Evaluation of prolyl-4-hydroxylase subunit beta and special AT-rich region-binding protein-1 immunoexpression in bladder transitional-cell carcinoma

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Background
Prolyl-4-hydroxylase subunit beta (P4HB) and special AT-rich region-binding protein-1 (SATB1) have been implicated in tumorigenesis and progression in many cancers, but their significance in bladder urothelial carcinoma remains to be elucidated. This study aimed to investigate the correlation and prognostic value of P4HB and SATB1 expression along with clinicopathological features in bladder transitional-cell carcinoma.
Patients and methods
This is a retrospective, selected, controlled study carried on 50 cases of bladder urothelial carcinoma to detect the expression of P4HB and SATB1 immunohistochemistry and statistical correlation with various clinicopathological parameters, including molecular subtypes.
Results
Prolyl-4-hydroxylase subunit beta (P4HP) is highly expressed in 48% of the study cases. P4HP expression was significantly associated with size of the tumor (*P*=0.002), muscle invasion (*P*=0.000), the grade of tumor (*P*=0.000), and the depth of invasion of the primary tumor (T) (*P*=0.000). High SATB1 expression was detected in 46% of the study cases. A significant association was detected between SATB1 expression and molecular subtypes (*P*=0.001), size of the tumor (*P*=0.004), histopathological type (*P*=0.024), muscle invasion (*P*=0.000), the grade of tumor (*P*=0.000), and the depth of invasion of the primary tumor (T) (*P*=0.000). Receiver operating characteristic curve was carried on for P4HP and SATB1 in relation to molecular classification and showed that SATB-1 has the highest sensitivity (75%) and specificity (70%) in discrimination between luminal versus nonluminal subtypes with significant relation (*P*=0.01). There was significant association between P4HP and SATB1 expression in bladder urothelial transitional-cell carcinoma (*P*=0.000).
Conclusion
This study highlighted important information about the link between P4HB and SATB1 pathways during the progression of urinary bladder transitional-cell carcinoma. P4HB and SATB1 could be used as a prognostic marker in cases in urinary bladder transitional-cell carcinoma.

Keywords:
bladder urothelial carcinoma, prolyl-4-hydroxylase subunit beta, the special AT-rich region-binding protein

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Introduction

Urinary bladder cancer is ranked the 10th most common cancer in the world, and its incidence is steadily rising worldwide, especially in developed nations (Saginala *et al.*, 2020). In Egypt, bladder cancer is the third most common malignancy (6.9%) after breast cancer and hepatocellular carcinoma and constitutes a significant proportion of cancer morbidity and mortality (Zaghloul *et al.*, 2020; Lotfy et al., 2021).

Bladder cancer is characterized by being a heterogeneous disease. Most bladder cancers are urothelial carcinomas, and are classified as either nonuscle-invasive bladder cancer or muscle-invasive bladder cancer (Kamoun *et al.*, 2020). Genomic-profiling studies have demonstrated that bladder cancer can be divided into molecular subtypes referred to as luminal, basal, and double negative with distinct clinical behaviors and sensitivities to chemotherapy (Guo *et al.*, 2020).

The endoplasmic reticulum (ER) has an essential role in calcium dynamic balance and participates in folding of membrane-bound proteins and secretory proteins (Adams *et al.*, 2019). Various cellular stresses such as hypoxia lead to breakdown of ER and loss of its homeostasis, resulting in accumulation of abnormal and misfolded proteins (Grandjean and Wiseman, 2020).

A series of signaling pathways named unfolded protein response (UPR) is activated to restore the ER homeostasis (Selles *et al.*, 2017). Malignant tumors are well known to be associated with different cellular stresses such as hypoxia, acidosis, and nutrient deprivation. Growing evidence indicates that UPR occurs in different types of malignancy. Protein-disulfide isomerases (PDI) are one of these signaling pathways (Huang *et al.*, 2021).

Prolyl-4-hydroxylase subunit beta (P4HB), is a member of PDI. P4HB is a highly abundant multifunctional enzyme that acts as an ER chaperone to inhibit the aggregation of misfolded protein (Sun *et al.*, 2017). P4HB is approved to be upregulated in a variety of cancers such as hepatocellular carcinoma (Xia *et al.*, 2017), gastric carcinoma (Zhang *et al.*, 2018), and lymphoma (Jiang *et al.*, 2018).

