Original paper

The clinicopathological significance of *METTL3*/*SERPINE2* immunoexpression in urothelial carcinoma of the urinary bladder

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The aim of this study was to investigate the immunohistochemical expression of *METTL3* and *SERPINE2* in urothelial carcinoma and to assess their individual and combined associations with clinicopathological features and possible correlation. This study included 126 cases of urothelial carcinoma. Immunohistochemical staining was used to evaluate *METTL3* and *SERPINE2* expression in tumour tissues. Clinicopathological data including age, sex, bilharziasis, tumour grade and stage, lymph node status, lymphovascular invasion, soft tissue surgical margins, and tumour-infiltrating lymphocytes (TIL) were collected. Statistical analysis was performed to determine associations between marker expression and clinicopathological parameters. High nuclear *METTL3* expression was significantly associated with a higher tumour grade, advanced stage, increased TIL, and lymphovascular invasion, with no significant association with age, sex, bilharziasis, nodal stage, or surgical margins. High cytoplasmic *SERPINE2* expression was significantly associated with an advanced stage, lymph node metastasis, and positive soft tissue margins, while no association was found with age, sex, bilharziasis, grade, TIL, or lymphovascular invasion. Combined high *METTL3*/high *SERPINE2* expression correlated significantly with a high grade, advanced tumour and nodal stages, low TIL, and positive surgical margins, indicating a more aggressive tumour phenotype.
METTL3 and *SERPINE2* are associated with adverse pathological features in urothelial carcinoma, and their combined overexpression may serve as a potential prognostic biomarker.

Key words: *METTL3*, immunohistochemistry, urothelial carcinoma, *SERPINE2*, clinicopathological parameters.

Introduction

Urinary bladder cancer is the ninth most commonly diagnosed cancer worldwide, with estimated 614,298 new cases and approximately 220,596 deaths in 2022 [1], reflecting a substantial global health burden. In Egypt, bladder cancer ranks as the third most common malignant tumour after hepatocellular carcinoma and breast cancer, accounting for 8.7% of all cancers and representing the third leading cause of cancer-related mortality (7.9%) [1, 2]. Despite advances in diagnosis and treatment, the prognosis remains heavily dependent on the tumour stage and grade, necessitating the identification of reliable molecular markers for better risk stratification.

Methyltransferase 3 (*METTL3*) is the catalytic core of the N6-methyladenosine (m^6A) RNA methyltransferase complex and plays a central role in the post-transcriptional regulation of gene expression through the modulation of RNA stability, splicing,

and translation. Increasing evidence indicates that aberrant *METTL3* expression contributes to tumour initiation and progression in various malignancies by promoting cell proliferation, invasion, epithelial-mesenchymal transition, and metastatic potential. In urothelial carcinoma, dysregulation of *METTL3* has been implicated in aggressive tumour behaviour and unfavourable outcomes; however, its exact clinicopathological significance remains incompletely understood, warranting further investigation [3, 4].

SERPINE2, also known as protease nexin-1, is a member of the serine protease inhibitor family and is involved in extracellular matrix remodelling, tumour-stromal interactions, and regulation of proteolytic activity within the tumour microenvironment. Overexpression of *SERPINE2* has been associated with enhanced tumour invasion, angiogenesis, and disease progression in several solid tumours. In bladder cancer, *SERPINE2* is thought to facilitate tumour progression through modulation of the extracellular matrix and inflammatory microenvironment, particularly in tumours arising in chronically inflamed settings; nevertheless, its expression pattern and clinicopathological relevance have not been fully clarified [5].

Considering that *METTL3* is involved in the epigenetic regulation of tumour-related gene expression, while *SERPINE2* plays a role in extracellular matrix remodelling and tumour invasion, the simultaneous evaluation of both markers may provide complementary information regarding tumour behaviour and progression in urothelial carcinoma.

Material and methods

Case selection

The present study included 126 cases of urothelial carcinoma of the urinary bladder, selected from the archive of the Department of Pathology, Minia University Hospital in the period from 2020 to 2025. Tumour tissues were obtained from 83 patients who had undergone radical cystectomy and 43 patients who underwent transurethral resection of the bladder tumour.

