

Distinct protein-protein interactions define papillary and non-papillary urothelial carcinoma architectures

Charissa Chou, Yigit Baykara, Sean Hacking, Ali Amin, Liang Cheng, Alper Uzun, Ece Dilber Gamsiz Uzun

Submitted to: JMIR Bioinformatics and Biotechnology
on: April 29, 2025

Disclaimer: © The authors. All rights reserved. This is a privileged document currently under peer-review/community review. Authors have provided JMIR Publications with an exclusive license to publish this preprint on its website for review purposes only. While the final peer-reviewed paper may be licensed under a CC BY license on publication, at this stage authors and publisher expressly prohibit redistribution of this draft paper other than for review purposes.

Table of Contents

Original Manuscript.....	5
Supplementary Files.....	20

Preprint
JMIR Publications

Distinct protein-protein interactions define papillary and non-papillary urothelial carcinoma architectures

Charissa Chou¹ BS; Yigit Baykara^{2, 1} MD; Sean Hacking^{2, 1, 3} MD; Ali Amin^{2, 1} MD; Liang Cheng^{2, 1} MD; Alper Uzun^{2, 4, 5, 6*} MS, PhD; Ece Dilber Gamsiz Uzun^{1, 2, 5, 7, 8*} MS, PhD

¹ Department of Pathology and Laboratory Medicine Rhode Island Hospital Providence US

² Department of Pathology and Laboratory Medicine Alpert Medical School Brown University Providence US

³ Department of Pathology Grossman School of Medicine New York University New York US

⁴ Brown Center for Clinical Cancer Informatics and Data Science Providence US

⁵ Legorreta Cancer Center Brown University Providence US

⁶ Department of Pediatrics Brown University Providence US

⁷ Brown Center for Clinical Cancer Informatics and Data Science (CCIDS) Providence US

⁸ Center for Computational Molecular Biology (CCMB) Brown University Providence US

*these authors contributed equally

Corresponding Author:

Ece Dilber Gamsiz Uzun MS, PhD

Department of Pathology and Laboratory Medicine
Rhode Island Hospital
593 Eddy Street
Providence
US

Abstract

Background: Bladder cancer is a disease with complex perturbations in gene networks and heterogeneous in terms of histology, mutations, and prognosis. Advances in high-throughput sequencing technologies, genome-wide association studies, and bioinformatics methods have revealed greater insights into the pathogenesis of complex diseases. Network biology-based approaches have been used to demonstrate the complex physical or functional interactions between molecules which can lead to potential drug targets.

Objective: There is a need to better understand gene networks and protein-protein interactions (PPI) specific to urothelial carcinoma.

Methods: We performed a multi-sample PPI study comparing two urothelial carcinoma architectures: papillary and non-papillary. We used a novel PPI analysis tool, Proteinarium to identify clusters of patients with shared PPI networks in each architecture. The feature of this tool is to analyze the PPI networks of patients and visualize them in clusters based on their network similarities from any genomic data including Next Generation Sequencing (NGS).

Results: We observed distinct networks for the papillary and non-papillary groups. Proteins unique to the papillary urothelial carcinoma detected in two separate datasets included UBA52, RPS27A, UBR4, CUL1, UBE2K, and CDC5L. Proteins found in the non-papillary urothelial carcinoma specific PPI network were GNB1, UBC, RHOA, FPR2, GNGT1, PIK3CA, PIK3CG, HSP90AA1, SLC11A1, CCT7, ARHGEF1, PAK1, PAK2, PSMA7, and TRIO.

Conclusions: We identified distinct PPI networks specific to papillary and non-papillary urothelial carcinomas presenting unique molecular entities. Clinical Trial: N/A

(JMIR Preprints 29/04/2025:76736)

DOI: <https://doi.org/10.2196/preprints.76736>

Preprint Settings

1) Would you like to publish your submitted manuscript as preprint?

✓ Please make my preprint PDF available to anyone at any time (recommended).

Please make my preprint PDF available only to logged-in users; I understand that my title and abstract will remain visible to all users.
Only make the preprint title and abstract visible.

No, I do not wish to publish my submitted manuscript as a preprint.

2) If accepted for publication in a JMIR journal, would you like the PDF to be visible to the public?

✓ **Yes, please make my accepted manuscript PDF available to anyone at any time (Recommended).**

Yes, but please make my accepted manuscript PDF available only to logged-in users; I understand that the title and abstract will remain visible to all users.

Yes, but only make the title and abstract visible (see Important note, above). I understand that if I later pay to participate in <http://www.jmir.org/>

No. Please do not make my accepted manuscript PDF available to anyone. I understand that if I later pay to participate in <https://www.jmir.org/>



Original Manuscript

Distinct protein-protein interactions define papillary and non-papillary urothelial carcinoma architectures

Charissa Chou^{1*}, Yigit Baykara^{1,2*}, Sean Hacking^{1,2,3}, Ali Amin^{1,2}, Liang Cheng^{1,2,4}, Alper Uzun^{2,4, 5,6,7,#}, Ece D. Gamsiz Uzun^{1,2,4,5,6,7#}.

1. Department of Pathology and Laboratory Medicine, Rhode Island Hospital and Brown University Health, Providence, RI, United States of America 02903
2. Department of Pathology and Laboratory Medicine, Warren Alpert Medical School of Brown University, Providence, RI, United States of America 02903
3. Department of Pathology, NYU Grossman School of Medicine, New York, NY, United States of America 10016
4. Legorreta Cancer Center, Brown University, Providence, RI, United States of America 02903
5. Department of Pediatrics, Warren Alpert Medical School of Brown University, Providence, RI, United States of America 02903
6. Center for Computational Molecular Biology, Brown University, Providence, RI, United States of America 02912
7. Brown Center for Clinical Cancer Informatics and Data Science (CCIDS), Providence, RI, United States of America 02903