Special AT-rich-binding protein (SATB) family proteins have emerged as key regulators that integrate higher-order chromatin organization and consist of two proteins SATB-1 and SATB-2 (Guo *et al.*, 2017).

The SATB1 is a nuclear matrix-attachment region-binding protein that has been reported to be a 'genome organizer' that delineates specific epigenetic modifications at target gene (Frömberg *et al.*, 2018). SATB1 has been associated with the development of several types of cancer, including glioma, colorectal, breast, lung, and kidney cancers (Nüssing *et al.*, 2019).

The aim of this study was to investigate the immunoexpression of P4HB and SATB1 and correlate their immunoexpression with different clinicopathological parameters, including molecular subtypes of bladder transitional-cell carcinoma. The correlation between two markers will also be identified.

Patients and methods

This retrospective study was carried upon 50 selected cases of bladder transitional-cell carcinoma (28 cases with radical cystectomy and 22 cases with transurethral-resection biopsy). Six cases of normal urothelium were taken as control. The studied cases included archival formalin-fixed, paraffin-embedded blocks processed from January 2017 to December 2020 from the Pathology Department of Benha Faculty of Medicine and Early Cancer Detection Unit (ECDU), after obtaining approval from the Institutional Ethics Committee (77-2022).

Histopathological evaluation

Hematoxylin-stained and eosin-stained slides on all cases were reviewed to confirm the original diagnosis and classify the lesions into papillary and nonpapillary urinary-bladder transitional-cell carcinoma. According to muscle invasion, it is classified into nonmuscle-invasive bladder cancers and muscle-invasive bladder cancers (Smith *et al.*, 2014). Molecular subtyping was based on previous immunostaining of combined GATA3 and CK5/6 that classified the cases into luminal subtype (GATA3+ve, CK5/6–ve), basal subtype (GATA3–ve, CK5/6+ve), and double-negative subtype (GATA3–ve, CK5/6–ve) (Dadhania *et al.*, 2016).

Clinicopathological parameters, including age, sex, size of the tumor, carcinoma in situ (CIS), tumor grade based on pathologic findings following the 2016 WHO grading system (Compérat *et al.*, 2018), depth of tumor (T) lymph-node metastasis (N), and distant metastasis (M) (Wang and McKenney, 2018), were obtained from patient's files.

Immunohistochemical evaluation

In all, 4-μm tissue sections were obtained from formalin-fixed, paraffin-embedded tissue blocks on coated slides. The manufacturer's instructions were followed using a standard labeled streptavidin–biotin system (Dako Cytomation A/S, Glostrup, Denmark). Antigen retrieval was performed by using 10 mmol/l citrate monohydrate buffer (pH 6.0) and heated for 15 min in the microwave.

The slides of both markers then were incubated overnight at 4°C with optimal dilutions of anti-SATB1 (rabbit monoclonal antibody, 1: 100 dilution; Code ab92307, Abcam, Los Angeles, CA, USA) and P4HB antibody (rabbit polyclonal antibody, dilution 1: 250, ab137110, Abcam, Cambridge, UK). Immunoreaction was visualized by adding 3,3'-diaminobenzidine as a chromogen. A section of positive endometrial carcinoma was used as external positive control for SATB-1. A section of positive breast carcinoma was used as external positive control for P4HB. For negative controls, the primary antibodies were omitted from the staining procedure, replaced by saline or phosphate buffer.

Immunohistochemical assessment

Assessment of prolyl-4-hydroxylase subunit beta expression

For P4HB, cytoplasmic staining was defined as immunoreactions. The P4HB expression status of

immunostaining was evaluated according to staining intensity and the proportion of stained cells as follows: scoring for staining intensity of stained cells: 1 (no staining), 2 (weak staining), 3 (moderate staining), and 4 (strong staining); scoring for the proportion of stained cells: 1 (≥1 to <25%), 2 (≥25 to <50%), 3 (≥50 to <75%), and 4 (≥75%). The final score was obtained by multiplying the staining-intensity score and the staining-proportion score. Bladder-carcinoma patients were categorized as either the low P4HB-expression group (1–8) or the high P4HB-expression group (9–16) (Wang *et al.*, 2020).

