CHANGES IN PLASMA MIR-21 EXPRESSION LEVELS IN PATIENTS WITH ADVANCED COLON CANCER BEFORE AND AFTER TREATMENT WITH OXALIPLATIN AND CLINICAL SIGNIFICANCE

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ABSTRACT

Objective: *This work aimed to investigate the changes in plasma microRNA-21 and its clinical significance in patients with advanced colon cancer before and after treatment with tiggio combined with oxaliplatin.*

Methods*: RT-PCR was used to detect the relationship between plasma microRNA-21 and the therapeutic effect of tiggio combined with oxaliplatin in 73 patients with advanced colon cancer before and after treatment.*

Results*: The level of miR-21 in the plasma of patients with advanced colon cancer was significantly higher than that of the control group before treatment (t=25.31, P<0.001). Compared with 1 week before treatment, miR-21 significantly decreased in the plasma of patients with advanced colon cancer 2 weeks and 6 weeks after treatment (F=219.48, P<0.001). The level of miR-21 in the CR + PR group was significantly lower than that of the SD + PD group (t=-8.56, P<0.001).*

Conclusion*: Plasma miR-21 may be the sensitive index for predicting the efficacy of tegafur combined with oxaliplatin in patients with advanced colon cancer, and it may guide the clinical treatment of advanced colon cancer.*

Keywords*: colon cancer, tiggio, oxaliplatin, miR-21, clinical significance.*

DOI: 10.19193/0393-6384_2019_2_110

Received *Septemper 17, 2018;* ***Accepted*** *December 20, 2018*

Introduction

Colon cancer is the third most common cancer among males and females in the United States. Owing to the prevention of risk factors, the introduction and spread of screening tests and the improvement of treatment modalities, the incidence and mortality of colon cancer have been declining for decades, although it still accounts for approximately 7% of all cancers and is one of the most common causes of cancer-related death.

The prognosis of patients depends mainly on TNM staging at diagnosis and radical surgical resection, and radical resection can only be done in patients with local disease(1). Colon cancer is an invasive disease that still has a considerable impact on global health. The number of colon cancer cases will continue to grow in the next few years, especially among young people according to predictions(2). Colon cancer is one of the malignant tumours with the highest incidence of gastrointestinal cancer and the highest mortality rate.

In recent years, the incidence of colon cancer in China has been increasing year by year, seriously jeopardizing people's health and quality of life. Various molecules, including DNA, proteins, and microRNAs, can be used as new molecular markers for non-invasive and accurate experiments for colon cancer screening[3-5]. MicroRNAs, endogenous non-protein-coding small RNAs, act as carcinogenic mediators or suppress tumours and cancer cell spreading[6], and miR-21, a protooncogene involved in many cellular processes and tumourigenesis, is frequently overexpressed in some cancer types[7]. MiR-21 is involved in the occurrence and development of tumours. Overexpression of miR-21 is a new prognostic biomarker for malignant tumours, which are characterized by high proliferation and high growth rate and is positively correlated with low apoptosis[8]. MiR-21 expression has been associated with clinical stage, lymph node metastasis and distant metastasis in colon cancer patients[9], but no studies have assessed the correlation between miR-21 expression and tiggio and oxaliplatin in the treatment of colon cancer. Therefore, in this paper, real-time fluorescence quantitative examination was used to dynamically monitor the level of miR-21 in plasma of patients with colon cancer receiving tiggio combined with oxaliplatin 1 week before treatment, 2 weeks after treatment and 4 weeks after treatment. Determining whether the clinicopathological features are related is intended to provide a reference for the diagnosis and treatment of colon cancer.

Materials and Methods

General information

The 73 patients with advanced colon cancer who received treatment in hospital from January 2016 to January 2018 were selected (44 males and 29 females, aged 52~74 years). The Karnofsky score of each patient included in the study was over 60. The expected survival time of the patients was more than 6 months, and each patient had more than one tumour lesion. The liver and kidney functions of the patients were normal. The electrocardiogram examination showed no abnormality. All patients signed the informed consent and volunteered to participate. This study was approved by the Medical Ethics Committee of our hospital.

