Int J Clin Exp Pathol 2016;9(10):9798-9809
www.ijcep.com /ISSN:1936-2625/IJCEP0037190

Original Article

Differential expression of serum microRNAs in locally advanced esophageal squamous cell carcinoma patients undergoing radiochemotherapy in Xinjiang area

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Received August 3, 2016; Accepted August 21, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Objective: To investigate the changes of serum microRNA (miRNA) expressions in locally advanced esophageal squamous cell carcinoma (ESCC) patients before and after radiochemotherapy among different ethnic groups in Xinjiang area. Methods: Agilent microRNA gene chip was applied to detect serum miRNA levels of each subject. Results: 1. Compared with normal controls, 98 miRNAs were differentially expressed with dominant distinction of has-miR-483-5p and has-miR-5703 (3 times difference) in the ESCC patients; 2. Prior to the treatment, the Han patients had 160 differentially expressed miRNAs, Uygur had 92 and Kazak had 115 compared with the normal controls; 3. After the treatment, 124 miRNAs were differentially expressed in the Han patients, 103 in Uygur patients and 129 in Kazak patients compared with the normal controls; 4. Compared with the miRNA levels before the treatment, the Han patients exhibited 6 differentially expressed miRNAs, Uygur exhibited 2 and Kazak exhibited 4 after the treatment; 5. Overall, the Han and Kazak patients shared 6 differentially expressed miRNAs, the Kazak and Uygur patients shared 10, while Uygur and Han patients shared 17. Conclusions: Serum miRNAs are differentially expressed in ESCC patients, and those miRNAs may function as biomarkers for diagnosis of ESCC. Radiochemotherapy may alter serum levels of part miRNAs. In addition, there are different miRNAs profiles in ESCC patients among the three ethnic groups.

Keywords: Nationality, esophageal squamous cell carcinoma, serum, microRNA, expression difference

Introduction

Esophageal cancer (EC) is reported to be one of the commonest malignant digestive tumors, which possesses very high morbidity and mortality, resulting in 288000 new cases diagnosed each year and 208000 cancer-related deaths [1]. Surprisingly, esophageal squamous cell carcinoma (ESCC) accounts for approximately 90% of EC, and the highest rate of ESCC is in Iran, followed by other countries including China, South Africa as well as France [2]. In China, EC exhibits remarkable ethnic and regional differences. The Xinjiang Uygur Autonomous Region is situated in the northwestern part of China, which is the place with highest incidence of EC, and Uygur and Kazak populations also had highest occurrence rate of EC [3]. EC is able to be treated through surgery with radiochemotherapy as an auxiliary treatment regime [4]. Although the prognosis has improved over the last decade, the 5-year survival rate of EC is still only 20 to 35% with tumor recurrences occurring within the first year after surgery due to approximately 80% of patients diagnosed at advanced stage [5]. According to the National Cancer Comprehensive Network (NCCN) clinical practice guidelines in oncology, the advanced EC is mainly treated with radiochemotherapy, but the overall effective rate is not satisfactory and high invasion and migration of cancer cells are still problem in EC [6, 7]. Due to high inva-

Table 1. Characteristic information of Han subjects

Items	Case group (n=15)	Control group (n=15)	t/χ^2	*P*
Median age	64 ± 3.5	60 ± 3.2	0.772	0.441
Gender (M/F)	10/5	7/8	1.222	0.269
Smoking history (Y/N)	6/9	7/8	0.136	0.713
Alcohol history (Y/N)	7/8	8/7	0.133	0.715

Note: M, male; F, female; Y: yes; N: no.

Table 2. Characteristic information of Uygur subjects

Items	Case group (n=15)	Control group (n=15)	t/χ^2	*P*
Median age	65 ± 3.5	59 ± 3.4	0.692	0.347
Gender (M/F)	7/8	7/8	0.133	0.715
Smoking history (Y/N)	6/9	7/8	0.136	0.713
Alcohol history (Y/N)	8/7	6/9	0.536	0.464

Note: M, male; F, female; Y: yes; N: no.

Table 3. Characteristic information of Kazak subjects

Items	Case group (n=15)	Control group (n=15)	t/χ^2	*P*
Median age	61 ± 3.5	60 ± 3.3	0.832	0.561
Gender (M/F)	9/6	8/7	0.136	0.713
Smoking history (Y/N)	7/8	9/6	0.536	0.464
Alcohol history (Y/N)	10/5	9/6	0.144	0.705

Note: M, male; F, female; Y: yes; N: no.

Table 4. Characteristic information of Han, Uygur and Kazak esophageal squamous cell carcinoma patients

Items	Han subjects (n=15)	Uygur subjects (n=15)	Kazak subjects (n=15)	F
Median age	64 ± 3.5	65 ± 3.5	61 ± 3.5	1.920
Gender (M/F)	10/5	7/8	9/6	0.613
Smoking history (Y/N)	6/9	6/9	7/8	0.085
Alcohol history (Y/N)	7/8	8/7	10/5	0.778

Note: M, male; F, female; Y: yes; N: no.

sion, poor prognosis, and multiple therapy patterns, it is difficult to make effective prognosis and predict treatment efficacy to improve survival rate for EC patients. Therefore, the biological behavior of the activation, development and progression of EC must be elucidated to offer predictive markers for efficient diagnosis, and favorable prognosis of radiochemotherapy.

