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 1.3.2 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
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
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 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.
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.
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.

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.

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.
Training Program for the Young 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](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](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).

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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