Original Article

TP53 and CDKN2A mutations in patients with early-stage lung squamous cell carcinoma: an analysis of the correlations and prognostic outcomes

Peiyuan Wang[1,2,3#], Feng Wang[1#], Hao He[1], Yujie Chen[1], Hui Lin[1], Peng Chen[1], Xiaofeng Chen[1], Shuoyan Liu[1]

[1]Department of Thoracic Oncology Surgery, Fujian Medical University Cancer Hospital, Fujian Cancer Hospital, Fuzhou, China; [2]Fujian Key Laboratory of Translational Cancer Medicine, Fuzhou, China; [3]Fujian Provincial Key Laboratory of Tumor Biotherapy, Fuzhou, China

Contributions: (I) Conception and design: P Wang; (II) Administrative support: S Liu, F Wang; (III) Provision of study materials or patients: H He, Y Chen; (IV) Collection and assembly of data: H Lin, P Chen, X Chen; (V) Data analysis and interpretation: P Wang, F Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Shuoyan Liu. Department of Thoracic Oncology Surgery, Fujian Medical University Cancer Hospital, Fujian Cancer Hospital, Fuzhou, China. Email: shuoyanliu2010@163.com.

Background: Lung squamous cell carcinoma (LUSC) is characterized by frequent mutations of tumor protein p53 (TP53) and cyclin dependent kinase inhibitor 2A (CDKN2A). However, to date, the impact of TP53/CDKN2A status on the clinical outcome of patients with early-stage LUSC is unclear.

Methods: Tissue samples from 16 early-stage, surgically resected LUSCs were analyzed by next-generation sequencing (NGS). Information regarding TP53 and CDKN2A alterations and patient survival time was downloaded from The Cancer Genome Atlas (TCGA) database. The associations between TP53 and CDKN2A status and tumor characteristics, outcomes including overall survival (OS) and disease-free survival (DFS), and mutation counts were investigated.

Results: TP53 and CDKN2A exhibited a high frequency of somatic mutations in early-stage LUSC in our center. Data for 1,176 samples were collected from TCGA. CDKN2A mutation status was associated with TP53 mutation status (P=0.040). TP53 mutation was a favorable prognostic factor for early-stage LUSC. The OS times of patients with wild-type and mutated TP53 were 28.94 and 60.48 months, respectively (P=0.002). In contrast, CDKN2A mutations were significantly associated with a shorter survival time in early-stage LUSC. The OS times for wild-type and mutated CDKN2A patients were 62.81 and 37.55 months, respectively (P=0.026). Patients with TP53 mutations had higher total mutation counts compared to patients with wild-type TP53. Furthermore, OS was significantly shorter in patients with a low mutation count compared to patients with a median or high mutation count.

Conclusions: Early-stage LUSC patients with TP53 mutations had a longer OS, while those with CDKN2A mutations had a shorter OS. Furthermore, patients with TP53 mutation/CDKN2A wild-type status had a longer OS. CDKN2A mutation is a vital indicator for prognostic assessment according to TP53 status. The prolonged survival of patients with TP53 mutations may be due to their high mutation counts. Larger datasets are required to validate these observations.

Keywords: Tumor protein p53 (TP53); cyclin dependent kinase inhibitor 2A (CDKN2A); lung squamous cell carcinoma (LUSC); mutation; prognosis

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^ ORCID: 0000-0001-5544-7457.

Introduction

Lung squamous cell carcinoma (LUSC) accounts for 20–30% of non-small-cell lung cancers (NSCLCs) and results in approximately 400,000 deaths annually in the United States. Unfortunately, to date, very few personalized therapies have been developed for LUSC due to the limited understanding of the molecular targets (1). Previous profiling efforts have demonstrated that mutations in tumor protein p53 (TP53) represent the most frequent (81%) genomic alteration found in LUSC (2). TP53 encodes the tumor suppressor protein p53, binds directly to chromatin in the nucleus, and plays an important role in the regulation of the cell cycle, apoptosis, autophagy, and DNA repair in response to oncogenic stress (3). The position, nature, and functional effects of mutations on protein structure and activity have led to a recent classification of TP53 mutations, and it is now recognized that various classes of mutations have differential prognostic effects. However, data on the prognostic or predictive effects of TP53 status in NSCLC are limited and inconclusive (4). To date, there is still a paucity of drugs approved for targeting TP53 mutations in cancer patients, and the prognostic value of TP53 in early-stage LUSC is unclear. Thus, this study examined the prognostic value of TP53 in early-stage LUSC.

Cyclin dependent kinase inhibitor 2A (*CDKN2A*), a known tumor suppressor gene that encodes the $p16^{INK4A}$ and $p14^{ARF}$ proteins, is inactivated in 72% of LUSC cases (2). Patients who are carriers of certain CDKN2A mutations show increased risks of malignant neoplasms, particularly pancreatic, lung, and head and neck cancers (5). LUSC is characterized by frequent TP53 mutations and CDKN2A alterations (2,6). Previous studies have reported that the degradation of the p53 protein by the ubiquitin pathway is mediated by its binding to mouse double minute 2 (MDM2). However, the expression of MDM2 mRNA and protein is negatively regulated by $p14^{ARF}$ in the nucleus (7,8). While these alterations have increased our understanding of the molecular pathology of LUSC, the impact of TP53/CDKN2A status on the clinical outcomes of patients with early-stage LUSC is unclear.

This study analyzed the mutational landscape of 16 early-stage, surgically resected LUSC patients using targeted next-generation sequencing (NGS) encompassing 59–1,021 cancer-related genes. Furthermore, we utilized a well annotated specimen set that permits analysis of mutations, alone or in combination, with outcome. This study aimed to evaluate the association of TP53 and CDKN2A status, as well as the prognostic value of these two genes combined in early-stage, surgically resected LUSC. We present the following article in accordance with the REMARK reporting checklist (available at https://dx.doi.org/10.21037/atm-21-3709).

Methods

Patients and samples

Sixteen early-stage, surgically resected LUSC samples were obtained from the Fujian Cancer Hospital in Fuzhou, China, from August 2018 to August 2019. All patients provided written informed consent and received NGS testing at the Geneplus-Beijing Institute. NGS testing covered approximately 1.4 Mbp genomic regions of 1,021 cancer-related genes (or approximately 230 Kbp genomic regions of 59 genes for some patients) (Table S1). All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by regional ethics board of Fujian Cancer Hospital (No.: SQ2020-055-01) and informed consent was taken from all the patients.

Gene expression databases

Information regarding TP53 and CDKN2A alterations and survival times in patients with LUSC was downloaded from The Cancer Genome Atlas (TCGA), an open access database that is publicly available at http://www.cbioportal.org. The Lung Squamous Cell Carcinoma (TCGA, Firehose Legacy), Lung Squamous Cell Carcinoma (TCGA, Nature 2012), and Lung Squamous Cell Carcinoma (TCGA, PanCancer Atlas) datasets were selected as the data source as they contained only early-stage, surgically resected LUSC samples. There was a total of 1176 LUSC samples (available online: https://cdn.amegroups.cn/static/public/atm-21-3709-1.xlsx). The gene set of interest, "TP53 CDKN2A", was entered in the input box. Mutation and survival data were downloaded from the cBioPortal website after submitting the query regarding "TP53 CDKN2A" in the input box. Data were merged according to the unique patient ID, such as "TCGA-18-3406-01". Altogether, 841 pieces of mutation data and 979 pieces of survival data were downloaded. Analysis of the data revealed 349 pieces of duplicated patient data, which were discarded. The duplication was largely due to overlapping data with another

