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Mutational status of RAS, SMAD4 and APC predicts survival after resection of colorectal liver metastases in Chinese patients: prognostic stratification based on genetic sequencing data of multiple somatic genes

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Abstract

Background This study aimed to investigate the impact of the mutational status of multiple genes on survival in Chinese patients with colorectal liver metastases (CRLM) undergoing liver resection.

Methods This study included 519 Chinese patients undergoing curative liver resection for CRLM between 2011 and 2021 and had genomic sequencing data of 620 genes available for analysis. The genes associated with overall survival (OS) were identified using Cox regression analyses. The patients were stratified according to a novel scoring system based on the number of genes with a deleterious status (mutation or wild type), and OS was compared among the groups. The prognostic capacity of the scoring system was assessed using Harrell's C-index.

Results Twelve genes were mutated in more than 10% of the patients. RAS mutation, SMAD4 mutation, and APC wild-type status were significantly associated with worse OS. A scoring system was built based on the mutational status of RAS, SMAD4, and APC. Higher scores were significantly associated with worse OS (HR > 1, $p < 0.05$, for any two groups), and the patients with a score of 3 had poor survival with a median OS of only 17.1 months. The scoring system demonstrated moderate discriminative capacity (Harrell's C-index = 0.627).

Conclusions In Chinese patients, the mutational status of RAS, SMAD4, and APC was significantly associated with survival after CRLM resection. The three-gene scoring system provided information on prognostic stratification for survival, which can be used to improve precision surgery for CRLM.

Keywords Colorectal liver metastases, Liver resection, Multiple somatic genes, Prognostic stratification

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Background

Liver resection is now widely accepted as the standard of care for colorectal liver metastases (CRLM), providing a 5-year overall survival (OS) rate of approximately 50% [1–3]. The increasing use of neoadjuvant and conversion therapies [4, 5] and the demonstrated effectiveness of ablation and parenchymal-preserving resection [6–9] have led to a higher resectable rate. However, CRLM is a heterogeneous disease, and survival after liver resection varies greatly. Multidiscipline team (MDT) management and precision medicine are important in CRLM treatment [10, 11].

Researchers have been trying to develop a risk stratification system for the optimal selection of patients and their specific surgery strategy, termed precision surgery [10]. Many scoring systems are based on clinicopathological risk factors, among which the Fong score [12] is the most widely used. However, with the increasing use of neoadjuvant chemotherapy and the expansion of the resectable population, the performance of the clinical risk scores declined in the molecular era [13–16]. With the development of next-generation sequencing, genomic information helped improve prognostic stratification. KRAS and BRAF mutations were reported to be associated with resistance to anti-EGFR treatment [17, 18] and poor survival of patients with CRLM [19–21]. Discordance of KRAS mutational status between primary tumors and liver metastases was also reported to be associated with worse survival [22]. The scoring systems combining RAS or RAS/BRAF mutations with clinical risk factors, such as the GAME score [13] and m-CS [14], showed better discriminatory capacity than that of the traditional clinical risk score (Fong score).

Studies showed that other genes, including SMAD4 [23–25] and TP53 [24, 26], are also associated with survival besides RAS and BRAF. Further, the co-mutational status of somatic genes has also been reported to improve prognostic stratification. It was reported that RAS co-mutated with TP53 or SMAD4 is associated with worse survival in patients undergoing liver resection for CRLM. In contrast, the subset with only RAS mutations had similar survival to those that were RAS wild-type [23, 27]. Kawaguchi et al. also developed a pathway-centric approach based on the mutational status of TP53, APC, RAS/BRAF, and SMAD4 and successfully stratified the patients into four groups [24].

However, to date, no study has explored the impact of the status of multiple somatic genes on survival after liver resection for CRLM in Chinese patients. This study aimed to investigate the genes that influence OS after CRLM resection in Chinese patients and to develop a prognostic scoring system based on these genes, which may be helpful for precision surgery in the management of CRLM.

Methods

Patients

Patients who underwent liver resection for CRLM with curative intent at the Hepato-Pancreato-Biliary Surgery Department I, Peking University Cancer Hospital, from 2011 to 2021 and had genetic sequencing data of 620 genes from primary tumor tissues or CRLM specimens were included from a prospectively compiled cohort. Patients with the following conditions were excluded: (1) R2 surgical margin of liver metastases or primary tumor, (2) repeated liver resection for recurrence of CRLM, (3) staged liver resection, and (4) extrahepatic metastasis that was not removed radically. Data on demographic information, clinicopathological characteristics, and survival outcomes were collected. The last follow-up date was June 1, 2023.

