Genetic variation in an MiRNA-1827 binding site in MYCL1 alters susceptibility to small cell lung cancer

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Running title: MYCL1 polymorphism and small-cell lung cancer risk

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Abstract

Genetic variations in microRNAs (miRNAs) that affect control of their target genes may alter individual susceptibilities to cancer. In this study, we took an in silico approach to identify single nucleotide polymorphisms (SNPs) within the 3′ UTR of miRNA genes deregulated in human small-cell lung cancer (SCLC), and then investigated their associations with SCLC susceptibility in 666 SCLC patients and 758 controls. Odds ratios (ORs) were estimated by multivariate logistic regression and biochemical assays was performed to investigate SNP functions. We identified two SNPs, rs3134615 and rs2291854, which were located in the 3′ UTR of the L-MYC gene MYCL1 and the neuronal development Achaete-Scute Complex homolog ASCL1. Case-control analyses showed that the rs3134615T allele was associated with a significantly increased risk of SCLC, with the OR for carrying the GT or TT genotype being 2.08 (95% CI, 1.39–3.21; \( P = 0.0004 \)) compared with the GG genotype. In support of the likelihood that these 3′ UTR SNPs may directly affect miRNA binding sites, reporter gene assays indicated MYCL1 as the target of hsa-miR-1827 and the rs3134615 G>T change resulted in altered regulation of MYCL1 expression. Our findings define a 3′ UTR SNP in the human L-MYC oncogene that may increase susceptibility to SCLC, possibly resulting from attenuated interaction with the miRNA hsa-miR-1827.
Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide (1). Small-cell lung cancer (SCLC) is the most aggressive subtype of lung cancer, characterized by rapid doubling time, high growth fraction, and early development of widespread metastases (2). As other common cancers, SCLC is a complex trait caused by both genetic and environmental factors. Smoking is a well-known risk environmental factor for SCLC; however, little has been known about the genetic factors associated with the development of SCLC. It has been reported previously that the *CYP3A4*1B variant and *MPO* promoter −463G variant are associated with increased risk of developing SCLC (3, 4). Recently, several genome-wide association studies have been conducted to explore the common genetic variations associated with susceptibility to lung cancer (5-9); however, most of the cases were limited to non-small cell lung cancer (NSCLC). Among the identified risk loci, only one variant at 5q15.33 was found to significantly influence the risk of lung cancer differentially by histology, implying that genetic factors associated with the risk of SCLC and NSCLC may be different (10, 11). Therefore, it is warranted that more efforts should be made to identify the risk genetic factors involved in the etiology of SCLC.

The specific molecular mechanisms leading to the development of SCLC have not been fully elucidated. However, aberrant expression of some genes taking part in the processes that mediate proliferation, anti-apoptosis, angiogenesis, and metastasis has been implicated in tumorigenesis of SCLC (12). MicroRNAs (miRNA) are a class of small noncoding RNA molecules that regulate gene expression through binding the 3′untranslated region (3′UTR) of mRNAs of their target genes, resulting in mRNA cleavage or translation repression (13). It is estimated that about 30% of human genes are regulated by miRNAs (14). As a result, miRNAs may play important roles in tumorigenesis by regulating the expressions of proto-oncogenes or tumor suppressor genes (15). It has been proposed that single nucleotide polymorphisms (SNPs) located in the 3′UTR of miRNA target genes might affect...
miRNA-mediated gene regulation and are thus associated with individual susceptibility to cancer development (16). On the basis of this postulation, we hypothesized that SNPs located in the 3′UTR of genes deregulated in SCLC might be associated with susceptibility to SCLC.

In this study, we used public databases to identify SNPs in the 3′UTR of genes deregulated in SCLC that have potential to affect miRNA binding, and examined their association with risk of developing SCLC in a case-control analysis. Furthermore, we conducted a set of functional assays to assess the effects of these SNPs on the regulation of target genes.

