Tumor Mutation Burden May be a Prognostic Biomarker of Long-Term Survival in Resected Small-Cell Lung Cancer

Jing Qin
The Cancer Hospital of the University of Chinese Academy of Sciences（Zhejiang Cancer Hospital）
Qiuyue Pan
The Cancer Hospital of the University of Chinese Academy of Sciences（Zhejiang Cancer Hospital）
Lei Wang
The Cancer Hospital of the University of Chinese Academy of Sciences（Zhejiang Cancer Hospital）
Chenghui Li
The Cancer Hospital of the University of Chinese Academy of Sciences（Zhejiang Cancer Hospital）
Hongyang Lu (luhy@zjcc.org.cn)
Zhejiang Cancer Hospital

Research

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Abstract

Background

Small cell lung cancer (SCLC) has a poor prognosis. The majority of SCLC patients do not survive within a few months of diagnosis, however, a sub-group of patients have a long-term survival. Individual differences in prognosis remain elusive. We present the first comprehensive comparative genomic profiling and tumor mutation burden (TMB) analyses of SCLC on patients with long-term survival (LTS) and short-term survival (STS) after surgery.

Methods

The present study included 52 patients with SCLC who underwent surgery in Zhejiang Cancer Hospital from April 2008 to December 2017. A total of 6 LTS patients (≥4 years) with stage IIB or IIIA SCLC and 5 STS patients (<2 years) with stage IA or IB SCLC were included. The latter subjects were used as control subjects. All subjects underwent resection without neoadjuvant therapy. We assessed their genomic profile and calculated TMB using next-generation sequencing (NGS). Moreover, we assessed the correlation between TMB and prognosis. Subsequently, we analyzed and compared the molecular characteristics of LTS and STS.

Results

The data indicated that the LTS harbored high TMB. The median TMB for LTS was approximately 16.4 Mutations/Mb, while STS harbored low TMB which was approximately 8.5 Mutations/Mb. A differential trend on the median TMB was revealed between LTS and STS (p = 0.08). Moreover, we discovered that TMB exhibited significant effects on the survival of the patients (p=0.007). In the LTS group, the median number was 10 (range: 6-13) in mutated genes per sample and the samples harbored collectively 60 gene mutations in the following 28 genes: *TP53* (n=6), *RB1* (n=4), *FAT3* (n=4), *KMT2D* (n=3), *NOTCH1* (n=3), *FAM135B* (n=3), *LRP1B* (n=3), *CDKN2C* (n=3), *H3F3A* (n=3) . In the mutated genes per sample of STS, the median number was 7 (range: 3-8) and 30 mutations were found in the 17 genes as followed: *TP53* (n=5), *RB1* (n=4), *KMT2D* (n=2), *NOTCH1* (n=2), *FAT4* (n=2), *MUC16* (n=2), *PTEN* (n=2), *FAM135B* (n=1), *LRP1B* (n=1), *KMT2C* (n=1). Gene alteration represented the survival difference between the two groups. The *FAT3* gene was only noted in LTS and the P-value was 0.06 as determined by the Fisher's exact test. Interestingly, the *FAT3* gene could impact the patients' survival (p=0.11) though no significant difference were noted.

Conclusions

High nonsynonymous TMB was associated with improved prognosis on patients with resected SCLC. The *FAT3* gene may impact disease prognosis. The data may provide valuable information of differences between individuals in terms of prognosis and guide treatment. Studies involving larger groups are required to confirm these findings.

Background

Lung cancer remains the leading cause of cancer mortality worldwide and in China [1, 2]. Small cell lung cancer (SCLC) nearly makes up 15% of all lung cancers, and it is recognized as a highly aggressive and lethal malignancy often seen with early development, extensive metastasis and rapid recurrence following treatment. SCLC patients can be divided into limited diseases (LD) and extensive diseases (ED) with clinical stage. LD patients have a chance being cured with a long-term survival rate of 20–25% when treated with the standard chemoradiotherapy and prophylactic cranial irradiation (PCI). ED patients are theoretically incurable. The median overall survivals (OS) for patients with LD and ED are approximately 15–20 months and 8–10 months, respectively. The median OS is recorded to range from 29 to 91 months among patients who take comprehensive treatment including surgery [3–6], with an extended therapeutic window, which is worthy of consideration.

Despite the existence of rare long-term survivors of SCLC, there is still relatively a lack of prognostic factors apart from the disease staging and the performance status (PS). Furthermore, it is difficult to explain the ability of certain patients in late stage to get long-term survival, as well as the ability of patients in early stage to relapse among the patients having optimal PS scores. Consequently, in the absence of an adjusted multivariate analysis, it is difficult to assess the prognostic significance of the reported biomarkers beyond clinical variables. However, the determinants of long-term survival in SCLC remain largely unknown. Comprehensive genomic profiling is probably required to establish more robust prognostic markers in SCLCs.

In order to explore the potential genetic alterations and identify prognostic biomarkers in long-term survival (LTS) we analyzed a case control study comparing surgically resected tumors of late-stage (Ⅱ or IIB) long-term survival with tumors of early-stage (I) short-term survival (STS). Here, we investigate the genomic profiling and tumor mutation burden (TMB) of LTS and STS using a gene panel (OrigiMed, Shanghai, China) in resected SCLCs, covering all the coding exons of 450 cancer-related genes and 64 selected introns of 39 genes that are frequently rearranged in solid tumors. We expect to find the genetic differences that represent a contributor to survival.

