**<0.0001**[a]	1	–	0.005[a]
	Positive (N1-N2)	28 (8.2)	19 (5.6)		4.030	2.149–7.558		**15** (4.4)		2.453	1.312–4.584	
Histological grade	Low grade	56 (16.5)	4 (1.2)	0.0215[a]	1	–	0.008*	3 (0.9)	0.0027[a]	1	–	0.040[a]
	High grade	284 (83.5)	57 (16.8)		3.803	1.425–10.151		67 (19.7)		2.279	1.039–4.999	
Vascular invasion	Absent	234 (68.8)	24 (7.1)	**<0.0001**[a]	1	–	0.347	26 (7.6)	**<0.0001**[a]	1	–	0.007[a]
	Present	106 (31.2)	37 (10.9)		1.341	0.727–2.474		44 (12.9)		2.299	1.260–4.196	
Perineural invasion	Absent	321 (94.4)	50 (14.7)	**<0.0001**[a]	1	–	0.002*	61 (17.9)	**<0.0001**[a]	1	–	0.032[a]
	Present	19 (5.6)	11 (3.2)		3.773	1.508–6.565		9 (2.6)		2.254	1.073–4.735	
Mitotic rate (per 10 high power fields)	<10	173 (50.9)	27 (7.9)	0.167	–	–		30 (8.8)	0.0823	–	–	
	>=10	167 (49.1)	34 (10.0)		–	–		40 (11.8)		–	–	
CHI3L1 expression	Low	170 (50.0)	9 (2.6)	**<0.0001**[a]	1	–	**<0.001**	17 (5.0)	**<0.0001**[a]	1	–	0.002[a]
	High	170 (50.0)	52 (15.3)		3.940	1.850–8.390		53 (15.6)		2.511	1.396–4.516	

HR = hazard ratio, CI = confidence interval.
[a] Statistically significant.

