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Mutation Status of *RAS*, *TP53*, and *SMAD4* is Superior to Mutation Status of *RAS* Alone for Predicting Prognosis after Resection of Colorectal Liver Metastases

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Abstract

Purpose—Somatic gene mutations have been increasingly recognized to impact prognosis following resection of colorectal liver metastases (CLM). We aimed to determine the impact of combinations of somatic mutations on survival in patients undergoing CLM resection.

Experimental Design—We identified patients who underwent initial CLM resection during 2007–2017 and had genetic sequencing data available. Risk factors for overall survival (OS) and recurrence-free survival (RFS) were determined using Cox proportional hazards models.

Results—Of 1460 patients who underwent CLM resection during the study period, 507 met the inclusion criteria. Multigene testing revealed mutation rates greater than 10% for *TP53* (mutated in 70.8% of patients), *APC* (53.5%), *RAS* (50.7%), *PIK3CA* (15.8%), and *SMAD4* (11.0%). *BRAF* was mutated in 2.0% of patients. *BRAF*, *RAS*, *TP53*, and *SMAD4* mutations were significantly associated with OS, and *RAS*, *TP53*, and *SMAD4* mutations were significantly associated with RFS. Coexisting mutations in *RAS*, *TP53*, and *SMAD4* were associated with significantly worse OS and RFS than coexisting mutations in any 2 of these genes and mutations in 1 or none of these genes. Coexisting mutations in 2 genes conferred significantly worse OS and RFS than single mutation or no mutations. OS and RFS did not differ significantly between patients with *RAS* mutation and wild-type *TP53* and *SMAD4* and patients with wild-type *RAS* ($P = 0.858$ and 0.729, respectively).

Conclusions—*RAS* mutation status alone is not sufficient for precisely predicting prognosis after CLM resection.

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Keywords

molecular marker; somatic mutation; co-existing mutation; triple mutation; colorectal liver metastasis

Introduction

Understanding of tumor biology and risk stratification based on clinicopathologic prognostic factors have improved the selection of patients undergoing resection of colorectal liver metastases (CLM) [1, 2]. Recent studies have focused on response to preoperative chemotherapy [3–5] and molecular alterations in CLM for risk stratification. Specifically, gene mutation analysis has been studied for its utility in identifying patients who may benefit from CLM resection [6–9].

Previous studies have shown that mutations in *BRAF* and *KRAS* are associated with a poor outcome after CLM resection [6, 10–14]. Mutations in *NRAS*, *TP53*, *PIK3CA*, and *SMAD4* were reported to be potential prognostic factors after CLM resection [15–20]. However, the association between mutations in *TP53* or *PIK3CA* and survival has not been consistent across reported studies [15–18]. Our group previously reported that coexisting mutations in *RAS* and *TP53* were associated with worse prognosis [18]. We hypothesized that co-existence of frequently mutated genes would predict prognosis after CLM resection more precisely than mutation in only 1 of the genes.

To test this hypothesis, we sought to determine the impact of combinations of high-frequency somatic mutations and clinicopathologic factors on survival among patients undergoing liver resection for CLM.

Materials and Methods

Study population

Patients who underwent initial liver resection for CLM with curative intent in the Department of Surgical Oncology at The University of Texas MD Anderson Cancer Center from January 2007 through March 2017 were identified from a prospectively-compiled database. Patients without genetic sequencing data were excluded. Demographic and clinicopathologic characteristics, results of mutational analysis, and outcomes were collected. In order to validate our study, we analyzed the influence of multiple somatic mutations on patients who had unresectable colorectal metastases. Patients who had genetic sequencing data were identified from a prospectively-compiled database of the Department of Medical Oncology between 2005 and 2015. All research was conducted under an appropriate institutional review board protocol (PA18–0295) and waiver of consent from patients. The study was conducted in accordance with the Declaration of Helsinki.

Institutional approach to surgical management of CLM

This approach has been previously published [21]. At our institution, the vast majority of patients with CLM receive preoperative chemotherapy. During preoperative chemotherapy,

restaging is performed. CLM are deemed resectable when a hepatectomy can achieve a negative margin while preserving more than 20% to 30% of the total estimated liver volume, sparing 2 contiguous hepatic segments, and maintaining vascular inflow, vascular outflow, and biliary drainage [22]. In the case of disease progression or suboptimal tumor response after first-line chemotherapy, second-line chemotherapy is considered [23]. For patients with synchronous CLM and an intact primary tumor, decisions regarding the treatment sequence (primary tumor resection first, combined, or liver resection first) are discussed at a multidisciplinary conference, where decisions are primarily based on the extent of the primary tumor and CLM [7]. For patients with an anticipated insufficient future liver remnant, preoperative portal vein embolization and staged hepatectomy are proposed. Perioperative chemotherapy is typically administered to complete a total of 12 cycles, including those given preoperatively [24]. Patients are routinely followed after resection with history, physical examination, laboratory evaluation, and axial imaging every 3 to 4 months for the first 2 years and every 4 to 6 months for the subsequent 3 years.