Materials and Methods

Study subjects

We designed a case-control analysis consisting of 666 patients with SCLC and 758 controls from a Han Chinese population. Patients were recruited between June 1999 and May 2008 at the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing). All patients with cytologically or histologically confirmed SCLC were enrolled with a response rate of 93%. Population controls were accrued from a community nutritional survey conducted in the same region during the period of patient collection. The controls were randomly selected from a database consisting of 2,500 individuals based on a physical examination with the participation response rate being 91%. The selection criteria of control subjects included no history of cancer and frequency-matching to patients with SCLC in sex and age (±5 years). At recruitment, informed consent was obtained from each participant and each participant was then interviewed to collect detailed information on demographic characteristics, the history of tobacco smoking exposure and clinical characteristics. This study was approved by the Institutional Review Board of the Chinese Academy of Medical Sciences Cancer Institute.

SNP selection and genotyping

In 2009 we queried against the cancer-related microarray databases using web-based
NextBio software (www.nextbio.com) to find genes deregulated at the RNA level in SCLC and used existing Patrocles database (www.patrocles.org) to identify putative SNPs within the miRNA-targeting site of the 3′-UTR of interest genes (17, 18). We identified 26 genes that are considerably deregulated in SCLC when set the cutoff score as >85 (using Nextbio, a higher score means that a gene is more substantially associated with a disease). Among these genes, 17 had a total of 53 putative SNPs located in the 3′UTR that might create or destroy miRNA-binding sites (Supplementary Table 1). We chose the SNPs for further analysis according to their minor allelic frequency (MAF) ≥5% in Han Chinese population (HapMap data release 21a) and with this criterion, only two SNPs, rs3134615 G>T in the 3′UTR of MYCL1 (L-MYC) and rs2291854 C>T in the 3′UTR of mammalian achaete-scute complex homologue 1 (MASH1 or ASCL1), were finally selected. Genomic DNA isolated from peripheral blood lymphocytes of the study subjects were used for genotyping by PCR-based restriction fragment length polymorphism (RFLP) assays. The PCR primers for amplifying DNA containing rs3134615 G>T or rs2291854 C>T were F5′-gtactgctgcctttccagct-3′/R5′-agccagcaagctccttggaatt-3′ and F5′-gtactgagacgaaagacac-3′/R5′-tatgaagagcaactgggac-3′, respectively. PCR products containing rs3134615 G>T or rs2291854 C>T were respectively digested with PvuII or MseI (New England BioLabs, Beverly, MA) and separated on 3.0% agarose gel. Genotypes revealed by PCR-RFLP were further confirmed by directly DNA sequencing of the PCR products. Genotyping was performed without knowledge of subjects’ case/control status. A 10% random sample of cases and controls was tested twice by different investigators (F. Xiong and J. Chang) to ensure quality control, and the results were identical.

**MYCL1 3′UTR reporter gene constructs**

The full-length of human MYCL1 3′UTR containing the rs3134615 G allele was amplified with primer pair of 5′-ctgaccaaaaagcctgacagttct-3′/5′-catgatatgacatccttttattaga-3′ from a rs3134615 GG homozygous human genomic DNA sample. The PCR products were
separated in agarose gel and extracted, purified and cloned with TA cloning kit (TaKaRa, Dalian, China). The insert containing the G allele was confirmed by sequencing. It was subsequently used as a template to generate the insert containing the T allele by using site-specific mutagenesis kit (Sbsgene, Shanghai, China) with primers of
\[5'-\text{tgccctttcacctgctcaatcct}-3' \quad \text{and} \quad 5'-\text{aaggattgagatgaggca}-3'.\]
The vector pGL3m modified from pGL3-control (Promega, Madison, WI) by creating EcoRV, ApaI, SacII, NdeI, PstI, EcoRI, and NruI sites immediately downstream the XbaI site was kindly provided by Prof. Z. Liang of Peking University (19). The TA clone containing the rs3134615 G or T allele was digested with NdeI and EcoRI, and then directionally cloned into pGL3m vector. The resulting constructs were named as pGL3m-MYCL1-3'UTR-T and pGL3m-MYCL1-3'UTR-G. Both constructs used in this study were restriction mapped and sequenced to confirm their authenticity.

