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RAS mutation status predicts survival and patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases

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Abstract

Objective—To determine the impact of *RAS* mutation status on survival and patterns of recurrence in patients undergoing curative resection of colorectal liver metastases (CLM) after preoperative modern chemotherapy.

Summary Background Data—*RAS* mutation has been reported to be associated with aggressive tumor biology. However, the effect of *RAS* mutation on survival and patterns of recurrence after resection of CLM remains unclear.

Methods—Somatic mutations were analyzed using mass spectroscopy in 193 patients who underwent single-regimen modern chemotherapy before resection of CLM. The relationship between *RAS* mutation status and survival outcomes was investigated.

Results—Detected somatic mutations included *RAS* (*KRAS/NRAS*) in 34 patients (18%), *PIK3CA* in 13 (7%), and *BRAF* in 2 (1%). At a median follow-up of 33 months, 3-year overall survival (OS) rates were 81% in patients with wild-type vs 52.2% in patients with mutant *RAS* (P=0.002); 3-year recurrence-free survival (RFS) rates were 33.5% with wild-type vs 13.5% with mutant *RAS* (P=0.001). Liver and lung recurrences were observed in 89 and 83 patients, respectively. Patients with *RAS* mutation had a lower 3-year lung RFS rate (34.6% vs 59.3%, P<0.001), but not a lower 3-year liver RFS rate (43.8% vs 50.2%, P=0.181). In multivariate

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analyses, *RAS* mutation predicted worse OS (hazard ratio [HR] 2.3, *P*=0.002), overall RFS (HR 1.9, *P*=0.005), and lung RFS (HR 2.0, *P*=0.01), but not liver RFS (*P*=0.181).

Conclusions—*RAS* mutation predicts early lung recurrence and worse survival after curative resection of CLM. This information may be used to individualize systemic and local tumor-directed therapies and follow-up strategies.

INTRODUCTION

For patients with colorectal liver metastases (CLM), hepatic resection combined with systemic therapy has been reported to be the most effective treatment in terms of improving survival.[1] To date, various prognostic scoring systems have been proposed to stratify patient prognosis after resection of CLM.[2–4] However, in the era of modern systemic therapy, these conventional scoring systems are becoming less relevant because of inconsistent predictive power and lack of reproducibility due to selection bias.[5]

Preoperative systemic therapy has increasingly been used as part of a multidisciplinary approach for patients with CLM to test the biologic aggressiveness of the tumor and select optimal candidates for surgery.[6] Our group has reported that pathologic response[7–9] and radiologic response[10, 11] to preoperative chemotherapy are powerful predictors of long-term outcomes for patients with CLM. However, in patients with CLM, there is strong variability in clinical presentation, biologic aggressiveness, and patterns of treatment failure, and no biomarker predicts these phenotypic differences.

During the past decade, mutation status of *RAS* family genes (predominantly *KRAS* and *NRAS*) has been shown to correlate with the effectiveness of anti-EGFR agents against unresectable metastatic colorectal cancer,[12, 13] and a possible prognostic role of these somatic gene mutations after resection of CLM in an era predating the use of preoperative chemotherapy has been reported.[14] Our group has recently reported that metachronous CLM detected after modern chemotherapy for the primary colorectal tumor are associated with a higher incidence of somatic gene mutations and worse survival.[15] In addition, while recently reported studies have indicated a per-patient concordance of mutation type between primary tumor and metastases, a higher rate of mutation has been identified in patients with metastases at particular sites (e.g., peritoneum, lung and brain metastases), suggesting that tumors with mutations have a propensity to metastasize to the lungs and brain.[16–19]

On the basis of these clinical findings, we hypothesized that somatic mutation status predicts survival outcomes and types of recurrence after curative resection of CLM. In this study, we investigated the impact of *RAS* mutation status on survival and patterns of recurrence in patients who underwent curative resection of CLM after preoperative modern chemotherapy.

PATIENTS AND METHODS

Study Population

The Institutional Review Board of The University of Texas MD Anderson Cancer Center approved this study protocol (PA11-0653). The prospectively maintained liver resections database of the Department of Surgical Oncology was queried to identify all patients who underwent liver resection for CLM during the period from November 1997 through October 2011. We studied patients undergoing curative hepatectomy without concomitant radiofrequency ablation. Patients with a history of previous treatment for metastatic disease (chemotherapy, radiofrequency ablation, or resection) were excluded.

All patients included in this study received preoperative oxaliplatin- or irinotecan-based chemotherapy including the anti-VEGF agent bevacizumab. Patients who received anti-

EGFR agents were excluded. Patients who received 2 or more regimens of preoperative chemotherapy because of disease progression during first-line chemotherapy were excluded from the current study, as were patients who died within 90 days after hepatectomy and patients who had less than 5% viable tumor cells in the CLM specimen.

Preoperative, Intraoperative, and Postoperative Management

Before operation, all patients underwent a medical history, physical examination, laboratory evaluation, and imaging studies, including helical computed tomography of the chest, abdomen, and pelvis with a triphasic liver protocol. In selected patients, fluorodeoxyglucose positron emission tomography was used to rule out extrahepatic disease and confirm the metastatic nature of atypical lesions. Only patients with hepatic and extrahepatic disease amenable to complete and safe resection were considered for hepatectomy. In patients with an anticipated insufficient future liver remnant, preoperative portal vein embolization was used to induce hypertrophy.

