Therapeutic Strategies Targeting PIK3CA Mutations in Bladder Cancer

Yueming Wan[1], Chunwang Liao[1], Keliang Peng[1], Hong Shan[2]

Background: *Bladder cancer (BLCA) remains a significant global health burden with diverse incidence rates and mortality patterns. The advent of immunotherapy, particularly immune checkpoint inhibitors (ICIs), has transformed BLCA treatment paradigms. However, challenges persist, including resistance and biomarker identification.The phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) gene, implicated in BLCA pathogenesis, warrants investigation for its role in immune modulation and therapeutic responses.*

Methods*: This study integrated multi-omics data from The Cancer Genome Atlas (TCGA) to dissect PIK3CA mutations' impact on BLCA.*

Results: *PIK3CA mutations were associated with higher overall and disease-free survival rates. Transcriptomic analyses revealed differential gene expression, enriching immune-related pathways. PIK3CA mutations correlated with altered immune cell infiltration and immune checkpoint gene expression, indicating potential immunotherapeutic implications. Moreover, drug susceptibility analyses identified compounds showing differential responses based on PIK3CA mutation status.*

Conclusion: *These findings underscore PIK3CA's clinical relevance and its potential as a therapeutic Strategies in BLCA, offering insights for precision medicine approaches to enhance patient outcomes.*

BACKGROUND

BLCA remains a formidable health burden globally, with its incidence and mortality rates exhibiting significant variations across geographical regions and demographic profiles[1,2]. Accounting for a substantial proportion of urinary tract malignancies, BLCA manifests as a heterogeneous disease entity, encompassing diverse histological subtypes and clinical trajectories[3]. Risk factors for BLCA encompass a complex interplay of environmental exposures, lifestyle habits, and genetic predispositions, with cigarette smoking representing prominent etiological determinants[4,5]. Clinically, the main symptom of BLCA is blood in the urine[6]. Diagnosis relies on a combination of clinical evaluation, imaging modalities, and histopathological examination, with transurethral resection of bladder tumor (TURBT) serving as the cornerstone of both diagnostic and therapeutic interventions[7,8].

The advent of immunotherapy has revolutionized the landscape of cancer treatment, offering a paradigm shift from conventional cytotoxic regimens towards harnessing the host immune response to combat malignancies. In the context of BLCA, ICIs targeting programmed cell death protein 1 (PD-1) and its ligand (PD-L1), as well as

[1]Department of Urology, Yueyang Hospital Affiliated to Hunan Normal University, Yueyang People's Hospital,Yueyang, Hunan, China.
[2]Department of Emergency Medicine, The Affiliated Changsha Central Hospital, Hengyang Medical School, University of South China,Changsha, Hunan, China.
Address for correspondence to: Hong Shan, Department of Emergency Medicine, The Affiliated Changsha Central Hospital, Hengyang Medical School, University of South China, Changsha, Hunan, China. E-mail: shirleyv5@163.com.
Financial Disclosures: This work was funded by the Natural Science Foundation of Hunan Province of China (No. 2022JJ40249), Changsha Municipal Natural Science Foundation (No. kq2202463).

The Journal of Reproductive Medicine®

cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), have emerged as cornerstone therapies in both advanced and localized disease settings[9,10,11,12,13,14,15,16]. The fundamental principle of immunotherapy strategies in BLCA entails harnessing the potential of the immune system to selectively target and eradicate cancer cells, thus serving as a crucial adjunct to organ-preserving approaches[17]。 Despite the unprecedented success of ICIs in a subset of BLCA patients, challenges persist, including primary and acquired resistance, biomarker identification, and optimal patient selection criteria. Thus, there exists a compelling need for comprehensive molecular profiling and mechanistic insights to unravel the complexities of immune evasion and therapeutic response modulation in BLCA.

The PIK3CA gene represents a key player in the intricate network of signaling pathways governing cellular proliferation and apoptosis[18]. As a principal component of the phosphatidylinositol 3-kinase (PI3K) pathway, PIK3CA serves as a hotspot for genetic alterations across a spectrum of human malignancies, including BLCA[19,20,21,22,23]. Point mutations within the helical and kinase domains of PIK3CA engender constitutive activation of downstream signaling cascades, driving tumorigenesis, and conferring therapeutic resistance[24,25].

In the context of BLCA, PIK3CA mutations have been implicated in disease progression, metastasis, and therapeutic response modulation, underscoring their clinical relevance as potential biomarkers and therapeutic targets[26,27,28,29]. However, the precise mechanistic underpinnings of PIK3CA-mediated oncogenesis and its interplay with the tumor microenvironment remain incompletely understood, necessitating further elucidation through integrated genomic and immunogenomic
analyses.