**Transient transfection and luciferase assays**

H446 (a human small-cell lung cancer cell line) and HeLa (a human cervical carcinoma cell line) cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) in 2003 and 2000, respectively. Cells were resuscitated and cultured according to ATCC guidelines for less than 4 months before use. The authentication of these cell lines was provided by ATCC through short tandem repeat profiling, karyotyping, and cytochrome c oxidase I testing. Cells cultured in RPMI 1640 medium with 10% fetal bovine serum were seeded in 96-multiwell plates and when grown to 80% confluence, they were transfected with the constructed reporter plasmid (200 ng), 0 or 40 nM hsa-miR-1827 mimic, and the negative control RNA duplex (GenePharma, Brussels, Belgium), respectively, using lipofectamine 2000 (Invitrogen, Karlsbad, CA). The pRL-SV40 (2 ng; Promega), containing Renilla reniformis luciferase, was cotransfected to standardize transfection efficiency. Reporter expression was analyzed using the Dual-Luciferase Reporter Assay (Promega). For each plasmid construct, three independent transfection experiments were conducted, and each was
done in six replicates. The empty pGL3m vector cotransfected with pRL-SV40 plasmid was served as a reference. Fold increase was calculated by defining the activity of empty pGL3m vector as 1. Differences were determined by $t$-test and $P<0.01$ was considered significant.

**Transfection of hsa-miR-1827 and real-time analysis of MYCL1 RNA**

Hsa-miR-1827 mimic and the negative control RNA duplex were transfected into H446 cells seeded in 6-well plates using lipofectamine 2000 (Invitrogen) and cells were harvested 48 h after transfection and RNAs were isolated. cDNA was synthesized using PrimeScript™ 1st strand cDNA Synthesis Kit (TaKaRa). MYCL1 RNA was measured by real-time quantitative reverse transcription PCR using the ABI Prism7900 sequence detection system (Applied Biosystems, Foster City, CA) based on the SYBR-Green method (TaKaRa). Each assay was done in triplicate from two independent transfection experiments, and the expression levels of MYCL1 RNA was calculated relative to expression of GAPDH.

**Statistical analysis**

We used $\chi^2$ or Student's $t$ test to examine the differences in the distributions of demographic characteristics, selected variables, and genotype frequencies between cases and controls. Light and heavy smokers were categorized by using the 50th percentile pack-year values [pack-years = (cigarettes per day/20) × (years smoked)] of the controls as the cutoff points. The SCLC risk associated with genotype was estimated as odds ratios (ORs) and 95% confidence intervals (CIs) computed using logistic regression model adjusted for age, sex, and smoking status where it was appropriate. A homogeneity test was done to compare the difference between smoking-related ORs among different genotypes. A multiplicative gene-smoking interaction was evaluated by logistic regression analysis including main effect variables and their product terms. The differences in the expression levels of MYCL1 among subgroups were examined using $t$-test. All statistical tests were two-sided with $P<0.05$ as the significant level unless those specified. Statistical analyses were done using Statistical Analysis System software (version 9.0; SAS Institute, Cary, NC).
Results

The distribution of demographic and clinical characteristics of 666 SCLC cases and 758 controls are shown in Table 1. No differences in the distributions of age and sex were found between case patients and controls. However, more smokers and heavy smokers were presented in case patients than in controls.