During laparotomy, intraoperative sonography of the liver was performed to confirm the location of known CLM and their relation to the portal pedicles or the hepatic veins and to rule out the presence of previously undetected CLM. Parenchymal transection was carried out under total or selective hepatic inflow occlusion using the Cavitron Ultrasonic Surgical Aspirator (Valleylab, Boulder, CO) and saline-linked cautery (dissecting sealer DS 3.0, Tissuelink Medical, Inc, Dover, NH) as reported previously.[20]

All specimens were subjected to histologic evaluation to confirm the diagnosis of metastatic colorectal cancer and determine the width of the tumor-free surgical margin. The degree of pathologic response of CLM to preoperative chemotherapy was defined according to the percentage of the CLM tumor surface area composed of viable tumor cells: major pathologic response was defined < 50% viable cells and minor pathologic response ≥ 50% viable cells.[7] After surgery, chemotherapy was usually reintroduced to complete a total of 12 cycles including both preoperative and postoperative chemotherapy. Patients were reassessed every 4 months after completion of the second stage of liver resection. Radiological evidence or positive biopsy was required to confirm recurrence, and time and site of relapse were systematically recorded. Further treatment was decided according to the findings at reassessment.

DNA Extraction and Somatic Gene Mutation Profiling

Hematoxylin-eosin-stained slides from all CLM were reviewed by a gastrointestinal pathologist (DMM). Tumor viability in the CLM specimens was checked in order to exclude specimens with tumor viability less than 5%. Areas with maximum amount of available tumor were selected for macrodissection. Tumor tissue was scraped from the glass slides under direct visualization or under a dissecting microscope, and DNA was extracted from tumor tissue using a QIAmp DNA Mini Kit (Qiagen, Valencia, CA).

Somatic gene mutations were assessed using mass spectrometry. DNA extracted from formalin-fixed, paraffin-embedded resected CLM was quantified and analyzed with Sequenom MassARRAY technology (Sequenom, Inc., San Diego, CA).[21] Sequenom's MassARRAY system utilizes polymerase chain reaction amplification and single-base primer extension for mutation detection.[22–24] The MassARRAY system offers a highly effective method for profiling hundreds of somatic mutations in parallel. A high-throughput analysis of 159 point mutations in 33 genes commonly involved in solid tumors was performed in MD Anderson's Characterized Cell Line Core Facility. The genes tested for this study were *AKT1, AKT2, AKT3, ALK, BRAF, CDK4, CTNNB1, DEAR1, EGFR, ERA, FRAP, GNAS, HIF1A, IDH1, IDH2, IGFR1R, JAK2, KIT, KRAS, MEK1, MET, NRAS,*

PDGFRA, *PDPK1*, *PHLPP2*, *PIK3CA*, *PIK3R1*, *PRKAG1*, *PRKAG2*, *RET*, *RICTOR*, *STK11*, and *TNK2*.

Statistical Analysis

Qualitative variables were expressed as frequencies. Patients were stratified according to *KRAS* or *NRAS* mutation status into 2 groups: mutant *RAS* and wild-type *RAS*. Clinicopathological features were compared between these 2 groups using chi-square or Fisher's exact tests, as appropriate. Overall survival (OS), overall recurrence-free survival (RFS), lung RFS, and liver RFS were calculated using the Kaplan–Meier method from the date of liver resection to the date of death, first recurrence at any site, lung recurrence, or liver recurrence, respectively. Patients without an event during the follow-up period were censored at the date of last follow-up. These survival outcomes were compared using the log-rank test.

To identify factors associated with OS and RFS in the entire study cohort, we evaluated the following clinicopathologic variables in a univariate analysis: disease-free interval after the primary tumor diagnosis (<12 months vs ≥12 months), primary tumor location (rectum vs colon), regional lymph node status of the primary tumor (positive vs negative), *RAS* mutation status (mutant vs wild-type), number of cycles of preoperative chemotherapy for CLM (>6 vs ≤6), pathologic response to preoperative chemotherapy (major vs minor), number of CLM in the pathologic specimen (multiple vs solitary), diameter of the largest of the CLM in the pathologic specimen (>5 vs ≤5 cm), major postoperative complications were defined as complications of grade 3 or higher (necessitating a surgical, endoscopic or radiological procedure) in the Dindo classification[25], and liver resection margin status on microscopic analysis (positive vs negative),.

All variables associated with OS, overall RFS, lung RFS, and liver RFS with $P<0.1$ in the univariate proportional hazards models were entered into a Cox multivariate regression model with backward elimination. $P<0.05$ was considered statistically significant. Statistical analyses were performed using the software IBM SPSS Statistics, version 19 (IBM, Armonk, NY).

RESULTS

Among 1406 consecutive patients treated for CLM at MD Anderson during the study period, 621 were excluded because of concomitant radiofrequency ablation or nonreceipt of preoperative chemotherapy. An additional 497 patients were excluded because they received multiple lines of preoperative chemotherapy, did not receive bevacizumab, received anti-EGFR agents before or after liver resection, had less than 5% viable tumor cells in the specimen, died within 90 days after surgery, or underwent a noncurative hepatectomy. Among the 288 patients eligible for the genetic testing, 95 patients were excluded because they did not have available paraffin blocks or had insufficient DNA for genetic analysis. The remaining 193 patients were studied in detail (Figure 1).