Assessment of special AT-rich region-binding protein-1 expression

For STAB-1, nuclear staining was defined as immunoreactions. According to the intensity and the percentage of stained cells, the score was calculated. The predominant intensity was evaluated as follows: no nuclear staining (0), weak nuclear staining (1), moderate nuclear staining (2), and strong staining (3). The percentage of stained cells was evaluated as follows: less than 5% (0), 5–25% (1), 25–50% (2), and more than 50% (3). The final score was calculated by adding the two above scores. Scores of 0–2 were defined as low-expression scores, and 3–6 were defined as high scores (Hussein *et al.*, 2021).

Statistical analysis

The collected data were tabulated and analyzed using SPSS, version 16 software (SPSS Inc., Chicago, Illinois, USA). Receiver operating characteristic curve analysis was applied to assess optimum sensitivity and specificity of study markers in diagnosis of molecular subtypes. Categorical data were presented as number and percentages using χ^2 test or Fisher's exact test for their analysis, while quantitative data were expressed as mean±SD, median, and range. Quantitative data were tested for normality using Shapiro–Wilks test assuming normality at P value more than 0.05, using Student t test to analyze parametric variables.

Results

Clinicopathological results

All clinicopathological data are shown in Table 1.

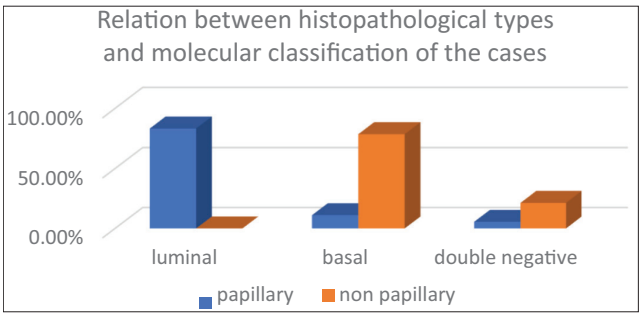
Regarding the histopathological types, it showed significant positive statistical relations with molecular classification of the cases (P<0.01) (Graph 1). No significant statistical relations were found between histopathological types and other parameters, including age (P>0.05), sex (P>0.05), muscle invasion (P>0.05), size of the tumor (P>0.05), grade (P>0.05), presence of CIS (P>0.05), T (depth of invasion)

Table 1 Clinicopathological variables of urinary-bladder transitional-cell carcinoma studied cases

Variables	*n* (%)
Age (mean±SD)=57.4±9.47	
<60	14 (28)
=or >60	36 (72)
Sex	
Male	45 (90)
Female	5 (10)
Size	
≥3	36 (72)
<3	14 (28)
Histopathology	
Papillary	36 (72)
Nonpapillary	14 (28)
Grade	
Low	22 (44)
High	28 (56)
Molecular classification	
Luminal	30 (60)
Basal	15 (30)
Double negative	5 (10)
CIS	
Present	13 (26)
Absent	37 (74)
Muscle invasion	
Present	28 (56)
Absent	22 (44)
T	
T1	22 (44)
T2	15 (30)
T3	10 (20)
T4	3 (6)
N	
N0	15 (53.6)
N1	9 (32.2)
N2	2 (7.1)
N3	2 (7.1)
M	
M0	23 (82.1)
M1	5 (17.9)

CIS, carcinoma in situ; M, distant metastasis; N, lymph-node metastasis; T, depth of invasion.

Graph 1



Relation between histopathological types and molecular classification of the studied cases.

Table 2 Relation between clinicopathological variables and prolyl-4-hydroxylase subunit beta expression in studied cases

