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Deleterious Effect of *RAS* and Evolutionary High Risk *TP53* Double Mutation in Colorectal Liver Metastases

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Abstract

Objective: To assess the impact of somatic gene mutations on survival among patients undergoing resection of colorectal liver metastases (CLM).

Summary Background Data: Patients undergoing CLM resection have heterogeneous outcomes, and accurate risk stratification is necessary to optimize patient selection for surgery.

Methods: Next-generation sequencing of 50 cancer-related genes was performed from primary tumors and/or liver metastases in 401 patients undergoing CLM resection. Missense *TP53* mutations were classified by the Evolutionary Action Score (EAp53), a novel approach that dichotomizes mutations as low or high risk.

Results: The most frequent somatic gene mutations were *TP53* (65.6%), followed by *KRAS* (48.1%) and *APC* (47.4%). Double mutation in *RAS*/*TP53*, identified in 31.4% of patients, was correlated with primary tumor location in the right colon ($P = 0.006$). On multivariable analysis, *RAS/TP53* double mutation was an independent predictor of shorter overall survival (hazard ratio, 2.62; 95% confidence interval 1.41 to 4.87; $P = 0.002$). In patients with co-mutated *RAS*, EAp53 high risk mutations were associated with shorter 5-year overall survival of 12.2%, compared with 55.7% for *TP53* wild-type ($P < 0.001$). The negative prognostic effects of *RAS* and *TP53* mutations were limited to tumors harboring mutations in both genes.

Conclusions: Concomitant *RAS* and *TP53* mutations are associated with decreased survival after CLM resection. A high EAp53 predicts a subset of patients with worse prognosis. These

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preliminary analyses suggest that surgical resection of liver metastases should be carefully considered in this subset of patients.

INTRODUCTION

Colorectal cancer is the second leading cause of cancer mortality in the United States and is estimated to account for over 49,000 deaths in 2016.[1] Most patients dying from colorectal cancer will have liver metastases. Surgical resection of colorectal liver metastases (CLM) is associated with a 5-year overall survival (OS) rate of 58% and is accepted as standard of care.[2] Owing to advances in systemic therapy, radiology, and surgical technique, more patients are eligible for hepatic resection. However, colorectal cancer is a heterogeneous disease, and subsets of patients undergoing surgery will succumb early to disease recurrence.[3] With systemic therapy alone, median OS rates exceeding 2 years are reported for metastatic colorectal cancer.[4] Therefore, accurate prognostic markers are needed for risk stratification and optimization of patient selection for hepatic resection.

Currently, few biologic markers are used to guide therapy and prognostication in CLM. Defective DNA mismatch repair (MMR) is established as an important factor in colorectal cancer pathogenesis, treatment, and outcome.[5] However, MMR-deficient tumors are found in a minority of patients undergoing CLM resection.[6] Also uncommon, occurring in < 5% of patients, *BRAF* mutations are associated with particularly poor outcomes, with median overall survival (OS) of 22.6 months after CLM resection.[7] To date, *RAS* mutations are the most prevalent predictive and prognostic genetic alterations in CLM, detected in 18%−52% of patients undergoing CLM resection.[7, 8] In addition to predicting resistance to anti-epidermal growth factor receptor therapies, *RAS* mutations are associated with disease recurrence in the lungs, higher rate of positive margins, and worse survival after CLM resection.[9, 10]

As next-generation sequencing technology becomes widely available, multigene analysis is expected to inform clinical decisions in CLM beyond *BRAF* and *RAS*. *TP53* is the most frequently mutated gene in metastatic colorectal cancer, but previous studies on *TP53* mutations and colorectal cancer prognosis have yielded conflicting results.[11–13] Preclinical studies have demonstrated that cooperation between mutated *TP53* and *RAS* is critical for malignant transformation of colorectal cancer cells.[14, 15] The malignant state of colorectal cancer cells depends upon genes controlled synergistically by loss of wild-type p53 function and *RAS* activation.[14] In this article, we evaluated cooperativity between somatic mutations in *RAS* and *TP53* among patients undergoing CLM resection. Since *TP53* mutations are not all functionally equivalent, we categorized mutations according to the Evolutionary Action (EA) score, a novel computational approach to assess the functional impact of genetic mutations.[16] We hypothesized that integrating the mutational status of *RAS* and *TP53* provides information on patients' prognosis after CLM resection that is complementary to standard clinicopathologic factors to guide treatment decisions.

