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Original Article

Top 250 gene set enrichment to screen the metastasis key genes of renal cell carcinoma

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Abstract: The goal of this study was to investigate the key metastasis genes in renal cell carcinoma (RCC) in order to establish the scientific foundation for therapy. GEO2R, an online analysis tool in Gene Expression Omnibus website (GEO), was used to analyze the derivatives of the top 250 genes of GSE47352, downloaded from GEO. Key metastasis genes were then screened out by Gene Ontology (GO), Kyoto encyclopedia of Genes and Genomes (KEGG), protein-protein interaction (PPI), and Venn methods. Summing up the results of enrichment analysis by PPI and Venn, cellular response to stimulus, cellular focal adhesion, tube development, and cellular communication were the main significant changes between metastasis RCC (meta-RCC) and non-metastasis RCC. JUN and LAMA4 may be key metastasis genes for RCC metastasis, and further studies are needed to determine the biological function of these key metastasis genes.

Keywords: RCC, GEO, metastasis, GO, Key gene

Introduction

Renal cell carcinoma (RCC) is a common urinary neoplasm, which accounts for 3% of adult malignant tumors. Clear cell renal cell carcinoma is the predominant form, accounting for about 85%-90%. It is worth to note that the incidence and mortality are increasing year by year [1-3]. Surgical resection is mainly performed for the treatment of early RCC. Minimally invasive surgery represented by laparoscope has been widely carried out in China in recent years, which has brought hope to patients with early RCC. Unfortunately, 30% of the patients still have tumor recurrence or distant metastasis after surgery, and some patients even have distant metastases just at the first visit. Currently, small molecular targeted drugs, such as tyrosine kinase inhibitors (sunitinib, sorafenib) and mTOR inhibitors (temsirolimus, everolimus), are first-line drugs for metastatic kidney cancer (metastatic RCC, mRCC). But mRCC are highly resistant to clinical radiotherapy, chemotherapy, and immunotherapy, with poor clinical prognosis. About 50% of the patients have a short survival time less than 1 year, and the 5-years survival rate is only 10% [4, 5]. Although many studies have proved that the VHL-HIF signaling pathway is the main factor for the development of kidney cancer and metastasis [6-8], Young AC et al., found that VHL mutations and deletions have no relationship with prognosis of RCC patients [9]. So, further study is urgently needed to explore new targets and strategies for the treatment of kidney cancer.

The traditional "one disease, one gene" research model cannot clarify the occurrence and development of diseases under the multi-level and multi-gene cooperation model. High-throughput sequencing and gene expression chip technology can achieve the expression level of all genes in the disease genome [10, 11]. By using high-throughput data and using gene function enrichment analysis, researchers can comprehensively and systematically elucidate signal pathways playing key roles in disease development or progression, and reveal the underlying molecular mechanisms of diseases [11, 12]. In our study, GSE47352 was downloaded from the Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/gds/), including 5 non-metastatic RCC patients and 4 metastatic RCC patients. The patients'

tumor tissue was extracted for gene expression profiling, and bioinformatics analysis tools were used to analysis molecular mechanisms of renal cancer metastasis and key biological signaling pathways, and to find new targets for the treatment of renal cancer.

Materials and methods

DNA microarray data

Microarray data of GSE47352 was downloaded from the official website of GEO database Dong et al. [13]. Gene expression profiling was obtained from 5 non-metastatic RCC patients and 4 metastatic RCC patients using GeneChip U133 Plus 2.0 array [13].

DNA microarray data extraction

RCC patients were divided into two groups, non-metastatic (5 patients) and metastatic (4 patients). GEO2R, an online analysis software of the GEO database, was used to extract the top 250 significant changed genes (Top 250) between non-metastatic and metastatic groups, the information including: Gene.symbol, Gene.title, *P*.Value, adj.P.Val, logFC, etc., meanwhile, deleting the uncertain gene results.

