Cancer Management and Research

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

The association of polymorphisms in miRNAs with nonsmall cell lung cancer in a Han Chinese population

This article was published in the following Dove Press journal:
Cancer Management and Research

Chuanyin Li[1]*
Yu Zhang[1]*
Yingfu Li[2]
Qianli Ma[3]
Shuyuan Liu[1]
Yueting Yao[1]
Fang Tan[2]
Li Shi[1]
Yufeng Yao[1]

[1]Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Kunming, People's Republic of China; [2]Department of Geriatrics, The No. 1 Affiliated Hospital of Kunming Medical University, Kunming, People's Republic of China; [3]Department of Thoracic Surgery, The No. 3 Affiliated Hospital of Kunming Medical University, Kunming, People's Republic of China

*These authors contributed equally to this work

Background: MicroRNAs (miRNAs) have been demonstrated to play important roles in cancer progression. Recently, studies have revealed that polymorphisms in miRNAs might be associated with cancer susceptibility.

Materials and methods: In the current study, we investigated the associations of single nucleotide polymorphisms (SNPs) in miRNAs (rs11134527 in *pri-miR-218-2*, rs74693964 in *pri-miR-145*, rs6062251 in *pri-miR-133a-2,* and rs4705343 in *pri-miR-143*) with nonsmall cell lung cancer (NSCLC) in a Han population from Yunnan Province, Southwest China using a binary logistic regression analysis. A total of 452 patients with NSCLC and 452 healthy individuals were recruited for polymorphism genotyping using the TaqMan assay.

Results: Our results showed that the allelic frequencies of rs11134527 and rs4705343 were significantly different between the NSCLC and control groups (P=0.025 and 0.029). Additionally, the genotypic frequencies of rs11134527 were significantly different between the NSCLC and control groups (P=0.045). The mode of inheritance analysis showed that genotypes A/G+G/G of rs11134527 were associated with a lower risk of NSCLC under the dominant model (OR=0.69; 95% CI: 0.51–0.94). In addition, genotypes 2C/C+C/T of rs4705343 were associated with an increased risk of NSCLC under the log-additive model (OR=1.25; 95% CI: 1.01–1.53). However, there was no significant difference in the other SNPs between the NSCLC and control groups (P>0.05). Moreover, the association analysis of these SNPs between adenocarcinoma and squamous cell carcinoma (SCC) showed that allele A of rs11134527 was associated with SCC (OR=0.65; 95% CI: 0.48–0.88).

Conclusion: Our results indicated that the A allele of rs11134527 might be a risk factor (OR=1.24; 95% CI: 1.03–1.50) and that the T allele of rs4705343 might be a protective factor (OR=0.80; 95% CI: 0.66–0.98) for NSCLC in a Han Chinese population.

Keywords: microRNA, single nucleotide polymorphism, lung cancer, Chinese population, genetic variation

Introduction

Lung cancer is one of the most common cancers and is the leading cause of cancer-related deaths worldwide. There were 1.8 million new diagnoses of lung cancer in 2012, accounting for 13% of the global cancer burden.[1] The incidence and mortality rates of lung cancer in China were estimated to be 0.7 and 0.6 million cases in 2015.[2] Although smoking is the key risk factor for lung cancer, there are lung cancer patients without a smoking history.[3] An increasing number of studies have proven that genetic factors play a key role in the development of nonsmall cell lung cancer (NSCLC), which accounts for approximately 80% of lung cancer cases.

Correspondence: Li Shi; Yufeng Yao
Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, 935# Jiaoling Rd, Kunming 650118, Yunnan, People's Republic of China
Tel +86 871 6833 5632
Fax +86 871 6818 1483
Email: shili.imb@gmail.com; yufeng_yao@imbcams.com.cn

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/CMAR.S154040

MicroRNAs (miRNAs) are a group of short RNAs with 20–23 noncoding nucleotides that play important modulatory roles in various biological processes through posttranscriptional regulation. It is estimated that miRNAs regulate more than 30% of all human genes[4,5] and are involved in crucial physiological processes, such as proliferation, apoptosis, differentiation, tumorigenesis, and cancer metastasis.[5–8] Recently, studies have indicated the aberrant expression of miRNAs in human cancers,[9–11] including lung cancer.[12] Moreover, Sheervalilou et al[13] showed that aberrant expression of miRNAs in lung cancer might induce lung tumorigenesis. On the other hand, functional studies have shown that single nucleotide polymorphisms (SNPs) in miRNA genes may be associated with miRNA expression[14,15] and target gene expression.[16] Moreover, several studies have shown that SNPs in miRNA are associated with human cancers,[17–21] for example, in 2014, Wang et al reported that rs3746444 in *miR-499a* and rs11614913 in *miR-196a2* were associated with lung cancer.[22]

In the current study, we evaluated the association of SNPs (rs11134527 in *pri-miR-218-2*; rs74693964 in *pri-miR-145*; rs6062251 in *pri-miR-133a-2*; rs4705343 in *pri-miR-143*) in miRNA genes with NSCLC in a Han Chinese population. Our results revealed the role of genetic variations in the miRNA genes in the development of NSCLC in a Han Chinese population.