METHODS

Patients

From 1151 patients undergoing CLM resection at the University of Texas MD Anderson Cancer Center between 2005 and 2015, 401 patients underwent multigene panel testing from their primary tumors and/or hepatic metastases. For patients undergoing initial CLM resection before 2012, genomic analysis was performed from archived tumor samples or repeat hepatectomy specimens. Computerized medical records were queried for data on clinicopathologic factors, disease recurrence, and patient survival. Synchronous metastases were defined as metastases diagnosed within 6 months of primary tumor diagnosis. Positive surgical margin was defined as tumor cells at the line of transection and included patients undergoing radiofrequency ablation at time of hepatic resection. The number and diameter of liver metastases were determined from surgical pathology specimens. Major hepatectomy was defined as resection of 3 or more contiguous Couinaud liver segments.[17]

Primary tumor location was classified as right colon if the vascular supply originated from the superior mesenteric artery, including the proximal transverse colon. Primary tumors in the left were defined by vascular supply from the inferior mesenteric artery, including distal transverse colon and rectal cancers. MMR status was determined as previously described.[5] Primary tumor T stage was classified according to the American Joint Committee on Cancer, 7th edition.[18]

Preoperative and postoperative chemotherapy regimens were selected at the discretion of treating medical oncologists. Our institutional practice is to administer short-course preoperative chemotherapy to most patients before liver resection, followed by postoperative chemotherapy to complete 6 months total of systemic therapy. Preoperative chemotherapy was administered to 338 of the 401 patients (84.3%) and included regimens containing oxaliplatin (63.6%), irinotecan (12.0%), or both oxaliplatin and irinotecan (4.7%). Anti-epidermal growth factor receptor (EGFR) therapy was administered to 20 patients (5.0%) before liver resection and 16 patients (4.0%) after liver resection.

Selection criteria for liver resection included radiologic response or stable disease after preoperative chemotherapy, sufficient remnant liver volume, and technical resectability, as previously described.[19] Patients with anticipated insufficient future liver remnant volume underwent portal vein embolization. Two-stage hepatectomy was performed for patients with bilateral disease that could not be safely resected in one stage. Patients with extrahepatic disease were considered for surgery if all sites of disease could be resected, or if patients had indeterminate or disappearing lung lesions.

This study was approved by the institutional review board at the University of Texas MD Anderson Cancer Center.

Genomic Analysis

Since 2012, metastatic colorectal cancer samples submitted for *KRAS* and/or *BRAF* testing are routinely analyzed by next-generation sequencing to detect somatic mutations in the

coding sequence of 50 genes from DNA extracted from formalin-fixed paraffin-embedded tumor samples, as previously described.[20]

Sequence analysis of *RAS* included *KRAS* exons 2–4 (codons 5–66 and 114–150), *NRAS* exons 2–4 (codons 3–31, 43–69, and 124–150), and *HRAS* exons 2–3 (codons 5–35 and 42–82). For *TP53*, the exons (codons) tested were 2 (1–20), 4 (68–113), 5 (126–138), 5–6 (149–223), 7 (225–258), 8 (263–307), and 10 (332–367).

Mutation diagrams (lolliplots) were generated using the cBioPortal.[21, 22]

Classification of TP53 Mutations

Missense *TP53* mutations were classified as low or high risk according to the Evolutionary Action (EAp53) score.[16] EA measures the fitness effect of coding mutations. It models evolution as a mapping of genotypes (γ) to phenotypes (φ) in the fitness landscape via an evolutionary function $f: \gamma \rightarrow \varphi$ Assuming evolvability, f is differentiable, and following calculus, the action of a mutation $d\gamma$ on fitness is $d\varphi = f'(\gamma)\, d\gamma$, where $f'(\gamma)$ is the sensitivity of the mutated site. In practice, $d\gamma$ is approximated with inverse amino acid substitution log-odds and $f'(\gamma)$ with Evolutionary Trace ranks of importance. The fitness effect $d\varphi$, or EA, of coding mutations is then computable and correlates with experimental loss of function and morbidity. A training cohort of head and neck squamous cell cancer patients defined a threshold EAp53 value of > 75% that was associated with poor prognosis.[23] Evolutionary Action and EAp53 are available for non-profit use at http://mammoth.bcm.tmc.edu/EvolutionaryAction and http://mammoth.bcm.tmc.edu/EAp53/, respectively.

Statistical Analysis

Group comparisons were performed using chi-square tests for categorical variables and Mann-Whitney for continuous variables. The Kaplan-Meier method was used to estimate probability of OS and recurrence-free survival (RFS) from date of liver resection. The log-rank test was used to compare OS and RFS between subgroups. Factors significant on univariable analysis were entered into a multivariable Cox analysis of OS. All P values were two-sided, and $P < 0.05$ was considered statistically significant. All analyses were performed with SPSS Statistics 23.0 (IBM Corp., Chicago, IL).

