TP53 Gain-of-Function and Non–Gain-of-Function Mutations Are Differentially Associated With Sidedness-Dependent Prognosis in Metastatic Colorectal Cancer

Minggui Pan, MD, PhD[1,2]; Chen Jiang, PhD[2]; Pam Tse, BA[2]; Ninah Achacoso, MS[2]; Stacey Alexeeff, PhD[2]; Aleyda V. Solorzano, MD[2]; Elaine Chung, MA[2]; Wenwei Hu, MD, PhD[3]; Thach-Giao Truong, MD[4]; Amit Arora, MD[5]; Tilak Sundaresan, MD[6]; Jennifer Marie Suga, MD[4]; Sachdev Thomas, MD[4]; and Laurel A. Habel, PhD[2]

abstract

PURPOSE To examine the association of gain-of-function (GOF) and non–gain-of-function (non-GOF) *TP53* mutations with prognosis of metastatic right-sided (RCC) versus left-sided colorectal cancer (LCC).

METHODS This cohort study included patients with metastatic colorectal cancer (CRC) who had next-generation sequencing performed from November 2017 to January 2021. We defined R175H, R248W, R248Q, R249S, R273H, R273L, and R282W as GOF and all other mutp53 as non-GOF. We used Cox regression modeling to examine the association between GOF and non-GOF mutp53 and overall survival (OS), adjusting for age, sex, ethnicity, performance status, Charlson comorbidity index and receipt of chemotherapy.

RESULTS Of total 1,043 patients, 735 had tumors with mutp53 and 308 had wild-type p53 (wtp53). GOF was associated with worse OS than non-GOF mutp53 only in LCC (hazard ratio [HR] = 1.66 [95% CI, 1.20 to 2.29]), but not in RCC (HR = 0.79 [95% CI, 0.49 to 1.26]). Importantly, RCC was associated with worse OS than LCC only in the subset of patients whose CRC carried non-GOF (HR = 1.76 [95% CI, 1.30 to 2.39]), but not GOF mutp53 (HR = 0.92 [95% CI, 0.55 to 1.53]) or wtp53 (HR = 0.88 [95% CI, 0.60 to 1.28]). These associations were largely unchanged after also adjusting for RAS, BRAF, and PIK3CA mutations, and microsatellite instability-high.

CONCLUSION Poorer survival of patients with metastatic RCC versus LCC appeared to be restricted to the subset with non-GOF mutp53, whereas GOF versus non-GOF mutp53 was associated with poorer survival only among patients with LCC. This approach of collectively classifying mutp53 into GOF and non-GOF provides new insight for prognostic stratification and for understanding the mechanism of sidedness-dependent prognosis. If confirmed, future CRC clinical trials may benefit from incorporating this approach.

J Clin Oncol 40:171-179. © 2021 by American Society of Clinical Oncology

INTRODUCTION

TP53 mutation is present in more than 50% of colorectal cancer (CRC),[1-5] and is implicated in the majority of Li-Fraumeni syndrome cases, a hereditary cancer syndrome associated with early onset of leukemia and solid tumors.[6,7] p53 regulates numerous biologic processes including DNA repair, cell cycle arrest, apoptosis, cell senescence, metabolic pathways, and others primarily by modulating the expression of its target genes.[4,8-11]

The spectrum of p53 mutation (mutp53) is broad and varies in various malignancies, including missense mutation, nonsense mutation, deletion, frameshift, insertion, etc. Approximately 80% of these mutations occur in the DNA binding domain (DBD), most of which are missense mutations.[3,10,12,13] Approximately 30% of the mutations cluster within six mutation hotspots in the DBD: R175, G245, R248, R249, R273, and R282.[3,12,14] Several of these mutp53 have been shown to exhibit gain-of-function (GOF) properties, possessing novel functions that are not part of the wild-type p53 protein (wtp53).[3,12,15,16] The concept of GOF was first demonstrated by Dittmer et al who ectopically expressed mutp53 R175H and R273H in p53-null cells and showed enhanced tumorigenic potential in nude mice, higher efficiency in forming colonies in soft agar, and increased expression of drug-resistant genes.[3,12,17] This was consistent with the observation of patients with Li-Fraumeni syndrome whose onset of malignancies was significantly earlier when a missense mutation such as R175H was responsible compared with *TP53* deletion that causes the loss of

CONTEXT

Key Objective

The prognostic significance of p53 mutations in colorectal cancer (CRC) has not been established. This study investigates whether p53 mutations, when classified into gain-of-function (GOF) and non–gain-of-function (non-GOF) on the basis of review of current literature, could stratify the prognosis of right-sided versus left-sided metastatic CRC.

Knowledge Generated

Our study reveals that non-GOF p53 mutations were associated with worse prognosis of metastatic right-sided versus left-sided colorectal cancer, while GOF versus non-GOF p53 mutations were associated with worse prognosis in the left-sided but not right-sided colorectal cancer.