Materials And Methods

Sample collection

Postoperative tissue specimens were retrospectively obtained from 52 patients with SCLC. All included 52 patients who underwent surgery at Zhejiang Cancer Hospital (Hangzhou, China) between April 2008 to December 2017 [7] in view of at least 2-year follow-up. All patients were diagnosed with conventional SCLC and the pathological diagnosis was based on the standard criteria defined by the World Health Organization classification [8]. Tumor stage was defined according to the lung cancer TNM classification, eighth edition [9].

The selection process of the SCLC patients is demonstrated in Figure 1. Patients who experienced neoadjuvant chemotherapy or chemoradiotherapy were excluded from our research due to potential puzzling effects of treatment-induced DNA damage. The two special subsets were selected from 52 patients. Patients screened in the study based on the following selection criteria: LTS: Stage Ⅱ or IIB and OS≥4 years, sufficient tumor tissue for testing; STS: Stage Ⅱ and OS<2 years, sufficient tumor tissue for testing. A total of 6 subjects met the standard of LTS and 5 that of STS (Fig. 1). The present research was approved by the Medical Ethics Committee of Zhejiang Cancer Hospital. The overwhelming majority of the specimens in the study were obtained from the Biological Sample Bank of Zhejiang Cancer Hospital, and the patients signed the written informed consents to have their specimens preserved in the Biological Sample Bank of Zhejiang Cancer Hospital for use in the research study. A limited number of patients were deceased.

Sample preparation

From each tumor-rich formalin-fixed paraffin-embedded (FFPE) and matched normal lung tissue block, 4 μm of sections were cut, deparaffinized and dissected to isolate 1 cm^2 of tumor tissue. DNA was isolated using the Cobas R DNA Sample Preparation Kit, according to the manufacturer protocol (Roche Molecular Systems, Pleasanton, CA, USA). The DsDNA concentration was determined using the Qubit R _ 2.0 Fluorometer and the Qubit R _ 2.0 dsDNA HS Assay Kit (ThermoFisherScientific, Waltham, MA, USA). The quality of the sample DNA was evaluated using a specimen control size ladder test (Invivoscribe Technologies, San Diego, CA, USA).

Next-generation sequencing

The genomic information was produced by NGS-based YuanSu^{TM} 450 gene panel (OrigiMed, Shanghai, China) which covers all the coding exons of 450 cancer-related genes and 64 selected introns of 39 genes that are frequently rearranged in solid tumors. The genes were captured and sequenced with a mean depth of 800X, using Illumina NextSeq 500 (Illumina, Inc). Genomic alterations (GAs) were identified by the alignment of sequences from tissues and matched normal lung tissue, following the previously reported methods [10]. Tumor mutation burden (TMB) was estimated by counting the somatic mutations, containing SNVs and Indels, per megabase of the sequence examined on each patient. The driver mutations and recorded germline alterations were not counted.

Statistical analysis

Statistical analyses were performed with the SPSS version 22.0 (SPSS Inc). The significance of the differences was analyzed with the Fisher's exact test. A P value lower than 0.05 (p<0.05) was considered to indicate a significant difference.

Follow-up

The follow-up deadline was March 17, 2020. Five patients are still alive and no patient was lost to follow-up and six patients were deceased. The survival time was counted from the date of pathological diagnosis.

Results

Patient characteristics

The median overall survival (mOS) was 87 months (range: 51-143) in the LTS group and 18 months (range: 16-22) in the STS group. The patient characteristics are presented in table 1. The LTS group included 3 male and 3 female subjects, whereas the STS (n=5) patients were all male subjects. Patients in the LTS group aged from 49 to 63 years old (57 ± 6), while that of STS ranged from 38 to 76 years old (57.8±15 years old). Four patients were heavy smokers (≥20 packs/year) and the 5 remaining patients did not have a smoking history (Table 1).

The association between TMB and prognosis

The median TMB rates of the LTS and STS groups were 16.4 Mutations/Mb (19.25±9.48) and 8.5 Mutations/Mb (9.88±5.35) respectively. We defined 10 mutations/Mb as the cut-off value. A value higher than 10 (TMB>10 mutations/Mb) was considered high and a value lower than and/or equal to 10 (TMB≤10 mutations/Mb) low. The differences between the LTS and STS groups were assessed via the Fisher's exact test. The P-value was 0.08 (Figure 2). Moreover, univariate analysis indicated significantly longer overall survival (OS) with high TMB than that with low TMB (NA vs. 22 months, p=0.007) (Figure 3). Univariate analysis indicated that high TMB was an independent prognostic factor for OS with adjustment for age, sex, and smoking status (Table 2). The clinical and TMB characteristics are provided for each patient in Supplementary Table S1.

