İstanbul Med J 2026; 27(3): 233-7
DOI: 10.4274/imj.galenos.2026.73920

Core Circadian Clock Proteins (CLOCK and BMAL1) in Non-Muscle-Invasive Urothelial Carcinoma: Links to Histopathology and Tumor Progression

Begüm Çalım Gürbüz[1], Başak Bekiroğlu[1], Dilara Yitiz[1], Şeyma Aker[1], Emre Korkmaz[2], Halil Lütfi Canat[2]

[1]University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of Pathology, İstanbul, Türkiye
[2]University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of Urology, İstanbul, Türkiye

ABSTRACT

Introduction: This study aims to evaluate the immunohistochemical expression of core circadian clock proteins, circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like protein 1 (BMAL1), in patients with non-muscle-invasive urothelial carcinoma (NMIBC) and to determine their potential role in predicting disease progression.

Methods: A total of 34 patients diagnosed with NMIBC who subsequently developed either progressive (n=20) or non-progressive (n=14) recurrence were included. Sixty-eight specimens from initial and recurrent biopsies, with a minimum 3-month interval, were analyzed. The expression of CLOCK and BMAL1 was quantified by independently assessing staining intensity and the percentage of positive cells, both of which were then used to calculate the H-score for each antibody. Statistical analyses were performed using SPSS 25.0, with significance set at $p<0.05$.

Results: The mean age of 34 patients was 64.4±10.8 years. Significant stage migration was observed between initial and subsequent biopsies ($p=0.01$), with 9% of cases progressing to muscle-invasive disease. Progressive recurrence was significantly associated with initial pathological stage and grade ($p=0.02$), particularly in non-invasive papillary urothelial carcinoma (pTa) high-grade tumors ($p=0.01$). Peritumoral inflammatory infiltration was identified in 37% of cases and was significantly more frequent in high-grade stages ($p=0.02$) and invasive stages ($p<0.01$). Strong CLOCK expression was found in 99% of cases, while BMAL1 expression was significantly milder in pTa tumors ($p=0.04$). However, changes in CLOCK or BMAL1 H-scores (ΔH-score) and shift work history were not significantly associated with time to disease progression ($p>0.05$).

Conclusion: Our findings suggest that CLOCK protein activation occurs early and remains stable during the pathogenesis of urothelial carcinoma, while BMAL1 expression correlates with the pT stage. However, within this cohort, these circadian regulators did not serve as independent prognostic markers for disease progression. Further studies with larger sample sizes are needed to elucidate the complex role of the circadian system in tumor evolution.

Keywords: Circadian rhythm, CLOCK, BMAL1, urothelial carcinoma, disease progression, immunohistochemistry, NMIBC

Introduction

Bladder cancer is one of the most common malignancies of the urinary tract, with urothelial carcinoma representing the vast majority of cases (1). At the time of initial diagnosis, approximately 75% of patients present with non-muscle-invasive urothelial carcinoma (NMIBC). Despite various treatment modalities, NMIBC is characterized by a high rate of recurrence and a significant risk of progression to muscle-invasive disease, which necessitates lifelong surveillance and impacts patient quality of life (2). Identifying reliable biomarkers that can predict the risk of progression is therefore crucial for optimizing management strategies (3).

In recent years, the role of the circadian rhythm—the internal biological clock that regulates physiological processes over a 24-hour cycle—has gained prominence in cancer research (4-7). At the molecular level, this system is governed by a set of core clock genes, including circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like 1 (BMAL1). These genes form a transcriptional-translational feedback loop that regulates cell cycle progression, DNA repair, and apoptosis. Disruptions in this system, often caused by lifestyle factors such as shift work, have been classified as potential carcinogens and are increasingly linked to the development and aggressiveness of various cancers (8).

Address for Correspondence: Begüm Çalım Gürbüz, MD, University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of Pathology, İstanbul, Türkiye
E-mail: calimbegum@gmail.com **ORCID ID:** orcid.org/0000-0001-6720-0662
Cite this article as: Çalım Gürbüz B, Bekiroğlu B, Yitiz D, Aker Ş, Korkmaz E, Canat HL. Core circadian clock proteins (CLOCK and BMAL1) in non-muscle-invasive urothelial carcinoma: links to histopathology and tumor progression. İstanbul Med J. 2026; 27(3): 233-7

Received: 30.03.2026
Accepted: 01.07.2026
Publication Date: 03.08.2026

In the context of bladder cancer, the expression patterns of CLOCK and BMAL1 and their association with tumor behavior remain an area of active investigation (9). On the other hand, the molecular influence of circadian regulators on disease progression remains incompletely understood.