Somatic Gene Mutation Status

Of the 193 patients included in the study, 43 (22.3%) had one or more somatic mutations in tested genes. Thirty-four patients (17.6%) had *RAS* mutations (27 *KRAS* and 7 *NRAS*), 13 patients (6.7%) had *PIK3CA* mutations, 2 patients (1%) had *BRAF* mutations, and 2 patients (1%) had rare mutations—one had a *CTNNB1* mutation, and the other had an *AKT1* mutation. Among the 34 patients with *RAS* mutations, 29 (85%) exhibited a mutation at codon 12 (nucleotide changes: G→A in 17 patients, G→T in 8 patients, and G→C in 4 patients), 3 (9%) exhibited a mutation at codon 61 (A→G in 1 patient, A→C in 1 patient,

and C→G in 1 patient), and 2 (6%) exhibited a mutation at codon 13 (G→A in both of them). Among the 13 patients with *PIK3CA* mutations, 6 (46%) exhibited a mutation at codon 20 and 7 (54%) at codon 9.

Patient Characteristics by *RAS* Mutation Status

Clinicopathologic characteristics by *RAS* mutation status are shown in Table 1. Patients with *RAS* mutation had a lower rate of major pathologic response (< 50% viable tumor cells) than patients with wild-type *RAS* (38.2% vs 58.5%; *P*=0.037). The remaining characteristics did not differ significantly between patients with mutant and wild-type *RAS*.

Long-term Survival and Predictors of Outcomes

At a median follow-up time of 33 months, 51 patients had died (48 of cancer and 3 of other causes), 54 were alive with disease recurrence, and 88 were alive with no evidence of disease at last follow-up. For the entire cohort, 3- and 5-year RFS rates were 29.9% and 26.9%, respectively, and 3- and 5-year OS rates were 76.4% and 61.8%, respectively. Patients with mutant *RAS* had worse long-term outcomes than those with wild-type *RAS* (3-year RFS: 13.5% vs 33.5%, *P*=0.001; 3-year OS: 52.2% vs 81%, *P*=0.002) (Figure 3).

In multivariate analysis, independent risk factors of worse RFS were *RAS* mutation (hazard ratio [HR], 1.9; 95% CI, 1.2–3.0; *P*=0.005) and minor pathologic response to preoperative chemotherapy (HR, 2.0; 95% CI, 1.4–3.0; *P*<0.001) (Table 2). Independent risk factors of worse OS also were *RAS* mutation (HR, 2.3; 95% CI, 1.1–4.5; *P*=0.002) and minor pathologic response (HR, 2.1; 95% CI, 1.1–4.0; *P*=0.022) (Table 3).

Patterns of Recurrence and Predictors of Recurrence Pattern

Of the 126 patients who had tumor recurrence during the follow-up period, 83 patients had a lung recurrence and 89 patients had a liver recurrence. Patients with mutant *RAS* had worse 3-year lung-RFS than those with wild-type RAS (34.6% vs 59.3%, *P*<0.001) (Figure 4A, Table 4). In contrast, 3-year liver-RFS was not influenced by RAS mutation (43.8% for mutant *RAS* vs 50.2% for wild-type *RAS*, *P*=0.181) (Figure 4B, table 4). At the last follow-up, lung recurrence was observed in 64.7% of patients with *RAS* mutation (22/34) versus 38.3% of patients with wild-type *RAS* (61/159) (*P*=0.005). The incidence of liver recurrence did not correlate significantly with *RAS* mutation status (44.7% of patients with *RAS* mutation vs 52.9% of those with wild-type *RAS*; *P*=0.379).

Multivariate analysis indicated that *RAS* mutation (HR, 2.0; 95% CI, 1.1–3.4; *P*=0.01) and minor pathologic response (HR, 1.9; 95% CI, 1.1–3.0; *P*=0.009) were independent predictors of lung RFS, while minor pathologic response was the only independent predictor of liver RFS (HR, 2.2; 95% CI, 1.4–3.5; *P*=0.001) (Tables 4 and 5).

DISCUSSION

In this study, we analyzed the prognostic impact of *RAS* mutation status in 193 patients who underwent curative resection of CLM after single-regimen modern systemic therapy. Consistent with previous oncogene profiling studies for primary colorectal cancers, *RAS*, *BRAF*, and *PIK3CA* were identified as the most common point-mutated genes in CLM.[23, 26] Our analysis indicates that *RAS* mutation status is an independent predictor of OS, overall RFS, and lung RFS, but not liver RFS after resection of CLM. The current study also confirms our previous study indicating the pre-eminence of response to chemotherapy as a dynamic biological predictor outcome superior to traditional clinical pathological predictors such as number of liver metastases, size of liver metastases, lymph node status of primary tumor or surgical margins.[7, 27] Over the past decade, the prognostic role of

somatic mutations has been actively investigated, especially in primary colorectal cancer. The most common somatic gene mutations reported in previous studies were *KRAS, NRAS, PIK3CA*, and *BRAF*, with mutation rates around 40%, 2.6%, 14.5%, and 4.7%, respectively.[13] The previous studies of molecular alterations in colorectal cancer included patients with advanced and unresectable metastatic disease. The current study indicates that *KRAS* mutation rates are lower in patients selected to undergo curative resection of CLM than in medical series reporting on *KRAS* mutation in patients with advanced metastatic colorectal cancer not amenable to curative resection. This suggests that among patients affected by metastatic colorectal cancer, those amenable to curative resection of CLM represent a preselected population with better tumor biology and longer survival. This hypothesis is supported by the fact that the only study reporting on rates of *KRAS* mutations indicated a 25% rate of mutations in patients undergoing resection after receiving preoperative modern chemotherapy.[28]

