BRIEF REPORT

**TSC2 genetic variant and prognosis in non-small cell lung cancer after curative surgery**

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**Abstract**
This study was conducted to investigate the associations between polymorphisms of genes involved in the LKB1 pathway and the prognosis of patients with non-small cell lung cancer (NSCLC) after surgical resection. Twenty-three single nucleotide polymorphisms (SNPs) in the LKB1 pathway were investigated in 782 patients with NSCLC who underwent curative surgery. The association of SNPs with overall survival (OS) and disease-free survival (DFS) were analyzed. Among the 23 SNPs investigated, **TSC2 rs30259G > A** was associated with significantly worse OS and DFS (adjusted hazard ratio for OS 1.88, 95% confidence interval 1.21–2.91, \(P = 0.005\); adjusted hazard ratio for DFS 1.65, 95% confidence interval 1.15–2.38, \(P = 0.01\), under codominant models, respectively). Subgroup analysis showed that SNPs were significantly associated with survival outcomes in squamous cell carcinoma, ever-smokers, and stage I, but not in adenocarcinoma, never-smokers, and stage II–IIIA. The results suggest that **TSC2 rs30259G > A** may be useful to predict prognosis in patients with NSCLC, especially squamous cell carcinoma, after curative surgery.

**Introduction**
Lung cancer is the leading cause of cancer death worldwide.\(^1\) Despite complete resection as a potentially curative treatment in early stage non-small cell lung cancer (NSCLC), many patients experience recurrence and death during follow-up. Furthermore, patients with the same pathologic stage, the single most important prognostic factor, exhibit different recurrence and mortality rates.\(^2\) Thus, the identification of novel biomarkers for more precise prognostication after curative surgery in NSCLC patients would be very helpful.

The molecular mechanism associated with a connection between cellular metabolism and tumorigenesis is an active area of investigation in cancer research. The serine/threonine kinase liver kinase B1 (LKB1) is one of the recently discovered links connecting cell metabolism and cancer.\(^3\) LKB1, also known as serine/threonine kinase 11 (STK11), acts as a master upstream activator of AMP-activated protein kinase (AMPK) upon metabolic stress, such as energy...
starvation, playing a crucial role in cell growth, polarity, and energy metabolism. The LKB1-AMPK pathway functions as a metabolic checkpoint in the cell, regulating cell growth and proliferation according to the availability of nutritional supplies. LKB1 was first identified as the tumor suppressor gene associated with Peutz–Jeghers syndrome, a cancer predisposition syndrome, and its somatic mutation has been implicated in multiple sporadic cancers, including lung cancer. More recent studies have suggested that LKB1 loss has a considerable impact not only on tumorigenesis, but also on cancer invasion and metastasis. Although not yet fully elucidated, the relationship between LKB1 dependent molecular pathways and cancer may help us to better understand the pathogenesis of cancer, providing potential prognostic biomarkers or therapeutic targets.

In this study, we investigated if genetic variants in the LKB1 pathway could predict the survival outcomes of NSCLC patients undergoing surgical resection.

Methods

Study population

A total of 782 patients with pathologic stages I, II, or IIIA (micro-invasive N2) NSCLC who underwent curative surgical resection at Kyungpook National University Hospital (KNUH, n = 354) and Seoul National University Bundang Hospital (SNUBH, n = 428) were enrolled in this study. None of patients received chemotherapy or radiotherapy prior to surgery. Written informed consent was obtained from all patients prior to surgery at each of the participating institutions. This study was approved by and performed in accordance with the research protocol of the institutional review boards of KNUH and SNUBH.

