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Genetic profiling and pathway analysis in bladder carcinoma: Implications for therapeutic targeting

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ABSTRACT

Objective: Bladder carcinoma represents a significant challenge in oncology due to its heterogeneous molecular nature. This study aimed to identify key genetic factors and molecular pathways involved in bladder carcinoma pathogenesis to facilitate the development of targeted therapies.

Material and Methods: The top 30 genes associated with bladder carcinoma were retrieved from the disease gene network database. Comprehensive bioinformatic analysis was performed using various enrichment tools, including gene ontology biological process, cellular component, molecular function analyses, and pathway mapping through WikiPathways and metabolite associations through human metabolome database. Drug interactions were evaluated using DrugMatrix data.

Results: Gene ontology analysis revealed significant enrichment of cancer-related biological processes, cellular components, and molecular functions. Pathway analysis identified strong associations with head and neck squamous cell carcinoma, cancer pathways, pleural mesothelioma, endometrial cancer, and bladder cancer pathways. Key genes including *CDKN2A*, *PTEN*, *EGFR*, *PIK3CA*, *HRAS*, *FGFR3*, and *TP53* were implicated across multiple pathways. Metabolite analysis showed significant associations with phosphatidylinositol derivatives, highlighting the importance of the PI3K pathway. Drug interaction analysis revealed potential modulatory effects of several compounds including sertraline, valproic acid, and hydroxyurea on gene expression patterns in bladder carcinoma.

Conclusion: This study provides comprehensive insights into the molecular underpinnings of bladder carcinoma, highlighting interconnected pathways and potential therapeutic targets. The significant overlap with other cancer types suggests common oncogenic mechanisms that could be exploited for therapeutic intervention. Further validation of these findings in clinical samples may facilitate the development of personalized treatment approaches for bladder carcinoma patients.

Keywords: Bladder carcinoma, gene expression, pathway analysis, therapeutic targets, molecular oncology

INTRODUCTION

Bladder carcinoma represents one of the most common malignancies of the urinary tract, with significant morbidity and mortality worldwide (1). According to global cancer statistics, bladder cancer ranks as the tenth most commonly diagnosed cancer, with an estimated 573,000 new cases and 213,000 deaths annually (2). The disease disproportionately affects males, with a male-to-female ratio of approximately 3:1, and incidence rates vary significantly across different geographical regions (3).

The etiology of bladder carcinoma involves complex interactions between genetic and environmental factors. Established risk factors include tobacco smoking, occupational exposure to aromatic amines, arsenic in drinking water, and chronic urinary tract infections (4). Additionally, genetic predisposition plays a crucial role in determining individual susceptibility to the disease (5). Understanding the interplay between these factors is essential for developing effective preventive and therapeutic strategies.

From a pathological perspective, bladder carcinomas are classified into two major categories: Non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) (6). NMIBC accounts for approximately 75% of cases at initial diagnosis and is generally associated with better prognosis, although recurrence rates remain high (7). In contrast, MIBC represents a more aggressive form with poorer outcomes and higher risk of metastasis, necessitating more aggressive treatment approaches (8).

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Despite advances in diagnostic techniques and treatment modalities, the management of bladder carcinoma remains challenging, with limited improvement in survival rates over the past decades (9). Conventional treatment options include surgery, intravesical therapy, chemotherapy, and radiation therapy, each with its own limitations and side effects (10). Recent developments in targeted therapies and immunotherapies have shown promise, but their efficacy varies significantly among patients, highlighting the need for better understanding of the underlying molecular mechanisms (11).

Molecular characterization has revealed distinct genomic alterations associated with bladder carcinoma, including mutations in tumor suppressor genes such as tumor protein p53 (*TP53*) and *RB1*, as well as oncogenes like *FGFR3* and *RAS* family members (12). These genetic alterations contribute to dysregulation of critical cellular processes, including cell cycle control, apoptosis, and DNA damage response, ultimately leading to tumor development and progression (13).

Recent advances in high-throughput genomic technologies have enabled comprehensive profiling of bladder carcinomas, revealing complex molecular landscapes and potential therapeutic targets (14). Integrated analyses of genomic, transcriptomic, and proteomic data have identified distinct molecular subtypes with different clinical behaviors and treatment responses (15). Furthermore, epigenetic alterations, including DNA methylation and histone modifications, have emerged as important regulators of gene expression in bladder cancer, adding another layer of complexity to the disease (16).

Understanding the intricate network of signaling pathways involved in bladder carcinogenesis is crucial for identifying potential therapeutic targets (17). Key pathways implicated in bladder cancer development include phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR, MAPK, WNT/β-catenin, and JAK/STAT signaling, which regulate various cellular functions such as proliferation, survival, and invasion (18). Targeting these pathways represents a promising approach for developing novel therapeutic strategies with improved efficacy and reduced toxicity (19).

In this study, we aimed to comprehensively analyze the genetic and molecular landscape of bladder carcinoma by examining the top 30 genes associated with the disease from the disease gene network (DisGeNET) database (20). Through detailed pathway analysis, gene ontology (GO) enrichment, and exploration of metabolite and drug interactions, we sought to identify key molecular mechanisms and potential therapeutic targets for bladder carcinoma.

Objectives

1. To identify and characterize the key genetic factors associated with bladder carcinoma through comprehensive bioinformatic analysis of DisGeNET data.

2. To elucidate the significant biological processes, cellular components, and molecular functions associated with bladder carcinoma-related genes using GO enrichment analysis.