MicroRNAs (miRNAs) is defined as a series of non-coding small RNAs with 20 to 22 nucleotides and are able to govern the post-transcriptional level by modulating gene expression through binding to the 3'-untranslated region (UTR) of the targeted mRNAs, which can lead to mRNA translation, inhibition or degradation [8]. MiRNAs exhibit abnormal expressions in various types of cancers and can act as a new class of oncogenes or tumor suppressor genes relying on their targets [9]. Interestingly, miRNAs have been found to participate in the progression and development of esophageal adenocarcinoma [10], prostatic cancer [11], colon cancer [12], pancreatic cancer [13] and EC [14], which indicates that serum miRNA may be a potential biomarker for the early diagnosis, prognosis evaluation and therapy efficacy prediction in the cancer management [1].

Up till now, researches related with chemotherapy and radiotherapy efficacy of EC among different ethnic groups in Xinjiang Uygur Autonomous Region are limited. The present study aims to explore the changes of serum miRNA levels among different ethnic groups before and after chemotherapy and radiotherapy, as well as the differential expression in comparison with the normal controls with Agilent miRNA gene chip, which may give rise to an intriguing possibility for EC management.

Materials and methods

Inclusion criteria and treatment regime

The experiment was approved by the Ethics Committee of our hospital and all subjects received informed consents. Based upon the tumor node and metastasis (TNM) staging system released by American Joint Committee on Cancer (AJCC), the ESCC patients in stage IIIB~IV were included after pathological verification. All subjects were aged between 45 and 75 years, with measurable lesions. According to World Health Organization, the performance status (PS) of the patients was less than 0~2

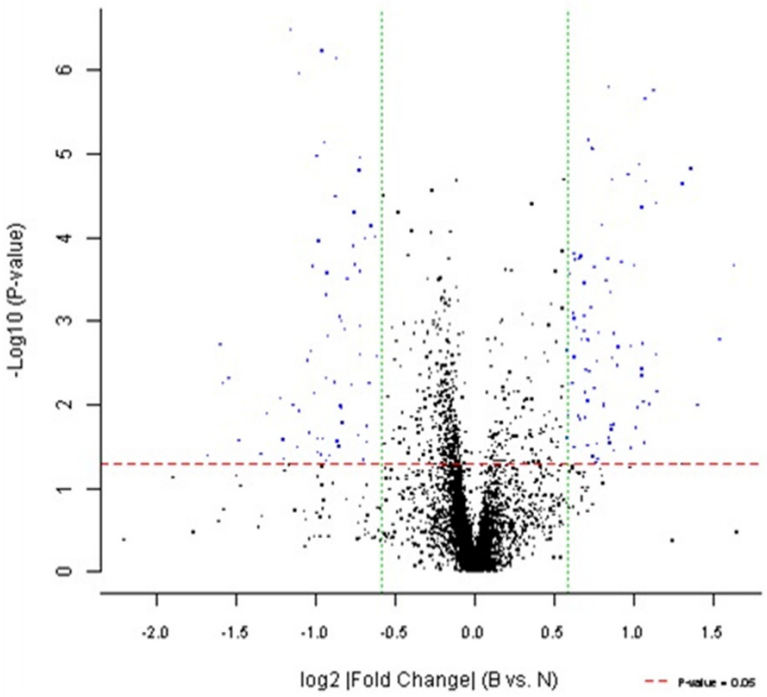

Figure 1. Volcanic diagram of serum microRNA expressions between esophageal squamous cell carcinoma patients and normal controls (blue point refers to the differentially expressed microRNAs). Note: B, esophageal squamous cell carcinoma patients; N, normal controls.

scores. Eligible patients in the study were sensitive to radiotherapy or chemotherapy; the expected survival time was more than 6 months; no dysfunction was observed in major organs; the blood routine, liver, renal and cardiac functions were in normal range.

Combination regime of chemotherapy and radiotherapy: 1. chemotherapy: paclitaxel/docetaxel + carboplatin/cisplatin, once in 3~4 weeks, 4~6 cycles; 2. radiotherapy: intensity-modulated radiation therapy (IMRT), DT50-66Gy/25-33f.

Experimental methods entrusted to Beijing protein innovation Co., Ltd

The blood samples of ESCC patients before and after the treatment were collected. The separated serum was stored in ultra-low temperature freezer at -80°C for reservation. Total RNA was isolated from 400 μL of frozen serum/plasma using Total RNA Purification Kit (Norgen Biotek Corp, Ontario, Canada, 17200) in accordance with instructions. The obtained RNA solution was 60 μL.

Labeling and hybridization were achieved with application of the Human microRNA Microarray Kit (Agilent Technologies, Inc., Santa Clara, CA, US) in conformity with the manufacturer's protocol. Briefly, total RNA of 30 μL was labeled by Cyanine3 (Cy3), re-suspended in hybridization buffer and hybridized to the array platform overnight (20 hours) at 55°C in a rotating Agilent hybridization oven by using Agilent's recommended hybridization chamber. Next, the microarrays were washed with the Agilent Gene Expression Wash Buffer 1 for 5 min at room temperature. Subsequently, the microarrays were washed for 5 min again using Agilent Gene Expression Wash Buffer 2 which was warmed to 37°C. After hybridization, the fluorescence signals were measured with the help of a DNA microarray scanner G2505C (Agilent Technologies, Inc., Santa Clara, CA, US) with one color scan setting for 8 × 60 K array slides (Scan resolution 5 μm, Scan Area 61 × 21.6 mm, Dye channel: Green, Green PMT: 100%).