*Equal authors

#Equal corresponding authors

Corresponding Authors: Ece D. Gamsiz Uzun – dilber_gamsiz@brown.edu

Alper Uzun – alper_uzun@brown.edu

Abstract

Bladder cancer is a disease with complex perturbations in gene networks and heterogeneous in terms of histology, mutations, and prognosis. Advances in high-throughput sequencing technologies, genome-wide association studies, and bioinformatics methods have revealed greater insights into the pathogenesis of complex diseases. Network biology-based approaches have been used to demonstrate the complex physical or functional interactions between molecules which can lead to potential drug targets. There is a need to better understand gene networks and protein-protein interactions (PPI) specific to urothelial carcinoma. We performed a multi-sample PPI study comparing two urothelial carcinoma architectures: papillary and non-papillary. We used a novel PPI analysis tool, Proteinarium to identify clusters of patients with shared PPI networks in each architecture. The feature of this tool is to analyze the PPI networks of patients and visualize them in clusters based on their network similarities from any genomic data including Next Generation Sequencing (NGS). We observed distinct networks for the papillary and non-papillary groups. Proteins unique to the papillary urothelial carcinoma detected in two separate datasets included *UBA52*, *RPS27A*, *UBR4*, *CUL1*, *UBE2K*, and *CDC5L*. Proteins found in the non-papillary urothelial carcinoma specific PPI network were *GNB1*, *UBC*, *RHOA*, *FPR2*, *GNGT1*, *PIK3CA*, *PIK3CG*, *HSP90AA1*, *SLC11A1*, *CCT7*, *ARHGEF1*, *PAK1*, *PAK2*, *PSMA7*, and *TRIO*. We identified distinct PPI networks specific to papillary and non-papillary urothelial carcinomas presenting unique molecular entities.

Running Title: Protein-protein interactions in urothelial carcinoma

Key Words: Urothelial Carcinoma, Comprehensive Genomic Profiling, Proteinarium, Protein-Protein Interactions

Introduction

In 2025, there were 17420, deaths from urothelial carcinoma, which is four times more prevalent in men than women[1]. Urothelial carcinomas can be divided into papillary and non-papillary based on the architecture. Papillary urothelial carcinomas grow in slender projections with fibrovascular cores, and while most do not penetrate the deeper layers of the bladder (non-invasive papillary carcinoma), others can become invasive. Non-invasive papillary urothelial carcinoma has higher rates of recurrence than non-papillary tumors, but rarely metastasize. Non-papillary urothelial carcinomas do not grow towards the hollow center of the bladder and can be invasive or non-invasive (flat carcinoma in situ, CIS)[2]. Invasive non-papillary urothelial carcinomas can aggressively infiltrate the bladder wall, leading to metastasis in lymph nodes and invade adjacent surrounding anatomical structures such as the prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, and the abdominal wall. Previous research on the genetic architecture of bladder cancer analyzed the relative rates of mutations in specific genes using data from high throughput sequencing[3]. Previous studies have used mRNA expression profiling and whole genome comparative genomic hybridization to provide evidence that papillary and non-papillary urothelial carcinomas have distinct molecular origins and characteristics[4].

Network biology is an emerging paradigm that aims to understand the complex interactions of molecules at the cellular level[5]. Experimental studies describing biological networks demonstrate that they are not random, and in fact characterized by well-known organizing principles. By the advancement of high throughput screening methods, extensive protein-protein interaction (PPI) networks have been discovered[6]. In addition, large scale protein-protein interaction maps show that proteins involved in related phenotypes often interact physically. Moreover, it was also shown that similar phenotypes share neighboring network environments not only in model organisms but also in humans[7-10]. In this regard, by integrating high throughput omics data, network biology can be used to discover reliable molecular biomarkers for cancer identification and progression. At the genomic level, assays such as the Achilles Project screen for loss of function mutations on a genome scale for hundreds of cancer cell lines[11]. At the transcriptomic level, gene co-expression networks (GCNs) are applied to infer cell-cell interactions and potential cancer biomarkers. At the proteomic level, PPI network analysis is used for novel biomarker discovery and disease-related functional modules[12]. Applying a network biology approach to discover how cellular pathways interact provides better understanding in the genetic architecture of cancer types and subtypes. There are several studies based on transcriptomic or microarray data for bladder cancer identifying potential networks based on differentially expressed genes or co-expressed genes in clusters[13-16]. Most of the time, differentially expressed genes between patients and healthy subjects or among patients with different tumor stages are used as an input for building gene or protein interaction networks. Despite the advances in gene pathway or PPI analysis algorithms, none of the current network analysis tools are designed to identify clusters of patients based on their PPI networks by using the data from individual patient samples. In order to address this, we have developed a PPI analysis and visualization tool, Proteinarium[17]. Proteinarium is a multi-sample PPI tool that identifies clusters of samples with shared networks[17]. Proteinarium builds protein networks from high-dimension data for individual samples, measures network similarity between samples using the Jaccard distance, clusters them based on these similarities, and identifies shared networks associated with a disease phenotype. In this study, we investigated the PPI networks using Proteinarium to identify the PPI networks specific to papillary and non-papillary urothelial carcinoma.

Materials and Methods

Study Design

We studied two datasets of patients with urothelial carcinoma. The RNA-seq data from the

TCGA Cell 2017[18] and TCGA Nature 2014[19] studies were obtained from cBioPortal for Cancer Genomics[20, 21]. Only patients with mRNA z-score data were included in the analysis. Patient groups were identified based on urothelial carcinoma architecture (papillary or non-papillary), sex, patient status (alive, recurrent, or deceased), and cancer stage (stage I, II, III, and IV) using custom-made R scripts (R version 4.0.4). The TCGA Cell 2017 dataset[18] included 133 papillary and 273 non-papillary urothelial carcinoma patients. Of these patients, 132 papillary and 270 non-papillary patients had mRNA data and were thus included in the PPI network analysis. In the TCGA Nature 2014 dataset[19], there were 39 papillary and 88 non-papillary urothelial carcinoma patients with mRNA data. For both studies, mRNA data was downloaded as gene expression z-scores.

In the phenotypic data analysis, sex, age, follow-up time, overall survival, disease free time, pM stage, survival status, disease free status, histologic grade, pT stage, pN stage, and stage grouping data were included in the analysis of the TCGA Cell 2017 dataset. For the TCGA Nature 2014 dataset, patients with sex, age, survival status, overall survival, disease free status, and disease-free time data were included. 346 and 129 urothelial carcinoma patients were analyzed in the TCGA Cell 2017 and TCGA Nature 2014 datasets, respectively. Patients without phenotypic data were excluded from the phenotypic data analysis. Thus, there are differences in the number of patients in the PPI network analysis and phenotypic data analysis.