Against this backdrop, the present study endeavors to unravel the intricate interplay between PIK3CA gene mutations and the immune microenvironment in BLCA, elucidating their clinical implications and therapeutic relevance. Through comprehensive analyses of transcriptomic data from TCGA, we aim to delineate the molecular landscape of PIK3CA-mutated BLCA, focusing on differential gene expression patterns, signaling pathway alterations, and immune regulatory mechanisms. By integrating multi-omics data and bioinformatics approaches, we aspire to elucidate the functional implications of PIK3CA mutations in shaping the tumor immune microenvironment and modulating therapeutic responses. Ultimately, our findings hold promise for advancing precision medicine approaches in BLCA management, facilitating the development of targeted therapies and immunomodulatory interventions tailored to the molecular signatures of individual patients.

MATERIALS AND METHODS

Data preparation

We obtained transcriptome data for Bladder Cancer samples from TCGA database (https://portal.gdc.cancer.gov/), comprising a total of 431 cases. Among these, 406 samples were derived from tumor tissues, while 25 samples represented normal urothelial tissues. Subsequently, we systematically curated and organized the data into an expression matrix for further analytical investigations.

Genetic mutation and clinical prognostic analysis

We conducted an analysis of PIK3CA mutation frequency and mutation sites in patients diagnosed with bladder cancer utilizing the cBioPortal database (www.cbioportal.org). The database was queried to retrieve pertinent information regarding the occurrence and specific loci of PIK3CA mutations in the patient cohort. Subsequently, the patients were dichotomized into two distinct groups based on their PIK3CA mutation status, distinguishing those harboring PIK3CA mutations from those with wild-type PIK3CA.

Survival analysis was performed for overall survival (OS) and disease-free survival (DFS) using Kaplan-Meier curves. These survival curves were constructed to depict the survival probabilities of the two groups over time.

Differential expression gene analysis

We retrieved sample names containing mutation information in the PIK3CA gene from the cBioPortal database. Subsequently, the samples were stratified based on their PIK3CA mutation status into two distinct groups: those harboring PIK3CA mutations in tumor tissues and those exhibiting wild-type PIK3CA in tumor tissues. The corresponding expression data for these groups were extracted for subsequent analyses. Differential gene expression analysis between the two groups was conducted utilizing the Limma package. Specifically, we employed stringent criteria for identifying differentially expressed genes, setting the threshold at $|Log2FC| > 0.5$ and $P < 0.05$. The outcomes of this analysis were visually represented through the generation of a volcano plot and a heatmap.

GO and KEGG enrichment analysis

For investigating the functional implications and pathway alterations associated with PIK3CA gene mutations, we performed differential gene analysis using the clusterProfiler and enrichplot packages. Enrichment analysis was conducted for Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, with a significance threshold set at $P < 0.05$. The results were visually presented using bar plots and bubble charts.

ceRNA and transcription factor network analysis

To gather information on target genes and transcription factors from the htfTarget database (https://guolab.wchscu.cn/hTFtarget), we extracted all transcription factors associated with the PIK3CA gene. Subsequently, we performed Spearman correlation analysis in transcriptomic data to explore the correlation between the PIK3CA gene and identified transcription factors. A significance threshold was set at $P < 0.05$, and the correlation coefficient was required to exceed an absolute value of 0.4. For BLCA tumor samples from TCGA, we downloaded transcriptomic data for LncRNA and miRNA.

Employing Spearman correlation analysis, we investigated the associations between PIK3CA and both LncRNA and miRNA. Selection criteria included a p-value less than 0.05 for Lnc-PIK3CA and a correlation coefficient greater than 0.4, while for mir-PIK3CA, a P -value less than 0.05 and a correlation coefficient less than 0 were considered.Finally, we utilized the starbase database to analyze candidate miRNA-mRNA and miRNA-LncRNA relationships.

PPI analysis

The obtained set of differentially expressed genes was imported into the STRING database (https://string-db.org/), with a species restriction set to "Homo sapiens" and a confidence score threshold of ≥0.4. Subsequently, nodes without connections were removed, resulting in a refined network representing the protein-protein interaction relationships. The refined interaction data were then imported into Cytoscape software. Utilizing the MCODE module, we applied specific parameters for subnetwork selection, including a Degree Cutoff of 5, Node Score Cutoff of 0.2, and a K-Core of 2. These parameters were employed to filter and identify relevant subnetworks within the larger protein-protein interaction network.

GSEA analysis

To gain deeper insights into the pathway alterations associated with PIK3CA gene mutations, the BLCA tumor samples downloaded from TCGA were stratified into two groups: wild-type (WT) comprising 318 cases and mutant type (MT) comprising 88 cases, based on the PIK3CA gene status. Subsequently, Gene Set Enrichment Analysis (GSEA) version 4.3.2 software was employed for enrichment analysis using the c2.cp.kegg_medicus.v2023.2.Hs.symbols.gmt gene set.

