

Predictive value and changes of miR-34a after concurrent chemoradiotherapy and its association with cognitive function in patients with nasopharyngeal carcinoma

WEIYU DENG¹, ANQI LIN² and JIALIN YANG³

Departments of ¹Neurology, ²Pneumology and ³Oncology, Shunde Hospital, Guangzhou University of Traditional Chinese Medicine, Foshan, Guangdong 528300, P.R. China

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Abstract. This study explored the changes and predictive value of miR-34a in nasopharyngeal carcinoma (NPC) after concurrent chemoradiotherapy (CCRT), and its association with cognitive function. Fifty NPC patients admitted to Shunde Hospital, and another fifty healthy individuals were assigned into treated group and control group, respectively. Patients in the treated group received 3 courses of CCRT. The relative expression of miR-34a in the two groups was detected, and the cognitive function of patients was assessed. Diagnostic and predictive values of miR-34a in advanced NPC were analyzed. The expression of miR-34a in the control group was significantly higher than that in the treated group ($t=13.364$, $P<0.001$), with an area under the curve (AUC) of 0.979. The expression of miR-34a was significantly upregulated after treatment ($t=4.559$, $P<0.001$). After treatment, there were 32 complete remission (CR) patients and 18 partial remission (PR) patients. According to efficacy, CR patients were classified as significant group and PR patients as general group. The expression of miR-34a in the significant group was higher than that in the general group before treatment ($t=4.704$, $P<0.001$), with an AUC of 0.852. The Montreal Cognitive Assessment (MoCA) score was significantly decreased after treatment ($t=13.042$, $P<0.001$). The expression of miR-34a was positively correlated with the MoCA score after treatment, that is, MoCA score gradually increased with the upregulation of miR-34a expression ($r=0.379$, $P=0.006$). There is a positive correlation between miR-34a and cognitive function of patients. Moreover, the expression of miR-34a can be used as a potential predictor of the efficacy of CCRT in patients with NPC.

Introduction

Nasopharyngeal carcinoma (NPC) is a highly malignant tumor arising from epithelial cells (1). In 2015, there were 60,600 newly diagnosed NPC and 34,100 deaths in China (2). Despite the low morbidity and mortality, the treatment of NPC has always been difficult in clinical practice. NPC is occult and adjacent to optic nerve, trigeminal nerve, and internal carotid artery, so it is difficult to be treated by surgery (3). With the exception of a small number of patients with early NPC who can be cured by radical surgery, most patients are treated with palliative chemoradiotherapy to improve the condition (4). At present, there are few efficacy assessment indicators for NPC, and imaging examinations are mainly applied to assess the efficacy of chemoradiotherapy (5). However, serological test is less harmful, faster and more convenient. Therefore, it is of great significance to find a specific serological index (6).

Patients with NPC always suffer from cognitive decline after chemoradiotherapy. Currently, Montreal Cognitive Assessment (MoCA) score and Das-Naglieri cognitive assessment system are commonly used to assess the cognitive function of patients (7). However, subjective bias may occur in artificial scoring, so it is very important to find a new observation index for cognitive function assessment (8). MicroRNAs (miRs) is a class of newly discovered, highly conserved, endogenous, non-coding, hairpin nucleotide transcripts that widely exist in eukaryotic cells and are approximately 19-25 bases in length and 18-25 nucleotides in size (9). Some miRNAs are reported to be closely related to the occurrence and progression of NPC (10). As an important member of the miR-34 family, miR-34a is located at 1p36.23 and mainly distributed in brain tissue under normal circumstances. A study found that it has a significantly reduced expression in NPC and was expected to become a potential diagnostic indicator for NPC (11). Another study suggested that miR-34a was closely associated to cognitive function, that is, cognitive function could be improved by downregulating the expression of miR-34a (12). Therefore, we speculate that there may be a correlation between miR-34a and cognitive function, but whether miR-34a is related to the cognitive impairment in patients with NPC has not been confirmed yet.