Gene enrichment analysis

Top 250 genes in GSE47352 were submitted to Omicsbean (http://www.omicsbean.com:88//) system to do GO (Gene Ontology) enrichment analysis, including Biological Process, Cell Component, Molecular Function enrichment; KEGG pathway enrichment; and protein-protein interaction (PPI) model construction. GO analysis provides a comprehensive model of biological systems, based on the comprehensive resource currently available for computable knowledge regarding the functions of genes and gene products. KEGG pathway drawn pathway networks, including metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, human diseases, drug development, based on knowledge about molecular interaction, reaction and relation. PPI provides an investigation of protein complex structures and gaining insights into various bio-processes by combining bioinformatics and structural biology. Omicsbean online system was developed and maintained by Jinfukang Biotechnology (Shanghai) Co., Ltd.

Venn analysis

In Dong's study, 6 key RCC metastasis genes were screen out by considering signal-net analysis, differential gene *P* value, differential gene variation range, and betweenness centrality. In our study, a Venn diagram was used to find out the core genes for RCC metastasis by combining Top250 genes and 6 key genes.

Results

Molecular function enrichment

In our study, GO molecular enrichment analysis was used to analyze Top 250 genes between non-metastatic RCC and mRCC patients. The results showed that 36 gene products, such as SOX21, EPAS1, TP63, JUN, and TEAD2, are involved in nucleic acid binding, tumor cell proliferation, drug resistance, and angiogenesis. Furthermore, 35 gene products function by binding to intracellular cations to regulate cellular physiological processes, and 12 gene products are involved in regulation of RNA polymerase II transcription factor activity (**Figure 1**).

Cell composition enrichment

In order to figure out the genes that were changed significantly in the cell composition during metastasis, cellular components enrichment analysis showed that there were 86 gene products, accounting for about 60% of organelle-related genes, involved in regulation of intracellular organelle functions in the process of renal cell metastasis, such as TNF, ADAM8, and RAB25. An additional 9 gene products were extracellular matrix proteins, for example, LAMA4, MMP28, ADAMTSL3, LAMA1 etc. and 39 gene products were involved in storage and transport of cell vesicles (**Figure 2**).

Biological pathway enrichment

Top 250 genes enrichment analysis results showed that most of top 250 genes, are enriched in cell growth and development, signal transduction, and cellular chemical stress response, etc. It is worthy to note that SOX21, TNF, IL2, EPAS1, TP63, and JUN are involved in regulation of multiple signaling pathways (**Figure 3**). The direct pathways associated with