Selection of single nucleotide polymorphisms (SNPs) and genotyping

We searched the public SNP database (http://www.ncbi.nlm.nih.gov/SNP) for all SNPs in LKB1 pathway genes to collect potentially functional polymorphisms for this study. Next, using the FuncPred utility for functional SNP prediction and TagSNP utility for linkage disequilibrium (LD) tag SNP selection in the SNPinfo web server (http://snpinfo.niehs.nih.gov/), a total of 23 potentially functional SNPs with minor allele frequency ≥0.05 in the HapMap JPT data were collected after excluding those in linkage disequilibrium ($r^2 ≥ 0.8$). Genomic DNA was extracted from peripheral blood lymphocytes using a blood Quick-DNA whole blood kit S (Fujifilm, Tokyo, Japan). Genotyping was performed using the MassARRAY iPLEX assay (Sequenom Inc., San Diego, CA, USA).

Statistical analysis

Differences in the distribution of genotypes according to clinicopathologic factors were compared using $\chi^2$ tests. Overall survival (OS) was measured from the date of surgery until the date of death or last follow-up. Disease-free survival (DFS) was estimated from the date of surgery until recurrence or death. The Kaplan–Meier method was used to calculate survival estimates. Differences in OS and DFS across different genotypes were compared by the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using multivariate Cox proportional hazards models with adjustments for age, gender, smoking status, tumor histology, pathologic stage, and adjuvant therapy. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics and clinical predictors

The clinical and pathologic characteristics of the patients and association with OS and DFS are shown in Table 1. Univariate analysis showed that age (log-rank $P \left[ P_{L-R} \right] = 2 \times 10^{-3}$), gender ($P_{L-R} = 4 \times 10^{-4}$), smoking status ($P_{L-R} = 3 \times 10^{-4}$), and pathologic stage ($P_{L-R} = 1 \times 10^{-11}$) were significantly associated with OS. Only pathologic stage was significantly associated with DFS ($P_{L-R} = 2 \times 10^{-15}$).

Associations between SNPs and survival outcomes

The SNP information, genotype distribution, and log-rank $P$ values for OS and DFS of the 23 SNPs are shown in Table 2. Of the 23 SNPs analyzed, TSC2 rs30259G > A was significantly associated with poor OS (adjusted HR [aHR] 1.88, 95% CI 1.21–2.91; $P = 0.005$, under a codominant model) and DFS (aHR 1.65, 95% CI 1.15–2.38; $P = 0.01$, under a codominant model) when adjusted for age, gender, smoking status, tumor histology, pathologic stage, and adjuvant therapy (Table 3 and Fig 1). The effect of the rs30259 genotypes on survival outcomes was then evaluated according to tumor histology, smoking status, and pathologic stage. rs30259G > A was significantly associated with OS and DFS in squamous cell carcinoma (SCC) (aHR 2.36, 95% CI 1.37–4.07, $P = 0.002$; aHR 3.21, 95% CI 2.01–5.14, $P = 1 \times 10^{-6}$, under codominant models, respectively) but not in adenocarcinoma (AC) (Table 3 and Fig 1). When stratified according to smoking status, SNPs were significantly associated with OS and DFS in ever-smokers (aHR 2.08, 95% CI 1.30–3.32, $P = 0.002$; aHR 2.07, 95% CI 1.38–3.12, $P = 5 \times 10^{-4}$, 2019) 335–340 © 2018 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd.
# Table 1 Univariate analysis of survival outcomes by clinicopathological features