Tumour staging

Tumour (T) and nodal (N) stages were determined according to the TNM classification system of the American Joint Committee on Cancer, 8th edition. The T stage was assigned based on the depth of tumour invasion, while the N stage was evaluated according to the presence and extent of regional lymph node metastasis [6].

Tumour grading

Tumour grade was assigned according to the 2016 World Health Organization/International Society of Urological Pathology classification of urothelial carcinoma into low-grade and high-grade categories [7].

Tumour-infiltrating lymphocyte assessment

Tumour-infiltrating lymphocytes (TIL) were assessed on hematoxylin and eosin stained sections within the tumour stroma surrounding invasive tumour foci, according to published recommendations. Areas with necrosis or significant artifacts were excluded. The percentage of stromal TIL was semi-quantitatively estimated by two independent pathologists who were blinded to the clinicopathological data, with discrepancies resolved by consensus. Tumour-infiltrating lymphocytes were categorized into three groups: $< 1\%$, 1–5%, and $> 5\%$, in line with previously reported cut-off values used in urothelial and other epithelial malignancies [8, 9].

Immunohistochemical procedure

Immunohistochemical (IHC) staining was performed using an automated staining platform (Ventana BenchMark XT, Roche Diagnostics). Formalin-fixed, paraffin-embedded tissue sections were prepared for staining. Sections were deparaffinized and rehydrated, followed by heat-induced antigen retrieval using citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked using hydrogen peroxide. A monoclonal mouse anti-METTL3 antibody (0.1 ml of concentrated stock; Servicebio, China) used at a 1 : 200 dilution and a polyclonal rabbit anti-*SERPINE2* antibody (0.1 ml of concentrated stock; BIOSS, USA) used at a 1 : 150 dilution were used as primary antibodies applied according to the manufacturers' instructions. Detection was performed using a polymer-based detection system, and the reaction was visualized using 3,3′-diaminobenzidine as the chromogen. Sections were then counterstained with haematoxylin.

Appropriate positive and negative controls were included in each staining run. Mouse testicular tissue was used as a positive control for *METTL3*, while placental tissue served as a positive control for *SERPINE2*. Negative controls were obtained by replacing the primary antibody with phosphate-buffered saline.

Scoring of immunohistochemical staining

All slides were independently evaluated by two pathologists who were blinded to the clinicopathological data. Immunohistochemical expression of *METTL3* and *SERPINE2* was assessed using a semi-quantitative scoring system based on staining intensity and the proportion of positively stained tumour cells. *METTL3* expression was evaluated in the nuclei of tumour cells. The immunoreactivity score was calculated by multiplying the staining intensity score by the percentage of positive cells. Staining intensity was graded as follows: 0 (negative), 1 (weak), 2 (moderate),

and 3 (strong). The proportion of positive cells was scored as 0 (0%), 1 (≤ 30%), 2 (> 30 to ≤ 60%), and 3 (> 60%). A total score > 3 was considered high *METTL3* expression, while scores ≤ 3 were considered low expression [10]. *SERPINE2* expression was assessed in the cytoplasm of tumour cells using a similar semi-quantitative approach based on staining intensity and the proportion of positive cells. Staining intensity was scored as 0 (no staining), 1 (yellow), 2 (brownish-yellow), and 3 (tawny-brown). The proportion of positive cells was scored as 0 (< 5%), 1 (6–25%), 2 (26–50%), 3 (51–75%), and 4 (> 75%). The final immunoreactivity score was obtained by multiplying the intensity score by the proportion score, resulting in values ranging from 0 to 12. This semi-quantitative scoring system, which combines staining intensity and the proportion of positive tumour cells, has been widely used in IHC studies to provide a reproducible and reliable assessment of protein expression in tumour tissues. Scores were categorised as 0–1 (negative), 2–4 (+), 5–8 (++), and 9–12 (+++). Low *SERPINE2* expression was defined as a score between 2 and 4, while scores ≥ 5 were considered high expression [11].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 25. The associations between *METTL3* and *SERPINE2* expression and clinicopathological features were analysed using the χ^2 test and Fisher's exact test. Pearson's rho coefficient was used to test the correlation between the two markers for continuous variables. Statistical significance was set at a p-value of ≤ 0.05.