Proteinarium Analysis and Network Visualization

We used Proteinarium to identify clusters of patients with shared PPI networks associated in patient groups with papillary and non-papillary bladder cancer using the RNA-seq data from the TCGA Cell 2017 and TCGA Nature 2014 datasets. For each patient, we ranked the genes based on their z-scores to identify the most upregulated genes. To determine the number of seed genes used to build the PPI networks of each patient, we applied cophenetic correlation scores to the dendrogram output from the Proteinarium comparison of papillary and non-papillary patients using the top 50, 100, 125, 150, 200, 250, and 300 seed genes. For papillary versus non-papillary comparisons in both the TCGA Cell 2017 and TCGA Nature 2014 datasets, the change in cophenetic correlation coefficients decreased after increasing the number of seed genes to 125, indicating that increasing the number of seed genes did not greatly change the dendrogram structure (Supplemental Figures 1, 2, and 3). Hence, we used the top 125 most upregulated seed genes for each patient as inputs for Proteinarium. We performed the same cophenetic correlation score analysis for the downregulated genes in the TCGA Cell 2017 dataset (Supplemental Figure 3). Downregulated genes were chosen by ranking the genes from lowest to highest z-score, then choosing the top N number of seed genes.

As a first step, Proteinarium converts gene names to their protein names and uses PPI information from the STRING Database [22]. Then Proteinarium builds the network graphs based on the seed genes for each patient. It calculates the similarity between each pair of graphs by using the Jaccard distance and records in a matrix. Proteinarium uses this similarity matrix as the input for clustering graphs. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) is used for clustering. Patients are clustered based on their network similarities as an output. Significance of clusters is calculated by using Fisher exact test, identified by the abundance of cases and controls. Networks of the significant clusters can be visualized and analyzed. In the Proteinarium analysis, we used the default parameters of the tool for building the graphs and the maximum path length parameter set to 2. By applying this, we included only the seed proteins connected directly to each other and/or via a single intermediary protein. The intermediary connecting proteins were referred as “imputed proteins”.

Network Annotation and Modularity

Networks of the significant clusters were annotated by g:Profiler, a web server for identifying biological categories enriched in gene lists[23]. We entered the list of unique genes for the papillary

and non-papillary PPI networks in g:Profiler to identify the Gene Ontology (GO) terms and KEGG pathways associated with these networks. Only those with a False Discovery Rate (FDR) less than 0.05 were included. We used GEPHI version 0.9.2, a network visualization tool to visualize the networks and their network specific modularity. [24]. We used the modularity function that was based on Blondel's algorithm on GEPHI[25]. Modularity is defined as a measure of network structure which identifies the strength of division of a network into modules.

Separation Test

Separation testing is a computational method to determine the genetic similarity between two groups by comparing their PPI networks in the interactome. We performed separation tests to determine the distance between the networks specific to papillary and non-papillary groups in the interactome using a Python script adapted from Menche et.al.[26]. The interactome is composed of 141,296 experimentally determined physical interactions between 13,460 proteins. s_{AB} is a measure of separation between the networks of two groups in the interactome. $s_{AB} > 0$ indicates a topological separation of the networks specific to two groups and that the those groups can be regarded as distinct molecular entities. $s_{AB} < 0$ indicates that the networks specific to two groups overlap in the interactome.

Connectivity Map and Drug Repurposing Tool

We used Connectivity Map (CMap) from Broad Institute and applied the repurposing tool to explore potential drugs that may target the hub genes of Networks A, B, and C for connectivity map[27].

Statistical Analysis

Chi-square or Fisher's exact test was used for categorical variables when appropriate. Paired t-test (two-sided) was used to compare the means of two groups. All statistical analyses were carried out using JMP Pro 16.0 software (SAS Institute, Cary, NC). P-values less than 0.05 were considered to be statistically significant.

Results

Clinical Profile of the Patient Sample

Of all the 346 urothelial carcinomas in the TCGA Cell 2017 dataset[18], no significant differences were found between the papillary and non-papillary groups regarding sex, age, follow-up time, overall survival, disease free time and pM stage. However, statistically significant differences were observed in survival status, disease free status, histologic grade, pT stage, pN stage and stage grouping (Table 1). Of all the 129 urothelial bladder carcinomas in the TCGA Nature 2014 dataset[19], no significant differences were found between the papillary and non-papillary groups regarding age, overall survival, disease free status and disease-free time. However, statistically significant differences were observed in sex and survival status (Table 2).

Table 1. Clinical parameters of urothelial carcinomas in the TCGA Cell 2017 dataset.

Parameter	Papillary	Non-papillary	P-value
Case Number	133	213	
Sex			
Female	27	60	0.1261
Male	106	153	
Age			
Mean $\pm$ SEM	66.9 $\pm$ 1.0	68.4 $\pm$ 0.7	0.2064

Follow-up Time (Days) Mean $\pm$ SEM	521.8 $\pm$ 69.8	652.5 $\pm$ 72.5	0.2084
Survival Status			
Living	90	104	
Deceased	43	109	0.0008
Overall Survival (Months) Mean $\pm$ SEM	24.4 $\pm$ 2.0	27.1 $\pm$ 1.9	0.3442
Disease Free Status			
Disease Free	75	79	
Recurred/Progressed	33	88	0.0003
Disease Free Time (Months) Mean $\pm$ SEM	22.8 $\pm$ 2.2	26.8 $\pm$ 2.3	0.2311
Histologic Grade			
Low Grade	18	1	
High Grade	113	212	< 0.0001
pT Stage			
T1/T2	56	53	
T3	41	117	
T4	21	29	0.0001
pN Stage			
N0	89	117	
N1	8	27	
N2	19	43	0.0278
pM Stage			
M0	75	89	
M1	4	4	1
Stage Grouping			
Stage I/II	61	53	
Stage III	34	84	
Stage IV	34	76	0.0001

* SEM: Standard error of mean

+ Follow-up time was not available for 26 patients in papillary group and 68 patients in non-papillary group

† Overall survival was not available for 3 patients in papillary group

‡ Disease free status was not available for 25 patients in papillary and 46 patients in non-papillary group

▪ Disease free time was not available for 26 patients in papillary and 46 patients in non-papillary group

◇ Histologic grade is not available for 2 patients in papillary group

● There is 1 T1, 15 Tx, 12 Nx, 54 Mx in papillary group; and 1 T1, 14 Tx, 24 Nx, 120 Mx in non-papillary group

△ Stage not available for 2 patients in papillary

Only 1 Stage I in papillary and non-papillary group

Table 2. Clinical parameters of urothelial carcinomas in the TCGA Nature 2014 dataset.

