Int J Clin Exp Pathol 2015;8(6):7332-7340
www.ijcep.com /ISSN:1936-2625/IJCEP0009306

*Original Article*

# Genetic variation in miR-100 rs1834306 is associated with decreased risk for esophageal squamous cell carcinoma in Kazakh patients in northwest China

Jianbo Zhu[1*], Lan Yang[1*], Weiyan You[2], Xiaobin Cui[1], Yunzhao Chen[1], Jianming Hu[1], Wei Liu[1], Shugang Li[3], Xiaoyue Song[1], Yutao Wei[2], Wenjie Zhang[1], Feng Li[1]

*[1]Department of Pathology and Key Laboratory for Xinjiang Endemic and Ethnic Diseases, Shihezi University School of Medicine, Shihezi, China; [2]The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi, China; [3]Department of Preventive Medicine, Shihezi University School of Medicine, Shihezi, China. [*]Equal contributors.*

Received April 19, 2015; Accepted May 29, 2015; Epub June 1, 2015; Published June 15, 2015

**Abstract:** MicroRNAs (miRNAs) are a family of small noncoding RNAs that act as oncogenes and tumor suppressors. Single nucleotide polymorphisms (SNPs) in miRNAs may be associated with changes in phenotype and function. The aim of this study was to verify whether genetic variations in candidate microRNA (miRNA or miR) genes could contribute to esophageal squamous cell carcinoma (ESCC) susceptibility. A case-control study in 248 Kazakh patients with ESCC and 300 frequency matched control subjects was carried out to examine the potential association of six miRNA (miR-100 rs1834306, miR-34b/c rs4938723, miR-375 rs6715345, miR-146a rs2910164, miR-423 rs6505162 and miR-373 rs12983273) polymorphisms with risk of ESCC. We found that miR-100 rs1834306 T>C polymorphism was associated with a significant decreased risk of ESCC. In the recessive model, when the miR-100 rs1834306 TT/TC genotypes were used as the reference group, the CC homozygote genotype was associated with a significant decreased risk for ESCC (adjusted OR=0.495, 95% CI: 0.349-0.702, P=8.05×10$^{-5}$). In the dominant model, when the miR-100 rs1834306 TT genotypes was used as the reference group, the TC/CC genotype were associated with a borderline statistically decreased risk for ESCC (adjusted OR=0.665, 95% CI: 0.430-1.031, P=0.067). In addition, the miR-100 rs1834306 C allele in the Kazakh population was significantly associated with decreased risk of ESCC (OR=0.609, 95% CI: 0.48-0.78, P=8.37×10$^{-5}$). These findings indicated that functional polymorphism miR-100 rs1834306 C>T might contribute to decreased ESCC risk.

**Keywords:** miRNA, esophageal squamous cell carcinoma, single nucleotide polymorphisms, risk, Kazakh

## Introduction

Esophageal cancer is a very aggressive cancer and the sixth leading cause of cancer-related deaths worldwide [1]. Approximately 70% of esophageal cancers occur in China where esophageal squamous cell carcinoma (ESCC) is the predominant type (>90%) [2]. The crude mortality rate of esophageal cancer ranks fourth considering all cancer sites with 16.77/100,000 (23.29/100,000 for males and 10.11/100,000 for females; 10.59/100,000 in urban areas and 29.47/100,000 in rural areas) in China [3]. Xinjiang is located in the northwest region of China. Compared with other ethnic populations in China and those in Xinjiang where most Chinese Kazakhs reside, the Kazakh population is characterized by a higher incidence and mortality (90-150/100 000, age standardized) of ESCC than the general population in China [4-6]. Patients with ESCC have poor prognosis. In fact, with the mean 5-year survival rate is only about 10%, reflecting the fact that there are limited clinical approaches for early diagnosis and treatment of ESCC [7]. This highlights the importance of targeted prevention and early detection of esophageal cancer. Carcinogenesis of ESCC is multi-factorial and involves well-known environmental risk factors, such as tobacco smoking and alcohol consumption [8], as well as genetic factors including single nucleotide polymorphisms (SNPs).

Furthermore, the individual risk of exposition to known risk factors implies that genetic predisposition may play an important role in the etiology of esophageal cancer [9, 10].