Results

A total of 126 patients with urothelial carcinoma were included in the present study. The associations between nuclear *METTL3* expression, cytoplasmic *SERPINE2* expression, and their combined expression patterns with clinicopathological features are summarised in Tables I–III.

Association between nuclear *METTL3* expression and clinicopathological features

Nuclear *METTL3* expression was evaluated immunohistochemically in all studied cases. Negative to low nuclear *METTL3* expression was observed in 71 cases (56.3%), while high nuclear expression was detected in 55 cases (43.7%). High nuclear *METTL3* expression showed significant associations with several adverse clinicopathological parameters (Table I).

A statistically significant association was identified between nuclear *METTL3* expression and tumour grade, as high *METTL3* expression was significantly more frequent in high-grade tumours compared to low-grade tumours ($p < 0.001$) (Figure 1A). In addition, nuclear *METTL3* expression was significantly associated with tumour stage, with a higher proportion of *METTL3* overexpression observed in advanced T stages (T3–T4) compared to early stages (T1–T2) ($p = 0.001$) (Figures 2A, B).

A significant inverse association was noted between nuclear *METTL3* expression and TIL. Tumours with high *METTL3* expression were significantly associated with low TIL density, whereas tumours with negative or low *METTL3* expression more frequently demonstrated higher TIL levels ($p < 0.001$) (Figure 3B). Furthermore, lymphovascular invasion (LVI) was significantly more common in cases exhibiting high nuclear *METTL3* expression ($p = 0.03$) (Figure 3A).

No statistically significant associations were found between nuclear *METTL3* expression and patient age, gender, bilharziasis status, nodal stage, or soft tissue surgical margin status ($p > 0.05$).

Association between cytoplasmic *SERPINE2* expression and clinicopathological features

Cytoplasmic *SERPINE2* expression was negative or low in 61 cases (48.4%) and high in 65 cases (51.6%). As shown in Table II, high cytoplasmic *SERPINE2* expression demonstrated significant associations with tumour progression parameters.

A significant association was observed between *SERPINE2* expression and tumour stage, with high *SERPINE2* expression being more frequently detected in advanced T stages ($p = 0.03$) (Figure 1B). Moreover, cytoplasmic *SERPINE2* expression was strongly associated with nodal status, as high *SERPINE2* expression was significantly more common in cases with nodal metastasis (N1–N3) compared to node-negative or Nx cases ($p < 0.001$).

Soft tissue surgical margin involvement was also significantly associated with high *SERPINE2* expression, with positive margins occurring more frequently in tumours exhibiting *SERPINE2* overexpression ($p = 0.04$). However, no statistically significant associations were found between *SERPINE2* expression and patient age, gender, bilharziasis, tumour grade, TIL, or LVI ($p > 0.05$) (Figures 4A, B).

Association between combined *METTL3* and *SERPINE2* expression patterns and clinicopathological features

Further analysis of combined *METTL3* and *SERPINE2* expression patterns revealed distinct associations with clinicopathological parameters (Table III). Cases were categorized into four groups based on *METTL3* and *SERPINE2* expression status: high *METTL3*/high *SERPINE2*, high *METTL3*/low *SERPINE2*, low *METTL3*/high *SERPINE2*, and low *METTL3*/low *SERPINE2*.

Table I. Association between nuclear *METTL3* expression and clinicopathological features for patients with urothelial carcinoma ($N = 126$)