Therefore, this study explored the potential value of miR-34a in assessing cognitive function and radiotherapy

Correspondence to: Dr Jialin Yang, Department of Oncology, Shunde Hospital, Guangzhou University of Traditional Chinese Medicine, Daliang Jinsha Avenue, Foshan, Guangdong 528300, P.R. China
E-mail: jpxr71@163.com

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efficacy in patients with NPC by determining its expression before and after radiotherapy, so as to provide reference for clinicians.

Patients and methods

Clinical data. A total of 89 patients with NPC admitted to Shunde Hospital (Foshan, China) from July 2015 to February 2017 were enrolled, 39 patients who failed to complete treatment were excluded, and the remaining 50 served as a treated group. Moreover, 50 healthy individuals who underwent physical examination in the hospital during the same period were collected as a control group. Laboratory test indexes and imaging tests of patients in the control group were normal. The study was approved by the Ethics Committee of Shunde Hospital. Signed informed consents were obtained from the patients and/or guardians. Inclusion criteria: Patients aged over 18 years; patients conforming to AJCC 7th version (13) and staged III-IVB; patients diagnosed with NPC through imaging and pathological examinations; Eastern Cooperative Oncology Group (ECOG) score ≤ 1 . Exclusion criteria: Patients complicated with other tumors; patients receiving corresponding anti-cancer treatment before this treatment; patients intolerant to chemoradiotherapy; breast-feeding women and patients with cognitive dysfunction.

Main reagents and drugs. Cisplatin sodium chloride injection (Guizhou Hanfang Pharmaceutical Co., Ltd.); total RNA extraction kit EasyPure miRNA kit - TransScript Green miRNA Two-Step qRT-PCR SuperMix (Beijing TransGen Biotech Company; AQ 202-01, ER601-01); PCR instrument (ABI 7500; Applied Biosystems; Thermo Fisher Scientific, Inc.).

Treatment. All the patients were treated with concurrent chemoradiotherapy (CCRT), and 6 MV-X-ray, 9-field three-dimensional conformal radiotherapy (3D-CRT) and intensity modulated radiotherapy (IMRT) were performed. The specific steps were as follows: The tumor volume was measured by MRI and CT localization images, prescription dose: PGTVnx, 70.4-72.3 Gy/32 f; PGTVnd, 66-70.4 Gy/32 f; PCTV 1, 60-62 Gy/30 f; PCTV 2, 54-55.8 Gy/30 f, once a day, five times a week. The radiation dose to temporal lobes was obtained using dose-volume histogram (DVH). Chemotherapy was performed with cisplatin at the same time. The specific protocol was as follows: One cycle of cisplatin (100 mg/m²) was given on the 1st, 22nd and 43rd days, respectively, 21 days each cycle (14).

Expression of miR-34a in serum of patients. Samples of peripheral blood (5 ml) were collected from patients before and after 3 cycles of CCRT (after treatment) and from subjects in the control group, placed at room temperature for 30 min, and centrifuged at 1,500 x g for 10 min to collect the serum. Total RNAs were extracted using a EasyPure miRNA kit, and the purity, concentration and integrity were detected using an ultraviolet spectrophotometer and agarose gel electrophoresis. The RNAs were reverse transcribed into cDNAs by 2X TS miRNA reaction mix in transScript Green miRNA two-step qRT-PCR supermix kit, and the specific procedure was performed according to the manufacturer's kit instructions. Then PCR

Table I. Efficacy evaluation.

| Efficacy grade | Evaluation criteria |
|----------------|---|
| CR | After treatment, the lesions disappeared completely for >4 weeks |
| PR | After treatment, the total maximum diameter of the lesions decreased by $\geq 50\%$ |
| SD | After treatment, the total maximum diameter of the lesions decreased by <50% |
| PD | After treatment, the total maximum diameter of the lesions decreased by $\geq 25\%$ or new lesions appeared |

CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

amplification was carried out. PCR reaction system: 1 μ l of cDNA, 0.4 μ l of each upstream and downstream primers, 10 μ l of 2X TransTaq[®] Tip Green qPCR SuperMix, 0.4 μ l of Passive Reference Dye (50X), finally made up to 20 μ l with ddH₂O. PCR reaction conditions: Pre-denaturation at 94°C for 30 sec, denaturation at 94°C for 5 sec, annealing at 60°C for 15 sec, extension for 10 sec, for a total of 40 cycles. Each sample was tested in 3 repeat wells, and the experiment was carried out 3 times. In this study, U6 was used as an internal reference and the 2^{- $\Delta\Delta$ C_q} was used to analyze the data (15). miR-34a upstream primer 5'-GTGCAGGGTCCGAGGT-3', downstream primer 5'-GCCGCTGGCAGTGTCTTAGCTG-3', U6 upstream primer 5'-CTCGCTTCGGCAGCACA-3', downstream primer 5'-AACGCTTCACGAATTTGCGT-3'.

Efficacy evaluation criteria. According to Response Evaluation Criteria in Solid Tumors, the short-term efficacy was evaluated and divided into 4 grades: Complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD) (Table I).

Outcome measures. Main outcome measures: The expression of miR-34a in the control and the treated group before treatment was compared. The diagnostic value of miR-34a was assessed using a receiver operating characteristic (ROC) curve, and the changes of miR-34a before and after treatment were compared. The changes of MoCA score (all the subjects were scored by a senior psychiatrist in the hospital) before and after treatment were compared. A higher score indicated a better cognitive function of the patients (30 points in total).

Secondary outcome measures: CR patients were classified as significant group and PR patients as general group. The expression of miR-34a before treatment was compared between the two groups, and the area under the ROC curve (AUC) was obtained. The correlation between miR-34a and MoCA score after treatment was analyzed.

Statistical analysis. In this study, SPSS 20.0 software package (Guangzhou Pomine Information Technology Co., Ltd.) was used to carry out statistical analysis on the collected

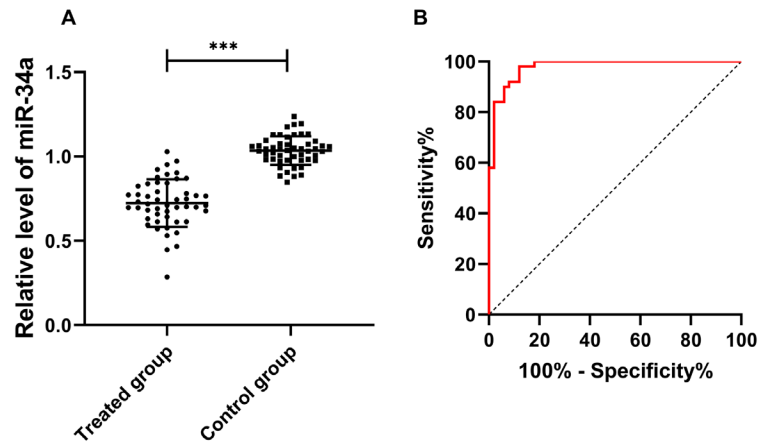


Figure 1. Expression and diagnostic value of miR-34a in NPC. (A) Comparison of serum miR-34a expression in normal individuals and in NPC patients. (B) Diagnostic value of miR-34a in the diagnosis of advanced NPC. When cut-off was 0.879, the optimal specificity, sensitivity and Youden index were 88.00, 98.00 and 86.00%, respectively. *** $P < 0.001$. miR, microRNA; NPC, nasopharyngeal carcinoma.