| Variables          | No. of cases | No. of deaths (%)† | 5Y-OSR (%)‡ | Log-rank P | No. of events (%)† | 5Y-DFSR (%)‡ | Log-rank P |
|--------------------|--------------|--------------------|-------------|------------|--------------------|-------------|------------|
| Overall            | 782          | 208 (26.6)         | 62          |            | 340 (43.5)         | 45          |            |
| Age (years)        |              |                    |             |            |                    |             |            |
| < 65               | 383          | 88 (23.0)          | 69          | 2 × 10⁻³   | 162 (42.3)         | 48          | 0.14       |
| ≥ 65               | 399          | 120 (30.1)         | 55          |            | 178 (44.6)         | 41          |            |
| Gender             |              |                    |             |            |                    |             |            |
| Male               | 572          | 173 (30.2)         | 59          | 4 × 10⁻⁴   | 261 (45.6)         | 42          | 0.10       |
| Female             | 210          | 35 (16.7)          | 71          |            | 79 (37.6)          | 52          |            |
| Smoking status     |              |                    |             |            |                    |             |            |
| Never              | 232          | 40 (17.2)          | 74          | 3 × 10⁻⁴   | 90 (38.8)          | 50          | 0.15       |
| Ever               | 550          | 168 (30.6)         | 57          |            | 250 (45.5)         | 43          |            |
| Histological type  |              |                    |             |            |                    |             |            |
| SCC                | 341          | 103 (30.2)         | 60          | 0.17       | 146 (42.8)         | 48          | 0.22       |
| AC                 | 425          | 99 (23.3)          | 63          |            | 184 (43.3)         | 42          |            |
| LCC                | 16           | 6 (37.5)           | 59          |            | 10 (62.5)          | 35          |            |
| Pathologic stage   |              |                    |             |            |                    |             |            |
| I                  | 378          | 59 (15.6)          | 76          | 1 × 10⁻¹¹  | 107 (28.3)         | 60          | 2 × 10⁻¹⁵  |
| II                 | 227          | 81 (35.7)          | 52          |            | 116 (51.1)         | 39          |            |
| IIIA               | 177          | 68 (38.4)          | 47          |            | 117 (66.1)         | 20          |            |
| Adjuvant therapy§  |              |                    |             |            |                    |             |            |
| No                 | 184          | 72 (39.6)          | 49          | 0.58       | 102 (56.0)         | 37          | 0.36       |
| Yes                | 220          | 77 (34.7)          | 50          |            | 131 (59.0)         | 25          |            |

†Row percentage. ‡Five year-overall survival rate (OSR) and five-year disease-free survival rate (DFSR), proportion of survival derived from Kaplan–Meier analysis. §In pathologic stages II + IIIA: 182 cases received adjuvant chemotherapy alone, 11 cases received adjuvant radiotherapy alone, and 27 cases received both chemotherapy and radiotherapy. AC, adenocarcinoma; LCC, large cell carcinoma; SCC, squamous cell carcinoma.

