INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide, and 22% and 13.8% of cancer deaths in 2018 were estimated to be caused by lung cancer in men and women, respectively. The corresponding percentages in China were 28% and 23% in 2012, making it the most common cause of cancer death. Lung cancer is classified into two main categories: non-small cell lung cancer (NSCLC), accounting for...
approximately 80% of all lung cancer cases, and small-cell lung cancer (SCLC). Although tobacco smoking is the major risk factor, the etiology of lung cancer is multifactorial, including inherited genetic characteristics, such as single nucleotide polymorphisms (SNPs), which explains individual’s susceptibility to the development of lung cancer. During the past decade, genome-wide association studies (GWAS) have identified many common SNPs associated with the risk and outcome of lung cancer. However, heritability analysis indicated that the identified genetic loci could explain only a small fraction of lung cancer susceptibility. Additional efforts are needed to search for more lung cancer-related genetic factors, especially those rare variants and loci in non-coding regions.

Long non-coding RNAs (lncRNAs) are a class of RNA transcripts with more than 200 nucleotides in length and without translational capability. LncRNAs have been found to have diverse biological functions, some of which are involved in various tumorigenic processes. A number of dysregulated lncRNAs have also been demonstrated to be potential diagnostic or prognostic biomarkers for lung cancer, such as metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and HOX antisense intergenic RNA (HOTAIR) which are overexpressed in NSCLC and recognized as onco-lncRNAs. In contrast, maternally expressed gene 3 (MEG3), lncRNA (TUG1), and BRAF-activated non-protein coding RNA (BANCIR) which are downregulated in NSCLC are considered as tumor suppressors. These dysregulated lncRNAs are found to be involved in regulation of cell growth, proliferation, migration, and invasion.

Evidence also indicates that SNPs in the lncRNA genes affected tumorigenic process and chemotherapy response. Gong et al. found that SNPs in HOTTIP, H19, and CCAT2 were associated with lung cancer risk, and SNPs in MALAT1, H19, CCAT2, HOTAIR, and ANRIL were related to lung cancer patients’ response to platinum-based chemotherapy. Yuan et al. conducted a meta-analysis of eight GWAS on subjects with European ancestry and discovered rs114020893 in the lncRNA NEXN-AS1 associated with lung cancer risk. This SNP’s influence on lung cancer susceptibility may be achieved through its genotype-specific secondary structure stability. Hu et al. reported a SNP in CASC8 associated with both lung cancer risk and chemotherapy response and toxicity.

Findings from the above studies indicate that identifying SNPs in the lncRNA genes associated with lung cancer may help to elucidate the biological mechanisms of lncRNAs in lung cancer. Currently, our knowledge on lncRNA’s involvement in lung cancer is still limited; more studies are needed to discover SNPs in lncRNAs which are associated with lung cancer risk or outcome. Based on the findings of our previous study on lncRNAs in NSCLC, we conducted a case-control study on SNPs of the lncRNAs which showed different expression between tumor and matched adjacent normal tissues. In this study, we analyzed the association of lung cancer with 17 SNPs in 13 selected lncRNAs. We also investigated these SNPs in relation to lung cancer survival. Results of our association study are described in this report.

### 2 MATERIALS AND METHODS

#### 2.1 Study subjects

The case-control study included 1294 NSCLC cases and 1729 healthy controls who were recruited between April 2011 and July 2015 from the China Medical University. The cases were newly diagnosed