RESULTS

Clinical Characteristics

Baseline characteristics of 401 patients who underwent CLM resection are listed in Table 1. Among 381 patients who underwent evaluation of microsatellite instability (MSI), 376 (98.7%) had MSI-stable tumors. Overall, 155 (38.7%) patients underwent resection of a solitary hepatic metastasis, and 246 (61.3%) resection of multiple hepatic metastases. Median duration of follow-up was 35 months (range, 4–144 months).

Somatic DNA Mutations

Overall, 383 (95.5%) of 401 patients had at least one genetic mutation identified in their tumors. *TP53* (65.6%) was the most frequently mutated gene, followed by *KRAS* (48.1%),

APC (47.4%), *PIK3CA* (15.0%), and *SMAD4* (11.7%) (Figure 1A). Three patients had mutations in both *KRAS* and *NRAS*. Among 16 (4.0%) patients with *BRAF* mutations, 6 had V600E mutations, and the remainder had mutations in BRAF codons 466, 469, 581, or 594.

Multigene panel testing was performed from the liver metastases in 173 patients, primary colorectal tumor in 144 patients, and lung metastases in 24 patients. Multigene panel testing from both the primary tumor and liver metastases was performed in 60 patients. Discordant results for *KRAS* were observed in 2 patients, *NRAS* in 1 patient, and *TP53* in 1 patient.

The most frequent concurrent mutations with *TP53* were *RAS* and *APC* (Figure 1B). Mutated *TP53* and *RAS* were observed in 126 patients (31.4%), mutated *TP53* and *APC* in 132 patients (32.9%), and triple mutation of *TP53*, *RAS*, and *APC* in 64 patients (16.0%).

Potential Prognostic Factors by Mutational Status

Correlations between potential prognostic factors and double mutation in *RAS/TP53* are presented in Table 2. Compared with patients lacking double mutations, those with mutated *RAS* and *TP53* were more likely to have primary tumors in the right colon ($P = 0.006$). There was not a statistically significant association between double mutation in *RAS/TP53* and site of disease recurrence.

Survival by Mutational Status

RAS mutations were associated with worse median and 5-year OS rates of 48 months and 34.1%, compared with 71 months and 61.6% for *RAS* wild-type ($P < 0.001$, Figure 2A). When considered in isolation, *TP53* mutations were not associated with OS, with median OS rates of 55 and 62 months, with and without *TP53* mutations, respectively ($P = 0.27$, Figure 2B).

Among patients with co-mutated *RAS*, *TP53* mutations were associated with median and 5-year OS rates of 41 months and 20.6%, compared with 62 months and 55.7% for *TP53* wild-type ($P = 0.003$, Figure 2C). The negative prognostic effect of mutated *RAS* was restricted to patients with concurrent *TP53* mutations. RFS was also shorter in patients with co-mutations in *RAS* and *TP53*, with median and 2-year RFS rates of 9 months and 5.2%, compared with 11 months and 23.6% without co-mutations ($P = 0.001$, Figure 2D).

After excluding the 20 patients (5.0%) who received anti-EGFR therapy before or after liver resection, the poor prognostic effect of concurrent mutations in *RAS* and *TP53* remained significant for overall survival, with *P*-value of < 0.001.

In multivariable analysis, factors independently associated with OS were size of liver metastases ≥ 3 cm and double mutation in *RAS/TP53* (Table 3).

Distribution and Classification of TP53 Mutations

Among 263 patients with *TP53* mutations, 9 patients harbored two distinct *TP53* mutations. Overall, 202 missense, 68 truncating (nonsense, splice site, or frameshift), and 2 in-frame deletion *TP53* mutations were identified. Among the missense mutations, 121 mutations

(59.9%) resulted in amino acid substitutions at six residues established in the literature as hotspots most frequently mutated in human cancers, R175, G245, R248, R249, R273, and R282 (Figure 3A).

Survival analysis was performed after classifying *TP53* mutations as truncating or missense, and further stratifying missense mutations as high or low risk according to the Evolutionary Action score (EAp53). Median OS rates were not significantly different between *TP53* wild-type, truncating, EAp53 low, and EAp53 high risk mutations (wild-type: 62 months vs truncating: 54 months vs low risk: 63 months vs high risk: 52 months; Figure 3B). In patients with co-mutated *RAS*, OS was shorter with EAp53 high risk mutations, with median and 5-year OS rates of 41 months and 12.2%, compared with 62 months and 55.7% for *TP53* wild-type ($P < 0.001$, Figure 3C). Median and 5-year OS rates were similar between EAp53 low risk (45 months and 28.4%) and truncating mutations (42 months and 33.0%, $P = 0.71$).