TME analysis

The transcriptome data obtained from BLCA tumor samples were stratified into two groups based on the PIK3CA mutation status: the PIK3CA mutant group and the wild-type group.

Subsequently, the "estimate" package was utilized to compute stromal and immune scores for each sample, aiming to analyze the differences in immune infiltration between the two groups.

Immunoinfiltration analysis

To further investigate the immune cell infiltration in each sample, CIBERSORT analysis was performed on the transcriptome data of tumor samples from the PIK3CA mutant and wild-type groups. This analysis encompassed 22 distinct immune cell types, allowing an assessment of their infiltration levels in tumor tissues. Differential analysis was conducted to compare the proportions of each immune cell type between the two groups.

Immune checkpoint correlation analysis

To further investigate the relationship between the mutated PIK3CA gene and immune checkpoints, correlation analysis was conducted on 47 immune checkpoint genes and PIK3CA. Transcriptome data from PIK3CA-mutant tumor tissues were initially extracted, and the expression data for both immune checkpoint genes and PIK3CA were subsequently isolated. Pearson correlation analysis was then performed separately on these datasets, with a threshold set at $P < 0.001$.

Drug susceptibility analysis

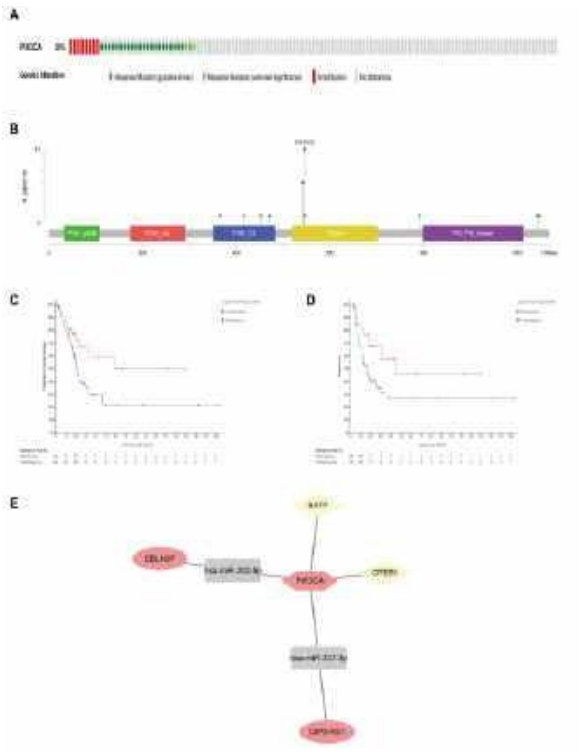
Utilizing the pRRophetic R package, sensitivity scores for each patient to various small-molecule compounds were calculated in both the PIK3CA-mutant and wild-type groups. The cpg2016 dataset, comprising 251 drugs, was selected as the training data. The IC50 values were employed to assess the impact of PIK3CA mutations on the sensitivity of patients to different drugs within these two groups, thereby identifying drugs for which PIK3CA mutations may confer enhanced sensitivity.

RESULTS

PIK3CA gene mutation and clinically relevant analysis results

The mutation frequency of the PIK3CA gene in bladder cancer accounts for 26% (Figure 1A). Most PIK3CA mutations occur primarily in the helical domain, particularly the E545K hotspot mutation (Figure 1B). To investigate the impact of PIK3CA gene mutations on the prognosis of bladder cancer patients, we categorized patients into PIK3CA mutation and non-mutation groups and evaluated their overall survival and disease-free survival rates. The results indicate that patients with PIK3CA mutations exhibit higher overall survival rates (Figure 1C) and disease-free survival rates (Figure 1D) at different time points. To gain further insights into the regulatory role of the PIK3CA gene in bladder cancer, we constructed a regulatory network centered around PIK3CA using bladder cancer transcriptome data from TCGA (Figure 1E).

Figure 1:



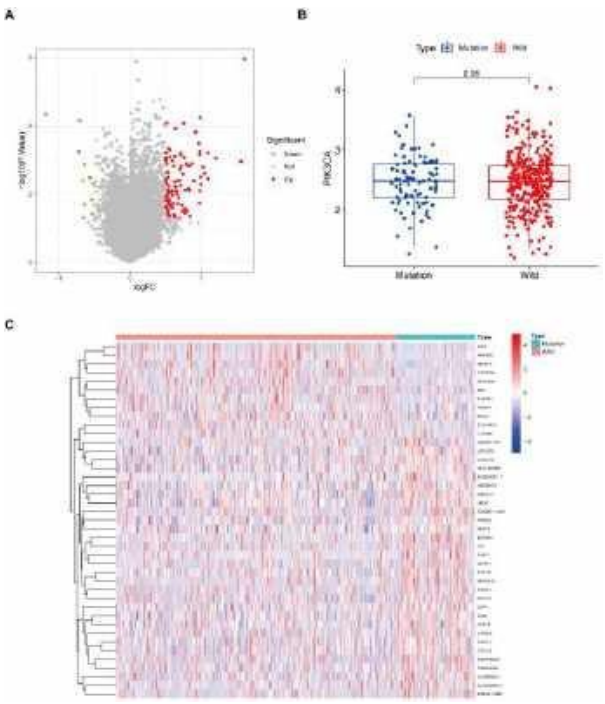
Initially, we screened transcription factors potentially related to the PIK3CA gene from the htfTarget database and identified BATF and CREB1 as major factors that positively correlate with PIK3CA expression. Furthermore, utilizing miRNA and LncRNA transcriptome data from TCGA, we analyzed the correlation between PIK3CA and these non-coding RNAs.

Through the starBase database, we found that the LncRNAs EBLN3P and OIP5-AS1 may positively regulate PIK3CA expression and potentially act as competing endogenous RNAs (ceRNAs) by sequestering miRNAs such as hsa-miR-202-5p and hsa-miR-337-3p. This comprehensive analysis sheds light on the complex regulatory mechanisms involving the PIK3CA gene in bladder cancer and provides valuable insights into potential therapeutic targets.

Analysis of differentially expressed genes associated with IK3CA mutations

To elucidate the series of phenotypic and signaling pathway changes induced by PIK3CA gene mutations in bladder cancer, we stratified patients into PIK3CA mutation and wild-type groups. Differential analysis was conducted using the Limma package, with a threshold set at $|LogFC| > 0.5$ and $p < 0.05$. A total of 110 differentially expressed genes were identified, with 99 genes upregulated and 11 genes downregulated in the mutation group (Figure 2A). It is noteworthy that there is no significant difference in PIK3CA gene expression between the mutant group and the non-mutant group (Figure 2B).

Figure 2:

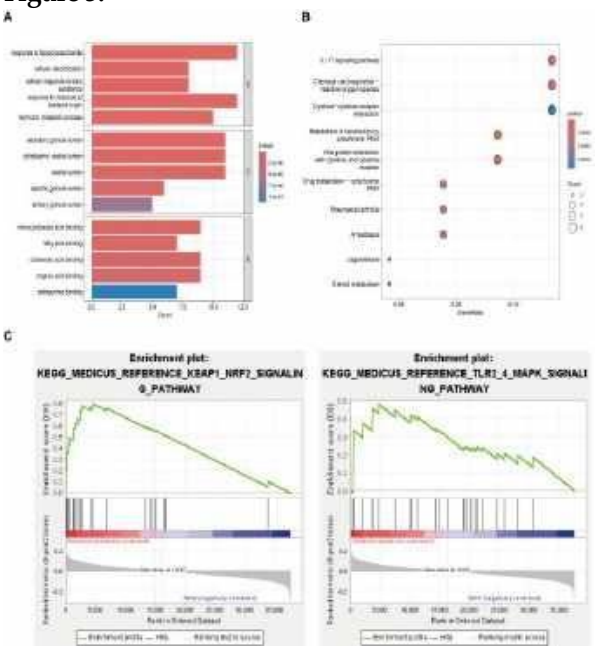


To visually depict the differential gene expression between the two groups, we generated a heatmap for selected genes. Among these, downregulated genes in the mutation group included IGF2, MIR483, and MGAT3, while upregulated genes comprised CXCL17, LRG1, and HSPB1P1 (Figure 2C). This comprehensive analysis provides valuable insights into the molecular alterations associated with PIK3CA mutations in bladder cancer, potentially informing targeted therapeutic strategies for improved patient outcomes.

Functional enrichment analysis of DEG

The GO enrichment analysis results (Figure 3A) revealed significant involvement of biological processes in the response to lipopolysaccharide. Cellular components included the secretory granule lumen and cytoplasmic vesicle lumen. Molecular functions encompassed monocarboxylic acid binding and fatty acid binding. KEGG analysis indicated that these genes are primarily involved in IL−17 signaling pathway and Cytokine−cytokine receptor interaction (Figure 3B).