Materials and methods

Ethics statement

This study was approved by the Institutional Review Board of the No. 1 and No. 3 Affiliated Hospitals of Kunming Medical University. The methods used in this investigation were in accordance with the approved guidelines and the principles expressed in the Helsinki Declaration of 1975, which was revised in 2008. All participants provided written informed consent.

Subjects

The NSCLC group included 452 patients (308 males and 144 females) who were diagnosed with NSCLC at the No. 1 and No. 3 Affiliated Hospitals of Kunming Medical University from July 2014 to May 2016. The histological type of NSCLC was identified according to the World Health Organization (WHO 2004) classifications.[23] The pathologic stage was determined according to the International System for Staging Lung Cancer.[24] Based on the pathomorphological reports, the NSCLC cases included adenocarcinoma (AC), squamous cell carcinoma (SCC), and both AC and SCC. NSCLC patients with a prior history of primary cancer other than lung cancer were excluded from the current study. Clinical characteristics and data, such as gender, age, family history of cancer, and histological type of cancer, were obtained. The healthy control group included 452 subjects (318 males and 134 females) who had no family history of NSCLC and were recruited from a population undergoing routine health checkups at the No. 1 and No. 3 Affiliated Hospitals of Kunming Medical University. All participants self-reported as ethnic Hans and lived within approximately the same geographic region (Yunnan Province, Southwest China).

SNP genotyping

Genomic DNA was extracted from peripheral lymphocytes using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany). Four SNPs in miRNA genes, namely, rs11134527, rs74693964, rs6062251, and rs4705343, were genotyped using PCR amplification with a TaqMan assay. Primers and probes were purchased from Applied Biosystems (Foster City, CA, USA). To identify the accuracy of SNP genotyping by the TaqMan assay, three positive controls and one negative control were genotyped via TaqMan assay at the same time.

Statistical analysis

SPSS 19.0 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel were used to conduct the statistical analyses. The Student's *t*-test or one-way analysis of variance was used to test the quantitative variables, and the categorical data were compared by chi-square test. The allelic and genotypic frequencies of the four SNPs were calculated by the direct counting method. Hardy–Weinberg equilibrium (HWE) was tested for the SNPs in both the NSCLC and control groups. The genetic association between the SNPs in miRNA genes and NSCLC susceptibility was calculated using a binary logistic regression with gender and age as the covariates, and the ORs with the associated 95% CIs of allele-specific risks were also calculated. In addition, the Hosmer–Lemeshow test and the Nagelkerke R^2 were employed to establish the optimal model. The association between genotypes of the SNPs and NSCLC was analyzed using the inheritance model analysis of SNPStats software.[25] Five models including the codominant, dominant, recessive, overdominant, and log-additive were analyzed. The best-fit inheritance model was determined using the Akaike information criterion and Bayesian information criterion, which possesses the minimal Akaike information criterion and Bayesian information criterion values. A P value less than 0.05 was considered statistically significant.

Results

Subject characteristics

Table 1 lists the characteristics of the enrolled subjects. There were no age or gender differences between the NSCLC and

control groups (P>0.05). In the NSCLC individuals, 271 had AC, 169 had SCC, and 12 had both AC and SCC. There were 70 patients in pathological stage I, 77 in stage II, 161 in stage III, and 144 in stage IV.

Association of the four SNPs with NSCLC

The allelic and genotypic frequencies of the four SNPs in the miRNAs are displayed in Table 2. Genotypic frequencies of the four SNPs were in HWE for the case and control groups (P>0.05). The allelic and genotypic frequencies of rs11134527 were significantly different in the NSCLC and control groups (P=0.025 and 0.045, respectively). The A allele of rs11134527 occurred more frequently in the NSCLC group compared with the control group (OR=1.24; 95% CI: 1.03–1.50). The allelic frequencies for rs4705343 were significantly different between the NSCLC and control groups (P=0.029). The frequency of the A allele for rs4705343 was significantly higher in the control group than that in the NSCLC group (OR=0.80; 95% CI: 0.66–0.98). The allelic and genotypic frequencies of rs74693964 and rs6062251 showed no significant difference between the NSCLC and control groups (P>0.05).