### TABLE 1 Information on 17 SNPs in the 13 lncRNA genes

| Rs number | Gene     | Locus  | Location | Base change | MAF in controls | HWE P |
|-----------|----------|--------|----------|-------------|-----------------|-------|
| rs10889184 | LINC01748 | 1p32.1 | 60540378 | G/A         | 0.45             | 0.622 |
| rs3113503 | LINC00607/LINC01614 | 2q35 | 215719150 | G/C         | 0.33             | 0.849 |
| rs498238   | LINC01833 | 2p21  | 44921691 | C/T         | 0.12             | 0.624 |
| rs496467   | LINC01833 | 2p21  | 44921864 | A/G         | 0.49             | 0.702 |
| rs13431201 | LINC01833 | 2p21  | 44922015 | C/G         | 0.06             | 0.440 |
| rs1992825  | LINC01833 | 2p21  | 44923139 | G/C         | 0.32             | 0.242 |
| rs517055   | LINC01833 | 2p21  | 44923338 | A/T         | 0.49             | 0.573 |
| rs1466099  | RNF144A-AS1 | 2p25.2 | 6917071 | G/A         | 0.26             | 0.819 |
| rs62288095 | LINC00887 | 3q29  | 194303359 | C/A         | 0.11             | 0.512 |
| rs6830064  | LINC02466 | 4q28.2 | 129725387 | T/G         | 0.18             | 0.694 |
| rs7678341  | Inc-RCHY1-3:1 | 4q13.3 | 75269312 | G/A         | 0.23             | 0.541 |
| rs16901995 | Inc-NDUF36-5:5 | 5p15.33 | 1933867 | C/T         | 0.42             | 0.978 |
| rs4077205  | LOC100128340 | 5q35.3 | 177975648 | A/G         | 0.70             | 0.107 |
| rs35132843 | CASC21/CASC8 | 8q24.21 | 127289874 | T/G         | 0.37             | 0.087 |
| rs10734387 | BBOX1-AS1 | 11p14.2 | 27151108 | T/C         | 0.30             | 0.638 |
| rs1867299  | HOXC13-AS | 12q13.13 | 53936191 | T/C         | 0.18             | 0.135 |
| rs219741   | LOC105369301 | 21q22.13 | 36480738 | G/A         | 0.10             | 0.716 |

HWE, Hardy-Weinberg equilibrium; MAF, Minor allele frequency.
Using the ABI 7900 FAST real-time polymerase chain reaction (PCR) system (Thermo Fisher Scientific, Waltham, MA, USA). All primers and probes were purchased from Thermo Fisher Scientific. Ten percent of the DNA samples were randomly selected for replication, and the results of the repeats were in complete concordance.

2.3 | Statistical analysis

Distributions of subject characteristics and genetic polymorphisms were compared between cases and controls using the chi-square test. Student t test was used for comparison of continues variables between groups. Hardy-Weinberg equilibrium was calculated for each SNP in the control subjects. In order to balance the distributions of age and gender in case and control groups, propensity score matching (PSM) analysis was conducted. Associations between SNPs and NSCLC risk were analyzed using the unconditional logistic regression model. Odds ratios (OR) and 95% confidence interval (CI) were calculated in the regression model, and the analyses were adjusted for confounding factors (age, gender, and smoking status). Subgroup analyses were also performed for each polymorphism to assess potential gene-environment interaction or joint effect. Survival time was defined as the time interval from the date of NSCLC diagnosis to the date of death or end of follow-up. Median survival time (MST) was the time point when 50% of the patients were dead. Kaplan-Meier survival analysis and log-rank test were used to compare differences in survival time by SNP genotypes. Associations between SNPs and overall survival were analyzed using the Cox proportional hazards regression model in which hazard ratios (HR) and 95%CI were estimated. P values reported were two-tailed, and P < 0.05 was considered statistically significant. All data analyses were performed using the SPSS software version 19.0 (IBM, Armonk, NY, USA). We also selected the NCBI data sets, GSE19804 and GSE18842, for analysis of gene expression. The scatter plots were generated using the GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA).

3 | RESULTS

3.1 | Study population

The demographic characteristics of the initial 1294 cases and 1729 controls were summarized in Table S1. In order to balance the age and gender differences between cases and controls, we conducted PSM. First, we deleted subjects with missing values in gender and age, which left us with 1169 NSCLC cases and 1354 controls. Then, a propensity score (PS) was constructed to quantify each subject’s gender and age. The cases were later matched to controls by PS. After PSM, we obtained well-balanced distributions of demographic characteristics between cases and controls (Table 2). The age (P = 0.310) and gender (P = 0.326) were no longer significantly different. There were more smokers in cases than in controls (48.07% vs 25.37%).

| Variables      | Case (n = 1169) | Control (n = 1005) | P value* |
|----------------|----------------|--------------------|----------|
| Gender         |                |                    |          |
| Male           | 1169 (100%)    | 1005 (100%)        | 0.326    |
| Female         | 579 (49.53%)   | 519 (51.64%)       |          |
| Age            |                |                    |          |
| <60            | 584 (49.96%)   | 524 (52.14%)       | 0.310    |
| ≥60            | 585 (50.04%)   | 481 (47.86%)       |          |
| Smoking status |                |                    |          |
| Non-smoker     | 605 (51.93%)   | 747 (74.63%)       | <0.001   |
| Ever-smoker    | 560 (48.07%)   | 254 (25.37%)       |          |

*Due to the missing values, the numbers of cases and controls were less than 1169 and 1005, respectively. Two-side chi-squared test.

