Original Article

Comprehensive genomic profiling of combined small cell lung cancer

Jing Zhang[1#], Liping Zhang[2#], Jie Luo[3#], Tao Ge[1#], Pengyu Fan[1], Liangdong Sun[1], Likun Hou[2], Junqiang Li[4], Huansha Yu[1], Chunxiao Wu[5], Yuming Zhu[1], Chunyan Wu[2], Gening Jiang[1], Giancarlo Troncone[6], Jyoti Malhotra[7], Katsuhiro Okuda[8], Mariacarmela Santarpia[9], Rita Zamarchi[10], Taichiro Goto[11], Andrés F. Cardona[12,13,14], Jianfang Xu[3], Qiankun Chen[1], Zhonghong Zhang[15], Peng Zhang[1]; written on behalf of the AME Lung Cancer Collaborative Group

[1]Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China; [2]Department of Pathology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China; [3]Department of Medical Oncology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China; [4]D1Med, Building 6, No. 28 Xiangle Road, Jiading District, Shanghai, China; [5]Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China; [6]Department of Public Health, University of Naples Federico II, Naples, Italy; [7]Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA; [8]Department of Oncology, Immunology and Surgery, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan; [9]Medical Oncology Unit, Department of Human Pathology of Adult and Evolutive Age "G. Barresi", University of Messina, Messina, Italy; [10]Veneto Institute of Oncology IOV-IRCCS, Padua, Italy; [11]Lung Cancer and Respiratory Disease Center, Yamanashi Central Hospital, Yamanashi, Japan; [12]Clinical and Translational Oncology Group, Clínica del Country, Bogotá, Colombia; [13]Foundation for Clinical and Applied Cancer Research-FICMAC, Bogotá, Colombia; [14]Molecular Oncology and Biology Systems Research Group (Fox-G), El Bosque University, Bogotá, Colombia; [15]Respiration Department II, the First Affiliated Hospital of Shihezi University Medical College, Xinjiang, China

Contributions: (I) Conception and design: J Xu, Q Chen, Z Zhang, P Zhang; (II) Administrative support: Y Zhu, C Wu, G Jiang; (III) Provision of study materials or patients: C Wu; (IV) Collection and assembly of data: J Zhang, L Zhang, J Luo, T Ge; (V) Data analysis and interpretation: J Zhang, L Zhang, J Luo, T Ge; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Jianfang Xu, MD. Department of Medical Oncology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China. Email: xujianfang63@aliyun.com; Qiankun Chen, MD. Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China. Email: drchen2016@126.com; Zhonghong Zhang, MD. Respiration Department II, the First Affiliated Hospital of Shihezi University Medical College, Xinjiang, China. Email: 1064564778@qq.com; Peng Zhang, MD, PhD. Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine, No. 507 Zhengmin Road, Shanghai, China. Email: zhangpeng1121@tongji.edu.cn.

Background: Combined small cell lung cancer (CSCLC) is an uncommon and heterogeneous subtype of small cell lung cancer (SCLC). However, there is limited data concerning the different molecular changes and clinical features in CSCLC compared to pure SCLC.

Methods: The clinical and pathological characteristics of pure SCLC and CSCLC patients were analyzed. Immunohistochemistry and microdissection were performed to isolate the CSCLC components. Further molecular analysis was carried out by next-generation sequencing (NGS) in 12 CSCLC and 30 pure SCLC.

Results: There were no significant differences in clinical features between CSCLC and pure SCLC. Overall survival (OS) of CSCLC patients was worse than pure SCLC (P=0.005). NGS results indicated that *TP53* and *RB1* were the most frequently mutated genes in both CSCLC (83.33% and 66.67%) and pure SCLC (80.00% and 63.33%) groups. However, less than 10% common mutations were found in both CSCLC and pure SCLC. When analyzing the data of SCLC and non-small cell lung cancer (NSCLC) components of CSCLC, more than 50% common mutations, and identical genes with mutations were detected. Moreover, there were also common biological processes and signaling pathways identified in CSCLC and pure SCLC, in addition to SCLC and NSCLC components.

Conclusions: There were no significant differences in terms of clinical features between CSCLC and pure SCLC. However, the prognosis for CSCLC was worse than pure SCLC. NGS analysis suggested that

CSCLC components might derive from the same pluripotent single clone with common initial molecular alterations and subsequent acquisitions of other genetic mutations.

Keywords: Combined small cell lung cancer (CSCLC); small cell lung cancer (SCLC); comprehensive genomic profiling; targeted gene sequencing

Submitted Oct 08, 2020. Accepted for publication Dec 01, 2020.
doi: 10.21037/tlcr-20-1099
View this article at: http://dx.doi.org/10.21037/tlcr-20-1099

Introduction

Lung cancer is the leading cause of cancer mortality worldwide and has the highest morbidity among all cancers (1). Overall, approximately 15% of lung cancer cases are diagnosed as small cell lung cancer (SCLC) (1,2). Combined small cell lung cancer (CSCLC), a rare subtype of SCLC, consists of a heterogeneous neoplasm with SCLC plus a non-small cell cancer (NSCLC) component. In this setting, the NSCLC component may manifest as adenocarcinoma (AD), squamous cell carcinoma (SCC), large cell neuroendocrine carcinoma (LCNC), or sarcomatoid lung cancer (3-5). To date, despite standard chemotherapy and radiation regimens, the survival of SCLC is poor (6). As for CSCLC, few studies have been conducted to characterize the features of this rare subtype.

It is well acknowledged that heavy smoking contributes greatly to the development of SCLC, however, 2–3% of SCLC patients are reported to be never smokers (7,8). Molecular studies have demonstrated that genomic aberrations, including tumor protein P53 (*TP53*), retinoblastoma 1 (*RB1*) mutations, and Myc proto-oncogene protein (*c-Myc*) amplification, are associated with the tumorigenesis and aggressive features of SCLC (9-11). Moreover, SCLC has been shown to be associated with a higher tumor mutational burden (TMB), which may favor the use of the chemo-immunotherapy as first-line treatment (IMPOWER-133 and CASPIAN trials) or immunotherapy in treatment for further lines (12-14). In addition, immunotherapy seems to be more effective in patients with SCLC who have primary resistance to platinum, a phenomenon that is associated with the presence of mutations in DNA damage response (DDR) and DNAH10 with a higher TMB (15-17). However, owing to the lower rate of CSCLC and the rarity of resected samples, the exact genomic landscape of this entity remains unknown.

Emerging evidence shows that NSCLC originates from either basal cells in the bronchial membrane or bronchoalveolar junction, whereas SCLC generally arises from neuroendocrine cells underneath the basal bronchial membrane and alveolar type 2 (AT2) cells of the most distal region of the lung (18-20). Diverse studies of *in vitro* and murine models aimed at exploring the origin of the transformation of epidermal growth factor receptor (EGFR) positive ADs in SCLC after tyrosine kinase exposure have evaluated the role of type II pneumocytes (21). Sutherland *et al.* previously reported that targeted disruption of Tp53 and Rb1 in AT2 cells led to the development of SCLC, albeit at a much lower frequency than that of neuroendocrine cells (19). Nonetheless, the cellular origin of CSCLC remains unclear. Some reports have suggested that the components of CSCLC derive from a single pluripotent clone before random genetic aberrations occur, whereas other evidence indicate that the components of CSCLC may co-exist in a single tumor (22,23).

Due to the paucity of reports, this current study aimed to explore the clinical, pathological, and molecular differences between CSCLC and pure SCLC and the prognosis of these tumor subtypes. To explore the possible origin of the different components of CSCLC, we applied a next-generation sequencing (NGS) approach for comprehensive genomic profiling.

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037tlcr-20-1099).

