Original Article

Gene network screening of bladder cancer via modular analysis

Xiaodong Li[1,2,3#], Ye Wu[2#], Ye Yuan[4]

[1]Outpatient Department, The Third Affiliated Hospital of Soochow University, Changzhou, China; [2]The First School of Clinical Medicine, Nanjing University of Traditional Chinese Medicine, Nanjing, China; [3]Department of Nutrition and Food Hygiene, School of Public Health, Soochow University, Suzhou, China; [4]Department of Oncology, The Third Affiliated Hospital of Soochow University, Changzhou, China

Contributions: (I) Conception and design: Y Yuan; (II) Administrative support: Y Yuan; (III) Provision of study materials or patients: Y Yuan; (IV) Collection and assembly of data: X Li, Y Wu; (V) Data analysis and interpretation: X Li, Y Wu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Dr. Ye Yuan, MD, PhD. Department of Oncology, The Third Affiliated Hospital of Soochow University, 185 Juqian St., Changzhou, Jiangsu Province, China. Email: yuanye.198705@163.com.

Background: Bladder cancer (BC) is one of the most common cancers of the urinary system. Negative regulation of apoptotic pathways is of the most significant biological process in cancer. More accurate tumor characterization and stratification of BC patients for selection of more appropriate treatments are required.

Methods: The data for this study are from the National Center for Biotechnology Information (NCBI)'s Online Mendelian Inheritance in Man (OMIM) database. Disease-associated genes were performed via multiple text-based Searching in Agilent Literature Search software version 3.2.2. MCODE version 1.32 was used for computational analysis of network for the gene complex detection. Genes with common biological processes or pathways were divided into the same module. DAVID was used for Gene ontology (GO) and pathway analysis. The OS time of hub gene expression was analyzed by GEPIA. The study used Pearson Correlation Coefficient for correlated calculation of the hub genes in the same module (Bladder Urothelial Carcinoma samples compared with normal samples). We enriched the modules and predict the regulated miRNAs by Cluepedia. Interactions within each pathway can be investigated and new potential associations are revealed through gene/miRNA enrichments.

Results: A total of 187 BC-associated genes were got from OMIM and used for network construction. A total of seventy-five modules were found in the network. *EGFR*, *AR*, *MET*, *RELA*, *TP53*, *TSG101* are hub genes (edges above 10) of the largest 3 modules. The results demonstrate that BC patients with low-expressed *TSG101* have longer OS, and are associated with *TP53*. Low-expressed *RELA* and over-expressed *AR* patients have a higher survival time. Low-expressed *TSG101* patients have a longer survival time.

Conclusions: In our study, we found that miRNA17, miRNA20a, miRNA15a, has-let-7b and miRNA16 were miRNAs regulating the top 3 modules.

Keywords: Bladder cancer (BC); gene network screening; modular analysis

Submitted Sep 02, 2020. Accepted for publication Nov 27, 2020.
doi: 10.21037/tcr-20-2822
View this article at: http://dx.doi.org/10.21037/tcr-20-2822

Introduction

Bladder cancer (BC) is one of the most common cancers of the urinary system. BC is the 13th most common cause of cancer-related death worldwide (1). The majority of BC is non-muscle invasive bladder cancers (NMIBC), which tend to have a high rate of recurrence after primary tumor resection. The 5-year overall survival (OS) for NMIBC patients is nearly 90%, and 60% to 70% for muscle invasive bladder cancers (MIBC) patients (2). Approximately 50% of patients occurred distant metastasis after radical cystectomy (3). For decades, the outcome or treatment for

BC has not progressed much (4). Cisplatin was tested in neoadjuvant chemotherapy for MIBC since 1980s, and still used after cystectomy or metastatic patients as the first line option (5). The use of platinum-based chemotherapy has been limited because of neutropenia, peripheral neuropathy and mucositis (5). The progression, metastasis and drug resistance also barricade the treatment of BC.

Therefore, more accurate tumor characterization and stratification of BC patients for selection of more appropriate treatments are required. BC is a very heterogeneous disease due to clinical history, the pathological features and the molecular mechanisms involved in each case differ (6). *FGFR* is an oncogene and play important roles in cell proliferation, migration and invasion (7). FGFR3 mutations are highly associated with low-grade non-muscle invasive urothelial carcinoma (8). Medicines such as FGFR1 and FGFR3 inhibitors have been developed to treat BC, but these drugs are still in the continuation phase of clinical trials (9,10).

Gene amplifications have been found in EGFR and MET (11). miRNAs are long non-coding RNA gene products which can serve as oncogenes or tumor suppressors, it regulates target genes by binding to specific sites.

An increasing number of studies have implied that miRNAs might be the potential biomarkers and molecular therapeutic targets for BC. Gene pairs such as EGFR and c-MET are regulated by microRNA-23b/27b which contribute to BC oncogenesis and metastasis (12).

Numerous genes and miRNAs are involved in the occurrence and development of BC, the complicated regulatory mechanism remains unclear. Previous study has constructed a protein-protein interaction network by differentially expressed genes of BC. *PCNA*, *TOP2A*, *CCND1* and *CDH1* were found to be hub genes in the network (13).

Although much has been known about single gene or miRNA in BC, much less is on the roles of paired significant genes and miRNAs. In this study, we utilized genetic associated genes network construction to identify the gene correlation and OS time in BC, and analyzed the miRNAs which might regulate the significant modules and hub genes. Multi-level molecular mechanism was also explored.

We present the following article in accordance with the MDAR checklist (available at http://dx.doi.org/10.21037/tcr-20-2822).

Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Gene collection and network construction

The data for this study are from the National Center for Biotechnology Information (NCBI)'s Online Mendelian Inheritance in Man (OMIM) database http://www.ncbi.nlm.nih.gov/omim), which is a knowledge database of human genes and genetic disorders. Disease-associated genes were performed via multiple text-based Searching in Agilent Literature Search software version 3.2.2 (http://www.agilent. com/labs/research/litsearch.html), by which the gene network was constructed.

Module division

MCODE (http://baderlab.org/Software/MCODE) version 1.32 was used for computational analysis of network for the gene complex detection. Genes with common biological processes or pathways were divided into the same module.

Functional enrichment

DAVID (http://david.abcc.ncifcrf.gov) was used for Gene ontology (GO) and pathway analysis (14). Parameters: Count, 2; EASE, 0.01; and species and background, Homo sapiens. The biological processes and pathways were ranked by P values.

Overall survive time and correlation analysis of hub genes

The OS time of hub gene expression was analyzed by GEPIA (15). Parameters: hazards ratio (HR): yes, 95% CI: yes, axis units: month. The study used Pearson Correlation Coefficient for correlated calculation of the hub genes in the same module (Bladder Urothelial Carcinoma samples compared with normal samples).

miRNA prediction

We enriched the modules and predict the regulated miRNAs by Cluepedia (edge score =0.6, threshold =3). Interactions within each pathway can be investigated and new potential associations are revealed through gene/miRNA enrichments (16).

Statistical analysis

The study used Pearson Correlation Coefficient for correlated calculation of the hub genes in the same module (Bladder Urothelial Carcinoma samples compared with normal samples). The correlation of hub genes was used by non-log scale, and log-scale axis for visualization. Mantel–Cox test was used for the hypothesis evaluation of OS analysis.

Results

General gene information

A total of 187 bladder cancer-associated genes (including NMIBC and MIBC) were got from OMIM (Appendix 1). And 177 of which link to homologene based on a common GeneID, 23 genes link to UniSTS which based on markers cited in the OMIM record, 56 genes link to variation data in dbSNP (https://www.ncbi.nlm.nih.gov/snp). UniSTS is a large STS database comprised of both GenBank STS sequence entries and published STS maps (17). dbSNP contains human single nucleotide variations, microsatellites, and small-scale insertions for both common variations and clinical mutations (https://www.ncbi.nlm.nih.gov/snp).

