Original article

Involvement of erbB4 and tumor marker genes in renal carcinoma regulatory network

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A B S T R A C T

Background: Renal carcinoma is a common urologic tumor, and there is no ideal tumor marker for clinical diagnosis except for imaging diagnosis. This study aims to screen the serum tumor markers closely related with the benign and malignant of renal carcinoma out and chart out the regulatory network that involves renal carcinoma-related genes.
Methods: Based on 96 pathologically diagnosed renal cancer patients, factors strongly linked to renal carcinoma character were selected using Fisher discriminant analysis. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were utilized to manipulate function annotation of erbB4 and the selected genes and pathway analysis.
Results: Four essential tumor markers CYFRA21-1, CA125, VHL and HIF-1β were successfully screened out. Using GO and KEGG databases, the regulatory network of renal cancer cell escaping apoptosis was charted out on the basis of erbB4 signaling pathway.
Conclusion: Serum tumor marker genes play a certain part in the genesis and development of renal carcinoma. We preliminarily illustrated the molecular mechanism of these markers to predict tumor, laying a foundation for further exploration in renal carcinoma.
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1. Introduction

Renal carcinoma is a malignant tumor originating from renal tubular epithelial cells, accounting for more than 85% of all kidney tumors. Renal cell carcinoma (RCC) accounts for about 2–3% of adult malignancies. Its incidence ranks the second place in urinary tract tumors, second only to bladder cancer (Siegel et al., 2016). In countries with high incidence of renal carcinoma, 40% of cases are associated with smoking and obesity (Kokabi et al., 2015). With the increase in medical level, most patients can achieve early discovery and timely treatment of the disease, but there are still about 1/4 patients with tumor metastasis when it is discovered. It is reported that about 40% patients with renal carcinoma eventually die from

tumor and its complications (Bianchi et al., 2011). At present, clinically, domestic and foreign preoperative diagnosis of renal carcinoma still mainly depends on MRI/CT imaging data. However, there are still many clinical renal occupied lesions whose tumor nature cannot be determined simply by imaging data, surgical exploration and even nephrectomy are needed. Therefore, it is of important significance to explore the auxiliary tool to determine benign and malignant nature of renal tumor outside imaging data (such as tumor markers). Tumor markers are chemical substances that reflect the presence of tumor. The commonly used tumor markers for tumor diagnosis include carcino-embryonic antigen (CEA), carbohydrate antigen (CA125, CA199, CA153), etc. Rational use of these markers can help early diagnosis and treatment of renal carcinoma. Liu (2013) measured serum CEA, CA125 and Cyfra21-1 content of renal carcinoma patients before and after treatment. The result showed that positive rate of serum CEA, CA125 and Cyfra21-1 combined detection in renal carcinoma patients was higher than that of single detection. There are also studies showing that combined detection of serum P53 antibodies and CEA can improve the diagnosis rate of renal carcinoma, and it is found that CEA has relevance with clinical and pathological indicators (Shi, 2009). Domestic and foreign scholars have found

that combined detection of CEA and β-MG in patients with nephropathy has strong specificity and sensitivity in early diagnosis, identification, efficacy and prognosis evaluation of renal carcinoma (Zhao et al., 2016). At present, few researchers have undertaken in-depth study in internal molecular mechanism for these markers' involvement in renal cancer cell regulation. erbB4 gene is a regulatory gene in the growth of renal carcinoma. HER4 protein plays an important role in maintaining the growth of renal cancer cell and may become the target of tumor immunotherapy. Therefore, it is proposed to supplement the tumor marker gene into the regulatory network on the basis of the original signal pathway of erbB4, to reveal the internal molecular regulation mechanism for predicting tumorigenesis and development, which will lay the foundation for further deeper research on tumor suppression.

