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Investigating the Role of Escherichia coli Infection in the Pathogenesis of Bladder Cancer: A Comparative Genomics Interaction

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Abstract

Background: Bladder cancer (BC) is the 10th most common cancer worldwide. Microorganisms, including bacteria, may contribute to urological tumor development. Escherichia coli, responsible for 70% of urinary tract infections, has been linked to BC progression. This study investigated the potential role of Escherichia coli in BC pathogenesis using a bioinformatics approach.

Method: This computational study used human gene expression and host-pathogen protein interaction data related to BC. Gene expression data were sourced from The Cancer Genome Atlas, while host-pathogen interactions were obtained from Host-Pathogen Interaction Database. Differentially expressed genes/proteins were identified, filtered based on interactions, and analyzed for their significance in BC. Differentially expressed genes/proteins were extracted using EdgeR. EdgeR primarily uses the negative binomial distribution to model count data, which is common in RNA-Seq experiments. A protein-protein interaction network was constructed to identify hub genes/proteins, and pathway enrichment analysis assessed the relevance of these genes/proteins in BC.

Results: We identified 118 interactions between differentially expressed genes/proteins of the human host and E. coli proteins in BC. Network analysis highlighted E. coli genes O52302 and Q8XAJ5 as having the most interactions with human genes. Additionally, FADD, RIPK1, TRADD, LRRK2, and CDC42 were significant in the interaction network. Pathway enrichment analysis indicated involvement in pathways such as TNF Signaling and Extrinsic Apoptotic Signaling.

Conclusion: This study identifies key hub genes and pathways in BC potentially influenced by E. coli, highlighting the need for in vitro and in vivo validation of these findings through comparative genomics interactions.

Keywords: Cancer of Bladder, Escherichia coli, Computational biology, Gene expression

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Introduction

Bladder cancer (BC), a prevalent malignancy worldwide, with 573,278 newly diagnosed cases and an estimated 212,536 deaths, is the 10th most common cancer globally. In 2022, the United States alone reported approximately 81,180 new cases and 17,100 deaths related to BC.[1,2] Among BC patients, approximately 75% present with non-muscle invasive disease (NMIBC), confined to the mucosa or submucosa, whereas 25% have muscle-invasive disease (MIBC).[3] BC is a prevalent urinary tract tumor in clinical practice, but there remains a need for deeper insights into its occurrence, diagnosis, and treatment. Apart from genetic factors, environmental influences, such as smoking and occupational exposure, also play a significant role in BC development.[4,5] Interestingly, these environmental factors can impact changes in the microbiota.[6] The microbiota, a diverse group of microorganisms that reside in the body, has a significant impact on cancer development through mechanisms like inflammation, carcinogen metabolism, and immune modulation.[7] Chronic inflammation, caused by dysbiosis, is commonly associated with cancer risk, can lead to chronic inflammation, which creates a tumor-promoting environment by releasing pro-inflammatory cytokines and reactive oxygen species that damage DNA and promote cellular proliferation. Certain bacteria, such as Fusobacterium nucleatum in colorectal cancer and Helicobacter pylori in gastric cancer, are directly associated with tumorigenesis.[8] The microbiota has the potential to influence the immune response to tumors, which could have an impact on cancer progression and treatment effectiveness.[9] Additionally, certain microbes can modulate the immune response, either enhancing anti-tumor immunity or suppressing it, thereby affecting the body's ability to detect and eliminate cancer cells. Collectively, these mechanisms illustrate the multifaceted role of microorganisms in cancer biology, highlighting their potential impact on tumorigenesis and progression.[10] The full understanding of these complex interactions and their implications for cancer prevention and therapy requires ongoing research. Evidence pointed out that twenty percent of malignant tumor tissues had microbial infiltration. This suggested that BC tissues might have been contaminated by bacteria. A contributing factor or cofactor in the development of urological tumors may be bacteria, fungi, or viruses in the genitourinary tract. The microbiota might be crucial in the treatment of BC.[11] For instance, seventy percent of urinary tract infections originate from infection with Escherichia coli (E. coli). In a study focusing on E. coli, it was demonstrated that this bacterium can facilitate the advancement of BC T24 cells by inducing epithelial-mesenchymal transformation and metabolic reprogramming.[12]

