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ORIGINAL ARTICLE

# Correlations of an Insertion/Deletion Polymorphism (rs10680577) in the RERT-lncRNA with the Susceptibility, Clinicopathological Features, and Prognosis of Lung Cancer

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## Abstract

The aim of this study was to investigate the correlations of an Ins/Del polymorphism (rs10680577) in the RERT-lncRNA with the susceptibility, clinicopathological features, and prognosis of lung cancer. A total of 376 patients with lung cancer and 419 healthy subjects were enrolled in this study. The genotype of rs10680577 was performed using polymerase chain reaction (PCR) followed by polyacrylamide gel electrophoresis. Quantitative real-time PCR was used to measure RERT-lncRNA and *EGLN2* expressions. Subjects with Del allele of rs10680577 exhibited an elevated risk of lung cancer. The expressions of RERT-lncRNA and *EGLN2* in tumor tissues were higher than adjacent normal tissues, manifesting a positive correlation. Compared to patients with Ins/Ins genotype carriers, those with Ins/Del + Del/Del genotype carriers had upregulated expressions of RERT-lncRNA and *EGLN2*. Moreover, Ins/Del + Del/Del genotype and expressions of RERT-lncRNA and *EGLN2* were associated with age, smoking habits, and TNM stage in lung cancer patients. Besides, patients with Ins/Ins genotype of rs10680577 had a longer OS than those with Ins/Del + Del/Del genotype carriers, and patients with lower expressions of RERT-lncRNA and *EGLN2* presented a shorter OS than those with higher expressions. COX multivariate analysis demonstrated that Ins/Del + Del/Del genotype and higher expressions of RERT lncRNA and *EGLN2* were risk factors affecting the prognosis of lung cancer. The Ins/Del polymorphism (rs10680577) in RERT-lncRNA was correlated with the risk, major clinicopathological features, and prognosis of lung cancer patients, and the patients with Ins/Del + Del/Del genotype carriers had higher expressions of RERT-lncRNA and *EGLN2* than those with Ins/Ins carriers.

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**Keywords** Lung cancer · RERT-lncRNA · rs10680577 · *EGLN2*

## Introduction

Lung cancer, as one of the leading cancer killers worldwide, is a malignant lung tumor with the characteristics of uncontrolled cell growth in lung tissues, threatening the health and life of human beings (Qiu et al. 2015; Siegel et al. 2013; Xia et al. 2016). To the best of our knowledge, lung cancer is mainly divided into two types including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), in which the former accounts for nearly 80% of all lung cancer patients (Jiang et al. 2016). Based on the prevalent opinions, lung cancer is traditionally suggested as a kind of complicated disease caused by genetic and environmental risk factors, which is a consequence of the multifactorial and multistep process resulting in a malignant genotype (Ansari et al. 2016). With respect to the importance of genetic differences in the susceptibility to the development of lung cancer among individuals, polymorphisms as the common form of genetic variations affecting individuals' susceptibility to cancer have been identified as candidates to date (Chandra et al. 2016). At present, considerable evidences have accumulated to support the close relation between polymorphisms and lung cancer.

Long noncoding RNA (lncRNA), a kind of functional noncoding RNA molecule larger than 200 nucleotides, regulates gene expressions via three levels, including epigenetic modification, transcription, and posttranscription, thereby participating in the biological events, like embryonic development and cell differentiation (Mestdagh et al. 2016; Paytuvi Gallart et al. 2016). A large number of studies have discovered the correlations of the polymorphisms of lncRNAs with the susceptibility, progression, and prognosis of lung cancer. For example, Hu et al. pointed out that lncRNA CASC8 rs10505477 could not only serve as the potential indicator in diagnosis of lung cancer, but also could predict the adverse responses to chemotherapy for lung cancer (Hu et al. 2016). RERT-lncRNA, with 2849 base pairs (bp) in length, is located within the proximal promoter of prolyl hydroxylases (PHD1, also known as *EGLN2*), and a 4-bp (base pairs) Ins/Del polymorphism (rs10680577) was discovered within this novel lncRNA (Zhu et al. 2012). Of note, this polymorphism was closely associated with the susceptibility to gastric cancer, and exerted functions in the progression of colorectal carcinoma and hepatocellular carcinoma via modulation of *EGLN2* expression (Li et al. 2017; Zhu et al. 2012). It should be noted that *EGLN2* was a key adjustment factor which could mediate the degradation of hypoxia-inducible factor (HIF-1), thus involving in the process of multiple tumors (Che et al. 2014), including in lung cancer (Wang et al. 2015). Thus, in this study, we analyze the correlations of rs10680577 with the susceptibility, clinicopathological features, and prognosis of patients with lung cancer. Moreover, the expression levels of RERT-lncRNA and *EGLN2* in different genotypes of rs10680577 were compared to further explore the potential role of lncRNA in the development and progression of lung cancer, in the hope of developing a proper individualized diagnosis and treatment strategy for lung cancer in the future.

