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Analysis of NudCD1 and NF-κB in the early detection and course evaluation of renal cancer

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Abstract. – OBJECTIVE: Early detection and effective evaluation are helpful for renal cancer diagnosis and treatment. NudCD1 and NF-κB are abnormally expressed in tumors and inflammations. However, their role in early detection and course evaluation of renal cancer has not been reported.

PATIENTS AND METHODS: The serum of clinically diagnosed renal cancer patients and healthy volunteers (control group) were collected to measure the expressions of NudCD1 and NF-κB mRNA by Real time PCR.

RESULTS: NudCD1 and NF-κB mRNA in renal cancer patients were significantly upregulated compared to controls ($p<0.05$). NudCD1 was positively correlated with tumor diameter, TNM stage, lymph node metastasis, degree of differentiation, and distant metastasis ($p<0.05$); whereas, NF-κB was positively related to TNM stage, lymph node metastasis, and distant metastasis ($p<0.05$) but not to tumor diameter and differentiation degree. NudCD1 and NF-κB were positively correlated. The combined detection improved the diagnostic specificity and sensitivity of renal cancer.

CONCLUSIONS: The expression of NudCD1 and NF-κB is increased in renal cancer and is correlated with renal cancer clinicopathological characteristics. The combined detection of NudCD1 and NF-κB can improve the early diagnosis of kidney cancer.

Key Words:
Renal cancer, NudCD1, NF-κB, Evaluation.

Introduction

The incidence of renal cancer ranks second among malignant tumors of the urinary system. Due to lifestyle changes, dietary habits and stress in the working environment, the incidence of tumors is increasing year by year[1,2]. Renal cancer often occurs mainly in the renal parenchymal urothelial epithelial system, which usually originates from the different positions of the urinary tubule. According to the pathological classification, it can be divided into renal tubular epithelial cells, including renal papillary adenocarcinoma, clear cell carcinoma and other pathological types[3]. Renal cancer can be seen in all ages, accounting for 2% of global malignancies. Its high incidence is found in middle and middle-aged people and is predominantly found in males[4,5]. With the changes in lifestyles and eating habits, the incidence of renal cancer has been increasing year by year and the incidence trend has gradually become younger[6]. At present, the main treatment is surgery, but because the early symptoms of renal cancer are not obvious, patients often have advanced renal cancer at the time of consultation and some patients have recurrence or metastasis after surgery[7,8]. The main clinical symptoms of patients with renal cancer include hematuria, low back pain, and abdominal masses. For patients in advanced stage, adrenal glands, lymph nodes and other nearby tissues and organs metastasize with distant metastases of lung, liver and even bone[9]. On the other hand, in addition to surgical treatment, renal cancer is not sensitive to adjuvant treatments such as chemotherapy and radiation therapy, leading to poor prognosis[10]. Early detection and effective evaluation of its the condition are conducive to the diagnosis and treatment of the disease. Therefore, many studies focus on the exploration of molecular markers for the diagnosis and treatment of kidney cancer.

Nucleus distribution gene C domain 1 (NudCD1) is a nuclear distribution gene C (NudC)

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family[11] and can participate in cell proliferation, mitosis, cell cycle, intracellular material transport, and cell migration by regulating related signaling pathways[12,13]. The abnormal expression of NudCD1 can lead to the emergence of malignant biological behaviors such as abnormal cell proliferation and differentiation[14]. Transcription regulator NF-κB can initiate gene transcription and then regulate the biological and pathological processes with abnormal expression in various tumors[15,16]. However, the application of NudCD1 and NF-κB in early detection and evaluation of renal cancer has not been reported.

Patients and Methods

Patients

This study was approved by the Medical Ethics Committee of The Fifth Hospital of Wuhan. All selected participants signed an informed consent. From January 2018 to December 2018, clinical data of 56 patients with renal cancer diagnosed by pathological histological examination according to diagnostic guideline[17] who were admitted to our hospital for surgery were selected. All patients were surgically treated, aged 41-69 years, mean age 47.2 ± 9.2 years old, including 32 males and 24 females. The clinical TNM stage was 11 in stage I, 15 stage II, 21 stage III, and 9 stage IV. The materials were collected during the operation for pathological diagnosis. Inclusion and exclusion criteria included[10]: confirmed by pathological diagnosis; for the first time, primary renal cancer was found without surgery or chemotherapy or other treatments such as radiotherapy. Exclude recurrent or metastatic renal cell carcinoma; previous surgery or radiotherapy or chemotherapy; combined with other diseases and systemic immune diseases and malignant tumor complications at admission; patients who were unwilling to cooperate or could not cooperate with this study and follow-up. 60 healthy volunteers were selected for physical examination, including 31 males and 29 females, average age: 63.8 ± 6.7 years (ranging: aged 51-77). There was no statistical difference in general clinical data such as age and gender between the study groups and they were comparable.

