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Research Article

BioinformaticsAnalysis of the Muscle-invasive Bladder Cancer Subtypes

Xu Wenbin，Zheng Weiying，Xia Hong，Hua Lin

School of Biomedical Engineering, Capital Medical University, Beijing 100069

Abstract: Objective In order to improve the accuracy in distinguishing subtypes of bladder cancer and to explore its potential therapeutic targets, we identify differences between two kinds of bladder cancer subtypes (basal-like and luminal) in molecular mechanism and molecular characteristics based on the bioinformatics analysis. Methods In this study, the RMA (robust multichip averaging) was applied to normalize the mRNA profile which included 22 samples from basal-like subtype and 132 from luminal subtype, and the differential expression analysis of genes with top 1000 highest standard deviation was performed. Then, the Gene Ontology and KEGG pathway enrichment analysis of differentially expressed genes was performed. In addition, the protein-protein interactions networks analysis for the top 100 most significant differentially expressed genes was performed. Results A total of 742 differentially expressed genes distinguishing basal-like and luminal subtypes were found, of which 405 were up-regulated and 337 genes were down-regulated in basal-like subtype. GO enrichment analysis showed that differentially expressed genes were significantly enriched in the extracellular matrix, chemotaxis and inflammatory response. KEGG pathway enrichment analysis showed that the differentially expressed genes were significantly enriched in the pathway of extracellular matrix receptor interaction. The hub proteins we founded in protein-protein interaction networks were LNX1, MSN and PPARG. Conclusion In this study, the mainly difference of molecular mechanism between basal-like and luminal subtypes are alteration in extracellular matrix region, cell chemotaxis and inflammatory response. Genes such as LNX1, MSN and PPARG were forecast to play important roles in the classification of bladder carcinoma subtypes.

Key words: bladder carcinoma; molecular subtype; differentially expressed gene; enrichment analysis; protein-protein interaction network

Corresponding Author: Hua Lin (E-mail: hualin7750 @ 139. com)

0 Introduction

Bladder carcinoma is a complex malignant tumor with multiple pathogenic factors.Its incidence rate ranks 9th among malignant tumors. The incidence of bladder carcinoma in men is 3 times higher than that in women, and the incidence rate increases with age. Bladder carcinoma can be divided into urothelial carcinoma, squamous cell carcinoma and adenocarcinoma, of which 75% to 80% are urothelial carcinoma. Clinically, bladder carcinoma can be classified into superficial bladder (Ta-T1) and in situ carcinoma (Tis) and the muscle-invasive bladder cancer (T2-T4) based on the degree of invasion or infiltration of primary tumors into the bladder wall. The muscle-invasive bladder cancer has rapid progression, high degree of malignancy, easy metastasis, and high recurrence and mortality rate. Although there are many therapy methods, the prognosis is poor and the effect is not ideal. The 6-year survival rate accounts for about 25%.

The pathogenic factors of bladder carcinoma can be summarized as the following 3 points: chemical environmental exposure, chronic irritation and related genetic abnormalities. Among them, there are aromatic amines, aniline dyes,nitrities and nitrates, acrolein and arsenic in the chemical environment exposure, but the most important environmental factor is smoking.

Chronic irritation includes indwelling catheter, infection and pelvic radiotherapy and so on. Genetic abnormalities include variation in genes such as FGFR3, TP53, and TSC1, the amplification of genes such as PPARG, CCND1, EGFR and the deletion of genes such as CDKN2A and RB1.

The genome-wide analysis shows that the major RNA-expressing subtypes of the muscle-invasive bladder cancer are basal-like and luminal, and different cancer subtypes have different biomarkers to promote the development of new therapy and auxiliary diagnostic tools. Compared with luminal, basal-like bladder cancer has a higher degree of malignancy and metastasis rate, a poorer prognosis, and a shorter survival period. Although basal-like bladder cancer is more invasive, it is more sensitive to cisplatin combined with chemotherapy, and p53-like tumors in luminal bladder cancer subtypes have a certain degree of resistance to chemotherapy. This may cause some problems for patients who need therapy. By comparing two subtypes of bladder carcinoma genes, it is found that the overexpressed genes in basal-like are EGFR, ZEB2, and the overexpressed genes in luminal bladder cancer are PPARG, FGFR3, etc. These genes can help to study the types of cancer and help to develop new therapeutic strategies and molecular targets for specific types of cancer.

