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COL6A1 expression as a potential prognostic biomarker for risk stratification of T1 high grade bladder cancer: Unveiling the aggressive nature of a distinct non-muscle invasive subtype

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Purpose: T1 high grade (T1HG) bladder cancer (BC) is a type of non-muscle invasive BC (NMIBC) that is recognized as an aggressive subtype with a heightened propensity for progression. Current risk stratification methods for NMIBC rely on clinicopathological indicators; however, these approaches do not adequately capture the aggressive nature of T1HG BC. Thus, new, more accurate biomarkers for T1HG risk stratification are needed. Here, we enrolled three different patient cohorts and investigated expression of collagen type VI alpha 1 (*COL6A1*), a key component of the extracellular matrix, at different stages and grades of BC, with a specific focus on T1HG BC.

Materials and Methods: Samples from 298 BC patients were subjected to RNA sequencing and real-time polymerase chain reaction.

Results: We found that T1HG BC and muscle invasive BC (MIBC) exhibited comparable expression of *COL6A1*, which was significantly higher than that by other NMIBC subtypes. In particular, T1HG patients who later progressed to MIBC had considerably higher expression of *COL6A1* than Ta, T1 low grade patients, and patients that did not progress, highlighting the aggressive nature and higher risk of progression associated with T1HG BC. Moreover, Cox and Kaplan–Meier survival analyses revealed a significant association between elevated expression of *COL6A1* and poor progression-free survival of T1HG BC patients (multivariate Cox hazard ratio, 16.812; 95% confidence interval, 3.283–86.095; p=0.001 and p=0.0002 [log-rank test]).

Conclusions: These findings suggest that *COL6A1* may be a promising biomarker for risk stratification of T1HG BC, offering valuable insight into disease prognosis and guidance of personalized treatment decisions.

Keywords: Collagen type VI; Non-muscle invasive bladder neoplasms; Progression-free survival; Risk assessment

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INTRODUCTION

Bladder cancer (BC), the most common malignant tumor of the urinary system, has the highest incidence among all genitourinary system tumors [1]. BC is divided into non-muscle invasive (NMIBC) and muscle invasive (MIBC) types based on the depth of tumor infiltration into the bladder wall. In clinical practice, 75%–85% of initial BC cases are NMIBC, with 20% of patients having T1 high grade (T1HG) BC [2]. T1HG BC is classified as NMIBC based on pathological staging; however, due to high heterogeneity and difficulty in predicting the prognosis, it is generally considered as a highly malignant tumor. The recurrence rate of NMIBC is 45%–55%, and the progression rate is 6%–17%, depending on patient-specific disease characteristics [3]. Unlike other NMIBC types, T1HG BC has a recurrence rate of up to 80%, and approximately half of patients progress to MIBC within 3 years [4].

The main treatment options for T1HG BC are instillation of high-dose intravesical Bacillus Calmette–Guérin (BCG) or radical cystectomy (RC), with RC being particularly recommended for patients at high risk of disease progression [5]. However, pathological stage and grade cannot reliably predict progression of T1HG BC, leading to some patients being either overtreated or missing the optimal treatment window. In other words, accurate prediction of which patients are suitable for intravesical BCG instillation and which should undergo bladder removal is a challenge. Therefore, there is an urgent need for more precise strategies to guide treatment of T1HG BC in clinical practice. Studies demonstrate that the prognosis of T1HG BC can be predicted based on factors such as gene expression levels, specific gene mutations, and abnormal expression of the immune checkpoint molecule programmed death ligand 1 (PD-L1) [6-11]; however, there is still no consensus.

In a previous study, we showed that expression of the collagen type VI alpha 1 (*COL6A1*) gene was higher in MIBC patients than in NMIBC patients [12]. Collagen is one of the main components of the extracellular matrix (ECM), which is part of the tumor microenvironment (TME) and plays a crucial role in tumor invasion and metastasis. Changes in collagen levels within the TME lead to release of biomechanical signals that are sensed by tumor cells and stromal cells, thereby triggering a series of alterations. Degradation of collagen can affect the TME by regulating ECM remodeling to promote tumor progression [13]. Research on the role of COL6A1 in BC is limited, with only a few informatics-based studies demonstrating an increase expression of certain biomarkers, including COL6A1, in BC patients [14,15]. The specific molecular mechanisms underlying expression of *COL6A1* in BC remain unclear. It is worth noting that the two types of BC (NMIBC and MIBC) exhibit different molecular characteristics in terms of pathogenesis and progression, which necessitates separate analyses of the two types. In particular, T1HG BC falls under the classification of NMIBC, but demonstrates biological and clinical prognostic features resembling those of MIBC; therefore, it is crucial to conduct separate analyses to determine the role of *COL6A1* in progression of T1HG BC. The aim of this study was to investigate the role of *COL6A1* in T1HG BC, and to evaluate its potential as a prognostic marker for predicting disease progression.