In our previous study,16 we found 153 lncRNAs, which had significant differences in expression (fold change >2) between tumor and matched adjacent tissues. Based on the list, we searched NCBI dbSNP (http://www.ncbi.nlm.nih.gov/), HapMap (http://www.hapmap.org), and IncRNAsNP (http://bioinfo.life.hust.edu.cn/IncRNAsNP/) and identified 3765 SNPs. Considering that polymorphisms in the non-coding regions may affect the binding of other transcripts such as microRNAs,17 we selected SNPs located in the binding sites which may alter the binding affinity of lncRNAs to other molecules. The following selection criteria were established to choose SNPs for genotyping: (a) minor allele frequency (MAF) reported in HapMap ≥5% in Chinese Han, Beijing (CHB); (b) located in the regulatory region of genes; and (c) affecting the binding with microRNAs. Following the criteria, we selected 17 SNPs for study (Table 1).

Our genotyping method has been described elsewhere.18 In brief, genomic DNA in peripheral blood leukocytes was extracted from cases and controls using the standard phenol-chloroform method. SNP genotyping was determined by the TaqMan assay.
**TABLE 3** Associations between selected SNPs and NSCLC risk after PSM

| Genotypes           | N (%)                  | P value* | Crude OR (95%CI) | Adjusted OR (95%CI)a |
|---------------------|------------------------|----------|------------------|----------------------|
| **rs3113503 (G>C)** |                        |          |                  |                      |
| GG                  | 432 (42.69%)           |          | 1.00             | 1.00                 |
| GC                  | 489 (48.32%)           |          | 1.20 (1.00-1.44) | 1.22 (1.01-1.49)     |
| CC                  | 91 (8.99%)             |          | 0.87 (0.64-1.19) | 0.81 (0.59-1.13)     |
| Dominant model      | 1012 (100%)            |          | 0.167            |                      |
| GG                  | 432 (42.69%)           |          | 1.00             | 1.00                 |
| GC + CC             | 580 (57.31%)           |          | 1.13 (0.95-1.35) | 1.14 (0.94-1.37)     |
| Recessive model     | 1012 (100%)            |          | 0.129            |                      |
| GG + GC             | 921 (91.01%)           |          | 1.00             | 1.00                 |
| CC                  | 91 (8.99%)             |          | 0.80 (0.59-1.07) | 0.74 (0.54-1.00)     |
| **rs498238 (C>T)**  |                        |          |                  |                      |
| CC                  | 791 (78.71%)           |          | 1.00             | 1.00                 |
| CT                  | 209 (20.80%)           |          | 0.97 (0.78-1.20) | 0.97 (0.77-1.22)     |
| TT                  | 5 (0.50%)              |          | 0.39 (0.14-1.12) | 0.33 (0.11-0.97)     |
| Dominant model      | 1005 (100%)            |          | 0.526            |                      |
| CC                  | 791 (78.71%)           |          | 1.00             | 1.00                 |
| CT + TT             | 214 (21.29%)           |          | 0.93 (0.75-1.16) | 0.93 (0.74-1.16)     |
| Recessive model     | 1005 (100%)            |          | 0.072            |                      |
| CC + CT             | 1000 (99.50%)          |          | 1.00             | 1.00                 |
| TT                  | 5 (0.50%)              |          | 0.40 (0.14-1.13) | 0.33 (0.11-0.97)     |
| **rs16901995 (C>T)**|                       |          |                  |                      |
| CC                  | 380 (34.67%)           |          | 1.00             | 1.00                 |
| CT                  | 532 (48.54%)           |          | 0.95 (0.78-1.15) | 0.94 (0.77-1.15)     |
| TT                  | 184 (16.79%)           |          | 0.84 (0.66-1.09) | 0.78 (0.59-1.01)     |
| Dominant model      | 1096 (100%)            |          | 0.348            |                      |
| CC                  | 380 (34.67%)           |          | 1.00             | 1.00                 |
| CT + TT             | 716 (65.33%)           |          | 0.92 (0.76-1.10) | 0.89 (0.74-1.08)     |
| Recessive model     | 1096 (100%)            |          | 0.230            |                      |
| CC + CT             | 912 (83.21%)           |          | 1.00             | 1.00                 |
| TT                  | 184 (16.79%)           |          | 0.87 (0.70-1.09) | 0.80 (0.63-1.02)     |
| **rs219741 (G>A)**  |                        |          |                  |                      |
| GG                  | 813 (76.99%)           |          | 1.00             | 1.00                 |
| GA                  | 235 (22.25%)           |          | 1.14 (0.92-1.41) | 1.08 (0.86-1.35)     |
| AA                  | 8 (0.76%)              |          | 0.67 (0.27-1.68) | 0.60 (0.23-1.56)     |
| Dominant model      | 1056 (100%)            |          | 0.316            |                      |
| GG                  | 813 (76.99%)           |          | 1.00             | 1.00                 |
| GA + AA             | 243 (23.01%)           |          | 1.11 (0.90-1.38) | 1.05 (0.84-1.31)     |
| Recessive model     | 1056 (100%)            |          | 0.361            |                      |
| GG + GA             | 1048 (99.24%)          |          | 1.00             | 1.00                 |
| AA                  | 8 (0.76%)              |          | 0.66 (0.26-1.64) | 0.59 (0.23-1.53)     |

