Single nucleotide polymorphism in the 3′ untranslated region of LPP is a risk factor for lung cancer: a case-control study

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Abstract

Background: Single nucleotide polymorphisms (SNPs) in 3′-untranslated region (UTR) of genes related with cell-matrix adhesions and migration might affect miRNA binding and potentially affect the risk of cancer. The present study aimed to screen SNPs in 3′ UTR of cancer-related genes and investigate their contribution to the susceptibility of lung cancer.

Methods: Seven SNPs were selected and genotyped in a case-control study (322 lung cancer patients and 384 controls) among Chinese Han population. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated by logistic regression adjusted for age and gender in multiple genetic models.

Results: In stratified analyses by gender, three (rs1064607, rs3796283 and rs2378456) of LPP gene were associated with a significantly increased susceptibility for lung cancer among male population. Besides, LPP rs2378456 weakened lung cancer risk in female. LPP rs1064607 polymorphism was significantly correlated with increased risk of lung adenocarcinoma. Furthermore, AA genotype of TNS3 rs9876 polymorphism was associated with lymphatic metastasis.

Conclusion: Our results provides evidence for the impact of LPP polymorphisms on the susceptibility to lung cancer in Chinese population.

Keywords: Lung cancer, LPP, Polymorphism, Genetic susceptibility

Background

Lung cancer is the most frequently diagnosed cancers and the leading causes of cancer death in men, both overall and in less-developed countries. According to Global Cancer Statistics 2018, approximately 2.1 million new lung cancer cases were diagnosed, accounting for 11.6% of total cancer diagnoses made last year [1]. Lung cancer is also the most prevalent cancer and the main leading cause of cancer death in Chinese with significantly high age-standardized incidence and mortality rates [2]. Despite significant treatment breakthroughs, the five-year survival rate for patients with lung cancer is less than 10%, and most lung patients are diagnosed at an advanced stage [3]. Tobacco exposure is the commonly accepted primary cause of lung cancer, while and other occupational and environmental factors, such as asbestos, heavy metal, radiation and air pollution also contribute to its incidence. Additionally, individual variation, including age, sex, ethnicity and body weight and genetic susceptibility exert an important influence on the etiology of lung cancer [4]. Genetic polymorphisms are now recognized as a major cause of the disease, and the causal association between lung cancer and genetic polymorphisms has been proven in many experimental and epidemiological studies [5–7].

Cell migration plays a critical role in many biological processes and is implicated in cancer invasion and
metastasis [8]. Specifically, LPP (lipoma preferred partner/LIM domain containing preferred translocation partner in lipoma), TNS3 (Tensin 3) and NR5A2 (nuclear receptor subfamily 5 group A member 2) encode proteins important in cell adhesion and migration. Recent data have shown that LPP participates in the formation of disorganized micro-vessels within tumor tissue, leading to increased migration and invasion following epithelial-mesenchymal transition [9, 10]. It has previously been shown that TNS3 is deregulated in cancer and has implications in cell migration, invasion and tumorigesis [11]. NR5A2, also known as LRH-1 (liver receptor homolog-1) is essential for diverse biological activities, including cell proliferation and differentiation, embryonic development, and cholesterol metabolism [12]. Expression dysregulation and certain variants of these genes are associated with the risk of various cancers, including lung cancer [13–15].

Variations in the 3′-untranslated region (3′-UTRs) of genes may affect their expression by reinforcing, weakening, or disrupting miRNA-mRNA interactions [16]. Many studies of 3′UTR single nucleotide polymorphisms (SNPs) and lung cancer have provided insight into lung carcinogenesis, development of diagnostic and prognostic markers, and discovery of novel therapeutic approaches [17]. However, we did not find previous reports on the association between the 3′UTRs of LPP, TNS3, and NR5A2 and lung cancer risk. Thus, we sought to identify relevant 3′UTR SNPs within these genes and to assess their effects on lung cancer susceptibility in the Han Chinese population using a case-control study approach.

Materials and methods

Study subjects

Our case-control study included 322 patients with lung cancer and 384 cancer-free control subjects. All participants were genetically unrelated Han Chinese. Lung cancer patients were enrolled in the First Affiliated Hospital of Xi’an Jiaotong University (Xi’an, China). The inclusion criteria for patients were patients with primary lung cancer who was newly diagnosed and histologically confirmed according to International Classification of Diseases for Oncology, no familial history and no cancer history. The exclusion criteria of the patient group were patients with any other malignancy and pulmonary diseases, such as chronic obstructive pulmonary disease, pneumothorax, and asthma. No age, gender, tumor histology, or stage restrictions were applied; however, patients with prior cancer history were excluded from this study. No lung cancer patients had received either radiotherapy or chemotherapy before blood sampling. Healthy controls without history of any cancer were randomly recruited from the physical examination center of the same hospital during the similar period, when they had visited for an annual health examination. The exclusion criteria for the control group were any lung cancer family history of more than three generations, tuberculosis and chronic respiratory disease. At the time of recruitment, each subject was personally interviewed by trained personnel using a structured questionnaire to obtain information on the demographic information.