MATERIALS AND METHODS

1. Ethics statement

The Ethics Committee of Chungbuk National University approved the protocol, and written informed consent was obtained from each subject. The collection and analysis of all samples were approved by the Institutional Review Board (IRB) of Chungbuk National University (IRB approval number CBNU-202301-BR-0285), and informed consent was obtained from each subject.

2. Study design

The workflow and overall study design are shown in Fig. 1. A previous study reported differential expression of *COL6A1* mRNA in normal control (normal tissue surrounding BC), NMIBC, and MIBC tissues (all $p<0.05$). Specifically, expression of *COL6A1* was significantly lower in both NMIBC and MIBC tissues than in controls ($p<0.0001$), while it was expressed at higher levels in MIBC than in NMIBC ($p<0.05$) [12]. To identify the relationship between *COL6A1* levels and progression of T1HG BC, the present study evaluated expression of the *COL6A1* gene in patients with BC of different pathological stage and grade, as well as differing prognoses. This was done by conducting RNA sequencing (RNA-seq) and real-time polymerase chain reaction (PCR) in BC samples. In the test cohort, expression of *COL6A1* was first examined in patients with BC of varying pathological stage and grade. Subsequently, expression of *COL6A1* in T1HG BC cases with disease progression was compared with that in those without progression. Afterwards, *COL6A1* gene expression was subjected to a comprehensive analysis using real-time PCR of samples from the first validation cohort. A second validation cohort comprising 82 T1HG BC patients was utilized to demonstrate the performance of *COL6A1* as a prospective marker for predicting disease progression.

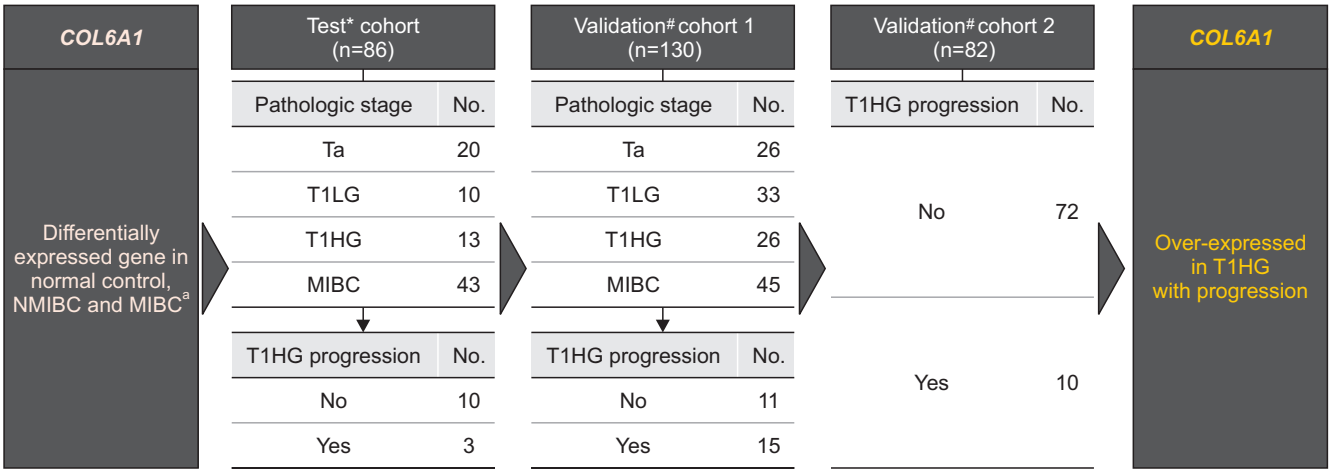

Fig. 1. Overall study design. [a]:Denotes reference [12]. *RNA-seq-based cohort. #Real-time polymerase chain reaction-based cohort. *COL6A1*, collagen type VI alpha 1; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle invasive bladder cancer; T1LG, T1 low grade; T1HG, T1 high grade.

Comprehensive details regarding patient information and tissue sample descriptions can be found in Table 1 and the online only Supplementary material.

3. RNA extraction

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen) and stored at -80℃. Next, cDNA was synthesized from 1 μg total RNA using the First Strand cDNA Synthesis Kit (Clontech, Takara), according to the manufacturer's protocol.

4. RNA-sequencing analysis

The value of RNA Integrity Number (RIN) and the DV200 metric were measured using an RNA 6000 Nano Kit and an Agilent 2100 Bioanalyzer (Agilent Technologies) to confirm the quality and integrity of the RNA. RNA samples with a RIN >7 were designated as "good total RNA quality" and were selected for downstream applications. Total RNA (500 ng) was processed to prepare a whole transcriptome sequencing library. Enrichment of whole transcriptome RNA was conducted by depleting ribosomal RNA (rRNA) prior to preparation of the whole transcriptome sequencing library using the MGIEasy RNA Directional Library Prep Kit (MGI Tech Co., Ltd.) as described previously [16]. Reference genome sequence data from Homo sapiens were obtained from the NCBI Genome database (assembly ID: GRCh38). Reference genome indexing and read mapping of tissue samples were performed using STAR software (ver. 2.5.4b).