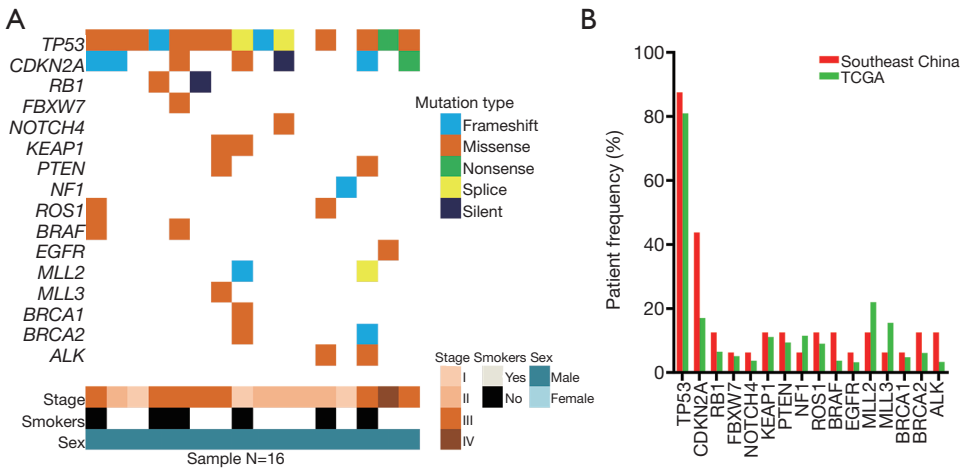

Figure 1 Significantly mutated genes observed in early-stage, surgically resected lung squamous cell carcinoma (LUSC) samples obtained in our center. (A) The top 16 significantly mutated genes in LUSC samples. (B) A comparison of the mutation frequencies of significantly mutated genes between the southeastern China cohort and The Cancer Genome Atlas (TCGA) cohort of LUSC patients.

selected study. After the merge, there were 492 pieces of data from early-stage LUSC patients. Each piece of data contained the mutation type of CDKN2A and TP53 as well as the survival time of the patient. The TP53 and CDKN2A mutations were divided into different groups based on the different exons containing the mutations. However, due to limited information from the cBioPortal database, there were 13 cases without mutation counts in TP53-wild-type patients and 10 cases without mutation counts in TP53-mutated patients. No statements of approval or informed consent were required for this section of the study, as all data was obtained from an open access database.

Statistical methods

Fisher's exact test and the Mann-Whitney test were utilized to analyze the categorical and continuous variables. Survival curves were analyzed using the Kaplan-Meier method and log-rank tests. The Cox proportional hazards model was used to evaluate associations between clinicopathological characteristics and patient survival. Overall survival (OS) and disease-free survival (DFS) data were obtained from the cBioPortal website directly. Statistical analyses were performed using GraphPad Prism 5.0. The statistical significance (alpha-value) threshold was fixed at 0.05, and all P values were three-sided.

Results

TP53 and CDKN2A exhibited a higher frequency of somatic mutations than other cancer-related genes

A retrospective study was conducted on 16 LUSC patients involving genomic profiling via targeted NGS encompassing 59–1,021 cancer-related genes. Among LUSC patients, the rate of TP53 mutation was 87.5% (14/16), while the rate of CDKN2A mutation was 43.8% (7/16). Interestingly, CDKN2A mutations were accompanied by TP53 mutations. TP53 and CDKN2A were among the most frequently mutated genes, whereas F-box and WD repeat domain containing 7 (FBXW7), notch receptor 4 (NOTCH4), epidermal growth factor receptor (EGFR), BRCA1 DNA repair associated (BRCA1) had lower mutation rates in our study cohort (*Figure 1A*). The mutation frequencies of TP53, NOTCH4, kelch like ECH associated protein 1 (KEAP1), phosphatase and tensin homolog (PTEN), ROS proto-oncogene 1, receptor tyrosine kinase (ROS1), EGFR, and BRCA1 were comparable with those in the TCGA

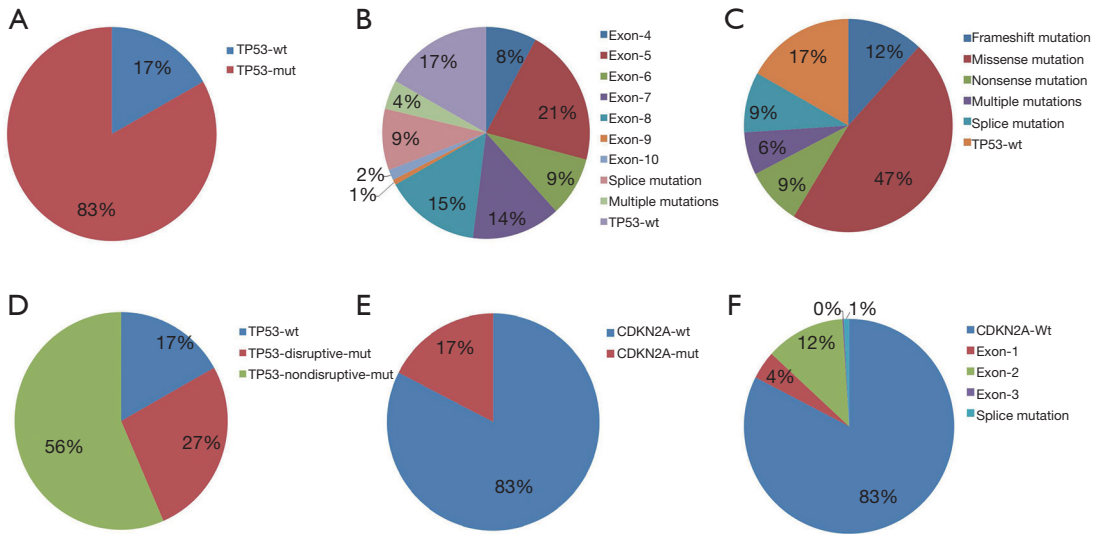

Figure 2 The distribution of tumor protein p53 (TP53) and cyclin dependent kinase inhibitor 2A (CDKN2A) mutations in early-stage lung squamous cell carcinoma (LUSC). (A) The distribution of TP53 wild type (wt) or mutated type (mut). (B) The distribution of different TP53 mutation sites. (C) The distribution of different TP53 mutation types. (D) The distribution of TP53 mutation status divided into wild type, disruptive mutation, and nondisruptive mutation groups. (E) The distribution of CDKN2A wild type (wt) or mutated type (mut). (F) The distribution of different CDKN2A mutation sites.

data (*Figure 1B*). Patients with early-stage LUSC exhibited higher TP53 and CDKN2A mutation frequencies compared to other cancer-related genes.

TP53 mutation and CDKN2A mutation profiling and patient characteristics

In the 492 early-stage LUSC patients, the mutation rate of TP53 was 83.13% (409/492). Exons 4–8 were the most frequent mutation sites for TP53, accounting for 66.9% of all mutations (329/492). Exons 9 and 10 were rarely mutated, accounting for 2.4% of all mutations (12/492). Multiple mutations occurred in 4.5% of patients (22/492), and 9.3% (46/492) of mutations could not be classified (*Figure 2A,2B*). TP53 mutations, mainly missense mutations, were the most common mutations in early-stage LUSC (*Figure 2C*).

In another classification, TP53 mutation status was divided into wild type, disruptive mutations, and nondisruptive mutations, as previously described (9). A total of 131 patients (27%) had TP53 disruptive mutations, and 278 patients (56%) showed nondisruptive TP53 mutations (*Figure 2D*). Patient and tumor characteristics based on TP53 status are shown in Table S2. Dual TP53/CDKN2A mutations were observed in 78 patients (15.8%). CDKN2A mutation status was associated with TP53 mutation status (P=0.040).

Of the 492 patients, 406 patients did not have CDKN2A mutations, 21 patients had exon 1 mutations, 60 had exon 2 mutations, 1 had exon 3 mutations, and 4 had splice mutations (*Figure 2E,2F*). Patient and tumor characteristics based on CDKN2A status are shown in Table S3. There were no statistically significant differences in tumor characteristics.