The genotype distributions of rs3134615 G>T and rs2291854 C>T in control subjects conformed to the Hardy-Weinberg equilibrium. The frequencies of rs3134615 GG, GT, and TT genotypes were 89.2%, 10.5%, and 0.3% in patients compared with 94.6%, 5.4%, and 0% in controls (P=0.0005; Table 2). Logistic regression analysis revealed that subjects with the rs3134615 T allele (TG and TT genotypes) had 2.08-fold increased risk for the development of SCLC (95% CI, 1.39–3.12; P=0.0004) compared with the rs3134615 GG genotype. However, the genotype distributions of rs2291854 SNP in case patients and controls was not significantly different (P=0.409).

Stratification analysis showed an increased risk of SCLC associated with the rs3134615 T allele (GT and TT genotypes) in both smokers and non-smokers (Table 3). In smokers, the OR for combined rs3134615 GT and TT genotypes was 3.15 (95% CI, 1.86–5.32), significantly higher than that for the rs3134615 GG genotype (OR=1.63, 95% CI, 1.27–2.07; P=0.0042 for homogeneity test). In non-smokers, the variant GT and TT genotypes of rs313461 SNP contributed to 2.01-fold (95% CI, 1.03–3.92) increased risk for the cancer. These findings suggest that rs3134615 SNP is a risk factor for the development of SCLC independent on smoking. No significant differences were detected in terms of odds ratio for rs2291854 genotypes in both smokers and non-smokers, consistent with the result showing no association between this SNP alone and risk of SCLC. No significant interaction between these two SNPs and smoking was found in this study (Table 3).

In silico analysis showed that the rs3134615 SNP lies within a binding site for the seed region of hsa-miR-1827 and the G allele matches the predicted hsa-miR-1827 seed binding
domain, whereas the T allele represents a C:U mismatch base pairing (Figure 1a). We hypothesized that hsa-miR-1827 would bind *MYCL1* mRNA transcripts containing the G allele, negatively regulating *MYCL1* expression. On the other hand, binding of hsa-miR-1827 to mRNA containing the T allele would be disrupted, allowing upregulation of *MYCL1* expression. To test this postulation, we cloned the full length of *MYCL1* 3′UTR fragments containing rs3134615 T or G allele into the luciferase reporter vector pGL3m to compare the luciferase activities between the two alleles (Figure 1a). We found that H446 cells transfected with vectors containing the rs3134615 G allele of the *MYCL1* 3′UTR yielded a 22% reduced luciferase activity compared to cells transfected with vectors containing the T allele counterpart. However, no significant difference in the luciferase activity was found between the two vectors transfected into HeLa cells (Figure 1b).

To further verify whether the rs3134615 SNP may affect the ability of the *MYCL1* 3′UTR to interact with hsa-miR-1827, the pGL3m vectors containing the rs3134615 T or G allele of the *MYCL1* 3′UTR were cotransfected in parallel with hsa-miR-1827 mimic or the negative control into the cells. Luciferase assays showed that hsa-miR-1827 suppressed luciferase expression more efficiently for the rs3134615G-containing vector than the rs3134615T-containing counterpart in H446 cell line (56% vs. 44%, *P*<0.001). Similar effect was also observed in HeLa cell line (Figure 1c). We also transiently transfected hsa-miR-1827 mimic or the negative control into H446 cell line, a rs3134615 GG genotype carrier, and measured the endogenous *MYCL1* transcript levels in these cells. Compared to transfection with the negative control, transfection with hsa-miR-1827 mimic significantly decreased the level of *MYCL1* mRNA expression in H446 cells (*P*<0.001; Figure 1d).

**Discussion**

In the present study, we have examined two SNPs, rs3134615 and rs2291854, located respectively in the 3′UTR of the *MYCL1* and *ASCL1* genes that are deregulated in SCLC, and
identified rs3134615 SNP in the hsa-miR-1827 complementary site of MYCL1 3’UTR associated with increased risk for developing SCLC in a Chinese population. A set of functional assays in vitro and in cells demonstrated that MYCL1 might be targeted by hsa-miR-1827, which negatively regulates MYCL1 expression. The rs3134615 G to T change may inhibit the interaction of hsa-miR-1827 with MYCL1 3'UTR, resulting in higher constitutive expression of MYCL1. Because MYCL1 is a member of MYC oncogene family that play a critical role in carcinogenesis, individuals carrying the rs3134615 T allele would be expected to have elevated risk for the development of SCLC.

