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Characterization of the genomic landscape in liver oligometastatic NSCLC

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Abstract

Objectives Emerging data have shown that local treatment could provide clinical benefit for non-small cell lung cancer (NSCLC) patients with oligometastasis. Liver metastases have the worst prognosis in advanced NSCLC, but the genomic characteristics of liver oligometastasis remain unclear. The aim of our study was to elucidate the molecular features of liver oligometastatic NSCLC.

Methods Paired liver metastatic tissue samples and peripheral blood from 32 liver oligometastatic NSCLC patients were concurrently collected for comprehensive genomic analysis using next-generation sequencing.

Results A total of 206 mutated genes in 32 patients were detected, with a median of 4 mutations per sample. The most frequent alterations (> 10%) in liver oligometastasis were TP53 (72%), EGFR (50%), RB1 (19%) and SMARCA4 (12%). The co-occurrence rate of TP53 and RB1 in our cohort was significantly higher than that in the TCGA-LUAD cohort. Age, APOBEC, homologous recombination deficiency (HRD) and deficient mismatch repair (dMMR) established the mutational signature of liver oligometastatic NSCLC. The median tumor mutation burden (TMB) was 4.8 mutations/Mb. A total of 78.12% patients harbored at least one potentially actionable molecular alteration that may guide further targeted therapy according to the OncoKB evidence.

Conclusions Our study comprehensively delineated the genomic characteristics of liver oligometastatic NSCLC - such findings were helpful to better understand the distinct clinic-biological features of oligometastasis and optimize personalized treatment of this population.

Keywords NSCLC, Liver oligometastasis, Genomic profiling, Next-generation sequencing, Tumor mutational burden

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Introduction

Lung cancer has the highest cancer mortality and incidence in the world [1, 2]. Approximately two-thirds of non-small cell lung cancer (NSCLC) patients present with metastatic disease at diagnosis. Liver is one of the most frequent sites of tumor metastases in advanced NSCLC patients, together with a poor overall survival (OS) [3]. It is also considered an organ of immune tolerance, characterized by immuno- suppressive signals and T-cell anergy. With improved understanding of tumor biology, the oligometastatic state has been regarded as a unique subgroup. Liver oligometastasis refers to the metastases confined to the liver with a limited number of metastatic lesions as detected by computed tomography, magnetic resonance imaging and/or positron emission tomography-CT. Emerging evidence have demonstrated that local treatment, such as surgery or radiotherapy, could provide clinical benefit for NSCLC patients with oligometastasis [4–6]. However, little is known about the genomic characteristics of liver oligometastatic NSCLC. Since liver metastases remain the worst prognosis in advanced NSCLC, it is therefore important to investigate the molecular features of liver oligometastatic NSCLC, which will provide a better understanding basis for the biology of this distinct lung cancer subtype.

The outcomes of NSCLC patients have been prolonged with the development of targeted therapies and immune checkpoint inhibitors (ICIs). Targetable mutant genes were often used to guide individualized targeted therapies under the tumor genome sequencing for primary lung cancer in the current age of precision medicine. However, the frequency and evidence level for actionable mutations in liver oligometastasis remains largely unclear since previous researches on targeted therapy have mostly focused on patients with conventional NSCLC. Moreover, tumor mutation burden (TMB) serves as a predictive biomarker for immunotherapy of multiple tumors [7], but the TMB expression pattern in liver oligometastasis is still unknown. Thus, a re-evaluation on the actionability of targetable alterations and exploration of the predictive biomarkers of immunotherapy in liver oligometastasis could provide new insights into the clinical application of targeted therapy and ICIs among this patient population.

In the present study, we report the unique genomic features of liver oligometastatic NSCLC and reveal its potential clinical significance in guiding therapeutic strategies to help clinicians in personalized cancer treatment selection.

Method and material

Study population and sample collection

We enrolled 32 liver oligometastatic NSCLC patients in the Oncology Center of the Second Affiliated Hospital of Chongqing Medical University between February 2017 and September 2022. The major inclusion criteria for this study were: pathologically confirmed NSCLC, stage IV disease based on the 8th edition of the American Joint Committee on Cancer staging system, with only one metastasis confined to liver for three months or longer to ensure the true oligometastatic state. Involved regional lymph nodes were classified as the primary tumors and were not counted as metastatic lesions. Associated clinical and histopathological data were obtained from electronic medical records. The study was approved by the ethical committee of Chongqing Medical University. Written informed consent was obtained from each patient before inclusion.

DNA extraction and target capture sequencing

Tissue specimens from 32 patients were sequenced by a 1021 gene panel in the Geneplus-Beijing Institute (Beijing, China) and were all taken from metastatic lesions. Gene lists of targeted sequencing panel may be found in supplementary Table 1. Genomic DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tumor tissues and matched peripheral blood using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). Then, the Qubit dsDNA BR assay (Life Technologies, USA) was used to quantify the extracted DNA. The custom-designed probes covering ~ 1.4 Mb of genomic sequences for 1021 cancer-related genes (Supplementary Table 1) were used to capture DNA. To construct the library, 1.0 μg of tissue DNA and matched peripheral blood were sheared into 300-bp fragments using a Covaris S2 ultrasonicator. Libraries were constructed using the KAPA DNA Library Preparation Kit. An Applied Biosystems 7500 real-time PCR system and an Agilent 2100 Bioanalyzer were used to measure the captured libraries. DNA sequencing was performed on the HiSeq3000 Sequencing System using 2 × 100 bp paired-end reads.