All participants were voluntary recruited and provided written informed consent before taking part in this research. This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Xi’an Jiaotong University, and in compliance with the Declaration of Helsinki. The design and performance of this study involving human subjects were obviously described in a research protocol.

SNP selection

We selected three cancer-related genes (NR5A2, LPP and TNS3 gene) to explore the relationship between their 3′-UTR SNPs and lung cancer risk in our study population. The 3′UTR sequences of these candidate genes were identified using NCBI database of nucleotide (https://www.ncbi.nlm.nih.gov/nuccore/). The online software of miRBase (http://www.mirbase.org/), miRDB (http://www.mirdb.org) and TargetScan 7.1 (http://www.targetscan.org) were used to choose SNPs in putative miRNA binding sites within 3′UTR of each gene. Information regarding genetic variations of these 3′UTR SNP was obtained by an extensive search of the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/), and UCSC genome browser (http://genome.ucsc.edu/). SNPs with a minor allele frequency (MAF) greater than 5% in Chinese Han population were selected based on the HapMap Han Chinese in Beijing (CHB) database. Seven SNPs (rs2246209 and rs1056426 in NR5A2; rs1064607, rs3796283 and rs2378456 in LPP; rs3750163 and rs9876 in TNS3) were ultimately chosen as the candidate SNPs for the further evaluation. Using these bioinformatics tools, two miRNAs (hsa-miR-144-3p, and hsa-miR-182-5p) were identified that potentially bind to a stretch of sequence harboring candidate SNPs in the 3′ UTR of NR5A2, LPP and TNS3.

SNP genotyping

Peripheral blood (5 mL) from each participant was collected and stored in Vacutainer tubes (BD Franklin Lakes, NJ) containing anticoagulant of EDTA. Genomic DNA was isolated using the QIAGEN DNA Extraction Kit (QIAGEN, Valencia, CA, USA), following the manufacturer’s protocol. Purity and concentration of the DNA samples were determined by a NanoDrop2000c Spectrophotometer (ThermoFisher Scientific, Wilmington, DE, EUA). All DNA samples were suspended in TE buffer and stored at −80 °C for later analysis.
Genotypes of SNPs were detected using the Agena MassARRAY system (Agena, San Diego, CA, U.S.A.) by two laboratory personnel independently in a double-blinded fashion. Primers for amplification and single base extension were designed with Agena MassARRAY Assay Design 3.0 Software. Sequence data was collected and analyzed by Agena Typer 4.0 Software. Meanwhile, approximately 10% of samples were randomly selected to repeat genotyping, and the reproducibility was 100%.

**Statistical analysis**

Independent sample Student’s t test (for continuous variables) and U Mann Whitney test (for categorical variables) were used to evaluate the differences in the distribution of demographic characteristics between cases and controls. Hardy-Weinberg equilibrium (HWE) was assessed using the Chi-square test to compare the observed and expected genotype frequencies in controls. The distribution of SNPs allele and genotype frequencies of cases and controls were compared with a Pearson Chi-squared test or Fisher’s exact test. Multiple inheritance models (genotype, dominant, recessive and log-additive) was estimated using logistic regression analysis with adjustments for the potential lung risk factors (age and gender) by SNPstats online tools software (http://bioinfo.iconcologia.net/snpstats/start.htm). The analyses of joint effects were further stratified by age (≤ 50 and > 50 years) and gender (male and female). Power and Sample Size (PS) Calculation software (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize) was used to calculate the power of the significant difference. Haplotypes were reconstructed with Haploview software package (version 4.2) and SNPstats software. In haplotype analysis, haplotype frequencies less than 0.01 were omitted. Besides, multifactor dimensionality reduction (MDR) (version 3.0.2) was employed to identify the potential interactions of these SNPs in LPP on the risk of lung cancer. All the statistical analyses were performed with SPSS v17.0 (IBM Analytics, Chicago, IL, USA), and two-sided p value < 0.05 indicated statistical significance.

**Results**

Our study included 706 subjects including 322 patients with lung cancer (245 males and 77 females) and 384 healthy controls (278 males and 106 females). There were no statistically significant differences (p = 0.265) on the gender distribution between the case and control groups. The average age among cases and controls was 59.00 ± 9.83 years and 51.16 ± 11.50 years, respectively. However, the result revealed the age distribution was statistically significant differences (p < 0.001). A summary of the participants’ demographic characteristics was summarized in Table 1. Lung cancer patients were consisted of 150 adenocarcinomas, 98 squamous cell carcinomas, and 74 small cell adenoscarcinomas.

Details of selected SNPs were described in Table 2. These 3’UTR SNPs were successfully genotyped for further analysis, and the call rate was above 99%. MAF of all SNPs was greater than 5% and the observed genotype frequencies of all SNPs in the control groups were in HWE (p > 0.05).