Clinicopathological features	*N* (%)	Nuclear *METTL3* expression		*p*- value
		Negative/low expression, $n = 71$ (%)	High expression, $n = 55$ (%)	
Age [years]				
< 55	20 (34.3)	14 (19.7)	6 (10.9)	0.136
≥ 55	106 (65.7)	57 (80.3)	49 (89.1)	
Gender				
Male	98 (77.8)	53 (74.6)	45 (81.8)	0.229
Female	28 (22.2)	18 (25.4)	10 (18.2)	
Bilharziasis				
Present	69 (54.8)	42 (59.2)	27 (49.1)	0.172
Absent	57 (45.2)	29 (40.8)	28 (50.9)	
Tumour grade				
High grade	34 (27)	9 (12.7)	25 (45.5)	< 0.001*
Low grade	92 (73)	62 (87.3)	30 (54.5)	
T stage				
T1	16 (12.7)	11 (15.5)	5 (9.1)	0.001*
T2	85 (67.5)	55 (77.5)	30 (54.5)	
T3	17 (13.5)	4 (5.6)	13 (23.6)	
T4	8 (6.3)	1 (1.4)	7 (12.7)	
N stage				
Nx	43 (34.1)	25 (53.2)	18 (32.7)	0.828
N0	52 (41.3)	30 (42.3)	22 (40)	
N1–N3	31 (24.6)	16 (22.5)	15 (27.3)	
TIL (%)				
< 1	37 (29.4)	14 (19.7)	23 (41.8)	< 0.001*
1–5	33 (26.2)	14 (19.7)	19 (34.5)	
> 5	56 (44.4)	43 (60.6)	13 (23.6)	
STSM				
Positive	6 (4.8)	2 (2.8)	4 (7.3)	0.5
Negative	77 (61.1)	44 (62)	33 (60)	
Not present	43 (34.1)	25 (35.2)	18 (32.7)	
LVI				
Present	24 (19)	9 (12.7)	15 (27.3)	0.03*
Absent	102 (81)	62 (87.3)	40 (72.7)	

LVI – lymphovascular invasion, STSM – soft tissue surgical margin, TIL – tumour infiltrating lymphocytes
** Tests of significance: χ^2 test and Fisher's exact test*
p-value < 0.05 is considered statistically significant.

Table II. Association between cytoplasmic *SERPINE2* expression and clinicopathological features for patients with urothelial carcinoma ($N = 126$)

Clinicopathological features	*N* (%)	Cytoplasmic serpine2 expression		*p*-value
		Negative/low expression, $n = 61$ (%)	High expression, $n = 65$ (%)	
Age [years]				
< 55	20 (34.3)	11 (18)	9 (13.8)	0.345
≥ 55	106 (65.7)	50 (82)	56 (86.2)	
Gender				
Male	98 (77.8)	44 (72.1)	54 (83.1)	0.103
Female	28 (22.2)	17 (27.9)	11 (16.9)	
Bilharziasis				
Present	69 (54.8)	38 (62.3)	31 (47.7)	0.07
Absent	57 (45.2)	23 (37.7)	34 (52.3)	
Tumour grade				
High grade	34 (27)	15 (24.6)	19 (29.2)	0.350
Low grade	92 (73)	46 (75.4)	46 (70.8)	
T stage				
T1	16 (12.7)	5 (8.2)	11 (16.9)	0.03*
T2	85 (67.5)	49 (80.3)	36 (55.4)	
T3	17 (13.5)	5 (8.2)	12 (18.5)	
T4	8 (6.3)	2 (3.3)	6 (9.2)	
N stage				
Nx	43 (34.1)	24 (39.3)	19 (29.2)	< 0.001*
N0	52 (41.3)	32 (52.5)	20 (30.8)	
N1–N3	31 (24.6)	5 (8.2)	26 (40)	
TIL (%)				
< 1	37 (29.4)	13 (21.3)	24 (36.9)	0.148
1–5	33 (26.2)	17 (27.9)	16 (24.6)	
> 5	56 (44.4)	31 (50.8)	25 (38.5)	
STSM				
Positive	6 (4.8)	0 (0)	6 (9.2)	0.04*
Negative	77 (61.1)	37 (60.7)	40 (61.5)	
Not present	43 (34.1)	24 (39.3)	19 (29.2)	
LVI				
Present	24 (19)	8 (13.1)	16 (24.6)	0.08
Absent	102 (81)	53 (86.9)	49 (75.4)	

LVI – lymphovascular invasion, STSM – soft tissue surgical margin, TIL – tumour infiltrating lymphocytes
** Tests of significance: χ^2 test and Fisher's exact test*
p-value < 0.05 is considered statistically significant.