Statistical analysis

The Agilent 60-mer SurePrint chip probe was applied for the database design. The experiment was based on the Sanger miRBase 21 version which included a total of 2549 human mature miRNA genes containing 100% miRBase r21. The chip image file (Tiff) was extracted with Agilent Feture Extraction (v10.7.3.1) and Grid template (046064_D_20121223) for the raw data of gMedianSignal value. The filter, merge, Quantile normalization, homogenization and statistical calculation for data were grounded on R set (2.12.1). Pair-wise T-test was performed for the *P*-value and differences between two groups and among multiple groups. Differential gene screening was set at |log2 ratio| ≥ 0.585 (1.5 times difference) and *P*-value < 0.05.

Table 5. Differential expressions of serum microRNA in normal controls and esophageal squamous cell carcinoma patients

ID	Name	log2 (Ratio) B/N	*P*-value B/N	Up/down-regulated
A_25_P00015596	hsa-miR-3202	0.872687915	2.05E-05	Up
A_25_P00015747	hsa-miR-4270	0.759175573	0.006409769	Up
A_25_P00017131	hsa-miR-4499	0.74804838	8.85E-06	Up
A_25_P00016725	hsa-miR-4669	1.064908967	0.001891438	Up
A_25_P00012459	hsa-miR-483-5p	1.130708187	1.75E-06	Up
A_25_P00017948	hsa-miR-6124	0.694418732	0.000885208	Up
A_25_P00017641	hsa-miR-642a-3p	0.906871565	0.002085159	Up
A_25_P00017401	hsa-miR-5703	-0.953435677	5.87E-07	Down

Note: N, normal controls; B, esophageal squamous cell carcinoma patients.

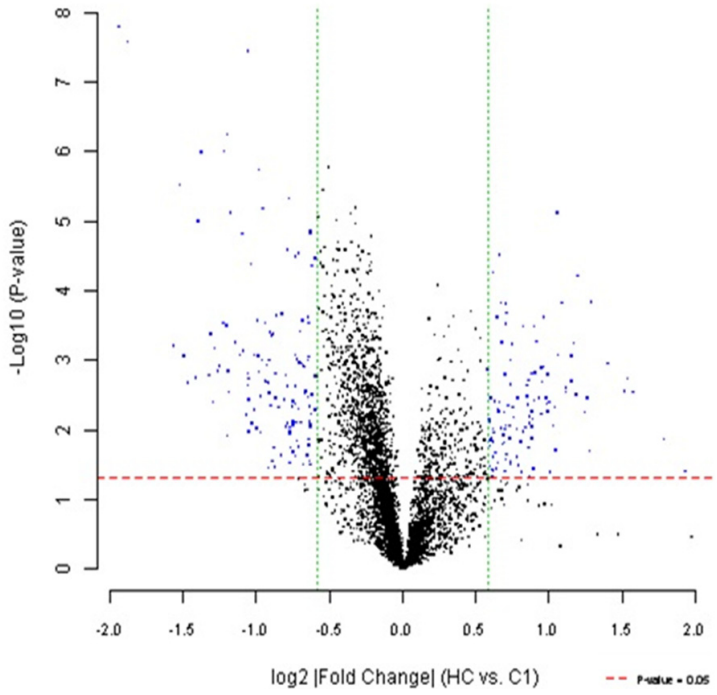

Figure 2. Volcanic diagram of serum microRNA expressions between the Han case group before the treatment and the Han control group (blue point refers to the differentially expressed microRNAs). Note: HC, Han case group before the treatment; C1, Han control group.

Results

General information of all subjects

Our study consisted of three case groups including Han, Uygur and Kazak (n=15 for each group), and three corresponding control groups including Han, Uygur and Kazak (n=15 for each group) (**Tables 1**-**4**). With regard to age, gender and smoking and alcohol history, no significant difference was found between the case group and control groups in each nationality, as well as among the three case groups (*all P > 0.05).*

Chip analysis results for changes of miRNA expressions between the ESCC patients and normal controls, between the Han, Uygur and Kazak ESCC patients and the corresponding normal controls before and after the radiochemotherapy, and between every two ethnic groups

Differential gene screening was set at |log2 ratio| ≥ 0.585 (1.5 times difference), $P < 0.05$).

Comparison between the ESCC patients and normal controls: in total, 98 miRNAs were differentially expressed in the patients with ESCC compared with the normal controls, among which 50 miRNAs were upregulated, such as hsa-miR-3202, hsa-miR-4270, hsa-miR-4499, hsa-miR-4669, hsa-miR-483-5p, hsa-miR-6124, hsa-miR-642a-3p, and 48 were down-regulated, such as hsa-miR-5703 (**Figure 1**; **Table 5**).

Comparison between the Han case group and Han control group: before the treatment, the expressions of 160 miRNAs were differentially expressed in the Han case group in comparison with the Han control group, in which 73 miRNAs were increased, such as hsa-miR-3202, hsa-miR-4270, hsa-miR-4669 and hsa-miR-483-5p, and 87 were reduced, such as hsa-miR-5703. After the treatment, when the Han