BC gene network

Inputting 187 BC-associated genes into the Agilent Literature Search, the BC gene network contains 1,289 nodes and 7,164 edges (*Figure 1*). The average number of node neighbors is 10.438, and the isolated nodes number is 76.

Module analysis

Dense regions of the BC gene network were divided by MCODE. Totally, 35 modules found in the network (Appendix 2). Three modules (modules 1, 2 and 3) have the largest nodes were detected (*Figure 2*). *EGFR*, *AR*, *MET*, *RELA*, *TP53* and *TSG101* are hub genes (edges above 10) of the largest 3 modules.

Enrichment analysis

A total of 216 functional annotations and 95 pathways were found in the enrichment analysis of the most significant top 3 modules (https://cdn.amegroups.cn/static/public/TCR-20-2822-1.pdf). Negative regulation of apoptotic process and pathways in cancer are the most significant biological process and pathway separately (*Figure 3*). The hub genes in the top 3 modules involved in the significant processes such as regulation of cell cycle and positive regulation of transcription from RNA polymerase II promoter (*Table 1*).

OS time and correlation to hub genes

The results demonstrate that BC patients with low-expressed TSG101 have longer OS, and are associated with TP53. Low-expressed RELA and over-expressed AR patients have a higher survival time. Low-expressed TSG101 patients have a longer survival time (*Figure 4*).

miRNA prediction

miRNA17, miRNA20a and miRNA15a were found to regulate module 1, miRNA15a and has-let-7b regulating module 2, miRNA15a and miRNA16 regulating module 3 (*Figure 5*).

Discussion

By the investigation of human genome-wide functional microarray or RNA-seq gene expression in pathway databases, *TP53*, *AR* and *RELA* were found as transcriptional targets (18). In the present study, the hub genes, miRNAs and pathways associated with BC were identified.

TP53 and *TSG101* are hub genes of module 1, there is a positive correlation of gene expression between them. *TP53* is involved in the regulation of cell cycle and apoptosis. The expressions of *TP53* in NMIBC cells (KK47 and RT4) were lower than those in MIBC cells (T24, 5637, and UM-UC-3) (19). Overexpression of *TP53* is related to poor survival in patients with advanced BC (20). Mutations in the *TP53* have been observed more frequently in invasive high grade BC compared with low grade BC (21). TSG101 is a common target of splicing defects, the stress-activated TP53 can regulate TSG101 splicing process (22). Meanwhile, TSG101 attenuates p53 signaling (23), and the TSG101 transcripts is correlated with tumor grade and p53 mutation in breast cancer (24). The GO analysis for The TOP 3 modules demonstrated that TP53 and TSG101 are involved in the processes including regulation of cell cycle, positive regulation of protein transport and nucleolus.

AR and RELA are hub genes of module 2. AR is a nuclear steroid hormone receptor and play key roles in

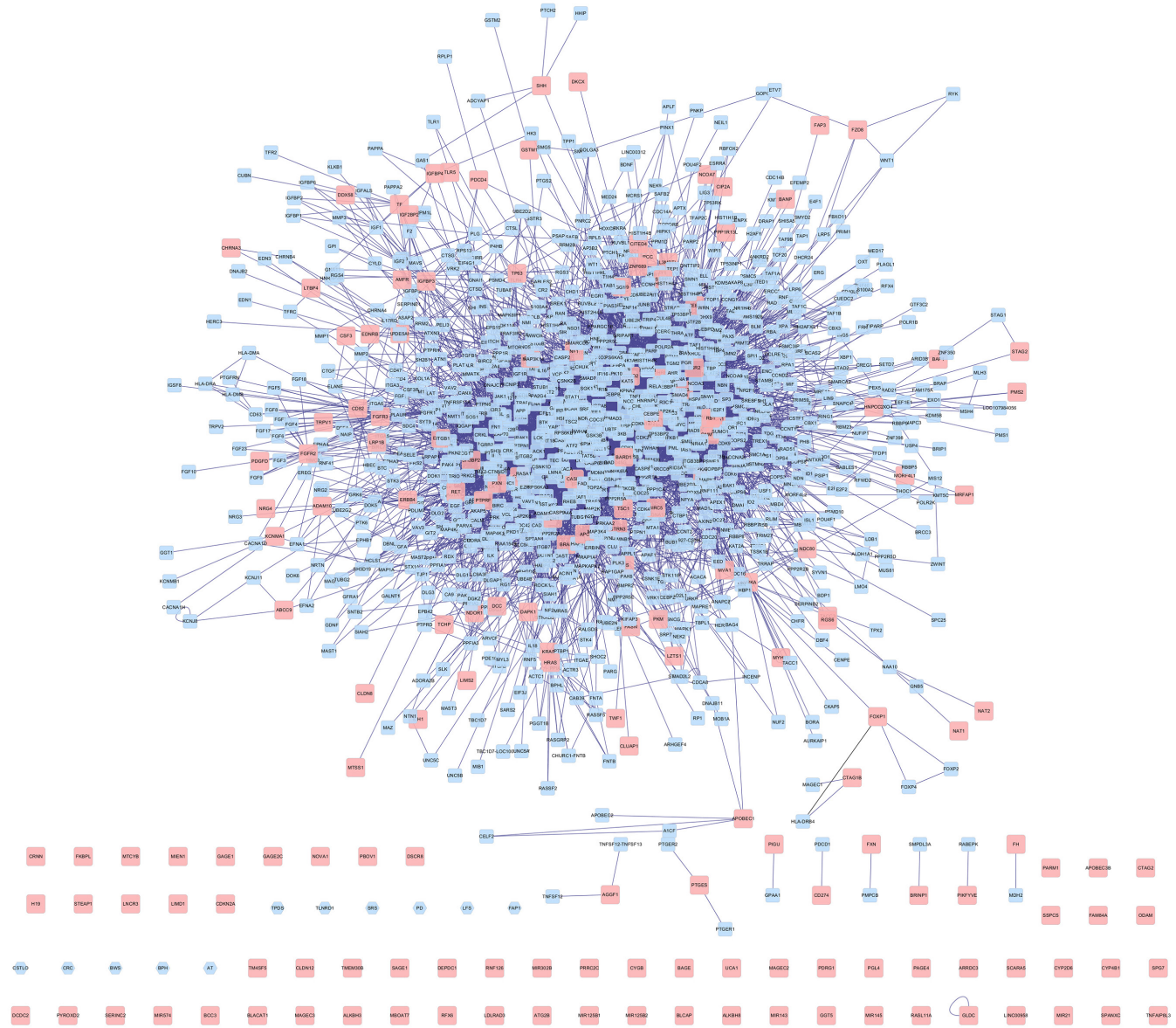

Figure 1 A landscape of bladder cancer gene network. The bladder cancer gene network contains 1,289 nodes and 7,164 edges. The red nodes are 187 bladder cancer genes, the blue nodes are genes found by the text-based searching.

the occurrence and progression of many cancers (25). Systems biology modeling demonstrated that *RELA* and *AR* are hub genes of the radiation-specific biomarkers and related to radio-sensitization drugs (26). Interleukin-1 (IL-1) is implicated in prostate cancer initiation and progression, RELA can regulate IL-1-mediated AR repression in prostate cancer cells (27). Meanwhile, AR declined the angiogenic potential of cancer cells. The activation of AR decreases the expression of RELA, and reduced its transcriptional activity which is an anti-tumor mechanism (28). AR together with RELA involved in 5 biological processes, the most significant is positive regulation of transcription from RNA polymerase II promoter, which is equal to MET and EGFR.