Variables	P4HB expression		Total	*P* value
	Low	High		
Age				
<60	8 (30)	6 (25)	14 (28)	*P*=0.658
=or >60	18 (69.2)	18 (75)	36 (72)	
Sex				
Male	24 (92.3)	21 (87.5)	45 (90)	*P*=0.580
Female	2 (7.7)	3 (12.5)	5 (10)	
Size				
≥3	14 (53.8)	22 (91.7)	36 (72)	*P*=0.002 (HS)
<3	12 (46.2)	2 (8.3)	14 (28)	
Histopathology				
Papillary	20 (76.9)	16 (66.7)	36 (72)	*P*=0.430
Nonpapillary	6 (23.1)	8 (33.3)	14 (28)	
Muscle invasion				
Present	6 (23.1)	22 (91.7)	28 (56)	*P*=0.000 (HS)
Absent	20 (76.9)	2 (8.3)	22 (44)	
Grade				
Low	21 (80.8)	1 (4.2)	22 (44)	*P*=0.00 (HS)
High	5 (19.2)	23 (95.8)	28 (56)	
Molecular classification				
Luminal	17 (65.4)	13 (54.2)	30 (60)	*P*=0.309 (NS)
Basal	6 (23.1)	9 (37.5)	15 (30)	
Double negative	3 (11.5)	2 (8.3)	5 (10)	
CIS				
Present	5 (19.2)	8 (33.3)	13 (26)	*P*=0.265 (NS)
Absent	21 (80.8)	16 (66.7)	37 (74)	
T				
T1	21 (80.8)	1 (4.2)	22 (44)	*P*=0.00 (HS)
T2	1 (7.7)	13 (54.2)	15 (30)	
T3	2 (7.7)	8 (33.3)	10 (20)	
T4	1 (3.8)	2 (8.3)	3 (6)	
N				
N0	2 (40)	13 (56.5)	15 (53.6)	*P*=0.159 (NS)
N1	1 (20)	8 (43.8)	9 (32.1)	
N2	1 (20)	1 (4.3)	2 (7.1)	
N3	1 (20)	1 (4.3)	2 (7.1)	
M				
M0	3 (60)	20 (87)	23 (82.1)	*P*=0.165 (NS)
M1	2 (40)	3 (13)	5 (17.9)	

CIS, carcinoma in situ; HS, highly significant; M, distant metastasis; N, lymph-node metastasis; NS, nonsignificant; P4HB, prolyl-4-hydroxylase subunit beta; S, significant; T, depth of invasion.

(*P*>0.05), lymph-node metastasis (*P*>0.05), nor distant metastasis (*P*>0.05).

Regarding the relation between molecular subtyping and muscle invasion of the tumors, no significant statistical relation was found between them (*P*>0.05).

Immunohistochemical staining results of prolyl-4-hydroxylase subunit beta

A significant association between P4HB expression and size of the tumor (*P*=0.002), muscle invasion (*P*=0.000), the grade of tumor (*P*=0.000), and the depth of invasion of the primary tumor (T) (*P*=0.000) was found. However, the age of the patient, sex, histopathology types, CIS, molecular classification, lymph-node metastasis (N), and distant metastasis (M) showed no significant relation with P4HB expression (Table 2 and Figure 1).

Immunohistochemical staining results of special AT-rich region-binding protein-1

A significant association was detected between SATB1 expression and molecular subtypes (*P*=0.001), size of the tumor (*P*=0.004), histopathological type (*P*=0.024), muscle invasion (*P*=0.000), the grade of tumor (*P*=0.000), and the depth of invasion of the primary tumor (T) (*P*=0.000) (Table 3 and Figure 2).

Table 3 Relation between clinicopathological variables and special AT-rich region-binding protein-1 expression in studied cases

Variables	SATB1 expression		Total	*P* value
	Low	High		
Age				
<60	6 (29.6)	8 (26.1)	14 (28)	*P*=0.786
=or >60	19 (70.4)	17 (73.9)	36 (72)	
Sex				
Male	23 (85.2)	22 (95.7)	45 (90)	*P*=0.227
Female	4 (4.8)	1 (4.3)	5 (10)	
Size				
≥3	15 (55.6)	21 (58.3)	36 (72)	*P*=0.004 (HS)
<3	12 (44.4)	2 (8.7)	14 (28)	
Histopathology				
Papillary	23 (85.2)	13 (56.5)	36 (72)	*P*=0.024 (S)
Nonpapillary	4 (14.8)	10 (43.5)	14 (28)	
Muscle invasion				
Present	8 (29.6)	20 (87)	28 (56)	*P*=0.000 (HS)
Absent	19 (70.4)	3 (13)	22 (44)	
Grade				
Low	20 (74.1)	2 (8.7)	22 (44)	*P*=0.00 (HS)
High	7 (25.9)	21 (91.3)	28 (56)	
Molecular classification				
Luminal	27 (100)	3 (13)	30 (60)	*P*=0.001 (HS)
Basal	0	15 (65.2)	15 (30)	
Double negative	0	5 (21.7)	5 (10)	
Carcinoma in situ				
Present	5 (18.5)	8 (34.8)	13 (26)	*P*=0.199 (NS)
Absent	22 (81.5)	15 (56.2)	37 (74)	
T				
T1	20 (74.1)	2 (8.7)	22 (44)	*P*=0.00 (HS)
T2	6 (22.2)	9 (39.1)	15 (30)	
T3	1 (3.7)	9 (39.1)	10 (20)	
T4	0	3 (13)	3 (6)	
N				
N0	6 (58.7)	9 (42)	15 (53.6)	*P*=0.190 (NS)
N1	0	9 (42)	9 (32.1)	
N2	1 (14.3)	1 (4.8)	2 (7.1)	
N3	0	2 (9.5)	2 (7.1)	
M				
M0	6 (85.7)	17 (81)	23 (82.1)	*P*=0.786 (NS)
M1	1 (14.3)	4 (19)	5 (17.9)	