Institutional approach to surgical management of CRLM

Preoperative chemotherapy is recommended for patients with unresectable CRLM and those presenting with risk factors of recurrence (clinical risk score ≥ 2). First-line chemotherapy regimens encompass oxaliplatin- or irinotecan-based chemotherapy, with or without a targeted agent (bevacizumab or cetuximab). During preoperative therapy, restaging is conducted in accordance with Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [28]. Chemotherapy response was categorized as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Second-line therapy is considered for patients with PD after first-line chemotherapy. For patients receiving multiple lines of chemotherapy, chemotherapy response to the last-line regimen was included for analysis. Resectability is deliberated by MDT. It can be summarized as the feasibility of surgical resection, with or without ablation of all the lesions, while preserving > 30% of liver remnant and maintaining vascular inflow, vascular outflow, and biliary drainage. Resection of ≥ 3 segments of the liver is regarded as a major hepatectomy. Right colon cancer is defined as the primary tumor located in the cecum, ascending colon, or the hepatic flexure of the transverse colon. Left colorectal cancer is defined as the primary tumor located in the splenic flexure of the transverse colon, descending colon, sigmoid colon, or rectum.

Genomic sequencing

Multigene panel testing of 620 cancer-related genes (Additional file 1) was performed on primary tumor tissues or liver metastases from the included patients. As previously described [29], DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissues and white blood cells. A custom-designed IDT capture panel (Integrated DNA Technologies, Coralville, IA, USA) was used to capture the coding regions of 620 genes, followed

by library preparation and quantification using KAPA Hyper Prep protocols (Kapa Biosystems, Wilmington, MA, USA). Purification was performed using AMPure XP (Beckman Coulter, Brea, CA), and quantification was done using a Qubit™ dsDNA HS Assay Kit (Thermo Fisher, Waltham, MA). Finally, the library was sequenced to a depth of at least 500 × on a NovoSeq 6000 platform (Illumina, San Diego, CA, USA).

Prognostic scoring system development

Univariate and multivariate Cox proportional hazards models were fitted to identify the genes and clinical factors that were predictors of OS. Univariate analysis included the following factors: (1) recurrently mutated genes with a mutational frequency of more than 10%, (2) age (>65 versus ≤65), (3) sex, 4)primary tumor location, 5) T stage (T3–4 versus T1–2), 6) lymph node status of the primary tumor, 7) disease-free interval (<12 months versus ≥12 months), 8) the number of CRLM (>1 versus 1), 9) the largest diameter of CRLM as a categorical variable, 10) chemotherapy response, 11) preoperative CEA concentration as a categorical variable, 12) major hepatectomy, and 13) resection margin (R1 versus R0). Factors with a p-value<0.1 in the univariate analysis were included in the multivariate analysis. The genomic factors with a p-value<0.05 in the multivariate analysis were used to develop a scoring stratification system in which the risk score was equal to the number of genes with a deleterious status (mutation or wild type). The Kaplan–Meier method and hazard ratios, after adjusting for other risk factors, were used to compare OS among the groups.

Statistical analysis

Categorical variables are represented as numerical values and percentages and were compared between groups using the chi-square test or Fisher's exact test. Continuous variables are represented as median values and interquartile ranges (IQR). Receiver operating characteristic (ROC) curve analysis was used to determine the cut-off values for CEA concentration and the largest diameter of CRLM. Cox proportional hazard models were employed to identify risk factors associated with OS. The Kaplan–Meier method was used to estimate time-related events. The hazard ratios and the log-rank test were used to compare OS. Harrell's concordance statistic was used to test the capacity of prognostic stratification [30]. Statistical significance was set at $p<0.1$ for univariate Cox regression analysis and $p<0.05$ for other analyses. Statistical analyses were conducted using R version 4.3.2 (2023-10-31).

