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HLA-DQB1 REFLECTS AN ANTIGEN-PRESENTATION-ASSOCIATED IMMUNE MICROENVIRONMENT IN BLADDER CANCER AND SHOWS RESPONSE-RELATED SIGNALS IN AN ATEZOLIZUMAB-TREATED COHORT

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Abstract: Bladder cancer is a heterogeneous malignancy with variable immune infiltration and limited response rates to immune checkpoint blockade. HLA-DQB1, a major histocompatibility complex class II gene involved in antigen presentation, may participate in tumor immune regulation. However, its expression pattern, prognostic relevance, immune microenvironmental associations, and immunotherapy-related significance in bladder cancer remain incompletely characterized. Transcriptomic and clinical data of bladder cancer were obtained from TCGA-BLCA. HLA-DQB1 expression was compared between tumor and normal tissues, and its association with overall survival was evaluated using Kaplan–Meier and Cox regression analyses. Single-sample gene set enrichment analysis was used to estimate immune-cell infiltration. Correlations between HLA-DQB1 and immune checkpoints, antigen-presentation genes, and immune-related modules were assessed using Spearman correlation analysis. Differential expression analysis between HLA-DQB1-high and HLA-DQB1-low tumors was performed using limma, followed by GO and Hallmark GSEA. External validation was conducted using GSE13507. Immunotherapy-related analyses were further performed in the IMvigor210 atezolizumab-treated urothelial carcinoma cohort. Public single-cell visualization was used as exploratory evidence of HLA-DQB1 cellular heterogeneity. In TCGA-BLCA, HLA-DQB1 expression did not differ significantly between tumor and normal tissues and was not significantly associated with overall survival. However, HLA-DQB1 showed strong positive associations with multiple immune-cell infiltration scores, including CD8 T cells, Tfh cells, macrophages, Th1 cells, NK cells, T cells, and Treg cells. HLA-DQB1 was also strongly correlated with antigen-presentation genes and immune-related markers, including HLA-DRA, HLA-DPA1, CD74, CIITA, PDCD1, CTLA4, HAVCR2, CXCL9, and CXCL10. GSEA revealed enrichment of immune activation, antigen presentation, interferon response, and inflammatory pathways in the HLA-DQB1-high group. In GSE13507, although tumor tissues showed lower HLA-DQB1 expression than normal bladder mucosa, the immune associations of HLA-DQB1 were externally reproduced. HLA-DQB1 was significantly correlated with antigen-presentation, myeloid/APC, T-cell-inflamed, and checkpoint-exhaustion module scores in both TCGA-BLCA and GSE13507. In the IMvigor210 cohort, HLA-DQB1 expression tended to be higher in responders, and after adjustment for tumor mutational burden, higher HLA-DQB1 expression was significantly associated with increased odds of response to atezolizumab. HLA-DQB1 expression showed cohort-dependent tumor-versus-normal patterns and was not significantly associated with overall survival in TCGA-BLCA. Across independent transcriptomic cohorts, HLA-DQB1 was consistently associated with MHC-II antigen-presentation-related and immune-inflamed transcriptional programs. Exploratory analysis of an atezolizumab-treated urothelial carcinoma cohort suggested an association with treatment response after adjustment for tumor mutational burden, although this finding requires further validation. Overall, HLA-DQB1 appears to reflect a broader immune-related transcriptional state rather than a validated independent prognostic or immunotherapy response biomarker.
Keywords: Bladder cancer; HLA-DQB1; Antigen presentation; Immune microenvironment; TCGA; GSE13507; IMvigor210; Immunotherapy

1 INTRODUCTION

Bladder cancer is one of the most common malignancies of the urinary system and is characterized by substantial molecular and immunological heterogeneity [1-2]. Despite advances in surgery, chemotherapy, and immune checkpoint blockade, clinical outcomes remain variable, particularly in patients with advanced or recurrent disease [3-5]. The tumor immune microenvironment has emerged as a key determinant of bladder cancer progression and therapeutic response [6]. Tumors with active antigen presentation, cytotoxic T-cell infiltration, interferon signaling, and immune checkpoint co-expression are often considered immune-inflamed tumors, but the molecular markers reflecting this immune state remain incompletely defined [7].

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Major histocompatibility complex class II molecules are central components of antigen presentation [8]. They primarily present extracellular antigens to CD4-positive T cells and are commonly expressed by professional antigen-presenting cells, including dendritic cells, macrophages, and B cells [9]. HLA-DQB1 encodes the beta chain of the HLA-DQ heterodimer and participates in MHC-II-mediated antigen presentation [10-11]. In cancer, altered expression of MHC-II-related genes may influence immune surveillance, T-cell activation, and immunotherapy sensitivity [12-13]. However, the biological interpretation of HLA-DQB1 expression in bulk tumor transcriptomes is complex because its signal may arise from tumor cells, infiltrating immune cells, or both [7].

Previous studies have suggested that HLA-related genes may be associated with immune checkpoint blockade response and tumor immune phenotypes in urothelial carcinoma [14]. Nevertheless, whether HLA-DQB1 represents a tumor-intrinsic prognostic factor or a marker of immune microenvironmental composition in bladder cancer remains unclear. In particular, the expression pattern of HLA-DQB1 between tumor and non-tumor bladder tissues, its survival relevance, and its association with immune-cell infiltration and antigen-presentation modules require systematic investigation.

In this study, we integrated TCGA-BLCA and GSE13507 transcriptomic datasets to characterize the expression, prognostic significance, immune associations, pathway enrichment, and external reproducibility of HLA-DQB1 in bladder cancer. We further used public single-cell visualization as exploratory evidence to assess cellular heterogeneity of HLA-DQB1 expression. Our findings suggest that HLA-DQB1 is better interpreted as a marker of antigen-presentation-associated immune microenvironmental heterogeneity rather than as a conventional prognostic biomarker.