# Table 2 List of analyzed SNPs and associations with survival outcomes

| SNP ID  | Gene | Base change | MAF  | Dominant | Reccessive | Codominant | Dominant | Reccessive | Codominant |
|---------|------|-------------|------|----------|------------|------------|----------|------------|------------|
| rs30259 | TSC2 | G>A         | 0.04 | 0.01     | 0.15       | 0.005      | 0.03     | 0.8 × 10⁻⁶ | 0.01       |
| rs1130214 | Akt1 | G>T          | 0.13 | 0.90     | 0.65       | 0.94       | 0.23     | 0.65       | 0.35       |
| rs2494750 | Akt1 | A>G          | 0.38 | 0.33     | 0.88       | 0.61       | 0.33     | 0.56       | 0.36       |
| rs17036508 | MOR | T>C          | 0.12 | 0.36     | 0.97       | 0.20       | 0.72     | 0.71       | 0.82       |
| rs1135172 | MOR | C>T          | 0.16 | 0.48     | 0.98       | 0.60       | 0.22     | 0.51       | 0.29       |
| rs1034528 | MOR | G>C          | 0.19 | 0.81     | 0.35       | 0.75       | 0.40     | 0.99       | 0.41       |
| rs1057079 | MOR | A>G          | 0.18 | 0.90     | 0.34       | 0.79       | 0.19     | 0.97       | 0.29       |
| rs37658594 | MOR | T>C          | 0.01 | 0.32     | 0.46       | 0.33       | 0.33     | 0.46       |            |
| rs11121691 | MOR | C>T          | 0.07 | 0.97     | 0.98       | 0.86       | 0.87     | 0.98       | 0.73       |
| rs7711806 | PRKAA1 | T>C         | 0.24 | 0.50     | 0.99       | 0.57       | 0.24     | 0.85       | 0.30       |
| rs1342382 | PRKAA2 | A>T         | 0.25 | 0.93     | 0.17       | 0.73       | 0.32     | 0.20       | 0.68       |
| rs11581010 | PRKAA2 | A>G         | 0.11 | 0.88     | 0.98       | 0.72       | 0.66     | 0.96       | 0.54       |
| rs857148 | PRKAA2 | G>T         | 0.42 | 0.94     | 0.99       | 0.85       | 0.81     | 0.96       | 0.82       |
| rs9803799 | PRKAA2 | T>G         | 0.16 | 0.54     | 0.23       | 0.98       | 0.43     | 0.71       | 0.63       |
| rs4912411 | PRKAA2 | C>A         | 0.38 | 0.39     | 0.25       | 0.25       | 0.47     | 0.06       | 0.18       |
| rs37385684 | PRKAA2 | T>C         | 0.36 | 0.31     | 0.50       | 0.35       | 0.26     | 0.28       | 0.21       |
| rs739441 | TSC1 | A>G         | 0.26 | 0.12     | 0.31       | 0.16       | 0.56     | 0.45       | 0.48       |
| rs1050700 | TSC1 | A>G         | 0.25 | 0.14     | 0.77       | 0.14       | 0.62     | 0.84       | 0.70       |
| rs2809244 | TSC1 | C>A         | 0.39 | 0.47     | 0.49       | 0.31       | 0.62     | 0.99       | 0.66       |
| rs4962225 | TSC1 | A>C         | 0.12 | 0.76     | 0.24       | 0.45       | 0.66     | 0.56       | 0.54       |
| rs2074969 | TSC2 | G>C         | 0.22 | 0.92     | 0.09       | 0.60       | 0.96     | 0.17       | 0.59       |
| rs3806317 | PRKAA2 | C>T       | 0.12 | 0.71     | 0.51       | 0.37       | 0.50     | 0.48       | 0.32       |
| rs701848 | PTEN | C>T         | 0.47 | 0.55     | 0.89       | 0.63       | 0.51     | 0.77       | 0.68       |

*P values calculated using multivariate Cox proportional hazard models, adjusted for age, gender, smoking status, tumor histology, pathologic stage, and adjuvant therapy. MAF, minor allele frequency.
Table 3 Overall and disease-free survival according to TSC2 rs30259G > A genotypes