MET and EGFR are hub genes of module 3. MET is associated with the progression, treatment effect and OS of cancers. Urinary soluble MET level of BC patients is higher than patients without BC (29). EGFR and c-Met signaling

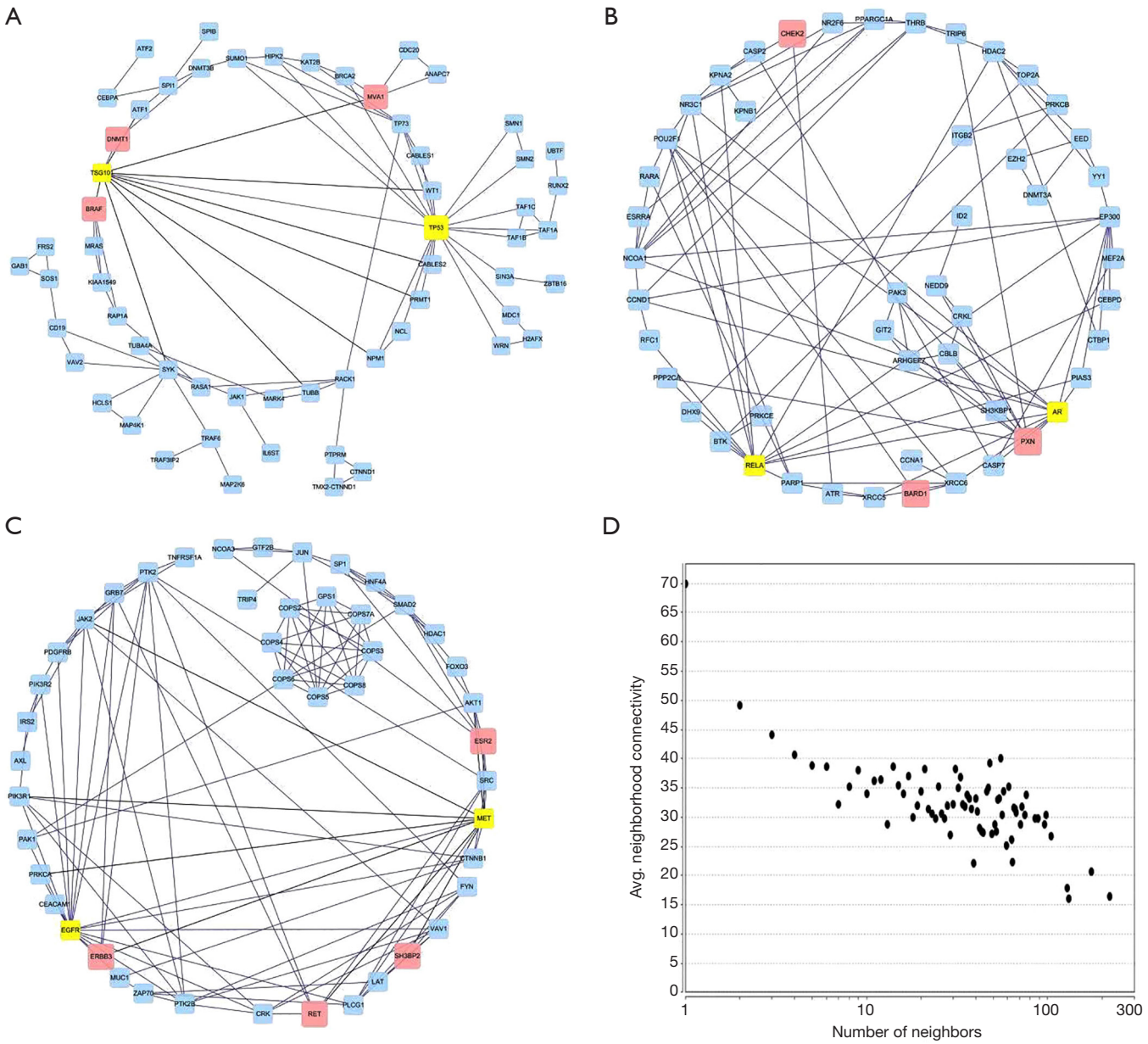

Figure 2 The three largest modules of bladder cancer gene network. (A) Module 1; (B) module 2; (C) module 3. Yellow nodes are hub genes of the modules.

pathways can be regulated by miR-23b/27b, the decreased expression of which may enhance cancer cell proliferation and migration (12). MET together with EGFR involve in 3 biological processes, including positive regulation of transcription from RNA polymerase II promoter, tissue morphogenesis, cellular lipid metabolic process.

Previous study confirmed that miRNAs can be critical players in the prognosis and diagnosis of BC (30). miRNA16 inhibited the proliferation, migration, and invasion of CRC cells by downregulating ITGA2 (31).

MiRNA-17, miR-20a, miR-15a, let-7b, miR-16 were predicted regulating the top 3 modules of BC. mir-17 and mir-15a were found significantly correlated with the OS of BC (32).

Pathways in cancer (hsa05200) is the most significant pathway according to P value. Hub genes, such as *AR*, *TP53* and *EGFR*, were found to take part in the apoptosis and