3. To map the molecular pathways implicated in bladder carcinoma pathogenesis and identify potential points of therapeutic intervention.

4. To explore associations between bladder carcinoma genes and metabolites, as well as drug interactions, to facilitate the development of targeted therapeutic approaches.

MATERIAL and METHODS

Data Acquisition

The top 30 genes associated with bladder carcinoma were retrieved from the DisGeNET database (https://www.disgenet.org/). DisGeNET is a comprehensive platform that integrates information on gene-disease associations from various expert-curated databases and text-mining-derived associations. The selection of genes was based on the association score provided by DisGeNET, which considers various factors including the number and type of sources supporting the gene-disease association and the number of publications.

GO Enrichment Analysis

GO enrichment analysis was performed to identify the biological processes, cellular components, and molecular functions associated with the selected genes. The analysis was conducted using the GO_Biological_Process_2023, GO_Cellular_Component_2023, and GO_Molecular_Function_2023 databases. The enrichment analysis identified statistically significant associations between the gene set and specific GO terms.

The statistical significance of enrichment was calculated using a hypergeometric test, which compares the observed overlap between the gene set and each GO term to the expected overlap by chance. The resulting p-values were adjusted for multiple testing using the Benjamini-Hochberg method to control the false discovery rate (FDR). Terms with adjusted p-values less than 0.05 were considered statistically significant. The results were visualized as bar graphs depicting the -log10 (adjusted p-value) for each significant term.

Pathway Analysis

To identify the signaling pathways and biological networks associated with bladder carcinoma, the gene set was analyzed using the WikiPathways_2024 database. WikiPathways is a community-curated resource of biological pathways that provides comprehensive coverage of various biological processes and disease mechanisms.

The pathway enrichment analysis was performed using a similar approach to the GO enrichment analysis, with statistical

significance determined by a hypergeometric test and p-values adjusted using the Benjamini-Hochberg method. For each significantly enriched pathway, the analysis provided information on the overlap (number of genes from the input set that are part of the pathway), p-value, adjusted p-value, odds ratio, combined score, and the specific genes involved.

Subcellular Localization Analysis

To understand the subcellular distribution of the bladder carcinoma-associated genes, enrichment analysis was performed using the Jensen_COMPARTMENTS database. This database provides information on protein localization based on experimental evidence, annotations, and predictions. The analysis identified cellular compartments where the proteins encoded by the selected genes are significantly enriched, offering insights into their functional roles within the cell.

Tissue Expression Analysis

Tissue-specific expression patterns of the bladder carcinoma-associated genes were examined using the Jensen_TISSUES database. This analysis helped identify tissues where the selected genes are predominantly expressed, providing context for their role in bladder carcinoma pathogenesis and potential implications for tissue-specific therapeutic targeting.

Transcription Factor Analysis

The regulatory mechanisms governing the expression of bladder carcinoma-associated genes were investigated using the ChIP-X Enrichment Analysis (ChEA_2022) database. This analysis identified transcription factors that significantly regulate the expression of the selected genes, offering insights into the upstream regulatory networks involved in bladder carcinoma.

Metabolite Association Analysis

To explore the metabolic aspects of bladder carcinoma, the gene set was analyzed for associations with specific metabolites using the human metabolome database (HMDB) database. The HMDB provides detailed information on small molecule metabolites found in the human body. This analysis identified metabolites that are significantly associated with the selected genes, suggesting potential metabolic alterations in bladder carcinoma.

Drug Interaction Analysis

The potential interactions between the bladder carcinoma-associated genes and various drugs were examined using the DrugMatrix database. This analysis identified drugs that significantly modulate the expression of the selected genes, offering insights into potential therapeutic agents for bladder carcinoma. The results included information on the drug name, concentration, vehicle, organism, tissue, treatment duration, direction of gene expression change (up or down), and the specific genes affected.

Data Visualization and Interpretation

The results of the various analyses were visualized using bar graphs and tables to facilitate interpretation. The bar graphs depicted the -log10 (adjusted p-value) for each significant term, facilitating the comparison of the statistical significance of different terms. The tables provided detailed information on each significant finding, including the specific genes involved.

The interpretation of the results focused on identifying key biological processes, pathways, and potential therapeutic targets relevant to bladder carcinoma. Particular attention was given to patterns and commonalities across different analyses, as well as to findings with strong statistical significance and biological relevance. The results were contextualized within the current understanding of bladder carcinoma biology and compared with findings from previous studies to identify novel insights and confirm established knowledge.

Statistical Analysis

Combining R version 4.0.1 with many Bioconductor programs guarantees thorough data evaluation by means of statistical analysis. Using the limma program, which performed empirical Bayes moderation to enhance variance estimates across genes, genes with a modified p-value of 0.05 and an absolute log2 fold change ≥1 were regarded as significant, and differentially expressed genes were discovered. Hierarchical clustering heatmaps and volcano plots created using ggplot2 and pheatmap, respectively helped visualize patterns of gene expression. Functional enrichment studies for GO and KEGG pathways were conducted using the clusterProfiler software with the Benjamini-Hochberg correction to control the FDR at 5%. Furthermore, a protein-protein interaction network built utilizing STRING database data was shown in Cytoscape, and a statistically significant evaluation based on enrichment p-values was presented. Using TARGETSCAN and MIRBASE, microRNA-target interactions were evaluated. Metabolomic pathway enrichment was done with Enrichr and MetaboAnalyst 6.0, where enrichment statistics, including p-values and adjusted p-values, were computed and visualized using bubble plots based on -log10 transformation of the p-values.