Another study was conducted to assess the role of E. coli infection in BC development. They examined histopathological changes in bladder tissue and measured nuclear factor kappa p65 (NF-κBp65), Bcl-2, and interleukin 6 (IL-6) levels in different groups of male albino rats. The results indicated that E. coli infection alone could cause some histopathological changes in the bladder, and when combined with a nitrosamine precursor, it showed the highest incidence of urinary bladder lesions, suggesting a significant additive and synergistic role of E. coli in bladder carcinogenesis.[13]

The association between E. coli infection and urinary tract cancers, with a particular focus on BC, remains an area of active research and ongoing debate in the scientific community. While there have been indications of a possible link, the exact mechanisms and extent of E. coli's involvement in promoting or influencing cancer development in the urinary tract have not been entirely elucidated.

A bioinformatics approach is particularly suitable for investigating the role of E. coli infection in the pathogenesis of bladder cancer due to its numerous advantages in handling complex biological data. Bioinformatics enables the integration and analysis of large datasets generated from high-throughput techniques, allowing to uncover patterns and correlations that may not be evident through traditional methods.[14] Also, bioinformatics tools facilitate rapid data

processing and analysis, significantly reducing the time required to derive meaningful insights from experimental data. Bioinformatics approaches are often more cost-effective compared with extensive laboratory experiments, as they minimize the need for expensive reagents and extensive biological replicates.[15]

In this study, we applied a bioinformatics approach to explore potential correlations between cancer mechanisms and the presence of specific microbial species. Through this comprehensive and innovative approach, we aimed to advance our knowledge of the potential role of E. coli in BC pathogenesis. This study aimed to investigate the potential role of E. coli in bladder cancer pathogenesis through a bioinformatics approach.