Table II. Comparison of baseline data.

| Factor | Observation group (n=50) | Control group (n=50) | t/ χ^2 | P-value |
|--|--------------------------|----------------------|-------------|---------|
| Sex | | | | |
| Male | 39 (78.00) | 42 (84.00) | 0.585 | 0.444 |
| Female | 11 (22.00) | 8 (16.00) | | |
| Age (years) | 52.1±7.2 | 53.4±6.3 | 0.961 | 0.339 |
| BMI (kg/m ²) | 21.25±1.47 | 21.48±1.55 | 0.761 | 0.448 |
| Clinical staging | | | | |
| III | 5 (10.00) | | | |
| IVa | 36 (72.00) | | | |
| IVb | 9 (18.00) | | | |
| T staging | | | | |
| T2 | 3 (6.00) | | | |
| T3 | 4 (8.00) | | | |
| T4 | 43 (86.00) | | | |
| N staging | | | | |
| N0 | 4 (8.00) | | | |
| N1 | 8 (16.00) | | | |
| N2 | 27 (54.00) | | | |
| N3 | 11 (22.00) | | | |
| Pathological type | | | | |
| Keratinizing squamous cell carcinoma | 25 (50.00) | | | |
| Non-keratinizing squamous cell carcinoma | 25 (50.00) | | | |

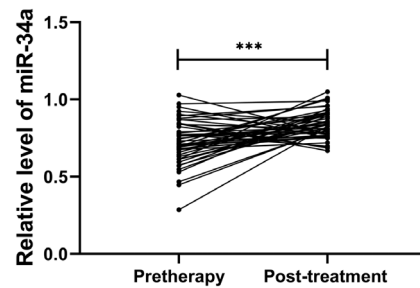


Figure 2. Expression of miR-34a in the treated group before and after treatment. *** $P < 0.001$. miR, microRNA.

were analyzed using the Chi-square test (denoted by χ^2). Measurement data conforming to normal distribution and variance homogeneity were expressed as the mean \pm SD. Independent t-test was used for analysis between the two groups, while paired t-test was used for comparison before and after treatment within the group (denoted by t). ROC was used to assess the predictive and diagnostic values of miR-34a for NPC. Pearson test was used for determining the correlation between miR-34a and MoCA score after treatment. $P < 0.05$ indicates a statistically significant difference.

Results

Comparison of baseline data. There was no significant difference in sex, age and body mass index (BMI) ($P > 0.05$) between the two groups, as shown in Table II.

Expression and diagnostic value of miR-34a in NPC. The expression of miR-34a in the control group (1.035 ± 0.085) was significantly higher than that in the treated group (0.723 ± 0.141) ($t = 13.364$, $P < 0.001$). ROC curve showed that the AUC of miR-34 was 0.979, showing a high diagnostic value (Fig. 1).

miR-34a expression before and after treatment. The expression of miR-34a in the treated group increased significantly after treatment compared with that before treatment ($t = 4.559$, $P < 0.001$), as shown in Fig. 2.

data. GraphPad Prism 7 (Shanghai Cabit Information Technology Co., Ltd.) was used to draw the figures. Kolmogorov-Smirnov (K-S) test was used to analyze the data distribution. Counting data expressed as percentage (%)

