**Acta Medica Ruha**
*International Journal of Medicine and Health Sciences*
E-ISSN: 2980-1184

**LETTER TO THE EDITOR**
Volume:2 Issue:3 Year:2024
https://doi.org/10.5281/zenodo.13784506

# Prognostic Significance of Tumor Budding in Bladder Cancer: A Call for Molecular Insights

Mesane Kanserinde Tümör Tomurcuklanmasının Prognostik Önemi: Moleküler Perspektifte Bir Çağrı

**Tunay Doğan**[1,2]

[1]Istinye University, Vocational School of Health Care Services, Department of Medical Laboratory Techniques
[2]Istinye University, Molecular Cancer Research Center (ISUMKAM), Istanbul, Türkiye

Dear Editor,

We wish to share our insights on the critical prognostic significance of tumor budding (TB) in bladder cancer (BC) and underscore the urgent necessity for further molecular-level investigations in this domain, prompted by recent studies. Tumor buds, also referred to as "sprouts," are defined as isolated single tumor cells and/or small clusters comprising fewer than five tumor cells, which originate from the invasive tumor margin and infiltrate the stroma. These entities were first characterized by Imai in the 1950s (1). The TB scoring system, established by the "International Tumour Budding Consensus Conference" (ITBCC) in 2016, has been validated as an independent predictor of lymph node metastasis in pT1 colorectal cancer cases and poor survival outcomes in stage 2 colon cancer cases, and it is now routinely reported by pathologists (2). Tumor buds are intimately associated with epithelial-mesenchymal transition (EMT) and engage in interactions with the tumor microenvironment (TME), tumor stroma, and immune system cells (3). This dynamic interaction at the molecular level in budding tumor cells establishes a distinctive signature characterized by: upregulation of MMP-7 and MMP-9 expressions, which play a role in extracellular matrix degradation; anoikis resistance through the enhanced expression of TrkB; frequent upregulation of stem cell markers such as LGR5, ALDH1, and CD44; immune evasion facilitated by the loss of MHC class I expression; increased TGFβ expression and regulation of TGFβ signaling; regulation of WNT signaling; a decrease in miRNA-200 expression, accompanied by the epigenetic upregulation of EMT-associated transcription factors, including ZEB, TWIST, and SNAIL; reduced expression of E-cadherin, particularly at the cell membrane, and β-catenin; an increase in mesenchymal markers like Vimentin, alongside a reduction in Cytokeratin expression; low levels of Ki-67 and Caspase-3 expression; and a relatively spindle-shaped morphology with podia formation (3). While it is generally accepted in solid tumors that "an increase in tumor buds correlates with a poorer clinical outcome" (3), the body of research examining TB as a prognostic marker in non-gastrointestinal tumors—particularly in BC—remains limited. As of August 8, 2024, a PubMed search using the MeSH terms "tumor budding AND bladder cancer*" yielded 34 studies, of which only 10 were found to be directly relevant to this subject.

Fukumoto and colleagues investigated the prognostic effects of TB in 121 cases of pT1 non-muscle-invasive bladder cancer (NMIBC) and demonstrated that TB positivity was statistically significantly associated with pT1 sub-staging (microinvasion/extensive lamina propria invasion) (p=0.002), tumor architecture (papillary/nodular) (p=0.023), and lymphovascular invasion (LVI) positivity (p=0.001) (4). Additionally, it was reported that the 5-year progression-free survival rate was statistically significantly higher (p=0.001) in TB-negative pT1 BC cases (88.4%), and that TB was an independent risk predictor for progression to muscle-invasive bladder cancer (MIBC) in both the entire pT1 BC cohort and the subgroup receiving intravesical BCG instillation according to Cox regression analysis (4). Building on these findings, the researchers also observed that in 86% of TB-positive cases, E-cadherin immunoexpression in the tumor center was higher than in the TB areas, highlighting the relationship between TB and EMT in pT1 BC cases (4). While Fukumoto et al. focused on the clinical implications