Kaplan-Meier analyses of survival time according to TP53 status in early-stage LUSC

Consistent with the prognostic capacity of tumor staging, among the entire cohort of 492 patients, the Kaplan-Meier survival curve indicated that patients with different tumor stages had significantly different OS times (P=0.009; *Figure 3A*). Similarly, there was a significant difference in OS based on TP53 mutant or wild-type status with distinct tumor staging (P=0.020 and P=0.025, respectively) (*Figure 3B,3C*). However, the year of initial diagnosis did not significantly affect prognosis (P=0.595; Figure S1A).

Furthermore, TP53 mutation was a positive prognostic factor for OS and DFS in early-stage LUSC (*Figure 3D* and Figure S1B). The estimated OS times for patients with

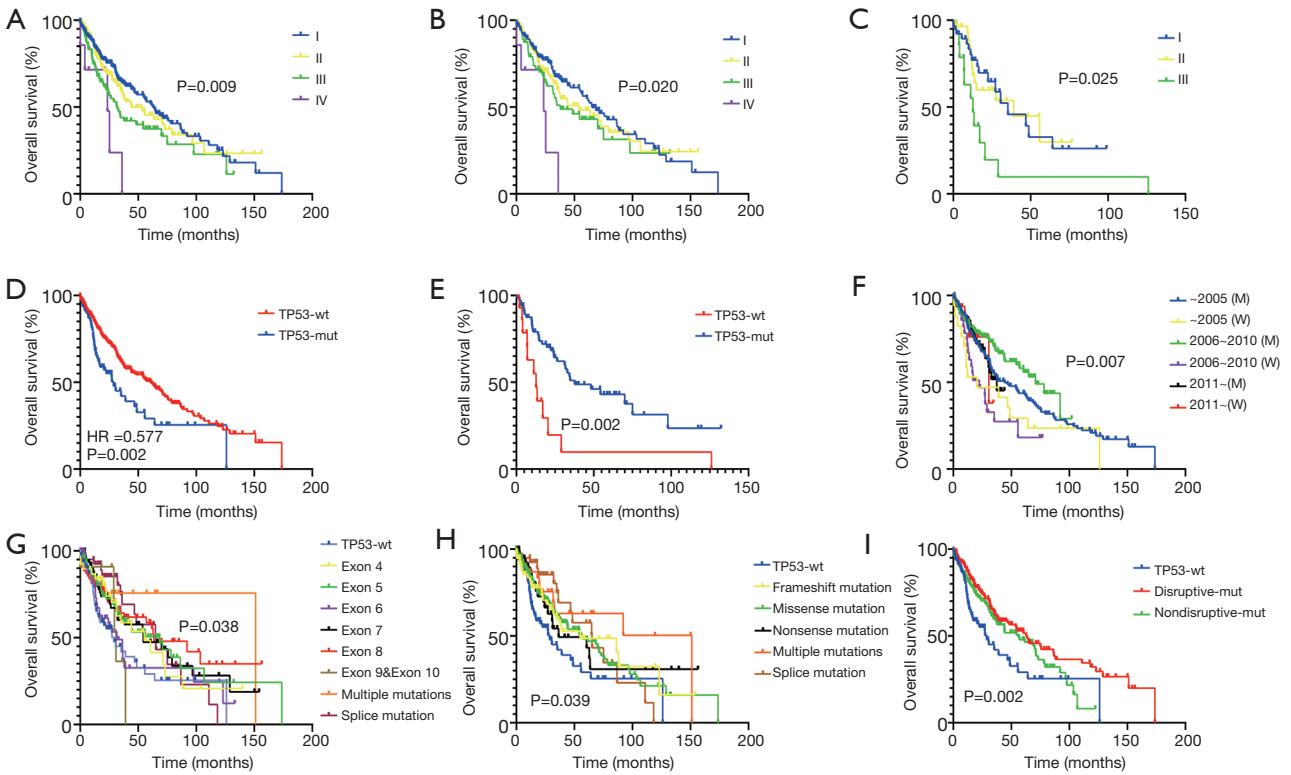

Figure 3 Survival curves of patients carrying tumor protein p53 (TP53) mutations. (A) Overall survival (OS) in different tumor stages. (B) OS in TP53-mutated tumors of different stages. (C) OS in TP53 wild-type tumors of different stages. (D) OS in TP53 wild-type and TP53-mutated patients. (E) OS in stage III patients carrying wild-type or mutated TP53. (F) OS in patients carrying wild-type or mutated TP53 depending on the year of initial diagnosis. (G) OS in patients with wild-type and mutated TP53 subdivided according to mutation site. (H) OS in patients with wild-type and mutated TP53 subdivided according to mutation type. (I) OS in patients with wild-type and mutated TP53 subdivided into disruptive or nondisruptive mutation types.

wild-type TP53 and mutated TP53 were 28.94 months and 60.48 months, respectively [hazard ratio (HR) 0.577; 95% confidence interval (CI), 0.390 to 0.878; P=0.002]. This prompted us to investigate the association between patient survival time and tumor stage as well as TP53 mutations. In stage III patients, OS was affected by TP53 status (HR 4.21; 95% CI, 1.68 to 10.56; P=0.002 for OS), but no significant difference was identified in stage I–II patients (*Figure 3E* and Figure S1C,S1D). In addition, OS for the entire cohort was influenced by the year of initial diagnosis according to TP53 status (P =0.007; *Figure 3F*).

TP53 mutations were divided according to the affected exons. Patients with different mutated exons had significantly different OS times (P=0.038; *Figure 3G*). However, the difference in DFS was not statistically significant (Figure S1E). Diverse types of TP53 mutations can occur, and the TP53 mutation type can affect the prognosis of LUSC patients (P=0.045 for OS and P=0.039 for PFS; *Figure 3H* and Figure S1F). In another classification, TP53 mutation status was divided into wild type, disruptive mutations, and nondisruptive mutations. The difference in survival between these mutation groups was also statistically significant (P=0.002 for OS and P=0.039 for PFS; *Figure 3I*).

Survival analysis of TP53 and CDKN2A status in early-stage LUSC

Few studies have demonstrated the prognostic impact of CDKN2A mutations in early-stage LUSC. The results demonstrated that the CDKN2A mutation was a negative prognostic factor in early-stage LUSC (*Figure 4A*). The estimated OS times for patients with wild-type CDKN2A and mutated CDKN2A were 62.81 months and 37.55 months,

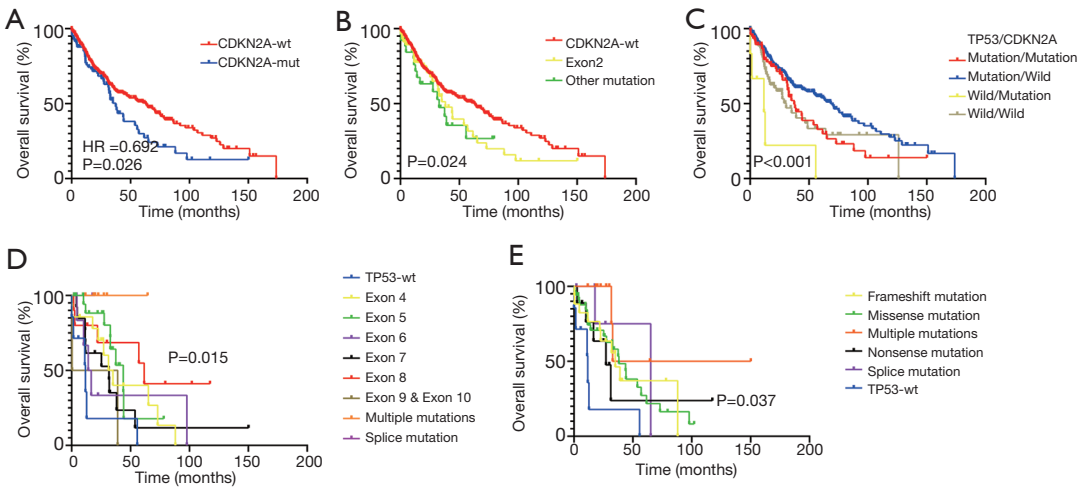

Figure 4 Survival curves of patients carrying cyclin dependent kinase inhibitor 2A (CDKN2A) and tumor protein p53 (TP53) mutations. (A) Overall survival (OS) in patients with wild-type and mutated CDKN2A. (B) OS in patients with wild-type and mutated CDKN2A subdivided according to mutation site. (C) OS of patients in different CDKN2A/TP53 mutation groups. (D) OS according to different TP53 mutation sites in CDKN2A-mutated patients. (E) OS according to different TP53 mutation types in CDKN2A-mutated patients.