Bold OR values indicated P < 0.05.

*aAdjusted for age, gender, and smoking status.

*Two-side chi-squared test.
### 3.2 Associations of SNPs and NSCLC risk

Allele distributions of the 17 SNPs selected for study were all in Hardy-Weinberg equilibrium in the control group ($P > 0.05$, Table 1). After PSM, genotype distributions of the 17 SNPs and their associations with NSCLC risk in different inheritance models (dominant, recessive, and additive) are shown in Tables S2 and 3. Potential gene-environment interaction was assessed for each polymorphism in the initial study population stratified by the environmental factor of interest (Table 4). Significant associations with NSCLC were suggested for three SNPs, including rs498238, rs16901995, and rs219741.

#### TABLE 4 Associations between SNPs and NSCLC risk stratified by selected variables

| Genetic Variant | Variables | Genotypes (Cases/Controls) | P value | Dominant model (95%CI) |
|-----------------|-----------|---------------------------|---------|-----------------------|
|                 |           | AA | AB + BB | P | (AB + BB)/AA |
| rs3113503       | Gender    | Male | 231/235 | 280/338 | 0.370 | 0.88 (0.67-1.16) |
|                 |           | Female | 202/463 | 301/511 | 0.054 | 1.29 (1.00-1.66) |
|                 | Age       | <60 | 225/390 | 273/458 | 0.877 | 0.98 (0.74-1.30) |
|                 |           | ≥60 | 207/204 | 307/269 | 0.196 | 1.20 (0.91-1.57) |
|                 | Smoking status | Non-smoker | 221/641 | 302/765 | 0.373 | 1.11 (0.88-1.39) |
|                 |           | Ever-smoker | 215/120 | 276/148 | 0.589 | 1.09 (0.80-1.48) |
| rs498238        | Gender    | Male | 391/426 | 114/139 | 0.662 | 0.93 (0.67-1.29) |
|                 |           | Female | 402/754 | 100/192 | 0.711 | 0.94 (0.69-1.29) |
|                 | Age       | <60 | 390/648 | 107/171 | 0.518 | 0.89 (0.63-1.26) |
|                 |           | ≥60 | 401/362 | 107/108 | 0.623 | 0.92 (0.67-1.27) |
|                 | Smoking status | Non-smoker | 410/1081 | 107/295 | 0.936 | 0.99 (0.75-1.31) |
|                 |           | Ever-smoker | 385/200 | 105/64 | 0.334 | 0.84 (0.58-1.20) |
| rs16901995      | Gender    | Male | 177/180 | 371/396 | 0.918 | 0.99 (0.74-1.31) |
|                 |           | Female | 204/333 | 346/648 | 0.202 | 0.85 (0.66-1.09) |
|                 | Age       | <60 | 195/278 | 346/577 | 0.076 | 0.77 (0.58-1.03) |
|                 |           | ≥60 | 185/154 | 370/321 | 0.698 | 1.06 (0.80-1.40) |
|                 | Smoking status | Non-smoker | 212/469 | 354/950 | 0.035 | 0.78 (0.62-0.98) |
|                 |           | Ever-smoker | 168/89 | 364/180 | 0.679 | 1.07 (0.78-1.48) |
| rs219741        | Gender    | Male | 407/456 | 127/109 | 0.617 | 1.09 (0.79-1.50) |
|                 |           | Female | 408/747 | 116/168 | 0.651 | 1.07 (0.79-1.46) |
|                 | Age       | <60 | 405/676 | 119/125 | 0.033 | 1.47 (1.03-2.10) |
|                 |           | ≥60 | 408/351 | 124/119 | 0.433 | 0.88 (0.65-1.20) |
|                 | Smoking status | Non-smoker | 420/1096 | 121/238 | 0.400 | 1.13 (0.86-1.48) |
|                 |           | Ever-smoker | 395/210 | 122/59 | 0.933 | 1.02 (0.71-1.45) |