2 MATERIALS AND METHODS

2.1 Data Acquisition and Preprocessing

RNA-seq transcriptomic data and clinical survival data for TCGA-BLCA were downloaded from the UCSC Xena platform. Gene expression values were analyzed as log2-transformed TPM values. Ensembl gene identifiers were converted to HGNC gene symbols using the biomaRt package. When multiple Ensembl identifiers mapped to the same gene symbol, expression values were summarized at the gene-symbol level using the median value.

For external validation, the GSE13507 dataset was obtained from the Gene Expression Omnibus using the GEOquery package. Platform annotation was used to map probes to gene symbols. HLA-DQB1 was identified using the platform annotation, and when multiple probes were available, the probe with the largest expression variance was selected. Samples were classified according to the source information into normal bladder mucosa, bladder mucosa surrounding cancer, primary bladder cancer, recurrent non-muscle-invasive tumor, and excluded non-bladder MPP samples. For the main tumor-versus-normal comparison, primary bladder cancer and recurrent non-muscle-invasive tumor samples were combined as tumor samples, while adjacent mucosa samples were not included in the normal reference group. The overall analytical workflow is summarized in Figure 1.

2.2 Expression Analysis

HLA-DQB1 expression was compared between tumor and normal samples in TCGA-BLCA and GSE13507. The Wilcoxon rank-sum test was used for two-group comparisons. For detailed GSE13507 tissue group comparisons, the Kruskal–Wallis test followed by pairwise Wilcoxon tests with Benjamini–Hochberg correction was used.

2.3 Survival Analysis

For TCGA-BLCA, tumor samples were matched to patient-level survival data. Overall survival was defined using OS time and OS event status. Patients were divided into HLA-DQB1-high and HLA-DQB1-low groups according to the median HLA-DQB1 expression value. Kaplan–Meier survival analysis and log-rank testing were performed. Cox proportional hazards regression was used to evaluate the association between HLA-DQB1 expression and overall survival.

2.4 Immune Infiltration Analysis

Immune-cell infiltration was estimated using single-sample gene set enrichment analysis. A curated immune-cell gene set panel covering B cells, CD8 T cells, CD4 T cells, T helper cells, Treg cells, Th1 cells, Th2 cells, Th17 cells, Tfh cells, NK cells, dendritic cells, macrophages, monocytes, neutrophils, mast cells, eosinophils, cytotoxic cells, and total T cells was used. Spearman correlation analysis was performed to assess associations between HLA-DQB1 expression and immune-cell infiltration scores. Wilcoxon tests were used to compare immune-cell scores between HLA-DQB1-high and HLA-DQB1-low groups. P values were adjusted using the Benjamini–Hochberg method.

2.5 Immune-Related Gene Correlation Analysis

Associations between HLA-DQB1 and immune-related genes were evaluated using Spearman correlation analysis. Genes of interest included MHC-II and antigen-presentation genes, T-cell and cytotoxicity markers, B-cell markers,

myeloid/APC markers, chemokines, and immune checkpoint genes. The analyzed genes included CD74, HLA-DRA, HLA-DRB1, HLA-DPA1, HLA-DPB1, CIITA, CD3D, CD3E, CD8A, CD8B, PRF1, IFNG, CD79A, CD79B, CD68, LST1, C1QA, C1QB, CXCL9, CXCL10, PDCD1, CD274, CTLA4, LAG3, TIGIT, and HAVCR2.

2.6 Immune Module Scoring

To evaluate immune-related transcriptional programs, four immune modules were constructed: antigen presentation, myeloid/APC, T-cell-inflamed, and checkpoint-exhaustion modules. For each module, genes present in the expression matrix were z-score transformed, and the module score was calculated as the mean z-score across included genes. Spearman correlation analysis was used to assess associations between HLA-DQB1 expression and each immune module score in both TCGA-BLCA and GSE13507.

2.7 Differential Expression and Enrichment Analysis

TCGA-BLCA tumor samples were divided into HLA-DQB1-high and HLA-DQB1-low groups according to median expression. Differential expression analysis was performed using the limma package. Genes with absolute log2 fold change greater than 1 and adjusted P value less than 0.05 were considered significantly differentially expressed. Gene set enrichment analysis was performed using clusterProfiler. GO Biological Process and Hallmark gene sets were used to identify pathways enriched in the HLA-DQB1-high group.

2.8 Immunotherapy Cohort Analysis

To further investigate the immunotherapy relevance of HLA-DQB1, we analyzed the IMvigor210 urothelial carcinoma cohort using the easierData package. The Mariathasan2018_PDL1_treatment dataset was retrieved as a SummarizedExperiment object containing transcriptomic and clinical information from patients treated with atezolizumab. TPM expression values were transformed to log2(TPM + 1) for downstream analyses. HLA-DQB1 expression was extracted at the gene-symbol level.

Best overall response (BOR) was used to classify patients into responder and non-responder groups. Samples annotated as “R” were defined as responders, while samples annotated as “NR” were defined as non-responders. HLA-DQB1 expression was compared between groups using the Wilcoxon rank-sum test. Patients were further divided into HLA-DQB1-high and HLA-DQB1-low groups according to the median HLA-DQB1 expression value, and the difference in response rate between groups was assessed using Fisher’s exact test.