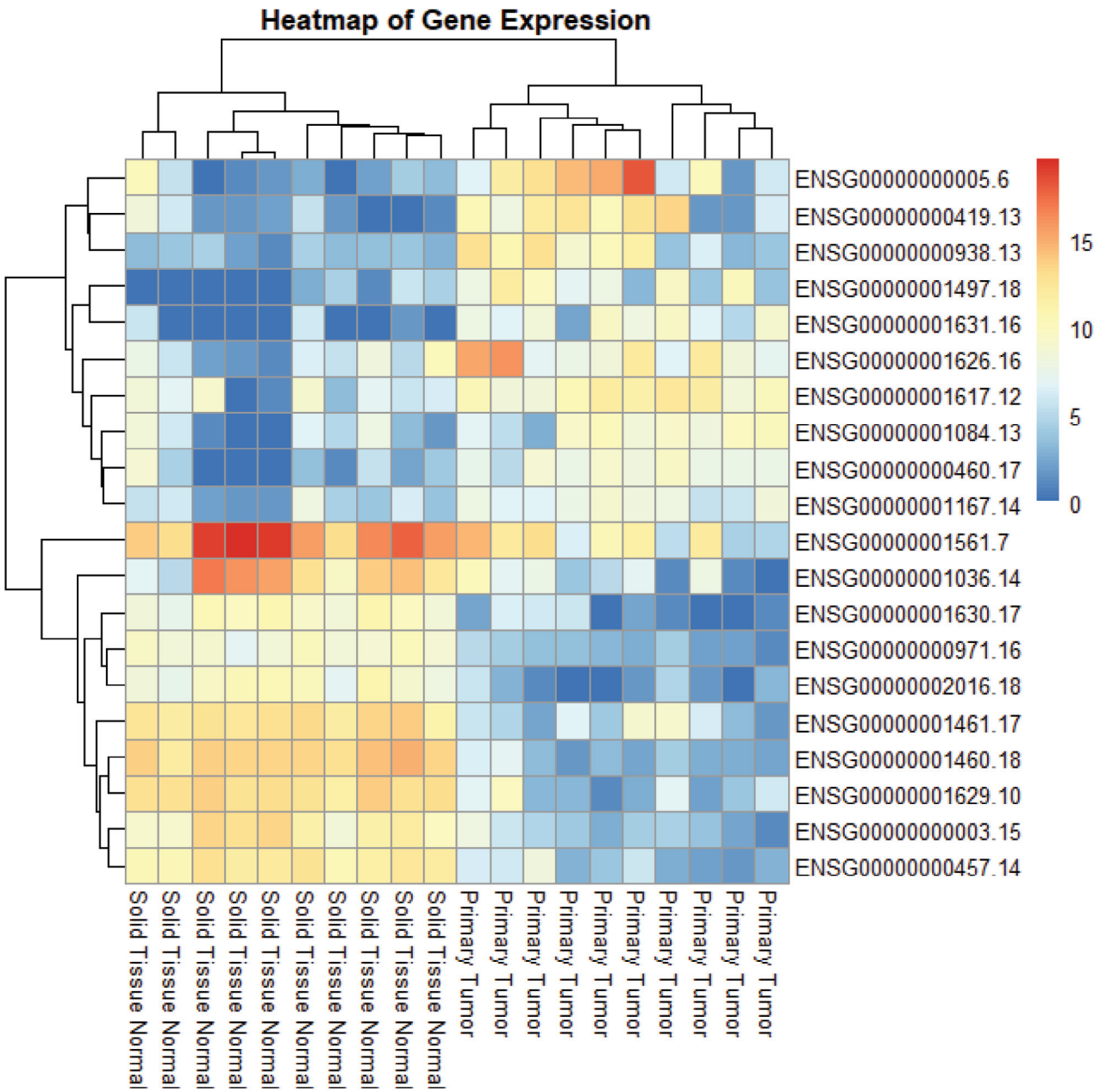

Figure 1. This figure depicts the Heatmap of selected gene expression profiles in BC.
BC: Bladder cancer

Table 1. Effective and candidate genes in the interaction between human and pathogen

Name	Degree	Stress	Topological coefficient
O52302	10	90	0
Q8XAJ5	7	42	0
B7UM99 TIR	6	208	0.259259
Q7DB77 TIR	6	208	0.259259
Q8XA11	5	20	0

Material and Methods

Data selection

The present study was a computational analysis using transcriptomics data by RNA-seq data. The dataset used in this study consists of two sets of data, one related to the host (human) and the other to the pathogen. The human host gene expression data associated with BC is obtained from The Cancer Genome Atlas (TCGA) database, accessible at https://portal.gdc.cancer.gov/. On the other hand, for the collection of E. coli-associated host proteins, the host-pathogen-protein-protein interactions (HP-PPIs) of E. coli were retrieved from the Host-Pathogen Interaction Database (HPIDB v.3.0), which is a publicly available biological repository known for collecting experimentally verified HP-PPIs. The database can be accessed at https://hpidb.igbb.msstate.edu/.

Data analysis

The gene expression data for BC was obtained from the TCGA database.[16] Using the R package TCGAbiolinks (Version Release 3.20), we were able to freely download all the BC data along with the corresponding clinical information.[17] Following the data acquisition, we performed normalization to ensure that the gene expression levels were comparable across samples. This involved using the DESeq2 package for variance stabilization and normalization of raw counts, which helps to account for differences in sequencing depth and other technical variations. After normalization, we applied log2 transformation to stabilize the variance and facilitate downstream analyses. A total of 431 bladder tissue samples were included in the study, comprising 412 BC samples and 19 normal samples. Differentially expressed genes/proteins (DEGs/DEPs) were extracted using the EdgeR library (Version 4.0) in the R programming language, as well as the DGEList function.[18] EdgeR primarily uses the negative binomial distribution to model count data, which is common in RNA-Seq experiments. Finally, DEGs were filtered based on an absolute log fold change greater than 2 and an adjusted p-value of less than 0.05. Only the genes that encode proteins were retained.

