Int J Clin Exp Pathol 2024;17(8):236-244
www.ijcep.com /ISSN:1936-2625/IJCEP0157025

Original Article

Expression and clinicopathologic significance of HER2 and PD-L1 in high grade urothelial carcinoma of the urinary tract

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Received April 4, 2024; Accepted July 5, 2024; Epub August 15, 2024; Published August 30, 2024

Abstract: Background: Urothelial carcinoma (UC) is an aggressive tumor with high recurrence rates and poses a great challenge for clinical management. Programmed death ligand-1 (PD-L1) inhibitors and human epidermal growth factor receptor 2 (HER2) blockers have been approved for the treatment of advanced urothelial carcinoma. PD-L1 and HER2 expression in UC will determine whether patients are likely to respond to these targeted treatments. This study assessed the expressions of HER2 and PD-L1 in UC at our institution and investigated their correlations with gender, tumor location (upper genitourinary (GU) tract vs. lower GU tract), tumor stage, and histologic divergent subtypes. Design: Patients with UC who had PD-L1 or HER2 immunostains performed in the past 3 years at our institution were included in our analysis. A total of 97 cases were identified. PD-L1 and HER2 scores were provided by two experienced GU pathologists. HER2 scores were given according to the criteria used in breast cancer, while PD-L1 scores were reported as the combined positive score. We assessed correlation of the scores with the patients' gender, tumor location, tumor stage, and histologic divergent subtypes. The data for PD-L1 expression were analyzed using the Mann-Whitney U Test for gender and urinary tract location, and one-way analysis of variance (ANOVA) for stage and histology. The data for HER2 expression were analyzed using the chi-square test. For all analyses, significance was set at $P<0.05$. Results: Of the 97 patients, the average age was 69 years. There were 95 patients who had previously reported HER2 results and 86 patients who had PD-L1 results. PD-L1 expression did not show a significant difference among the histological divergent subtypes ($P=0.36$). However, HER2 status exhibited a significant difference, with more HER2-positive cases observed in the conventional histology ($P=0.008$). No correlation between HER2 status and either gender or tumor stage was identified. The median PD-L1 combined positive score was significantly higher in lower urinary tract UC than upper (10 and 2, respectively; $P=0.049$). No significant differences were observed for gender or pathologic stage. Conclusion: Our data suggest that HER2 is more frequently expressed in conventional UC than in divergent subtypes. Additionally, PD-L1 has a higher expression level in lower urinary tract UC compared to upper. However, PD-L1 and HER2 expression are not related to gender or tumor stage in UC.

Keywords: PD-L1, HER2, high grade urothelial carcinoma

Introduction

Urothelial carcinoma (UC) originates from the urothelium and can arise in the renal pelvis, ureter, bladder, and urethra [1]. The renal pelvis and ureter are defined as the upper urinary tract, while the bladder and urethra are considered the lower urinary tract. UC is the most common type of bladder cancer (BC), constituting approximately 90% of all BC cases. Globally, BC ranks as the 10th most common cancer, with an estimated 573,278 new cases reported in 2020 [2]. In China, BC is the 13th most prevalent cancer, with around 85,694 new cases diagnosed in the same year [3]. Locally advanced and metastatic urothelial carcinoma (la/mUC) account for 7% and 5% of all UC cases, respectively. Despite advancements in treatment, the five-year survival rate remains dishearteningly low, with only 34% survival for locally advanced UC and a mere 5.4% for metastatic UC cases. Predicting the risk of

https://doi.org/10.62347/AAPB6946

recurrence or disease progression remains challenging due to the lack of reliable biomarkers.

Both clinical and morphologic characteristics have been reported to be associated with the prognosis and predictive of outcomes in UC, including gender, primary site, tumor grade, pathologic stage, histologic divergent subtypes, time to recurrence, and the consideration of early radical cystectomy [4]. As a result of ongoing investigations into the molecular pathways of UC, new therapeutic targets have been identified including human epidermal growth factor receptor 2 (HER2/neu) and programmed death ligand 1 (PD-L1).