To evaluate whether HLA-DQB1 provided response-related information beyond tumor mutational burden, logistic regression analysis was performed using response as the dependent variable and HLA-DQB1 as the predictor, with or without adjustment for TMB. HLA-DQB1 expression and TMB were standardized before logistic regression; the reported odds ratios represent associations per one-standard-deviation increase. Odds ratios and 95% confidence intervals were calculated. In addition, immune module scoring was performed in the IMvigor210 cohort using the same antigen-presentation, myeloid/APC, T-cell-inflamed, and checkpoint-exhaustion gene sets as in the TCGA-BLCA and GSE13507 analyses. Spearman correlation analysis was used to assess associations between HLA-DQB1 expression and module scores.

2.9 Public Single-Cell Visualization

Public single-cell datasets available in CELLxGENE were explored to assess the cellular heterogeneity of HLA-DQB1 expression. Because the available urothelial carcinoma dataset was derived from organoid models and lacked complete immune-cell annotations, this analysis was interpreted as exploratory and was not used to infer definitive cellular origin.

2.10 Statistical Analysis

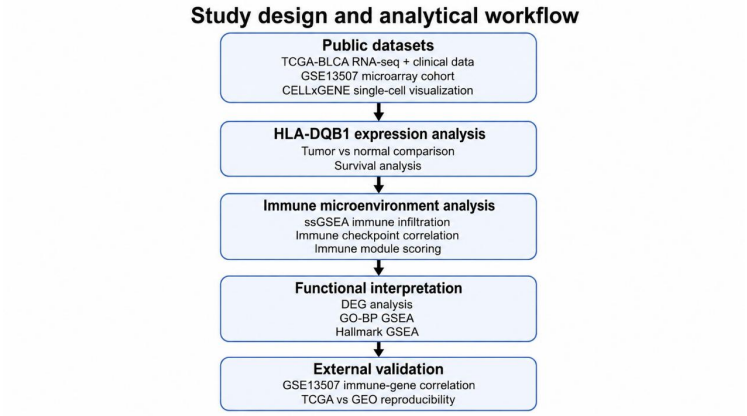


Figure 1 Study Design and Analytical Workflow

TCGA-BLCA transcriptomic and clinical data were used to evaluate HLA-DQB1 expression, survival relevance, immune infiltration, immune-related gene correlations, differential expression, and pathway enrichment. GSE13507 was used for external validation of expression and immune associations. Public single-cell visualization was used as exploratory evidence of cellular heterogeneity.

All analyses were performed in R. Continuous variables were compared using the Wilcoxon rank-sum test or Kruskal–Wallis test as appropriate. Correlations were assessed using Spearman correlation coefficients. Survival analysis was performed using Kaplan–Meier curves, log-rank tests, and Cox regression models. P values were adjusted using the Benjamini–Hochberg method where appropriate. A two-sided P value less than 0.05 was considered statistically significant.

3 RESULTS

3.1 HLA-DQB1 Expression Shows Cohort-Dependent Differences between Bladder Tumor and Non-Tumor Tissues

We first examined HLA-DQB1 expression in TCGA-BLCA. A total of 409 tumor samples and 19 normal bladder tissue samples were included. HLA-DQB1 expression showed broad inter-sample variability in tumor tissues. The median expression was slightly higher in tumor tissues than in normal tissues, but the difference was not statistically significant by Wilcoxon rank-sum test.

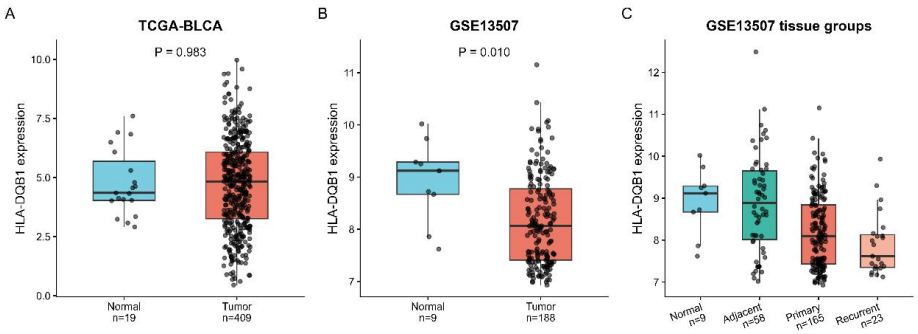


Figure 2 HLA-DQB1 Expression in TCGA-BLCA and GSE13507: (A) TCGA-BLCA HLA-DQB1 Tumor vs Normal Boxplot; (B) GSE13507 Normal vs Tumor HLA-DQB1 Boxplot; (C) GSE13507 Four-Group Expression Boxplot

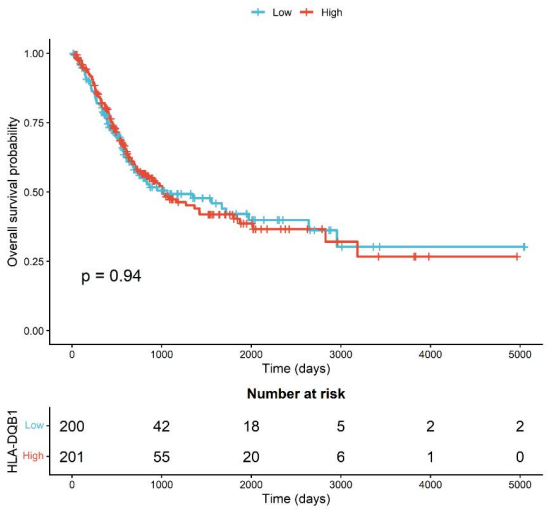


Figure 3 Kaplan–Meier Overall Survival Curve

To externally validate the expression pattern of HLA-DQB1, we analyzed GSE13507. After excluding non-bladder MPP samples, the dataset included normal bladder mucosa, bladder mucosa surrounding cancer, primary bladder cancer, and recurrent non-muscle-invasive tumor samples. Because adjacent mucosa may be affected by field effects and local inflammation, it was not used as the normal reference in the main analysis. Primary bladder cancer and recurrent non-muscle-invasive tumor samples were combined as tumor samples. In contrast to TCGA-BLCA, HLA-DQB1 expression was significantly lower in tumor tissues than in normal bladder mucosa in GSE13507.