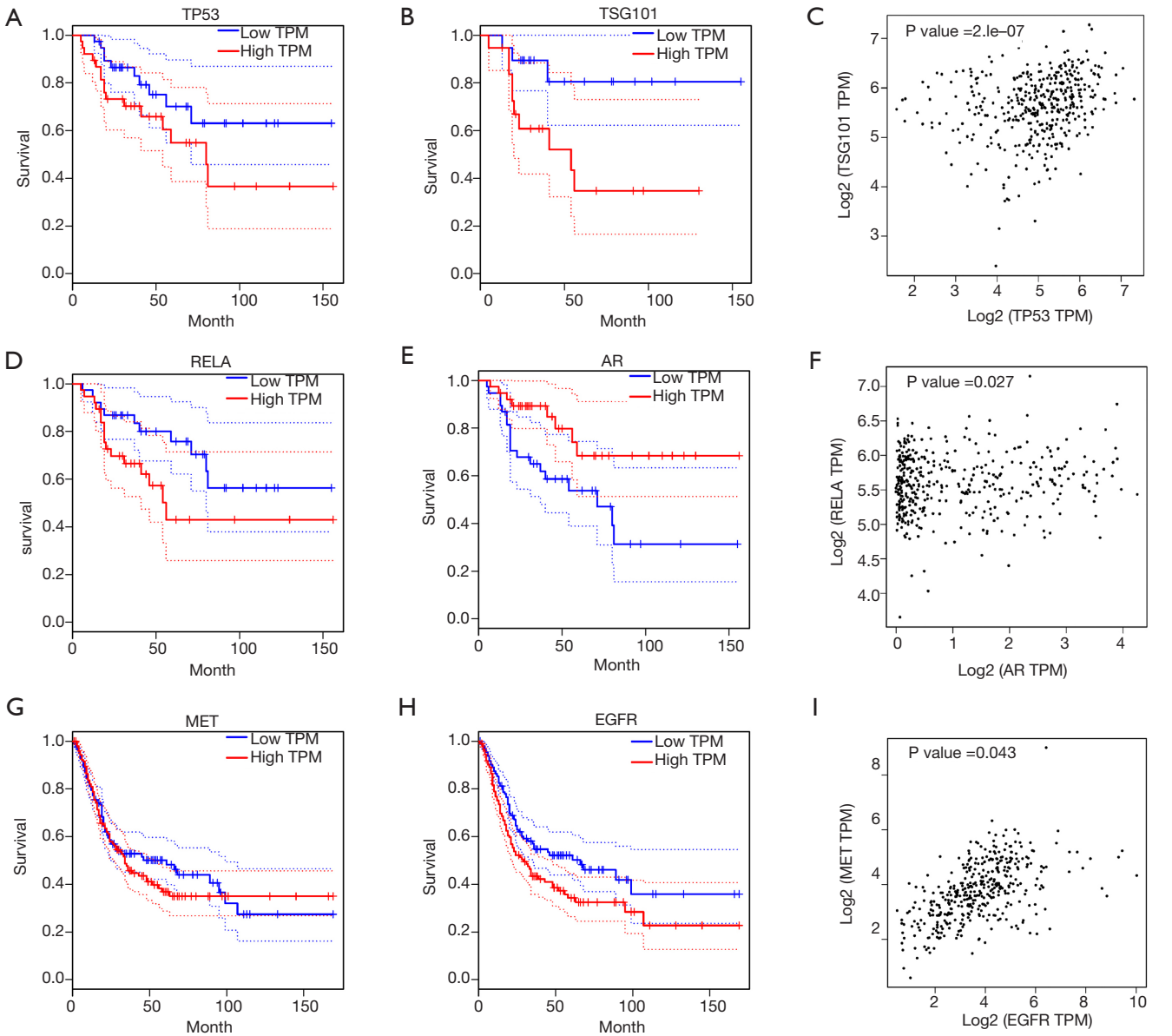

Figure 3 Overall survival of bladder cancer patients with 6 hub genes is evaluated by Kaplan-Meier curve with high and low expression of *TP53* (A), *TSG101* (B), *RELA* (D), *AR* (E), *MET* (G), *EGFR* (H). Log-rank test is used to evaluate difference between the two curves. The Pearson test is used to evaluate correlation between the hub genes in a same module. C, *TP53* and *TSG101*; F, *RELA* and *AR*; I, *MET* and *EGFR*.

Table 1 Significant gene ontology of the hub genes in the top 3 modules

TP53-TSG101	*AR-RELA*	*MET-EGFR*
GO: 0051726 regulation of cell cycle (1.08E-14)	GO: 0045944 positive regulation of transcription from RNA polymerase II promoter (7.56E-10)	GO: 0045944 positive regulation of transcription from RNA polymerase II promoter (7.56E-10)
GO: 0051222 positive regulation of protein transport (5.27E-7)	GO: 0008284 positive regulation of cell proliferation (1.22E-5)	GO: 0048729 tissue morphogenesis (4.60E-4)
GO: 0005730 positive regulation of protein transport and nucleolus (8.40E-3)	GO: 0051092 positive regulation of NF-κB transcription factor activity (1.40E-2)	GO: 0044255 cellular lipid metabolic process (5.50E-3)

GO, gene ontology.

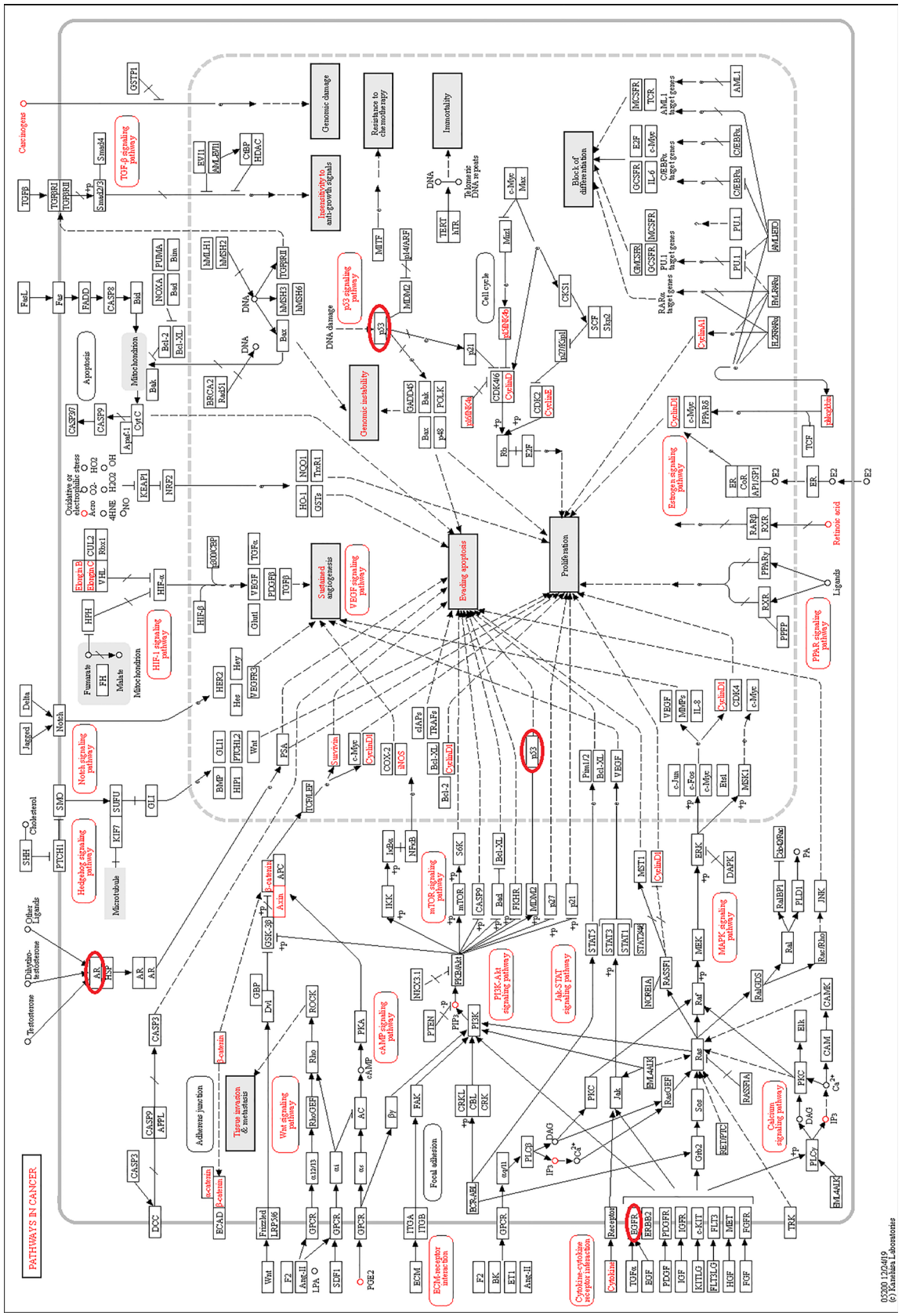

Figure 4 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of bladder cancer.