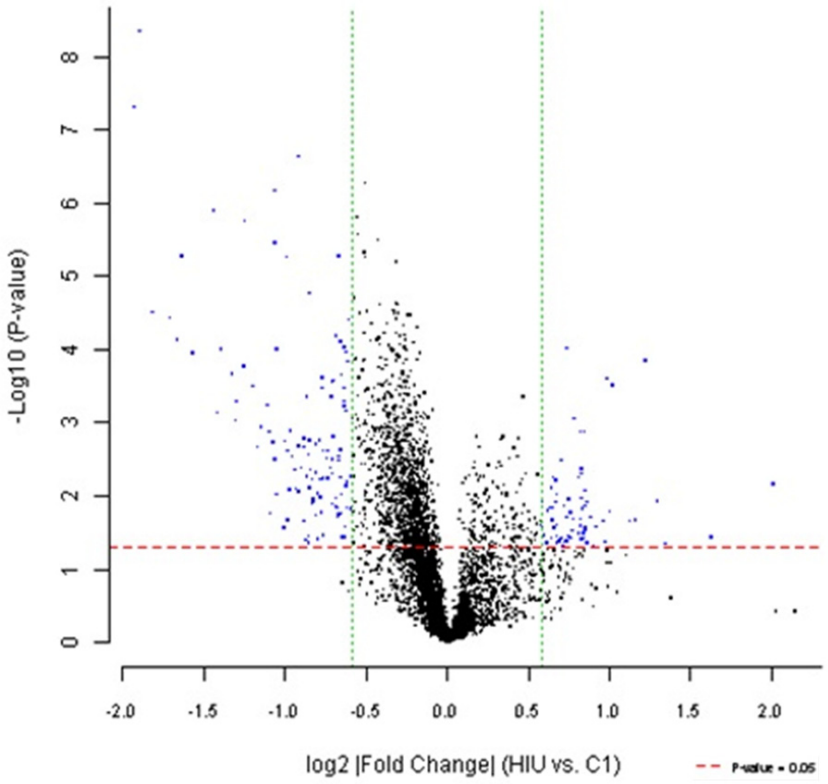

Figure 3. Volcanic diagram of serum microRNA expressions between the Han case group after the treatment and the Han control group (blue point refers to the differentially expressed microRNAs). Note: HIU, Han case group after the treatment; C1, Han control group.

Table 6. Differential expressions of serum microRNA in Han case and control groups before the treatment

ID	Name	log2 (Ratio) HC/C1	*P*-value HC/C1	Up/down-regulated
A_25_P00015596	hsa-miR-3202	1.043756973	0.004770351	Up
A_25_P00015748	hsa-miR-4270	0.713317012	0.000212609	Up
A_25_P00016725	hsa-miR-4669	1.201289136	0.0000629	Up
A_25_P00012459	hsa-miR-483-5p	1.407809805	0.00113979	Up
A_25_P00017400	hsa-miR-5703	-1.211745486	0.00000102	Down

Note: C1, Han control group; HC, Han case group before the treatment.

case group was compared with the Han control group, a total of 124 miRNAs was differentially expressed; 42 miRNAs like hsa-miR-4669 and hsa-miR-483-5p were elevated, while 82 were declined with hsa-miR-5703 as an example in the Han case group. Additionally, when the levels of miRNAs in the Han case group were compared before and after the treatment, 6 miRNAs were down-regulated in the Han case group (**Figures 2**, **3** and **Tables 6**-**8**).

Comparison between the Uygur case group and Uygur control group

Before the treatment, 92 miRNAs were abnormally expressed, 40 of which were up-regulated, such as hsa-miR-3202, hsa-miR-4669 and hsa-miR-483-5p, and 52 were down-regulated, such as hsa-miR-5703 in the Uygur case group compared with the Uygur control group. After the treatment, 103 miRNAs were differentially expressed, 48 of which were elevated, such as hsa-miR-3202, hsa-miR-4669, hsa-miR-623 and hsa-miR-483-5p, while 55 were declined, such hsa-miR-5703 in the Uygur case group compared with the Uygur control group. In addition, compared with miRNA levels before the treatment, 2 miRNAs were changed after the treatment, with hsa-miR-623 up-regulated and hsa-miR-185-5p down-regulated (**Figures 4**, **5** and **Tables 9**-**11**).

Comparison between the Kazak case group and Kazak control group

Before the treatment, 115 miRNAs were dysregulated, 30 of which were up-regulated, such as hsa-miR-4270 and hsa-miR-4669, and 85 were down-regulated, such as hsa-miR-5703. After the treatment, 129 miRNAs were differentially expressed, 30 of which were increased, such as hsa-miR-3202, hsa-miR-3911, hsa-miR-4669 and hsa-miR-623, while 99 were reduced, such as hsa-miR-5703 in the Kazak case group compared with the Kazak control

Table 7. Differential expressions of serum microRNA in Han case and control groups after the treatment

ID	Name	log2 (Ratio) HIU/C1	*P*-value HIU/C1	Up/down-regulated
A_25_P00016725	hsa-miR-4669	0.761684566	0.038234726	Up
A_25_P00012459	hsa-miR-483-5p	0.922862554	0.026485328	Up
A_25_P00017400	hsa-miR-5703	-1.390920369	0.000100986	Down

Note: C1, Han control group; HIU, Han case group after the treatment.

Table 8. Differential expressions of serum microRNA (down-regulated) in Han case group before and after the treatment

ID	Name	log2 (Ratio) HIU/HC	*P*-value HIU/HC
A_25_P00013941	hsa-miR-125a-3p	-0.615472956	0.008923132
A_25_P00016354	hsa-miR-451b	-0.791655632	0.018803465
A_25_P00017219	hsa-miR-4730	-0.822959179	0.012421029
A_25_P00017967	hsa-miR-6132	-0.650286528	0.013944169
A_25_P00017853	hsa-miR-6514-3p	-0.625545578	0.025367141
A_25_P00017874	hsa-miR-6716-3p	-0.729847923	0.012563562

Note: HC, Han case group before the treatment; HIU, Han case group after the treatment.