Parameter	Papillary	Non-papillary	P-value
Case Number	41	88	
Sex			
Female	5	27	0.0284
Male	36	61	
Age			
Mean $\pm$ SEM	67.5 $\pm$ 1.8	69.0 $\pm$ 1.0	0.4447
Survival Status			
Living	33	49	0.0064
Deceased	8	39	
Overall Survival (Months)			
Mean $\pm$ SEM	14.6 $\pm$ 3.3	19 $\pm$ 2.7	0.3295
Disease Free Status			
Disease Free	20	37	0.1454
Recurred/Progressed	12	43	
Disease Free Time (Months)			
Mean $\pm$ SEM	9.0 $\pm$ 1.8	11.0 $\pm$ 1.5	0.4878

* SEM: Standard error of mean

+ Disease free was not available for 9 patients in papillary and 8 patients in non-papillary group

† Disease free time was not available for 30 patients in papillary and 54 patients in non-papillary group

Protein-protein Interaction Analysis and Network Visualization

We performed Protein-protein Interaction analysis using RNA-seq data from patients with papillary and non-papillary urothelial carcinomas to determine the differences in the PPI networks within these two architectures. We analyzed the top 125 upregulated genes obtained from RNA-seq data from TCGA Cell 2017[18] and TCGA Nature 2014[19] as well as the top 125 downregulated genes from the TCGA Cell 2017 dataset.

Upregulated Gene Analysis

In the analysis of the TCGA Cell 2017 dataset, one significant cluster (N = 393, p-value = 0.000757), was identified for further analysis as this cluster included 89 of 132 total patients with papillary urothelial carcinoma and 214 of 270 patients with non-papillary urothelial carcinoma (Figure 1). In the network specific to the papillary urothelial carcinoma patient group (Network A), *RPS27A*, *UBA52*, and *VAMP8* were the most connected or hub proteins identified. In the network specific to the non-papillary patient group (Network B), *GNB1*, *RHOA*, *UBC*, and *FPR2* were found to be the hub proteins (Figure 1). In the analysis of the TCGA Nature 2014 dataset, one cluster of six papillary urothelial carcinoma patients, was identified as a significant cluster based on Fisher's exact test (p = 0.000631) (Figure 2). In Network C, *CCDC22*, *ANAPC4*, and *UBR4* were identified as hub proteins unique to papillary urothelial carcinoma. There were six proteins shared between the two papillary urothelial carcinoma consensus networks (Networks A and C), including *UBR4*, *CUL1*, *UBE2K*, *CDC5L*, *UBA52*, and *RPS27A*.

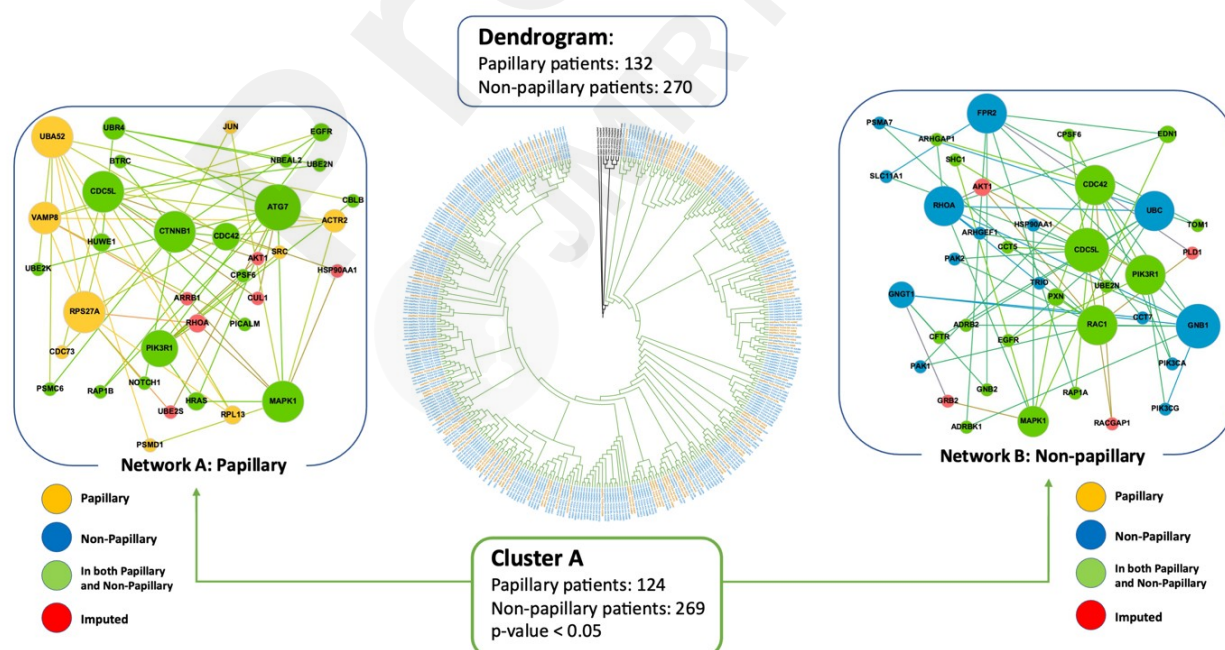


Figure 1: Dendrogram and consensus networks specific to papillary and non-papillary urothelial carcinoma from the TCGA Cell 2017 dataset (Upregulated genes). The dendrogram shows 402 patients, which were clustered based on their network similarities. Patients with green

branches are part of the significant cluster whose networks are next to the dendrogram. In the networks, larger circles represent more connected nodes. Proteins specific to the papillary and non-papillary groups are shown in yellow and blue, respectively. Proteins in both groups were shown in green. Proteins in red were imputed from the network analysis.