HS, highly significant; M, distant metastasis; N, lymph-node metastasis; NS, nonsignificant; S, significant; SATB1, special AT-rich region-binding protein-1; T, depth of invasion.

The relation between prolyl-4-hydroxylase subunit beta and special AT-rich region-binding protein-1 expression in urinary transitional-cell carcinoma

There was a significant association between P4HB and SATB1 immunohistochemical expression (*P*=0.000) as shown in Graph 2.

Receiver operating characteristic curve for the validity and predictivity of P4HP and special AT-rich region-binding protein-1 expression in relation to molecular subtypes

SATB1 has sensitivity (75%) and specificity (70%) in discrimination between all molecular subtypes of the study of bladder urothelial-carcinoma cases (luminal vs. nonluminal types). P4HB has sensitivity (56.3%) and specificity (54%) (Table 4, Graph 3).

Discussion

Bladder cancer is currently the 10th most commonly diagnosed malignancy worldwide. Urothelial cell carcinoma is the predominant histologic subtype of bladder cancer, contributing to more than 90% of bladder cancer cases. Approximately, 70% of patients are diagnosed with non-muscle-invasive bladder cancer (NMIBC), whereas the remaining have muscle-invasive bladder cancer (MIBC) (Yang *et al.* 2019). Bladder cancer is a biologically heterogeneous disease with complicated molecular alterations during cancer progression and metastasis (Meeks *et al.* 2020).

The present study aims to detect immunohistochemical expression of P4HB1 and SATB1 in urothelial bladder carcinoma and its relevance to the various clinicopathological features and molecular sybtypes of urothelial carcinoma. Besides, the study revealed the correlation between P4HB and SATB1.

Once ER homeostasis is disturbed, misfolded proteins accumulate, eventually leading to ER stress. In order to restore protein-folding capacity with protein-synthesis requirements, a coordinated transcriptional and translational network termed the UPR is initiated. The UPR allowed cancer cells to survive outside their normal environment (Adams *et al.*, 2019).

P4HB, also known as PDI, is a multifunctional protein that catalyzes the formation and rearrangement of disulfide bonds. It can act as a molecular chaperone to refine misfolded proteins in response to ER stress. P4HB is significantly increased in several solid tumors (Xie *et al.*, 2020).

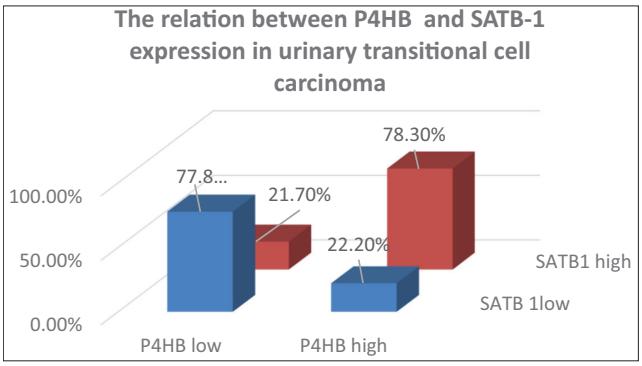
In this study, P4HB high expression was detected in 48% of urothelial-carcinoma cases, while it showed a negative expression in normal urothelial mucosa with a statistically highly significant difference, suggesting its oncogenic role in carcinogenesis of urothelial carcinoma.