Results

Clinicopathologic characteristics

From 2011 to 2021, 1503 Chinese patients underwent liver resection for CRLM in our center, and 641 patients had genetic sequencing data of 620 genes available. Among the 641 patients, 519 patients undergoing liver resection with curative intent were included in the analysis, and 373 patients (71.9%) in this study underwent liver resection from 2019 to 2021. The median follow-up was 37.2 months (IQR, 27.3–50.7), during which 201 patients died. The 1-, 3- and 5-year survival rates after liver resection were 95%, 62.8%, and 49.9%, respectively. Clinicopathological characteristics are summarized in Table 1. The median age was 58 (IQR, 52–64) years. The most common primary tumor sites were the left colon and rectum (81.9%). Further, 390 patients (75.1%) were diagnosed with liver metastases within 12 months following the initial diagnosis of primary cancer. In total, 425 (82.1%) patients received pre-hepatectomy treatments, and 104 (20%) were treated with more than six cycles of chemotherapy. Anti-EGFR agents were administered to 149 patients (28.7%), and anti-VEGF agents were administered to 158 patients (30.4%). Among the patients undergoing preoperative chemotherapy, CR, PR, SD, and PD occurred in 0 (0%), 199 (47.5%), 200 (47.7%), and 20 (4.8%) patients, respectively. Nearly one-quarter of the resections were major hepatectomies (24.5%). R0 resection occurred in 83.0% of the patients. The most appropriate cut-off values were 44 ng/mL for CEA concentration and 36 mm for the largest diameter of CRLM.

Genomic landscape of the 519 patients who underwent liver resection

Of the 620 genes examined, 33 were mutated in >5% of patients (Fig. 1). Twelve genes had frequency of somatic mutation higher than 10%: TP53 (78%), APC (76%), KRAS (38%), FLG (16%), TCF7L2 (15%), SMAD4 (14%), PIK3CA (12%), FBXW7 (12%), ZFHX3 (12%), LRP1B (12%), FAT4 (11%), and SOX9 (10%). KRAS and NRAS mutations were grouped into the RAS mutation category, which occurred in 40.3% of patients. KRAS and PIK3CA were mutated more frequently in patients with right colon cancer than in those with left colorectal cancer (right colon cancer vs. left colorectal cancer: KRAS, 61.6% vs. 32.1%, $p<0.001$; PIK3CA, 31.3% vs. 7.14%, $p<0.001$) (Additional file 2).

Predictors of overall survival after liver resection

To identify the predictors of OS after hepatectomy for CRLM, univariate and multivariate Cox proportional hazard models were fitted based on clinicopathological factors and the 12 genes with a mutational frequency of over 10% (Table 2). Of the genes analyzed, RAS and SMAD4 mutations were significantly associated with

Table 1 Demographic and clinicopathologic characteristics of 519 Chinese patients with CRLM undergoing liver resection

characteristics		value
Age, median (IQR)		58 (52–64)
Sex, male: female		358:161
Primary tumor site	Right colon	99 (19.1%)
	Left colon or rectum	420 (80.9%)
T stage	T1-T2	42 (8.4%)
	T3-T4	460 (91.6%)
	NA	17
Lymph node metastases of primary tumor	negative	162 (32.1%)
	positive	343 (67.9%)
	NA	14
Disease-free interval (months)	< 12	390 (75.1%)
	≥ 12	129 (24.9%)
Therapy before surgery	No treatment	94 (18.1%)
	Chemotherapy	268 (51.6%)
	Chemotherapy with anti-VEGF agents	158 (30.4%)
	Chemotherapy with anti-EGFR agents	149 (28.7%)
Cycles of treatments before surgery	> 6	104 (20.0%)
	≤ 6	321 (61.8%)
Response to preoperative chemotherapy n (% in patients receiving chemotherapy)	PR	199 (47.5%)
	SD	200 (47.7%)
	PD	20 (4.8%)
	NA	6
Preoperative CEA (ng/ml), median (IQR)		7.14 (3.32–20.5)
Number of liver metastases	> 1	361 (69.6%)
	1	158 (30.4%)
Diameter of the largest CRLM (cm), median (IQR)		2.5 (1.70–3.75)
Major hepatectomy		127 (24.5%)
Resection margin	R0	431 (83.0%)
	R1	88 (17.0%)

CRLM, colorectal liver metastases; IQR, interquartile range; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor

worse OS after liver resection (HR = 1.88 for RAS, $p < 0.001$; HR = 1.52 for SMAD4, $p = 0.034$), while APC mutation was associated with better OS (HR = 0.57, $p = 0.001$). The number of liver metastases (> 1 vs. 1, HR = 1.63, $p = 0.015$), lymph node status of the primary tumor (positive vs. negative, HR = 1.50, $p = 0.021$), and major hepatectomy (HR = 1.48, $p = 0.027$) were also independent prognostic factors of OS. Patients with SD exhibited a trend of worse survival than those with PR, although the survival difference was not significant (HR = 1.39 [95% CI, 0.98–1.97], $p = 0.066$). Patients with PD had significantly worse OS compared to patients with PR (HR = 3.14, $p = 0.001$).