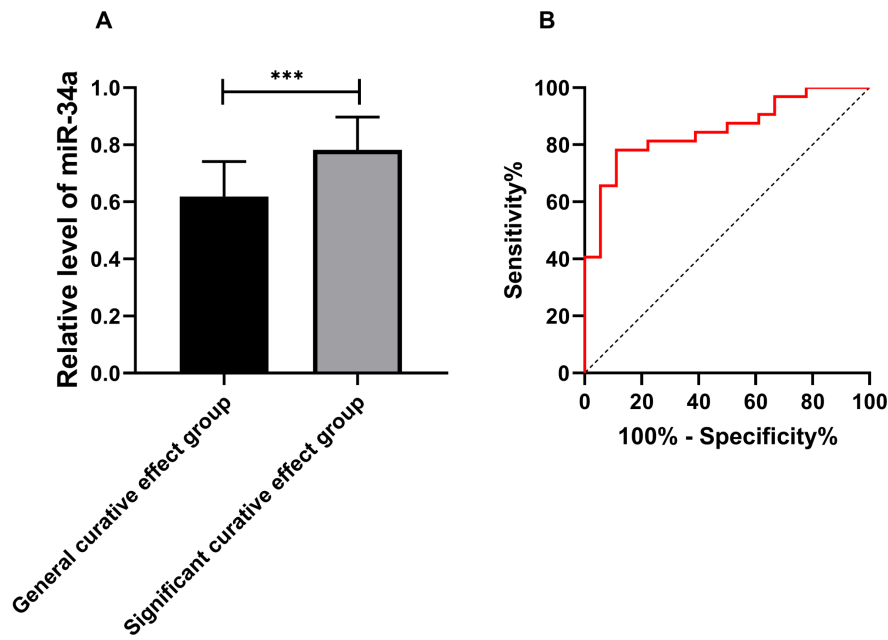


Figure 3. Predictive value of miR-34a in NPC. (A) Expression of miR-34a in significant group and general group before treatment. (B) When cut-off was 0.706, the optimal specificity, sensitivity and Youden index were 88.89, 78.13 and 67.01%, respectively. *** $P < 0.001$, miR, microRNA; NPC, nasopharyngeal carcinoma.

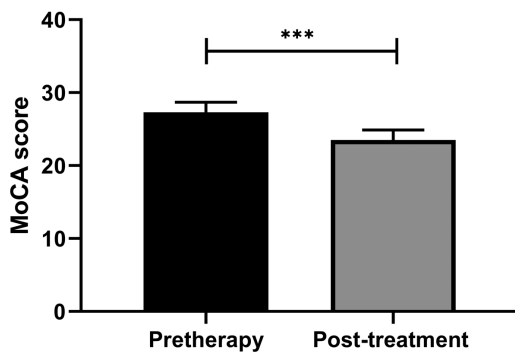


Figure 4. MoCA score changes before and after treatment in treated group. *** $P < 0.001$. MoCA, Montreal Cognitive Assessment.

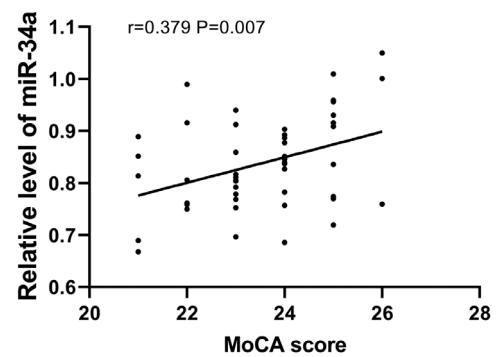


Figure 5. Correlation between miR-34a expression and MoCA score after treatment. miR, microRNA; MoCA, Montreal Cognitive Assessment.

Predictive value of miR-34a in NPC. Evaluation of the clinical efficacy of patients in the treated group showed that there were 32 CR patients and 18 PR patients after treatment. The CR patients were classified as the significant group and PR patients as the general group. The expression of miR-34a in the significant group was higher than that in the general group before treatment ($t=4.704$, $P < 0.001$). The ROC curve showed that the AUC was 0.852 (Fig. 3).

MoCA score changes before and after treatment. The MoCA score of patients after treatment (23.52 ± 1.37) was significantly lower than that before treatment (27.32 ± 1.36) ($t=13.042$, $P < 0.001$) (Fig. 4).

Correlation between miR-34a expression and MoCA score after treatment. Pearson correlation analysis showed that the expression of miR-34a was positively correlated with the MoCA score after treatment, that is, MoCA score increased gradually with the increase of miR-34a expression ($r=0.379$, $P=0.006$), as shown in Fig. 5.

