Original Article

Correlation of *PIK3CA* mutation with programmed death ligand-1 (PD-L1) expression and their clinicopathological significance in colorectal cancer

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Background: The prognostic significance of *PIK3CA* mutations in colorectal cancer (CRC) remains controversial. Recently, an association between programmed death ligand-1 (PD-L1) and *PIK3CA* mutations has been reported. The study presented here was conducted to investigate the effect of *PIK3CA* mutations on the prognosis of CRC patients and the association between *PIK3CA* mutations and PD-L1.

Methods: *PIK3CA* mutations were analyzed by targeted next-generation sequencing using formalin-fixed paraffin-embedded specimens from 224 primary CRC patients. PD-L1 expression was evaluated by immunohistochemical staining.

Results: *PIK3CA* mutations and PD-L1 expression were detected in 21.4% and 10.3% of CRC patients, respectively. *PIK3CA* mutations were significantly correlated with right-side colon cancer (P=0.011) and were correlated inversely with lymph node metastasis (P=0.026), distant metastasis (P=0.047), and high TNM stage (P=0.036). In univariate analysis, *PIK3CA* mutations were correlated with longer relapse-free survival in CRC patients. PD-L1 expression was correlated significantly with *PIK3CA* mutations (P<0.001).

Conclusions: *PIK3CA* mutations were associated with favorable prognostic factors, longer relapse-free survival, and expression of PD-L1. Further investigation is needed to identify whether *PIK3CA* mutations are a good prognostic factor. Additionally, further studies are needed to understand the mechanisms behind the correlation between *PIK3CA* mutations and PD-L1 expression.

Keywords: Colorectal cancer (CRC); PIK3CA; programmed death ligand-1 (PD-L1)

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Introduction

Colorectal cancer (CRC) is a common type of cancer. The number of new CRC cases worldwide has exceeded 1.8 million, and the number of deaths was estimated to be 881,000 in 2018 (1). Tumorigenesis and progression of CRC are multifactorial and dependent on lifestyle, the immune system, and genetic mutations. The main treatment for CRC is surgery and adjuvant chemotherapy.

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The relapse rate of CRC is approximately 30% (2). Due to the high relapse rate, recent studies have emphasized the importance of individually tailored treatments for better patient outcomes. The molecular characteristics of CRC provide important information for development of targeted therapies.

PIK3CA is a commonly mutated gene in CRC and encodes the p110 alpha (p110α) protein, which is a subunit of an enzyme called phosphatidylinositol 3-kinase (PI3K). *PIK3CA* mutations activates the PI3K/protein kinase B (PI3K-AKT) pathway and contribute to cellular growth, proliferation, and survival of the tumor cells (3). Early studies have suggested that *PIK3CA* mutations can influence tumor development and progression (4-6). *PIK3CA* mutations are thought to play an oncogenic role and act as a marker for poor prognosis and as a negative predictor for response to anti-epidermal growth factor receptor (EGFR) treatment in CRC (7). However, other reports have revealed that *PIK3CA* mutations are a favorable prognostic factor in CRC (8,9), or that the prognostic relevance of *PIK3CA* mutations remains controversial (10,11). Analysis of the cBioPortal database has revealed a significant correlation between *PIK3CA* mutations and prolonged disease-free survival in patients with CRC (12). *PIK3CA* mutations are observed in 24.7% (147 of 594) of CRC cases in The Cancer Genome Atlas (TCGA). Survival analysis of cases from the TCGA database disclosed no prognostic value for *PIK3CA* mutations in CRC (13).

Programmed death ligand-1 (PD-L1), a ligand for programmed death receptor-1 (PD-1), is expressed in tumor infiltrating immune cells and tumor cells. PD-1 is an inhibitory receptor expressed on activated T-cells. When PD-L1 expressed on tumor cells binds to its receptor, PD-1, it suppresses T cell function, downregulating the immune response. Targeting the PD-1/PD-L1 blockade has been proposed as a promising breakthrough in cancer treatment including that of CRC.

Previous studies have shown that genetic alterations within tumors can influence the engagement of the immune system. *EGFR* mutations or EML4-ALK fusions activate the PD-1/PD-L1 pathway via PD-L1 upregulation. *PIK3CA* mutations were associated with PD-L1 overexpression in gastric cancer (14). In this study, we analyzed *PIK3CA* mutations and PD-L1 expression in 224 CRC specimens and examined the correlation between the two. We also investigated their correlation with clinicopathologic features of CRC. We present the following article in accordance with the REMARK reporting checklist (available at https://dx.doi.org/10.21037/atm-21-2315).