Method

Treatment

All patients took oral tiggio (manufacturer: Shandong New Times Pharmaceutical Co., Ltd., production batch number: 20160408, national standard number: H20080802) 40-60 mg per time, 2 times per day, repeating from day 1 to 14 for 3 weeks and intravenous infusion of 130 mg/m2 oxaliplatin (manufacturer: Jiangsu Hengrui Pharmaceutical Co., Ltd., production batch number: 20161208, national standard number: H20000337) for 3 h on the first day, repeated for 3 weeks. All patients were evaluated for clinical effects after 2 cycles of treatment.

Blood sample collection

In the control group, 73 healthy volunteers and 73 patients with advanced colon cancer gave 5 mL peripheral venous blood on an empty stomach one week before treatment. Blood was re-extracted 2 weeks and 6 weeks after treatment. The blood samples were underloaded by a vacuum glass tube containing EDTA anticoagulant. After standing for 10 min, the plasma was centrifuged to separate in a test chamber (2000 r/min at 4 °C for 10 min). The enzyme was centrifuged to separate the plasma in the 1.5 mL centrifuge tube of RNA-free nucleic acid and stored at -80 °C for examination.

Plasma total RNA extraction

The frozen plasma samples were thawed at room temperature. Each 400 μL sample was transferred into clean 1.5 mL EP tube, centrifuged (2000 r/min at 4 °C for 10 min) and collected by centrifugation. The upper layer of plasma and QIA amp Viral RNA Mini Kit reagent were added at a ratio of 2:1 (v/v) to a new EP tube. The tube was mixed well by shaking, placed in an ice-water mixture for 15 min, then centrifuged (12000 r/min at 4 °C for 30 min), followed by removing the supernatant, washing twice with the cartridge''s own adsorption column and buffer, and finally dissolving the total RNA with the solution. The concentration and purity of the RNA were determined using a nucleic acid protein meter.

Plasma miR-21 detection

The miR-21 was detected by the RT-PCR, and the reverse transcription reaction was carried out in strict accordance with the instructions attached to the kit (Hairpin-it TM miRNAs q PCR Quantitation

Kit was purchased from Shanghai Jima Pharmaceutical Technology Co., Ltd.). Real-time PCR: target miRNAs were transcribed using universal reverse transcription conditions. Amplification conditions: 95 °C 30 s; 95 °C 10 s; 55 °C 15 s; 72 °C 20 s; 95 °C 10 s; cycle 35 times. Each test table specimen were tested 3 times. of U6snRNA was amplified as an internal reference gene for PCR amplification, and the level of miR-21 was represented by $2^{-\Delta\Delta Ct}$.

Evaluation criteria

According to RECIST 1.1, the evaluation criteria for the therapeutic effect of solid tumours are divided into[10]

CR: colon cancer tumour lesions disappear; PR: the total length of the colon tumour lesions is reduced by more than 30%;

PD: the total length of the colonic tumour lesions increased by more than 20% or new colon cancer tumour lesions appeared;

SD: the total length of the colon cancer tumour lesions decreased but did not reach PR or increased but did not reach PD.

Statistical processing

Statistical analysis was performed by SPSS 21.0. The level of miR-21 is expressed as the mean ± standard deviation ($\bar{x}\pm s$). The levels of miR-21 (1 week before treatment, 2 weeks after treatment and 6 weeks after treatment) were compared by the t-test. The correlation between the level of miR-21 and clinical efficacy was measured by the independent-sample t test. $P < 0.05$ was considered statistically significant.

Results

Comparison of plasma miR-21 between patients with advanced colon cancer and control group before treatment

Before treatment, miR-21 was significantly higher in patients with advanced colon cancer than that in the control group (t=25.31, P<0.001) (Table 1).

Changes in miR-21 from before to after treatment of advanced colon cancer

Compared with one week before treatment, miR-21 decreased significantly in patients with advanced colon cancer 2 weeks and 6 weeks after treatment (F=219.48, P<0.001), as shown in Table 2.

Group	n	miR-21 level	t	P
Colon cancer patient	73	8.56±2.53	25.31	< 0.001
Control	73	1.04±0.21		

Table 1: Comparison of plasma miR-21 between patients with advanced colon cancer and control group (x±s).

Variable	1 week before treatment	2 weeks after treatment	6 weeks after treatment	F	P
miR-21	8.56± 2.53	5.41±1.22[a]	2.64±0.93[ab]	219.48	< 0.001

Table 2: Changes in miR-21 from before to after treatment of advanced colon cancer ($\bar{x}\pm s$, n=73).

The level of miR-21 was significantly lower in the CR + PR group than that in the SD + PD group (t=-8.56, P<0.001), as shown in Table 3.