The genomic profiling of UC revealed actionable genomic alterations, indicating the potential for targeted therapies that specifically address driver mutations such as HER2 [5-7]. A range of antibody-drug conjugates (ADCs) have been devised, including enfortumab vedotin (EV) targeting nectin-4, RC48-ADC targeting HER2, and sacituzumab govitecan targeting TROP-2. These ADCs have demonstrated promising efficacy in clinical trials, indicating their use as valuable therapeutic options for UC patients [8].

According to the updated European Association of Urology (EAU) guidelines for metastatic urothelial carcinoma (mUC), the first-line therapy for patients is platinum-based chemotherapy [9]. In recent years, immune checkpoint inhibitors (ICIs) have been approved for patients who are ineligible for chemotherapy or have progressed despite chemotherapy. For patients with PD-L1 expression who are unable to receive cisplatin, immunotherapy options such as atezolizumab or pembrolizumab may be administered [10-12]. If the disease shows no signs of progression while on platinum-based chemotherapy, subsequent maintenance immunotherapy with avelumab is advised [13, 14].

In our study, we evaluated the expression of HER2 and PD-L1 in UC in a large medical institution, and investigated their correlations with gender, tumor location, tumor stage, and histologic divergent subtypes. The results may fill in the knowledge gap in these associations and provide further directions for treatment.

Methods

Tissue samples

A total of 97 cases with a diagnosis of high grade urothelial carcinoma of the urinary tract from 2018 to 2024 were retrieved from the internal pathology system of Mount Sinai Hospital. The corresponding HER2 and PD-L1 immunostain results were collected if available. Clinicopathologic parameters including age, gender, submitted tissue type, tumor location (upper urinary tract or lower urinary tract), grade, and stage were obtained from the patients' medical records. Cases with tumors other than urothelial carcinoma, cases of metastatic tumor with primary site other than urothelium, or cases of recurrence and prior chemotherapy were excluded from this study. Cases with urothelial carcinoma involving both upper and lower urinary tract were excluded only from the site analysis.

Definition and diagnostic criteria

Grade and stage were determined based on World Health Organization (WHO) classification and College of American Pathologists (CAP) Cancer Protocol TNM staging system [4]. Recurrence was defined as the emergence of a new tumor, confirmed by biopsy, three months after the initial transurethral resection of the bladder tumor, within the follow-up period [15].

Immunohistochemical (IHC) analysis

Both HER2 IHC and PD-L1 IHC were previously performed on whole section slides from formalin-fixed, paraffin-embedded tissue. HER2 IHC staining was performed using the Roche anti-HER2/neu antibody, Clone 4B5. IHC scoring was independently performed by two experienced pathologists to reach an agreed consensus. The HER2 scoring was analyzed according to the CAP protocol for assessing HER2 in breast cancer [16, 17]. Four scoring categories were utilized: Score 0 (no staining), Score 1+ (characterized by incomplete membrane staining that is faint or barely perceptible in more than 10% of tumor cells), Score 2+ (showing incomplete and/or weak to moderate membrane staining in more than 10% of tumor cells), and Score 3+ (demonstrating circumferential, intense, complete membrane staining in