In this study, using the gene expression profile data of two subtypes of the muscle-invasive bladder cancer, the basal-like and luminal bladder cancer, the screening for differentially expressed genes, functional annotations, and pathway enrichment analysis, the construction of protein interaction networks and other bioinformatics analysis are conducted to further study the differences in the molecular mechanisms of two subtypes of bladder cancer.

1 Materials and Methods

1.1 Data sources and data preprocessing

The data source in this study was the gene expression profile of bladder cancer samples in theCartes d'Identite'des Tumeurs (CIT) program in France, data numbered E-MTAB-1940. Available from Bioconductor's MineICA package (http://www.bioconductor.org/packages/release/bioc/html/MineICA.html).The dataset consisting of two bladder cancer subtypes included 22 samples from basal-like subtype and 132 from luminal subtype. The RMA (robust multichip averaging) was applied to standardized data preprocessing and the analysis of genes with top 1000 highest standard deviation was performed.

1. 2 Screening of differentially expressed genes

BRB-ArrayTools was an integrated data package for visualization and statistical analysis of microarray gene expression. This study used the BRB-ArrayTools 4.5.1 version to identify differentially expressed genes in two bladder cancer subtypes. The method used was t-test of two samples with a test standard of $P<0.05$.

1.3 Enrichment analysis of differentially expressed genes

In order to further identify the differences between two subtypes of the muscle-invasive bladder cancer at the functional genomic level, the online analysis tools (https://david.ncifcrf.gov/) of DAVID (The Database for Annotation, Visualization and Integrated Discovery) was used to perform GO (gene ontology) functional annotation (including biological processes, molecular functions, and cellular components) and KEGG (Kyoto encyclopedia of genes and genomes) enrichment analysis on the differential expression analysis of genes. The screening values were $P < 0.001$ and FDR <0.05.

1.4 Construction of the protein-protein interactions networks and identification of hub proteins

The current research has found that the systematic analysis of interaction of a large number of proteins in biological systems is of great significance in understanding the working principles of proteins in biological systems, understanding the response mechanism of biological signals and energy metabolism in special physiological states such as diseases, as well as understanding the functional links between proteins. In this study, the first 100 genes with the most significant differences were selected and the interactions with the target genes were extracted in the human protein interactions database HPRD (Human Protein Reference Database, http://www.hprd.org/), and thenCytoscape 3. 4. 0 bioinformatics analysis software was used to build the protein-protein interactions networks.

2 Results

2.1 Identification of differentially expressed genes

Through the test standard of $P < 0.05$, 742 differentially

expressed genes were screened in 1000 genes, of which 405 were up-regulated, and 337 genes were down-regulated.Heatmap of genes is shown in Figure 1.

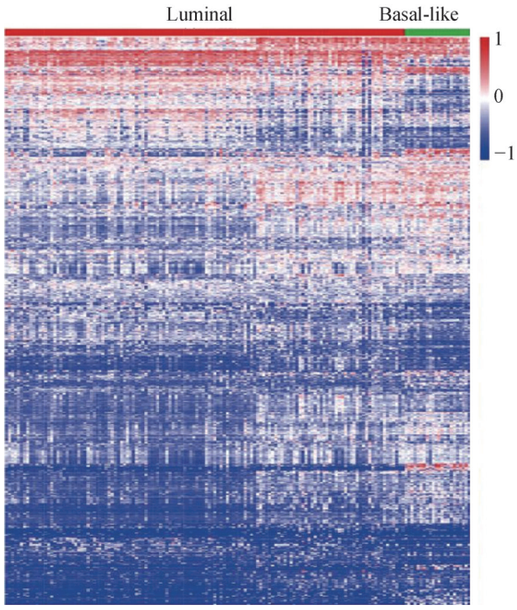
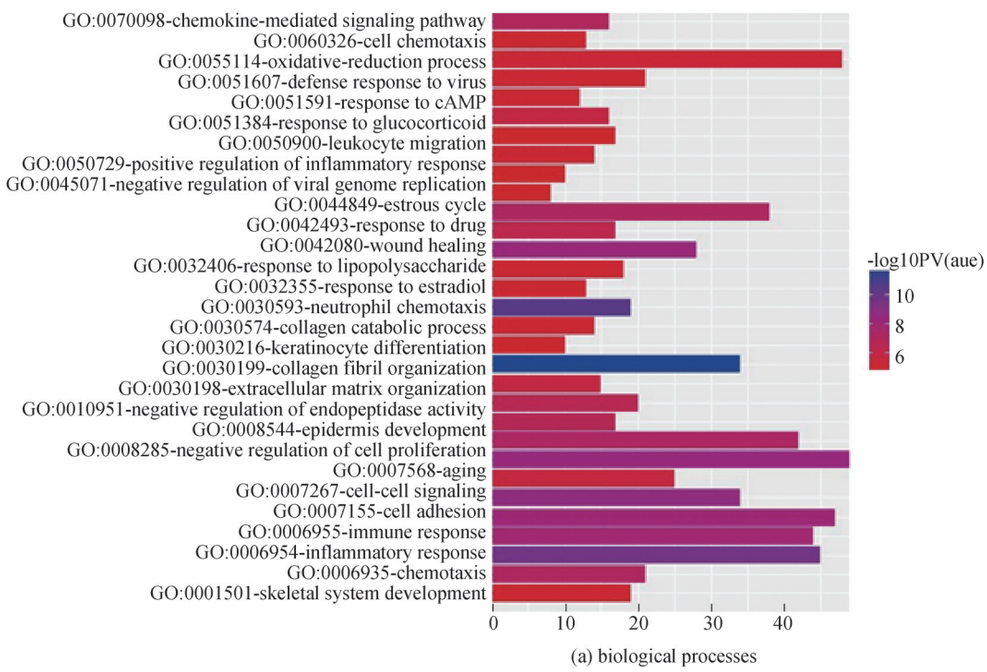