Sequencing data analysis

From the raw sequencing data, adaptor sequences and low-quality reads were first removed. The human GRCh37 reference genome was used to perform the alignment using BWA (a Burrows-Wheeler aligner) [8]. PCR duplicates were marked by Picard tools (http://broadinstitute.github.io/picard/). Variations were called by GATK (version 3.4-46-gbc02625) or MuTect (version 1.1.4) [9, 10]. Germline mutations were filtered out by PBL sequencing. All candidate mutations were then reviewed manually with the Integrated Genome Viewer (IGV) [11]. Variation sites were annotated with ANNOVAR software [12].

Analysis of mutational signature

Mutation signatures were characterized according to the six substitution patterns (T > A, T > C, and T > G, C > A, C > G, C > T) and 3′- and 5′- flanking nucleotides. The Catalogue of Somatic Mutations in Cancer (COSMIC) [13] mutation signature was used as a reference to extract potential mutational signatures (R package MutationalPatterns) [14]. Subsequently, the relative contribution of different mutational signatures in liver oligometastasis has been analyzed.

Clinical actionability based on OncoKB

The Precision Oncology Knowledge Database (OncoKB) [15] predicts drug actionability according to available clinical evidence, including regimens approved by Food and Drug Administration (FDA) and those still in clinical trials. This clinical support tool established an evidence classification system that could categorize potentially actionable mutations into different levels based on the evidence strength. Alterations with level 1–2 are FDA-approved or considered biomarkers that can predict the response to FDA-approved drugs. Level 3–4 alterations signify compelling clinical or biological evidence that can predict the response to an existing drug. "Actionable mutations" are defined as genomic alterations corresponding to evidence level 1–4 in OncoKB that have therapeutic implications. Annotation of the genomic mutations using the OncoKB database was performed on January 29th, 2023.

Table 1 Clinical characteristics of 32 liver-only oligometastatic NSCLC patients

Characteristics	No. Of patients (%)
Total	32(100.0)
Gender	
Male	15(46.9)
Female	17(53.1)
Age (years)	
Median (range)	65(34–88)
Tumor histology	
Adenocarcinoma	32(100)
Squamous cell carcinoma	0(0.0)
Smoking	
Yes	13(40.6)
No	19(59.4)
Family history	
Yes	5(15.6)
No	27(84.4)
Type of liver oligometastasis	
Synchronous	24(75.0)
Metachronous	8(25.0)
Nodal status	
N0-1	9(28.1)
N2-3	23(71.9)

Statistical analysis

The analyses of Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment were performed for all somatic mutations by the ClusterProfiler package [16]. SPSS 26.0 software was used for statistical analysis. Categorical variables were compared by the Fisher's exact or Chi-square test, while continuous data were compared by the Mann–Whitney U test or two-sample t test. Results were recognized as statistically significant when the two-sided p-value was less than 0.05.

Results

Patient characteristics

Table 1 presented the clinical characteristics of the 32 liver oligometastatic NSCLC patients included in this study. Histologically, lung adenocarcinoma was diagnosed in all oligometastatic patients, and less than half of them were male (15/32, 46.9%). The median age of all included patients was 65 years (range, 34 to 88 years). The majority of patients were non-smokers (19/32, 59.4%), without a family history (27/32, 84.4%), with synchronous liver oligometastasis (24/32, 75.0%) and with a lymph nodal status of N2-N3 (23/32, 71.9%).

Genomic characteristics of liver oligometastatic NSCLC

The landscape of genomic mutations in 32 liver oligometastatic NSCLC is shown in Fig. 1A. The most frequent alterations (> 10%) in liver oligometastasis were TP53 (72%), EGFR (50%), RB1 (19%) and SMARCA4 (12%). A total of 206 mutated genes in 32 patients were detected by targeted sequencing, with a median of 4 mutations per sample (range, 0–24) (Fig. 1A). Among them, missense mutations were the most common type of mutation, followed by nonsense and frame shift deletions (Fig. 1B), while insertions and deletions occurred less frequently than single nucleotide polymorphisms (Supplementary Fig. 1A). We also described the CNV landscape of our cohort and found that the most frequent CNV changes were EGFR amplification (25.00%), followed by CDKN2A deletion (9.38%), CDKN2B deletion (9.38%), CDK4 amplification (6.25%) and MYC amplification (6.25%) (Supplementary Fig. 1B). For each sample, we counted and calculated the proportion of six-base substitution for the single nucleotide variation (SNV) spectrum (Supplementary Fig. 1D), and found that C > A transversion accounted for the most common substitution type in our patients (Fig. 1C), with a Ti/Tv ratio of 1.13 (Supplementary Fig. 1C).

Considering that all liver oligometastatic tumors in our cohort were lung adenocarcinoma (LUAD), we further compared the high-frequency mutated genes in our study with mutations in East Asian LUAD in Singapore Oncology Data Portal (OncoSG) cohort, Chinese LUAD cohort and TCGA LUAD cohort (Fig. 1E). The frequency