Sequence analysis and identification of mutations

In all 11 samples (6 LTS and 5 STS), 29 somatic mutated genes were recognized. All the SCLC samples carried a minimum of 5.5 mutations. The top 10 genes with the highest mutation frequency were as followed: *TP53* (N=11, 100%), *RB1* (N=8, 73%)、*KMT2D* (N=5, 45%)、*NOTCH1*(N=5, 45%)、*FAM135B*、N=4, 36%), *FAT3* (N=4, 36%)、*LRP1B* (N=4, 36%), *CDKN2C* (N=3, 27.3%), *H3F3A* (N=3, 27.3%) and *FAT4* (N=3, 27.3%)(Figure 4). In these mutated genes per sample, the median number was 10 (range: 6-13) in the LTS and 7 (range: 3-8) in the STS, whereas the mutated genes number was 28 and 17 in the LTS and STS groups respectively. *FAT3, CDKN2C, H3F3A, ARAF, B2M, CUL3, EPHA3, FAT1, FLT4, PRKDC* and *STAT3* were discovered only in LTS and *PTEN* was unique to the STS (Table 3). Only one gene alteration was noted that represented the survival difference between the two groups. The *FAT3* gene was only found in the 4 LTS patients and the corresponding P-value was 0.06 as determined by the Fisher's exact test. The association of the *FAT3* gene with survival is shown in Figure 5.

Discussion

The research aimed to investigate whether genomic alterations in SCLC may differentiate LTS and STS who experienced surgical resection for early-stage SCLC. The data demonstrated that high TMB may be a prognostic biomarker of long-term survival in resected SCLC, regardless of the disease stage. A tendency was noted for the *FAT3* gene to separate between the LTS and STS groups. Although the present study contains a limited number of samples, our data suggests a discovery that warrants further investigation.

The association between TMB and disease prognosis in lung cancer is still unclear. Whether TMB is a prognostic factor for postoperative non–small-cell lung cancer (NSCLC) is controversial. Devarakonda et al. proved that high nonsynonymous TMB was significantly connected with a favorable prognosis in resected NSCLC [11]. In contrast to this study, Owada-Ozaki et al. demonstrated opposite results suggesting that high TMB could be related to a poor prognostic in lung cancer and in resected NSCLC [12]. However, the former study contained 908 samples, while the latter only 90 samples. As a consequence, the former conclusion seems more convincing. McGranahan et al. observed an association between higher OS and high neo-epitope burden on early-stage lung adenocarcinoma patients [13]. Yu et al. assessed 255 specimens from patients suffering early-stage squamous cell lung cancers (SqCLC) to evaluate the potential role of PD-L1 protein expression and TMB, and the putative identification of an immune gene signature [14]. The authors of that study found that TMB was not associated with OS. SCLC is characterized by high TMB because of its association with smoking. However, whether TMB affects the prognosis of resected SCLC has not been studied. Zhou et al analyzed the correlations between clinical outcomes and genomic alterations in 53 SCLC samples [15]. The authors of this study reported that high TMB (> 21 mutations/Mb) was connected with a favorable prognosis in OS (21.7 vs. 10.4 months, p = .012). In our study, we found that LTS exhibited higher median TMB (16.4 mutations/Mb, ranging from 10.8 to 35.6 compared with 8.5, from 5.2 to 17.9). There was no significant difference in the median TMB between the LTS and STS groups ($p = 0.08$) (Fig. 2). However, exploratory analyses suggested that TMB may have a significant predictive effect on OS ($p = 0.007$) (Fig. 3). For patients with high TMB and low TMB, The former group did not reach mOS and the latter one survived 22 months. These findings provide evidence that high TMB exhibits optimal prognostic value.

The genetic mutational landscape of SCLC is complex and heterogeneous. However, the most common genetic alterations include inactivation of the tumor suppressor genes *TP53* and *RB1* [16, 17]. Hu et al. demonstrated that the most frequently altered genes in small cell lung cancer were as followed: *TP53* (93.4%), *RB1* (78.7%), *LRP1B*(18.9%), *KMT2D* (15.6%), *FAT1* (11.5%), *KMT2C* (11.5%),*STK24* (11.5%), *FAM135B* (10.7%), and *NOTCH1* (10.7%) [10]. The results were derived from genomic profiling of 122 Chinese patients. In the study, with the exception of *TP53* and *RB1*, the remaining high frequency mutated genes were those that encoded enzymes involved in histone modification, notably those that participate in the NOTCH and Wnt signaling pathways. With the Fisher's exact test, we found a significant correlation between LTS and gene mutations on *FAT3*. The human *FAT* gene family consists of the *FAT1*, *FAT2*, *FAT3* and *FAT4* genes [18–21]. Hong et al reported the partial coding sequence of *FAT3* in 2004, whereas Katoh et al reported the complete coding sequence of *FAT3* and *FAT4* in 2006. *FAT3* is found on chromosome 11q14.3-q21 and has 26 exons encoding a protein of 4,557 amino acids [22]. The *FAT1* and *FAT3* genes adjoin the *MTNR1A* and *MTNR1B* genes, respectively. *FAT1* exhibits a higher homology with *FAT3*, while

