Iran Red Crescent Med J. 2020 January; 22(1):e97983. doi: 10.5812/ircmj.97983.

Published online 2020 February 2. **Research Article**

A Clinical Study on the Expression of miR-195 in Cardiac Carcinoma

Hao Wang [1], Cai-Xia Ba [1,*], Xue-Feng Bai [2], Pei-Long Zhang [1], Gang-Ling Zhang [1], Fei Yu [2], Bing-Chang Tian [1], Zuo-Jun Li [3] and Shui-Ying Zhou [3]

[1] Department of Surgical Oncology, Baotou Cancer Hospital, Baotou, China
[2] Department of Pathology, Baotou Cancer Hospital, Baotou, China
[3] Department of Digestive System Disease, Baotou Cancer Hospital, Baotou, China

[*] *Corresponding author*: Department of Surgical Oncology, Baotou Cancer Hospital, No. 18, Tuanjie St., Qingshan District, Baotou 014030, China. Tel: +861-04725913866, Fax: +861-04725156681, Email: bacx658410@163.com

Received 2019 September 10; **Revised** 2020 November 26; **Accepted** 2019 December 23.

Abstract

Background: Recently, some studies have revealed that microRNAs (miRs; miRNAs) mainly regulate gene expression in the transcription of microRNAs. However, the role of microRNAs in cell development, differentiation, proliferation, and other physiological processes remains unclear.
Objectives: The present study aimed to investigate the expression level of microRNA-195 in the pathological tissues of patients with cardiac carcinoma and its clinical effects.
Methods: Patients with primary cardiac carcinoma were enrolled as the study subjects. The tumor and adjacent tissue samples for cancer pathology were obtained during the operation. Reverse transcription polymerase chain reaction (RT-PCR) was used to detect the expression of microRNA-195 and its correlation with the clinicopathological features of cardiac carcinoma was analyzed.
Results: A total of 64 patients were included in this study. Firstly, the expression rate of miR-195 in cardiac carcinoma tissues showed a significant decreasing trend (3.65 ± 0.42 vs. 2.05 ± 0.33) ($P < 0.001$) and the actual expression level of miR-195 in pathological tissues of cardiac carcinoma was negatively correlated with the malignant degree of pathological tissues ($P = 0.028$), invasion ($P = 0.037$), and lymph node metastasis of cardiac carcinoma ($P = 0.023$), but miR-195 was not correlated with age ($P = 0.615$) and gender ($P = 0.465$).
Conclusions: The expression of microRNA-195 is closely correlated to the severity of cardiac carcinoma and the occurrence of lymph node metastasis.

Keywords: miRNA-195, Cardiac Carcinoma, RT-PCT, Adenocarcinoma, Mucosa Tissues

1. Background

Primary cardiac carcinoma is a type of adenocarcinoma that occurs in the tissue structure of the human cardia. Its anatomic location is within 2 cm below the boundary between the esophageal tissue and gastric organs. When compared with patients with lower esophageal cancer and gastric cancer, it has relatively independent and typical anatomical and histological characteristics, clinical symptoms, and treatment measures (1-3). Since cardiac carcinoma has the characteristics of high malignancy, strong invasiveness to the surrounding tissues, and being prone to cervical lymph node metastasis, the prognosis for patients is generally poor. Therefore, exploring a new individualized treatment for cardiac carcinoma is of great significance to the prognosis of patients (2, 3). In recent years, some studies have revealed that microRNAs (miRs; miRNAs) mainly regulate gene expression in the transcription of microRNAs, thus, playing a role in cell development, differentiation, proliferation, and other physiological processes (4-6).

In recent years, a number of studies have revealed that the expression of miR-195 decreased in tissues of many tumors including bladder cancer, breast cancer, liver cancer, colon cancer, and gastric cancer. From this finding, it is determined that miR-195 may be a tumor suppressor gene. A scholar considered that miR-195 could induce cell arrest in the G1/s phase and could inhibit the proliferation and invasion of tumor cells and promote its apoptosis by directly inhibiting apoptotic factor Bdl-2, thereby inhibiting the formation of tumors.

2. Objectives

In the present study, 64 patients with primary cardiac carcinoma who were admitted, diagnosed, and treated at

the Baotou Cancer Hospital of Inner Mongolia were enrolled as the study subjects, and the correlation between the expression level of microRNA-195 (miR-195) and the condition of patients with primary cardiac carcinoma was calculated and analyzed in order to clarify the important role of miR-195 in the occurrence and development of cardiac carcinoma. The details are reported as follows.

3. Methods

3.1. General Information

This study was a cross sectional study. Patients with cardiac carcinoma who were admitted to China Mongolian Baotou Cancer Hospital from January 2014 to December 2016 were enrolled in this study. This study has been approved by the Ethics Committee of our hospital, and all patients have signed the informed consent.

