JBUON 2020; 25(4): 2017-2022
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

The effect of neo-adjuvant chemotherapy on the improvement of oral cancer patients and the predictive value of miR-182 on its efficacy

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Summary

Purpose: *This study aimed to observe the effect of neo-adjuvant chemotherapy on the improvement of oral cancer patients and investigate the predictive value of miR-182 on its efficacy.*

Methods: *A total of 143 patients with advanced oral cancer admitted to Yidu Central Hospital of Weifang from September 2015 to July 2017 formed the study group. Among them, there were 62 cases in the control group (surgery+postoperative radiotherapy) and 81 cases in the study group (preoperative neo-adjuvant chemotherapy+surgery+postoperative radiotherapy). The treatment effect and adverse reactions of patients were compared between the two groups. RT-PCR was used to detect the expression levels of serum miR-182 of patients before and after treatment. The 1-year survival of patients in the two groups was recorded and compared by follow-up.*

Results: *The total effective rate of patients in the study group was significantly higher than that of patients in the control group ($p<0.05$). The incidence of adverse reactions of patients in the study group was significantly higher than in the control group ($p<0.05$). There was no significant difference in the prognostic 1-year survival rate between the two groups. After treatment, the expression of miR-182 was lower than before treatment and in the study group it was significantly lower than the control group ($p<0.05$). ROC curve analysis showed that the area under the curve of miR-182 in the predictive value of oral cancer was 0.756. When the cut-off value was less than 1.823, the optimal specificity was 70.18% and the sensitivity was 75.86%.*

Conclusion: *Neo-adjuvant chemotherapy can significantly improve the therapeutic effect, but the incidence of adverse reactions increases. miR-182 may be involved in the occurrence and deterioration of oral cancer and is a good indicator for predicting the treatment efficacy of patients with oral cancer.*

Key words: *preoperative neo-adjuvant chemotherapy, oral cancer, miR-182, prognosis*

Introduction

Oral cancer is a disfiguring malignant tumor of the head and neck [1] and is the sixth leading cause of cancer-related death in the world [2]. At present, surgery is the main treatment for oral cancer in the clinic [3], accompanied by conservative treatment such as chemotherapy and radiotherapy at the same time [4]. However, due to the late disease detection in some patients, their condition is serious, so it is difficult to define the safe range of surgery [5]. Chemotherapy is the use of chemical drugs to prevent the proliferation, infiltration and metastasis of cancer cells [6]. It can

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Received: 18/03/2020; Accepted: 15/04/2020

eliminate the residual minimum cancer lesions after surgery, improve the surgical treatment effect [7] and has gradually been applied in the treatment of advanced oral cancer.

MicroRNAs (miR's), as a kind of non-coding short interfering RNA with a length of about 22nt, have become one of the most important research topic in the field of life sciences in the past 20 years [8,9]. Previous studies have shown that miRs are closely related to the occurrence and deterioration of oral cancer [10]. miR-182 is one of the important members of the miR family. Studies have found that miR-182 is involved in the occurrence and deterioration of various tumors and abnormal expression status was observed in retinoblastoma, chronic myeloid leukemia, head and neck squamous cell carcinoma and other malignant tumors [11-13]. Chou et al found that miR-182 was up-regulated in head and neck squamous cell carcinoma (HNSCC) [14]. Li et al found that miR-182-5p was up-regulated in cell lines *in vitro* and clinical oral squamous cell carcinoma samples *in vivo*. miR-182-5p promoted the growth of oral squamous cell carcinoma by inhibiting CAMK2N1 [15]. Prognosis factors of oral cancer are still limited to clinicopathological indicators and it is difficult to make accurate estimates of prognosis.

In this study RT-PCR was used to detect the expression levels of miR-182 in oral cancer and the relationship between it and prognosis was analyzed, so as to provide references for further rational selection of treatments.