MTNR1A exhibits a higher homology with *MTNR1B*. A previous study in mice indicated that *FAT3* expression restricted the development of central nervous system (CNS), with highest expression found at the olfactory bulb and retina [23]. These findings led to the hypothesis that murine *FAT3* plays significant roles in the development of CNS, possibly in axon organization and interaction [23, 24]. Sadeqzadeh et al reported that on patients suffering breast and ovarian cancer exhibited high frequency *FAT3* mutations and FAT3 impacts the development of the central nervous system [22, 25, 26]. Ji-Yeon Kim et al examined 119 patients of breast cancer in an exploratory biomarker study: a total of 40 subjects from that study exhibited an available biomarker. With targeted deep sequencing, *FAT3* (48%) was found to be the most frequently mutated gene. However, further survival analysis indicated that *FAT3* mutations seemed to be associated with poor prognosis, though the results were statistically insignificant [27]. The information regarding the association between *FAT3* and the prognosis in SCLC was lacking in the present study. Interestingly, *FAT3* mutation occurred solely in LTS and the P-value from the comparison with the STS group was 0.06 as determined by the Fisher's exact test. The survival curve analysis (Fig. 3) also showed that *FAT3* mutation may be associated with optimal prognosis among SCLC patients, although there was no notable difference between them ($p = 0.11$) (Fig. 3). This may be the first report regarding the effects of *FAT3* mutations on the prognosis of SCLC patients. However, the reports that exist on *FAT3* are limited and it remains unknown whether the genetic changes occurring in *FAT3* can significantly affect the pathophysiology of the cancer [22]. Further studies that can prove the implication of *FAT3* in SCLC would be of significant value.

This study has a few limitations. The sample size was small and this was retrospective study. Surgical specimens were scarce and precious due to the less opportunity for surgery. For this reason, scientific studies about SCLC molecular profiles are hampered by a lack of tissue availability. Moreover, in order to thoroughly ignore the effect of stage on prognosis, we selected two extreme cohorts of LTS with stage IIB-III and STS with stage I as the objects of study. All the patients were followed up for more than 2 years. Therefore, the number of patients who qualified is very small. Despite these limitations, our study offered some new discoveries.

Conclusions

We studied the genomic alteration profile and TMB of LTS and STS. High nonsynonymous TMB may be considered an optimal prognostic biomarker of long-term survival in resected SCLC. The *FAT3* gene may exert an impact on prognosis and it may be a potential and interesting gene to study. Further studies are warranted to confirm these observations and explore the mechanisms underlying this association.

Abbreviations

SCLC: Small cell lung cancer; TMB: tumor mutation burden; LTS: long-term survival; STS: short-term survival; NGS: next-generation sequencing; LD: limited diseases; ED: extensive diseases; PCI: prophylactic cranial irradiation; OS: overall survival; PS: performance status; FFPE: formalin-fixed paraffin-embedded;

Gas:Genomic alterations; NSCLC: non–small-cell lung cancer; SqCLC: squamous cell lung cancers; CNS :central nervous system.

Declarations

Ethics approval and consent to participate:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The Medical Ethical Committee of Zhejiang Cancer Hospital approved this study. This study is a retrospective study, and the overwhelming majority of the specimens in the study were obtained from the Biological Sample Bank of Zhejiang Cancer Hospital, and the patients signed the written informed consents to have their specimens preserved in the Biological Sample Bank of Zhejiang Cancer Hospital for use in the research study.

Consent for publication:

Not applicable.

Availability of data and materials:

Not applicable.

Competing interests:

The authors have declared that no competing interest exists.

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Authors' contributions:

Hongyang Lu and Jing Qin conceived and designed the study. Jing Qin wrote the manuscript and collected the clinical data. Qiuyue Pan and Lei Wang analyzed genetic informations. Chenghui Li collected the clinical and follow-up data .All authors read and approved the final manuscript.

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Authors' information:

[1]The Cancer Hospital of the University of Chinese Academy of Sciences（Zhejiang Cancer Hospital）， Institute of Basic Medicine and Cancer（IBMC），Chinese Academy of Sciences，Hangzhou，Zhejiang 310022，China. [2]Zhejiang Key Laboratory of Diagnosis & Treatment Technology on Thoracic Oncology (lung and esophagus), Zhejiang Cancer Hospital, Hangzhou, 310022, P.R. China.[3]OrigiMed, Shanghai, China

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Tables

Table1: The characteristics of the participants.

	LTS	**STS**
	N=6	N=5
Age Median（range）	57 (49-63)	57.8 (38-76)
Sex		
Male	3(50%）	5 (100%)
Female	3(50%)	0
TMB （mean±SD）	19.25±9.48	9.88±5.35
Median	16.4	8.5
Smoking		
Yes	2（33.3%）	4 (80%)
No	4（66.7%）	1 (20%)
Over survival （mean±SD）	95.7±40.1	19±2.83
Median	87	18

Table 2: Univariate analysis of OS

	OS		
	HR	95%CI	P value
Age≥60	1.75	0.32-9.62	0.517
Sex	2.98	0.34-26	0.322
Smoking	3.64	0.42-31.28	0.24
TMB≥10 mutations/Mb	0.11	0.02-0.69	0.018*