| Polymorphism/genotype | No. of Cases(%)† | No. of deaths(%)‡ | 5Y-OSR (%)§ | HR (95% CI)¶ | P† | No. of deaths(%)‡ | 5Y-DFSR (%)§ | HR (95% CI)¶ | P¶ |
|-----------------------|------------------|------------------|-------------|-------------|----|------------------|-------------|-------------|----|
| All cases††           |                  |                  |             |             |    |                  |             |             |    |
| GG                    | 715 (92.9)       | 184 (74.3)       | 63          | 1.00        |    | 302 (57.8)       | 46          | 1.00        |    |
| GA                    | 53 (6.9)         | 19 (64.2)        | 45          | 1.79 (1.12–2.89) | 0.02 | 28 (47.2)       | 36          | 1.44 (0.98–2.13) | 0.07 |
| AA                    | 2 (0.3)          | 1 (50.0)         | 50          | 4.43 (0.61–32.15) | 0.14 | 2 (0.0)         | 50          | 26.0 (6.31–107.26) | 7 × 10⁻⁶ |
| Dominant              | 55 (7.1)         | 20 (63.6)        | 45          | 1.85 (1.16–2.94) | 0.01 | 30 (45.5)       | 35          | 1.54 (1.06–2.25) | 0.03 |
| Recessive             | 768 (99.7)       | 203 (73.6)       | 62          | 4.25 (0.59–30.86) | 0.15 | 330 (57.0)      | 45          | 25.43 (6.17–104.86) | 8 × 10⁻⁶ |
| Codominant            | 1.88 (1.21–2.91) | 0.005            |             |             |    |                  |             |             |    |
| Squamous cell carcinoma |               |                  |             |             |    |                  |             |             |    |
| GG                    | 314 (93.2)       | 90 (71.3)        | 62          | 1.00        |    | 126 (59.9)       | 50          | 1.00        |    |
| GA                    | 21 (6.2)         | 11 (47.6)        | 32          | 2.34 (1.23–4.46) | 0.01 | 15 (28.6)       | 23          | 2.74 (1.58–4.75) | 0.0003 |
| AA                    | 2 (0.6)          | 1 (50.0)         | 50          | 4.46 (0.61–32.78) | 0.14 | 2 (0.0)         | 50          | 20.03 (4.74–84.60) | 5 × 10⁻⁵ |
| Dominant              | 23 (6.8)         | 12 (47.8)        | 33          | 2.45 (1.32–4.54) | 0.005 | 17 (26.1)       | 21          | 3.07 (1.82–5.16) | 2 × 10⁻⁵ |
| Recessive             | 335 (99.4)       | 101 (69.9)       | 60          | 4.35 (0.59–31.94) | 0.15 | 141 (57.9)      | 48          | 18.70 (4.43–78.88) | 7 × 10⁻⁵ |
| Codominant            | 2.36 (1.37–4.07) | 0.002            |             |             |    |                  |             |             |    |
| Adenocarcinoma        |                  |                  |             |             |    |                  |             |             |    |
| GG                    | 386 (92.6)       | 88 (77.2)        | 65          | 1.00        |    | 166 (57.0)       | 43          | 1.00        |    |
| GA                    | 31 (7.4)         | 8 (74.2)         | 56          | 1.22 (0.59–2.54) | 0.59 | 13 (58.1)       | 46          | 0.96 (0.55–1.70) | 0.89 |
| AA                    | 0 (0.0)          | 0 (0.0)          | 0           | —           | —  | —                | —           | —           | —  |
| Dominant              | 31 (7.4)         | 8 (74.2)         | 56          | 1.22 (0.59–2.54) | 0.59 | 13 (58.1)       | 46          | 0.96 (0.55–1.70) | 0.89 |
| Recessive             | 417 (100.0)      | 96 (77.0)        | 64          | —           | —  | 179 (57.1)      | 92          | —           | —  |
| Codominant            | 1.16 (0.55–2.42) | 0.70             |             |             |    |                  |             |             |    |

†Column percentage. ‡Row percentage. §Five-year overall survival rate (OSR) and five-year disease-free survival rate (DFSR), proportion of survival derived from Kaplan-Meier analysis. ¶Hazard ratios (HRs), 95% confidence intervals (CIs), and their corresponding P values were calculated using multivariate Cox proportional hazard models adjusted for age, gender, smoking status, tumor histology, pathologic stage, and adjuvant therapy for all cases, and adjusted for age, gender, smoking status, pathologic stage, and adjuvant therapy for squamous cell carcinoma and adenocarcinoma. ††Genotype failures: 12 cases for rs30259.
under codominant models, respectively), but not in never-smokers (Table S1 and Figure S1). We then evaluated the effect of SNPs according to pathologic stage. The association between SNPs and survival outcomes remained significant in stage I (aHR 2.30, 95% CI 1.21–4.39, \(P = 0.01\); aHR 2.02, 95% CI 1.13–3.60, \(P = 0.02\), under codominant models, respectively), but not in stage II–IIIA (Table S1 and Figure S1).