This study aims to evaluate the immunohistochemical expression of CLOCK and BMAL1 in patients who were diagnosed with NMIBC at their first biopsy and who subsequently developed either progressive or non-progressive recurrence. By comparing the H-scores of these markers in sequential biopsies and analyzing the relationship of these scores with clinical parameters—such as shift-work history and time to progression—we seek to determine whether circadian clock proteins have potential as prognostic indicators in the clinical course of urothelial carcinoma.

Methods

Case Selection and Preparation

This study was approved by the Scientific Research Ethics Committee No. 2 of Başakşehir Çam and Sakura City Hospital (decision no: 84, date: February 4, 2026). Due to the retrospective design of the study and the use of anonymized data, informed consent was not required in accordance with the Ethics Committee's decision and institutional regulations.

The study included 34 patients over the age of 18 who were diagnosed with NMIBC at their initial biopsy. These patients presented with either progressive or non-progressive recurrences between June 1, 2020, and January 1, 2026, at the Clinic of Pathology of University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital. The progression was defined solely by an increase in stage [e.g., non-invasive papillary urothelial carcinoma (pTa) to pT1/pT2]. Cases were included regardless of sex. A minimum interval of three months between the two biopsies was required, while transurethral resection (TUR) procedures performed solely for residual tumor sampling were excluded. Consequently, 68 bladder TUR and radical cystectomy specimens with sufficient tumor tissue were identified for analysis.

Clinical and Histopathological Parameters

Clinical data and archival records were retrospectively analyzed for the following parameters: age, sex, shift work history (<1 year), and survival status. Specimen-related data included the type (TUR or cystectomy) and tumor dimensions (mm), with the latter derived from cystoscopic or macroscopic measurements. Recurrence status (non-progressive vs. progressive) was determined by comparing the first and second biopsies. Other recorded variables included the inter-biopsy interval (months), pT stage, histological grade (low/high), presence of carcinoma *in situ* (CIS), and peritumoral inflammatory infiltration (PTI), categorized according to the presence of lymphocytes and plasma cells.

Immunohistochemical Staining and Evaluation

The paraffin block most representative of the tumor was selected. Sections of 2 µm thickness were prepared from these blocks and placed on positively charged slides. Staining was performed with CLOCK (JA12-33) rabbit monoclonal antibody (1/50 dilution) and BMAL1 rabbit polyclonal antibody (1/50 dilution) on the Ventana Medical System BenchMark ULTRA/ISH automated stainer, using the Ultraview Universal DAB Detection Kit. For each case, two CLOCK slides and two BMAL1 slides were obtained from both the first and second biopsies. Neural tissue served as a positive control for BMAL1, and colon adenocarcinoma served as a positive control for CLOCK.

Immunohistochemical evaluation of tumor cells was based on cytoplasmic and/or membranous staining for the CLOCK antibody and nuclear staining for the BMAL1 antibody. For both antibodies, the extent of staining in tumor cells was determined as a percentage. Staining intensity was scored as 0 (no staining), 1 (mild), 2 (moderate), and 3 (strong). The H-score was calculated using the formula (1 × 1%+) + (2 × 2%+) + (3 × 3%+), taking into account the percentage of cells corresponding to each intensity level (10). The resulting scores ranged from 0 to 300. Immunohistochemical scoring was performed by two pathologists, and interobserver agreement was assessed.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics (version 25.0). Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean and standard deviation or median (minimum–maximum), as appropriate. The chi-square and Fisher's exact tests were employed to compare categorical data. Normality of the data was assessed using the Shapiro–Wilk test. Accordingly, the Mann–Whitney U test was used to compare non-normally distributed continuous variables. For paired categorical data, the McNemar–Bowker test of marginal homogeneity was performed. Correlation coefficients were evaluated using Spearman's rho. A p value <0.05 was considered statistically significant.