Interactions between DEGs/DEPs of host and E. coli proteins

As mentioned in the previous step, HP-PPIs of E. coli were obtained from HPIDB.[19] In this step, DEGs/DEPs were filtered based on the HP-PPIs of E. coli to retain only common interactions between the DEGs/DEPs of the human host and E. coli proteins in the context of BC. The interactions of genes/proteins between the human host and E. coli in BC were imported to Cytoscape and visualized.[20] Then, this network was analyzed using the NetworkAnalyzer plugin, employing metrics such as Degree, Stress, and Topological Coefficient to assess the significance and roles of specific genes/proteins in the context of the interactions between the human host and E. coli in BC.

Interactions of DEGs/DEPs in host

The DEGs/DEPs of the host that interact with E. coli proteins were considered for this step. At first, the protein-protein interactions (PPIs) network was created using both STRING (Version v10) and Cytoscape (Version 3.7.0) software.[21] The parameters in STRING were maintained with a minimum required interaction score of 0.4. Then, the network underwent analysis using the NetworkAnalyzer plugin, where metrics such as degree, stress, and topological coefficient were employed to determine the hub genes/proteins concerning the human host in BC.

Pathway enrichment analysis of DEGs/DEPs in host

KEGG pathway as a valuable database for systematically analyzing high-level genome pathways related to genes. To perform pathway enrichment analysis for DEGs/DEPs, Enrichr software was used,[22] and a statistical significance threshold of adjusted p-value less than 0.05 was applied.

Results

Data analysis

After downloading and processing the gene expression data for BC from TCGA, DEGs/DEPs were extracted from the data. Based on ourresults, a total of 2141 DEGs/DEPs were identified, comprising 650 up-regulated and 1491 down-regulated genes/proteins. To visualize the expression patterns of a subset of 20 genes was selected for analysis across 20 samples, a heatmap was generated, highlighting the differences in expression levels. This heatmap is depicted in (Figure 1), offering a clear representation of the expression profiles of these genes in the context of BC.

Interactions between DEGs/DEPs of host and E. coli proteins

After identifying DEGs/DEPs, HP-PPIs of E. coli were retrieved from HPIDB. The DEGs/DEPs were subjected to filtering, based on the HP-PPIs of E. coli. A total of 118 unique interactions were identified as shared between the DEGs/DEPs of the human host and E. coli proteins within the context of BC. These interactions were subjected to Cytoscape for visualization (Figure 2). Next, this network was analyzed using NetworkAna-