DISCUSSION

High-throughput genomic analysis is elucidating the molecular complexity underlying heterogeneous outcomes of patients with metastatic colorectal cancer. MMR status, *KRAS*, and *BRAF* are currently used as predictive and prognostic biomarkers.[5, 7, 10] In this study, we analyzed results of next-generation sequencing of 50 cancer-related genes in patients undergoing CLM resection. *TP53* mutations were the most frequent somatic gene mutations and associated with poor OS and RFS in the subset of patients with co-mutated *RAS*. Similarly, *RAS* mutations correlated with worse prognosis only in patients with co-occurring *TP53* mutations. Three-quarters of *TP53* mutations were missense, and when stratified by the EA score, high risk mutations were associated with 5-year OS of only 12.2% after liver resection.

According to the current study, the most frequent somatic gene mutation in patients with CLM is *TP53*, followed by *KRAS* and *APC.* These results are consistent with the distribution of molecular alterations identified in metastatic colorectal cancer patients screened for targeted therapy trials.[24] Whole genome sequencing of primary colorectal cancers by The Cancer Genome Atlas identified 32 somatic recurrently mutated genes, including *RAS* and *TP53*.[25] Only 1.3% of patients undergoing CLM resection had MSI-high tumors, reflecting the low frequency of MSI in metastatic colorectal cancer. Similarly, only 4.0% had *BRAF*-mutated tumors, and less than half were V600E mutations. As shown by previous reports, *BRAF*-mutated tumors are associated with peritoneal metastases and rarely liver-only metastases.[26]

On multivariable analysis, double mutation in *RAS*/*TP53* was independently associated with worse OS, with a HR of 2.62. Prior studies have shown discordant results on the impact of *TP53* mutations on colorectal cancer prognosis and response to systemic therapy.[12, 27, 28] However, these studies did not analyze *TP53* in the context of *RAS*. In this report, the negative prognostic effects of *RAS* and *TP53* mutations were limited to tumors harboring both mutations, suggesting cooperativity between the two mutated genes. Cooperation between co-occurring genetic events is an important factor in cancer progression.

Concurrent mutations in *RAS* and *TP53* have been shown to drive carcinogenesis in vitro and in animal models.[14, 15] In locally advanced rectal cancer, combined *KRAS* and *TP53* mutations promote resistance to neoadjuvant chemoradiation.[29] We previously showed that double mutation of *APC* and *PIK3CA* predicted poor survival in patients with CLM.[30] In the present study, double *APC-PIK3CA* mutations were significantly associated with overall survival on univariable but not multivariable analysis. Thirty-two patients had double *APC-PIK3CA* mutations, compared to 126 patients with double *RAS-TP53* mutations.

Co-mutations in *RAS* and *TP53* were associated with primary tumor location in the right colon, suggesting their potential biologic significance. The embryologic origin of colorectal cancers from midgut or hindgut has emerged as an important biologic and prognostic factor.[31, 32] Co-mutations in *RAS* and *TP53* were associated with a trend toward higher rate of extrahepatic metastases (23.8% with co-mutations vs 15.6% without, $P = 0.052$), which may have not reached statistical significance due to the small number of patients with extrahepatic metastases. Given the heterogeneity of systemic therapy regimens administered, we did not evaluate the effect of co-mutations on response and resistance to systemic therapy. All of the patients in this study underwent CLM resection, and our practice is to consider liver resection for patients who do not progress on chemotherapy.

This report demonstrates that in patients undergoing CLM resection, most *TP53* mutations are missense and occur at established *TP53* hotspots, consistent with previously published studies.[33] To identify potentially non-deleterious *TP53* mutations, we classified mutations as truncating and missense, and further stratified missense mutations by EAp53, which is based upon the evolutionary importance of a mutation site and the relative odds of an amino acid substitution.[16] EAp53 was previously shown to stratify outcomes in head and neck cancer.[23] The current report demonstrates that EAp53 is also prognostic in CLM, highlighting the applicability of the EA model across tumor types. Patients with co-occurring *RAS* and EAp53 high risk, but not low risk, mutations had significantly worse OS than patients with *TP53* wild-type tumors.

This study has several limitations. Patients undergoing CLM resection before 2012 did not initially undergo multigene panel testing, which was performed in these earlier patients from archived tumor or repeat hepatectomy specimens. In most patients, mutations were determined from DNA sequencing of either primary tumor or metastases samples. However, among 60 patients who underwent DNA sequencing from both the primary tumor and liver metastases, a high mutational concordance rate of 93.3% was observed for *RAS* and *TP53*, consistent with prior studies.[34] These results suggest that in most patients, multigene panel testing from either the primary tumor or metastases is sufficient, and additional biopsies for molecular analyses are not necessary.