RESULTS

GO Analysis

GO biological process analysis (Figure 1)

GO analysis for biological processes revealed significant enrichment of pathways crucial for cancer development and progression. The top enriched terms included processes related

to cell proliferation, signal transduction, apoptosis regulation, and response to external stimuli. These findings suggest that the selected bladder carcinoma-associated genes are primarily involved in cellular processes that regulate growth, survival, and response to environmental factors, which are critical aspects of cancer biology.

4.1.2 GO cellular component analysis (Figure 2)

Analysis of cellular components showed significant enrichment of terms related to membrane structures, cytoplasmic components, and nuclear regions. The enrichment of membrane components suggests that many bladder carcinoma-associated proteins are involved in cell-cell interactions, signal transduction, and membrane-bound receptor functions. The presence of both cytoplasmic and nuclear components indicates the involvement of these genes in diverse cellular processes spanning multiple compartments.

4.1.3 GO molecular function analysis (Figure 3)

The molecular function analysis revealed significant enrichment of terms related to protein binding, kinase activity, transcription factor activity, and receptor function. These findings highlight the diverse functional roles of bladder carcinoma-associated genes, particularly in signalling pathways, gene regulation, and cellular response mechanisms.

4.2 Pathway Analysis (Table 1)

The WikiPathways analysis identified multiple significantly enriched pathways associated with bladder carcinoma genes (Table 1). The most significantly enriched pathway was "head and neck squamous cell carcinoma" (adjusted p-value $=3.09\times10^{-27}$), with 15 out of 30 genes mapping to this pathway. Other significantly enriched pathways included "cancer pathways" (20/30 genes, adjusted p-value $=3.51\times10^{-23}$), "pleural mesothelioma" (18/30 genes, adjusted p-value $=7.36\times10^{-18}$), "endometrial cancer" (11/30 genes, adjusted p-value $=5.60\times10^{-13}$), and notably "bladder cancer" (9/30 genes, adjusted p-value $=1.37\times10^{-10}$).

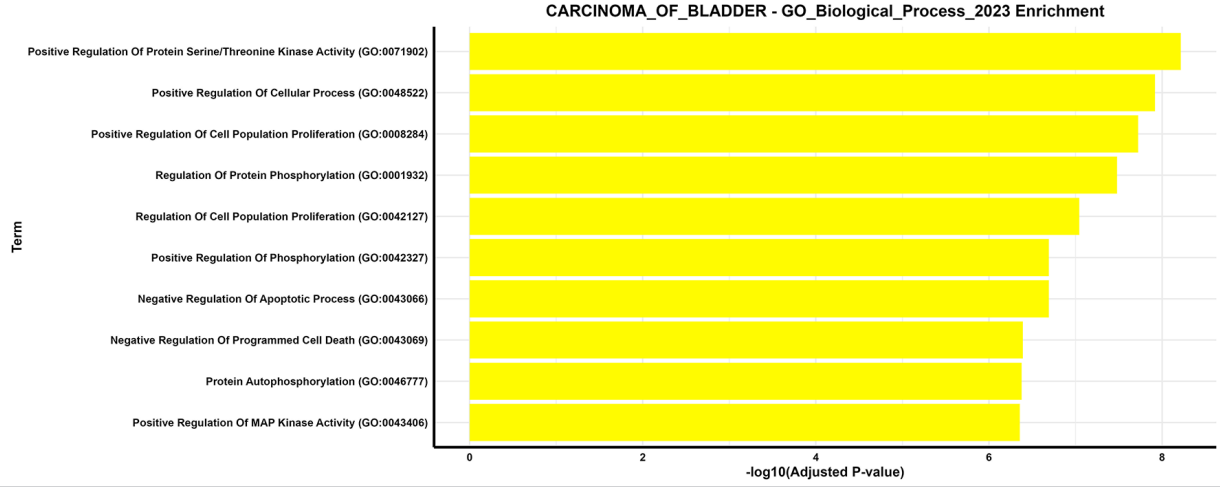

Figure 1. This bar graph for GO_Biological_Process_2023 depicts the relationship between -log10 (adjusted p-value) and terms as listed.

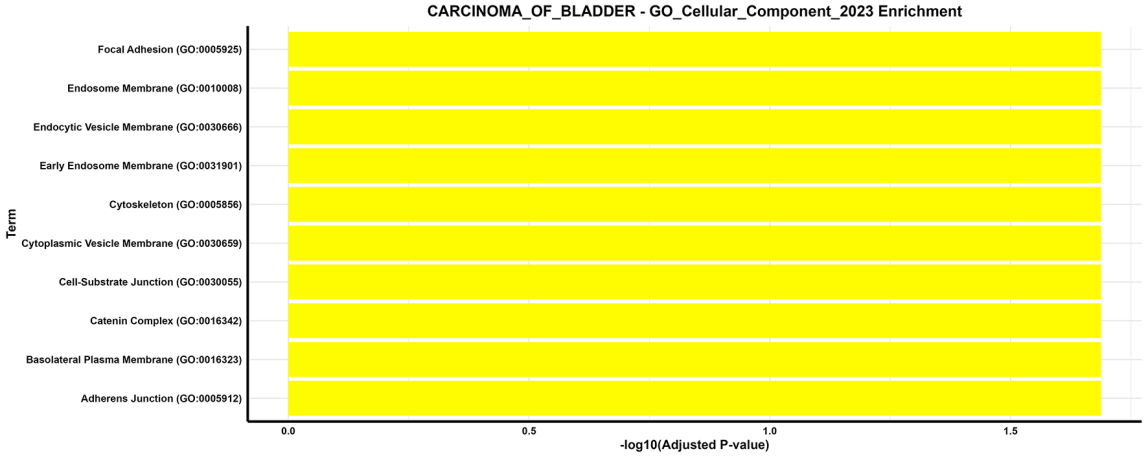

Figure 2. The bar graph for GO_Cellular_Component_2023 demonstrates -log10 (adjusted p-value) versus terms arranged sequentially.