Table 3: Number of mutated samples per gene in the LTS and STS

Gene	Mutated samples				Gene	Mutated samples			
	LTS		STS			LTS		STS	
	N=6	(%)	N=5	%		N=6	(%)	N=5	%
TP53	6	(100)	5	(100)	FLT4	2	(33)	0	(0)
RB1	4	(67)	4	(80)	PRKDC	2	(33)	0	(0)
FAT3	4	(67)	0	(0)	STAT3	2	(33)	0	(0)
KMT2D	3	(50)	2	(40)	FAT4	1	(17)	2	(40)
NOTCH1	3	(50)	2	(40)	MUC16	1	(17)	2	(40)
FAM135B	3	(50)	1	(20)	BCORL1	1	(17)	1	(20)
LRP1B	3	(50)	1	(20)	ERBB4	1	(17)	1	(20)
CDKN2C	3	(50)	0	(0)	LRP1	1	(17)	1	(20)
H3F3A	3	(50)	0	(0)	LRP2	1	(17)	1	(20)
KMT2C	2	(33)	1	(20)	PIK3CB	1	(17)	1	(20)
ARAF	2	(33)	0	0	SMARCA4	1	(17)	1	(20)
B2M	2	(33)	0	(0)	TEK	1	(17)	1	(20)
CUL3	2	(33)	0	(0)	TRIO	1	(17)	1	(20)
EPHA3	2	(33)	0	(0)	PTEN	0	(0)	2	(40)
FAT1	2	(33)	0	(0)					

Figures

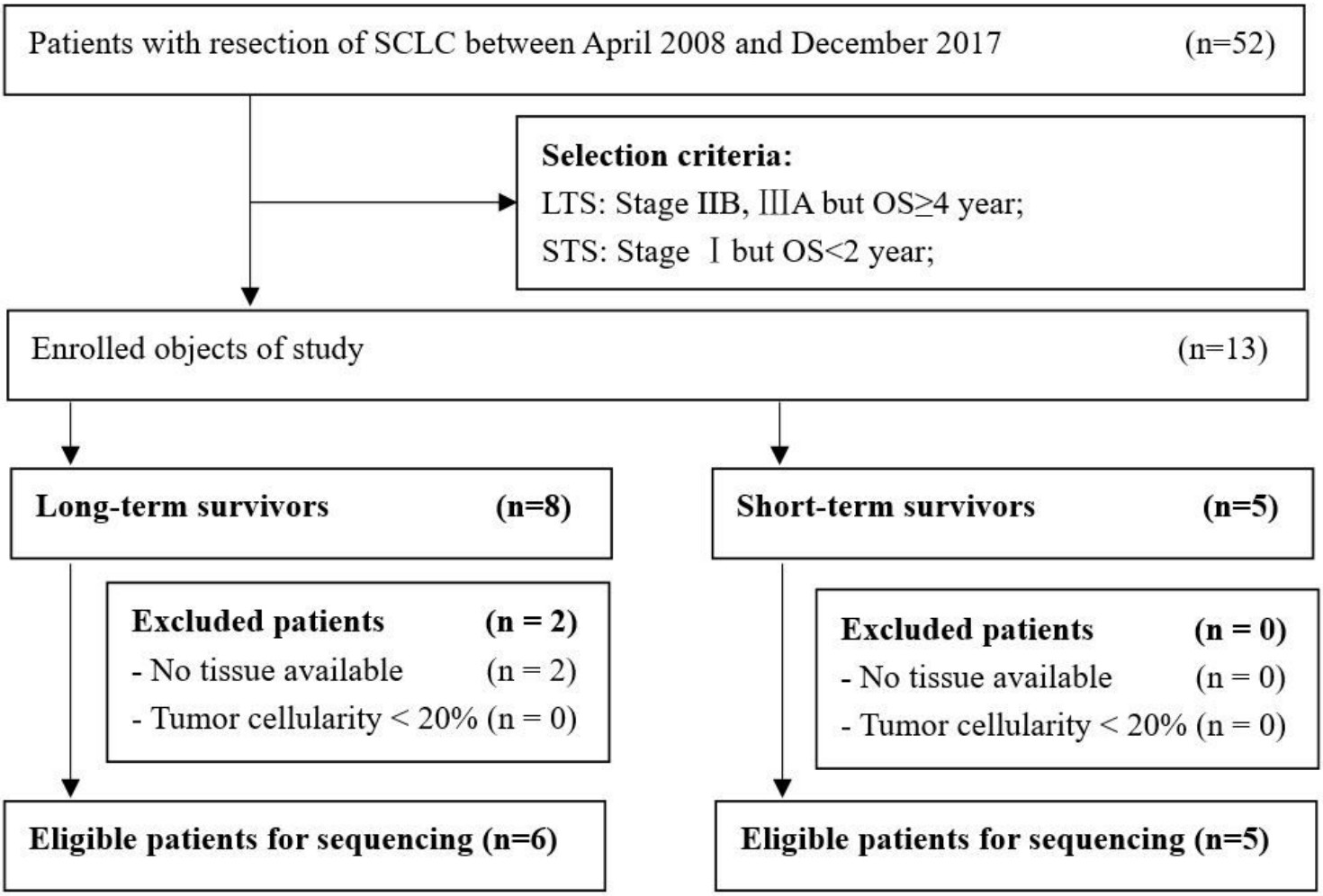

Figure 1

Selection of patients. The patients who underwent resection of SCLC were included on the basis of clinical stage and survival time. The patients were eligible for sequencing when tissue with sufficient tumor cellularity was available.

