Original Article

Somatic *KMT2D* loss-of-function mutations in lung squamous cell carcinoma: a single-center cohort study

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Background: The significant progress has been made in targeted therapy for lung adenocarcinoma (LUAD) in the past decade. Only few targeted therapeutics have yet been approved for the treatment of lung squamous cell carcinoma (LUSC). Several higher frequency of gene alterations are identified as potentially actionable in LUSC. Our work aimed to explore the complex interplay of multiple genetic alterations and pathways contributing to the pathogenesis of LUSC, with a very low frequency of a single driver molecular alterations to develop more effective therapeutic strategies in the future.

Methods: We retrospectively analyzed the targeted next-generation sequencing (NGS) data (approximately 600 genes) of 335 patients initially diagnosed with non-small cell lung cancer (NSCLC) at our institution between January 2019 and March 2023 and explored the somatic genome alteration difference between LUSC and LUAD.

Results: We analyzed that the presence of loss-of-function (LoF) mutations (nonsense, frameshift, and splice-site variants) in histone-lysine N-methyltransferase 2D (*KMT2D*) was much more prevalent in LUSC (11/53, 20.8%) than in LUAD (6/282, 2.1%). Moreover, our data indicated TP53 co-mutated with *KMT2D* LoF in 90.9% (10/11) LUSC and 33.3% (2/6) LUAD. Notably, the mutation allele fraction (MAF) of *KMT2D* was very similar to that of *TP53* in the co-mutated cases. Genomic profiling of driver gene mutations of NSCLC showed that 81.8% (9/11) of the patients with LUSC with *KMT2D* LoF mutations had *PIK3CA* amplification and/or *FGFR1* amplification.

Conclusions: Our results prompted that somatic LoF mutations of *KMT2D* occur frequently in LUSC, but are less frequent in LUAD and therefore may potentially contribute to the pathogenesis of LUSC. Concurrent *TP53* mutations, *FGFR1* amplification, and *PIK3CA* amplification are very common in LUSC cases with *KMT2D* LoF mutations. It needs more deeper investigation on the interplay of the genes and pathways and uses larger cohorts in the future.

Keywords: Histone-lysine N-methyltransferase 2D (*KMT2D*); lung squamous cell carcinoma (LUSC); non-small cell lung cancer (NSCLC); next-generation sequencing (NGS); retrospective study

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Introduction

Despite the advent of molecular targeted therapy and immunotherapy, lung cancer remains the leading cause of cancer-related death worldwide (1). Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all new lung cancer cases (1). Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the most common NSCLC subtypes. Over the past decade, significant progress has been made in the targeted treatment of LUAD harboring driver mutations, such as epidermal growth factor receptor (*EGFR*), kirsten rats arcomaviral oncogene homolog (*KRAS*), V-Raf Murine Sarcoma Viral Oncogene Homolog B (*BRAF*), proto-oncogene receptor tyrosine kinase (*MET)*, anaplastic lymphoma kinase (*ALK*), ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*), and rearranged during transfection (*RET*) gene aberrations, which have more frequently genetic alterations in LUAD than in LUSC (2-5). However, a vast majority of patients with NSCLC who receive targeted therapies will eventually develop drug resistance and experience tumor relapse (6,7). Recently, immune checkpoint inhibitors [e.g., anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1)] have shown significant clinical benefits for patients with advanced NSCLC and a high level of PD-L1 expression. However, two-third of NSCLC either do not express or express PD-L1 at a low level. The benefit of immunotherapy in this subset of NSCLC is rather modest (8-10).

In current clinical practice, NSCLC driver mutations can be relatively easy to detect using next-generation sequencing (NGS) technology from small tumor biopsy samples or blood by analyzing circulating-free DNA (5). The single driver oncogenes are much more common in LUAD and the prognosis for LUSC is poorer (1,11). Only a few targeted therapeutics have yet been approved for the treatment of LUSC, largely due to complex interplay of co-occurring genetic alterations driving the pathogenesis of LUSC as opposed to a clear single driver gene alteration (<20% in European patients) (12). The Cancer Genome Atlas Research Network (13) identified a higher frequency of alterations in *NFE2L2*, *PTEN*, *NOTCH*, *TP53*, and *Rb1* genes in LUSC samples. Additionally, alterations in FGFR, PIK3CA and DDR2 are also identified as potentially actionable in LUSC (14-18). There is an urgent need to gain insight into the molecular profiles of LUSC to dissect the role of individual gene in carcinogenic process to develop more effective therapeutic strategies.

