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A common polymorphism in *pre-microRNA-146a* is associated with lung cancer risk in a Korean population

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ABSTRACT

Introduction: MicroRNAs (miRs) play important roles in the development and progression of human cancers. MiR-146a down-regulates *epidermal growth factor receptor* and the nuclear factor-κB regulatory kinase *interleukin-1 receptor-associated kinase 1* genes that play important roles in lung carcinogenesis. This study was conducted to evaluate the association between rs2910164C>G, a functional polymorphism in the *pre-miR-146a*, and lung cancer risk.

Material and methods: The rs2910164C>G genotypes were determined in 1094 patients with lung cancer and 1100 healthy controls who were frequency matched for age and gender.

Results: The rs2910164 CG or GG genotype was associated with a significantly decreased risk for lung cancer compared to that of the CC genotype (adjusted odds ratio = 0.80, 95% confidence interval = 0.66–0.96, P = 0.02). When subjects were stratified according to smoking exposure (never, light and heavy smokers), the effect of the rs2910164C>G genotype on lung cancer risk was significant only in never smokers (adjusted odds ratio = 0.66, 95% confidence interval = 0.45–0.96, P = 0.03, under a dominant model for the C allele) and decreased as smoking exposure level increased (P_{trend} < 0.001). In line with this result, the level of *miR-146a* expression in the tumor tissues was significantly higher in the GG genotype than in the CC or CG genotype only in never-smokers (P = 0.02).

Conclusions: These findings suggest that the rs2910164C>G in *pre-miR-146a* may contribute to genetic susceptibility to lung cancer, and that miR-146a might be involved in lung cancer development.

1. Introduction

MicroRNAs (miRs) are endogenous small (~22 nucleotides) non-coding RNAs that down-regulate gene expression by complimentary binding to the 3′-untranslated region of target messenger RNAs, thereby repressing translation or decreasing mRNA stability (Valencia-Sanchéz et al., 2006). More than 1000 miRs have been identified in the human genome, each of which can regulate multiple genes (Griffiths-Jones et al., 2006). Increasing evidence indicates that miRs play important roles in the development and progression of human cancers, largely by targeting genes that are key regulators of cell proliferation and survival, DNA repair, and the immune response (Esquela-Kerscher and Slack, 2006). MiRs represent two opposing roles, by behaving as tumor promoters or tumor suppressors depending on the tissue type and the presence of specific targets (Fabbri et al., 2007). In addition, miR profiling studies have demonstrated that an abnormal miR expression pattern is different based on tumors originating from various tissues (Wouters et al., 2011).

Single nucleotide polymorphisms (SNPs) in miR sequences could affect miR processing and/or target selection, and thereby contribute to cancer development and progression (Hu et al., 2008; Wu et al., 2008). miR-146a down-regulates several cancer-related genes, including *epidermal growth factor receptor* (*EGFR*), *breast cancer 1, early onset*

Abbreviations: miRs, microRNAs; SNPs, single nucleotide polymorphisms; EGFR, epidermal growth factor receptor; BRCA1, breast cancer 1, early onset; NF-κB, nuclear factor-κB; IRAK1, interleukin-1 receptor-associated kinase 1; RT-PCR, reverse transcription-polymerase chain reaction; HWE, Hardy–Weinberg equilibrium; aORs, adjusted odds ratios; CIs, confidence intervals; SCCs, squamous cell carcinomas; ACs, adenocarcinomas; SCLC, small cell lung cancers; P_H, *P*-values of the homogeneity test.

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(*BRCA1*), and the nuclear factor (NF)-κB regulatory kinase *interleukin-1 receptor-associated kinase 1* (*IRAK1*) (Garcia et al., 2011; Y. Li et al., 2010). In addition, the rs2910164C>G polymorphism in *pre-miR-146a*, which results in a change from a C:U pair to a mismatched G:U pair in its stem region, affects the expression of mature miR-146a and miR-146a target genes (Jazdzewski et al., 2008, 2009; Shen et al., 2008). Several studies have reported that this SNP affects susceptibility to various human cancers (Guo et al., 2010; Jazdzewski et al., 2008; Permuth-Wey et al., 2011; Shen et al., 2008; Xu et al., 2008, 2010). However, the results of these previous studies are heterogeneous across different cell types of cancer: the 2910164C allele has been associated with early-onset familial breast and ovarian cancer (Shen et al., 2008) and an increased risk of adult glioma (Permuth-Wey et al., 2011), whereas the 2910164C allele has been associated with a decreased risk of esophageal squamous cell carcinoma, hepatocellular carcinoma and prostate cancer (Guo et al., 2010; Xu et al., 2008, 2010). Because the over-expression of EGFR and activation of NF-κB are common in lung cancer, we carried out a case–control study to evaluate the effect of the *pre-miR-146a* rs2910164G>C polymorphism on lung cancer risk.