## Materials and Methods

### Ethical Statement

Experimental protocols and clinical trials corresponding to this study were approved by the Ethic Committee of Jingzhou Central Hospital and performed in strictly accordance with the *Declaration of Helsinki* (World Medical Association 2013). AQThe samples were acquired following informed consents obtained from all those patients who had volunteered to participate in this study.

### Subjects

A total of 376 patients with lung cancer admitted to this hospital from February 2012 to February 2015 were enrolled as the Case group, consisting of 246 males and 130 females, with the average age of ($64.30 \pm 12.20$) years old. In this group, 346 patients had smoking habits, and 30 cases had no smoking history. There were 94 patients with SCLC (42 cases in limited stage and 52 cases in extensive stage) and 282 cases with NSCLC according to the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) tumor–node-metastasis (TNM)-staging system staging system (Jin et al. 2016). Among them, 35 cases were in stage I, 49 cases in stage II, 119 cases in stage III, and 79 cases in stage IV. Tumor tissues and adjacent normal tissues were obtained followed by snap freezing in liquid nitrogen and preservation in − 80 °C for later use. Inclusion criteria: the samples were confirmed by at least two pathologists; and patients had no prior history of chemotherapy, radiotherapy, or surgical treatment before the operation. Exclusion criteria: patients had diseases in heart, brain, liver, kidney, or hemopoietic system; or patients had mental disorders. Meanwhile, 419 subjects with no relative relationship to each other, who underwent the physical examination in our hospital were enrolled as the control group, including 269 males and 150 females (average age: $63.35 \pm 10.71$ years old). Of these subjects, 372 individuals had smoking habits, while 47 had no smoking history. There was no statistical significance between two groups in age, gender ratio, and smoking habits (all $P > 0.05$).

### Genotyping

With the promoter sequence of *EGLN2* downloaded from NCBI, the primer of rs10680577 locus was designed using the Primer Premier 5.0 as follows: forward, 5′-TGATTCCCACAATCGTCC-3′; reverse, 5′-ATGGCTACGCAGTTAGTTGA-3′. Synthesis of primers was contracted to the Sangon Biotech Co., Ltd. (Shanghai, China). Peripheral blood samples were collected from all the subjects, and the genome DNA was extracted using the DNA extraction kit (Qiagen, Hilden, Germany). The concentration and purity of DNA were measured by means of the ultraviolet spectrometer (UV-1800, Shimadzu, Kyoto, Japan), while the integrity of DNA was determined through agarose gel electrophoresis. Gene amplification consisted of

pre-denaturation (94 °C, 7 min), 34 cycles of amplification (94 °C, 30 s; 58 °C, 30 s; 72 °C, 30 s) and extension (72 °C, 7 min). The product was loaded for 7% native polyacrylamide gel electrophoresis (600 V for 60 min). Gel was then collected and washed using double distilled water, in which 1% glacial acetic acid was added, followed by shaking for 3 min. Then, with 1% glacial acetic acid being abandoned, gel was placed in 0.1% silver nitrate for staining for 10 min, and after three washes, gel was placed in the 30% sodium carbonate-formaldehyde for band development. The genotypes of genes in samples were identified by the position and number of bands, and the results are shown in Fig. 1.