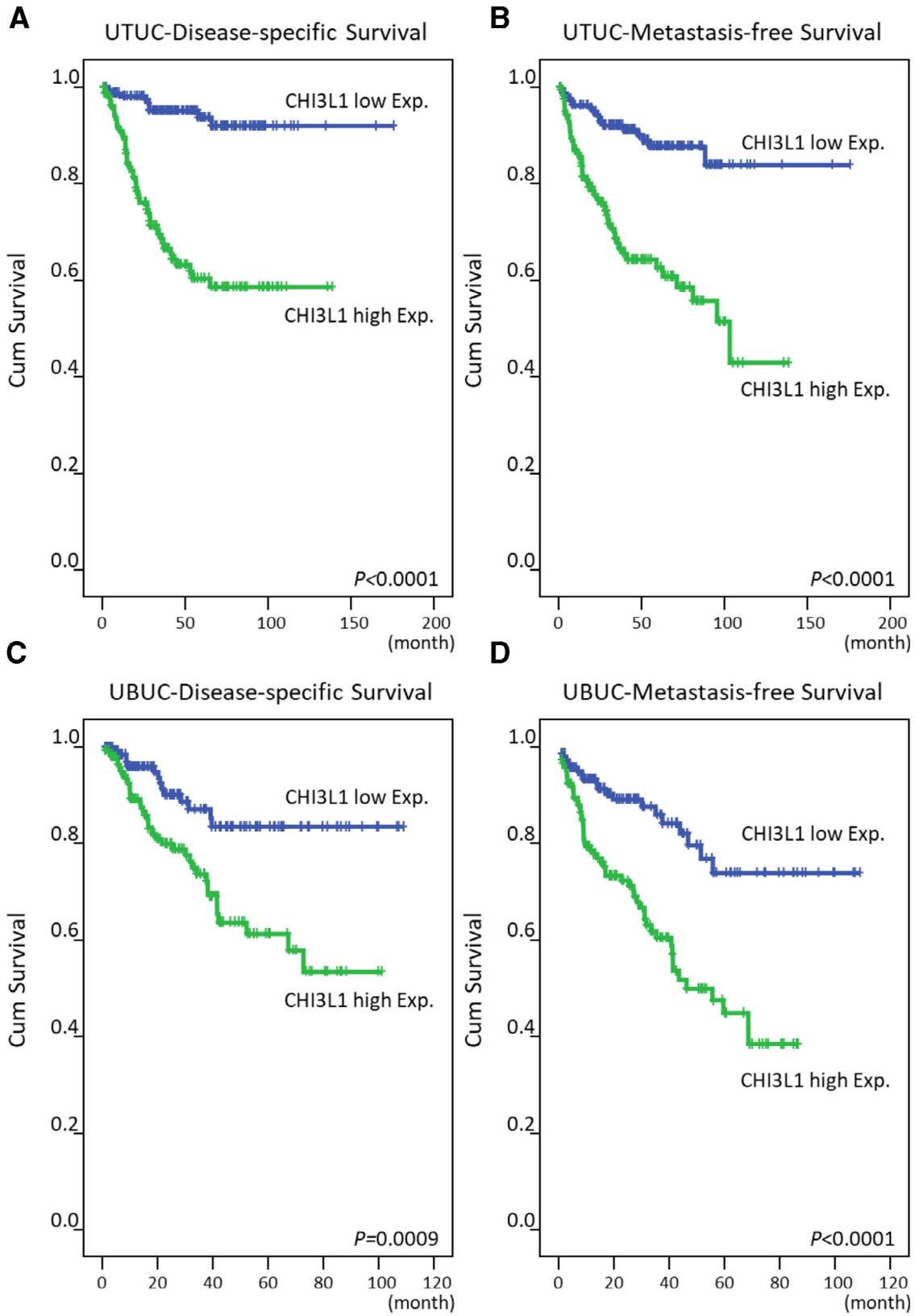

Fig. 3. Kaplan-Meier analysis demonstrated that patients with high expression of CHI3L1 had shorter disease-specific survival and metastasis-free survival than those with low expression, both in the UTUC (A and B) and UBUC (C and D) groups.

Table 4
Univariate log-rank and multivariate analyses for disease-specific and metastasis-free survivals in urinary bladder urothelial carcinoma

			Disease-specific survival					Metastasis-free survival				
			Univariate analysis		Multivariate analysis			Univariate analysis		Multivariate analysis		
Parameter	Category	Case no.*n* (%)	No. (%) of event	*P* value	HR	95% CI	*P* value	No. (%) of event	*P* value	HR	95% CI	*P* value
Gender	Male	216 (73.2)	41 (13.9)	0.4446	–	–	–	60 (20.3)	0.2720	–	–	–
	Female	79 (26.8)	11 (3.7)		–	–	–	16 (5.4)		–	–	–
Age (years)	<65	121 (41.0)	17 (5.8)	0.1136	–	–	–	31 (10.5)	0.6875	–	–	–
	≥65	174 (59.0)	35 (11.9)		–	–	–	45 (15.3)		–	–	–
Primary tumor (T)	Ta	84 (28.5)	1 (0.3)	**<0.0001**[a]	1	–	**<0.001**[a]	4 (1.4)	**<0.0001**[a]	1	–	0.014[a]
	T1	88 (29.8)	9 (3.1)		5.707	0.605–53.844		23 (7.8)		4.385	1.248–15.408	
	T2-T4	123 (41.7)	42 (14.2)		22.507	2.480–204.294		49 (16.6)		6.377	1.805–22.525	
Nodal metastasis	Negative (N0)	266 (90.2)	41 (13.9)	0.0002[a]	1	–	0.673	61 (20.7)	**<0.0001**[a]	1	–	0.117
	Positive (N1-N2)	29 (9.8)	11 (3.7)		1.169	0.566–2.415		15 (5.1)		1.652	0.882–3.095	
Histological grade	Low grade	56 (19.0)	2 (0.7)	0.0013[a]	1	–	0.922	5 (1.7)	0.0007[a]	1	–	0.775
	High grade	239 (81.0)	50 (16.9)		0.923	0.187–4.555		71 (24.1)		0.851	0.282–2.570	
Vascular invasion	Absent	246 (83.4)	37 (12.5)	0.0024[a]	1	–	0.200	54 (18.3)	0.0001[a]	1	–	0.915
	Present	49 (16.6)	15 (5.1)		1.565	0.789–3.103		22 (7.5)		0.968	0.527–1.776	
Perineural invasion	Absent	275 (93.2)	44 (14.9)	0.0001[a]	1	–	0.016[a]	66 (22.4)	0.0007[a]	1	–	0.048[a]
	Present	20 (6.8)	8 (2.7)		2.843	1.214–6.655		10 (3.4)		2.134	1.008–4.517	
Mitotic rate (per 10 high power fields)	<10	139 (47.1)	12 (4.1)	**<0.0001**[a]	1	–	0.009[a]	23 (7.8)	**<0.0001**[a]	1	–	0.019[a]
	>=10	156 (52.9)	40 (13.6)		2.443	1.247–4.787		53 (18.0)		1.861	1.109–3.124	
CHI3L1 expression	Low	147 (49.8)	14 (4.7)	0.0009[a]	1	–	0.036[a]	20 (6.8)	**<0.0001**[a]	1	–	0.003[a]
	High	148 (50.2)	38 (12.9)		2.008	1.045–3.860		56 (19.0)		2.264	1.324–3.873	