In the present study, P4HB immunoexpression was significantly associated with size of the tumor (P=0.002), muscle invasion (P=0.000), the grade of tumor differentiation (P=0.000), and the depth of invasion of the primary tumor (T). However, the age of the patient, sex, histopathology types, CIS, molecular classification, lymph-node metastasis (N), and distant metastasis (M) showed no significant relation with P4HB expression.

These findings are consistent with previous studies. Wang *et al.* (2020) found that P4HB protein expression was upregulated in bladder-carcinoma tissues compared with adjacent normal tissues and that the high P4HB expression was also associated with higher tumor grade, age, and stage. Lyu *et al.* (2020) found that P4HB expression was associated with age, sex, cancer stage, and pathological TNM stage in bladder carcinoma. Wu *et al.* (2021) declared that expression of P4HB in bladder carcinoma was significantly higher at advanced pathological T stage, pathological N stage, and poor survival. Difference in the results concerning age and stage may be explained by that heterogeneity of urothelial carcinoma concerning different genetic basis may play a role. In addition, the different number of studied cases.

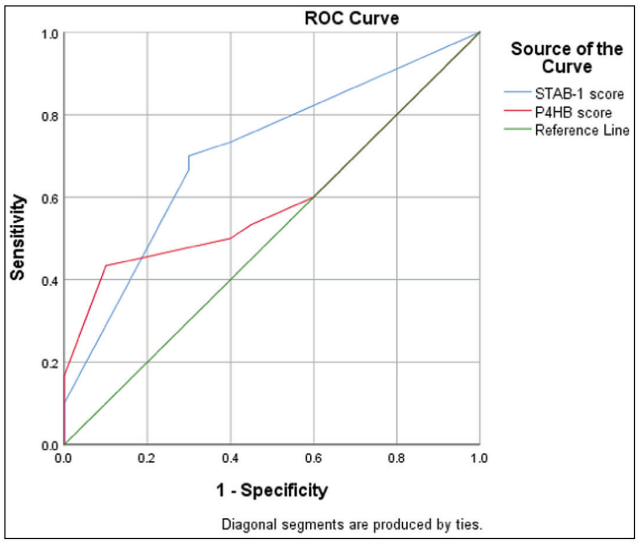
In the same context, Zou *et al.* (2018) declared that P4HB high expression was detected significantly in high-grade astrocytomas and associated with poor prognosis. Xia *et al.* (2017) found that P4HB levels were significantly correlated with the grade, stage, number

Graph 2



Relation between P4HB-1 and SATB1 expression.

Graph 3



The receiver-operating characteristic curve for the validity of P4HP and SATB1 expression in relation to molecular subtypes of the studied urinary-bladder carcinoma cases. SATB1, special AT-rich region-binding protein-1.

Table 4 Receiver operating characteristic curve for the validity and predictivity of P4HP and special AT-rich region-binding protein-1 expression in relation to molecular subtypes

Marker	AUC	Cutoff point	*P* value	Sensitivity	Specificity	PPV	NPV	Accuracy
SATB1	0.707	>4.0	0.014*	75.0	70.0	60.9	77.8	70.0
P4HB	0.601	10.5	0.23	56.3	54.0	64.0	44.0	54.0

AUC, area under the curve; NPV, negative predictive value; P4HB, prolyl-4-hydroxylase subunit beta; PPV, positive predictive value; SATB1, special AT-rich region-binding protein-1.

of tumors, and vascular invasion in hepatocellular carcinoma.

Zhu *et al.* (2019) found that P4HB is correlated with TNM staging and poor overall survival in human clear-cell renal-cell carcinoma. Zhang *et al.* (2020) stated that expression of P4HB was correlated with age, depth of tumor invasion, lymph-node metastasis, and postoperative adjuvant chemotherapy in gastric carcinoma.

The oncogenic roles of P4HB and its biological function in cancer remain elusive. Xia *et al.* (2017) had shown that P4HB promotes epithelial-to-mesenchymal transition in HCC through downregulation of glucose-regulatory protein 78. Zhou *et al.* (2019) had shown that P4HB knockdown can induce the apoptosis of human HT29 colon-cancer cell through generating reactive oxygen species and inhibiting STAT3 signaling.

Ma *et al.* (2020) had found that the knockdown of P4HB significantly decreased the expression of total and nuclear β-catenin, and downregulated the expression of Snail, indicating that P4HB may influence the epithelial–mesenchymal transition (EMT) process via the β-catenin/Snail pathway. In fact, further studies are required to detect the oncogenic role of P4HB in carcinogenesis.