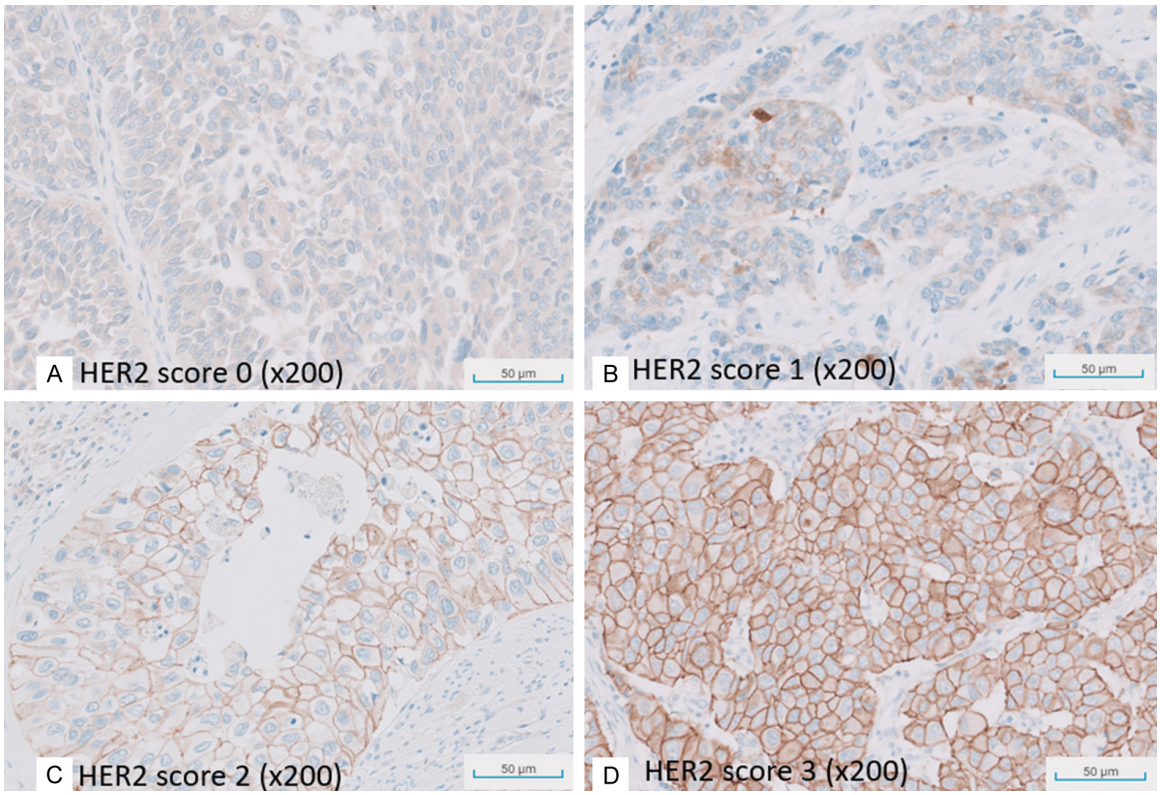

Figure 1. Urothelial carcinomas with (A) HER2 score 0 (×200), (B) HER2 score 1+ (×200), (C) HER score 2+ (×200) and (D) HER2 score 3+ (×200).

more than 10% of tumor cells) (**Figure 1**) [17-19]. A HER2 IHC score of 3+ was regarded as positive, HER2 IHC scores of either 0 or 1+ were regarded as negative; and a HER2 score of 2+ was further confirmed by Fluorescence In Situ Hybridization (FISH) to determine if it was positive or negative [18]. PD-L1 staining utilized the polyclonal anti-PD-L1 antibody clone 22C3, performed on the Dako autostainer. The PD-L1 IHC interpretation was performed following the guidelines of the PD-L1 IHC 22C3 pharmDx Interpretation Manual for Urothelial Carcinoma and recorded as a combined positive score (CPS) [18, 20, 21]. The PD-L1 staining ranged from absent or weak to moderate or strong (**Figure 2**).

Statistical analysis

HER2 scoring analysis was performed on all the collected data using Stata Statistical Software. The Stata program utilizes the Chi-square test to assess the significance of associations between categorical variables, i.e. the association between gender, location, stage, and histologic divergent subtypes with HER2 scoring results. The Analysis of Variance (ANOVA) analysis was used for HER2 pairwise comparison among the different histology groups with Bonferroni Correction. The continuous data of the PD-L1 CPS is presented as median with interquartile range (IQR). The Mann-Whitney U test was used to compare two groups, while the Kruskal-Wallis test was utilized for comparisons involving more than two groups. A *p*-value of less than 0.05 was considered significant.

Results

General characteristics

A total of 97 patients with a diagnosis of UC from 2018 to 2024 were included in the study, made up of 32 females (32.9%) and 65 males (67.1%) with a mean age of 69.1 years.