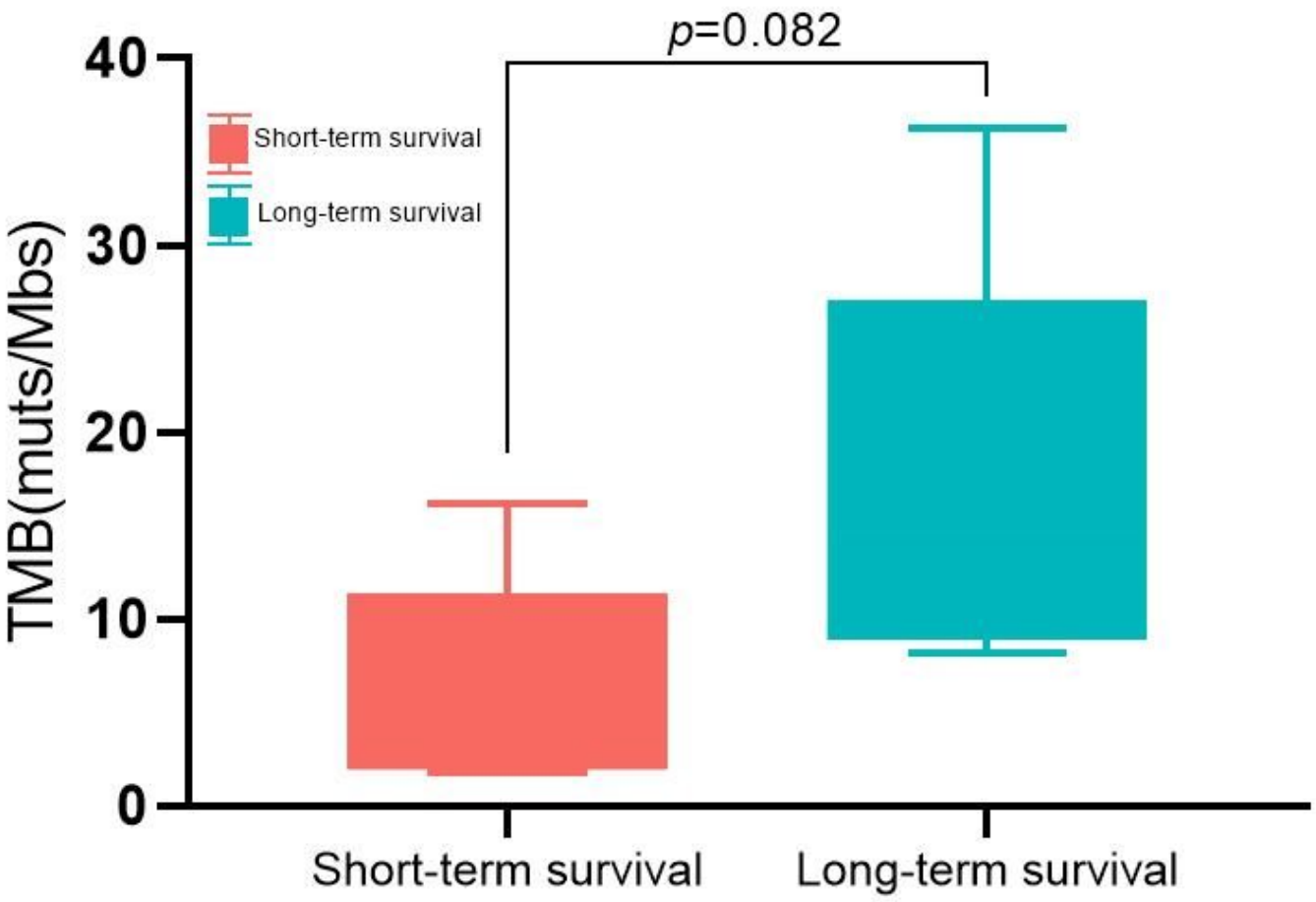

Figure 2

TMB in LTS versus STS. The data indicate no significant correlation for TMB-L in STS with TMB-H in LTS.