Relevance

Our findings suggest that different p53 mutations may differentially affect survival of patients with metastatic CRC on the basis of anatomic location and shed new light on the potential oncogenic mechanisms of p53 mutations in CRC. GOF versus non-GOF p53 mutations may collectively serve as useful biomarkers in guiding clinical practice with prognostic stratification and in the design of future CRC clinical trials.

function.[7,18] This observation could be replicated in knock-in mice carrying R175H, R273H, or R248Q that developed epithelial cancer with more rapid progression and shorter survival compared with the knock-out mice (with deletion of both *TP53* alleles) that primarily develop lymphoma.[19-21] Numerous experimental studies have found that GOF mutants increase cell invasion and proliferation, chemoresistance, colony formation, genomic instability, angiogenesis, etc.[3,12,15] GOF mutants can bind to novel proteins, form new interactions, enhance the signaling of receptor tyrosine kinases, and drive the expression of NF-kB, etc.[3,12,15,22-26]

In addition to the established molecular features (RAS, BRAF, and PIK3CA mutations, and microsatellite instability-high [MSI-high]) that are closely related to the survival of metastatic CRC, anatomic location (sidedness) of the primary tumor has emerged in recent years as an important prognostic factor.[27,28] Many studies have shown that metastatic right-sided CRC (proximal, RCC) is associated with worse survival than metastatic left-sided CRC (distal, LCC), including findings from several randomized clinical trials and a large meta-analysis.[29-32] The poorer survival of patients with RCC appears to be independent of the molecular features; however, its underlying mechanism remains unclear.[27,28]

Despite many lines of experimental evidence for p53 GOF in cell lines and animals, the data in human malignancies are limited. Our goal was to investigate the p53 GOF concept in human malignancy using the next-generation sequencing (NGS) data within Kaiser Permanente Northern California (KPNC) and to explore the relations between the sidedness of metastatic CRC, p53 mutation, and overall survival (OS).

METHODS

Study Population

Our data set includes KPNC patients with stage IV CRC with StrataNGS (Ann Arbor, MI) performed from November 2017 to January 2021. Patient data on demographics,

Charlson comorbidity index (CCI), performance status (PS), and lines of chemotherapy received were retrieved from the electronic medical record (Epic) and cancer registry database. CCI was based on the 12 month-period before the date of diagnosis of metastatic CRC. This study was approved by the KPNC institutional review board with waiver of consent.

StrataNGS

StrataNGS of advanced malignancies began in November 2017 in KPNC and initially included approximately 90 commonly mutated genes including *TP53*, *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, and MSI, expanded to include other genes in August 2019, and is currently a 429-gene, pan-solid tumor, NGS assay for formalin-fixed, paraffin-embedded tumor tissue, performed on coisolated DNA and RNA.[33]

Definition of GOF and Non-GOF p53 Mutation

There are no common established criteria for all the GOF p53 mutations in the literature. We defined the GOF and non-GOF in our data set on the basis of our literature review. There were 299 different individual p53 mutations identified in our data set. We focused on the six mutation hotspots in the DBD to examine the impact of mutp53 GOF: R175H, G245S, R248Q and R248W, R249S, R273H and R273L, and R282W.[3,12] Although a few other mutp53 have been shown to possess some GOF properties, the strength of evidence does not appear strong.[3,12] We further excluded G245S because the evidence was weak for it to be considered a GOF. For example, in a mouse knock-in experiment, G245S knock-in mice exhibited later onset of tumors and longer survival compared with the R248Q knock-in, similar to the mice that carried *TP53* deletion.[20] Also, patients with Li-Fraumeni syndrome carrying a germline G245S mutation had later onset of tumors compared with R175, R248, and R282 mutations.[19] Multiple GOF properties have been demonstrated with mutp53 R175H, R248Q, R248W, R249S, R273H, R273L, and R282W,

including increased invasion, cell proliferation, chemoresistance, and tumor growth in xenograft.[3,12,14,15] We identified a total of 204 patients with GOF in our cohort, including 83 patients with an R175H, 39 with an R248Q, 21 with an R248W, 4 with an R249S, 35 with an R273H, one with R273L, and 21 with an R282W. We classified all other mutp53 as non-GOF.

Definition of RCC and LCC

RCC was defined as colon cancer of ascending and transverse colon, and LCC defined as CRC from splenic flexure to rectum.

Statistical Analysis

OS was measured from the time of diagnosis of stage IV CRC to the time of death or end of follow-up (April 16, 2021, last OS data pull), whichever came first. Patients who were still alive at the end of follow-up were censored. Pearson's chi-squared test was used to determine statistical difference on demographics and distribution of p53, RAS, BRAF, and PI3K mutations, and MSI-high. We used the one-way analysis of variance test to assess differences in continuous variables. We used Kaplan-Meier plot to perform unadjusted OS analysis and estimate median OS. The number of patients at risk under the OS curves reflects delayed entry into the cohort at the time of receipt of NGS results (ie, left truncation, with study entry ranging from 0 to 9 years after diagnosis, median 4.0 months).[34] The prespecified alpha error was set at .05 in two-tailed test. To adjust for multiple testing, we controlled the false discovery rate at the level α = .05 and determined statistical significance by using the Benjamini-Hochberg procedure.[35] Cox proportional hazards regression models were used to estimate adjusted hazard ratios (HRs) and 95% CIs for the association between p53 mutations and OS, adjusted for covariates. Time since diagnosis of stage IV CRC was the time scale used in the regression models, allowing for left truncation. In our primary analysis, covariates included in our main regression models were age (continuous), sex (male and female), ethnicity (non-Hispanic White, Black, Asian, Hispanic, and other or unknown), PS (0-1, 2-3), CCI (continuous), and chemotherapy received (yes or no). In our secondary models, we also included the molecular features (RAS [yes or no], BRAF [yes or no], and PIK3CA [yes or no] mutations, and MSI-high [yes or no]). PS was the only variable with missing or unknown data, which we included as a dummy variable. The statistical analysis was performed using SAS software version 9.4, R (R Core Team, 2020).