Methods

Baseline data

A total of 143 cases with advanced oral cancer admitted to Yidu Central Hospital of Weifang from September 2015 to July 2017 were collected as the study group. There were 62 patients in the control group (surgery+postoperative radiotherapy) and 81 in the study group (preoperative neo-adjuvant chemotherapy+ surgery+postoperative radiotherapy). This study was approved by the ethics committee of Yidu Central Hospital of Weifang. All the above participants signed informed consent forms.

Inclusion and exclusion criteria

Inclusion criteria: all patients met the diagnostic criteria and were diagnosed as advanced oral cancer; patients treated in Yidu Central Hospital of Weifang; patients 18-70 years old; patients able to cooperate with the investigation; informed consent forms were signed by patients or immediate family members; all patients had complete medical records.

Exclusion criteria: patients with important organ injury such as heart, liver, spleen, lung, kidney; patients with mental disorders and speech dysfunction; pregnant and lactating women; patients with surgical contraindications; patients with drug allergy.

Methods of treatment

Study group: first, neo-adjuvant chemotherapy was used: chemotherapy lasted for one week and was administered for 2-3 cycles before surgery. Cisplatin injection (Qilu Pharmaceutical co., LTD., SFDA Approval No. H37021358) 100 mg/m^2 was used for i.v. drip before treatment for 2 days and 5-fluorouracil (Qilu Pharmaceutical co., LTD., SFDA Approval No. H37021281) 750 mg/m^2 was used for i.v. drip after treatment for 5 days. After the end of chemotherapy, the patient condition was evaluated. If the surgical conditions were met, the primary lesion and comprehensive neck dissection were excised radically. Radiotherapy started after the operation for 4 to 6 weeks. The permissible dose was 1.8 to 2 Gy / day, 5 days per week for 6 weeks (54 to 60Gy in total).

Control group: patients were treated with a simple radiotherapy regimen only 4 to 6 weeks after surgery. The radiotherapy method was the same as the combination therapy group.

Detection method of serum miR-182

In the morning, 5 mL of fasting venous blood of patients in the two groups was collected before and after treatment,placed in a vacuum tube and then centrifuged at 3000 RPM for routine separation. Total RNA was extracted from serum using a Trizol extraction kit (Invitrogen, Carlsbad, CA, USA). The concentration and purity of RNA were detected by a Nano-Drop 2000 ultraviolet spectrophotometer (Beijing Keyu Xingye Science and Technology Development co. LTD, China). RNA was reversely transcribed into cDNA according to Takara reverse transcription kit (Invitrogen, Carlsbad, CA, USA) and the synthesized cDNA was stored at -20°C for later use. Primers were designed and synthesized by Shanghai GenePharma Co.,Ltd, China (Table 1). The reaction was carried out on an ABI PRISM 7500 fluorescence PCR instrument (Applied Biosystems, Foster City, CA, USA). The PCR amplification cycle conditions were : 90°C for 5 min, 90°C for 5 s, 60°C for 30 s, 72°C for 5 s, for a total of 40 cycles. Each sample was repeatedly tested 3 times and the relative expression of the gene was expressed after calculation by $2^{-\Delta CT}$ software.

Table 1. Primer sequence

	F	*R*
U6	5'-GCTTCGGCAGCACATATACTAAAT-3'	5'-CGCTTCACGAATTTGCGTGTCAT-3'
mir-182	5'-ACTTTTGGCAATGGTAGAACTCAC-3'	5'-AATCCATGAGAGATCCCTAGCG-3'

Criteria of efficacy evaluation

The assessment was carried out in accordance with the World Health Organization's relevant efficacy judgement standard: complete remission (CR): tumor lesions completely disappeared after the end of treatment and maintained for more than 4 weeks; partial remission (PR): the length-diameter of tumor lesions were reduced by more than 50% and maintained after the end of treatment for more than 4 weeks; stable disease (SD): after the end of treatment, the length-diameter of tumor lesions decreased by less than 25% or increased by less than 20%; disease progression (PD): tumor lesions increased by more than 20% or new lesions appeared. Overall response (RR) = good efficacy = CR+PR. The side effects of the patients and the patients' satisfaction with the treatment in the two groups were observed and compared. Patients were followed up and followed by telephone communication for one year.