Risk stratification based on RAS, SMAD4, and APC

Based on the results of multivariate Cox regression, a deleterious status was defined as RAS mutation, SMAD4 mutation, or APC wild-type status (since APC wild-type status was associated with worse OS). A novel scoring system was developed based on the number of genes with a deleterious status. The formula for calculating the score was as follows: score = 1 point (if RAS is mutated) + 1 point (if SMAD4 is mutated) + 1 point (if APC is wild-type). Therefore, the patients were divided into four groups (score 0–3). The multivariate hazard ratios by the score, adjusted for other risk factors, are presented in Table 3. The Kaplan–Meier curves and hazard ratio after adjusting other risk factors showed that a higher score was significantly associated with worse survival than a lower score (HR > 1 and $p < 0.05$, for any two groups) (Fig. 2; Table 3). The 5-year survival rates of scores 0, 1, and 2 were 65.1%, 47.3%, and 28.3%, respectively. The seven patients with a score of 3 had poor survival with a median OS of 17.1 months. All seven patients died within 36 months, except for one patient censored at 34.2 months (Fig. 2). Harrell's C-index of the scoring system was 0.627, which was comparable to that of the GAME score (C-index = 0.625, in the primary study) [13].

Discussion

This study demonstrated that mutational statuses of RAS, APC, and SMAD were significantly associated with survival after liver resection for CRLM. A higher score, equal to the number of genes with a deleterious status, was significantly associated with worse survival. The subset of patients with mutated RAS, mutated SMAD4, and wild-type APC had poor survival, with a median OS of 17.1 months.

In this study, the sequencing data of 620 genes from 519 patients who underwent liver resection for CRLM were analyzed. The mutational frequency of the 620 genes was similar to that in our previous study [29]' as TP53, APC, and KRAS were the three most frequently mutated genes. These data provided the genomic landscape of Chinese patients with CRLM, which also resembled the cohorts from the University of Texas MD Anderson Cancer Center [27] and the Memorial Sloan Kettering Cancer Center [31]. However, there were differences for individual genes. For example, APC mutation frequency is 79% in the cohort of Memorial Sloan Kettering Cancer Center, which is similar to that of our cohort (76%), while it is lower in the cohort of the University of Texas MD Anderson Cancer Center (47.4%). The differences may be due to the population-specific backgrounds. Additionally, genomic sequencing was performed on either primary