Results

Demographic and Clinical Findings

The mean age of the 34 patients was 64.4±10.8 years (range: 41-86), and 85% (n=29) were male. Seven patients (20%) had a history of shift work. Additionally, there were two deaths (6%) in the series, and the causes of death were unrelated to the primary malignancy. Of the 68 specimens collected from the initial and subsequent biopsies, 64 were obtained via TUR, and 4 were cystectomy specimens (all from the second biopsy). The mean tumor diameter was 30.4±13.4 mm. In terms of progression, 59% (n=20) of cases were classified as non-progressive and 41% (n=14) as progressive. The median interval between biopsies was 4 months (range: 3-20 months) for non-progressive cases and 3 months (range: 3-29 months) for progressive cases.

Histopathological Findings

Analysis of the 68 specimens revealed that 70% (n=48) were pTa, 26% (n=17) were invasive urothelial carcinoma involving the lamina propria (pT1), and 4% (n=3) were invasive urothelial carcinoma with muscularis propria involvement (pT2, all from cystectomy specimens). Significant stage migration was observed between the initial and

subsequent diagnoses (p=0.01). Specifically, while only 12% (n=4) of cases were classified as pT1 at the initial diagnosis, this proportion increased to 39% (n=13) at the second diagnosis. Additionally, 9% (n=3) of cases progressed to muscle-invasive bladder cancer (pT2) during follow-up.

Among the 48 pTa cases, 61% (n=29) were high-grade and 39% (n=19) were low-grade. A statistically significant association was observed between pathological stage/grade and disease progression (p=0.02). Specifically, progressive recurrence was significantly more prevalent in the pTa high-grade group than in the pTa low-grade group (p=0.01). The detailed distribution of pathological stages and grades at initial diagnosis and at recurrence is summarized in Table 1.

CIS was identified in 13 cases (19%). PTI was detected in 25 cases (37%). On initial biopsy, PTI was significantly more frequent in high-grade than in low-grade pTa cases (p=0.02) (Figure 1). Furthermore, when all biopsies were evaluated, PTI was significantly lower in pTa cases than in invasive stages (p<0.01). However, no significant associations were found between disease progression and age, sex, shift work history, survival status, or the presence of CIS or PTI (p>0.05).

Immunohistochemical Findings

CLOCK expression was similar between the first and second biopsies. Strong expression was observed in the majority of cases (99%, n=66), with mean H-scores of 281.4±37.5 in the first biopsy and 278.2±56.5 in the second.

BMAL1: The mean H-score was 94.7±85.4 in the initial biopsy and 83.5±105.7 in the subsequent biopsy. Notably, pTa cases demonstrated significantly higher rates of mild expression compared with other stages (p=0.04).

Representative images illustrating strong CLOCK and mild BMAL1 expression in a non-invasive low-grade pTa are provided in Figure 2.

Progression and H-Score Correlation

Statistical analyses (Mann-Whitney U and univariate logistic regression) showed no significant difference or association between the change in H-score (ΔH-score) and disease progression. Furthermore, Cox regression analysis indicated that CLOCK and BMAL1 ΔH-scores had no significant effect on the time to progression.

Discussion

This study is a pioneering effort examining the expression levels of CLOCK and BMAL1, core components of the circadian rhythm, and the impact of changes in these levels (ΔH-score) on disease progression in patients with NMIBC. The risk of progression remains one of the most critical factors in the surveillance and treatment planning of bladder cancer patients.

Limited epidemiological evidence exists; however, basic research consistently links circadian disruption to bladder cancers (11). In this context, circadian syndrome—exemplified by night-shift workers—has been suggested as a potential risk factor influencing the recurrence and prognosis of NMIBC (12). Despite existing literature associating circadian

Table 1. Distribution of pathological stage and grade in initial and second diagnoses, and their association with tumor progression status

Pathological stage and grade	Initial diagnosis (n=34)	Subsequent diagnosis (n=34)	p value	Non-progressive recurrence (n=20)	Progressive recurrence (n=14)	p value
pTa low-grade	10 (29%)	9 (26%)	0.01*	9 (45%)	1 (7%)	0.01**
pTa high-grade	20 (59%)	9 (26%)		8 (40%)	12 (86%)	
pT1	4 (12%)	13 (39%)		3 (15%)	1 (7%)	
pT2	0 (0%)	3 (9%)		0 (0.0%)	0 (0%)	

