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Sub-millimeter surgical margin is acceptable in patients with good tumor biology after liver resection for colorectal liver metastases

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ABSTRACT

Background: The definition of R1 resection in colorectal cancer liver metastases (CRLM) remains debatable. This retrospective study was conducted to clarify the impact of R1 margin on patient survival after liver resection for CRLM, taking into consideration tumor biology, including RAS status and chemotherapy response.

Methods: We retrospectively analysed the clinical and survival data of 214 CRLM patients with initially resectable liver metastases who underwent liver resection after receiving neoadjuvant chemotherapy between January 2006 and December 2016.

Results: R1 resection significantly impacted patients' overall survival (OS) and disease-free survival (DFS) in the overall patient cohort (5-year OS: 53.2% for R0 vs 38.2% for R1, P = 0.001; 5-year DFS: 26.5% for R0 vs 10.5% for R1, P = 0.002). In the RAS wild-type subgroup and respond to chemotherapy (RC) subgroup, R1 reached a similar OS to those who underwent R0 resection (RAS wild-type, P = 0.223; RC, P = 0.088). For the RAS mutated subgroup and no response to chemotherapy (NRC) subgroup, OS was significantly worse underwent R1 resection (RAS mutant, P = 0.002; NRC, P = 0.022). When considering tumor biology combining RAS and chemotherapy response status, R1 resection was only acceptable in patients with both RAS wild-type and RC (5-year OS: 66.4% for R0 vs 65.2% for R1, p = 0.884), but was significantly worse in those with either RAS mutation or NRC.

Conclusions: Tumor biology plays an important role in deciding the appropriate resection margin in patients with CRLM undergoing radical surgery. R1 resection margin is only acceptable in RAS wild-type patients who respond to chemotherapy.

Introduction

Surgical resection is the most effective treatment for colorectal cancer liver metastases (CRLM) [1,2]. With the development of effective systemic chemotherapy and surgical techniques, the indications for hepatectomy in CRLM patients have been expanding [3]. The current criteria for resection of CRLM is to completely remove the liver metastases, leaving a remnant of more than 30% liver volume to allow adequate vascular inflow and outflow to the liver [4]. Since liver resection provides the only chance for cure, more intensive surgical strategies have been applied in CRLM. A submillimeter margin is frequently technically unavoidable in patients who have multiple tumors. The proposed concept of preserving liver parenchyma (PSH) was developed in order to completely remove those tumors larger in size or closely located around major blood vessels [5,6]. Despite this proposal, the marginal width of liver resection is still controversial. In 2015, the EGOSLIM (Expert Group on OncoSurgery management of Liver Metastases) group suggested that a minimal surgical clearance margin of 1 mm is sufficient for CRLM [7]. However, several recent studies have found that submillimeter margin R1 clearance may be acceptable for CRLM patients with good biologic behavior attributed to genetic status and response to systematic chemotherapy management [8–10].

Chemotherapy response and RAS status are currently recognized as the most important factors reflecting the biologic behavior of tumors [11,12]. Neoadjuvant chemotherapy could destroy the micro-metastases around the tumor margin and prolong disease-

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free survival, besides that, the tumor response to chemotherapy is also very important in predicting patients' long-term survival [13,14]. Studies have found that in chemotherapy-responsive patients, submillimeter margins provide a similar long-term outcome compared to greater than 1 mm resection margins [10,15]. RAS status is another important factor reflecting the inherent tumor behavior. Submillimeter margins are more commonly seen in RAS mutant patients. A greater margin width or even anatomic resection may improve survival in these patients [16,17]. These facts show the impact of submillimeter margins on survival differs in CRLM patients with different biologic behavior. It is of vital important to evaluate the biologic behavior ahead so that the most appropriate surgical margin required could be known before surgery. Therefore, we designed this study to explore the minimal marginal width required for patients with different biologic behavior combining chemotherapy response and RAS status.

Materials and methods

Patient selection. Pathologically confirmed CRLM patients with initially resectable disease after neoadjuvant chemotherapy with confirmed RAS gene status who underwent liver resection between January 2006 and December 2016 in the HPB Surgery Ward I at the Beijing Cancer Hospital (Beijing, China) were identified from our prospective patient database. Patient exclusion criteria included those: (1) initially unresectable and underwent conversion therapy, (2) received anti-EGFR drugs in neoadjuvant chemotherapy; (3) underwent ablation procedures; (4) underwent palliative surgery (R2 resection), (5) underwent repeated hepatectomy due to intrahepatic recurrences, (6) lost in follow-up, (7) had double primary malignancies. All study participants provided written consent. The study design was approved by the Ethical Review Board committee of the Beijing Cancer Hospital and Institute (Beijing, China).

