Original Article

Functional miRNA variants affect lung cancer susceptibility and platinum-based chemotherapy response

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Background: Platinum-based chemotherapy is widely used as the first-line treatment of lung cancer. MicroRNAs have an important role in lung carcinogenesis and progression. Single-nucleotide polymorphisms (SNPs) in miRNA involved in miRNA biogenesis and structural alteration may affect miRNA expression. In this study, we aimed to investigate the association of functional miRNA variants with the lung cancer susceptibility and platinum-based chemotherapy response.

Methods: Nine genetic polymorphisms in miR-605, 146a, 149, 196a-2, 27a, 499, 30c-1, 5197 and let-7a-2 were selected with comprehensive collection strategy and genotyped by MALDI-TOF mass spectrometry in a total of 215 health control and 507 lung cancer patients (386 patients received at least two consecutive cycles of platinum-based chemotherapy).

Results: We found that an allele carriers of miR-146a rs2910164 (P=0.022, OR=1.315) and C allele carriers of miR-149 rs71428439 (P=0.042, OR=1.372) performance a high risk of lung cancer. Mir-30c-1 rs928508 (P=0.005, in recessive model) and let-7a-2 rs629367 (P=0.030 and P=0.021, in additive and dominant models, respectively) showed strong relationship with lung cancer risk in age under 57 years. The rs11614913 (miR-196a-2) C allele or rs9280508 (miR-30c-1) G allele carriers shown more sensitive to platinum both in additive (P=0.010, P=0.022, respectively) and dominant models (P=0.001, P=0.018, respectively).

Conclusions: These findings suggested that SNPs rs71428439 (miR-149), rs2910164 (miR-146a), rs928508 (mir-30c-1) and rs629367 (let-7a-2) were associated with the lung cancer prevalence, polymorphisms of rs11614913 (miR-196a-2) and rs9280508 (miR-30c-1) significantly influenced the patients' response to platinum-based chemotherapy, which may serve as potential clinical biomarkers to predict lung cancer risk and platinum-based chemotherapy response.

Keywords: Genetic polymorphism; miRNA; lung cancer; susceptibility; chemotherapy response

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Introduction

Lung cancer is the most frequently diagnosed cancer and the leading cause of cancer related death in the world (1). In China, over the past 30 years, lung cancer has taken the place of liver cancer as the highest fatality rate of malignant tumors, becoming the top of the list of cancer deaths. According to the World Health Organization (WHO) "Histological Typing of Lung and Pleural Tumours", depending on the degree of differentiation and morphological characteristics, lung cancer can be classified into non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). Over 80% are NSCLC patients, which comprise squamous-cell carcinoma (SCC), adenocarcinoma (ADC), adenosquamous carcinoma (AC), and large-cell carcinoma. SCLC is comparatively simple, but grows quickly and has a tendency of early transfer. Surgery is the mainstay of therapy for early stage of lung cancer, while most of lung cancer (over 70%) typically treated by platinum-based chemotherapy, are advanced or metastatic at the time of diagnosis (2). But large individual differences occurs in the chemotherapy efficacy of platinum-based drugs with the overall 5-year relative survival for all lung cancer patients remaining low (15%) and the recurrence rate still high, even in early-stage groups (3-6). Therefore, there is urgent need for the early prediction and therapeutic strategy guideline for lung cancer, which underlines the importance of developing new diagnostic approaches and useful tumor molecular markers for drug sensitivity to guide clinical chemotherapy regimens.

MicroRNAs (miRNAs) are a class of conserved endogenous small molecule non-protein-coding RNAs with sizes of 17–25 nucleotides. Which functional as post-transcription regulators and widely exists in the life progress of animals and plants (7). They can negatively regulate gene expression by perfect or nearly perfect complementarity to 3'untranslated region (3'-UTR) of protein-coding mRNA in a sequence specific manner, which induces mRNA degradation or protein translation repression (8). Bioinformatics database shows that one miRNA can target a variety of gene sequences, also one gene may also receive the control of multiple miRNAs. Those progress could be implicated in the regulation of almost every biological processes of organisms, especially in processes during tumorigenesis, such as proliferation, apoptosis, metastasis, and angiogenesis (9).

Mutations occur in pre-miRNA and mature miRNA, especially in the seed region, may result in deregulation of target gene expression by altering miRNAs expression or maturation (10-13), consequently contributing to cancer susceptibility, prognosis and chemotherapy sensitivity (14-17). Recently, an increasing number of researches have discussed the associations between miRNAs genetic variants and human cancers susceptibility, including lung cancer (18-21). However, the roles of genetic variants of miRNAs in platinum-based chemotherapy resistance in lung cancer are still unknown. Our study tried to verify the impact of pre-miRNA SNPs mutation on the lung cancer risk and explore the effect of miRNA mutation on platinum-based chemotherapy response.