Main Reagents and Instruments

RNA extraction kits, RT-PCR primers, reverse transcription (RT) kits, and real-time PCR reagents were purchased from Axygen (Union City, CA, USA). The ultra-clean bench was purchased from Suzhou Sutai Purification Equipment Engineering Co., Ltd (Suzhou, China).

Patient Data Collection

Clinical data of patients were recorded, including gender, age, TNM stage, pathological grade, tumor diameter, etc.

Specimen Collection

2 ml early morning fasting blood was collected into vacuum biochemical tubes and left for 30 min followed by centrifugation at 4°C at 3600 rpm for 10 min to obtain the supernatant.

Real-Time PCR

Under aseptic conditions, mRNA was extracted followed by reverse transcription synthesis according to the kit instructions. The primers were designed by Primer Premier 6.0 based on each gene sequence and synthesized by Shanghai Yingjun Biotechnology Co., Ltd. (Table I). Real-time PCR detects the expression of the gene of interest with reaction conditions: 52 °C for 1 min, 90 °C for 30 s, 58 °C for 50 s, and 72 °C for 35 s for a total of 35 cycles. Relevant data were collected to calculate the standard starting cycle number (CT) according to the internal reference GAPDH followed by drawing a standard curve. The 2-ΔCt method was used for quantitative analysis.

Statistical Analysis

SPSS 19.0 software (IBM, Armonk, NY, USA) was adopted for processing data which were displayed as mean ± standard deviation (SD) and assessed by one-way ANOVA. Counting data were tested by chi-square test. Correlation analysis was performed using the Pearson correlation analysis. $p < 0.05$ indicates a statistical difference.

Table I. Primer sequences.

Factor	Upstream primer	Downstream primer
BANCR	5'-ACAGGACTCCATGGCAAACG-3'	5'- ATGAAGAAAGCCTGGTGCAGT-3'
GAPDH	5'-GGGAGCCAAAAGGGTCAT-3'	5'-GAGTCCTTCCACGATACCAA -3'

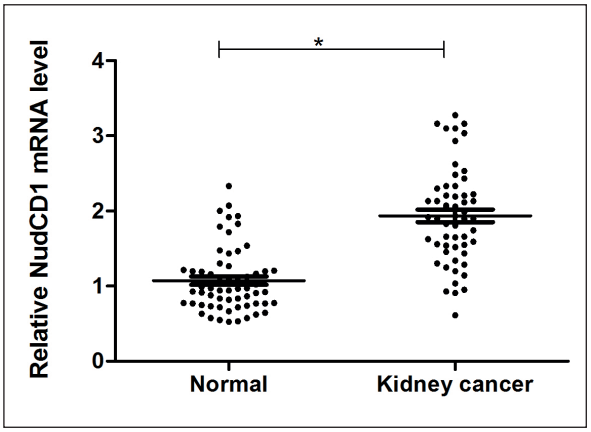

Figure 1. NudCD1 expression in renal cancer patients. Compared with the control group, *p<0.05.

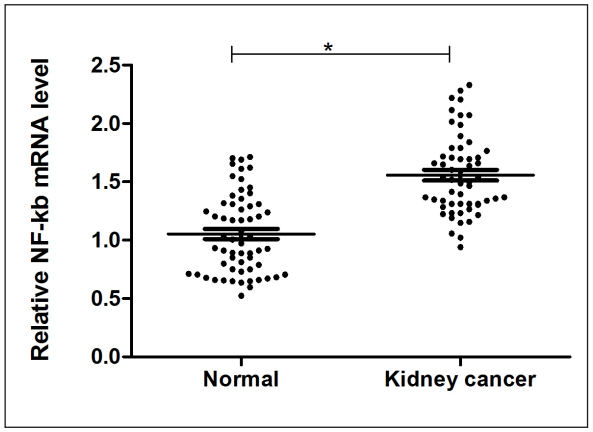

Figure 3. Expression of NF-κB in renal cancer patients. Compared with the control group, *p<0.05.

Results

NudCD1 Expression in Renal Cancer Patients

NudCD1 in renal cancer patients was significantly upregulated compared to controls (p < 0.05) (Figure 1).