Study design. Marginal width was defined as the distance from the lesion to the closest transected liver surface based on final pathological analysis. The clinical pathologist involved was blinded to the other significant factors for the study. The closest marginal width was recorded as final margin when there were multiple metastases. R1 resection was defined as margin clearance less than 1 mm (<1 mm or submillimeter). R0 resection was defined as margin clearance with no tumor cells of more than 1 mm ($\geq$1 mm) [18,19]. Response to neoadjuvant chemotherapy (RC) was defined as tumor shrinkage after chemotherapy, including complete response (CR), partial response (PR) and those with tumor shrinkage within stable disease (SD); No response to neoadjuvant chemotherapy (NRC) was defined as when the tumor size increased after chemotherapy, including progressive disease (PD) and tumor progression within SD (RECIST 1.1) [20]. The tumor response was measured by the clinical radiologist and surgeon, who were blinded to the other factors significant for the study. All H & E slides of every tumor resection were reviewed for confirmation of pathological diagnosis to identify the appropriate tissue block for molecular studies. DNA was extracted from formalin-fixed paraffin-embedded tumors. Once a block was selected for KRAS mutation analysis, 10–20 consecutive sections were then made from the block. Forty-five patients had their primary tumor resected at other institutions. For these patients, we would ask them to borrow their H & E slides and formalin-fixed paraffin-embedded tumors or consecutive sections of the primary tumors from the institution where they underwent primary tumor resection for genetic testing. If the tissue cannot be borrowed from that hospitals, we would do the genetic testing using the liver metastasis tissue after hepatectomy. The quality of whole-genome amplified DNA was verified by polymerase chain reactions (PCR) using 2 control amplicons [21]. Mutations in KRAS (codons 12, 13 and 61), NRAS (codons 12, 13 and 61) were detected. Primary tumors located in the cecum, ascending colon, and transverse colon were defined as right side (RS) tumors, and those located in the splenic flexure, descending colon, sigmoid colon, and rectum were defined as left side (LS) tumors [22].

Perioperative management and follow-up. Multidisciplinary team meetings were scheduled weekly in our center for every patient with CRLM. Gadoxetic acid/contrast-enhanced MRI combined with diffusion-weighted MRI were routinely performed on CRLM patients to prevent small lesions going undetected. Radiologists assisted the identification and measurement of each tumor before and after chemotherapy. Chest and pelvic CT scans were routinely performed. Positron emission tomography-CT scans were not routinely performed unless patients were suspected as having extrahepatic disease. All patients received neoadjuvant chemotherapy, including oxaliplatin- or irinotecan-based chemotherapy (mFOLFOX6/XELOX/FOLFIRI) regimens in combination with or without targeted agents (Anti-VEGF, Bevacizumab). The time interval between the date of the last chemotherapy session and hepatic surgery was usually 2–4 weeks, extending to 6–8 weeks with the addition of bevacizumab. Contrast-enhanced CT scans or MRI, liver function tests, and measurements of carcinoembryonic antigen levels were performed 4 weeks after surgery and thereafter every 3 months. For patients with liver-limited recurrence, localized treatment, including surgical or ablation techniques, was the treatment option of choice.

Patient selection for liver resection and operative technique. For some patients, the primary tumor was resected at another institution, however the surgical treatment of all LMs was conducted at our center. LMs were considered resectable provided the following criteria were met: (1) the possibility of R0/R1 resection with a liver remnant of $\geq$30.0% and sufficient hepatic blood inflow and outflow and (2) no evidence of unresectable extrahepatic metastases [23,24]., Intra-operative ultrasound was routinely performed during the hepatectomy to detect the presence of any further as yet undetected lesions.

Statistical analyses. Continuous variables were presented as the mean and standard deviation, or the median and interquartile range. Discrete variables were presented as numbers and percentages. Categorical variables were compared using the Chi-squared test and continuous variables were compared using Student's *t*-test or a non-parametric test, as appropriate. Disease-free survival (DFS) and overall survival (OS) were calculated from the date of hepatectomy until the date of radiographic detection of recurrence, death, or the latest follow-up date. Follow-up time was calculated from the day of liver resection to death or the last follow-up date using reverse Kaplan-Meier (KM) method. Survival curves were plotted using the Kaplan-Meier method and compared by the log-rank test. Variables that were statistically significant in the univariate analysis ($p < 0.05$) were included in the multivariate analysis using a Cox proportional hazards model. All statistical analyses were conducted using Statistical Package for the Social Sciences for Windows, software version 21.0 (IBM Corp., Armonk, NY, USA). A $p < 0.05$ was considered statistically significant.