Methods

Subjects

From November 2011 to August 2014, a total of 507 Chinese Han lung cancer patients were enrolled from the Affiliated Cancer Hospital and Xiangya Hospital of Central South University (Changsha, Hunan, China), and 215 healthy controls without any diseases physically examined in the Xiangya hospital were selected. Our study was approved by the Ethics Committee of Xiangya School of Medicine, Central South University and the registration numbers are CTXY-110008-2 and CTXY-110008-3. The clinical research admission was approved by Chinese Clinical Trial Registry with registration numbers of ChiCTR-RO-12002873 and ChiCTR-RCC-12002830, and written informed consent was obtained from all participants. The inclusion criteria were as follows: patients were histopathologically or cytologically diagnosed as lung cancer; patients were received at least two cycle of platinum-based chemotherapy. Exclusion criteria contain pregnancy or lactation, active infection, symptomatic brain or leptomeningeal metastases, and/or previous or concomitant malignancies.

A total of 386 patients treated with at least 2 cycle of platinum-based chemotherapy were selected in our Chemotherapy sensitivity analyses. According to the Response Evaluation Criteria (RECIST) guideline (version 1.1) for solid tumors (22), the selected objects were classified as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). In our study, patients with CR or PR were designated platinum sensitivity, while PD or SD were regarded as platinum resistance.

DNA extraction, SNP selection and genotyping

Peripheral blood samples (5 mL) were obtained from each participating individuals and stored at –20 ℃ less than one week. The genomic DNA was isolated using a commercially available Genomic DNA Purification Kit (Wizard Genomic DNA Purification Kit, A1620; Promega, Madison, WI, USA) according to the standard protocols, the genomic DNA solution were stored at –20 ℃ orthonormal until use. For the target SNPs selection, two basic principles were considered. One was that, the minor allele frequency (MAF) larger than 5% in Chinese Han population. The other was that the SNPs might be associated with lung cancer susceptibility, prognosis or toxicity. To achieve these two criteria, we searched the published data concern about miRNA mutations and lung cancer. In addition, the databases of dbSNP (http://www.ncbi.nlm.nih.gov/snp), International HapMap Project (http://www.hapmap.org), and the online miRNA polymorphism databases (http://www.bioguo.org/miRNASNP/) were browsed. Eventually, 9 potentially functional SNPs were selected in this work.

All the polymorphisms were conducted by the Sequenom Mass Array Genotype Platform (Sequenom, San Diego, California, USA). Primers were designed in the soft of AssayDesigner (version 3.1).

Statistical analysis

The Hardy-Weinberg equilibrium analysis was carried out for the study participants using the χ^2-test. The relationship of genotypes with lung cancer risk and chemotherapy response were examined by logistic regression analysis, the odds rations (OR) and their 95% confidence intervals (CI) were used to evaluate the results. And correction of potential confounder analysis was also made by adjusting for age, sex, smoking status, stage, histological type and chemotherapy regimens. Differences were considered statistically significant when two-tailed P value was less than 0.05. The statistical analyses above were performed by PLINK 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) and SPSS 18.0 for Windows (IBM, Inc., Chicago, IL, USA). The figures were performed using StataSE version 12 for Windows (StataCorp, CollegeStation, TX, USA).

Results

Demographic and clinical characteristics of the study population

The genotyping was taken in a total of 507 lung cancer patients (398 males and 109 females) and 215 (84 males and 131 females) health controls. Among the lung cancer patients, only 386 patients, including 143 sensitive and 243 resistant individuals, received at least two cycles of platinum-based chemotherapy, and were enrolled in our study to investigate the role of miRNA polymorphisms in platinum-based chemotherapy response. The detailed information of clinical characteristics were summarized in *Table 1*. A significant difference was found in sex and histology between response and non-response (P=0.020 and 0.000, respectively). Other clinical characteristics, such as age, smoking status, stage, chemotherapy regimen were comparable in both groups. The information of 9 polymorphisms genotyped in this study were shown in *Table 2*, and the call rates of all the SNPs were above 95%, except rs2043556 with a call rate of 93.78%. The primer sequences for all the selected mutations were given in *Table 3*.