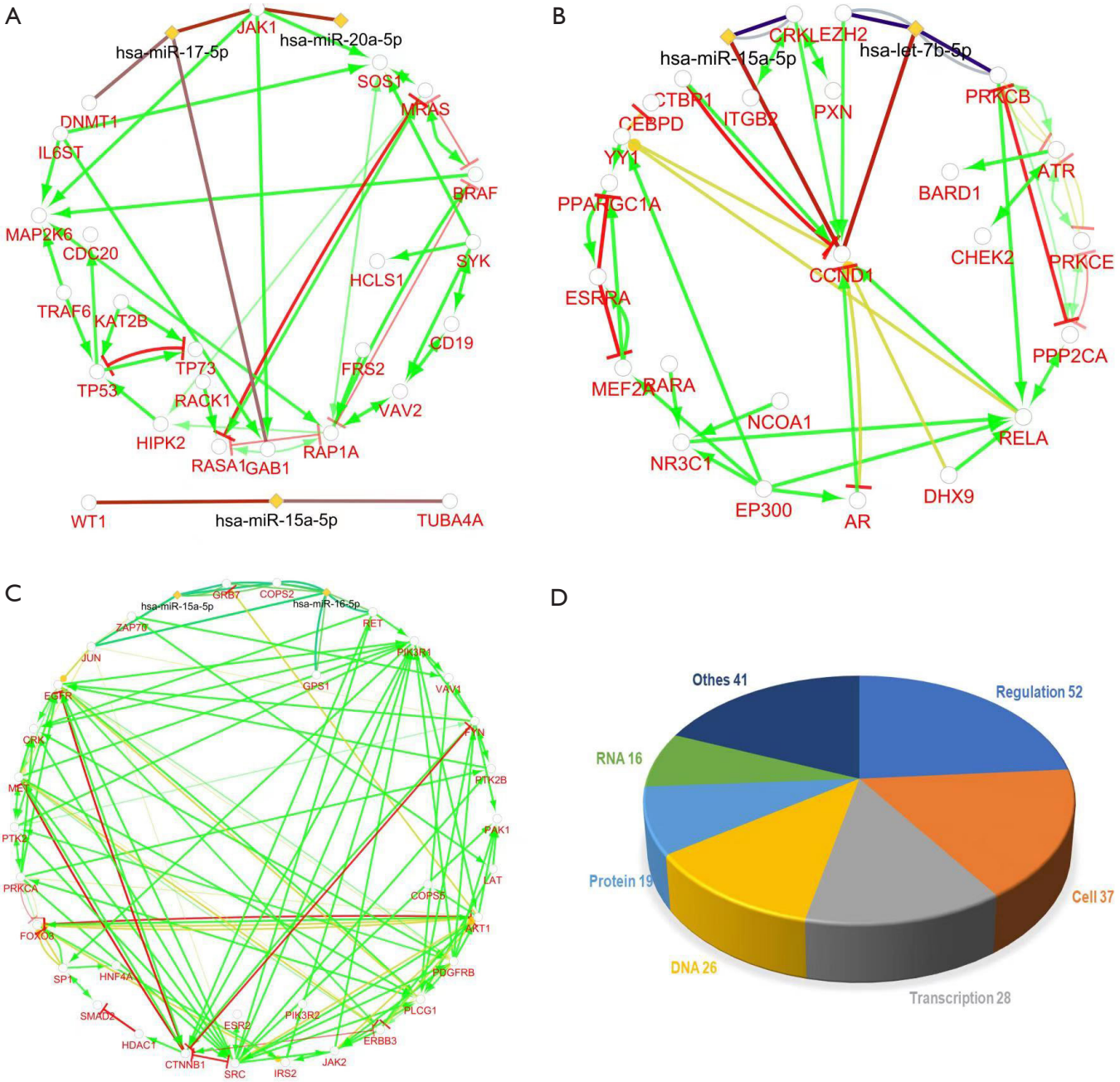

Figure 5 (A-C) Five microRNAs were predicted regulating the top 3 modules of bladder cancer. (D) The biological process of the top 3 modules.

cytokine-cytokine receptor interaction of the pathway.

In conclusion, our study revealed multiple possible significant functional mechanisms in the BC development. The combined pattern of hub genes, miRNAs, significant processes and pathways supply new drug targets and treatments for further study.

Acknowledgments

We sincerely appreciate Dr. Hanze Zhang for his valuable language editing for this manuscript.

Funding: This study was supported by grants from Jiangsu Province Postdoctoral Science Research Founding Project

(2020Z280, to Dr. Xiaodong Li), Training Program for the Youth Talent of the Changzhou Commission of Health (CZQM2020016, to Dr. Xiaodong Li), Program for the Young Talents of the Changzhou Commission of Health (QN201902, to Dr. Xiaodong Li), and the Basic Research Project of Changzhou (YB2017059, to Dr. Ye Yuan).

Footnote

Reporting Checklist: The authors have completed the MDAR checklist. Available at http://dx.doi.org/10.21037/tcr-20-2822

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-2822). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7-34.
2. Magers MJ, Lopez-Beltran A, Montironi R, et al. Staging of bladder cancer. Histopathology 2019;74:112-34.
3. Yafi FA, Aprikian AG, Chin JL, et al. Contemporary outcomes of 2287 patients with bladder cancer who were treated with radical cystectomy: a Canadian multicentre experience. BJU Int 2011;108:539-45.
4. Ebrahimi H, Amini E, Pishgar F, et al. Global, Regional and National Burden of Bladder Cancer, 1990 to 2016: Results from the GBD Study 2016. J Urol 2019;201:893-901.
5. Flaig TW, Spiess PE, Agarwal N, et al. Bladder Cancer, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2020;18:329-54.
6. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nature Reviews Cancer 2015;15:25-41.
7. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010;10:116-29.
8. Tomlinson DC, Baldo O, Harnden P, et al. FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer. J Pathol 2007;213:91-8.
9. Krook MA, Lenyo A, Wilberding M, et al. Efficacy of FGFR Inhibitors and Combination Therapies for Acquired Resistance in FGFR2-Fusion Cholangiocarcinoma. Mol Cancer Ther 2020;19:847-57.
10. Nogova L, Sequist LV, Perez Garcia JM, et al. Evaluation of BGJ398, a Fibroblast Growth Factor Receptor 1-3 Kinase Inhibitor, in Patients With Advanced Solid Tumors Harboring Genetic Alterations in Fibroblast Growth Factor Receptors: Results of a Global Phase I, Dose-Escalation and Dose-Expansion Study. J Clin Oncol 2017;35:157-65.
11. Reis H, van der Vos KE, Niedworok C, et al. Pathogenic and targetable genetic alterations in 70 urachal adenocarcinomas. Int J Cancer 2018;143:1764-73.
12. Chiyomaru T, Seki N, Inoguchi S, et al. Dual regulation of receptor tyrosine kinase genes EGFR and c-Met by the tumor-suppressive microRNA-23b/27b cluster in bladder cancer. Int J Oncol 2015;46:487-96.
13. Tang F, He Z, Lei H, et al. Identification of differentially expressed genes and biological pathways in bladder cancer. Mol Med Rep 2018;17:6425-34.
14. Huang L, Xie D, Yu Y, et al. TCMID 2.0: a comprehensive resource for TCM. Nucleic Acids Res 2018;46:D1117-d20.
15. Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45:W98-W102.
16. Bindea G, Galon J, Mlecnik B. CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. Bioinformatics 2013;29:661-3.
17. Rotmistrovsky K, Jang W, Schuler GD. A web server for performing electronic PCR. Nucleic Acids Res 2004;32:W108-12.
18. Shmelkov E, Tang Z, Aifantis I, et al. Assessing quality and completeness of human transcriptional regulatory