Somatic gene mutation profiling

As previously described [21], tumor DNA was isolated from 5-mm-thick unstained sections from tumor tissue blocks or slides from primary colorectal cancer or CLM specimens. Macrodissection was performed in cases of low tumor cellularity. Next-generation sequencing was performed with an AmpliSeq 50-gene panel related to cancer (Supplementary Table 1) using the Ion Torrent Personal Genome Machine (Life Technologies, CA) in a Clinical Laboratory Improvement Amendment–certified molecular diagnostic laboratory [25].

Definitions

As previously described [21], synchronous metastases were defined as metastases diagnosed within 12 months of primary tumor diagnosis. A positive surgical margin was defined as the presence of tumor cells within 1 mm of the transection line. Pathologic response was defined as less than 50% residual cancer cells remaining. [26] The number and diameter of liver metastases were determined from examination of surgical pathology specimens. T category of the primary tumor was classified according to the staging system in the *AJCC Cancer Stating Manual*, eighth edition.[27] Preoperative chemotherapy regimens were recorded and further categorized according to whether they included an anti-VEGR or anti-EGFR agent.

Statistical analysis

For statistical analyses, we chose to group *KRAS* and *NRAS* mutations in a single category, *RAS* mutation. This choice is in line with our previously published analyses [9, 18] and is supported by the fact that previous studies by other groups suggest that *NRAS* mutations are associated with worse survival in patients with metastatic colorectal cancer [19, 28, 29].

Sample size calculation was done based on the method reported by Lakatos [30] using the following parameters: α, 0.05; beta, 0.20; mutation rate, 10%; 5-year OS in *RAS* wild-type patients, 62%; 5-year OS in *RAS* mutant patients, 34% [18]. With these assumptions, a sample of 500 patients would be able to detect approximately 18% or more decrease in 5-year OS after CLM resection. Categorical variables were expressed in numbers and

percentages and were compared among groups using Fisher's exact test or the chi-square test, as appropriate. Continuous variables were expressed as median values with the interquartile range. A Cox proportional hazards model analysis initially included the following factors: age (continuous variable), sex, primary tumor location, T category, primary lymph node metastasis, pre-hepatectomy carcinoembryonic antigen level (continuous variable), timing of metastasis (synchronous vs. metachronous), preoperative chemotherapy, extrahepatic disease, postoperative adjuvant chemotherapy, number of CLM (continuous variable), largest liver metastasis diameter (continuous variable), surgical margin status (R1 vs. R0), *BRAF* mutation, *RAS* mutation, *TP53* mutation, *APC* mutation, *PIK3CA* mutation, and *SMAD4* mutation. A backward elimination with a threshold *P* value of 0.05 was used to select variables for the final models. Hazard ratios and 95% confidence intervals were calculated for each factor. The performance of a Cox proportional hazards model was internally validated through a 10-fold cross validation approach [31]. Discrimination was evaluated using a Harrell's concordance statistic [32]. We estimated the median overall survival (OS) time and survival curves adjusted for covariates by using direct adjusted survival estimation [33, 34]. This method uses the Cox regression model to estimate survival probabilities at each time point for each individual and averages them to obtain an OS estimate. The proportional hazards assumption was tested by using Schoenfeld residuals. $P \leq 0.05$ was considered to indicate statistical significance. Statistical analysis was conducted with SAS (SAS Institute, Cary, NC).

Results

Study population

Of 1460 patients who underwent CLM resection during the study period, 507 patients met inclusion criteria (Supplementary Figure 1). Demographic and clinicopathologic characteristics are summarized in Table 1. Prehepatectomy chemotherapy was delivered to 455 (89.7%) patients including 142 (28.0%) with more than 6 cycles, and an anti-EGFR agent was delivered preoperatively to 39 (7.7%) patients. The median duration of follow-up was 3.0 years (interquartile range, 1.9–4.5 years). During the follow-up period, 166 (32.7%) patients died and 397 (78.3%) patients experienced recurrence.

Mutational analyses

Of the 50 cancer-related genes examined, 13 were mutated in more than 1% of patients (Supplementary Figure 2). Five genes had frequency of somatic mutation higher than 10%: *TP53* (mutated in 359 patients; 70.8%), *APC* (271 patients; 53.5%), *KRAS* (237 patients; 46.7%), *PIK3CA* (80 patients; 15.8%), and *SMAD4* (56 patients; 11.0%). *NRAS* was mutated in 22 patients (4.3%), and *BRAF* was mutated in 10 patients (2.0%). Two patients had concurrent mutations of *KRAS* and *NRAS*. *RAS* (*KRAS* and/or *NRAS*) mutation was found in 257 patients (50.7%). *RAS* mutation and *PIK3CA* mutation differed significantly by location of colorectal cancer: right colon vs. left colon vs. rectum; *RAS* mutation, 71.9% vs. 39.2% vs. 51.7%, $P < 0.001$; *PIK3CA* mutation, 14.8% vs. 24.0% vs. 10.7%, $P = 0.013$. *TP53* mutation ($P = 0.053$), *APC* mutation ($P = 0.479$), and *SMAD4* mutation ($P = 0.612$) were not significantly different by location of colorectal cancer.