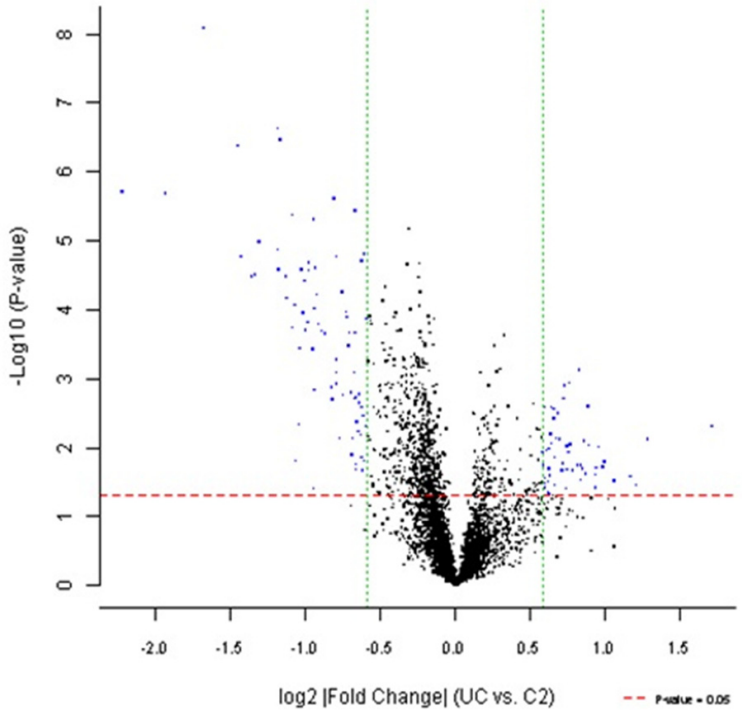

Figure 4. Volcanic diagram of serum microRNA expressions between the Uygur case group before the treatment and the Uygur control group (blue point refers to the differentially expressed microRNAs). Note: UC, Uygur case group before the treatment; C2, Uygur control group.

group. In addition, compared with miRNA levels before the treatment, 4 miRNAs were up-regulated after the treatment (**Figures 6**, **7** and **Tables 12**-**14**).

miRNA expression differences among the Han, Uygur and Kazak ESCC patients

Between the Kazak and Han patients, 6 miRNAs were differentially expressed with 4 up-regualted and 2 down-regulated; between the Uygur and Han patients, 17 miRNAs were differentially expressed with 3 up-regulated and 14 down-regulated; between the Kazak and Uygur patients, 10 miRNAs were differentially expressed with all up-regulated (**Tables 15**-**17**).

Discussion

ESCC is a malignant and aggressive tumor in digestive tract with high incidence in Xinjiang Uygur Autonomous Region, which reveals regional differences among different ethnic groups. invasion and migration of cancer cells is still a problem in ESCC for lack of effective biomarkers for diagnosis and prognosis of ESCC [7]. Recently, miRNAs have attracted much attention for its participatory role in tumor progression and management [15-19]. Due to the stability and accessibility of serum miRNAs, the present study aims to investigate the serum miRNA differential expressions before and after radiochemotherapy in locally advanced ESCC patients among different ethnic groups in Xinjiang area. Our study re-

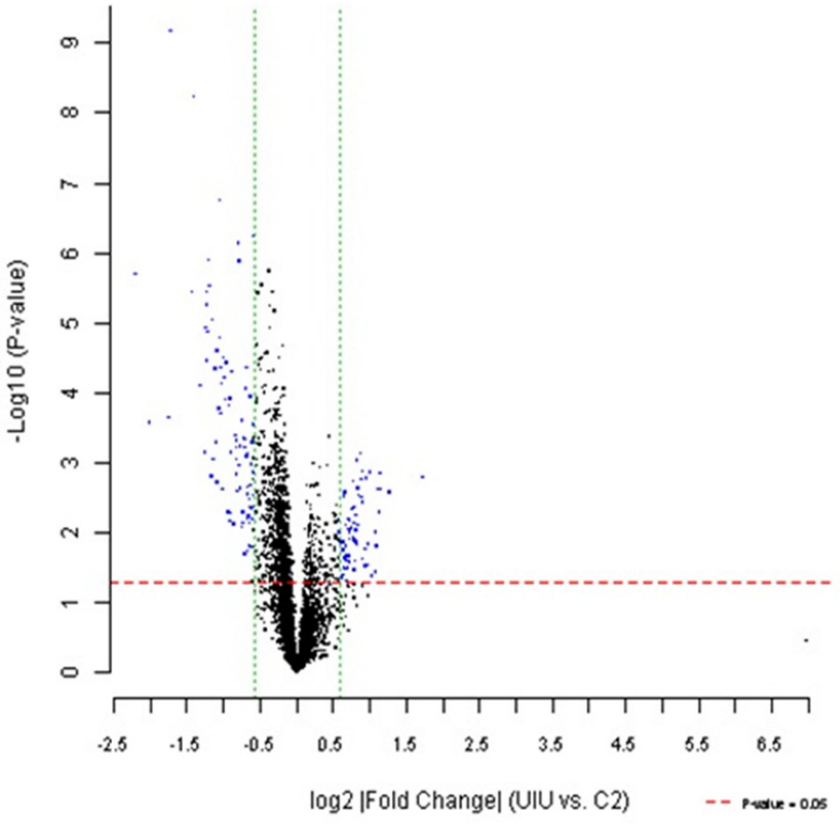

Figure 5. Volcanic diagram of serum microRNA expressions between the Uygur case group after the treatment and the Uygur control group (blue point refers to the differentially expressed microRNAs). Note: UIU, Uygur case group after the treatment; C2, Uygur control group.