*McNemar-Bowker/Marginal homogeneity test, **Fisher's exact test. pTa: Non-invasive papillary urothelial carcinoma

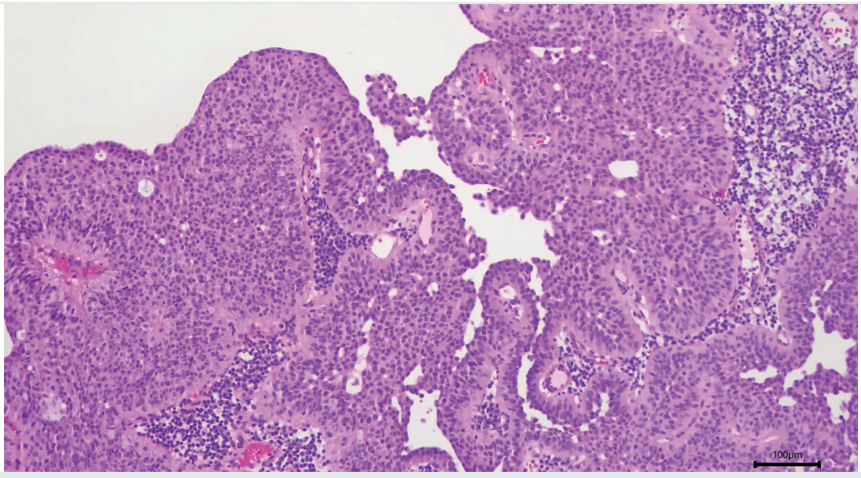

Figure 1. Lymphoplasmacytic inflammatory infiltration surrounding the peritumoral area within the papillary fibrovascular core in a non-invasive high-grade papillary urothelial carcinoma (x10)

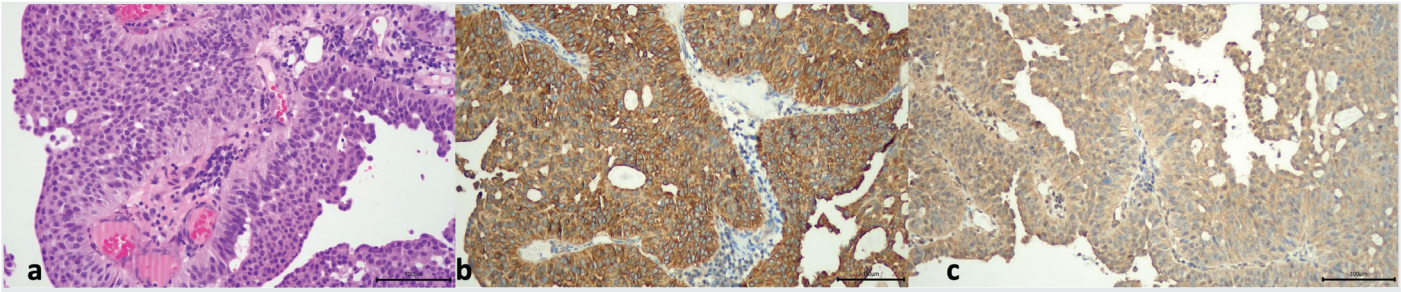

Figure 2. (a) H&E staining of non-invasive low-grade papillary urothelial carcinoma (x20). Immunohistochemical analysis showing (b) strong expression of CLOCK and (c) mild expression of BMAL1 in non-invasive low-grade papillary urothelial carcinoma (x20). H&E: Hematoxylin and eosin

rhythm disruption with increased cancer risk, our cohort did not demonstrate a statistically significant relationship between a history of night-shift work and disease progression. This discrepancy may suggest that the impact of circadian disruption is more pronounced during the tumor initiation phase than during the progression phase.

Non-muscle-invasive bladder carcinoma is characterized by a high recurrence rate; approximately 15%–61% of patients experience recurrence within one year, and 31%–78% within five years following TUR of bladder tumor (13). Non-invasive high-grade urothelial carcinoma, in particular, exhibits a high recurrence rate of up to 80% and can rapidly advance despite its initially non-invasive nature (14). Conversely, non-invasive low-grade pTa is associated with a similarly high recurrence risk (30%-80%) but carries a significantly lower risk (≤5%) of progression to muscle-invasive disease (15). A key observation in our study was the strong association between the initial pathological status and the risk of progressive recurrence. This finding aligns with established literature, which suggests that while pTa low-grade tumors frequently recur, their risk of progression to muscle-invasive disease remains relatively low. Conversely, pTa high-grade tumors represent a more heterogeneous and unpredictable group, exhibiting a significantly higher propensity for upstaging and aggressive clinical behavior.

