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Expressions and effects of G250, Bax and Bcl-2 in rats with renal clear cell carcinoma

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Abstract. – OBJECTIVE: To investigate the expressions and effects of G250, B-cell lymphoma-2 associated X protein (Bax) and Bcl-2 in rats with renal clear cell carcinoma (RCCC).

MATERIALS AND METHODS: A total of 66 male Sprague-Dawley (SD) rats were selected, among which 56 were selected to establish RCCC rat model and the remaining 10 were selected as control group. Three weeks after modeling, 4 rats failed in the modeling. Expressions of G250 in RCCC rat model group and healthy rat model control group were detected by Reverse Transcription-Polymerase Chain Reaction (RT-PCR); expressions of Bcl-2 and Bax in each group were detected by Western blot and their effects were analyzed.

RESULTS: The positive expression rates of G250 in 52 RCCC rats in model group and 10 healthy rats in control group were 83.3% and 0%, respectively. The results showed that expression of G250 had a certain correlation with the pathological changes of RCCC ($p < 0.01$). Expressions of Bax and Bcl-2 were up-regulated in the RCCC group, while expressions were down-regulated in the healthy control group ($p < 0.05$).

CONCLUSIONS: G250, as a new specific marker of renal cell carcinoma, is involved in the pathological changes of renal cell carcinoma. Joint detection of Bax and Bcl-2 can be used as an important index for the diagnosis of RCCC.

Key Words:
Renal clear cell carcinoma, Rat model, G250, Bax, Bcl-2.

Introduction

Renal clear cell carcinoma (RCCC), stemming from the adenocarcinoma of renal tubular epithelial cells[1], is one of the most common forms of renal carcinoma, accounting for 82.5% of all renal carcinoma patients in the world[2]. Zhang et al[3] showed that an average of 300 thousand patients with RCCC is increased in China every year and the incidence rate is rising year by year. As there are no evident symptoms in the early period of RCCC, it is apt to be neglected by patients, thereby missing the best timing of treatment. Patients have mostly been in intermediate and advanced stage with great difficulty to be cured and once diagnosed the prognosis will be poor[4,5]. Now surgical excision combined with radiotherapy and chemotherapy is mainly used in the clinical treatment for RCCC, but it has a long treatment period and will bring great harm to patients[6]. Armstrong et al[7] indicated that the death rate of RCCC patients within 5 years after surgery is as high as 47.6%. Due to the high incidence rate, difficulty to be cured and poor prognosis, RCCC has become the main research object in clinic practice. Now there have been reports[8-10] demonstrating that relevant research has been done on the diagnosis, treatment, and nursing of RCCC patients. However, there are no targeted research viewpoints for patients in China in terms of molecular biology due to its complex pathogenesis. G250, B-cell lymphoma-2 associated X protein (Bax) and Bcl-2 can act as the factors regulating and controlling the occurrence and progression of cancer[11]. So, it is conjectured in this paper that whether the features of RCCC can be analyzed through molecular expression, thus providing targeted treatment and achieving the better effect of diagnosis and treatment. Therefore, expressions of G250, Bax, and Bcl-2 were investigated, and their effects were analyzed by establishing RCCC rat models. The results could provide reference and guiding suggestions for RCCC patients in future clinical practice.

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Materials and Methods

Animal Models

A total of 66 male Sprague-Dawley (SD) rats were selected (provided by Animal Experiment Center of Anhui Medical University, Hefei, Anhui Province, China), among which 56 were randomly selected for RCCC modeling, and the remaining 10 were selected as control group and fed normally. The study was approved by the Ethics Committee of The Affiliated Yantai Hospital of Qingdao University Medical College

Methods

Modeling methods: RCCC rat models were established according to the methods described by Shroff et al[12]: 10% fetal calf serum culture medium (produced by Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to culture the pathological specimen obtained from RCCC patients until it was in logarithmic growth phase. Cell number was calculated after the medium was digested by 0.25% trypsin solution (produced by SPH No. 1 Biochemical & Pharmaceutical co., Ltd., Shanghai, China), and cells were diluted to 5×10^7/mL with phosphate buffered saline (PBS) (0.01 mol/L, pH7.4, produced by Thermo Fisher Scientific Inc., Waltham, MA, USA) before standing. Then, the upper-layer suspended cells were taken to inoculate into the dorsal part of left axilla of rats for 20 consecutive days. At the 5th week, half of modeled rats and control rats were selected randomly and were killed by decapitation, among which the modeling rats were classified into experimental group A and the control rats were classified into control group A. Hematoxylin-eosin (HE) staining was used to detect the existence of RCCC and diameter of tumor > 8 mm suggested that the model was established successfully. All the remaining rats were killed after 10 weeks of culture, among which the modeled rats were classified into experimental group B and the healthy rats were classified into control group B.