2. Materials and methods

2.1. Study population

This case–control study consisted of 1094 patients with lung cancer and 1100 healthy controls. The subjects were recruited from an ongoing lung cancer molecular epidemiological study, as described previously (Bae et al., 2012; Lee et al., 2010). In brief, eligible cases included all patients newly diagnosed with primary lung cancer between January 2000 and December 2003 at Kyungpook National University Hospital, Daegu, Korea. There were no age, gender, histological, or stage restrictions, but patients with a prior history of cancer were excluded from this study. Control subjects were randomly selected from a pool of healthy volunteers who visited the general health check-up center at Kyungpook National University Hospital during the same period. The control subjects were frequency matched (1:1) to the cases based on gender and age (±5 years). All case and control subjects were ethnic Koreans residing in Daegu or the surrounding regions. This study was approved by the Institutional Review Board of Kyungpook National University Hospital, Daegu, Korea, and written informed consent was obtained from all participants. Genomic DNA samples of the cases and healthy controls were provided by the National Biobank of Korea, which is supported by the Ministry of Health, Welfare, and Family Affairs. Never smokers were defined as subjects who had smoked less than 100 cigarettes during their lifetime. A former smoker was defined as one who had stopped smoking at least 1 year before either a diagnosis of lung cancer (cases) or the date the informed consent form was signed (controls). The cumulative cigarette dose (pack-years) was calculated using the formula: pack-years = packs per day × years smoked.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion and phenol/chloroform extraction. The genotypes were determined by a polymerase chain reaction–restriction fragment length polymorphism assay. All genotyping analyses were blinded with respect to the case/control status to ensure quality control. Two researchers independently examined the gel images and performed a repeat assay if they did not reach a consensus on the tested genotype. Approximately 10% of the samples were randomly selected to be genotyped again by a different investigator, and the results were 100% concordant. Selected polymerase chain reaction-amplified DNA samples (n = 10 for each genotype) were examined by DNA sequencing, and the results were also 100% concordant.

2.3. Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Quantitative RT-PCR was performed to determine the expression of *miR-146a* according to the *miR-146a* rs2910164C>G genotypes. RNAs from NSCLC tissues and paired non-malignant lung tissues (n = 69; genotype distribution: 14 CC, 43 CG, and 12 GG in all cases; 12 CC, 28 CG, and 7 GG in smokers; 2 CC, 15 CG, and 5 GG in never-smokers) were isolated using Trizol (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Expression levels of *miR-146a* were determined using TaqMan MicroRNA Assays (Applied Biosystems, CA, USA). The $2^{-\Delta\Delta CT}$ (where CT is threshold cycle) method (Livak and Schmittgen, 2001) was used to calculate relative expression level of *miR-146a*, using small nuclear RNA U6 as an endogenous control to normalize the expression of mature miRs. All real-time PCR was performed in triplicate using a LightCycler 480 (Roche Applied Science, Mannheim, Germany) according to the manufacturer's protocol.

2.4. Statistical analysis

Differences in demographic characteristics, selected variables, and the frequencies of the genotypes between the cases and controls were compared using Student's *t*-test for continuous variables or the χ^2 test for categorical variables. Deviations of the genotype frequencies in the controls from those expected under the Hardy–Weinberg equilibrium (HWE) were assessed by a goodness-of-fit χ^2 test, as implemented through SAS Genetics. Unconditional logistic regression analysis was used to calculate adjusted odds ratios (aORs) and 95% confidence intervals (CIs) with adjustment for possible confounders (age and pack-years of smoking as continuous variables; and gender as a nominal variable). In addition to the overall association analysis, we performed a stratified analysis by age (median age), gender, smoking status, and tumor histology to further explore the association between the genotypes and lung cancer risk in each stratum. A homogeneity test was performed to compare the difference between genotype-related ORs of the different groups. All statistical analyses were performed using Statistical Analysis System software version 9.1.3 (SAS Institute, Cary, NC, USA).

Table 1
Characteristics of the study population.

Variables	Cases (n = 1094)	Controls (n = 1100)	*P*
Age (years)			
Mean ± SD	60.7 ± 9.3	60.6 ± 9.3	0.70[a]
Sex			
Male	837 (76.5)[b]	840 (76.4)	0.94[b]
Female	257 (23.5)	260 (23.6)	
Smoking status			
Current	618 (56.5)	394 (35.8)	<0.0001[b]
Former	247 (22.6)	336 (30.6)	
Never	229 (20.9)	370 (33.6)	
Pack-years[c]			
Mean ± SD	39.6 ± 20.1	30.4 ± 16.6	<0.0001[a]
Stage			
I	239 (21.8)		
II	63 (5.8)		
III	372 (34.0)		
IV	420 (38.4)		
Histological types			
Squamous cell ca.	461 (42.1)		
Adenoca.	466 (42.6)		
Large cell ca.	28 (2.6)		
Small cell ca.	139 (12.7)		

[a] *t*-test.
[b] $\chi 2$ test.
[c] In current and former smokers.