5. Real-time PCR analysis

Relative gene expression was analyzed by real-time PCR using the 2-ΔΔCq method [17]. Amplification of mRNA was performed using a Rotor Gene 6000 instrument (Qiagen GmbH). For the real-time PCR reactions, microtubes (Qiagen GmbH) containing SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Inc.) were utilized. The following primers were used to amplify the candidate gene: *COL6A1* sense, 5'-CGTGGACCTGTTCTTTGTG-3', and *COL6A1* antisense, 5'-CGTCACTGTAGTGCAGCG-3'. GAPDH, used for normalization, was amplified using the following primers: *GAPDH* sense, 5'-CATGTTCGTCATGGGTGTGA-3', and *GAPDH* antisense, 5'-ATGGCATGGACTGTGGTCAT-3'.

6. Statistical analysis

Continuous variables are expressed as the mean±standard deviation. The normality of the data was estimated by the One-Sample Kolmogorov-Smirnov test. Gene expression values were natural log-transformed and median-centered across samples. One-way ANOVA, followed by Tukey's honestly significant difference test, was used to examine *COL6A1* gene expression in patients with different stage, grade, and prognoses in each of the three different cohorts. Homogeneous subset tables show which groups have the same mean and which groups have a different mean. Student's t-test was used to compare *COL6A1* expression in T1HG BC patients with or without progression. The significance of various clinicopathological variables was evaluated using univariate and multivariate Cox proportional hazard regression models. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to investigate relative risk. Survival curves were plotted using the Kaplan–Meier method and used to determine the prognostic value of the genetic biomarker. Data were compared using the log-rank test. The optimal cut-off point for stratifying patients into low-*COL6A1* and high-*COL6A1* was calculated by receiver operating characteristic (ROC) curve analysis using the Youden index

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Table 1. Clinicopathological features of the primary BC patients

Variable	Test cohort (RNA-seq)	Validation cohort1 (real-time PCR)	Validation cohort2 (real-time PCR)
		BC	T1HG BC
No.	86	130	82
Age (y)	68.58±12.77	65.67±11.65	68.17±12.17
Sex			
Male	63 (73.3)	106 (81.5)	72 (87.8)
Female	23 (26.7)	24 (18.5)	10 (12.2)
Operation			
TUR-BT	69 (80.2)	97 (74.6)	72 (87.8)
RC	17 (19.8)	33 (25.4)	10 (12.2)
Tumor size (cm)			
≤1	33 (38.4)	24 (18.5)	38 (46.3)
2–3	50 (58.1)	43 (33.1)	41 (50.0)
>3	3 (3.5)	63 (48.5)	3 (3.7)
Multiplicity			
Single	32 (37.2)	83 (63.8)	29 (35.4)
2–7	31 (36.0)	33 (25.4)	37 (45.1)
>7	23 (26.8)	14 (10.8)	16 (19.5)
Grade, 2004 WHO grading system			
Low	26 (30.2)	65 (50.0)	-
High	60 (69.8)	65 (50.0)	82 (100.0)
Stage			
TaN0M0	20 (23.3)	26 (20.0)	-
T1N0M0	23 (26.7)	59 (45.4)	82 (100.0)
≥T2 or ≥N1 or M1	43 (50.0)	45 (34.6)	-
Progression			
No	77 (89.5)	93 (71.5)	72 (87.8)
Yes	9 (10.5)	37 (28.5)	10 (12.2)
Mean follow-up (mo)	51.95 (1–227.33)	62.19 (1–172.20)	73.65 (1–239.17)

Values are presented as number only, mean±standard deviation, number (%), or number (range).
BC, bladder cancer; PCR, polymerase chain reaction; T1HG, T1 high grade; TUR-BT, transurethral resection of bladder tumor; RC, radical cystectomy; WHO, World Health Organization.

[18]. Statistical analysis was performed using SPSS v24.0 (IBM Co.), GraphPad Prism 8 (GraphPad Software), and MedCalc software ver. 20.0 (MedCalc Software). p-values <0.05 were considered significant.

RESULTS

1. Expression of *COL6A1* mRNA in BC tissues from the test RNA-seq cohort

Expression of *COL6A1* mRNA in T1HG BC samples was comparable with that in MIBC samples, and both showed higher expression than Ta and T1 low grade (T1LG) BC (p<0.05, respectively) (Fig. 2A). Table 2 lists the homogeneous subsets identified by one-way ANOVA with Tukey's post-hoc analysis. There are two distinct homogeneous subsets. One includes Ta and T1LG BC patients, with no significant difference in *COL6A1* expression between the two groups. The other subset comprises T1HG and MIBC patients, who show higher expression of *COL6A1* than the former subset and form a separate homogeneous subset, again with no significant difference in *COL6A1* expression between the two groups. These findings support the notion that T1HG BC is more similar to MIBC than NMIBC in terms of inherent characteristics. Consequently, we created a combined group comprising Ta and T1LG BC patients, and subdivided the T1HG group into two: T1HG patients with progression and T1HG patients without progression. Analysis of *COL6A1* mRNA expression within each of these groups revealed significantly higher expression in the progression group (p<0.0001). Furthermore, expression of *COL6A1* mRNA in MIBC was significantly higher than that in Ta, T1LG, and T1HG without progression (p<0.05, respectively) (Fig. 2B).