Table 1 Clinical characteristics of the subjects enrolled in the current study

Characteristics	NSCLC	Control	*P*-value
N	452	452	
Ages, mean ± SD (years)	55.71±10.01	55.96±10.72	0.112
Gender (male/female)	308/144	318/134	0.471
Adenocarcinoma	271 (59.9%)		
Squamous cell carcinoma	169 (37.4%)		
Adenocarcinoma and squamous cell carcinoma	12 (2.7%)		
Clinical stage			
I	70 (15.5%)		
II	77 (17.0%)		
III	161 (35.6%)		
IV	144 (31.9%)		

Abbreviation: NSCLC, nonsmall cell lung cancer.

Model of inheritance analysis of the four SNPs with NSCLC

Tables 3–6 present the results of the analyses of the different inheritance model for the four SNPs. The genotypic frequency of rs11134527 A/G+G/G was significantly different from A/A (P=0.011) under the dominant model between the NSCLC and control groups. The A/G+GG genotype was associated with a decreased risk of NSCLC (OR=0.69; 95% CI: 0.52–0.92). The genotypic frequencies of rs4705343 were significantly different under the log-additive model between the NSCLC and control groups (P=0.036). The 2CC+T/C genotype was associated with a higher risk of NSCLC (OR=1.25; 95% CI: 1.01–1.53).

Association analysis of the four SNPs with different pathologic stages

Table 7 shows the results of the association analysis of the four SNPs between pathologic stages I+II and III+IV. There were no significant differences in the allelic and genotypic frequencies for the four SNPs between pathologic stages I+II and III+IV (P>0.05).

Association analysis of the four SNPs with AC and SCC

Our results showed that the allelic and genotypic frequencies of rs11134527 were significantly different between AC and SCC (P=0.005 and 0.015, respectively). The A allele was associated with SCC (OR=0.65; 95% CI: 0.48–0.88) (Table 8).

Table 2 Comparison of genotypic and allelic distribution of the four SNPs between NSCLC and control groups (after adjusting for gender and age)

SNP	Genotype, n (%)			*P*-value	Allele, n (%)		*P*-value	OR (95% CI)
rs11134527	A/A	A/G	G/G		A	G		
NSCLC	181 (40.0)	195 (43.1)	76 (16.8)	0.045	557 (61.6)	347 (38.4)	0.025	1.24 (1.03–1.50)
Control	145 (32.1)	220 (48.7)	87 (19.2)		510 (56.4)	394 (43.6)		
rs74693964	C/C	C/T	T/T		C	T		
NSCLC	422 (93.4)	30 (6.6)	0 (0.0)	0.264	874 (96.7)	30 (3.3)	0.319	0.76 (0.44–1.31)
Control	430 (95.1)	21 (4.6)	1 (0.2)		881 (97.5)	23 (2.5)		
rs6062251	C/C	C/T	T/T		C	T		
NSCLC	43 (9.5)	217 (48.0)	192 (42.5)	0.338	303 (33.5)	601 (66.5)	0.395	1.90 (0.89–1.33)
Control	46 (10.2)	195 (43.1)	211 (46.7)		287 (31.7)	617 (68.3)		
rs4705343	T/T	T/C	C/C		T	C		
NSCLC	204 (45.1)	196 (43.4)	52 (11.5)	0.092	604 (66.8)	300 (33.2)	0.029	0.80 (0.66–0.98)
Control	232 (51.3)	183 (40.5)	37 (8.2)		647 (71.6)	257 (28.4)		

Abbreviations: NSCLC, nonsmall cell lung cancer; SNP, single nucleotide polymorphism.

Table 3 Inheritance analysis of rs11134527 in *pri-miR-218-2* between NSCLC and control groups (after adjusting for gender and age)

Model	Genotype	Control, n (%)	NSCLC, n (%)	OR (95% CI)	*P*-value	AIC	BIC
Codominant	A/A	145 (32.1)	181 (40)	1.00	0.039	1334.6	1603.7
	A/G	220 (48.7)	195 (43.1)	0.69 (0.51–0.94)			
	G/G	87 (19.2)	76 (16.8)	0.69 (0.46–1.02)			
Dominant	A/A	145 (32.1)	181 (40)	1.00	0.011	1332.6	1596.9
	A/G-G/G	307 (67.9)	271 (60)	0.69 (0.52–0.92)			
Recessive	A/A-A/G	365 (80.8)	376 (83.2)	1.00	0.340	1338.1	1602.5
	G/G	87 (19.2)	76 (16.8)	0.84 (0.59–1.20)			
Overdominant	A/A-G/G	232 (51.3)	257 (56.9)	1.00	0.086	1336.1	1600.5
	A/G	220 (48.7)	195 (43.1)	0.79 (0.60–1.04)			
Log-additive	–	–	–	0.81 (0.66–0.98)	0.026	1334.1	1598.5

Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion; NSCLC, nonsmall cell lung cancer.

