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Correlation of miRNA-21 and Blood Cr Levels with Tumor Infiltration and Distant Metastasis in Renal Cancer Patients

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ABSTRACT

The object of this study was to explore the correlation analysis between miRNA-21 and blood Cr levels with tumor invasion and distant metastasis in renal cancer patients. For this purpose 49 cases of renal cancer patients treated in our hospital from February 2018 to March 2020 were selected as the study group, and another 165 cases of renal benign tumors that were pathologically confirmed in our hospital during the same period were selected as the control group. MiRNA-21 and blood Cr levels, miRNA-21 and blood Cr levels at different stages, and miRNA-21 and blood Cr levels when tumor invasion and distant metastasis were present were compared between the two groups. Results showed that compared with the control group, the levels of miRNA-21 and blood Cr in the study group increased, the difference was significant ($P < 0.05$); compared with stage I patients, the levels of miRNA-21 and blood Cr in stage II patients increased, compared with stage II patients, the levels of miRNA-21 and blood Cr in stage III patients increased, compared with stage III patients, the levels of miRNA-21 and blood Cr in stage IV patients increased, the difference was significant Compared with the case without tumor invasion, the levels of miRNA-21 and blood Cr in the case of tumor invasion were increased, the difference was statistically significant ($P < 0.05$); compared with the case without distant metastasis, the levels of miRNA-21 and blood Cr in the case of tumor distant metastasis were increased, the difference was significant ($P < 0.05$) When distant metastasis, miRNA-21 and blood Cr levels increased significantly, which showed that miRNA-21 and blood Cr levels were positively correlated with tumor invasion and distant metastasis; compared with the control group, TIMP1, TIMP2, MACC1 protein expression in the study group increased, and the three showed a positive correlation, the difference was significant ($P < 0.05$). It is concluded when renal cancer patients were tested, we found that miRNA-21 and blood Cr levels were abnormally increased, and the two had a certain correlation with renal cancer tumor invasion and distant metastasis, which showed a positive correlation.

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Introduction

Renal cancer is a malignant tumor originating from the urinary tubular epithelial system of the renal parenchyma. Its full academic term is renal cell carcinoma, which may also be called renal adenocarcinoma (1-2). Renal cancer includes various subtypes of renal cell carcinoma originating from different parts of the urinary tract but excludes tumors of interstitial origin and renal pelvis tumors. As one of the most common malignant tumors in the urinary system, kidney cancer is second only to bladder tumors, accounting for about 85% of renal malignancies and seriously threatening the lives of patients (3-4). MicroRNAs are a class of small non-coding RNAs of 19-25 nucleotides in length that have been discovered only in recent years and are capable of complete or incomplete pairing mainly through the 3' untranslated region of the target gene mRNA (5-6). Some clinical studies have shown that abnormal miRNA expression is closely associated with disease development, such as miR-145, miR-200 and miR-21 play an important role in the pathogenesis of kidney cancer. Blood Cr is a product of human muscle metabolism (7). In this study, we measured miRNA-21 and blood Cr in kidney cancer patients, aiming to investigate the correlation between miRNA-21 and blood Cr levels and tumor infiltration and distant metastasis in kidney cancer patients.

Materials and methods

Subjects

149 cases of renal cancer patients admitted to our hospital from February 2018 to March 2020 were selected and recorded as the study group. Another 165

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cases with pathologically confirmed benign renal tumors in our hospital during the same period were selected as the control group. The study group included 89 male patients and 60 female patients, aged 46-64 years, with a mean of (55. 0±7. 2) years. Pathological types: 102 cases of clear cell carcinoma, 23 cases of papillary carcinoma, 15 cases of chromophobic cell carcinoma, 6 cases of cystic cell carcinoma, and 3 cases of other types. TNM stage: 78 cases in stage I, 34 cases in stage II, 15 cases in stage III, and 7 cases in stage IV. Stage I also included T1a 35 cases and T1b 43 cases. The control group included 85 male patients and 80 female patients aged 47-65 years, with a mean of (56. 2±7. 1) years. The general data of all patients were compared with no statistical difference. Inclusion criteria: all patients met the diagnostic criteria for lung cancer in the Chinese Medical Association (8). Exclusion criteria: ① those with combined blood disorders; ② those with recent anticoagulation or platelet therapy; ③ those with combined cardiac, hepatic, renal and other organ insufficiencies; ④ those with poor compliance and unable to cooperate. All patients and their families were informed of this study and approved by the ethics committee of our hospital.