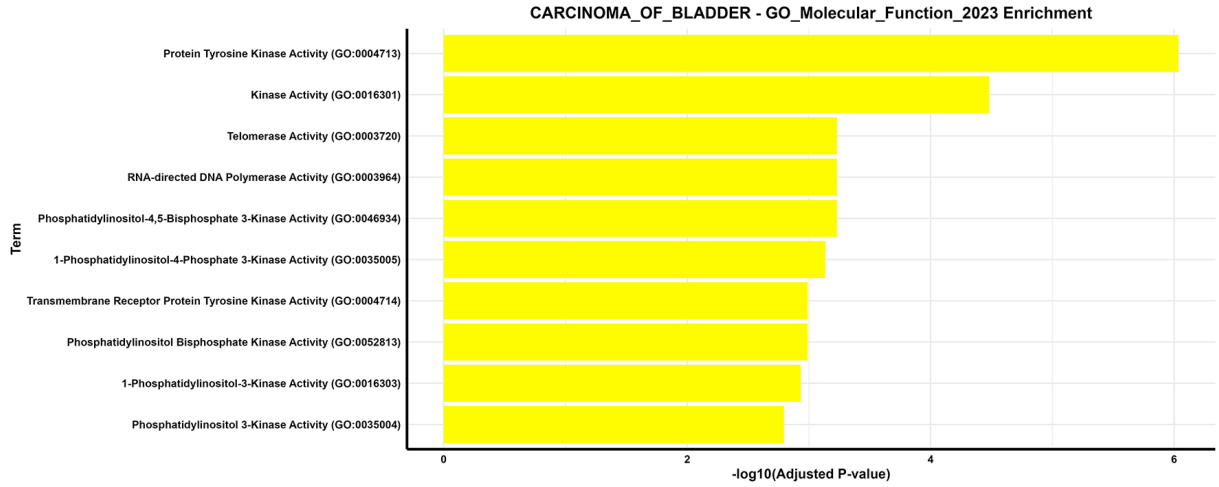

Figure 3. GO_Molecular_Function_2023 is illustrated with a bar graph that maps -log10 (adjusted p-value) against terms.

Table 1. Presented here is a table for WikiPathways_2024_

Term	Overlap	p-value	Adjusted p-value	Old p-value	Old adjusted p-value	Odds ratio	Combined score	Genes
Head and neck squamous cell carcinoma WP4674	15/73	0.000000000000000000008668776	0.000000000000000000003094753	0	0	343.31034	22,973.582	*CDKN2A;PTEN;EGFR;PIK3CG;MTOR;VEGFA;TERT;CCND1;PIK3CA;ERBB2;AKT1;CTNNB1;HRAS;FGFR3;TP53*
Cancer pathways WP5434	20/507	0.000000000000000000019674549 7899	0.000000000000000000035119071375	0	0	80.01232	4,551.732	*GSTM1;CDKN2A;WNT5A;PTEN;EGFR;MTOR;VEGFA;AR;TERT;CCND1;PIK3CA;CDH1;ERBB2;AKT1;BIRC5;CTNNB1;HRAS;FGFR3;TP53;WNT3*
Pleural mesothelioma WP5087	18/437	0.000000000000000000082996077 0625	0.000000000000000000735776 5331704	0	0	69.99165	3,579.209	*CD274;CDKN2A;WNT5A;PTEN;EGFR;PIK3CG;MTOR;VEGFA;TERT;CCND1;PIK3CA;CDH1;AKT1;CTNNB1;HRAS;FGFR3;TP53;WNT3*
Endometrial cancer WP4155	11/63	0.000000000000000000036709133 0970	0.000000000000000000056016576 2901289	0	0	221.75911	10,315.678	*CCND1;PIK3CA;CDH1;ERBB2;PTEN;AKT1;CTNNB1;HRAS;FGFR3;TP53;EGFR*
DNA damage response only ATM dependent WP710	12/109	0.000000000000000000022878566 1902	0.000000000000000000209225053 5296260	0	0	136.58419	6,143.091	*CCND1;PIK3CA;CDKN2A;ERBB2;WNT5A;PTEN;AKT1;CTNNB1;HRAS;TP53;PIK3CG;WNT3*
Breast cancer pathway WP4262	12/154	0.000000000000000000035258803 9960	0.000000000000000000012826304047 8987992	0	0	93.08920	3,786.722	*CCND1;PIK3CA;ERBB2;WNT5A;PTEN;AKT1;CTNNB1;HRAS;TP53;EGFR;WNT3;MTOR*
Bladder cancer WP2828	9/40	0.000000000000000000025252110 0024	0.000000000000000000013746049647 8576001	0	0	275.65438	11,151.604	*CCND1;CDH1;CDKN2A;ERBB2;HRAS;FGFR3;TP53;EGFR;VEGFA*