Detailed tissue-group analysis further demonstrated heterogeneity of HLA-DQB1 expression across normal mucosa, adjacent mucosa, primary tumors, and recurrent non-muscle-invasive tumors.

Together, these findings indicate that HLA-DQB1 does not show a uniform tumor-versus-normal expression pattern across independent bladder cancer cohorts. Instead, its bulk expression appears to be context dependent and may be

influenced by cohort composition, tissue source, platform differences, and immune-cell content. These cohort-dependent expression patterns are illustrated in Figure 2A–C.

3.2 HLA-DQB1 Is Not Significantly Associated with Overall Survival in TCGA-BLCA

We next investigated whether HLA-DQB1 expression was associated with patient survival in TCGA-BLCA. Patients were divided into HLA-DQB1-high and HLA-DQB1-low groups according to the median expression value. Kaplan–Meier analysis showed no significant difference in overall survival between the two groups. The Kaplan–Meier survival curves are shown in Figure 3.

Consistently, Cox regression analysis did not support a significant prognostic effect of HLA-DQB1. In the median-split analysis, the hazard ratio for the high-expression group was approximately 0.99, with no statistical significance. When HLA-DQB1 was modeled as a continuous variable, it also showed no significant association with overall survival. These findings indicate that HLA-DQB1 expression was not significantly associated with overall survival in the TCGA-BLCA cohort. The corresponding Cox regression estimates are provided in Table S1.

3.3 HLA-DQB1 Is Strongly Associated with Immune-Cell Infiltration in TCGA-BLCA

Given that HLA-DQB1 is a major MHC-II gene, we next assessed whether its expression was related to immune-cell infiltration. ssGSEA analysis revealed that HLA-DQB1 expression was strongly and positively correlated with multiple immune-cell infiltration scores in TCGA-BLCA.

The strongest correlations were observed for CD8 T cells, Tfh cells, macrophages, Th1 cells, NK cells, total T cells, Treg cells, M2 macrophages, and cytotoxic cells. These results indicate that HLA-DQB1-high tumors exhibit a broadly immune-infiltrated phenotype.

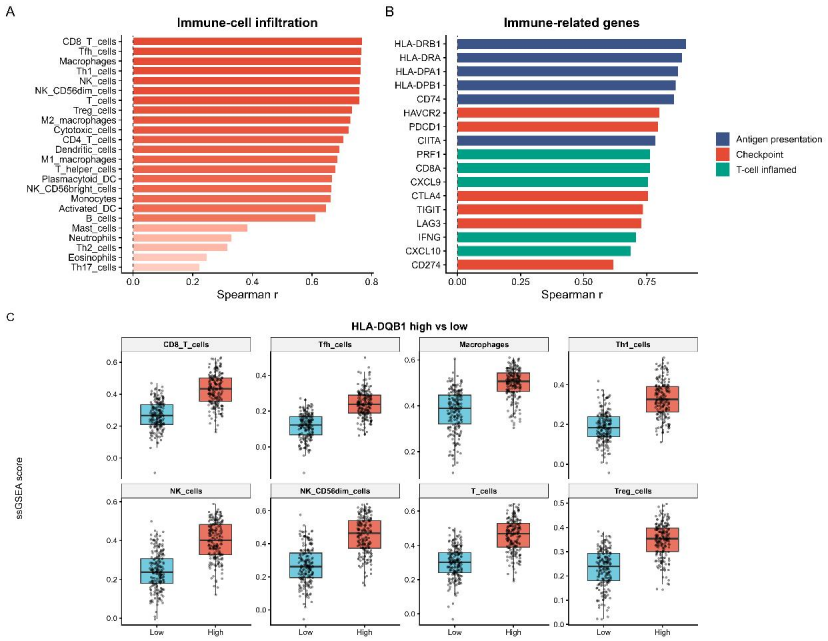


Figure 4 (A) Bar Plot of Spearman Correlations between HLA-DQB1 and Immune-Cell ssGSEA Scores; (B) Immune Checkpoint and Antigen-Presentation Gene Correlation Bar Plot; (C) Boxplots comparing immune-cell ssGSEA scores between HLA-DQB1-high and HLA-DQB1-low groups.

Comparison between HLA-DQB1-high and HLA-DQB1-low tumors further supported this finding. HLA-DQB1-high tumors showed significantly higher immune infiltration scores for multiple immune-cell populations, particularly Tfh cells, CD8 T cells, total T cells, Th1 cells, NK cells, macrophages, Treg cells, cytotoxic cells, and CD4 T cells.

These data suggest that HLA-DQB1 expression in bulk bladder cancer transcriptomes primarily reflects an immune-inflamed tumor microenvironment rather than a simple tumor-cell-intrinsic expression change. The immune-cell correlations and high-versus-low expression comparisons are shown in Figures 4A and 4C, respectively.

3.4 HLA-DQB1 Correlates with Antigen-Presentation and Immune Checkpoint Genes in TCGA-BLCA

We next evaluated the relationship between HLA-DQB1 and immune-related genes. In TCGA-BLCA, HLA-DQB1 was strongly correlated with other MHC-II and antigen-presentation genes, including HLA-DQA1, HLA-DRB1, HLA-DRA, HLA-DPA1, HLA-DPB1, and CD74. This indicates that HLA-DQB1 is part of a coordinated antigen-presentation transcriptional program.