In this study, we analyzed the mutation status of multiple somatic genes and found that of 43 patients with at least one somatic gene mutation, the majority had a *RAS* mutation (n=34), while only a small minority had mutations of *PIK3CA* (n=5), *BRAF* (n=2), *CTTNB1* (n=1), and *AKT1* (n=1). This finding confirms the dominance of *RAS* mutations in colorectal cancer and suggests that the less common mutations (of *PIK3CA, BRAF, CTTNB1*, and *AKT1*) are unlikely to contribute significantly to future overall outcome analyses of patients undergoing resection of CLM. However, it is still possible that, in future studies, these rare mutations could help to predict outcomes of specific subsets of patients with poor prognosis or specific metastatic patterns.

Only a few prior studies[16–18] have focused on mutation rates and recurrence patterns in patients with colorectal cancer. Tie et al[16] reported higher rates of *RAS* mutations in colorectal lung metastases than in CLM. In the current study, we specifically looked at patterns of recurrence following hepatectomy. The results indicate that, compared to wild-type *RAS, RAS* mutation is associated with a shorter 3-year lung RFS rate (34.6% vs 59.3%, $P<0.001$), but not with a shorter liver RFS rate (43.8% vs 50.2%, $P=0.181$) (Figure 4). These results suggest a propensity for *RAS*-mutated tumors to metastasize to lungs and are in line with the results of previous studies that have shown higher *KRAS* mutation rates in lung (62%) and brain (57%) metastases from colorectal cancer than in CLM (32%).[16, 17] This discordance in mutation rates suggests the possibility that *KRAS*-mutant tumors are biologically versatile and able to grow in different visceral organs and have higher capacity for systemic vascular (as opposed to portal vascular) tumor spread. These findings argue in favor of studying molecular heterogeneity and differences in biologic interaction between colon cancer tumor cells and host organ factors at different metastatic sites, in in vitro and in vivo models, comparing *KRAS*-mutant and *KRAS*-wild-type colorectal cancer.

In a previous study, indeterminate lung nodules were reported in 43% of patients undergoing chest computed tomography before resection of CLM. Only 35% of indeterminate nodules proved to be lung metastases, and their presence was not associated with worse survival, leading the authors to conclude that the presence of indeterminate lung nodules should not preclude resection of CLM.[29] The findings from our current study, while supporting the role of preoperative chest computed tomography before resection of CLM, suggest that nonspecific lung nodules in patients with *RAS* mutations may be more likely to represent metastatic disease. This information may help physicians select, among patients with multiple and bilobar CLM requiring extensive resection, those who may benefit from an aggressive surgical approach. In addition, our data favor the use of chest computed tomography surveillance after resection of CLM in patients harboring a *RAS* mutation.

The *RAS* mutation rate in the current study (18%) is lower than the 47% *RAS* mutation rate reported in our previous study in patients undergoing resection of metachronous CLM.[15] We interpret the findings of our previous study as indicating that adjuvant chemotherapy for 6 months after resection of the primary tumor resulted in a selection pressure favoring the onset of metachronous liver metastases enriched for *KRAS* mutations and prevented metastases in a number of patients with primary tumors with wild-type *KRAS* - similar to the phenomenon by which antibiotic therapy can select for treatment-resistant bacteria. These interpretations are consistent with findings from other studies indicating that adjuvant FOLFOX for the primary tumor does not cause mutations in CLM.[30, 31] and that the mutation types remain concordant between the primary tumor and CLM in more than 90% of patients when the primary and the metastases are compared.[32–36]

In contrast with our previous study, our current work focused on patients who underwent single-regimen preoperative modern chemotherapy for 2–3 months and excluded patients who had received multiple lines of chemotherapy. Therefore, the current study focused on a clinically preselected population of patients with favorable tumor biology accounting for the low mutation rate. In addition, the analysis of clinical and pathological differences between patients with mutant *RAS* and those with wild-type *RAS* indicated similar median numbers of chemotherapy cycles in the two groups (Table 1). This data supports the concept that preoperative chemotherapy for liver metastases does not affect the *RAS* mutation rate.[37] Taken together, these mutational data do not argue against the use of modern chemotherapy as adjuvant therapy for primary tumors or neoadjuvant therapy for liver metastases.

The limitations of this study include its retrospective nature and the selected patient population due to limited availability of specimens suitable for genetic analysis. However, the present analysis was based on a patient population with similar pathologic and clinical characteristics, and *RAS* mutation status well stratified patients with respect to prognosis and patterns of recurrence, even after exclusion of patients with very good pathologic response to chemotherapy (% of residual tumor cells < 5%), in whom genetic profiling was impossible due to very low proportion of residual viable tumor cells in the specimen and consequent insufficient DNA for genetic analysis.