NudCD1 Expression in Different TNM Stages of Renal Cancer

NudCD1 expression in different TNM stages of renal cancer was significantly different and increased with the increase of stage (p < 0.05; p < 0.01) (Figure 2).

NF-κB *Expression in Renal Cancer Patients*

Renal cancer patients had significantly higher NF-κB level than controls (p < 0.05) (Figure 3).

Expression of NF-κB *in Patients with Different TNM Stages of Renal Cancer*

NF-κB level in different TNM stages was different and elevated with the increase of stage (p < 0.05; p < 0.01) (Figure 4).

Analysis of the Correlation Between NudCD1 and Clinicopathological Characteristics of Renal Cancer

NudCD1 was positively correlated with tumor diameter, TNM stage, lymph node and distant metastasis, differentiation degree (p < 0.05) but not to age, gender, and BMI (Table II).

Relationship between NF-κB *and clinicopathological characteristics of renal cancer*

NF-κB was positively related to TNM staging, lymph node and distant metastasis (p < 0.05) but

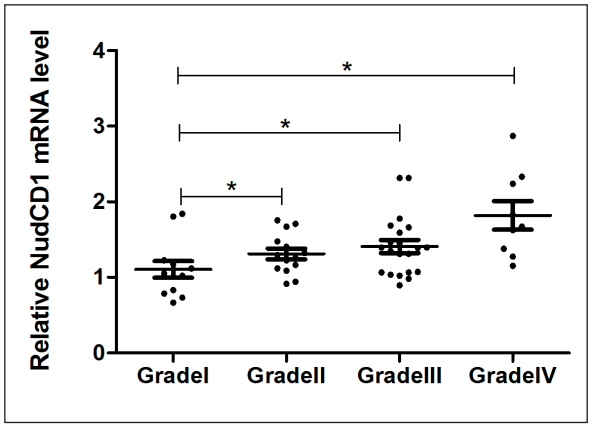

Figure 2. NudCD1 expression in patients with different TNM stages of renal cancer. Compared with Phase I, *p<0.05; **p<0.01.

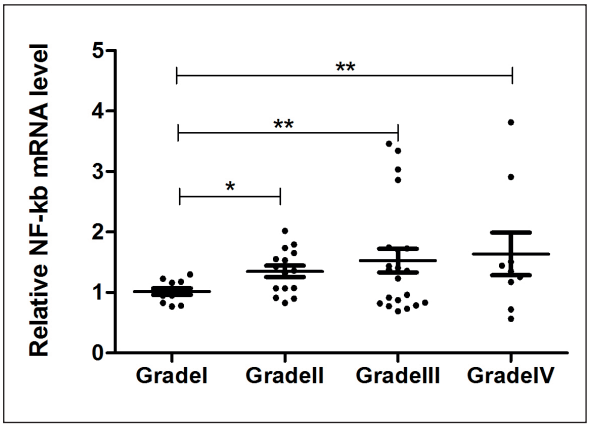

Figure 4. Expression of NF-κB in renal cell carcinoma patients with different TNM stages. Compared with Phase I, *p<0.05; **p<0.01.

Table II. General data.

Factors	CG n=82	TG n=87	t/χ^2	p
Gender			0.026	0.873
Male	50 (60.98)	52 (59.77)		
Female	32 (39.02)	35 (40.23)		
Age (years)	63.34±6.37	63.04±6.41	0.305	0.761
BMI (kg/m²)	23.24±2.11	23.34±2.17	0.304	0.761
Course of disease (year)	3.11±0.32	3.14±0.34	0.591	0.555
Drinking history			0.009	0.926
With	43 (52.44)	45 (51.72)		
Without	39 (47.56)	42 (48.28)		
Smoking history			0.054	0.816
With	40 (48.78)	44 (50.57)		
Without	42 (51.22)	43 (49.43)		
Hypertension			0.006	0.963
With	54 (65.85)	57 (65.52)		
Without	28 (34.15)	30 (34.48)		
Diabetes mellitus			0.001	0.969
With	45 (54.88)	48 (55.17)		
Without	37 (45.12)	39 (44.83)		

not to tumor diameter and differentiation, age, gender, and BMI (Table III). NF-κB and NudCD1 were positively correlated ($r = 0.616$, $p < 0.05$).