Corresponding Author: Tunay Doğan, e-mail: tunay.dogan@istinye.edu.tr
Received: 23.08.2024, Accepted: 13.09.2024, Published Online: 20.09.2024
Cited: Doğan T. Prognostic Significance of Tumor Budding in Bladder Cancer: A Call for Molecular Insights. Acta Medica Ruha. 2024;2(3):228-230. https://doi.org/10.5281/zenodo.13784506

of TB, Miyake and colleagues delved into the underlying molecular mechanisms. In their comprehensive study, they used MGH-U3, UM-UC-14, and UM-UC-3 cells in an orthotopic bladder tumor model in SCID mice and suggested that COL4A1 and COL13A1 might play a primary role in the formation of the infiltrative pattern of TB (5). However, as accurate TB analysis can be complicated by peritumoral inflammatory infiltrate or reactive stromal cells, Brieu and colleagues adopted a different approach by targeting cytokeratin, which had previously been shown to effectively distinguish TBs. They enhanced their analysis by applying machine learning and automated image analysis to IF-stained samples, thereby achieving more accurate quantification in a sample of 100 MIBC cases (6). In addition to these efforts to improve prognostic assessments, Liu and colleagues conducted a bioinformatics-based study in which they evaluated the tumor stroma ratio (TSR) and TB together in MIBC cases. By developing a TSR-TB scoring system, they reported that increased TSR-TB might be an independent poor prognostic factor for overall survival (7). In contrast, in the study conducted by Kucuk and colleagues on a sample of 60 MIBC cases, no statistically significant relationship was found between TB and tumor necrosis ($p=1.000$), LVI ($p=0.114$), or perineural invasion ($p=0.712$) (8). However, a different perspective is offered by Seker and colleagues, who, in their study involving a sample of 108 MIBC cases, found that TB was statistically significantly associated with overall survival ($p=0.004$) (9). The researchers reported that TB could be a useful parameter for predicting prognosis in MIBC cases (9). Further emphasizing the prognostic importance of TB, Soriano and colleagues quantified TB in a sample of 108 MIBC cases that had undergone pancytokeratin staining and reported that TB is an independent risk predictor for mortality (10). The researchers further noted that MIBC cases with 14 or more TBs were associated with an increased risk of mortality as well as a higher tumor stage, suggesting that each additional TB increases the cancer-specific mortality risk by approximately 2% in these cases (10). Expanding on these findings, Busquets and colleagues, in their study involving 168 high-grade stage pT1 NMIBC cases, reported that TB (when present with a count of 6 or more) ($p=0.032$, HR: 2.1), along with the presence of carcinoma in situ (CIS), endoscopic tumor pattern (papillary/solid), and the absence of BCG induction, was significant in predicting disease progression according to multivariate variance analysis (11). The researchers also emphasized that the inclusion of TB in the TNM staging system should be carefully considered and that it could assist in the decision-making process for early radical cystectomy in high-grade stage pT1 NMIBC cases (11). Eckstein and colleagues built on this by studying 92 pT1 NMIBC cases with pancytokeratin staining. They reported that, according to Kaplan–Meier analysis, TB was statistically significantly associated with worsened recurrence-free survival ($p=0.005$), progression-free survival ($p=0.017$), and cancer-specific survival ($p=0.002$) (12). The researchers also found that the presence of TB was associated with multifocal tumors ($p=0.003$) and extensive lamina propria invasion when pT1 sub-staging was performed ($p<0.001$) (12). Interestingly, among the cases that received BCG instillation, those without TB not only had better recurrence-free survival ($p=0.012$), progression-free survival ($p=0.011$), and cancer-specific survival ($p=0.022$), but also no progression or disease-related deaths were observed in this group (12). Finally, Yang and colleagues contributed further by conducting a retrospective study involving 80 BC cases (36 NMIBC and 44 MIBC) (13). They investigated the prognostic effects of EPDR1 immunoexpression and TB quantification (13). The researchers quantified TB in 44 MIBC samples, considering those with six or more TBs as positive. The study found that EPDR1 immunoexpression varied statistically significantly ($p<0.05$) with tumor stage, and MIBC cases with high EPDR1 immunoexpression were statistically significantly more likely to have increased TB ($p<0.05$) (13). Furthermore, an increased TB count was associated with a tendency toward a worse clinical status in MIBC cases ($p<0.001$) (13).