Histone lysine methylation by lysine methyltransferase (KMT) is a posttranslational modification that plays important roles in the epigenetic regulation of a broad spectrum of biological processes, including development, differentiation, metabolism, and tumor suppression (19,20). Histone-lysine N-methyltransferase 2D (*KMT2D*), also known as *MLL2* or *MLL4* in some studies, belongs to a family of mammalian histone H3 lysine 4 (H3K4) methyltransferases (19,20). The human *KMT2D* gene is located on chromosome 12q13.12 and contains 54 exons, encoding a 5,537 amino acid protein including 7 plant homeodomain (PHD) domains, a high mobility group (HMG)-binding motif, an F/Y-rich C terminus (FYRC), an F/Y-rich N terminus (FYRN) motif, and a C-terminal SET

Highlight box

Key findings

- Our results demonstrate that the frequent occurrence of *KMT2D* somatic loss-of-function (LoF) mutations of *KMT2D* in lung squamous cell carcinoma (LUSC), while being uncommon in lung adenocarcinoma (LUAD) and therefore may potentially contribute to the pathogenesis of LUSC. LUSC cases harboring *KMT2D* LoF mutations frequently harbor *TP53* mutations, *FGFR1* amplification, and *PIK3CA* amplification.

What is known and what is new?

- The previous research identified a higher frequency of alterations in *NFE2L2*, *PTEN*, *NOTCH*, *TP53*, and *Rb1* genes in LUSC samples and the alterations in *FGFR*, *PIK3CA* and *DDR2* are also identified as potentially actionable in LUSC.
- We explored the Chinese LUSC cohort and found the KMT2D mutation has the potential to contribute to the pathogenesis of LUSC by working in concert with other common genetic alterations in LUSC, including *TP53* mutation, *FGFR1* amplification, and *PIK3CA* amplification.

What is the implication, and what should change now?

- Further studies are needed to understand the role of an individually altered genes in LUSC to explore their contribution towards LUSC carcinogenesis to effectively develop anti-tumor therapies.

domain. The KMT2D protein is a histone methyltransferase that monomethylates H3K4, a hallmark of an active transcription state. Additionally, the presence of the SET domain is responsible for the methyltransferase activity of the KMT2D protein (19,20). The KMT2D protein is essential for maintaining the level of H3K4 monomethylation via the enzymatic Su(var)3-9, Enhancer-of-zeste, Trithorax (SET) domain, which is correlated with transcriptionally engaged enhancer elements as an active transcription factor (21,22). Somatic loss-of-function (LoF) mutations in the *KMT2D* gene have been linked to many types of cancers, including lymphoma, leukemia, gastric cancer, esophageal squamous cell carcinoma (ESCC), lung cancer, prostate cancer, chordoid meningiomas, and adult granulosa cell tumor (22). Recent studies have indicated that the KMT2D protein functions as a tumor suppressor and might play an important role in carcinogenesis of LUSC (23,24), and perhaps may act as a driver alteration. The *NCOA6* and *KMT2C* or *KMT2D* were revealed to act as coactivators of the tumor suppressor and TF p53 in cell assays and the expression of endogenous p53 target genes needs the coactivators in response to doxorubicin, a DNA damaging agent (25). The *KMT2C* and *KMT2D* were demonstrated to act as a tumor suppressor in acute myeloid leukemia, follicular lymphoma, and diffuse large B cell lymphoma in three studies in mice (26-28). An important evidence about *KMT2D* as a key regulator of LUSC tumorigenesis was obtained in cell organoids of LUSC. *Kmt2d* loss activated receptor tyrosine kinases (RTKs) to a high level, partly through reprogramming the chromatin landscape to decrease the expression of protein tyrosine phosphatases. The study identified *KMT2D* functioned as a pivotal epigenetic modulator for LUSC oncogenesis and suggested that *KMT2D* loss leaded LUSC therapeutically vulnerable to RTK-RAS inhibition (24). However, LoF mutations in *KMT2D* have only been found in a small portion of the LUAD population. In this study, we examined the somatic genome alterations of patients with NSCLC to clarify the molecular mutation characteristics of *KMT2D* LoF mutations in patients with LUSC and LUAD. We present this article in accordance with the STROBE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-134/rc).

Methods

Patient cohorts and clinical characteristics

In this retrospective, single-institution cohort study, patients initially diagnosed with LUSC and LUAD at the Zhujiang Hospital between January 2019 and March 2023 were examined. Patients with lung mixed adenosquamous lung carcinoma were excluded. Before the administration of anti-cancer therapy, all included patients' samples underwent molecular genetic analysis with a targeted NGS gene panel that evaluated approximately 600 tumor-associated genes. The NGS data were reanalyzed to confirm LoF mutations in the *KMT2D* gene, while missense variants in this gene were not examined in this study due to the vast majority of variants being of uncertain significance. Our cohort consisted of 53 cases of LUSC and 322 cases of LUAD. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Zhujiang Hospital (No. 2024-KY-142-01). A waiver of patient consent was granted due to the retrospective, data-collection design of this study. Clinical data were obtained via an electronic medical record query, which included information on age, sex, smoking history, stage, specimen site, tumor histology, dates of diagnosis, and gene mutation analysis. Nonsmokers were defined as patients who had smoked fewer than 100 cigarettes in their lifetime. Smokers included former smokers, who were defined as those who quit >12 months before diagnosis, and current smokers, who were defined as those who quit <12 months before or still smoked at diagnosis.