Predictors of OS and RFS after CLM resection

Next, we sought to evaluate predictors of OS and RFS after resection of CLM. Specifically, we evaluated the 5 genes with mutational frequency over 10% (*TP53*, *APC*, *KRAS*, *PIK3CA*, and *SMAD4*) and known predictors, *BRAF* and *NRAS*, together with clinicopathologic factors. As explained above, *KRAS* and *NRAS* mutations were grouped together in a single category, *RAS* mutations. A multivariable Cox proportional hazards model analysis revealed that mutations of *BRAF*, *RAS*, *TP53*, and *SMAD4* were independent predictors of OS (Table 2). Additionally, largest liver metastasis diameter and surgical margin status were associated with OS. With respect to risk factors for RFS, mutations of *RAS*, *TP53*, and *SMAD4* were identified in addition to age, number of CLM, largest liver metastasis diameter, prehepatectomy chemotherapy > 6 cycles, extrahepatic disease, and surgical margin status (Table 2).

Multivariable hazard ratio of OS and RFS by mutation status of *RAS*, *TP53*, and *SMAD4*

Because somatic mutations of *RAS*, *TP53*, and *SMAD4* were independently associated with OS and RFS, we calculated multivariable hazard ratios for mutations in all 3, 2, or 1 of these genes (Table 3). Co-existence of mutations in all 3 genes (“triple mutation”) was significantly associated with worse OS and RFS than co-existence of mutations in any 2 of the genes (“double mutation”), mutation in only 1 of the genes (“single mutation”), and no mutation in any of the genes (“wild-type status”). Double mutation was also significantly associated with worse OS and RFS than single mutation and wild-type status. OS and RFS did not differ between patients with single mutation and those with wild-type status. Results of the internal validation revealed little overoptimism in the predictive discrimination of our Cox models for OS and RFS. The correction to the mean Harrell’s c-statistic that determined by cross validation was 0.02 (reducing the value from 0.672 to 0.652) in the Cox model for OS and 0.009 (reducing the value from 0.636 to 0.627) in the Cox model for RFS.

Although use of anti-EGFR agent in perioperative chemotherapy differed significantly among patients with triple mutation, double mutation, single mutation, and wild-type status (5.0% vs. 2.3% vs. 11.2% vs. 9.6%, $P = 0.002$), other regimens of perioperative chemotherapy were not significantly different: oxaliplatin-containing regimen, 70.0% vs. 75.1% vs. 72.1% vs. 78.9%, $P = 0.703$; irinotecan-containing regimen, 35.0% vs. 18.1% vs. 21.7% vs. 23.1%, $P = 0.358$; anti-VEGF agent, 80.0% vs. 71.8% vs. 66.3% vs. 80.8%, $P = 0.108$. Pathological response did not differ significantly among the groups: 60.0% vs. 45.2% vs. 49.6% vs. 50.0%, $P = 0.780$.

OS and RFS estimates by mutation status of *RAS*, *TP53*, and *SMAD4*

OS and RFS curves after adjustment for other prognostic factors are shown in Figure 1. Triple mutation was associated with shorter OS and RFS than double mutation, single mutation, and wild-type status. Also, double mutation was associated with shorter OS and RFS than single mutation and wild-type status. The rates of OS and RFS were not significantly different between patients with single mutation and those with wild-type status.

We then evaluated the impact of *RAS* mutation in combination with *TP53* and/or *SMAD4* mutations. Whereas OS and RFS were significantly worse in patients with *RAS* mutation

than in patients with wild-type *RAS* (Supplementary Figure 3), OS and RFS did not differ significantly between patients with *RAS* mutation and wild-type *TP53* and *SMAD4* and patients with wild-type *RAS* (Figure 2 and Supplementary Table 2). Patients with *RAS* mutation with *TP53* or *SMAD4* mutation (double mutation) and patients with *RAS* mutation with mutation of *TP53* and *SMAD4* (triple mutation) had shorter OS and RFS than patients with wild-type *RAS*. Similar results of OS and RFS were found when stratified by *TP53* mutation in combination with *RAS* and/or *SMAD4* mutations. OS and RFS did not differ significantly between patients with *TP53* mutation and wild-type *RAS* and *SMAD4* (n=178) and patients with wild-type *TP53* (n=148): hazard ratio (95% confidence interval); OS, 1.12 (0.73–1.72), $P = 0.614$; RFS, 0.97 (0.75–1.25), $P = 0.793$. We did not repeat this analysis by *SMAD4* mutation because the number of patients with *SMAD4* mutation and wild-type *RAS* and *TP53* was small (n = 4).