Table 1. Continued

Term	Overlap	p-value	Adjusted p-value	Old p-value	Old adjusted p-value	Odds ratio	Combined score	Genes
Gastrin signaling WP4659	11/115	0.000000000000000006931725707981400161	0.000000000000000309328259718670022	0	0	110.59008	4,369.461	*CCND1;PIK3CA;CDH1;CDKN2A;AKT1;BIRC5;CTNNB1;HRAS;EGFR;MTOR;VEGFA*
Glioblastoma signaling WP2261	10/82	0.000000000000000021363986678023000982	0.000000000000000847438138228244992	0	0	138.18056	5,304.036	*CCND1;PIK3CA;CDKN2A;ERBB2;PTEN;AKT1;HRAS;TP53;EGFR;PIK3CG*
EGFR tyrosine kinase inhibitor resistance WP4806	10/84	0.000000000000000027523047678472601241	0.000000000000000982572802121471009	0	0	134.43243	5,126.111	*CCND1;PIK3CA;ERBB2;PTEN;AKT1;HRAS;FGFR3;EGFR;MTOR;VEGFA*

Key genes that appeared across multiple pathways included *CDKN2A, PTEN, EGFR, PIK3CG, MTOR, VEGFA, TERT, CCND1, PIK3CA, ERBB2, AKT1, CTNNB1, HRAS, FGFR3*, and *TP53*. The high representation of these genes across different cancer pathways suggests common oncogenic mechanisms shared between bladder carcinoma and other cancer types.

The significant enrichment of DNA damage response pathways, particularly the "DNA damage response only ATM dependent" pathway (12/30 genes, adjusted p-value $=2.09\times10^{-12}$), highlights the importance of genomic instability in bladder carcinoma pathogenesis. Additionally, the presence of signalling pathways such as "gastrin signaling" and "EGFR tyrosine kinase inhibitor resistance" underscores the complexity of signalling networks involved in bladder carcinoma.

Subcellular Localization Analysis

The Jensen_COMPARTMENTS analysis indicated significant enrichment of bladder carcinoma-associated proteins in various cellular compartments. The results showed predominant localization in the cytoplasm, plasma membrane, and nucleus, consistent with the diverse functional roles of these proteins in cell signalling, gene regulation, and cellular architecture.

Tissue Expression Analysis (Figure 4)

Analysis of tissue expression patterns using Jensen_TISSUES revealed significant expression of bladder carcinoma-associated genes across multiple tissue types. In addition to the bladder tissue showed significant enrichment, other epithelial tissues also exhibited notable expression levels. This pattern suggests potential systemic effects of these genes beyond the primary site of carcinogenesis and may explain the comorbidities and secondary manifestations often observed in bladder carcinoma patients.

Transcription Factor Analysis (Figure 5)

The ChEA_2022 analysis identified several transcription factors that significantly regulate the expression of bladder carcinoma-

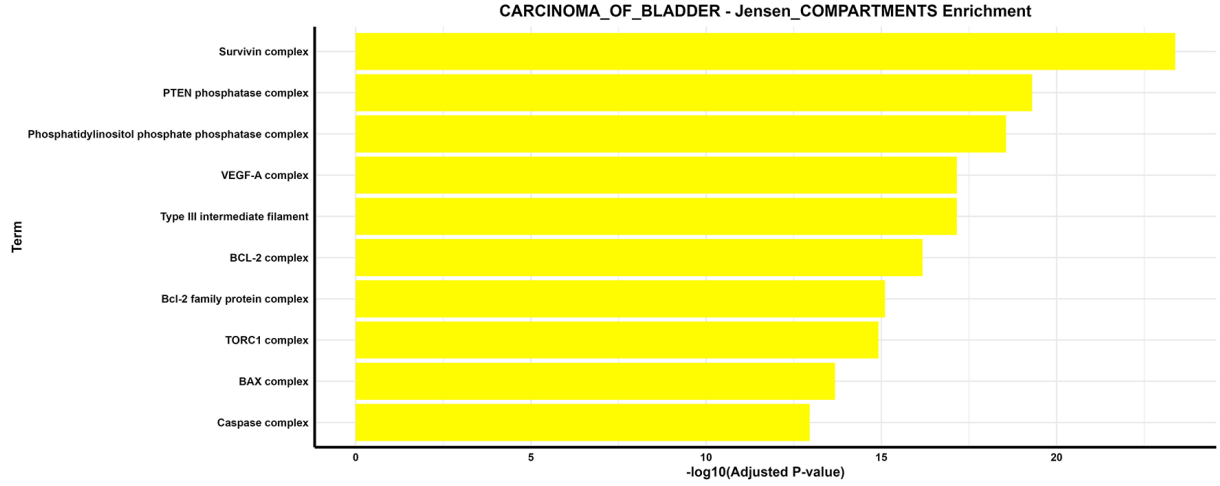

Figure 4A. The depicted bar graph for Jensen_COMPARTMENTS shows -log10 (adjusted p-value) plotted versus the ordered list of terms.