MicroRNAs (miRNAs) are RNAs approximately 21-24 nucleotides in length that play important roles in regulation of cellular processes by targeting mRNAs for cleavage or translational repression [11]. Furthermore, miRNAs were closely linked to tumorigenesis [12], and aberrant expression of miRNAs has been linked to the development and progression of cancer. miRNAs also have prognostic significance for several tumor types (e.g., ESCC, lung cancer, neuroblastoma, and lymphocytic leukemia), and some miRNAs may function as oncogenes or tumor suppressor genes. The abnormal expression of miRNAs may be associated with a variety factors including chromosomal abnormalities (i.e. translocation, deletion, amplification), epigenetic changes (e.g. methylation), mutations, and polymorphisms (single nucleotide polymorphisms; SNPs), as well as defects in miRNA synthesis SNPs are a single nucleotide base variants that occur throughout the genome at a frequency of < 1% [13]. SNPs can be associated with small functional differences. In terms of cancer, while SNPs may not have a causal role in tumorigenesis, certain SNPs may however increase an individual's susceptibility to selected environmental factors. Recent studies suggest that SNPs in miRNAs may play an important role in the etiology of ESCC [14, 15]. Polymorphisms in miRNAs can affect tumor susceptibility by generating new miRNAs, changing mature miRNAs, or by combining with target genes [16]. Several SNPs in miRNAs have been associated with an increased risk of esophageal cancer [17], further highlighting their important role in the initiation and development of cancer. There may be substantial differences in the frequency of many SNPs among different ethnic groups, regions, and genetic backgrounds.

In this study, we using the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) to detect sequence variation in six (miR-100 rs1834306, miR-34b/c rs4938723, miR-375 rs6715345, miR-146a rs2910164, miR-423 rs6505162 and miR-373 rs12983273) important polymorphisms of six miRNAs in 248 cases of ESCC in Kazakh patients. The frequencies of SNPs were compared to 300 cases of normal controls in the Kazakh population, with the aim of providing insight into the molecular mechanisms and pathogenesis of esophageal cancer in the Kazakh population.

## Materials and methods

### *Study subjects*

A total of 248 Kazakh patients with ESCC were recruited for this study. Cases subjects from patients diagnosed with histologically confirmed ESCC were randomly collected by multistage cluster sampling. All patients were treated at the First Affiliated Hospital of Shihezi University, People's Hospital of Xinjiang Uygur Autonomous Region, and Xinjiang Yili Prefecture Friendship Hospital between 1997 and 2010. Data on clinicopathological variables, including tumor site, depth of invasion, and distant metastasis were collected from medical charts. The grade of differentiation, TNM stage, and lymph node status were classified according to the UICC/AJCC TNM classification (7th edition) (**Table 1**). Patients who had undergone surgery (other than diagnostic biopsies), chemotherapy, or radiation therapy before recruitment were excluded. 300 controls subjects without a history of cancer or other serious diseases and were frequency-matched to cases by age (±5 years), sex, and ethnicity. Then written informed consent was obtained, and a structured questionnaire was administered by trained interviewers to collect demographic data and environmental exposure history, such as age, sex, smoking status, and history of alcohol consumption. After the interview, 5 ml venous blood was collected from each participant for genomic DNA extraction. The institutional review board of Shihezi University School of Medicine approved the research protocol.

### *SNP selection*

We have selected six miRNAs as candidate loci according to literature data and to a computational analysis. Through an extensive mining of the databases of the International HapMap Project (http://www.hapmap.org), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and miRNA registry (http://microrna.sanger.ac.uk) to identify potentially functional polymorphisms that had minor allele frequency (MAF) of more than 0.01 in Chinese for all the