Table 4 Inheritance analysis of 74693964 in *pri-miR-145* between NSCLC and control groups (after adjusting for gender and age)

Model	Genotype	Control, n (%)	NSCLC, n (%)	OR (95% CI)	*P*-value	AIC	BIC
Codominant	C/C	430 (95.1)	422 (93.4)	1.00	0.110	1336.6	1605.8
	C/T	21 (4.7)	30 (6.6)	1.66 (0.91–3.05)			
	T/T	1 (0.2)	0 (0.0)	0.00 (0.00–NA)			
Dominant	C/C	430 (95.1)	422 (93.4)	1.00	0.130	1336.8	1601.2
	C/T-T/T	22 (4.9)	30 (6.6)	1.57 (0.87–2.86)			
Recessive	C/C-C/T	451 (99.8)	452 (100)	1.00	0.200	1337.4	1601.8
	T/T	1 (0.2)	0 (0.0)	0.00 (0.00–NA)			
Overdominant	C/C-T/T	431 (95.3)	422 (93.4)	1.00	0.094	1336.2	1600.6
	C/T	21 (4.7)	30 (6.6)	1.67 (0.91–3.06)			
Log-additive	–	–	–	1.47 (0.82–2.62)	0.190	1337.4	1601.7

Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion; NSCLC, nonsmall cell lung cancer.

Table 5 Inheritance analysis of rs6062251 in *pri-miR-133a-2* between NSCLC and control groups (after adjusting for gender and age)

Model	Genotype	Control, n (%)	NSCLC, n (%)	OR (95% CI)	*P*-value	AIC	BIC
Codominant	T/T	211 (46.7)	192 (42.5)	1.00	0.340	1338.9	1608.1
	C/T	195 (43.1)	217 (48)	1.23 (0.92–1.64)			
	C/C	46 (10.2)	43 (9.5)	1.01 (0.63–1.63)			
Dominant	T/T	211 (46.7)	192 (42.5)	1.00	0.220	1337.5	1601.9
	C/T-C/C	241 (53.3)	260 (57.5)	1.19 (0.90–1.56)			
Recessive	T/T-C/T	406 (89.8)	409 (90.5)	1.00	0.690	1338.9	1603.3
	C/C	46 (10.2)	43 (9.5)	0.91 (0.58–1.44)			
Overdominant	T/T-C/C	257 (56.9)	235 (52)	1.00	0.140	1336.9	1601.3
	C/T	195 (43.1)	217 (48)	1.23 (0.93–1.61)			
Log-additive	–	–	–	1.08 (0.88–1.34)	0.450	1260.4	1279.6

Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion; NSCLC, nonsmall cell lung cancer.

Discussion

Recently, many studies have reported the association of miRNA genes and NSCLC. In addition, several studies have been carried out to evaluate the association of SNPs located in miRNA genes with the susceptibility of cancers, including NSCLC. In the current study, we analyzed the association of four SNPs (rs11134527 in *pri-miR-218-2*; rs74693964 in *pri-miR-145*; rs6062251 in *pri-miR-133a-2*; rs4705343 in *pri-miR-143*) in miRNAs with NSCLC susceptibility in a Han Chinese population. Our results showed that the A allele of rs11134527 might be a risk factor and that the T allele of rs4705343 might be a protective factor for NSCLC in this Han Chinese population.

miR-218 has been reported to be downregulated in various cancers.[26–28] In 2010, Wu et al[29] reported that reduced expression of miR-218 was associated with worse survival in lung cancer. Then, Kumamoto et al[30] found that ectopic expression of miR-218 significantly inhibited cancer cell migration and invasion in lung cancer. In addition, some studies have found associations between rs11134527, located at *pri-miR-218-2*, and the risk of different human cancers, such as esophageal squamous cell carcinoma (ESCC),[31]

Table 6 Inheritance analysis of rs4705343 in *pri-miR-143* between NSCLC and control groups (after adjusting for gender and age)

Model	Genotype	Control, n (%)	NSCLC, n (%)	OR (95% CI)	*P*-value	AIC	BIC
Codominant	T/T	232 (51.3)	204 (45.1)	1.00	0.110	1336.7	1605.8
	T/C	183 (40.5)	196 (43.4)	1.25 (0.94–1.67)			
	C/C	37 (8.2)	52 (11.5)	1.54 (0.96–2.48)			
Dominant	T/T	232 (51.3)	204 (45.1)	1.00	0.056	1335.4	1599.8
	T/C-C/C	220 (48.7)	248 (54.9)	1.30 (0.99–1.71)			
Recessive	T/T-T/C	415 (91.8)	400 (88.5)	1.00	0.160	1337.1	1601.4
	C/C	37 (8.2)	52 (11.5)	1.39 (0.88–2.19)			
Overdominant	T/T-C/C	269 (59.5)	256 (56.6)	1.00	0.280	1337.9	1602.2
	T/C	183 (40.5)	196 (43.4)	1.16 (0.88–1.53)			
Log-additive	–	–	–	1.25 (1.01–1.53)	0.036	1334.7	1599.0

Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion; NSCLC, nonsmall cell lung cancer.