In conclusion, double mutation in *RAS*/*TP53* is a strong independent predictor of worse survival after CLM resection. Five-year OS after resection of CLM with concurrent *RAS* and high EAp53 mutations is only 12.2%, and future studies are needed to validate these results. On the other hand, patients with traditionally poor prognostic factors, such as multiple liver metastases and extrahepatic disease, can be considered for surgery if their

mutational status is favorable. Further investigations are needed to determine the mechanistic and downstream effects of co-occurring *RAS* and *TP53* mutations in CLM.

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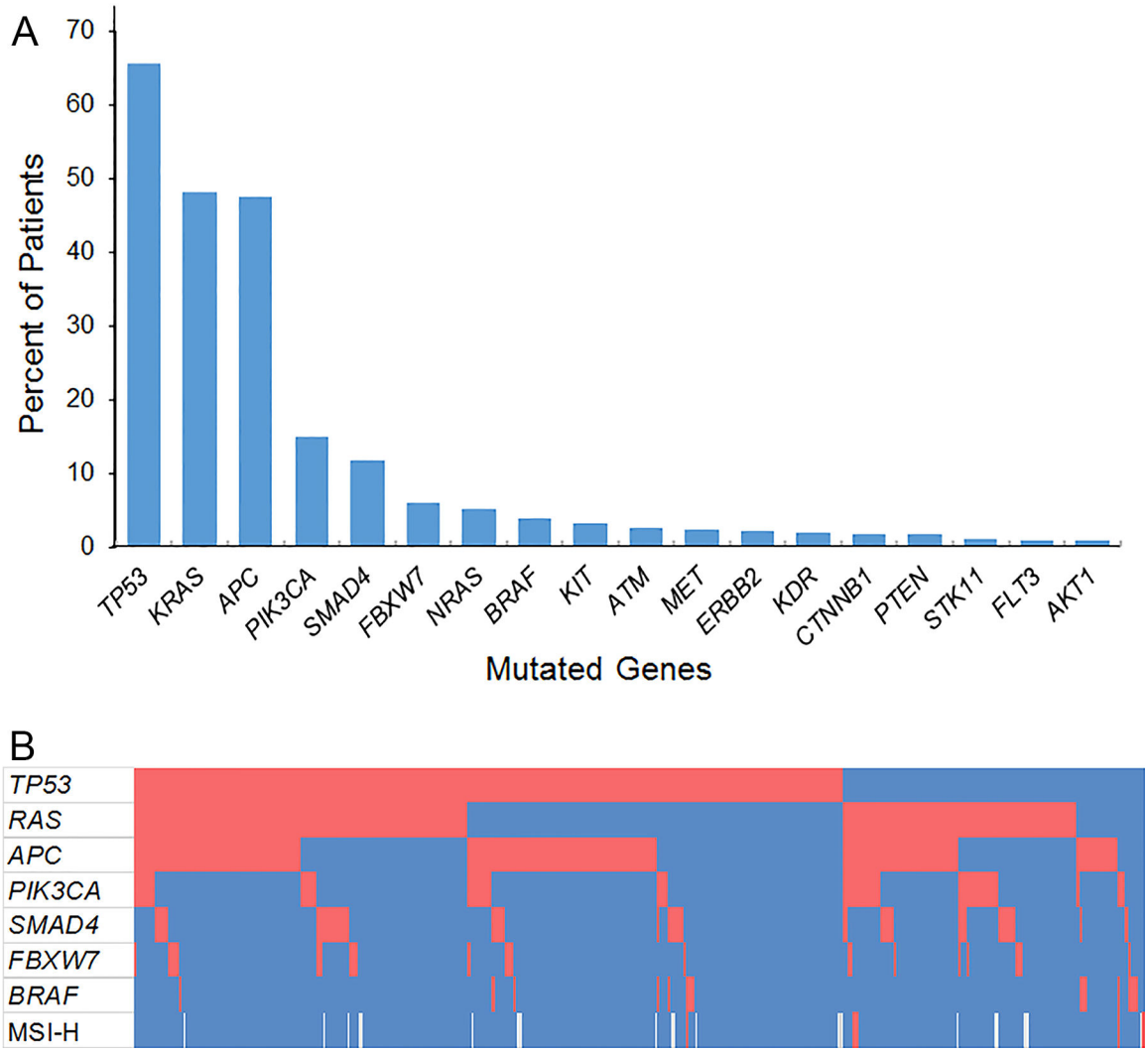

Figure 1.

(A) Prevalence of somatic gene mutations among 401 patients undergoing resection of colorectal liver metastases. (B) Co-mutation plot showing patients in columns and genes in rows. Red blocks represent somatic mutations or deficient mismatch repair status. Blue blocks represent absence of somatic mutations or intact mismatch repair. Microsatellite instability status was not available for 20 patients, represented by white blocks. MSI-H, microsatellite instability-high.