Patient characteristics and survival in validation cohort

Supplementary Table 3 shows demographic and clinicopathologic characteristics of a validation cohort including 313 patients with unresectable colorectal metastases. Median (IQR) age was 52 (43–61) years. Site of first metastasis was liver for 97 patients (31.0%), lung for 32 patients (10.2%), peritoneum for 44 patients (14.1%), and multiple organ metastases for 124 patients (39.6%). The median duration of follow-up was 2.3 years (IQR, 1.5–3.5 years). During the follow-up period, 272 (86.9%) patients died. Supplementary Figure 4 shows OS curves after adjustment for site of first metastasis. Double mutation was significantly associated with shorter OS than single mutation. OS did not differ between patients with wild-type status and those with single mutation and between patients with triple mutation and those with double mutation.

Discussion

We demonstrated in this study that multiple somatic mutations in *RAS*, *TP53*, and *SMAD4* were associated with worse prognosis than mutation in only 1 or none of these genes after resection of CLM. This finding was confirmed in a separate validation cohort of patients with unresectable colorectal metastases. We also found that *RAS* mutation in patients with wild-type *TP53* and *SMAD4* was not associated with worse prognosis than wild-type *RAS*. Our findings indicate that although mutant *RAS* is a prognostic factor for survival and recurrence, *RAS* mutation status alone is not sufficient for predicting prognosis in patients undergoing resection of CLM.

In this era of increasingly personalized therapeutic strategies for patients with CLM, ascertainment of individual tumor biology is paramount for accurate prediction of prognosis. For example, it has been established that *RAS* mutation status stratifies patients with respect to outcomes after resection of CLM following various systemic and locoregional therapies [35, 36] in addition to implying lack of response to anti-EGFR therapy [37]. We previously reported the importance of *RAS* mutation status in combination with *TP53* mutations classified as high or low risk according to the evolutionary action score [18]. In the work reported here, we built upon our prior work with analysis of 5 frequently mutated genes and

BRAF and found that *TP53* mutation status and *SMAD4* mutations status were independent prognostic factors, together with *RAS* and *BRAF* mutation status.

The main strength of the current study is the comprehensive assessment of the 5 most frequently mutated genes in CLM, along with *BRAF* and clinicopathologic factors, to predict survival and recurrence in patients with CLM who underwent resection. In contrast, prior publications have focused on the prognostic value of single gene mutations or mutations in only 2 genes of interest rather than more broadly evaluating molecular markers. The findings of our study revealed that mutations of *RAS*, *TP53*, and *SMAD4* were independently associated with prognosis after adjustment for other prognostic factors.

Interestingly, these genes are member of independent signaling pathways (Figure 3). The *RAS* oncogene is a key member of the MAPK (mitogen-activated protein kinase) pathway and contributes to the deregulation of tumor-cell growth, programmed cell death and invasiveness, and ability to induce new blood-vessel formation [38]. *TP53* is a tumor suppressor gene in the P53 pathway and prevents cells from progressing through the cell cycle under conditions that could generate or perpetuate DNA damage [39]. *SMAD4* is also a tumor suppressor gene in the transforming growth factor-β pathway and regulates cell proliferation, differentiation, morphogenesis, and apoptosis [40]. Our data can not explain the interaction of pathway alterations although studies reported crosstalk between the signaling pathways [41–43]. It may be that mutation of any 1 of these genes exerts a deleterious effect on survival through the corresponding signaling pathway and that the hazard for survival increases from single to double mutation and from double to triple mutation in a stepwise fashion. Our findings that patients with triple mutation of *RAS, TP53,* and *SMAD4* had the worst survival following resection suggest that these patients may deserve careful consideration for resection and may be considered for alternative strategies for systemic and local therapy. Additionally, our findings may help clinicians tailor the surveillance intensity after CLM resection and change decisions regarding repeat resection vs. medical therapy after recurrence. Lastly, the data presented here represents a clinical demonstration of the complex interplay of independent pathways within tumors leading to worse outcomes for our patients.

BRAF mutation is a well-known predictor of prognosis after CLM resection [10–14]. However, *RAS* and *BRAF* mutations are almost always mutually exclusive [44], the number of patients with *BRAF* mutation in our study was small, and *BRAF* mutation was not identified as a risk factor for RFS in a Cox proportional hazards model. Thus, we focused on *RAS*, *TP53*, and *SMAD4* mutations, which were independently associated with prognosis, and assessed multivariable hazard ratios for OS and curves of OS after adjusting for *BRAF* mutation status together with other risk factors.