Differences in SNPs genotype and allele frequencies between cases and controls were compared by Chi-squared test and odds ratios (ORs) to evaluate the associations with the risk of lung cancer, as showed in Table 3. The minor allele of each SNP as a risk factor was compared to the wild-type (major) allele. However, we had not found that any SNPs were significantly different of genotypes and allele frequencies between lung cancer cases and healthy population (Table 3). Multiple inheritance models (dominant, recessive, and additive models) were applied to analyze potential association by logistic regression analysis adjusted for age and gender. Again, we observed no statistically significant differences between patients and controls (p > 0.05, Additional file 1: Table S1).

**Table 1** Characteristics of patients with lung cancer and controls

| Characteristics       | Patients (n = 322) | Controls (n = 384) |
|-----------------------|-------------------|--------------------|
| Age, years            | 59.00 ± 9.83      | 51.16 ± 11.50      |
| ≤ 50 years            | 58 (18.01%)       | 156 (40.62%)       |
| > 50 years            | 264 (91.99%)      | 228 (59.38%)       |
| Gender                |                   |                    |
| Male                  | 245 (76.1%)       | 278 (72.4%)        |
| Female                | 77 (23.9%)        | 106 (27.6%)        |
| Histology             |                   |                    |
| Adenocarcinoma        | 150               |                    |
| Squamous cell         | 98                |                    |
| small cell carcinoma  | 74                |                    |
| Stage                 |                   |                    |
| I-II                  | 95                |                    |
| III-IV                | 213               |                    |
| Missing               | 34                |                    |
| Lymphatic metastasis  |                   |                    |
| Yes                   | 193               |                    |
| No                    | 127               |                    |
Table 2 Basic Information about the candidate SNPs in this study

| Gene  | SNP ID      | Chr Position | Role       | Alleles (minor/major) | Nucleotide change | MAF       | O(HET) | E(HET) | p-value for HWE | Call rate (%) |
|-------|-------------|--------------|------------|-----------------------|-------------------|-----------|--------|--------|-----------------|---------------|
| NRS2A | rs2246209   | 1:200145533  | UTR-3      | A/G                   | 2195G>A           | 0.301     | 0.313  | 0.417  | 0.430           | 0.553         |
| NRS2A | rs1056426   | 1:20014603   | UTR-3      | C/T                   | 3065T>C           | 0.225     | 0.238  | 0.367  | 0.363           | 0.889         |
| LPP   | rs1064607   | 3:188595672  | UTR-3      | C/G                   | 3405G>C           | 0.405     | 0.365  | 0.453  | 0.463           | 0.661         |
| LPP   | rs3796283   | 3:188602952  | UTR-3      | G/A                   | 10684A>G          | 0.447     | 0.414  | 0.474  | 0.485           | 0.674         |
| LPP   | rs2378456   | 3:188603007  | UTR-3      | C/G                   | 10739C>G          | 0.473     | 0.453  | 0.483  | 0.496           | 0.608         |
| TNS3  | rs3750163   | 7:47317510   | UTR-3      | A/G                   | 164G>A            | 0.087     | 0.079  | 0.148  | 0.146           | 1.000         |
| TNS3  | rs9876      | 7:47315291   | UTR-3      | A/G                   | 2383G>A           | 0.475     | 0.487  | 0.464  | 0.500           | 0.154         |

SNP single nucleotide polymorphism, MAF minor allele frequency, O(HET) observed heterozygosity, E(HET) expected heterozygosity, HWE Hardy-Weinberg equilibrium. p values were calculated with Pearson’s χ² tests; p < 0.05 indicates statistical significance.

We next performed a stratified analysis according to age and gender to evaluate the effect of these 3’UTR SNPs on lung cancer risk. Stratified analysis by gender revealed significant associations between three SNPs and the risk of lung cancer, as displayed in Table 4 and Additional file 1: Table S2. These three polymorphisms (rs1064607, rs3796283 and rs2378456) in LPP, which increased the risk of lung cancer among males, were identified using the dominant model (rs1064607, GC-CC vs GG, OR = 1.57, CI = 1.06–2.33, p = 0.024; rs3796283, GA-GG vs AA, OR = 1.67, CI = 1.10–2.51, p = 0.014; and rs2378456, GC-CC vs GG, OR = 1.55, CI = 1.02–2.38, p = 0.041), with power values of 0.799, 0.866, and 0.718, respectively. We also determined that the genotype “GA” of rs3796283 increased the risk of lung cancer in males under genotype model (OR = 1.76, CI = 1.14–2.71, p = 0.036, power = 0.955). Conversely, rs2378456 polymorphism in LPP was associated with reduced susceptibility of lung cancer in females under genotype (GC vs GG, OR = 0.37, 95% CI = 0.18–0.76, p = 0.022, power = 1.000) and dominant (GC-CC vs GG, OR = 0.44, 95% CI = 0.23–0.84, p = 0.012, power = 0.998) model. Interaction analysis did not reveal any significant associations between these SNPs and lung cancer risk with respect to age (Additional file 1: Table S3).