RESULTS

OS of Patients With Metastatic CRC Bearing Individual mutp53 GOF

OS associated with each individual GOF mutp53 of interest is shown in the Data Supplement (online only). Median OS was 24.3, 48.2, 14.5, 3.1, 26.4, and 23.8 months, respectively, for R175H, R248Q, R248W, R249S, R273H and R273L, and R282W. Median OS for the entire GOF subset was 24.3 months.

Comparison of Demographics and the Molecular Features Between mutp53 and wtp53 and Between mutp53 GOF and Non-GOF Patients

As depicted in Table 1, of 1,043 patients with metastatic CRC with StrataNGS performed, 308 had tumors with wtp53 and 735 had mutp53. The estimated median OS was 25.1 months for the entire cohort. Median age of wtp53 patients was higher than that of mutp53 patients, although the two groups were generally similar with respect to sex, ethnicity, PS, CCI, or chemotherapy received. A higher percent of wtp53 patients had RCC, and a higher percent had RAS (including both KRAS and NRAS), BRAF, and PIK3CA mutations, and MSI-high. Between the GOF and the non-GOF subsets, there was no statistically significant difference in median age, sex, PS, CCI, or chemotherapy received, but more GOF patients were White, whereas more non-GOF patients were Asian (P = .04). There was no significant difference in the percent of RAS, BRAF, and PIK3CA mutations, and MSI-high.

Comparison of Demographics and the Molecular Features Between LCC and RCC

As depicted in Table 2, compared with patients with LCC, patients with RCC were on average older, and a larger percent had PS of Eastern Cooperative Oncology Group 2-3, higher CCI, fewer patients received and have received second-line chemotherapy, and had more RAS, BRAF, and PIK3CA mutations, and MSI-high, even when separated by GOF, non-GOF, and wtp53. Patients with either BRAF or RAS mutation showed worse OS than patients with both BRAF and RAS wild-type (Data Supplement), with HR of 2.10 and 1.26, respectively, after adjusting for age, sex, ethnicity, PS, CCI, and chemotherapy received (Data Supplement). OS of RCC was worse than OS of LCC (Data Supplement), with HR of 1.2 after adjusting for age, sex, ethnicity, PS, CCI, and chemotherapy received (Fig 1).

Mutp53 GOF Was Associated With Worse Survival Than mutp53 Non-GOF Only in Patients With LCC But Not RCC

In the entire cohort, unadjusted and adjusted OS were similar between patients with wtp53 and patients with mutp53 (Data Supplement and Fig 1). Unadjusted OS curves showed little difference between patients with GOF and patients with non-GOF mutp53 (Data Supplement). However, after adjusting for age, sex, ethnicity, PS, CCI, and chemotherapy received, OS of patients with GOF was approximately 30% worse than OS of patients with non-GOF (HR = 1.30 [95% CI, 1.00 to 1.68]), and this difference remained after further adjusting for the molecular features (RAS, BRAF, and PIK3CA mutations, and MSI-high; HR = 1.29 [95% CI, 0.99 to 1.67]; Fig 1). Intriguingly, OS of patients with LCC that bore mutp53 GOF was significantly worse than OS of patients with LCC that bore mutp53 non-GOF (median OS 24.6 *v* 33.5 months, P = .01;

TABLE 1. Comparison of Demographics and Molecular Features of Patients With Metastatic Colorectal Cancer by p53 Mutation

Demographics	wtp53 (n = 308)	mutp53 (n = 735)	P	GOF (n = 204)	Non-GOF (n = 531)	P
Median age, years	63.8 (27.1-91.1)	59.8 (18.1-92.2)	.001	59.2 (22.2-90.2)	60.3 (18.1-92.2)	.22
Female, No. (%)	145 (47.4)	328 (44.6)	.41	100 (49.3)	228 (42.9)	.11
Race, No. (%)						
White	173 (56.2)	371 (50.4)	.16	121 (59.6)	250 (46.9)	.04
Black	26 (8.4)	58 (7.9)		13 (6.4)	45 (8.5)	
Asian	47 (15.2)	162 (22.0)		33 (16.2)	129 (24.2)	
Hispanic	49 (15.9)	117 (15.9)		29 (14.3)	88 (16.5)	
Other	13 (4.2)	27 (3.7)		7 (3.4)	20 (3.7)	
PS, No. (%)						
0-1	154 (50.0)	388 (52.8)	.25	105 (51.5)	273 (51.4)	.78
2-3	37 (12.0)	64 (8.7)		16 (7.8)	48 (9.0)	
Unknown	117 (38.0)	283 (38.5)		83 (40.7)	210 (39.5)	
CCI (median)	3 (0-15)	3 (0-16)	.46	3 (0-14)	3 (0-15)	.87
Primary tumor location, No. (%)						
RCC	152 (49.3)	215 (29.3)	< .001	65 (32.0)	150 (28.2)	.34
LCC	156 (50.7)	520 (70.7)		139 (68.0)	381 (71.8)	
Chemotherapy received, No. (%)						
First line	280 (90.9)	668 (90.9)	.99	186 (91.2)	482 (90.8)	.87
Second line	164 (53.2)	423 (57.6)	.20	118 (57.8)	305 (57.4)	.92
Third line	72 (23.4)	167 (22.7)	.82	47 (23.0)	120 (22.6)	.90
RAS mutation, No. (%)	198 (63.3)	335 (45.5)	< .001	91 (44.8)	244 (45.8)	.82
BRAF mutation, No. (%)	31 (10.1)	45 (6.1)	.03	13 (6.4)	32 (6.0)	.84
PIK3CA mutation, No. (%)	100 (32.5)	79 (10.7)	< .001	25 (12.3)	54 (10.1)	.39
MSI-high, No. (%)	25 (8.1)	8 (1.1)	< .001	2 (1.0)	6 (1.1)	.87