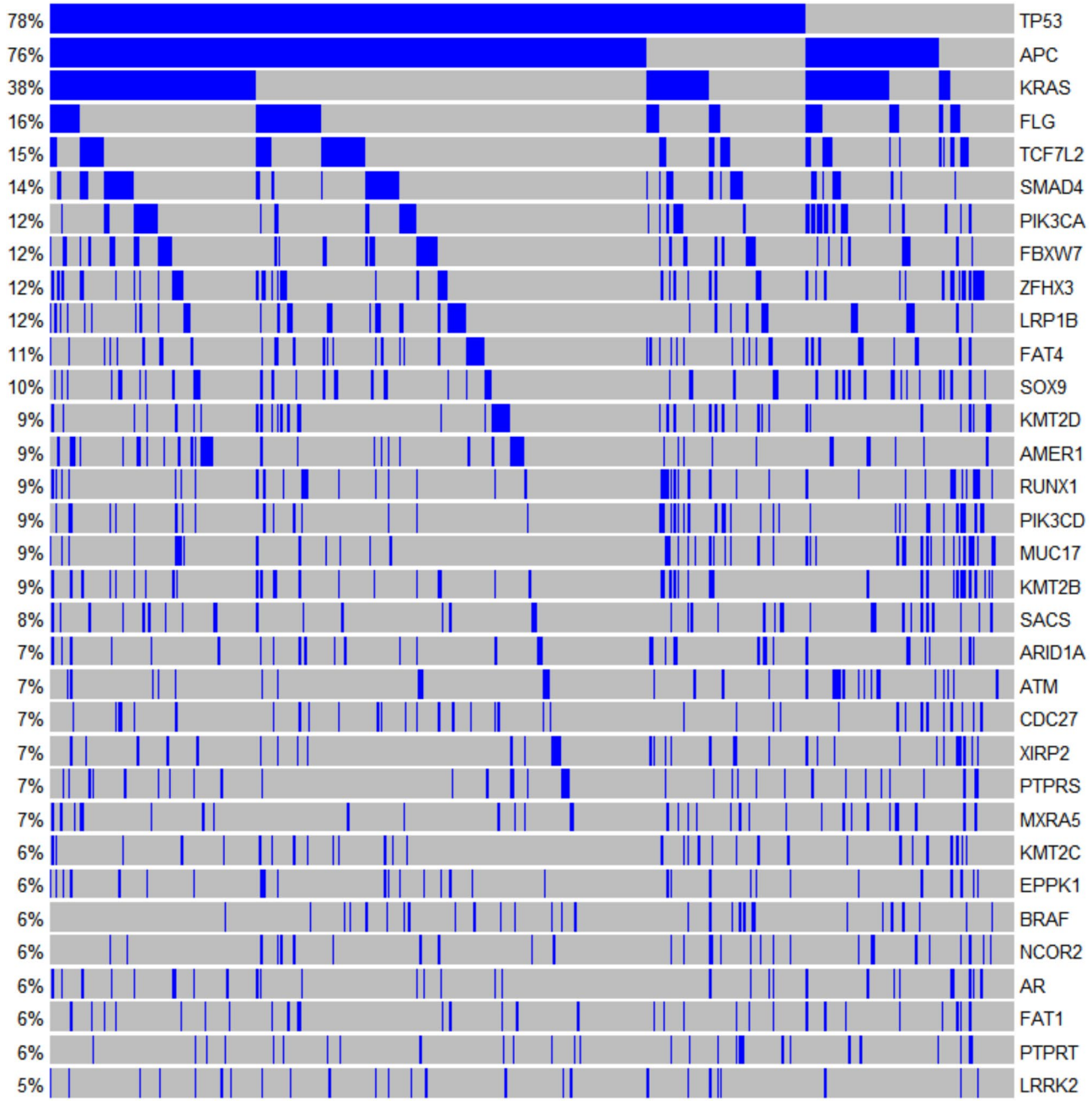

Fig. 1 Genomic landscape of colorectal liver metastases (CRLM)
Mutational frequency of the recurrently mutated genes in 519 Chinese patients with CRLM undergoing liver resection

tumor tissues or liver metastases but not a single source, which may also result in the differences.

CRLM is a heterogeneous disease; precision surgery [10] is meaningful for its management. However, clinicopathological factors are insufficient for prognostic stratification in the molecular era. Data from genetic sequencing provide prognostic information. In the Chinese patients, RAS and SMAD4 mutations were found to have a deleterious impact on OS. RAS proteins (KRAS, NRAS, and HRAS) are GTPases, which are involved in various cellular signal pathways, regulating cell processes such as proliferation, differentiation, and apoptosis. RAS mutations drive constitutive activation of downstream signaling cascades (e.g., MAPK pathway), promoting uncontrolled cell proliferation and poor prognosis [32]. SMAD4 mutations disrupt the transforming growth factor (TGF)-β signaling pathway [33] and are reported to promote metastasis and epithelial-to-mesenchymal transition, playing a critical role in the progression of colorectal cancer [34, 35]. RAS mutation [13, 14, 19, 20]

Table 2 Univariate and multivariate hazard ratios for OS after liver resection for CRLM