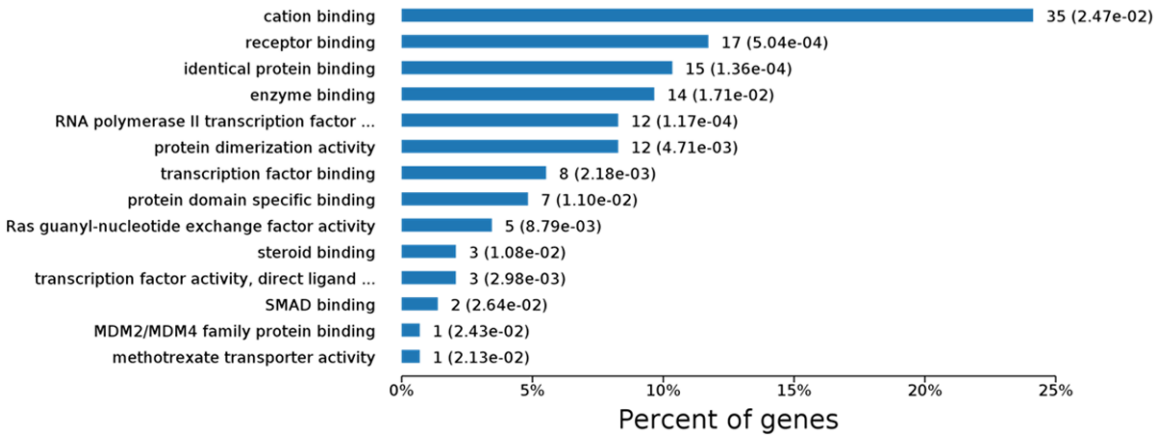

Figure 1. Molecular Function enrichment of Top 250 genes set: The Top 250 genes derived from 5 non-metastatic and 4 metastatic RCC patient tissues were subjected to GO analysis using Omicsbean software to putative molecular activities of gene products, function molecular clusters were graphed on y-axis.

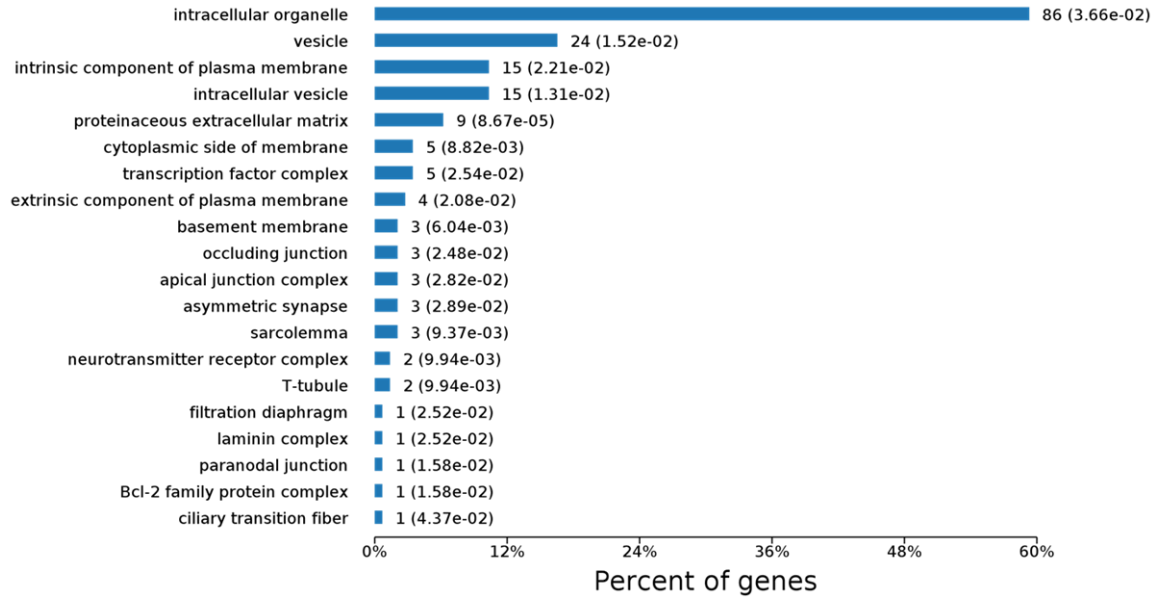

Figure 2. Cell Component enrichment of Top 250 genes set: The Top 250 genes derived from 5 non-metastatic and 4 metastatic RCC patient tissues were subjected to GO analysis using Omicsbean software to predict where gene products are active, genes cell component clusters were graphed on y-axis.

invasion and metastasis of RCC were vascular morphology, endothelial cell growth, cell differentiation, and tubule formation.

KEGG pathway enrichment

KEGG, a biological information database, combined with gene chip technology can identify deterministic signaling pathways during disease progression. In our study, pathway enrichment analysis revealed that top 250 genes were mainly concentrated in tumor, renal cell carcinoma, inflammation, lung cancer, tumor MicroRNA pathway, etc. The significantly changed pathways between metastatic and non-metastatic patients were MAPK and JAK-STAT signaling pathways, and cell adhesion ability was also significantly changed between metastatic and non-metastatic patients (**Figure 4**).