### Quantitative Real-Time PCR (qRT-PCR)

The total RNAs were extracted from the tissues by using RNA extraction kit (Omega Bio-Tek, Norcross, USA). Ultraviolet spectrometer was used to determine the purity and concentration of RNA, and agarose gel electrophoresis was performed to evaluate the integrity of RNA. The primers of RERT-lncRNA (forward: 5′-CGGAGA GGATGGGCTCTGGCATT-3′; reverse: 5′-AGGGACCCTTCAGGGTGGCTG-3′) and *EGLN2* (forward: 5′-AGGCTGTCGAAGCATTGGTG-3′; reverse: 5′-GGGATT GTCAACGTGCCTTAC-3′) were designed using Primer 5.0 software and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). cDNA was prepared using the Primescript™ RT reagent Kit (Takara, Japan) in reverse transcription system (10 μL) in following conditions: 16 °C for 30 min, 42 °C for 30 min, and 85 °C for 10 min. Real-time quantitative PCR (qRT-PCR) was carried out with the SYBR® premix Ex Taq TM qRT-PCR kit (Takara, Dalian, China) in following conditions: pre-denaturation at 95 °C for 2 min, and 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The relative expressions of RERT-lncRNA and *EGLN2* were calculated using $2^{-\Delta\Delta C_t}$ with GAPDH as internal reference.

### Follow-Up

Within a 5-year follow-up, subjects who were lost to the follow-up due to death of other reasons, or any other events, or still alive at the end of follow-up were counted as censored data with the time of last clinic visit being recorded. The follow-up information was obtained through clinic visit, telephone, or medical records, and

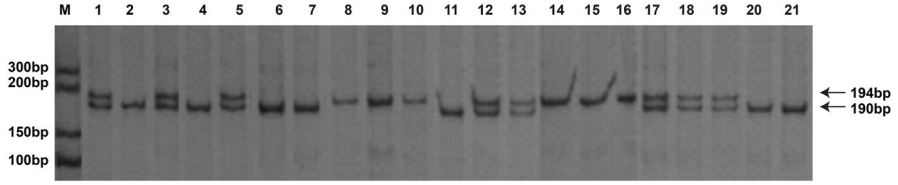


**Fig. 1** The genotyping results of rs10680577 polymorphism. *Note M* marker, *lanes 8, 9, 10, 14, 15, and 16* Ins/Ins genotype, *lanes 2, 4, 6, 7, 11, 20, and 21* Del/Del genotype, *lanes 1, 3, 5, 12, 13, 17, 18, and 19* Ins/Del genotype

the survival time of patients was counted in months. Overall survival (OS) initiated from diagnosis time to the time of endpoint event for any patients.

### Statistical Analysis

All data were analyzed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Intergroup comparison of measurement data, in form of mean ± SD, was carried out using *t* test. The Pearson analysis was conducted to examine relationship between RERT lncRNA expression and *EGLN2* expression in tumor tissues from patients with lung cancer. Intergroup comparison of the enumeration data in % or rate was performed using $\chi^2$ test. Predicted and observed values of genotype frequencies were analyzed with Hardy–Weinberg equilibrium. Odds ratio (OR) and 95% confidence interval (95% CI) representing the relative risk were calculated by nonparameter logistic regression models. Survival analysis was performed using Kaplan–Meier curve, and multivariate analysis using Cox proportional hazards regression model. $P < 0.05$ was bconsidered as statistically significant.

## Results

### The Correlation Between rs10680577 in the RERT-lncRNA and the Risk of Lung Cancer

The distribution of genotypes in RERT-lncRNA rs10680577 in the control group conformed to the Hardy–Weinberg equilibrium (HWE) (Table 1). Unconditional logistic regression analysis showed (Table 2) that the Del/Del genotype of rs10689577 could increase the risk of lung cancer (Del/Del vs. Ins/Ins: OR 4.288, 95% CI 2.138–8.599, $P < 0.001$). In the dominant models, significant differences were found in the genotype frequencies between the case group and the control group (Ins/Del + Del/Del vs. Ins/Ins: OR 1.443, 95% CI 1.080–1.929, $P = 0.013$). Moreover, the frequencies of Del allele in the case group and the control group were 25.40 and 17.54%, respectively, and subjects with Del allele carriers faced increased risk of lung cancer (Del vs. Ins: OR 1.600, 95% CI 1.256–2.039, $P < 0.001$).