Another interesting finding of our study was that a subset of patients with *RAS* mutation has the same long-term outcomes as patients with *RAS* wild-type. Similar to previous studies [6–8, 11, 13], our study showed that *RAS* mutation was associated with worse survival than wild-type *RAS*. However, OS and RFS in patients with *RAS* mutation and wild-type *TP53* and *SMAD4* were not significantly different from OS and RFS in patients with *RAS* wild-type. This finding likely explains the fact that some studies demonstrated worse OS and RFS

in patients with *RAS* mutation than in patients with wild-type *RAS* [6–8, 11], whereas others failed to confirm a significant difference between these patient groups in OS and RFS [10, 13, 15] or in RFS [45, 46] using a multivariable Cox model. The survival analysis of these studies may have been influenced by other gene mutations hiding behind *RAS* mutation. It should be noted that this finding needs to be understood in the context of type II error as more than 18,000 events would be required to detect a true difference which may exist according to the sample size analysis. Nonetheless, our findings from the current study, taken together with the findings from previous studies of the prognostic impact of *RAS* mutation, suggest that testing of a multigene mutation profile, rather than simply testing *RAS* and *BRAF*, is needed to precisely predict prognosis on the basis of molecular markers.

This study is limited by its retrospective design and the fact that it was conducted at a single, albeit a high-volume, institution. Because comprehensive multigene testing was rarely performed prior to 2012, the majority of the patients studied underwent liver resection after 2012 and had relatively short follow-up. Mutational analysis was performed on either primary tumor tissue or CLM depending on availability. We do not believe this is likely to have impacted the results as many studies suggest a high rate of concordance for *RAS*, *TP53*, *APC*, *PIK3CA*, and *SMAD4* mutations between primary tumors and metastases [20, 46–49]. We did not assess gene mutations occurring in fewer than 10% of patients because the small numbers may weaken statistical power. However, the genes that we studied—*KRAS* and *NRAS* (analyzed together as *RAS), TP53, APC, PIK3CA,* and *SMAD4*—are active in 5 different major signaling pathways, and the result is a more representative prognostic sample of cellular drivers associated with oncogenesis and/or tumor suppression. The prognostic effect of *RAS* on OS may be influenced by the use of anti-EGFR therapy. However, the prognostic effect of *RAS* on RFS is probably not biased by the use of anti-EGFR therapy because anti-EGFR therapy is not used in the adjuvant setting [50].

In conclusion, double or triple gene mutations in *RAS*, *TP53*, and *SMAD4* are associated with worse survival and recurrence than mutation in only 1 or none of these genes after resection of CLM. Thus, *RAS* mutation status alone is not sufficient for predicting survival and recurrence after CLM resection. These findings may be useful for clinical decision making in patients with tumor characteristics associated with poor prognosis and for risk stratification of patients in future clinical studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CLM	colorectal liver metastases
OS	overall survival
RFS	recurrence-free survival

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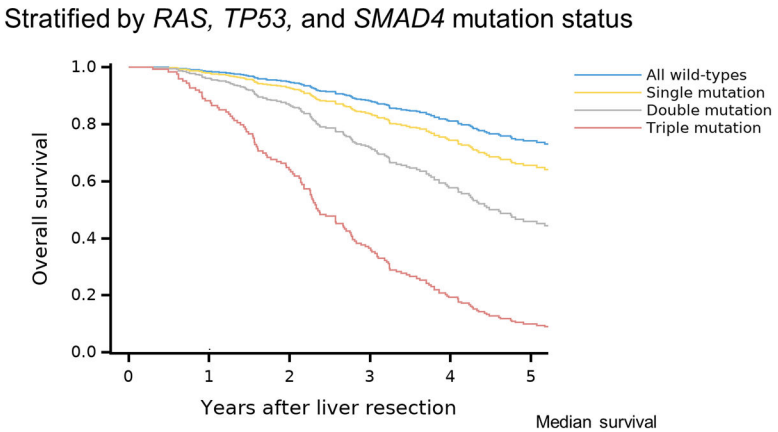
Translational Relevance

Somatic gene mutations have been increasingly recognized to impact prognosis following resection of colorectal liver metastases (CLM). We studied the association of 5 frequently mutated genes (*TP53*, *APC*, *RAS*, *PIK3CA*, and *SMAD4*) and *BRAF* with survival in patients who underwent CLM resection. We found that multiple somatic mutations in *RAS*, *TP53*, and *SMAD4* were associated with worse prognosis than mutation in only 1 or none of these genes. Additionally, *RAS* mutation in patients with wild-type *TP53* and *SMAD4* was not associated with worse prognosis than wild-type *RAS*. Thus, *RAS* mutation status alone is not sufficient for predicting prognosis in patients undergoing CLM resection. Our findings may be useful for clinical decision making in patients with tumor characteristics associated with poor prognosis and for risk stratification of patients in future clinical studies.