pathways on a genome-wide scale. Biol Direct 2011;6:15.
19. Yan L, Li Q, Yang J, et al. TPX2-p53-GLIPR1 regulatory circuitry in cell proliferation, invasion, and tumor growth of bladder cancer. J Cell Biochem 2018;119:1791-803.
20. Lorenzo-Romero JG, Salinas-Sánchez AS, Giménez-Bachs JM, et al. Prognostic implications of p53 gene mutations in bladder tumors. J Urol 2003;169:492-9.
21. Proctor I, Stoeber K, Williams GH. Biomarkers in bladder cancer. Histopathology 2010;57:1-13.
22. Moyret-Lalle C, Duriez C, Van Kerckhove J, et al. p53 induction prevents accumulation of aberrant transcripts in cancer cells. Cancer Res 2001;61:486-8.
23. Gray TA, Alsamman K, Murray E, et al. Engineering a synthetic cell panel to identify signalling components reprogrammed by the cell growth regulator anterior gradient-2. Mol Biosyst 2014;10:1409-25.
24. Turpin E, Dalle B, de Roquancourt A, et al. Stress-induced aberrant splicing of TSG101: association to high tumor grade and p53 status in breast cancers. Oncogene 1999;18:7834-7.
25. Chang C, Lee SO, Yeh S, et al. Androgen receptor (AR) differential roles in hormone-related tumors including prostate, bladder, kidney, lung, breast and liver. Oncogene 2014;33:3225-34.
26. Eschrich S, Zhang H, Zhao H, et al. Systems biology modeling of the radiation sensitivity network: a biomarker discovery platform. Int J Radiat Oncol Biol Phys 2009;75:497-505.
27. Thomas-Jardin SE, Dahl H, Kanchwala MS, et al. RELA is sufficient to mediate interleukin-1 repression of androgen receptor expression and activity in an LNCaP disease progression model. Prostate 2020;80:133-45.
28. Nelius T, Filleur S, Yemelyanov A, et al. Androgen receptor targets NFkappaB and TSP1 to suppress prostate tumor growth in vivo. Int J Cancer 2007;121:999-1008.
29. Matsumoto K, Umitsu M, De Silva DM, et al. Hepatocyte growth factor/MET in cancer progression and biomarker discovery. Cancer Sci 2017;108:296-307.
30. Usuba W, Urabe F, Yamamoto Y, et al. Circulating miRNA panels for specific and early detection in bladder cancer. Cancer Sci 2019;110:408-19.
31. Xu Y, Shen L, Li F, et al. microRNA-16-5p-containing exosomes derived from bone marrow-derived mesenchymal stem cells inhibit proliferation, migration, and invasion, while promoting apoptosis of colorectal cancer cells by downregulating ITGA2. 2019;234:21380-94.
32. Yin XH, Jin YH, Cao Y, et al. Development of a 21-miRNA Signature Associated With the Prognosis of Patients With Bladder Cancer. Front Oncol 2019;9:729.

Cite this article as: Li X, Wu Y, Yuan Y. Gene network screening of bladder cancer via modular analysis. Transl Cancer Res 2021;10(2):1043-1052. doi: 10.21037/tcr-20-2822

Appendix 1 Bladder cancer associated genes

601439 - ATP-BINDING CASSETTE, SUBFAMILY C, MEMBER 9
602192 - A DISINTEGRIN AND METALLOPROTEINASE DOMAIN 10
608464 - ANGIOGENIC FACTOR WITH G-PATCH AND FHA DOMAINS 1
610603 - AlkB HOMOLOG 3, ALPHA-KETOGLUTARATE-DEPENDENT DIOXYGENASE
613306 - AlkB HOMOLOG 8, tRNA METHYLTRANSFERASE
603243 - AUTOCRINE MOTILITY FACTOR RECEPTOR
611731 - APC GENE
600130 - APOLIPOPROTEIN B mRNA-EDITING ENZYME, CATALYTIC POLYPEPTIDE 1
607110 - APOLIPOPROTEIN B mRNA-EDITING ENZYME, CATALYTIC POLYPEPTIDE-LIKE 3B
612464 - ARRESTIN DOMAIN-CONTAINING 3
208900 - ATAXIA-TELANGIECTASIA
616226 - AUTOPHAGY 2, S. CEREVISIAE, HOMOLOG OF, B
607585 - ATAXIA-TELANGIECTASIA MUTATED GENE
603072 - AURORA KINASE A
605167 - B MELANOMA ANTIGEN
611564 - BTG3-ASSOCIATED NUCLEAR PROTEIN
603089 - BRCA1-ASSOCIATED PROTEIN 1
601593 - BRCA1-ASSOCIATED RING DOMAIN 1
613059 - BASAL CELL CARCINOMA, SUSCEPTIBILITY TO, 3
603352 - BACULOVIRAL IAP REPEAT-CONTAINING PROTEIN 5
615480 - BLADDER CANCER-ASSOCIATED TRANSCRIPT 1, NONCODING
613110 - BLADDER CANCER-ASSOCIATED PROTEIN
600082 - PROSTATIC HYPERPLASIA, BENIGN
164757 - B-RAF PROTOONCOGENE, SERINE/THREONINE KINASE
113705 - BREAST CANCER 1 GENE
602965 - BONE MORPHOGENETIC PROTEIN/RETINOIC ACID-INDUCIBLE NEURAL-SPECIFIC PROTEIN 1
130650 - BECKWITH-WIEDEMANN SYNDROME
600636 - CASPASE 3, APOPTOSIS-RELATED CYSTEINE PROTEASE
605402 - CD274 MOLECULE
600623 - CD82 ANTIGEN
192090 - CADHERIN 1
600160 - CYCLIN-DEPENDENT KINASE INHIBITOR 2A
604373 - CHECKPOINT KINASE 2
118503 - CHOLINERGIC RECEPTOR, NEURONAL NICOTINIC, ALPHA POLYPEPTIDE 3
610643 - CELL PROLIFERATION-REGULATING INHIBITOR OF PROTEIN PHOSPHATASE 2A
606815 - CBP/P300-INTERACTING TRANSACTIVATOR, WITH GLU/ASP-RICH CARBOXY TERMINAL DOMAIN, 4
611232 - CLAUDIN 12
611231 - CLAUDIN 8
616787 - CLUSTERIN-ASSOCIATED PROTEIN 1
615134 - MELANOMA, CUTANEOUS MALIGNANT, SUSCEPTIBILITY TO, 9
114500 - COLORECTAL CANCER
611312 - CORNULIN
157800 - CARDIOSPONDYLOCARPOFACIAL SYNDROME
138970 - COLONY-STIMULATING FACTOR 3
218040 - COSTELLO SYNDROME
300156 - CANCER/TESTIS ANTIGEN 1B
300396 - CANCER/TESTIS ANTIGEN 2
158350 - COWDEN SYNDROME 1
608759 - CYTOGLOBIN
124030 - CYTOCHROME P450, SUBFAMILY IID, POLYPEPTIDE 6
124075 - CYTOCHROME P450, SUBFAMILY IVB, MEMBER 1
600831 - DEATH-ASSOCIATED PROTEIN KINASE 1
120470 - DCC NETRIN 1 RECEPTOR
605755 - DOUBLECORTIN DOMAIN-CONTAINING PROTEIN 2
609631 - DEAD BOX POLYPEPTIDE 58
612002 - DEP DOMAIN-CONTAINING PROTEIN 1
305000 - DYSKERATOSIS CONGENITA, X-LINKED
126375 - DNA METHYLTRANSFERASE 1
613396 - DOWN SYNDROME CRITICAL REGION GENE 8
131244 - ENDOTHELIN RECEPTOR, TYPE B
190151 - ERB-B2 RECEPTOR TYROSINE KINASE 3
600543 - ERB-B2 RECEPTOR TYROSINE KINASE 4
133430 - ESTROGEN RECEPTOR 1
601663 - ESTROGEN RECEPTOR 2
600541 - ETS VARIANT GENE 1
611234 - FAMILY WITH SEQUENCE SIMILARITY 84, MEMBER A
175100 - FAMILIAL ADENOMATOUS POLYPOSIS 1