Association of functional miRNA polymorphisms with lung cancer susceptibility

Preliminary analyses revealed that miR-149 rs71428439 and miR-146a rs2910164 were associate with the occurrence of lung cancer (P=0.042 and 0.022, respectively). As shown in *Table 4*, after adjusting age and sex, rs2910164 still has significantly association with lung cancer susceptibility in dominant model (OR=0.67, P=0.041) and has a marginal statistical effect in additive model (OR=0.77, P=0.052). While the rs71428439 have marginal statistical effect in dominate model with P value equal to 0.057. The other results were listed in *Table 5* (mark A). In order to explore the deeper influence of miRNA mutation on the risk of lung cancer susceptibility, subgroup analyses were performed and the results (*Figure 1*) shown that rs71428439 was associated with NSCLC in dominant mode, the female patient or younger than 56 years subjects with this polymorphism may have lower risk of lung cancer in both additive and dominant modes. miR-146a rs2910164 polymorphism was

Table 1 Demographic and clinical characteristics of lung cancer patients and normal controls

Characteristics	Lung cancer, (n=507), n (%)	Response (n=143), n (%)	Non-response (n=243), n (%)	Controls (n=215), n (%)
Sex				
Male	398 (78.5)	120 (83.92)	179 (73.66)	84 (39.07)
Female	109 (21.5)	23 (16.08)	64 (26.34)	131 (60.93)
Age (years)				
≤56	244 (48.13)	69 (48.25)	125 (51.44)	138 (64.19)
>56	263 (51.87)	74 (51.75)	118 (48.56)	77 (35.81)
Smoking				
Smokers	307 (60.55)	92 (64.34)	139 (57.2)	
Nonsmokers	200 (39.45)	51 (35.66)	104 (42.8)	
Stage (NSCLC)				
I, II	17 (3.35)	1 (0.7)	7 (2.88)	
III, IV	429 (84.62)	110 (76.92)	213 (87.65)	
Stage (SCLC)				
Limited	34 (6.71)	17 (11.89)	11 (4.53)	
Extensive	27 (5.33)	15 (10.49)	12 (4.94)	
Histology				
Adenocarcinoma	222 (43.79)	46 (31.47)	133 (54.73)	
Squamous carcinoma	185 (36.49)	59 (41.26)	79 (32.51)	
Adenosquamous	19 (3.75)	6 (4.2)	8 (3.29)	
SCLC	69 (13.61)	32 (22.38)	23 (9.47)	
Other	12 (2.37)	–	–	
Chemotherapy regimen				
Cisplatin based		114 (79.72)	198 (81.48)	
Carboplatin based		29 (20.28)	45 (18.52)	

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

associated with susceptibility of lung cancer of SCC patients in a dominant model. In subgroup under 57 years miR-30c-1 rs928508 polymorphism in recessive model and miR-let-7a-2 rs629367 polymorphism in dominant model were associated with lung cancer susceptibility (P=0.005 and 0.021, respectively). Let-7a-2 rs629367polymorphism was also associated with susceptibility of lung cancer of NSCLC and SCC individuals in a dominant model (P=0.043 and 0.034, respectively).

Effects of functional miRNA polymorphisms on the therapeutic efficacy of platinum-based chemotherapy in lung cancer patients

A total of 386 patients who received at least two cycles of platinum-based cure had been studied in the chemotherapy sensitivity analyses. After adjustment for age, sex, smoking status, stage, histology, and chemotherapeutic regimen, we found that carriers with T allele of miR-196a-2 rs11614913

Table 2 The information of 11 gene polymorphisms examined in this study

miRNAs	dbSNP	Chr	Position	Call rate (%)	Allele	MAF
hsa-mir-605	rs2043556	chr10	53059406	93.78	T/C	0.2894
hsa-mir-146a	rs2910164	chr5	159912418	98.19	C/G	0.3465
hsa-mir-149	rs71428439	chr2	241395500	98.19	A/G	0.1559
hsa-mir-196a-2	rs11614913	chr12	54385599	98.70	C/T	0.5271
hsa-mir-27a	rs895819	chr19	13947292	98.19	T/C	0.2847
hsa-mir-499	rs3746444	chr20	33578251	97.67	A/G	0.1318
hsa-let-7a-2	rs629367	chr11	122017014	98.45	A/C	0.7765
hsa-mir-378	rs1076064	chr5	149112166	98.19	A/G	0.5099
hsa-mir-30c-1	rs928508	chr1	41223414	98.96	G/A	0.4877
hsa-mir-218-1	rs11134527	chr4	168195356	98.19	G/A	0.5916
hsa-mir-5197	rs2042253	chr5	143059433	98.45	T/C	0.3901

MAF, minor allele frequency.