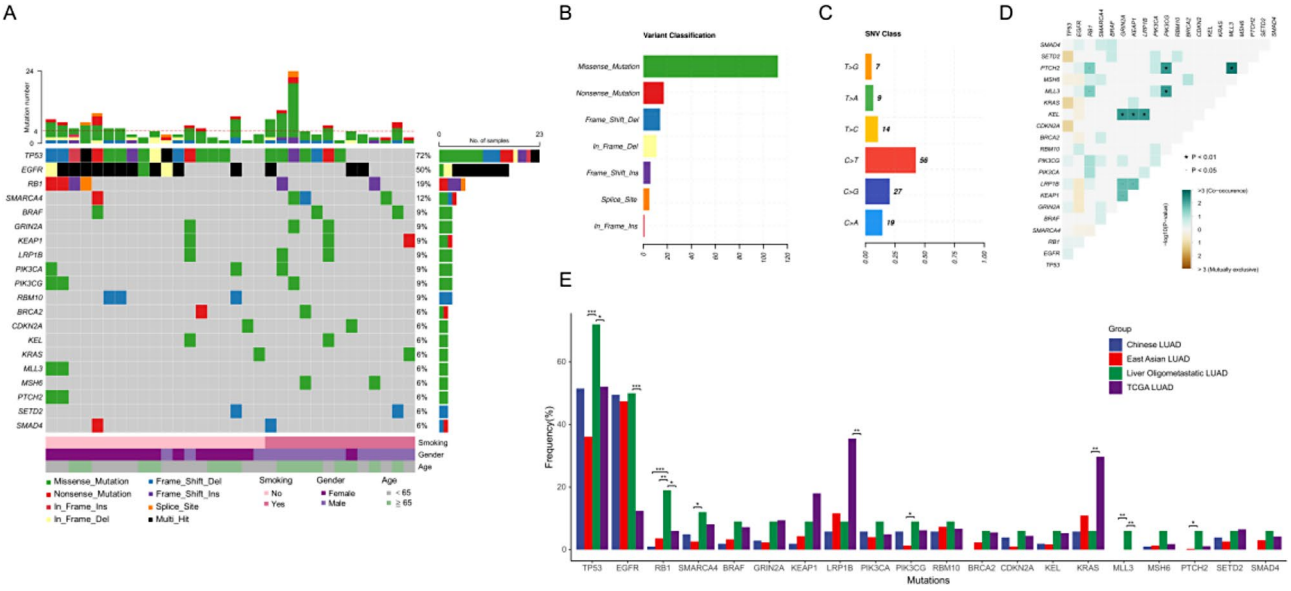

Fig. 1 (**A**) Mutation landscape of 32 liver oligometastatic NSCLC patients. The heatmap shows the top 20 genes across all samples, with genes ranked by mutation frequency. Top bar summarizes the total number of alterations in each patient (columns), and the dashed line indicates the median number of alterations. Side bar (rows) summarizes the percentage of tumors with mutation in each gene and mutation composition for each gene in the entire cohort. Bottom heatmap, smoking, gender and age information. Different colors denote different types of mutations and different clinical features. (**B**) Variant classification, (**C**) single nucleotide variations, (**D**) mutual exclusivity and co-occurrence analysis, (**E**) Comparison of the frequency of 20 high-frequency mutations identified in liver oligometastatic LUAD with that in the East Asian and TCGA LUAD. TCGA, The Cancer Genome Atlas. *$p < 0.05$, and **$p < 0.01$, ***$p < 0.001$

of alterations in TP53 and MLL3 in the liver oligometastatic group was found to be significantly higher than that reported in East Asian and TCGA LUAD. Notably, RB1 mutations were more frequent in patients with liver oligometastasis compared to all other cohorts (Fig. 1E). Because TP53 and EGFR were the most frequently mutations in this cohort and the subtypes of the two genes were strongly correlated with response to therapy and treatment option, they were analyzed separately to reveal the heterogeneity and mapped to corresponding protein sequences by MutationMapper. We found that the most frequently mutated sites of TP53 and EGFR were Q331 and T790M (Supplementary Fig. 2A and B), respectively. And the mutation sites of the other top 20 highly mutated genes were also displayed in supplementary Fig. 2C to T. TP53 was the most common mutation in our cohort. A total of 25 mutations in TP53 were identified among 72% (23/32) of the patients, and 44% of the alterations were truncated mutations leading to TP53 inactivation. Furthermore, we observed 16 patients (16/32 = 50%) harbored 41 EGFR mutations, of which EGFR p.L858R and exon 19del comprised 14.63% and 17.07%, respectively (Supplementary Fig. 1E). EGFR/TP53 co-alterations are correlated with shortened response to EGFR tyrosine kinase inhibitors (TKIs) and poor survival outcomes in EGFR-mutant NSCLC patients [17–19]. It is worth noting that the co-occurrence of EGFR and TP53 mutations accounted for 81.25% of EGFR-mutant oligometastatic tumors (Supplementary Fig. 1F). Previous studies have shown that the inactivation of both TP53 and RB1 was associated with the histologic transformation of LUAD into small-cell lung cancer (SCLC). Of note, we found that that 5 (15.63%) of the 32 patients with oligometastatic LUAD had mutations in both TP53 and RB1, which was significantly higher than that in the TCGA-LUAD cohort (15.63% vs. 4.81%, $p = 0.026$) (Supplementary Fig. 1G). Finally, we further performed the somatic interaction analysis to show the mutual exclusivity and co-occurrence of genetic alterations in liver oligometastasis. Mutations in KEL/GRIN2A, KEL/KEAP1, KEL/LRP1B, MLL3/PIK3CG, PTCH2/PIK3CG and PTCH2/MLL3 were significantly co-occurring (p<0.01), but mutually exclusive mutations were not found in our research (Fig. 1D).