Detection methods: Expressions of G250 in RCCC rats with successful modeling and healthy rats in control model group were detected by RT-PCR using the RNeasyMinikit RNA extraction reagent (QIAGEN Inc., Hilden, German) in strict accordance with operation instructions, and primer designs are shown in Table I. Expressions of Bcl-2 and Bax in each group were detected by Western blot, and their differences were compared, and their effects were analyzed.

Table I. Primer design of G250.

	Sequence
Forward	5′-GTCTCGCTTGGAAGAAATCG-3′
Reverse	5′-AGAGGGTGTGGAGCTGTTA-3′

Statistical Analysis

Statistical Product and Service Solutions (SPSS; IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Armonk, NY, USA) 22.0 software was used for data analysis, and enumeration data were presented as `x ± s, and measurement data were presented as ratio, and chi-square test was used. $p < 0.05$ suggested that the difference was statistically significant.

Results

Results of Modeling

Among 56 rats, 52 rats were successful in modeling with a successful rate of 92.9%. The 52 RCCC rats were randomly divided into experimental group A (n=26) and experimental group B (n=26). Another 10 healthy rats were divided into control group A (n=5) and control group B (n=5)..

Detection Results in Experimental Group A and Control Group A

The detection results of G250, Bax, Bcl-2, and Bcl-2/Bax in experimental group A were (0.33 ± 0.20), (0.26 ± 0.13), (0.39 ± 0.17), and (1.52 ± 0.39), respectively. And the detection results of G250 and Bcl-2 in control group A were (0.01 ± 0.01) and (0.02 ± 0.01), respectively. All the indexes in experimental group A were significantly higher than those in control group A ($p < 0.05$) (Table II).

Detection Results in Experimental Group B and Control Group B

The detection results of G250, Bax, Bcl-2, and Bcl-2/Bax in experimental group B were (0.42 ± 0.21), (0.18 ± 0.15), (0.49±0.24), and (1.75 ± 0.24), respectively, and those in control group B were (0.01 ± 0.02), (0.01 ± 0.01), (0.02 ± 0.01), and (0.01± 0.01), respectively. All the indexes in experimental group B were significantly higher than those in control group B ($p < 0.05$) (Table III).

Table II. Detection results of G250, Bax, Bcl-2 and Bcl-2/Bax in experimental group A and control group A.

	G250	Bax	Bcl-2	Bcl-2/Bax
Experimental group A (n=26)	0.33 ± 0.20	0.26 ± 0.13	0.39 ± 0.17	1.52 ± 0.39
Control group A (n=5)	0.01 ± 0.01	0	0.02 ± 0.01	0
t	2.51	2.36	2.38	2.45
p	0.028	0.017	0.019	0.022

Table III. Detection results of G250, Bax, Bcl-2 and Bcl-2/Bax in experimental group B and control group B.

	G250	Bax	Bcl-2	Bcl-2/Bax
Experimental group B (n=26)	0.42 ± 0.21	0.18 ± 0.15	0.49 ± 0.24	1.75 ± 0.24
Control group B (n=5)	0.01 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
t	2.36	2.21	2.88	2.58
p	0.015	0.028	0.012	0.021

Comparisons of Detection Results Between Experimental Group A and Experimental Group B

The detection results of G250 and Bcl-2 in experimental group B were apparently higher than those in experimental group A, while the detection result of Bax was apparently lower than that in experimental group A ($p < 0.05$). There was no significant difference in Bcl-2/Bax between the two groups ($p > 0.05$) (Table IV).