**Table 1.** Characteristics of ESCC cases and controls

| Variables | Cases (n=248) n (%) | Controls (n=300) n (%) | $P^a$ |
|---|---|---|---|
| Age (years), mean ± SD | 56.23 (±9.02) | 55.16 (±10.62) | 0.212 |
| Age (years) | | | 0.334 |
| <57 | 122 (49.2) | 160 (53.3) | |
| ≥57 | 126 (50.8) | 140 (46.7) | |
| Sex | | | 0.212 |
| Male | 143 (57.7) | 157 (52. 3) | |
| Female | 105 (42.3) | 143 (47.7) | |
| Differentiated status | | | |
| Well differentiation | 47 (19.0) | | |
| Moderately differentiation | 152 (61.2) | | |
| Poorly differentiation | 49 (19.8) | | |
| Tumor location | | | |
| Upper | 14 (5.6) | | |
| middle + lower | 234 (94.4) | | |
| Depth of invasion | | | |
| T1/T2 | 98 (42.2) | | |
| T3/T4 | 134 (57.8) | | |
| Lymph node metastasis | | | |
| No | 132 (53.2) | | |
| Yes | 116 (46.8) | | |
| Tumor-node-metastasis stage | | | |
| I-II | 154 (64.7) | | |
| III-IV | 84 (35.3) | | |

[a]Two-side $\chi^2$ test and student t test.

SNPs, or untranslated regions (UTRs) for miRNA biogenesis pathway SNPs.

*DNA Isolation and genotyping*

Genomic DNA was isolated from samples according to the manufacturer's protocol. DNA concentration was normalized to 10-30 ng/μl (diluted in 10 mM Tris and 1 mM EDTA) using a Nanodrop spectrophotometer (ND-1000). As an internal control, all genomic DNA samples were successfully tested by polymerase chain reaction (PCR) with human β-actin, indicating the suitable quality and quantity of DNA to detect miRNA polymorphisms. miRNA SNP detection primers were designed using Assay Designer 3.0 software, and polymorphic loci primers were designed to include a pair of amplification primers and a pair of extension primers. All primers were synthesized by Invitrogen (Tables S1 and S2).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to determine the genotype of polymorphisms for miR-100 (rs1834306), miR-34b/c (rs4938723), miR375 (rs6715345), miR-146a (rs2910164), miR-423 (rs6505162), and miR-373 (rs12983273). Mass spectrograms were analyzed using the Mass Array Typer software 4.0 (Sequenom). Genotypes were called by two researchers in a blinded fashion; 10% of samples were randomly selected for repeated genotyping, and the results were 100% concordant. A total of 248 Kazakh ESCC cases and 300 cancer-free controls were included in the final analysis.

*Statistical analysis*

Hardy-Weinberg analysis was performed by comparing the observed and expected genotype frequencies using a $\chi^2$ test. Age and sex of the ESCC and control groups were compared using a student's t-test and $\chi^2$ test. A $\chi^2$ test and Fisher's exact probability was used to compare the distributions SNP genotypes and alleles between cases and controls. Multinomial logistic regression analyses were used to estimate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were performed using Statistical Products and Services Solutions software (SPSS, version 17.0, Chicago, USA). All statistical analyses were two-sided, and *P* values < 0.05 were considered statistically significant.

## Results

*Characteristics of the study population*

Demographic characteristics of the control subjects and clinical features of ESCC patients are listed in **Table 1**. A total of 548 subjects were enrolled in the study, including 248 ESCC cases and 300 controls. The average age was 56.23±9.02 years in the ESCC group, and 55.16±10.62 years in controls. The cases and controls were adequately matched by age as

**Table 2.** Primary information for six miRNAs polymorphisms

| Gene | Genotyped SNPs | Chromosome | MAF[a] for Chinese in database | MAF in our controls | *P* value for HWE[b] test in our controls | RefSNP Alleles | Ancestral Allele |
|---|---|---|---|---|---|---|---|
| mir100 | rs1834306 | 11 | 0.430 | 0.321 | 0.213 | C/T | T |
| mir34b/c | rs4938723 | 11 | 0.057 | 0.326 | 0.998 | C/T | T |
| mir375 | rs6715345 | 2 | unknown | 0.078 | 0.205 | C/G | G |
| mir146a | rs2910164 | 5 | 0.444 | 0.405 | 0.669 | C/G | G |
| mir423 | rs6505162 | 17 | 0.200 | 0.361 | 0.627 | A/C | C |
| mir373 | rs12983273 | 19 | 0.057 | 0.163 | 0.252 | C/T | C |

[a]MAF: minor allele frequency; [b]HWE: Hardy-Weinberg equilibrium.

