Identification of GSN and LAMC2 as Key Prognostic Genes of Bladder Cancer by Integrated Bioinformatics Analysis

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Abstract

Bladder cancer is a common malignancy with mechanisms of pathogenesis and progression. This study aimed to identify the prognostic hub genes, which are the central modulators to regulate the progression and proliferation in the specific subtype of bladder cancer. The identification of the candidate hub gene was performed by weighted gene co-expression network analysis to construct a free-scale gene co-expression network. The gene expression profile of GSE97768 from the Gene Expression Omnibus database was used. The association between prognosis and hub gene was evaluated by The Cancer Genome Atlas database. Four gene-expression modules were significantly related to bladder cancer disease: the red module (human adenocarcinoma lymph node metastasis), the darkturquioise module the lightgreen module (grade 3 carcinoma), and the royalblue module (transitional cell carcinoma lymphatic metastasis). Based on betweenness centrality and survival analysis, we identified laminin subunit gamma-2 in the grade 2 carcinoma, gelsolin in the grade 3 carcinoma, and homeodomain-interacting protein kinase 2 in the transitional cell carcinoma lymphatic metastasis. Subsequently, the protein levels of LAMC2 and GSN were respectively down-regulated and up-regulated in tumor tissue with the Human Protein Atlas database. Our results suggested that LAMC2 and GSN are the central modulators to transfer information in the specific subtype of the disease.

Keywords: **bladder cancer**; **weighted gene coexpression network analysis**; **prognosis**; **GEO**; **TCGA**; **HPA**

Introduction

Bladder cancer ranks 13th in cancer death worldwide and has heterogeneous subtypes, in which the urothelial carcinoma is the most common [1,2]. According to the degree of muscle invasion and metastasis, urothelial carcinomas can be classified into three types: non-muscle invasive, muscle-invasive, and metastasis bladder cancer [2]. Resection and radical cystectomy are the mainstream treatment for non-muscle invasive and muscle-invasive bladder cancer, respectively. On the other hand, the cisplatin-based or gemcitabine-based combination therapies would be used as first-line treatment for metastasis or recurrent bladder cancer [3,4,5]. Most bladder patients were diagnosed with non-muscle invasive bladder cancer at presentation and with favorable prognosis [2]. However, 50% of recurrences and 20% of case progressions were observed in 5 years for patients with NMIBC [6]. In addition, patients can still present a poor prognosis when treated with standard treatment or immune checkpoint inhibitors following the failure of the standard cancer treatment [3,4,5]. Although the fibroblast growth factor receptor inhibitor was newly approved in 2019 and proved to increase the survival rates, only patients with FGFR genetic alterations can be treated [7].

Materials and Methods

4.1. Dataset Collection

A flowchart of this study is presented in **Figure 11**. Gene expression profiles of Dataset GSE97768 were downloaded from Gene Expression Omnibus database. This dataset contains the RNA-sequencing profiling of 30 human urothelial cancer cell lines. Except for BV cell lines with unknown origin, we included 29 cell lines, which were clustered according to the disease type (**Figure 12**). The disease type of human urothelial cancer cell line was retrieved from the American Type Culture Collection and ExPASy Bioformatics Resources Portal [59].he normalized data was retrieved from the GEO database. All the cancer cell lines on were categorized unclear inform with the disease.

Results

Identification of Candidate Genes with High Weighted Degree Score

Highly connected hub genes were defined by module connectivity (ModuleMembership > 0.8) and clinical trait relationship The hub genes of each module were visualized as networks in Gephi and screened out the top candidate gene by in-rank ordering of betweenness score for further analysis

Network analysis of the modules obtained from WGCNA was focused on the betweenness centrality (BC) of the genes within the modules. Since this measure reflects influence over the information transfer between different genes, we identified genes for which betweenness is considerably changed between the four networks (red module, darkturquioise module, lightgreen module, and royalblue module), as shown in **Figure 9**. Using the betweenness value to rank genes in the human adenocarcinoma lymph node metastasis network, we identified EIF5B as the gene with the highest betweenness (BC = 1.0), suggesting that it has a central role in information transfer in this module. The role of EIF5B in bladder cancer was unknown, but it was reported as an antagonist of the G0 phase. The overexpression of EIF5B caused cell death [32]. On the other hand, LAMA3 and LAMC2 were identified in the network analysis of the grade 2 carcinoma.

Discussion

Bladder cancer is a disease with high recurrence and variable prognosis. Although several prognostic models were proposed, most lacked accuracy .Better and more accurate biomarkers for cancer-specific prognosis are highly needed. On the other hand, due to the high recurrence rate of the standard treatment of bladder, exploring the molecular mechanisms involved in the development and progression of bladder cancer is important. Here, we used an integrated analysis to screen the prognostic biomarkers and potential molecular pathways in different disease types.

With the WGCNA analysis, we identified potential genes related to the survival of bladder cancer. Among the modules, clinical characteristic correlated subtypes as follows: human adenocarcinoma lymph node metastasis, grade 2 carcinoma, grade 3 carcinoma, and transitional cell carcinoma lymphatic metastasis. Confirmed by the TCGA database, 11 hub genes were found. In human adenocarcinoma lymph node metastasis, EPCAM, ACLY, ADD3, and SNRPD3 were identified. On the other hand, three genes were found related to the survival of bladder cancer in grade 2 carcinoma. GSN, HTRA1, and PDLIM5 were the prognostic genes in the grade 3 carcinoma. Moreover, we also found that patients with a high expression level of HIPK2 had a higher mortality risk.

ATP citrate lyase epithelial cell adhesion molecule HtrA Serine Peptidase 1 and laminin subunit gamma 2 were reported as a potential biomarker

Conclusions

We constructed a gene co-expression network and used betweenness centrality to identify and validate the hub genes associated with the prognosis of bladder cancer. The several potential genes and molecular mechanisms in different disease types found in this study require further investigation.

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This work is partly presented at Joint Event on 24th World Congress on Pharmacology & 7th World Heart Congress, August19- 20, 2019 at Vienna, Austria