Statistics

All the experimental results were statistically calculated by SPSS24.0 statistical package (Shanghai Yuchuang Network Technology Co, Ltd). All the graphs were drawn by Graphpad8 (Shenzhen Tianruiqi Software Technology Co, Ltd) and the results were checked twice. The count data were expressed in the form of rates. The chi-square test was used for comparison among groups. The measurement data were expressed in the form of mean number±standard deviation. The t-test was used for comparison among groups. Kaplan-Meier method was used to draw survival curves for the control group and the study group. The Log-rank test was used to evaluate the difference of survival curves between the two groups. The difference was statistically significant when $p<0.05$.

Results

Comparison of the baseline data

Comparison of clinical data in the control and the study group showed no significant difference in gender, age, TNM stage, grade of differentiation, smoking history and drinking history (all $p>0.05$), which proved that the two groups of patients were comparable. More details are shown in Table 2.

Comparison of clinical effect of patients between the two groups

In the control group, the number of RR was 43, the number of SD 14, the number of PD 5. In the study group, the number of RR was 71, the number of SD was 9 and the number of PD was 1. There was a statistically significant difference in comparison of the number of RR between the two groups (all $p<0.05$). More details are shown in Table 3.

Comparison of adverse reactions of patients between the two groups

In the control group, there were 26 cases of nausea and vomiting, 17 cases of oral mucosa damage and one case of hematological toxicity, febrile neutropenia and elevated SGPT. In the study group, there were 45 cases of nausea and vomiting, 49 cases of oral mucosal damage, 19 cases of hematological toxicity, 4 cases of febrile neutropenia and 5 cases of elevated SGPT. More details are shown in Table 4.

Table 2. Comparison of the clinical data

	Control group (n=62) n (%)	*Study group (n=81) n (%)*	*t/F*	*P*
Gender			0.134	0.714
Male	31 (50.00)	43 (53.09)		
Female	31 (50.00)	38 (46.91)		
Age/years old	43.3±15.3	41.4±14.3	0.763	0.446
TNM stage			0.153	0.695
Stage I+II	21 (33.87)	30 (37.04)		
Stage III+IV	41 (66.13)	51 (62.96)		
Pathological differentiation			0.514	0.773
Well differentiated	13 (20.97)	18 (22.22)		
Moderately differentiated	32 (51.61)	45 (55.56)		
Poorly differentiated	17 (27.42)	18 (22.22)		
Smoking history			0.687	0.406
Yes	28 (45.16)	31 (38.27)		
No	34 (54.84)	50 (61.73)		
Drinking history			0.160	0.689
Yes	25 (40.32)	30 (37.04)		
No	37 (59.68)	51 (62.96)		

Table 3. Comparison of clinical effect of patients between the two groups

Clinical effect	*Control group (n=62)*	*Study group (n=81)*	x^2	*P*
CR	17	19		
PR	26	52		
SD	14	9		
PD	5	1		
RR	43	71	7.274	<0.05

Table 4. Comparison of adverse reactions of patients between the two groups

	Nausea and vomiting	*Oral mucosal damage*	*Hematologic toxicity*	*Febrile neutropenia*	*Elevated SGPT*
Control group (n=62)	26	17	1	1	1
Study group (n=81)	45	49	19	4	5
x^2	2.606	15.462	13.931	1.151	1.817
P	0.106	<0.05	<0.05	0.283	0.177

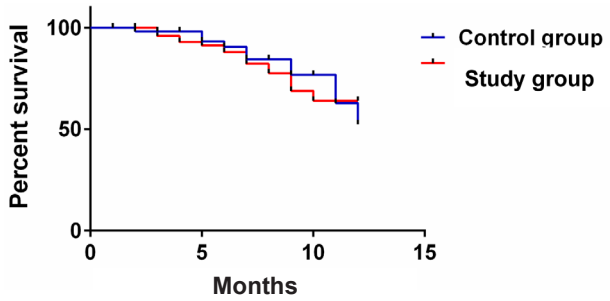

Figure 1. 1-year survival curve between the two groups. The difference was not statistically significant (p>0.05).