We further assessed the association between these SNPs and clinic-pathological features, including histological subgroups, clinical stage (I-II vs. III-IV) and lymphatic metastatic stats (non-metastasis vs. metastasis). The results indicated that LPP rs1064607 polymorphism was significantly correlated with increased risk of lung adenocarcinoma (allele model, OR = 1.35, 95% CI: 1.03–1.77, p = 0.030; and additive model, OR = 1.38, 95% CI: 1.04–1.83, p = 0.026, Table 5). Moreover, AA genotype of TNS3 rs9876 polymorphism had a significantly lower risk of lymphatic metastasis (AA vs. GG-AG, OR = 0.52, 95% CI: 0.30–0.89, p = 0.017, Table 6). Nevertheless, we failed to observe significant differences between clinic-pathological features and other polymorphisms (Additional file 1: Table S4, S5, S6, S7, S8).

Subsequently, haplotype analysis was used to explore the association of LPP gene with lung cancer susceptibility. Linkage Disequilibrium (LD) analysis demonstrated SNPs rs1064607, rs3796283 and rs2378456 composed a LD block, as shown in Fig. 1. To examine the effect of haplotypes on the risk of lung cancer, the haplotype-based logistic regression method adjusted by age and gender was carried out. However, the results did not reveal a significant association between common haplotypes and the risk of lung cancer (p > 0.05, Additional file 1: Table S9).

Then, MDR was used to analyze the interactions of these SNPs in LPP. Table 7 summarizes the results of MDR for analyzing interactions of these SNPs in their influences on risk of lung cancer. The best model was between rs1064607and rs2378456 with a testing accuracy of 0.513 and a maximum CVC of 10 out of 10 followed by a statistical significance of p < 0.05.

Discussions
We genotyped the seven 3’UTR SNPs in NRS2A, LPP, and TNS3 to determine the potential association with the susceptibility to lung cancer. Noticeably, LPP polymorphisms (rs1064607, rs3796283 and rs2378456) were associated with increased susceptibility to lung cancer in males. LPP rs1064607 polymorphism was significantly correlated with increased risk of lung adenocarcinoma. Moreover, TNS3 rs9876 polymorphism was associated with the lymphatic metastasis of lung cancer patients. To our best knowledge, this is the first study to explore the relationship between these 3’UTR polymorphisms and lung cancer risk in Chinese Han population. Our findings further highlight the biological significance of the genetic variations in 3’UTR region, which may play an important role in the development of lung cancer.
### Table 3: The genotype and allele frequencies of seven 3′UTR polymorphisms in lung cancer patients and controls

| Gene | SNP ID   | Genotype | Adjusted analysis | Crude analysis | Allele |
|------|----------|----------|-------------------|----------------|--------|
|      |          |          | Control           | Case           | OR (95% CI) | p     | Control | Case | OR (95% CI) | p     |
|      |          |          | OR (95% CI)       |                | OR          | (95% CI) | p         |       | OR          | (95% CI) | p           |
|      |          |          |                   |                | p           |         |           |       | p           |         |             |
| NR5A2 | rs1056426 | TT       | 197 (61.6%)       | 222 (57.8%)    | 1.00        | 0.270   | T         | 585 (76.2%) | 496 (77.5%) | 1.00 | 0.560       |
|       |          | CT       | 102 (31.9%)       | 141 (36.7%)    | 0.78 (0.55–1.09) | 0.82 (0.59–1.12) | 0.380 |
|       |          | CC       | 21 (66%)          | 21 (55%)       | 1.15 (0.59–2.24) | 1.13 (0.60–2.13) |       |
| NR5A2 | rs2246209 | GG       | 160 (49.7%)       | 184 (47.9%)    | 1.00        | 0.940   | G         | 528 (68.8%) | 450 (69.9%) | 1.00 | 0.650       |
|       |          | GA       | 130 (40.4%)       | 160 (41.7%)    | 0.98 (0.70–1.37) | 0.93 (0.68–1.28) | 0.890 |
|       |          | AA       | 32 (9.9%)         | 40 (10.4%)     | 0.91 (0.53–1.56) | 0.92 (0.55–1.53) |       |
| LPP   | rs1064607 | GG       | 111 (34.6%)       | 157 (40.9%)    | 1.00        | 0.280   | G         | 488 (63.5%) | 382 (59.5%) | 1.00 | 0.120       |
|       |          | GC       | 160 (49.8%)       | 174 (45.3%)    | 1.25 (0.89–1.77) | 1.30 (0.94–1.80) | 0.230 |
|       |          | CC       | 50 (15.6%)        | 53 (13.8%)     | 1.41 (0.87–2.30) | 1.33 (0.85–2.11) |       |
| LPP   | rs3796283 | AA       | 94 (29.3%)        | 134 (34.9%)    | 1.00        | 0.490   | A         | 450 (58.6%) | 355 (55.3%) | 1.00 | 0.210       |
|       |          | GA       | 167 (52%)         | 182 (47.4%)    | 1.24 (0.86–1.77) | 1.31 (0.93–1.83) | 0.280 |
|       |          | GG       | 60 (18.7%)        | 68 (17.7%)     | 1.20 (0.76–1.92) | 1.26 (0.81–1.99) |       |
| LPP   | rs2378456 | GG       | 88 (27.9%)        | 117 (30.6%)    | 1.00        | 0.890   | G         | 419 (54.7%) | 333 (52.7%) | 1.00 | 0.450       |
|       |          | GC       | 157 (49.7%)       | 185 (48.3%)    | 1.08 (0.74–1.56) | 1.13 (0.80–1.60) | 0.730 |
|       |          | CC       | 71 (22.5%)        | 81 (21.1%)     | 1.11 (0.71–1.74) | 1.17 (0.76–1.78) |       |
| TNS3  | rs3750163 | GG       | 270 (83.8%)       | 325 (84.6%)    | 1.00        | 0.370   | G         | 707 (92.1%) | 588 (91.3%) | 1.00 | 0.610       |
|       |          | GA       | 148 (49.9%)       | 57 (14.8%)     | 0.93 (0.60–1.44) | 1.01 (0.67–1.54) | 0.580 |
|       |          | AA       | 4 (12%)           | 2 (0.5%)       | 3.25 (0.56–18.93) | 2.41 (0.44–13.24) |       |
| TNS3  | rs9876   | GG       | 86 (26.8%)        | 108 (28.1%)    | 1.00        | 0.430   | G         | 394 (51.3%) | 337 (52.5%) | 1.00 | 0.660       |
|       |          | AG       | 165 (51.4%)       | 178 (46.4%)    | 1.15 (0.79–1.67) | 1.16 (0.82–1.66) | 0.360 |
|       |          | AA       | 70 (21.8%)        | 98 (25.5%)     | 0.89 (0.57–1.38) | 0.90 (0.59–1.36) |       |