respectively (HR 0.692; 95% CI, 0.479 to 0.998; P=0.026). DFS was not influenced by CDKN2A status (HR 0.823; 95% CI, 0.596 to 1.138; P=0.209; Figure S2A). The mutation type was further divided according to the affected exons, and the difference between exon groups was statistically significant for OS (P=0.024; *Figure 4B*). Again, DFS was not significantly different between the exon groups (P=0.197; Figure S2B). The patients were further divided into different groups based on the mutation type of CDKN2A (wild type or mutated) and TP53 (wild type or mutated). The results revealed that the survival of the 4 different groups was significantly different (P<0.001 for OS and DFS; *Figure 4C* and Figure S2C). Patients with TP53 mutated/CDKN2A wild-type status showed longer OS and DFS compared to patients in the other 3 groups. This suggested that TP53 mutation and CDKN2A mutation types are prognostic factors in early-stage LUSC.

The prognostic value of CDKN2A and TP53 mutation types was further investigated. Survival curves of CDKN2A-mutated patients indicated that TP53 wild-type patients had a poor prognosis (P=0.015 for different mutation sites; P=0.037 for different mutation types; *Figure 4D,4E*). However, in CDKN2A wild-type patients, OS was not influenced by TP53 status (P=0.219 for different mutation sites in different exons; P=0.154 for different mutation types; Figure S2D,S2E). Interestingly, when TP53 mutation status was divided into wild type, disruptive mutations, and nondisruptive mutations, OS was not influenced by CDKN2A status (Figure S2F,S2G).

Correlation of mutation counts and survival time

Oncogenic stress triggers the DNA damage response which involves p53-mediated DNA repair to trigger cell cycle arrest and cell death by apoptosis or senescence (10). When TP53 is mutated, more mutations may occur. The association between mutation counts during early-stage LUSC and at different tumor stages was investigated. Different stages exhibited similar mutation frequencies. Total mutation counts were not influenced by stage regardless of TP53 status (Figure S3A-S3C). Interestingly, patients with mutated TP53 harbored more total mutations (*Figure 5A*). However, the CDKN2A wild-type group and CDKN2A-mutated group exhibited similar mutation counts (Figure S3D). In addition to the exon 4 mutation, different TP53 mutation sites were related to higher mutation counts compared to wild-type TP53 (*Figure 5B*). Similarly, patients with TP53 frameshift mutation, missense mutation, and multiple mutations had more mutations, with the exception of splice mutations and nonsense mutations (*Figure 5C*). In addition, patients with disruptive and nondisruptive TP53 mutations all presented with higher mutation counts than TP53 wild-type patients (*Figure 5D*). Moreover, compared to the TP53 and CDKN2A wild-type cohorts, the TP53

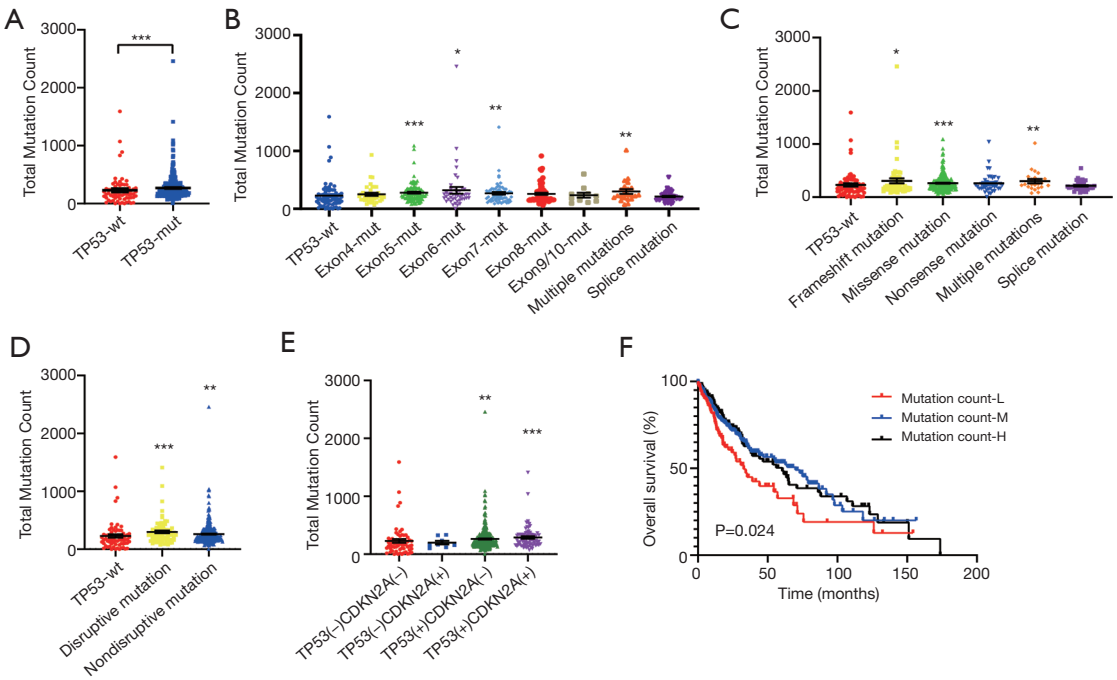

Figure 5 The correlation between mutation counts and tumor protein p53 (TP53) status and survival time. (A) Total mutation count in LUSC patients with wild-type and mutated TP53. (B) Total mutation count in lung squamous cell carcinoma (LUSC) patients with wild-type and mutated TP53 subdivided according to mutation site. (C) Total mutation count in LUSC patients with wild-type and mutated P53 subdivided according to mutation type. (D) Total mutation count in LUSC patients with wild-type and mutated P53 subdivided into disruptive or nondisruptive mutation types. (E) Total mutation count in LUSC patients with different cyclin dependent kinase inhibitor 2A (CDKN2A)/TP53 mutations. (F) Overall survival (OS) of early-stage LUSC patients subdivided into a low mutation count group (mutation count-L), a medium mutation count group (mutation count-M), and a high mutation count group (mutation count-H). For (A-E), each dot represents a patient [mean ± standard deviation (SD)]; *, P<0.05; **, P<0.01; ***, P<0.001.

mutation cohort had more mutations irrespective of CDKN2A mutation status. These results suggested that mutation count is associated with TP53 status, independent of CDKN2A status (*Figure 5E*). The number of mutations was divided into 3 cohorts, namely, the low mutation count cohort , which included patients with 1–150 mutations; the medium mutation count cohort, which included patients with 151–300 mutations; and the high mutation count cohort, which included patients with over 301 mutations. OS was significantly shorter in the low mutation count cohort compared to patients in the medium and high mutation count groups (P=0.024; *Figure 5F*).

Discussion

In LUSC, recurrent mutations of TP53, FGFR1, FGFR2, FGFR3, DDR2, and genes of the PI3K pathway have been detected, as have quantitative gene abnormalities of PTEN and CDKN2A (1,6). This current study reviewed 16 patients with surgically resected LUSC and identified that TP53 and CDKN2A exhibited a higher frequency of somatic mutations than other cancer-related genes. These results were compared with those from the TCGA dataset, which is mainly composed of the Western population. Therefore, it is essential to further elucidate the association of TP53 status and CDKN2A status, as well as the prognostic value of these two genes together in early-stage, surgically resected LUSC patients in the Chinese population .