Table 3 Primers of all the selected SNPs

SNP ID	2nd-PCRP	1st-PCRP
rs2042253	ACGTTGGATGCAGTCTCTTCTTGAACTGGC	ACGTTGGATGCTGATCTACAGCTAGGGAAG
rs2043556	ACGTTGGATGGAGAGCAATATACCTGTGGC	ACGTTGGATGAACAGAGAAGGCACTATGAG
rs2910164	ACGTTGGATGAAGCCGATGTGTATCCTCAG	ACGTTGGATGCACGATGACAGAGATATCCC
rs3746444	ACGTTGGATGGGAAGCAGCACAGACTTG	ACGTTGGATGGGCTGTTAAGACTTGCAGTG
rs71428439	ACGTTGGATGTCTTCACTCCCGTGCTTGTC	ACGTTGGATGGCCCGGCGACCTGCGTTGT
rs928508	ACGTTGGATGAAGAGTGCTGCCTATTTGGG	ACGTTGGATGCTGAGAACAGTGCTAATTGC
rs11614913	ACGTTGGATGCTGATCTGTGGCTTAGGTAG	ACGTTGGATGTCGACGAAAACCGACTGATG
rs895819	ACGTTGGATGACTTAGCCACTGTGAACACG	ACGTTGGATGAGCAGGGCTTAGCTGCTTGT
rs629367	ACGTTGGATGTATGCAGCATTTTTGTGAC	ACGTTGGATGATTCTGTTTCCTCGGGTTAG
rs1076064	ACGTTGGATGAGATCACCAGAAGATCCTCG	ACGTTGGATGGCAGTGAAAGTTAATCTGGG
rs11134527	ACGTTGGATGAGAGGAAGCAGCGTGGAGAA	ACGTTGGATGAAGTGTTCCAGTGGAACCCC

SNP, single nucleotide polymorphism; PCRP, polymerase chain reaction primer.

Table 4 Associations of the common miRNA polymorphisms and lung cancer risk

Gene	Polymorphisms	Allele	Controls (DD/Dd/dd)	Lung cancers (DD/Dd/dd)	Additive		Dominant		Recessive	
					OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
hsa-mir-149	rs71428439	A/G	133/66/5	365/115/13	0.75 (0.55–1.04)	0.087	0.69 (0.47–1.01)	0.057	0.93 (0.30–2.88)	0.898
hsa-mir-146a	rs2910164	C/G	67/110/32	204/230/58	0.77 (0.59–1.00)	0.052	0.67 (0.47–0.98)	0.041*	0.78 (0.47–1.32)	0.362

*, P<0.05.

Table 5 The relationship between other miRNA polymorphisms of lung cancer risk or platinum-based chemotherapy response

Gene	Category	Polymorphisms	Additive		Dominant		Recessive	
			OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
hsa-mir-499	A	rs3746444	0.92 (0.63–1.35)	0.681	0.95 (0.63–1.41)	0.792	0.52 (0.10–2.74)	0.442
	B		1.00 (0.63–1.61)	0.983	0.98 (0.60–1.61)	0.951	1.80 (0.14–2.61)	0.654
hsa-let-7a-2	A	rs629367	1.22 (0.89–1.66)	0.212	1.42 (0.98–2.06)	0.062	0.70 (0.31–1.55)	0.373
	B		0.87 (0.61–1.24)	0.443	0.89 (0.57–1.38)	0.593	0.67 (0.26–1.69)	0.386
hsa-mir-149	A	rs71428439	0.75 (0.53–1.04)	0.087	0.69 (0.47–1.01)	0.058	0.93 (0.30–2.88)	0.902
	B		1.15 (0.76–1.72)	0.514	1.30 (0.81–2.11)	0.284	0.62 (0.19–2.04)	0.441
hsa-mir-27a	A	rs895819	1.12 (0.84–1.48)	0.450	1.12 (0.79–1.60)	0.522	1.23 (0.61–2.48)	0.562
	B		0.94 (0.68–1.31)	0.721	1.00 (0.65–1.55)	0.983	0.73 (0.35–1.51)	0.404
hsa-mir-5197	A	rs2042253	0.88 (0.67–1.14)	0.323	0.92 (0.64–1.34)	0.668	0.73 (0.45–1.18)	0.193
	B		1.25 (0.90–1.74)	0.183	1.28 (0.82–2.00)	0.284	1.45 (0.75–2.80)	0.272
hsa-mir-146a	A	rs2910164	0.77 (0.59–1.00)	0.053	0.68 (0.47–0.98)	0.041	0.79 (0.47–1.32)	0.364
	B		1.18 (0.86–1.60)	0.311	1.29 (0.84–1.98)	0.253	1.12 (0.58–2.16)	0.731
hsa-mir-218-1	A	rs11134527	0.95 (0.73–1.22)	0.684	0.90 (0.62–1.31)	0.594	0.98 (0.61–1.58)	0.943
	B		0.87 (0.64–1.18)	0.383	0.82 (0.53–1.29)	0.392	0.86 (0.49–1.51)	0.602
hsa-mir-196a-2	A	rs11614913	1.03 (0.80–1.33)	0.832	1.04 (0.71–1.52)	0.847	1.04 (0.67–1.60)	0.847
hsa-mir-30c-1	A	rs928508	1.09 (0.84–1.42)	0.503	1.35 (0.89–2.04)	0.151	0.93 (0.62–1.41)	0.742
hsa-mir-378	B	rs1076064	1.14 (0.83–1.57)	0.411	1.08 (0.65–1.78)	0.783	1.33 (0.79–2.25)	0.285
hsa-mir-605	B	rs2043556	1.03 (0.72–1.47)	0.872	0.82 (0.53–1.28)	0.385	2.87 (1.02–8.04)	0.055