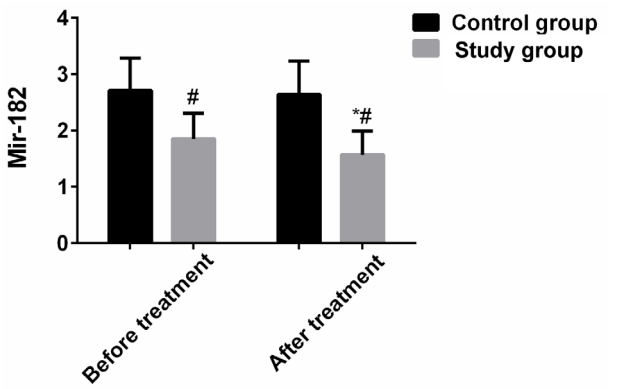

Figure 2. Comparison of miR-182 expression before and after treatment in both groups. The results of RT-PCR detection showed that there was no difference in the miR-182 expression of patients between the two groups before treatment. The expression after treatment was lower than that before treatment and in the study group it was significantly lower than in the control group (p<0.05). # indicates comparison before treatment. * indicates comparison with the control group (p<0.05).

Comparison of survival of patients between the two groups

The patients were followed up and investigated for 1 year by telephone, visits, letters, etc. The follow-up success rate was 100%. In the control group, the 1-year survival rate was 82.26% (51/62) while in the study group the 1-year survival rate was 79.01% (64/81). The difference was not statistically significant in comparison of prognostic 1-year survival rate between the two groups (p>0.05). More details are shown in Figure 1.

Comparison of miR-182 expression of patients before and after treatment in the two groups

The results showed that there was no difference in the miR-182 expression of patients between the two groups before treatment. The expression after treatment was lower than that before treatment and was significantly lower in the study group than in the control group (p<0.05). More details are shown in Figure 2.

Predictive value of miR-182 in patients with poor treatment efficacy in oral cancer

All patients were divided into good efficacy group (n=114) and poor efficacy group (n=29) according to different outcomes of oral cancer. According to the miR-182 expression of patients in the two groups, ROC curve was drawn to analyze the predictive value of miR-182 efficacy in oral cancer. After detection, it was found that the area under the miR-182 curve was 0.756 and the 95% CI was 0.659-0.853. When the cut-off value was less than 1.823, the optimal specificity was 70.18%,

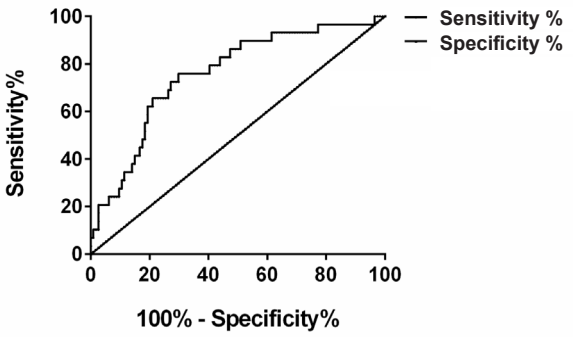

Figure 3. ROC curve of serum miR-182 expression in the diagnostic value of oral cancer efficacy. All patients were divided into good efficacy group (n=114) and poor efficacy of oral cancer group (n=29) according to different outcomes of oral cancer. After detection, it was found that the area under the miR-182 curve was 0.756 and the 95% CI was 0.659-0.853. When the cut-off value was less than 1.823, the optimal specificity was 70.18%, the sensitivity was 75.86% and the Youden index was 46.03.

the sensitivity 75.86% and the youden index 46.03. More details are shown in Figure 3.