Result

Patient characteristics of study group

A total of 326 patients with initially resectable CRLM who received neoadjuvant chemotherapy underwent curative intent hepatectomy between January 2006 and December 2016 in our center. One hundred and twelve patients were excluded from the study, including: 39 who received neoadjuvant chemotherapy combined with cetuximab; 18 who underwent repeated liver surgery; 29 whose RAS status were not available; 13 who received

ablation radiofrequency in combination with surgery; 7 who underwent palliative surgery; 4 with double primary tumor and 2 lost to follow-up. Finally, a total of 214 patients were included in the study. The clinicopathologic characteristics of the cohort stratified by R0 and R1 resection are summarised in Table 1. The proportion of patients with RAS mutation were much higher in CRLM patients who underwent R1 resection than R0 resection (61.2% vs. 40.1%, P = 0.005). A higher proportion of T3/T4 primary tumors were also seen in patients who underwent R1 resection. Since only one patient was detected with BRAF V600E mutation in RS tumor, these data were not included in the table. Other clinicopathologic characteristics were comparable between the two groups (Table 1).

Survival analysis

A total of 148 patients (69.2%) suffered tumor recurrence. The median follow-up duration was 30 months. The OS and DFS were significantly better in patients who underwent R0 resection compared with R1 resection in the overall cohort, the 1-, 3-, and 5-year OS and DFS rates were 97.3%, 59.6%, 53.2% and 49.3%, 31.9%, 26.5% for R0 resection and 79.4%, 41.7%, 38.2% and 28.5%, 14.0%, 10.5% for R1 resection, respectively (OS P = 0.001, Fig. 1A; DFS P = 0.002, Fig. 1B). When the patients were stratified according to RAS status, there was no difference between OS in patients who underwent R0 resection or R1 resection in RAS wild-type patients (1-, 3-, 5- year OS: 96.6%, 61.0%, 54.4% for R0 and 85.2%, 52.0%, 46.2% for R1, P = 0.223) (Fig. 2A). On the contrary, patients underwent R0 resection showed significant better OS than R1 resection among patients with RAS mutation (1-, 3-, 5- year OS: 98.3%, 57.8%, 52.0% for R0 and 75.6%, 35.5%, 35.5% for R1, P = 0.002) (Fig. 2B). Similarly, when the patients were stratified according to tumor response to neoadjuvant chemotherapy, the OS was similar between R1 and R0 resections among patients with RC (1-, 3-, 5- year OS: 100.0%, 67.8%, 63.6% for R0 and 90.7%, 59.1%, 53.7% for R1, P = 0.088) (Fig. 2C), but significantly worse in R1 resections among patients with NRC (1-, 3-, 5- year OS: 88.6%, 32.6%, 24.4% for R0 and 60.0%, 12.8%, 12.8% for R1, P = 0.022) (Fig. 2D).

Since the impact of R1 resection on survival differs significantly in different RAS and tumor response status, we further explored the influence of R0 and R1 resections on survival, considering both these factors (Table 2). For patients with RAS wild-type and RC, there was no difference in survival for patients who underwent R0 or R1 resection (1-, 3-, 5- years OS: 100.0%, 69.5%, 66.4% for R0; 100.0%, 73.3%, 65.2% for R1, p = 0.884) (Fig. 3A). For patients with RAS mutation and RC, the OS of patients who underwent R1 was significantly worse than those who underwent R0 resection (1-, 3-, 5- years OS: 100.0%, 65.0%, 58.5% for R0 and 84.0%, 48.7%, 48.7% for R1, p = 0.043) (Fig. 3B). Similar results were also found in patients with RAS wild-type and NRC (1-, 3-, 5- years OS: 85.0%, 32.8%, 24.6% for R0 and 55.6%, 11.1%, 11.1% for R1, p = 0.024) (Fig. 3C). For patients with the worst tumor biology, with both RAS mutation and NRC, OS was also significantly worse in those who underwent R1 resection (1-, 3-, 5- years OS: 93.3%, 19.5%, 19.5% for R0 and 58.3%, 0%, 0% for R1, p = 0.022) (Fig. 3D). We then classified all patients into two groups; those with good tumor biology, including patients with RAS wild-type and RC; and the bad tumor biology group, including patients with either RAS mutation or NRC. In the uni/multivariate analysis, R1 resection was not an independent factor for OS in the good tumor biology group. On the other hand, R1 resection was significantly associated with worse OS among patients with bad tumor biology (HR 1.770; 95% CI: 1.088–2.879, P = 0.022). In addition, liver metastases ≥50 mm in size (HR 2.026; 95% CI: 1.169–3.510, P = 0.012) were also shown to be independent prognostic factors contributing to OS in patients with bad tumor biology (Table 3).