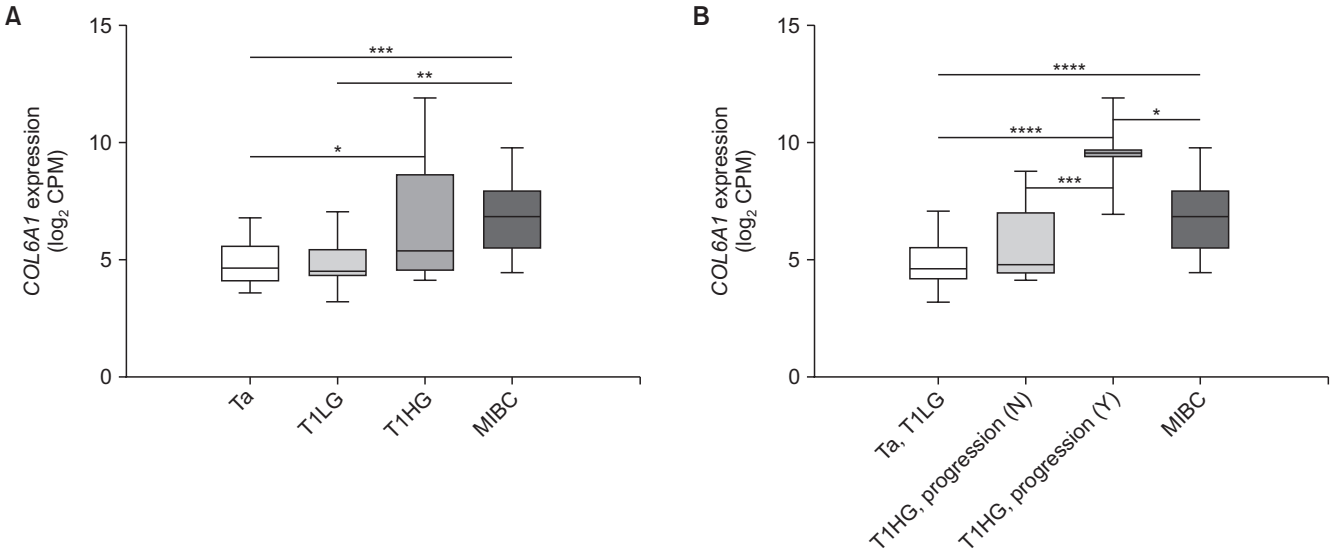

Fig. 2. Expression of *COL6A1* mRNA in BC tissues from the test RNA-seq cohort. (A) Comparison of *COL6A1* mRNA expression in the Ta, T1LG, T1HG, and MIBC groups. (B) Comparison of *COL6A1* mRNA expression in the Ta and T1LG, T1HG without progression, T1HG with progression, and MIBC groups. p-values were calculated using one-way ANOVA followed by the Tukey's honestly significant difference test. ****p<0.0001, ***p<0.001, **p<0.01, and *p<0.05. *COL6A1*, collagen type VI alpha 1; BC, bladder cancer; T1LG, T1 low grade; T1HG, T1 high grade; MIBC, muscle invasive bladder cancer.

Table 2. Homogeneous BC subsets identified by Tukey's honestly significant difference test of *COL6A1* gene expression data

BC groups according to diverse stage and grade	N	Homogeneous subsets	
		1	2
Ta	20	4.8887[a]	
T1LG	10	4.8945[a]	
T1HG	13		6.5387[a]
MIBC	43		6.8026[a]
Sig.		1.000	0.963

BC groups according to diverse stage, grade, and prognosis	N	1	2	3
Ta, T1LG	30	4.8907[a]		
T1HG without future progression	10	5.6599[a]	5.6599[a]	
MIBC	43		6.8026[a]	
T1HG with future progression	3			9.4677[a]
Sig.		0.691	0.367	1.000

BC, bladder cancer; T1LG, T1 low grade; T1HG, T1 high grade; MIBC, muscle invasive bladder cancer; Sig., statistical significance.
[a]:Mean expression value of *COL6A1* in each group.

Tukey's post-hoc analysis suggested that there are three distinct homogeneous subsets. Notably, the T1HG group with progression formed a separate homogeneous subset with significantly higher expression of *COL6A1* than the other groups (Table 2). This suggests that *COL6A1* is a predictive marker for identifying patients at high risk of disease progression. Subsequent to the initial findings, further validation tests were carried out to confirm the prognostic value of *COL6A1* overexpression in T1HG BC.