Taking materials

All patients underwent nephrectomy. After surgery, lung cancer tissue and 5 mm of tissue adjacent to the lung cancer were excised and randomly divided into two parts. One part was homogenized and filtered using a glass homogenizer followed by centrifugation, after which individual cell nuclei were extracted and stored at 0°C for use. The other portion was serially frozen, sectioned immediately after excision, subsequently fixed and placed at -20°C for storage until use. 5 mL of fasting venous blood was drawn from all patients and centrifuged. The supernatant was extracted and stored at -40°C until use.

Tumor infiltration detection

Specimens to be tested were dewaxed and hydrated using immunohistochemistry. They were soaked in 0. 01 mol/L, 95°C citrate buffer for thermal repair and incubated for 10 min in 3% H_2O_2 environment. Goat serum was dropped in and incubated at 26°C for 30 min. The blocking solution was withdrawn, a primary antibody was added, and the culture was placed at 5°C overnight. The secondary antibody was added the next day and incubated in a 37°C incubator for 30 min. After washing, DAB color development and blocking were performed. The subsequent inspection was performed using light microscopy.

Tumor staging and metastasis detection

Kidney cancer tumor staging is performed according to TNM and can be divided into stage I, stage II, stage III and stage IV. Whether metastasis is classified according to the stage as N_x: regional lymph nodes could not be evaluated; N_o: no regional lymph node metastasis; N_1: with regional lymph node metastasis; M_X: distant metastasis could not be evaluated; M_o: no distant metastasis; M_1:preoperative imaging suggested distant tumor metastasis.

miRNA-21 expression detection

Firstly, the total RNA of individual cell nuclei was extracted. The purity and content of the extracted total RNA were tested. Subsequently, reverse transcription was performed, and cDNA was obtained after reverse transcription processing. After obtaining cDNA, primers were designed using Primer 5. 0 software and calculated using the 2-ΔΔCt method with internal reference U6.

miRNA-21 primers, upstream: 5'GTGCAGGGTCCGAGGT-3', downstream: 5'-GCCGCTAGCTTATCAGACTGATGT-3'. U6 primer, upstream 5'-CTCGCTTCGGCAGCACA-3'; downstream: 5'-AACGCTTCACGAATTTGCGT-3'. The reverse transcription reaction conditions were set to 25°C for 10 min, 40°C for 60 min, and 85°C for 5 min. The amplification conditions were set to 94°C for 20s, 72°C for 30s, 60°C for 30s, and 35 cycles. The 2-ΔΔCt method was used to calculate the amount of miRNA-21 expression.

Blood Cr level detection

Blood Cr levels were measured by ELISA. Specific antibody globulin was diluted to the most appropriate concentration (1-10 Ug/ml) by the coating buffer. Subsequently, 0. 3 ml was added to each well and stored overnight at 0°C. On the second day, the wells were rinsed at 5-min intervals with washing buffer after removal of the coating solution, and the wells were rinsed three times. 0. 2 ml of antigen-containing specimens were added to each well for 2 h at 37°C.

Subsequent washes were performed again, 3 times using wash buffer (containing 0. 05% Tween-20). 0. 2 ml of antibody solution was added (Enzyme-labeled specific antibody solution after dilution with dilution buffer) at 37°C for 2h. Three washes were performed again. After washing, 0. 2 ml of substrate solution was added to each concave well, and $2MH_2SO_4$ or 2M citric acid 0. 05 ml was added to each concave well after 30 min at room temperature. The blood Cr levels were evaluated using an enzyme-labeled colorimeter.