A



Patients at risk					Median survival time, year
All wild-types	52	50	43	23	7.8
Single mutation	258	247	192	87	6.9
Double mutation	177	168	126	50	4.5
Triple mutation	20	17	8	1	2.3

B

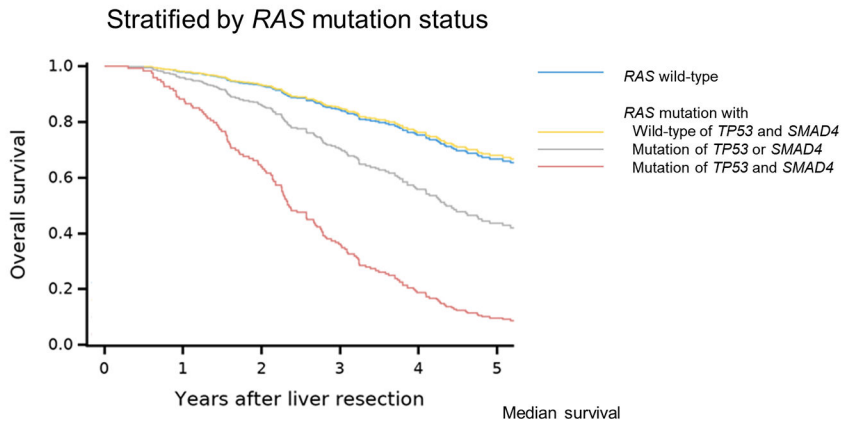
Patients at risk					Median survival time, year
All wild-types	52	29	17	3	1.2
Single mutation	258	131	58	20	1.1
Double mutation	177	74	32	7	0.7
Triple mutation	20	3	1	0	0.4

Figure 1. Overall survival (OS) and recurrence-free survival (RFS) by *RAS*, *TP53*, and *SMAD4* mutation status.

(A) OS curves after adjustment for *BRAF* mutation status, largest liver metastasis diameter, and surgical margin status.

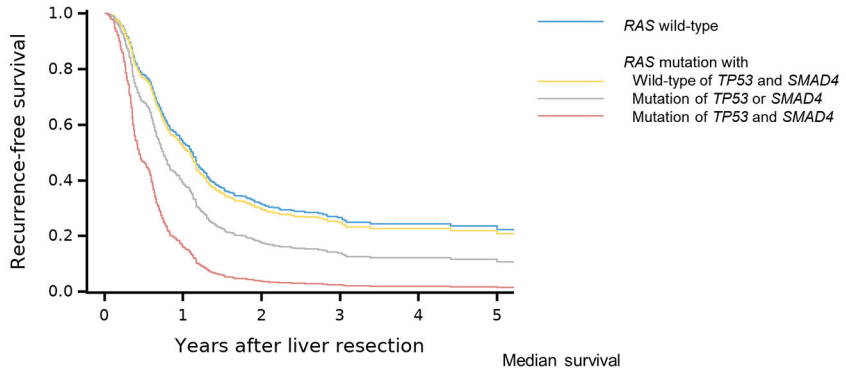
(B) RFS curves after adjustment for age, number of CLM, largest liver metastasis diameter, prehepatectomy chemotherapy (> 6 cycles vs. ≤ 6 cycles or no prehepatectomy chemotherapy), extrahepatic disease, and surgical margin status.

A



Patients at risk					Median survival time, year
RAS wild-type	250	238	189	95	7.0
RAS mutation with					
Wild-type of *TP53* and *SMAD4*	76	75	60	24	7.3
Mutation of *TP53* or *SMAD4*	161	152	112	41	4.3
Mutation of *TP53* and *SMAD4*	20	17	8	1	2.3

B



Patients at risk					Median survival time, year
RAS wild-type	250	129	59	18	1.1
RAS mutation with					
Wild-type of *TP53* and *SMAD4*	76	37	19	6	1.1
Mutation of *TP53* or *SMAD4*	161	68	29	6	0.7
Mutation of *TP53* and *SMAD4*	20	3	1	0	0.4

Figure 2. Overall survival (OS) and recurrence-free survival (RFS) by *RAS* mutation status.

(A) OS curves after adjustment for *BRAF* mutation status, largest liver metastasis diameter, and surgical margin status.

(B) RFS curves after adjustment for age, number of CLM, largest liver metastasis diameter, prehepatectomy chemotherapy (> 6 cycles vs. ≤ 6 cycles or no prehepatectomy chemotherapy), extrahepatic disease, and surgical margin status.