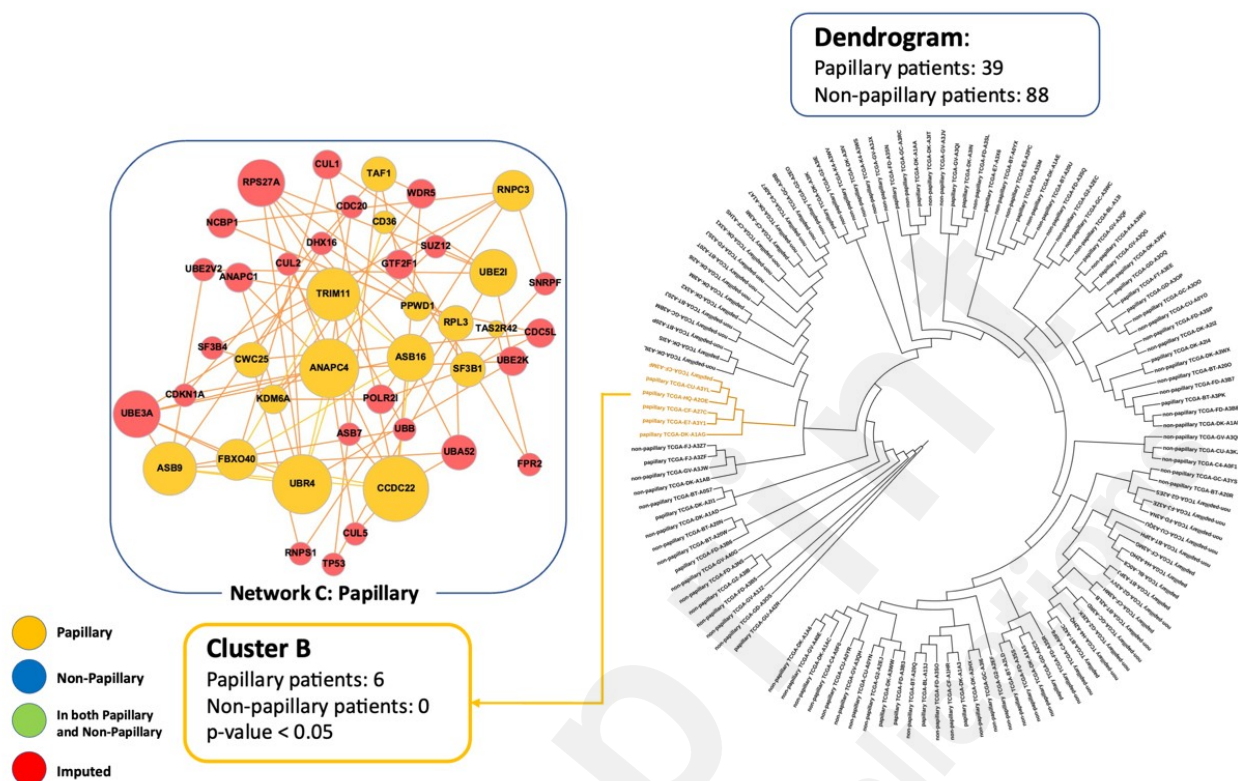


Figure 2: Dendrogram and consensus networks specific to papillary and non-papillary urothelial carcinoma from the TCGA Nature 2014 dataset (Upregulated genes). The dendrogram shows 127 patients, which were clustered based on their network similarities. Patients with yellow branches are part of the significant cluster, whose network is shown to the left of the dendrogram. In the networks, larger circles represent more connected nodes. Proteins specific to the papillary and non-papillary groups are shown in yellow and blue, respectively. Proteins shared by both groups were shown in green (none was identified in this dataset). Proteins in red were imputed from the network analysis.

The separation score for the distance between the papillary and non-papillary specific networks of the first dataset (Networks A and B) was calculated using the papillary and non-papillary genes from their respective networks. Including only these seed genes unique to either network, the separation score was calculated as $s_{AB} = 0.1722$, indicating that there are modules of genes in the papillary and non-papillary consensus networks that do not overlap in the interactome and can be considered as distinct entities. The separation score comparing Networks B and C was calculated as $s_{AB} = -0.0661$. The negative s_{AB} value shows that there is an overlap between the papillary consensus patient networks for these two independent datasets, indicating that our results were replicated in two independent datasets.

We visualized and annotated network modularity for non-papillary groups in both datasets by using GEPHI, a network and graph visualization tool. We found five modules whose genes are grouped by common functionality for each network in Networks A and C, and six modules in Network B. We used the default setting in GEPHI. gProfiler was used to annotate the modules for Networks A, B, and C to identify significant GO terms and KEGG pathways [23] was used to

annotate the modules for Networks A, B, and C to identify significant GO terms and KEGG pathways. Notably, the papillary networks of both datasets – Networks A and C – each contained one module enriched in proteins involved in ubiquitin-related activities. The KEGG pathway *Ubiquitin mediated proteolysis* and Molecular Function GO terms *Ubiquitin-like protein conjugating enzyme activity* and *Ubiquitin protein ligase binding* were common to the module in Network A and Network C.

Pathway and Gene Set Enrichment Analysis

We used the g:GOST functional profiling tool from g:Profiler to visualize significant GO terms and KEGG pathways for the genes in Networks A, B, and C [23] to visualize significant GO terms and KEGG pathways for the genes in Networks A, B, and C. The proteins unique to non-papillary urothelial carcinoma patients in Network B are enriched in the *PI3K-Akt signaling pathway* (KEGG pathway, $p = 0.0040$), with the most significant Molecular Function GO term being *Phosphatidylinositol-4,5-bisphosphate-3-kinase activity*. The GO terms and KEGG pathways related to the PI3K-Akt signaling pathway were unique to Network B, while ubiquitin-related processes were unique to the papillary Networks A and C. *Ubiquitin mediated proteolysis* was the most significant KEGG pathway for Network C ($p\text{-value} = 2.742e-12$) and *Ubiquitin-like protein ligase binding* was the most significant Molecular Function term for Network A ($p = 0.00110187$).

The sixth most significant KEGG pathway for Network A ($p\text{-value} = 1.294e-7$).