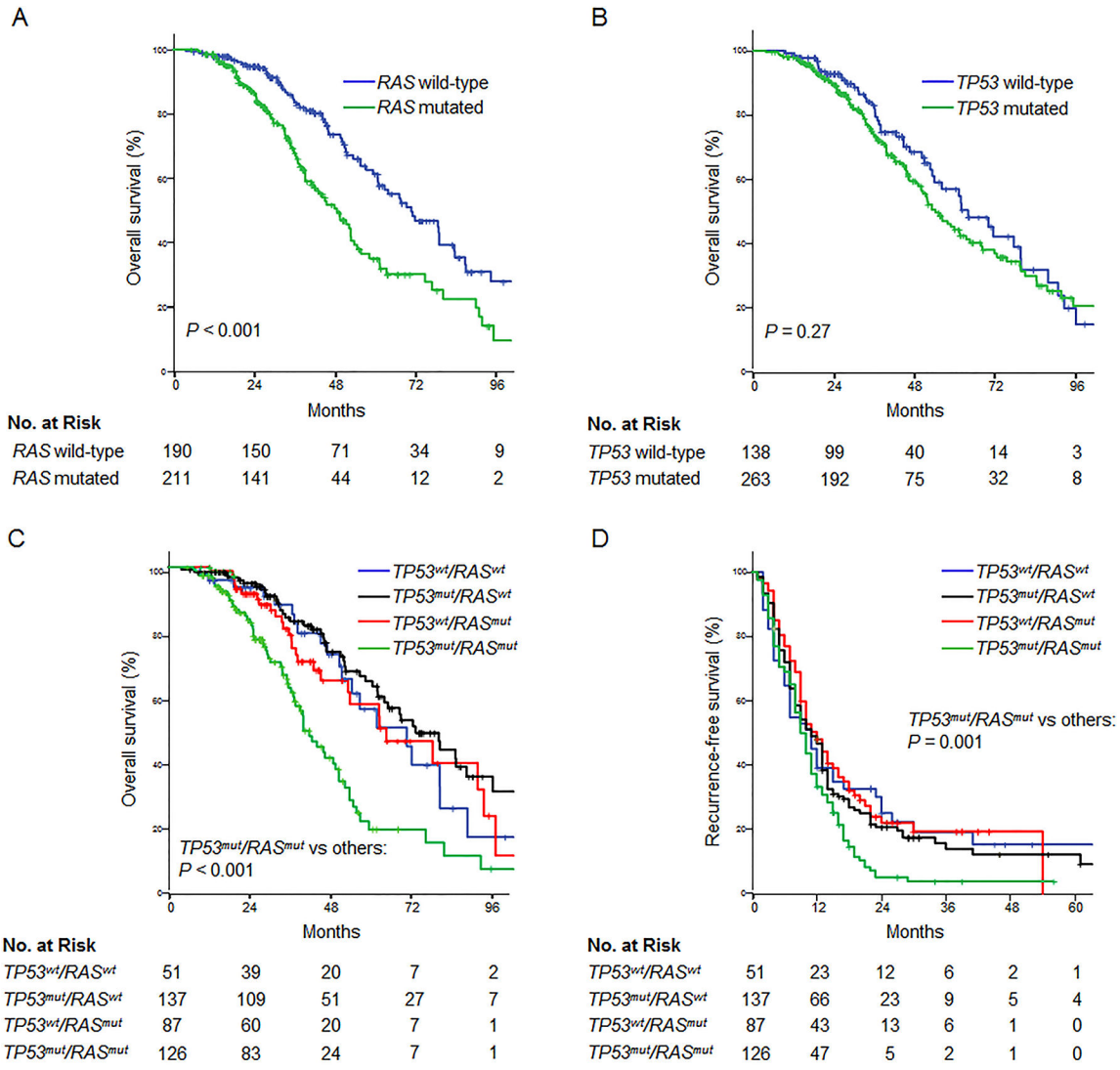

Figure 2.

Relationship between *RAS* and *TP53* mutations and overall survival after resection of colorectal liver metastases. Analysis of the entire patient cohort demonstrated a significant association between overall survival and *RAS* mutations (A) but not *TP53* mutations (B). Stratification by both mutations revealed that *TP53* mutations were significantly associated with overall survival among patients with concomitant *RAS* mutations (C). Similarly, the negative prognostic effect of *RAS* mutations was limited to patients with concurrent *TP53* mutations. Recurrence-free survival was decreased in patients with double mutation in *RAS/TP53* (D). Wt, wild-type; mut, mutated.