Bold OR values indicated $P < 0.05$.  
*Adjusted for age, gender, and smoking status when properly.  
*A stands for major allele and B stands for minor allele.
For SNP rs498238, individuals with the TT homozygous genotype had a lower risk of NSCLC compared to those with the CC homozygous genotype after age, gender, and smoking status were adjusted in the analysis (adjusted OR = 0.33, 95%CI: 0.11-0.97, P = 0.043; Table 3). The association between rs498238 and NSCLC mainly came from the recessive model, and no significant association was seen in the dominant model.

SNP rs16901995 was not associated with NSCLC in overall analysis, but in the stratified analysis it was shown that in non-smokers, individuals with CT or TT genotypes had a reduced risk for NSCLC compared...
to those with CC genotype (adjusted OR = 0.78, 95% CI: 0.62–0.98, \( P = 0.035 \); Table 4). Similarly, when analyzing the relationship in subgroups, we found that SNP rs219741 was associated with increased risk of NSCLC among younger subjects (age < 60 years). The adjusted OR was 1.47, and 95% CI was between 1.03 and 2.10 (\( P = 0.033 \)).

SNP rs3113503 showed controversial results. Individuals with GC genotype had an increased risk compared to those with wild GG genotype (adjusted OR = 1.22, 95% CI: 1.01–1.49, \( P = 0.035 \)). But subjects with CC genotype had a reduced risk in a recessive model (adjusted OR = 0.74, 95% CI: 0.54–1.00, \( P = 0.050 \)). There was no significant difference in the dominant model, nor in stratified analyses.

### 3.3 Associations of SNPs and NSCLC outcome

Patient characteristics and clinical features are shown in Table S3. Survival analysis was performed to assess the genotypes of the four selected SNPs in association with the NSCLC outcome (Table 5). The analysis showed no significant associations between these genotypes and NSCLC overall survival before or after adjustment for age, gender, smoking status, disease stage, and histology type. To further investigate the association of SNPs with NSCLC survival in patients with different clinical characteristics, we conducted stratification analyses in the dominant model (Table S4). The results showed that only in patients with lung adenosquamous carcinoma (ASC), rs219741 was associated with survival. However, the sample size (deaths/patients: 19/23 vs 5/6, in GG vs GA + AA genotypes, respectively) was too small to draw a conclusion.

### 4 DISCUSSION

In this study, we evaluated 17 SNPs in 13 lncRNAs with regard to their associations with NSCLC risk and survival. We found that NSCLC risk was significantly associated with SNP rs3113503, rs498238, rs16901995, and rs219741. These SNPs are located in

![FIGURE 1](image)

**FIGURE 1** Scatter plots of relative lncRNA levels in NSCLC tumor and adjacent non-tumor tissues. LOC100130502 in GSE19804 (A) and GSE18842 (B), LINC00607 in GSE19804 (C) and GSE18842 (D), Rs219741 G>A change in Inc-CHAF1B-3:1, genotype G (WT) (E), and genotype A (MT) (F). *** \( P < 0.0001 \)
different lncRNA genes and appeared to have different associations with NSCLC. While SNP rs219741 was associated with an increased risk in younger population, SNP rs498238 and rs16901995 were linked to a reduced risk of NSCLC. SNP rs3113503 had a conflicting relationship with NSCLC risk.