A, polymorphisms and risk of lung cancer; B, polymorphisms and evaluation of platinum-based chemotherapy response.

were more resistance to platinum-based chemotherapy both in additive and dominant models (OR=0.67, 95% CI: 0.50–0.91, P=0.010 and OR=0.43, 95% CI: 0.26–0.72, P=0.001, respectively). And patients with A allele of miR-30c-1 rs928508 shown higher sensitivity to platinum-based chemotherapy in additive and dominant models (OR=0.69, 95% CI: 0.50–0.95, P=0.022 and OR=0.53, 95% CI: 0.31–0.90, P=0.018, respectively) (*Table 6*). The other results were listed in *Table 5* (marked as B). In subgroup analyses, rs11614913 and rs928508 were affect chemotherapy response on certain level (summarized in *Figure 2*). For NSCLC, III and IV stage NSCLC, AC and limited-stage SCLC, the rs11614913 mutant individuals were more resistance to platinum-based chemotherapy in dominant model. We also found that in both additive and dominant models, rs11614913 polymorphism affected cisplatin-based chemotherapy response in male, SCLC, non-smoking, and over 56 years patients. MiR-30c-1 rs928508 was associated with chemotherapy response in additive model and dominant models for NSCLC, III IV stage of NSCLC, and AC subgroups, as well as in dominant model for male subgroup.

Discussion

In the current study, we investigated the association of 9 functional polymorphisms in pre-miRNAs on the risk of the lung cancer and the influence of platinum-based chemotherapy response in Chinese lung cancer patients. Our results showed that SNPs rs71428439 (miR-149), rs2910164 (miR-146a), rs629367 (let-7a-2) and rs928508 (miR-30c-1) might be related with the lung cancer prevalence, and polymorphisms of rs11614913 (miR-196a-2), rs9280508 (miR-30c-1) were significantly influence the response to platinum-based chemotherapy. To our knowledge, this is the first investigation about the association between functional polymorphisms of miRNAs and platinum-based chemotherapy response in Chinese lung cancer patients. These made it possible to do a rapid screening of lung cancer risk and drug resistance about