Discussion

Due to the concealed onset of NPC, majority of patients are in advanced stage when diagnosed, missing the optimal treatment timing. At present, early NPC patients are mainly treated with radiotherapy alone, and patients in advanced stage are treated with platinum plus radiotherapy (16,17). Efficacy evaluation is mainly based on imaging tests after treatment that determine the size of solid tumors. However, the tests have a certain impact on the human body, and the imaging results take time (18). Therefore, we questioned whether it is possible to predict the efficacy by performing tests on the patient before treatment, in order to assess the clinical effect in advance and to improve the treatment efficiency. However, there are few relevant indicators for clinical prediction of clinical efficacy, so it is very important to find one.

miRs are a class of highly conserved non-coding small RNAs widely distributed in eukaryotic cells, which play an important role in the development and progression of malignant tumors (19). Studies have shown that (20,21) a

variety of miRs are capable of regulating the occurrence and development of NPC. Previous studies have found that miR-34a, a member of the miRs family, regulates biological function in prostate cancer and renal cell cancer (22,23). Recent studies have shown that miR-34a has a low expression in NPC (24). However, whether it can be used as an efficacy predictor has not been studied. Therefore, in this study, the predictive value of miR-34a in efficacy evaluation of NPC patients after CCRT was explored to provide indicators for the clinic. The expression of serum miR-34a was first detected, and the results showed that the expression in the treated group was significantly lower than that in the control group. Moreover, the ROC curve showed that miR-34a had extremely high diagnostic value in patients with advanced NPC. In the study of Long *et al* (25), it was found that hyperfraction radiotherapy enhanced cell apoptosis and upregulated miR-34a expression. Moreover, the expression of miR-34a before and after treatment through clinical trials was compared, and it was found that the expression was significantly increased after treatment, which was consistent with the results of Long *et al* (25), indicating that the expression of miR-34a increased after CCRT. Furthermore, the patients were divided into the significant group and the general group according to the curative effect, the expression of miR-34a before treatment between the two groups was compared. The results indicated that the expression in significant group was significantly higher than that in general group. ROC curve showed that the AUC was >0.8, suggesting that observing the expression of miR-34a before treatment was a potential predictor for assessing the efficacy of CCRT in patients with NPC. The higher the expression of miR-34a before treatment, the more significant improvement of the clinical curative effect.

Although the condition of the patient and survival are improved, the cognitive function of patients may be declined after radiotherapy, which reduces the quality of life of patients. At present, various scores and scale are preferred means to assess cognitive function, subjective errors are inevitable. A study (26) found that upregulation of miR-34a alleviated total abdominal irradiation (TAI)-mediated cognitive impairment by restoring BDNF expression in hippocampus. Therefore, we speculated whether the expression of miR-34a is related to the cognitive function of patients. Therefore, the changes of cognitive function of patients before and after treatment were compared, and it was found that the MoCA score after treatment was significantly lower than that before treatment. Qiu *et al* (27) stated that the MoCA score of NPC patients after radiotherapy was significantly lower than that before treatment, which was consistent with our research results. Moreover, Pearson correlation analysis showed that there was a positive correlation between miR-34a and MoCA score, indicating that the expression of miR-34a can be used as a potential observation indicator for patients with cognitive dysfunction. However, MoCA score may be affected by cancer staging, side effects, nutritional status and other factors, interfering with our findings. Thus, we will supplement more cognitive function related scales to investigate the association between miR-34a and cognitive function.

This study found that miR-34a can be used as a predictor of clinical efficacy and an observation indicator of cognitive

function in NPC patients after CCRT. However, there are still limitations. The survival of NPC patients was not followed up, so whether miR-34a is related to the survival remains unknown. This is a clinical experiment, the relevant mechanism between miR-34a and patients' cognitive function is unclear. Only samples of patients with advanced NPC were collected, the expression of miR-34a in patients with early NPC and those without CCRT is not clear.

In conclusion, there is a positive correlation between miR-34a and cognitive function of patients. Moreover, observing the expression of miR-34a before treatment can be used as a potential observation predictor of the efficacy of CCRT in patients with NPC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

WD conceived and designed the study, and drafted the manuscript. WD, AL and JY collected, analyzed and interpreted the experimental data, and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shunde Hospital (Foshan, China). Signed informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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