Figure 3:



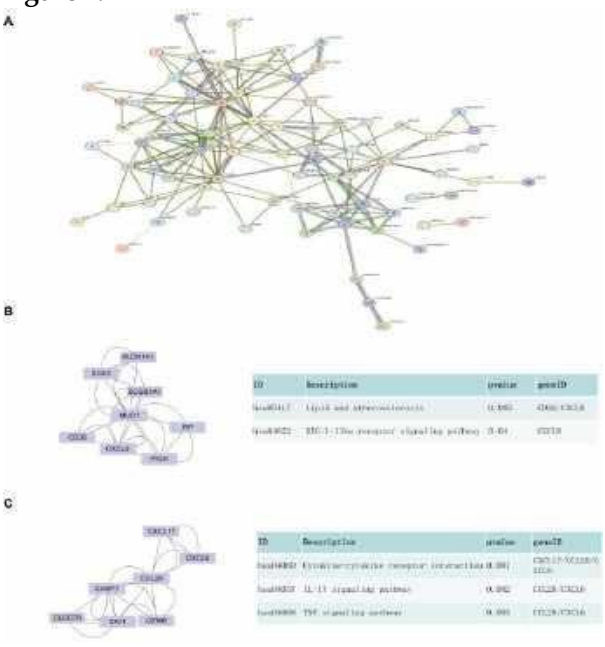
The GSEA aimed to discern notable differences in pathway enrichment between the PIK3CA wild-type and mutant groups, shedding light on the potential impact of PIK3CA mutations.

Utilizing the c2.cp.kegg_medicus.v2023.2.Hs.symbols.gmt gene set enabled a focused exploration of related pathways. The GSEA enrichment analysis results unveiled that PIK3CA mutations may trigger pathways associated with KEAP1/NRF2 signaling pathway and TLR2/4/MAPK signaling pathway (Figure 3C). This comprehensive analysis provides valuable insights into the molecular mechanisms underlying the effects of PIK3CA mutations in bladder cancer, particularly in modulating immune-related pathways, thus informing potential therapeutic strategies targeting immune responses in PIK3CA-mutated tumors.

The PPI network of DEGs

To further elucidate the differential protein-protein interaction networks between the PIK3CA mutation and wild-type groups, the differentially expressed genes were inputted into the STRING database for analysis. After removing unconnected protein nodes, the interactome network of all proteins was visualized in Figure 4A. Subsequently, all connected nodes were subjected to molecular complex detection (MCODE) in Cytoscape to identify functional subnetworks, followed by KEGG enrichment analysis of these subnetworks.

Figure 4:

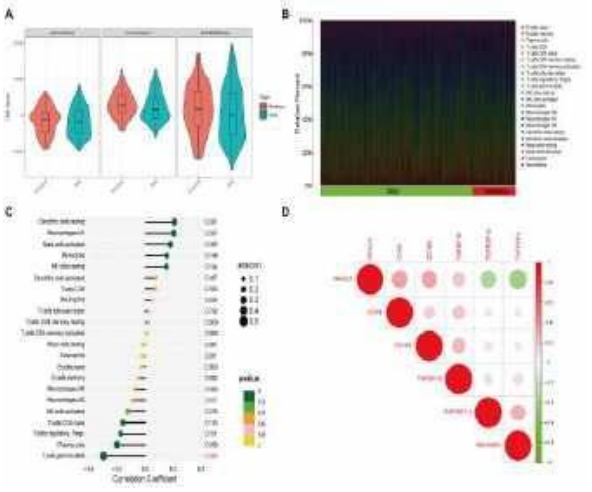


The results revealed that Cluster 1 primarily participates in signaling pathways such as Lipid and atherosclerosis and RIG-I-like receptor signaling pathway (Figure 4B). On the other hand, Cluster 2 primarily participates in immune response processes, exhibiting associations with cytokine receptor-related signaling pathways, as well as IL-17 and TNF signaling pathways (Figure 4C). This in-depth analysis provides insights into the intricate molecular mechanisms underlying the differential protein interactions associated with PIK3CA mutation status. The identification of distinct functional clusters highlights the involvement of specific signaling pathways that may contribute to the observed phenotypic differences between PIK3CA mutation and wild-type groups.

Correlation analysis between PIK3CA mutation and tumor immune invasion

In our quest to elucidate the functional ramifications of PIK3CA gene mutations, a pronounced association with immune cell modulation has been discerned. To further interrogate the immune and stromal cellular milieu within tumor tissues, we harnessed the ESTIMATE algorithm for comprehensive assessment. Employing characteristic gene signatures, we evaluated tumor specimens from each patient, revealing a conspicuous trend towards heightened StromalScore, ImmuneScore, and ESTIMATEScore in samples harboring PIK3CA mutations relative to their non-mutated counterparts (Figure 5A). Intrigued by the specific nuances in immune cell infiltration between these cohorts, we undertook a meticulous analysis via CIBERSORT to scrutinize the spectrum of 22 distinct immune cell types. This scrutiny unveiled a noteworthy augmentation in the infiltration levels of CD8+ T cells and resting NK cells within the PIK3CA mutation cohort, juxtaposed with a pronounced decline in the infiltration level of resting CD4+ T memory cells (Figure 5B). In further analysis, we focused on the correlation between PIK3CA gene and immune cells in the mutation group (Figure 5C). The results showed that PIK3CA gene was significantly negatively correlated with T cells (gamma delta type).