Table 7 Comparison of genotypic and allelic distribution of the four SNPs between pathologic stages I+II and III+IV (after adjusting for gender and age)

SNP	Genotype, n (%)			*P*-value	Allele, n (%)		*P*-value	OR (95% CI)
rs11134527	A/A	A/G	G/G		A	G		
I+II	63 (42.9)	60 (40.8)	24 (16.3)	0.693	186 (63.3)	108 (36.7)	0.462	0.90 (0.67–1.20)
III+IV	118 (63.3)	135 (44.3)	52 (17.0)		371 (60.8)	239 (39.2)		
rs74693964	C/C	C/T	T/T		C	T		
I+II	138 (93.9)	9 (6.9)	0 (0.0)	0.760	285 (96.9)	9 (3.1)	0.749	0.88 (0.40–1.94)
III+IV	284 (93.1)	21 (6.9)	0 (0.0)		589 (96.6)	21 (6.9)		
rs6062251	C/C	C/T	T/T		C	T		
I+II	17 (11.6)	74 (50.3)	56 (38.1)	0.334	108 (36.7)	186 (63.3)	0.156	0.81 (0.60–1.08)
III+IV	26 (8.5)	143 (46.9)	136 (44.6)		195 (32.0)	415 (68.0)		
rs4705343	T/T	T/C	C/C		T	C		
I+II	63 (42.9)	63 (42.9)	21 (14.3)	0.422	189 (64.3)	105 (35.7)	0.248	1.19 (0.89–1.59)
III+IV	141 (46.2)	133 (43.6)	31 (10.2)		415 (68.0)	195 (32.0)		

Abbreviation: SNP, single nucleotide polymorphism.

Table 8 Comparison of genotypic and allelic distribution of the four SNPs between AC and SCC (after adjusting for gender and age)

SNP	Genotype, n (%)			*P*-value	Allele, n (%)		*P*-value	OR (95% CI)
rs11134527	A/A	A/G	G/G		A	G		
AC	96 (35.4)	124 (45.8)	51 (18.8)	0.015	316 (58.3)	226 (41.7)	0.005	0.65 (0.48–0.88)
SCC	82 (48.5)	67 (39.6)	20 (11.8)		231 (68.3)	107 (31.7)		
rs74693964	C/C	C/T	T/T		C	T		
AC	253 (93.4)	18 (6.6)	0 (0.0)	0.853	524 (96.7)	18 (3.3)	0.870	1.07 (0.49–2.32)
SCC	157 (92.9)	12 (7.1)	0 (0.0)		326 (96.4)	12 (3.6)		
rs6062251	C/C	C/T	T/T		C	T		
AC	25 (9.2)	121 (44.6)	125 (46.1)	0.344	171 (31.5)	371 (68.5)	0.364	0.87 (0.64–1.18)
SCC	17 (10.1)	86 (50.9)	66 (39.1)		120 (35.5)	218 (64.5)		
rs4705343	T/T	T/C	C/C		T	C		
AC	124 (45.8)	116 (42.8)	31 (11.4)	0.984	364 (67.2)	178 (32.8)	0.961	0.99 (0.73–1.34)
SCC	76 (45.0)	73 (43.2)	20 (11.8)		225 (66.6)	113 (33.4)		

Abbreviations: AC, adenocarcinoma; SCC, squamous cell carcinoma; SNP, single nucleotide polymorphism.

cervical cancer,[32,33] and hepatocellular carcinoma. Our results also showed that the genotypic and allelic frequencies of rs11134527 were significantly different between the NSCLC and control groups. Allele A of rs11134527 was associated with a higher risk of NSCLC. Our results are consistent with previous studies performed on cervical cancer that found that the rs11134527 GG genotype was associated with a decreased risk of cervical carcinoma[32,33] and ESCC,[31] which indicated that rs11134527 might alter the expression of miR-218 and be associated with cancer. However, Zhang et al[34] and Zhang et al[35] found that there was no association of this SNP with ESCC and hepatocellular carcinoma, respectively. The reason for these different results might be the different cancer types.

miR-143, a tumor suppressor of various types of human cancer, has been demonstrated to play crucial roles in tumor growth, migration, and invasion.[36–38] In lung cancer, miR-143 can inhibit the genesis and development of tumors through directly suppressing the expression of its target genes.[39,40] Recently, an integrated bioinformatics analysis showed that miR-143 was downregulated in NSCLC.[41] These data indicate that miR-143 is important for the initiation and development of human cancers. Based on this, other studies have investigated the association of SNPs in miR-143 with human cancers and found that SNPs (such as rs4705343 and rs353292) in *miR-143* are associated with several cancers.[42–44] In the current study, we found that allele T of rs4705343 was associated with a decreased risk of NSCLC, which is in agreement with the results of a case–control study on cervical SCC.[45] These results also indicate that rs4705343 in miR-143 plays an important role in NSCLC and cervical SCC.