TP53 has been shown to be one of the most frequently mutated genes in lung cancers irrespective of histological type, with the vast majority of mutations clustering in exons 4 to 8 (11), which is consistent with our study. In addition, similar to the results of previous studies (3),

missense mutations were the most common mutations observed in our early-stage LUSC cohort. In contrast to other studies (11), we demonstrated that TP53 mutations were not significantly associated with age or stage. As we only analyzed the influence of TP53 mutations in relation to early-stage, surgically resected LUSC rather than NSCLC, this may account for the difference observed between studies. Interestingly, CDKN2A mutation status was shown to be associated with TP53 mutation status, and in fact, CDKN2A mutations were present in 17% of early-stage LUSCs. However, there was no significant relationship between CDKN2A mutation status and tumor characteristics.

To date, data on the prognostic or predictive effect of TP53 in NSCLC have been limited and inconclusive. In a study cohort of 35 patients with NSCLC from a prospective phase II trial, TP53 mutation was predictive of resistance to induction therapy (cisplatin/etoposide plus radiation) (12). However, Schiller et al. failed to identify prognostic or predictive value in 197 patients with completely resected tumors enrolled in a randomized trial of postoperative radiotherapy plus chemotherapy (13). Negative results were also observed in JBRonchus (JBR), a randomized trial of patients with stage IB and II NSCLC assigned to treatment with cisplatin-based adjuvant chemotherapy (ACT) versus observation (OBS) (14). Another randomized trial of ACT versus OBS in patients with stage I to III NSCLC, the International Adjuvant Lung Cancer Trial (IALT), showed that TP53 mutation was neither prognostic nor predictive for OS after 8 years of follow-up. Ma et al. performed a pooled analysis of four randomized trials of ACT versus OBS and reported that TP53 mutation had no prognostic effect but was marginally predictive for survival from ACT (4).

This current investigation examined the prognostic value of TP53 in early-stage LUSC. Analysis of the TCGA data revealed a trend towards decreased OS with progressing tumor stage, regardless of TP53 status. Our study indicated that TP53mutation is a favorable prognostic factor in early-stage LUSC patients. This effect was only significant in stage III patients and not in stage I–II patients. OS was also significantly affected by the year of initial diagnosis, especially before 2010, and TP53 status. This discrepancy might be attributable to the development of ACT for use after surgery. TP53 mutations should be considered not only in terms of mutation status but also in terms of mutation site and mutation type. We found that the TP53 mutation site and mutation type were clinically meaningful. Similar to previous studies (15), patients with TP53 exon 4 or exon 6 mutations demonstrated poorer prognosis compared to patients with TP53 exon 5, exon 7, or exon 8 mutations. In addition, patients with multiple mutations demonstrated better prognosis than those with nonsense mutations. The former study divided TP53 mutation types into disruptive and nondisruptive, and found that nondisruptive mutations of TP53 are an independent prognostic factor of shorter survival time in EGFR-mutated NSCLC (16). However, our study showed that disruptive mutation of TP53 seemed to confer a longer survival time in early-stage LUSC, in agreement with Hou et al. (17).

CDKN2A alterations are frequent in all lung cancer expression subtypes (6). However, few reports have investigated the predictive or prognostic significance of CDKN2A in NSCLC. Our study indicated that CDKN2A mutations in early-stage LUSC are significantly associated with poor survival time. This was also the first study to analyze the association between TP53 status and CDKN2A status in early-stage, surgically resected LUSC patients. Patients with mutated TP53 and wild-type CDKN2A demonstrated a longer survival time compared with other early-stage LUSC patients. When CDKN2A status was divided into wild-type and mutated groups, survival curves of the mutated CDKN2A group showed that TP53 wild-type patients had a poorer prognosis. There were no significant differences between wild-type CDKN2A and TP53 status in terms of OS. The results suggested that CDKN2A mutation is a vital indicator for prognostic assessment according to TP53 status.

This investigation demonstrated that patients with TP53 mutations have longer OS and DFS among early-stage LUSC patients. Patients with TP53 mutation had more total mutations than those with wild-type TP53. Specifically, patients with different TP53 mutation sites and mutation types harbored different mutation counts and had higher mutation counts than those with wild-type TP53. Interestingly, patients with higher mutation counts had a longer survival time, which was consistent with the results demonstrating that patients with TP53 mutations had a longer survival time. Previous reports have suggested that the measurement of mutation counts is representative of tumor mutation burden (TMB) (18,19). Tumors with high TMB are thought to express more cancer-specific antigens (neoantigens) that can be recognized by the immune system (20). In the present study, data from the TCGA database included information on patients with early-stage, surgically resected LUSC from 1992 to 2013 who had good performance status and an active immune

system. Moreover, patients with TP53 mutations diagnosed before 2010 who accepted limited therapy after surgery had a longer survival time than P53-wild-type patients. This discrepancy might be caused by differences in stage III patients and stage I–II patients. As resectable stage III LUSC has more circulating tumor cells, and patients with TP53 mutations may harbor higher mutation counts and express more neoantigens which can be recognized by the immune system compared to patients without TP53 mutations.

There were several limitations to this investigation including the small sample size of the cohort and inadequate information from the cBioPortal database. Future work should verified these results using other cohorts, such as data from the TCGA cohort. Further investigations regarding TP53 and CDKN2A mutations, and the prognosis of LUSC patients are required to fully evaluate the role of TP53/CDKN2A status as a prognostic and predictive variable in patients with LUSC. Though many biochemical aspects of p53 and CDKN2A regulation and activity were elucidated and it have demonstrated their inhibition of tumorigenesis (21,22), p53 and CDKN2A mutants differ considerably in form and function need furthermore investigation. We hope that different treatment strategies were adopted according to TP53 and CDKN2A status.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by regional ethics board of Fujian Cancer Hospital (No.: SQ2020-055-01) and informed consent was taken from all the patients.

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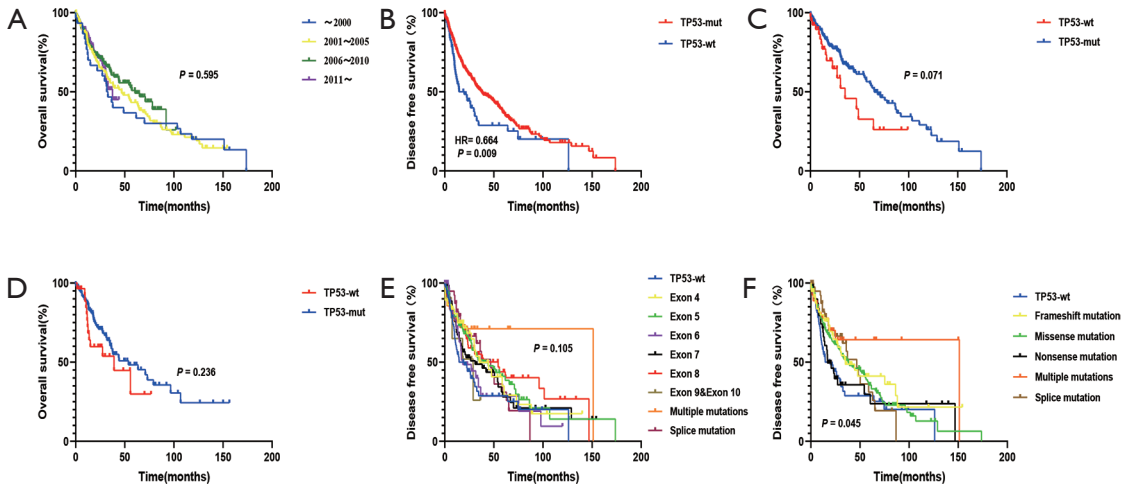

Figure S1 Survival curves of patients carrying tumor protein p53 (TP53) mutations. (A) Overall survival (OS) of patients with different year of initial diagnosis according to tumor staging. (B) Disease free survival (DFS) in TP53 wild-type and mutated patients. (C) OS in stage I patients carrying wild-type or mutated TP53. (D) OS in stage II patients carrying wild-type or mutated TP53. (E) DFS in patients with wild-type and mutated TP53 subdivided according to mutation site. (F) DFS in patients with wild-type and mutated TP53 subdivided according to mutation type.