Table 1
Patient clinicopatholigical characteristics.

	R0 resection	R1 resection	P value
Age, n (%)			
<60	92 (62.6%)	43 (64.2%)	0.879
≥60	55 (37.4%)	24 (35.8%)	
Gender, n (%)			
Male	96 (65.3%)	47 (70.1%)	0.534
Female	51 (34.7%)	20 (29.9%)	
RAS status, n (%)			
Wild-type	88 (59.9%)	26 (38.8%)	**0.005**
Mutant	59 (40.1%)	41 (61.2%)	
Chemotherapy response*, n (%)			
Response	111 (75.5%)	43 (64.2%)	0.102
No response	36 (24.5%)	24 (35.8%)	
Primary tumor location, n (%)			
Left-sided	123 (83.7%)	53 (79.1%)	0.443
Right-sided	24 (16.3%)	14 (20.9%)	
Tumor size (mm) (IQR)	25 (23)	30 (20)	0.220
Tumor number (IQR)	2 (3)	3 (6)	0.072
Primary tumor N stage, n (%)			
Lymphnode negative	56 (38.1%)	23 (34.3%)	0.648
Lymphnode positive	91 (61.9%)	44 (65.7%)	
Primary tumor T stage, n (%)			
<T3	28 (19.0%)	4 (6.0%)	**0.013**
≥T3	119 (81.0%)	63 (94.0%)	
DFI, n (%)			
>12 months	18 (12.2%)	7 (10.4%)	0.821
≤12 months	129 (87.8%)	60 (89.6%)	
CEA (ng/mL) (IQR)	6.9 (27.1)	8.3 (26.7)	0.368
Combined with BEV, n (%)			
Yes	39 (26.5%)	27 (40.2%)	0.055
No	108 (73.5%)	40 (59.8%)	
Adjuvant chemotherapy*, n (%)			
Yes	107 (72.8%)	52 (77.6%)	0.503
No	40 (27.2%)	15 (22.4%)	

DFI, Disease free interval from the primary disease to liver metastases; CEA, carcinoembryonic antigen; IQR, interquartile range; Response, tumor shrinkage after chemotherapy; No response, tumor size increased after chemotherapy.
*Tumor response refers to the response to the last line chemotherapy.
Bold values indicate statistically significant differences.

Discussion

One centimeter has been recognized as the standard marginal width for CRLM resection since Ekberg et al. proposed it in 1985 [25,26]. In 2005, Pawlik et al. found that there was no different on survival between patients with 1–4 mm, 5–9 mm or >10 mm marginal width, indicating that <1 cm margin should not be a contraindication for resection [19]. Since then, a 1 mm surgical margin has been gradually recognized as the standard margin for CRLM [27,28]. The underlying mechanism of 1 mm marginal width is that micro-metastases occur predominantly within 2–4 mm around the tumor, in particular within 1 mm [29,30]. However, with the development of surgical techniques and more effective chemotherapy regimens, the 1 mm marginal standard has also been challenged in recent years. According to data from MSKCC, submillimeter margins (0.1–0.9 mm) can provide about 20% 10-year survival rate for patients with CRLM, which was significantly superior to those who underwent palliative chemotherapy [9]. Besides, submillimeter or even positive margin might be the only chance to obtain radical resection for patients with deeply located or large tumors, and it can also help to preserve liver parenchyma (PSH). Several studies have even found that the long-term survival of patients who underwent PSH is superior to those who underwent major resection, since PSH provided more opportunities for local treatment after liver-limited recurrence [6,31]. In addition, a submillimeter marginal width provides a chance to detach tumors