In conclusion, *RAS* mutation status is a powerful predictor of OS, RFS, and lung recurrence after curative resection of CLM. These data indicate that the genetic profile of CLM can be used to improve selection of patients with CLM for surgery and predict outcome of patients with CLM. In addition, the finding of a higher rate of pathologic response in patients with wild-type *RAS* sets the stage for further studies focusing on somatic gene mutations and pattern of response associated with preoperative chemotherapy.

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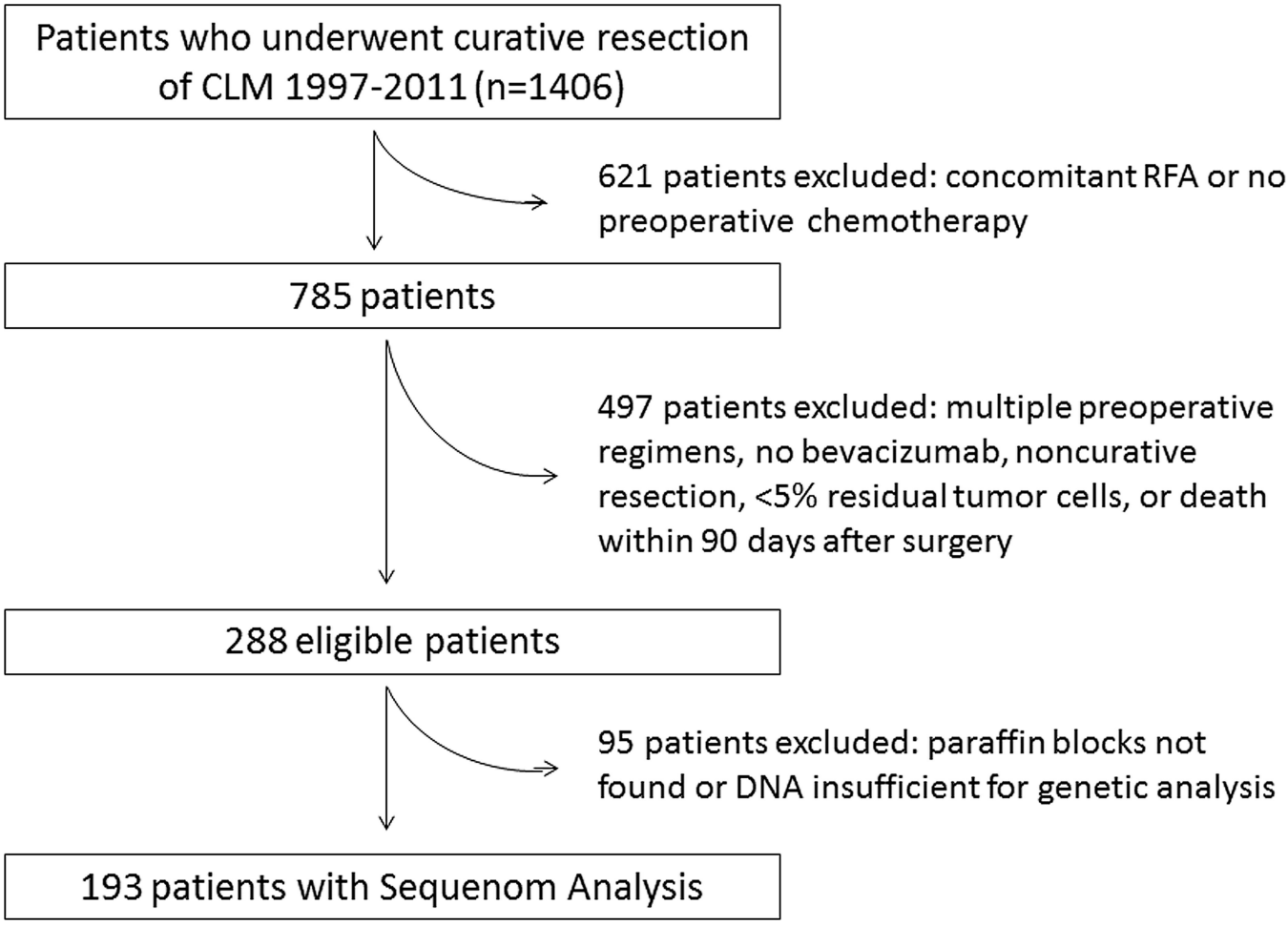

FIGURE 1.
Selection of study population.

Mutational status	n (%)
Study cohort	193 (100)
(*K* or *N*) *RAS* + /- *PIK3CA* + /- *BRAF*	43 (22.3)
(*K* or *N*) *RAS*	34 (17.6)
PIK3CA	13 (6.7)
BRAF	2 (1)
Single rare mutations (*CTNNB1* (1), *AKT1* (1))	2 (1)

FIGURE 2.
Somatic gene mutations.

