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Diagnostic Utility of Immunohistochemical Markers Gata3 and P63 in Urothelial Carcinoma: A Prospective Study

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Abstract

Background: Urothelial carcinoma (UC) is the most common malignancy of the urinary bladder and shows wide morphological heterogeneity. Immunohistochemistry (IHC) is a valuable adjunct in diagnostically challenging cases such as poorly differentiated tumors, small biopsies, and metastatic deposits. GATA3 and p63 are two nuclear markers commonly used to confirm urothelial differentiation, but their expression patterns differ considerably across tumor grade and stage.

Aim: To evaluate the immunohistochemical expression of GATA3 and p63 in urothelial carcinoma and to correlate their expression with tumor grade and muscle invasion, thereby comparing their diagnostic utility.

Materials and Methods: This prospective observational study was conducted in the Department of Pathology, MGM Medical College and Hospital, Navi Mumbai. Thirty-five histopathologically confirmed cases of urothelial carcinoma, predominantly transurethral resection of bladder tumor (TURBT) specimens, were evaluated. Hematoxylin and eosin (H&E) stained sections were assessed for tumor grade and muscle invasion. Immunohistochemical staining for GATA3 and p63 was performed and scored using a semi-quantitative immunoreactivity score based on staining intensity and percentage of positive tumor cells.

Results: The majority of patients were in the 50-70 year age group (mean age 61 years) with a clear male predominance. High-grade tumors constituted 74.3% of cases, and muscle invasion was present in 42.9% of

cases. GATA3 showed diffuse nuclear positivity in 100% of cases with expression preserved across both low-grade and high-grade tumors, including muscle-invasive lesions. p63 was positive in approximately 74% of cases, with strong staining largely confined to low-grade tumors and weak, patchy, or absent staining in high-grade and invasive tumors.

Conclusion: GATA3 demonstrated greater sensitivity, more uniform staining, and more consistent expression across tumor grade and invasion status than p63. GATA3 can therefore be regarded as a superior primary marker for confirming urothelial differentiation, while p63 is best used as a supportive marker within an immunohistochemical panel, particularly in high-grade and invasive urothelial carcinoma.

Keywords: Urothelial carcinoma; GATA3; p63; Immunohistochemistry; Tumor grade; Muscle invasion.

Introduction

Urothelial carcinoma (UC) is the most common malignancy of the urinary bladder, arising from the transitional epithelium lining the urinary tract.[1–3] Bladder cancer ranks among the most frequently diagnosed malignancies worldwide, shows a clear male predominance, and predominantly affects individuals in the sixth and seventh decades of life.[5,6] A key feature of the disease is its high recurrence rate, which necessitates long-term follow-up and repeated interventions.[7]

UC is a biologically heterogeneous group of tumors ranging from low-grade, non-invasive lesions to high-grade, invasive tumors with metastatic potential.[1,4] Its wide morphological spectrum - papillary, flat, nested, or infiltrative growth patterns, together with variant histologies such as micropapillary, plasmacytoid, and sarcomatoid types - can closely mimic other malignancies, creating diagnostic difficulty, especially in small or fragmented biopsy samples.[1,13–15]

While hematoxylin and eosin (H&E) staining remains the diagnostic gold standard, it has recognized limitations in poorly differentiated tumors, limited TURBT material, and variant histology.[2,3,14,15] Immunohistochemistry (IHC) has therefore become an essential adjunct, particularly for confirming urothelial origin, differentiating UC from morphological mimickers, and increasing diagnostic confidence in small or metastatic specimens.[15,16]

GATA3, a zinc-finger transcription factor involved in epithelial differentiation, shows strong, diffuse nuclear staining that is characteristically maintained in both low-grade and high-grade urothelial carcinomas, including poorly differentiated tumors.[19,20] p63, a nuclear transcription factor of the p53 family associated with basal cell differentiation, is typically strongly expressed in low-grade tumors but becomes patchy, reduced, or lost in high-grade and invasive disease, reflecting loss of basal differentiation during tumor progression.[22,23]

Since both markers are widely used but demonstrate differing expression patterns, there is a need to systematically compare their diagnostic utility, evaluate their consistency across tumor grades, and assess their reliability in routine diagnostic practice. This study was therefore undertaken to evaluate the immunohistochemical expression of GATA3 and p63 in urothelial carcinoma and to determine which marker is more sensitive, consistent, and reliable for diagnosis.

Aim and Objectives

The aim of this study was to evaluate the immunohistochemical expression of GATA3 and p63 in urothelial carcinoma and to correlate their expression with clinicopathological parameters, thereby assessing

their diagnostic utility. The specific objectives were: (1) to study GATA3 and p63 expression in urothelial carcinoma, and (2) to assess their diagnostic utility and compare their respective roles.