Methods

Patients

Patient data from a total of 324 patients with SCLC resected between January 2014 to July 2019 were obtained from Shanghai Pulmonary Hospital. Clinical data about gender, age, smoking history, pathological characteristics, postoperative treatment etc. were collected from the medical records. Telephone follow-up and outpatient records were

used for survival data, and the cutoff date for follow-up of the current study was September 30, 2019. Pathological features of all samples were revised and confirmed independently by two certified pathologists (L Zhang and L Hou). The pathological stage was determined based on the eighth edition of the American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system for lung cancer (24). This study was approved by the Institutional Review Board of Shanghai Pulmonary Hospital (ethical approval number: K18-066), Moreover, all procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all the patients.

Immunohistochemistry (IHC)

First, 4-μm slices were dyed with hematoxylin and eosin (HE), and the histological type was analyzed by selecting different markers using IHC. After dewaxing in xylene and rehydrating in gradient ethanol, the slides were prepared for antigen retrieval by boiling them in 10-mM sodium citrate buffer. Then, 3% hydrogen peroxide was used to block the endogenous peroxidase activity of the slides. Thereafter, the samples were incubated with the following primary antibodies at 4 ℃ overnight using thyroid transcription factor 1 (TTF-1) (clone 8G7G3/1, DAKO; dilution 1:200), napsin A (polyclone, Abcam; dilution 1:250), P40 (polyclone, Abcam; dilution 1:200), cluster of differentiation 56 (CD56, clone 123C3, DAKO; dilution 1:100), synaptophysin (Syn, clone DAK-SYNAP, DAKO; dilution 1:50), cytokeratin 5/6 (CK5/6, clone D5/16 B4, DAKO; dilution 1:100), and chromogranin A (CgA, clone DAK-A3; dilution 1:200). The slides were subsequently incubated with an anti-primary antibody and then stained with diaminobenzidine and hematoxylin. Finally, they were dehydrated in ethanol, washed in xylene, and then covered with coverslips for further microscopic observation.

Sample collection and preparation

The formalin fixed paraffin-embedded (FFPE) samples were obtained from patients who underwent surgery, and detailed information were reported in Tables S1,S2. After staining with HE and with different markers of IHC, the morphologies of all of the samples were confirmed by two senior pathologists, independently. The CSCLC cases with a clear morphological boundary of disparate contents were selected for microdissection and further subjected to genomic profiling performed in the OrigiMed laboratory (Yuansu, OrigiMed Inc., China).

Targeted NGS and genetic analysis

NGS was performed as previously described (16,25). Briefly, FFPE tumor tissues were collected for the detection of genetic alterations. Yuansu 466 panel covered all the coding exons of the 466 cancer-related genes and selected introns of 36 genes that were frequently rearranged in solid tumors (table online: https://cdn.amegroups.cn/static/public/tlcr-20-1099-3.docx). Then, 50–250 ng of DNA was extracted, and libraries were constructed and sequenced on an Illumina NextSeq 500 (San Diego, CA, USA) with a mean coverage of at least 700×. Genomic alterations consisting of single-nucleotide variations (SNV), copy-number variations (CNV), gene fusions/rearrangements, and short or long insertions/deletions (indels) were identified for advanced analysis. All genomic alterations for the examined tissues in the current study are summarized in https://cdn.amegroups.cn/static/public/tlcr-20-1099-3.docx. Normally, identical genes with the same DNA change indicated a common mutation (26). And we also defined identical genes with a different var type as identical genes with mutations in current study (Figures S1,S2).

Statistical analysis

SPSS 21.0 software (SPSS Inc., USA) was conducted for statistical analysis. The significances of the parameters between CSCLC and pure SCLC were determined by the χ^2 test or Fisher's exact test, as appropriate. The survival curves were calculated using the Kaplan-Meier method, and significance was analyzed by the log-rank test. A P value <0.05 was defined as statistically significant.

Results

Clinical characteristics and survival

Among all patients with resected SCLC, 52 patients (16%) were diagnosed as CSCLC, while the remaining 272 patients were confirmed as pure SCLC. The median age for all SCLC patients was 63 years (range, 31 to 83 years), while for CSCLC and pure SCLC the median age was 65 years (range, 31 to 78 years) and 63 years (range, 31 to 83 years), respectively. Upon comparing clinico-

pathological characteristics between the CSCLC and SCLC groups, no significant difference was revealed (*Table 1*). The median time for follow-up was 17 months (range, 2 to 66 months). We found that the median overall survival (OS) for pure SCLC and CSCLC was 60 and 22.9 months, respectively. Additionally, the OS of pure SCLC (95% CI: 42.79 to 51.07) was better in respect to CSCLC (95% CI: 27.00 to 47.47) as shown in *Figure 1*.

IHC characteristics

Among the 12 cases of CSCLC, SCC components were discovered in 6 cases, while AD components were identified in the remaining 6 cases (*Figure 2*). Neuroendocrine markers (CD56, Syn, and CgA) were positive-stained in 83.33% [10/12], 83.33% [10/12], and 41.67% [5/12] of the SCLC elements, respectively. P40 staining was positive in the SCC components [5/6] and negative in the SCLC components. TTF-1 staining was positive in 83.33% [5/6] of the AD components and 58.33% [7/12] of the SCLC components. Napsin A was expressed positively in 50.00% [3/6] of the AD components and negatively in the SCLC components (Table S3).

The mutational landscape in CSCLC and pure SCLC

To illustrate the mutational landscape in CSCLC and pure SCLC, a targeted NGS panel was adopted. In all, 12 cases diagnosed as CSCLC and 30 cases diagnosed as pure SCLC were successfully dissected, and the DNA from each sample was extracted, qualified, and sequenced. This yielded 267 mutations within 192 genes in CSCLC cases, and 385 mutations within 200 genes in pure SCLC samples (*Figure 3*, online material: https://cdn.amegroups.cn/static/public/tlcr-20-1099-1.docx and https://cdn.amegroups.cn/static/public/tlcr-20-1099-2.docx). To determine the possible role of the mutated genes in CSCLC and pure SCLC, we further analyzed the biological processes and pathways involved using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) in The Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/summary.jsp). We found that the mutated genes might be involved in the following common biological processes: GO:0018108 (peptidyl-tyrosine phosphorylation), GO:0048015 (phosphatidylinositol-mediated signaling), GO:0046777 (protein autophosphorylation), GO:0007169 (transmembrane receptor protein tyrosine kinase signaling pathway), GO:0045944 (positive regulation of transcription from RNA polymerase II promoter), GO:0014066 (regulation of phosphatidylinositol 3-kinase signaling), GO:0045893 (positive regulation of transcription, DNA-templated), GO:0008284 (positive regulation of cell proliferation), GO:0007165 (signal transduction), and GO:0007050 (cell cycle arrest) (*Figure 4A*,*B*). Moreover, the mutated genes in the two groups of tumors have been implicated in some common signaling pathways, such as Wnt, p53, and notch signaling pathways (*Figure 4C*,*D*). Further analysis found that 22 common mutations and 104 identical genes with mutations were determined in CSCLC and pure SCLC, occupying 8.24% and 54.17% of CSCLC and 5.71% and 52.00% of pure SCLC, respectively (Figures S1A,S2A). Among the top 10 genes with mutations in CSCLC and pure SCLC, 9 genes in CSCLC [*TP53*, *RB1*, SRY-box transcription factor 2 (*SOX2*), *EGFR*, telomerase reverse transcriptase (*TERT*), notch receptor 3 (*NOTCH3*), histone-lysine N-methyltransferase 2D (*KMT2D*), FAT tumor suppressor homolog 1 (*FAT1*), and family with sequence similarity 135 member B (*FAM135B*)] and 9 genes in pure SCLC (*TP53*, *RB1*, spectrin alpha, erythrocytic 1 (*SPTA1*), low-density lipoprotein receptor-related protein 1B (*LRP1B*), *TERT*, NK2 homeobox 1 (*NKX2-1*), *NOTCH1*, *FAM135B*, and *FAT4*] appeared in the abovementioned 104 genes (Figure S3). Additionally, the amplification of *SOX2*, *MYC*, AKT serine/threonine kinase 2 (*AKT2*), RAD54 homolog B (*RAD54B*), protein kinase C iota (*PRKCI*), cyclin D1 (*CCND1*), cyclin E1 (*CCNE1*) and fibroblast growth factors 4/3/12 (*FGF4*, *FGF3*, *FGF12*) were found only in CSCLC, while amplification of *TERT*, *NKX2-1*, succinate dehydrogenase complex flavoprotein subunit A (*SDHA*), and *FGF10* could be detected in both CSCLC and pure SCLC (Figure S3).