Table III. Association between the expression of both *METTL3* and *SERPINE2* and clinicopathological features for patients with urothelial carcinoma

Clinicopathological features	*N* (%)	*METTL3* and *SERPINE2* expression				*p*-value
		High *METTL3*/high *SERPINE2*, *n* (%)	High *METTL3*/low *SERPINE2*, *n* (%)	Low *METTL3*/high *SERPINE2*, *n* (%)	Low *METTL3*/low *SERPINE2*, *n* (%)	
Age [years]						
< 55	20 (34.3)	5 (25)	1 (5)	4 (20)	10 (50)	0.125
≥ 55	106 (65.7)	27 (25.5)	22 (20.8)	29 (27.4)	28 (26.4)	
Gender						
Male	98 (77.8)	28 (28.6)	17 (17.3)	26 (26.5)	27 (27.6)	0.397
Female	28 (22.2)	4 (14.3)	6 (21.4)	7 (25)	11 (39.3)	
Bilharziasis						
Present	69 (54.8)	14 (20.3)	13 (18.8)	17 (24.6)	25 (36.2)	0.308
Absent	57 (45.2)	18 (31.6)	10 (17.5)	16 (28.1)	13 (22.8)	
Tumour grade						
High grade	34 (27)	12 (35.3)	13 (38.2)	7 (20.6)	2 (5.9)	< 0.001*
Low grade	92 (73)	20 (21.7)	10 (10.9)	26 (28.3)	36 (39.1)	
T stage						
T1	16 (12.7)	1 (6.3)	4 (25)	10 (62.5)	1 (6.3)	< 0.001*
T2	85 (67.5)	18 (21.2)	12 (14.1)	18 (21.2)	37 (43.5)	
T3	17 (13.5)	8 (47.1)	5 (29.4)	4 (23.5)	0 (0)	
T4	8 (6.3)	5 (62.5)	2 (25)	1 (12.5)	0 (0)	
N stage						
Nx	43 (34.1)	13 (30.2)	5 (11.6)	6 (14%)	19 (44.2)	< 0.001*
N0	52 (41.3)	8 (15.4)	14 (26.9)	12 (23.1)	18 (34.6)	
N1–N3	31 (24.6)	11 (35.5)	4 (12.9)	12 (23.1)	18 (34.6)	
TIL (%)						
< 1	37 (29.4)	17 (45.9)	6 (16.2)	7 (18.9)	7 (18.9)	0.001*
1–5	33 (26.2)	10 (30.3)	9 (27.3)	6 (18.2)	8 (24.2)	
> 5	56 (44.4)	5 (8.9)	8 (14.3)	20 (35.7)	23 (41.1)	
STSM						
Positive	6 (4.8)	4 (66.7)	0 (0)	2 (33.3)	0 (0)	0.007*
Negative	77 (61.1)	15 (19.5)	18 (23.4)	25 (32.5)	19 (24.6)	
Not present	43 (34.1)	13 (30.2)	5 (11.6)	6 (14)	19 (44.2)	
LVI						
Present	24 (19)	9 (37.5)	6 (25)	7 (29.2)	2 (8.3)	0.07
Absent	102 (81)	23 (22.5)	17 (16.7)	26 (25.5)	36 (35.3)	

LVI – lymphovascular invasion, STSM – soft tissue surgical margin, TIL – tumour infiltrating lymphocytes
** Tests of significance: χ^2 test and Fisher's exact test*
p-value < 0.05 is considered statistically significant.