600212 - FATTY ACID SYNTHASE
176943 - FIBROBLAST GROWTH FACTOR RECEPTOR 2
134934 - FIBROBLAST GROWTH FACTOR RECEPTOR 3
136850 - FUMARATE HYDRATASE
617076 - FK506-BINDING PROTEIN-LIKE
605515 - FORKHEAD BOX P1
606829 - FRATAXIN
606146 - FRIZZLED CLASS RECEPTOR 8
300594 - G ANTIGEN 1
300595 - G ANTIGEN 2C
137168 - GAMMA-GLUTAMYLTRANSFERASE 5
238300 - GLYCINE DECARBOXYLASE
138350 - GLUTATHIONE S-TRANSFERASE, MU-1
103280 - H19, IMPRINTED MATERNALLY EXPRESSED NONCODING TRANSCRIPT
609310 - COLORECTAL CANCER, HEREDITARY NONPOLYPOSIS, TYPE 2
190020 - HRAS PROTOONCOGENE, GTPase
147700 - ISOCITRATE DEHYDROGENASE 1
608289 - INSULIN-LIKE GROWTH FACTOR 2 mRNA-BINDING PROTEIN 2
146732 - INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN 3
146733 - INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN 4
600150 - POTASSIUM CHANNEL, CALCIUM-ACTIVATED, LARGE CONDUCTANCE, SUBFAMILY M, ALPHA MEMBER 1
190070 - KRAS PROTOONCOGENE, GTPase
608802 - L3MBTL3
617986 - LOW DENSITY LIPOPROTEIN RECEPTOR CLASS A DOMAIN-CONTAINING PROTEIN 3
151623 - LI-FRAUMENI SYNDROME
604543 - LIM DOMAIN-CONTAINING PROTEIN 1
607908 - LIM AND SENESCENT CELL ANTIGEN-LIKE DOMAINS 2
618335 - LONG INTERGENIC NONCODING RNA 958
612571 - LUNG CANCER SUSCEPTIBILITY 3
608766 - LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1B
604710 - LATENT TRANSFORMING GROWTH FACTOR-BETA-BINDING PROTEIN 4
606551 - LEUCINE ZIPPER, PUTATIVE TUMOR SUPPRESSOR 1
300468 - MELANOMA ANTIGEN, FAMILY C, 2
300469 - MELANOMA ANTIGEN, FAMILY C, 3
606048 - MEMBRANE-BOUND O-ACETYLTRANSFERASE DOMAIN-CONTAINING PROTEIN 7
611802 - MIGRATION AND INVASION ENHANCER 1
610104 - MICRO RNA 125B1
610105 - MICRO RNA 125B2
612117 - MICRO RNA 143
611795 - MICRO RNA 145
611020 - MICRO RNA 21
614597 - MICRO RNA 302B
615469 - MICRO RNA 574
607303 - MORTALITY FACTOR 4-LIKE PROTEIN 1
616905 - MORF4 FAMILY-ASSOCIATED PROTEIN 1
516020 - CYTOCHROME b OF COMPLEX III
608486 - METASTASIS SUPPRESSOR 1
257300 - MOSAIC VARIEGATED ANEUPLOIDY SYNDROME 1
160745 - MYOSIN, HEAVY CHAIN 11, SMOOTH MUSCLE
108345 - N-ACETYLTRANSFERASE 1
612182 - N-ACETYLTRANSFERASE 2
609752 - NUCLEAR RECEPTOR COACTIVATOR 7
607272 - NDC80, S. CEREVISIAE, HOMOLOG OF
606073 - NADPH-DEPENDENT DIFLAVIN OXIDOREDUCTASE 1
162200 - NEUROFIBROMATOSIS, TYPE I
602157 - NEUROONCOLOGIC VENTRAL ANTIGEN 1
610894 - NEUREGULIN 4
602656 - ENDONUCLEASE III-LIKE 1
614843 - ODONTOGENIC AMELOBLAST-ASSOCIATED PROTEIN
300287 - P ANTIGEN FAMILY, MEMBER 4
617688 - PROSTATE ANDROGEN-REGULATED MUCIN-LIKE PROTEIN 1
605669 - PROSTATE AND BREAST CANCER OVEREXPRESSED 1
168600 - PARKINSON DISEASE, LATE-ONSET
608610 - PROGRAMMED CELL DEATH 4
609673 - PLATELET-DERIVED GROWTH FACTOR D
610789 - p53 AND DNA DAMAGE-REGULATED 1
115310 - PARAGANGLIOMAS 4
608528 - PHOSPHATIDYLINOSITOL GLYCAN ANCHOR BIOSYNTHESIS CLASS U PROTEIN
609414 - PHOSPHOINOSITIDE KINASE, FYVE FINGER-CONTAINING
175200 - PEUTZ-JEGHERS SYNDROME
179050 - PYRUVATE KINASE, MUSCLE
600259 - PMS1 HOMOLOG 2, MISMATCH REPAIR SYSTEM COMPONENT
607463 - PROTEIN PHOSPHATASE 1, REGULATORY SUBUNIT 13-LIKE
617373 - PROLINE-RICH COILED-COIL PROTEIN 2C
605172 - PROSTAGLANDIN E SYNTHASE
179590 - PROTEIN-TYROSINE PHOSPHATASE, RECEPTOR-TYPE, F
602505 - PAXILLIN
617889 - PYRIDINE NUCLEOTIDE-DISULPHIDE OXIDOREDUCTASE DOMAIN-CONTAINING PROTEIN 2
612403 - RAS-LIKE, FAMILY 11, MEMBER A
614041 - RB TRANSCRIPTIONAL COREPRESSOR 1
144700 - RENAL CELL CARCINOMA, NONPAPILLARY
164761 - REARRANGED DURING TRANSFECTION PROTOONCOGENE
612659 - REGULATORY FACTOR X, 6
603894 - REGULATOR OF G PROTEIN SIGNALING 6
615177 - RING FINGER PROTEIN 126
300359 - SARCOMA ANTIGEN 1
611306 - SCAVENGER RECEPTOR CLASS A, MEMBER 5
185470 - SUCCINATE DEHYDROGENASE COMPLEX, SUBUNIT B, IRON SULFUR PROTEIN
614549 - SERINE INCORPORATOR 2
602104 - SH3 DOMAIN-BINDING PROTEIN 2
600725 - SONIC HEDGEHOG SIGNALING MOLECULE
300330 - SPANX FAMILY, MEMBER C
602783 - SPG7 GENE
180860 - SILVER-RUSSELL SYNDROME
617108 - SESSILE SERRATED POLYPOSIS CANCER SYNDROME
604328 - STRUCTURE-SPECIFIC RECOGNITION PROTEIN 1
300826 - STROMAL ANTIGEN 2
604415 - STEAP FAMILY MEMBER 1
614766 - STRIATIN, CALMODULIN-BINDING PROTEIN 3
605303 - TRANSFORMING, ACIDIC, COILED-COIL-CONTAINING PROTEIN 3
612654 - TRICHOPLEIN
187270 - TELOMERASE REVERSE TRANSCRIPTASE
190000 - TRANSFERRIN
615466 - TALIN ROD DOMAIN-CONTAINING PROTEIN 1
603031 - TOLL-LIKE RECEPTOR 5
604657 - TRANSMEMBRANE 4 SUPERFAMILY, MEMBER 5
611029 - TRANSMEMBRANE PROTEIN 30B
616438 - TUMOR NECROSIS FACTOR-ALPHA-INDUCED PROTEIN 8-LIKE 3
191170 - TUMOR PROTEIN p53
603273 - TUMOR PROTEIN p63
614327 - TUMOR PREDISPOSITION SYNDROME
602076 - TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL, SUBFAMILY V, MEMBER 1
605284 - TSC1 GENE
610932 - TWINFILIN, DROSOPHILA, HOMOLOG OF, 1
605046 - UBIQUILIN 1
617500 - UROTHELIAL CANCER-ASSOCIATED GENE 1
189909 - ZINC FINGER E BOX-BINDING HOMEOBOX 1
618033 - ZINC FINGER PROTEIN 689
616186 - H19/IGF2-IMPRINTING CONTROL REGION