The mutational landscape in NSCLC and SCLC components of CSCLC

To further investigate the correlation between NSCLC and SCLC components of CSCLC, the mutated genes of the unique component of CSCLC were analyzed. We observed 211 mutations linked to 159 genes in the SCLC component of CSCLC and 169 mutations linked to 125 genes in the NSCLC component of CSCLC (online material: https://cdn.amegroups.cn/static/public/tlcr-20-1099-1.docx and https://cdn.amegroups.cn/static/public/tlcr-20-1099-2.docx). GO analysis in DAVID showed that most of the biological processes associated with the

Table 1 Clinical and pathological parameters of CSCLC and pure SCLC

Parameters	Category	Combined SCLC	Pure SCLC	χ^2	P
Gender	Male	46	230	0.5269	0.4679
	Female	6	42		
Age	>60	36	160	1.9784	0.1596
	≤60	16	112		
Smoking	≥400	19	97	0.0146	0.9038
	<400	33	175		
Surgery type	Limited resection	5	15	4.3073	0.1161
	lobectomy	46	231		
	Pneumonectomy	1	26		
Tumor site	Left	26	147	0.2869	0.5922
	Right	26	125		
Pleural invasion	Yes	4	12	0.4240	0.5150
	No	48	260		
Nerve invasion	Yes	4	5	3.5840	0.0583
	No	48	267		
Vessel invasion	Yes	9	31	1.4093	0.2351
	No	43	241		
T	1	18	121	3.6781	0.2887
	2	28	108		
	3	4	33		
	4	2	10		
N	0	27	134	1.9829	0.5733
	1	11	44		
	2	14	93		
	3	0	1		
TNM	I	23	108	0.9455	0.8108
	II	11	51		
	III	18	109		
	IV	0	4		
Postoperative adjuvant chemotherapy	Yes	34	189	0.3421	0.5586
	No	18	83		
Postoperative adjuvant radiotherapy	Yes	11	75	0.9227	0.3368
	No	41	197		
NSE	Normal	43	187	2.9473	0.0860
	Abnormal	4	48		
CEA	Normal	40	217	2.5369	0.1112
	Abnormal	7	18		

CSCLC, combined small cell lung cancer; SCLC, small cell lung cancer; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen.

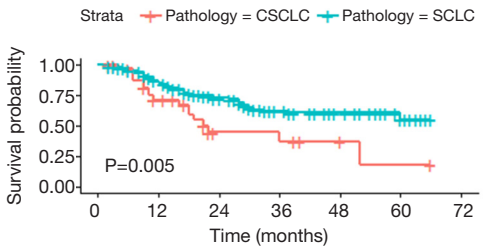

Figure 1 The overall survival of pure small cell lung cancer (SCLC) was better than combined small cell lung cancer (CSCLC) (P=0.005).

mutated genes of both components of CSCLC were similar (*Figure 5A,B*). Additionally, the mutated genes of the two CSCLC components were both implicated in classical signaling pathways, including PI3K-Akt, p53, notch, and others (*Figure 5C,D*). When comparing the common mutations and mutated genes in the two components, we discovered 112 common mutations and 92 identical genes with mutations in both components. Also, the proportion of common mutations and identical genes with mutations was 53.08% and 57.86% for the SCLC component, respectively, and 66.27% and

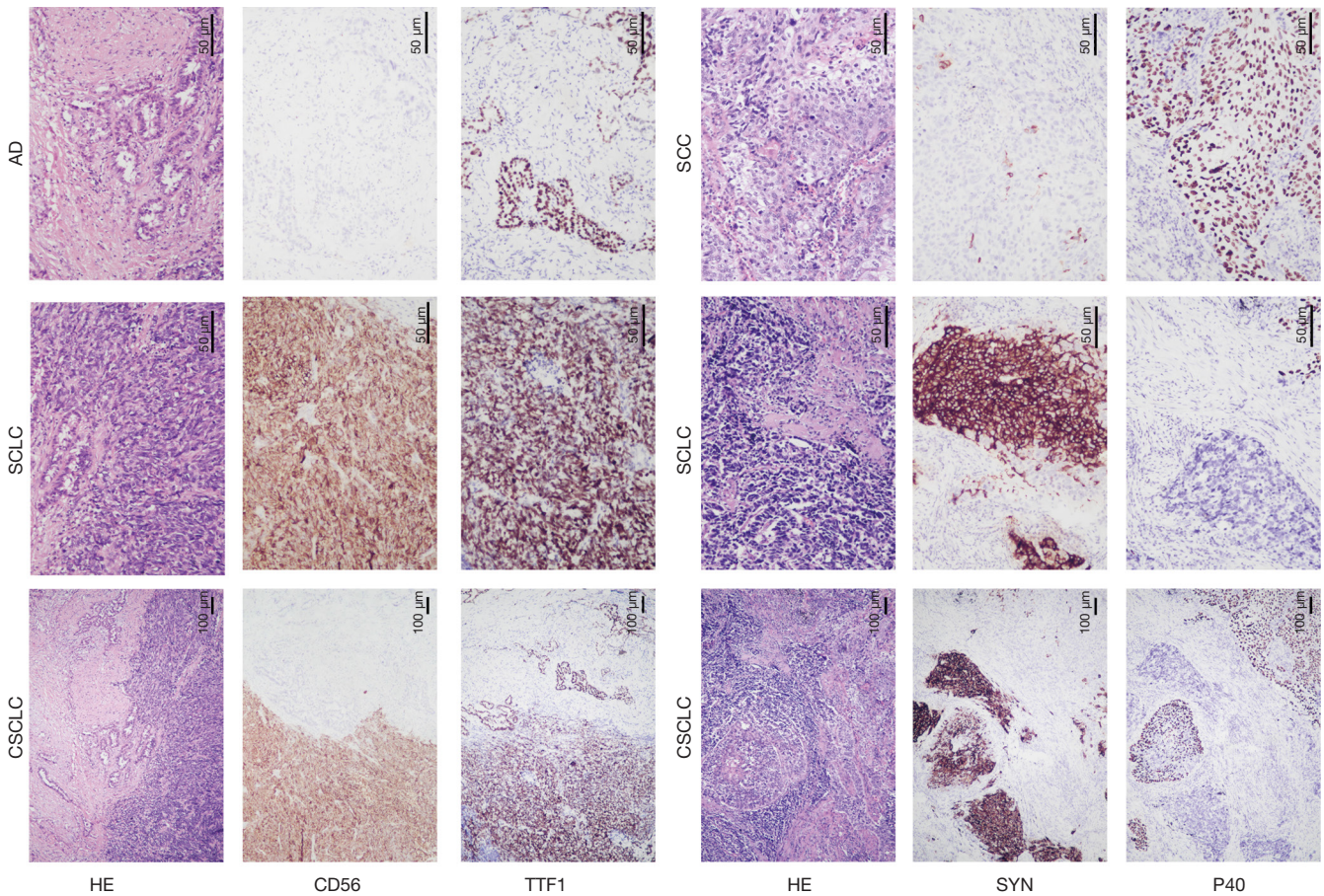

Figure 2 Representative images of specific markers and the boundary of the different components of combined small cell lung cancer (CSCLC). The left panel shows the staining of small cell lung cancer (SCLC) and adenocarcinoma (AD), while the right panel shows the staining of SCLC and squamous cell carcinoma (SCC).