The SATB1 is a genome organizer that facilitates the organization of chromatin and regulates gene expression. SATB1 plays crucial roles in tumor growth, migration, and metastasis by reprogramming of the expression of various genes by recruiting chromatin-remodeling enzymes and transcription factors to genomic DNA (Ramanujam *et al.*, 2021).

In this study, SATB1 high expression was detected in 46% of urothelial-carcinoma cases, while it showed a negative expression in healthy mucosa. Difference in SATB1 expression among the adjacent normal mucosa and malignant tumors, was statistically highly significant, suggesting that SATB1 might act as an oncoprotein and may have a role in carcinogenesis of urothelial carcinoma.

These findings are consistent with previous studies provided by Han *et al.* (2013) and Wan *et al.* (2015), who found that expression of SATB1 was significantly overexpressed in bladder-cancer tissues compared with normal bladder tissue. In addition, Han *et al.* (2013) approved the possible oncogenic role of SATB1 in bladder cancer through regulation of both cyclin D1 and cyclin E, thus, SATB1 played an important role in cell-cycle control of bladder-cancer cells.

In the present study, SATB1 immunoexpression was significantly associated with size of the tumor (P=0.004), histopathological type (P=0.024), muscle invasion (P=0.000), the grade of tumor differentiation (P=0.000), and the depth of invasion of the primary tumor (T) (P=0.000). This means that SATB1 expression is associated with poor prognostic features, suggesting its potential role as an independent prognostic factor in transitional-cell carcinoma.

This is matching with many studies in bladder carcinoma, such as Heussein *et al.* (2021) and Lotfy *et al.* (2021). Heussein *et al.* (2021) found significant correlation between SATB1 high expression and higher grade, depth of invasion of the primary tumor, lymph-node metastasis, and TNM stage. Lotfy *et al.* (2021) declared that overexpression of SATB1 was statistically associated with tumor stage, tumor grade, and tumor size. Consequently, our results might provide further evidence of being SATB1 as a biomarker of aggressiveness and poor prognosis in transitional-cell carcinoma of bladder.

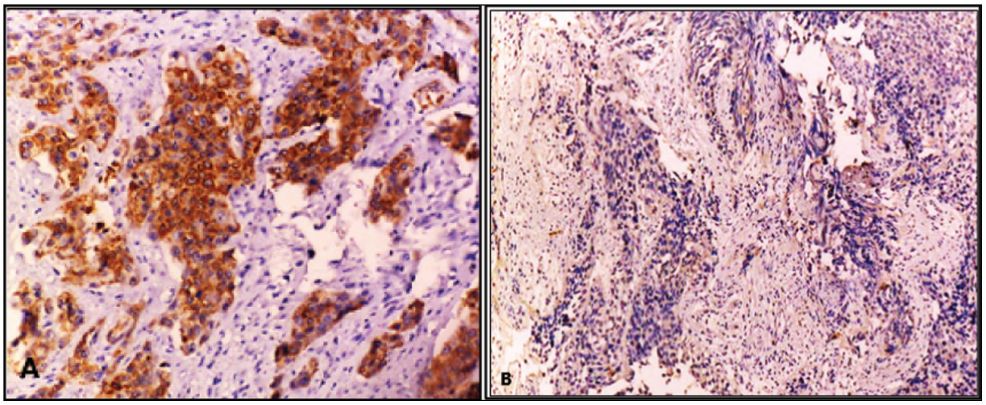
Previous studies found a correlation between SATB1 expression and poor prognostic factors of other malignant tumors such as hepatocellular carcinoma (Wu *et al.*, 2016) and breast cancer (Wang *et al.*, 2017). The underlying mechanism of the SATB1 role in affecting the behavior of the malignant tumors could be attributed to its role as chromatin organizers that modulate higher-order chromatin organization, thereby reprogramming gene-expression patterns, leading to tumorigenesis and tumor progression. SATB1 also mediates histone modifications that regulate gene expression, leading to cancer progression (Naik and Galande, 2019).

In addition, SATB1 overexpression was found to induce EMT through upregulation of E-cadherin transcription repressors Snail and Slug, and downregulation of epithelial marker E-cadherin in an established human bladder-cancer cell line (Wan *et al.*, 2015). They also declared that SATB1 promoted cell-cycle progression, proliferation, migration, and increased invasive capability in bladder carcinoma.