2. Materials and methods

2.1. Research objects

This study collected the patients with primary renal carcinoma between 2014 and 2016. All patients were in line with WHO diagnostic criteria for renal carcinoma and confirmed by surgical biopsy. Excluding cases with acute inflammation, abnormal vascular hyperplasia, a total of 96 cases are included. There were 41 cases of high differentiated carcinoma, 35 cases of moderate differentiated carcinoma and 20 cases of poor differentiated carcinoma, including 52 males and 44 females. All the cases were clearly diagnosed via CT imaging and pathology by studying their medical records. Aged 42–78 years, the patients were in the mean age of (58.63 ± 9.31) years. The 80 cases of healthy volunteers in our hospital for the same period were included, including 45 males and 35 females. Aged 46–75 years, the group were in the mean age of (59.26 ± 9.42) years. There being no statistically significant difference in baseline information such as gender, age between the two groups ($P > .05$), the results are comparable.

2.2. Determination of serum tumor marker content

In this study, eight serological parameters of blood samples were examined. The eight indicators were carcino-embryonic antigen (CEA), cytokeratin 19 (CYFRA21-1), carbohydrate antigen 125 (CA125), tumor antigen 199 (CA199), carbohydrate antigen 724 (CA724), hypoxia-inducible factor (HIF-1α, HIF-1β), VHL gene product VHL protein. The detection of the indicators followed the operation steps in kit instructions.

2.3. Research method

2.3.1. Fisher discriminant analysis

Fisher discriminant analysis was proposed by British statistic scientist Fisher in the 1930s. For the first time, the definition of

Fisher discriminant analysis was applied to iris analysis. Until now, Fisher discriminant analysis is still in constant improvement, which is recognized as one of the best feature extraction methods. The basic idea of Fisher discriminant is to linearly project the sample data, so that inter-class dispersion of the post-projection data is maximized while the intra-class dispersion is minimized. In this study, SPSS 23.0 was used to screen all serum tumor markers by Fisher discriminant analysis, in an effort to screen out the factors closely related to benign and malignant nature of renal carcinoma.

2.3.2. GO analysis of renal carcinoma related genes

The gene corresponding to tumor markers closely related to benign and malignant nature of renal carcinoma was searched by literature search. Go to AmiGO homepage (http://geneontology.org/) for GO analysis. The screening condition Taxon was "Homo sapiens". Respective preliminary analysis was done for the screened serum tumor marker gene and renal carcinoma related gene erbB4. Function annotation of the genes was performed from cell components, molecular function and biological process to determine respective function and lay the foundation for follow-up study.

2.3.3. KEGG analysis of renal carcinoma related genes

Enter the KEGG database homepage (http://www.kegg.jp/kegg/pathway.html), enter the keyword "renal cell carcinoma", "erbB4" to find the signal pathway; find key factors for action of gene corresponding to tumor markers, then take these key factors as a breakthrough, link the signal path with key nodes on the basis of erbB4 signal pathway, and then add the role of the tumor markers.

3. Results

3.1. Determination of serum tumor marker content

The examination results of tumor markers in 176 patients of malignant renal carcinoma and control groups are shown in Table 1. Indicators for the eight serum tumor markers are CEA, CYFRA21-1, CA125, CA199, CA724, HIF-1α, HIF-1β and VHL protein. According to the table, the serum levels of the six serum tumor markers are higher in the malignant renal carcinoma group than in the control group ($P < .05$) except CA199 and CA724.

3.2. Fisher discriminant screening results

According to the clinical test results, the eight serological influencing factors of CEA, CYFRA21-1, CA125, CA199, CA724, HIF-1α, HIF-1β, VHL protein were used as variables for the discriminant analysis model. The Wilks' lambda method was used to analyze these variables step by step, and four statistically significant predictors, CYFRA21-1, CA125, HIF-1β, VHL, were selected by using F

Table 1
Detection results of 8 serum tumor markers for renal carcinoma ($\overline{X} \pm S$).