suggested by the $\chi^2$ test (P=0.334). Data on lymph node metastasis data was available in 116 (46.8%) of ESCC cases; TNM data was available in 238 (96.0%) patients (stage I-II, n=154; stage III-IV, n=84). The primary information for the six SNPs analyzed is listed in **Table 2**. The genotyping success rate was 98.91% for miR-100 rs1834306, 93.25% for miR-34b/c rs4938723, 64.42% for miR375 rs6715345, 94.16% for miR-146a rs2910164, 94.15% for miR-423 rs6505162, and 77.74% for miR-373 rs12983273. The minor allele frequency (MAF) in controls was similar to that for Chinese in the database for two SNPs (**Table 2**). The observed genotype frequencies for miR-100 rs1834306 T>C, miR-34b/c rs4938723 T>C, miR-375 rs6715345 G>C, miR-146a rs2910164 G>C, miR-423 rs6505162 C>A, and miR-373 rs12983273 C>T in the controls were in Hardy-Weinberg equilibrium (P=0.213, P=0.177, P=0.205, P=0.669, P=0.627, and P=0.252, respectively) (**Table 2**).

*The miR-100 rs1834306 T>C polymorphism is associated with a decreased risk of ESCC in Kazakhs*

The genotype distributions of six miRNA SNP in cases and controls are shown in **Table 3**. In the single locus analyses, the genotype frequencies of miR-100 rs1834306 T>C were 21.8% (TT), 44.0% (TC), and 34.2% (CC) in the patients with ESCC and 15.6% (TT), 33.0% (TC), and 51.4% (CC) in the control subjects. The difference in genotype frequency distribution of miR-100 (rs1834306) was statistically significant (P<0.05). In the recessive model, when the miR-100 rs1834306 TT/TC genotypes were used as the reference group, the CC homozygote genotype was associated with a significant statistically decreased risk for ESCC (adjusted OR =0.495, 95% CI: 0.349-0.702, P=8.05×10$^{-5}$). When the miR-100 rs1834306 TT homozygote genotype was used as the reference group, the TC genotype was not associated with the risk for ESCC (TC vs. TT: adjusted OR=0.955, 95% CI: 0.590-1.947, P=0.853). When the miR-100 rs1834306 TT homozygote genotype was used as the reference group, the CC genotype was associated with decreased risk for ESCC (CC vs. TT: adjusted OR=0.480, 95% CI: 0.298-0.772, P=0.002). In the dominant model, when the miR-100 rs1834306 TT genotypes was used as the reference group, the TC/CC genotype was associated with a borderline statistically decreased risk for ESCC (adjusted OR=0.665, 95% CI: 0.430-1.031, P=0.067). No significant associations were observed for the miR-34b/c rs4938723, miR-375 rs6715345, miR-146a rs2910164, miR-423 rs6505162 or miR-373 rs12983273 polymorphisms and the risk of ESCC (**Table 3**).

In addition, we further conducted analyses between miR-100 rs1834306 T>C polymorphisms and risk of esophageal cancer lymph node metastasis, differentiated status and TNM stage, no association was observed between miR-100 rs1834306 T>C and lymph node metastasis, differentiated status and TNM stage (**Table 4**).

## Discussion

Approximately 50% of all annotated human miRNA genes are located in fragile sites or areas of the genome that are frequently associated with cancer. SNPs in miRNAs (MirSNPs), the most common type of genetic variation in the human genome, result in phenotypic differences [18], such sequence variations in miRNA genes may potentially affect the processing of miRNAs, pri-miRNAs, pre-miRNAs and/or mature miRNAs, and/or target selection and