Table 9. Differential expressions of serum microRNA in Uygur case and control groups before the treatment

ID	Name	log2 (Ratio) UC/C2	*P*-value UC/C2	Up/down-regulated
A_25_P00015596	hsa-miR-3202	0.82339905	0.018414131	Up
A_25_P00016725	hsa-miR-4669	0.668599812	0.036101632	Up
A_25_P00012459	hsa-miR-483-5p	0.76334367	0.012035189	Up
A_25_P00017400	hsa-miR-5703	-0.865827418	0.000226323	Down

Note: C2, Uygur control group; UC, Uygur case group before the treatment.

Table 10. Differential expressions of serum microRNA in Uygur case and control groups after the treatment

ID	Name	log2 (Ratio) UIU/C2	*P*-value UIU/C2	Up/down-regulated
A_25_P00015885	hsa-miR-3192	0.6250249	0.040297507	Up
A_25_P00015596	hsa-miR-3202	0.822791329	0.005761763	Up
A_25_P00016725	hsa-miR-4669	0.821434984	0.013744036	Up
A_25_P00010228	hsa-miR-623	0.903901699	0.023908908	Up
A_25_P00012459	hsa-miR-483-5p	0.859378393	0.002937745	Up
A_25_P00017401	hsa-miR-5703	-0.828088807	0.000415505	Down

Note: C2, Uygur control group; UIU, Uygur case group after the treatment.

veals that serum miRNAs are differentially expressed in ESCC patients and may act as biomarkers for diagnosis of ESCC, and radiochemotherapy may change the serum levels of part miRNAs.

The Agilent microRNA gene chip is applied for preliminary screening of ESCC serum miRNAs and reveals that there are a number of miRNAs with differential expression in ESCC patients. Moreover, differentially expressed miRNAs are also observed in ESCC patients between the three ethnic groups and the corresponding control groups before and after radiochemotherapy. In all of the assessed miRNAs, some are jointly up-regulated, such as has-miR-483-5p, while some are down-regulated with has-miR-5703 as an example. Different ethnic groups also present distinct differentially expressed miRNAs like hsa-miR-4454, hsa-miR-144-3p and hsa-miR-4459. The findings of our study imply that miRNAs deliver complicated expressions in serum. After software analysis and reference to literature reviews, we preliminarily screen out several dominant genes with distinct expressions in serum of ESCC patients, namely hsa-miR-483-5p and hsa-miR-5703, among which hsa-miR-483-5p

Table 11. Differential expressions of serum microRNA in Uygur case group before and after the treatment

ID	Name	log2 (Ratio) UIU/UC	*P*-value UIU/UC	Up/down-regulated
A_25_P00010229	hsa-miR-623	1.011170082	0.012871273	Up
A_25_P00012239	hsa-miR-185-5p	-0.686896229	0.015796039	Down

Note: UC, Uygur case group before the treatmen; UIU, Uygur case group after the treatment.

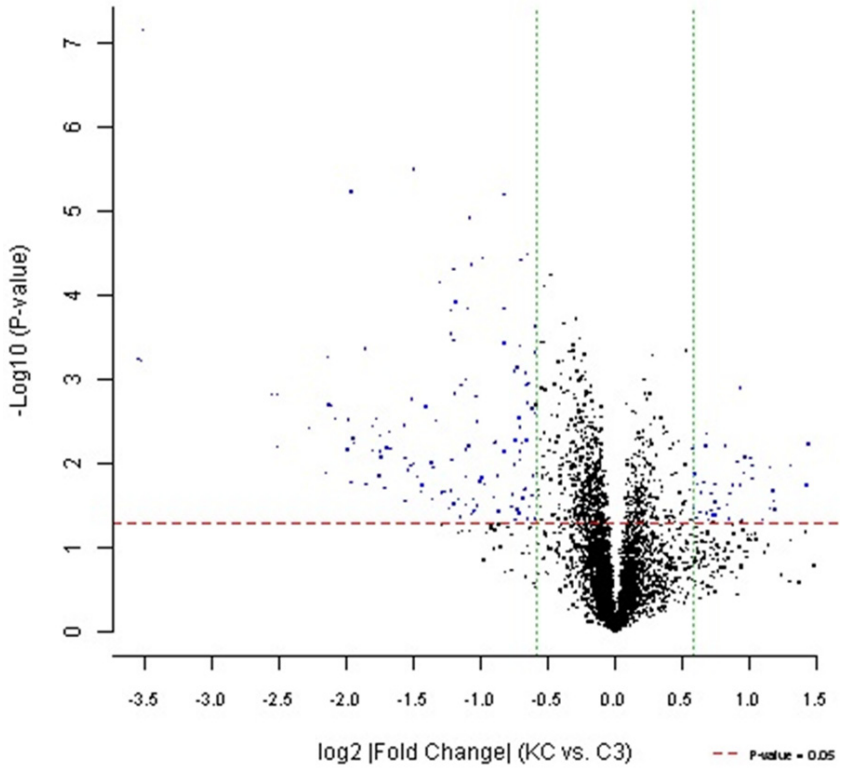

Figure 6. Volcanic diagram of serum microRNA expressions between the Kazak case group before the treatment and the Kazak control group (blue point refers to the differentially expressed microRNAs). Note: KC, Kazak case group before the treatment; C3, Kazak control group.