Sample preparation and target sequencing

DNA extraction from tumor samples and targeted NGS were performed in a third-party laboratory (Mygene Diagnostics Co., Ltd., Guangzhou, China). DNA from either frozen (n=36) or formalin-fixed paraffin-embedded (FFPE) (n=299) tumor samples was extracted using the MagPure FFPE DNA LQ Kit C (Magen Biotechnology, Waltham, MA, USA). Germline DNA was extracted from blood using a Surbiopure Blood Genomic DNA kit (GuangZhou Surbiopure Biotechnology Co., Ltd., Guangzhou, China) as a reference for detecting somatic alterations. DNA quantity and purity were assessed using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). A total of 50 ng of each genomic DNA (gDNA) sample based on Qubit quantification was fragmented and subjected to end repair, A-tailing, and adapter ligation via the Universal Plus DNA Library Prep Kit (Vazyme, Nanjing, China) according

Table 1 Baseline clinical characteristics of patients initially diagnosed with NSCLC (n=335)

Characteristics	LUSC (n=53)	LUAD (n=282)	P value
Age (years)	68 [37–91]	61 [20–88]	<0.001
≥60	43 (81.1)	157 (55.7)	
<60	10 (18.9)	125 (44.3)	
Sex			<0.001
Male	43 (81.1)	151 (53.5)	
Female	10 (18.9)	131 (46.5)	
Smoking status			<0.001
Smoker (current/former)	39 (73.6)	84 (29.8)	
Non-smoker	14 (26.4)	198 (70.2)	
Clinical stage			0.09
I + II	9 (17.0)	79 (28.0)	
III + IV	44 (83.0)	203 (72.0)	
KMT2D LoF mutations	11 (20.8)	6 (2.1)	<0.001

Data are presented as median [range] or number (percentage). NSCLC, non-small cell lung cancer; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; LoF, loss of function.

to the manufacturer's instructions. Subsequently, libraries were captured using 2.6 M probes from TargetSeq Hyb & Wash Kit v. 2.0 (iGeneTech, Beijing, China) and finally amplified. After quality control with a Qsep 100 analyzer (BIOptic, New Taipei City, Taiwan) confirmed DNA without degradation and quantification with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific), the libraries were sequenced on an MGISEQ-2000 platform (BGI Group, Shenzhen, China).

Data analysis

Clean reads were obtained by filtering adapter, low-quality, and reads with a proportion of N>5 using a fastp v. 0.21.0. Clean reads were aligned to the reference human genome hg19 (GRCh37) using Burrows-Wheeler aligner maximum exact matches (BWA-MEM; v. 0.7.17). Somatic single-nucleotide variants (SNVs) and insertions/deletions were detected using VarDict 1.8.3 based on mapped consensus in binary alignment map (BAM) files for tumor and control tissue. Somatic SNVs and indels were further filtered according to the flowing criteria: read depth ≥100 in tumor samples, mapping quality ≥40 and base quality ≥20, variant allele frequency (VAF) ≥1%, supporting reads ≥3 in the tumor, and VAF in the tumor ≥5 times that of the matched normal VAF. Variant annotation for gene consequence was performed using Ensembl Variant Effect Predictor (VEP) 103.1. Somatic SNVs and indels were excluded when their population allele frequency >0.5% according to the 1000 Genomes Project, the Genome Aggregation Database (gnomAD), and the Exome Aggregation Consortium (ExAC) annotations. The copy number was determined using CNVkit 0.9.9 tool. Copy number homozygous deletion (copy number <0.5 and region of deletion >74%) and amplification (copy number >2.5 and amplification region >60%) were included in the analysis. Candidate structural variants (SVs) were determined using lumpy 0.2.13 under default parameters. Potential false SVs were identified and then excluded based on the following criteria: read depth <100 or supported by fewer than 3 split reads or 15 supported read pairs.

Statistical analysis

Descriptive statistics are presented as the median and range for continuous variables and as the number and percentage for categorical variables. Differences between groups were compared using the Chi-squared test or Fisher exact test. The P value less than 0.05 was considered statistically significant.