Figure 1 Heatmap of genes

2.2 Enrichment analysis of differentially expressed genes

The result of GO enrichment analysis for differentially expressed genes is shown in Figure 2. Each column in the figure represents the GO term. The length of the column indicates the number of genes enriched in this term (the higher the column, the greater the number of genes enriched in this term), the column color indicates the P value (the P value becomes smaller from red to blue). In the study, differentially expressed genes are most significantly enriched in extracellular matrix-associated life processes, including the assembly, arrangement of extracellular matrix components, and the breakdown of extracellular matrix. A large part is enriched in cell chemotaxis-related molecular mechanisms, such as cell chemotaxis, neutrophil chemotaxis, chemokine-mediated signaling pathways, and chemokine activity. In addition, the differentially expressed genes are also significantly enriched in response to glucocorticoids, which can play an anti-inflammatory and anti toxic effect, and to cyclic adenosin monophosphate (cAMP). Among them, cAMP can regulate the synthesis of neurotransmitters and promote the secretion of hormones. It is the second messenger in cells. cAMP also regulates cell proliferation, differentiation, and tissue development cAMP induces differentiation by downregulating the expression of RAS and MYC genes and inhibits the malignant phenotype of bladder cancer cells, which has an important role in the prevention and treatment of tumors.



(a) biological processes

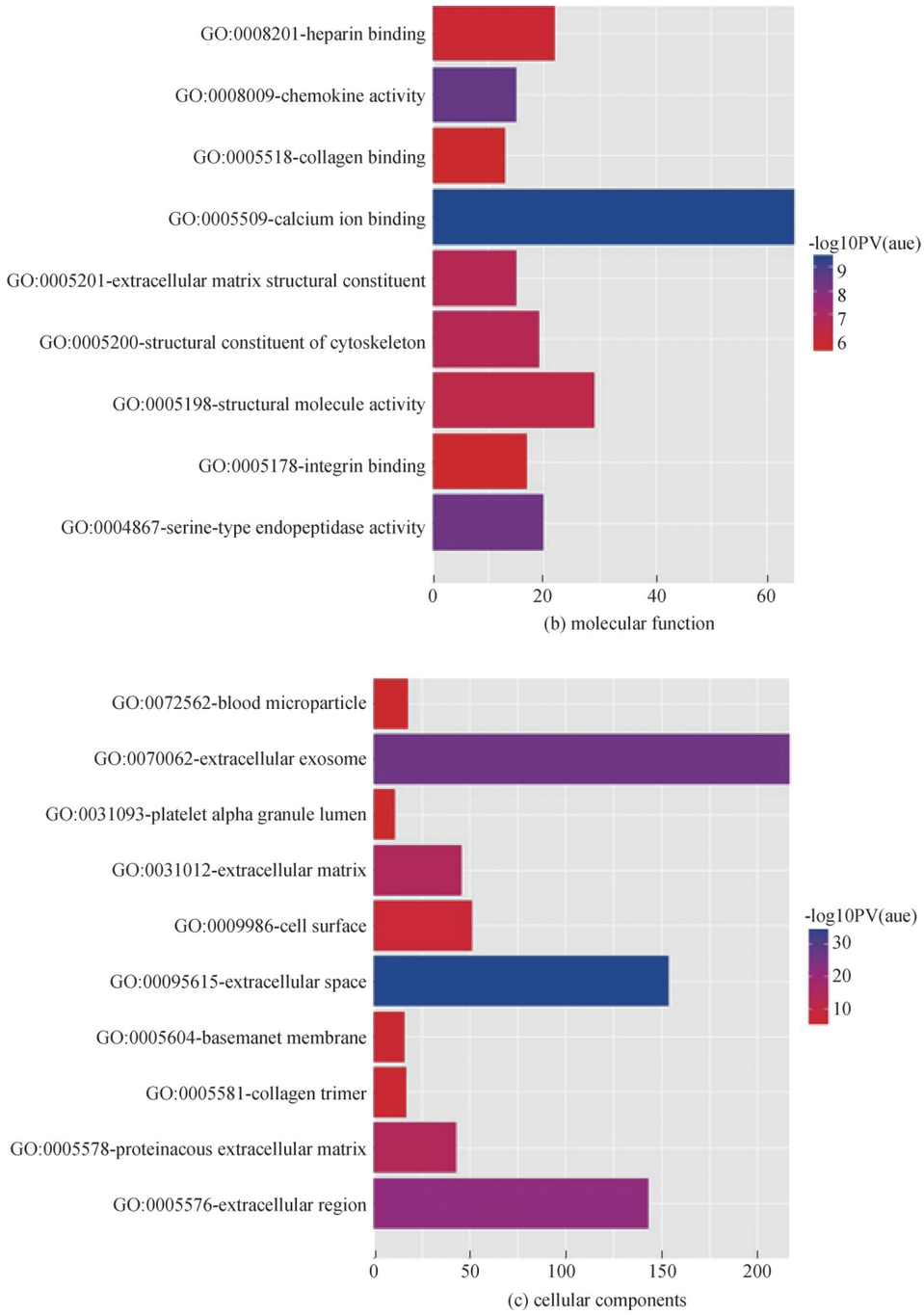
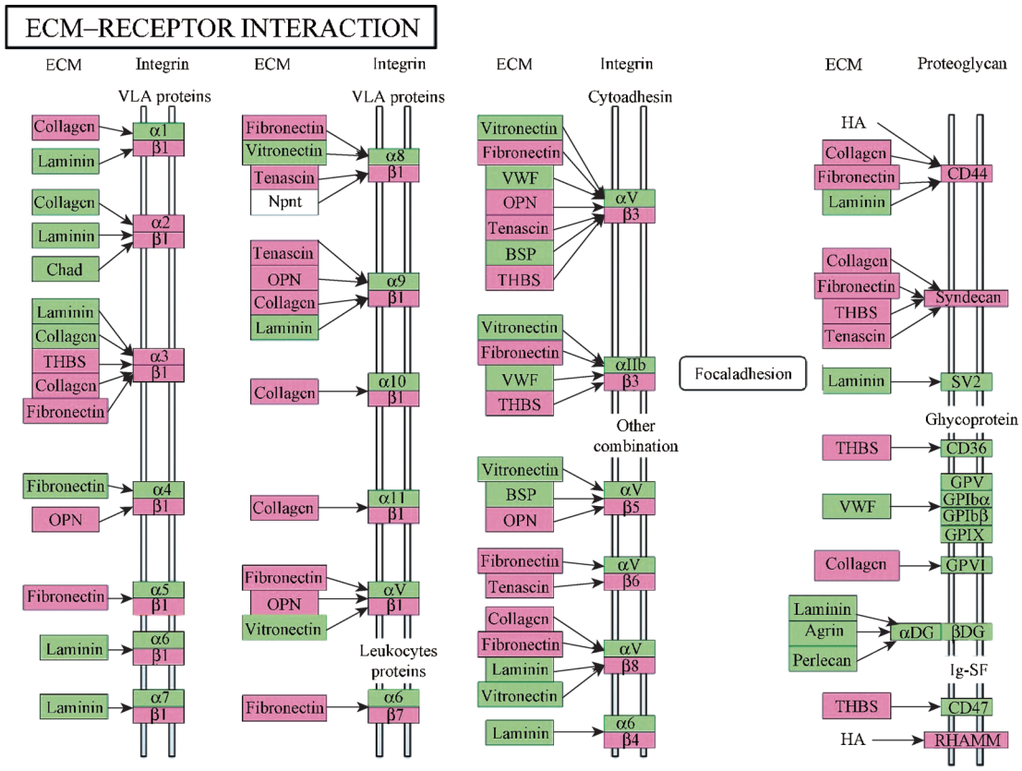