Inclusion criteria: (1) histological or cytological diagnosis of tumors; (2) tumors that could be surgically resected after clinical evaluation; (3) patients who could tolerate abdominal or thoracic surgery; (4) patients without a history of other digestive system tumors; (5) patients without a history of chemotherapy or radiotherapy before the operation.

Exclusion criteria: patients that had severe complications. The analysis was carried out according to the 2010 UICC/staging criteria for pathological Tumor Node Metastasis (TNM) of cardiac carcinoma and patients that underwent preoperative chemotherapy/radiotherapy with incomplete treatment and medical records were excluded.

The sample size formula: $N = Z^2 \times (P \times (1 - P))/E^2$

N: sample size; Z: statistics, Z = 1.64 when the confidence is 90%; E: error value; P: probability value.

Waxed specimens of pathological tissues from all included patients were selected. Normal cardiac tissue at 5 cm outside the tumor were taken and confirmed as normal tissues. The Pathology Department of Baotou Tumor Hospital carried out the detection.

3.2. Reagents and Instruments

LEICA RM2245 microtome (LEICA, Germany); quantitative PCR machine ABI 7300 plus (Applied Biosystems, USA); miR-195 extraction kit (TIAN GEN, China); miR-enhanced miRNAcDNA first chain synthesis kit (TIAN GEN, China); miR-enhanced miRNA quantitative fluorescence kit (TIAN GEN, China); has-U6 detection primers and miR-195 primers (Thermo Fisher, USA) were used in the present study. ABI7300Plus (Applied Biosystems, USA): Error range: ABI 7300 real-time fluorescent quantitative PCR system can achieve the following performance objectives after verification: linear range of 9 orders of magnitude, the 50ul reaction volume can be detected for a single template reporting only 10 initial copy numbers in the fluorescence TaqMan experiment, with a confidence of 99.7%.

MiR-195 primer sequences: 5'-ACA CTC CAG CTG GGT AGC AGC ACA GAAAT-3'. Total RNA was extracted by the TRIzol method. Reverse transcription of cDNA and quantitative fluorescence PCR were performed. U6 was used as an internal control gene. Three threshold cycles (Ct) were set for each reaction. After integrating the reactions, the Cts of each reaction was analyzed by quantitative fluorescence PCR. The formula of relative expression is $F = 2^{-\Delta\Delta Ct}$. $\Delta\Delta Ct = (Ct_{miR\text{-}195} - Ct_{U6})_{cancer\ tissue} - (Ct_{miR\text{-}195} - Ct_{U6})_{paracancerous\ depression}$. Each reaction had internal control and blank control. The amplification was repeated three times in both the experimental group and the control group.

3.3. Statistical Analysis

The statistical analysis was conducted using statistical software SPSS version 20. The continuous variable was expressed as mean $\pm$ standard deviation and the binary variable was expressed as percentages. Non-normal data was expressed as median and interquartile range (IQR). The W test was used for normality assessment. Normally distributed data were compared between two groups using a *t*-test, and non-normally distributed data were compared between two groups using a nonparametric test. The correlation between the expression of miR-195 and the clinicopathological characteristics of patients with primary cardiac carcinoma was evaluated using χ^2 test. Furthermore, the prognostic analysis and process were carried out in accordance with standardized procedures. A $P < 0.05$ was considered statistically significant.

4. Results

4.1. The General Characteristics

From January 2014 to December 2016, there were 105 patients with cardiac cancer. According to the exclusion criteria, 41 patients did not meet the requirements of this study and were excluded. The remaining 64 patients with cardiac carcinoma were included in the study. The participants comprised of 52 male patients and 12 female patients. The ages of the patients ranged between 45 - 78 years, with an average age of 62.3 years.

4.2. The expression Rate of miR-195 in Cardiac Carcinoma and Normal Mucosa Tissues

The detection results revealed that, when compared with the adjacent tissue with normal physiological states, the expression rate of miR-195 in cardiac carcinoma tissues showed a significant decreasing trend (3.65 ± 0.42 vs. 2.05 ± 0,33), the difference was statistically significant (P < 0.001, Table 1).

Table 1. The Expression Rate of miR-195 in Cardiac Carcinoma and Normal Mucosa Tissues

Index	Cases	miR-195
Expression in cardiac carcinoma tissue	64	2.05 ± 0,33
Expression in normal mucosa tissues	64	3.65 ± 0.42
***t* value**		3.245
P value		0.000

4.3. Correlation of Expression of miR-195 and Clinicopathological Features of Cardiac Carcinoma

In order to evaluate the correlation of expression of miR-195 with clinicopathological features and the prognosis of cardiac carcinoma, according to the expression of miR-195 in the tissues, the patients were divided into two groups: low expression group and high expression group. The grouping basis was as follows: in patients with relative expression of miR-195 < the median was assigned to the low expression group and in patients with relative expression of miR-195 > the median was assigned to the high expression group.