MYCL1 is located on chromosome 1p34 with structural similarity to MYC (C-MYC) and MYCN (N-MYC) (20, 21). The expression of MYCL1 is limited to normal lung tissues (22) and some types of cancer including SCLC and Merkel cell carcinoma (23, 24); both are neuroendocrine cancer. It has been shown that a majority of human SCLC cell lines have MYCL1 amplification and expression (25). The specific expression of MYCL1 in lungs suggests that it may have a special role in the etiology of SCLC. Although the function of MYCL1 in human carcinogenesis remains to be fully elucidated, a growing body of evidence suggests that it may function differently from MYC and MYCN, two well-characterized oncogenes in the same family. It has been shown in transgenic mice that constitutive expression of MYC inhibits proliferative arrest of lens fiber cells, while overexpression of MYCL1 directly affects differentiation processes of the cells (26). The impact of MYCL1 on cell differentiation might also be relevant to the development of SCLC because poor differentiation or undifferentiation is a major nature of this cancer.

Our reporter gene assays revealed that the presence of a hsa-miR-1827 binding site in the 3'UTR of MYCL1 significantly reduces the expression of luciferase carrying the rs3134615 G 3'UTR compared with that carrying the rs3134615 T 3'UTR, which is consistent with in silico analysis assuming the functional interaction between hsa-miR-1827 and MYCL1 mRNA. Furthermore, transfection of H446 cells carrying the rs3134615 G allele with hsa-miR-1827
mimic also showed substantial inhibition of the constitutive expression of MYCL1 mRNA. These biochemical findings suggest that hsa-miR-1827 is a post-transcriptional regulator of MYCL1 expression. These findings are also in agreement with the results of case-control analysis showing that the MYCL1 3'UTR rs3134615 T is the risk allele for the development of SCLC. Taken together, we conclude that the association between risk of developing SCLC and the rs3134615 SNP in the 3'UTR of the oncogene MYCL1 may be mediated by the miRNA hsa-miR-1827.

Although ASCL1 also displays high expression in SCLC and is thought to be involved in the development of this cancer (27, 28), we did not found any significant association between the rs2291854 SNP in the 3'UTR of this gene and risk of developing SCLC. This result may indicate that despite of located in the 3'UTR, the rs2291854 SNP is not functionally relevant to ASCL1 post-transcriptional regulation and therefore is not associated with the disease susceptibility. Alternatively, this result might imply that although ASCL1 is overexpressed in SCLC, it may not be involved in carcinogenesis but contribute to the promotion and biological behavior of the cancer, as it has been proposed by other investigators (29, 30).

Increasing evidence demonstrates that SNPs in the 3'UTR of genes targeted by miRNAs can disturb or obstruct miRNAs binding and consequentially influence regulation of target genes, which might be associated with susceptibility to certain diseases including cancer (31). In the present study, we referred to the databases of gene expression prolife to identify genes that are deregulated in SCLC and their SNPs in the 3'UTR. Because we queried these deregulated genes in 2009, new candidate SCLC dysregulated genes such as SCLC1 and IGHG1 identified afterward were not assessed in this study. Among the 53 putative SNPs, we only found two SNPs having MAF ≥5% in Chinese population based on the HapMap CHB database. Genotyping of 1424 subjects (666 cases and 758 controls) revealed that the MAFs for rs3134615 and rs2291854 in our study population were 4.2% and 10.2%. Previous studies have shown that the vast majority of SNPs in miRNAs target sequences appear to be evolving...
under neutrality and to be at relatively low population frequencies (32, 33). This might be relevant to the observation that none of SNPs in miRNAs target sequences has ever been identified as susceptibility locus for common diseases including lung cancer in genome-wide association studies based on common SNPs. The inclusion of rare SNPs in miRNAs target sequences in future genome-wide association studies would help to unveil low-penetrance susceptibility loci of diseases.