Drug Repurposing Tool

We identified several potential drugs targeting the hub proteins of Network B. *GNB1*, the most highly connected gene unique to non-papillary patient networks, encodes the beta subunit of G proteins, which modulate transmembrane signaling, including that of the PI3K/AKT signaling pathway[28]. Using the drug repurposing tool, we identified Y16 as a potential drug for non-papillary urothelial carcinoma treatment. Y16 is a Rho-associated kinase inhibitor in preclinical trials. Other Rho-associated kinase inhibitors, including Y-27632 and HA-1077, were shown to inhibit proliferation and invasion of bladder cancer cells and induce apoptosis, respectively[29, 30]. Y16 may be efficacious in treating patients with non-papillary urothelial carcinoma by targeting the hub gene *GNB1*. *FPR2*, another hub protein unique in the networks specific to non-papillary urothelial carcinoma, encodes the formyl peptide receptor 2 and is involved in the response to amyloid beta, regulation of the defense response, and positive regulation of monocyte chemotaxis[31]. TC-FPR2-43 is a formyl peptide receptor agonist in preclinical trials, which may be considered for use in treating non-papillary urothelial carcinoma. We also identified drugs targeting other proteins specific to Network B, including *PIK3CA*, and *PAK1*. *PIK3CA*, which encodes the p100 α catalytic subunit of PI3K[32], is the target of several PI3K inhibitors used to treat breast cancer (Alpelisib), follicular lymphoma (Copanlisib), and chronic lymphocytic leukemia (Idelalisib). *PAK1*, a member of the serine/threonine p2-activating kinase family, is targeted by several drugs in preclinical trials: serine/threonine kinase inhibitor FRAX486, p21 activated kinase inhibitors IPA-3 and NVS-PAK1-1, and Rho associated kinase inhibitor RKI-1447.

Discussion

The genomic landscape distinguishing papillary and non-papillary urothelial carcinoma architectures is not completely understood. Using a network biology approach, we sought to understand how the PPI networks differed between patients with papillary and non-papillary urothelial carcinoma. Proteinarium, a multi-sample PPI analysis tool was used to analyze gene expression data from patients with papillary and non-papillary urothelial carcinoma. and create clusters of patients with shared networks and genes specific to these two architectures. Our results showed that *RPS27A*, *UBA52*, and *VAMP8* were the hub proteins in Network A, the papillary consensus network, which shared six genes with Network C, the papillary consensus network of the validation dataset. These six genes - *UBR4*, *CUL1*, *UBE2K*, *CDC5L*, *UBA52*, AND *RPS27A* – were enriched in gProfiler for ubiquitination processes, including ubiquitin mediated proteolysis.

Dysregulation of the ubiquitin-proteasome system, which regulates tumor suppressors and oncogenic proteins, is seen across cancer types[33]. In the non-papillary urothelial carcinoma consensus network, Network B, we identified *GNB1*, *RHOA*, *UBC*, and *FPR2* as hub proteins and that the Network B was enriched for proteins involved in the PI3K/AKT signaling pathway.

Of the genes shared by Network A and Network C, the two papillary urothelial carcinoma consensus networks, *CDC5L* and *CUL1* have been found by previous studies to be upregulated in bladder cancer tissues. It has been shown that their overexpression is significantly associated with poorer prognosis in patients diagnosed with urothelial carcinoma[34, 35]. Knockdown of *CDC5L* inhibited the proliferation of bladder cancer cells by inducing apoptosis and limiting bladder cancer cell migration, invasion, and EMT[35]. There are currently no drugs in development that target *CDC5L* or *CUL1*. *RPS27A* and *UBA52*, which are hub genes unique to papillary urothelial carcinoma in Network A, have been found to be overexpressed in several different cancers, including colon cancer[36, 37], prostate cancer[38], and leukemia[39]. *RPS27A* and *UBA52* are both ribosomal fusion proteins composed of ubiquitin conjugated to the ribosomal proteins S27a and L40, respectively. *RPS27A*, an RNA-binding protein part of the ribosomal 40S subunit[40], plays a role in inhibiting apoptosis, regulating the progression of the cell cycle, and promoting proliferation[39]. *UBA52* is required for embryonic development and regulates protein synthesis and the cell cycle by modulating the expression of cyclins D1 and D3[41]. Further studies should be done to elucidate the role of overexpression of *RPS27A* and *UBA52* in papillary urothelial carcinoma.

The PI3K/AKT signaling pathway is activated alongside the MAPK signaling pathway upon activation of mitogens binding RTKs. PI3K/AKT pathway regulates homeostatic metabolism and cell growth, regulating processes such as glucose metabolism and the biosynthesis of macromolecules essential for cell growth through both direct regulation of nutrient transporters and enzymes or regulation of transcription factors controlling gene expression of key components of the metabolic pathways[42, 43]. *PIK3CA* and *PIK3CB* are genes in the non-papillary urothelial carcinoma specific network. *PIK3CA* encodes the p100 α catalytic subunit of PI3K while *PIK3CB* encodes a catalytic subunit of PI3K-beta[32]. *PIK3CA* upregulation has been implicated in bladder cancer as promoting cell growth, regulating metastasis[44-46]. Specifically, knockdown of *PIK3CA* was found to substantially inhibit cell proliferation[46] while overexpression of *PIK3CA* promoted bladder cancer cell growth, migration, invasion, and metastasis[45]. Higher levels of *PIK3CA* expression were associated with worse prognosis[45]. Subsequent activation of PI3K at the plasma membrane by dimerized RTKs causes phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to produce the secondary messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3). Downstream of the secondary messenger, proteins including AKT are activated and further promote additional activity of AKT. *AKT*, controls cell growth and metabolism either by directly phosphorylating metabolic enzymes or by modulating expression of transcription factors[32]. The 11th most significant KEGG term for Network B genes, the PI3K-Akt signaling pathway (adj. p-value = 0.004), was unique to Network B genes and was not found in the network annotations of either papillary Networks A or C. This provides evidence that the PI3K/AKT pathway is an important mechanism for non-papillary urothelial carcinoma and use of PI3K inhibitors may effectively treat patients with non-papillary urothelial carcinoma. Several PI3K inhibitors have been launched to treat breast cancer (Alpelisib), follicular lymphoma (Copanlisib), and chronic lymphocytic leukemia (Idelalisib). PI3K inhibition has been found to suppress growth, migration, and colony formation of bladder cancer cells in vitro[46].