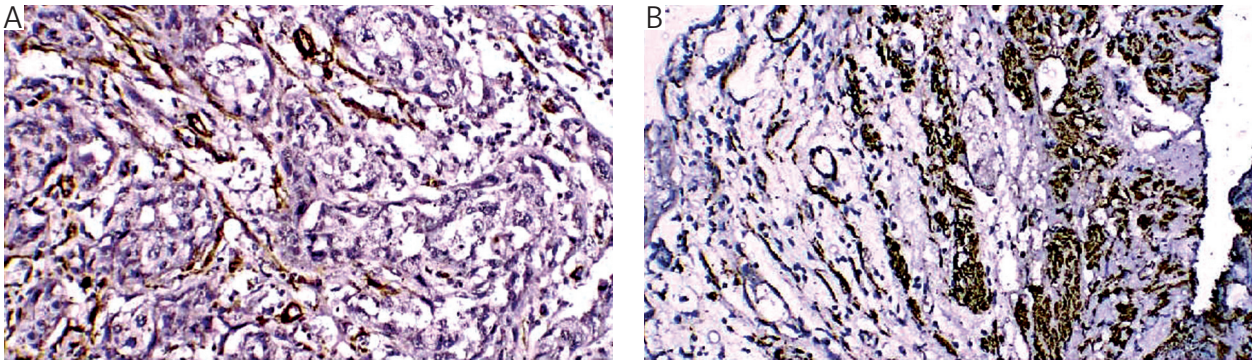

Figure 1. A) High nuclear *METTL3* expression in urothelial carcinoma invading the muscle (400×). **B)** High nuclear *METTL3* expression in urothelial carcinoma invading the fat (200×)

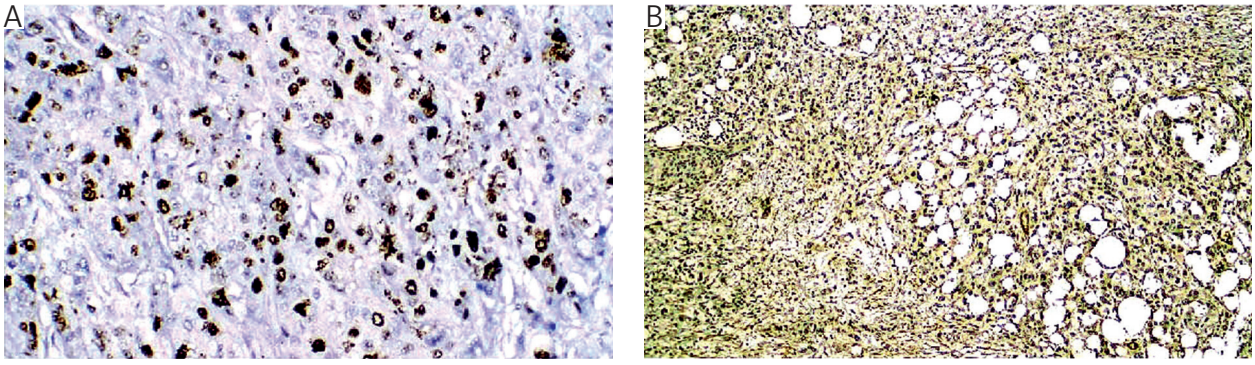

Figure 2. A) Low cytoplasmic *SERPINE2* expression in high grade urothelial carcinoma (400×). B) Low cytoplasmic *SERPINE2* expression in low grade urothelial carcinoma (200×)

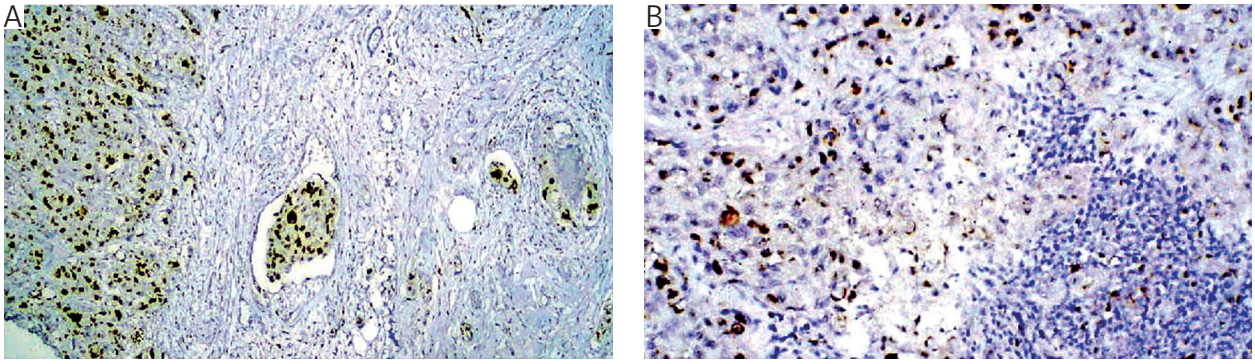

Figure 3. A) High nuclear *METTL3* expression in urothelial carcinoma with lymphovascular invasion (200×). **B)** Low nuclear *METTL3* expression in urothelial carcinoma with high tumour-infiltrating lymphocytes (200×)