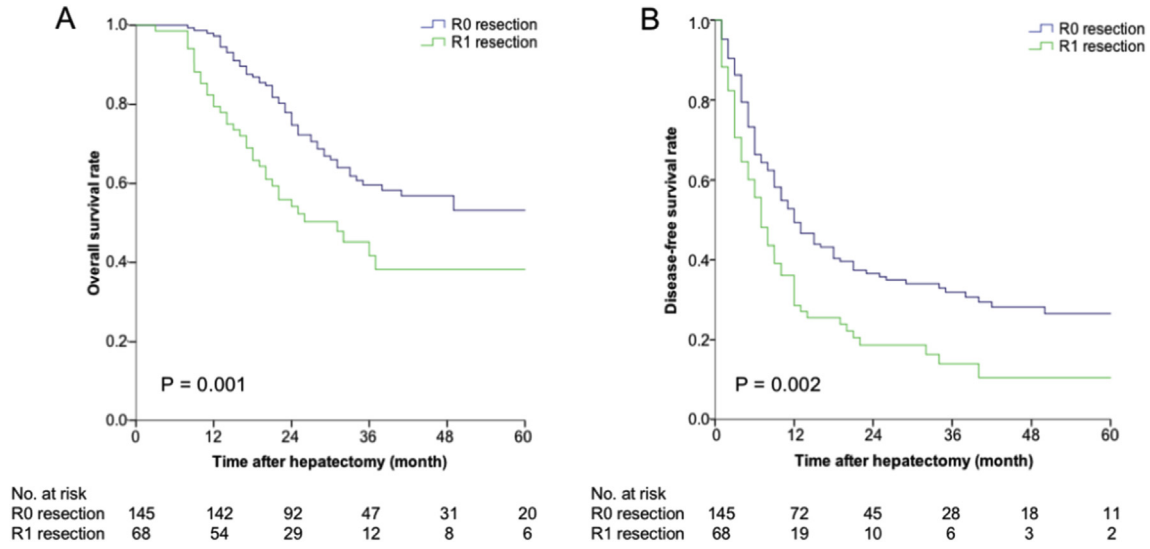

Fig. 1. (**A**) Overall survival and (**B**) disease-free survival of patients stratified by R0/R1 resection in the whole cohort (OS: P = 0.001, DFS: P = 0.002) (log-rank test).