Serum tumor marker	Renal carcinoma group	Control group	t	P
CEA (ng/mL)	16.12 ± 4.85[*]	3.87 ± 0.58	3.83	<.01
CA125 (U/mL)	50.46 ± 6.59[*]	16.95 ± 2.96	5.46	<.01
CA199 (U/mL)	28.42 ± 4.23	14.28 ± 2.08	3.58	.059
CA724 (U/mL)	7.62 ± 1.09	4.51 ± 1.29	0.59	.637
VHL (U/mL)	9.98 ± 0.97[*]	20.72 ± 4.31	5.07	<.01
CYFRA21-1 (ng/mL)	8.58 ± 1.06[*]	2.38 ± 0.21	6.089	<.01
HIF-1α (ng/mL)	15.01 ± 0.64[*]	12.03 ± 0.52	3.61	<.01
HIF-1β (ng/mL)	12.04 ± 0.17[*]	8.06 ± 0.06	6.49	<.01

[*] Significant difference between the two groups.

Table 2
Serological variables [a,b,c,d] input/deleted by Fisher discriminant analysis.

Step	Input	Wilks' Lambda							
		Statistics	df1	df2	df3	Accurate F			
						Statistics	df1	df2	P
1	CYFRA21-1	0.986	1	1	245.000	27.899	1	245.000	<.01
2	HIF-1β	0.846	2	1	245.000	31.093	2	244.000	<.01
3	VHL	0.759	3	1	245.000	26.997	3	243.000	<.01
4	CA125	0.712	4	1	245.000	24.068	4	242.000	<.01

Note: In each step, minimized Wilks' Lambda is input.
[a] The maximum number of steps is 16.
[b] The minimum partial F to be input is 3.84.
[c] The maximum partial F to be deleted is 2.71.
[d] F level, tolerance or VIN is insufficient for the next step.

Table 3
Serological variables in Fisher discriminant analysis.

Step		Tolerance	F to be deleted	Wilks' Lambda
1	CYFRA21-1	1.000	27.899	
2	CYFRA21-1	0.991	32.331	0.912
	HIF-1β	0.991	27.151	0.896
3	CYFRA21-1	0.989	32.096	0.848
	HIF-1β	0.991	25.358	0.828
	VHL	0.998	19.497	0.811
4	CYFRA21-1	0.988	29.358	0.810
	HIF-1β	0.983	27.157	0.803
	VHL	0.931	10.973	0.757
	CA125	0.925	9.565	0.753

value as the standard for discriminant statistic, as shown in Tables 2 and 3.

3.3. GO and KEGG analysis of renal carcinoma-related gene

Search CYFRA21-1, CA125, HIF-1β and VHL in NCBI and Wanfang, VIP, CNKI databases. CYFRA21-1, a soluble fragment of cytokeratin 19, can be used as one indicator for clinical efficacy test and tumor prognosis judgment, which is also often used in diagnosis of renal carcinoma. Cell keratin 19 is the expression product of gene KRT19 (Sun and Zhao, 2014). CA125, a carbohydrate antigen, can be used as an independent prognostic factor for renal carcinoma in auxiliary diagnosis of the disease. Its gene is MUC16 (Bai et al., 2016). VHL gene, a representative tumor suppressor gene in renal carcinoma, is closely related to the occurrence and development of renal carcinoma with special significance for diagnosis of the disease. The erbB4 gene, also known as HER4 gene, is an oncogene that encodes the fourth human epidermal growth factor receptor (HER4). Studies show that it is overexpressed in renal carcinoma and is associated with lymph node metastasis, TNM staging and postoperative survival rate of renal carcinoma (Weng et al., 2016). However, no gene annotation related to HIF-1β was found in the GO database.

The annotation results of gene KRT19 indicate that KRT19 plays an important role in cell components, molecular functions and biological processes. Its protein products are distributed in intermediate filament, glycoprotein complex, plasma membrane, etc. mainly for protein binding or cytoskeleton function and involved in multiple biological processes like Notch signal pathway. According to MUC16 GO analysis results, the gene is mainly involved in cell components and biological processes, with its products seen in plasma membrane, Golgi body cavity and playing an important role in several biological processes like cell adhesion, modification after protein translation, cell protein metabolism. VHL gene annotation shows that: VHL also involves cell components, molecular