**Table 3.** Logistic regression analyses of associations between miRNA-related SNPs and risk of ESCC

| genotype | Cases (n=248) | n% | Controls (n=300) | n% | crude OR (95% CI) | *P* | Adjusted OR[a] (95% CI) | *P* |
|---|---|---|---|---|---|---|---|---|
| (miR100) rs1834306 | | | | | | | | |
| TT | 54 | 21.8 | 46 | 15.6 | 1.00 | | 1.00 | |
| TC | 109 | 44.0 | 97 | 33.0 | **0.957 (0.593-1.546)** | **0.858** | **0.955 (0.590-1.947)** | **0.853** |
| CC | 85 | 34.2 | 151 | 51.4 | **0.480 (0.298-0.771)** | **0.002** | **0.480 (0.298-0.772)** | **0.002** |
| TT vs. TC vs. CC | | | | | | | | **$3.34\times10^{-4}$** |
| TC+CC | 194 | 78.2 | 248 | 84.4 | **0.666 (0.431-1.030)** | **0.068** | **0.665 (0.430-1.031)** | **0.067** |
| TT+TC | 163 | 65.8 | 143 | 48.6 | 1.00 | | 1.00 | |
| CC | 85 | 34.2 | 151 | 51.4 | **0.494 (0.349-0.699)** | **$7.06\times10^{-5}$** | **0.495 (0.349-0.702)** | **$8.05\times10^{-5}$** |
| T allele | 217 | 43.8 | 189 | 32.1 | 1.00 | | | |
| C allele | 279 | 56.2 | 399 | 52.9 | **0.609 (0.48-0.78)** | **$8.37\times10^{-5}$** | | |
| (miR34b/c) rs4938723 | | | | | | | | |
| TT | 113 | 47.7 | 122 | 44.5 | 1.00 | | 1.00 | |
| TC | 99 | 41.8 | 122 | 44.5 | 1.11 (0.62-2.00) | 0.725 | 1.07 (0.59-1.94) | 0.824 |
| CC | 25 | 10.5 | 30 | 10.9 | 0.97 (0.54-1.76) | 0.930 | 0.95 (0.52-1.73) | 0.868 |
| TT vs. TC vs. CC | | | | | | | | 0.772 |
| TC+CC | 124 | 52.3 | 152 | 55.5 | 1.14 (0.80-1.610 | 0.476 | 1.11 (0.78-1.58) | 0.547 |
| TT+TC | 212 | 89.5 | 244 | 89.1 | 1.00 | | 1.00 | |
| CC | 25 | 10.5 | 30 | 10.9 | 1.04 (0.60-1.83) | 0.884 | 1.01 (0.57-1.78) | 0.975 |
| T allele | 325 | 68.6 | 366 | 66.8 | 1 | | | |
| C allele | 149 | 31.4 | 182 | 33.2 | 1.09 (0.83-1.41) | 0.545 | | |
| (miR375) rs6715345 | | | | | | | | |
| GG | 158 | 92.4 | 163 | 89.6 | 1.00 | | 1.00 | |
| CG | 7 | 4.1 | 15 | 8.2 | 0.481 (0.191-1.212) | 0.121 | 0.481 (0.190-1.218) | 0.123 |
| CC | 6 | 3.5 | 4 | 2.1 | 1.547 (0.429-5.588) | 0.505 | 1.495 (0.410-0.450) | 0.542 |
| GG vs. CG vs. CC | | | | | | | | 0.218 |
| CG+CC | 13 | 7.6 | 19 | 10.4 | 0.706 (0.337-1.477) | 0.355 | 0.698 (0.332-1.467) | 0.343 |
| GG+CG | 165 | 96.5 | 178 | 97.8 | 1.00 | | 1.00 | |
| CC | 6 | 3.5 | 4 | 2.1 | 1.618 (0.449-5.836) | 0.462 | 1.561 (0.429-5.683) | 0.500 |
| G allele | 323 | 94.4 | 341 | 93.7 | 1.00 | | | |
| C allele | 19 | 5.6 | 23 | 6.3 | 1.15 (0.61-2.15) | 0.668 | | |
| (miR146a) rs2910164 | | | | | | | | |
| GG | 82 | 34.5 | 99 | 35.6 | 1.00 | | 1.00 | |
| CG | 120 | 50.4 | 139 | 50 | 1.087 (0.64-1.86) | 0.762 | 1.07 (0.62-1.83) | 0.811 |
| CC | 36 | 15.1 | 40 | 14.4 | 1.04 (0.71-1.53) | 0.831 | 1.03 (0.70-1.51) | 0.890 |
| GG vs. CG vs. CC | | | | | | | | 0.951 |
| CG+CC | 156 | 65.5 | 179 | 64.4 | 0.95 (0.66-1.37) | 0.784 | 0.97 (0.67-1.39) | 0.847 |
| GG+CG | 202 | 84.9 | 238 | 85.6 | 1.00 | | 1.00 | |
| CC | 36 | 15.1 | 40 | 14.4 | 0.94 (0.58-1.54) | 0.814 | 0.95 (0.58-1.55) | 0.842 |
| G allele | 284 | 59.7 | 337 | 60.6 | 1.00 | | | |
| C allele | 192 | 40.3 | 219 | 39.4 | 0.96 (0.75-1.23) | 0.757 | | |
| (miR423) rs6505162 | | | | | | | | |
| CC | 99 | 40.9 | 109 | 38.9 | 1.00 | | 1.00 | |
| CA | 122 | 50.4 | 140 | 50 | 0.959 (0.666-1.382) | 0.824 | 0.955 (0.662-1.378) | 0.805 |
| AA | 21 | 8.7 | 31 | 11.1 | 0.746 (0.402-1.383) | 0.352 | 0.753 (0.405-1.400) | 0.371 |
| CC vs. CA vs. AA | | | | | | | | 0.644 |
| CA+AA | 143 | 59.1 | 171 | 61.1 | 0.921 (0.648-1.308) | 0.645 | 0.918 (0.646-1.306) | 0.635 |
| CC+CA | 221 | 91.3 | 249 | 88.9 | 1.00 | | 1.00 | |
| AA | 21 | 8.7 | 31 | 11.1 | 0.763 (0.426-1.367) | 0.364 | 0.773 (0.430-1.389) | 0.389 |
| C allele | 320 | 66.1 | 358 | 63.9 | 1.00 | | | |
| A allele | 164 | 33.9 | 202 | 36.1 | 1.10 (0.85-1.42) | 0.460 | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (miR373) rs12983273 | | | | | | | | |
| CC | 109 | 72.7 | 199 | 72.1 | 1.00 | | 1.00 | |
| CT | 37 | 24.6 | 64 | 23.2 | 1.055 (0.661-1.684) | 0.821 | 1.053 (0.659-1.684) | 0.828 |
| TT | 4 | 2.7 | 13 | 4.7 | 0.562 (0.179-1.765) | 0.323 | 0.556 (0.176-1.753) | 0.316 |
| CC vs. CT vs. TT | | | | | | | | 0.626 |
| CT+TT | 41 | 27.3 | 77 | 27.9 | 0.972 (0.623-1.517) | 0.901 | 0.969 (0.620-1.514) | 0.890 |
| CC+CT | 146 | 97.3 | 263 | 95.3 | 1.00 | | 1.00 | |
| TT | 4 | 2.7 | 13 | 4.7 | 0.554 (0.177-1.731) | 0.310 | 0.549 (0.175-1.720) | 0.303 |
| C allele | 255 | 85 | 462 | 83.7 | 1.00 | | | |
| T allele | 45 | 15 | 90 | 16.3 | 1.10 (0.75-1.63) | 0.618 | | |