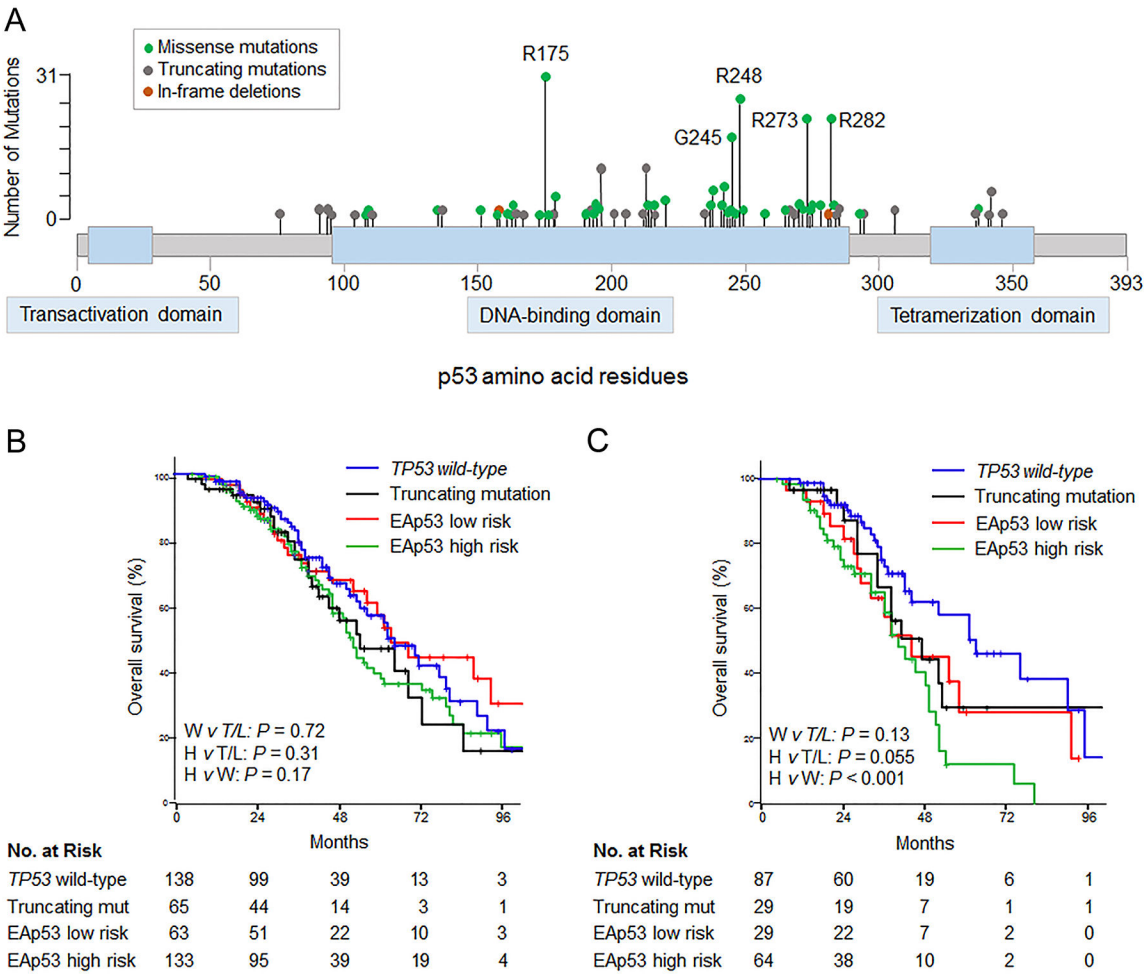

Figure 3.

(A) Lolliplots showing distribution of somatic mutations in *TP53* in 263 patients undergoing resection of colorectal liver metastases. (B) Overall survival by type of *TP53* mutation (n = 399). Two patients with *TP53* in-frame deletions were omitted from analysis. (C) Overall survival among *RAS* mutated patients, by type of *TP53* mutation (n = 209). EAp53, Evolutionary Action of p53; W, *TP53* wild-type; T/L, truncating or EAp53 low risk mutation; H, EAp53 high risk mutation; mut, mutation.

Table 1.