**Table 1** Tests for the genotype distributions of rs10680577 in the RERT-lncRNA with Hardy–Weinberg equilibrium (HWE)

| Genotype | Control group ($n = 419$) | | | | Case group ($n = 376$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Practical value | Theoretical value | $\chi^2$ | *P* | Practical value | Theoretical value | $\chi^2$ | *P* |
| Ins/Ins | 283 | 284.89 | 0.409 | 0.523 | 222 | 209.26 | 12.031 | 0.001 |
| Ins/Del | 125 | 121.21 | | | 117 | 142.49 | | |
| Del/Del | 11 | 12.89 | | | 37 | 24.26 | | |

**Table 2** The correlation between rs10680577 in the RERT-lncRNA and the risk of lung cancer

| Genotype | Case group ($n=376$) | Control group ($n=419$) | $\chi^2$ | $P$ | OR | 95% CI |
|---|---|---|---|---|---|---|
| Ins/Ins | 222 (59.04%) | 283 (67.54%) | | | Refer. | |
| Ins/Del | 117 (31.12%) | 125 (29.83%) | 1.270 | 0.260 | 1.193 | 0.877–1.623 |
| Del/Del | 37 (9.84%) | 11 (2.63%) | 19.310 | < 0.001 | 4.288 | 2.138–8.599 |
| Ins/Del + Del/Del | 154 (40.96%) | 136 (32.46%) | 6.178 | 0.013 | 1.443 | 1.080–1.929 |
| Allele | | | | | | |
| Ins | 561 (74.60%) | 691 (82.46%) | | | Refer. | |
| Del | 191 (25.40%) | 147 (17.54%) | 14.620 | < 0.001 | 1.600 | 1.256–2.039 |

*Refer.* reference

## The Correlation of rs10680577 in the RERT-lncRNA with the Clinicopathological Features of Patients with Lung Cancer

In the dominant models, the genotype frequencies of Ins/Del + Del/Del of rs10680577 were positively related to older age and smoking (all $P < 0.05$), but were unrelated to the histology or gender of patients with lung cancer (all $P > 0.05$). In addition, the Ins/Del + Del/Del genotype in rs10680577 was significantly associated with the tumor stages of NSCLC and SCLC (all $P < 0.05$, Table 3).

## Correlations of RERT-lncRNA and *EGLN2* Expressions with the Clinicopathological Features of Patients with Lung Cancer

In comparison with the adjacent normal tissues (ANT), the expressions of RERT-lncRNA and *EGLN2* were significantly elevated in a positive correlation (all $P < 0.05$, Fig. 2). Compared to the Ins/Ins genotype of rs10680577, lung cancer patients with the Ins/Del+Del/Del genotype carriers had increased expressions of RERT-lncRNA and *EGLN2* ($P < 0.05$, Fig. 3). Moreover, the expressions of RERT-lncRNA and *EGLN2* in tumor tissues were highly associated with age, smoking habits, and tumor stages (both SCLC and NSCLC) of lung cancer patients (all $P < 0.05$, Table 4).

## Correlations of Lung Cancer Patient's Prognosis with rs10680577 in RERT-lncRNA, and Expressions of RERT-lncRNA and *EGLN2*

During a 5-year follow-up of 376 patients, there were 12 patients loss to the follow-up (3.19%), with a median follow-up period of 41 month ($38.55 \pm 16.95$ months), and the 5-year-survival rate was 11.96%. Kaplan–Meier survival analysis found that the patients with the Ins/Ins genotype of rs10680577 in RERT-lncRNA had a longer OS than those patients carried with the Ins/Del + Del/Del genotype ($P < 0.05$, Fig. 4a). In addition, according to the median expressions of RERT-lncRNA and *EGLN2* in tumor tissues, the patients were divided into the high-expression group and low-expression group