TIMP1, TIMP2 and MACC1 assays

TIMP1, TIMP2 and MACC1 expression was detected using Western blot assay. The specimens were washed with PBS buffer and then lysed for 30 min, after which the protein concentration was measured. 20 μg/well protein was selected and 10 min electrophoresis was started after the addition of completed protein buffer, followed by immersion of the electrotransfer membranes in 10% milk and blocking for 90 min at ambient temperature. Then the primary antibody is conjugated, diluted and incubated for one day. After removal and rinsing with TBST solution, the secondary antibody was later conjugated and kept for 60 min then washed with color development. The expression of apoptosis-associated proteins P38 MAPK and MMP-3 was examined.

Statistical processing

SPSS 20. 0 software was used for analysis. The measurement data were described using mean ± standard deviation ($\bar{x} \pm s$), and independent samples t-test was performed for comparison between groups. The statistical data were expressed as %, and the x^2 test was performed for comparison between groups, with P < 0. 05 indicating a statistically significant difference.

Results and discussion

Immunohistochemical section staining of kidney cancer cells

As shown in Figure 1, Figure 1A shows a benign tumor section, which was found to be mostly round in shape and with few dendritic projections and fewer mature DC cells. Figure 1B shows a section of a malignant tumor with T cells distributed in clusters, with more dendritic projections and more mature DC cells.

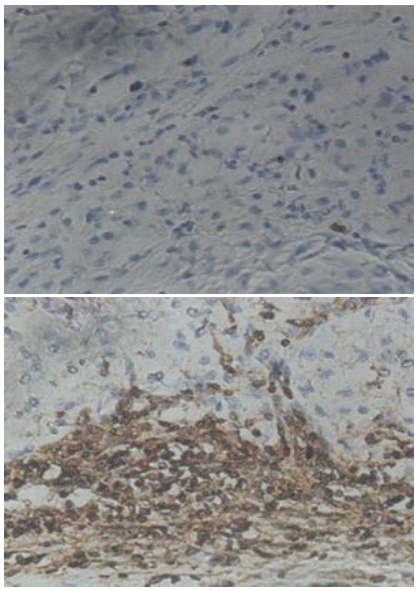

Figure 1. Immunohistochemical section staining of kidney cancer cells

Comparison of miRNA-21 and blood Cr levels between two groups of patients

As shown in Table 1, miRNA-21 and blood Cr levels were increased in the study group compared with the control group, and the difference was statistically significant (P < 0. 05).

Table 1. Comparison of miRNA-21 and blood Cr levels between two groups of patients

Group	Cases (n)	miRNA-21	Blood Cr (μmol/L)
Control group	165	1. 25±0. 16	75. 23±10. 26
Research Group	149	4. 25±0. 48	115. 23±20. 16
t		13. 264	12. 251
P		0. 001	0. 001

Comparison of miRNA-21 and blood Cr levels under different kidney cancer stages

As shown in Table 2, miRNA-21 and blood Cr levels increased in stage II patients compared with stage I patients, miRNA-21 and blood Cr levels increased in stage III patients compared with stage II patients, and miRNA-21 and blood Cr levels increased in stage IV patients compared with stage III patients, with statistically significant differences (P < 0. 05).

Table 2. Comparison of miRNA-21 and blood Cr levels under different kidney cancer stages

Staging	Cases (n)	miRNA-21	Blood Cr
Stage I	78	3. 15±0. 15	105. 26±12. 23
Stage II	34	3. 85±0. 24	110. 23±15. 26
Stage III	15	4. 07±0. 34	118. 25±17. 48
Stage IV	7	5. 66±0. 75	130. 26±187. 46
F		16. 238	21. 534
P		0. 001	0. 001

Comparison of miRNA-21 and blood Cr levels under infiltration and metastasis

As shown in Figure 2, miRNA-21 and blood Cr levels were increased under tumor infiltration compared with the tumor non-infiltrated case, and the differences were statistically significant (P < 0. 05). Compared with the case without distant metastasis, miRNA-21 and blood Cr levels increased under tumor distant metastasis, and the difference was statistically significant (P < 0. 05).