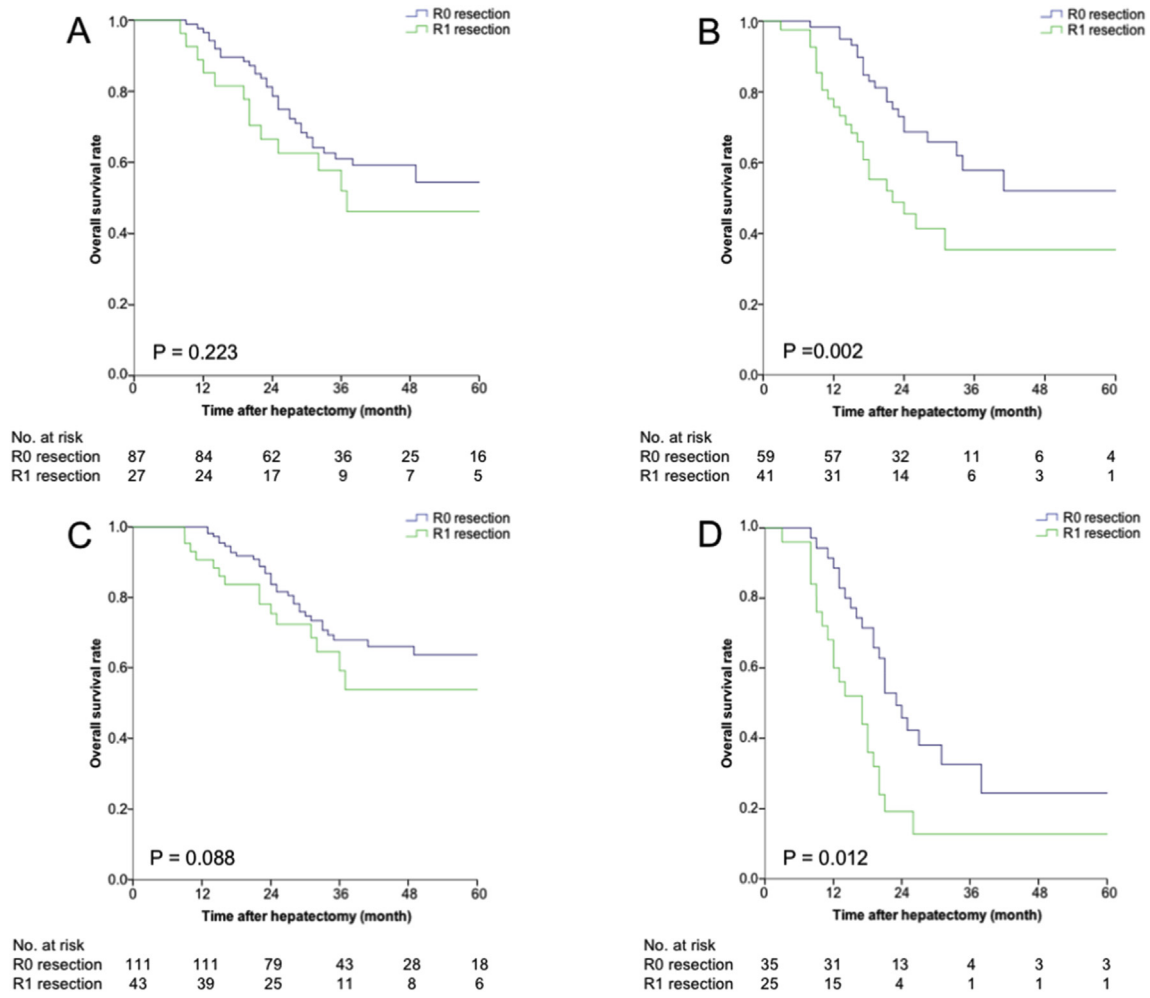

Fig. 2. Overall survival stratified by with R0/R1 resection in CRLM patients with (**A**) RAS wild-type (P = 0.223), (**B**) RAS mutant (P = 0.002), (**C**) RC (P = 0.088), (**D**) NRC (P = 0.022) (log-rank test).

Table 2
The influence of R0 and R1 resections on survival considering both RAS status and the tumor response status.

Tumor biology	RAS status	Tumor response	No. of patients	5-year OS		P value
				R0 resection	R1 resection	
Good	WT	RC	85	66.4%	65.2%	0.884
Bad	Mutation	RC	69	58.5%	48.7%	**0.043**
	WT	NRC	29	24.6%	11.1%	**0.024**
	Mutation	NRC	31	19.5%	0%	**0.022**

WT: RAS wild type; Mutation: RAS mutation; RC: response to chemotherapy, tumor shrinkage after chemotherapy; NRC: no response to chemotherapy, tumor size increased after chemotherapy.
Bold values indicate statistically significant differences.