Association of clinicopathological characteristics with expression of HER2

Among 97 cases, 95 cases had a HER2 score. There were 25 cases (27.8%) with a site of

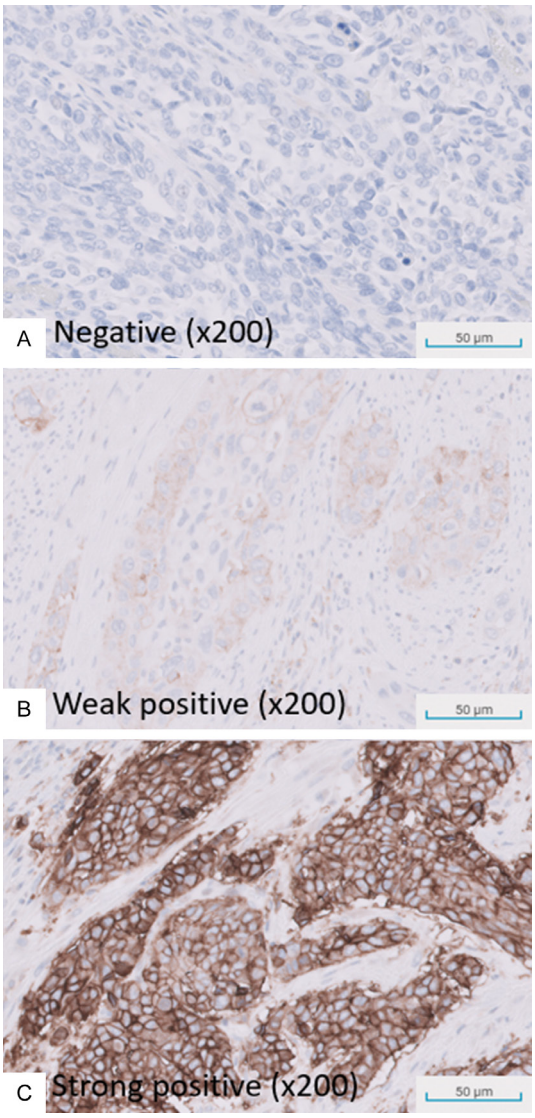

Figure 2. PD-L1 immunohistochemical staining in urothelial carcinoma. A. Negative expression of PD-L1 (×200). B. Weak positive expression of PD-L1 (×200). C. Strong positive expression of PD-L1 (×200).

upper urinary tract. Among these 25 cases, 9 cases (36%) had a HER2 IHC score of 2+ (showing incomplete and/or weak to moderate membrane staining in more than 10% of tumor cells) and 9 cases (36%) had a HER2 IHC score of 3+ (showing circumferential, intense, complete membrane staining in more than 10% of tumor cells). Among the 65 cases (72.2%) in the lower urinary tract, 24 cases (36.9%) had a HER2 IHC score of 2+. There were 5 remaining cases which involved both the upper and lower urinary tracts and were excluded from this analysis. Overall, HER2 expression showed no significant difference based on location.

Regarding pathologic staging, 72 out of 97 cases had TNM staging recorded. Three cases fell into the category of pTa (Non-invasive papillary carcinoma) or pTis (urothelial carcinoma in situ), while all other 69 cases were invasive urothelial carcinoma (pT1 to pT4) with 36 cases (50%) belonging to pT3. There was no statistically significant association between HER2 expression and stage. For the histologic divergent subtypes, HER2 expression showed a significant difference among the different variants of invasive urothelial carcinoma (P=0.008), indicating histologic divergent subtypes have different HER2 expression (**Table 1**). After performing the pairwise HER2 scoring analysis among different histologic variants with Bonferroni Correction, there was a statistically significant difference in HER2 scores according to histologic type (P=0.0012). Squamous histology had significantly lower HER2 expression compared to cases with conventional histology (P=0.011), micropapillary histology (P=0.004) or mixed histology (P=0.03). However, there was no significant difference found between the glandular and conventional or micropapillary histology groups (P=1.000). There was also no significant difference between the poorly differentiated divergent subtypes and any other histologic group (**Table 2**).