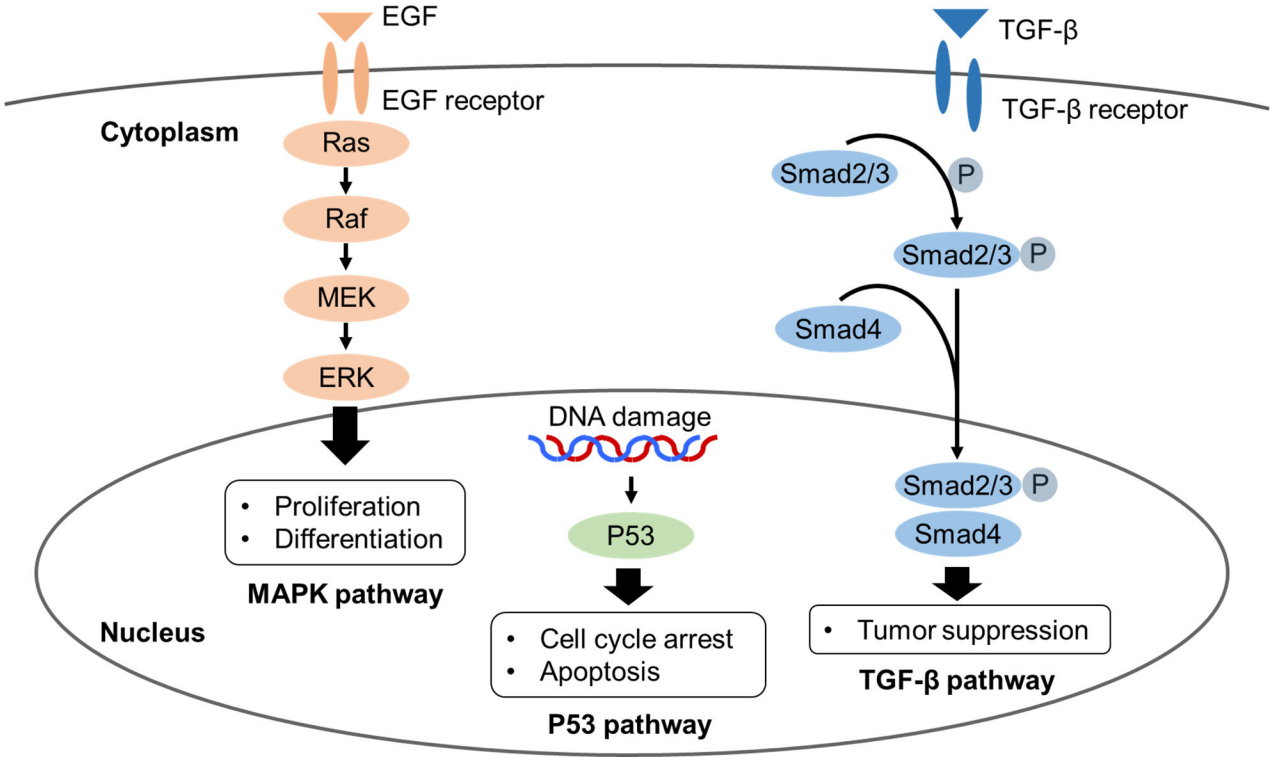


Figure 3. Overview of three signaling pathways involved in colorectal cancer

Abbreviations: EGF, epidermal growth factor; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; TGF-β, transforming growth factor-β; P, phosphorylation.

Table 1.

Demographic and clinicopathologic characteristics in 507 patients who underwent resection of CLM

Characteristic	Value
Patient factors	
Age, median (IQR), yr	55 (46–62)
Sex, male : female, n	286 : 221
ASA score ≥ 3, n (%)	442 (87.2%)
Primary lesion factors	
Location, colon : rectum, n	358 : 149
T category ≥ 3, n (%) *	436 (87.4)
Lymph node metastasis, n (%) *	354 (71.8)
Liver metastases clinical factors	
Prehepatectomy CEA level, median (IQR), ng/mL	4.0 (2.2–12.5)
Synchronous metastasis, n (%)	382 (75.3%)
Extrahepatic metastasis, n (%)	83 (16.4%)
Prehepatectomy chemotherapy, n (%)	455 (89.7%)
> 6 cycles, n (%)	142 (28.0%)
Oxaliplatin-containing regimen	374 (73.8%) †
Irinotecan-containing regimen	107 (21.1%) ‡
With anti-VEGF agent, n (%)	356 (70.2%) §
With anti-EGFR agent, n (%)	39 (7.7%) ¶
Liver metastases histopathologic factors	
Tumor number, median (IQR)	2 (1–4)
Maximum diameter, median (IQR), cm	2.5 (1.5–4.0)
R1 surgical margin, n (%)	104 (20.5%)

Abbreviations: IQR, interquartile range; ASA, American Society of Anesthesiologists; CEA, carcinoembryonic antigen; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor.