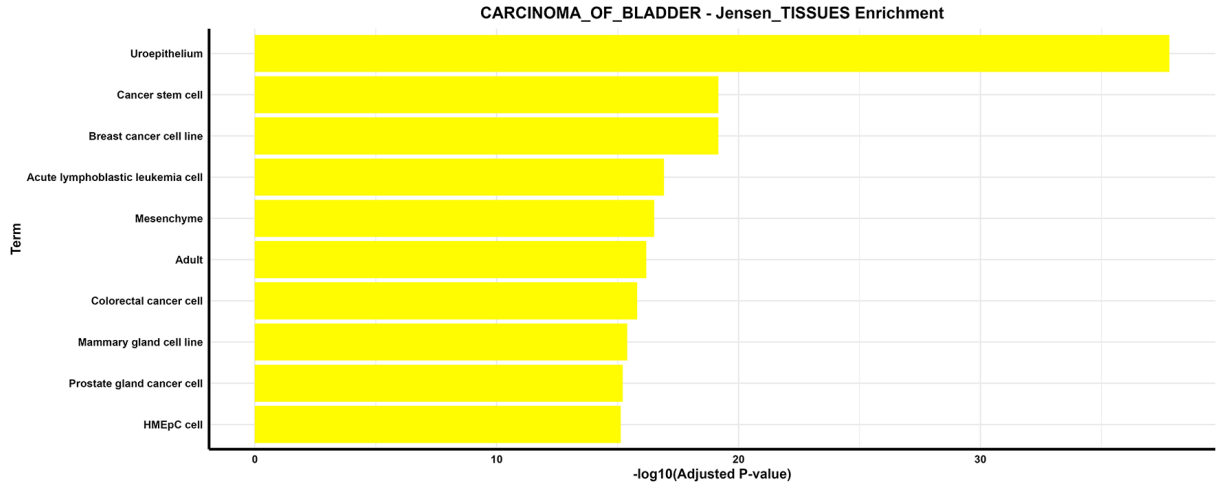

Figure 4B. The depicted bar graph for Jensen_TISSUES shows -log10 (adjusted p-value) plotted versus the ordered list of terms.

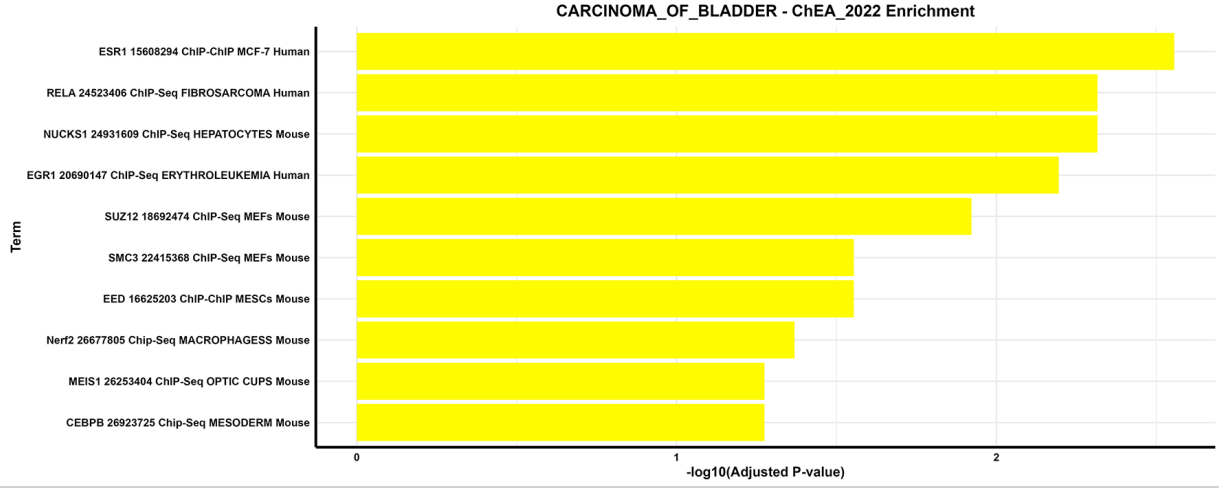

Figure 5. The depicted bar graph for ChEA_2022 shows -log10 (adjusted p-value) plotted versus the ordered list of terms.

associated genes. These transcription factors represent potential upstream regulators in bladder carcinoma pathogenesis and may serve as novel therapeutic targets for modulating the expression of key oncogenes and tumor suppressor genes.

Metabolite Association Analysis (Table 2)

The HMDB_Metabolites analysis revealed significant associations between bladder carcinoma genes and various metabolites, particularly phosphatidylinositol derivatives (Table 2). The most significantly associated metabolite was C11H19O13P (HMDB06953), with an adjusted p-value of 0.00023 and an odds ratio of 92.34, with three genes (*PIK3CA, PTEN, PIK3CG*) showing significant association. Other significantly associated metabolites included various phosphatidylinositol species such as PI(16:0/16:1(9Z)) and PI(16:0/18:1(9Z)).

The consistent association with phosphatidylinositol derivatives highlights the importance of the PI3K pathway in bladder carcinoma. This pathway is known to regulate cell growth, proliferation, survival, and motility, and its dysregulation is a common feature in many cancer types, including bladder carcinoma.

Drug Interaction Analysis (Table 3)

The DrugMatrix analysis identified several drugs that significantly modulate the expression of bladder carcinoma-associated genes (Table 3). Sertraline (23 µM) showed significant downregulation of five genes (*GSTM1, CDH1, AKT1, TTN, VEGFA*) with an adjusted p-value of 0.07 and an odds ratio of 13.34. Other drugs with significant gene expression modulation included valproic acid, stavudine, hydroxyurea, amoxicillin, pentobarbital, phenylhydrazine, chlortetracycline, and colistin.

The diverse range of drugs identified in this analysis, including antidepressants (sertraline), anticonvulsants (valproic acid), and antibiotics (amoxicillin, chlortetracycline), suggests potential off-target effects that could be exploited for bladder carcinoma treatment. Particularly interesting is the downregulation of genes like *AKT1*, *VEGFA*, and *EGFR*, which are key players in cancer signalling pathways and established therapeutic targets.