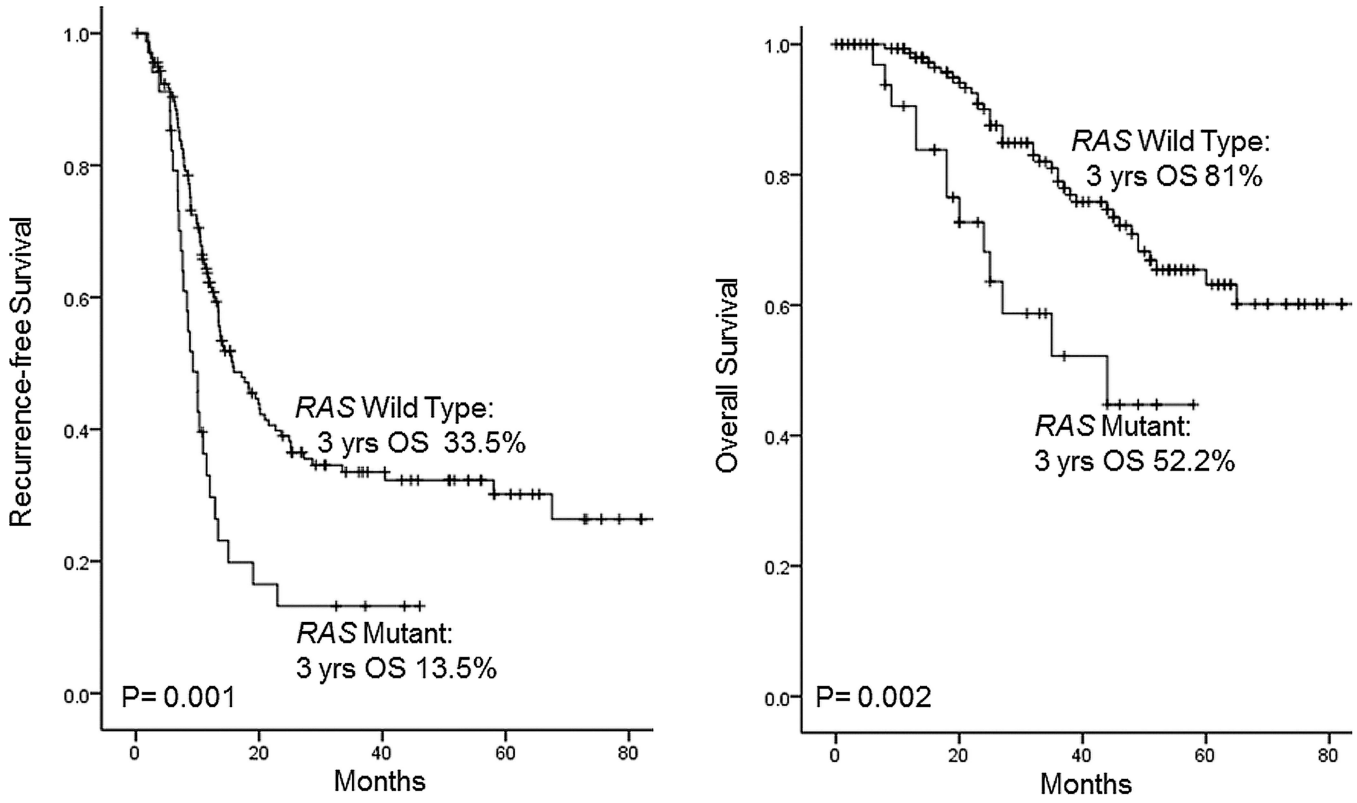

FIGURE 3.
Overall survival (OS) (A) and recurrence-free survival (RFS) (B) according to *RAS* mutation status.