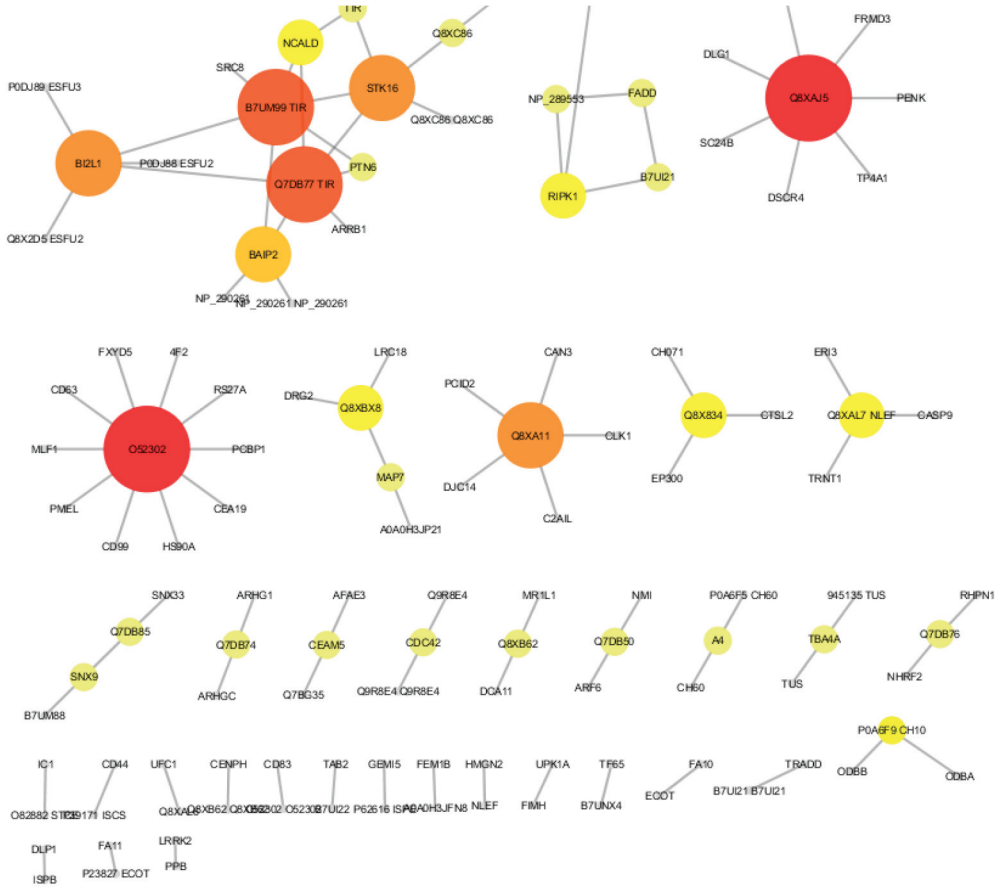

Figure 2. Interaction network between host and pathogen genes. In this figure, colors ranging from red to yellow indicate the degree of the nodes, ranging from high to low, in terms of genes.

Table 2. Key genes obtained from human interaction network analysis

Name	Degree	Stress	Topological coefficient
FADD	7	182	0.5
RIPK1	6	130	0.537037
TRADD	6	130	0.537037
LRRK2	5	520	0.4
CDC42	5	434	0.266667

lyzer. According to results, O52302 and Q8XAJ5 genes in E.coli exhibit the highest degree and most interactions with human genes (Table 1).

Interactions of DEGs/DEPs in host

As mentioned in the previous section, a total of 2141 DEGs/DEPs were identified in the host for BC. These DEGs/DEPs were subjected to STRING to assess interactions between proteins. The extracted PPIs were examined using NetworkAnalyzer. As revealed by the analysis, FADD, RIPK1, TRADD, LRRK2, and CDC42 genes play a more significant role in the network in terms of their degree of interaction with other genes compared with others (Figure 3 and Table 2).

Pathway enrichment analysis of DEGs/DEPs in host

To perform pathway enrichment analysis for DEGs/DEPs, Enrichr tool was used. The top five pathway terms are TNF Signaling, Defective RIPK1-mediated Regulated Necrosis, TNFR1-induced NFkappaB Signaling Pathway, Caspase Activation via Extrinsic Apoptotic Signaling Pathway, and Regulation of TNFR1 Signaling, respectively (Table 3).

Discussion

In this study, we identified 118 interactions between differentially expressed genes/proteins of the human host and E. coli proteins in BC. Also, FADD, RIPK1, TRADD, LRRK2, and CDC42 were significant in the interaction network. The study aimed to comprehensively investigate and innovate our understanding of the potential role of E. coli in the pathogenesis of BC using bioinformatics approach.

BC is a common and widespread malignancy, ranking as the 10th most prevalent cancer worldwide.[23] Most patients with BC (approximately 75%) are diagnosed NMIBC, while the remaining 25% have MIBC.[24] Apart from genetic factors, environmental influences, like smoking and occupational exposure, also significantly contribute to BC development.[25] Bacteria, fungi, or viruses present in the genitourinary tract might act as contributing factors or cofactors in the development of urological tumors.[26] Notably, E. coli is responsible for seventy percent of urinary tract infections, and has been implicated in the progression and advancement of BC.[27]