**Table 3** Correlation of rs10680577 in the RERT-lncRNA with the clinicopathological features of patients with lung cancer

| Variables | *N* | Ins/Ins ($n=222$) | Ins/Del + Del/Del ($n=154$) | $\chi^2$ | *P* | OR | 95% CI |
|---|---|---|---|---|---|---|---|
| Age (year) | | | | | | | |
| ≤ 64 | 207 | 132 (59.46%) | 75 (48.70%) | Refer. | | | |
| > 64 | 169 | 90 (40.54%) | 79 (51.30%) | 4.253 | 0.039 | 1.545 | 1.021–2.338 |
| Gender | | | | | | | |
| Female | 130 | 72 (32.43%) | 58 (37.66%) | Refer. | | | |
| Male | 246 | 150 (67.57%) | 96 (62.34%) | 1.099 | 0.294 | 0.795 | 0.517–1.222 |
| Smoking habits | | | | | | | |
| Nonsmokers | 30 | 23 (10.36%) | 7 (4.55%) | Refer. | | | |
| Smokers | 346 | 199 (89.64%) | 147 (95.45%) | 4.187 | 0.041 | 2.427 | 1.014–5.809 |
| Histology | | | | | | | |
| Small cell (SCLC) | 94 | 50 (22.52%) | 44 (28.57%) | Refer. | | | |
| Non-small cell (NSCLC) | 282 | 172 (77.48%) | 110 (71.43%) | 1.774 | 0.183 | 0.727 | 0.454–1.164 |
| Tumor stage (by histology) | | | | | | | |
| SCLC | | | | | | | |
| Limited | 42 | 28 (56.00%) | 14 (31.82%) | Refer. | | | |
| Extensive | 52 | 22 (44.00%) | 30 (68.18%) | 5.537 | 0.019 | 2.727 | 1.171–6.351 |
| NSCLC | | | | | | | |
| I | 35 | 25 (14.53%) | 10 (9.09%) | Refer. | | | |
| II | 49 | 30 (17.44%) | 19 (17.27%) | 0.940 | 0.332 | 1.583 | 0.624–4.021 |
| III | 119 | 80 (46.51%) | 39 (35.45%) | 0.220 | 0.639 | 1.219 | 0.533–2.788 |
| IV | 79 | 37 (21.51%) | 42 (38.18%) | 5.913 | 0.015 | 2.838 | 1.205–6.682 |

*Refer.* reference

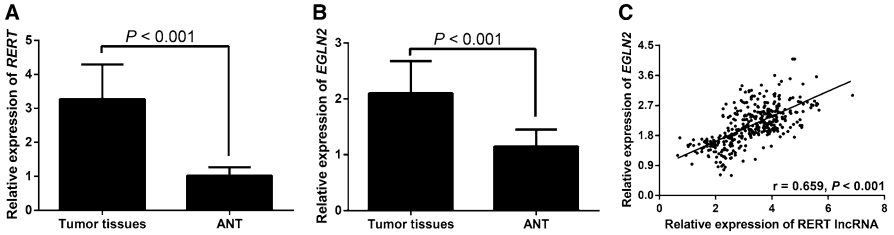


**Fig. 2** The expressions of RERT-lncRNA and *EGLN2* in tissues of patients with lung cancer. *Note* **a**, **b** The expressions of RERT-lncRNA (**a**) and *EGLN2* (**b**) in the tumor tissues and adjacent normal tissues (ANT) of patients with lung cancer; **c** the correlation analysis between the expressions of RERT-lncRNA and *EGLN2* in tumor tissues of patients with lung cancer

($\text{Median}_{\text{RERT-lncRNA}} = 3.285$, $\text{Median}_{EGLN2} = 2.090$), and the results indicated that the OS was shorter in patients with low expressions of RERT-lncRNA and *EGLN2* than that in patients with high expressions, respectively ($P < 0.05$, Fig. 4b, c). Multivariate analysis using Cox proportional hazards regression