is mostly studied in related researches. Hsa-miR-483-5p is expressed in several types of human cancers, such as lung cancer [20], colorectal cancer [21], ovarian cancer [22], gastric cancer [23], prostatic cancer [24] and pancreatic cancer [25]. Additionally, hsa-miR-483-5p is also found to participate in the progression and development of ESCC. Wu *et al.* reports that the serum hsa-miR-483-5p level is significantly reduced after surgery in comparison with the preoperative level, and hsa-miR-483-5p may serve as a molecular marker for the diagnosis and efficacy evaluation of ESCC [26]. Warnecke-Eberz U and his team propose that hsa-miR-483-5p is elevated in serum and tumor tissues of the patients with ESCC and esophageal adenocarcinoma, signaling hsa-miR-483-5p as an effective indicator of ESCC diagnosis [27]. Also, our study reveals that hsa-miR-483-5p is evidently increased in ESCC patients' serum with differential multiple |log2 ratio| ≥ 1.00 (3 times difference, $P < 0.000$), which also indicates that hsa-miR-483-5p may function as a serum biomarker for diagnosis of ESCC, consistent with the former reports. In terms of hsa-miR-5703, it presents increased expression following treatment of cancer cells with luteolin and/or gefitinib in prostatic cancer [28]. However, no literature has explored the role of hsa-miR-5703 in ESCC. Our experiment finds that the ESCC patients in the three ethnic groups all display decreased serum levels of hsa-miR-5703 before and after the radiochemotherapy, which reveals that the radiochemotherapy exerts no significant influence on its expression, and that hsa-miR-5703 may act as a molecular marker for diagnosis of ESCC. Our findings enrich the miRNA researches in ESCC and provide further evidence for exploration into the mechanism of miRNAs in ESCC progression and development.

The comparison of miRNA differential expressions between every two ethnic groups reveals that Han, Uygur and Kazak ESCC patients share down-regulated hsa-miR-5703 gene pre- and

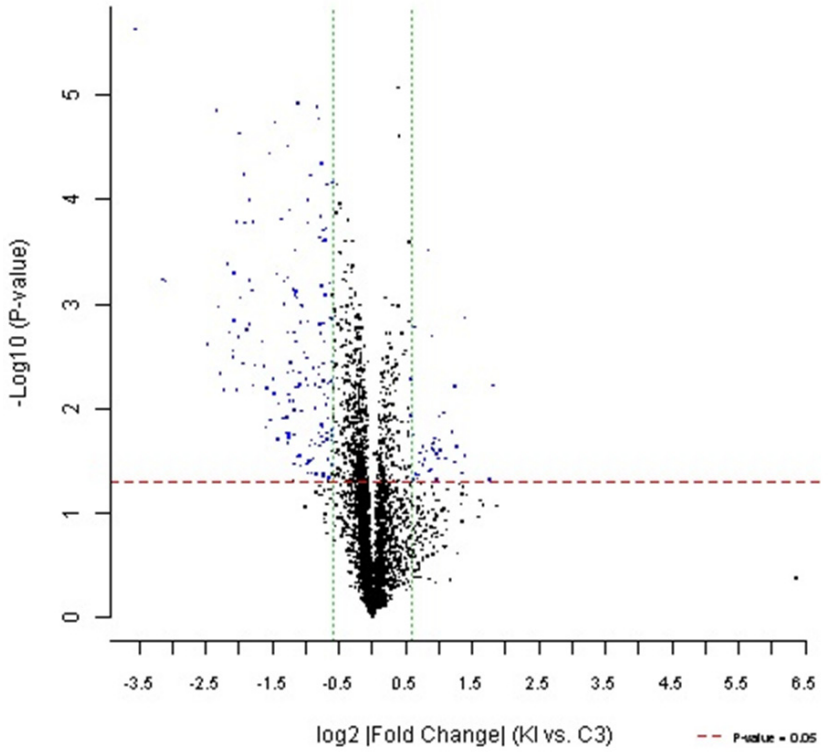

Figure 7. Volcanic diagram of serum microRNA expressions between the Kazak case group after the treatment and the Kazak control group (blue point refers to the differentially expressed microRNAs). Note: KI, Kazak case group after the treatment; C3, Kazak control group.

Table 12. Differential expressions of serum microRNA in Kazak case and control groups before the treatment

ID	Name	log2 (Ratio) KC/C3	*P*-value KC/C3	Up/down-regulated
A_25_P00015747	hsa-miR-4270	1.197770098	0.035652252	Up
A_25_P00016726	hsa-miR-4669	1.191693695	0.028126831	Up
A_25_P00017401	hsa-miR-5703	-0.645365338	0.00540332	Down

Note: C3, Kazak control group; KC, Kazak case group before the treatment.

postoperatively when compared with the corresponding control groups, explaining that nationality and radiochemotherapy exert no significant influence on the gene expression, which further demonstrates that hsa-miR-5703 can be used as a biomarker for ESCC diagnosis. However, our data also claims that has-miR-4270 expression can be changed by radiochemotherapy, and preoperative has-miR-4270 is differentially expressed in Han and Kazak ESCC patients, while postoperative level is not. Previous studies have not reported similar findings and our study needs further verification by the recruitment of larger samples.

Further comparisons of miRNA levels among the three ethnic groups imply that miRNAs are differentially expressed between every two ethnic groups with has-miR-4454 most dominant between Uygur and Kazak ESCC patients, has-miR-144-3p between Uygur and Han ESCC patients and has-miR-4459 between Kazak and Han ESCC patients, respectively. The differential multiple of |log2 ratio| is over 1.00 (3 times difference, $P < 0.05$). Collectively, these genes can serve as the respective biomarkers for Han, Uygur and Kazak ESCC patients. The ESCC patients in Han, Uygur and Kazak ethnic groups in Xinjiang area share the same differential expressions of miRNAs and also had specifically expressed miRNAs, which is related to the ESCC characteristics of each ethnic group. However, our study needs to be verified by further exploration based upon larger specimens.