**OR** odds ratio, **95% CI** 95% confidence interval

* p < 0.05 indicates statistical significance
| SNP ID | Model | Genotype | Male | Control | Case | OR (95%CI) | p | Female | Control | Case | OR (95%CI) | p |
|--------|-------|----------|------|---------|------|------------|---|---------|---------|------|------------|---|
| rs1064607 | Allele | G | 358 (64.4%) | 286 (58.6%) | 1.00 | 0.055 | | | 130 (61.3%) | 96 (62.3%) | 1.00 | 0.840 |
| | | C | 198 (35.6%) | 202 (41.4%) | 1.28 (0.99–1.64) | | | | 82 (38.7%) | 58 (37.7%) | 0.96 (0.62–1.47) | |
| | Codominant | G/G | 118 (42.5%) | 78 (32%) | 1.00 | 0.078 | 1.00 | 39 (36.8%) | 33 (42.9%) | 1.00 | 0.370 | 1.00 |
| | | G/C | 122 (43.9%) | 130 (53.3%) | 1.58 (1.04–2.39) | | | 52 (49.1%) | 30 (39%) | 0.68 (0.35–1.30) | | 0.68 (0.36–1.30) | 0.390 |
| | | C/C | 38 (13.7%) | 36 (14.8%) | 1.54 (0.85–2.80) | 1.43 (0.84–2.45) | | 15 (14.2%) | 14 (18.2%) | 1.12 (0.47–2.68) | | 1.10 (0.47–2.62) |
| | Dominant | G/G | 118 (42.5%) | 78 (32%) | 1.00 | 0.024 | 1.00 | 39 (36.8%) | 33 (42.9%) | 1.00 | 0.410 | 1.00 |
| | | G/C-C/C | 160 (57.5%) | 166 (68%) | 1.57 (1.06–2.33) | | | 67 (63.2%) | 44 (57.1%) | 0.77 (0.42–1.42) | | 0.78 (0.43–1.41) |
| | | C/C | 38 (13.7%) | 36 (14.8%) | 1.19 (0.69–2.05) | 1.09 (0.67–1.79) | | 15 (14.2%) | 14 (18.2%) | 1.38 (0.62–3.08) | | 1.35 (0.61–2.99) |
| | Log-additive | – | – | – | – | 1.00 | 0.053 | 1.29 (1.00–1.66) | 0.052 | – | – | 0.96 (0.63–1.47) | 0.870 |
| | | – | – | – | – | 1.00 | 0.058 | – | – | 1.00 | 0.440 |
| rs379628 | Allele | A | 332 (59.7%) | 263 (53.9%) | 1.00 | 0.036 | 1.00 | 30 (28.3%) | 30 (39%) | 1.00 | 0.160 | 1.00 |
| | | G | 224 (40.3%) | 225 (46.1%) | 1.27 (0.99–1.62) | | | 94 (44.3%) | 62 (40.3%) | 0.85 (0.56–1.29) | | 0.90 |
| | Codominant | A/A | 104 (37.4%) | 64 (26.2%) | 1.00 | 0.036 | 1.00 | 30 (28.3%) | 30 (39%) | 1.00 | 0.160 | 1.00 |
| | | G/A | 124 (44.6%) | 135 (53.3%) | 1.76 (1.14–2.71) | | | 58 (54.7%) | 32 (41.6%) | 0.52 (0.27–1.03) | | 0.55 (0.28–1.07) | 0.190 |
| | | G/G | 50 (18%) | 45 (18.4%) | 1.43 (0.81–2.50) | 1.46 (0.88–2.43) | | 18 (17%) | 15 (19.5%) | 0.80 (0.34–1.89) | | 0.83 (0.36–1.95) |
| | Dominant | A/A | 104 (37.4%) | 64 (26.2%) | 1.00 | 0.014 | 1.00 | 30 (28.3%) | 30 (39%) | 1.00 | 0.100 | 1.00 |
| | | G/A-G/A | 174 (52.6%) | 180 (73.8%) | 1.67 (1.10–2.51) | | | 76 (71.7%) | 47 (61%) | 0.59 (0.31–1.11) | | 0.62 (0.33–1.15) |
| | | G/G | 50 (18%) | 45 (18.4%) | 1.01 (0.61–1.65) | 1.03 (0.66–1.61) | | 18 (17%) | 15 (19.5%) | 1.17 (0.55–2.52) | | 1.18 (0.55–2.53) |
| | Log-additive | – | – | – | – | 1.26 (0.96–1.66) | 0.095 | 1.27 (0.99–1.63) | 0.056 | – | – | 0.83 (0.54–1.27) | 0.380 |
| | | – | – | – | – | 1.00 | 0.058 | – | – | 1.00 | 0.440 |
| rs2378456 | Allele | G | 312 (56.3%) | 245 (50.8%) | 1.00 | 0.077 | 1.00 | 107 (50.5%) | 88 (58.7%) | 1.00 | 0.120 |