**Discussion**

The present study was performed to examine whether genetic variants involved in the LKB1-dependent pathway affect the prognosis of patients with NSCLC undergoing curative surgery. Among the 23 SNPs evaluated, TSC2 rs30259G > A was significantly associated with survival outcomes. Subgroup analysis showed that SNPs were significantly associated with survival outcomes in SCC, ever-smokers, and stage I, but not in AC, never-smokers, and stage II–IIIA. These results suggest that TSC2 rs30259G > A may be useful to predict prognosis in patients with NSCLC, especially SCC, after curative surgery.

TSC/mammalian target of rapamycin (mTOR) signaling is one of the major downstream pathways of LKB1/AMPK and is associated with protein synthesis, cell growth, and viability.14 Phosphorylation of TSC2 by AMPK after ATP depletion results in activation of the TSC1:TSC2 complex, which regulates the activity of mTORC1, a complex comprised of mTOR, raptor, and mLST8.15–17 Several downstream effectors of mTORC1 play a key role in protein translation, angiogenesis, and autophagy.18–20 Thus, genetic alteration of either TSC1, TSC2, or other upstream regulators increases the level of mTOR activators, resulting in the inappropriate stimulation of protein translation and cell growth.21 In lung cancer, however, data regarding a pathogenic role of the TSC complex is sparse. Previous studies have shown that both the TSC1 locus (9q34) and the TSC2 locus (16p) are frequent targets of loss of heterozygosity in both lung AC and precursor lesions.22,23 Another study suggested that TSC1 loss synergizes with the KRAS mutation to enhance lung tumorigenesis in mice.24

Somatic mutations in the LKB1 gene are observed in 20~30% of white NSCLC patients, although less frequently in Asians, ranking LKB1 as the third most frequently mutated gene in lung adenocarcinoma.10,11 Interestingly, in our study, TSC2 rs30259G > A was significantly associated with survival outcomes in SCC but not in AC. A previous study showed that TSC1 expression was significantly associated with poor survival in SCC and small cell LC, but was not observed in AC.24 These results suggest that genetic variations in the LKB1 pathway may have differential biological effects that could modify the clinical outcome according to the histologic subtypes of NSCLC. Recent advances in the molecular biology of lung cancer have revealed that genetic alterations in SCC and AC are markedly different, leading to the development of histology-specific therapeutics and different clinical outcomes between SCC and AC.25 Therefore, the genetic biomarkers of the two different major subtypes of NSCLC may be different. Subgroup analysis by smoking status showed that the effect of TSC2 rs30259G > A was limited to ever-smokers, in line with the histology-specific effect of the variant given that SCC is a smoking-related histological subtype of lung cancer.26 Further studies are needed to investigate the role of the LKB1 pathway in the development and progression of NSCLC, and also the biologic mechanism of the observed associations between the variant and survival, especially differential effects according to histologic subtypes.

**Figure 1** Overall survival according to TSC2 rs30259G > A genotypes in (a) all cases, (b) squamous cell carcinoma, and (c) adenocarcinoma, and disease-free survival in (d) all cases, (e) squamous cell carcinoma, and (f) adenocarcinoma. \(P\) values from the multivariate Cox proportional hazard model. —GG, —GA, —AA.
In conclusion, analysis of the TSC2 polymorphism may be useful to predict patient prognosis after surgery, thereby helping to improve therapeutic decisions for NSCLC, especially SCC. Future studies are warranted to understand the biological mechanism of our findings and to confirm our results in a larger patient cohort including diverse ethnic groups.

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Disclosure

No authors report any conflict of interest.