Materials and Methods

This prospective observational study was conducted in the Histopathology section of the Department of Pathology, MGM Medical College and Hospital, Kamothe, Navi Mumbai. Patients presenting with clinical suspicion of urothelial malignancy were included, and relevant clinical and demographic data were recorded on a structured proforma. A total of 35 histopathologically confirmed cases of urothelial carcinoma, received during the study period, were evaluated.

Inclusion Criteria

Specimens from the bladder, urethra, and renal pelvis showing features suggestive of urothelial malignancy, in patients aged over 18 years who provided informed consent, were included.

Exclusion Criteria

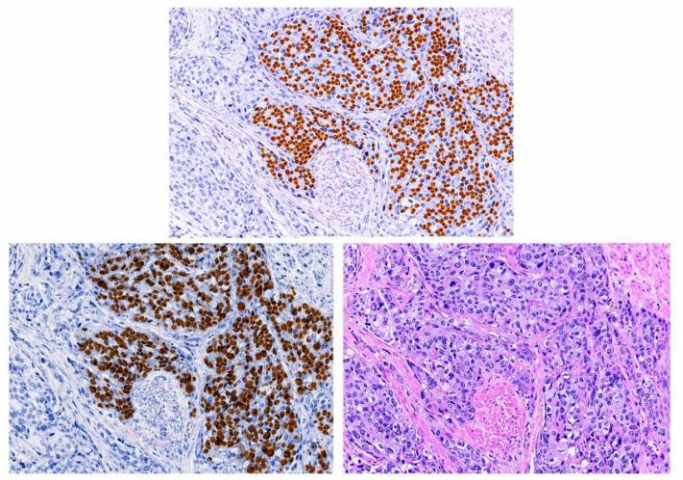
Specimens diagnosed as non-urothelial malignancies or benign lesions, inadequate or insufficient tissue samples, and specimens with poor preservation, autolysis, or improper fixation were excluded.

Specimen Processing and Histopathological Evaluation

The study primarily included transurethral resection of bladder tumor (TURBT) specimens, along with other biopsy and cystectomy specimens where available. All tissue was fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Sections of 3-4 µm thickness were stained with H&E and examined for architectural pattern, cytological atypia, and depth of invasion. Tumors were classified and graded according to the WHO classification of urothelial carcinoma. [1]

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks of confirmed cases were stained for GATA3 and p63 using standard IHC protocols with appropriate positive and negative controls. Only well-preserved, viable tumor areas were evaluated, with a minimum of 3-5 representative high-power fields examined per case. The percentage of tumor cells showing nuclear positivity was scored as focal (<10%), patchy (10-50%), or diffuse (>50%), and staining intensity was graded as negative (0), weak (1+), moderate (2+), or strong (3+). An immunoreactivity score (IRS) was derived as the product of the intensity score and the percentage score, and cases were categorized as negative (0-1), weakly positive (2-4), moderately positive (5-8), or strongly positive (9-12). p63 expression was further classified by distribution pattern as basal, suprabasal, or diffuse, with particular attention to loss of the basal staining pattern in high-grade and invasive tumors.



Statistical Analysis

All findings were compiled in Microsoft Excel, and statistical analysis was performed using SPSS software. The chi-square test was applied to categorical variables,

and a p-value < 0.05 was considered statistically significant.

Results

A total of 35 cases of urothelial carcinoma were included in the study. The majority of patients were in the fifth to seventh decade of life (mean age 61 years), and a clear male predominance was noted. Painless hematuria was the most common presenting complaint, occasionally accompanied by irritative urinary symptoms such as dysuria and increased frequency. Most specimens were obtained by TURBT, with a smaller proportion received as biopsy or cystectomy specimens. All cases were diagnosed as urothelial carcinoma, not otherwise specified, with occasional variant differentiation.

Age Distribution

Table 1 summarizes the age-wise distribution of cases according to tumor grade.

Age group (years)	High grade	Low grade
<50	3	1
50-70	14	8
>70	9	0

Tumor Grade and Muscle Invasion

High-grade tumors accounted for 74.3% (26/35) of cases, while low-grade tumors comprised 25.7% (9/35), reflecting a clear predominance of high-grade lesions. Muscle invasion was present in 42.9% (15/35) of cases, with the remaining 57.1% (20/35) being non-muscle-invasive, indicating that a substantial proportion of cases in this cohort had invasive disease at the time of diagnosis.

GATA3 Expression

GATA3 positivity was observed in 100% (35/35) of cases, with a predominantly diffuse nuclear staining pattern and moderate to strong staining intensity in most cases. Expression was consistently retained across both low-grade and high-grade tumors and was preserved in muscle-invasive lesions, with no significant reduction in staining noted with increasing tumor grade or depth of invasion.

p63 Expression

p63 expression was seen in approximately 74% of cases, with the staining pattern varying from diffuse to focal. Strong, diffuse p63 positivity was mainly observed in low-grade tumors, whereas high-grade and muscle-invasive tumors more frequently showed weak, patchy, or absent staining, indicating a decline in expression with increasing tumor grade and invasion.