The actual expression level of miR-195 was negatively correlated with the degree of malignancy of pathological tissues of the tumor in the patients. It is usually considered that the higher the degree of malignancy, the lower the expression level of miR-195 in the pathological tissues of patients (P < 0.05). The actual expression level of miR-195 was directly correlated to the actual lymph node metastasis. Moreover, the expression level of miR-195 in pathological tissues of cardiac carcinoma in patients with lymph node metastasis was significantly lower than that in patients without lymph node metastasis and the number of lymph node metastases was negatively correlated with the expression level of miR-195 (P < 0.05). The actual expression level of miR-195 was not correlated to gender, age, T-stage, and M-stage in patients with cardiac carcinoma (P > 0.05, Table 2).

5. Discussion

The results of this study revealed that, compared with the adjacent tissue with normal physiological states, the actual expression level of miR-195 in pathological tissues of cardiac carcinoma was negatively correlated with the degree of malignancy of pathological tissues and the expression rate showed a significant decreasing trend; the expression of miR-195 was negatively correlated with the degree of malignancy, invasion, and lymph node metastasis of cardiac carcinoma and was not correlated with age and gender.

Primary cardiac carcinoma is a common malignant tumor occurring in the human gastric organs; its incidence and mortality rate has been at a high level for a long time. Diagnosis and intervention in the early stage of the disease are of great significance to provide patients with the best effect of clinical treatment and intervention activities (3, 7, 8).

The MiRs are small non-coding RNAs that participate in tissue-specific gene regulation of the eukaryotic cells. Recent studies have proposed that miRs may play a dual regulatory role in the development of tumors, where some miRs are oncogenes, while others are anti-oncogenes. Therefore, the classification of miRs is conducive to further study in the occurrence and development of tumors (5).

MicroRNA-203 (miR-203) is located on chromosome 14, which negatively regulates cell proliferation in tumors such as head and neck squamous cell carcinoma, liver cancer, chronic myeloid leukemia, and B-cell leukemia. A previous study revealed that, compared with normal control tissues, in tumor tissues, the expression of miR-203 was significantly decreased while the expression level of Np63 was significantly increased. Therefore, increasing the expression of miR-203 has a certain inhibitory effect on esophageal squamous cell carcinoma. Furuta et al. confirmed in their study that miR-203 inhibited the proliferation of esophageal squamous cell carcinoma cells by mediating the Np63 signaling pathway (9).

MicroRNA-21 (miR-21) is considered to be a microRNA with oncogene function, which is over-expressed in tissues of a variety of tumors. The functional target of miR-21 in various aspects of tumor progression (cell proliferation, invasion, metastasis, and mass formation) is PDCD4. Hiyoshi et al. revealed in their study that the expression of miR-21 was negatively correlated with the level of PDCD4 protein (its mechanism is to regulate the expression of PDCD4 in the late stage of translation by binding to the 3' non-coding region of messenger RNA of PDCD4). Yao et al. also revealed in their study that miR-21 was involved in many genes directly regulating the proliferation and flow of cancer cells in fibroblasts and also promoted the interaction of tumor mechanisms and induced cell transformation (10).

MicroRNA-16-2 (miR-16-2) may target retinoic acid receptor B2 (RAR-B2). A previous study revealed that ben-

Table 2. The Correlation of Expression of mir-195 and Clinicopathological Features of Cardiac Carcinoma

Index	Cases	Mir-195 Expression (Cases)		χ^2	P Value
		Low Expression	High Expression		
Sex				1.746	0.465
Male	52	33	19		
Female	12	10	2		
Age, y				1.063	0.615
$\geq$ 60	24	18	6		
< 60	40	25	15		
Degree of tumor differentiation				5.316	0.028
High and middle	16	7	9		
Low	48	36	12		
Invasion depth				4.363	0.037
Invading mucous membrane and muscular layer	12	5	7		
Invading the outer membrane and above	52	38	14		
Lymph node metastasis				5.429	0.023
Yes	43	33	10		
No	21	10	11		
TNM stage				2.849	0.074
I II	11	5	6		
III IV	53	38	15		

zopyrenediol epoxide (BPDE) could inhibit the expression of RAR-B2 and induce the expression of miR-16-2. In addition, RAR-B 2 is a major member of the RAR-B family and also the main subtype induced by retinoic acid. Also, RAR-B 2 is considered as a tumor suppressor gene in many cancer studies. In vitro animal and cell experiments confirmed that cells transfected with miR-16-2 could inhibit the expression of RAR-B 2 and miR-16-2 induced proliferation, colony formation, and apoptosis of the esophageal cancer cells (11).