Results

Patient cohort description

A total of 335 patients diagnosed with NSCLC were included in this study. Among them, there were 53 cases (15.8%) of LUSC and 282 cases (84.2%) of LUAD. The clinical characteristics of patients with LUSC or LUAD are summarized in *Table 1*. Briefly, patients with LUSC or LUAD had a median age of 68 years (range, 37–91 years) and 61 years (range, 20–88 years) at diagnosis, respectively. There were significantly more male patients and smokers in the LUSC group than the in LUAD group (P<0.001). The clinical stage did not differ significantly between the two patient groups (P>0.05). Moreover, reanalysis of sequencing data revealed a higher prevalence of somatic LoF mutations (nonsense, frameshift, and splice-site variants) for *KMT2D* in the LUSC group than in the LUAD group (20.8% *vs.* 2.1%; P<0.001).

Table 2 KMT2D (NM_003482) LoF mutations detected in patients with LUSC and LUAD

Case ID	Variant	Amino acid change	Abbreviation	Exon/intron	Variant type
LUSC (n=11)					
P3	c.840-2A>G	N/A	N/A	Intron 6	sp
	c.3190dup	p.(Val1064Glyfs*4)	p.(V1064Gfs*4)	Exon 11	ins_fs
P10	c.4418G>A	p.(Trp1473*)	p.(W1473*)	Exon 15	non
P76	c.7539del	p.(Gln2514Serfs*29)	p.(Q2514Sfs*29)	Exon 31	del_fs
P96	c.14734G>T	p.(Glu4912*)	p.(E4912*)	Exon 48	non
	c.15433G>T	p.(Glu5145*)	p.(E5145*)	Exon 48	non
P136	c.839+1_839+2del	N/A	N/A	Intron 6	sp
P142	c.12688C>T	p.(Gln4230*)	p.(Q4230*)	Exon 39	non
P181	c.4302_4312del	p.(Gln1435Profs*8)	p.(Q1435Pfs*8)	Exon 15	del_fs
	c.6109+1G>A	N/A	N/A	Intron 28	sp
P229	c.14710C>T	p.(Arg4904*)	p.(R4904*)	Exon 48	non
P242	c.7807G>T	p.Glu2603*)	(p.E2603*)	Exon 31	non
P266	c.2605G>T	p.(Glu869*)	p.(E869*)	Exon 10	non
P313	c.1468G>T	p.(Glu490*)	p.(E490*)	Exon 10	non
LUAD (n=6)					
P64	c.2350G>T	p.(Glu784*)	p.(E784*)	Exon 10	non
P162	c.1036dup	p.(Cys346Leufs*18)	p.(C346Lfs*18)	Exon 8	ins_fs
P188	c.6184-16_6198del	N/A	N/A	Intron 29–exon 30	del_sp
P240	c.4472G>A	p.(Trp1491*)	p.(W1491*)	Exon 16	non
P253	c.11266C>T	p.(Gln3756*)	p.(Q3756*)	Exon 39	non
	c.15079C>T	p.(Arg5027*)	p.(R5027*)	Exon 48	non
P318	c.13840-1G>A	N/A	N/A	Intron 41	sp
	c.14000-1G>A	N/A	N/A	Intron 42	sp

LoF, loss of function; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; sp, splice; ins, insertion; fs, frameshift; non, nonsense; del, deletion; N/A, not available.

KMT2D LoF mutation pattern in NSCLC

LoF mutations in *KMT2D* were detected in 11 cases of LUSC and 6 cases of LUAD, respectively (*Table 2*). Of these 17 patients, there were 10 males and 1 female with LUSC and 5 males and 1 female with LUAD. Double LoF mutations in *KMT2D* were detected in 3 cases of LUSC (P3, P96, and P136) and 2 cases of LUAD (P253 and P318). The types of LoF mutations included 12 nonsense, 4 frameshift, and 6 splice-site mutations. Classical splice-site mutations (exon-intron junctions) were predicted to disrupt messenger RNA (mRNA) splicing, potentially leading to protein dysfunction (*Figure 1A*). Nonsense and frameshift mutations were predicted to result in truncated proteins of KMT2D lacking the SET domain (*Figure 1B*). All the LoF mutations in all types were spread throughout the whole gene, while no recurrent mutations or mutation hotspots were identified. The distribution of *KMT2D* LoF mutations between LUSC and LUAD was very similar.