Table 2. Shown is a table for HMDB_Metabolites

Term	Overlap	p-value	Adjusted p-value	Old p-value	Old adjusted p-value	Odds ratio	Combined score	Genes
C11H19O13P (HMDB06953)	3/27	0.000008693467	0.0002323831	0	0	92.34259	1,076.0626	*PIK3CA;PTEN;PIK3CG*
1,2-dihexadecanoyl-sn-glycero-3-phospho-(1'-myo-inositol) (HMDB09778)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*
PI(16:0/16:1(9Z)) (HMDB09779)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*
C41H75O13P (HMDB09780)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*
PI(16:0/18:0) (HMDB09781)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*
PI(16:0/18:1(11Z)) (HMDB09782)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*
PI(16:0/18:1(9Z)) (HMDB09783)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*
PI(16:0/18:2(9Z,12Z)) (HMDB09784)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*
PI(16:0/20:0) (HMDB09785)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*
PI(16:0/20:2(11Z,14Z)) (HMDB09786)	3/77	0.000206692166	0.0002323831	0	0	29.87387	253.4583	*PIK3CA;PTEN;PIK3CG*

Table 3. The table presents DrugMatrix

Term	Overlap	p-value	Adjusted p-value	Old p-value	Old adjusted p-value	Odds ratio	Combined score	Genes
Sertraline-23 uM in DMSO-Rat-Primary rat hepatocytes-0.67d-dn	5/300	0.00007693805	0.07015019	0	0	13.33898	126.35365	*GSTM1;CDH1;AKT1; TTN;VEGFA*
Valproic Acid-1500 mg/kg in Water-Rat-Liver-3d-dn	5/307	0.00008577889	0.07015019	0	0	13.02517	121.96423	*GSTM1;CDH1;AKT1; EGFR;TTN*
Stavudine-58 mg/kg in Water-Rat-Liver-0.25d-dn	4/215	0.00028579270	0.07015019	0	0	14.40685	117.56344	*GSTM1;NUMA1; CDH1;TTN*
Hydroxyurea-400 mg/kg in Saline-Rat-Liver-1d-dn	4/221	0.00031731410	0.07015019	0	0	14.00425	112.81293	*GSTM1;CDH1;EGFR;V EGFA*
Amoxicillin-1100 mg/kg in Water-Rat-Liver-3d-dn	4/267	0.00064750804	0.07015019	0	0	11.52793	84.64245	*GSTM1;CDH1;EGFR; VEGFA*
Pentobarbital-20 mg/kg in Water-Rat-Liver-1d-dn	4/267	0.00064750804	0.07015019	0	0	11.52793	84.64245	*CDH1;EGFR;TTN; VEGFA*
Hydroxyurea-400 mg/kg in Saline-Rat-Liver-3d-dn	4/269	0.00066585467	0.07015019	0	0	11.43977	83.67549	*GSTM1;CDH1;EGFR; VEGFA*
Phenylhydrazine-78 mg/kg in Water-Rat-Liver-0.25d-dn	4/272	0.00069406357	0.07015019	0	0	11.30999	82.25695	*CDH1;EGFR;TTN; VEGFA*
Chlortetracycline-1500 mg/kg in CMC-Rat-Liver-5d-dn	4/273	0.00070365233	0.07015019	0	0	11.26737	81.79240	*GSTM1;CDH1;TTN; VEGFA*
Colistin-121 mg/kg in Water-Rat-Liver-5d-up	4/273	0.00070365233	0.07015019	0	0	11.26737	81.79240	*GSTM1;CDH1;EGFR; TTN*