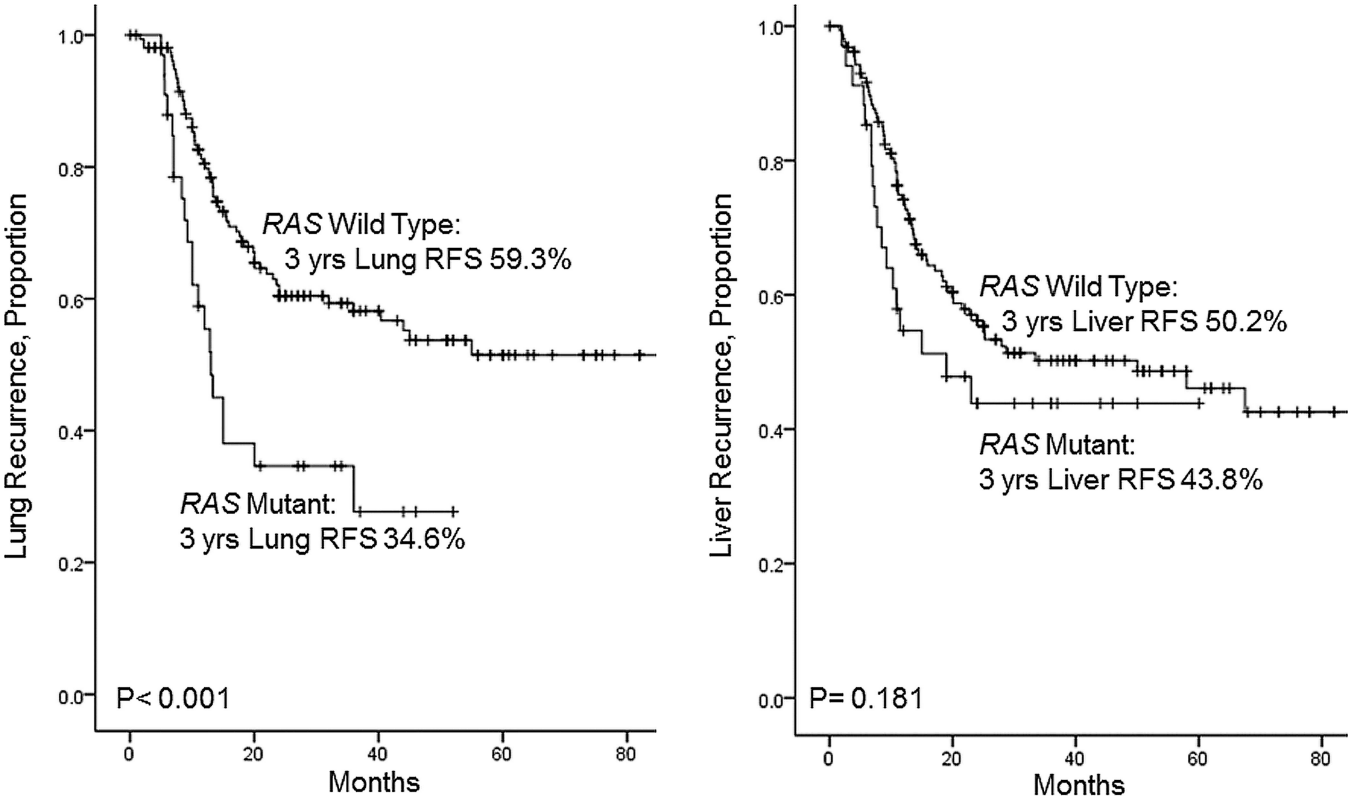

FIGURE 4.
Lung (A) and liver (B) recurrence-free survival (RFS) according to *RAS* mutation status.

Table 1

Clinicopathologic Characteristics by *RAS* Mutation Status

Characteristic	Wild-type *RAS* (n=159)	Mutant*RAS* (n=34)	Univariate Analysis *P* Value
Disease-free interval <12 months, no. (%) (n=130)	107 (67.3)	23 (67.6)	0.968
Rectal primary tumor, no. (%) (n=39)	32 (20.1)	7 (20.6)	0.951
Primary tumor positive nodal status, no. (%) (n=133)	107 (67.3)	26 (76.5)	0.294
No. of cycles of preoperative chemotherapy, median (range)	6 (2–23)	6 (3–24)	0.345
% viable tumor cells <50%, no. (%) (n=106)	93 (58.5)	13 (38.2)	0.037
No. of CLM, median (range)	2 (1–80)	3 (1–18)	0.569
Diameter of largest of CLM, mm, median (range)	25 (5–150)	22.5 (5–100)	0.484

CLM indicates colorectal liver metastases.

Table 2

Univariate and Multivariate Analysis of Factors Associated with Recurrence-free Survival (RFS) in 193 Patients Who Underwent Somatic Gene Mutation Analysis

Factor	3-year RFS (%)	5-year RFS (%)	Univariate Analysis *P* Value	Multivariate Analysis	
				P Value	Hazard Ratio (95% CI)
Disease-free interval[a]					
<12 months (n=130)	27.8	22.4	0.147		
≥12 months (n=63)	34.2	34.2			
Primary tumor location					
Rectum (n=39)	16.7	16.7	0.063	**NS**	
Colon (n=154)	32.5	26.7			
Primary tumor nodal status					
Positive (n=133)	24.6	24.6	0.067	**NS**	
Negative (n=60)	42	33.5			
RAS mutation status					
Mutant (n=34)	13.5	-	**0.001**	**0.005**	**1.92 (1.21 – 3.03)**
Wild-type (n=159)	33.5	32.1			
No. of cycles of preoperative chemotherapy					
>6 (n=65)	21.5	16.6	**0.027**	**NS**	
≤6 (n=128)	34.5	27.6			
Pathologic response					
%VTC ≥50% (n=87)	13.5	13.5	**<0.001**	**<0.001**	**2.02 (1.36 – 2.99)**
%VTC <50% (n=106)	33.5	30.5			
No. of CLM					
Multiple (n=125)	24.9	23.4	**0.007**	**NS**	
Single (n=68)	41.3	36.1			
Diameter of largest of CLM					
>5 cm (n=24)	25.2	16.8	0.240		
≤5 cm (n=169)	30.7	28.3			
Major complication					
Yes (n=32)	16.7	11.1	0.085	**NS**	

Factor	3-year RFS (%)	5-year RFS (%)	Univariate Analysis *P* Value	Multivariate Analysis	
				P Value	Hazard Ratio (95% CI)
No (n=161)	32.8	28.6			
Surgical margin					
Poitive (n=19)	24.6	24.6	0.833		
Negative (n=174)	30.1	26.7			

CLM indicates colorectal liver metastases; NS, not significant; %VTC, percentage viable tumor cells.

[a] From diagnosis of primary tumor to diagnosis of CLM.