Overexpression of *RHOA*, a member of the Rho family of small GTPases that regulates cell attachment, motility, and shape[32], has been associated with bladder cancer cell proliferation and metastasis[47-49]. *RHOA* is a hub gene unique to the non-papillary urothelial carcinoma consensus network. Ras-induced RhoA and NF-kappaB activation was implicated in promoting the invasion and migration of bladder cancer cells[50]. PAK proteins, including *PAK1* which was unique to the non-papillary consensus network, critically link Rho GTPases, including RhoA, to cytoskeletal reorganization and nuclear signaling[51]. *PAK1* has been found to be significantly overexpressed in

bladder cancer tissue compared to normal bladder tissue[52], and inhibition of *PAK1* by miR-145 decreased bladder cancer cell invasion[53]. Thus, while there are no current drugs targeting *RHOA*, inhibition of *PAK1* may limit bladder cancer progression. There are several drugs targeting *PAK1* in preclinical trials – serine/threonine kinase inhibitor FRAX486, p21 activated kinase inhibitors IPA-3 and NVS-PAK1-1, and Rho associated kinase inhibitor RKI-1447. These drugs are potential drug candidates for treating non-papillary urothelial carcinoma. Three of the four hub proteins of Network B, the non-papillary consensus network – *GNB1*, *RHOA*, and *FPR2* – have been associated with worse patient prognosis in other cancers, including gastric cancer[54], bladder cancer[48], lung cancer[55], and cervical squamous cell carcinoma[56].

Conclusions

In conclusion, two distinct networks specific to urothelial carcinoma architectures identified in this study suggests that papillary and non-papillary urothelial carcinomas present unique molecular entities with different biological drivers that can be used as both therapeutic targets and predictors of disease development. Our findings support that the non-papillary subtype of urothelial carcinomas may proliferate and thrive via the PI3K/AKT signaling pathway.

Acknowledgements

CC was supported by Brown University SPRINT award. EDGU and AU were supported by the Brown University Legorreta Cancer Center, Brown University Department of Pathology and Laboratory Medicine and Rhode Island Foundation.

Conflicts of Interest

Authors do not have any conflicts of interests.

Author Contributions

CC, YB, SH, AU, EDGU interpreted data, AU, EDGU performed study concept and design, CC acquired the study materials, CC and SH performed analysis, CC, YB, SH, AA, LC, AU, EDGU read, LC, AU, EDGU revised, and AU, EDGU approved the final paper.

Data Availability

All data collected and analyzed in this study is available at cBioPortal:
https://www.cbioportal.org/study/summary?id=blca_tcga_pub_2017
https://www.cbioportal.org/study/summary?id=blca_tcga_pub

References

1. Siegel, R.L., et al., *Cancer Statistics, 2025*. CA Cancer J Clin, 2025. **75**(1): p. 10-45.
2. Edge, S.B. and C.C. Compton, *The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM*. Ann Surg Oncol, 2010. **17**(6): p. 1471-4.
3. Luis, N.M., E. Lopez-Knowles, and F.X. Real, *Molecular biology of bladder cancer*. Clin Transl Oncol, 2007. **9**(1): p. 5-12.
4. McConkey, D.J., et al., *Molecular genetics of bladder cancer: Emerging mechanisms of tumor initiation and progression*. Urol Oncol, 2010. **28**(4): p. 429-40.
5. Zhang, B., Y. Tian, and Z. Zhang, *Network biology in medicine and beyond*. Circ Cardiovasc Genet, 2014. **7**(4): p. 536-47.
6. Vidal, M., M.E. Cusick, and A.L. Barabasi, *Interactome networks and human disease*. Cell, 2011. **144**(6): p. 986-98.
7. Gavin, A.C., et al., *Proteome survey reveals modularity of the yeast cell machinery*. Nature, 2006. **440**(7084): p. 631-6.
8. Li, S., et al., *A map of the interactome network of the metazoan C. elegans*. Science, 2004. **303**(5657): p. 540-3.
9. Oti, M., et al., *Predicting disease genes using protein-protein interactions*. J Med Genet, 2006. **43**(8): p. 691-8.
10. Stark, C., et al., *BioGRID: a general repository for interaction datasets*. Nucleic Acids Res, 2006. **34**(Database issue): p. D535-9.
11. Cowley, G.S., et al., *Parallel genome-scale loss of function screens in 216 cancer cell lines for the identification of context-specific genetic dependencies*. Sci Data, 2014. **1**: p. 140035.
12. L'Vov K, M., et al., *[Relative size of collagen body-peptides of the human cornea in the normal state and in keratoconus]*. Biofizika, 1989. **34**(3): p. 478-80.
13. Bi, D., et al., *Gene expression patterns combined with network analysis identify hub genes associated with bladder cancer*. Comput Biol Chem, 2015. **56**: p. 71-83.
14. Deng, S.P., L. Zhu, and D.S. Huang, *Mining the bladder cancer-associated genes by an integrated strategy for the construction and analysis of differential co-expression networks*. BMC Genomics, 2015. **16 Suppl 3**: p. S4.
15. Li, S., et al., *Identification of Biomarkers Correlated with the TNM Staging and Overall Survival of Patients with Bladder Cancer*. Front Physiol, 2017. **8**: p. 947.
16. Yuan, L., et al., *Overexpression of COL3A1 confers a poor prognosis in human bladder cancer identified by co-expression analysis*. Oncotarget, 2017. **8**(41): p. 70508-70520.
17. Armanious, D., et al., *Proteinarium: Multi-sample protein-protein interaction analysis and visualization tool*. Genomics, 2020. **112**(6): p. 4288-4296.
18. Robertson, A.G., et al., *Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer*. Cell, 2017. **171**(3): p. 540-556 e25.
19. Cancer Genome Atlas Research, N., *Comprehensive molecular characterization of urothelial bladder carcinoma*. Nature, 2014. **507**(7492): p. 315-22.
20. Cerami, E., et al., *The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data*. Cancer Discov, 2012. **2**(5): p. 401-4.
21. Gao, J., et al., *Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal*. Sci Signal, 2013. **6**(269): p. p11.
22. Szklarczyk, D., et al., *The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets*. Nucleic Acids Res, 2021. **49**(D1): p. D605-D612.
23. Raudvere, U., et al., *g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update)*. Nucleic Acids Res, 2019. **47**(W1): p. W191-W198.
24. Bastian, M., S. Heymann, and M. Jacomy, *Gephi: An Open Source Software for Exploring and Manipulating Networks*. Proceedings of the International AAAI Conference on Web and