Figure 2 GO enrichment analysis

The KEGG pathway enrichmentwas performed on the differentially expressed genes using the on-line analysis tool of DAVID. The enrichment pathways obtained are shown in Table 1. It can be seen that the most significantly enriched pathway of differentially expressed genes is the extracellular matrix (ECM) receptor interaction. In addition, they are significantly enriched in adhesion plaques, complement and coagulation cascades, and infections (includes Staphylococcus aureus infection and amebiasis). The extracellular matrix receptor interaction pathway includes 18 differentially expressed genes, as shown in Figure 3.

Table 1 KEGG pathway names and P values of differentially expressed genes

KEGG pathway names	P
hsa04512: ECM-receptor interaction	2. 79E-07
hsa04610: Complement and coagulation cascades	3. 31E-07
hsa05150: Staphylococcusaureus infection	3. 99E-06
hsa05146:Amoebiasis	4. 99E-06
hsa05323:Rheumatoid arthritis	8. 43E-06
hsa04510: Focal adhesion	1. 92E-05
hsa00980: Metabolism ofxenobiotics by cytochrome	P450 2. 42E-05



The pink frame represents the differentiall expressed genes. The green frame represents the non-differentially expressed genes

Figure 3 Metabolic pathway of extracellular matrix receptor interaction

2.3 Construction of the protein-protein interactions networks and identification of hub protein

The selected differentially expressed genesare mapped to human protein-protein database HPRD and constructed the protein-protein interactions networks. The protein-protein interactions networks identified a total of 355 genes and 332 interactions. In protein-protein interaction networks, a node with a large degree of connectivity is called hub, which supports the important genes of basic life activities or their translation products. At the same time, the hub is significantly enriched with genes related to genetic diseases such as cancer.

In the construction of protein-protein interactions networks, the hub has LNX1 (connectivity is 47), PPARG (connectivity is 33) and MSN (connectivity is 20), so it is considered that these genes play an important role in subtypes of bladder cancer. The LNX1 gene is one of the four members of the LNX ubiquitin ligase family, which structurally includes the N-terminal RING domain and the four PDZ domains in the C-terminus. This gene participates in important signaling pathways such as Notch, neuregulin-1 /ErbB, participates in the reorganization of tight junctions, and plays an important role in tumorigenesis. MSN, or Moesin (for membrane tissue extension spike proteins), is a member of the ERM family, including

Ezrin and Radixin. The ERM protein acts as a cross-linker between the plasma membrane and the actin-based cytoskeleton. Moesin is located in filopodia and membranous protuberances and plays an important role in cell-cell recognition and signal transduction and cell movement. The PPARG gene encodes a member of PPAR-γ protein in the subgroup pf peroxisome proliferator-activated receptor (PPAR). PPARG can regulate cell growth, immune surveillance and adipocyte differentiation. In urinary system, PPARG activates the changes of urothelial cells and eventually leads to the expression of specific markers of terminal urothelial differentiation.

3 Discussion

Bladder cancer is a heterogeneous disease characterized by complex molecular alterations and abnormal gene expression. The muscle-invasive bladder cancer is often accompanied by cancer metastasis, which causes high mortality. Thegenome-wide mRNA expression profiling is widely applied to study the molecular heterogeneity of human bladder cancer and to explore the gene expression characteristics associated with cancer progression, metastasis and survival. The basal-like and luminal subtypes of the muscle-invasive bladder cancer respectively have different clinical features, molecular characteristics and molecular targets. Therefore, through bioinformatics analysis, it is of great significance to reveal the occurrence and development of two subtypes of bladder cancer as a molecular mechanism at the molecular level, thereby improving the diagnosis and therapy level of bladder cancer.