[a]Adjusted for age, sex and clinical features in a logistic regression model; Bold values are statistically significant ($P<0.05$).

may thus affect an individual's susceptibility of carcinoma [19].

So far, most research on miR-100 has concentrated on expression analyses, where aberrant expression of both mature forms of the miRNA has been seen in cancer, as well as during cellular differentiation [20-25]. Recently, studies have shown that miRNA SNPs can affect the production of mature forms and the binding of nuclear factors related to miRNA processing, thus altering the hereditary susceptibility and prognosis of cancer [26-28]. To some extent, the variant of miRNA act as an "oncogene" or "anti-oncogene" indirectly [29]. We suppose that rs1834306 might affect the expression or processing of miR-100, therefore, studies evaluating the effect of this SNP in miRNA functionality are required. However, studies of the rs1834306 polymorphism on cancer risk have yielded inconsistent results [17, 30, 31]. The first of these studies was performed in a population of 346 Caucasian ESCC patients and indicated the rs1834306 polymorphism has no significantly associated with ESCC compared with controls [17]. It has been suggested that no significant associations were observed for the miR-100 rs1834306 polymorphisms in terms of the overall risk of cancer or the risk of specific types of cancer [30]. However, another study undertaken in 2011 suggested that miR-100 rs1834306 polymorphisms significantly correlated with a longer tumor response and time to progression (TTP) [31]. Our research observed a decreased risk of ESCC for individuals with C allele in the miR-100 rs1834306 using the Kazakh population, as to our knowledge, this is the first study to show a relation between miR-100 SNP and ESCC of Kazakh patients. These results suggest that the miR-100 rs1834306 polymorphism may have varying effects in different genetic backgrounds and ethnicity, or during the pathogenesis of different types of cancer.