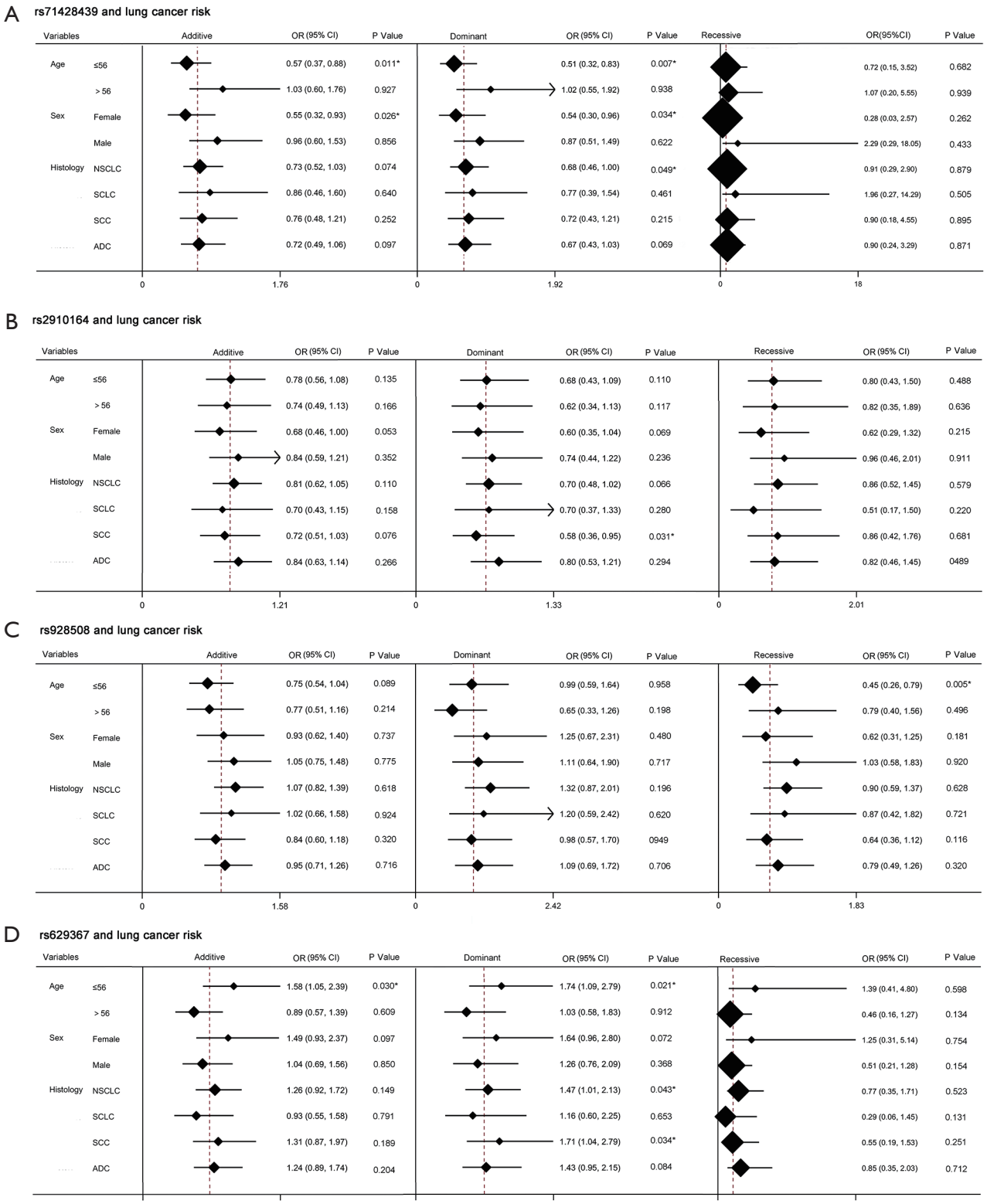

Figure 1 Subgroup analyses of rs71428439 (A), rs2910164 (B), rs928508 (C), and rs629367 (D) polymorphisms with lung cancer risk in Additive, Dominant, Recessive models. Each black box and horizontal line represent OR value and 95% CI.

Table 6 Association of the single nucleotide polymorphisms and platinum-based chemotherapy response in all lung cancer patients

Gene	Polymorphisms	Allele	Responders (DD/Dd/dd)	Non-responders (DD/Dd/dd)	Additive		Dominant		Recessive	
					OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
hsa-mir-196a-2	rs11614913	C/T	37/76/28	53/106/81	0.67 (0.50–0.91)	0.010*	0.43 (0.26–0.72)	0.001*	0.79 (0.48–1.30)	0.353
hsa-mir-30c-1	rs928508	G/A	25/79/37	67/126/48	0.69 (0.50–0.95)	0.022*	0.53 (0.31–0.90)	0.018*	0.71 (0.43–1.18)	0.183

*, P<0.05.