In this study, the TNF signaling pathway emerges as a key link between chronic inflammation and cancer, being one of the most well-known factors associated with tumor necrosis. Tumor necrosis factors, a group of cytokines, play a role in inducing cell apoptosis. By activating the TNF inflammatory pathway, all the genes and downstream pathways involved in the process of inflammation, cell death, and ultimately cancer, are triggered, providing direct evidence of the connection between this infection and cancer. TNF-α can initiate various pathways, leading to apoptosis, cell survival, or inflammation. The tumor necrosis factor induces apoptosis by binding caspase-8 to FADD, while also enhancing inflammation and survival through TRAF2, JNK-dependent kinase cascade, MEKK kinase cascade, and NF-kB activation via the RIP mediator.[28,29]

The subsequent key pathway is the Defective RIPK1-mediated Regulated Necrosis, which belongs to a family of protein kinase receptor interaction (RIP) kinases comprised of seven serine/threonine kinases that play a crucial role in cell survival and cell death signaling. RIP1 and RIP3 are well-known for their vital roles in necroptosis, programmed necrosis, and inflammatory cell death. Dysregulation of RIP kinases contributes to inflammatory diseases, neurological disorders, and cancer. In cancerous cells, changes in RIP kinases at different levels

have been observed, acting as factors for tumor progression and metastasis, evasion of anti-tumor immune responses, and therapeutic resistance. However, RIP kinases exhibit dual functionality as either tumor protectors or tumor suppressors, contingent on the tumor types and cellular contexts involved. Clinical trials for inflammatory diseases have mainly assessed therapeutic agents that target RIP kinases.[30,31]

Another key pathway is the TNFR1-induced NF-κB signaling pathway is a crucial inflammatory pathway activated by TNF through its receptor TNFR1. This pathway plays a significant role in cell survival and cell death signaling. Upon TNF binding to TNFR1, a trimeric complex is formed, leading to the recruitment of adaptor proteins TRADD and RIP1, followed by the formation of the membrane-bound receptor complex I involving cIAP1/2 and TRAF2. In the context of cancer, alterations in the TNFR1-induced NF-κB signaling pathway have been observed, impacting tumor progression, metastasis, and evasion of anti-tumor immune responses. Dysregulation of RIP kinases, such as RIP1 and RIP3, which are key components of this pathway, can contribute to cancer development and therapeutic resistance. The dysregulated expression of these kinases has been observed in various cancer types, including urinary cancers, suggesting their potential as therapeutic targets.[32,33]

In this study, two genes, O52302 and Q8XAJ5 in E. coli, showed the highest degree of interaction with human genes. O52302 (EspZ) is a type III effector protein secreted by pathogenic E. coli strains, including enterohaemorrhagic and enteropathogenic E. coli (EHEC and EPEC). The researchers demonstrated that EspZ interacts with host CD98, a transmembrane protein that is involved in amino acid transport and cell signaling. This finding suggests that the interaction between EspZ and CD98 is important for host cell pro-survival signaling during E. coli infection, which could potentially be a factor in cancer development.[34]

On the other hand, Q8XAJ5 (nleA/espI) is involved in the type III secretion system. NleA is a bacterial virulence factor produced by pathogenic bacteria such as EPEC and EHEC. It