NOTE. Comparisons between patients whose tumor bore wtp53 or mutp53, and between patients whose tumor bore GOF mutp53 or non-GOF mutp53. Molecular features: RAS, BRAF, and PIK3CA mutations, and MSI-high. The first- and second-line chemotherapy were primarily a combination of fluorouracil, leucovorin, or capecitabine, plus oxaliplatin or irinotecan (FOLFOX, CAPOX, FOLFIRI), with or without bevacizumab, or cetuximab, or panitumumab. The third and later lines included primarily capecitabine, regorafenib, trifluridine, and tipiracil hydrochloride or an immune checkpoint inhibitor.

Abbreviations: CCI, Charlson comorbidity index; GOF, gain-of-function; LCC, left-sided colorectal cancer; MSI-high, microsatellite instability-high; mutp53, p53 mutation; non-GOF, non–gain-of-function; PS, performance status; RCC, right-sided colon cancer; wtp53, wild-type p53.

Fig 2A), and this difference remained after adjusting for age, sex, ethnicity, PS, CCI, and chemotherapy received (P = .002; HR = 1.66 [95% CI, 1.20 to 2.29]) and after further adjusting for the molecular features (P = .002; HR = 1.66 [95% CI, 1.20 to 2.30]; Fig 1). By contrast, OS of patients with RCC that bore mutp53 GOF was not worse than OS of patients with RCC that bore mutp53 non-GOF (median OS 23.6 v 13.8 months, P = .08; Fig 2B), even after adjusting for age, sex, ethnicity, PS, CCI, and chemotherapy received (HR = 0.79 [95% CI, 0.49 to 1.26]) and after further adjusting for the molecular features (HR = 0.73 [95% CI, 0.47 to 1.21]; Fig 1).

Mutp53 Non-GOF But Not GOF Was Associated With Worse Survival of Patients With Metastatic RCC Versus LCC

There was no meaningful OS difference between patients with RCC and patients with LCC that bore wtp53, either

unadjusted or adjusted (Data Supplement and Fig 1). However, there was a significant OS difference between patients with RCC and patients with LCC that bore mutp53 (median OS 17.6 v 30.7 months, P < .001; Fig 3A), with HR of 1.48 (P = .004; 95% CI, 1.14 to 1.91) after adjusting for age, sex, ethnicity, PS, CCI, and chemotherapy received, and HR of 1.33 (P = .03; 95% CI, 1.02 to 1.73) after further adjusting for the molecular features (Fig 1). Interestingly, there was no meaningful OS difference between patients with RCC and LCC whose CRC bore mutp53 GOF, either unadjusted (Fig 3B), or adjusted for age, sex, ethnicity, PS, CCI, and chemotherapy received or further adjusted for the molecular features (Fig 1). By contrast, there was a dramatic OS difference between patients with RCC and LCC whose CRC bore mutp53 non-GOF (median OS 13.8 v 33.5 months, P < .001; Fig 3C), and this OS difference remained highly significant after adjusting for age, sex, ethnicity, PS, CCI, and chemotherapy received (HR = 1.76 [95% CI, 1.30 to 2.39])

TABLE 2. Comparison of Demographics and Molecular Features of Patients With Metastatic LCC Versus RCC

All Patients	LCC (n = 676)	RCC (n = 367)	P
Median age, years	59.1 (23.3-92.2)	65.6 (18.1-90.7)	< .001
Female, No. (%)	301 (44.5)	172 (46.9)	.47
Ethnicity, No. (%)			
White	351 (51.9)	193 (52.6)	.24
Black	48 (7.1)	36 (9.8)	
Asian	146 (21.6)	63 (17.2)	
Hispanic	108 (16.0)	58 (15.8)	
Other	23 (3.6)	17 (4.6)	
PS, No. (%)			
0-1	371 (54.9)	171 (46.6)	.002
2-3	51 (7.5)	50 (13.6)	
Unknown	254 (37.6)	146 (39.8)	
CCI (median)	3 (0-15)	4 (0-16)	.01
Chemotherapy received, No. (%)			
First line	621 (91.9)	327 (89.1)	.14
Second line	399 (59.0)	188 (51.2)	.02
Third line	162 (24.0)	77 (21.0)	.27
RAS mutation, No. (%)	326 (48.2)	207 (56.4)	.01
BRAF mutation, No. (%)	22 (3.3)	54 (14.7)	< .001
PIK3CA mutation, No. (%)	94 (13.9)	85 (23.2)	< .001
MSI-high, No. (%)	11 (1.6)	22 (6.0)	< .001
Non-GOF (n = 531)	**LCC (n = 381)**	**RCC (n = 150)**	**P**
RAS, No. (%)	163 (42.8)	80 (53.3)	.03
BRAF, No. (%)	10 (2.6)	21 (14.0)	< .001
PIK3CA, n (%)	34 (8.9)	20 (13.3)	.13
MSI-high, No. (%)	2 (0.5)	4 (2.7)	.04
GOF (n = 204)	**LCC (n = 139)**	**RCC (n = 65)**	**P**
RAS, No. (%)	60 (43.2)	32 (49.2)	.42
BRAF, No. (%)	5 (3.6)	9 (13.8)	.01
PIK3CA, No. (%)	15 (10.8)	10 (15.4)	.35
MSI-high, No. (%)	1 (0.7)	1 (1.5)	.58
Wtp53 (n = 308)	**LCC (n = 156)**	**RCC (n = 152)**	**P**
RAS, No. (%)	103 (66.0)	95 (62.5)	.52
BRAF, No. (%)	7 (4.5)	24 (15.8)	.001
PIK3CA, No. (%)	45 (28.8)	55 (36.2)	.17
MSI-high, No. (%)	8 (5.1)	17 (11.2)	.05