Association of clinicopathological characteristics with expression of PD-L1

Out of the 86 cases with a PD-L1 CPS, 85 had a clearly identified tumor location. Among these, 22 cases (25.89%) involved the upper urinary tract, 60 cases (70.59%) were in the lower urinary tract, and 3 cases (3.53%) involved both and were excluded from the statistical analysis of sites. The PD-L1 CPS was significantly higher (showing staining as moderate or strong) in the lower urinary tract tumors compared to the upper urinary tract tumors (P=0.048). Among the 67 cases with recorded pathologic stages, no significant difference in PD-L1 CPS was observed among the various stages. Finally, no significant difference was observed for PD-L1 CPS among the histologic divergent subtypes (See **Table 1**).

Table 1. HER2 and PD-L1 expression

Category	Case number for HER-2	Her-2 (score 0)	Her-2 (score 1)	Her-2 (score 2)	Her-2 (score 3)	*P*-value for HER-2	Case number for PDL-1	PDL-1 Median (IQR)	*P*-value for PDL-1
Age	69.1 ± 1.1						71 ± 6.5		
Gender						0.242			0.50926
Female	32 (33.7%)	4 (4.21%)	6 (6.32%)	12 (12.63%)	10 (10.53%)		31 (36.05%)	5 (1-20)	
Male	63 (66.3%)	14 (14.74%)	4 (4.21%)	24 (25.26%)	21 (22.11%)		55 (63.95%)	5 (1-30)	
Location						0.928			0.04884
Upper	25 (27.78%)	5 (5.56%)	2 (2.22%)	9 (10.00%)	9 (10.00%)		22 (25.89%)	2 (1-10)	
Lower	65 (72.22%)	13 (14.44%)	8 (8.89%)	24 (26.67%)	20 (22.22%)		60 (70.59%)	10 (1.5-35)	
Stage						0.394			0.5446
pTa, pTis	3 (4.17%)	0 (0)	0 (0)	2 (2.78%)	1 (1.39%)		2 (2.99%)	NA	
pT1	6 (8.33%)	1 (1.39%)	0 (0)	1 (1.39%)	4 (5.56%)		5 (7.46%)	10 (3.5-17.5)	
pT2	14 (19.44%)	4 (5.56%)	3 (4.17%)	4 (5.56%)	3 (4.17%)		13 (19.40%)	30 (2.5-77.5)	
pT3	36 (50.00%)	6 (8.33%)	4 (5.56%)	16 (22.22%)	10 (13.89%)		34 (50.75%)	4 (1-25)	
pT4	13 (18.06%)	2 (2.78%)	2 (2.78%)	2 (2.78%)	7 (9.72%)		13 (19.40%)	4 (1.5-17.5)	
Histology						0.008			0.3663
Conventional	44 (49.44%)	6 (6.74%)	4 (4.49%)	18 (20.22%)	16 (17.98%)		40 (47.62%)	5 (1-15)	
Squamous	15 (16.85%)	8 (8.99%)	2 (2.25%)	3 (3.37%)	2 (2.25%)		15 (17.86%)	20 (2-60)	
Glandular	4 (4.49%)	0 (0)	1 (1.12%)	3 (3.37%)	0 (0)		4 (4.76%)	5.5 (0.5-35)	
Micropapillary	8 (8.99%)	0 (0)	1 (1.12%)	1 (1.12%)	6 (6.74%)		8 (9.52%)	5 (1.5-27.5)	
Poorly differentiated	12 (13.48%)	4 (4.49%)	1 (1.12%)	5 (5.62%)	2 (2.25%)		11 (13.10%)	10 (1-60)	
Mixed differentiation	6 (6.74%)	0 (0)	1 (1.12%)	1 (1.12%)	4 (4.49%)		6 (7.14%)	15 (2-25)	

Table 2. *P*-values for ANOVA comparing HER2 results by histology (with Bonferroni correction)

Histologic subtype	Conventional	Glandular	Micropapillary	Multiple (>1 type of differentiation)	Poorly differentiated
Glandular	1.000				
Micropapillary	1.000	1.000			
Multiple (>1 type of differentiation)	1.000	1.000	1.000		
Poorly differentiated	1.000	1.000	0.165	0.543	
Squamous	0.011	1.000	0.004	0.030	1.000