Combination of NF-κB and NudCD1 in Detecting Renal Cancer

The sensitivity and specificity of combined detection of serum tumor markers to detect renal cancer were determined by analyzing the expression of the two serum markers. The results showed that the combined sensitivity of the two serum markers NF-κB and NudCD1 was the highest (90.3%); the detection sensitivity of the individual markers was 51.2% and 65.2%, respectively. In terms of specificity, the combined detection of two serum markers was highest (81.5%) and the specificity of individual marker detection was 65.2% and 71.7%, respectively (Table IV).

Table III. Comparison of therapeutic effect between the two groups.

Therapeutic effective	CG n=82	TG n=87	t/χ^2	p
Markedly effective	41 (50.00)	53 (60.92)	2.039	0.153
Effective	28 (34.15)	29 (33.33)	0.012	0.911
Invalid	13 (15.85)	5 (5.75)	4.531	0.033
Total effective rate	69 (84.15)	82 (94.25)	4.531	0.033

Table IV. Comparison of ADR between the two groups.

Factors	CG n=82	TG n=87	t/χ^2	p
Vomiting	2 (2.44)	0	2.147	0.143
Bleeding	3 (3.66)	0	3.240	0.072
Ecchymoma	3 (3.66)	1 (1.15)	1.150	0.284
Ecchymosis	3 (3.66)	1 (1.15)	1.150	0.284
Total incidence rate (%)	11 (13.41)	2 (2.30)	7.346	0.007

Table V. Comparison of ADR between the two groups.

Factors	CG n=82	TG n=87	t/χ^2	p
Angina pectoris	6 (7.32)	2 (2.30)	2.357	0.125
High risk arrhythmia	7 (8.54)	2 (2.30)	3.258	0.071
Acute left ventricular failure	7 (8.54)	2 (2.30)	3.258	0.071
Total incidence rate	20 (24.39)	6 (6.90)	9.924	0.001

Discussion

Renal cancer is a very common malignant tumor in urinary system tumors worldwide. At present, the disease prognosis is very unsatisfactory. Even if surgery is combined with chemoradiotherapy or radiotherapy, the postoperative recurrence rate is high and the average survival time is short[18,19]. The pathogenesis and molecular biology are complicated and have not been fully elucidated so far. The occurrence and development of renal cancer are rapid with histological and morphological diversity[20]. In recent years, with the in-depth research on the biological characteristics and pathogenesis of renal cancer, the diagnosis and treatment of renal cancer have been improved. However, there is still a lack of reliable markers for the prevention, early diagnosis and treatment in clinical practice. Early detection of kidney cancer, improvement of treatment effect, prognosis, and survival rate have become urgent problems[21]. The selection of new molecular markers can be widely used in clinical diagnosis and evaluation of curative effect to monitor its recurrence and prognosisr[22].

NudCD1 regulates cell apoptosis and immune response[11]. NudCD1 overexpression promotes HO-8910 cell proliferation, invasion, and survival via IGF-1R-MAPK signaling; NudCD1 overexpression can confer NIH3T3 cells tumorigenicity and inhibit the apoptosis of human tumor cells induced by chemotherapeutic drugs through activation of PI3K-AKT and ERK1/2-MAPK signal pathways[23,24]. NudCD1 as a cancer protein promotes Hela cell proliferation, invasion, and survival[25]. Our study confirmed that NudCD1 was upregulated in renal cancer cells and increased significantly with the increase of TNM stage, and positively related to tumor diameter, TNM stage, lymph node and distant metastasis and differentiation degree, indicating that NudCD1a in serum can be used as a tumor marker for the diagnosis of renal cancer.

NF-κB is a regulatory factor, which exists in a variety of tissues and cells, and has been shown to be abnormally expressed in a variety of tumors, including colon cancer. Viral infections, toxins, cytokines, mitogens, etc., can activate NF-κB and NF-κB itself is a key regulator between cell survival and apoptosis. NF-κB activation inhibits apoptosis and promotes tumor formation[26-28]. This study indicated that NF-κB in renal cancer cells was elevated with the increase of TNM stage, and positively related to TNM staging, lymph node and distant metastasis. Further, this study reveals that the combined detection of NF-κB and NudCD1 has significantly improved the sensitivity and specificity of early detection of renal cancer, which is helpful for disease evaluation.

Conclusions

Our study for the first time demonstrates that the expression of NudCD1 and NF-κB in renal cancer patients is increased and related to its clinicopathological characteristics, which is the novelty of our study. The combined detection of NudCD1 and NF-κB can improve the early diagnosis of renal cancer.

Conflict of Interests

The authors declare that they have no conflict of interest.

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