Association of KMT2D and TP53 co-mutations in NSCLC

TP53 mutations occurred concurrently with *KMT2D* LoF

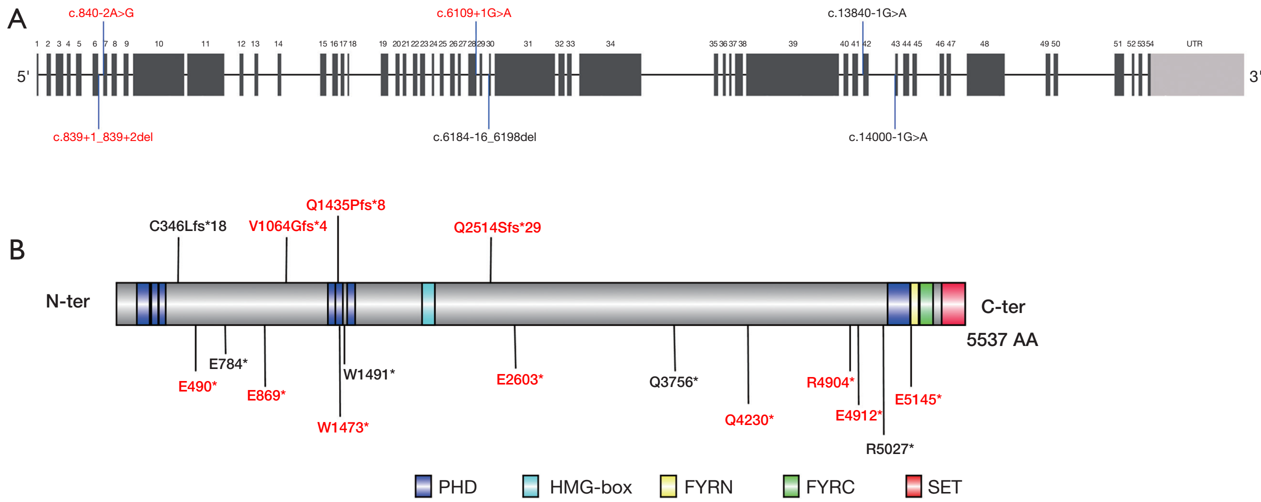

Figure 1 The distribution of *KMT2D* LoF mutations in the LUSC and LUAD cohorts. (A) Exon-intron structure map showing the distribution of *KMT2D* splice-site mutations (exon-intron junctions) in LUSC and LUAD. (B) Nonsense and frameshift mutations distribution throughout the KMT2D protein. Mutations in LUSC are labeled in red font. Mutations in LUAD are labeled in black font. LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma. PHD, plant homeodomain; HMG-box, high mobility group box; FYRN, F/Y-rich N terminus; FYRC, F/Y-rich C terminus; SET, Su(var)3-9, Enhancer-of-zeste, Trithorax domain.

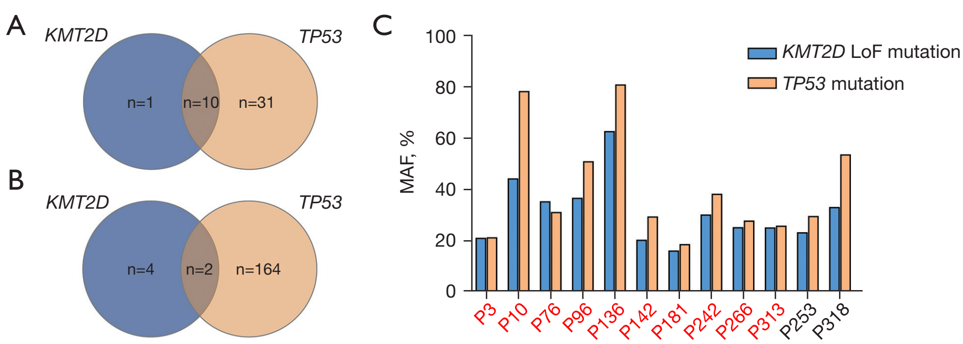

Figure 2 Association of *KMT2D* and *TP53* comutations in NSCLC. (A) Venn diagram of 10 cases with LUSC harboring *KMT2D* and TP53 comutations; (B) Venn diagram of 2 cases with LUAD harboring *KMT2D* and *TP53* comutations; (C) the MAF of *KMT2D* was very similar to that of *TP53* in the comutated cases. Comutations in LUSC are labeled in red font. Comutations in LUAD are labeled in black font. NSCLC, non-small cell lung cancer; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; MAF, mutation allele fraction.

mutations in 90.9% (10/11) of LUSC and 33.3% (2/6) of LUAD cases, respectively. Notably, the mutation allele fraction (MAF) of *KMT2D* was very similar to that of *TP53* in the co-mutated cases (*Figure 2*). In a 57-year-old male patient (P76) diagnosed with LUSC, sequencing DNA from FFPE tumor tissue showed that the MAFs of *KMT2D* mutation (c.7539del, p.Q2514Sfs*29) and *TP53* mutation (c.1121del, p.G374Vfs*48) were 35.3% and 31.1%, respectively. Subsequent post-treatment monitoring was performed through sequencing peripheral blood circulating tumor DNA (ctDNA), which detected an MAF of 2.08% in the *KMT2D* mutation and 1.52% in the *TP53* mutation. Similarly, in a 53-year-old male patient with LUSC (P313), sequencing from FFPE tumor tissue indicated MAFs of 25.1% in the *KMT2D* mutation (c.1468G>T, p.E490*) and 25.8% in the *TP53* mutation (c.536A>C, p.H179P), while the MAFs of the *KMT2D* and *TP53* mutations in the plasma ctDNA samples were 2.02% and 1.86%, respectively.