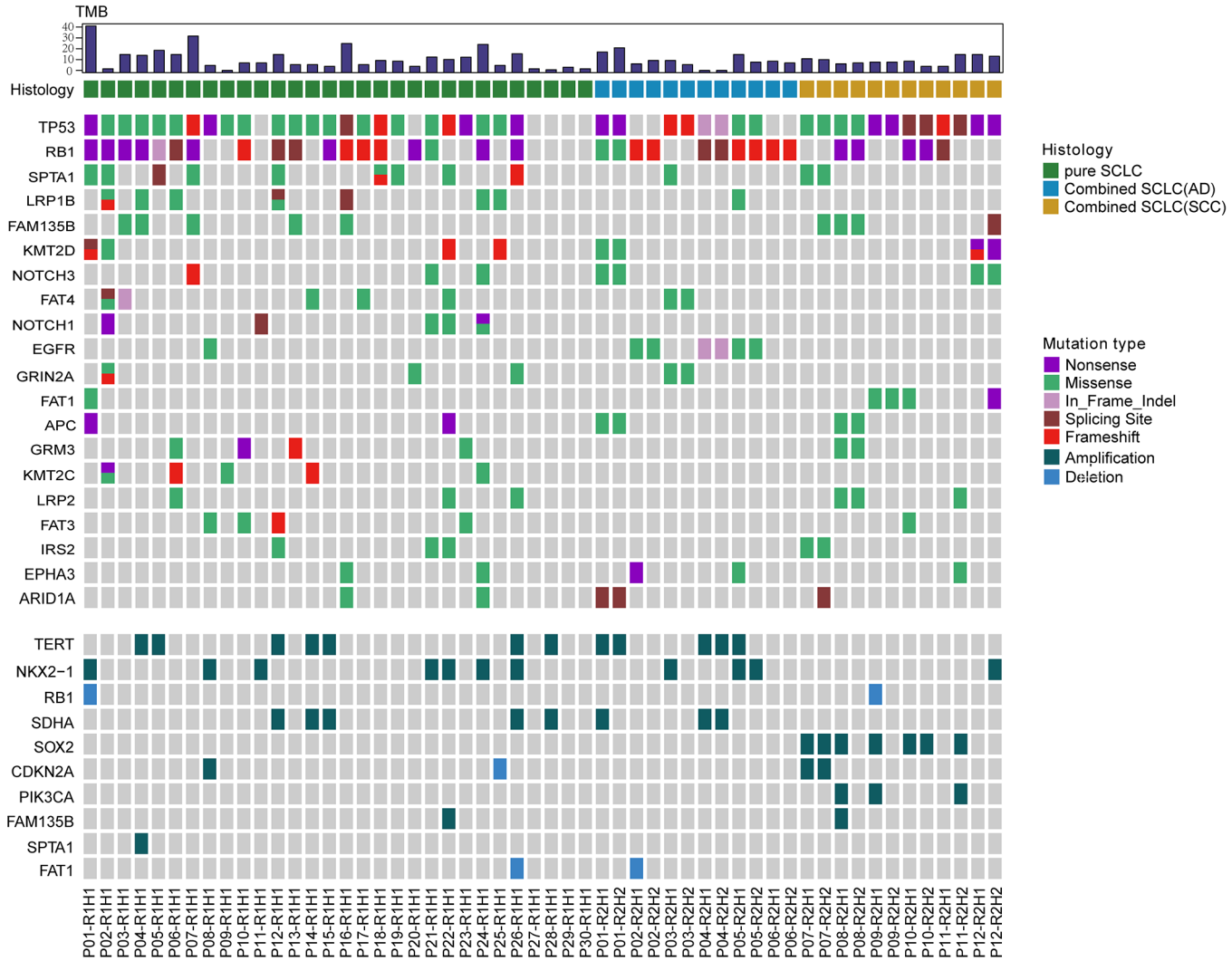

Figure 3 Summary of gene mutations in combined small cell lung cancer (CSCLC) and pure small cell lung cancer (SCLC). The distribution patterns of single nucleotide variants (top) and copy number variations (bottom) in our study assessed by next-generation sequencing (NGS). Histology and tumor mutation burden (TMB) values of each patient are depicted at the top. H1 in the sample identity (ID) (bottom) represents the component of SCLC; H2 in the sample ID represents the component of non-small cell lung cancer (NSCLC).

73.60% for the NSCLC component, respectively (Figures S1B,S2B). In terms of the common mutations in both components, 10 *TP53* common mutations, followed by *RB1* [7], phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*, 3), and *EGFR* [2] common mutations, were found in 12 samples of SCLC and NSCLC components of CSCLC (Table S4). Meanwhile, among the top 10 mutated genes of SCLC and NSCLC components of CSCLC, 10 mutated genes in SCLC [*TP53*, *RB1*, *SOX2*, *PIK3CA*, *TNK2*, *TERT*, nuclear factor, erythroid 2-like 2 (*NFE2L2*), *MYC*, *KMT2D*, and Kelch-like family member 6 (*KLHL6*)] and 9 mutated genes in NSCLC [*TP53*, *RB1*, *SOX2*, *PIK3CA*, *NOTCH3*, *FAM135B*, *EGFR*, cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and *TERT*] were found in the 92 identical genes with mutations (Figure S4). The unique amplifications of *FGF12*, *FGF10*, serine/threonine kinase 24 (*STK24*), RPTOR-independent companion of MTOR complex 2 (*RICTOR*), mitogen-activated protein kinase kinase kinase 13 (*MAP3K13*), *FGF14*, ETS variant transcription factor 5 (*ETV5*), retinoid X receptor alpha (*RXRA*), and TNF superfamily member 13b (*TNFSF13B*) were discovered in the SCLC component