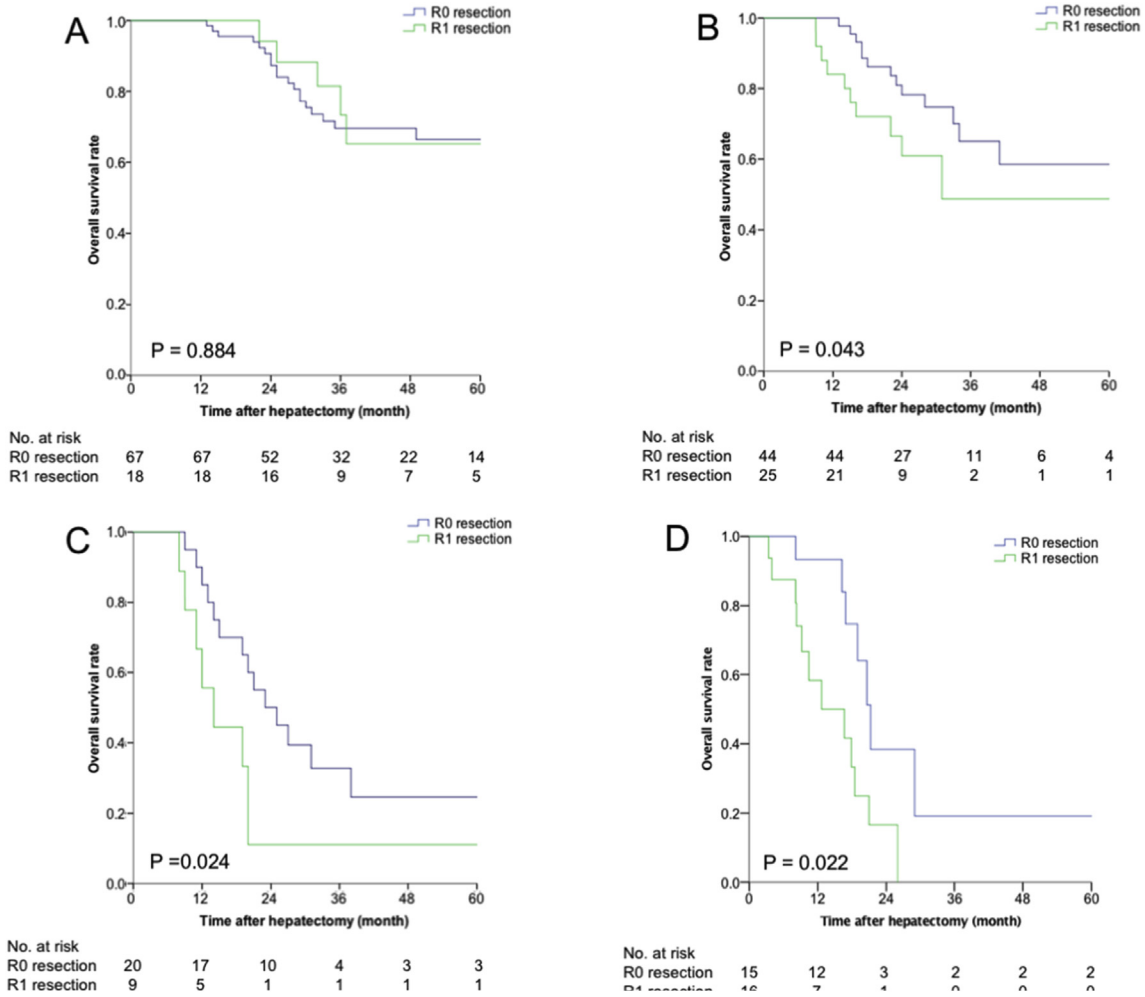

Fig. 3. Overall survival stratified by with R0/R1 resection in CRLM patients with (**A**) RAS wild-type and RC (P = 0.884), (**B**) RAS mutation and RC (P = 0.043), (**C**) RAS wild-type and NRC (P = 0.024) and (**D**) RAS mutation and NRC (P = 0.022) (log-rank test).

from intrahepatic vessels, avoiding having to resect the entire vessel along with a huge liver volume. An Italian study showed that for patients with tumors adjacent to large vessels, the long-term survival and local recurrence rate were similar between those who underwent tumor detachment from intrahepatic vessels and those who underwent major resection [32]. Several reasons were proposed to explain why submillimeter margins has been well accepted. Firstly, liver tissue outside the tumor could be removed by surgical devices such as ultrasonic scalpel and CUSA, and then sucked away by an aspirator [5,19]. Thus, the real marginal width is actually greater than 1 mm. Secondly, electrosurgical devices are now widely used to stop bleeding on the surgical margin after resection. This procedure could actually result in cauterizing coagulation necrosis about 2–3 mm from the resection margin, just as radiofrequency ablation. Therefore, even though some tumors may have insufficient margin for resection, electrosurgical devices could destroy the remaining tumor cells [33].

Over the recent years, studies have found that tumor biology rather than surgical technique dictates prognosis in colorectal cancer liver metastases [5,9,16,34]. Up to now, RAS status and chemotherapy response were considered the most important factors reflecting the biological behavior of the patients. In this study, we found that R1 resection was more commonly seen in RAS mutant patients. Brudvik et al. had similar findings, that patients

Table 3
Uni/multivariate analyses of factors associated with overall survival stratified by different tumor biology.

Prognostic factor		Good tumor biology (RAS wild-type and response to chemotherapy)			Bad tumor biology (RAS mutation or no response to chemotherapy)		
		Univariable P	Multivariable analysis HR (95% CI) P value		Univariable P	Multivariable analysis HR (95% CI) P value	
Primary T stage	T1-2/T3-4	0.745			0.773		
Primary N stage	N negative/N positive	0.212			0.124		
Liver metastases number	Single/Multiple	0.084			0.431		
Liver metastases size	<50 mm/≥50 mm	0.541			**0.001**	2.026 (1.169–3.510)	**0.012**
Primary tumor location	Left-sided/Right-sided	0.436			0.232		
CEA before hepatectomy	<200 ng/mL/≥200 ng/mL	0.842			**0.025**	1.403 (0.850–2.313)	0.185
DFI	<12 month/≥12 month	0.348			0.871		
Combined with BEV	Yes/No	0.082			0.288		
Adjuvant chemotherapy	Yes/No	**0.005**	2.904 (1.370–6.157)	**0.005**	0.228		
Resection margin	R0/R1	0.212			**0.004**	1.770 (1.088–2.879)	**0.022**

DFI, Disease free interval from the primary disease to liver metastases; CEA, carcinoembryonic antigen; BEV, Bevacizumab; CI, confidence interval; HR, hazard ratio.
Bold values indicate statistically significant differences.

with RAS mutation tended to have narrower margins. Rajaganesha et al. also found that surgical margins of patients with RAS mutation were prone to hypoxia, resulting in high expressions of HIF-1, leading to increased rates of local recurrence [35]. Radiological imaging also showed that the tumor margin of RAS mutant patients are more invasive and more susceptible to micro-metastasis [16]. Our study confirmed that surgical margins should be expanded to more than 1 mm in RAS mutant patients. Margonis et al. even believed that anatomical resection could improve the survival of RAS mutant patients [17]. Although this conclusion might be considered too aggressive, submillimeter margins is still insufficient for long term survival in RAS mutant patients [34]. Chemotherapy response is another important factor predicting survival and reflecting the biological behavior of patients with CRLM [13]. With the development of targeted agents in recent years, patients are receiving more effective chemotherapy regimens. Our study found that the survival of submillimeter margin is equivalent to more than 1 mm margin in the RC group, but was significantly worse in the NRC group. Several studies reported similar findings, that a narrow surgical margin (<1 mm) was associated with a poor prognosis in patients with suboptimal pathologic or radiologic response to chemotherapy; while the width of the surgical margin did not correlate with survival outcomes in patients showing excellent pathologic or radiologic responses [10,15,36]. The scattered tumor cells or micro-metastases around the tumor could be destroyed when there is good tumor response, which is an important indicator for a good morphological response, resulting in a sharp tumor liver interface (TNI) [37,38]. When there tumor budding remains around the metastasis, this indicates that the patients did not response well to chemotherapy and an ill-defined TNI is shown. Thus, submillimeter margins may not guarantee radical removal of micro-metastases or “budding” tumors, resulting in an increased recurrence rate [15,39,40].