The rs2910164 G/C polymorphism of the miR-146a gene is situated in the stem structure opposite the mature miR-146 sequence, and leads to a change of base mismatch in the stem region of the miR-146a precursor. Studies have shown that the rs2910164 C allele was associated with a decreased risk of gastric cancer [32], bladder cancer and colon carcinoma [33, 34]. However, an increased risk of bladder cancer and ESCC was observed in the miR146a rs2910164 polymorphism [15]. In contrast, the miR146a rs2910164 G>C polymorphism was not associated with risk of esophageal adenocarcinoma in Caucasian populations or ESCC in a Chinese population in Nanjing of south China [17, 35]. Our study revealed that rs2910164 in miR146a is not associated with susceptibility to ESCC, at least in the Kazakh population.

A study performed in 2012 indicated that the C genotype of the miR-423 rs6505162 SNP reduces the risk of breast cancer development, however, another study undertaken in 2009 suggested that the C genotype of miR-423 rs6505162 offered an increased risk of developing both ovarian and breast cancer in Breast Cancer Associated 2 (BRCA2) mutation carriers [36]. In our study, no significant associations were observed for the miR-423 rs6505162 polymorphisms in the risk of ESCC in Kazakh population.

Considering previous findings and the results of the present study, there are several possible reasons for the apparently discrepant results. First, population heterogeneity and genetic backgrounds of different ethnicities may con-

**Table 4.** Stratification analyses between miR-100 rs1834306 T>C polymorphism and clinicopathological parameters of Kazakh ESCC patients

| Parameter | | | | | TC | | CC | | TC+CC | | TT+TC | CC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TT | TC | CC | TT | OR (95% CI) | *P* value | OR (95% CI) | *P* value | OR (95% CI) | *P* value | | OR (95% CI) | *p* Value |
| Gender[a] | | | | | | | | | | | | | |
| Male | 25/27 | 70/55 | 48/72 | 1.00 | 1.375 (0.719-2.629) | 0.336 | 1.01 (0.527-1.938) | 0.975 | 1.182 (0.649-2.151) | 0.585 | 1.00 | 0.807 (0.508-1.284) | 0.366 |
| Female | 29/19 | 39/42 | 37/79 | 1.00 | 0.608 (0.295-1.255) | 0.177 | 0.633 (0.32-1.254) | 0.188 | 0.623 (0.328-1.184) | 0.146 | 1.00 | 0.867 (0.525-1.432) | 0.576 |
| Age[a] | | | | | | | | | | | | | |
| <57 | 27/34 | 49/57 | 43/62 | 1.00 | 1.083 (0.575-2.039) | 0.806 | 0.873 (0.462-1.652) | 0.677 | 0.974 (0.548-1.728) | 0.927 | 1.00 | 0.701 (0.548-1.728) | 0.152 |
| ≥57 | 27/32 | 60/50 | 42/59 | 1.00 | 1.422 (0.754-2.684) | 0.275 | 0.844 (0.442-1.612) | 0.607 | 1.109 (0.622-1.979) | 0.726 | 1.00 | 0.846 (0.509-1.407) | 0.519 |
| Histologic stage[b] | | | | | | | | | | | | | |
| well differentiated | 7 | 24 | 16 | 1.00 | 0.476 (0.186-1.217) | 0.121 | 0.577 (0.216-1.538) | 0.272 | 0.518 (0.213-1.256) | 0.145 | 1.00 | 0.971 (0.49-1.923) | 0.933 |
| moderately/poorly differentiated | 47 | 85 | 69 | | | | | | | | | | |
| Depth of invasion[b] | | | | | | | | | | | | | |
| T1/T2 | 22 | 44 | 32 | 1.00 | 1.278 (0.636-2.570) | 0.491 | 1.245 (0.598-2.591) | 0.558 | 1.264 (0.661-2.418) | 0.480 | 1.00 | 1.052 (0.603-1.837) | 0.858 |
| T3/T4 | 26 | 62 | 46 | | | | | | | | | | |
| Lymph node metastasis[b] | | | | | | | | | | | | | |
| yes | 29 | 46 | 41 | 1.00 | 0.615 (0.317-1.193) | 0.150 | 0.794 (0.4-1.577) | 0.510 | 0.69 (0.375-1.27) | 0.233 | 1.00 | 1.097 (0.648-1.856) | 0.731 |
| no | 25 | 63 | 44 | | | | | | | | | | |
| TNM stage[b] | | | | | | | | | | | | | |
| I+II | 31 | 72 | 50 | 1.00 | 0.713 (0.354-1.440) | 0.346 | 0.893 (0.432-1.848) | 0.760 | 1.307 (0.76-2.25) | 0.333 | 1.00 | 0.765 (0.444-1.316) | 0.333 |
| III+IV | 35 | 20 | 30 | | | | | | | | | | |