HLA-DQB1 was also positively associated with immune activation and immune checkpoint-related genes, including HAVCR2, PDCD1, CTLA4, LAG3, TIGIT, IFNG, CXCL9, CXCL10, GZMB, PRF1, CD8A, and CD8B.

These findings suggest that HLA-DQB1-high tumors are characterized by antigen-presentation activity, T-cell inflammation, cytotoxic immune features, and immune checkpoint co-expression. This pattern is consistent with an immune-inflamed but potentially immunoregulatory microenvironment. Correlations with immune-related genes are shown in Figure 4B.

3.5 HLA-DQB1-High Tumors Show Enrichment of Immune and Antigen-Presentation Pathways

To further characterize the biological processes associated with HLA-DQB1 expression, we performed differential expression analysis between HLA-DQB1-high and HLA-DQB1-low TCGA-BLCA tumors. A total of 880 genes were identified as significantly differentially expressed using the threshold of absolute log2 fold change greater than 1 and adjusted P value less than 0.05. As expected, HLA-DQB1 and other MHC-II-related genes were among the most upregulated genes in the HLA-DQB1-high group.

GSEA of GO Biological Process terms demonstrated enrichment of immune-related pathways in the HLA-DQB1-high group. These included antigen processing and presentation, MHC class II-related processes, T-cell activation, leukocyte-mediated immunity, cytokine-mediated signaling, and interferon-related immune responses.

Hallmark GSEA further showed enrichment of immune and inflammatory pathways, including interferon-gamma response, inflammatory response, allograft rejection, TNF-alpha signaling via NF-kB, and related immune activation signatures.

A representative GSEA enrichment curve for the interferon-gamma response is shown in Figure 5E, further supporting the association between high HLA-DQB1 expression and an immune-related transcriptional program.

Together, these results indicate that high HLA-DQB1 expression is associated with coordinated activation of antigen-presentation, interferon response, and inflammatory immune pathways. Differentially expressed genes, GO-BP and Hallmark pathway enrichment, the top 50 differentially expressed genes, and a representative GSEA enrichment curve are presented in Figure 5A–E, respectively.

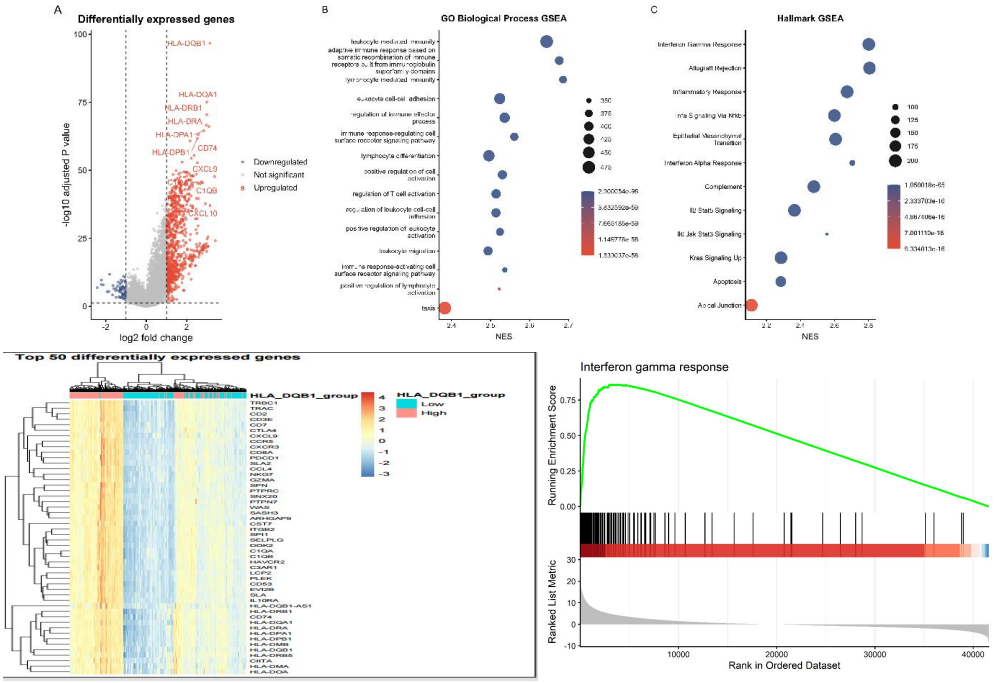


Figure 5 (A) Volcano Plot of Differentially Expressed Genes; (B) GO-BP GSEA Dotplot; (C) Hallmark GSEA Dotplot; (D) Heatmap of the Top 50 Differentially Expressed Genes between HLA-DQB1-High and HLA-DQB1-Low Bladder Cancer Samples in TCGA-BLCA; (E) Representative GSEA enrichment curve for the interferon-gamma response. Rows represent genes and columns represent tumor samples. Colors indicate relative gene expression levels after row-wise standardization. Samples are grouped according to the median HLA-DQB1 expression level.

3.6 External Validation Confirms the Association between HLA-DQB1 and Immune-Related Transcriptional Programs in GSE13507

Because HLA-DQB1 expression showed cohort-dependent tumor-versus-normal differences, we next asked whether its immune associations were reproducible in an independent dataset. Using tumor samples from GSE13507, we assessed the correlation between HLA-DQB1 and immune-related genes.

HLA-DQB1 showed strong positive correlations with MHC-II antigen-presentation genes, including HLA-DRA, HLA-DPA1, CD74, HLA-DPB1, and CIITA. HLA-DQB1 was also positively associated with myeloid/APC-related

markers, including LST1, C1QA, and C1QB. In addition, selected T-cell-inflamed and checkpoint-related genes, including CD8A, PRF1, PDCD1, CXCL9, CXCL10, CTLA4, LAG3, and HAVCR2, were significantly positively correlated with HLA-DQB1.