Discussion

Oral cancer is the 11th most common cancer in the world [16] and has a significant impact on the patient quality of life [17]. At present, the treatment technology, including surgery, radiotherapy and chemotherapy, has been improved to some extent [18], but the overall prognosis of this disease is poor and the 5-year survival rate is only 50% [19]. Therefore, it is very important to find a biological index in predicting the prognosis of oral cancer for choosing treatment methods, improving the prognosis of patients and increasing the survival rate.

The results of this study showed that the number of overall response in the control group was significantly lower than in the study group and indicated that preoperative neo-adjuvant chemotherapy can effectively reduce the volume of tumor lesions before surgery and improve the effect of surgical treatment to a certain extent. There were 4 patients in the study group who could not be operated. The surgical conditions were optimized and the lesions were reduced by preoperative chemotherapy. We speculate that the tumor has good blood flow without surgical interference. The chemotherapeutic drugs achieve steady blood concentration in the lesion and have good effects. This is similar to the conclusion of Hawkins et al who reported that preoperative chemotherapy can improve the survival rate of radical resection for the treatment of large-diameter tumors in the case analysis of anorectal gastrointestinal stromal tumors resection [20]. Comparing the different adverse reactions of patients after operation in the two groups, the incidence of adverse reactions in the study group was higher than in the control group. It was found that there were 19 cases of hematological toxicity in the study group and 11 cases were grade I hematological toxicity. The low incidence of severe toxicity suggests that the chemotherapy dose may be feasible. Cisplatin is a key drug for chemotherapy in patients with head and neck squamous cell carcinoma [21]. Cisplatin combined with 5-fluorouracil can enhance the break of DNA strand and the inhibition of human chorionic gonadotropin. The combination of the two drugs shows a synergistic effect that is effective in the treatment of oral cancer [22]. Cervical lymph node metastasis appears in the early stage of oral cancer [23] and general metastatic spread appears in the late stage. It is necessary to inhibit and reduce cancer metastasis as early as possible. In the Cats et al study, the chemotherapy compliance of the patients was poor in the treatment group after operation, suggesting that we should focus on optimization of preoperative treatment strategies [24]. So we chose preoperative neo-adjuvant chemotherapy to help slow down the deterioration trend of cancer. The difference of one-year survival rate between the two groups was analyzed and there was no statistical difference, suggesting that preoperative neo-adjuvant chemotherapy combined with surgery did not reduce the survival rate. Then we analyzed the expression of miR-182 in both groups and found that its expression after treatment was lower than before treatment and was significantly lower in the study group than in the control group ($p<0.05$). The expression of miR-182 in the study group was significantly lower than in the control group. This indicated that miR-182 may be involved in the occurrence and deterioration of oral cancer. Wang et al reported that miR-182 expression was significantly up-regulated in oral cancer tissues compared with adjacent non-malignant tissues [25], which supports our findings. ROC curve was used to analyze the efficacy and found that the area under the miR-182 curve was 0.756, the optimal specificity was 70.18% and the sensitivity was 75.86%. This suggests that miR-182 has a certain value in the prediction of the therapeutic efficacy of oral cancer and provides a good prognostic biomarker.

This study found that neo-adjuvant chemotherapy before operation had a good outcome in oral cancer prognosis, but there were still some limitations. First, for the purpose of not delaying surgical treatment, we only performed 2-3 cycles of chemotherapy for all patients. However, individual differences in the time of induction chemotherapy may have an impact on the treatment outcome.

Secondly, miR-182 was not explored in-depth on the migration and invasion ability of oral cancer cells and its regulatory mechanism is still unclear. These limitations need to be further addressed in future research and complement the results in this study.

In summary, neo-adjuvant chemotherapy can significantly improve the therapeutic effect, but the incidence of adverse reactions increases. miR-182 may be involved in the occurrence and deterioration of oral cancer. It is a good indicator for predicting the therapeutic efficacy in oral cancer.

Conflict of interests

The authors declare no conflict of interests.

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