HR = hazard ratio.
[a] Statistically significant.

analysis [23]. There was no significant difference in urinary CHI3L1 levels between bladder cancer patients and healthy controls. Interestingly, another study revealed that urinary CHI3L1 levels were significantly elevated in all invasive (T1-T4) cancers except for low stage (Ta) and low grade ones, compared to controls [24]. Moreover, published microarray data also disclosed that increased gene expression of CHI3L1 was found in muscle-invasive comparing with superficially invasive bladder cancer [25]. These findings implied that CHI3L1 could acted as a marker for risk stratification or disease progression.

Understanding the molecular mechanism of carcinogenesis and disease progression is a crucial step for the identification of potential therapeutic targets and the development of anticancer targeted treatments. In prostatic cancer cells, the expression levels of CHI3L1 were higher in metastatic prostatic cancer cells when compared with less invasive and normal prostatic epithelial cell lines. In in vitro functional study, overexpression of CHI3L1 increased cell migration and invasion abilities, as well as promoted anchorage-independent growth of prostatic cancer cells [15]. The oncogenic role of CHI3L1 was confirmed in another study that suppression of CHI3L1 by short-hairpin RNA reduced glioma cell invasion, anchorage-independent growth, and increased cell death triggered by certain chemotherapeutic drugs, including cisplatin, etoposide, and doxorubicin [26]. Moreover, the expression of CHI3L1 was mediated by certain transcription factors, nuclear factor I-X3, and STAT3 [27]. In gastric cancer cells, CHI3L1 has been found to bind to CD44, and thus activated Erk and Akt, along with Wnt/β-catenin signaling by inducing β-catenin phosphorylation. The data in that study further demonstrated that CHI3L1 promoted cell proliferation, colony formation, and invasion in gastric cancer cells. Notably, these effects were significantly abrogated by CD44 blockage [13]. The epithelial-mesenchymal transition is a biologic process by which polarized epithelial cells lose their cell polarity and cell-cell adhesion to become a mesenchymal cell phenotype, characterized by enhancing invasive, migratory, and metastatic properties [28,29]. In gastric cancer cells, CHI3L1 could promote epithelial-mesenchymal transition by enhancing mesenchymal markers, snail and vimentin expression [13].