KMT2D mutation concurrence with other actionable gene alterations in NSCLC

Genomic profiling of driver gene mutations of NSCLC indicated that 9 cases of LUSC with *KMT2D* LoF mutations also exhibited *PIK3CA* gene amplification (n=5), *FGFR1* gene amplification (n=2), and both *PIK3CA* and *FGFR1* gene amplifications (n=2) (*Table 3*). The presentation in the table were the ones with identifiable mutations/other alterations of interest. There were two patients with LUSC (P3 and P136) who had both the *KMT2D* mutation and *TP53* mutation that lacked known driver mutations. Moreover, a 68-year-old male patient with LUSC (P229) who harbored a *KMT2D* nonsense mutation (c.14710C>T, p.R4904*) had no *TP53* mutation but did have both the *PIK3CA* and *FGFR1* amplifications.

Of the 6 cases of LUAD with *KMT2D* LoF mutations, 4 cases without the *TP53* mutation had the *EGFR* mutation (n=2), *KRAS* mutation (n=1), or *EML4-ALK* fusion (n=1). A 66-year-old male diagnosed with LUAD (P253) harbored two *KMT2D* mutations (p.R5027* and p.Q3756*) and the *TP53* mutation (*p.R158L*) and *EGFR* mutation (p.E746_A750del). The other patient with LUAD (P318) without a known driver gene mutation was a 71-year-old male who had the *KMT2D* mutation (c.13840-1G>A, c.14000-1G>A) and *TP53* mutation (p.H179R).

PIK3CA amplification (18/20) or mutation (2/20) were detected in 20 cases of LUSC, *PIK3CA* mutation (11/12) or amplification (1/12) in 12 cases of LUAD, respectively (*Table 3*). Of these 20 cases of LUSC, 14 patients with *PIK3CA* amplification and *TP53* mutation. Of these 12 cases of LUAD, 6 patients with *PIK3CA* mutation and *TP53* mutation, 9 cases co-mutated with *EGFR* mutations (9/12) or *KRAS* mutations (3/12). There were only 1 patient with *FGFR1* amplification in LUAD cohort and 6 cases in in LUSC cohort (*Table 3*). Of these 6 patients of LUSC, 4 cases also exhibited *FGFR1* gene amplification and mutations, 4 cases co-mutated with *PIK3CA* gene amplification.

Discussion

In this study, we analyzed targeted sequencing data from a cohort of 335 patients diagnosed with NSCLC. The frequency of *KMT2D* somatic LoF mutations was found to be 20.8% in LUSC and 2.1% in LUAD. We explored more about the characterization of squamous carcinoma driver genes, especially in terms of co-mutations. High frequency of *KMT2D* and *TP53* co-mutations occur in the LUSC cohort. Notably, the MAF of *KMT2D* was very similar to that of *TP53* in the co-mutated cases which need to be confirmed in larger cohorts. Moreover, genomic profiling of actionable gene mutations of NSCLC showed that *PIK3CA* and/or *FGFR1* gene amplification was detected in 81.8% (9/11) of the patients with LUSC and *KMT2D* LoF mutations. In a recent study, KMT2D protein was identified as a key regulator of LUSC tumorigenesis, and Kmt2d deletion transformed lung basal cell organoids to LUSC (24). However, the characteristics of the co-occurrence gene with *KMT2D* gene prompted that *KMT2D* may play important role and interact with the stronger driver genes in the tumor development.

Numerous studies have shown that the *KMT2D* mutation is closely related to congenital developmental disorders and various types of tumors (27-31). It is well known that *KMT2D* or its binding partner *KDM6A* is the major causative gene for autosomal dominant Kabuki syndrome (KS), although cancer has been reported in several individuals with KS (e.g., neuroblastoma, hepatoblastoma, Wilms tumor, Burkitt lymphoma), there is no clear association between KS and an increased risk for cancer (27-31). Heterozygous germline mutations in *KMT2D* are detected in 56% to 75% of patients with KS, the majority of which are LoF variants.