When tumor infiltration and distant metastasis appeared in kidney cancer patients, miRNA-21 and blood Cr levels increased substantially. Thus, miRNA-21 and blood Cr levels were correlated with tumor infiltration and distant metastasis and showed a positive correlation.

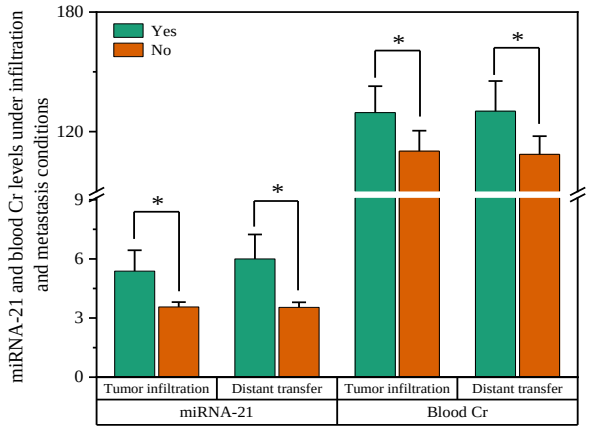

Figure 2. Comparison of miRNA-21 and blood Cr levels under infiltration and metastasis. Note: Compared with the Yes, *P < 0. 05

TIMP1, TIMP2, MACC1 protein expression

Figure 3 show that TIMP1, TIMP2 and MACC1 protein expression increased in the study group compared with the control group, and Figure 4 showed a positive correlation with statistically significant differences (P < 0. 05).

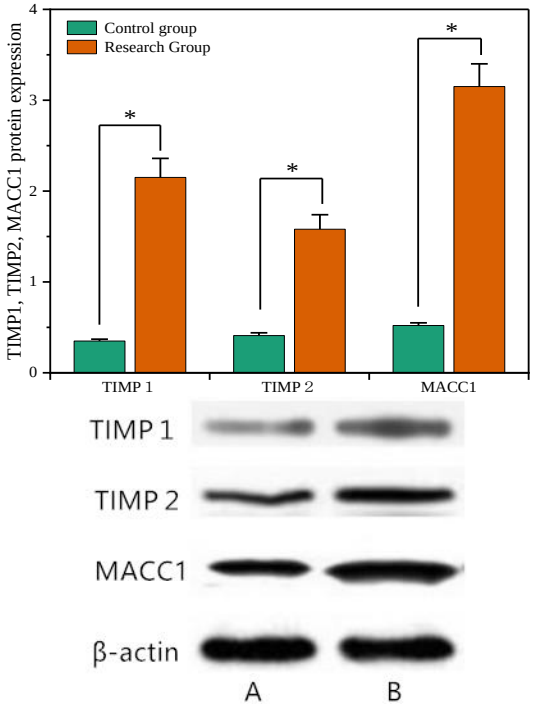

Figure 3. WB map of TIMP1, TIMP2, and MACC1 protein expression. Compared with the control group (up). Western blot assay. Note: A: control group; B: study group. (down). Note: Compared with the control group, *P < 0. 05

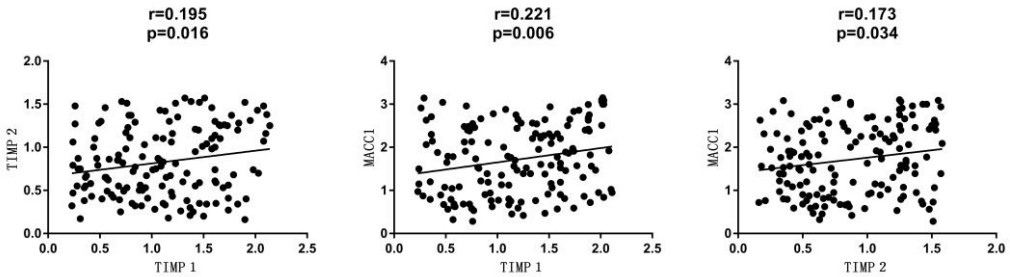

Figure 4. TIMP1, TIMP2, MACC1 expression correlation graph.