Discussion

Urothelial carcinoma remains a burdensome malignancy globally in terms of incidence and mortality [22, 23]. HER2 over-expression in tumor tissues has been recognized across various cancers, including breast, colon, gastric, lung, and bladder cancer [24]. HER2 has also been a valuable prognostic and predictive biomarker in breast cancer and advanced gastric cancer [25]. Given its potential as a therapeutic target and predictive biomarker, there is growing interest in evaluating HER2 status in urothelial carcinoma [26]. Immune checkpoint inhibitors (ICIs) have also emerged as promising tools to combat urothelial carcinoma by blocking the interaction between programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1), thereby enhancing the immune function of anti-tumor T cells [27]. As research continues to explore anti-HER2 and PD-L1 inhibitors as treatment options for urothelial carcinoma, it becomes crucial to gather data on HER2 and PD-L1 expression and their correlation with clinical or pathologic features of UC.

Our study evaluated the status of PD-L1 and HER2 expression in UC and investigated their correlations with gender, tumor site (upper GU tract vs. lower GU tract), stage, and histologic divergent subtypes.

Histopathologic diagnosis remains the gold standard for diagnosing and staging urothelial carcinoma. The management of this condition relies on clinicopathological data such as grade, stage, and whether the tumor has recurred. These findings also play a pivotal role in prognostic assessment. However, even for tumors with a similar grade or stage, patient outcomes remain unpredictable [28, 29]. This unpredictability regarding recurrence, progression to later stages, or response to available therapy has prompted investigations into prognostic biomarkers, with HER2 emerging as a key player. This is largely due to the improved outcomes observed in HER2-positive breast carcinoma patients who received HER2-targeted therapy, as well as the ongoing development of anti-HER2 therapy for urothelial carcinoma [30]. A recent study cohort demonstrated a correlation between positive HER2 expression and higher tumor grade, but not with tumor stage [26, 31]. In our study, we observed an association between HER2 status and histologic divergent subtypes of UC. The squamous variant exhibited lower HER2 scores compared to conventional, micropapillary and mixed differentiation types. This variation in expression across divergent subtypessuggests that certain subtypes may be more responsive to HER2 targeted treatment.

The expression of PD-L1 in tumor cells can be assessed through IHC staining. High levels of PD-L1 expression are associated with a higher likelihood of response to ICI therapies, such as anti-PD-1 or anti-PD-L1 antibodies. Since PD-L1 status is often used as a biomarker to predict the response to immunotherapy in UC, the importance of PD-L1 expression has increased significantly in recent years. Although several ICIs have been approved for cases with resistance to platinum chemotherapy, there is still uncertainty surrounding the effectiveness and safety of immune checkpoint inhibitors (ICIs) [11, 12]. Objective response rates for first-line patients have been reported to range from 24% to 30%, and patients may also experience immune-related adverse events (irAEs). Recognizing the perceived gaps in current treatment options, ongoing research is focused on exploring more novel therapies [7, 16, 32].

There are several limitations of our study. Due to the absence of standardized HER2 scoring

protocols in urothelial carcinoma, our study, along with numerous others investigating urothelial carcinoma, adopted the commonly used HER2 evaluation criteria established for breast cancer [16]. Nonetheless, inconsistencies persist among studies regarding the designated cut-off value for determining the percentage of tumor cells exhibiting HER2 positivity [15, 33]. This ambiguity regarding definitions of HER2 overexpression may contribute to the variability observed in the correlation between HER2 expression and pathologic data. We also did not perform an analysis based on disease progression or recurrence, and did not consider the treatment given such as intravesical therapy, radical cystectomy or chemotherapy.

Conclusion

Our study explores associations between the demographic and clinicopathologic data of invasive UC with HER2 and PD-L1 expression. We found that the squamous subtype has significantly lower HER2 expression compared to cases with conventional, micropapillary, or mixed histology. PD-L1 expression was significantly higher in lower urinary tract cases (bladder or urethra) compared with upper (renal pelvis or ureter). These findings may be useful for treatment and prognosis of high grade UC.

Disclosure of conflict of interest

None.

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