Multiomic Characterization of *RCC1* and *RCC2* Expression and Their Association With Molecular Alterations, Immune Phenotypes, and Cancer Outcomes

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ABSTRACT

PURPOSE Regulator of chromosome condensation 1 (*RCC1*) and *RCC2* have been shown to play important roles in the regulation of cell cycle, DNA damage response, and nucleocytoplasmic transport.

MATERIALS AND METHODS DNA (592-gene or whole exome) and RNA (whole transcriptome) sequencing was performed at Caris Life Sciences (Phoenix, AZ). Samples were stratified by *RCC1* expression quartile thresholds (Q1: low, Q4: high) for small cell lung cancer (SCLC; n = 876), non–small cell lung cancer (NSCLC; n = 21,603), gastric cancer (GC; n = 1,908), pancreatic cancer (PC; n = 5,071), and colorectal cancer (CRC; n = 14,892). Statistical significance was determined using chi-square and Wilcoxon rank-sum tests and adjusted for multiple comparisons (*$P < .05$). Corresponding analyses were run for *RCC2*.

RESULTS Median *RCC1* mRNA expression was highest in SCLC (14.3 transcript per million [TPM]), followed by GC (9.9), NSCLC (9.9), CRC (9.8), and PC (6.9). Similar to *RCC1*, the median *RCC2* expressions were highest in SCLC (36.2 TPM). Tumor mutational burden-high rates were positively associated with increasing *RCC1* expression quartiles (Q1-4) in NSCLC (31%-41%), GC (7%-22%), and CRC (5%-17%) and with increasing *RCC2* expression in NSCLC and CRC only. Higher expression with *RCC1* and *RCC2* was associated with worse overall survival in NSCLC (hazard ratio [HR] for *RCC1* and *RCC2* were 1.3 and 1.3, respectively), PC (HR for *RCC1* and *RCC2* were 1.5 and 1.12, respectively), and CRC (HR for *RCC1* and *RCC2* were 1.3 and 1.03, respectively).

CONCLUSION *RCC1* and *RCC2* expression is a negative prognostic marker in NSCLC, PC, and CRC. Further studies to investigate *RCC1* and *RCC2* function at the molecular level may provide opportunities for novel targeted drug development.

ACCOMPANYING CONTENT

Data Supplement

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INTRODUCTION

Regulator of chromosome condensation 1 (*RCC1*) is the only identified guanine nucleotide exchange factor (GEF) for the nuclear Ras-like G protein Ran. Ran is a GTPase that cycles between GDP-bound and GTP-bound states.[1] Ran is found to be highly concentrated in the nucleus where it is predominantly present in the Ran-GTP form. In the cytoplasm, lower concentrations of Ran are found and are mainly in the GDP-bound form, thus creating a gradient of Ran-GTP in the cells.[1] This is regulated by the asymmetric and mutually exclusive distribution of cytosolic GTPase activating proteins (GAPs), such as Ran GAP1, and nuclear *RCC1* which is the sole identified GEF for Ran.[2] The many roles[3-9] of *RCC1* are summarized in Figures 1A-1F.

Studies have shown that *RCC1* is involved in multiple critical cellular processes such as cell cycle regulation, DNA damage, and aging, in addition to its Ran-related functions.[10-12]

RCC2, a second member which belongs to the *RCC* family, contains seven *RCC1*-like domains. As with *RCC1*, *RCC2* has guanine exchange activity and plays a role in cell cycle regulation[13,14] (Fig 1). *RCC2* was identified originally for its role in mitosis[15] and has been postulated to be involved in the regulation of cell cycle during interphase.[13,16-18] In addition, *RCC2* prevents the activation of RAC1 by its GEFs, thereby suppressing RAC1-mediated reorganization of actin cytoskeleton.[19,20]

Published data support a strong role of *RCC1* and *RCC2* in carcinogenesis. To identify the landscape of coexpressed

CONTEXT

Key Objective
What can we learn from molecularly characterizing tumors with regulator of chromosome condensation 1 (*RCC1*) and *RCC2* expression?

Knowledge Generated
Important genomic coalterations were identified, such as progressive increase in the rates of *TP53* mutation with higher expression of *RCC1* and *RCC2* was seen in non–small cell lung cancer (NSCLC), pancreatic cancer (PC), and colorectal cancer (CRC). *RCC1* and *RCC2* expression is a negative prognostic marker in NSCLC, PC, and CRC.

Relevance *(P.L. Kunz)*
RCC1 and *RCC2* have been shown to play important roles in carcinogenesis. *RCC1* and *RCC2* expression is a negative prognostic marker in NSCLC, PC, and CRC. Further studies to investigate RCC1 and RCC2 function at the molecular level may provide opportunities for novel targeted drug development.*

Plain Language Summary *(M. Lewis)*
Genes called *RCC1* and *RCC2* play an important role in how cells divide and become cancer. When these genes are expressed, they indicate a worse prognosis for patients with lung cancer, prostate cancer and colon cancer. Future studies may help us determine if *RCC1* and *RCC2* are potential targets for cancer treatments.†

*Relevance section written by *JCO Oncology Advances* Editor-in-Chief Pamela L. Kunz, MD.
†Plain Language Summary written by *JCO Oncology Advances* Associate Editor Mark Lewis, MD.

mutations and amplifications of other genes activated in the tumor microenvironment, we analyzed the association of *RCC1* and *RCC2* expression with PD-L1 expression, tumor mutational burden (TMB), and immune cell count in tumor microenvironments and characterized genomic coalterations in *RCC1*- and *RCC2*-overexpressing tumors. We also analyzed the association of *RCC1* and *RCC2* overexpression with patient outcomes.

MATERIALS AND METHODS

Tissue Samples

Consecutive tumors were analyzed using DNA (592-gene or whole exome)/RNA (whole transcriptome) sequencing by Caris Life Sciences (Phoenix, AZ) as part of routine profiling. Samples were stratified by *RCC1* and *RCC2* expression quartile thresholds (Q1: low, Q4: high) for small cell lung cancer (SCLC; n = 876), non–small cell lung cancer (NSCLC; n = 21,603), gastric cancer (GC; n = 1,908), pancreatic cancer (PC; n = 5,071), and colorectal cancer (CRC; n = 14,892). These tumor types were specified at the start of the project as cancer types of interest as it was not feasible to perform biomarker associations in all cancer types.

Next-Generation Sequencing

Genomic DNA was isolated from formalin-fixed paraffin-embedded (FFPE) samples that underwent microdissection to enrich tumor purity and subjected to next-generation sequencing (NGS) using the NextSeq or NovaSeq 6000 platforms (Illumina, Inc, San Diego, CA). For NextSeq-sequenced tumors, a custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA). For NovaSeq-sequenced tumors, whole-exome sequencing was performed. The full list of genes assayed can be found as previously described.[21]

NGS of RNA used a hybrid capture method to pull down the full transcriptome from FFPE tumor samples using the Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies, Santa Clara, CA) and the Illumina NovaSeq platform (Illumina, Inc, San Diego, CA). A full 22,948-gene data set of expression data was produced by the Salmon and was further processed for RNA variants using a proprietary custom detection pipeline.[22]

Immunohistochemistry

Immunohistochemistry was performed on full FFPE sections of glass slides and validated per Clinical Laboratory Improvement Amendments/College of American Pathologists and International Organization of Standardization requirements.

Gene Fusion Detection

Gene fusions were detected by RNA sequencing using either the ArcherDx fusion assay (Archer FUSIONPlex Solid Tumor panel, Coralville, IA) or whole-transcriptome sequencing assay (Illumina NovaSeq platform; Illumina, Inc, San Diego, CA).[23]

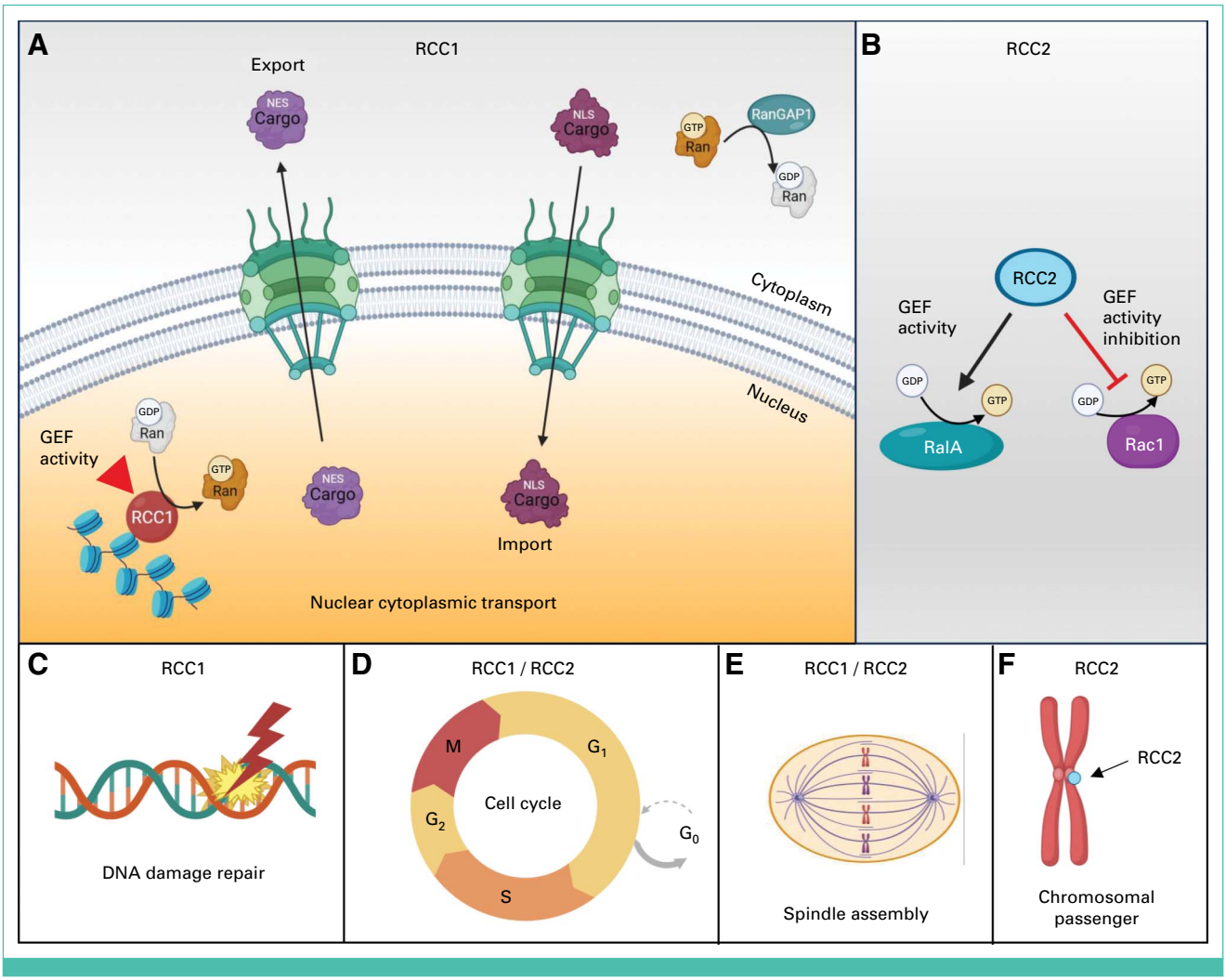


FIG 1. Functions of RCC1 and RCC2. RCC1 family proteins have various functions including guanine nucleotide exchange on small GTPases. RCC1 and RCC2 are two members of this family which play important roles in cell cycle and have been linked to several cancers. (A) RCC1 acts as a GEF on Ran GTPase, thereby acting within Ran-mediated nuclear cytoplasmic transport. (B) RCC2 acts as a GEF for RalA GTPase, whereas it inhibits guanine nucleotide exchange on Rac1. (C) RCC1 plays a role in the import of DNA damage repair factors and their recruitment to DNA damage sites. (D) RCC1 and RCC2 are involved in the regulation of cell cycle progression. (E) Both RCC1 and RCC2 play roles in the regulation of spindle assembly during mitosis. (F) RCC2 associates with the chromosomal passenger complex and may be required for the integration of kinetochores with the mitotic spindle. GEF, guanine nucleotide exchange factor; RCC, regulator of chromosome condensation.

Tumor Microenvironment

Tumor microenvironment cell fractions were assessed using the previously published quanTIseq bioinformatic package for devolution of bulk tumor RNAseq data.[24]

Survival Analysis

Overall survival (OS) was calculated using a repository of real-world evidence (RWE) insurance claim data and defined from the date of biopsy or start of therapy until last contact. High (Q4) and low (Q1) categorical subgroups were compared in the survival analyses, consistent with the thresholds used in the earlier analyses. The *P* value reflects the log-rank test.

Statistical Analysis

Molecular associations were tested using chi-square and Fisher's exact tests for categorical variables and the Mann-Whitney *U* test for continuous variables, where appropriate. Statistical analyses were performed using JMP V13.2.1 (SAS Institute) and open-source Python libraries (Pandas, NumPy, Seaborn, and Matplotlib). To adjust for multiple comparisons, the Benjamini-Hochberg procedure was applied.

Ethics Statement

This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont report, and U.S. Common

rule. In line with 45 CFR 46.101(b) (4), this study was performed using retrospective, deidentified clinical data.

RESULTS

In this study, there were 876 patients with SCLC, 21,603 with NSCLC, 1,908 with GC, 5,071 with PC, and 14,892 with CRC. The median age was 67.0 years, 69.0 years, 65.0 years, 67.0 years, and 62.0 years, respectively. Table 1 shows overall demographics.

RCC1

The median *RCC1* expressions were highest in SCLC (14.3 transcript per million [TPM]), followed by GC (9.9 TPM), NSCLC (9.9 TPM), CRC (9.8 TPM), and PC (6.9 TPM). Further stratification of GI cancers according to microsatellite instability (MSI) status demonstrated that the highest median *RCC1* expression was found in CRC-MSI cancers with 15.3 TPM, followed by GC-MSI cancers with 14.3 TPM and CRC-MSS and GC-MSS cancers both with 9.6 TPM.

The association with TMB-high rates and *RCC1* expression differed between cancer types. TMB-high rates increased progressively across subgroups stratified by *RCC1* expression quartiles (Q1-4) in NSCLC (31%-41%), GC (7%-22%), and CRC (5%-17%), whereas dMMR/MSI-H rates also progressively increased with *RCC1* Q1-4 in GC (5%-17%) and CRC (2%-13%). PDL1+ rates progressively increased with *RCC1* Q1-4 subgroups in NSCLC (55%-58%), PC (13%-20%), and CRC (2%-5%). Prevalence of immunotherapy (IO)-related biomarkers is shown in Figure 2A.

Several coalterations were associated with *RCC1* expression in a cancer type–specific manner (Figs 3A and 3E). In SCLC, *TP53* mutation rates progressively increased with *RCC1* Q1-4 subgroups, ranging from 83.6% to 95.9%. In NSCLC, the rates of *TP53* (57.1%-77.0%), *RB1* (6.8%-13.4%), and *EGFR* (9.9%-15.1%) mutations, along with genomic loss of heterozygosity (LOH; 14.3%-21.4%), progressively increased across *RCC1* Q1-4 subgroups. Conversely, higher frequencies of *STK11* and *KRAS* mutations in NSCLC were associated with lower *RCC1* expression, with Q1-4 ranging from 15.3% to 10.1% and 33.0% to 20.5%, respectively. In gastric MSS, *CTNNB1* mutations were associated with higher *RCC1* expression (Q1-4 1.2%-6.1%). In PC, *TP53* mutations, along with genomic LOH and *MYC* amplifications, were associated with higher *RCC1* expression, with Q1-4 ranging from 69.5% to 84.9%, from 8.8% to 16.1%, and from 1.1% to 3.6%, respectively. *ATM* mutations in PC were negatively associated with *RCC1* Q1-4 subgroups (5.6%-2.8%). In CRC-MSS, coalteration rates for several genes increased across *RCC1* Q1-4 subgroups, including *TP53* (Q1-4 70.1%-81.4%), *APC* (71.9%-81.4%), *FBXW7* (6.4%-13.1%), *PIK3CA* (14.9%-18.2%), *MYC* amplification (1.2%-2.5%), *SETD2* (0.3%-1.3%), *POLE* (0.3%-0.8%), and *MSH2* (0.1%-0.5%).

There were many associations found between tumor immune cell microenvironment composition and *RCC1* expression. In both SCLC and NSCLC, higher *RCC1* expression subgroups were associated with increasing dendritic cell (5.0%-6.3% and 0.8%-1.3%, respectively) and NK cell fractions (4.6%-6.3% and 2.5%-3.2%, respectively), whereas Treg fractions decreased across *RCC1* Q1-4 subgroups (1.8%-1.3% and 3.0%-2.6%, respectively). In NSCLC but not SCLC, *RCC1* Q1-4 subgroups were associated with progressively increased proportions of M1 macrophages (5.6%-6.2%), monocytes (0.1%-0.2%), and neutrophils (5.8%-7.0%). In PC, higher *RCC1* expression was associated with increased M1 macrophage (6.0%-6.8%), NK cell (2.7%-3.0%), and neutrophil fractions (5.7%-6.7%) and decreasing CD4 T-cell (1.0%-0.7%) and CD8 T-cell abundance (0.54%-0.51%). In GC-MSI, higher *RCC1* expression was associated with increased NK cell abundance

TABLE 1. *RCC1* and *RCC2* Overall Demographics

Cancer Type	SCLC	NSCLC	Gastric Cancer	Pancreatic Cancer	CRC
Count, No.	876	21,603	1,908	5,071	14,892
Age, years, median (range)	67.0 (18 to ≥90)	69.0 (21 to ≥90)	65.0 (19 to ≥90)	67.0 (13 to ≥90)	62.0 (14 to ≥90)
Female, No. (%)	456 (52.1)	10,901 (50.5)	1,114 (58.4)	2,693 (53.1)	8,092 (54.3)
Male, No. (%)	420 (47.9)	10,702 (49.5)	794 (41.6)	2,378 (46.9)	6,800 (45.7)
RCC1 expression quartile thresholds, No.					
25th percentile	8.85	6.12	6.13	4.17	6.21
50th percentile	14.31	9.86	9.923	6.85	9.82
75th percentile	21.09	15.18	15.09	10.42	14.87
RCC2 expression quartile thresholds, No.					
25th percentile	24.78	12.42	13.41	11.16	14.12
50th percentile	36.20	18.85	20.41	16.892	20.71
75th percentile	55.00	27.73	28.71	24.73	29.04

Abbreviations: CRC, colorectal cancer; NSCLC, non–small cell lung cancer; RCC, regulator of chromosome condensation; SCLC, small cell lung cancer.

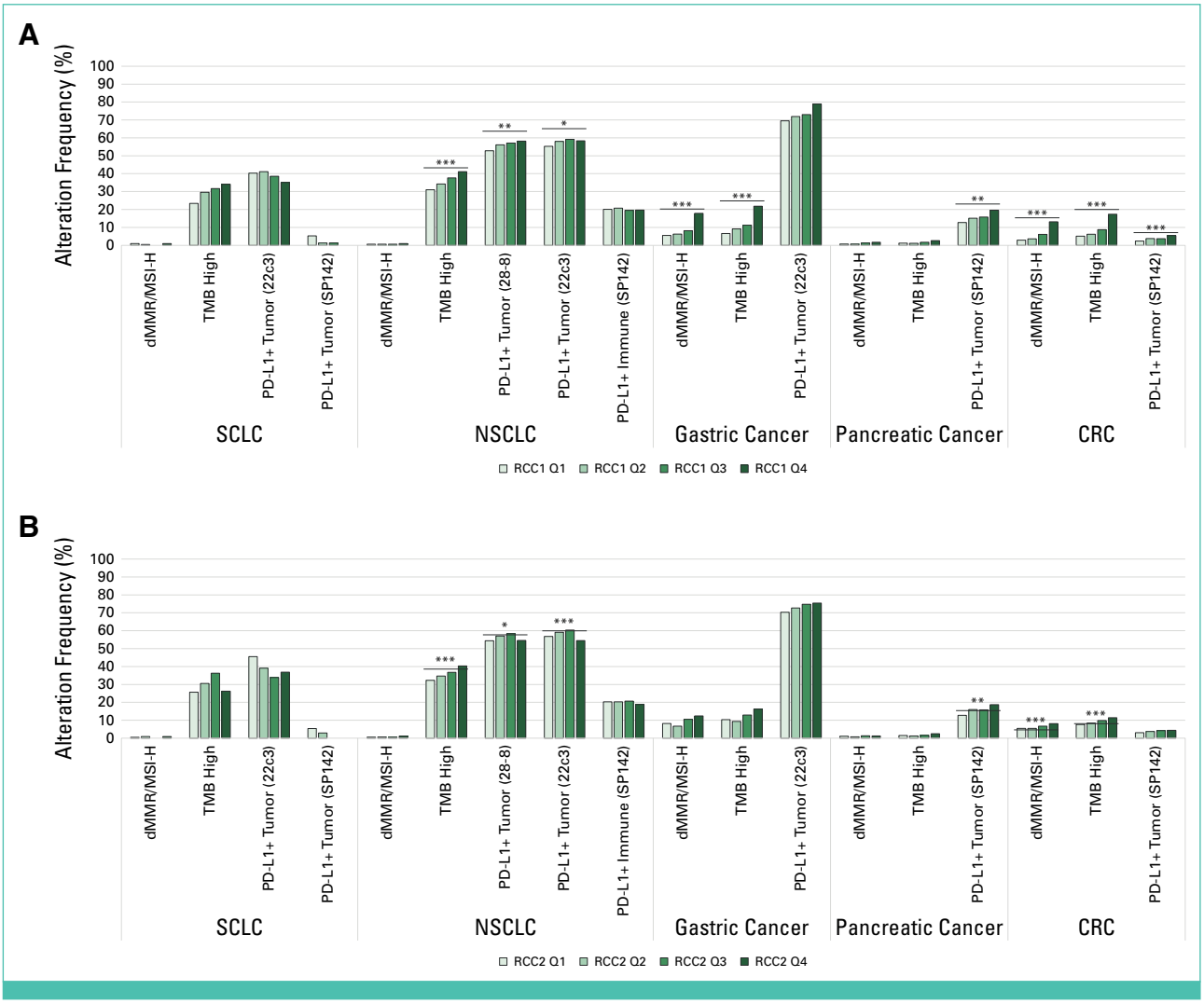


FIG 2. Prevalence of biomarkers associated with response to immunotherapy in cohorts stratified by (A) RCC1 expression and (B) RCC2, including dMMR/MSI-H, TMB-high, and PDL1 + expression on tumor or immune cells tested by immunohistochemistry. **P* < .05, ***P* < .01, ****P* < .001. CRC, colorectal cancer; dMMR/MSI-H, defective mismatch repair/microsatellite instability high; NSCLC, non–small cell lung cancer; RCC, regulator of chromosome condensation; SCLC, small cell lung cancer; TMB, tumor mutational burden.

(2.7%-3.5%). In CRC-MSI, higher *RCC1* expression was associated with increased dendritic cell (0.4%-0.6%), M1 macrophage (6.9%-8.8%), NK cell (2.8%-4.0%), and neutrophil abundance (4.9%-5.5%). The above data all represent statistically significant findings.

The association between *RCC1* expression and OS varied across cancer types. In SCLC, *RCC1* expression was not associated with differences in OS (hazard ratio [HR], 1.018 [95% CI, 0.799 to 1.296]; $P = .888$) and the median OS was 11.05 months for patients with low *RCC1* Q1 and 11.12 months for patients with high RCC1 Q4. In NSCLC, higher *RCC1* expression was associated with worse OS (HR, 1.183 [95% CI, 1.119 to 1.25]; $P < .0001$) and the median OS was 18.292 months for *RCC1* Q1 and was 14.838 months for *RCC1* Q4. Increased *RCC1* expression in NSCLC was associated with worse OS from the start of chemotherapy (HR, 1.18 [95% CI, 1.066 to 1.307]; $P = .001$) but not from IO (HR, 1.087 [95% CI, 0.969 to 1.22]; $P = .155$).

In GC-MSS, higher *RCC1* expression was associated with worse OS (HR, 1.206 [95% CI, 1.01 to 1.439]; $P = .038$) and the median OS was 13.65 months for *RCC1* Q1 and was 9.97 months for Q4. In GC-MSI, high *RCC1* expression correlated with better OS in patients (HR, 0.512 [95% CI, 0.268 to 0.98]; $P = .04$). The median OS was 14.11 months for low *RCC1* Q1, whereas the median OS was 25.761 high *RCC1* Q4.

In PC, higher *RCC1* expression was associated with worse OS (HR, 1.406 [95% CI, 1.282 to 1.543]; $P < .00001$) and the median OS was 13.36 months for *RCC1* Q1 and 8.126 months for *RCC1* Q4. Increased *RCC1* expression in PC was associated with worse OS from the start of chemotherapy (HR, 1.274 [95% CI, 1.112 to 1.459]; $P < .001$). In CRC-MSS, higher *RCC1* expression was associated with worse OS (HR, 1.169 [95% CI, 1.088 to 1.256]; $P < .0001$) and the median OS was 29.45 months for *RCC1* Q1 and 25.859 months for *RCC1* Q4.

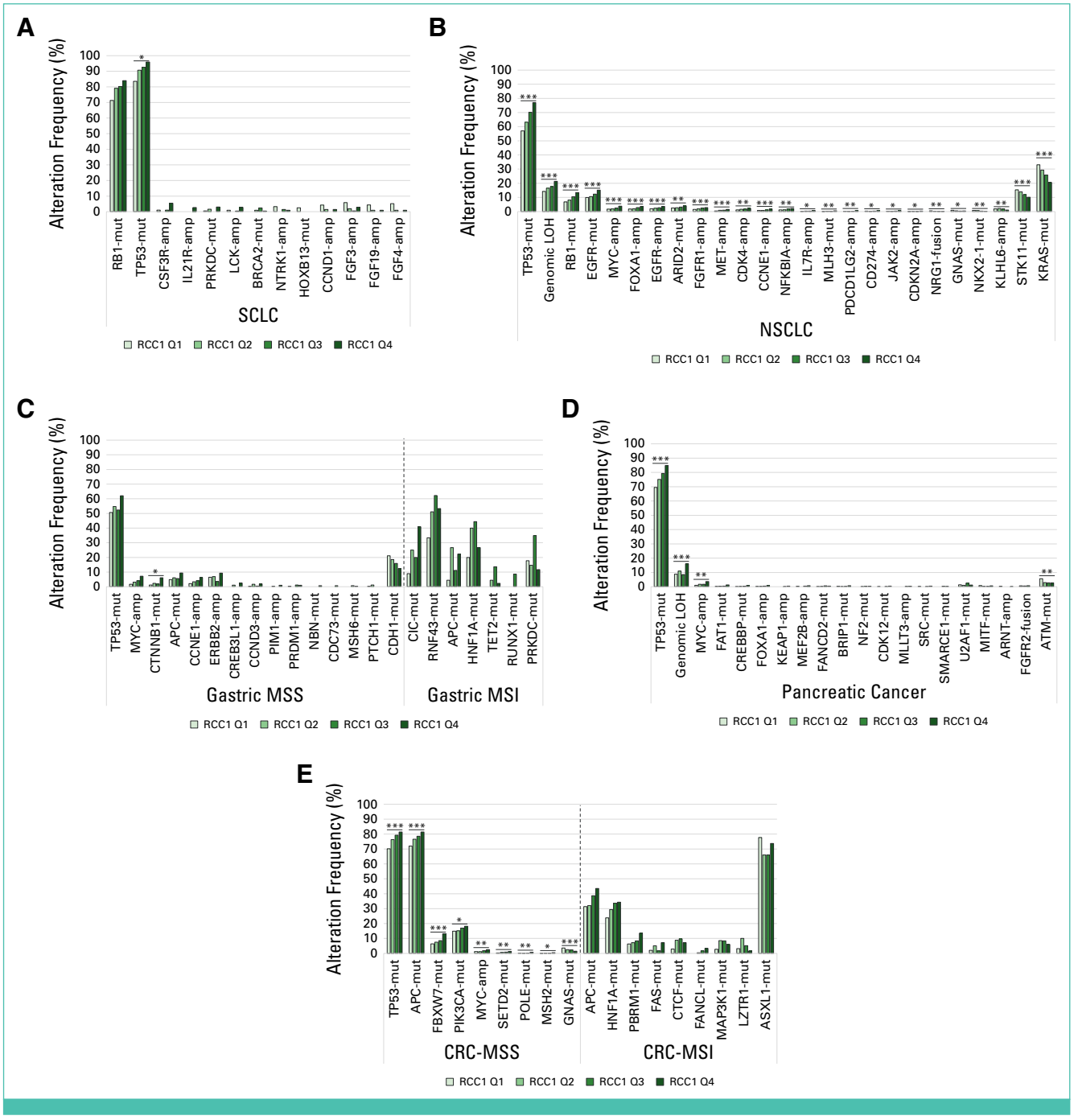
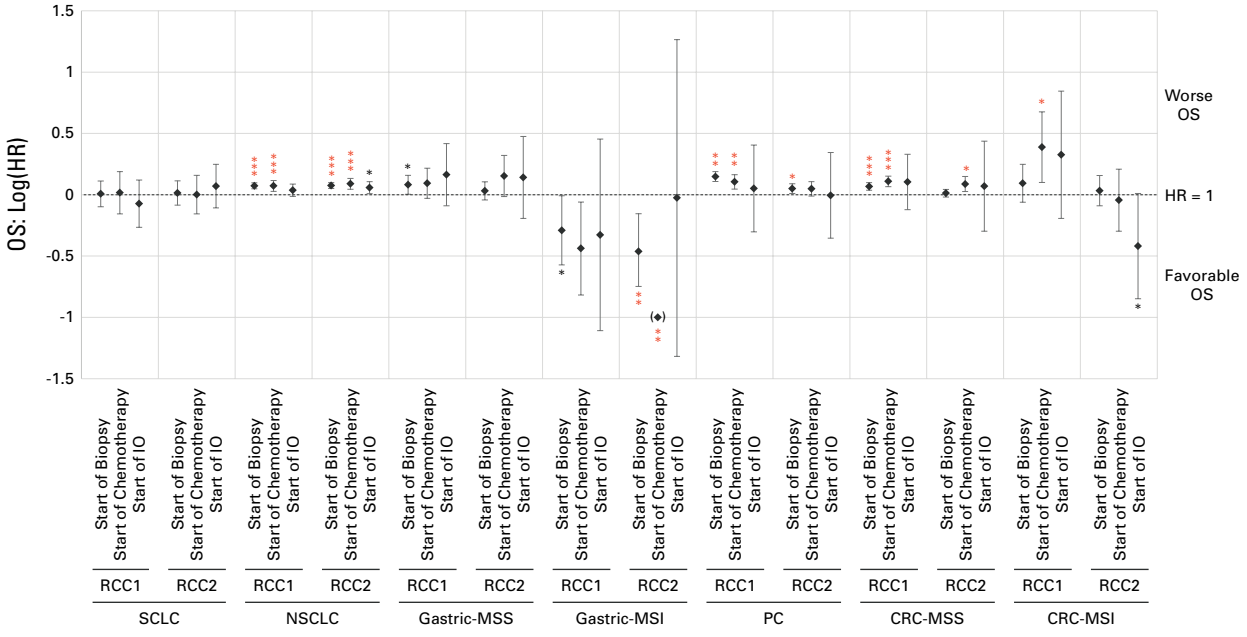


FIG 3. Prevalence of coalterations associated with RCC1 expression in (A) SCLC, (B) NSCLC, (C) gastric cancer, (D) pancreatic cancer, and (E) CRC. Biomarkers shown include pathogenic/likely pathogenic SNV/indel mut, copy number amp, gene fusions (fusion), and genomic LOH with significantly different prevalences across RCC1 expression subgroups based on raw *P* value. **P* < .05, ***P* < .01, ****P* < .001 after correction for multiple hypothesis testing. Biomarkers sorted by Q1 versus Q4 difference in prevalence. amp, amplifications; CRC, colorectal cancer; LOH, loss of heterozygosity; MSI, microsatellite instability; MSS, microsatellite stable; mut, mutations; NSCLC, non–small cell lung cancer; RCC, regulator of chromosome condensation; SCLC, small cell lung cancer; SNV, single nucleotide variant.

Increased *RCC1* expression in CRC-MSS was associated with worse OS from the start of chemotherapy (HR, 1.286 [95% CI, 1.165 to 1.419]; *P* < .00001). A comprehensive comparison of outcomes between *RCC1* and *RCC2* is illustrated in Figure 4. Individual Kaplan Meier curves are shown in the Data Supplement.

RCC2

The IQRs of *RCC2* expression were 24.8-55.0 TPM in SCLC, 12.4-27.7 TPM in NSCLC, 13.4-28.7 TPM in GC, 11.1-24.7 TPM in PC, and 14.1-29.0 TPM in CRC. Similar to *RCC1*, the median *RCC2* expression was highest in SCLC (36.2 TPM).



Cancer Type	Gene	Survival Metric	HR	95% CI Lower	95% CI Upper	*P*	Adj *P*
SCLC	RCC1	Biopsy to last contact	1.018	0.799	1.296	.8880	.9563
		Start of Chemotherapy	1.039	0.698	1.545	.8520	.9417
		Start of IO	0.846	0.543	1.318	.4600	.6038
	RCC2	Biopsy to last contact	1.033	0.823	1.298	.7800	.8854
		Start of Chemotherapy	1.003	0.699	1.438	.9870	.9870
		Start of IO	1.176	0.782	1.77	.4370	.5921
NSCLC	RCC1	Biopsy to last contact	1.183	1.119	1.25	.0000	.0001
		Start of Chemotherapy	1.18	1.066	1.307	.0010	.0047
		Start of IO	1.087	0.969	1.22	.1550	.2959
	RCC2	Biopsy to last contact	1.19	1.127	1.256	.0000	.0001
		Start of Chemotherapy	1.228	1.11	1.358	.0010	.0047
		Start of IO	1.143	1.02	1.28	.0210	.0588
Gastric MSS	RCC1	Biopsy to last contact	1.206	1.01	1.439	.0380	.0988
		Start of Chemotherapy	1.242	0.936	1.648	.1320	.2640
		Start of IO	1.458	0.813	2.613	.2030	.3622
	RCC2	Biopsy to last contact	1.075	0.906	1.275	.4090	.5908
		Start of Chemotherapy	1.421	0.966	2.091	.0730	.1614
		Start of IO	1.384	0.642	2.986	.4050	.5908
Gastric MSI	RCC1	Biopsy to last contact	0.512	0.268	0.98	.0400	.0988
		Start of Chemotherapy	0.365	0.152	0.873	.0180	.0540
		Start of IO	0.47	0.078	2.843	.4000	.5908
	RCC2	Biopsy to last contact	0.345	0.179	0.7	.0020	.0084
		Start of Chemotherapy	0	0	inf	.0010	.0047
		Start of IO	0.943	0.048	18.381	.9690	.9870
PC	RCC1	Biopsy to last contact	1.406	1.282	1.543	.0000	.0001
		Start of Chemotherapy	1.274	1.112	1.459	.0010	.0047
		Start of IO	1.125	0.498	2.543	.7770	.8854
	RCC2	Biopsy to last contact	1.122	1.024	1.231	.0140	.0452
		Start of Chemotherapy	1.117	0.976	1.279	.1080	.2268
		Start of IO	0.988	0.442	2.209	.9770	.9870
CRC-MSS	RCC1	Biopsy to last contact	1.169	1.088	1.256	.0001	.0008
		Start of Chemotherapy	1.286	1.165	1.419	.0000	.0001
		Start of IO	1.271	0.756	2.137	.3670	.5908
	RCC2	Biopsy to last contact	1.029	0.959	1.104	.4220	.5908
		Start of Chemotherapy	1.223	1.061	1.409	.0050	.0191
		Start of IO	1.175	0.504	2.736	.7060	.8721
CRC-MSI	RCC1	Biopsy to last contact	1.24	0.868	1.771	.2350	.3948
		Start of Chemotherapy	2.441	1.258	4.736	.0060	.0210
		Start of IO	2.117	0.641	6.995	.2070	.3622
	RCC2	Biopsy to last contact	1.08	0.812	1.435	.5960	.7585
		Start of Chemotherapy	0.903	0.505	1.614	.7290	.8748
		Start of IO	0.381	0.142	1.024	.0480	.1120

FIG 4. Forest plot of HRs for OS from the start of biopsy, chemotherapy, or IO among patients stratified by RCC1/2 expression. **P* < .05, ***P* < .01, ****P* < .001. CRC, colorectal cancer; HR, hazard ratio; IO, immunotherapy; MSI, microsatellite instability; MSS, microsatellite stable; NSCLC, non–small cell lung cancer; OS, overall survival; PC, pancreatic cancer; RCC, regulator of chromosome condensation; SCLC, small cell lung cancer.

While the TMB-high rates increased progressively across *RCC1* Q1-4 in NSCLC, GC, and CRC, a similar trend was observed for *RCC2* Q1-4 subgroups only in NSCLC and CRC. Although the PDL1+ rates progressively increased among *RCC1* Q1-4 subgroups in NSCLC, PC, and CRC, no significant trend was associated with *RCC2* expression. Prevalence of IO-related biomarkers across *RCC2* subgroups is shown in Figure 2B.

As was in the case with *RCC1* expression, coalteration rates associated with *RCC2* expression varied by cancer type (Figs 5A and 5E). In SCLC, *TP53* mutation rates progressively increased among *RCC2* Q1-4 subgroups (83.2%-94.0%), along with *RB1* mutations (67.5%-85.5%). In NSCLC, the rates of *TP53*, *RB1*, *KMT2D*, *CDKN2A*, and genomic LOH progressively increased across *RCC2* Q1-4 subgroups. Conversely, higher

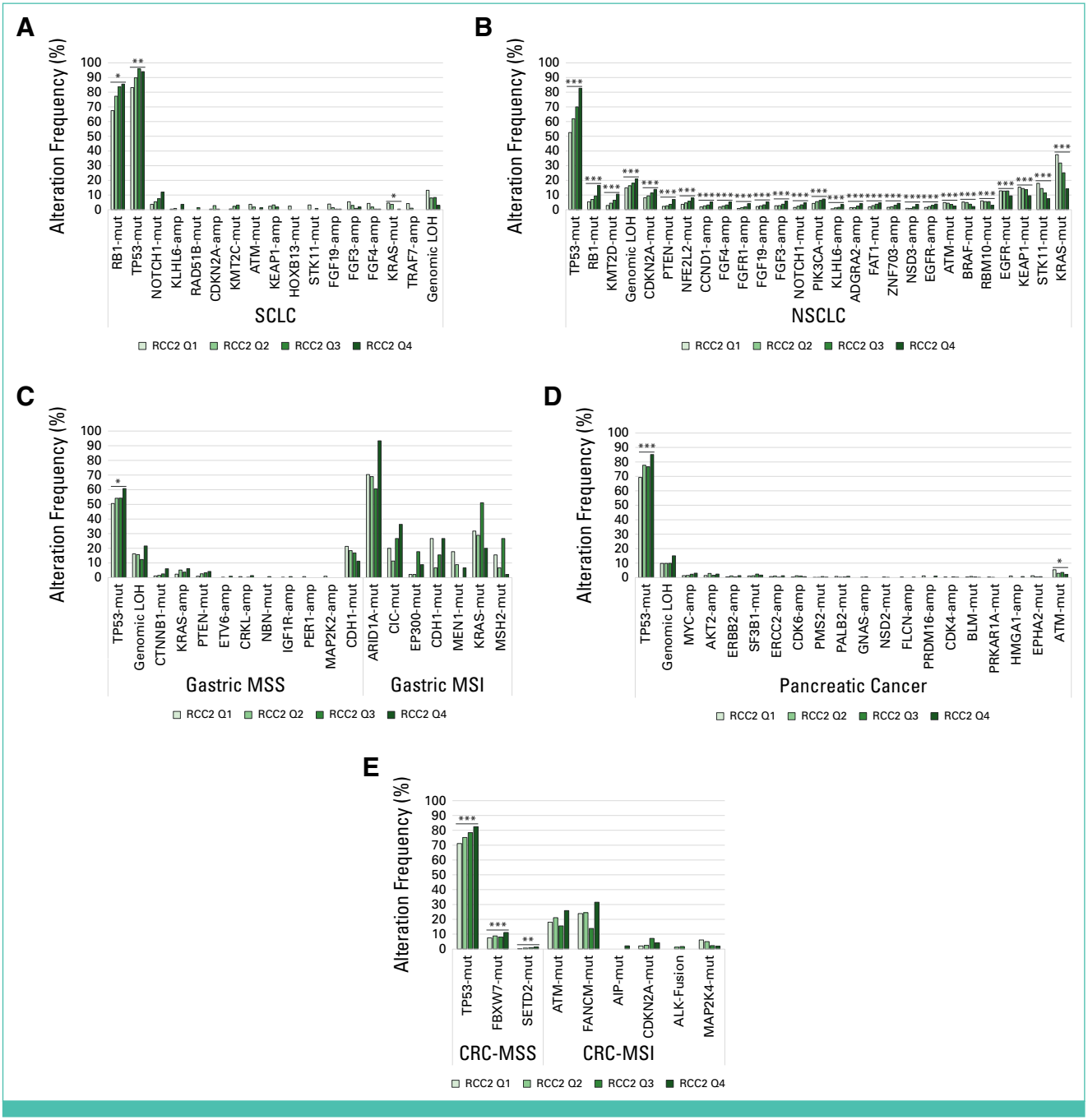


FIG 5. Prevalence of coalterations associated with RCC2 expression in (A) SCLC, (B) NSCLC, (C) gastric cancer, (D) pancreatic cancer, and (E) CRC. Biomarkers shown include pathogenic/likely pathogenic SNV/indel mut, copy number amp, gene fusions (fusion), and genomic LOH with significantly different prevalences across RCC1 expression subgroups based on raw *P* value. **P* < .05, ***P* < .01, ****P* < .001 after correction for multiple hypothesis testing. Biomarkers sorted by Q1 versus Q4 difference in prevalence. amp, amplifications; CRC, colorectal cancer; LOH, loss of heterozygosity; MSI, microsatellite instability; MSS, microsatellite stable; mut, mutations; NSCLC, non–small cell lung cancer; RCC, regulator of chromosome condensation; SCLC, small cell lung cancer; SNV, single nucleotide variant.

frequencies of *STK11*, *KEAP1*, and *KRAS* mutations in NSCLC were associated with lower *RCC2* expression, similar to *RCC1* Q1-4 subgroups. In PC, *TP53* mutations were associated with higher *RCC2* expression, whereas *ATM* mutations were associated with lower *RCC2* expression, similar to *RCC1*. In CRC-MSS, several coalterations were positively associated with higher *RCC2* expression, including *TP53*, *FBXW7*, and *SETD2*.

There were many associations found between tumor immune cell microenvironment factors and *RCC2* expression. In both SCLC and NSCLC, high *RCC2* expression was associated with increased B cells, dendritic cells, monocytes, and NK cells, whereas decreased CD4 T-cell and Treg fractions were observed. In PC, higher *RCC2* expression was associated with increased M1 macrophage, NK cells, and neutrophil proportions. In CRC-MSI, higher *RCC2* expression was associated with increased M1 macrophage and NK cell fractions.

The association between *RCC2* expression and OS varied across cancer types. Higher *RCC2* expression was associated with worse OS in NSCLC, PC, and CRC, but not in SCLC or GC. Increased *RCC2* expression in NSCLC was associated with worse OS from the start of chemotherapy (HR, 1.228; $P < .0001$) or IO (HR, 1.143; $P = .021$). In PC, higher *RCC2* expression was associated with worse OS (HR, 1.122; $P = .014$), with a similar trend observed for OS from the start of chemotherapy (HR, 1.117; $P = .108$). In CRC, higher *RCC2* expression was associated with worse OS (CRC-MSS: HR, 1.029; $P = .422$; CRC-MSI: HR, 1.08; $P = .596$). Increased *RCC2* expression in CRC-MSS was associated with worse OS from the start of chemotherapy (HR, 1.223; $P = .005$). In CRC-MSI, increased *RCC2* expression was associated with improved OS from the start of IO (HR, 0.381; $P = .048$; Data Supplement, Fig S5).

DISCUSSION

In our multiomic study, we demonstrated both *RCC1* and *RCC2* expression to be negative prognostic markers in NSCLC, PC, and CRC. Indeed, *RCC1* is highly expressed in tumor tissues compared with normal tissues, and high *RCC1* expression has also been previously reported to be correlated with a worse prognosis across many cancer subtypes. Using The Cancer Genome Atlas Program data set, Wu et al[25] demonstrated that high expression of *RCC1* was associated with poor OS in adrenocortical carcinoma, breast invasive adenocarcinoma, kidney renal papillary cell carcinoma, low-grade glioma, liver hepatocellular carcinoma, lung adenocarcinoma, stomach adenocarcinoma/GC, and skin cutaneous melanoma. Recently, Gong et al[26] performed a pan-cancer analysis for *RCC2* expression using published gene expression data sets and showed that *RCC2* was highly expressed in the majority of cancer types, with higher expression linked to poor overall prognosis.

RCC1 is the only identified GEF for Ran GTPase, and it has function in nuclear transport, cell cycle, and DNA damage response.[11,27] *RCC1* overexpression may drive proliferation and resistance to DNA damage, whereas the disruption of *RCC1* activity, and thus Ran gradient disruption, would result in defects in the DNA damage response. As expected, *RCC1* is implicated in tumor development and progression.[12] For example, Lin et al[28] demonstrated that *RCC1* expression was associated with tumor differentiation and depth of invasion ($P < .05$) from three GC cell lines (AGS, MKN45, and TSGH9201) and 85 pairs of matched human gastric carcinoma samples. In their study, they found that *RCC1* silencing was associated with more aggressive disease. Consistent with their findings, we have also found, in this study, that lower *RCC1* expression is associated with worse OS in microsatellite instable GC. Interestingly, Deng et al[29] used 129 pairs of matched specimens from primary CRC and liver metastasis in those with oligometastases to the liver and showed that high *RCC1* expression in liver oligometastases was an independent prognostic marker for unfavorable recurrence-free survival and OS ($P = .036$ and $P = .016$). Gene expression profiles generated from microarray analysis showed that genes correlated with *RCC1* were enriched in Myc targets, E2F targets, and DNA repair pathways.[30]

Our study also exhibited similar findings in MSS-CRC, including *MYC* amplification, *MSH2* mutations, and other coalteration rates that positively increased across *RCC1* Q1-4 expression subgroups. While carcinogenesis is a complex process involving numerous pathways, these findings, at least partially, may explain the implications of *RCC1* in tumor development and progression. Similarly, our study also demonstrated *RCC2* expression to be associated with immune suppression, TMB, and MSI, which has been previously described.[26]

In addition, *RCC1* and *RCC2* expression might also have predictive implications. In our study, high *RCC1* expression was associated with worse OS from the start of chemotherapy or IO in NSCLC, with similar results observed for *RCC2*. With PC and CRC, high *RCC1*, as well as *RCC2* expression, was associated with worse OS from the start of chemotherapy. OS correlation was not seen with IO therapy in PC and CRC, which is comprehensible, as there are limited indications of IO use in these cancers. Although the median *RCC1* and *RCC2* expression was highest in SCLC, high *RCC1* or *RCC2* expression was not associated with worse OS in SCLC, which may be related to the overall poor prognosis at baseline for these patients.

RCC1 and *RCC2* silencing may result in sensitivity not only to chemotherapy and radiation but also to IO. Interestingly, preclinical studies published by Zeng et al[31] displayed that in vivo, knockdown of *RCC1* significantly reduced the growth rate of NSCLC tumor in mouse xenografts and further decreased the volume of tumor treated with a PD-L1 monoclonal antibody, suggesting that downregulation of RCC1 may sensitize IO by upregulating PD-L1 via the $p27^{kip1}$/CDK4 axis.

Interestingly, although increased *RCC1* expression in NSCLC was associated with high rates of PDL1+ and TMB-high and

tumor immune microenvironment changes, including increased dendritic cells, NK cells, M1 macrophages, monocytes, and neutrophils, as well as decreased Treg cells, higher *RCC1* was associated with worse OS in those who received chemotherapy and IO. Although increased *RCC1* was associated with multiple factors traditionally thought to predict response to IO, it was also associated with poor prognostic factors, such as coalteration of *TP53* and *RB1*, which might play a larger role in overall tumorigenesis and disease progression.

Our study is not without limitations because of the nature of retrospective evaluation and limited scope of cancer types. This study used RWE data that lack some elements of clinical history (such as tumor stage, tumor location, and sites of metastasis as well as detailed treatment regimen and sequence), and survival outcomes were inferred based on time from tissue collection to date of last contact, with NGS performed at varying time points during the course of the disease and treatments. Furthermore, this work was conducted as an exploratory study, and we acknowledge that the univariable/unadjusted framework used has its limitations as the absence of adjustments for potential confounders may affect the accuracy and comprehensiveness of the prognostic assessments.

Despite these limitations, we evaluated multiomic characteristics associated with *RCC1* and *RCC2* expression in SCLC, NSCLC, GC, PC, and CRC. Further studies to investigate the underlying biological and molecular mechanisms that account for these associations are warranted. Specifically, beyond the Q1-Q4 comparisons by different mutations, further analyses could include gene expression profiling to identify multiple genes associated with *RCC1* and *RCC2* expression levels. Finally, our findings are likely only part of the bigger picture, and a more detailed examination of the clinical effects of other coalterations, including upstream, downstream, and related pathways, as well as tumor immune microenvironments, may provide a rationale for developing therapeutics that target vulnerabilities in the *RCC1/2* signaling axis. Collectively, our data suggest that *RCC1* and *RCC2* play central roles in cancer resistance and may be potential targets for novel drug development. Future studies using multivariable-adjusted analyses to validate the findings of this study in a larger, more diverse cohort of patients would be warranted.

In conclusion, *RCC1* and *RCC2* expression is a negative prognostic marker in NSCLC, PC, and CRC. Further studies to investigate this at the molecular level may provide opportunities for novel targeted drug development.

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