To further evaluate cross-cohort reproducibility, we compared HLA-DQB1 immune-gene correlation coefficients between TCGA-BLCA and GSE13507. Most antigen-presentation and myeloid/APC-related genes showed positive correlations with HLA-DQB1 in both cohorts. Although several T-cell and checkpoint genes showed weaker or inconsistent correlations in GSE13507, the overall pattern supported reproducible immune associations across independent cohorts.

Module-level analysis further confirmed this pattern. In TCGA-BLCA, HLA-DQB1 was strongly correlated with the antigen-presentation module, myeloid/APC module, T-cell-inflamed module, and checkpoint-exhaustion module. In GSE13507, HLA-DQB1 remained significantly positively correlated with all four modules, although the correlation strengths were generally lower than those observed in TCGA-BLCA.

These findings demonstrate that the association between HLA-DQB1 and antigen-presentation-associated immune programs is reproducible across independent bladder cancer cohorts. The external validation results are summarized in Figure 6A–C.

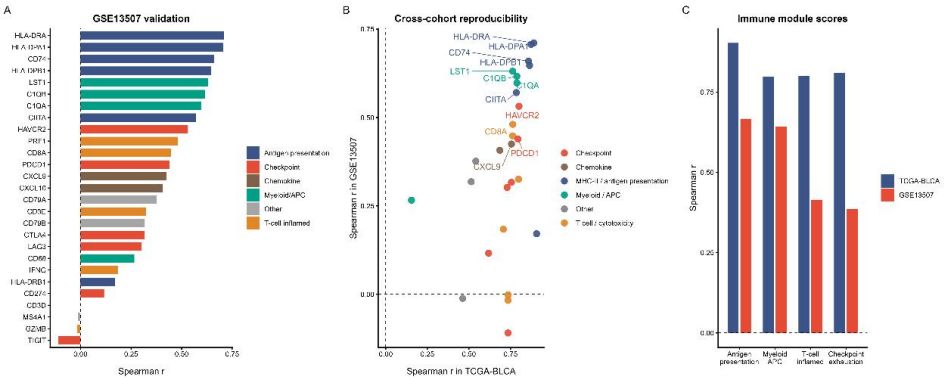


Figure 6 (A) GSE13507 Immune-Gene Correlation Bar Plot; (B) TCGA vs GEO Correlation Scatter Plot; (C) Immune Module Score Correlation Bar Plot for TCGA and GEO

3.7 HLA-DQB1 Shows Immunotherapy-Related Signals in the IMvigor210 Cohort

To further evaluate the immunotherapy relevance of HLA-DQB1, we analyzed the IMvigor210 urothelial carcinoma cohort treated with atezolizumab. In unadjusted comparison, HLA-DQB1 expression tended to be higher in responders than in non-responders, although the difference did not reach statistical significance (median 4.77 vs. 4.19, Wilcoxon P = 0.265).

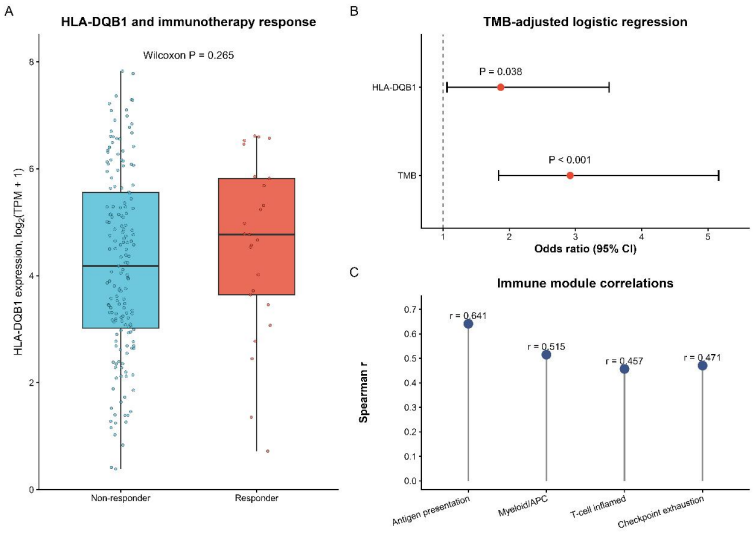


Figure 7 HLA-DQB1 Shows Immunotherapy-Related Signals in the IMvigor210 Cohort

(A) HLA-DQB1 expression in responders and non-responders in the IMvigor210 atezolizumab-treated urothelial carcinoma cohort. HLA-DQB1 expression tended to be higher in responders, although the difference was not statistically significant in the unadjusted comparison.

(B) Forest plot of logistic regression adjusted for tumor mutational burden. After TMB adjustment, higher HLA-DQB1 expression was significantly associated with increased odds of response, while TMB also remained an independent predictor.

(C) Spearman correlations between HLA-DQB1 expression and immune module scores in the IMvigor210 cohort. HLA-DQB1 was significantly associated with antigen-presentation, myeloid/APC, T-cell-inflamed, and checkpoint-exhaustion modules.

When patients were dichotomized according to median HLA-DQB1 expression, the HLA-DQB1-high group showed a numerically higher response rate than the low-expression group. However, this difference was not statistically significant by Fisher's exact test (odds ratio = 1.93, P = 0.198), indicating that HLA-DQB1 alone was insufficient to robustly stratify response in simple unadjusted group-wise comparisons.

We next performed logistic regression analysis to determine whether HLA-DQB1 remained associated with response after accounting for tumor mutational burden. In the univariate model, HLA-DQB1 was not significantly associated with response. However, after adjustment for TMB, higher HLA-DQB1 expression was significantly associated with increased odds of response (OR = 1.87, 95% CI 1.06–3.51, P = 0.038), while TMB was also independently associated with response (OR = 2.92, 95% CI 1.84–5.16, P < 0.001).