Inactivating mutations in the *KMT2D* have been reported in approximately 11% of patients with NSCLC (32). A comparison of the somatic profiles of LUAD and LUSC based on The Cancer Genome Atlas (TCGA) database showed that *KMT2D* is one of the most commonly mutated genes in LUSC but not in LUAD (33). In a cohort of 105 Korean patients with LUSC, *KMT2D* was identified as a high frequent mutation with a mutation rate of 24% (34). In addition to NSCLC, SCLC also exhibits frequent inactivating mutations in the *KMT2D* gene (35,36). However, the *KMT2D* mutation is associated with reduced survival in NSCLC but not in SCLC (37). Interestingly, in a previous study of a small number of tumors-normal tissue pairs from patients with NSCLC, *KMT2D* gene mRNA expression was significantly reduced in tumor tissues compared with adjacent nontumor lung tissues, regardless of the mutation status (32). In the present study, we confirmed that *KMT2D* LoF mutations occur much more frequently in LUSC, we collected the cohorts retrospectively and many patients didn't accept the administration in the same hospital, it is difficult to perform survival analysis. Whether there is a link between *KMT2D* mutations and survival in

Table 3 The representation of a wider genomic landscape for PIK3CA amplification and FGFR1 amplification or mutations detected in patients with LUSC and LUAD

Case ID	Sample type	KMT2D variant	TP53 variant	Other variants
LUSC (n=23)				
P3	Peripheral blood (cfDNA)	KMT2D LoF mutation	TP53 mutation	–
P10	FFPE	KMT2D LoF mutation	TP53 mutation	FGFR1 amplification
P76	FFPE	KMT2D LoF mutation	TP53 mutation	PIK3CA amplification; FGFR1 amplification
P96	FFPE	KMT2D LoF mutation	TP53 mutation	PIK3CA amplification
P136	FFPE	KMT2D LoF mutation	TP53 mutation	–
P142	FFPE	KMT2D LoF mutation	TP53 mutation	PIK3CA amplification
P181	Peripheral blood (cfDNA)	KMT2D LoF mutation	TP53 mutation	PIK3CA amplification
P229	FFPE	KMT2D LoF mutation	–	FGFR1 amplification; PIK3CA amplification
P242	FFPE	KMT2D LoF mutation	TP53 mutation	PIK3CA amplification
P266	FFPE	KMT2D LoF mutation	TP53 mutation	FGFR1 amplification
P313	FFPE	KMT2D LoF mutation	TP53 mutation	PIK3CA amplification
P19	FFPE	–	TP53 mutation	PIK3CA amplification
P24	FFPE	–	TP53 mutation	PIK3CA amplification
P69	FFPE	–	–	PIK3CA amplification
P186	FFPE	–	TP53 mutation	PIK3CA amplification
P263	FFPE	–	TP53 mutation	PIK3CA mutation
P265	FFPE	–	TP53 mutation	FGFR1 amplification; PIK3CA amplification
P270	FFPE	–	TP53 mutation	PIK3CA amplification
P271	FFPE	–	–	PIK3CA amplification
P272	FFPE	–	TP53 mutation	FGFR1 amplification; PIK3CA amplification
P277	FFPE	–	–	PIK3CA mutation
P282	FFPE	–	–	PIK3CA amplification
P315	FFPE	–	TP53 mutation	PIK3CA amplification
P320	FFPE	–	TP53 mutation	PIK3CA amplification
LUAD (n=19)				
P64	FFPE	KMT2D LoF mutation	–	KRAS mutation
P162	Peripheral blood (cfDNA)	KMT2D LoF mutation	–	EGFR mutation
P188	FFPE	KMT2D LoF mutation	–	EGFR mutation
P240	FFPE	KMT2D LoF mutation	–	ALK fusion
P253	FFPE	KMT2D LoF mutation	TP53 mutation	EGFR mutation
P318	FFPE	KMT2D LoF mutation	TP53 mutation	–
P6	FFPE	–	–	EGFR mutation; PIK3CA mutation
P32	FFPE	–	TP53 mutation	FGFR1 amplification

Table 3 (*continued*)

Table 3 (*continued*)

Case ID	Sample type	KMT2D variant	TP53 variant	Other variants
P38	FFPE	–	–	EGFR mutation; PIK3CA mutation
P53	FFPE	–	TP53 mutation	EGFR mutation; PIK3CA mutation
P61	Peripheral blood (cfDNA)	–	TP53 mutation	EGFR mutation; PIK3CA mutation
P67	FFPE	–	TP53 mutation	KRAS mutation; PIK3CA mutation
P71	FFPE	–	–	EGFR mutation; PIK3CA mutation
P127	FFPE	–	–	EGFR mutation; PIK3CA mutation
P143	Peripheral blood (cfDNA)	–	TP53 mutation	EGFR mutation; PIK3CA mutation
P145	FFPE	–	–	EGFR mutation; PIK3CA amplification
P226	FFPE	–	TP53 mutation	KRAS mutation; PIK3CA mutation
P291	FFPE	–	TP53 mutation	EGFR mutation; PIK3CA mutation
P303	FFPE	–	–	KRAS mutation; PIK3CA mutation

LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; FFPE, formalin fixed paraffin embedded; TP53, cellular tumor antigen p53; KMT2D, histone lysine methyltransferase 2D; FGFR1, fibroblast growth factor receptor 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog.