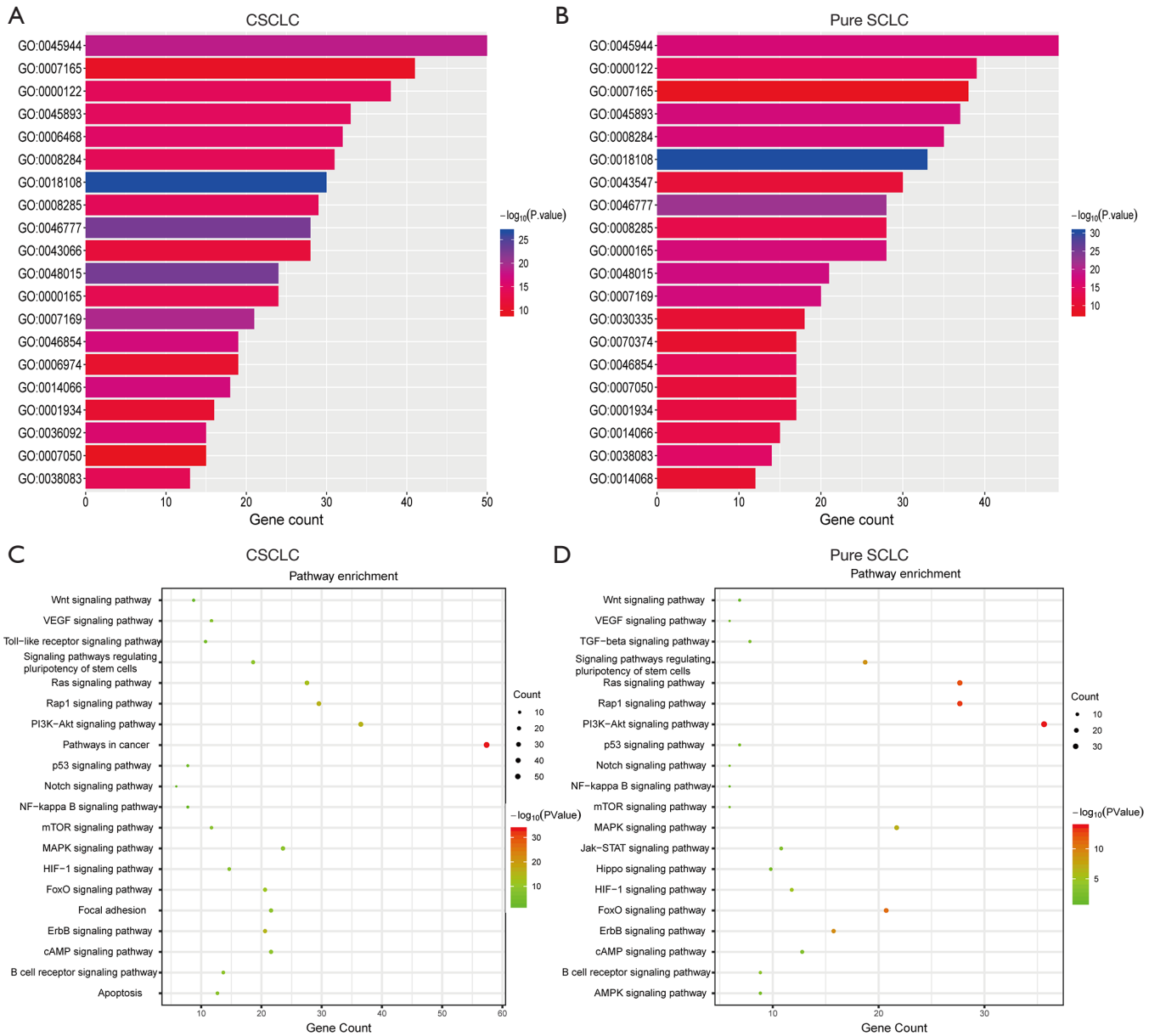

Figure 4 The biological process and the pathways of the mutated genes in different components predicted by the Database for Annotation, Visualization and Integrated Discovery (DAVID). (A,B) Images of the biological processes of the mutated genes in the combined small cell lung cancer (CSCLC) and pure small cell lung cancer (SCLC). (C,D) Images of signaling pathways involved in the mutated genes in combined small cell lung cancer (CSCLC) and pure SCLC.

of CSCLC, whereas amplifications of *SOX2*, *TERT*, *NKX2-1*, *SDHA*, *FGF3*, *FGF4*, *FGF19*, *CCND1*, *CCNE1*, *AKT2*, *AKT1*, X-ray repair cross complementing 3 (*XRCC3*), *TNK2*, serine/threonine kinase 11 (*STK11*), *RAD54B*, *PRKCI*, Rac family small GTPase 1 (*RAC1*), PMS1 homolog 2, and mismatch repair system component (*PMS2*) were found in both components of CSCLC (Figure S4).

However, when analyzing the mutated genes between 6 CSCLC cases containing AD and SCLC and 6 CSCLC cases containing SCC and SCLC, we found that 137 mutations were associated with 111 genes in 6 CSCLC cases expressing AD and SCLC, while 144 mutations were associated with 113 genes in 6 CSCLC cases expressing SCC and SCLC (Figure S1C and online material: https://

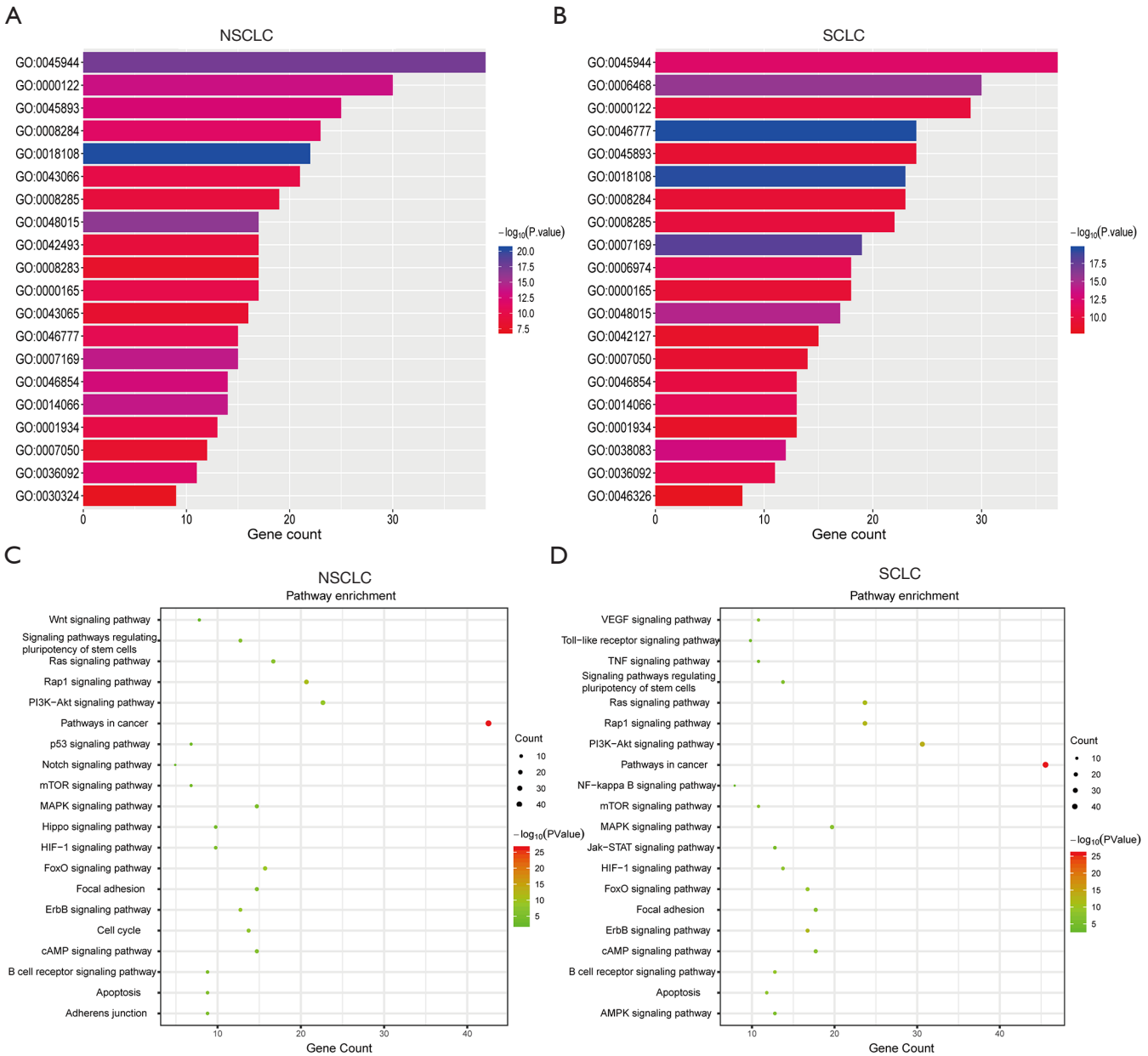

Figure 5 The biological process and the pathways of the mutated genes in different components predicted by the Database for Annotation, Visualization and Integrated Discovery (DAVID). (A,B) Images of the biological processes of the mutated genes in the non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) components of combined small cell lung cancer (CSCLC). (C,D) Images of the signaling pathways of the mutated genes in NSCLC and SCLC components of CSCLC.

cdn.amegroups.cn/static/public/tlcr-20-1099-1.docx and https://cdn.amegroups.cn/static/public/tlcr-20-1099-2.docx). Moreover, there were only 13 common mutations and 32 identical genes with mutations in both groups. The proportion of common mutations and identical genes with the mutation was 9.49% and 28.83% for CSCLC cases with AD and SCLC components respectively, and 9.03% and 28.32% for CSCLC cases with SCC and SCLC components, respectively (Figures S1C,S2C). Interestingly, amplifications of *TERT*, *AKT1*, *XRCC3*, *SDHA*, DOT1-like histone lysine methyltransferase (*DOT1L*), *STK11*, *RAC1*, *PMS2*, caspase recruitment