- Social Media, 2009. **3**(1): p. 361-362.
25. Blondel, V.D., Guillaume, J.L., Lambiotte, R., & Lefebvre, E., *Fast unfolding of communities in large networks*. Journal of statistical mechanics: theory and experiment, 2008.
 26. Menche, J., et al., *Disease networks. Uncovering disease-disease relationships through the incomplete interactome*. Science, 2015. **347**(6224): p. 1257601.
 27. Subramanian, A., et al., *A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles*. Cell, 2017. **171**(6): p. 1437-1452 e17.
 28. O'Leary, N.A., et al., *Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation*. Nucleic Acids Res, 2016. **44**(D1): p. D733-45.
 29. Abe, H., et al., *The Rho-kinase inhibitor HA-1077 suppresses proliferation/migration and induces apoptosis of urothelial cancer cells*. BMC Cancer, 2014. **14**: p. 412.
 30. Jiang, L., J. Wen, and W. Luo, *Rhoassociated kinase inhibitor, Y27632, inhibits the invasion and proliferation of T24 and 5367 bladder cancer cells*. Mol Med Rep, 2015. **12**(5): p. 7526-30.
 31. Alliance of Genome Resources, C., *Alliance of Genome Resources Portal: unified model organism research platform*. Nucleic Acids Res, 2020. **48**(D1): p. D650-D658.
 32. Sayers, E.W., et al., *Database resources of the national center for biotechnology information*. Nucleic Acids Res, 2022. **50**(D1): p. D20-D26.
 33. Morrow, J.K., et al., *Targeting ubiquitination for cancer therapies*. Future Med Chem, 2015. **7**(17): p. 2333-50.
 34. Mao, S.Y., et al., *Expression of CUL1 correlates with tumour-grade and recurrence in urothelial carcinoma*. ANZ J Surg, 2017. **87**(7-8): p. 624-629.
 35. Zhang, Z., et al., *Depletion of CDC5L inhibits bladder cancer tumorigenesis*. J Cancer, 2020. **11**(2): p. 353-363.
 36. Barnard, G.F., et al., *Ubiquitin fusion proteins are overexpressed in colon cancer but not in gastric cancer*. Biochim Biophys Acta, 1995. **1272**(3): p. 147-53.
 37. Mu, Q., et al., *Apolipoprotein M promotes growth and inhibits apoptosis of colorectal cancer cells through upregulation of ribosomal protein S27a*. EXCLI J, 2021. **20**: p. 145-159.
 38. Zhang, Y., et al., *Network analysis of ChIP-Seq data reveals key genes in prostate cancer*. Eur J Med Res, 2014. **19**: p. 47.
 39. Wang, H., et al., *RPS27a promotes proliferation, regulates cell cycle progression and inhibits apoptosis of leukemia cells*. Biochem Biophys Res Commun, 2014. **446**(4): p. 1204-10.
 40. Chen, L. and H. Wang, *Nicotine Promotes Human Papillomavirus (HPV)-Immortalized Cervical Epithelial Cells (H8) Proliferation by Activating RPS27a-Mdm2-P53 Pathway In Vitro*. Toxicol Sci, 2019. **167**(2): p. 408-418.
 41. Kobayashi, M., et al., *The ubiquitin hybrid gene UBA52 regulates ubiquitination of ribosome and sustains embryonic development*. Sci Rep, 2016. **6**: p. 36780.
 42. Friesen, B.J., et al., *Caring for severely emotionally disturbed children and youth. Improving services for families*. Child Today, 1988. **17**(4): p. 18-22.
 43. Fruman, D.A., et al., *The PI3K Pathway in Human Disease*. Cell, 2017. **170**(4): p. 605-635.
 44. Liu, X., et al., *The CUL4B-miR-372/373-PIK3CA-AKT axis regulates metastasis in bladder cancer*. Oncogene, 2020. **39**(17): p. 3588-3603.
 45. Wang, Z., et al., *PIK3CA Is Regulated by CUX1, Promotes Cell Growth and Metastasis in Bladder Cancer via Activating Epithelial-Mesenchymal Transition*. Front Oncol, 2020. **10**: p. 536072.
 46. Zhu, S., et al., *Synergistic antitumor activity of pan-PI3K inhibition and immune checkpoint blockade in bladder cancer*. J Immunother Cancer, 2021. **9**(11).
 47. Chang, H.R., et al., *The suppressive effect of Rho kinase inhibitor, Y-27632, on oncogenic Ras/RhoA induced invasion/migration of human bladder cancer TSGH cells*. Chem Biol Interact, 2010. **183**(1): p. 172-80.
 48. Kamai, T., et al., *Significant association of Rho/ROCK pathway with invasion and metastasis*

- of bladder cancer. Clin Cancer Res, 2003. **9**(7): p. 2632-41.
49. Volanis, D., et al., *Expression profile of Rho kinases in urinary bladder cancer*. J BUON, 2011. **16**(3): p. 511-21.
 50. Rosenblum, M.G., et al., *Antibody-mediated delivery of tumor necrosis factor (TNF-alpha): improvement of cytotoxicity and reduction of cellular resistance*. Cancer Commun, 1991. **3**(1): p. 21-7.
 51. Benninger, Y., et al., *Essential and distinct roles for cdc42 and rac1 in the regulation of Schwann cell biology during peripheral nervous system development*. J Cell Biol, 2007. **177**(6): p. 1051-61.
 52. Huang, K., et al., *[Expression of PAK1 in bladder cancer and its influence on invasion of bladder cancer cells]*. Zhonghua Yi Xue Za Zhi, 2016. **96**(40): p. 3227-3231.
 53. Kou, B., et al., *miR-145 inhibits invasion of bladder cancer cells by targeting PAK1*. Urol Oncol, 2014. **32**(6): p. 846-54.
 54. Hou, X.L., et al., *FPR2 promotes invasion and metastasis of gastric cancer cells and predicts the prognosis of patients*. Sci Rep, 2017. **7**(1): p. 3153.
 55. Fan, Z., et al., *CircRNA circ_POLA2 promotes lung cancer cell stemness via regulating the miR-326/GNB1 axis*. Environ Toxicol, 2020. **35**(10): p. 1146-1156.
 56. Cao, Y., et al., *CircRNA circ_POLA2 Promotes Cervical Squamous Cell Carcinoma Progression via Regulating miR-326/GNB1*. Front Oncol, 2020. **10**: p. 959.
 57. Asaduzzaman, M., et al., *Tumour suppressor EP300, a modulator of paclitaxel resistance and stemness, is downregulated in metaplastic breast cancer*. Breast Cancer Res Treat, 2017. **163**(3): p. 461-474.
 58. Bi, Y., et al., *EP300 as an oncogene correlates with poor prognosis in esophageal squamous carcinoma*. J Cancer, 2019. **10**(22): p. 5413-5426.

Supplementary Files