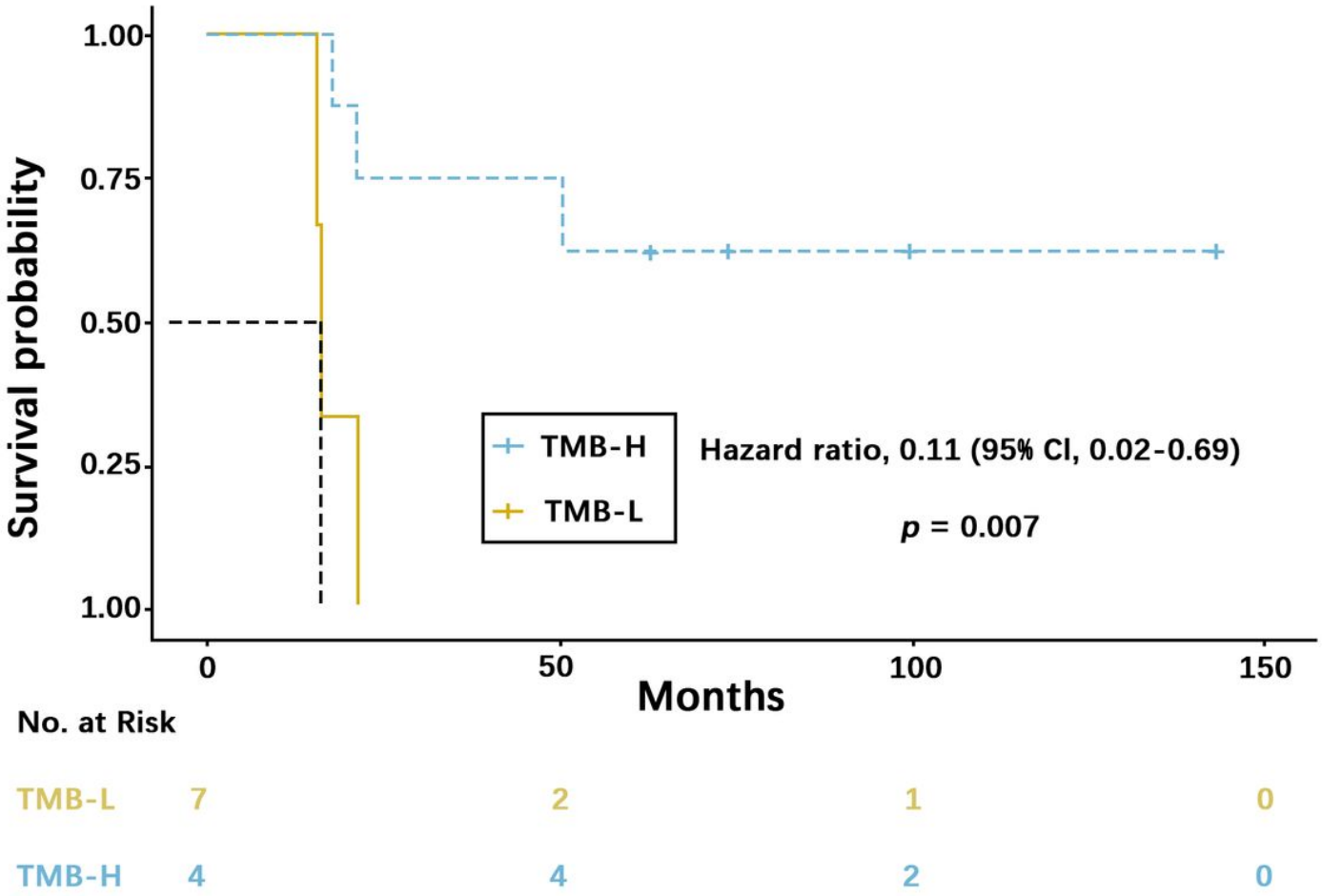

Figure 3

Kaplan-Meier analysis of the effect of nonsynonymous tumor mutation burden (TMB) on overall survival (OS). The data indicated that TMB was significantly associated with disease prognosis.