domain family member 11 (*CARD11*), *NFSF13B*, and src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristylation sites (*SRMS*) were found only in CSCLC containing AD and SCLC components, while amplifications of *SOX2*, *PIK3CA*, *RAD54B*, *PRKCI*, *FGF4*, *FGF3*, *FGF19*, *CCND1*, paired box 5 (*PAX5*), nuclear factor I B (*NFIB*), MET proto-oncogene, receptor tyrosine kinase (*MET*), *MAP3K13*, *KLHL6*, FA complementation group G (*FANCG*), *ETV5*, EMSY transcriptional repressor, BRCA2-interacting protein (*EMSY*), and *CDKN2B* were only detected in CSCLC containing SCC and SCLC components (Figure S5). In addition, when eliminating the mutated genes of the SCLC component, we discovered that 84 mutations were correlated with 65 genes in the AD components, and 89 mutations were associated with 73 genes in the SCC components (online material: https://cdn.amegroups.cn/static/public/tlcr-20-1099-1.docx and https://cdn.amegroups.cn/static/public/tlcr-20-1099-2.docx); of these only 4 common mutations and 13 identical genes with mutations were detected. Meanwhile, the rate of common mutations and identical genes with mutations was 4.76% and 20.00% for AD, respectively, and 4.49% and 17.81% for SCC, respectively (Figures S1D,S2D). Moreover, the amplifications of *TERT*, *NOTCH3*, *DOT1L*, *AKT1*, *XRCC3*, *TNK2*, *STK11*, *SDHA*, *RXRA*, *RAC1*, *PMS2*, *PDCD1*, *NOTCH1*, *FGFR3*, *CCNE1*, and *CARD11* were only examined in the AD component. Meanwhile, the amplifications of *SOX2*, *CDKN2A*, *FGF4*, *FGF3*, *FGF19*, *CCND1*, *RAD54B*, *PRKCI*, *PRK3CA*, *PAX5*, *NFKBIA*, *NFIB*, *MYC*, *MET*, *FANCG*, and *EMSY* were found exclusively in the SCC component (Figure S6).

The mutational landscape in AD and SCLC components of CSCLC

Among the 12 CSCLC cases examined with NGS, six samples showed AD and SCLC features. Further analysis demonstrated that 84 mutations correlated with 65 genes in the AD component of CSCLC, and 111 mutations were linked with 93 genes in the SCLC component of CSCLC (Figure S1E and online material: https://cdn.amegroups.cn/static/public/tlcr-20-1099-1.docx and https://cdn.amegroups.cn/static/public/tlcr-20-1099-2.docx). GO and KEGG analysis indicated that certain common biological processes and classical signaling pathways were present in both components (Figure S7A,B,C,D). Further analysis indicated that 58 common mutations and 47 identical genes with mutations were detected in both components, respectively accounting for 52.25% and 50.54% of the SCLC components, and 69.05% and 72.31% of the AD components (Figures S1E,S2E). When analyzing the individual sample, the respective proportion of common mutations in case 1 to case 6 was 71.88%, 50.00%, 83.33%, 92.31%, 76.47% and 33.33% in AD components, and 56.10%, 26.67%, 71.43%, 75.00%, 56.52% and 20.00% in the SCLC components (Figure S1G). As displayed in Table S5, five *RB1* common mutations were detected in five SCLC and AD components, followed by *TP53* [4] common mutations.

The mutational landscape in SCC and SCLC components of CSCLC

The result of an examination of 6 samples with SCC and SCLC features showed that 89 mutations were associated with 73 genes in the SCC components and 110 mutations were related to 91 genes in the SCLC components (online material: https://cdn.amegroups.cn/static/public/tlcr-20-1099-1.docx and https://cdn.amegroups.cn/static/public/tlcr-20-1099-2.docx). Additionally, some common biological processes and classical signaling pathways were evident in both components as demonstrated by GO and KEGG analysis (Figure S8A,B,C,D). Moreover, 54 common mutations and 51 identical genes with mutations were detected in both components, respectively occupying 49.09% and 56.04% of the SCLC components, and 60.67% and 69.86% of the SCC components (Figure S1F). As shown in Figure S1H, the proportion of common mutations (case 1 to case 6) was 80.00%, 76.92%, 90.91%, 57.14%, 66.67%, and 4.35%, respectively in the SCC components and 95.24%, 38.46%, 45.45%, 20.00%, 53.85%, and 8.33% in the SCLC components, respectively. Additionally, five *TP53* common mutations were discovered in five SCLC and SCC components, followed by *RB1*, *SOX2*, *CDKN2A*, *FGF19*, etc. (Table S6).

TMB analysis

In terms of TMB, we identified that TMB in patients with CSCLC (8.89±4.93) was lower than that in patients with pure SCLC (10.70±9.49) (Figure S9A), and TMB in the SCLC component and NSCLC component of CSCLC was 8.93±4.965 and 8.86±5.20, respectively (Figure S9B); However, this difference was not significant. A previous

study showed that the DDR pathway associated with platinum sensitivity was related with TMB level (27). In current study, the level of TMB in pure SCLC patients with genetic mutation in DDR pathway was higher than that in patients without a genetic mutation in DDR pathway (P=0.008). Furthermore, the level of TMB in CSCLC patients with a genetic mutation in DDR pathway was higher, but not significantly so (P=0.711) (Figure S9C). Further analysis concerning the different components of CSCLC revealed that the level of TMB in the SCLC and NSCLC components with genetic mutation was elevated (Figure S9D); however, no significant difference was found. We then analyzed the role of TMB and DDR status on prognosis. Considering the limited number of CSCLC cases, the data of pure SCLC was used. We found there to be no significant differences in pure SCLC cases with different TMB and in pure SCLC cases receiving chemotherapy with different DDR status (Figure S9E,F).

Discussion

SCLC, an aggressive malignancy with frequent recurrence and poor prognosis cancer, accounts for about 15% of all lung cancers. In 1999, a deeper understanding of SCLC had led to the International Association for the Study of Lung Cancer (IASLC) to classify SCLC into two subtypes: pure SCLC and CSCLC (28). It has been generally understood that the NSCLC components of CSCLC might cover one or more different elements (usually SCC, AD, LCNC, and so on). Previous research has revealed that CSCLC constitutes about13% of autopsy specimens of SCLC and 12–26% of surgical specimens of SCLC (29-31). In the current study, the incidence of CSCLC within resected specimens was 16%, and the prognosis for CSCLC was worse than that for pure SCLC. Further NGS results indicated that *TP53* and *RB1* were the most frequent mutated genes in both the CSCLC and pure SCLC groups. Moreover, fewer than 10% common mutations were found in both CSCLC and pure SCLC, while more than 50% common mutations were detected in both SCLC and NSCLC components of CSCLC, which may explain the tumor heterogeneity and common ancestral cells.

It is well known that the accumulation of sequential genomic alterations, as discovered by diverse methods, including loss of heterozygosity (LOH) analysis, comparative genomic hybridization (CGH), and DNA or whole-genome sequencing, leads to the tumorigenesis and progression of SCLC (9,22,32,33). A previous study demonstrated that allelic loss manifesting as LOH can be considered to result from the inactivation of a tumor suppressor via point mutations or other mechanisms and is associated with tumorigenesis (32). Murase *et al.* observed the role of point mutation in *TP53* gene and allelic deletion of chromosome 3p in SCLC development (22). Based on the NGS technique, a recent study found *TP53* to be the most frequently mutated gene, followed by *RB1*. Further analysis has suggested that the mutations of *TP53*, *RB1*, capping protein inhibiting regulator of actin dynamics (*KIAA1211*), and collagen type XXII alpha 1 chain (*COL22A1*) are implicated in G-protein-coupled receptor signaling and that high levels of delta-like non-canonical notch ligand 1 (*DLK1*) and achaete-scute family BHLH transcription factor 1 (*ASCL1*) contribute to low notch pathway activity (10,34). These findings have identified many of the key mutated genes and several of the significant signaling pathways involved in SCLC that may provide candidate therapeutic targets for this lethal cancer. In our current study, we found that *TP53* had the highest frequency of mutations in both CSCLC (83.33%) and pure SCLC (80.00%), followed by RB1 in CSCLC (66.67%) and pure SCLC (63.33%), and these were mainly responsible for the tumorigenesis of SCLC. Furthermore, the aberrations of notch family genes (*Notch1*, *Notch3*), *CREBBP*, *CDKN2A*, *MYC*, and others were also crucial for the development of SCLC. The involvement of PI3K-Akt, MAPK, Wnt, and other signaling pathways in both CSCLC and pure SCLC also play a critical role in SCLC. Taken together, we can conclude that both the key mutations of *TP53* and *RB1*, along with the abnormalities of a diverse array of mutated genes involved in various signaling pathways, might exert pro-tumorigenic roles in the development of SCLC.