In conclusion, we have identified the rs3134615 SNP in the miRNA binding site of oncogene MYCL1 that is associated with the risk for the development of SCLC. This SNP may prevent miRNAs hsa-miR-1827 from binding to MYCL1 mRNA, resulting in altered regulation of oncogene MYCL1 expression. Based on these findings, it would be reasonable to analogize that miR-1827 may play a role in lung carcinogenesis functioned as a tumor suppressor and further studies of this miRNA in cancer are warranted. Because SCLC is one of the most aggressive cancers with very poor prognosis, clear understanding of genetic factors for the development of this cancer is important. Our results provide a new insight into SCLC tumorigenesis and have potential implication in early detection and target treatment of SCLC.

Reference


Figure Legend

Figure 1. Characterization and functional analysis of the MYCL1 3′UTR. a, predicted effect of allelic variation at rs3134615 on hsa-miR-1827 recognition and the construct of pGL3m-MYCL1-3′UTR-G/T containing renilla luciferase gene and full-length 3′UTR of MYCL1 gene with different alleles of rs3134615. SNP rs3134615 occurs in the 8 bp seed sequence of complementarity at hsa-miR-1827 5′ end. Base pairing is indicated by a solid (Watson-Crick) vertical line. b, luciferase reporter assays to measure G/T allele difference at rs3134615. H446 or HeLa cells were transiently transfected with G- or T-containing reporters. After 48 h, Renilla luciferase activity was measured and normalized to Firefly luciferase. Results are shown as percentage relative to luciferase activity. Data are from three independent transfection experiments with assays performed in six replications. *, P<0.05. c, luciferase reporter assays to measure G or T allele difference at rs3134615 with the presence of hsa-miR-1827. Cells were transiently cotransfected with constructs and 40 nM hsa-miR-1827 mimic or negative control (NC). Results are shown as relative luciferase activity versus NC. Data are from three independent transfection experiments with assays performed in six replications. **, P<0.001. d, down regulation of MYCL1 mRNA by hsa-miR-1827 in H446 cells. Cells were transfected with hsa-miR-1827 mimic and RNAs were harvested 48 h after transfection. cDNA was synthesized and used for real-time RT-PCR analysis of MYCL1 mRNA normalized to a GAPDH standard. Expression levels are relative to the expression of H446 cells. Data are from two independent transfection experiments with assays performed in triplicate. **, P<0.001.
Table 1. Baseline clinical characteristics of patients with small-cell lung cancer and controls in a Chinese population, Cancer Hospital, Beijing, 1999–2008

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 666)</th>
<th>Controls (n = 758)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>483 (72.5)</td>
<td>563 (74.3)</td>
</tr>
<tr>
<td>Female</td>
<td>183 (27.5)</td>
<td>195 (25.7)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>181 (27.2)</td>
<td>235 (31.0)</td>
</tr>
<tr>
<td>50–65</td>
<td>356 (53.4)</td>
<td>383 (50.5)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>129 (19.4)</td>
<td>140 (18.5)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>252 (37.8)</td>
<td>359 (47.4)</td>
</tr>
<tr>
<td>Ever</td>
<td>414 (62.2)</td>
<td>399 (52.6)</td>
</tr>
<tr>
<td><strong>Smoking level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;27 pack-years</td>
<td>133 (32.1)</td>
<td>200 (50.1)</td>
</tr>
<tr>
<td>≥27 pack-years</td>
<td>281 (67.9)</td>
<td>199 (49.9)</td>
</tr>
<tr>
<td><strong>Tumor stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limited Stage</td>
<td>227 (34.1)</td>
<td></td>
</tr>
<tr>
<td>Extensive Stage</td>
<td>327 (49.1)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>112 (16.8)</td>
<td></td>
</tr>
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</table>