Downregulation of miR-145 and miR-133a has been reported in cancers. Several SNPs located in the flanking region of the *miR-145* gene were demonstrated to be associated with the expression of miR-145 and disease susceptibility.[43,45–47] In 2016, Xiao et al[48] identified miR-133 as a biomarker for lung cancer. In addition, several studies have found an association between SNPs in the *miR-133* gene (rs8089787, rs9948906, rs13040413, and rs200375711) with different diseases.[49,50] In the current study, we analyzed the association of rs74693964 in *miR-145* and rs6062251 in *miR-133a2* with NSCLC. However, we did not find an association of rs74693964 in *pri-miR-145* or rs6062251 in *pri-miR-133a-2* with NSCLC in the current population.

A limitation of the present study is that we did not ascertain the smoking status of the control individuals, making it difficult to perform future analyses of such exposure variables or a gene–smoking interaction analysis. This limitation may neutralize the effect of smoking and expose the effects of genetic variants in our study.

Conclusion

In the current study, we investigated the association of four SNPs located in four tumor suppressor miRNA genes (*pri-miR-218-2*, *pri-miR-143*, *pri-miR-145*, and *pri-miR-133a-2*) with NSCLC in a Chinese Han population. Our results showed that the A allele of rs11134527 in *pri-miR-218-2* might be a risk factor for lung cancer. In addition, the T allele of rs4705343 in *pri-miR-143* might be associated with a decreased risk of NSCLC. Our findings provide evidence that rs11134527 in *pri-miR-218-2* and rs4705343 in *pri-miR-143* may be new biomarkers for NSCLC. However, we analyzed the distribution of the SNPs between Asian and European populations (http://asia.ensembl.org/Homo_sapiens/Info/Index) and found that the allelic frequencies selected in the current study were significantly different between these two populations (Table 9). This result might suggest that it is necessary to evaluate the association of these SNPs with NSCLC in different populations before these SNPs are used as new biomarkers for NSCLC.

Acknowledgments

We are grateful for the participation of the patients and control subjects in this study. Appreciation is also owed to Xiaona Wang and Xinwen Zhang who participated in the experiments and data checking. This work was supported by a grant from the National Natural Science Foundation of China (81573206 and 31270030), Yunnan Applied Basic Research Projects (2016FA034), Fundamental Research Funds for the Central Universities and the PUMC Youth Fund (3332015149), The Association Foundation Program of Yunnan Provincial Science and Technology Department and Kunming Medical University (2017FE468-193, 2013FB169), and Special Funds for high-level health talents of Yunnan Province (D-201669 and L-201615). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

References

1. McGuire S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. *Adv Nutr.* 2016;7(2):418–419.

Table 9 Comparison of the SNP distribution between European and Asian populations

SNP in current study	Allelic frequencies in European population	Allelic frequencies in Eastern Asian population	*P* value
rs11134527	G: 76%; A: 24%	G: 43%; A: 57%	<0.001
rs74693964	C: 100%	C: 96%; T: 4%	0.043
rs6062251	T: 38%; C: 62%	T: 67%; C: 33%	<0.001
rs4705343	T: 83%; C: 17%	T: 68%; C: 32%	0.014

Abbreviation: SNP, single nucleotide polymorphism.