Discussion

RCCC is a very common malignant tumor in the urogenital system with a very complicated mechanism. Deml et al[13] indicated that RCCC is a polygene-associated tumor, and the occurrence and progression of cancer are directly affected by the proliferation, apoptosis, and regulator factors of cancer cells. Therefore, finding a new tumor marker has now become a challenging task in clinical practice. Divgi et al[14] showed that G250 is a kind of antigen not expressed in healthy people, but expressed in most renal cancers. The results suggested that G250 could be used as a new potential target for the treatment of RCCC. Moreover, Bcl-2, as a mitochondrial inner membrane protein associated with the suppression of apoptosis, has a close relation with chromosome t18 and t14, and can effectively inhibit the apoptosis of programmed cells[15]. However, Delbridge et al[16] indicated that Bcl-2 can effectively extend the lifetime of cells, cells with a high positive expression of Bcl-2 have a long lifetime and low apoptosis rate, and the long-time accumulation of cells will result in the occurrence and progression of the tumor. Moreover, Bax, as a factor opposite to Bcl-2, has a great apoptosis-promoting effect[17-19]. These two factors can form a heterodimer in cancer to regulate cells, so whether the heterodimer plays a role in promoting apoptosis or inhibiting apoptosis[20] depends on which factor is in the majority. The contents of G250, Bcl-2, and Bax in RCCC rats and their effects were investigated in this paper to study the molecular genetic

Table IV. Comparisons of detection results of G250, Bax, Bcl-2 and Bcl-2/Bax between experimental group A and experimental group B.

	G250	Bax	Bcl-2	Bcl-2/Bax
Experimental group A (n=26)	0.33 ± 0.20	0.26 ± 0.13	0.39 ± 0.17	1.52 ± 0.39
Experimental group B (n=26)	0.42 ± 0.21	0.18 ± 0.15	0.4 ± 0.24	1.75 ± 0.24
t	2.35	2.01	2.12	1.54
p	0.016	0.032	0.027	0.061

features of RCCC and provide more effective and better reference opinions for the diagnosis and treatment of RCCC in future clinical practice. The expressions of G250, Bax, and Bcl-2 were found to be significantly higher than those in healthy rats, which agree with Muselaers et al[21]. As indicated by regulating the modeling time of rats, differences were also observed in the expressions of G250, Bax, and Bcl-2 in experimental group B with more serious RCCC ($p < 0.05$). The expressions of G250 and Bcl-2 were increased with the severity of disease, but the expression of Bax declined with the severity of disease. The results suggested that G250 probably has a certain effect on the cell proliferation of RCCC. However, due to the small sample size, further studies are still needed. In terms of tumor node metastasis (TNM) staging, the expression level of G250 was correlated positively with TNM staging as G250 is a membrane antigen on membrane surfaces. With the aggravation of RCCC, the lesion caused by tumor necrosis factor α to cell surfaces and internal frame of membrane antigen became more severe, as a result of which the expression of G250 became higher and higher. The expressions of Bcl-2 in RCCC rats with different pathological stages were significantly up-regulated and the expressions of Bax were significantly down-regulated. The results indicated that the occurrence and progression of RCCC are mainly manifested as cell proliferation, which is also consistent with the findings of Delbridge et al[22]. It showed that Bcl-2 and Bax both have certain effects on the occurrence and progression of RCCC. Tumor necrosis of cells resulting from the proliferation of cells, caused by the expression dysregulation of Bcl-2, can cause a great damage to local tissue. By regulating the related downstream target gene, Bcl-2 is involved in the transcription, and exerts a direct effect on the cycle regulation, proliferation and differentiation, and programmed death of cells. The lower the ratio of Bcl-2 and Bax is, the more normal the cell metabolism and proliferation will become, and the higher the possibility of better prognosis will also be.

By establishing RCCC rat model, expression levels of G250, Bcl-2, and Bax were investigated in this paper. However, due to the differences between rats and human, the experimental results might have some errors. Thus, the opinions put forward in this experiment will be analyzed and proofed in next experiment through RCCC patients.

Conclusions

We showed that G250, Bcl-2, and Bax are all closely correlated with RCCC and can be used as new markers for renal carcinoma. Moreover, the detection of the expressions of G250, Bcl-2 and Bax can be used as an important reference index for the diagnosis of RCCC in clinical practice.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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