To determine whether HLA-DQB1 retained its immune contextual relevance in an immunotherapy-treated setting, we further assessed its correlation with immune module scores in the IMvigor210 cohort. HLA-DQB1 remained strongly and significantly correlated with the antigen-presentation module (r = 0.641), myeloid/APC module (r = 0.515), T-cell-inflamed module (r = 0.457), and checkpoint-exhaustion module (r = 0.471), all with FDR-adjusted significance. These findings indicate that HLA-DQB1 is closely linked to an immunotherapy-relevant immune microenvironment and may provide response-related information beyond TMB alone. The immunotherapy response analyses and immune-module associations are presented in Figure 7A–C.

3.8 Public Single-Cell Visualization Suggests Heterogeneous HLA-DQB1 Expression in Urothelial Carcinoma Organoid Cells

To explore HLA-DQB1 expression at the single-cell level, we examined publicly available single-cell data through CELLxGENE. In a urothelial carcinoma organoid dataset, HLA-DQB1 expression was detectable in a subset of cells rather than being uniformly expressed across all cells. HER2-high and HER2-low cell states occupied partially distinct transcriptional regions, while HLA-DQB1-positive cells were observed in limited regions of the UMAP space.

Because this dataset was derived from organoid models and lacked complete immune-cell annotations, it could not be used to determine the precise immune-cell origin of HLA-DQB1 expression. Therefore, this single-cell visualization was interpreted as exploratory evidence of transcriptional heterogeneity rather than definitive cell-type localization. The exploratory single-cell visualizations are presented in Supplementary Figure S1.

4 DISCUSSION

In this study, we systematically investigated the expression pattern, prognostic significance, immune associations, pathway enrichment, external reproducibility, and exploratory single-cell features of HLA-DQB1 in bladder cancer. Our findings demonstrate that HLA-DQB1 is not a robust prognostic marker in TCGA-BLCA and does not show a consistent tumor-versus-normal expression pattern across cohorts. However, HLA-DQB1 is strongly and reproducibly associated with antigen-presentation-related immune programs, myeloid/APC-related markers, T-cell-inflamed features, and immune checkpoint co-expression [10, 12]. These results suggest that HLA-DQB1 primarily reflects immune microenvironmental heterogeneity rather than tumor-cell-intrinsic aggressiveness [15].

One important observation of this study is the cohort-dependent expression pattern of HLA-DQB1. In TCGA-BLCA, HLA-DQB1 expression did not differ significantly between tumor and normal tissues. In contrast, GSE13507 showed lower HLA-DQB1 expression in tumor tissues compared with normal bladder mucosa. This apparent discrepancy may reflect differences in sample composition, platform technology, normal tissue definition, tumor purity, immune-cell content, and cohort-specific clinical characteristics. Importantly, this result argues against interpreting HLA-DQB1 as a universally upregulated or downregulated tumor marker in bladder cancer.

The lack of significant association between HLA-DQB1 and overall survival further suggests that HLA-DQB1 should not be considered a conventional prognostic biomarker. Negative survival results are biologically meaningful in this context. They indicate that although HLA-DQB1 is closely linked to immune activity, its expression alone may not be sufficient to stratify patient survival. The effect of immune infiltration on prognosis in bladder cancer is complex and may depend on the balance between cytotoxic immunity, antigen presentation, immune suppression, checkpoint activation, stromal context, and treatment history.

The strongest and most consistent finding of this study is the association between HLA-DQB1 and antigen-presentation-related immune programs. In TCGA-BLCA, HLA-DQB1 was strongly correlated with multiple MHC-II genes, including HLA-DRA, HLA-DQA1, HLA-DRB1, HLA-DPA1, and HLA-DPB1. In GSE13507, HLA-DQB1 remained significantly correlated with HLA-DRA, HLA-DPA1, CD74, HLA-DPB1, and CIITA. Module-level analysis confirmed that the antigen-presentation score showed the strongest and most reproducible association with HLA-DQB1 in both cohorts. These findings support the interpretation that HLA-DQB1 represents a coordinated MHC-II antigen-presentation transcriptional program [16].

In addition to antigen-presentation genes, HLA-DQB1 was associated with myeloid/APC-related markers, including LST1, C1QA, C1QB, and CD68. This suggests that bulk HLA-DQB1 expression may partly reflect the abundance or activation state of antigen-presenting immune cells, such as macrophages, monocytes, dendritic cells, and other myeloid

populations. This interpretation is also consistent with the known biology of MHC-II molecules, which are typically expressed by professional antigen-presenting cells.

HLA-DQB1 was also associated with T-cell-inflamed immune features. In TCGA-BLCA, HLA-DQB1 showed strong correlations with CD8 T-cell infiltration, cytotoxic markers, chemokines, and multiple immune checkpoints. In GSE13507, the associations with CD8A, PRF1, PDCD1, CXCL9, CXCL10, CTLA4, LAG3, and HAVCR2 were externally validated. These results indicate that HLA-DQB1-high tumors may represent an immune-inflamed phenotype characterized by antigen presentation, immune-cell recruitment, and checkpoint co-expression. However, not all immune genes were consistently validated in GSE13507. For example, CD274, TIGIT, GZMB, MS4A1, and CD3D were not significantly associated with HLA-DQB1 in the external cohort. Therefore, HLA-DQB1 should not be interpreted as a universal marker of all immune-cell populations or all immune checkpoint pathways. Its most stable association appears to be with MHC-II antigen presentation and myeloid/APC-related immune programs.