NSCLC will be explored in a future study, needs further exploration.

In addition to its tumor-suppressing candidates' genes in various tumors, the *KMT2D* mutations have been found to be closely associated with the development of squamous cell carcinomas, such as head and neck squamous cell carcinoma, ESCC, cutaneous squamous cell carcinoma, cervical squamous cell carcinoma, and LUSC (38-42). The *KMT2D* acts as a tumor repressor since *KMT2D* loss of function modestly increased cell proliferation and colony formation in one disrupted *KMT2D* study. Cells lacking *KMT2D* showed increased rates of migration and faster cell cycle progression (41). Similarly, when compared with esophageal adenocarcinoma (EAC), ESCC showed a significantly more frequent mutational rate within *KMT2D* (11.9% *vs.* 0.8%; P<0.001). A study on urothelial carcinoma (UC) found that *KMT2D* mutations occurred frequently in UC with squamous differentiation (UCS) compared to UC (48.4% *vs.* 0%, P<0.001) (43). Notably, LoF mutations in *KMT2D* were also reported in a case of histologic transformation of LUAD to LUSC after targeted treatment. The patient with LUAD and *EML4-ALK* fusion treated in sequence with four different tyrosine kinase inhibitors (TKIs) after drug resistance, and developed a well-known ALK-TKI resistance mutation and underwent a histological transformation from LUAD to LUSC. Upon development of resistance, a resistant mutation in *ALK*: p.I1171N was detected, as well as two LoF mutations in *KMT2D* were detected (c.4379dupC, p.L1461Tfs*30; c.1940delC, p.P647Hfs*283) (44). The molecular mechanisms through which this gene contributes to histological differentiation and carcinogenesis are still poorly understood.

H3K4 methylation in mammals occurs via an evolutionarily conserved SET1 family of methyltransferases known as complex proteins associated with SET1 (COMPASS). KMT2D forms a multiprotein complex with other co-actors including WDR5, RbBP5, ASH2L, DPY30, NCOA6, PTIP, PA1, and KDM6A (21). The KMT2D core complex predominantly consists of H3K4 mono-methyltransferases on enhancer regions and displays partial functional redundancy with KMT2C (19). The absence of KMT2D protein leads to the collapse of the multiprotein complex and the destabilization of KMT6A. One study showed that KMT2D knockout in bladder cancer cells reduced the activity of H3K4 monomethylation and effectively decreased PTEN and p53 expressions while suppressing STAG2 expression (45). In other research, KMT2D binding sites were found to be highly overlapped with p53-targeted regions, and a wide range of genes involved in the p53 pathway and cAMP-mediated signaling were significantly downregulated in KMT2D knockout cells (46). It was also reported that KMT2D interacts with the transcription factor TP63 on chromatin and regulates TP63 target enhancers to coordinate epithelial homeostasis (47). Moreover, lung-specific deletion of KMT2D was shown to significantly promote *KRAS*-driven lung tumorigenesis in mice and to shorten the survival of mice bearing *KRAS*-driven tumors, suggesting that KMT2D loss cooperates with other oncogenic aberrations (e.g., KRAS activation) to increase LUAD tumorigenicity (20). KMT2D loss has been found to suppress the expression of multiple receptor protein tyrosine phosphatases (RPTPs) and promote activation of EGFR and ERBB2 (21). Here, our results indicated that *TP53* mutations occurred concurrently with *KMT2D* LoF mutations in 90.9% of patients with LUSC, and *PIK3CA* and/or *FGFR1* amplification was detected in 81.8% of the patients with LUSC and *KMT2D* LoF mutations. However, patients with LUAD and *KMT2D* LoF mutations usually associate with genes alterations in *EGFR*, *KRAS*, and *ALK*.

Conclusions

Collectively, our results prompted that the frequent occurrence of *KMT2D* somatic LoF mutations in LUSC, while being uncommon in LUAD. Our study is the first Chinese cohort where frequency of *KMT2D* in LUSC and LUAD is estimated and explored the different frequency of *KMT2D* between LUSC and LUAD and hinted *KMT2D* as tumor-suppressing function in LUSC. Moreover, the *KMT2D* mutation has the potential to contribute to the pathogenesis of LUSC by working in concert with other commonly mutated genes in LUSC, including *TP53* mutation, *FGFR1* amplification, and *PIK3CA* amplification. Our work brought the direct evidence for mutation frequency in Chinese population. Further studies are needed to understand the role of an individually altered genes in LUSC to decipher their contribution towards LUSC carcinogenesis to effectively develop anti-tumor therapies.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-134/rc

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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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Zhujiang Hospital (No. 2024-KY-142-01). A waiver of patient consent was granted since this was a retrospective study from data collection only.

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