KEGG and GO pathway enrichment analysis

In order to disclose the significance and biological feature of mutated genes in liver oligometastasis, the enrichment analysis of KEGG and GO were conducted. Both somatic mutations and/or copy number alterations were analyzed for KEGG and GO pathway enrichment. The results were ranked based on the p value, and the top 20 enriched KEGG pathways (Fig. 2A) and GO (Fig. 2B) terms were presented in Fig. 2. Significantly altered pathways included central carbon metabolism in cancer, EGFR tyrosine kinase inhibitor resistance, PI3K-Akt signaling,

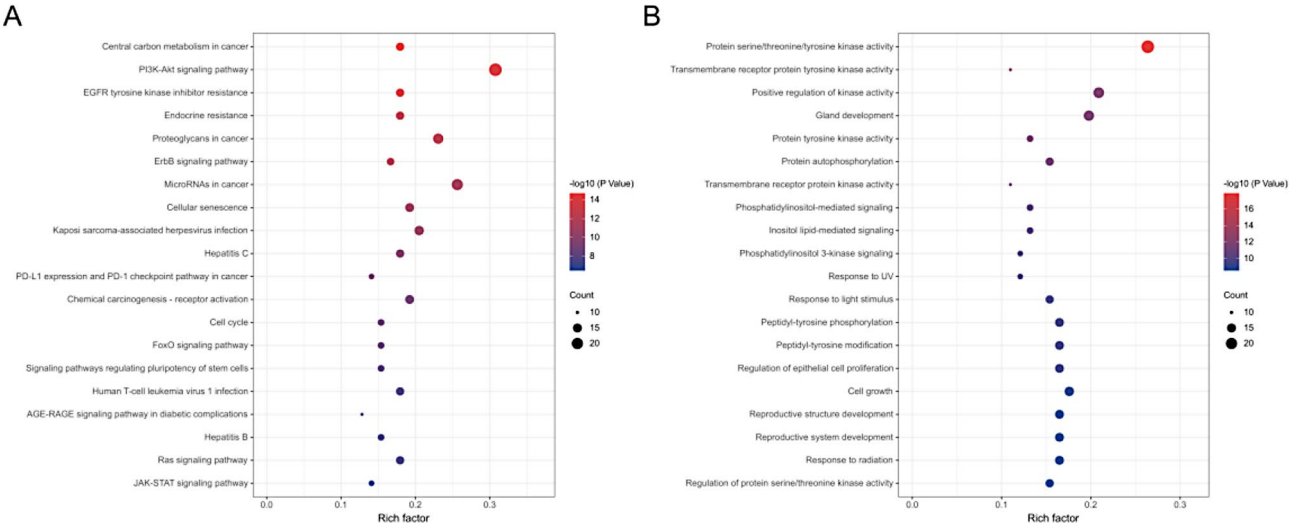

Fig. 2 Top 20 enriched pathways by (**A**) KEGG and functional terms by (**B**) GO enrichment of somatic mutations in liver oligometastasis. Count: the number of mutations enriched in this signaling pathway or functional term

ErbB signaling, FoxO signaling, Ras signaling, JAK-STAT signaling and other well-known pathways. Based upon these findings, we focused on the PI3K-AKT signaling because activation of this pathway may predict a higher risk of histologic transformation of LUAD to small cell lung cancer (SCLC) [20]. A total of 90.6% ($n=29$) of the patients carried alterations in genes of the PI3K-AKT pathway. In addition, we observed that the JAK-STAT signaling was significantly enriched in our cohort, which could alter the fibrotic and immune microenvironment of the liver to establish a pre-metastatic niche and strongly suppressed the antitumour immune response [21]. 71.9% ($n=23$) of liver oligometastatic patients harbored 54 genomic mutations in the JAK-STAT pathway. GO analysis revealed enrichment of genes were mainly related to kinase activity, phosphorylation and reproductive development.

Analysis of mutational signature and TMB

In addition to genetic mutations and signaling pathways, the profile of mutational signature can help to better understand the specific mutagenesis process of liver oligometastatic tumors. To extract the potential mutational signatures from our tumor samples, we used the non-negative matrix factorization (NMF) approach to identify etiological mutational processes by decomposing the base substitution matrix. We found that the mutational signatures of oligometastasis included signature 1 A (40.0%), signature 2 (27.4%), signature 20 (9.2%), signature 3 (16.6%) and unknown (4.8%) (Fig. 3A). Signature 1 A characterized by C > T transitions contributed most to the mutational process in our cohort, which was proposed to be caused by an endogenous mutational process initiated by spontaneous deamination of 5-methylcytosine and correlated with the age at cancer diagnosis. The particular feature of patients in this research whose median age was relatively old (median 65, range 34 to 88 years) may account for the abundance of signature 1 A. Signature 2 was related to APOBEC cytidine deaminase activity making up 27.4% of the observed signatures. Previous studies have demonstrated that the enrichment of APOBEC signatures in adenocarcinomas that may contribute to the transformation process of LUAD to SCLC [22, 23]. Signature 20 was associated with defective DNA mismatch repair (dMMR), an abnormal DNA repair mechanism that ultimately lead to frequent genomic mutations and instability. Signature 3 contributing to 16.6% of the total signatures in our study was relevant to homologous recombination deficiency (HRD). The presence of multiple somatic mutations in our tumor samples, including BRCA2 ($n=2$, 6.3%) and RAD51C ($n=1$, 3.1%), could support the DNA damage caused by HRD. The etiology of the remaining mutational signature (4.8%) in oligometastasis still remains unclear. In summary, age, APOBEC, HRD and dMMR established the mutational signature of liver oligometastatic NSCLC.

TMB has been identified as a prognostic and predictive biomarker for immunotherapy in a variety of solid tumors. In our study, the median TMB of liver oligometastasis was 4.8 mutations/Mb (range 0–24.96 mutations/Mb). The density plot in Fig. 3B shows the TMB distribution of all oligometastatic patients. Notably, we found that one patient had 24.96 mutations/Mb, significantly deviating from a normal distribution. Multiple alterations relevant to defective DNA repair genes, such as ATR, ERCC5, RECQL4 and TP53, were present in this sample. The median TMB in females was slightly higher than that in males (7.68 versus 3.84 mutations/Mb,