http://dx.doi.org/10.21037/tcr-20-2822

Appendix 2 Modules of bladder cancer network

Cluster	Score (Density*#Nodes)	Nodes	Edges	Node IDs
1	2.78	60	83	TUBB, IL6ST, TAF1C, CD19, TAF1B, SMN2, KIAA1549, BRAF, SMN1, BRCA2, TAF1A, TP53, H2AFX, TP73, CEBPA, DNMT3B, TUBA4A, DNMT1, MAP2K6, WRN, WT1, TRAF6, ATF1, SOS1, JAK1, MVA1, HCLS1, SPI1, SPIB, MARK4, MRAS, RAP1A, KAT2B, SUMO1, RASA1, UBTF, GAB1, RUNX2, TSG101, CTNND1, PRMT1, SYK, CABLES2, RACK1, NPM1, HIPK2, CDC20, TMX2-CTNND1, ZBTB16, NCL, FRS2, CABLES1, VAV2, ANAPC7, MAP4K1, PTPRM, SIN3A, MDC1, TRAF3IP2, ATF2
2	3.837	50	125	CRKL, ESRRA, ITGB2, PXN, TOP2A, CEBPD, DNMT3A, BTK, EZH2, SH3KBP1, CASP2, NCOA1, TRIP6, HDAC2, NEDD9, XRCC5, CASP7, THRB, RARA, YY1, CTBP1, PAK3, POU2F1, PARP1, ARHGEF7, XRCC6, CBLB, ATR, CCNA1, DHX9, EED, PPARGC1A, PIAS3, RELA, GIT2, NR3C1, BARD1, RFC1, EP300, KPNB1, CCND1, KPNA2, CHEK2, ID2, NR2F6, PPP2CA, AR, PRKCB, MEF2A, PRKCE
3	6.844	46	179	CRK, LAT, GRB7, MET, ESR2, PTK2B, AXL, COPS6, PLCG1, COPS5, RET, SMAD2, COPS7A, VAV1, MUC1, COPS8, SP1, COPS3, PDGFRB, HDAC1, SH3BP2, IRS2, JAK2, HNF4A, FOXO3, NCOA3, AKT1, CEACAM1, JUN, PAK1, FYN, ZAP70, ERBB3, COPS4, GPS1, COPS2, TRIP4, CTNNB1, PIK3R1, GTF2B, EGFR, PIK3R2, SRC, PTK2, TNFRSF1A, PRKCA
4	4.045	45	114	PRKCQ, MYOD1, INSR, BCAR1, CCNB1, ARNT, SUV39H1, MAP3K14, IGF1R, LYN, PTPN1, STAT5A, CEBPB, CSF3R, FGFR1, TRAF1, CSK, TAF1, NCOR1, GADD45G, DOK1, PML, NCOR2, FHL2, RAD51, LCK, ABL1, FOXO4, RPS6KA5, AKT2, CFLAR, MYC, FOXO1, HDAC3, GADD45B, DAXX, CAV1, CREBBP, NCOA2, NMI, EGR1, ERBB4, RBBP4, MDM2, KAT5
5	2.848	34	72	NFYB, MAP3K5, SREBF2, PRKDC, MAPK3, ESR1, TNFRSF14, MAPK1, TRIM28, ISL1, USF1, SNCG, ITGB3BP, TRIM24, RPA1, ATF3, SMAD1, EIF2AK2, TAF10, XPA, GNAI1, AOVD2, HOXC8, RAN, UBE2I, STRN, DAPK1, PEBP1, RLIM, TBP, LDB1, IKBKB, NFKB1, NFYA
6	2.7	21	35	PIK3R3, MCM4, CHUK, PLA2G4A, THRA, ARID3A, E2F1, MAPK14, MAPK8, IRS1, E2F4, YWHAB, CDK2, MAPK9, MAPK8IP1, YWHAH, TSC2, CREB1, RB1, CDK7, TSC1
7	2.111	19	34	APEX1, YBX1, PSEN2, CAD, POLB, MSN, BCL2L1, TOP1, CASP6, PCNA, NEK2, PPP1CA, CASP10, HIP1, TGFBR1, APAF1, CDK6, BAK1, BCAP31
8	2.111	19	33	USP7, CSNK2B, CCND2, NR4A1, PAK4, NEFL, BID, RAP1GAP, YWHAG, CDK5, PRKACA, YWHAZ, FOS, APC, MDM4, CDC25B, CDH1, PSEN1, CDKN1A
9	2.133	16	27	SMAD7, HSPD1, PPP2R1A, PKD1, AXIN1, MAGI2, TRADD, BCL10, BTRC, JUP, DLG4, FN1, ITGA3, CTNND2, ERBIN, GNA12
10	2.308	14	25	CSCF, XIAP, CASP9, RAF1, BCL2, AATF, LIMK1, EZR, MAPT, ROCK1, CHEK1, RPS6KA3, CDC25A, PIN1
11	2	12	21	SRF, CDK1, CDC42, GSK3A, RPS6KB1, MAPKAPK2, IRAK1, PRKCZ, RAC1, MTOR, RPS6KA1, MCL1
12	4.6	11	32	SHC1, STAT1, ERBB2, PTPN6, STAT5B, GRB2, PTPN11, SMAD3, SMAD4, BRCA1, CBL
13	2.5	9	17	SMARCB1, SMARCA4, AHR, STAT3, PJS, XPO1, HSP90AA1, PTGES3, NOS3
14	2	9	16	GRIN1, GRIN2D, GRIPAP1, CASP3, ATN1, IL16, STK4, VIM, GORASP1
15	3.143	8	14	NDC80, MAD2L1, AURKB, BIRC5, INCENP, CDCA8, CDC27, CDC16
16	2.667	4	7	CALR, LRP1B, SERPINE1, PLAT
17	2.667	4	5	KAT7, CASP8, CDK11B, CDK11A
18	2.667	4	4	RALGDS, HRAS, KRAS, RASSF2
19	3.333	4	5	HLA-DMA, HLA-DRA, HLA-DMB, CD63
20	2.667	4	6	ATM, FANCD2, RBBP8, MRE11
21	2	4	6	PGR, MSX1, PIAS1, PRMT2
22	4	4	9	PPFIA2, PPFIA3, PPFIA1, PTPRD
23	2.667	4	6	TLN1, VCL, ACTA1, S100A4
24	2	3	4	MAP2K1, DIABLO, BIRC6
25	2	3	4	STRAP, SUMO4, NFKBIA
26	3	3	4	CMM9, SMG6, SMG5
27	3	3	3	A1CF, APOBEC1, CELF2
28	2	3	4	WEE1, CCNT1, SKP2
29	3	3	4	KCNJ8, ABCC9, KCNJ11
30	2	2	3	PTPRA, PTPRF
31	2	2	3	CSNK1E, LOC400927-CSNK1E
32	2	2	3	HSP90AB1, MAP3K3
33	2	2	3	ITGB7, ITGA4
34	2	2	3	PLCG2, TEC
35	2	2	3	CD44, NF2
36	2	2	3	EIF4B, PABPC1
37	2	2	3	ITGB1, NME1
38	2	2	3	BMPR2, TOPBP1

http://dx.doi.org/10.21037/tcr-20-2822