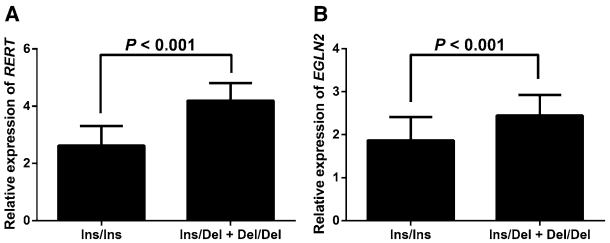


**Fig. 3** Comparisons of the RERT-lncRNA (**a**) and *EGLN2* (**b**) expressions in tumor tissues between lung cancer patients with different genotypes (Ins/Del +Del/Del vs. Ins/Ins) of rs10680577 in the RERT-lncRNA

**Table 4** Correlations of the expressions of RERT-lncRNA and *EGLN2* with the clinicopathological features of patients with lung cancer

| Variables | *N* | RERT lncRNA expression | *P* | *EGLN2* expression | *P* |
|---|---|---|---|---|---|
| Age (year) | | | | | |
| ≤ 64 | 207 | 2.88 ± 0.90 | | 1.92 ± 0.51 | |
| > 64 | 169 | 3.75 ± 0.96 | 5.22E−18 | 2.32 ± 0.58 | 7.00E−12 |
| Gender | | | | | |
| Female | 130 | 3.37 ± 1.02 | | 2.18 ± 0.57 | |
| Male | 246 | 3.22 ± 1.02 | 0.169 | 2.06 ± 0.58 | 0.061 |
| Smoking habits | | | | | |
| Nonsmokers | 30 | 2.36 ± 1.01 | | 1.85 ± 0.58 | |
| Smokers | 346 | 3.35 ± 0.98 | 2.15E−07 | 2.12 ± 0.58 | 0.015 |
| Histology | | | | | |
| Small cell (SCLC) | 94 | 3.32 ± 0.99 | | 2.08 ± 0.56 | |
| Non-small cell (NSCLC) | 282 | 3.25 ± 1.03 | 0.548 | 2.11 ± 0.59 | 0.675 |
| Tumor stage (by histology) | | | | | |
| SCLC | | | | | |
| Limited | 42 | 2.78 ± 0.92 | | 1.88 ± 0.6 | |
| Extensive | 52 | 3.76 ± 0.82 | 4.23E−07 | 2.24 ± 0.48 | 0.002 |
| NSCLC | | | | | |
| I | 35 | 2.23 ± 0.96 | | 1.75 ± 0.61 | |
| II | 49 | 2.66 ± 0.91* | | 1.83 ± 0.51 | |
| III | 119 | 3.30 ± 0.82*$^{\#}$ | | 2.12 ± 0.56*$^{\#}$ | |
| IV | 79 | 4.00 ± 0.80*$^{\#\&}$ | 9.64E−24 | 2.42 ± 0.5*$^{\#\&}$ | 1.02E−10 |

Compared with stage I, *$P < 0.05$; compared with stage II, $^{\#}P < 0.05$; compared with stage III, $^{\&}P < 0.05$

model suggested that the Ins/Del + Del/Del genotype of rs10680577 and higher expressions of RERT-lncRNA and *EGLN2* were the risk factors influencing the prognosis of patients with lung cancer ($P < 0.05$, Table 5).