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Table 3

Univariate and Multivariate Analysis of Factors Associated with Overall Survival (OS) in 193 Patients Who Underwent Somatic Gene Mutation Analysis

Factor	3-year OS (%)	5-year OS (%)	Univariate Analysis *P* Value	Multivariate Analysis	
				P Value	Hazard Ratio (95% CI)
Disease-free interval[a]					
<12 months (n=130)	72.7	58.3	0.197		
≥12 months (n=63)	84	69			
Primary tumor location					
Rectum (n=39)	71.8	54.2	0.611		
Colon (n=154)	77.2	63.4			
Primary tumor nodal status					
Positive (n=133)	72.5	53	**0.004**	**NS**	
Negative (n=60)	85	81.3			
RAS mutation status					
Mutant (n=34)	52.2	44.7	**0.002**	**0.002**	**2.26 (1.13 – 4.51)**
Wild-type (n=159)	81	65.4			
No. of cycles of preoperative chemotherapy					
>6 (n=65)	65.5	51.2	**0.017**	**NS**	
≤6 (n=128)	83.2	69			
Pathologic response					
%VTC ≥50% (n=87)	65.2	45.2	**<0.001**	**0.022**	**2.10 (1.11 – 3.97)**
%VTC <50% (n=106)	86.1	64.5			
No. of CLM					
Multiple (n=125)	73	58.4	0.160		
Single (n=68)	82.8	67.8			
Diameter of largest of CLM					
>5 cm (n=24)	58.9	46.4	**0.030**	**NS**	
≤5 cm (n=169)	79.1	64.3			
Major complication					
Yes (n=32)	64.1	53	0.108		
No (n=161)	77.6	62			

Factor	3-year OS (%)	5-year OS (%)	Univariate Analysis *P* Value	Multivariate Analysis	
				P Value	Hazard Ratio (95% CI)
Surgical margin					
Negative (n=174)	75.1	59.1	0.312		
Positive (n=19)	86.5	86.5			

CLM indicates colorectal liver metastases; NS, not significant; %VTC, percentage viable tumor cells.

[a] From diagnosis of primary tumor to diagnosis of CLM.

Table 4

Univariate and Multivariate Analysis of Factors Associated with Lung Recurrence after Resection of CLM

Factor	3-year Lung RFS (%)	Univariate Analysis *P* Value	Multivariate Analysis	
			***P* Value**	**Hazard Ratio (95% CI)**
Disease-free interval[a]				
<12 months (n=130)	52.7	0.680		
≥12 months (n=63)	59.7			
Primary tumor location				
Rectum (n=39)	34.3	**0.016**	0.069	
Colon (n=154)	59.4			
Primary tumor nodal status				
Positive (n=133)	52.8	0.511		
Negative (n=60)	59.7			
RAS mutation status				
Mutant (n=34)	34.6	**<0.001**	**0.01**	**2.01 (1.20 – 3.41)**
Wild-type (n=159)	59.3			
No. of cycles of preoperative chemotherapy				
>6 (n=65)	54.6	0.976		
≤6 (n=128)	55.9			
Pathologic response				
%VTC ≥50% (n=87)	39.7	**0.001**	**0.009**	**1.91 (1.17 – 3.10)**
%VTC <50% (n=106)	64.5			
No. of CLM				
Multiple (n=125)	50.3	0.062	**NS**	
Single (n=68)	66			
Diameter of largest of CLM				
>5 cm (n=24)	45.5	**0.04**	**NS**	
≥5 cm (n=169)	56.4			
Major complication				
Yes (n=32)	34.2	0.164		
No (n=161)	57.7			
Surgical margin				
Positive (n=19)	81.3	0.295		
Negative (n=174)	73.1			

CLM indicates colorectal liver metastases; NS, not significant; RFS, recurrence-free survival; %VTC, percentage viable tumor cells.

[a]From diagnosis of primary tumor to diagnosis of CLM.

Table 5

Univariate and Multivariate Analysis of Factors Associated with Liver Recurrence after Resection of CLM

Factor	3-year Liver RFS (%)	Univariate Analysis *P* Value	Multivariate Analysis	
			***P* Value**	**Hazard Ratio (95% CI)**
Disease-free interval[a]				
<12 months (n=130)	45	**0.016**	NS	
≥12 months (n=63)	58.4			
Primary tumor location				
Rectum (n=39)	44.3	0.533		
Colon (n=154)	50.3			
Primary tumor nodal status				
Positive (n=133)	42.3	**0.018**	**0.072**	**1.64 (0.96 – 2.83)**
Negative (n=60)	64.6			
RAS mutation status				
Wild-type (n=159)	43.8	0.181		
Mutant (n=34)	50.2			
No. of cycles of preoperative chemotherapy				
>6 (n=65)	43.2	0.164		
≤6 (n=128)	52.6			
Pathologic response				
%VTC ≥50% (n=87)	33.1	**<0.001**	**0.001**	**2.22 (1.41 – 3.52)**
%VTC <50% (n=106)	58.8			
No. of CLM				
Multiple (n=125)	42.1	**0.015**	NS	
Single (n=68)	63.9			
Diameter of largest of CLM				
>5 cm (n=24)	35.1	0.127		
≥5 cm (n=169)	51.2			
Major complication				
Yes (n=32)	37.1	0.066		
No (n=161)	51.7			
Surgical margin				
Positive (n=19)	38.2	0.402		
Negative (n=174)	49.1			

CLM indicates colorectal liver metastases; NS, not significant; RFS, recurrence-free survival; %VTC, percentage viable tumor cells.

[a]From diagnosis of primary tumor to diagnosis of CLM.