Although the biological implications of these SNPs in the lncRNA genes are unknown, our understanding of lncRNA’s involvement in cancer is rapidly expanding in recent years. The biological function of lncRNA largely depends on their subcellular localization. In cytoplasm, lncRNAs behave like competitive endogenous RNA to bind mRNAs, suppressing translation or degradation of targeted mRNAs. When in nucleus, lncRNAs serve as scaffold to form, for example, a chromatin modification complex, or act as decoy to suppress the function of other transcripts, such as microRNAs. Some lncRNAs tether transcription factors to gene promoters. Recently, lncRNAs are found to contain codes for functional micropeptides based on small-ORFs (Open Reading Frames). LncRNAs may also play roles in intercellular communication. Since 80% of SNPs associated with cancer are located in the non-coding regions, many of them are likely to be in lncRNAs. Studies have shown that SNPs in the lncRNA genes can influence cancer through different biological mechanisms. For example, SNPs can affect the expression of their relevant lncRNAs.

Different SNP genotype in LINC00673 may affect its binding to miR-1231, which alters the miRNA’s activity and influences PTPN11 (protein tyrosine phosphatase, non-receptor type 11) degradation in an allele-specific manner. Genetic polymorphisms can also affect the expression of lncRNAs through allele-specific modulation of their distal regulatory elements. A SNP located in a distal enhancer of lncRNA PCAT1 (prostate cancer associated transcript 1) alters the binding of its transcription factors ONECUT2 (one cut homeobox 2) and androgen receptor (AR) to the enhancer and PCAT1 promoter, thereby affecting the expression of PCAT1 which is involved in the development and progression of prostate cancer.

SNP rs498238 is located in the fourth exon of the long intergenic non-coding RNA 1833 gene (LINC01833), and the lncRNA, initially named as loc100130502, is predicted to stay mainly in the nucleus of A549 cells. In the NCBI GEO database, loc100130502 was shown to be upregulated in NSCLC tumors compared to matched adjacent non-tumor tissues of non-smoking women in one dataset GSE19804 (Figure 1A), but no difference in another dataset GSE18842 (Figure 1B). The LINC01833 gene is located close to the gene SIX3, and this non-coding transcript is considered a Wnt/β-catenin pathway-related lncRNA. SIX3 was reported to inhibit the pathway in the development of vertebrate forebrain. Kumar et al. found that SIX3 acted as a corepressor of Wnt and suppressed its transcription in breast cancer. In addition, in vivo binding assay revealed that SIX3 repressed Wnt1 expression by binding to its 3’ enhancer and to the elements located within its 5’ promoter region. SIX3 was downregulated in lung adenocarcinoma tissues compared to their matched adjacent normal tissues. Restoration of SIX3 expression in lung cancer cells with low endogenous SIX3 resulted in suppressed cell proliferation and migration. Moreover, high expression of SIX3 was associated with improved overall and progression-free survival of patients with lung adenocarcinoma.

A similar finding was also observed in patients with glioblastoma. A meta-analysis suggests that SIX3 may play a role in suppressing the progression of lung cancer, especially in its early stage.

SNP rs3113503 is an intron variant which is located in a gene encoding two long non-coding transcripts, including a shorter lncRNA named LINC01614 and a longer one called LINC00607. LINC00607 is present mainly in cell nucleus, and significant downregulation was observed in NSCLC when we analyzed the online datasets GSE19804 (Figure 1C) and GSE18842 (Figure 1D). No expression information was found for lnc-NDUFS6-5:5 (rs16901995) and loc105369301 (rs219741). LncRNAsNP database indicates that SNP rs219741 may change the secondary structure of the lncRNA Inc-CHAF1B-3:1 (Figure 1E for wild type and Figure 1F for mutant type). Our data suggest that SNP rs498238 and rs3113503 may have allele-specific influences on lncRNA expression in NSCLC.