| | | C | 242 (43.7%) | 237 (49.2%) | 1.25 (0.98–1.59) | | | 105 (49.5%) | 62 (41.3%) | 0.72 (0.47–1.09) | | 0.72 (0.47–1.09) |
| | Codominant | G/G | 93 (33.6%) | 58 (24.1%) | 1.00 | 0.010 | 1.00 | 24 (22.6%) | 30 (40%) | 1.00 | 0.022 | 1.00 |
| | | G/C | 126 (45.5%) | 129 (53.5%) | 1.63 (1.04–2.56) | | | 59 (55.7%) | 28 (37.3%) | 0.37 (0.18–0.76) | | 0.38 (0.19–0.76) | 0.023 |
| | | C/C | 58 (20.9%) | 54 (22.4%) | 1.39 (0.80–2.40) | 1.49 (0.91–2.45) | | 23 (21.7%) | 17 (22.7%) | 0.60 (0.26–1.38) | | 0.59 (0.26–1.35) |
| | Dominant | G/G | 93 (33.6%) | 58 (24.1%) | 1.00 | 0.041 | 1.00 | 24 (22.6%) | 30 (40%) | 1.00 | 0.012 | 1.00 |
| | | G/C-C/C | 184 (66.4%) | 183 (75.9%) | 1.55 (1.02–2.38) | | | 82 (77.4%) | 45 (60%) | 0.44 (0.23–0.84) | | 0.44 (0.23–0.84) |
| | | C/C | 58 (20.9%) | 54 (22.4%) | 1.02 (0.64–1.62) | 1.09 (0.72–1.66) | | 23 (21.7%) | 17 (22.7%) | 1.08 (0.53–2.22) | | 1.06 (0.52–2.15) |
| | Log-additive | – | – | – | – | 1.21 (0.92–1.58) | 0.180 | 1.25 (0.97–1.59) | 0.078 | – | – | 0.73 (0.49–1.11) | 0.140 |

OR odds ratio, 95% CI 95% confidence interval
Bold indicates statistical significance (p < 0.05)
### Table 5: The relationship of seven 3′UTR polymorphisms with lung cancer according to the histology stratification

| SNP ID   | Model     | Genotype | Control | Adenocarcinoma | Squamous cell carcinoma | Small cell carcinoma |
|----------|-----------|----------|---------|-----------------|-------------------------|---------------------|
|          |           |          |         | Case           | OR (95% CI)             | p-value             |
| rs1064607| Allele    |          |         |                 |                         |                     |
|          | G         |          | 488 (63.5%) | 169 (56.3%)  | 1.00                     | 0.030               |
|          | C         |          | 280 (36.5%) | 131 (43.7%)  | 1.35 (1.03–1.77)         |                     |
|          |           | Codominant | G/G     | 157 (40.9%) | 47 (31.3%)  | 1.00                     | 0.085               |
|          |           |           | G/C     | 174 (45.3%) | 75 (50%)    | 1.39 (0.89–2.16)         |                     |
|          |           |           | C/C     | 53 (13.8%)  | 28 (18.7%)  | 1.90 (1.06–3.42)         |                     |
|          |           | Dominant  | G/G     | 157 (40.9%) | 47 (31.3%)  | 1.00                     | 0.053               |
|          |           |           | G/C/C   | 227 (59.1%) | 103 (68.7%) | 1.50 (0.99–2.28)         |                     |
|          |           | Recessive | G/G-G/C | 331 (86.2%) | 122 (81.3%) | 1.00                     | 0.096               |
|          |           |           | C/C     | 53 (13.8%)  | 28 (18.7%)  | 1.57 (0.93–2.66)         |                     |
|          |           | Log-additive | –   | –            | –                      | 1.38 (1.04–1.83)     | 0.026               |