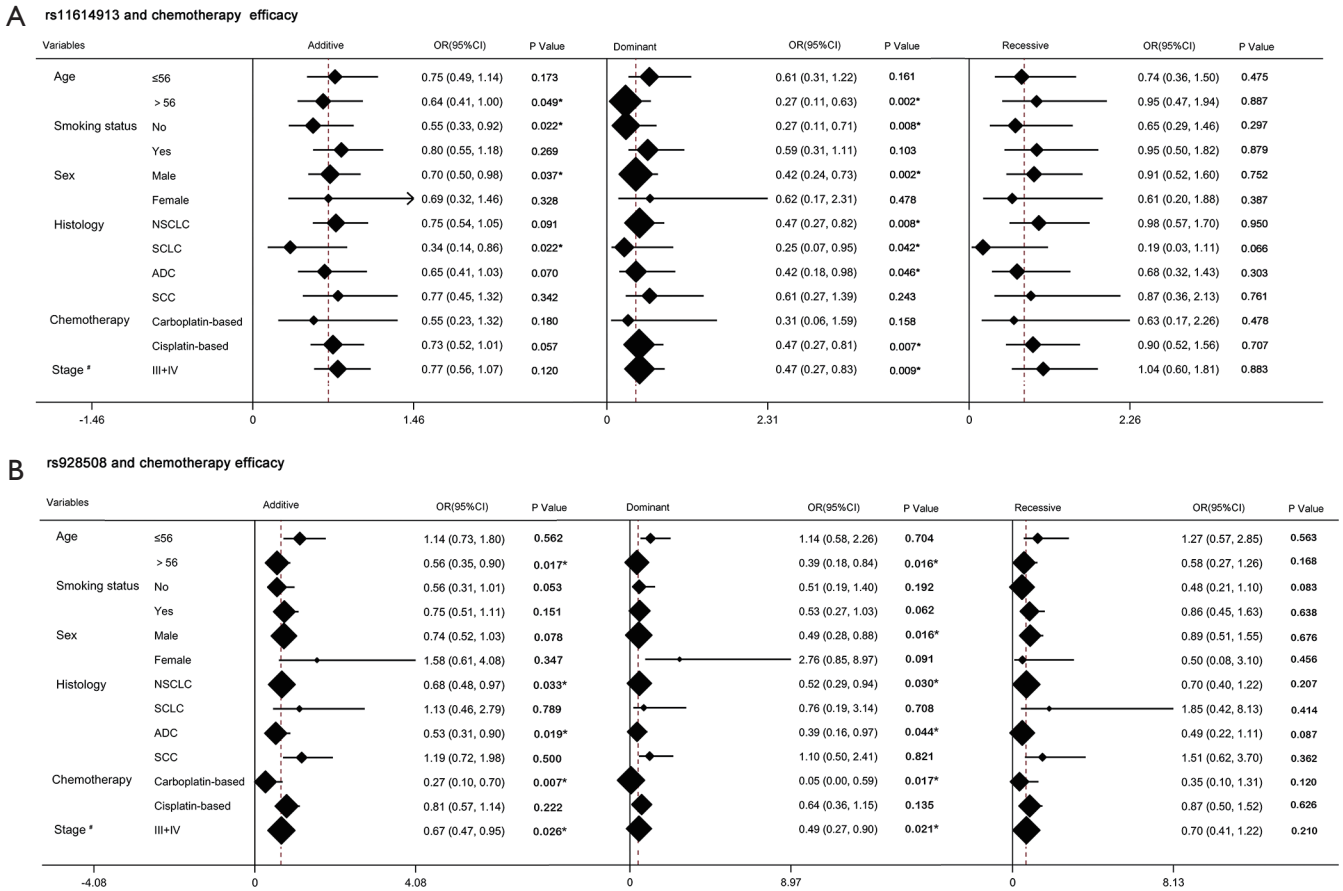

Figure 2 Subgroup analyses of the associations of 2 SNPs rs11614913 (A) and rs928508 (B) with the cisplatin-based chemotherapy response in Additive, Dominant, Recessive models. Each black box and horizontal line represents the OR value and 95% CI. #As the limited sample size of stage I and II (n=8), only stage III and IV individuals were analyzed

platinum-based chemotherapy in lung cancer parents without any invasive examination, only blood was needed.

MiRNAs are a class of small non-coding RNA molecules, play important roles in many biological and physiological processes, aberrant microRNA expression has been found in several diseases including lung cancer (23-28). MiR-149 is down regulated in several cancer types (29,30) and is believed to act as a versatile tumor-suppression miRNA by regulating the expression of many genes such as AKT1 (31), GIT1 (32), ZBTB2 (33), FOXM1 (34). Recently studies showed that miR-149 rs71428439 polymorphism was significantly associated with increased clear cell renal

cell carcinoma (CCRCC) and hepatocellular carcinoma (HCC) risk (28,35). We didn't find the association of this polymorphism with lung cancer risk in this study, but in NSCLC, female, age under 57 years subgroups, carriers with A allele of this mutation has a higher risk of lung cancer. Ding *et al.* observed that miR-149 can conquer mitochondrial related apoptosis through targeting the pro-apoptotic protein Puma, meanwhile the A allelic miR-149 precursor made a higher production of mature miR-149 (36), another study found that GG genotype expressed lower level of miRNA-149 compared with AA and AG genotypes in HCC tissues (35). All these data suggested that miRNA-149 A allele carriers may be more vulnerable carcinogenesis. Shi *et al.* (37) report that decreasing miR-146a is associated with the aggressiveness of human oral squamous cell carcinoma. Another research indicate that miR-146a expression levels were lower in lung cancer cells and could regulate COX-2 expression involved in the development of a metastatic condition (38). A functional mutation in miR-146a rs2910164 may act as a useful molecular marker for cardiovascular disease (39,40), and also for various cancer types (19,41,42). For Korean population rs2910164 may contribute to genetic susceptibility to lung cancer (43), it also can be used as a prognostic marker for NSCLC patients with surgically resected at early-stage in Chinese people (44) and another study revealed that rs2910164 CG genotype group had a higher risk of NSCLC than GG genotype group (45). Our results confirmed that polymorphism rs2910164 associated with the risk of lung cancer, the CC genotype and C allele distribution in the lung patient were significantly higher than that of the controls. As previous studies have found that rs2910164 G/C polymorphism affects both the efficiency of pri-miRNA processing and protein binding to the pre-miRNA product of this reaction (46) , and miR-146a CC genotypes have a lower expression level compare to GG/GC genotypes subgroup in tumor tissues (47), which may partly explain the tendency that rs2910164 C allele mutation subjects had a higher risk of lung cancer in our study.