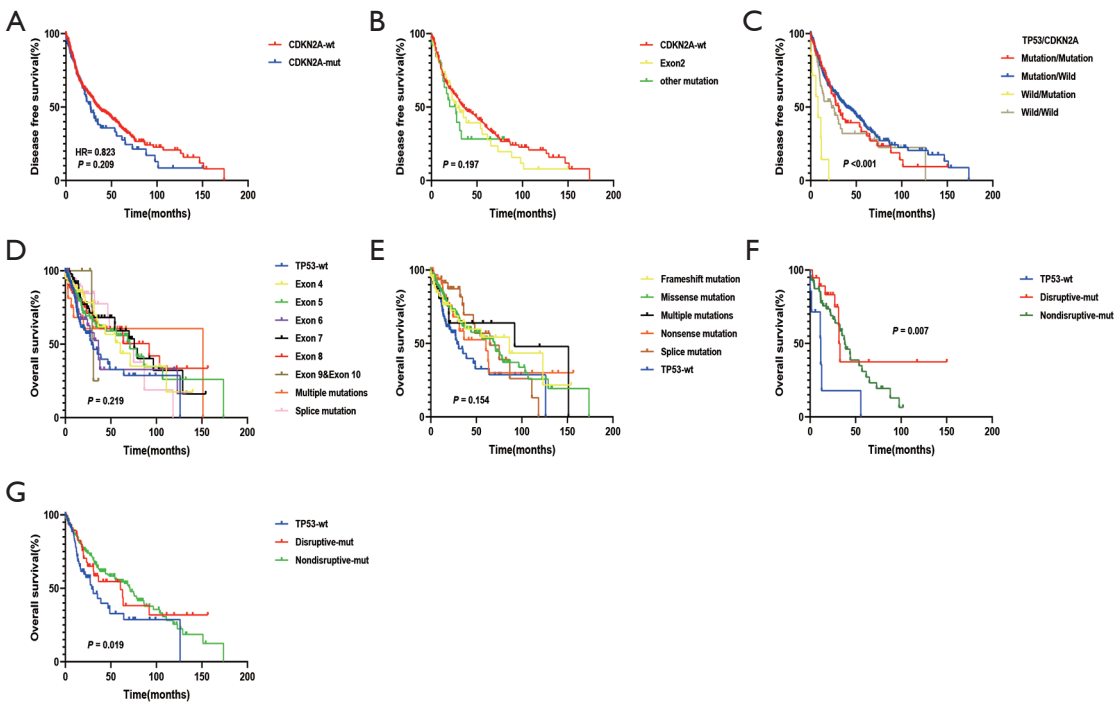

Figure S2 Survival curves of patients carrying cyclin dependent kinase inhibitor 2A (CDKN2A) and tumor protein p53 (TP53) mutations. (A) Disease free survival (DFS) in patients with wild-type and mutated CDKN2A. (B) DFS in patients with wild-type and mutated CDKN2A subdivided according to mutation site. (C) DFS of patients in different CDKN2A/TP53 mutation groups. (D) Overall survival (OS) of different TP53 mutation sites in CDKN2A wild-type patients. (E) OS of different CDKN2A mutation types in CDKN2A wild-type patients. (F) OS of patients with wild-type and mutated TP53 subdivided into disruptive or nondisruptive mutations in CDKN2A-mutated patients. (G) OS of CDKN2A wild-type patients with wild-type and mutated TP53 subdivided into disruptive or nondisruptive mutations.

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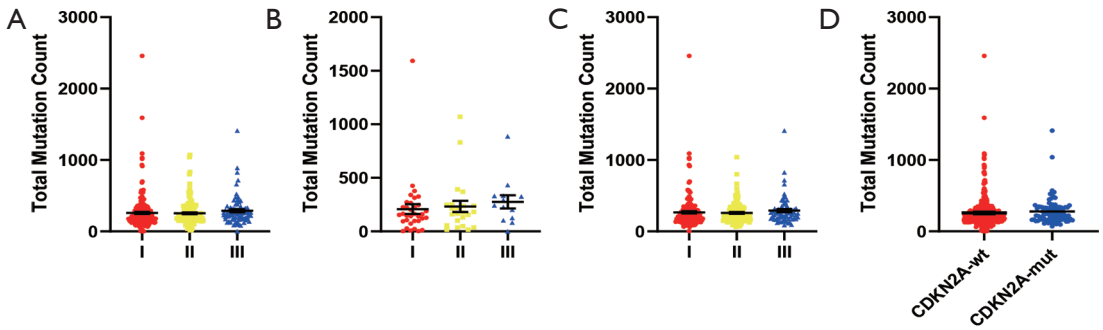

Figure S3 The mutation counts of LUSC patients. (A) Total mutation count of patients with lung squamous cell carcinoma (LUSC) at different tumor stages. (B) Total mutation count of TP53 wild-type patients with LUSC at different tumor stages. (C) Total mutation count of TP53-mutated patients with LUSC at different tumor stages. (D) Total mutation count of LUSC patients with wild-type and mutated CDKN2A. For (A-D), each dot represents a patient [mean ± standard deviation (SD)].

Table S1 1,021 gene panel

whole exons								
ABL1	ABL2	ACVR1B	AKT1	AKT2	AKT3	ALK	APC	AR
ARAF	ARID1A	ARID1B	ARID2	ASXL1	ATM	ATR	ATRX	AURKA
AURKB	AXIN1	AXIN2	AXL	B2M	BAP1	BARD1	BCL2	BCL2L1
BCOR	BLM	BMPR1A	BRAF	BRCA1	BRCA2	BRD4	BRIP1	BTK
C11orf30	CASP8	CBFB	CBL	CCND1	CCND2	CCND3	CCNE1	CD274
CDC73	CDH1	CDK12	CDK4	CDK6	CDK8	CDKN1A	CDKN1B	CDKN2A
CDKN2B	CDKN2C	CEBPA	CHEK1	CHEK2	CIC	CREBBP	CRKL	CSF1R
CTCF	CTNNA1	CTNNB1	CUL3	CYLD	DAXX	DDR1	DDR2	DICER1
DNMT3A	EGFR	ELAC2	EME2	EP300	EPAS1	EPCAM	EPHA2	EPHA3
EPHA5	EPHB2	EPHB6	ERBB2	ERBB3	ERBB4	ERCC1	ERCC3	ERG
ERRFI1	ESR1	EXT1	EXT2	EZH2	FAM123B	FAM175A	FANCA	FANCC
FANCD2	FANCG	FANCM	FAS	FAT1	FAT2	FBXW7	FCGR2A	FCGR3A
FGFR1	FGFR2	FGFR3	FGFR4	FH	FLCN	FLT1	FLT3	FLT4
FOXA1	FOXL2	FOXP1	FUBP1	GAB2	GALNT12	GATA3	GNA11	GNAQ
GNAS	GRIN2A	HDAC1	HDAC4	HGF	HNF1A	HOXB13	HRAS	HSP90AA1
IDH1	IDH2	IFNG	IFNGR1	IGF1R	IL7R	INPP4B	IRF2	IRS2
JAK1	JAK2	JAK3	KDM5A	KDM5C	KDM6A	KDR	KEAP1	KIT
KRAS	LRP1B	MAP2K1	MAP2K2	MAP2K4	MAP3K1	MAPK1	MAX	MCL1
MDM2	MDM4	MED12	MEN1	MET	MITF	MLH1	MLH3	MLL
MLL2	MLL3	MPL	MRE11A	MS4A1	MSH2	MSH3	MSH6	MTOR
MUTYH	MYC	MYCL1	MYCN	MYD88	NBN	NCOR1	NDUFA13	NF1
NF2	NOTCH1	NOTCH2	NOTCH3	NOTCH4	NPM1	NRAS	NSD1	NTHL1