As a common clinical malignant tumor of the kidney, kidney cancer has a high aggressiveness. Currently, the main and basic clinical treatment for kidney cancer is surgery, which is divided into radical nephrectomy and simple nephrectomy (9-10). Simple nephrectomy can maximize the preservation of residual kidney function and also reduce the incidence of end-stage renal disease, but simple nephrectomy has the potential for local recurrence (11-12). In addition, when the tumor is removed, the paracancerous tissue that is positive for tumor cells needs to be removed as much as possible. Some

clinical studies have confirmed that a variety of tumors, such as melanoma, bladder cancer, and colon cancer, are capable of leading to an increased probability of local recurrence after surgery with positive tumor cells at the cut edge (13-14).

In previous investigations, it was found that about 20%-35% of kidney cancers had already metastasized at the time of consultation, and about 15% were consulted because of metastatic symptoms, so it is particularly important to clinically investigate the molecular mechanisms of kidney cancer metastasis. miRNAs are a class of endogenous non-coding small RNAs of 18-25 nucleotides in length, and the "seed sequence" of 2-7 nucleotides in the 5' untranslated region of mature miRNAs can complementarily bind to the 3'-untranslated region of target mRNAs to inhibit the expression of target genes at the post-transcriptional level. miRNAs play an important role in tumors and can act as proto-oncogenes or oncogenes, which regulate a variety of important biological behaviors of the tumor surface (15-16). mRNA is involved in the regulation of individual development, apoptosis, proliferation and differentiation by regulating the expression and function of downstream genes. In recent years, miRNAs have rapidly developed into potentially important molecular markers for diseases such as tumors. miRNA, which is localized at 17q23. 1, with oncogene properties, shows high expression in malignant tumors such as pancreatic, esophageal, lung, and colorectal cancers and can be closely related to tumor development by inhibiting apoptosis (17-18). In this study, miRNA-21 was detected and found to show high expression in kidney cancer, and its expression became higher with the stage of the disease. Compared with the absence of tumor infiltration and distant metastasis, miRNA-21 expression was abnormally increased when tumor infiltration and distant metastasis of kidney cancer occurred, indicating that miRNA-21 was closely related to tumor infiltration and distant metastasis of kidney cancer and showed a positive correlation.

Blood Cr is blood creatinine, which is generally considered to be a product of muscle metabolism in the body. In muscle, creatine is formed slowly, mainly through an irreversible non-enzymatic dehydration reaction, after which it is put into the bloodstream and excreted in the urine. Therefore, blood creatinine is closely related to total muscle mass in the body and is less susceptible to the influence of diet. Changes in blood creatinine concentration are mainly determined by the filtration capacity of the glomerulus (19-20). When the filtration capacity decreases, the creatinine concentration increases. A higher than normal blood creatinine level in most cases indicates damage to the liver. Although creatinine is a more accurate indicator of renal parenchymal damage, it is not a sensitive indicator. Creatinine is a small molecule, which can be filtered by the glomerulus so that the renal tubules rarely absorb creatinine, and there is a small amount of creatinine in the renal tubules. Most of the creatinine produced in the human body daily will be excreted in the urine. Therefore, the blood creatinine test can be a better measurement of the body's kidney function in clinical practice, and it is an important indicator of kidney function. When there is an abnormal rise in blood creatinine in clinical tests, it can prove that the kidney function of the human body is impaired (21-23). In this study, blood Cr was measured and found to be high in kidney cancer, and its level increased with the stage of cancer. Compared with kidney cancer without tumor infiltration and distant metastasis, blood Cr levels increased when kidney cancer developed tumor infiltration and distant metastasis. This result indicates that blood creatinine is closely related to kidney cancer and shows a positive correlation with the appearance of tumor infiltration and distant metastasis.

In summary, it was found that miRNA-21 expression and blood Cr levels increased abnormally in patients with kidney cancer and that miRNA-21 expression and blood Cr levels changed with the development of the kidney cancer stage. When kidney cancer showed tumor infiltration and distant metastasis, miRNA-21 expression and blood Cr level increased abnormally, indicating that the two were closely related to kidney cancer tumor infiltration and distant metastasis, showing a positive correlation.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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