The MiR-195 belongs to the miR-16/15/195 family, which is located in human Chromosome 17pl3.19. In bladder cancer, miR195 inhibits the growth of bladder cancer cells by regulating CDK4, a G1 cell cycle regulator (12). The MiR-195 is highly conservative and regulates cell proliferation, differentiation, metastasis, apoptosis, and other processes by binding to the corresponding target sites in the 3'-UTR region of the target gene, thereby participating in the occurrence and development of tumors, cardiovascular diseases, central nervous system diseases, and other diseases. In addition, the expression of miR-195 is also regulated by upstream transcription factors and epigenetic modifications (13-17).

The present study revealed that the expression of miR-195 was decreased in patients with cardiac carcinoma and significantly decreased in patients with lymph node metastasis. The investigators consider that the low expression of miR-195 relieves cell cycle arrest, promotes cell proliferation, inhibits apoptosis, and induces cell dedifferentiation. The final manifestation is that the invasive potential of tumor cells increases and the tumors are likely to invade lymph nodes. The MiR-195is an anti-cancer factor, its expression is negatively correlated with the invasive potential of tumors and a lower expression level indicates a poor prognosis (18-21). Therefore, detection of miR-195 in tissue samples from patients with cardiac carcinoma can be used to predict the infiltration and lymph node metastasis of tumors and can also be used to further evaluate the prognosis of patients (22, 23).

Recently, some studies revealed that microRNAs (miRs; miRNAs) mainly regulate gene expression in the transcription of microRNAs. However, the role of microRNAs in cell development, differentiation, proliferation, and other physiological processes remains unclear. Our study has found out that the expression of microRNA-195 is closely correlated to the severity of cardiac carcinoma and the occurrence of lymph node metastasis. However, this study still has the following limitations. Firstly, this study is not a randomized controlled clinical trial and a blind sample was not used, thus, there is still a certain risk of bias. Sec-

ondly, this study is a single-center clinical trial and the number of included clinical samples is small, thus, further multi-center clinical trials with an enlarged sample size are still needed. Finally, the detection method in this study is relatively simple, in subsequent studies; it needs to combine with Western blot and immunohistochemistry for further investigation.

5.1. Conclusion

MicroRNAs are types of natural non-coding RNAs, which can regulate the corresponding gene expression by targeting the corresponding messenger RNAs and inhibit the process of oncogene translation accordingly. Different microRNAs can inhibit or promote the proliferation, metastasis, invasion, apoptosis, and progression of cardiac carcinoma. Therefore, microRNAs in serum can be used as non-invasive biomarkers for the detection of cardiac carcinoma, providing a basis for diagnosis, prevention, and treatment of cardiac carcinoma. In addition, the prognosis of cardiac carcinoma can be improved by regulating the expression of microRNAs or by removing the corresponding oncogenes. Relevant data results obtained in the course of this investigation fully revealed the correlation of the miR-195 expression with the severity of cardiac carcinoma and the occurrence of lymph node metastasis. The relevant research results have full clinical popularization value. Moreover, the expression level of miR-195 is closely correlated to the severity of cardiac carcinoma and the occurrence of lymph node metastasis. Therefore, the detection of the expression level of miR-195 has important guiding significance for the clinical diagnosis and treatment of primary cardiac carcinoma.

Footnotes

Authors' Contribution: Substantial contributions to the conception and design of the work, and draft of the work: Hao Wang and Cai-Xia Ba; the acquisition, analysis, and interpretation of data for the work: Hao Wang, Cai-Xia Ba, Xue-Feng Bai, Pei-Long Zhang, Gang-Ling Zhang, Fei Yu, Bing-Chang Tian, Zuo-Jun Li, and Shui-Ying Zhou; revising it critically for important intellectual content: Hao Wang, Cai-Xia Ba, Xue-Feng Bai, Pei-Long Zhang, Gang-Ling Zhang, Fei Yu, Bing-Chang Tian, Zuo-Jun Li, and Shui-Ying Zhou; final approval of the version to be published: Hao Wang, Cai-Xia Ba, Xue-Feng Bai, Pei-Long Zhang, Gang-Ling Zhang, Fei Yu, Bing-Chang Tian, Zuo-Jun Li, and Shui-Ying Zhou; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Hao Wang, Cai-Xia Ba, Xue-Feng Bai, Pei-Long Zhang, Gang-Ling Zhang, Fei Yu, Bing-Chang Tian, Zuo-Jun Li, and Shui-Ying Zhou.

Conflict of Interests: None of the authors had any personal, financial, commercial, or academic conflicts of interest separately.

Ethical Approval: This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Baotou Cancer Hospital.

Funding/Support: The project of social development supported by Science and Technology in Baotou city (No. 2017S2001-3-5).

Informed Consent: Written informed consent was obtained from the participants.

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