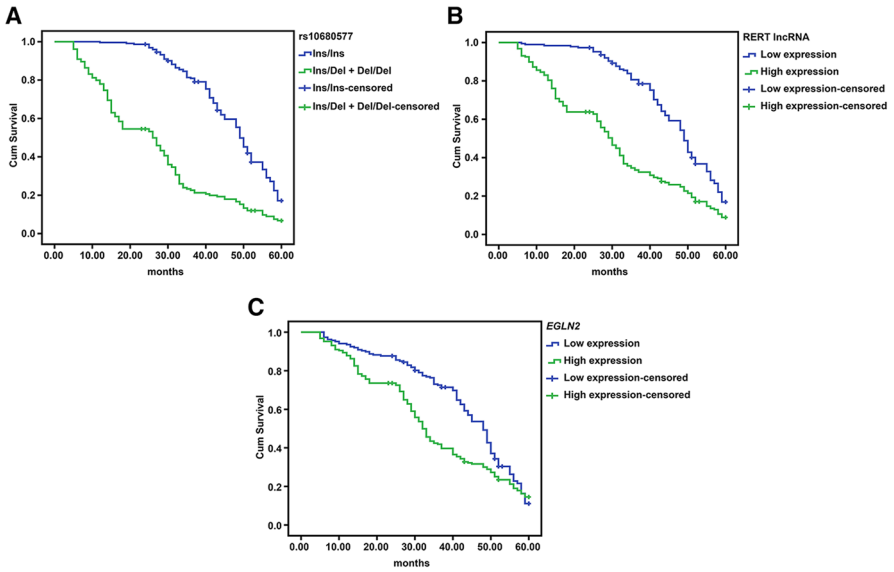


**Fig. 4** Correlations of rs10680577 in RERT-lncRNA (**a**) and the expressions of RERT-lncRNA (**b**) and *EGLN2* (**c**) with the prognosis of patients with lung cancer

**Table 5** Multivariate analysis of the risk factors influencing the prognosis of patients with lung cancer by means of Cox's proportional hazards regression model

| Variables | *B* | SE | Wald | *P* | OR | 95% CI for OR | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper |
| Age | 0.060 | 0.137 | 0.193 | 0.559 | 1.062 | 0.811 | 1.390 |
| Smoking (smokers vs. nonsmokers) | 0.170 | 0.216 | 0.618 | 0.437 | 1.185 | 0.776 | 1.809 |
| Gender | 0.169 | 0.120 | 2.003 | 0.129 | 1.185 | 0.937 | 1.498 |
| Histology (NSCLC vs. SCLC) | − 0.039 | 0.133 | 0.085 | 0.860 | 0.962 | 0.741 | 1.248 |
| *EGLN2* | 0.325 | 0.139 | 5.488 | 0.019 | 1.384 | 1.054 | 1.816 |
| RERT | 0.295 | 0.117 | 6.370 | 0.012 | 1.343 | 1.068 | 1.690 |
| rs10680577 (Ins/Del + Del/Del vs. Ins/Ins) | 0.794 | 0.195 | 16.614 | < 0.001 | 2.213 | 1.510 | 3.243 |

## Discussion

LncRNAs, a class of nonprotein-coding RNAs, are generally accepted as the crucial regulators involving in a wide range of biological functions in humans, and the dysregulated lncRNAs results in cancer development (Hamann et al. 2017). There were some evidences to suggest that polymorphisms located in the major region of these RNAs could severely affect the function of the corresponding RNAs (Hu et al. 2017; Pan et al. 2016), raising the possibility that polymorphism is likely one of the factors destructing the structure of lncRNAs to induce diseases.

In this study, the Del allele of RERT-lncRNA rs10680577 could increase the risk of lung cancer. Similar to this discovery, a previous study also found that in the co-dominant model, subjects with the heterozygous Ins/Del or homozygous Del/Del genotype could increase the CRC risk (Li et al. 2017). Moreover, a meta-analysis conducted by Hashemi et al. revealed the rs10680577 significantly increased risk of cancer in codominant, dominant, overdominant, and allele inheritance genetic models in Asian population (Hashemi et al. 2018b), including breast cancer in a southeast Iranian population (Hashemi et al. 2018a). In addition, rs10680577 was associated with the smoking habits in our study, which might be potentially caused by the location of rs10680577 on chromosome 19q13, while polymorphisms in 19q13 locus have been shown to be related to smoking behaviors, as indicated by the previous study (Cho et al. 2012). For instance, rs7937, a top genetic variant in 19q13, elevated the susceptibility to chronic obstructive pulmonary disease (COPD), since chromosome 19q13 region was relevant to both smoking and COPD (Nedeljkovic et al. 2018). In the study of UllaVogel et al., two regions (like XPD Asp312Asn and Lys751Gln) in chromosome 19q13 were suggested to be associated with risk of lung cancer (Vogel et al. 2004). On the other hand, smoking has been regarded as the leading risk factor giving rise to lung cancer. Taken together, the mutation of rs10680577 at 19q13 in our study might be associated with the smoking-induced diseases, including lung cancer. In addition, the patients older than 60 years showed stronger association between the genotype of rs10680577 and the risk of lung cancer, as shown by Che et al. (2014). Accordingly, our study indeed found that Ins/Del+Del/Del genotype of rs10680577 was correlated with the age, tumor stage, and prognosis of patients with lung cancer.