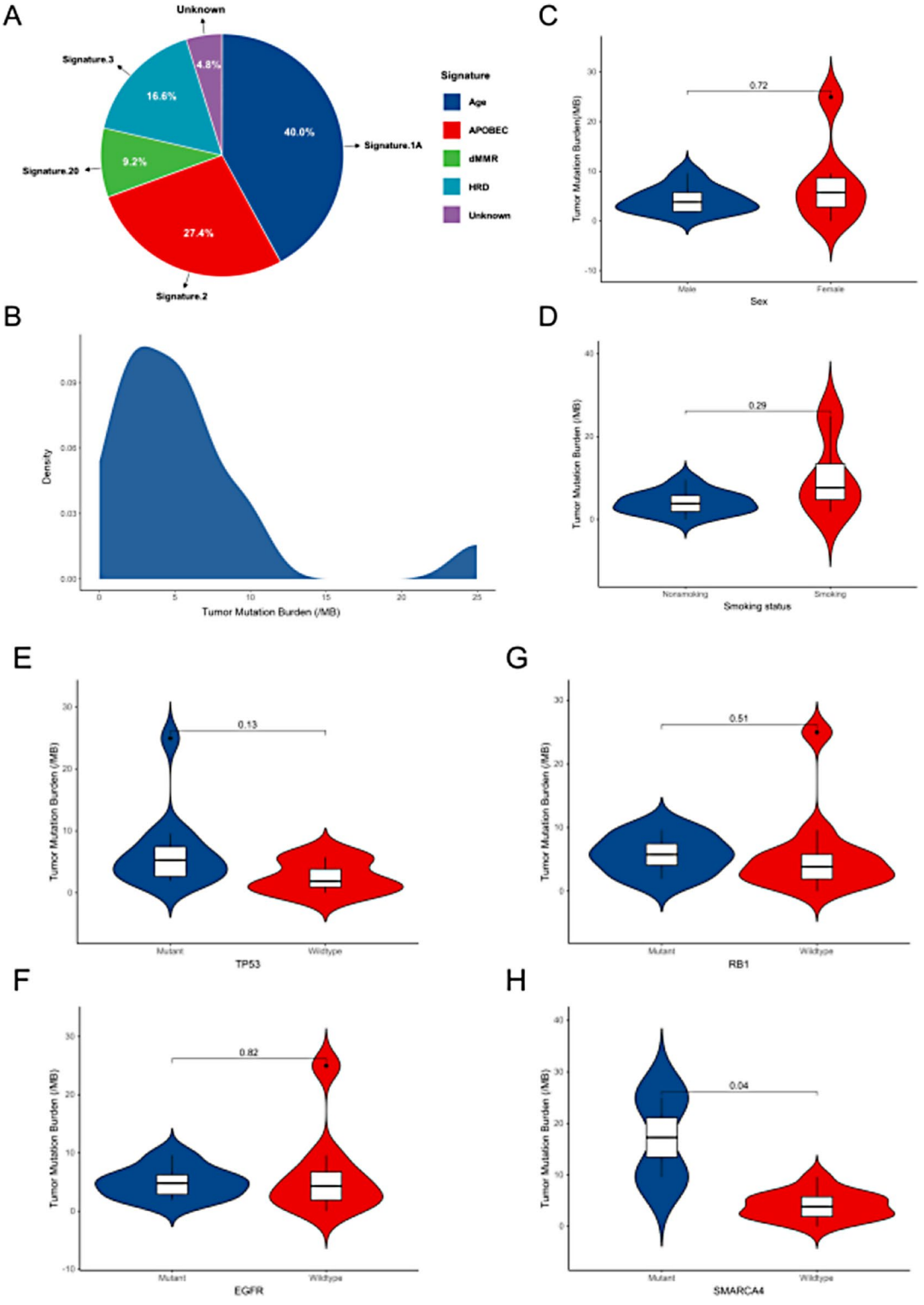

Fig. 3 (**A**) Mutational signatures in liver oligometastatic NSCLC. (**B**) Density plot of tumor mutational burden (TMB) in all cancer patients. TMB according to (**C**) gender, (**D**) smoking status, (**E**) TP53 genotype, (**F**) EGFR genotype, (**G**) RB1 genotype and (**H**) SMARCA4 genotype

$p = 0.72$) (Fig. 3 C). Meanwhile, for smoking patients, the median TMB was 7.68 mutations/Mb, which was higher than that in non-smoking patients (3.84 mutations/Mb, $p = 0.29$) (Fig. 3D). Since TP53 (72%), EGFR (50%), RB1 (19%) and SMARCA4 (12%) were the most common alterations (>10%) in liver oligometastasis, we further analysed the underlying relationship between these high-frequency mutations and TMB level. Tumors with mutations in TP53, EGFR, or RB1 had higher median TMB than those with wild type (5.28 versus 1.86 mutations/Mb, $p = 0.13$; 4.8 versus 4.32 mutations/Mb, $p = 0.82$; 5.76 versus 3.84 mutations/Mb, $p = 0.51$) (Fig. 3E, F and G). In addition, the median TMB of SMARCA4-mutant tumors was significantly higher than that of SMARCA4 wild-type tumors (17.28 versus 3.84 mutations/Mb, $p = 0.04$) (Fig. 3H).