Table 2. Genotype frequencies of the two SNPs in patients with small-cell lung cancer and controls in a Chinese population and their association with risk of SCLC, Cancer Hospital, Beijing, 1999–2008

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n = 666)</th>
<th>Controls (n = 758)</th>
<th>OR (95% CI)*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3134615</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>594 (89.2)</td>
<td>717 (94.6)</td>
<td>1.00 (Reference)</td>
<td></td>
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<tr>
<td>GT</td>
<td>70 (10.5)</td>
<td>41 (5.4)</td>
<td>2.03 (1.35–3.05)</td>
<td>0.0007</td>
</tr>
<tr>
<td>TT</td>
<td>2 (0.3)</td>
<td>0 (0.0)</td>
<td>NC†</td>
<td>NC</td>
</tr>
<tr>
<td>GT + TT</td>
<td>72 (10.8)</td>
<td>41 (5.4)</td>
<td>2.08 (1.39–3.12)</td>
<td>0.0004</td>
</tr>
<tr>
<td>rs2291854</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>529 (79.4)</td>
<td>623 (82.2)</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>127 (19.1)</td>
<td>126 (16.6)</td>
<td>1.24 (0.94–1.23)</td>
<td>0.128</td>
</tr>
<tr>
<td>TT</td>
<td>10 (1.5)</td>
<td>9 (1.2)</td>
<td>1.54 (0.62–3.85)</td>
<td>0.356</td>
</tr>
<tr>
<td>CT + TT</td>
<td>137 (20.6)</td>
<td>135 (17.8)</td>
<td>1.26 (0.96–1.64)</td>
<td>0.096</td>
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</tbody>
</table>

* Adjusted for age, sex and smoking status.
†Not calculated.
Table 3. Risk of small-cell lung cancer associated with the two SNPs by smoking in a Chinese population, Cancer Hospital, Beijing, 1999–2008

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nonsmokers</th>
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<th>Smokers</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.*</td>
<td>OR (95% CI) †</td>
<td>No.*</td>
<td>OR (95% CI) †</td>
</tr>
<tr>
<td>rs3134615</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>230/343</td>
<td>1.00 (Reference)</td>
<td>364/374</td>
<td>1.63 (1.27–2.07)</td>
</tr>
<tr>
<td>GT + TT</td>
<td>22/16</td>
<td>2.01 (1.03–3.92)</td>
<td>50/25</td>
<td>3.15 (1.86–5.32) §</td>
</tr>
<tr>
<td>rs2291854</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>193/286</td>
<td>1.00 (Reference)</td>
<td>336/337</td>
<td>1.77 (1.36–2.31)</td>
</tr>
<tr>
<td>CT + TT</td>
<td>59/73</td>
<td>1.21 (0.82–1.78)</td>
<td>78/62</td>
<td>2.13 (1.42–3.21) ‡</td>
</tr>
</tbody>
</table>

*Number of patients/number of controls.  
†Adjusted for age and sex.  
‡P = 0.9754, test for interaction between genotype and smoking.  
§P = 0.9173, test for interaction between genotype and smoking.
Xiong et al Fig. 1

(a) Hsa-miR-1827

MYCL1-G allele

MYCL1-T allele

Hsa-miR-1827

UAAGUUGAUAGACGGAGU

GCTGCTCTTTCCACCTGCTCTCA

GCTGCTCTTTCCACCTTCTCTCA

UAAGUUGAUAGACGGAGU

Luciferase → MYCL1 3'UTR → pGL 3m

Luciferase → MYCL1 3'UTR → pGL 3m

(b) Graph comparing T allele and G allele for HeLa and H446.

(c) Graph comparing NC and miR-1827 for HeLa and H446.

(d) Graph showing normalized expression for NC and miR-1827.
Genetic variation in an MiRNA-1827 binding site in MYCL1 alters susceptibility to small cell lung cancer

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