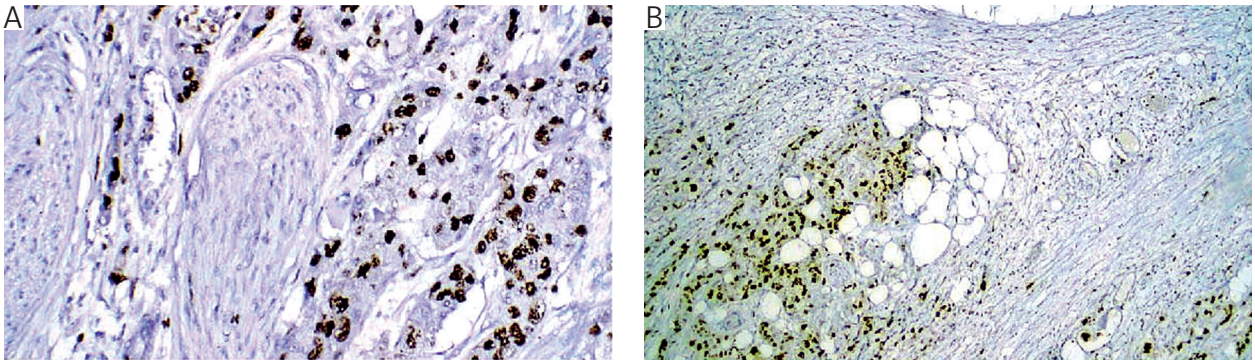

Figure 4. A) High nuclear *METTL3* expression in high grade urothelial carcinoma (200×). **B)** High cytoplasmic *SERPINE2* expression in high grade urothelial carcinoma invading the fat (200×)

A highly significant association was observed between combined expression patterns and tumour grade ($p < 0.001$). High-grade tumours were predominantly associated with high *METTL3*/high *SERPINE2* expression, whereas low-grade tumours more frequently demonstrated low *METTL3*/low *SERPINE2* expression. Tumour stage also showed a strong association with combined expression patterns ($p < 0.001$), with advanced stages (T3–T4) being enriched for tumours exhibiting concurrent high *METTL3* and *SERPINE2* expression.

Nodal stage demonstrated a highly significant association with combined expression patterns ($p < 0.001$). Tumours with nodal metastasis (N1–N3) were more frequently associated with high *METTL3* and/or high *SERPINE2* expression, while node-negative tumours more commonly showed low expression of both markers. In addition, TIL levels were significantly associated with combined expression patterns ($p = 0.001$), with high *METTL3*/high *SERPINE2* expression being associated with low TIL density and low *METTL3*/low *SERPINE2* expression correlating with higher TIL levels.

Soft tissue surgical margin positivity was significantly associated with high *METTL3*/high *SERPINE2* expression ($p = 0.007$). Although LVI tended to be more frequent in tumours with high *METTL3* and/or *SERPINE2* expression, this association did not reach statistical significance ($p = 0.07$). No significant associations were identified between combined *METTL3*/*SERPINE2* expression patterns and patient age, gender, or bilharziasis status.

Discussion

In this study, nuclear *METTL3* expression was observed in 43.7% of urothelial carcinoma cases, with high expression significantly associated with a high tumour grade, advanced T stage, low TIL, and the presence of LVI. These findings support the proposed oncogenic role of *METTL3* in bladder cancer, which has been reported to promote tumour proliferation, invasion, and metastasis *via* m^{6}A-dependent regulation of downstream targets, including cell cycle regulators and *EMT*-related genes [12].