2. Expression of *COL6A1* mRNA in BC tissues from the two different validation cohorts

Analysis of the first validation cohort revealed that expression of *COL6A1* in the T1HG with progression group was significantly higher than that in the Ta/T1LG, T1HG without progression, and MIBC groups (p<0.05, respectively; Fig. 3A). Analysis using Tukey's post-hoc analysis revealed two distinct homogeneous subsets. The first subset comprised Ta/T1LG BC, and T1HG BC patients without progression; there was no significant difference in *COL6A1* expression between these two groups. The second subset comprised

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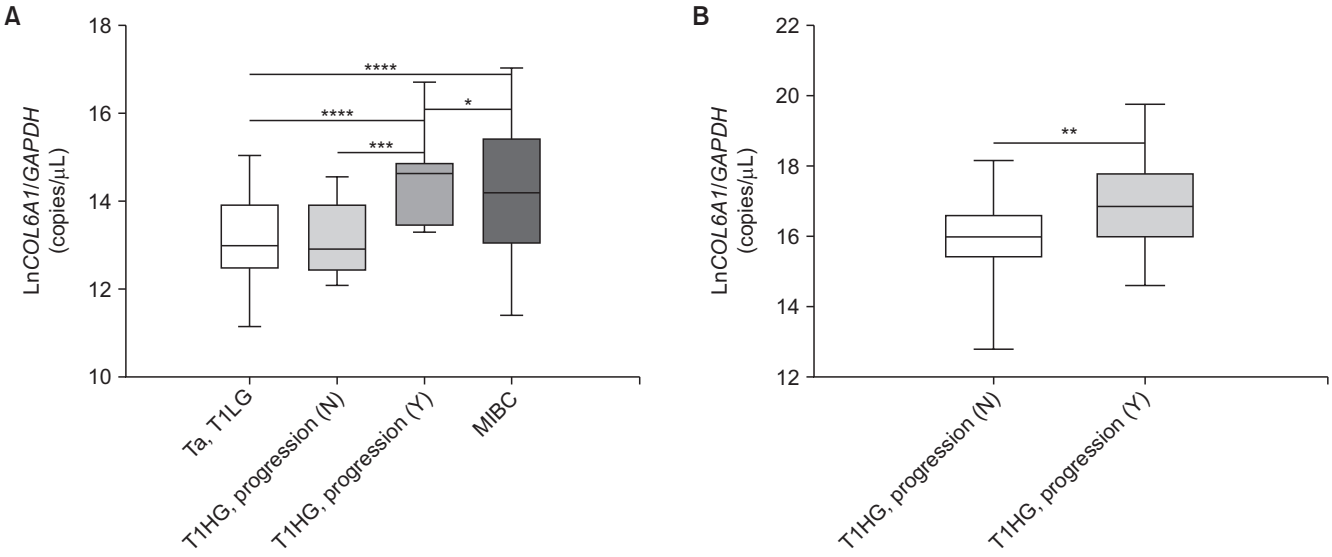

Fig. 3. Expression of *COL6A1* mRNA in BC tissues from the two different validation cohorts. (A) Comparison of *COL6A1* mRNA expression by the Ta and T1LG, T1HG without future progression, T1HG with future progression, and MIBC groups in the first validation cohort. (B) Comparison of *COL6A1* mRNA expression by T1HG BC patients with diverse prognoses. p-values were calculated using one-way ANOVA followed by the Tukey's honestly significant difference test and by Student's t-test. ****p<0.0001, ***p<0.001, **p<0.01, and *p<0.05. *COL6A1*, collagen type VI alpha 1; BC, bladder cancer; T1LG, T1 low grade; T1HG, T1 high grade; MIBC, muscle invasive bladder cancer.

Table 3. Homogeneous BC subsets based on *COL6A1* gene expression, as assessed by a Tukey's honestly significant difference test of data from the first validation cohort

Bladder cancer group according to stage, grade, and prognosis	N	Homogeneous subsets	
		1	2
Ta, T1LG	59	13.1437[a]	
T1HG without future progression	11	13.1739[a]	
MIBC	45		14.2761[a]
T1HG with future progression	15		14.4278[a]
Sig.		1.000	0.978

BC, bladder cancer; T1LG, T1 low grade; T1HG, T1 high grade; MIBC, muscle invasive bladder cancer; Sig., statistical significance.
[a]:Mean expression of *COL6A1* in each group.

primary MIBC patients and T1HG patients who later progressed to MIBC. Both groups demonstrated comparable expression of *COL6A1* and, importantly, expression levels of *COL6A1* in this second subset was higher than in the first subset (i.e., Ta/T1LG BC, and T1HG BC without progression). These findings provide compelling evidence supporting the notion that patients with T1HG BC, particularly those who experience progression later, share similarities with MIBC, and suggest a potential role for *COL6A1* in progression of T1HG BC towards an MIBC phenotype (Table 3). The second validation cohort comprised T1HG BC patients. We found a significant increase in expression of *COL6A1* mRNA among T1HG patients who later progressed to MIBC compared with those who did not (p<0.01) (Fig. 3B). This finding further supports the association between *COL6A1* expression and progression of T1HG BC, indicating that *COL6A1* is a potential biomarker for identifying patients at higher risk of disease advancement.