functions and biological processes. Cell components include cell nucleus, endoplasmic reticulum, mitochondria; molecular functions concern binding of protease and transcription factor, ubiquitin transferase activity; the involved biological processes include negative regulation of cell proliferation, negative regulation of RNA polymerase II transcription promoters, and involvement in transcriptional regulation. GO analysis results of erbB4 gene show that the gene involves a variety of functions. Cell components involve cell nucleus, mitochondria, plasma membrane; molecular function involves protein tyrosine kinase receptor signaling activation, epidermal growth factor receptor, etc. It is also involved in multiple signal pathways like cell proliferation, Ras protein signal transduction, mitogen-activated protein kinase (MAPK) cascade pathway and transmembrane receptor protein tyrosine kinase signaling pathway, epidermal growth factor receptor signaling pathway, insulin receptor, etc.

In the KEGG signal pathway database, enter the keyword erbB4, then definite signal pathway involving the gene can be found. It can participate in renal cancer cell evading apoptosis signal pathway via PI3K → PKB/AKt → MDM2 → p53, but MUC16, KRT19 gene related contents were not found. Through the gene annotation and literature search, these three genes are added to the renal cancer cell evading apoptosis signal pathway, as shown in Fig. 1. Gene annotation results show that KRT19 can act on Notch signaling pathway. Other literature (Gong, 2013) reports that MUC16 can promote transport of β-catenin (a key effect molecule of Wnt signaling pathway) from the cytoplasm to the nucleus, activate the Wnt signaling pathway, stimulate and enhance expression of downstream oncogenes through interaction with β-catenin.

4. Discussion

Renal carcinoma, also known as renal cell carcinoma, renal adenocarcinoma, is highly invasive. Renal carcinoma accounts for 85% of renal tumors, and its incidence ranks the 13th among all tumors. Early diagnosis of renal carcinoma is more and more valued, and it is expected that clinical tumor markers for diagnosis of renal carcinoma can be found as soon as possible (Mahoney et al., 2016). Serum tumor markers are important for the judgment of benign and malignant nature of renal carcinoma, of which, CYFRA21-1, CA125, HIF-1β and VHL are several commonly used tumor markers.

In this study, the 4 serum tumor markers CYFRA21-1, CA125, HIF-1β and VHL which are important for judgments of benign and malignant nature of renal carcinoma were screened from clinical cases. The CYFRA21-1, CA125, HIF-1β, VHL corresponding genes were determined to be KRT19, MUC16, HIF-1, VHL through literature search. Studies have shown that the oncogene erbB4, which encodes the fourth human epidermal growth factor receptor (HER4), is highly expressed in renal carcinoma (Kountourakis et al., 2006) and is closely related to cancer cell metastasis and patient
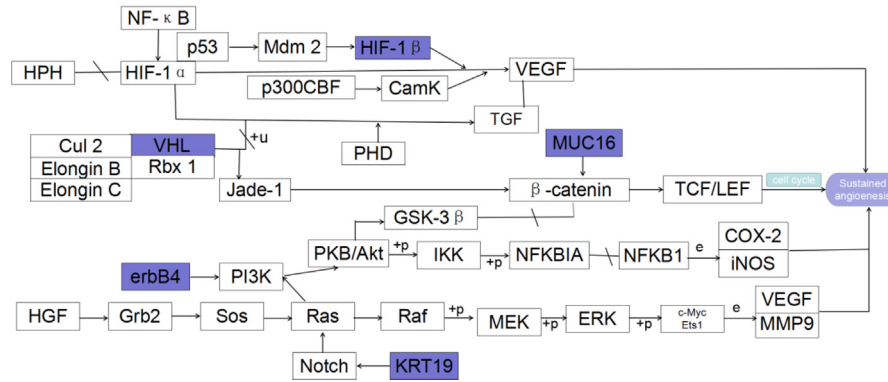
Fig. 1. Regulatory network of renal cancer cell evading apoptosis.

prognosis. It may be a candidate for molecular targeted therapy (Chen and Zhao, 2016). Therefore, GO function annotation and KEGG pathway analysis of YFRA21-1, CA125, HIF-1β, VHL and erbB4 were performed in this study, in an effort to further explore the occurrence and development of renal carcinoma.