PPI

The hub protein in the network and the key genes related to metastasis of RCC were screened out by top 250 genes PPI constructed. The PPI results showed that the main path-

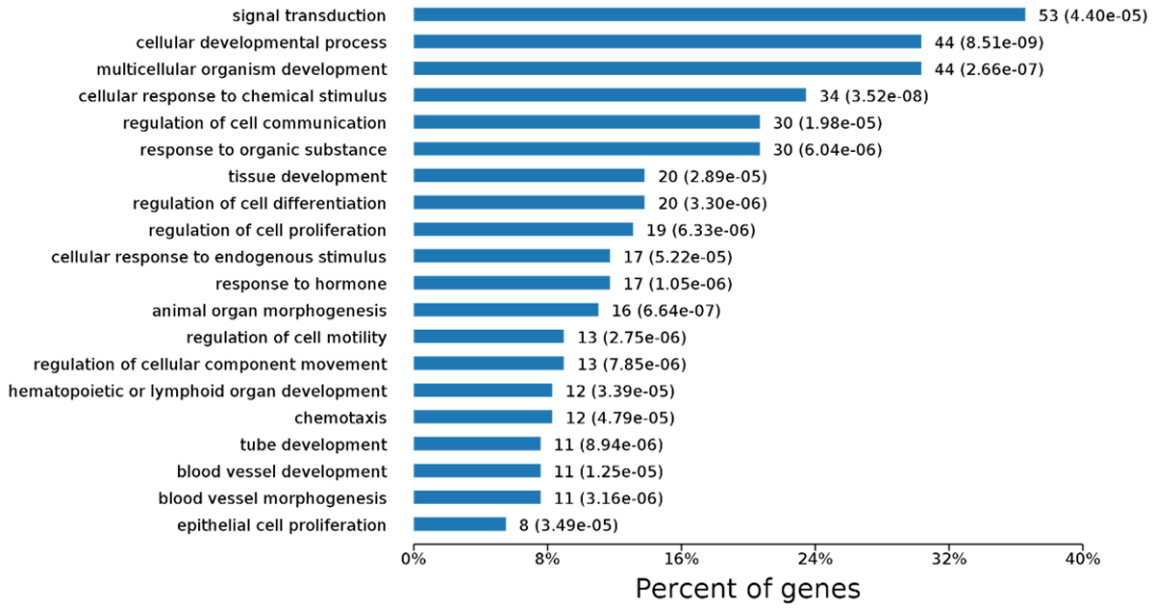

Figure 3. Biological Process enrichment of Top 250 genes set: The Top 250 genes derived from 5 non-metastatic and 4 metastatic RCC patient tissues were subjected to GO analysis using Omicsbean software to estimate processes made up of the activities of Top 250 genes products, processes clusters were graphed on y-axis.

way associated with renal cell metastasis is the renal cell carcinoma pathway, which encompasses six gene products: PTPN11, EPAS1, SOS2, TGFB2, ETS1, and JUN (**Figure 5**). It is worth nothing that there are two important transcription factors in RCC development, one is well-known EPAS1, another is JUN, which may play a crucial role in the RCC metastasis.

The core gene of RCC metastasis

To screen the key genes for metastasis of kidney cancer, the top 250 genes were combined for signal-net analysis results, and *P* values of the differentially expressed genes in metastatic and non-metastatic patients were analyzed, as well as betweenness centrality, and the magnitude of the differential expression genes. Altogether, these analyses obtained LAMA4 and JUN (**Figure 6**), which might be core genes for renal cancer metastasis.

Discussion

Metastasis of RCC is closely related to the malignant degree of RCC cells. Unfortunately, the molecular mechanism of renal cell metastasis is still unclear. In our study, software analysis was used to explore key genes for kidney cancer metastasis from the level of the gene cluster rather than the level of the single gene. Analyzing top 250 genes by GO assay, KEGG Pathway enrichment, and PPI analysis, we found that gene expression regulation, cell growth, cellular stress, cell adhesion properties, angiogenesis, and cellular out-migration transfer biological functions are the most significant changes between metastatic and non-metastatic patients.