The association between *METTL3* and advanced T stage in our study aligns with previous studies that have shown *METTL3* expression to correlate with tumour aggressiveness and poor clinical outcomes in bladder carcinoma [12, 13]. Interestingly, *METTL3* expression in our study did not significantly associate with nodal metastasis, suggesting its predominant role in local invasion rather than lymphatic spread. *METTL3* promotes invasion by regulating *EMT* and cell motility through m^{6}A-mediated modulation of *EMT*-related transcripts in various cancers, including gastric and breast cancers [14, 15]. However, in oral squamous cell carcinoma and breast carcinoma, *METTL3* overexpression was associated with lymph node metastasis [16, 17]. These findings suggest that the relationship between *METTL3* and nodal metastasis may be tumour type- and context-dependent, possibly influenced by subcellular localization and microenvironmental factors

Regarding cytoplasmic *SERPINE2* expression, high expression was detected in 51.6% of cases and was significantly associated with advanced T and N stages, positive surgical margins, and low TIL density. These results are consistent with prior reports demonstrating *SERPINE2* as a promoter of tumour invasion and metastasis through modulation of extracellular matrix remodelling and tumour-stroma interactions [18]. While *SERPINE2* has been less extensively investigated in bladder cancer, accumulating evidence from other epithelial malignancies supports its role in tumour aggressiveness and poor prognosis. In endometrial carcinoma, *SERPINE2* overexpression has been associated with increased tumour invasiveness, advanced stage, and unfavourable survival outcomes, suggesting a role in tumour progression and extracellular matrix remodelling [19]. Similarly, in colorectal cancer, *SERPINE2* has been implicated in enhancing tumour cell migration, invasion, and metastatic potential, with higher expression correlating with advanced TNM stage and lymph node metastasis [20]. In oral squamous cell carcinoma, *SERPINE2* expression was significantly associated with tumour depth of invasion, nodal metastasis, and reduced disease-free survival, highlighting its contribution to tumour aggressiveness and poor clinical outcomes [21]. Collectively, these findings support the potential prognostic value of *SERPINE2* and provide a biological rationale for investigating its clinicopathological relevance in urothelial carcinoma.

Importantly, the combined analysis of *METTL3* and *SERPINE2* revealed that tumours co-expressing high *METTL3* and high *SERPINE2* were more likely to exhibit high-grade histology, advanced T and N stages, and low TIL density, suggesting a synergistic effect between these markers in promoting tumour aggressiveness. The oncogenic role of *METTL3* is mediated through m^{6}A-dependent regulation of key signalling pathways involved in *EMT*, cell motility, and invasion, including the PI3K/AKT, Wnt/β-catenin, and TGF-β pathways. Through stabilization and enhanced translation of pro-invasive transcripts, *METTL3* reinforces malignant phenotypes and may amplify downstream effector molecules such as *SERPINE2*. Although the mechanistic link between these markers in urothelial carcinoma remains to be fully elucidated, Yan *et al*. [22] demonstrated that *METTL3* stabilizes *SERPINE2* mRNA *via* m^{6}A modification, thereby enhancing malignant behaviour in gastric signet ring cell carcinoma. This supports the existence of a potential *METTL3*/*SERPINE2* regulatory axis in bladder cancer, whereby

METTL3-mediated m^{6}A modification may upregulate *SERPINE2* expression, contributing to extracellular matrix remodelling, tumour invasion, and metastasis.

Notably, high TIL density was inversely associated with *METTL3* and *SERPINE2* expression, suggesting that these markers may contribute to an immunosuppressive tumour microenvironment, potentially impacting anti-tumour immune responses.

The main limitation of this study is the relatively small sample size, which may affect the generalizability of the findings. Nevertheless, the use of IHC scoring and blinded independent evaluation enhances the reliability of our data. Future studies with larger cohorts and functional assays are warranted to confirm the interplay between *METTL3* and *SERPINE2* and to explore their potential as therapeutic targets or biomarkers for prognosis in urothelial carcinoma.

Conclusions

High nuclear *METTL3* and cytoplasmic *SERPINE2* expression levels are significantly associated with aggressive clinicopathological features in urothelial carcinoma, including high tumour grade, advanced T and N stages, and low TIL density. Tumours co-expressing both markers exhibited the most aggressive phenotype, highlighting their potential utility as combined prognostic biomarkers. These findings underscore the importance of further research into the *METTL3/SERPINE2* axis for improved prognostication and potential targeted therapy in bladder cancer.

Disclosures

1. Institutional review board statement: This study was approved by the Ethics Committee of the Faculty of Medicine, Minia University, Egypt (approval decision no: 1602/07/2025, dated: 14.07.2025).
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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