DISCUSSION

This comprehensive analysis of bladder carcinoma-associated genes has provided valuable insights into the molecular underpinnings of this malignancy, revealing complex networks of genes, pathways, and potential therapeutic targets. The findings highlight the multifaceted nature of bladder carcinoma pathogenesis and offer several directions for future research and therapeutic development. The GO analysis revealed significant enrichment of biological processes related to cell proliferation, signal transduction, and response to external stimuli, consistent with the hallmarks of cancer (21). The involvement of these processes underscores the dysregulation of fundamental cellular functions in bladder carcinoma, contributing to uncontrolled growth, evasion of apoptosis, and altered response to environmental cues. Similarly, the enrichment of cellular components across various compartments, including membranes, cytoplasm, and nucleus, reflects the widespread impact of bladder carcinoma-associated genes on cellular architecture and function. These findings align with previous studies that have demonstrated the complex cellular alterations accompanying bladder carcinoma development and progression (22). One of the most striking observations from our pathway analysis is the significant overlap between bladder carcinoma and other cancer types, particularly head and neck squamous cell carcinoma, pleural mesothelioma, and endometrial cancer. This overlap suggests common oncogenic mechanisms that transcend tissue-specific boundaries, potentially offering opportunities for therapeutic approaches with broader applicability across multiple cancer types (23). Key genes implicated across numerous pathways include TP53, CDKN2A, PTEN, EGFR, PIK3CA, and HRas proto-oncogene, GTPase which represent central nodes in the oncogenic network and promising targets for therapeutic intervention. The involvement of these genes aligns with previous studies highlighting their crucial roles in bladder carcinoma pathogenesis (24). The significant enrichment of DNA damage response pathways, particularly the ataxia-telangiectasia mutated-dependent pathway, emphasizes the importance of genomic instability in bladder carcinoma development. This finding is consistent with the high mutation burden observed in bladder carcinoma, especially in patients with a history of tobacco exposure or occupational exposure to carcinogens (25). Targeting DNA damage response mechanisms represents a promising therapeutic strategy, as evidenced by the emerging role of poly(ADP-ribose) polymerase (PARP) inhibitors in various cancers with defects in DNA repair pathways (26). The metabolite association analysis revealed a strong link between bladder carcinoma genes and phosphatidylinositol derivatives, highlighting the central role of the PI3K pathway in this malignancy. The PI3K/AKT/mammalian target of rapamycin pathway is a key regulator of cell growth, proliferation, and survival, and its dysregulation is a common feature in many cancer types, including bladder carcinoma (27). The consistent association with phosphatidylinositol metabolites provides a metabolic perspective on this pathway and suggests potential opportunities for metabolic targeting in bladder carcinoma treatment. The drug interaction analysis identified several compounds that modulate the expression of bladder carcinoma-associated genes, offering insights into potential therapeutic repurposing opportunities. Particularly intriguing is the significant downregulation of cancer-related genes by drugs such as sertraline, valproic acid, and hydroxyurea, which are not conventionally used for cancer treatment. Sertraline, a selective serotonin reuptake inhibitor commonly used for depression and anxiety disorders, has shown anticancer properties in various preclinical studies, including inhibition of cell proliferation, induction of apoptosis, and modulation of signaling pathways (28). Similarly, valproic acid, an anticonvulsant and mood stabilizer, has demonstrated histone deacetylase inhibitor activity, which can alter gene expression patterns and exert anticancer effects (29). These findings suggest potential opportunities for drug repurposing in bladder carcinoma treatment, which could accelerate therapeutic development by leveraging existing drugs with established safety profiles. The transcription factor analysis identified key regulators of bladder carcinoma-associated genes, providing insights into the upstream control mechanisms governing gene expression in this malignancy. Targeting these transcription factors could offer a novel approach to modulating the expression of multiple oncogenes or tumor suppressor genes simultaneously, potentially enhancing therapeutic efficacy (30). This approach is particularly relevant in the context of bladder carcinoma, where complex genetic alterations often necessitate targeting multiple pathways for effective treatment. Our findings also have implications for personalized medicine approaches in bladder carcinoma management. The diverse molecular alterations observed in this study suggest that bladder carcinoma is not a homogeneous disease but rather a collection of molecularly distinct entities that may require tailored therapeutic strategies. This concept aligns with the emerging paradigm of molecular subtyping in bladder carcinoma, which has identified distinct subtypes with different clinical behaviors and treatment responses (31). Integrating the findings from our study with clinical and pathological features could facilitate the development of more precise prognostic and predictive models for bladder carcinoma patients. Despite these valuable insights, our study has several limitations that should be acknowledged. The analysis was based on data from the DisGeNET database, which, while comprehensive, may

not capture all relevant genes associated with bladder carcinoma. Additionally, the enrichment analyses provide statistical associations but do not necessarily establish causal relationships between genes, pathways, and bladder carcinoma. Furthermore, the drug interaction analysis was conducted using preclinical data, and the clinical relevance of these findings requires validation in appropriate models and eventually in clinical trials. Future studies should focus on validating these findings in larger cohorts of bladder carcinoma patients and exploring the functional significance of the identified genes and pathways in experimental models. Additionally, integrating multi-omics data, including genomics, transcriptomics, proteomics, and metabolomics, could provide a more comprehensive understanding of bladder carcinoma biology and facilitate the development of more effective therapeutic strategies (32).

Study Limitations

While the analysis that focused on the top 30 genes from the DisGeNET database provides valuable insights, it may not fully capture the molecular heterogeneity of bladder carcinoma. Additionally, drug interaction findings, particularly, those involving compounds like sertraline and valproic acid, are based on data from non-bladder tissues or animal models, necessitating validation in bladder-specific systems. The study also does not explore how the identified genes relate to established molecular subtypes of bladder cancer (e.g., luminal and basal), which limits the context for subtype-specific interpretation and therapeutic relevance.

CONCLUSION

This comprehensive bioinformatic analysis of bladder carcinoma-associated genes has revealed intricate networks of biological processes, signaling pathways, and potential therapeutic targets. The significant overlap with other cancer types suggests common oncogenic mechanisms that could be exploited for therapeutic intervention. Key genes including *CDKN2A*, *PTEN*, *EGFR*, *PIK3CA*, *HRAS*, *FGFR3*, and *TP53* emerged as central nodes in the bladder carcinoma gene network, representing promising targets for therapeutic development.

The metabolite association analysis highlighted the importance of the PI3K pathway in bladder carcinoma, offering a metabolic perspective on this key signaling network. The drug interaction analysis identified several compounds with potential off-target effects on bladder carcinoma-associated genes, suggesting opportunities for drug repurposing in bladder carcinoma treatment.

These findings contribute to a deeper understanding of bladder carcinoma biology and provide a foundation for future research aimed at developing more effective therapeutic strategies. Personalized medicine approaches that consider the molecular heterogeneity of bladder carcinoma may offer improved outcomes for patients with this challenging malignancy.

Ethics

Ethics Committee Approval: This is a bioinformatics manuscript involving secondary analysis. Henceforth, no ethical approval is required. However, we have followed the guidelines of the Declaration of Helsinki. Further, all the data sources were mentioned in the methodology.

Informed Consent: This study is based solely on publicly available data and does not involve human participants, animal subjects, or identifiable personal information.

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Footnotes

Author Contributions

Concept - U.S.A.; Design - U.S.A.; Data Collection or Processing - S.V.; Analysis or Interpretation - A.J.A., S.V.; Literature Search - A.J.A., S.V., U.S.A.; Writing - U.S.A.

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