In this study, we combined tumor response and RAS status to evaluate the impact of surgical margin on survival of CRLM patients with different tumor biology for the first time. Survival analysis showed that in RAS wild-type and chemotherapy-responsive patients, submillimeter margin is equivalent to more than 1 mm margin. On the other hand, the survival of patients with submillimeter margin was significantly worse in those with RAS mutation or no response to chemotherapy. Multivariate analysis also showed that the margin status was an independent risk factor only in patients with RAS mutation or no response to chemotherapy. This finding is in line with the current view, that resection margin is a reflection of tumor biology [34]. Patients with different biological behavior have different growth patterns, and therefore need different marginal status. For example, a compact growth pattern (confined by a capsule) is more commonly seen in patients with good tumor biology, and infiltrative growth patterns more commonly occur in patients with bad tumor biology [41,42]. Thus, a submillimeter marginal width can be acceptable in patients with good biological behavior. However, due to more invasive growth characteristic and higher occurrence of micro-metastasis surrounding the tumor, it would be unacceptable to perform a submillimeter margin for patients with poor biological behavior. The multivariate analysis found that adjuvant chemotherapy influences the survival independently only in patients with good tumor biology. This might be explained that for patients who were sensitive to preoperative chemotherapy, adjuvant chemotherapy could help them to obtain better tumor control and reduce the risk of tumor progression, thus gain more survival benefit than those with no response to chemotherapy. Another finding in multivariate analysis showed that tumor size was an important factor influencing survival only in patients with bad tumor biology, not in the patients with good tumor biology. Tumor size has been widely recognized as an important factor affecting patients' survival. For patients with bad tumor biology, tumor size has always been increasing. Thus the larger the tumor, the worse the prognosis. However, for patients with good response to chemotherapy, the tumor size after chemotherapy might not reflect patients' tumor condition very accurate since if we prolong the preoperative cycles, the tumor would keep shrink to smaller size. So larger tumor doesn't mean the same in response patients and in no-response patients.

There are some limitations to this study. Firstly, this retrospective study spanned across 10 years, which may lead to selection bias as more advanced surgical techniques and the concept of submillimeter margins developed more recently. But all patients were consecutively enrolled and selections were done strictly according to the aim of the study and the inclusion criteria, no special selection was made. Secondly, due to the small number within each subgroup, the results of survival may be biased when performing subgroup analysis. Future studies with a larger sample size is needed to further examine the impact of margin status in these patients. Thirdly, patients with unresectable disease who underwent conversion therapy were excluded from this study. These patients are more prone to have a heavier tumor burden and more effective tumor response, which will affect the survival of the chemotherapy-effective group, thus only patients with initially resectable disease were included. Fourthly, as anti-EGFR therapy is highly effective and could only be used in RAS wild-type patients, to unify the chemotherapy regime and balance the benefit of

chemotherapy in RAS wild-type and RAS mutant patients, we excluded patients receiving cetuximab to remove the bias from highly effective and ineffective cases on surgical margin. Lastly, we didn't include information on macrovascular invasion, thus there might be bias of vascular invasion between the 2 groups. But a previous study has found that those who underwent tumor detachment from intrahepatic vessels have similar survival outcome compared with those underwent major resection [32]. Thus it might have little impact on the result.

In conclusion, surgical resection margin has a variable impact on survival after resection of CRLM, based on differing tumor biology. Sub-millimeter margins are acceptable in RAS wild-type and chemotherapy-responsive patients, offering similar long-term survival to patients with more than 1 mm resection margin. However, in patients with either a RAS mutation or no response to chemotherapy, prognosis is significantly worse in those with sub-millimeter margins. A greater than 1 mm resection marginal width should be applied to patients with bad tumor biology, however, the most appropriate resection width for these patients is still inconclusive and needs further study.

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