Understanding the cellular origin of different lung cancer histotypes may aid in identifying the molecular differences of various cancers and help develop specific targeted drugs (35-37). Basal cells serving as tissue-specific stem cells of the club and ciliated epithelial cells were reported to be the candidate origin cell for the SCC type of lung cancer (38). AT2 cells, the major stem cells for alveolar epithelium, and club cells were implicated as the cellular origin for AD in lung cancer (39). In relation to SCLC, neuroendocrine and AT2 (SPC-expressing) cells losing *TP53* and *RB1* were found to be primarily responsible for the formation of SCLC (19). However, no consensus has been reached concerning the origin of CSCLC thus far. A few studies revealed that a single pluripotent clone might give rise to the different components of CSCLC in the course of

growth and proliferation after incurring genetic mutations (22,40,41). Murase *et al*. found that the components of SCLC and SCC of CSCLC showed the same *TP53* mutations and chromosomal abnormalities, which indicated that they might derive from the same clone before the *TP53* mutations and chromosomal abnormalities occur (22). Wagner *et al.* suggested that the components of SCLC and NSCLC in CSCLC displayed a similar immunophenotype and neuroendocrine differentiation, and thus might share a clonal precursor closely related to SCLC (41). Additionally, other researchers have put forward the hypothesis that the various components of CSCLC might grow in neighbor regions independently and then come together within the primary tumor (23,42). When comparing the mutations of CSCLC and pure SCLC in the current study, there were more than 50% of identical genes with mutations present in both groups, including the top frequent mutations of *TP53*, *RB1*, *TERT*, *FAM135B*, *NOTCH* family genes, and *FAT* family genes. This phenomenon may evince the close association between the development of CSCLC and pure SCLC.

It is generally accepted that the different components of a tumor might share a common clonal origin owing to the high frequency of shared mutations. Hepatocellular-cholangiocarcinoma (H-ChC), a rare subtype of liver cancer, is composed of hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA). Wang *et al.* reported that a number of common mutations exist in the HCC and iCCA components of H-ChC and concluded that the various components of H-ChC derive from a common clonal origin (26). Similarly, Zhao *et al.* found that the SCC and SCLC components of CSCLC, which shared a substantial number of common mutations, might have the same clonal origin (28). However, only three cases and a limited DNA panel were used in the analysis. In our current study, 10 *TP53* and 7 *RB1* common mutations were identified in 12 cases of CSCLC, suggesting that the CSCLC components might derive from common precursors. In addition, more than 50% of the common mutations were detected in the NSCLC and SCLC components of CSCLC. Additionally, we discovered that the proportion of common mutations in the AD and SCLC components of CSCLC, including *RB1*, *TP53*, *EGFR*, and *TERT*, surpassed 50% in AD and SCLC. A similar phenomenon was found in CSCLC containing SCC and SCLC, with the frequently mutated genes being *TP53*, *RB1*, and *SOX2*. We thus speculate that the different components of CSCLC may be derived from the same pluripotent single clone due to key mutations of *TP53* and *RB1* as well as a high percentage of common mutations.

A previous study in a mouse model with various genetic alterations established by Ferone *et al.* revealed that *SOX2* was pivotal for the shift to SCC from the original cells (38). In a comprehensive genomic study using NGS technologies to characterize 36 primary SCLC tumors and 17 SCLC cell lines, SOX2 amplification was found in approximately 27% of samples (43). Amplified SOX2 was first characterized as a driver oncogene in squamous lung cancer (44). SOX2 is a member of a large family of transcription factors involved in the maintenance of embryonic stem cells, induction of pluripotent stem cells, and lung cancer development, including the SCLC subtype (45). Meanwhile, *TP53*, *EGFR*, *TERT* and others were found to correlate with the development of AD (46,47). In contrast to the more common mutations mentioned above, less than 10% of the common mutations and 30% of the identical genes with mutations were found both in CSCLC cases with the AD and SCLC components and CSCLC cases with the SCC and SCLC components. Meanwhile, less than 5% of the common mutations and 20% of the identical genes with mutations were found in both the AD and SCC components. Notably, upon comparing the genes between AD and SCC, mutations of *TERT*, *EGFR*, *NKX2-1*, and *SDHA* were unique to AD, while mutations of *SOX2*, *CDKN2A*, and *FAM135B* were unique to SCC. We hypothesize that the different NSCLC components may undergo a separate progression from the primary clones, and the distinct genetic mutations and heterogeneity of the tumor may be responsible for this result.

Conclusions

Overall, our study identified no significant difference in the distribution of clinico-pathologic characteristics between the CSCLC and the pure SCLC group. However, the prognosis of CSCLC was worse than that of pure SCLC. Additionally, the results of NGS revealed that CSCLC and pure SCLC had a similar mutational profile. Further analysis revealed that different components of CSCLC might derive from the same pluripotent single clone. Nevertheless, little is known concerning the cellular and molecular abnormalities underlying CSCLC development, progression, and resistance to treatments.

Our study provides novel insights into the molecular features of CSCLC; however, further research is needed to confirm our findings. SCLC and CSCLC are heterogeneous

diseases, characterized by genomic alterations targeting a broad variety of genes. It has been widely established that genomic analyses in tissue samples could be limited by tumor heterogeneity. In contrast to tissue biopsy, liquid biopsy could provide a more complete representation of the entire genomic landscape by including primary tumor and multiple metastatic sites. Moreover, molecular profiling on liquid biopsy can be performed at various points, thus potentially offering a dynamic portrait of genomic changes during the course of disease. Therefore, more studies focusing on the use of liquid biopsy are needed to identify novel genetic alterations involved in CSCLC development which can potentially serve as targets for novel therapeutic agents.

Acknowledgments

Funding: This work was supported by the National Natural Science Foundation of China (Grant No. 81972172, 81902464), the Shanghai Municipal Health Commission (Grant No. 2017BR026, 20174Y0111), the Shanghai Hospital Development Center (Grant No. SHDC12018122, SHDC12017X03), Fundamental Research Funds for the Central Universities (Grant No. 22120180510, 22120180019), and the Health and Family Planning Commission of Shanghai Municipality (20184Y0090).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at http://dx.doi.org/10.21037tlcr-20-1099

Data Sharing Statement: Available at http://dx.doi.org/10.21037tlcr-20-1099

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037tlcr-20-1099). Dr. JM serves as an unpaid editorial board member of *Translational Lung Cancer Research* from Aug 2019 to Aug 2021. The authors have no other conflicts of interest to declare.

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. This study was approved by the Institutional Review Board of Shanghai Pulmonary Hospital (ethical approval number: K18-066). Moreover, all procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all the patients.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115-32.
3. Buys TP, Aviel-Ronen S, Waddell TK, et al. Defining genomic alteration boundaries for a combined small cell and non-small cell lung carcinoma. J Thorac Oncol 2009;4:227-39.
4. Jiang C, Shen F, Du J, et al. MicroRNA-564 is downregulated in glioblastoma and inhibited proliferation and invasion of glioblastoma cells by targeting TGF-beta1. Oncotarget 2016;7:56200-8.
5. Adelstein DJ, Tomashefski JF Jr, Snow NJ, et al. Mixed small cell and non-small cell lung cancer. Chest 1986;89:699-704.
6. Govindan R, Page N, Morgensztern D, et al. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. J Clin Oncol 2006;24:4539-44.
7. Varghese AM, Zakowski MF, Yu HA, et al. Small-cell lung cancers in patients who never smoked cigarettes. J Thorac Oncol 2014;9:892-6.
8. Ou SH, Ziogas A, Zell JA. Prognostic factors for survival in extensive stage small cell lung cancer (ED-SCLC): the importance of smoking history, socioeconomic and marital statuses, and ethnicity. J Thorac Oncol 2009;4:37-43.
9. Byers LA, Wang J, Nilsson MB, et al. Proteomic profiling

identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. Cancer Discov 2012;2:798-811.
10. George J, Lim JS, Jang SJ, et al. Comprehensive genomic profiles of small cell lung cancer. Nature 2015;524:47-53.
11. Santarpia M, Daffina MG, Karachaliou N, et al. Targeted drugs in small-cell lung cancer. Transl Lung Cancer Res 2016;5:51-70.
12. Hellmann MD, Callahan MK, Awad MM, et al. Tumor Mutational Burden and Efficacy of Nivolumab Monotherapy and in Combination with Ipilimumab in Small-Cell Lung Cancer. Cancer Cell 2018;33:853-861.e4.
13. Paz-Ares L, Dvorkin M, Chen Y, et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. Lancet 2019;394:1929-39.
14. Horn L, Mansfield AS, Szczesna A, et al. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. N Engl J Med 2018;379:2220-9.
15. Li M, Lin A, Luo P, et al. DNAH10 mutation correlates with cisplatin sensitivity and tumor mutation burden in small-cell lung cancer. Aging (Albany NY) 2020;12:1285-303.
16. Wang Y, Han X, Wang X, et al. Genomic based analyses reveal unique mutational profiling and identify prognostic biomarker for overall survival in Chinese small-cell lung cancer. Jpn J Clin Oncol 2019;49:1143-50.
17. Luo P, Lin A, Li K, et al. DDR Pathway Alteration, Tumor Mutation Burden, and Cisplatin Sensitivity in Small Cell Lung Cancer: Difference Detected by Whole Exome and Targeted Gene Sequencing. J Thorac Oncol 2019;14:e276-9.
18. Murgia C, Caporale M, Ceesay O, et al. Lung adenocarcinoma originates from retrovirus infection of proliferating type 2 pneumocytes during pulmonary post-natal development or tissue repair. PLoS Pathog 2011;7:e1002014.
19. Sutherland KD, Proost N, Brouns I, et al. Cell of origin of small cell lung cancer: inactivation of Trp53 and Rb1 in distinct cell types of adult mouse lung. Cancer Cell 2011;19:754-64.
20. Clarke DL, Johansson CB, Wilbertz J, et al. Generalized potential of adult neural stem cells. Science 2000;288:1660-3.
21. Oser MG, Niederst MJ, Sequist LV, et al. Transformation from non-small-cell lung cancer to small-cell lung cancer: molecular drivers and cells of origin. Lancet Oncol 2015;16:e165-72.
22. Murase T, Takino H, Shimizu S, et al. Clonality analysis of different histological components in combined small cell and non-small cell carcinoma of the lung. Hum Pathol 2003;34:1178-84.
23. Sugano M, Kawashima O, Nagashima T, et al. Collision cancer of squamous cell carcinoma and small cell carcinoma of the lung; report of a case. Kyobu Geka 2006;59:497-500.
24. Detterbeck FC, Nicholson AG, Franklin WA, et al. The IASLC Lung Cancer Staging Project: Summary of Proposals for Revisions of the Classification of Lung Cancers with Multiple Pulmonary Sites of Involvement in the Forthcoming Eighth Edition of the TNM Classification. J Thorac Oncol 2016;11:639-50.
25. Lin J, Shi J, Guo H, et al. Alterations in DNA Damage Repair Genes in Primary Liver Cancer. Clin Cancer Res 2019;25:4701-11.
26. Wang A, Wu L, Lin J, et al. Whole-exome sequencing reveals the origin and evolution of hepato-cholangiocarcinoma. Nat Commun 2018;9:894.
27. Park S, Lee H, Lee B, et al. DNA Damage Response and Repair Pathway Alteration and Its Association With Tumor Mutation Burden and Platinum-Based Chemotherapy in SCLC. J Thorac Oncol 2019;14:1640-50.
28. Zhao X, McCutcheon JN, Kallakury B, et al. Combined Small Cell Carcinoma of the Lung: Is It a Single Entity? J Thorac Oncol 2018;13:237-45.
29. Fukui T, Tsuta K, Furuta K, et al. Epidermal growth factor receptor mutation status and clinicopathological features of combined small cell carcinoma with adenocarcinoma of the lung. Cancer Sci 2007;98:1714-9.
30. Nicholson SA, Beasley MB, Brambilla E, et al. Small cell lung carcinoma (SCLC): a clinicopathologic study of 100 cases with surgical specimens. Am J Surg Pathol 2002;26:1184-97.
31. Babakoohi S, Fu P, Yang M, et al. Combined SCLC clinical and pathologic characteristics. Clin Lung Cancer 2013;14:113-9.
32. Shivapurkar N, Virmani AK, Wistuba, II, et al. Deletions of chromosome 4 at multiple sites are frequent in malignant mesothelioma and small cell lung carcinoma. Clin Cancer Res 1999;5:17-23.
33. Petersen I, Langreck H, Wolf G, et al. Small-cell lung cancer is characterized by a high incidence of deletions on chromosomes 3p, 4q, 5q, 10q, 13q and 17p. Br J Cancer 1997;75:79-86.
34. Meder L, Konig K, Ozretic L, et al. NOTCH, ASCL1,

p53 and RB alterations define an alternative pathway driving neuroendocrine and small cell lung carcinomas. Int J Cancer 2016;138:927-38.
35. Swanton C, Govindan R. Clinical Implications of Genomic Discoveries in Lung Cancer. N Engl J Med 2016;374:1864-73.
36. Foy V, Schenk MW, Baker K, et al. Targeting DNA damage in SCLC. Lung Cancer 2017;114:12-22.
37. Jiang T, Shi W, Wali VB, et al. Predictors of Chemosensitivity in Triple Negative Breast Cancer: An Integrated Genomic Analysis. PLoS Med 2016;13:e1002193.
38. Ferone G, Song JY, Sutherland KD, et al. SOX2 Is the Determining Oncogenic Switch in Promoting Lung Squamous Cell Carcinoma from Different Cells of Origin. Cancer Cell 2016;30:519-32.
39. Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature 2014;507:190-4.
40. Calbo J, van Montfort E, Proost N, et al. A functional role for tumor cell heterogeneity in a mouse model of small cell lung cancer. Cancer Cell 2011;19:244-56.
41. Wagner PL, Kitabayashi N, Chen YT, et al. Combined small cell lung carcinomas: genotypic and immunophenotypic analysis of the separate morphologic components. Am J Clin Pathol 2009;131:376-82.
42. Yamasaki M, Takenaka T, Matsumoto N, et al. Primary pulmonary collision tumor comprising squamous cell carcinoma and mucosa-associated lymphoid tissue lymphoma. Lung Cancer 2019;129:107-9.
43. Rudin CM, Durinck S, Stawiski EW, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 2012;44:1111-6.
44. Bass AJ, Watanabe H, Mermel CH, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 2009;41:1238-42.
45. Pietanza MC, Ladanyi M. Bringing the genomic landscape of small-cell lung cancer into focus. Nat Genet 2012;44:1074-5.
46. Parry EM, Gable DL, Stanley SE, et al. Germline Mutations in DNA Repair Genes in Lung Adenocarcinoma. J Thorac Oncol 2017;12:1673-8.
47. Zanetti KA, Wang Z, Aldrich M, et al. Genome-wide association study confirms lung cancer susceptibility loci on chromosomes 5p15 and 15q25 in an African-American population. Lung Cancer 2016;98:33-42.

Cite this article as: Zhang J, Zhang L, Luo J, Ge T, Fan P, Sun L, Hou L, Li J, Yu H, Wu C, Zhu Y, Wu C, Jiang G, Troncone G, Malhotra J, Okuda K, Santarpia M, Zamarchi R, Goto T, Cardona AF, Xu J, Chen Q, Zhang Z, Zhang P; written on behalf of the AME Lung Cancer Collaborative Group. Comprehensive genomic profiling of combined small cell lung cancer. Transl Lung Cancer Res 2021;10(2):636-650. doi: 10.21037/tlcr-20-1099