Smolińska *et al.* (2019) found positive correlation between the expression of SATB1 and NF-κB, the transcription factor that interacts with multiple upstream and downstream signaling pathways, and it is thought to play an important role in the invasion, angiogenesis, and metastasis in various neoplasms.

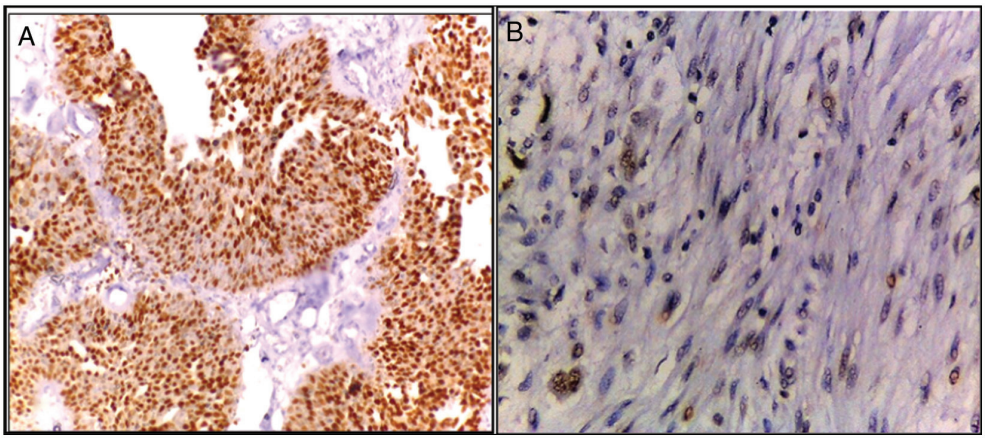
Concerning molecular subtypes of bladder urothelial carcinoma, significant correlation was detected

Figure 1



(a) Bladder urothelial carcinoma, muscle invasive, and high P4HB cytoplasmic expression score 16 (IHC, ×400). (b) Bladder urothelial carcinoma, muscle invasive, and low P4HB cytoplasmic expression score 8 (IHC, ×200) (avidin–biotin complex). IHC, immunohistochemistry; P4HB, prolyl-4-hydroxylase subunit beta.

Figure 2



(a) Bladder urothelial carcinoma, nonmuscle invasive, showing high nuclear expression for SATB1 score 6 (IHC, ×200). (b) Bladder urothelial carcinoma, muscle invasive, showing low nuclear expression for SATB1 score 2 (IHC, ×400). IHC, immunohistochemistry; SATB1, special AT-rich region-binding protein-1.

between SATB1 and different molecular subtypes, being the highest expression was in basal and double-negative subtypes and the lowest expression in luminal subtype. Receiver operating characteristic curve results indicated that SATB1 may have a diagnostic role through its validity in demarcation between different molecular subtypes of bladder carcinoma (luminal vs. nonluminal) with sensitivity (75%) and specificity (70%). To our knowledge, this may be the first study that documented significant correlation between SATB1 and different molecular subtypes of bladder carcinoma. This association may be attributed to the role of SATB1 as a chromatin organizer and a global transcriptional regulator affecting genes controlling molecular subtyping of bladder carcinoma.

Concerning the relation between P4HB1 and SATB1 in the present study, P4HB high expression showed high significant correlation with SATB1. SATB1 expression may govern genetic stimulation of P4HB1 in urothelial carcinoma. This may be explained by that as we noted before, SATB1 overexpression was found to induce EMT pathway, P4HB also induces EMT pathway as P4HP responding to ER stress by N-glycan biosynthesis. It was reported that N-glycans of certain proteins may have a vital role in metastatic process, including EMT, migration, invasion/intravasation, and extravasation of tumor cells (Wu *et al.*, 2021).

Conclusion

The current study showed that P4HB and SATB1 are overexpressed in bladder urothelial-carcinoma tissue in comparison with normal bladder mucosa. A positive correlation was detected between the expression of P4HB and SATB1. P4HB and SATB1 might have potential role as independent prognostic factors in urothelial carcinoma. SATB1 has validity in demarcation between different molecular subtypes of bladder carcinoma.

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Conflicts of interest
There are no conflicts of interest.

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