Table S1 (*continued*)

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Table S1 (*continued*)

whole exons								
NTRK1	NTRK3	PALB2	PAX5	PBRM1	PCK1	PDCD1LG2	PDGFRA	PDGFRB
PDK1	PHF6	PIK3CA	PIK3CB	PIK3CG	PIK3R1	PIK3R2	PMS1	PMS2
POLD1	POLE	POT1	PPM1D	PRKAR1A	PTCH1	PTCH2	PTEN	PTPN11
RAD50	RAD51	RAD51B	RAD51C	RAD51D	RAF1	RARA	RB1	RBM10
RET	RHEB	RHOA	RICTOR	RINT1	RNASEL	RNF43	ROS1	RPS6KB1
RUNX1	SDHA	SDHAF2	SDHB	SDHC	SDHD	SERPINB3	SERPINB4	SETD2
SLX4	SMAD2	SMAD4	SMARCA4	SMARCB1	SMARCE1	SMO	SOX2	SOX9
SRC	STAG2	STAT3	STK11	SUFU	SYK	TBX3	TCF7L2	TET2
TGFBR2	TMEM127	TMPRSS2	TNFAIP3	TOP1	TOP2A	TP53	TP73	TSC1
TSC2	VEGFA	VHL	WT1	XPO1	XRCC2	XRCC3	ZFHX3	ZMAT3
intron, promoter, fusion points/breakpoints								
ALK	BCL2L11	BRAF	BRCA1	BRD4	CD74	EGFR	EML4	ERG
ETV6	EZR	FGFR1	FGFR2	FGFR3	KIF5B	KIT	MAML2	MET
MSH2	MYC	MYCL1	NCOA4	NOTCH2	NTRK1	NTRK2	NTRK3	PDGFRA
PMS2	PPARG	RAF1	RET	ROS1	RSPO2	SLC34A2	TERT	TFE3
TMPRSS2	TPM3							
partial exons								
ABCA13	ABCB1	ABCC1	ABCC11	ABCC2	ABCG2	ACACA	ACIN1	ACTB
ACTG1	ACTG2	ACVR2A	ACVRL1	ADAM29	ADAMTS5	ADCY1	AFF1	AFF2
AFF3	AFF4	AHNAK	AKAP9	ALB	AMOT	ANGPT1	ANK3	ANKRD27
ANKRD30A	ANKRD30B	ANKRD36B	APEX1	APOBEC3B	ARAP3	ARFGEF1	ARFGEF2	ARHGAP26
ARHGAP29	ARHGAP35	ARID4B	ARNT	ASCL4	ASH1L	ASMTL	ASPM	ASTN1
ASXL2	ATIC	ATP12A	ATP11B	ATP1A1	ATP2B3	BAZ2B	BBS9	BCAS1
BCL11A	BCL11B	BCL2A1	BCL2L11	BCL3	BCL9	BCLAF1	BCORL1	BCR
BIRC2	BIRC3	BMPR2	BNC2	BPTF	BRD2	BRD3	BRSK1	BRWD1
BTLA	BUB1	C15orf23	C15orf55	C1QA	C1S	C3orf70	C7orf53	C8orf34
CACNA1D	CACNA1E	CADM2	CAMTA1	CAPN7	CARD11	CASP1	CASQ2	CBLB
CBR1	CBR3	CCDC168	CCNA1	CCNB3	CCT3	CCT5	CCT6B	CD22
CD33	CD5L	CDA	CDH11	CDH18	CDH23	CDK13	CHD1	CHD1L
CHD3	CHD4	CHD6	CHD8	CHD9	CHFR	CHI3L1	CHN1	CIITA
CKS1B	CLCC1	CLDN18	CLP1	CLSPN	CLTC	CNOT3	CNOT4	CNTN1
CNTN5	CNTNAP1	CNTNAP5	COL1A1	COL2A1	COL5A1	COL5A2	COL5A3	COPS2
CPS1	CREB3L1	CRIPAK	CRLF2	CRNKL1	CRTC1	CRYBG3	CSF1	CSF3R
CSMD1	CSMD3	CSNK1A1	CSNK1G3	CSNK2A1	CTLA4	CTNNA2	CTNND1	CUX1
CYBA	CYP19A1	CYP1B1	CYP1A1	CYP2A13	CYP2C19	CYP2C8	CYP2D6	CYP3A4

Table S1 (*continued*)

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Table S1 (*continued*)

whole exons								
CYP3A5	DCC	DDX3X	DDX5	DEK	DHX35	DHX9	DIAPH1	DIS3L2
DLC1	DMD	DNAH6	DNAJC11	DNM2	DNMT1	DOCK2	DOCK7	DOT1L
DPYD	DRGX	DTX1	DUSP22	DYSF	EBF1	ECT2L	EEF1A1	EGR3
EIF2AK3	EIF2C3	EIF3A	EIF4G3	ELF1	ELF3	ELF4	ELL	ELMO1
ELN	EMID2	EPC1	EPHA1	EPHA4	EPHA7	EPHB1	EPHB4	EPOR
EPPK1	EPS15	ERBB2IP	ERCC2	ESR2	ETS1	ETV1	ETV5	ETV6
EWSR1	EZR	F8	FAM131B	FAM135B	FAM157B	FAM22A	FAM46C	FAM5C
FAP	FASLG	FAT3	FAT4	FCGR1A	FCGR2B	FCRL4	FGF10	FGF14
FGF23	FGF3	FGF4	FGF6	FKBP5	FLG	FLI1	FLNC	FMN2
FMR1	FN1	FNDC4	FOXA2	FOXO3	FOXQ1	FRG1	FRMPD4	FUS
FXR1	FYN	FZD1	G3BP1	G3BP2	GABRA6	GATA2	GFRAL	GIGYF1
GKN2	GLB1L3	GLI1	GLI2	GLI3	GMPS	GNA12	GNA13	GNG2
GPC3	GPR124	GPX1	GRB7	GRM3	GSK3B	GSTM5	GSTP1	GUSB
H3F3A	H3F3C	HCLS1	HCN1	HDAC9	HECW1	HERC2	HEY1	HIP1
HIST1H1C	HIST1H1D	HIST1H1E	HIST1H2AC	HIST1H2AG	HIST1H2AL	HIST1H2AM	HIST1H2BC	HIST1H2BD
HIST1H2BJ	HIST1H2BK	HIST1H2BO	HIST1H3B	HIST1H4I	HLF	HMCN1	HNRPDL	HOXA11
HOXA13	HOXA3	HOXA9	HOXC13	HOXD11	HOXD13	HSD3B1	HSD3B2	HSP90AB1
HSPA8	HSPD1	HSPH1	ICK	IFITM1	IFITM3	IGF2	IGF2R	IGLL5
IGSF10	IKBKE	IKZF1	IKZF2	IKZF3	IL1RAPL1	IL21R	IL6	IL6ST
IMPG1	ING1	INHBA	INPP4A	INPP5D	INPPL1	IRF4	IRF6	ITGB3
ITK	ITSN1	JARID2	KALRN	KAT6A	KAT6B	KCNJ5	KCNQ2	KDM2B
KDM3B	KEL	KIF5B	KLB	KLF4	KLHL6	KLK1	KRTAP5-5	L3MBTL1
LAMA2	LCP1	LEF1	LGALS8	LIFR	LPHN2	LPP	LRP2	LRP4
LRP5	LRP6	LRRC7	LRRK2	LYN	LZTS1	MACF1	MAD1L1	MAGI2
MAGOH	MAML2	MAML3	MAP3K13	MAPK3	MCC	MCM3	MDH2	MECOM
MEF2C	MGA	MIB1	MIOS	MKI67	MKL1	MLL4	MLLT3	MLLT6
MMP2	MMP11	MN1	MNDA	MNX1	MPO	MSH4	MSN	MSR1
MTHFR	MTRR	MUC5B	MYB	MYBL2	MYH10	MYH11	MYH14	MYH9
MYO3A	NAP1L1	NAV3	NBPF1	NCAM2	NCF2	NCF4	NCK1	NCOA2
NCOR2	NCSTN	NDRG1	NEB	NFATC4	NFE2L2	NFE2L3	NIN	NKX3-1
NLRC3	NOD1	NOS3	NQO1	NR1I2	NR2F2	NR4A2	NRP2	NRXN1
NTM	NTRK2	NUMA1	NUP107	NUP210	NUP98	OBSCN	OGDH	OMD
OPCML	OR11G2	OR2T4	OR4A15	OR4C6	OR5L2	OR6F1	P2RY8	P4HB
PABPC1	PABPC3	PAG1	PAK1	PAK3	PARK2	PARP1	PASK	PAX3