3. Overexpression of *COL6A1* mRNA associates with T1HG progression

To assess the predictive value of *COL6A1* expression for determining progression-free survival (PFS) of patients with T1HG BC, we conducted ROC analysis using the Youden index to determine an optimal cut-off value for *COL6A1* expression. An ideal cut-off of 16.777 was established, with a sensitivity of 70% and a specificity of 79.2% for discriminating patients with progression from those without (Supplementary Table 1). Based on this cut-off value, T1HG patients in the second validation cohorts were divided into low-*COL6A1* and high-*COL6A1* groups. Univariate and multivariate Cox regression analyses revealed that T1HG patients

Table 4. Univariate and multivariate Cox regression analyses of factors predicting T1HG progression

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age				
≤70 (Ref.) vs. >70	2.096 (0.589–7.460)	0.253	2.621 (0.650–10.576)	0.176
Sex				
Male (Ref.) vs. female	24.193 (0.004–144,453.308)	0.472	705,957.008 (0.000–)	0.980
Tumor size				
≤3 cm (Ref.) vs. >3 cm	2.179 (0.559–8.4594)	0.262	3.636 (0.780–16.946)	0.100
Multiplicity				
Single (Ref.)				
2–7	2.634 (0.529–13.108)	0.237	4.353 (0.740–25.597)	0.104
Multiple	2.651 (0.367–19.179)	0.334	3.572 (0.409–31.203)	0.250
COL6A1 expression				
Low expression (Ref.) vs. high expression	8.520 (2.199–33.005)	0.002*	16.812 (3.283–86.095)	0.001*

T1HG, T1 high grade; HR, hazard ratio; CI, confidence interval; Ref., reference.
*p<0.05.

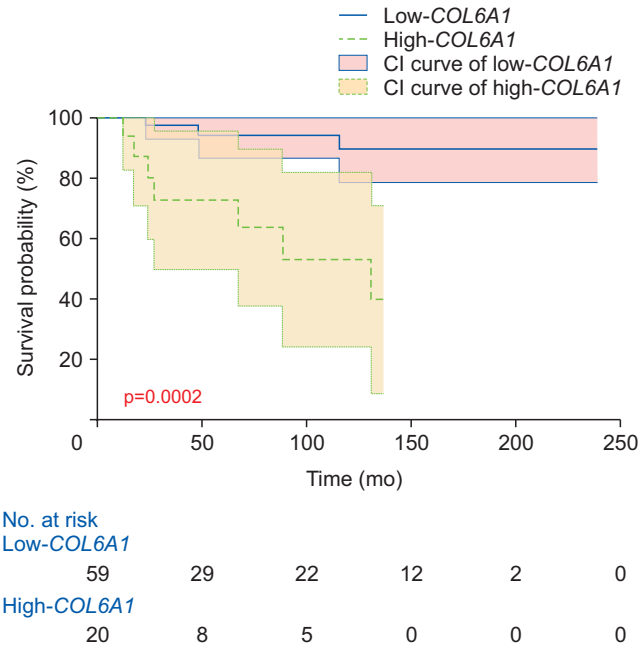

Fig. 4. Kaplan–Meier curves showing the effect of *COL6A1* on progression-free survival of patients with T1HG. T1HG BC patients were divided into low-*COL6A1* and high-*COL6A1*-expressing groups according to the receiver operating characteristic-based optimal cut-off value. The progression-free survival rate of T1HG BC patients with high expression of *COL6A1* was significantly lower than that of those with low *COL6A1* expression (log-rank test, p=0.0002). *COL6A1*, collagen type VI alpha 1; T1HG, T1 high grade; BC, bladder cancer; CI, confidence interval.

in the high-*COL6A1* group had a higher risk of progression than those in the low-*COL6A1* group (HRs, 8.520 and 16.812; 95% CIs, 2.199–33.005 and 3.283–86.095; respectively; both p<0.005; Table 4). This indicates that *COL6A1* expression can serve as an independent predictor of T1HG progression. No other clinico-pathological factors were significant predictors of the risk of T1HG progression (Table 4). Furthermore, Kaplan–Meier analysis revealed that T1HG BC patients in the low-*COL6A1* group had a significantly lower likelihood of progressing to MIBC than those in the high-*COL6A1* group (log-rank test, p=0.0002; Fig. 4). These findings highlight the potential prognostic value of *COL6A1* expression for predicting T1HG progression, and support its use as a predictive biomarker for clinical decision-making.

DISCUSSION

T1HG tumors are very aggressive, with high rates of progression (ranging from 30%–50%). These rates can increase during long-term follow-up, leading to lower survival rates [19-21]. The clinical heterogeneity of T1HG BC has led to an ongoing debate regarding the optimal treatment strategy for individual patients. For example, there are instances in which a patient with T1HG BC who received BCG therapy but progressed to MIBC or N1 was considered undertreated; by contrast, a patient who had the potential to respond well to BCG therapy, but underwent RC instead, was considered to be overtreated [19]. In recent times, early RC has gained increasing importance as a treatment for T1HG BC, although BCG therapy remains the current gold-standard adjuvant therapy [19-21]. However, a tool that can accurately predict prognosis of T1HG patients and provide valuable recommendations regarding the most appropriate course of action for each individual patient is needed urgently.