Besides, polymorphisms in lncRNAs genes may generate large effect on the gene structure and alter the regulation of downstream tumor-associated gene expressions, ultimately contributing to the malignant transformation of normal cells (Hu et al. 2017). For example, Pan et al. reported that the functional polymorphism in *HOTAIR* may affect HOTAIR expression and/or its function, and to be specific, the T allele carriers of rs920778 within *HOTAIR* results in an elevated expression of HOTAIR in both gastric cancer tissues and cell lines (Pan et al. 2016). In this study, lung cancer patientswith Ins/Del + Del/Del genotype carriers of rs10680577 exhibited the upregulation in RERT-lncRNA and *EGLN2* comparedto those with Ins/Ins genotype carriers. rs10680577, located in the intronic region of RERT-lncRNA, may alter the structure of RERT-lncRNA to regulate the RERT-lncRNA that, in turn, mediates the modulation of *EGLN2* (Zhu et al. 2012), one of the three prolyl hydroxylases that could mediate the ubiquity nation and HIF degradation through hydroxylation of α subunit of HIF. It is noteworthy that the role of *EGLN2*, as oncogenes or tumor suppressor, largely depends on cell type or types of tumors, which has been illustrated in previous studies. The expression of *EGLN2* was downregulated in CRC in the study of Rawluszko et al.(2013), but was increased in papillary subtype of renal cell carcinoma (pRCC) in comparison with the normal kidney (Kaufmann et al. 2013) and played an oncogenic role in prostate cancer (Zhang et al. 2017). Results of this study indicated that the expressions of RERT-lncRNA and *EGLN2* were increased in tumor tissues, exhibiting a positive correlation, as detected by a qRT-PCR analysis, highlighting the potential oncogene role of *EGLN2*

in lung cancer. In support of this finding, we supposed that *EGLN2* might increase HIF-1α to be involved in tumor development, leading to the upregulation of HIF-1α-regulated gene expression of VEGF, thereby contributing to the angiogenesis in tumors (Ma et al. 2014; Sasaki et al. 2008). Moreover, the expressions of RERT-lncRNA and *EGLN2* in tumor tissues were found to be related to the age, smoking habits, and tumor stages of lung cancer. Also, the Cox regression analysis revealed that rs10680577 polymorphism and the expressions of RERT lncRNA and *EGLN2* were risk factors affecting the prognosis of lung cancer patients, further implicating that rs10680577 affects the risk of development and prognosis of lung cancer possibly through altering the expressions of RERT lncRNA and *EGLN2*. However, this study also has some limitations: despite the strong correlation between rs10680577 and RERT-lncRNA expressions, as well as between the RERT-lncRNA and *EGLN2* expressions, we have not yet figured out how this polymorphism affects the expressions of RERT-lncRNA and *EGLN2*, which requires further studies on the genetic and functional levels.

In summary, the Del allele of rs10680577 increased the risk of lung cancer. In addition, RERT-lncRNA and *EGLN2* are upregulated in lung cancer tissues in a positive correlation. Moreover, patients with Ins/Del + Del/Del genotype had the higher expressions of RERT-lncRNA and *EGLN2* than those Ins/Ins genotype carriers, and rs10680577, as well as the expressions of RERT-lncRNA and *EGLN2* were linked to the major clinicopathological features of lung cancer patients, which were the risk factors affecting patients' prognosis.

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## Compliance with Ethical Standards

**Conflict of interest** The authors have no conflict of interests to declare.

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