Patient and Tumor Characteristics

Characteristic	N	%
Median age, years (range)	54 (23–79)	
Gender		
Male	223	55.6
Female	178	44.4
Primary tumor location		
Right colon	99	24.7
Left colon and rectum	299	74.6
Rectum only	113	28.2
NA	3	0.8
Primary tumor regional lymph node status		
Positive	274	68.3
Negative	114	28.4
NA	13	3.2
Primary tumor T stage		
T1–2	58	14.5
T3–4	327	81.5
NA	16	4.0
Microsatellite instability (MSI) status		
MSI-stable	376	93.8
MSI-high	5	1.2
NA	20	5.0
Synchronous diagnosis of liver metastases	278	69.3
Chemotherapy before liver resection	338	84.3
Extrahepatic metastases	73	18.2
Median number of liver metastases (range)	2 (1–40)	
Median size of largest liver metastasis, cm (range)	2.6 (0.0–18.0)	
Surgical resection margin		
R0	294	73.3
R1	107	26.7
Radiofrequency ablation at time of liver resection	28	7.0
Major hepatectomy	185	46.1
Two-stage hepatectomy	36	9.0
Postoperative chemotherapy	204	50.9
Disease recurrence	333	83.0

Abbreviation: NA, data not available.

Table 2.

Comparison of Potential Prognostic Factors by *TP53* and *RAS* Mutations, N (%)

Variable	*RAS/TP53* Co-mutation N = 126	Absence of *RAS/TP53* Co-mutation N = 275	*P*
Median age, years (range)	54 (23–79)	56 (26–79)	0.55
Male gender	66 (52.4)	157 (57.1)	0.39
Primary tumor location *			0.006
Right	43 (34.1)	56 (20.6)	
Left	83 (65.9)	216 (79.4)	
Node-positive primary tumor †	95 (76.6)	179 (67.8)	0.094
Primary tumor T stage ‡			0.91
T1–2	18 (14.8)	40 (15.2)	
T3–4	104 (85.2)	223 (84.8)	
Synchronous liver metastases	81 (64.3)	197 (71.6)	0.16
Extrahepatic metastases	30 (23.8)	43 (15.6)	0.052
Median number of liver metastases (range)	2 (1–17)	2 (1–40)	0.75
Median size of liver metastases, cm (range)	2.4 (0.0–11.0)	2.7 (0.0–18.0)	0.10
R1 resection margin	31 (24.6)	76 (27.6)	0.55
Major hepatectomy	53 (42.1)	132 (48.0)	0.28
Two-stage hepatectomy	11 (8.7)	25 (9.1)	1.00
Disease recurrence	115 (91.3)	220 (80)	0.005
Recurrence site			0.30
Liver	38 (33.0)	76 (34.5)	
Lungs	40 (34.8)	57 (25.9)	
Peritoneum	6 (5.2)	20 (9.1)	
Liver and lungs	8 (7.0)	15 (6.8)	

* Data not available in 3 patients.

† Data not available in 13 patients.

‡ Data not available in 16 patients.

Table 3.

Multivariable Overall Survival (OS) Analysis of 401 Patients Undergoing Resection of Colorectal Liver Metastases (CLM)

Variable	Median OS (months)	5-year OS (%)	Univariable P	Multivariable P	HR (95% CI)
Age, years	–	–	0.79		
Gender			0.037	0.42	
Female	51	41.3			
Male	63	54.9			
Primary tumor location *			0.004	0.27	
Right	45	28.9			
Left	62	54.0			
Primary tumor lymph node status †			0.009	0.084	
Positive	58	49.6			
Negative	72	54.2			
Primary tumor T stage ‡			0.006	0.10	
T1–2	80	72.9			
T3–4	56	46.3			
Synchronous CLM			0.90		
Yes	59	49.9			
No	56	47.5			
Extrahepatic metastases			0.76		
Yes	53	41.4			
No	60	50.1			
Number of CLM			0.005	0.30	
Solitary	77	58.9			
Multiple	51	43.0			
Size of CLM			0.012	0.006	1.66 (1.16−2.38)
≥ 3 cm	50	45.2			
< 3 cm	60	50.4			
Surgical margin			0.005	0.060	
Positive	50	38.9			
Negative	61	52.4			
Major hepatectomy			0.40		
Yes	56	47.7			
No	59	49.9			
Two-stage hepatectomy			0.61		
Yes	50	33.5			
No	59	50.0			
RAS and *TP53* status			< 0.001		
$RAS^{wt}/TP53^{wt}$	70	56.9		Ref	Ref

Variable	Median OS (months)	5-year OS (%)	Univariable P	Multivariable P	HR (95% CI)
RAS^{mut}/*TP53*^{mut}	41	20.6		0.002	2.62 (1.41–4.87)
RAS^{mut}/*TP53*^{wt}	62	55.7		0.79	
RAS^{wt}/*TP53*^{mut}	72	64.0		0.88	
Double mutation in *APC* and *PIK3CA*			0.033	0.073	
Yes (N = 32)	48	12.6			
No (N = 369)	61	50.7			

Abbreviations: HR, hazard ratio; CI, confidence interval; wt, wild-type; mut, mutated; Ref, reference variable.

[*] Data not available in 3 patients.

[†] Data not available in 13 patients.

[‡] Data not available in 16 patients.