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	**p**	**HR (95% CI)**	***p***
Gene mutation				
TP53	1.04 (0.74–1.46)	$p=0.814$		
APC	0.63 (0.47–0.84)	$p=0.002$	0.57 (0.41–0.78)	$p=0.001$
RAS	1.95 (1.48–2.58)	$p<0.001$	1.88 (1.35–2.61)	$p<0.001$
FLG	1.07 (0.75–1.51)	$p=0.722$		
TCF7L2	0.99 (0.67–1.46)	$p=0.952$		
SMAD4	1.51 (1.05–2.15)	$p=0.025$	1.52 (1.03–2.23)	$p=0.034$
PIK3CA	1.80 (1.21–2.68)	$p=0.004$	1.40 (0.89–2.21)	$p=0.148$
LRP1B	0.65 (0.39–1.08)	$p=0.093$	0.71 (0.41–1.24)	$p=0.233$
FBXW7	1.00 (0.65–1.55)	$p=0.982$		
FAT4	1.19 (0.78–1.81)	$p=0.411$		
ZFHX3	1.16 (0.79–1.71)	$p=0.454$		
SOX9	0.93 (0.58–1.49)	$p=0.767$		
Patient factors				
Age, > 65	1.00 (0.70–1.41)	$p=0.979$		
Sex, male	0.72 (0.54–0.96)	$p=0.025$	0.87 (0.63–1.20)	$p=0.385$
Clinicopathologic factors				
Primary site Left colon and rectum Right colon	Reference 1.38 (0.97–1.96)	$p=0.073$	Reference 1.06 (0.71–1.61)	$p=0.766$
Lymph node status of primary tumor Negative Positive	Reference 1.66 (1.20–2.30)	$p=0.002$	Reference 1.50 (1.06–2.12)	$p=0.021$
T stage T1-2 T3-4	Reference 1.93 (1.02–3.64)	$p=0.044$	Reference 1.37 (0.71–2.65)	$p=0.347$
Disease-free interval, < 12months	1.55 (1.10–2.28)	$p=0.012$	1.31 (0.89–1.93)	$p=0.175$
Number of CRLM, >1	1.82 (1.30–2.55)	$p=0.001$	1.63 (1.10–2.42)	$p=0.015$
Largest diameter of CRLM, > 36 mm	1.3 (0.96–1.8)	$p=0.095$	1.26 (0.90–1.77)	$p=0.186$
Preoperative CEA, > 44ng/ml	1.65(1.16–2.35)	$p=0.005$	1.13 (0.76–1.68)	$p=0.544$
Response to chemotherapy				
PR	Reference			
no preoperative chemotherapy	0.57 (0.35–0.94)	$p=0.028$	0.76 (0.43–1.35)	$p=0.350$
SD	1.44 (1.05–1.97)	$p=0.023$	1.39 (0.98–1.97)	$p=0.066$
PD	3.32 (1.93–5.74)	$p<0.001$	3.14 (1.63–6.05)	$p=0.001$
Resection margin R0 R1	Reference 1.26 (0.89–1.78)	$p=0.189$		
Major hepatectomy				
No	Reference		Reference	
Yes	1.70 (1.25–2.32)	$p<0.001$	1.48 (1.05–2.09)	$p=0.027$

OS, overall survival; CRLM, colorectal liver metastases

and SMAD4 mutation [23–25] have been reported to be associated with worse OS in patients undergoing liver resection for CRLM. KRAS is also a pivotal oncogene in metastatic colorectal cancer, independent of hepatic involvement, and its mutation is generally associated with a negative prognosis [19, 36, 37]. Notably, compared to poly-metastatic disease, oligo-metastatic disease (OMD) in colon cancer is characterized by lower rates of KRAS and SMAD4 mutations, suggesting these mutations may promote widespread metastases and predict aggressive biology [38].

The impact of APC on survival after CRLM resection was rarely reported. Yamashita et al. reported that double mutation of APC and PIK3CA predicts inferior response to preoperative chemotherapy and poor survival in patients with CRLM [39]. Here, a significant association was observed between APC mutation and better OS, which aligns with prior reports by Kawaguchi et al. and Lang et al. [24, 40]. The predictive value of APC

Table 3 Multivariate HRs for OS after surgery by the Three-gene score and the other risk factors

	Multivariate analysis	
	HR (95% CI)	***p***
score		
score 1		
vs. score 0	2.09 (1.46–2.97)	$p < 0.001$
score 2		
vs. score 0	3.08 (2.01–4.73)	$p < 0.001$
vs. score 1	1.48 (1.02–2.14)	$P = 0.038$
score 3		
vs. score 0	9.01 (3.49–23.31)	$p < 0.001$
vs. score 1	4.32 (1.71–10.90)	$P = 0.002$
vs. score 2	2.92 (1.13–7.55)	$p = 0.027$
Primary lymph node metastases	1.63 (1.17–2.28)	$p = 0.004$
Number of CRLM, >1	1.67 (1.14–2.45)	$p = 0.009$
Response to chemotherapy		
PR	Reference	
no preoperative chemotherapy	0.77 (0.44–1.32)	$p = 0.338$
SD	1.45 (1.04–2.02)	$p = 0.027$
PD	2.94 (1.63–5.31)	$p < 0.001$
Major hepatectomy	1.59 (1.13–2.23)	$p = 0.007$

HRs, hazard ratios; CI, confidence interval; OS, overall survival; CRLM, colorectal liver metastases

still needs to be further investigated in randomized controlled trials. APC mutations result in dysregulation of β-catenin and lead to continuous Wnt pathway activation [41, 42], which have been considered important mediators of colorectal neoplasia. The better prognosis of APC wild type may be due to other biological differences in patients with different APC status, which is worth further investigation. We observed no significant association of TP53 and PIK3CA with OS. The prognostic impact of TP53 is inconsistent across previous studies [26, 43, 44], and PIK3CA mutation was not associated with OS in several studies [20, 23, 24]. TCF7L2, mutated in 15% of patients in our cohort, was not significantly associated with OS in our analysis. However, Ottaiano et al. observed that diabetes-associated TCF7L2 variants were never present in patients with OMD affected by type II diabetes and suggested that TCF7L2 stability may suppress metastatic dissemination via Wnt pathway modulation [45]. The prognostic role of TCF7L2 in CRLM warrants further investigation.