Carcinoma *in situ* (CIS) is a flat tumor confined to the urothelium of the bladder. Like T1HG, it is a low stage, high grade, and highly malignant tumor. Research indicates that the presence of CIS is an important prognostic indicator for

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T1HG, and that T1HG patients with associated CIS have a higher rate of progression [22]. However, CIS as a prognostic indicator presents challenges due to a difficulty in diagnosing this tiny lesion. Some reports suggest that the presence of CIS is associated only with recurrence rate, and that it does not affect disease progression [23]. Another study points out that inconsistencies in reporting the coexistence of CIS and T1 tumor pathology could potentially impact the analysis of results [24]. Given the high heterogeneity of BC, the clinical and pathological indicators used for prognosis and treatment decisions remain the subject of debate. Therefore, recent studies have placed increasing emphasis on development/identification of molecular biomarkers. The European UROMOL project [25] suggested that the gene expression profiles of most T1, cT2, and CIS patients are similar, further indicating their high-risk nature. Another molecular study of T1 BC classified 149 patients into urobasal, genomically unstable (GU), and squamous cell-like (SCCL) subtypes. GU and SCCL tumors showed similar invasiveness, accompanied by higher rates of lymphovascular invasion, CIS, and deep invasion, suggesting that they have higher risk of progression than urobasal tumors [25]. The authors proposed the need for further validation to determine whether the gene profiles used to identify GU or SCCL tumors can serve as a prognostic biomarker for T1 BC [26]. In addition, Breyer et al. [27] investigated expression of the immune checkpoint receptor PD-L1 in paraffin tissue samples from T1 BC patients and found that those with higher expression of PD-L1 had relatively higher rates of recurrence-free survival, PFS, and tumor-specific survival than those with lower expression. These results support the significant value of molecular biomarkers for risk stratification of T1HG BC patients. However, further validation is needed to confirm and strengthen these findings. Regarding utilization of biomarkers in real clinical practice, it is desirable to have a limited number that are easy to measure and interpret. Simplifying the marker panel and adopting straightforward methods of analysis can facilitate integration into routine clinical workflows.

Previously, we identified differential expression of *COL6A1* among normal controls, NMIBC, and MIBC patients [12]. Collagen is a major component of the ECM, an important component of the TME that plays a key role in cancer development, metastasis, and resistance to treatment [28]. However, the role of type VI collagen in the specific pathological status of T1HG BC remains unclear. In the present study, we investigated expression of *COL6A1* in T1HG BC using three different patient cohorts. We observed that expression of *COL6A1* in T1HG BC was comparable with that in MIBC, and higher than that in NMIBC patients with Ta and T1LG disease (Fig. 2, Table 2). In particular, *COL6A1* expression was higher in T1HG patients who went on to experience progression than in those with Ta, T1LG, T1HG without progression, and MIBC (Fig. 3A). Furthermore, we found a significant association between high *COL6A1* expression and poor PFS of patients with T1HG BC (Fig. 4). Utilization of multiple cohorts increases the reliability of our findings because it allows assessment of *COL6A1* expression across diverse patient populations with diverse follow-up information. The heterogeneous expression of *COL6A1* among BC patients with different pathological stages and grades reflect the inherent heterogeneity of the disease. Specifically, the expression profile of *COL6A1* in T1HG BC is different from that in NMIBC, suggesting that T1HG should be treated differently due to its aggressive nature. In addition, the observed association between increased *COL6A1* expression and poor PFS suggests that *COL6A1* can serve as a potential prognostic biomarker for T1HG BC.

CONCLUSIONS

The findings reported herein increase our understanding of the molecular mechanisms underlying progression of T1HG BC, and highlight the potential clinical significance of *COL6A1* as a predictive marker for patient outcomes. Further research, along with more validation tests, is warranted to confirm these results and to explore the biological pathways associated with *COL6A1* expression in T1HG BC.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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SUPPLEMENTARY MATERIALS