NOTE. Molecular features: RAS, BRAF, and PIK3CA mutations, and MSI-high.
Abbreviations: CCI, Charlson comorbidity index; GOF, gain-of-function; LCC, left-sided colorectal cancer; MSI-high, microsatellite instability-high; PS, performance status; RCC, right-sided colon cancer.

and after further adjusting for the molecular features (HR = 1.63 [95% CI, 1.19 to 2.23]; Fig 1).

The comparisons between RCC versus LCC among patients with non-GOF mutp53, between GOF versus non-GOF among patients with LCC, and between RCC versus LCC among patients with mutp53 meet statistical significance on the basis of false discovery rate (Data Supplement).[35] We have illustrated the key findings in the flow diagram (Fig 3D).
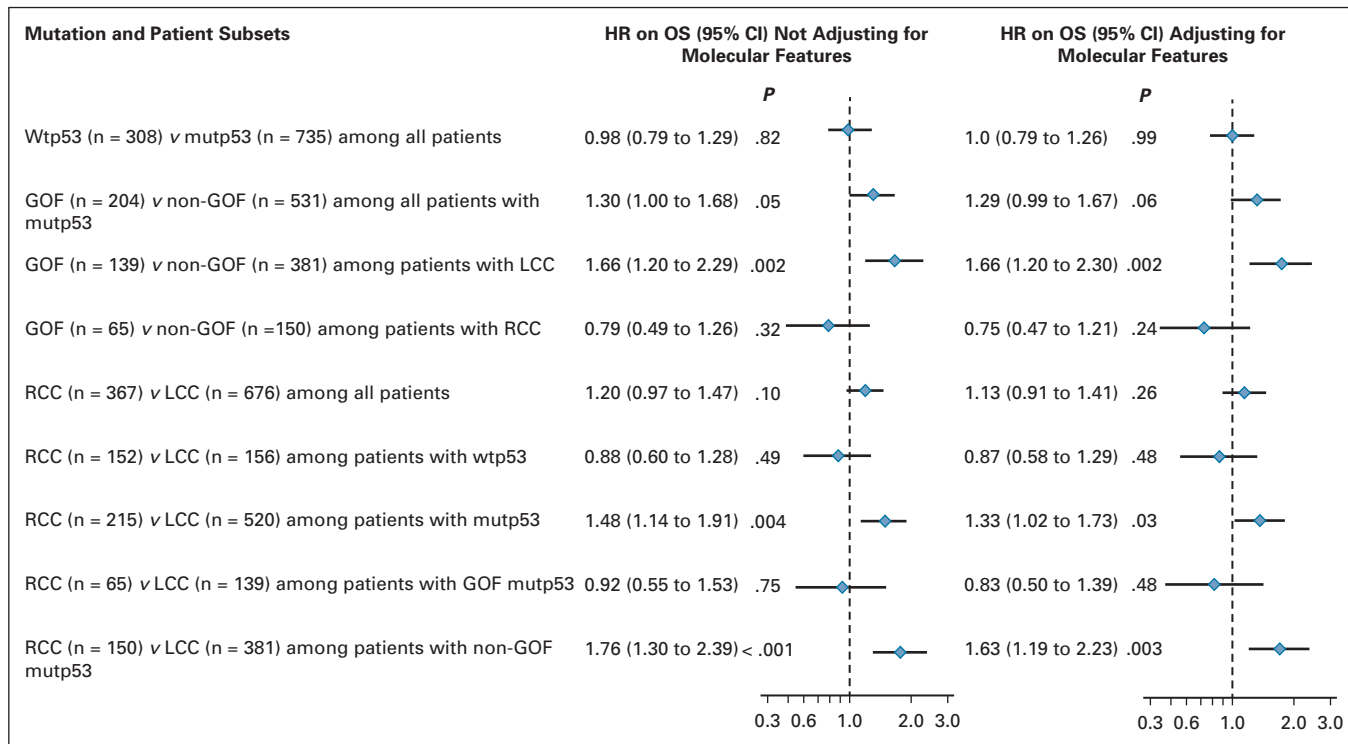
DISCUSSION

In this study in which p53 mutations were classified into GOF or non-GOF, we have uncovered unexpected associations between mutp53 and sidedness-dependent prognosis of patients with metastatic CRC: GOF versus non-GOF mutp53 was associated with inferior survival in patients with LCC but not RCC, whereas non-GOF but not GOF mutp53 was associated with poorer survival of RCC versus LCC. The magnitude of OS difference between patients with RAS or BRAF mutation versus RAS and BRAF wild-type, and between patients with RCC versus LCC was consistent with what has been reported in the literature,[28,29,32,36,37] which supports the validity of our data set.