Clinical actionability for targetable genes

With the rapid development of targeted drugs and cancer genomics, a clinical re-evaluation on the significance of comprehensive genomic profiles for targeted therapy in patients with liver oligometastasis according to the actionability of specific genetic mutations categorized by the OncoKB database is warranted (Supplementary Fig. 3). A total of 78.12% patients harbored at least one potentially actionable molecular alteration that may guide further therapeutic strategy. Furthermore, 56.25% tumors carried mutations ranked as level 1 including missense mutations of ALK, BRAF, EGFR, ERBB2 amplification, ALK fusion and in-frame deletion of EGFR. The final evidence level for each sample was defined as the highest level of all alterations detected in the patient. Thus, patients were not assigned to level 2 and 3 because these patients would all have higher-level targets. Level 2 mutations include missense mutations of FGFR and ERBB2 amplification. Level 3 actionable alterations were SNVs of EGFR and amplification of ERBB2. Level 4 mutations accounted for 21.88% including SNVs of BRAF, KRAS, PIK3CA and STK11, amplification of FGFR1 and deletion of CDKN2A (Fig. 4A, C, D). Drugs that target particular genomic mutations in our liver oligometastatic tumors were also presented in Fig. 4C. Given that better clinical outcomes can be achieved by combinations of various targeted drugs, we further profiled the distribution of patients carrying multiple actionable alterations. In general, 34.38% of liver oligometastatic patients harbored one actionable mutation, while 34.38% and 9.36% of those harbored two or more actionable mutations, respectively (Fig. 4B).

Discussion

NSCLC has historically been considered as a heterogeneous entity with a high degree of genomic diversity, resulting in variable responses to therapy and clinical outcomes [24]. Oligometastasis was recognized as a unique clinical disease with distinct molecular and clinic-biological features compared to widely metastatic disease, such as an indolent disease state that could benefit from local treatment. Liver metastases are common in advanced NSCLC patients, which could cultivate an immune desert that lead to inferior response to immunotherapy and shorter OS [25]. Emerging evidence has demonstrated that NSCLC patients with liver oligometastasis could benefit from local treatment and achieve better progression-free survival or overall survival [26, 27]. Until recently, current research has not reached a consensus on the exact number of metastases that define oligometastasis [28]. Considering that most clinical studies enrolled oligometastatic patients with all metastatic lesions adding up to five or less [29], we applied stricter inclusion criteria to include patients with only one distant metastasis confined to liver for three months or longer to ensure the true oligometastatic state. Comprehensive molecular profiling of cancers by next-generation sequencing methods is increasingly being used for therapeutic management decisions in oncology. Although rapid advances in local treatment have revolutionized cancer therapy in oligometastasis, little is known about the genomic characteristics of liver oligometastasis. Therefore, it is necessary to have a comprehensive and deeper understanding of molecular features of such patients to provide better insights into the underlying clinical and biological features of this disease.

In our research, we found that the co-occurrence rate of TP53 and RB1 in liver oligometastasis was significantly higher than that in the TCGA-LUAD cohort (15.63% vs. 4.81%, $p = 0.026$) (Supplementary Fig. 1G). The increase has profound clinical implications because previous studies have shown that the inactivation of both TP53 and RB1 may facilitate the histologic transformation of LUAD into SCLC, with an approximately 43-fold increase in the risk of promoting transformation [22]. In particular, the prognosis of transformed SCLC is significantly poor, even worse than de novo SCLC [30]. We also noticed that PIK3CA (9%) was one of the high-frequency mutations and 90.6% ($n = 29$) of the patients carried genomic alterations in the PI3K-AKT pathway. Emerging data also support that upregulation of PIK3CA gene mutation and PI3K/AKT signaling occurred earlier during the neuroendocrine transformation [20]. In addition, mutational signature 2 related to APOBEC (27.4%) as one of the dominant signatures in our cohort may promote the transformation process and drive drug resistance to EGFR TKIs [31]. These suggest that small cell histologic transformation occurs frequently in liver oligometastasis with a worse prognosis. Therefore, patients at the initial diagnosis of liver oligometastasis should be advised to undergo comprehensive genetic testing to clarify the