Figure 5:

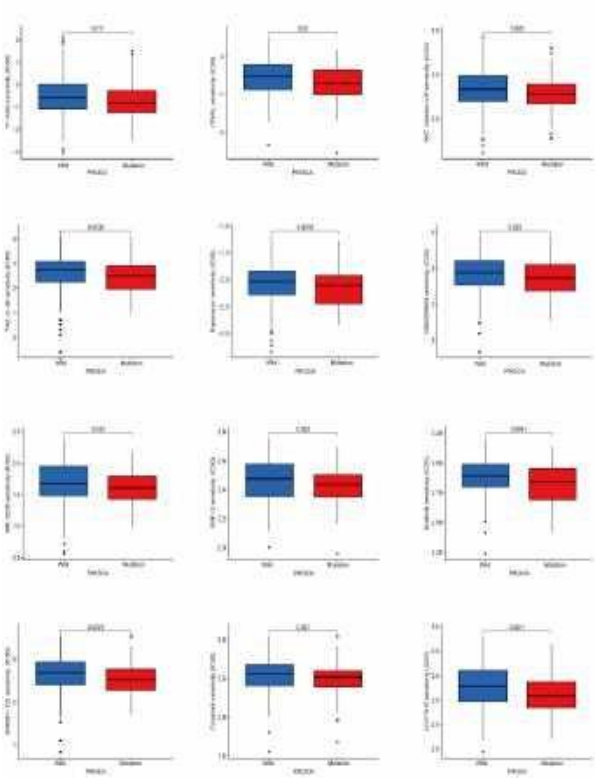


Of particular significance within the PIK3CA mutation cohort, were the noteworthy positive correlations observed between PIK3CA gene expression and the immune checkpoint gene CD44, CD160 and TNFSF18 coupled with significant negative correlations with TNFRSF14 and TNFRSF4 genes (Figure 5D). These findings collectively furnish a deeper understanding of the intricate immune landscape associated with PIK3CA mutations, offering pivotal insights into immune cell dynamics and potential regulatory crosstalk with immune checkpoint genes.

Evaluating the Therapeutic Response in the mutant and non-mutant groups

To assess the therapeutic sensitivity of patients with PIK3CA mutations to various drugs, we employed the pRRophetic algorithm to predict the differences in IC50 values between the PIK3CA mutation and wild-type groups in BLCA patients. A total of 251 drugs were evaluated for their IC50 values, revealing significant differences in the response to twelve compounds between the two groups (Figure 6). Patients harboring PIK3CA mutations may exhibit heightened sensitivity to certain drugs. These compounds warrant further investigation to expand the therapeutic repertoire available for patients with PIK3CA mutations in the future. The identification of specific drugs that exhibit divergent responses based on PIK3CA mutation status opens avenues for tailored treatment strategies, potentially enhancing

Figure 6:



treatment efficacy and patient outcomes in this subset of BLCA patients. Further validation studies and clinical trials are warranted to corroborate these findings and facilitate the translation of these insights into clinical practice.

DISCUSSION

Despite the significant efficacy demonstrated by immunotherapy in numerous cancer types, its application in the treatment of BLCA still faces several challenges[30]. Firstly, the response rate to immunotherapy remains suboptimal in BLCA patients[31]. While some patients exhibit durable clinical responses to immune checkpoint inhibitors, a considerable proportion remains insensitive to treatment or develops resistance[32,33]. This may partly be attributed to the heterogeneity of BLCA, resulting in varied responses to treatment among different patients[34,35]. Furthermore, the mechanism of immunotherapy for BLCA remains unclear at present. Thus, further research is still needed to address the uncertainties and resistance issues in immunotherapy responses among BLCA patients.

PIK3CA gene mutations are frequent in BLCA and closely associated with the clinical prognosis of patients[27]. In this study, we observed a significant advantage in overall survival and disease-free survival rates among patients with PIK3CA mutations. Furthermore, we found that the majority of PIK3CA mutations occur primarily in the helical domain, particularly the E545K hotspot mutation, further emphasizing the significance of PIK3CA in BLCA development. Transcriptomic analysis revealed that PIK3CA mutations lead to differential expression of a series of genes, involving various critical biological processes and signaling pathways[36,37,38]. These results suggest a pivotal role of PIK3CA mutations in the occurrence and progression of BLCA, providing important clues for further elucidating its underlying mechanisms of action.