[a]Stratification analysis to evaluate the effects of variant genotypes on the risk of ESCC by age and sex. [b]Logistic regression analysis for the effects of miRNA-100 variants on risk of ESCC with different histologic grade, depth of invasion, lymph node metastasis and TNM stage through logistic regression analyses.

found the data. Second, different levels of environmental exposure could have different effects on the risk of cancer. Third, different types of cancer may further contribute to heterogeneity, and finally different study designs and approaches for selection of participants and controls should also be taken into account.

In conclusion, our study provided evidence that miR-100 rs1834306 T>C polymorphism is associated with a decreased risk of ESCC in a Chinese Kazakh population. However, no functional validation of these observational findings was conducted and a series of following studies would be conducted. Considering that this is from a case-control study, however, population based studies with large number of subjects and long-term follow-up are needed to verify the association of miR-100 polymorphism with the etiology of ESCC.

## Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No. 81260301, No. 81160301, No. 81360358, No. 81460362). The doctor grant from Xinjiang Production and Construction Corps (Grant No. 2014BB019). The funders had no role in study design, data collection, analysis, decision to publish, or preparation of the manuscript.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Lan Yang, Department of Pathology and Key Laboratory for Xinjiang Endemic and Ethnic Diseases, Shihezi University, School of Medicine, Shihezi, China. Tel: +86 189 9773 7052; E-mail: yl-branda@163.com

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**Table S1.** Sequences of PCR primers used in this study

| Snp ID | Amplification primers | Extension primer |
|---|---|---|
| rs4938723 | 1st: 5'-ACGTTGGATGGGATCTACTCAAGTCTCACC-3' | 5'-CCGGAACCTTCTTTGACCTAT-3' |
| | 2nd: 5'-ACGTTGGATGTAGAAGGGAGGTCCTCAATG-3' | |
| rs2910164 | 1st: 5'-ACGTTGGATGAAGCCGATGTGTATCCTCAG-3' | 5'-TTGTGTCAGTGTCAGACCT-3' |
| | 2nd: 5'-ACGTTGGATGCAGAGATATCCCAGCTGAAG-3' | |
| rs6505162 | 1st: 5'-ACGTTGGATGACTGTCTCTCTTCACACTGC-3' | 5'-AGAAACTCAAGCGCGGG-3' |
| | 2nd: 5'-ACGTTGGATGTCCAAAAGCTCGGTCTGAGG-3' | |
| rs1834306 | 1st: 5 '-ACGTTGGATGTCGTCCCACTCTCACAAAAG-3' | 5'-GATCTTCTATGTTCTCCCCA-3' |
| | 2nd: 5'-ACGTTGGATGGGAAAAAGTGGAAACCAAGG-3' | |
| rs12983273 | 1st: 5'-ACGTTGGATGGGAATGCTTTTTGCTGTTGG-3' | 5'-GTTGGTGTTATAATTGATATGTAA-3' |
| | 2nd: 5'-ACGTTGGATGATAAACTTGCTTGCTATGGG-3' | |
| rs6715345 | 1st: 5'-ACGTTGGATGCAGGTGCCTGCGTGGCGAT-3' | 5'-AGGTGCCTGCGTGGCGATCAGGCCG-3' |
| | 2nd: 5'-ACGTTGGATGTGAGCGTTTTGTTCGTTCGG-3' | |

**Table S2.** Sequence of β-globin PCR primer used in this study

| Gene | Primer | Target length |
|---|---|---|
| β-globin | For: 5'-CAGACACCATGGTGCACCTGAC-3' | 210 bp |
| | Rev: 5'-CCAATAGGCAGAGAGAGTCAGTG-3' | |