* Data not available for T category in 8 patients and lymph node metastasis in 14 patients.

† Patients with *RAS* mutation vs. patients with *RAS* wild-type.191 (74.3%) vs. 183 (73.2%), $P = 0.840$.

‡ Patients with *RAS* mutation vs. patients with *RAS* wild-type.49 (19.1%) vs. 58 (23.2%), $P = 0.277$.

§ Patients with *RAS* mutation vs. patients with *RAS* wild-type.183 (71.2%) vs. 173 (69.2%), $P = 0.629$.

¶ Patients with *RAS* mutation vs. patients with *RAS* wild-type.4 (1.6%) vs. 35 (14.0%), $P < 0.001$.

Table 2.

Multivariable Cox proportional hazards model analysis for OS and RFS in 490 patients*

Factor	No. of patients	No. of events	Multivariable HR†	95% CI	*P* value
		OS			
Gene mutation					
BRAF	10	6	2.95	1.27–6.86	0.012
RAS	249	87	2.14	1.53–2.99	< 0.001
TP53	347	123	2.21	1.49–3.28	< 0.001
SMAD4	55	25	1.82	1.17–2.83	0.008
Clinicopathologic factors					
Largest liver metastasis diameter	–	–	1.07	1.01–1.13	0.033
Surgical margin, positive	97	33	1.83	1.23–2.72	0.003
		RFS			
Gene mutation					
RAS	249	206	1.47	1.20–1.82	< 0.001
TP53	347	283	1.40	1.11–1.78	0.005
SMAD4	55	51	1.62	1.20–2.20	0.002
Patients factors					
Age			1.01	1.00–1.02	0.024
Clinicopathologic factors					
Number of CLM	–	–	1.04	1.01–1.07	0.006
Largest liver metastasis diameter	–	–	1.08	1.04–1.13	< 0.001
Prehepatectomy chemotherapy‡	134	117	1.45	1.16–1.82	0.001
Extrahepatic disease	79	74	1.70	1.31–2.21	< 0.001
Surgical margin, positive	97	80	1.43	1.11–1.85	0.006

Abbreviations: HR, hazard ratio; CI, confidence interval, CLM, colorectal liver metastasis.

*Of the 507 patients, 490 patients were analysed because data were unavailable for T category in 8 patients and lymph node metastasis in 14 patients.

†The Cox proportional hazards model analysis initially included age (continuous variable), sex, primary tumor location, T category, primary lymph node metastasis, prehepatectomy carcinoembryonic antigen level (continuous variable), timing of metastasis (synchronous vs. metachronous), prehepatectomy chemotherapy, extrahepatic disease, number of CLM (continuous variable), largest liver metastasis diameter (continuous variable), surgical margin status (R1 vs. R0), *BRAF* mutation, *RAS* mutation, *TP53* mutation, *APC* mutation, *PIK3CA* mutation, and *SMAD4* mutation. A backward elimination with a threshold *P* value of 0.05 was used to select variables for the final models.

‡> 6 cycles vs. ≤ 6 cycles or no prehepatectomy chemotherapy.

Table 3.

Multivariable hazard ratios (HRs) for OS and RFS by mutation status of *KRAS*, *TP53*, and *SMAD4* in 507 patients

***KRAS*, *TP53*, and *SMAD4* mutation status**	**Reference**	**Multivariable HR**	**95% CI**	***P* value**
	OS [*]			
Triple mutation	vs. Double mutation	3.21	1.72–5.99	< 0.001
	vs. Single mutation	6.04	3.21–11.3	0.001
	vs. All wild-type	8.61	3.80–19.5	< 0.001
Double mutation	vs. Single mutation	1.88	1.35–2.63	< 0.001
	vs. All wild-type	2.68	1.45–4.97	0.002
Single mutation	vs. All wild-type	1.43	0.77–2.63	0.256
	RFS [†]			
Triple mutation	vs. Double mutation	2.06	1.28–3.29	0.003
	vs. Single mutation	3.17	1.97–5.07	< 0.001
	vs. All wild-type	3.72	2.14–6.46	< 0.001
Double mutation	vs. Single mutation	1.54	1.24–1.91	< 0.001
	vs. All wild-type	1.81	1.25–2.61	0.002
Single mutation	vs. All wild-type	1.18	0.82–1.68	0.378

Abbreviations: CI, confidence interval.

[*] Multivariable HR was assessed after adjustment for the following risk factors: *BRAF* mutation status, largest liver metastasis diameter, and surgical margin status (R1 vs. R0).

[†] Multivariable HR was assessed after adjustment for the following risk factors: age, number of CLM, largest liver metastasis diameter, prehepatectomy chemotherapy (> 6 cycles vs. ≤ 6 cycles or no prehepatectomy chemotherapy), extrahepatic disease, and surgical margin status (R1 vs. R0).