Our study further elucidated the impact of PIK3CA gene mutations on immune-related pathways through GO and KEGG enrichment analysis, as well as GSEA. The results indicated that PIK3CA mutations may modulate immune-related pathways, such as Cytokine−cytokine receptor interaction, potentially affecting the immune response to tumors. This suggests that PIK3CA mutations may regulate tumor immune responses by influencing immune pathways, providing a theoretical basis for developing immunotherapeutic strategies targeting PIK3CA-mutated BLCA. Changes in immune cell infiltration levels within tumor tissues are of significant importance for tumor progression and patient prognosis in BLCA.

Our results showed a significant increase in CD8+ T cell and resting NK cell infiltration levels in tumor tissues of patients with PIK3CA mutations, along with a notable decrease in resting CD4+ T memory cells infiltration levels. Additionally, we found significant positive correlations between PIK3CA gene expression and the immune checkpoint gene CD44, CD160 and TNFSF18, coupled with significant negative correlations with TNFRSF14 and TNFRSF4 genes. These findings further highlight the close relationship between PIK3CA gene mutations and the tumor immune microenvironment, providing important clues for a deeper understanding of the mechanisms of action of PIK3CA mutations in immunotherapy.

CONCLUSION

In conclusion, this study elucidates the important role of PIK3CA gene mutations in the occurrence and development of BLCA, as well as their potential impact on immune therapy response. This provides new directions for further research on personalized treatment strategies for BLCA. Future studies can further explore the relationship between PIK3CA mutations and immunotherapy resistance, and develop precise therapeutic strategies for PIK3CA-mutated BLCA to improve treatment outcomes and survival rates of patients.

DECLARATIONS

Data availability statement

All TCGA molecular and clinical data sets used in this study are publicly available and can be found here: https://portal.gdc.cancer.gov/. The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Financial support and sponsorship

This work was funded by the Natural Science Foundation of Hunan Province of China (No. 2022JJ40249), Changsha Municipal Natural Science Foundation (No. kq2202463).

Conflicts of interest

There are no conflicts of interest.

Author contributions

Yueming Wan, Chunwang Liao, Keliang Peng conceived the article and wrote the manuscript. Hong Shan reviewed and integrated it with additional data and references. All the remaining authors contributed with comments, adding data and references. All authors contributed to the article and approved the submitted version.

REFERENCES

1.Yue SY, Niu D, Liu XH, et al. BLCA prognostic model creation and validation based on immune gene-metabolic gene combination. Discov Oncol. 2023; 14:232.