Table S1 (*continued*)

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Table S1 (*continued*)

whole exons								
PAX7	PBX1	PC	PCDH18	PCLO	PCSK6	PCSK7	PDCD1	PDCD11
PDE4DIP	PDGFB	PDILT	PER1	PGR	PHF1	PIK3C2A	PIK3C2B	PIK3C2G
PIK3R3	PIP5K1A	PKD1L2	PKHD1	PLAC8	PLAG1	PLCB1	PLCG1	PLCG2
PLK1	PLXNA1	PLXNB2	POLQ	POLR2B	POM121	POM121L12	POTEG	POU2AF1
PPP1R17	PPP2R1A	PPP6C	PRAM1	PRDM1	PRDM16	PREX2	PRF1	PRKAA1
PRKCB	PRKCI	PRKDC	PRRX1	PRX	PSG2	PSIP1	PSMB1	PSMB5
PTGS1	PTGS2	PTK2	PTPN13	PTPN2	PTPRB	PTPRD	PTPRF	PTPRJ
PTPRK	PTPRO	PTPRT	PTPRU	RAB35	RAC1	RAC2	RAD21	RAD54B
RANBP2	RASA1	RASGRP1	RBL1	RECQL4	REL	RELN	RFC1	RGS3
RHOH	RHOT1	RIT1	RNF213	ROBO1	ROBO2	ROBO3	ROCK1	RPGR
RPL22	RPTOR	RSPO2	RSPO3	RUNX1T1	RUNX2	RXRA	RYR1	RYR2
SBDS	SCUBE2	SEC31A	SEMA3A	SEMA3E	SEMA6A	SERP2	SERPINA7	SETBP1
SETDB1	SF1	SF3A1	SF3A3	SF3B1	SFPQ	SGCZ	SH3PXD2A	SHH
SI	SIN3A	SLC16A1	SLC1A2	SLC22A16	SLC22A18	SLC22A2	SLC22A3	SLCO1B3
SLIT1	SLIT2	SMAD3	SMC1A	SMC1B	SMURF2	SNCAIP	SNTG1	SNX29
SOD2	SOS1	SOX10	SOX17	SPEN	SPOP	SPRR3	SPSB4	SPTA1
SRD5A2	SRGAP1	SRGAP3	SRSF2	SRSF7	SSX1	STAG1	STAT1	STAT5A
SUCLG1	SUCLG2	SULT1A1	SUZ12	SVEP1	SYNCRIP	SYNE1	TAF1	TAF15
TAF1L	TAL1	TBL1XR1	TBX15	TBX22	TCEB1	TCERG1	TCF12	TCF3
TCF4	TCL1A	TCP11	TEC	TENM3	TERT	TFDP1	TFDP2	TFE3
TGFBR1	TGFBR3	TGM2	THBS1	THBS2	THRAP3	TJP1	TLE1	TLL2
TLR4	TLX3	TMEM132D	TNN	TNPO1	TOP2B	TP53BP1	TP63	TPM3
TPR	TRAF5	TRERF1	TRIM24	TRIM58	TRIO	TRPC5	TRRAP	TSHR
TSHZ2	TSHZ3	TTF1	TTL	TUBA3C	TUBB3	TUSC3	TXNIP	TYMS
TYR	TYRP1	U2AF1	UBE2D2	UBR5	UGT1A1	UMPS	UPF3B	USH2A
USP6	USP8	VDAC2	VEZF1	VIM	WASF3	WDR90	WDTC1	WHSC1
WHSC1L1	WIPF1	WNK1	WNT5A	WSCD2	WWOX	WWP1	WWP2	XBP1
XPC	XRCC1	YBX1	YY1AP1	ZBTB16	ZC3H11A	ZFP36L1	ZFP36L2	ZFPM2
ZIC3	ZNF217	ZNF384	ZNF521	ZNF638	ZNF750	ZNF804B	ZNF814	
germline mutation								
ATM	BRCA1	BRCA2	MLH1	MLH3	MSH2			
MSH3	MSH6	PALB2	PMS1	PMS2				

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Table S2 Patient and tumor characteristics according to TP53 status

Variable	N	TP53 status		
		Mutation (n=409)	Wild (n=83)	P value
Age (years)				
<60	89	74	15	0.452
60-70	204	174	30	
>70	190	153	37	
Unknown	9	8	1	
Sex				
Male	363	307	56	0.152
Female	129	102	27	
Lymph node status				
pN0	316	265	51	0.695
pN1	130	105	25	
pN2/N3	46	39	7	
Tumor				
T1	110	87	23	0.355
T2	288	245	43	
T3	70	59	11	
T4	24	18	6	
Tumor stage				
I	239	198	41	0.682
II	160	132	28	
III	86	72	14	
IV	7	7	0	
Primary Tumor Site				
L-Upper	131	105	26	0.308
L-Lower	77	62	15	
R-Upper	128	107	21	
R-Middle	17	12	5	
R-Lower	108	96	12	
Bronchial	10	9	1	
Unknown	21	18	3	

Table S2 (*continued*)

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Table S2 (*continued*)

Variable	N	TP53 status		
		Mutation (n=409)	Wild (n=83)	P value
Year Initial Diagnosis				
–2000	30	24	6	0.583
2001–2005	96	85	11	
2006–2010	182	151	31	
2011–	167	135	32	
Unknown	17	14	3	
Surgical Margin Resection Status				
R0	390	326	64	0.862
R1+R2	17	14	3	
Unknown	85	69	16	
CDKN2A Mutation status				
Wild type	406	331	75	0.040
Mutated type	86	78	8	

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Table S3 Patient and tumor characteristics according to CDKN2A status

Variable	N	CDKN2A status		
		Mutation (n=86)	Wild (n=406)	P value
Age (years)				
<60	89	20	69	0.553
60-70	210	34	176	
>70	184	30	154	
Unknown	9	2	7	
Sex				
Male	363	65	298	0.418
Female	129	21	108	
Lymph node status				
pN0	316	53	263	0.225
pN1	130	28	102	
pN2/N3	46	5	41	
Tumor				
T1	110	17	93	0.844
T2	288	53	235	
T3	70	11	59	
T4	24	5	19	
Tumor stage				
I	239	38	201	0.344
II	160	34	126	
III	86	12	74	
IV	7	2	5	
Primary Tumor Site				
L-Upper	131	20	111	0.332
L-Lower	77	13	64	
R-Upper	128	22	106	
R-Middle	17	1	16	
R-Lower	108	20	88	
Bronchial	10	3	7	
Unknown	21	7	14	

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