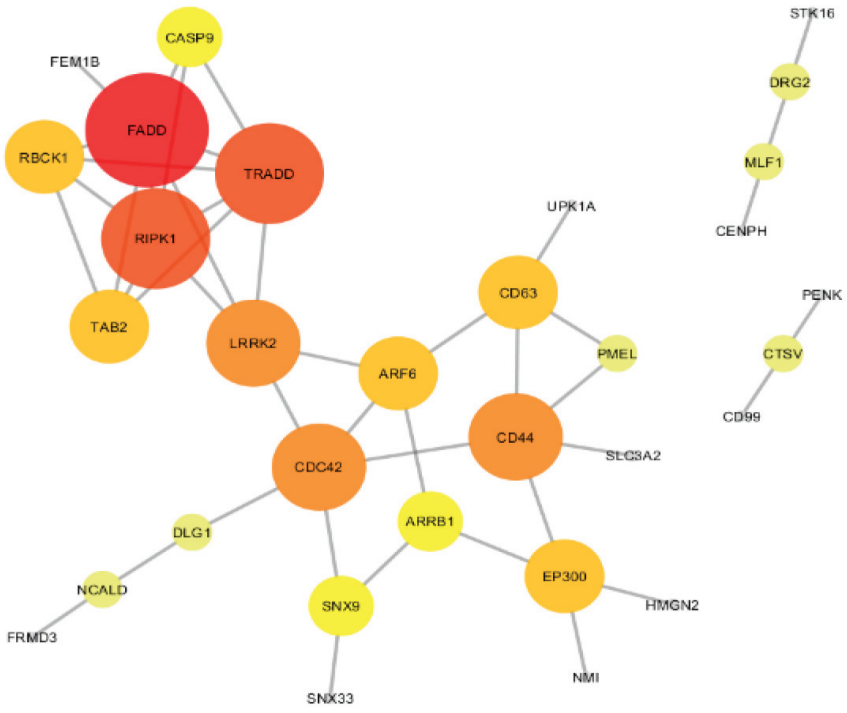

Figure 3. Network analysis on human genes. In this figure, colors ranging from red to yellow indicate the degree of the nodes, ranging from high to low, in terms of genes.

Table 3. Top 5 enriched biological pathways

Term	Adjusted *P*-value	Genes
TNF signaling R-HSA-75893	2.30E-06	TRADD;RIPK1;TAB2;FADD;RBCK1
Defective RIPK1-mediated regulated necrosis R-HSA-9693928	5.84E-06	TRADD;RIPK1;FADD
TNFR1-induced NFkappaB signaling pathway R-HSA-5357956	5.98E-06	TRADD;RIPK1;TAB2;RBCK1
Caspase activation via extrinsic apoptotic signaling pathway R-HSA-5357769	5.98E-06	CASP9;TRADD;RIPK1;FADD
Regulation of TNFR1 signaling R-HSA-5357905	1.66E-05	TRADD;RIPK1;FADD;RBCK1

uses a type III secretion system to transfer effective proteins into the host cytosol. These effectors manipulate host pathways to improve bacterial replication and survival. Studies have shown that this protein inhibits the secretion of cellular proteins by disrupting the function of the mammalian COPII complex. Moreover, COPII is a vital protein complex responsible for transporting newly synthesized proteins and lipids from the endoplasmic reticulum (ER) to the Golgi apparatus in cells for secretion. Disruption and mutations in COPII can lead to human diseases and cancer.[35]

This study for the first time, investigated the relation between E. coli and BC by bioinformatic approach and shown hot spot pathways and genes in patients with BC and E. coli infection. Further in vitro and in vivo studies and clinical trials are warranted to predict and prevent BC in patients with E. coli infection. While bioinformatics offers powerful tools for analyzing and interpreting large datasets, it is essential to acknowledge its limitations. One significant limitation is the reliance on in silico data, which may not always accurately reflect biological realities.

Computational models and algorithms can provide insights into potential mechanisms and associations. Additionally, findings derived from bioinformatics analyses require rigorous experimental validation to confirm their relevance and applicability.

In order to confirm our findings about the role of E. coli infection in the pathogenesis of bladder cancer, future research should prioritize both in vitro and in vivo studies. In vitro experiments can be performed to examine how E. coli infection affects host cell signaling pathways. The assessment may involve examining changes in essential signaling pathways, such as inflammation, apoptosis, and cell proliferation, in bladder epithelial cells exposed to E. coli. In addition, evaluating the physiological relevance of these findings requires in vivo studies using appropriate animal models.

Conclusion

The present study investigated the association between E. coli infection and BC development based on a comparative genomics interaction. It specifically focused on identifying hub genes and key pathways in BC that may be influenced by the potential role of E. coli in the pathogenesis of BC. The results of this study are based on bioinformatics methods and computational tools; therefore, this study requires in vitro and in vivo evaluation to confirm the findings.

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Authors' Contribution

MF, and MA participated in data curation, formal analysis, Methodology, Visualization, and writing- original draft preparation. MR, and MA contributed to conceptualization, review and editing. MR contributed to the supervision of the study, data validation, editing of the manuscript. All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest

None declared.

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