Let-7a is a member of the family of let-7 contains of let-7a-1/2/3, supposed to have antitumor effect in lung cancer (48). Guan *et al.* reported that 1,25-(OH)2VD3 could up-regulate the transcription of let-7a-2 in lung cancer cells, which might mediate the anti-proliferation effects in lung cancer cells (49). Recently Xu *et al.* reported that a polymorphism rs629367 could affect the mature of let-7a, further associated with gastric cancer risk and survival (50). In this study, we founded that rs629367 C allele could increase the risk of lung cancer in age no more than 56 years subgroup.

Researches shown that the expression level of miR-30c was lower in many cancers, including lung cancer, which affect the process of epithelial mesenchymal transition (51,52). Fang *et al.* made an evidence that miR-30c could promote the sensibility to doxorubicin in breast cancer cell by regulated p38 mitogen-activated protein kinase (p38MAPK) pathway (53). The genomics research revealed that genetic polymorphism rs928508 could influence the gastric cancer risk and the prognostic of NSCLC (54,55). In the present study we found that this polymorphism may associate with lung cancer risk in age under 57 years old person. Here, for the first time we found that the mutation of this SNP would increase platinum-based chemotherapy sensitivity in lung cancer. The subgroup analyses also made a same impact in NSCLC, III and IV stage NSCLC, AC, male subgroups. Previous study had testified that themiR-30c AA genotype had a higher expression than AG/GG genotypes (56), which given evidence to the chemotherapy sensitive analyses. As miRNAs could bind to various genes, including oncogenes and tumor suppressor genes, other targets of miR-30c needed to be figure out, which would give us a better understanding of the influence of rs928508 on chemotherapy response.

MiR-196a encoded at two paralogous locations in the B and C mammalian HOX clusters, contains miR-196a-1 located on chromosome 17 (17q21.32) and miR-196a-2 located on chromosome 12 (12q13.13), taken part in regulating cell differentiation (57,58). Accumulating number of studies showed miR-196a is highly expressed in various tumor tissues, including esophageal carcinoma, gastric carcinoma, pancreatic cancer (59) and lung cancer (45). Study shows that inhibition of miR-196a could reverse cisplatin resistance of A549/DDP cell lines (60). Recently a functional SNP in miR-196a-2 rs11614913 was researched. Shen *et al.* found that miR-196a2 rs11614913was associated with an increased esophageal squamous cell carcinoma (ESCC) risk in a Chinese population (61). Deng *et al.* revealed this polymorphism was associated with a decreased risk of bladder cancer (42). Unfortunately we didn't reveal the association between rs11614913 and lung cancer risk, however our study was the first to reveal that polymorphism rs11614913 was significantly influence platinum-based chemotherapy response in lung cancer patients. Another study confirmed that rs11614913 was associated with severe toxicity after platinum-based regimen in advanced NSCLC (62). Analysis of mature miRNA expression confirmed that

carriers with T allele in miR-196a-2 rs11614913 dramatically inhibited production of their mature products (63), while Vinci and colleagues found that rs11614913 CC genotype significant associated with high expression of miR-196a-2 (45). Thereby the alteration of miR-196a-2 expression may involve in cisplatin efficacy. On the other hand, the mutation may also influence genes binding effects (64), which confounding the drug resistance.

The data presented here suggested that miR-149 rs71428439, miR-146a rs2910164, let-7a-2 rs629367 and miR-30c-1 rs928508 were significantly associated with lung cancer susceptibility, what's more miR-30c-1 rs928508 and miR-96a-2 rs11614913 were significantly related to platinum-based chemotherapy response. It is conceivable that those genetic polymorphisms may be useful in predicting the occurrence risk of lung cancer and platinum-based chemotherapy response in lung cancer patients.

It should not be ignored that the current study had some limitations. On the one hand, after the multiple testing of False Discovery Rate (FDR) our results didn't make a statistical difference. On the other hand, an independent validation for these SNPs needs to be arranged to get more credible conclusion. After that, other in depth mechanism research needs to be carried out to figure out how these SNPs influence lung cancer risk and chemotherapy response.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the Ethics Committee of Xiangya School of Medicine, Central South University and the registration numbers are CTXY-110008-2 and CTXY-110008-3. The clinical research admission was approved by Chinese Clinical Trial Registry with registration numbers of ChiCTR-RO-12002873 and ChiCTR-RCC-12002830. Written informed consent was obtained from all participants.

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