|           |           |           | Squamous cell carcinoma | OR (95% CI) | p-value |           | OR (95% CI) | p-value |           | OR (95% CI) | p-value |
|----------|-----------|----------|-------------------------|-------------|---------|----------|-------------|---------|----------|-------------|---------|
|          |           |          |                         |             |         |          |             |         |          |             |         |
|          |           |          | Case                   | 121 (62.7%) | 1.00    | 0.762    | 56 (37.8%) | 1.06    | 0.74–1.53 |
|          |           |          | G/C                    | 73 (37.6%)  | 1.05    | 0.76–1.46| 38 (51.4%) | 1.20    | 0.69–2.10 |
|          |           |          | C/C                    | 13 (13.4%)  | 1.13    | 0.52–2.47| 9 (12.2%)  | 0.99    | 0.43–2.31 |
|          |           |          | G/G                   | 37 (38.1%)  | 1.00    | 0.76–1.46| 27 (36.9%) | 1.00    | 0.89–2.14 |
|          |           |          | G/C/G/C               | 60 (61.9%)  | 1.20    | 0.73–1.98| 47 (63.9%) | 1.16    | 0.68–1.97 |
|          |           |          | G/G-G/C               | 84 (86.6%)  | 1.00    | 0.970    | 65 (87.8%) | 1.00    | 0.89–1.95 |
|          |           |          | C/C                   | 13 (13.4%)  | 1.02    | 0.49–2.10| 9 (12.2%)  | 0.89    | 0.41–1.95 |
|          |           |          | Log-additive          | –           | 1.10    | 0.77–1.58| –           | 1.05    | 0.72–1.53 |

SNP: single nucleotide polymorphism, OR: odds ratio, 95% CI: 95% confidence interval. p-values were adjusted by gender and age. Bold indicates statistical significance (p < 0.05).
| SNP ID | Model   | Genotype | Non-Metastasis | Metastasis | Adjusted analysis | Crude analysis |
|--------|---------|----------|----------------|------------|------------------|---------------|
|        |         |          |                |            | OR (95%CI)       | p             |
|        |         |          |                |            |                  |               |
| rs9876 | Allele  | G        | 121 (48.0%)    | 216 (55.4%)| 1.00             | 0.068         |
|        |         | A        | 131 (52.0%)    | 174 (44.6%)| 0.74 (0.54–1.02) |               |
|        | Codominant | G/G     | 31 (24.6%)     | 55 (28.2%) | 1.00             | 0.059         |
|        |         | A/G     | 59 (46.8%)     | 106 (54.4%)| 1.01 (0.59–1.74) | 1.01 (0.59–1.74) | 0.065         |
|        |         | A/A     | 36 (28.6%)     | 34 (17.4%) | 0.52 (0.28–1.00) | 0.53 (0.28–1.01) |
|        | Dominant | G/G     | 31 (24.6%)     | 55 (28.2%) | 1.00             | 0.460         |
|        |         | A/G-A/A | 95 (75.4%)     | 140 (71.8%)| 0.83 (0.49–1.38) | 0.83 (0.50–1.39) |
|        | Recessive | G/G-A/G | 90 (71.4%)     | 161 (82.6%)| 1.00             | 0.017         |
|        |         | A/A     | 36 (28.6%)     | 34 (17.4%) | 0.52 (0.30–0.89) | 0.53 (0.31–0.90) | 0.019         |
|        | Log-additive | –      | –              | –          | 0.73 (0.53–1.01) | 0.068         |

OR: odds ratio, 95% CI: 95% confidence interval
Bold indicates statistical significance (p < 0.05)
LPP is located on chromosome 3q27.3-q28, which belongs to the zyxin family of LIM domain proteins that involves in cell-cell adhesion, cell migration or invasion, cell-substrate cytoskeletal interactions, and tumorigenesis [18]. Accumulating evidence suggests that the dysregulation of LPP expression is associated with various types of cancers, such as breast cancer, myeloma, and lung cancer [15, 19, 20]. For example, Kang et al. [21] demonstrated the LPP overexpression in non-small-cell lung cancer. In addition, LPP degraded N-cadherin during lung cancer, and loss of LPP in advanced-stage of cancer may trigger further dissemination and distant metastasis of lung adenocarcinoma [20]. TNS3 is located on chromosome 7p12.3, which encodes TNS3, an intracellular cytoskeletal-interacting protein that regulates cell motility and migration by anchoring actin to integrins [22]. Endogenous TNS3 contributes to cell migration, anchorage-independent growth, and tumorigenesis in cell lines derived from advanced lung cancer [23]. Thus, LPP and TNS3 may have an important role in the tumorigenesis and progression of lung cancer.