Angiogenesis is an important process for the survival and metastasis of tumor cells [14]. In our enrichment analysis, most Top 250 genes were involved in angiogenesis of renal cancer, and secretion of endothelial growth factors to promote endothelial cell growth and tubule formation, including Hif 2α, an important transcription factor promoting angiogenesis [6, 7]. The metastasis of tumor cells needs to change the adhesion and degradation of extracellular matrix, and studies have shown that metastasis of tumor cells needs to break through the basement membrane and transfer to other tissues. Therefore, changes in the tumor extracellular matrix are important changes in tumor cell invasion and migration. In our enrichment analysis, 9 gene products were extracellular matrix proteins, including MMP28, LAMA4 and LAMA1. MMP and LAMA family are both important components of extracellular matrix, and also closely related to metastasis. In recent

Figure 4. Enriched KEGG pathways: The Top 250 genes derived from 5 non-metastatic and 4 metastatic RCC patient tissues were subjected to GO analysis using Omicsbean software to drawn pathway map of Top 250 genes products based on our knowledge on the molecular interaction, reaction and relation networks for meta-RCC disease.

years, it has been determined that exosomes are important signaling transmitters for the tumor cells. Tumor cells can inhibit immunity by releasing exosomes. Exosomes can promote tumor growth, formation of blood vessels of endothelial cells, and carry specific membrane proteins to a specific organ, forming a metastasis "niche", waiting for tumor cells metastasis [15-17]. According to our study, 39 genes were involved in extracellular vesicles and vesicle transport. Among them, RAB25 and TNF have been reported to promote tumor cell proliferation, invasion, and metastasis in various tumors such as lung cancer, ovarian cancer, and gastric cancer [18-20]. The results of KEGG pathway enrichment and PPI showed that TNF, EPAS1, TP63, JUN and other genes might play crucial roles in the metastasis of renal cancer.

These above results indicate that there should be several key genes in top 250 for metastasis of kidney cancer. Then LAMA4 and JUN were screened out by considering signal-net analysis, *P* value, differential gene variation range, betweenness centrality, and top 250 genes. This result suggested that LAMA4 and JUN might be key core genes for kidney cancer metastasis, consistent with the PPI result.