2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin.* 2016;66(2):115–132.
3. Wakelee HA, Chang ET, Gomez SL, et al. Lung cancer incidence in never smokers. *J Clin Oncol.* 2007;25(5):472–478.
4. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell.* 2005;120(1):15–20.
5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 2004;116(2):281–297.
6. Sachdeva M, Mo YY. miR-145-mediated suppression of cell growth, invasion and metastasis. *Am J Transl Res.* 2010;2(2):170–180.
7. Winter J, Diederichs S. MicroRNA biogenesis and cancer. *Methods Mol Biol.* 2011;676:3–22.
8. Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA. MicroRNAs--the micro steering wheel of tumour metastases. *Nat Rev Cancer.* 2009;9(4):293–302.
9. Shrestha S, Hsu SD, Huang WY, et al. A systematic review of microRNA expression profiling studies in human gastric cancer. *Cancer Med.* 2014;3(4):878–888.
10. Wu X, Zeng R, Wu S, Zhong J, Yang L, Xu J. Comprehensive expression analysis of miRNA in breast cancer at the miRNA and isomiR levels. *Gene.* 2015;557(2):195–200.
11. Li MY, Hu XX. Meta-analysis of microRNA expression profiling studies in human cervical cancer. *Med Oncol.* 2015;32(6):510.
12. Yang C, Sun C, Liang X, Xie S, Huang J, Li D. Integrative analysis of microRNA and mRNA expression profiles in non-small-cell lung cancer. *Cancer Gene Ther.* 2016;23(4):90–97.
13. Sheervalilou R, Shirvaliloo S, Fekri Aval S, et al. A new insight on reciprocal relationship between microRNA expression and epigenetic modifications in human lung cancer. *Tumour Biol.* 2017;39(5):1010428317695032.
14. Mullany LE, Herrick JS, Wolff RK, Buas MF, Slattery ML. Impact of polymorphisms in microRNA biogenesis genes on colon cancer risk and microRNA expression levels: a population-based, case-control study. *BMC Med Genomics.* 2016;9(1):21.
15. Zhan JF, Chen LH, Chen ZX, et al. A functional variant in microRNA-196a2 is associated with susceptibility of colorectal cancer in a Chinese population. *Arch Med Res.* 2011;42(2):144–148.
16. Sibin MK, Harshitha SM, Narasingarao KV, Dhananjaya IB, Dhaval PS, Chetan GK. Effect of rs11614913 polymorphism on mature miR196a2 expression and its target gene HOXC8 expression in human glioma. *J Mol Neurosci.* 2017;61(2):144–151.
17. Fan L, Chen L, Ni X, et al. Genetic variant of miR-4293 rs12220909 is associated with susceptibility to non-small cell lung cancer in a Chinese Han population. *PloS One.* 2017;12(4):e0175666.
18. Rong GQ, Zhang XM, Chen B, Yang XD, Wu HR, Gong W. MicroRNA gene polymorphisms and the risk of colorectal cancer. *Oncology Lett.* 2017;13(5):3617–3623.
19. Cimpeanu RA, Popescu DM, Burada F, et al. miR-149 rs2292832 C>T polymorphism and risk of gastric cancer. *Rom J Morphol Embryol.* 2017;58(1):125–129.
20. Wang W, Qin H, Zhou L, Ma J. Meta-analysis of the relationship between microRNA-499 rs3746444 polymorphism and hepatocellular carcinoma risk in Asians. *J Cancer Res Ther.* 2016;12(2):676–680.
21. Li WJ, Wang Y, Gong Y, Tu C, Feng TB, Qi CJ. MicroRNA-124 rs531564 polymorphism and cancer risk: a meta-analysis. *Asian Pac J Cancer Prev.* 2015;16(17):7905–7909.
22. Wang G, Wang W, Gao W, Lv J, Fang J. Two functional polymorphisms in microRNAs and lung cancer risk: a meta-analysis. *Tumour Biol.* 2014;35(3):2693–2699.
23. Beasley MB, Brambilla E, Travis WD. The 2004 World Health Organization classification of lung tumors. *Semin Roentgenol*. 2005;40(2):90–97.
24. Groome PA, Bolejack V, Crowley JJ, et al. The IASLC Lung Cancer Staging Project: validation of the proposals for revision of the T, N, and M descriptors and consequent stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. *J Thorac Oncol.* 2007;2(8):694–705.
25. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics.* 2006;22(15):1928–1929.
26. Kinoshita T, Hanazawa T, Nohata N, et al. Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion through targeting laminin-332 in head and neck squamous cell carcinoma. *Oncotarget.* 2012;3(11):1386–1400.
27. Yamamoto N, Kinoshita T, Nohata N, et al. Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion by targeting focal adhesion pathways in cervical squamous cell carcinoma. *Int J Oncol.* 2013;42(5):1523–1532.
28. Yamasaki T, Seki N, Yoshino H, et al. MicroRNA-218 inhibits cell migration and invasion in renal cell carcinoma through targeting caveolin-2 involved in focal adhesion pathway. *J Urol.* 2013;190(3):1059–1068.
29. Wu DW, Cheng YW, Wang J, Chen CY, Lee H. Paxillin predicts survival and relapse in non-small cell lung cancer by microRNA-218 targeting. *Cancer Res.* 2010;70(24):10392–10401.
30. Kumamoto T, Seki N, Mataki H, et al. Regulation of TPD52 by antitumor microRNA-218 suppresses cancer cell migration and invasion in lung squamous cell carcinoma. *Int J Oncol.* 2016;49(5):1870–1880.
31. Jiang L, Wang C, Sun C, et al. The impact of pri-miR-218 rs11134527 on the risk and prognosis of patients with esophageal squamous cell carcinoma. *Int J Clin Exp Pathol.* 2014;7(9):6206–6212.
32. Shi TY, Chen XJ, Zhu ML, et al. A pri-miR-218 variant and risk of cervical carcinoma in Chinese women. *BMC Cancer.* 2013;13:19.
33. Zhou X, Chen X, Hu L, et al. Polymorphisms involved in the miR-218-LAMB3 pathway and susceptibility of cervical cancer, a case-control study in Chinese women. *Gynecol Oncol.* 2010;117(2):287–290.
34. Zhang LS, Liang WB, Gao LB, et al. Association between pri-miR-218 polymorphism and risk of hepatocellular carcinoma in a Han Chinese population. *DNA Cell Biol.* 2012;31(5):761–765.
35. Zhang J, Huang X, Xiao J, et al. Pri-miR-124 rs531564 and pri-miR-34b/c rs4938723 polymorphisms are associated with decreased risk of esophageal squamous cell carcinoma in Chinese populations. *PloS One.* 2014;9(6):e100055.
36. Wang F, Liu J, Zou Y, et al. MicroRNA-143-3p, up-regulated in H. pylori-positive gastric cancer, suppresses tumor growth, migration and invasion by directly targeting AKT2. *Oncotarget.* 2017;8(17):28711–28724.
37. Wang H, Li Q, Niu X, et al. miR-143 inhibits bladder cancer cell proliferation and enhances their sensitivity to gemcitabine by repressing IGF-1R signaling. *Oncol Lett.* 2017;13(1):435–440.
38. Sun X, Zhang L. MicroRNA-143 suppresses oral squamous cell carcinoma cell growth, invasion and glucose metabolism through targeting hexokinase 2. *Biosci Rep.* 2017;37(3). pii: BSR20160404.
39. Zhang HB, Sun LC, Ling L, Cong LH, Lian R. miR-143 suppresses the proliferation of NSCLC cells by inhibiting the epidermal growth factor receptor. *Exp Ther Med.* 2016;12(3):1795–1802.
40. Xia H, Sun S, Wang B, et al. miR-143 inhibits NSCLC cell growth and metastasis by targeting Limk1. *Int J Mol Sci.* 2014;15(7):11973–11983.
41. Li C, Yin Y, Liu X, Xi X, Xue W, Qu Y. Non-small cell lung cancer associated microRNA expression signature: integrated bioinformatics analysis, validation and clinical significance. *Oncotarget.* 2017;8(15):24564–24578.
42. Li L, Pan X, Li Z, et al. Association between polymorphisms in the promoter region of miR-143/145 and risk of colorectal cancer. *Human Immunol.* 2013;74(8):993–997.
43. Yuan F, Sun R, Li L, et al. A functional variant rs353292 in the flanking region of miR-143/145 contributes to the risk of colorectal cancer. *Sci Rep.* 2016;6:30195.
44. Sun R, Chen P, Li L, et al. A polymorphism rs4705341 in the flanking region of miR-143/145 predicts risk and prognosis of colorectal cancer. *Oncotarget.* 2016;7(38):62084–62090.
45. Liang Y, Sun R, Li L, et al. A functional polymorphism in the promoter of miR-143/145 is associated with the risk of cervical squamous cell carcinoma in Chinese women: a case-control study. *Medicine (Baltimore).* 2015;94(31):e1289.