The emergence of high-throughput data analysis technology has accelerated the development of cancer research as never before. Recently, genome studies have found a large number of biomarkers of basal-like and luminal bladder cancer. The Choiteam have found that the ΔNp63α gene is significantly enriched in basal-like bladder cancer and controls the expression of basal-like bladder cancer biomarkers. In basal-like bladder cancer, ΔNp63α controls the adhesion between epithelial cells and extracellular matrix of basal-like tumors, which is similar to the results of this study. In the GO enrichment analysis, it is found that two differentially expressed genes of bladder cancer subtypes are most significantly enriched in extracellular space-related life activities, whereas the KEGG pathway analysis reveals that the differentially expressed genes are also enriched in the extracellular matrix receptor interaction pathway. The occurrence, development, invasion and metastasis of malignant tumors are often accompanied by changes in the expression of extracellular matrix and its cell surface receptors. Specific interactions between cells and extracellular matrix directly or indirectly control cell activities such as adhesion, migration, differentiation, proliferation, and apoptosis. Metastatic tumor cells interact with stroma in many stages of tumor progression and metastasis. Some specific types of matrices and basement membranes play a key role in the progression of malignant tumors and blood-borne dissemination.

Since the discovery of HRAS as the first oncogene in bladder cancer cell lines, a number of genes that have been mutated in bladder cancer have been identified, suchas TP53, RB1, TSC1, FGFR3, and PIK3CA.

The researchers have found a significant association between the grades of bladder cancer subtypes and gene mutations. FGFR3 and TSC1 mutations frequently occur in luminal subtypes, while RB1 pathway changes including RB1 mutations/deletions, CCND1 amplification, SOX4/E2F3 expansion, and CCNE1 amplification frequently occur in basal-like subtype bladder cancer. The hub proteins in the constructed protein-protein interactions networks are LNX1, MSN and PPARG. It can be considered that the genes encoding these proteins play an important role in the grades of the muscle-invasive bladder cancer. PPARG is the most well-known biomarker of the three types of genes in bladder cancer subtypes research. Many studies have reported on this gene. In the copy number detection of bladder cancer, the PPARG gene can be found to be significantly amplified in luminal bladder cancer. Therefore, overexpression of PPARG gene is an important marker feature of luminal bladder cancer. PPARG is an oncogene associated with differentiation in bladder cancer, and the PPARG agonists inhibits the growth of bladder cancer cell lines in vivo and in vitro, which indicates that PPARG will be an important target for the treatment of bladder cancer if the molecular subtype specific effects of cancer cell biology are better understood. At present, there are few reports about the role of LNX1 and MSN in the grades of bladder cancer subtypes, but the mutations of these two genes play an important role in the occurrence of tumors. So far, there are not many studies on the molecular subtypes of bladder cancer. The LNX1 and MSN in this study may

be a new discovery and may become new therapeutic targets. This requires a large number of clinical trials and analysis to verify.

Recently, studies have found that bladder cancer and breast cancer share some genetic similarities. Through genome-wide mRNA expression profiling analysis, breast cancer can be roughly divided into five subtypes:claudin-low, basal, luminal A, luminal B and [HER2]-enriched. The claudin-low, basal subtype of breast cancer and the basal-like subtype of bladder cancer, the luminal A subtype of breast cancer and the luminal subtype of bladder cancer are highly similar, and have similar survival, prognosis and similar genetic signaling pathways. It is worth mentioning that the MSN gene found in this study also shows differential expression in the basal and luminal subtypes of breast cancer, which further illustrates the genetic similarity between bladder cancer and breast cancer. If bladder cancer subtypes can play a role in therapy grading like breast cancer subtypes, it will be particularly helpful for doctors to develop the best treatment process for patients, which is also one of the tasks to be done in the future.

4 Conclusion

In this study, the bioinformatics methodis applied to analyze the mRNA expression profiles of two subtypes of bladder cancer, and the differences in biological functions and signaling pathways between two different subtypes of bladder cancer are found, and some key genes of LNX1, MSN and PPARG that can be helpful to distinguish the subtypes of bladder cancer are found. However, because of the limited data set of the subtypes of bladder cancer, more data sets analysis is needed to ensure the stability of the research results. These results can be used as a basis for the design of the topic and can be used as a clue to further explore the molecular mechanism of bladder cancer subtypes.

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