References

1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7–30.
2 Dettebeck FC, Boffa DJ, Tanoue LT. The new lung cancer staging system. Chest 2009; 136: 260–71.
3 Shackelford DB, Shaw RJ. The LKB1–AMPK pathway: Metabolism and growth control in tumour suppression. Nat Rev Cancer 2009; 9: 563–75.
4 Alessi DR, Sakamoto K, Bayascas JR. LKB1-dependent signaling pathways. Annu Rev Biochem 2006; 75: 137–63.
5 Hawley SA, Boudeau J, Reid JL et al. Complexes between the LKB1 tumor suppressor, STRADα/β and MO25α/β are upstream kinases in the AMP-activated protein kinase cascade. J Biol 2003; 2: 28.
6 Gwinn DM, Shackelford DB, Egan DF et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell 2008; 30: 214–26.
7 Hemminki A, Markie D, Tomlinson I et al. A serine/threonine kinase gene defective in Peutz–Jeghers syndrome. Nature 1998; 391: 184–7.
8 Avizienyte E, Roth S, Loukola A et al. Somatic mutations in LKB1 are rare in sporadic colorectal and testicular tumors. Cancer Res 1998; 58: 2087–90.
9 Forbes S, Clements J, Dawson E et al. COSMIC. 2005. Br J Cancer 2006; 94: 318–22.
10 Ding L, Getz G, Wheeler DA et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008; 455: 1069–75.
11 Sanchez-Cespedes M, Parrella P, Esteller M et al. Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. Cancer Res 2002; 62: 3659–62.
12 Marcus AI, Zhou W. LKB1 regulated pathways in lung cancer invasion and metastasis. J Thorac Oncol 2010; 5: 1883–6.
13 Ji H, Ramsey MR, Hayes DN et al. LKB1 modulates lung cancer differentiation and metastasis. Nature 2007; 448: 807–10.
14 Shaw RJ, Bardeesy N, Manning BD et al. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell 2004; 6: 91–9.
15 Inoki K, Zhu T, Guan K-L. TSC2 mediates cellular energy response to control cell growth and survival. Cell 2003; 115: 577–90.
16 Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. Cancer Cell 2007; 12: 9–22.
17 Zhang Y, Gao X, Saucedo L, Ru B, Edgar B, Pan D. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. Nat Cell Biol 2003; 5: 578–81.
18 Jung CH, Jun CB, Ro SH et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 2009; 20: 1992–2003.
19 Hudson CC, Liu M, Chiang GG et al. Regulation of hypoxia-inducible factor 1α expression and function by the mammalian target of rapamycin. Mol Cell Biol 2002; 22: 7004–14.
20 Hara K, Yonezawa K, Kozlowski MT et al. Regulation of eIF-4E BP1 phosphorylation by mTOR. J Biol Chem 1997; 272: 26457–63.
21 Takamochi K, Ogura T, Suzuki K et al. Loss of heterozygosity on chromosomes 9q and 16p in atypical adenomatous hyperplasia concomitant with adenocarcinoma of the lung. Am J Pathol 2001; 159: 1941–8.
22 Takamochi K, Ogura T, Yokose T et al. Molecular analysis of the TSC1 gene in adenocarcinoma of the lung. Lung Cancer 2004; 46: 271–81.
23 Liang MC, Ma J, Chen L et al. TSC1 loss synergizes with KRAS activation in lung cancer development in the mouse and confers rapamycin sensitivity. Oncogene 2010; 29: 1588–97.
24 Fuchs A, König K, Heukamp LC et al. Tuberculous-sclerosis complex-related cell signaling in the pathogenesis of lung cancer. Diagn Pathol 2014; 9: 48.
25 Herbst RS, Morgensztern D, Boshoff C et al. The biology and management of non-small cell lung cancer. Nature 2018; 553: 446–54.
26 de Groot P, Munden RF. Lung cancer epidemiology, risk factors, and prevention. Radiol Clin North Am 2012; 50: 863–76.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website.

Table S1. Overall and disease-free survival according to TSC2 rs30259G > A genotypes, stratified by smoking status and pathologic stage

Figure S1. Overall survival according to TSC2 rs30259G > A genotypes in (a) ever-smokers, (b) never-smokers, (c) stage I, and (d) stage II–IIIA; disease-free survival in (e) ever-smokers, (f) never-smokers, (g) stage I, and (h) stage II–IIIA. P values from the multivariate Cox proportional hazard model.