Acknowledgements

This project was supported by Natural Science Foundation of Xinjiang Uygur Autonomous Region (No. 2013211B61). We thank the reviewers for critical comments.

Disclosure of conflict of interest

None.

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Table 13. Differential expressions of serum microRNA in Kazak case and control groups after the treatment

ID	Name	log2 (Ratio) KI/C3	*P*-value KI/C3	Up/down-regulated
A_25_P00014840	hsa-miR-124-3p	1.279964802	0.023481005	Up
A_25_P00016625	hsa-miR-1268b	1.396951685	0.001364614	Up
A_25_P00010078	hsa-miR-146a-5p	1.362819163	0.042105716	Up
A_25_P00015727	hsa-miR-3124-5p	1.403871516	0.028847438	Up
A_25_P00012407	hsa-miR-345-5p	1.821301339	0.006070611	Up
A_25_P00015644	hsa-miR-4271	1.250806969	0.033160115	Up
A_25_P00016709	hsa-miR-4634	1.20159913	0.016989643	Up
A_25_P00012861	hsa-miR-671-5p	1.774884341	0.04868518	Up
A_25_P00017800	hsa-miR-937-5p	1.253310256	0.006214761	Up
A_25_P00017401	hsa-miR-5703	-1.080355667	0.006019929	Down

Note: C3, Kazak control group; KI, Kazak case group after the treatment.

Table 14. Differential expressions of serum microRNA (up-regulated) in Kazak case group before and after the treatment

ID	Name	log2 (Ratio) KI/KC	*P*-value KI/KC
A_25_P00010635	hsa-miR-584-5p	0.743856015	0.016811154
A_25_P00015271	hsa-miR-320d	0.721519006	0.027315611
A_25_P00015448	hsa-miR-2276	0.597710672	0.01971283
A_25_P00015644	hsa-miR-4271	0.633701213	0.048346858

Note: KC, Kazak case group before the treatment; KI, Kazak case group after the treatment.

Table 15. Differential expressions of serum microRNA in Kazak and Han case groups

ID	Name	log2 (Ratio) KC/HC	*P*-value KC/HC	Up/down-regulated
A_25_P00012724	hsa-miR-574-5p	0.704916384	0.011158435	Up
A_25_P00012730	hsa-miR-574-3p	0.60649696	0.044601632	Up
A_25_P00016273	hsa-miR-4455	0.593116559	0.001663045	Up
A_25_P00016805	hsa-miR-4459	0.999009013	0.021873449	Up
A_25_P00015554	hsa-miR-3138	-0.666247163	0.034985453	down
A_25_P00015828	hsa-miR-3198	-0.652232982	0.01136029	down

Note: KC, Kazak case group before the treatment; HC: Han case group before the treatment.

Table 16. Differential expressions of serum microRNA in Uygur and Han case groups

ID	Name	log2 (Ratio) UC/HC	*P*-value UC/HC	Up/down-regulated
A_25_P00010434	hsa-miR-106b-5p	0.747738399	0.041531147	Up
A_25_P00010578	hsa-miR-151a-3p	0.776173749	0.003696845	Up
A_25_P00012189	hsa-miR-144-3p	1.089748869	0.02376712	Up
A_25_P00012375	hsa-miR-151a-5p	0.651790105	0.046550871	Down
A_25_P00010642	hsa-miR-601	-0.585568816	0.026599456	Down
A_25_P00012246	hsa-miR-188-5p	-0.659229945	0.007432581	Down
A_25_P00012618	hsa-miR-516a-5p	-0.671010994	0.041526364	Down
A_25_P00015537	hsa-miR-3137	-0.701953496	0.01024459	Down
A_25_P00015827	hsa-miR-3198	-0.673334414	0.009998068	Down
A_25_P00015875	hsa-miR-3156-5p	-0.609649082	0.00843159	Down
A_25_P00016218	hsa-miR-3622b-5p	-0.608788497	0.007964518	Down
A-25_p00016354	hsa-miR-451b	-0.610414216	0.034693434	Down
A_25_P00016433	hsa-miR-4755-3p	-0.807508311	0.020913068	Down

A_25_P00017218	hsa-miR-4730	-0.643677203	0.037194967	Down
A_25_P00017419	hsa-miR-5100	-0.607032654	0.021800345	Down
A_25_P00017930	hsa-miR-6129	-0.673372686	0.028412347	Down

Note: UC, Uygur case group before the treatment; HC: Han case group before the treatment.

Table 17. Differential expressions of serum microRNA in Uygur and Kazak case groups

ID	Name	log2 (Ratio) KC/UC	*P*-value KC/UC	Up/down-regulated
A_25_P00012505	hsa-miR-494	0.892553357	0.031646619	Up
A_25_P00015683	hsa-miR-3189-3p	0.801721812	0.01617351	Up
A_25_P00016273	hsa-miR-4455	0.646137349	0.020140928	Up
A_25_P00016354	hsa-miR-451b	0.838967445	0.006630652	Up
A_25_P00016804	hsa-miR-4459	0.715265988	0.003249553	Up
A_25_P00017025	hsa-miR-3591-3p	0.716636321	0.019555238	Up
A_25_P00017218	hsa-miR-4730	0.9129633	0.008830821	Up
A_25_P00017239	hsa-miR-4454	1.020525105	0.005907087	Up
A_25_P00017853	hsa-miR-6514-3p	0.665286713	0.020386178	Up
A_25_P00017875	hsa-miR-6716-3p	0.698970742	0.023354185	Up

Note: KC, Kazak case group before the treatment; UC, Uygur case group before the treatment.

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