Our definition of GOF and non-GOF is likely incomplete as many of the individual mutp53 have not been studied for the GOF properties; however, such an approach allowed us to define the patients with metastatic CRC into distinct subsets with different prognosis. GOF mutp53 occur in high frequency and exhibit extensive loss of transcriptional function, whereas non-GOF mutp53 occur in low frequency, many of them preserve certain level of transcriptional function, and are capable of activating some of the p53-regulated genes.[26,38]

Most intriguingly, despite that patients with non-GOF mutp53 collectively had significantly better OS than patients with GOF mutp53, non-GOF mutp53 uniquely marked a population of patients with RCC, but not LCC, with poor survival. The poor survival of RCC appears to be a function of the non-GOF mutp53 patients, independent of GOF or wtp53. Non-GOF mutp53 is not likely to be directly responsible for the poor survival of patients with RCC, since patients with LCC had similar percent of non-GOF mutp53 and yet better survival than patients with RCC. It is more likely that non-GOF mutp53 is a biomarker for RCC and contributes to promoting the oncologic behavior by cooperating with other unidentified molecular lesions that play major roles in its poor survival. These molecular lesions could include components of the pathways targeted by high-fat diet, bile acids, CRC-associated microbiome, and other carcinogenic insults.[39-41] In the non-GOF subset, the patients with RCC had significantly higher percent of RAS and BRAF mutations than the patients with LCC; however, this does not explain the worse OS of the non-GOF mutp53 RCC patients, because in the GOF mutp53 and the wtp53 subsets, the patients with RCC had similarly higher percent of BRAF mutation and yet similar OS compared with the patients with LCC. Importantly, even after further adjusting for RAS, BRAF, and PIK3CA mutations, and MSI-high, the

| Mutation and Patient Subsets	HR on OS (95% CI) Not Adjusting for Molecular Features		HR on OS (95% CI) Adjusting for Molecular Features	
		P		*P*
Wtp53 (n = 308) *v* mutp53 (n = 735) among all patients	0.98 (0.79 to 1.29)	.82	1.0 (0.79 to 1.26)	.99
GOF (n = 204) *v* non-GOF (n = 531) among all patients with mutp53	1.30 (1.00 to 1.68)	.05	1.29 (0.99 to 1.67)	.06
GOF (n = 139) *v* non-GOF (n = 381) among patients with LCC	1.66 (1.20 to 2.29)	.002	1.66 (1.20 to 2.30)	.002
GOF (n = 65) *v* non-GOF (n =150) among patients with RCC	0.79 (0.49 to 1.26)	.32	0.75 (0.47 to 1.21)	.24
RCC (n = 367) *v* LCC (n = 676) among all patients	1.20 (0.97 to 1.47)	.10	1.13 (0.91 to 1.41)	.26
RCC (n = 152) *v* LCC (n = 156) among patients with wtp53	0.88 (0.60 to 1.28)	.49	0.87 (0.58 to 1.29)	.48
RCC (n = 215) *v* LCC (n = 520) among patients with mutp53	1.48 (1.14 to 1.91)	.004	1.33 (1.02 to 1.73)	.03
RCC (n = 65) *v* LCC (n = 139) among patients with GOF mutp53	0.92 (0.55 to 1.53)	.75	0.83 (0.50 to 1.39)	.48
RCC (n = 150) *v* LCC (n = 381) among patients with non-GOF mutp53	1.76 (1.30 to 2.39)	< .001	1.63 (1.19 to 2.23)	.003

FIG 1. Forest plot for HR for OS associated with p53 mutation and sidedness. Multivariate analysis was performed using Cox regression models adjusting for age, sex, race, performance status, Charlson comorbidity index, chemotherapy received (left column), and further adjusting for molecular features including RAS, BRAF, and PIK3CA mutations, and MSI-high (right column). GOF, gain-of-function mutp53; HR, hazard ratio; LCC, left-sided colorectal cancer; MSI-high, microsatellite instability-high; mutp53, p53 mutation; non-GOF, non–gain-of-function mutp53; OS, overall survival; RCC, right-sided colon cancer; wtp53, wild-type p53.

OS of the RCC non-GOF patients remained dramatically worse than the OS of the LCC non-GOF patients.