Comparative Diagnostic Utility

Table 2 summarizes the comparative diagnostic performance of GATA3 and p63 observed in this study.

Parameter	GATA3	p63
Sensitivity	Very high (100%)	Moderate (~74%)
Staining pattern	Diffuse and consistent	Variable and patchy
Expression in high-grade tumors	Preserved	Reduced
Expression in muscle-invasive tumors	Preserved	Frequently decreased
Diagnostic reliability	Superior / primary marker	Supportive / adjunct marker

A strong concordance was observed between histopathological diagnosis and GATA3 expression across all tumor grades and invasion status. In contrast, discordance was noted more frequently with p63, chiefly due to reduced or absent staining in high-grade and invasive tumors, underscoring the greater reliability of GATA3 as a confirmatory marker of urothelial differentiation in this cohort.

Discussion

This study evaluated the immunohistochemical expression of GATA3 and p63 in 35 histopathologically confirmed cases of urothelial carcinoma and compared their diagnostic utility in relation to tumor grade and muscle invasion.

The predominance of cases in the 50-70 year age group, with a mean age of 61 years, is consistent with reports by Siegel et al.[6] and Cumberbatch et al.[9], who similarly described urothelial carcinoma as predominantly a disease of middle-aged and elderly individuals, likely reflecting cumulative carcinogenic exposure and reduced cellular repair mechanisms with age. The male predominance observed in this cohort corroborates findings by Cumberbatch et al.[9] and Freedman et al.[8], and is generally attributed to greater historical exposure to smoking and occupational carcinogens among men.

Painless hematuria was the most frequent presenting symptom in this study, consistent with observations by Babjuk et al.[7] and Epstein et al.[14], who described it as the classical presenting feature of bladder malignancy, occurring due to ulceration and friability of the tumor surface. Irritative urinary symptoms, when present, were more often associated with invasive disease, in agreement with Cheng et al.[4]

High-grade tumors predominated in this cohort (74.3%), consistent with the observations of Humphrey et al.[13] and Paner et al.[15], who attributed the predominance of high-grade lesions in tertiary care settings to relatively advanced disease at presentation. Muscle invasion was present in 42.9% of cases, underscoring the aggressive biological behavior frequently encountered in this setting, in keeping with the prognostic significance of muscle invasion described by Sylvester et al.[26] and Amin et al.[12]

GATA3 demonstrated 100% positivity with diffuse, moderate-to-strong nuclear staining that was preserved irrespective of tumor grade or muscle invasion. This finding closely mirrors the results of Liu et al.[20], Miettinen et al.[19], and Sangoi et al.[24], all of whom reported high sensitivity and consistent GATA3 expression even in high-grade and invasive urothelial carcinomas. This stability likely reflects the role of GATA3 as a lineage-determining transcription factor rather than a marker of proliferative or basal-cell activity, making it particularly valuable in poorly differentiated tumors and limited biopsy material.[19],[20]

In contrast, p63 was positive in only about 74% of cases, with a marked decline in staining intensity and extent in high-grade and muscle-invasive tumors. This is concordant with the findings of Di Como et al.[22], Urist et al.[23], and Paner et al.[15], who similarly reported strong p63 expression restricted largely to low-grade, basal-differentiated tumors, with progressive loss of expression accompanying tumor dedifferentiation and invasion. Since p63 reflects basal-cell phenotype, its reduction in high-grade disease is biologically consistent with loss of basal differentiation during tumor progression.[22],[23]

Taken together, these findings support GATA3 as the more sensitive, consistent, and diagnostically reliable marker of urothelial differentiation, while p63 - though

useful, particularly in well-differentiated tumors and for identifying squamous differentiation - is best regarded as a supportive marker within a broader immunohistochemical panel rather than a stand-alone diagnostic tool, especially in high-grade or muscle-invasive urothelial carcinoma.[19,20,22–24]

Conclusion

In this study of 35 cases of urothelial carcinoma, GATA3 showed diffuse and consistent nuclear positivity in all cases, irrespective of tumor grade or muscle invasion, confirming its high sensitivity and reliability as a marker of urothelial differentiation. p63, in contrast, showed variable expression with a marked decline in high-grade and muscle-invasive tumors, limiting its reliability when used in isolation. GATA3 can therefore be considered a superior primary immunohistochemical marker for confirming urothelial origin, particularly in poorly differentiated, invasive, or limited biopsy specimens, while p63 remains a useful adjunct marker within a panel-based diagnostic approach. The combined use of histopathology with GATA3-based immunohistochemistry can meaningfully improve diagnostic accuracy and confidence in the evaluation of urothelial carcinoma.

Ethical Approval

The study was approved by the Institutional Ethics Committee prior to commencement.

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