3′UTR polymorphisms may affect the binding of miRNA to target genes and exert effects on genes expressions and tumorigenesis [24, 25]. In this study, we found that three polymorphisms (rs1064607, rs3796283, and rs2378456) in the 3′-UTR of LPP and TNS3 rs9876 polymorphism were significantly associated with susceptibility to lung cancer. These SNPs were identified to putatively affects the binding sites of miR-144 and miR-182, whose abnormal expression in lung cancer was associated with the proliferation, migration, and invasion of tumor cells [26, 27]. We, therefore, propose that These SNPs may affect LPP or TNS3 expression in lung cancer by differential miRNA stability and binding activity of miR-144 and miR-182. While the functional relevance of this polymorphism has not yet been elucidated, our results might partially suggest a functional correlation between these polymorphisms and the risk of lung cancer, which may provide preliminary evidence of biological plausibility for the observed association in the current study.

Our study does have some limitations. First, the results of gene-to-environment interactions between LPP gene and lung cancer could not be obtained due to a lack of relevant information. Second, these SNPs located in the miRNA binding site were identified based on in silico analysis only; thus, additional studies are necessary to characterize the molecular mechanisms underlying the potential association with lung cancer development and progression.

Conclusions

In summary, we provided evidence that SNPs in the 3′-UTR region of LPP gene may have an effect on individual susceptibility to lung cancer among Chinese Han individuals, particularly males.

![Haplotype block map for three SNPs in LPP gene. The numbers inside the diamonds indicate the D′ for pairwise analyses.](Fig. 1)

Table 7  SNP-SNP interaction models analyzed by the MDR method

| Model                        | Training Bal. Acc. | Testing Bal. Acc. | CVC | OR (95% CI)       | p   |
|------------------------------|--------------------|-------------------|-----|-------------------|-----|
| rs1064607                    | 0.533              | 0.509             | 6/10| 1.31 (0.96–1.79)  | 0.080|
| rs1064607, rs2378456         | 0.539              | 0.513             | 10/10| 1.37 (1.01–1.87)  | 0.042|
| rs1064607, rs3796283, rs2378456 | 0.545              | 0.498             | 10/10| 1.43 (1.05–1.93)  | 0.022|

MDR multifactor dimensionality reduction, Bal. Acc. Balanced accuracy, CVC Cross-validation consistency, OR odds ratio, 95% CI 95% confidence interval. p values were calculated using χ² tests

Bold indicates statistical significance (p < 0.05)
Additional files

**Additional file 1:** Table S1. Relationship between selected 3′UTR polymorphisms and risk of lung cancer according to multiple inheritance models. Table S2. The relationship of selected 3′UTR polymorphisms with lung cancer according to the gender stratification. Table S3. The relationship of selected 3′UTR polymorphisms with lung cancer according to the age stratification. Table S4. Relationship between selected 3′UTR polymorphisms and risk of lung adenocarcinoma. Table S5. Relationship between selected 3′UTR polymorphisms and risk of lung squamous cell carcinoma. Table S6. Relationship between selected 3′UTR polymorphisms and risk of lung small cell carcinoma. Table S7. Relationship of clinical stage with selected 3′UTR polymorphisms in lung cancer patients. Table S8. Relationship of lymphatic metastatic status with 3′UTR polymorphisms in lung cancer patients. Table S9. Haplotype frequencies and their associations with lung cancer risk. (DOCX 130 kb)

Abbreviations
CIs: Confidence intervals; EMT: Epithelial-mesenchymal transition; HMGA2: High Mobility Group AT-Hook2; HWE: Hardy-Weinberg equilibrium; LPP: Lipoma preferred partner/LIM domain containing preferred translocation partner in lipoma; MAF: Minor allele frequency; NRS2A: Nuclear receptor subfamily 5 group A member 2; NSCLC: Non-small-cell lung cancer; OR: Odds ratio; SNPs: Single nucleotide polymorphisms; TNS3: Tensin3; UTR: 3′ untranslated region

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Availability of data and materials
All the data regarding the findings are available within the manuscript. Anyone who is interested in the information should contact the corresponding author.

Authors’ contributions
The work presented here was carried out in collaboration between all authors. SY and RS carried out the molecular genetic studies and drafted the manuscript. SW, TJ, and SZ designed the methods and experiments, performed the statistical analyse and interpreted the results. FN designed primers and performed the SNP genotyping experiments. JL collected clinical information about patients and performed the SNP genotyping experiments. MC conceived of the study, worked on associated data collection and their interpretation, participated in the design and coordination of the study, and funded the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All participants were voluntary and provided written informed consent before taking part in this research. This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Xian Jiaotong University, and in compliance with the Declaration of Helsinki. The design and performance of this study involving human subjects were obviously described in a research protocol.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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