Our study sheds new light for understanding the sidedness-dependent prognosis. Embryonically, right-sided colon arises from the mid-gut, whereas left-sided colon and the rectum from the hind-gut.[42] How this embryonic patterning mechanism is related to the distinct role of mutp53 GOF and non-GOF in these two anatomically defined CRC subsets would be interesting to investigate. The p53 mutation pattern, either somatic or germline, exhibits tissue-specific preferences.[38,43] There are differences in the gene expression profile between normal right colon epithelium and left colon epithelium; however, it is not known as to whether the intestinal stem cells of the right versus left
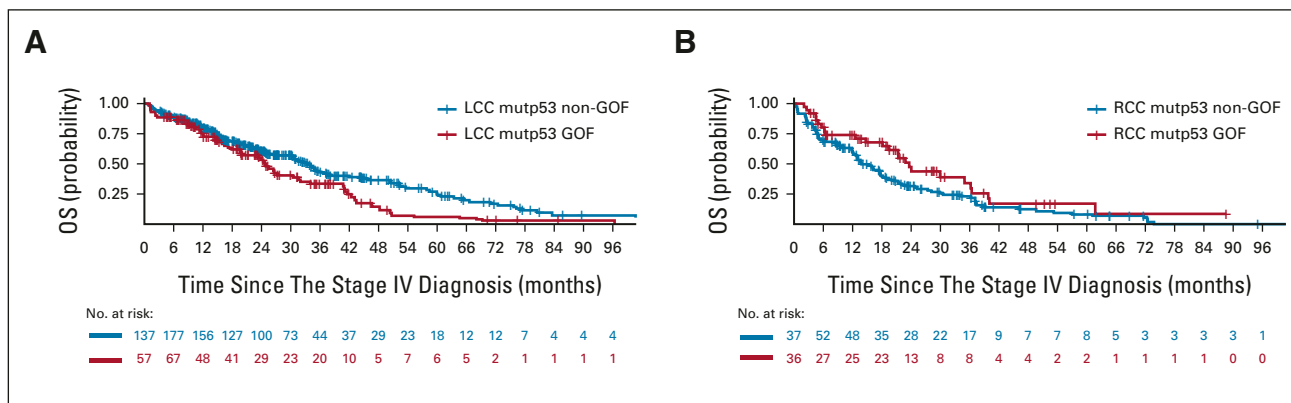
FIG 2. (A) Kaplan-Meier OS curves for patients with metastatic LCC that bore GOF versus non-GOF mutp53. Median OS was 24.6 (GOF, n = 139) versus 33.5 (non-GOF, n = 381) months, *P* = .01. (B) Kaplan-Meier OS curves for patients with metastatic RCC with GOF versus non-GOF p53 mutp53. Median OS was 23.6 (RCC mutp53 GOF, n = 65) versus 13.8 (RCC mutp53 non-GOF, n = 150) months, *P* = .08. GOF, gain-of-function; LCC, left-sided colorectal cancer; mutp53, p53 mutation; non-GOF, non–gain-of-function; OS, overall survival; RCC, right-sided colon cancer.
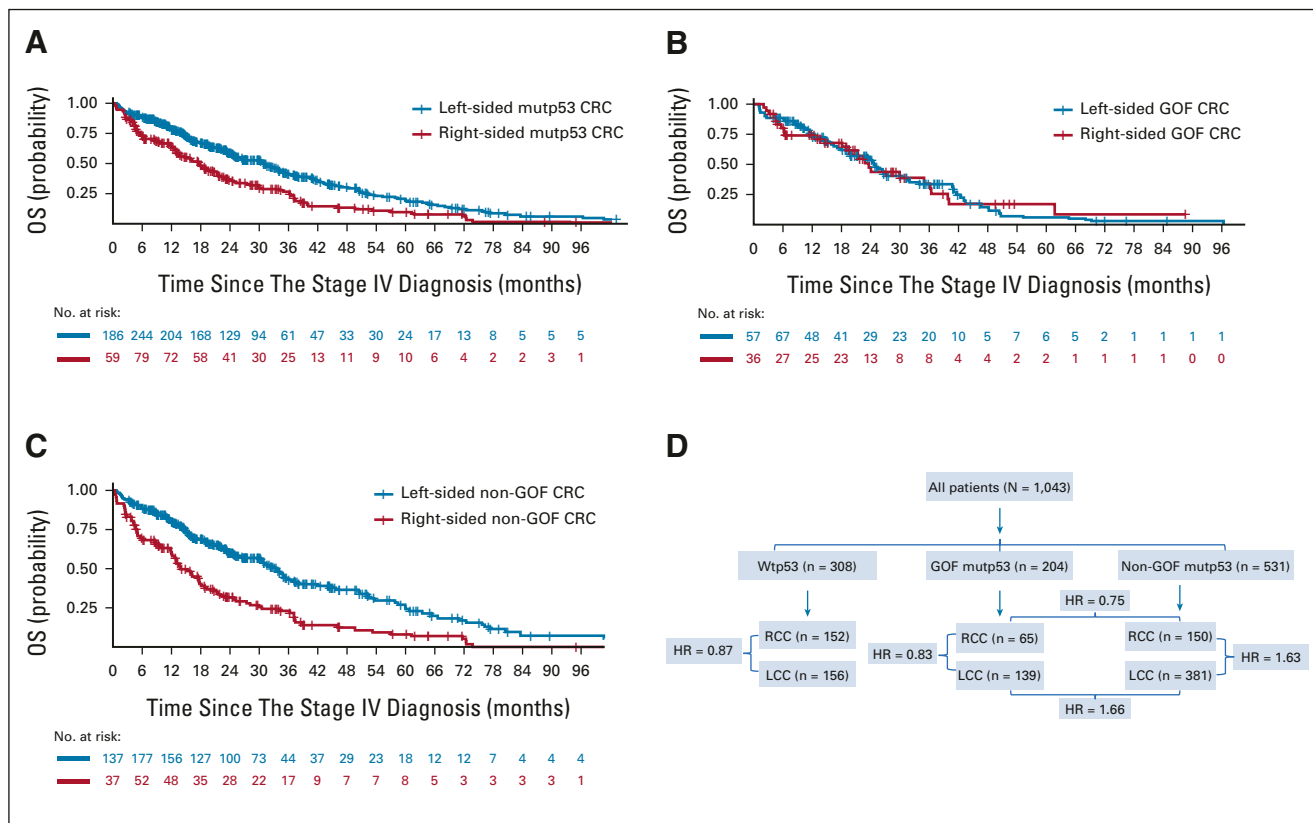
FIG 3. (A) Kaplan-Meier OS curves for patients with metastatic RCC versus LCC that bore mutp53. Median OS was 17.6 (right-sided mutp53 CRC, n = 215) versus 30.7 (left-sided mutp53 CRC, n = 520) months, *P* < .001. (B) Kaplan-Meier OS curves for patients with metastatic RCC versus LCC that bore gain-of-function p53 mutation (GOF). Median OS was 23.6 (right-sided GOF CRC, n = 65) versus 24.6 (left-sided GOF CRC, n = 139) months, *P* = .96. (C) Kaplan-Meier OS curves for patients with metastatic RCC versus LCC that bore non–gain-of-function p53 mutation (non-GOF). Median OS was 13.8 (right-sided non-GOF CRC, n = 150) versus 33.5 (left-sided non-GOF CRC, n = 381) months, *P* < .001. (D) Flow diagram illustrating key findings. HR = 0.83 for RCC versus LCC among patients with GOF mutp53, while 1.63 among patients with non-GOF mutp53; HR = 0.75 for GOF versus non-GOF among patients with RCC, while 1.66 among patients with LCC. CRC, colorectal cancer; GOF mutp53, gain-of-function p53 mutations; HR, hazard ratio; LCC, left-sided colorectal cancer; non-GOF mutp53, non–gain-of-function p53 mutations; OS, overall survival; RCC, right-sided colon cancer; Wtp53, p53 wild-type.

colon possess distinct sensitivity to GOF and non-GOF mutp53.[27] This could potentially be studied in mice by knocking-in GOF and non-GOF *mutp53* separately into LGR5+ cells or using organoids in cultures.[44] RCC is associated more frequently with BRAF mutation, MSI-high, CpG methylation, and resistance to anti-EGFR therapy, whereas LCC is associated more frequently with mutp53, ERBB2, and EGFR amplification, and response to anti-EGFR therapy.[27] There is evidence that RCC but not LCC is associated with mucosal bacteria biofilms in the gut.[45-48] Kadosh et al[49] showed that gut microbiome could switch the action of GOF mutp53 R172H from being tumor-suppressive to oncogenic by activating WNT pathways, but only in the mouse jejunum and not ileum. Our findings may also be valuable in aiding the classification of molecular subtypes of CRC.[27,50,51] In addition, our findings suggest that a reanalysis, using mutp53 GOF and non-GOF as additional biomarkers, of the previously completed large clinical trials evaluating the efficacy of cetuximab and bevacizumab plus chemotherapy may reveal additional insight.[31,32,36,37,52-54] Future CRC clinical trials may benefit from incorporating mutp53 GOF and non-GOF as biomarkers in the design.

This study has several strengths. Our data set is relatively large with more than 1,000 patients who received comprehensive primary and specialty services from a large integrated health care system that consists of 21 medical centers. In addition, the diverse membership is relatively stable, and electronic records capture virtually all encounters, diagnoses, and procedures. Our study also has limitations. It is a retrospective study, and a number of patients did not have StrataNGS until several months to years after their diagnosis of metastatic disease. Nonetheless, we used appropriate statistical methods to address this issue.[34] Also, the number of patients with some specific GOF mutp53 was small, limiting our ability to examine potential heterogeneity of OS findings across mutations. In addition, the follow-up length for some patients remained relatively short.

In summary, our study suggests that the functional classification of p53 mutations into GOF and non-GOF could help define the prognosis of metastatic CRC by sidedness and adds to the toolbox of the useful biomarkers. If confirmed, our findings could have important implications in clinical practice, future clinical trial design, and understanding the mechanism of CRC oncogenesis.

AFFILIATIONS

[1]Department of Oncology and Hematology, Kaiser Permanente, Santa Clara, CA
[2]Division of Research, Kaiser Permanente, Oakland, CA
[3]Department of Radiation Oncology, Rutgers Cancer Institute of New Jersey, Rutgers, State University of New Jersey, New Brunswick, NJ
[4]Department of Oncology and Hematology, Kaiser Permanente, Vallejo, CA
[5]Department of Oncology and Hematology, Kaiser Permanente, Fremont, CA
[6]Department of Oncology and Hematology, Kaiser Permanente, San Francisco, CA

CORRESPONDING AUTHOR

Minggui Pan, MD, PhD, Division of Research and Department of Oncology and Hematology, Kaiser Permanente, 710 Lawrence Expressway, Santa Clara, CA 95051; e-mail: minggui.pan@kp.org.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.21.02014.

DATA SHARING STATEMENT

Kaiser Permanente Northern California (KPNC) Institutional Review Board has not provided approval for StrataNGS data on individual patients used in this study to be placed in a public access repository. However, researchers can request access to use this study data by contacting the DOR Data Sharing Workgroup at DOR-DataSharingWorkgroup@kp.org.

AUTHOR CONTRIBUTIONS

Conception and design: Minggui Pan, Sachdev Thomas, Laurel A. Habel
Financial support: Minggui Pan
Administrative support: Minggui Pan, Pam Tse, Elaine Chung, Sachdev Thomas
Provision of study materials or patients: Minggui Pan, Chen Jiang, Ninah Achacoso, Thach-Giao Truong, Laurel A. Habel
Collection and assembly of data: Minggui Pan, Pam Tse, Ninah Achacoso, Aleyda V. Solorzano, Elaine Chung, Thach-Giao Truong, Amit Arora, Jennifer Marie Suga, Laurel A. Habel
Data analysis and interpretation: Minggui Pan, Chen Jiang, Ninah Achacoso, Stacey Alexeeff, Wenwei Hu, Amit Arora, Tilak Sundaresan, Sachdev Thomas, Laurel A. Habel
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

TP53 Gain-of-Function and Non–Gain-of-Function Mutations Are Differentially Associated With Sidedness-Dependent Prognosis in Metastatic Colorectal Cancer

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Stacey Alexeeff
Employment: The Permanente Medical Group

Wenwei Hu
Patents, Royalties, Other Intellectual Property: A patent application has been submitted (PCT/US20/20141). Title: LIF therapy for inducing intestinal epithelial cell regeneration

No other potential conflicts of interest were reported.