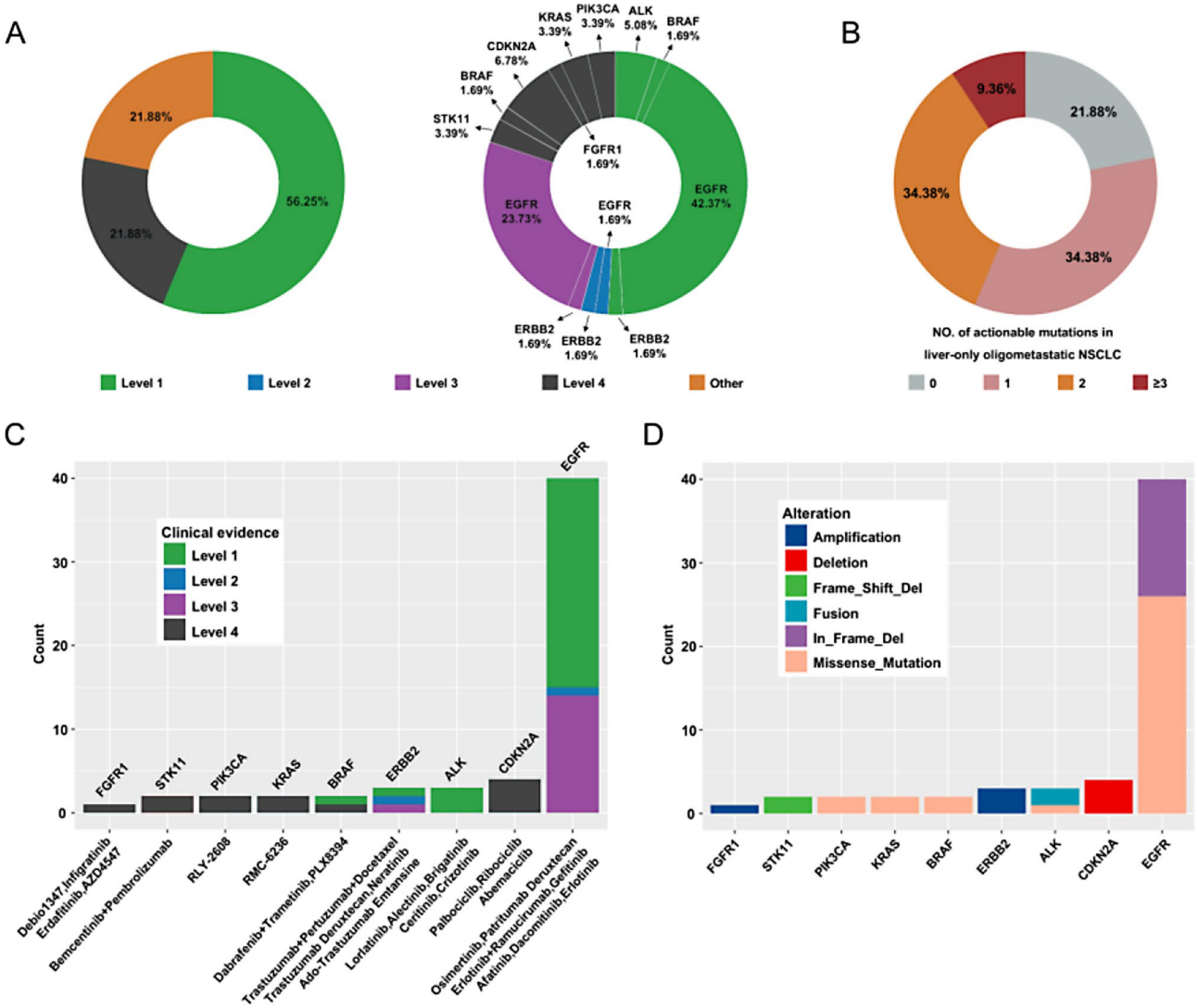

Fig. 4 Somatic alterations identified by the 1021-panel that are clinically actionable. (**A**) Clinical evidence based on OncoKB was used to define alterations. (**B**) Patients were classified according to their highest level of actionable alterations (left). Mutations in different grades (right). (**C**) The percentage of patients with a single actionable mutation or multiple actionable mutations. (**D**) Distribution of levels of actionable mutations and their corresponding potential targetable drugs. (**E**) Distribution of alteration types of actionable genes

presence of concurrent RB1 and TP53 alterations in order to provide guidance for future tumor evolution. Tumor tissues should also be retaken for pathology in time at the early stage of disease progression to determine whether the histological transformation occurs. Alpelisib, a PI3K-AKT inhibitor that significantly inhibits both TP53/RB1 expression in patient-derived cell model [32], can delay tumor growth in NSCLC undergoing histologic transformation. It may be a potential treatment option for liver oligometastatic NSCLC in future. However, these effects are currently the results of preclinical studies, and additional clinical trials are needed to validate the efficacy and safety of Alpelisib in inhibiting SCLC transformation in the real world.

TP53 was the most frequently mutated gene in our study and might have negative prognostic effects for NSCLC [33]. It co-occurred with EGFR mutations to reduce responsiveness to EGFR TKIs and was associated with poor survival outcomes [17–19]. The incidence of concurrent TP53/EGFR in liver oligometastasis (81.25%) was higher than that of EGFR-mutated NSCLC (55–65%) in other researches. Given the high ratio of concurrent TP53/EGFR, a role for APOBEC mutagenesis in the development of resistance to targeted therapies and the enrichment of EGFR tyrosine kinase inhibitor resistance signaling pathway, EGFR TKIs might have low efficiency in this particular population. However, preclinical studies indicate that inhibiting APOBEC mutagenesis through gene deletion or RNA interference could delay

resistance to targeted therapies in lung cancer cell lines [34]. Although direct inhibitors of APOBEC activity are currently unavailable, prior research has suggested a potential role for ataxia telangiectasia and Rad3-related (ATR) and PARP inhibitors [35, 36]. Specifically, studies have shown that activation of APOBEC may sensitize tumor cells to inhibitors of the DNA damage response pathway, such as ATR and PARP inhibitors. In vitro studies have demonstrated that ATR inhibition can effectively overcome EGFR-TKIs resistance [37]. Therefore, these findings suggest that DNA damage repair inhibitors in combination with EGFR-TKIs may hold promise for overcoming resistance and enhancing therapeutic efficacy in liver oligometastatic patients.

Patients with liver metastases have a lower response rate to immunotherapy and shorter survival time than those with metastases to other organs in lung cancer [25]. Of all the pathways enriched in oligometastasis, we focused on the JAK-STAT signaling signaling because activation of this pathway could alter the fibrotic and immune microenvironment of the liver to establish a pre-metastatic niche and lead to a highly immunosuppressive tumour microenvironment that severely hindered anti-tumour immunity [21]. Recent studies have shown that blockade or genetic ablation of components of JAK-STAT signaling could prevent establishment of a pre-metastatic niche and inhibit liver metastasis [21]. However, the PI3K pathway promotes tumour progression and growth independently of JAK-STAT3. Inhibitors of JAK-STAT3 signaling may not be effective as monotherapy in tumours with already activated PI3K pathway. Therefore, JAK-STAT3 inhibitors combined with PI3K signaling pathway inhibitors will be an effective therapeutic regimen for liver oligometastasis.