The SNPs we investigated in this study were selected from a list of lncRNAs which showed significant differences in expression between NSCLC tumor and matched adjacent normal tissues. The initial analysis of lncRNAs was accomplished with an expression microarray, and the study population was Chinese Han. Thus, the findings of our SNP analysis were likely to be limited to Chinese populations and the number of lncRNAs included in the microarray chip. In addition to these limitations, our sample size for analyzing the SNP association was relatively small, and there were no validation and P value adjustment during our evaluation. We also did not perform any functional evaluation and experiments to demonstrate the biological relevance of these SNPs in NSCLC. Despite these shortcomings, we were able to find some preliminary data to suggest that SNPs in non-coding regions, especially in the lncRNA genes, may have potential implications in cancer etiology. More studies are needed to characterize these non-coding region SNPs and elucidate their biological relevance and molecular mechanisms in relation to lncRNA’s function and tumorigenesis.

In summary, we analyzed 17 SNPs in the genes of lncRNAs with differential expression in NSCLC and identified three of them associated with the risk of NSCLC among Chinese. These findings suggest that SNPs in non-coding regions of the genome may also be important when comparing to those in the coding regions. Further analyzing this type of SNPs may provide new insights into the functions of lncRNAs and their involvement in cancer.

ACKNOWLEDGMENTS

This research was funded by the SJTU Interdisciplinary Research Key Grant (No. YG2015ZD01), SJTU Medicine-Engineering Research Fund (No. YG2016QN76), the Natural Science Foundation of Shanghai (No. 16ZR1418500).

ORCID

Biyun Qian https://orcid.org/0000-0003-1806-2026
REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.

2. Chen W, Zheng R, Zuo T, Zeng H, Zhang S, He J. National cancer incidence and mortality in China. 2012. Chin J Cancer Res. 2016;28(1):1-11.

3. Davidson MR, Gazdar AF, Clarke BE. The pivotal role of pathology in the management of lung cancer. J Thorac Dis. 2013;5(Suppl 5):S463-S478.

4. Vaz M, Hwang SY, Kagiampakis I, et al. Chronic cigarette smoke-induced epigenic changes precede sensitization of bronchial epithelial cells to single-step transformation by KRAS mutations. Cancer Cell. 2017;32(3):360-376.e6.

5. Li Y, Sheu CC, Ye Y, et al. Genetic variants and risk of lung cancer in never smokers: a genome-wide association study. Lancet Oncol. 2010;11(4):321-330.

6. Brennan P, Hainaut P, Boffetta P. Genetics of lung-cancer susceptibility. Lancet Oncol. 2011;12(4):399-408.

7. Hu X, Sood AK, Dang CV, Zhang L. The role of long noncoding RNAs in cancer: the dark matter matters. Curr Opin Genet Dev. 2018;48:8-15.

8. Gutschner T, Hammerle M, Eissmann M, et al. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Can Res. 2013;73(3):1180-1189.

9. Nakagawa T, Endo H, Yokoyama M, et al. Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. Biochem Biophys Res Comm. 2013;436(2):319-324.

10. Lu KH, Li W, Liu XH, et al. Long non-coding RNA MEG3 inhibits NSCLC cells proliferation and induces apoptosis by affecting p53 expression. BMC Cancer. 2013:13:461.

11. Zhang EB, Yin DD, Sun M, et al. P53-regulated long non-coding RNA TUG1 affects cell proliferation in human non-small cell lung cancer, partly through epigenetically regulating HOXB7 expression. Cell Death Dis. 2014;5:e1243.

12. Sun M, Liu XH, Wang KM, et al. Downregulation of BRAF activated non-coding RNA is associated with poor prognosis for non-small cell lung cancer and promotes metastasis by affecting epithelial-mesenchymal transition. Mol Cancer. 2014;13:68.

13. Gong WJ, Yin JY, Li XP, et al. Association of well-characterized lung cancer IncRNA polymorphisms with lung cancer susceptibility and platinum-based chemotherapy response. Tumour Biol. 2016;37(6):8349-8358.

14. Yuan H, Liu H, Liu Z, et al. A novel genetic variant in long non-coding RNA gene NEXN-AS1 is associated with risk of lung cancer. Sci Rep. 2016;6:34234.

15. Hu L, Chen SH, Lv QL, et al. Clinical significance of long non-coding RNA CASCB rs10505477 polymorphism in lung cancer susceptibility, platinum-based chemotherapy response, and toxicity. Int J Environ Res Public Health. 2016;13(6):E545.