Bidirectional communication between cancer cells and other cell types within the tumor microenvironment plays an important role in tumor progression [30]. The tumor microenvironment includes tumor cells, macrophages, immune suppressor cells, fibroblasts, blood vessels, and a wide variety of mesenchymal cells. Tumor-associated macrophages (TAMs) are key regulators of tumor progression by facilitating angiogenesis, extracellular matrix breakdown, and tumor cell motility [31]. TAMs have been identified to enhance tumor cell invasion via a colony-stimulating factor-1/epidermal growth factor paracrine loop in breast cancer and glioblastoma [32,33]. There are several subtypes of activated macrophages, and the 2 main groups are designed as M1 and M2 [34]. The M1 macrophages, previously referred to as classically activated macrophages, are responsible for antigen presentation and play an antitumorigenic role [35]. In contrast, the M2 macrophages have functions in wound healing, tissue repair, and putting the brake on damaging immune system by producing anti-inflammatory cytokines like IL-10. TAMs are mainly M2 macrophages and seem to promote tumor growth [36,37]. A recent study suggested that tumor-recruited M2 macrophages secreted CHI3L1 protein and promoted gastric and breast cancer metastasis [38]. The CHI3L1 protein interacted with interleukin-13 receptor α2 chain molecules on the cancer cell membranes, leading to activation of mitogen-activated protein kinase signaling pathway and up-regulation of matrix metalloproteinase that facilitate tumor metastasis [38]. In addition, CHI3L1 itself could promote macrophages recruitment via inducing secretion of monocyte chemoattractant protein-1 [39].

Angiogenesis is an important factor that aids in tumor growth and metastatic spreading by providing oxygen and nutrients. Stimulation of CHI3L1 significantly increased the tube formation of human umbilical vein endothelial cells in SW480 colon cancer cells [39]. CHI3L1 also acted as an angiogenic factor in breast cancer and glioblastoma cells [40,41]. CHI3L1 expression had a significant association with the increased microvessel density in colorectal cancer and clear cell renal cell carcinoma [18,39]. In addition to endothelial cell-mediated angiogenesis, an alternative process of microvascular formation known as vasculogenic mimicry (VM) has played an important role in tumor growth and metastasis [42]. Tumor cell VM was described as de novo formation of vascular networks lacking endothelial cells, thereby providing a perfusion pathway for rapidly growing tumors and an escape way for metastasis [43,44]. In cervical cancer, CHI3L1 expression was significantly associated with VM, and patients with VM tended to have shorter survival than those without VM [45]. These findings indicated that CHI3L1 was an enhancer for both endothelial cell-dependent angiogenesis and endothelial cell-independent VM. It raised the possibility of using antiangiogenic agent for treating UC patients with high expression levels of CHI3L1, but more studies are needed to confirm the therapeutic effect of these agents.

Some evidences have suggested that CHI3L1 is regarded as a potential therapeutic target. Blockade of CHI3L1 expression inhibited tumor growth, angiogenesis, and metastasis in glioblastoma xenografted animals [40]. A mouse anti-CHI3L1 monoclonal antibody enhanced cell death of glioblastoma cells U87 to γ-irradiation through decreased expression of pAKT and AKT [46]. Moreover, anti-CHI3L1 antibody and ionizing irradiation have synergistic effect that suppresses tumor vascularization and progression in glioblastoma [47]. Additionally, downregulation of CHI3L1 was an important factor to overcome temozolomide resistance in a glioblastoma cell line [48]. Though the effect of anti-CHI3L1 antibody on UC cells was still

unknown, it shed light on developing alternative treatment strategies for UC patients with high expression of CHI3L1.

5. Conclusion

Our data suggested that CHI3L1 expression is an independent and unfavorable prognostic factor associated with worse disease-specific survival and metastasis-free survival. High expression of CHI3L1 was significantly correlated with advanced pathological features. These findings also indicated that CHI3L1 had an oncogenic role in UC.

Conflict of interest

The authors have no conflicts of interest in this work.

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