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
2. Klaassen Z, Kamat AM, Kassouf W, Gontero P, Villavicencio H, Bellmunt J, et al. Treatment strategy for newly diagnosed T1 high-grade bladder urothelial carcinoma: new insights and updated recommendations. Eur Urol 2018;74:597-608.
3. Chang SS, Boorjian SA, Chou R, Clark PE, Daneshmand S, Konety BR, et al. Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline. J Urol 2016;196:1021-9.
4. Ping Z, Zhan X, Chen T, Zheng Y, Jiang M, Li Y, et al. Survival outcome of partial cystectomy versus transurethral bladder tumor resection in T1 high-grade bladder cancer patients: a propensity score matching study. J Oncol 2022;2022:3016725.
5. Babjuk M, Burger M, Capoun O, Cohen D, Compérat EM, Dominguez Escrig JL, et al. European Association of Urology guidelines on non-muscle-invasive bladder cancer (Ta, T1, and carcinoma in situ). Eur Urol 2022;81:75-94.
6. Yun SJ, Kim SK, Kim WJ. How do we manage high-grade T1 bladder cancer? Conservative or aggressive therapy? Investig Clin Urol 2016;57(Suppl 1):S44-51.
7. Kim WJ, Kim EJ, Kim SK, Kim YJ, Ha YS, Jeong P, et al. Predictive value of progression-related gene classifier in primary non-muscle invasive bladder cancer. Mol Cancer 2010;9:3.
8. Hurst CD, Platt FM, Taylor CF, Knowles MA. Novel tumor subgroups of urothelial carcinoma of the bladder defined by integrated genomic analysis. Clin Cancer Res 2012;18:5865-77.
9. Bartsch G Jr, Mitra AP, Mitra SA, Almal AA, Steven KE, Skinner DG, et al. Use of artificial intelligence and machine learning algorithms with gene expression profiling to predict recurrent nonmuscle invasive urothelial carcinoma of the bladder. J Urol 2016;195:493-8.
10. van Rhijn BW, Liu L, Vis AN, Bostrom PJ, Zuiverloon TC, Fleshner NE, et al. Prognostic value of molecular markers, substage and European Organisation for the Research and Treatment of Cancer risk scores in primary T1 bladder cancer. BJU Int 2012;110:1169-76.
11. Wankowicz SAM, Werner L, Orsola A, Novak J, Bowden M, Choueiri TK, et al. Differential expression of PD-L1 in high grade T1 vs muscle invasive bladder carcinoma and its prognostic implications. J Urol 2017;198:817-23.
12. Piao XM, Hwang B, Jeong P, Byun YJ, Kang HW, Seo SP, et al. Collagen type VI-α1 and 2 repress the proliferation, migration and invasion of bladder cancer cells. Int J Oncol 2021;59:37.
13. Chen P, Cescon M, Bonaldo P. Collagen VI in cancer and its biological mechanisms. Trends Mol Med 2013;19:410-7.
14. Zhu H, Chen H, Wang J, Zhou L, Liu S. Collagen stiffness promoted non-muscle-invasive bladder cancer progression to muscle-invasive bladder cancer. Onco Targets Ther 2019;12:3441-57.
15. Shi S, Tian B. Identification of biomarkers associated with progression and prognosis in bladder cancer via co-expression analysis. Cancer Biomark 2019;24:183-93.
16. Kim SK, Park SH, Kim YU, Byun YJ, Piao XM, Jeong P, et al. A molecular signature determines the prognostic and therapeutic subtype of non-muscle-invasive bladder cancer responsive to intravesical Bacillus Calmette-Guérin therapy. Int J Mol Sci

2021;22:1450.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 2001;25:402-8.
18. Ogłuszka M, Orzechowska M, Jędroszka D, Witas P, Bednarek AK. Evaluate cutpoints: adaptable continuous data distribution system for determining survival in Kaplan-Meier estimator. Comput Methods Programs Biomed 2019;177:133-9.
19. Morales A, Eidinger D, Bruce AW. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol 2017;197(2S):S142-5.
20. Jordan B, Meeks JJ. T1 bladder cancer: current considerations for diagnosis and management. Nat Rev Urol 2019;16:23-34.
21. Zhan X, Chen L, Jiang M, Fu B. Development and validation of a prognostic nomogram for predicting overall survival for T1 high-grade patients after radical cystectomy: a study based on SEER. Int J Gen Med 2022;15:3753-65.
22. Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 2006;49:466-5; discussion 475-7.
23. Fernandez-Gomez J, Solsona E, Unda M, Martinez-Piñeiro L, Gonzalez M, Hernandez R, et al.; Club Urológico Español de Tratamiento Oncológico (CUETO). Prognostic factors in patients with non-muscle-invasive bladder cancer treated with Bacillus Calmette-Guérin: multivariate analysis of data from four randomized CUETO trials. Eur Urol 2008;53:992-1001.
24. Martin-Doyle W, Leow JJ, Orsola A, Chang SL, Bellmunt J. Improving selection criteria for early cystectomy in high-grade t1 bladder cancer: a meta-analysis of 15,215 patients. J Clin Oncol 2015;33:643-50.
25. Hedegaard J, Lamy P, Nordentoft I, Algaba F, Høyer S, Ulhøi BP, et al. Comprehensive transcriptional analysis of early-stage urothelial carcinoma. Cancer Cell 2016;30:27-42.
26. Patschan O, Sjödahl G, Chebil G, Lövgren K, Lauss M, Gudjonsson S, et al. A molecular pathologic framework for risk stratification of stage T1 urothelial carcinoma. Eur Urol 2015;68:824-32; discussion 835-6.
27. Breyer J, Wirtz RM, Otto W, Erben P, Worst TS, Stoehr R, et al. High PDL1 mRNA expression predicts better survival of stage pT1 non-muscle-invasive bladder cancer (NMIBC) patients. Cancer Immunol Immunother 2018;67:403-12.
28. Quiles CG, Mallender R, More E, Humphries J, Humphries M, Whetton A, et al. Proteomic profiling of hypoxia-induced changes in cell-derived extracellular matrix from bladder cancer cell lines. Int J Radiat Oncol Biol Phys 2021;111(3 Suppl):E254.