The scoring of TB varies depending on the type of solid tumor. As a result, following the development of a standardized scoring system that can be adapted to BC, the inclusion of TB as a prognostic marker in routine histopathological evaluation could provide clinicians with significant advantages in risk stratification and treatment planning, particularly in managing patients with high-grade stage pT1 NMIBC who require accurate progression prediction. Although TB scoring is more easily and reliably performed on slides stained with pancytokeratin IHC, the fact that it can also be commonly done on routine H&E stained slides makes this cost-effective marker an attractive option for clinical use in BC as well. Since tumor budding is biologically closely related to EMT and the TME, detailed investigation of the underlying molecular mechanisms holds promise for the development of actionable targets. In this context, we believe that further multicenter prospective studies and more detailed molecular

analyses are necessary to validate the clinical utility of TB and evaluate its integration into existing prognostic models. These investigations could not only validate the clinical utility of TB but also pave the way for more personalized treatment strategies in BC.

Sincerely.

## DESCRIPTIONS

**No financial support.**

**No conflict of interest.**

## REFERENCES

1. Imai T. Growth patterns in human carcinoma. Their classification and relation to prognosis. PubMed. 1960;16:296-308. https://pubmed.ncbi.nlm.nih.gov/13717558.

2. Lugli A, Kirsch R, Ajioka Y, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Modern Pathology. 2017;30(9):1299-1311. doi:10.1038/modpathol.2017.46

3. Lugli A, Zlobec I, Berger MD, Kirsch R, Nagtegaal ID. Tumour budding in solid cancers. Nature Reviews Clinical Oncology. 2020;18(2):101-115. doi:10.1038/s41571-020-0422-y

4. Fukumoto K, Kikuchi E, Mikami S, et al. Tumor budding, a novel prognostic indicator for predicting stage progression in T1 bladder cancers. Cancer Science. 2016;107(9):1338-1344. doi:10.1111/cas.12990

5. Miyake M, Hori S, Morizawa Y, et al. Collagen type IV alpha 1 (COL4A1) and collagen type XIII alpha 1 (COL13A1) produced in cancer cells promote tumor budding at the invasion front in human urothelial carcinoma of the bladder. Oncotarget. 2017;8(22):36099-36114. doi:10.18632/oncotarget.16432

6. Brieu N, Gavriel CG, Nearchou IP, Harrison DJ, Schmidt G, Caie PD. Automated tumour budding quantification by machine learning augments TNM staging in muscle-invasive bladder cancer prognosis. Scientific Reports. 2019;9(1). doi:10.1038/s41598-019-41595-2

7. Liu J, Li C, Huang K, et al. A classification based on tumor-stroma ratio and tumor budding for patients with muscle-invasive bladder cancer. Expert Review of Anticancer Therapy. 2021;22(3):323-330. doi:10.1080/14737140.2022.2012158

8. Kucuk U, Ekmekci S, Cakir E, Ekin Z, Ergani B, Ekin GR. Prognostic significance of tumor budding in muscle invasive urothelial carcinomas of the bladder. Turkish Journal of Urology. 2019;45(4):273-278. doi:10.5152/tud.2018.58269

9. Seker NS, Tekin E, Özen A, Can C, Colak E, Acikalin MF. Prognostic significance of tumor budding in muscle invasive urothelial carcinomas of the urinary bladder. Annals of Diagnostic Pathology. 2021;54:151786. doi:10.1016/j.anndiagpath.2021.151786

10. Soriano LL, Jurado GO, Moreno JLP, et al. Tumor budding: Prognostic value in muscle-invasive bladder cancer. Urology. 2019;130:93-98. doi:10.1016/j.urology.2019.04.006

11. Busquets CXR, Semidey ME, Palacio FL, et al. Is tumor budding a new predictor for early cystectomy in PT1 High-Grade bladder cancer? Urologia Internationalis. 2021;106(2):154-162. doi:10.1159/000517543

12. Eckstein M, Kimmel C, Bruendl J, et al. Tumor budding correlates with tumor invasiveness and predicts worse survival in pT1 non-muscle-invasive bladder cancer. Scientific Reports. 2021;11(1). doi:10.1038/s41598-021-97500-3

13. Yang Y, Xu H, Zhu H, et al. EPDR1 levels and tumor budding predict and affect the prognosis of bladder carcinoma. Frontiers in Oncology. 2022;12. doi:10.3389/fonc.2022.986006