The improved prognostic stratification by considering the mutational status of multiple somatic genes has been previously reported. It was reported that RAS co-mutated with TP53 or SMAD4 is associated with worse survival, while the subset with only RAS mutation had similar survival to RAS wild-type [23, 27]. By evaluating the prognostic relevance of the predominant member genes of seven cancer-related signaling pathways, Kawaguchi et al. developed a pathway-centric approach based on the mutational status of TP53, APC, RAS/BRAF, and SMAD4. They successfully stratified the patients into four groups [24]. Here, we also developed a scoring system based on three genes involved in different signaling pathways, providing a simple yet powerful tool for prognostic stratification independent from clinicopathologic factors. The study by Kawaguchi et al. and our study underscore the importance of pathway-centric models in prognostic stratification.

The three-gene scoring system could be beneficial for clinical decision-making regarding patient selection for surgery, the choice of surgery strategy for a specific patient, and surveillance strategies after liver resection. Notably, patients with a score of 3 (mutated RAS, mutated SMAD4, and wild-type APC) exhibited a median OS of merely 17.1 months, significantly worse than other subgroups. We have the following recommendations for this subgroup of patients: first, due to the poor prognosis, we suggest intensified preoperative chemotherapy and postoperative surveillance. Second, high-risk surgical procedures, such as major hepatectomies and associating liver partition and portal vein ligation for staged hepatectomy, etc., are not advisable for such patients, as they bear the risk of the surgery but may not gain benefits from it. Third, in cases of disease progression after chemotherapy, the decision to proceed with hepatectomy should be carefully considered. Furthermore, patients with a score of 3 may not benefit from surgery since the median OS is more than 2 years for patients who received only systemic therapy [46] and approximately 5 years for patients undergoing surgery [1, 2]. It is worth further investigating whether such patients are oncologically suitable for hepatectomy.

The limitations of this study are as follows: First, it was a retrospective analysis of patients with CRLM undergoing liver resection. Second, the scoring system lacks validation in external cohorts, limiting its generalizability. While the current study is limited by its single-center design, the prognostic effects of RAS, SMAD4, and APC identified in this study were consistent with the findings in Western cohorts [24, 40]. Furthermore, despite being simpler, the discriminative capacity of our three-gene scoring system (C-index = 0.627) was comparable to that of the GAME score (C-index = 0.625) [13], supporting its clinical utility. A multicenter validation that can confirm these findings is required in the future. Third, the small subgroup of patients with a score of 3 ($n = 7$, 1.3%) reduced statistical power for this group. However, the median OS of 17.1 months highlights the poor prognosis of this subgroup. It is meaningful to validate the poor survival of this subgroup in prospective studies with larger cohorts and further investigate whether such patients can benefit from surgery.