Group	n	miR-21 level	t	P
CR + PR	32	7.16 ± 1.03	-8.56	<0.0001
SD + PD	41	9.46 ± 1.24		

Table 3: Correlation between miR-21 level and therapeutic effect ($\bar{x}\pm s$).

Conclusion

MicroRNA-21 is an endogenous non-protein-coding RNA molecule and one of the most commonly overexpressed microRNAs in gastric cancer, lung cancer, liver cancer, colon cancer and other tumours. It is closely related to the occurrence, development and treatment of tumours. The clinical treatment of tumours based on miR-21 has certain clinical effects both at home and abroad. Mir-21 can regulate the sensitivity of tongue squamous cell carcinoma cells to cisplatin, suggesting that miR-21 may be a potential target for the treatment of tongue squamous cell carcinoma[11]. Downregulation of microRNA-21 in peripheral blood of lung cancer patients can enhance the sensitivity of lung cancer cells to cisplatin[12]. Knocking out microRNA-21 in HT-29 colon cancer cells can increase their chemosensitivity to 5-fluorouracil[13]. The Naohide study found that high expression of microRNA-21 in colon cancer tissues was associated with poor prognosis and adjuvant chemotherapy response in colon cancer[14].

Tegafur is an anticancer preparation, mainly composed of tegafur, glimepiride and otiracycline, which is the precursor of 5-fluorouracil and the main anticancer component. The stability of uracil produced by tegafur is poor, and it is easily degraded by dihydropyrimidine dehydrogenase in vivo[15].

Gimeracil is a powerful dihydropyrimidine dehydrogenase inhibitor, which can slow down the decomposition of fluorouracil, prolonging the action time of drugs and improving the antitumour effect of tegafur. Otiracycline can block the phosphorylation of 5-fluorouracil, reducing the production of 5-fluorouracil-phosphodeoxyuridine, and alleviating the gastrointestinal side effects of tegafur[16].

Recently, tiggio has become the first-line drug for colon cancer and has been widely used in clinical treatment. Oxaliplatin is a third-generation platinum anticancer drug. It has obvious cytotoxicity that has a good effect on cloning drug resistance of many conventional chemotherapeutic drugs and a synergistic effect with 5-fluorouracil. At present, it is mainly used in the treatment of colorectal cancer[17,18].

Our results showed that the microRNA-21 was significantly higher in the plasma of patients with colon cancer than that in the control group before treatment, indicating that the plasma level of microRNA-21 in patients with colon cancer is closely related to the occurrence of colon cancer, which is consistent with the literature[19]. Compared with the first week before treatment, microRNA-21 decreased significantly in plasma of patients with colon cancer ($P<0.05$) after 2 and 6 weeks of treatment, suggesting that the microRNA-21 level in plasma of patients with colon cancer was affected by the treatment regimen of tiggio combined with oxaliplatin. In addition, plasma microRNA-21 was significantly lower in the CR + PR group than that in the SD + PD group, indicating that there was a correlation between the level of plasma microRNA-21 and the therapeutic effect of tiggio combined with oxaliplatin. The high level of plasma microRNA-21 in SD + PD patients may also indicate resistance to tiggio combined with oxaliplatin. Reducing plasma microRNA-21 may increase the sensitivity to tiggio combined with oxaliplatin[20].

In conclusion, the level of plasma microRNA-21 in patients with advanced colon cancer may be a sensitive biomarker for predicting the therapeutic effect of tiggio combined with oxaliplatin and judging the clinical therapeutic effect on advanced colon cancer. There are also some shortcomings in this work. The observation follow-up time was too short to calculate the total survival time of patients. The correlation between the level of microRNA-21 and the prognosis of patients with colon cancer needs to be further tracked and discussed.

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Acknowledgements
The therapeutic effect and mechanism of ultrasound microbubble leading multiple microRNAs timing expression system for colorectal cancer. (NO.:2015J01531), To investigate the effect and mechanism of targeted BMP-7 microbubbles on myocardial fibrosis (The Projects funded by overseas students in science and technology activities, Ministry of human resources and social security of the people's republic of china. 2015Year), To investigate the therapeutic effect and mechanism of ultrasound microbubble inducing BMP7 expression system reversing fibrosis of cardial and renal complication in the hypertension syndrome (NO.: 2016-ZQN-87).

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