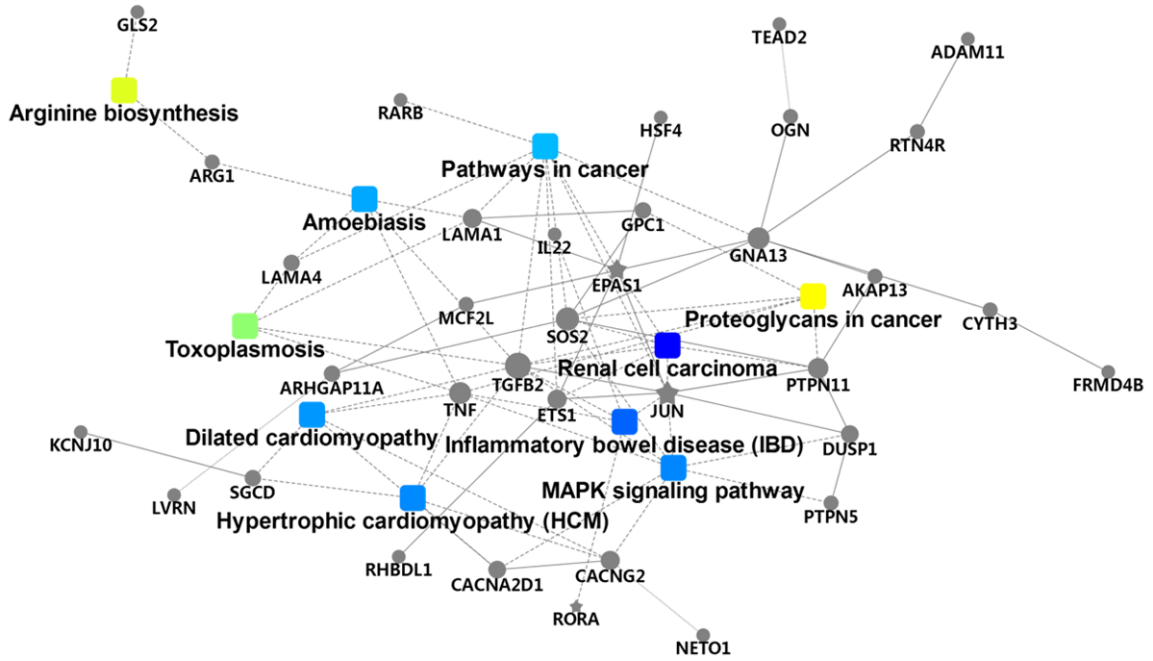

Figure 5. Protein-protein interaction: The Top 250 genes derived from 5 non-metastatic and 4 metastatic RCC patient tissues were subjected to GO analysis using Omicsbean software to construct the interaction of proteins based on biochemistry, quantum chemistry, molecular dynamics, signal transduction, among others.

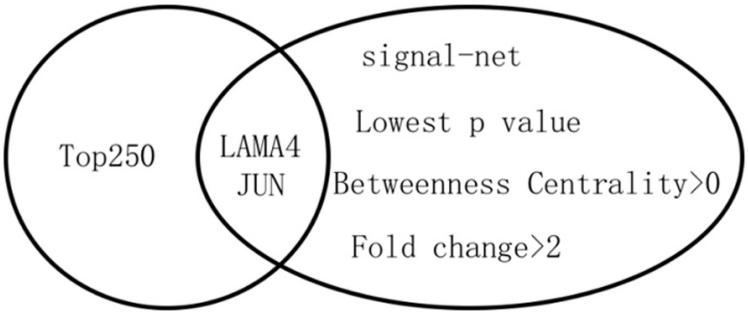

Figure 6. LAMA4 and JUN were the metastasis key genes: A Venn diagram was used to figure out the core genes for RCC metastasis by combining Top 250 genes and 6 key genes.

Furthermore, relevant information and biological functions of LAMA4 and JUN was searched in the Pubmed database. LAMA4 is a functional subunit of laminin 8, which is an important extracellular matrix and plays an important role in cell support, attachment, and maintenance of morphology. Simultaneously, LAMA4 also affects cell adhesion, growth, migration, and signal transduction [21]. Some researchers have reported that LAMA4 can affect the cell's basement membrane, the ability of cell invasion, and migration through regulating MMPs family [22]. Although there are few studies talking about the role of LAMA4 in tumor metastasis, gene chip and enrichment analysis results suggested that LAMA4 might play an important role in RCC metastatic process. We will evaluate its function in metastasis of kidney cancer in our future study. JUN, also known as c-Jun, is a subunit of the intracellular transcriptional activator AP-1 dimer [23]. AP-1 is an important factor in cell stress response (radiation, growth, stress, etc.). It has been reported that AP-1 can regulate cells responding to cytokines, growth factors, bacterial and viral infections, and stress, thereby controlling cell differentiation, proliferation, and apoptosis in a variety of tumors, playing an important role in the metastasis of tumor cells [23, 24]. These data indicate that JUN may be an important transcription factor for RCC metastasis.

In our study, JUN and LAMA4 were screened out by analyzing top 250 genes using a variety of bioinformatics methods. In future study, we

will detect the expression of LAMA4 and JUN in patient tissue samples, evaluate their relationships with the prognosis and survival of RCC patients, as well as the biological function in RCC metastasis, revealing potential new targets for clinical treatment of RCC.

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Disclosure of conflict of interest

None.

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