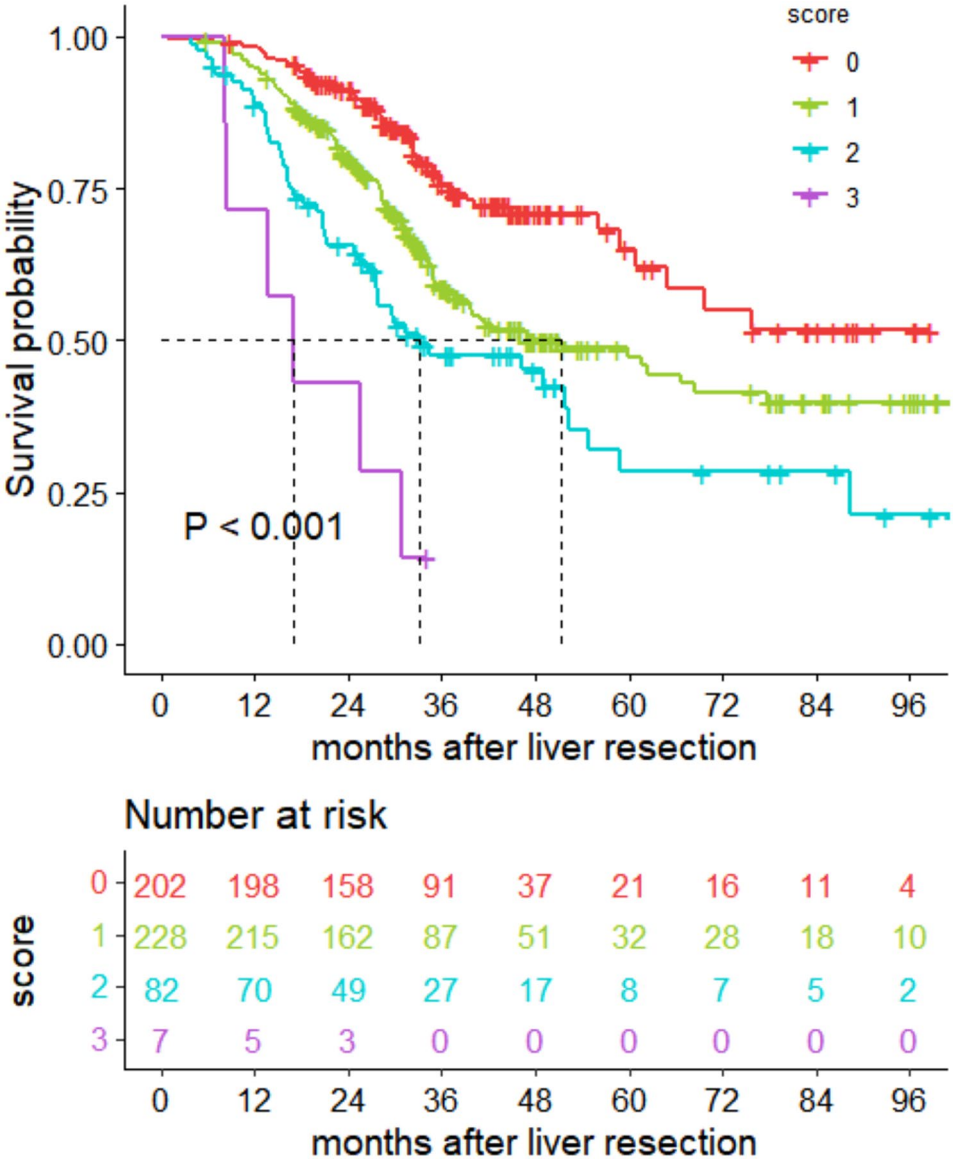

Fig. 2 The three-gene scoring system stratified overall survival (OS) after liver resection
Score = the number of the genes (RAS, SMAD4, and APC) with a deleterious status = 1 point (if RAS is mutated) + 1 point (if SMAD4 is mutated) + 1 point (if APC is wild-type). Kaplan–Meier analysis of OS by the three-gene scoring system in 519 Chinese patients with colorectal liver metastases (CRLM) undergoing liver resection

Conclusions

In Chinese patients, RAS and SMAD4 mutations were significantly associated with worse survival after CRLM resection, whereas APC mutation was significantly associated with better survival. The mutational status of multiple somatic genes improved prognostic stratification of patients undergoing liver resection for CRLM and may be used in precision surgery of CRLM. Patients with RAS mutation, SMAD4 mutation, and APC wild-type status had short survival time, and it is worth investigating whether this group of patients is oncologically suitable for surgery.

List of abbreviations

CEA	carcinoembryonic antigen
CRLM	colorectal liver metastases
EGFR	epidermal growth factor receptor
HR	hazard ratio
IQR	interquartile range
MDT	multidiscipline team
OMD	oligo-metastatic disease
OS	overall survival
VEGF	vascular endothelial growth factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12957-025-03755-9.

Additional file 1: 620 cancer-related genes in the targeted panel

Additional file 2: Mutation frequency by the primary tumor site in 519 Chinese patients with colorectal liver metastases (CRLM) undergoing liver resection

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Author contributions
BCX, ML, and WJC conceived and designed the study. WJC, HWW, LJW, and DX were involved in the acquisition of data. ML and WJC analyzed the data. BCX, ML, and WJC contributed to the interpretation of data. WJC wrote the manuscript. BCX and ML revised the manuscript. All authors read and approved the final manuscript.

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Data availability
Data and materials used during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Peking University Cancer Hospital. Informed consent was obtained from all patients included in this study.

Consent for publication
Informed consent was obtained from all participants for publication.

Competing interests
The authors declare no competing interests.

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