2. Caramelo B, Zagorac S, Corral S, et al. Cancer- associated Fibroblasts in Bladder Cancer: Origin, Biology, and Therapeutic Opportunities. Eur Urol Oncol. 2023; 6:366-375.
3. Minoli M, Cantore T, Hanhart D, et al. Bladder cancer organoids as a functional system to model different disease stages and therapy response. Nat Commun. 2023; 14:2214.
4. Lenis AT, Lec PM, Chamie K, et al. Bladder Cancer: A Review. Jama. 2020; 324:1980-1991.
5. Halachmi S, Madeb R, Kravtsov A, et al. Bladder cancer--genetic overview. Med Sci Monit. 2001; 7:164-168.
6. Kamat AM, Hahn NM, Efstathiou JA, et al. Bladder cancer. Lancet. 2016; 388:2796-2810.
7. Kirkali Z, Chan T, Manoharan M, et al. Bladder cancer: epidemiology, staging and grading, and diagnosis. Urology. 2005; 66:4-34.
8. Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. Lancet. 2009; 374:239-249.
9. Groeneveld CS, Fontugne J, Cabel L, et al. Tertiary lymphoid structures marker CXCL13 is associated with better survival for patients with advanced-stage bladder cancer treated with immunotherapy. Eur J Cancer. 2021; 148:181-189.
10. Zhu Z, Ma AH, Zhang H, et al. Phototherapy with Cancer-Specific Nanoporphyrin Potentiates Immunotherapy in Bladder Cancer. Clin Cancer Res. 2022; 28:4820-4831.
11. de Jong FC, Rutten VC, Zuiverloon TCM, et al. Improving Anti-PD-1/PD-L1 Therapy for Localized Bladder Cancer. Int J Mol Sci. 2021; 22:2800.
12. Goubet AG, Lordello L, Alves Costa Silva C, et al. Escherichia coli-Specific CXCL13-Producing TFH Are Associated with Clinical Efficacy of Neoadjuvant PD-1 Blockade against Muscle- Invasive Bladder Cancer. Cancer Discov. 2022; 12:2280-2307.
13. Lopez-Beltran A, Cimadamore A, Blanca A, et al. Immune Checkpoint Inhibitors for the Treatment of Bladder Cancer. Cancers (Basel) 2021; 13:131.
14. Rouanne M, Roumiguié M, Houédé N, et al. Development of immunotherapy in bladder cancer: present and future on targeting PD(L)1. 2020; 2020:8143158.
15. Mao F, Niu XB, Gu S, et al. CTLA-4 +49A/G
Polymorphism Increases the Susceptibility to Bladder Cancer in Chinese Han Participants: A Case-Control Study. Dis Markers. 2020; 2020:8143158.
16. Wołącewicz M, Hrynkiewicz R, Grywalska E, et al. Immunotherapy in Bladder Cancer: Current Methods and Future Perspectives. Cancers (Basel) 2020; 12:1181.
17. Michaud É, Mansure JJ, Kassouf W. Integrating novel immunotherapeutic approaches in organ- preserving therapies for bladder cancer. Br J Pharmacol. 2023.
18. Canaud G, Hammill AM, Adams D, et al. A review of mechanisms of disease across PIK3CA- related disorders with vascular manifestations. Orphanet J Rare Dis. 2021; 16:306.
19. Reinhardt K, Stückrath K, Hartung C, et al. PIK3CA-mutations in breast cancer. Breast Cancer Res Treat. 2022; 196:483-493.
20. Hao Y, Samuels Y, Li Q, et al. Oncogenic PIK3CA mutations reprogram glutamine metabolism in colorectal cancer. Nat Commun. 2016; 7:11971.
21. Jin N, Keam B, Cho J, et al. Therapeutic implications of activating noncanonical PIK3CA mutations in head and neck squamous cell carcinoma. J Clin Invest. 2021;131: e150335.
22. Herberts C, Murtha AJ, Fu S, et al. Activating AKT1 and PIK3CA Mutations in Metastatic Castration-Resistant Prostate Cancer. Eur Urol. 2020; 78:834-844.
23. Sathe A, Guerth F, Cronauer MV, et al. Mutant PIK3CA controls DUSP1-dependent ERK 1/2 activity to confer response to AKT target therapy. Br J Cancer. 2014; 111:2103-2113.
24. Delestre F, Venot Q, Bayard C, et al. Alpelisib administration reduced lymphatic malformations in a mouse model and in patients. Sci Transl Med. 2021;13: eabg0809.
25. Ross JS, Slodkowska EA, Symmans WF, et al. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist. 2009; 14:320-368.
26. Dueñas M, Martínez-Fernández M, García- Escudero R, et al. PIK3CA gene alterations in bladder cancer are frequent and associate with reduced recurrence in non-muscle invasive tumors. Mol Carcinog. 2015; 54:566-576.
33. Benjamin DJ, Lyou Y. Advances in Immunotherapy and the TGF-β Resistance Pathway in Metastatic Bladder Cancer. Cancers (Basel). 2021; 13:5724.
34. Kamoun A, de Reyniès A, Allory Y, et al. A Consensus Molecular Classification of Muscle- invasive Bladder Cancer. Eur Urol. 2020; 77:420-433.
35. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer. 2015; 15:25-41.
36. Chen H, Pan Y, Jin X, et al. An immune cell infiltration-related gene signature predicts prognosis for bladder cancer. Sci Rep. 2021; 11:16679.
37. Oh DY, Kwek SS, Raju SS, et al. Intratumoral CD4(+) T Cells Mediate Anti-Tumor Cytotoxicity in Human Bladder Cancer. Cell. 2020; 181:1612- 1625.e1613.
38. An Y, Sun JX, Xu MY, et al. Tertiary lymphoid structure patterns aid in identification of tumor microenvironment infiltration and selection of therapeutic agents in bladder cancer. Front Immunol. 2022; 13:1049884.
33. Benjamin DJ, Lyou Y. Advances in Immunotherapy and the TGF-β Resistance Pathway in Metastatic Bladder Cancer. Cancers (Basel). 2021; 13:5724.
34. Kamoun A, de Reyniès A, Allory Y, et al. A Consensus Molecular Classification of Muscle- invasive Bladder Cancer. Eur Urol. 2020; 77:420-433.
35. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer. 2015; 15:25-41.
36. Chen H, Pan Y, Jin X, et al. An immune cell infiltration-related gene signature predicts prognosis for bladder cancer. Sci Rep. 2021; 11:16679.
37. Oh DY, Kwek SS, Raju SS, et al. Intratumoral CD4(+) T Cells Mediate Anti-Tumor Cytotoxicity in Human Bladder Cancer. Cell. 2020; 181:1612- 1625.e1613.
38. An Y, Sun JX, Xu MY, et al. Tertiary lymphoid structure patterns aid in identification of tumor microenvironment infiltration and selection of therapeutic agents in bladder cancer. Front Immunol. 2022; 13:1049884.