PTI is a common immune response that frequently correlates with a more favorable prognosis and increased recurrence-free survival in high-grade urothelial carcinoma. While chronic inflammation may drive tumor progression, localized immune cell infiltration often serves as a host defense mechanism, potentially indicating better clinical outcomes (16,17). In our study, the significant association between PTI and high-grade tumors is consistent with existing literature. However, the finding that high-grade tumors with PTI did not demonstrate a significant difference in progression represents a departure from some previous reports. These results should be further validated in larger cohorts, as our current findings may be influenced by the limited sample size.

The immunohistochemical findings regarding CLOCK and BMAL1 in our study are particularly noteworthy. In contrast to the results of Litlekalsoy et al. (9), who reported a decrease in CLOCK expression and an increase in BMAL1 expression across 27 urothelial carcinoma cases—especially in high-grade instances—our study demonstrated consistently strong CLOCK expression and mild BMAL1 expression. Specifically, strong CLOCK expression was detected in 99% of cases in both the initial and recurrent biopsies. Conversely, BMAL1 expression was more variable and generally lower in pTa cases. These observations suggest that CLOCK activation in our patient group occurs early and remains a stable feature of the tumor's evolutionary process, regardless of histological grade or disease progression.

BMAL1 expression is closely associated with disease progression and prognosis in urothelial carcinoma. Generally, an increase in BMAL1 levels is linked to tumor aggressiveness and poor survival rates (18). Our finding in pTa cases with significantly reduced BMAL1 expression provides an important clue that circadian clock proteins may be associated with the invasive potential and stage of the tumor.

However, the lack of a significant difference in the change in CLOCK and BMAL1 H-scores between the progressive and non-progressive groups limits the utility of these markers standalone predictors of progression. These results indicate that although circadian proteins are present in urothelial carcinoma, progression is likely driven primarily by classical histopathological features and other molecular pathways that remain to be fully elucidated.

Study Limitations

Several limitations of this study should be noted, primarily the relatively small sample size, which limits statistical power and the generalizability of the results. The single-center retrospective design, combined with a short median interval between biopsies, may not fully reflect long-term molecular evolution or progression patterns of urothelial carcinoma. Additionally, the limited data on lifestyle factors and the semi-quantitative nature of the immunohistochemical H-score evaluation may have constrained the identification of significant associations between circadian regulators and disease progression.

Conclusion

This study explores the role of circadian rhythm components, CLOCK and BMAL1, in the progression of non-muscle-invasive bladder cancer. Our findings suggest that although these markers are widely expressed, changes in their expression over time do not independently predict disease progression. The consistently strong CLOCK expression observed across biopsies indicates that it may be an early and stable feature of bladder carcinogenesis, whereas the association of BMAL1 with pTa cases links circadian proteins to the tumor's invasive potential. Although PTI serves as a host defense mechanism in high-grade tumors, the initial pathological grade remains the most robust predictor of progressive recurrence. While circadian disruption may influence tumor initiation

rather than progression, these findings warrant further validation in larger cohorts to address the limitations of the current sample size.

Ethics

Ethics Committee Approval: This study was approved by the Scientific Research Ethics Committee No. 2 of Başakşehir Çam and Sakura City Hospital (decision no: 84, date: February 4, 2026).

Informed Consent: Due to the retrospective design of the study and the use of anonymized data, informed consent was not required in accordance with the Ethics Committee's decision and institutional regulations.