GSEA further supported this immune interpretation. HLA-DQB1-high tumors were enriched for immune-related biological processes, including antigen presentation, T-cell activation, interferon response, inflammatory response, leukocyte-mediated immunity, and cytokine signaling. Hallmark enrichment of interferon-gamma response, inflammatory response, and allograft rejection is consistent with an immune-activated microenvironment. These pathway-level findings strengthen the argument that HLA-DQB1 is embedded within a broader immune transcriptional program rather than acting alone.

In the IMvigor210 cohort, HLA-DQB1 expression was not significantly associated with treatment response in unadjusted analyses, whereas a positive association was observed after adjustment for tumor mutational burden. This finding suggests that HLA-DQB1 may capture immune-related information complementary to TMB. However, the result should be interpreted cautiously because of potential confounding and the lack of validation in an independent immunotherapy-treated cohort. The observed correlations with antigen-presentation and immune-related transcriptional modules further support the interpretation that HLA-DQB1 reflects a broader immune microenvironmental state rather than an established predictor of immunotherapy response [17-18].

The exploratory single-cell visualization provided limited but supportive evidence that HLA-DQB1 expression is heterogeneous across urothelial carcinoma organoid cells. However, because the available dataset lacked comprehensive immune-cell annotations and was derived from organoid models, it could not determine whether HLA-DQB1 expression originated from malignant epithelial cells or immune cells in patient tumors. Therefore, we treated the single-cell result cautiously and did not use it as a central conclusion. Future studies using patient-derived single-cell datasets with complete immune-cell annotation will be needed to define the precise cellular origin of HLA-DQB1 in bladder cancer.

This study has several limitations. First, the analysis was based primarily on retrospective public transcriptomic datasets. Second, bulk RNA-seq cannot distinguish tumor-cell-intrinsic expression from immune-cell-derived expression. Third, normal samples were limited in number, particularly in TCGA-BLCA and GSE13507, and adjacent mucosa may not represent truly normal tissue. Fourth, platform differences between RNA-seq and microarray datasets may contribute to cohort-dependent expression differences. Fifth, the available single-cell analysis was exploratory and based on an organoid dataset without full immune-cell annotation. Finally, experimental validation using clinical tissue specimens, such as immunohistochemistry for HLA-DQB1, CD8, and PD-L1, was not included in the current analysis.

Despite these limitations, the present study provides a coherent interpretation of HLA-DQB1 in bladder cancer. Rather than being a stable tumor-expression or prognostic marker, HLA-DQB1 appears to represent an antigen-presentation-associated immune microenvironmental state. This immune state includes MHC-II activation, myeloid/APC-related signals, T-cell inflammation, chemokine activity, and selected immune checkpoint features. These findings may provide a basis for future studies investigating HLA-DQB1 as a marker of immune contexture and antigen-presentation activity in bladder cancer.

5 CONCLUSION

HLA-DQB1 does not show a consistent tumor-versus-normal expression pattern across bladder cancer cohorts and is not significantly associated with overall survival in TCGA-BLCA. However, integrated analysis of TCGA-BLCA, GSE13507, and the IMvigor210 immunotherapy cohort demonstrates that HLA-DQB1 is reproducibly associated with MHC-II antigen presentation, myeloid/APC-related markers, T-cell-inflamed features, and checkpoint-related immune programs. In the IMvigor210 cohort, HLA-DQB1 also showed response-related signals after adjustment for tumor mutational burden. These findings suggest that HLA-DQB1 primarily reflects antigen-presentation-associated immune microenvironmental heterogeneity and may provide complementary immunotherapy-relevant information in bladder cancer.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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ETHICS APPROVAL

This study was based exclusively on secondary analyses of publicly available, de-identified datasets. No participants were directly recruited, and no identifiable personal information was accessed. In accordance with the institutional policy of the Second Affiliated Hospital of Guilin Medical University, ethics committee approval was not required for this study.

CONSENT TO PARTICIPATE

Not applicable. This study used previously collected, de-identified public datasets and involved no direct participant recruitment.

DATA AVAILABILITY

The datasets analyzed in this study were obtained from publicly accessible resources, including TCGA-BLCA via UCSC Xena, GSE13507 via the Gene Expression Omnibus, and the Mariathasan2018_PDL1_treatment dataset via easierData. The corresponding accession information and analysis procedures are described in the Methods section.

AUTHOR CONTRIBUTIONS

Fangming Xu: Methodology, data curation, formal analysis, visualization, and writing – original draft.
Zhongyi Qin: Investigation, data collection, data curation, formal analysis, and writing – original draft.
Jiaqiang Pan: Conceptualization, supervision, project administration, funding acquisition, and writing – review and editing.
Fangming Xu and Zhongyi Qin contributed equally to this work and share first authorship. All authors reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

Table S1 Cox Regression Estimates for HLA-DQB1

Model	HR	Lower95CI	Upper95CI	P_value
High vs Low	0.988842	0.735716	1.329056	0.940711
Continuous	0.980784	0.906722	1.060895	0.628129

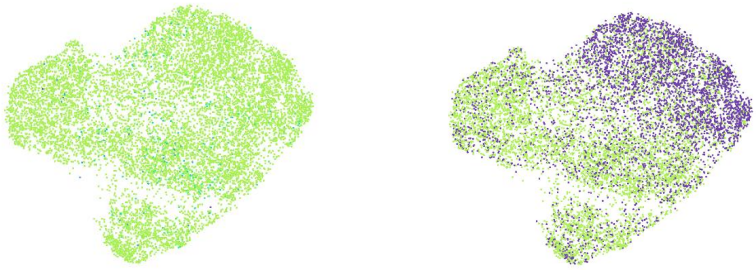


Figure S1 CELLxGENE HER2-Status UMAP and HLA-DQB1 Expression UMAP