16. Feng N, Ching T, Wang Y, et al. Analysis of microarray data on gene expression and methylation to identify long non-coding RNAs in non-small cell lung cancer. Sci Rep. 2016;6:37233.

17. Metwally M, Bayoumi A, Romero-Gomez M, et al. A polymorphism in the Irs1-encoding gene (FNDC5) associates with hepatic steatosis by differential miRNA binding to the 3’UTR. J Hepatol. 2018.

18. Zheng C, Li X, Qian B, et al. The IncRNA myocardial infarction associated transcript-centric competing endogenous RNA network in non-small-cell lung cancer. Cancer Manag Res. 2018;10:1155-1162.

19. Huang JZ, Chen M, Chen GX, et al. A peptide encoded by a putative IncRNA HOXB-AS3 suppresses colon cancer growth. Mol Cell. 2017;68(1):171-184.e6.

20. Peng Z, Liu C, Wu M. New insights into long noncoding RNAs and their roles in glioma. Mol Cancer. 2018;17(1):61.

21. Freedman ML, Monteiro AN, Gayther SA, et al. Principles for the post-GWAS functional characterization of cancer risk loci. Nat Genet. 2011;43(6):513-518.

22. Yan X, Hu Z, Feng Y, et al. Comprehensive genomic characterization of long non-coding RNAs across human cancers. Cancer Cell. 2015;28(4):529-540.

23. Iyer MK, Niknafs YS, Malik R, et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47(3):199-208.

24. Pandey GK, Mitra S, Subhash S, et al. The risk-associated long non-coding RNA NBAT-1 controls neuroblastoma progression by regulating cell proliferation and neuronal differentiation. Cancer Cell. 2014;26(5):722-737.

25. Zheng J, Huang X, Tan W, et al. Pancreatic cancer risk variant in LINC00673 creates a miR-1231 binding site and interferes with PTPN11 degradation. Nat Genet. 2016;48(7):747-757.

26. Guo H, Ahmed M, Zhang F, et al. Modulation of long noncoding RNAs by risk SNPs underlying genetic predispositions to prostate cancer. Nat Genet. 2016;48(10):1142-1150.

27. Mas-Ponte D, Carlevaro-Fita J. LncATLAS database for subcellular localization of long noncoding RNAs. RNA. 2017;23(7):1080-1087.

28. Chen J, Hu L, Chen J, et al. Detection and analysis of Wnt pathway related lncRNAs expression profile in lung adenocarcinoma. Pathol Oncol Res. 2016;22(3):609-615.

29. Carlin D, Sepich D, Grover VK, Cooper MK, Solnica-Krezel L, Inbal A. Six3 cooperates with Hedgehog signaling to specify ventral telencephalon by promoting early expression of Foxg1a and repressing Wnt signaling. Development. 2012;139(4):2614-2624.

30. Kumar R, Balasenthil S, Manavathi B, Rayala SK, Pakala SB. Metastasis-associated protein 1 and its short form variant stimulates Wnt1 transcription through promoting its derepression from Six3 corepressor. Can Res. 2010;70(16):6649-6658.

31. Lagutin OV, Zhu CC, Kobayashi D, et al. Six3 repression of Wnt signaling in the anterior neuroectoderm is essential for vertebrate forebrain development. Genes Dev. 2003;17(3):368-379.

32. Mo ML, Okamoto J, Chen Z, et al. Down-regulation of SIX3 is associated with clinical outcome in lung adenocarcinoma. PLoS One. 2013;8(8):e71816.

33. Zhang B, Shen C, Ge F, Ma T, Zhang Z. Epigenetically controlled Six3 expression regulates glioblastoma cell proliferation and invasion alongside modulating the activation levels of WNT pathway members. J Neurooncol. 2017;133(3):509-518.

34. Liu Q, Li A, Tian Y, et al. The expression profile and clinic significance of the SIX family in non-small cell lung cancer. J Hematol Oncol. 2016;9(1):119.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Wang R, Feng N, Wang Y, et al. SNPs in lncRNA genes are associated with non-small cell lung cancer in a Chinese population. J Clin Lab Anal. 2019;33:e22858. https://doi.org/10.1002/jcla.22858