Footnotes

Authorship Contributions: Surgical and Medical Practices - B.Ç.G., E.K., H.L.C.; Concept - B.Ç.G., B.B.; Design - B.Ç.G., B.B., D.Y.; Data Collection or Processing - B.Ç.G., B.B., D.Y., Ş.A., E.K., H.L.C.; Analysis or Interpretation - B.Ç.G., B.B., D.Y., Ş.A., E.K., H.L.C.; Literature Search - B.Ç.G., B.B., Ş.A.; Writing - B.Ç.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Zhang Y, Rumgay H, Li M, Yu H, Pan H, Ni J. The global landscape of bladder cancer incidence and mortality in 2020 and projections to 2040. J Glob Health. 2023; 13: 04109.
2. Manivasagam SS, Kassim A, Raman JD. Diagnosis, evaluation, and management of patients with non-muscle invasive bladder cancer. Future Sci OA. 2026; 12: 2622297.
3. Mihaila RI, Gheorghe AS, Zob DL, Stanculeanu DL. The importance of predictive biomarkers and their correlation with the response to immunotherapy in solid tumors-impact on clinical practice. Biomedicines. 2024; 12: 2146.
4. Xiao L, Chang AK, Zang MX, Bi H, Li S, Wang M, et al. Induction of the CLOCK gene by E2-ERα signaling promotes the proliferation of breast cancer cells. PLoS One. 2014; 9: e95878.
5. Aroca-Siendones MI, Moreno-SanJuan S, Puentes-Pardo JD, Verbeni M, Arnedo J, et al. Core circadian clock proteins as biomarkers of progression in colorectal cancer. Biomedicines. 2021; 9: 967.
6. Shen Z, Zhao Y, Xu X, Yang H, He S, Ma J, et al. Single-cell RNA sequencing integrated with bulk RNA sequencing analysis of clock circadian regulator with prognostic and immune microenvironment in thyroid cancer. Transl Oncol. 2025; 53: 102299.
7. Kuo KL, Chang SJ, Kwan AL, Chai CY. Correlation between levels of clock protein expression and effects on temozolomide-resistant glioblastoma and tumor progression. Hum Cell. 2025; 38: 75.
8. Fagiani F, Di Marino D, Romagnoli A, Travelli C, Voltan D, Di Cesare Mannelli L, et al. Molecular regulations of circadian rhythm and implications for physiology and diseases. Signal Transduct Target Ther. 2022; 7: 41.
9. Litlekalsoy J, Rostad K, Kalland KH, Hostmark JG, Laerum OD. Expression of circadian clock genes and proteins in urothelial cancer is related to cancer-associated genes. BMC Cancer. 2016; 16: 549.
10. Price P, Ganugapati U, Gatalica Z, Kakadekar A, Macpherson J, Quenneville L, et al. Reinventing nuclear histo-score utilizing inherent morphologic cutoffs: blue-brown color H-score (BBC-HS). Appl Immunohistochem Mol Morphol. 2023; 31: 500-6.
11. Li T, Jiang Y, Bai Y, Jiang K, Du G, Chen P, et al. A review for the impacts of circadian disturbance on urological cancers. Sleep Biol Rhythms. 2023; 22: 163-80.
12. Ren JW, Ye JJ, Bai YJ, Han P. The prognostic value of circadian syndrome in non-muscle-invasive bladder cancer: a retrospective cohort study. Int J Surg. 2026; 112: 3948-61.
13. Minato A, Jojima K, Watanabe S, Sugita Y, Mizushima Y, Matsukawa T, et al. Recurrence of low-risk non-muscle-invasive bladder cancer in patients who did not receive immediate intravesical chemotherapy. Cancer Diagn Progn. 2026; 6: 135-43.
14. Jeong SH, Han JH, Jeong CW, Kim HH, Kwak C, Yuk HD, et al. Clinical determinants of recurrence in pTa bladder cancer following transurethral resection of bladder tumor. BMC Cancer. 2022; 22: 631.
15. Isharwal S, Konety B. Non-muscle invasive bladder cancer risk stratification. Indian J Urol. 2015; 31: 289-96.
16. Offersen BV, Knap MM, Marcussen N, Horsman MR, Hamilton-Dutoit S, Overgaard J. Intense inflammation in bladder carcinoma is associated with angiogenesis and indicates good prognosis. Br J Cancer. 2002; 87: 1422-30.
17. Hodgson A, Xu B, Satkunasivam R, Downes MR. Tumour front inflammation and necrosis are independent prognostic predictors in high-grade urothelial carcinoma of the bladder. J Clin Pathol. 2018; 71: 154-60.
18. Wu YJ, Chang SJ, Huang YS, Chai CY. Overexpression of BMAL-1 is related to progression of urothelial carcinoma in arsenic exposure area. Int Urol Nephrol. 2025; 57: 1175-87.