In this study, the gene ontology database and the Kyoto gene and genome encyclopedia database were used to analyze the GO function and KEGG signal pathway of the five gene makers related to renal carcinoma. The regulatory network model of renal carcinoma was initially established to provide certain theoretical basis for gene therapy of renal carcinoma. In the process of GO analysis, the function annotation of HIF1 was mainly HIF1-α, and there was no annotation for HIF-1β. Therefore, GO analysis of KRT19, MUC16, VHL and erbB4 showed that these four genes played an important role in cell components, molecular functions and biology processes. For example, KRT19 was involved in Notch signaling pathway, and MUC16 was involved in modification after protein translation. Having been thoroughly studied, erbB4 participates in a variety of signaling pathways, and has a clear signaling pathway in KEGG pathway database. It can play a role in cancer cell evading apoptosis by PI3K-Akt signaling pathway. On this basis, KRT19 and MUC16 were supplemented in this study into the regulatory pathway in the KEGG signaling pathway by gene annotation and access to a large number of literatures, and the signal pathways of renal cancer cell evading apoptosis were charted out. KRT19 can act on the Notch signaling pathway, and the key gene Notch-encoded cell surface receptors in this pathway play an important regulatory role in the development of various biological cells. According to the literature, Notch signal can affect cell proliferation, cell boundary formation, apoptosis and multipotent progenitor specialization processes. Notch signaling pathway interacts with other critical pathways and plays an important role in the occurrence and development of tumors (Iso et al., 2003). It is reported that MUC16 can act on the key factor β-catenin of Wnt signaling pathway (Wang, 2010; Yin et al., 2002). Wnt signaling pathway is an evolutionally highly conserved signal transduction pathway that plays an important role in controlling embryonic development. The signaling pathway is connected with other pathways through complex networks, and its abnormal activation plays an important role in cell carcinogenesis, tumorigenesis and tumor invasion processes. Changes in Wnt gene itself or any other members of the pathway are likely to cause the occurrence of cancer. In the Wnt pathway, β-catenin plays a vital role and is considered as a key hub molecule in the entire Wnt pathway. Therefore, its transposition from the cytoplasm to the nucleus indicates that the signaling pathway is activated and function execution begins. At present, Wnt signal pathway as a target of tumor gene therapy has become a hotspot in research by global scientists (Tai et al., 2015). VHL gene is a representative tumor suppressor gene in renal

carcinoma, which is closely related to the occurrence and development of renal carcinoma. Recently, some new drugs such as Sorafenib (Haas et al., 2016); Bevacizumab (Yang et al., 2003) and Temsirolimus (Sankin et al., 2015; Leisz et al., 2015; Gao, 2017a, 2017b) have been developed to regulate the downstream genes of the VHL pathway. These new drugs have played a role in clinical gene therapy with good therapeutic effect achieved. At the same time, people have been making continuous in-depth explorations on the mechanism of VHL and pVHL. Leisz et al. (2015) first screened an amino acid sequence very similar to VHL protein β region, and then injected the amino acid sequence into the transplanted tumor of clear cell carcinoma on the dorsal side of the nude mice, resulting in partial regression of the tumor. The results suggest that prevention of tumor development and infiltration can rely on VHL amino acid fragments.

In summary, Fisher discriminant analysis was used in this study to screen out serum tumor markers CYFRA21-1, CA125, HIF-1β and VHL closely related to judgment of benign and malignant nature of renal carcinoma. The gene was identified by literature search. Then, GO and KEGG were used to analyze the regulatory pathway for renal carcinoma related genes to involve in the occurrence and development of renal carcinoma. Tumor marker genes were supplemented on the basis of erbB4 signal pathway, and the regulatory network of renal cancer cell evading apoptosis was charted out, which lays the foundation for continuous supplement and improvement of renal carcinoma signal pathways, and blazes a trail for future treatment of renal carcinoma with cancer gene as a target.

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