46. Chacon-Cortes D, Smith RA, Haupt LM, Lea RA, Youl PH, Griffiths LR. Genetic association analysis of miRNA SNPs implicates *MIR145* in breast cancer susceptibility. *BMC Med Genet.* 2015;16:107.
47. Wei YS, Xiang Y, Liao PH, Wang JL, Peng YF. An rs4705342 T>C polymorphism in the promoter of miR-143/145 is associated with a decreased risk of ischemic stroke. *Scientific Reports.* 2016;6:34620.
48. Xiao B, Liu H, Gu Z, Ji C. Expression of microRNA-133 inhibits epithelial-mesenchymal transition in lung cancer cells by directly targeting FOXQ1. *Arch Bronconeumol.* 2016;52(10):505–511.
49. Zhou PP, Li Y, Ma ZD, Li ZY, Chen FY, Jiang YX. Single nucleotide polymorphisms in the promoter region of mir-133a-1 and in pre-mir-152 rs1707 may contribute to the risk of asthma in a Chinese Han population. *Eur Rev Med Pharmacol Sci.* 2016;20(12):2642–2649.
50. Hedley PL, Carlsen AL, Christiansen KM, et al. MicroRNAs in cardiac arrhythmia: DNA sequence variation of MiR-1 and MiR-133A in long QT syndrome. *Scand J Clin Lab Invest.* 2014;74(6):485–491.