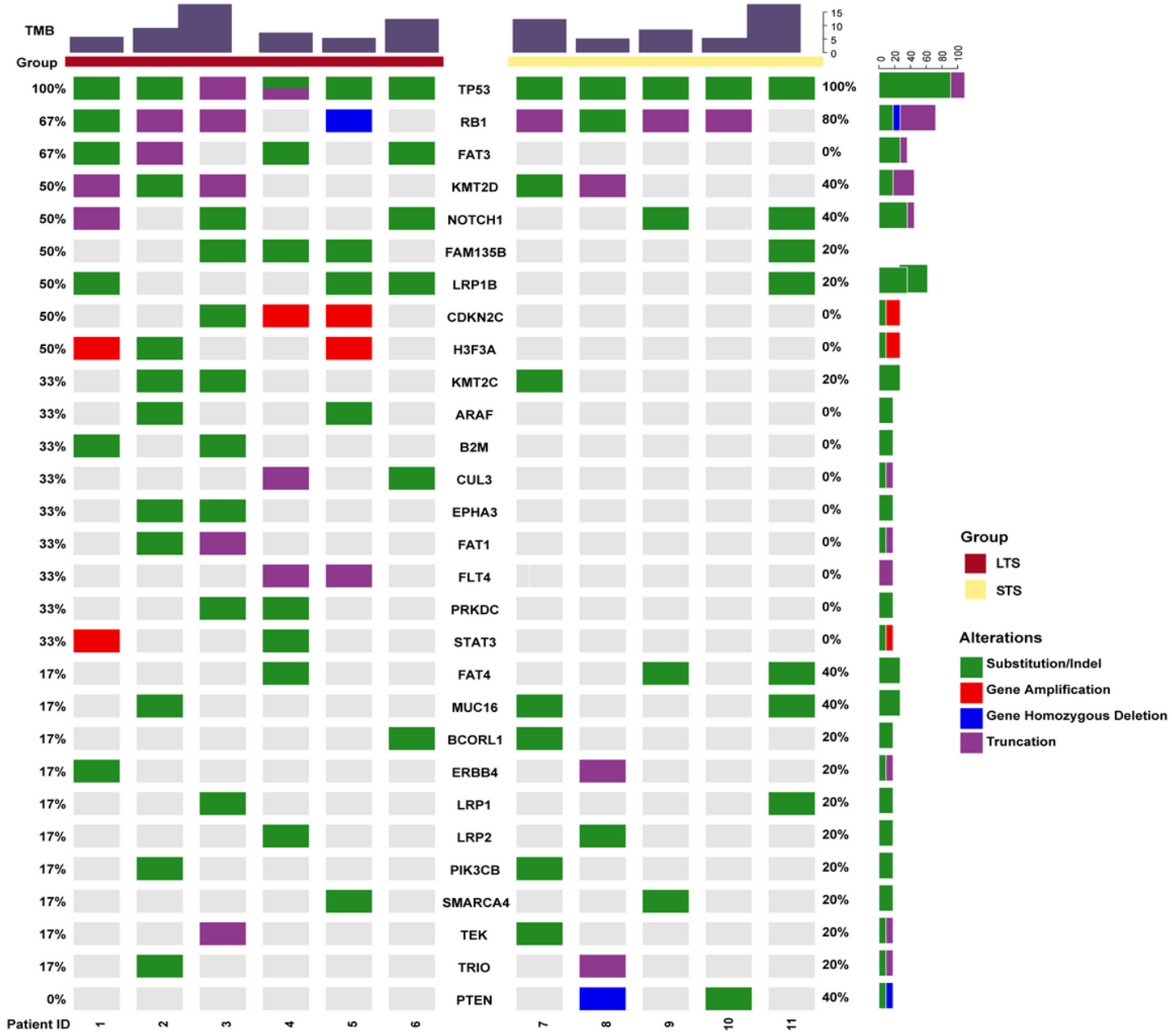

Figure 4

Genomic alterations in the LTS and STS groups. The left part denotes LTS and the right part STS. The abscissa denotes the tumor specimens and the ordinate the gene names. Genetic alterations were annotated according to the color panel on the right side of the image.

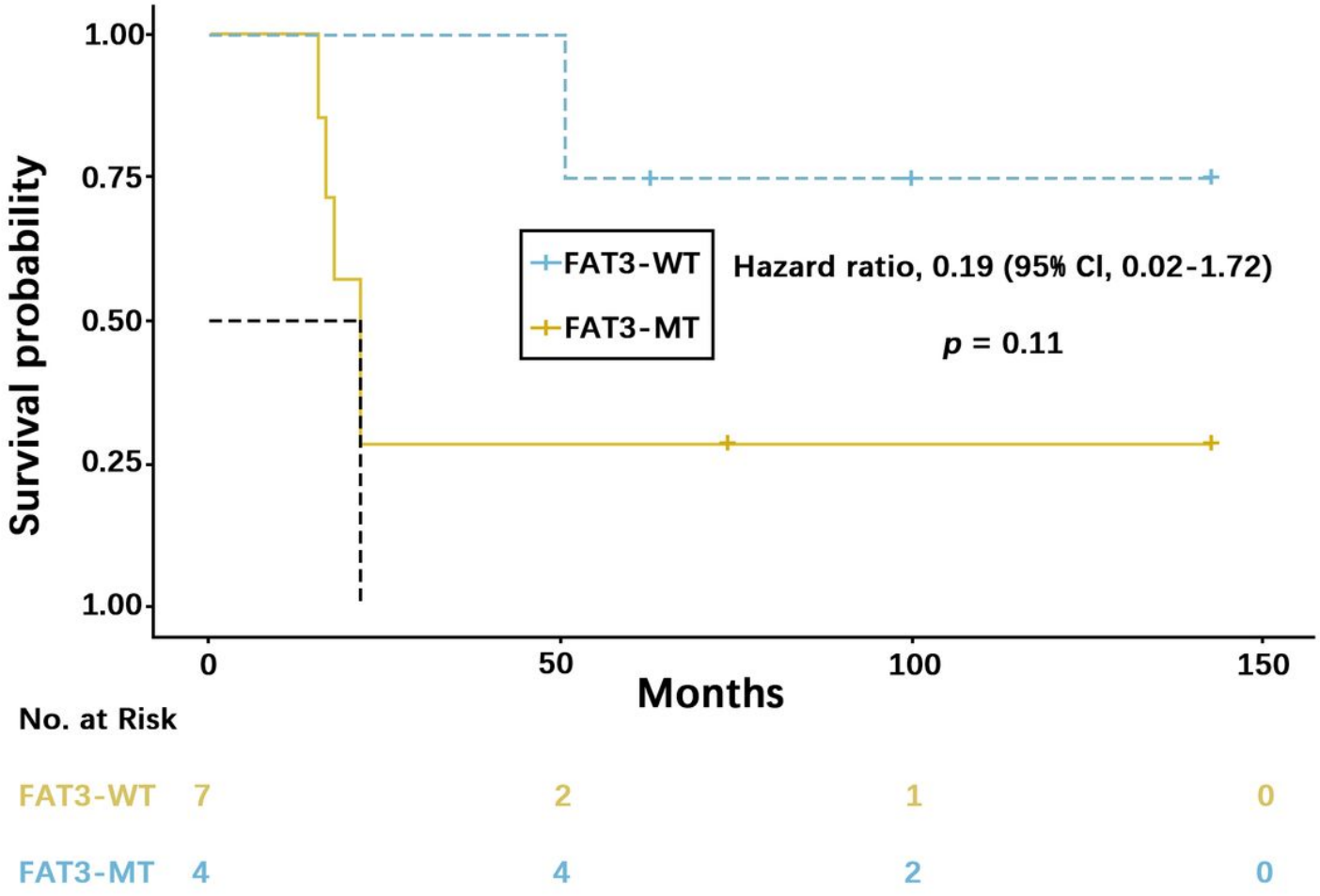

Figure 5

Kaplan-Meier analysis of FAT3 mutant and wild types. The data indicated no significant correlation for FAT3 with disease prognosis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx