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RECQ1 A159C Polymorphism Is Associated With Overall Survival of Patients With Resected Pancreatic Cancer: A Replication Study in NRG Oncology Radiation Therapy Oncology Group 9704

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Abstract

Purpose—To confirm whether a previously observed association between RECQ1 A159C variant and clinical outcome of resectable pancreatic cancer patients treated with preoperative chemoradiation is reproducible in another patient population prospectively treated with postoperative chemoradiation.

Methods and Materials—Patients were selected, according to tissue availability, from eligible patients with resected pancreatic cancer who were enrolled on the NRG Oncology Radiation Therapy Oncology Group 9704 trial of 5-fluorouracil (5-FU)-based chemoradiation preceded and followed by 5-FU or gemcitabine. Deoxyribonucleic acid was extracted from paraffin-embedded tissue sections, and genotype was determined using the Taqman method. The correlation between genotype and overall survival was analyzed using a Kaplan-Meier plot, log-rank test, and multivariate Cox proportional hazards models.

Results—In the 154 of the study's 451 eligible patients with evaluable tissue, genotype distribution followed Hardy-Weinberg equilibrium (ie, 37% had genotype AA, 43% AC, and 20% CC). The RECQ1 variant AC/CC genotype carriers were associated with being node positive compared with the AA carrier ($P = .03$). The median survival times (95% confidence interval [CI])

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for AA, AC, and CC carriers were 20.6 (16.3–26.1), 18.8 (14.2–21.6), and 14.2 (10.3–21.0) months, respectively. On multivariate analysis, patients with the AC/CC genotypes were associated with worse survival than patients with the AA genotype (hazard ratio [HR] 1.54, 95% CI 1.07–2.23, $P=.022$). This result seemed slightly stronger for patients on the 5-FU arm ($n = 82$) (HR 1.64, 95% CI 0.99–2.70, $P=.055$) than for patients on the gemcitabine arm ($n = 72$, HR 1.46, 95% CI 0.81–2.63, $P=.21$).

Conclusions—Results of this study suggest that the RECQ1 A159C genotype may be a prognostic or predictive factor for resectable pancreatic cancer patients who are treated with adjuvant 5-FU before and after 5-FU-based chemoradiation. Further study is needed in patients treated with gemcitabine to determine whether an association exists.

Introduction

Pancreatic cancer remains the fourth leading cause of cancer death for both men and women in the United States (1). It is estimated that approximately 39,590 Americans died of this highly lethal disease in 2014 (2). Although there are some improvements in radiation, systemic therapies, and targeted agents, the 5-year survival rate for early-stage resected patients remains less than 25% (3, 4). The poor prognosis of pancreatic cancer is due to the metastasis-prone and therapy-resistant characteristics, which can be partially explained by the frequent genetic and epigenetic alterations in this tumor. Moreover, individual differences in DNA repair or drug metabolism may also influence clinical response to therapy and overall survival (OS) for these patients (5).

Current accepted treatment of early-stage pancreatic cancer is adjuvant gemcitabine (GEM) and/or 5-fluorouracil (5-FU) combined with radiation after curative resection, but there have been no conclusions regarding the role or timing of the adjuvant chemoradiation (4). Whereas GEM and 5-FU have comparable activity in the chemoradiation therapy of pancreatic cancer, they likely affect different patient groups through different mechanisms of prodrug activation, drug catabolism, and cellular responses to the drug (3). Clearly, biomarkers are required to predict the treatment outcome and direct the choice of therapy for individual patients.

RECQ1 (also known as RECQL) A159C (rs13035) is a single-nucleotide polymorphism (SNP) located on the 3' untranslated region of the gene (6). It has been previously demonstrated that the RECQ1 A159C variant genotype, both alone and in combination with other genes, was associated with significantly decreased OS in pancreatic cancer patients (5–7). The hypothesis is that this single gene polymorphism may be a predictor of pancreatic cancer treatment response and survival. To confirm whether the previously observed association between RECQ1 A159C and OS is reproducible in another patient population, the prognostic value of this polymorphism was evaluated in association with the course of treatment and clinical outcome in patients who were enrolled in the NRG Oncology Radiation Therapy Oncology Group (RTOG) 9704 clinical trial, which used 5-FU–based chemoradiation preceded and followed by 5-FU or GEM (8).

Methods and Materials

Patient population

The study involved patients who were enrolled in the intergroup phase 3 trial NRG Oncology RTOG 9704, which was conducted by National Cancer Institute–sponsored cooperative groups: RTOG, the Eastern Cooperative Oncology Group, and the Southwest Oncology Group, inclusive of Canadian affiliates (8). All patients had localized adenocarcinoma of the pancreas with gross total resection. Patients could be T1 through T4, N0, or N1 and were required to have a Karnofsky performance score of at least 60 and be at least 18 years of age. Other entry requirements, as well as the detailed treatment plan, have been previously reported (8). Briefly, after undergoing tumor resection, patients were randomly assigned to either 5-FU (arm 1) or GEM (arm 2). Patients in arm 1 received continuous infusion of 5-FU at 250 mg/m²/d for 3 weeks. Arm 2 received GEM infusion at 1000 mg/m² once weekly for 3 weeks. Within 1 to 2 weeks after completion of the chemotherapy, patients on both arms received 28 fractions of 50.4 Gy radiation at 5 days per week and continuous infusion of 5-FU at 250 mg/m²/d throughout the chemoradiation treatment. Three to five weeks after completion of the chemoradiation treatment, arm 1 received 2 cycles of continuous infusion of 5-FU (4 weeks on and 2 weeks off). Arm 2 received 3 cycles of GEM (3 weeks on and 1 week off).

All patients consented to specimen submission, and residual surgical tissue was collected and stored at the RTOG Biospecimen Resource. The clinical trial was approved by the institutional review board of each participating institution. The present study was approved by the institutional review board of MD Anderson Cancer Center.

DNA extraction and genotyping

Paraffin-embedded tissue blocks were punched for DNA extraction. Extraction of DNA used the FFPE DNA purification kit (Promega, Madison, WI). Concentration of DNA was determined using NanoDrop (Thermo Scientific, Wilmington, DE). Genotyping used the Taqman SNP assay kit (Life Technologies, Grand Island, NY). Approximately 10% of the samples were analyzed in duplicate, and no discrepancy was observed. Call rate was 94%. All samples were blinded to the laboratory personnel, and genotype data were sent to RTOG for statistical analysis.

Statistical analysis

Failure for survival was defined as death due to any cause, and OS time was measured from the date of randomization to date of death or date of last follow-up for living patients.

Genotype distribution was tested for Hardy-Weinberg equilibrium using the goodness-of-fit χ^2 test. The association between OS and genotype was estimated univariately with the Kaplan-Meier method (9), and genotype groups were compared using the log-rank test. The following baseline characteristics were dichotomized: pathologic T stage (T1, T2 vs T3, T4), American Joint Committee on Cancer stage (I, II vs III, IV), primary tumor location (head vs non-head), and race (white vs African American/other). Univariate and multivariate Cox proportional hazards models (10) were used to identify the impact of RECQ1 genotype on

OS. For the multivariate analysis, only RECQ1 genotype was forced into the models, and a backwards selection procedure was used to choose other variables using $\alpha = 0.05$ level as the exit criteria for the model building. The following variables were included in the multivariate analyses along with the RECQ1 genotype: treatment arm, age (continuous), gender, race (white vs African American/other), nodal involvement (no vs yes), tumor diameter (<3 vs 3 cm), and surgical margin status (negative vs positive and negative vs unknown). All analyses were performed using SAS version 9.2 statistical software (SAS Institute, Cary, NC), and statistical significance was defined as a P value $< .05$.

Results

Patient characteristic and genotype distribution

A total of 154 patients (82 on the 5-FU arm and 72 on the GEM arm) were studied from 451 eligible patients according to tissue availability. The median age of the 154 patients in this study was 63 years (range, 35–80 years), with 80 (52%) males. White and non-white constituted 90% and 10% of the study population, respectively. There was no significant difference in baseline characteristics and in OS time between patients who were and were not evaluated for the RECQ1 genotype (data not shown).

The RECQ1 A159C genotype distribution followed Hardy-Weinberg equilibrium (ie, 37% had genotype AA, 43% AC, and 20% CC) (Table 1). No statistically significant associations existed between the RECQ1 genotype and baseline characteristics. However, when the RECQ1 genotype was investigated in the dominant inherit mode, AC/CC genotype carriers were more likely to be node positive compared with AA carriers (Table 2; $P = .03$).

RECQ1 genotype and OS

Follow-up time for the 154 patients was 18 months (range, 1.2–108 months), with a median survival time of 18.1 months (95% confidence interval [CI] 15.8–21.0) and 130 deaths (84.4%). An association of the RECQ1 A159 C genotype and OS was observed (Fig. 1). The median survival time was 20.6, 18.8, 14.2, and 16.8 months for the RECQ1 A159C AA, AC, CC, and AC/CC genotypes, respectively. Patients carrying the AC genotype had a 1.57-fold higher risk of death than those carrying the AA genotype after adjusting for age and race ($P = .027$; Table 3). Patients carrying the minor C allele (ie, the AC or CC genotypes) had a 1.54-fold higher risk of death than those carrying the AA genotype after adjusting for age and race ($P = .022$; Table 3).

Association of RECQ1 genotype and survival in different treatment arms

Multivariate analysis was performed separately for patients in each treatment arm. The association of C minor allele and increased risk of death was more definitive for patients on the 5-FU arm ($n = 82$) than for patients on the GEM arm ($n = 72$). The multivariate hazard ratio (95% CI) was 1.64 (0.99–2.70) in the 5-FU arm and 1.46 (0.81–2.63) in the GEM arm (Table 4).

Discussion

In this study, it was demonstrated that there is a positive association between RECQ1 A159C variant (rs13035) and OS in patients who were enrolled on the NRG Oncology RTOG 9704 trial. It was observed that patients carrying the C minor allele had a 4-month shorter OS and 1.5-fold increased risk of death compared with those carrying the A allele. Previously it was reported that the RECQ1 A159C variant genotype, both alone and in combination with other genes, was associated with significantly decreased OS in patients with resectable pancreatic cancer and who received neoadjuvant treatment (5–7). In the present study the same association was observed in patients with resected pancreatic cancer who received postsurgical 5-FU-based chemoradiation preceded and followed by 5-FU or GEM. The consistent findings from all these studies provide strong evidence supporting the hypothesis that the RECQ1 A159C genotype plays an important role in pancreatic tumor progression and/or response to clinical therapy.

RECQ1 belongs to the RECQ DNA helicases family, and it is the smallest but most abundant RECQ helicase in humans (11). Deoxyribonucleic acid helicases are crucial to DNA replication, recombination, and repair. Mutations in 3 of the 5 RECQ gene subfamily members (BLM, WRN, and RECQ4) have been associated with human diseases all characterized by chromosomal instability and predisposition to cancer (11). Although no inherited disease has been linked to RECQ1 mutations, accumulating evidence has proved its unique and important role in DNA metabolism (12) and in maintaining genomic stability (13). RECQ1 deficiency leads to increased frequency of sister chromatid exchange and hypersensitivity to ionizing radiation (14). RECQ1 silencing by small interfering RNA induced mitotic catastrophe, cancer cell-specific cytotoxicity, and reduced tumor growth (15, 16). RECQ1 functions as a Holliday junction-resolving enzyme (17) and is involved in the DNA mismatch repair pathway (18, 19) as well as in DNA replication fork restart and progression (20, 21). Furthermore, RECQ1 interacts with Ku70/80 and regulates end-joining of double-strand DNA breaks (22). On the basis of these observations, RECQ1 has been suggested as a potential therapeutic target in cancer treatment. It is known that GEM incorporates into DNA to cause a masked chain termination and DNA replication arrest (23), and 5-FU inhibits DNA synthesis by interrupting the action of thymidylate synthase (24). Deoxyribonucleic acid double-strand break is the major cell-killing DNA damage caused by radiation. Thus, the association between RECQ1 A159C variants and reduced survival observed in the present study may be explained by the role of RECQ1 in DNA damage repair. Although a stronger association of this SNP with OS in GEM-treated patients than in 5-FU group (6) was previously observed, the present study observed an opposite trend; both studies had a smaller sample size in the group with a weaker association. Thus it is not clear whether RECQ1 is predominantly involved in restarting the DNA replication in the fixation of the DNA double-strand breaks.

On the other hand, recent studies suggested that RECQ1 may play an important role in cancer progression (25). RECQ1 is overexpressed or amplified in rapidly dividing cancer cells (16, 25, 26) and in human cancer tissues (18, 27, 28). RECQ1 knockdown in cancer cells significantly reduces cell migration and invasion (29), and gene expression analysis has shown that RECQ1 actually regulates genes associated with cancer progression (30). The

present study found that patients carrying the RECQ1 A159C C allele were more likely to have node-positive tumors than those carrying the AA genotype. Therefore, there is a possibility that RECQ1 affected patient survival not only through its function on DNA damage repair but also via a mechanism of enhanced tumor progression.

The RECQ1 A159C SNP is located at the 3' untranslated region, and it may affect translation efficiency and messenger RNA stability. Previous DNA sequencing of this gene region did not reveal any significant association with other coding variants, and in silico analyses on this variant did not predict any functional significance (7). It is possible that this polymorphism may be in linkage with other important genes located in the same chromosome region. Further functional characterization of this SNP is warranted.

The strengths of this study included a homogenous patient population selected from a well-conducted clinical trial and the double-blind experimental design. The limitation of the study is the relatively small sample size in each arm and lack of functional validation. Further investigations to confirm the observations from this study in other larger patient populations, as well as on the biological functions of RECQ1 gene and its A159C polymorphism, are required. If confirmed, this genetic profile may provide opportunities that can direct the choice of therapy, predict the treatments' overall outcome, and provide the fundamental information required for future "individualized" therapy.

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Summary

RECQ1 A159C polymorphism has previously been associated with significantly reduced survival in resectable pancreatic cancer patients who were treated with preoperative chemoradiation. To confirm this association, this genotype was evaluated in resected pancreatic cancer patients who were treated with postoperative chemoradiation. The variant C allele was significantly associated with reduced survival and increased risk of death, which suggests a promising prognostic or predictive value for patients with resectable pancreatic cancer who are treated with chemoradiation.

Fig. 1. Kaplan-Meier plot of overall survival curves by genotype and treatment arm. (A, B) All patients; (C) 5-fluorouracil arm; (D) gemcitabine arm. Log-rank *P* values are .24, .093, .066, and .67 for A, B, C, and D, respectively.

Table 1

Patient characteristics by RECQ1 genotype (n = 154)

Characteristic	AA (n = 57)		AC (n = 66)		CC (n = 31)		P*
	n	%	n	%	n	%	
Age (y)							
Median	63		62		65		
Range	35–76		37–80		44–80		
Gender							.86 (.64)
Male	31	54.4	34	51.5	15	48.4	
Female	26	45.6	32	48.5	16	51.6	
Race							.21 (.09)
White	48	84.2	62	93.9	28	90.3	
African American/other	9	15.8	4	6.1	3	9.7	
Primary tumor location							.40 (.19)
Head	44	77.2	57	86.4	26	83.9	
Everything else	13	22.8	9	13.6	5	16.1	
KPS							.59 (.32)
60, 70, 80	20	35.1	28	42.4	14	45.2	
90, 100	37	64.9	38	57.6	17	54.8	
T-stage (surgical)							.84 (.76)
T1, T2	16	28.1	16	24.2	9	29.0	
T3, T4	41	71.9	50	75.8	22	71.0	
N-stage (surgical)							.07 (.03)
N0	25	43.9	16	24.2	10	32.3	
N1	32	56.1	50	75.8	21	67.7	
AJCC stage							.23 (.13)
I, II	22	38.6	16	24.2	10	32.3	
III, IV	35	61.4	50	75.8	21	67.7	
Largest tumor dimension of primary (cm)							.32 (.13)
<3	27	47.4	23	34.8	11	35.5	

Characteristic	AA (n = 57)		AC (n = 66)		CC (n = 31)		<i>P</i> [*] AA vs AC vs CC (AA vs [AC/CC])
	n	%	n	%	n	%	
3	30	52.6	43	65.2	20	64.5	
Surgical margins							.43 (.30)
Complete resection/negative margins	29	50.9	27	40.9	11	35.5	
Complete resection/positive margins	13	22.8	23	34.8	9	29.0	
Complete resection/unknown margins	15	26.3	16	24.2	11	35.5	
CRT							.59 (.58)
RT + 5-FU	32	56.1	36	54.5	14	45.2	
RT + gencitabine	25	43.9	30	45.5	17	54.8	

Abbreviations: 5-FU = 5-fluorouracil; CRT = chemoradiation therapy; KPS = Karnofsky performance score; RT = radiation therapy.
* *P*value from χ^2 test.

Table 2

Association of nodal stage (surgical) with RECQ1 genotype

N stage	AA (n=57)		AC/CC (n=97)		P*
	n	%	n	%	
N0	25	43.9	26	26.8	.03
N1	32	56.1	71	73.2	

* P-value from χ^2 test.

Table 3

Association of genotypes and survival

Genotype or variable	No. of deaths	Median survival time (mo) (95% CI)	Multivariate HR (95% CI)	P*
AA	46	20.6 (16.3–26.1)	1.00 (reference)	
AC	58	18.8 (14.2–21.6)	1.57 (1.05–2.33) [†]	.027
CC	26	14.2 (10.3–21.0)	1.49 (0.91–2.42) [†]	.11
AC/CC	84	16.8 (14.2–20.9)	1.54 (1.07–2.23) [†]	.022
Age (continuous)			0.98 (0.96–0.99)	.008
Race (white vs non-white)			2.39 (1.39–4.10)	.002

Abbreviations: CI = confidence interval; HR = hazard ratio.

* P values from χ^2 test using the Cox proportional hazards model.

[†] Hazard ratio after adjusting for age and race.

Table 4

Association of genotypes and survival by treatment arm

Arm [*]	Genotype	No. of deaths	Multivariate HR (95% CI)	P [†]
5-FU (n=82)	AC/CC	44	1.64 (0.99–2.70) [‡]	.055
Gemcitabine (n=72)	AC/CC	40	1.46 (0.81–2.63) [§]	.21

Abbreviations as in Tables 1 and 3.

^{*} Separate models were built within each arm.[†] P values from χ^2 test using the Cox proportional hazards model.[‡] Hazard ratio after adjusting for race (AA is the reference level).[§] Hazard ratio after adjusting for age, race, and surgical margin status (AA is the reference level).