TMB is a potential biomarker used to predict treatment response to ICIs in NSCLC. Tumours with liver oligometastasis in our study had a lower median TMB (4.8 mutations/Mb) compared with previously reported brain oligometastatic NSCLC (8.7 mutations/Mb). This finding is consistent with previous study that TMB is a site-specific biomarker correlated with tissue location and brain metastases have the highest TMB values compared to metastases in other organs in NSCLC [38]. Furthermore, SMARCA4 mutation was significantly positively associated with TMB in our cohort, similar to other study in conventional NSCLC [39]. Previous studies have shown that SMARCA4-mutant NSCLC tend to derive significant benefit from ICI treatment [39]. Although liver metastasis systemically restrains immunotherapy efficacy and remains an independent predictor of poor response to PD-(L)1 blockade, our study suggests that SMARCA4-mutant patients, an important subgroup of liver oligometastasis, may be more sensitive to and benefit well from immunotherapy compared with the overall population. Several researches have suggested SMARCA4 mutations were potential targets for lung cancer, but there are currently no effective targeted therapies for SMARCA4-mutant NSCLC. However, CDK4/6 inhibitors have recently been reported to show antitumor activity in SMARCA4 deficient tumors, so palbociclib (CDK4/6 inhibition) could be a promising option for the treatment of oligometastasis [40]. Notably, preclinical data suggest that increased ERK-driven mTOR pathway signalling is associated with resistance to palbociclib in lung cancer cell lines and upstream inhibition with a ERK inhibitor plus palbociclib increases cell apoptosis [41]. Based on these promising preclinical data, clinical trials are currently ongoing in advanced NSCLC investigating CDK4/6 inhibitors in combination with ERK inhibitors (NCT02857270, NCT03454035). Concurrent CDK4/6 and ERK inhibition may be more efficacious than CDK4/6 inhibitor monotherapy, which may be a better treatment option for liver oligometastatic patients.

With the discovery of novel targetable driver genes and the emergence of matched new targeted drugs, the clinical prognosis of lung cancer patients with specific gene mutations has been greatly improved. In the present study, we detected a total of 78.12% liver oligometastatic patients carried at least one potentially actionable alteration that may guide further treatment strategy based on OncoKB evidence. This proportion was higher than the 67% reported in a previous study that included 1564 conventional advanced NSCLC patients [42], indicating genomic mutations in liver oligometastasis are highly targetable. Targeted therapy matching level 1–2 genetic alterations could significantly extend progression-free survival and OS in NSCLC, but marked clinical advantage was not observed in patients with level 3–4 alterations [42]. The percentage of patients with level 1–2 and 3–4 mutations as their highest actionable targets was comparable to usual advanced NSCLC patients, respectively (56.25% vs. 57.1%; 21.88% vs. 19.2%).

In conclusion, our study comprehensively delineated the genomic characteristics of liver oligometastatic NSCLC – such findings were helpful to better understand the distinct clinic-biological features of these patients. We have also discussed promising and suitable therapeutic strategies for liver oligometastasis to optimize individualized treatment of this population. Finally, there are some limitations that require mentioning. First, we diagnosed oligometastasis in the absence of a precise diagnostic standard, so we might include polymetastatic patients. Second, the inclusion criteria for liver oligometastatic samples were more stringent than in other clinical studies, resulting in a limited sample size ($n = 32$). Third, this research was a retrospective study with risks of selection bias. Fourth, TMB was calculated using panel sequencing, which might be less accurate compared

with whole-exome sequencing. Fifth, while 1021-panel sequencing covers a large number of genes, targeted panels can still miss certain alterations due to technical limitations. In contrast, whole exome sequencing or whole genome sequencing would allow for more comprehensive analysis to further improve the accuracy and applicability of the results.

Abbreviations

NSCLC	Non-small cell lung cancer
LUAD	Lung adenocarcinoma
SCLC	Small-cell lung cancer
OS	Overall survival
TMB	Tumor mutation burden
FDA	Food and Drug Administration
FFPE	Formalin-fixed and paraffin-embedded
OncoKB	Precision Oncology Knowledge Database
SNV	Single nucleotide variation
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
ICI	Immune checkpoint inhibitor
TKIs	Tyrosine kinase inhibitors
HRD	Homologous recombination deficiency
dMMR	Deficient mismatch repair

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13478-5.

Supplementary Material 1: Supplementary Fig. 1. (A) variant type, (B) prevalence of CNV alterations, (C) Ti/Tv ratios, (D) Mutational fraction of the six-base substitution for each sample, (E) Frequency distributions of EGFR, (F) Frequency of concurrent EGFR/TP53 mutations, (G) Frequency of concurrent RB1/TP53 mutations in liver oligometastasis. Supplementary Fig. 2. The mutated sites of genes with high mutation frequency, including TP53 (A), EGFR (B), RB1 (C), SMARCA4 (D), BRAF (E), GRIN2A (F), KEAP1 (G), LRP1B (H), PIK3CA (I), PIK3CG (J), RBM10 (K), BRCA2 (L), CDKN2A (M), KEL (N), KRAS (O), MLL3 (P), MSH6 (Q), PTCH2 (R), SETD2 (S), SMAD4 (T). Supplementary Fig. 3. Clinical evidence based on OncoKB was used to define alterations

Supplementary Material 2: Supplementary Table 1. Gene list of the 1021-gene panel

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Author contributions

Rongxin Liao and Guangming Yi planned and carried out the majority of experiments, designed the figures, collected the clinical data and wrote the manuscript. Lu Shen and Xiao Xiao performed the targeted sequencing of all included samples. Chuan Zeng, Liangzhong Liu, Hongjun Tang and Shunping Huang did the statistical analysis. Xiaoyue Zhang, Zaicheng Xu, Yuan Peng and Zhenzhou Yang provided critical comments, suggestions, and revised the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

The variation data reported in this paper have been deposited in the Genome Variation Map (GVM) in National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences and China National Center for Bioinformation, under accession number GVM000844 that can be publicly accessible at https://bigd.big.ac.cn/gvm/getProjectDetail?Project=GVM000844.

Declarations

Ethics approval and consent to participate

The ethical committee of Chongqing Medical University approved the study and all methods were also performed in accordance with the relevant guidelines and regulations under the committee supervision. Written informed consent was obtained from each participant prior to enrollment. Clinical trial number: not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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