Methods

Patients and tissue samples

CRC patients who received surgical resection at Jeonbuk National University Hospital from January 2018 to May 2019, were enrolled in the study. For clinical stage I–III patients, radical resection was the first choice of treatment; for stage III patients, adjuvant chemotherapy (FOLFOX) was administered. Stage IV patients underwent palliative resection and received adjuvant chemotherapy. Clinicopathological characteristics were obtained from their medical records and are summarized in *Table 1*. The TNM classification of patients was based on the American Joint Committee on Cancer staging system (8th edition). Assessment of tumor-infiltrating lymphocytes (TILs) was performed using the International TILs Working Group (ITWG) methodology (15). Serum tumor markers, including carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA19-9), were collected preoperatively, and their cut-off levels were 5.0 ng/mL and 37 kU/L, respectively. The exclusion criterion was neoadjuvant chemotherapy or radiotherapy. For analysis, the entire colon was divided into the right colon and the left colon. The right colon was defined as the segment from the cecum to the proximal two-thirds of the transverse colon, and the left colon was defined as the segment from the distal one-third of the transverse colon to the rectum. Postoperative surveillance for CRC patients was performed every 3 months. Laboratory tests including serum tumor marker CEA and CA19-9 were performed. Abdominal computed tomography (CT) was performed to detect recurrence and metastasis.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Jeonbuk National University Hospital (IRB No. 2020-06-013-002), and individual consent for this retrospective analysis was waived.

Next generation sequencing

Targeted next-generation sequencing (NGS) was performed using formalin-fixed paraffin-embedded (FFPE) tumor tissue. Hematoxylin and eosin (H&E)-stained slides were reviewed, and the tumor area with enough viable tumor

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Table 1 Association between patterns of *PIK3CA* mutation, PD-L1 expression and clinicopathological characteristics

Characteristics	Subcategories	No.	*PIK3CA*, n (%)		PD-L1, n (%)	
			Mutation	P	Positive	P
Age, years	<70	103	19 (18.4)	0.316	10 (9.7)	0.799
	≥70	121	29 (24.0)		13 (10.7)	
Sex	Male	127	25 (19.7)	0.467	14 (11.0)	0.670
	Female	97	23 (23.7)		9 (9.3)	
Tumor site[†]	Right	73	23 (31.5)	0.011	12 (16.4)	0.034
	Left	151	25 (16.6)		11 (7.3)	
Histologic grade	Well	14	4 (28.6)	0.769	2 (14.3)	<0.001
	Moderated	193	40 (20.7)		14 (7.3)	
	Poorly	17	4 (23.5)		7 (41.2)	
TILs	Low	80	14 (17.5)	0.285	7 (8.8)	0.577
	High	144	34 (23.6)		16 (11.1)	
Serum CEA[‡]	Elevation	56	13 (23.2)	0.707	17 (30.4)	0.899
	Normal	168	35 (20.8)		6 (3.6)	
Serum CA19-9[‡]	Elevation	31	5 (16.1)	0.439	22 (71.0)	0.164
	Normal	193	43 (22.3)		1 (0.5)	
pT category	pT1–T3	188	42 (22.3)	0.447	19 (10.1)	0.856
	pT4	36	6 (16.7)		4 (11.1)	
pN stage	pN0	127	34 (26.8)	0.026	15 (11.8)	0.384
	pN1+2	97	14 (14.4)		8 (8.2)	
Metastasis	Absent	172	42 (24.4)	0.047	22 (12.8)	0.024
	Present	52	6 (11.5)		1 (1.9)	
TNM stage	I + II	129	34 (26.4)	0.036	15 (11.6)	0.435
	III + IV	95	14 (14.7)		8 (8.4)	
PD-L1 IHC	Negative	201	33 (16.4)	<0.001	–	–
	Positive	23	15 (65.2)		–	
Relapse	Absent	186	45 (24.2)	0.026	21 (11.3)	0.265
	Present	38	3 (7.9)		2 (5.3)	

[†], right colon: defined as the segment from the cecum to the proximal two-thirds of the transverse colon; left colon: defined as the segment from the distal one third of the transverse colon to the rectum. [‡], serum CEA elevation; defined as >5.0 ng/mL. Serum CA19-9 elevation defined as >37 kU/L. CA19-9, cancer antigen 19-9; CEA, carcinoembryonic antigen; IHC, immunohistochemistry; PD-L1, programmed death ligand-1; TILs, tumor infiltrating lymphocytes.

cells was marked and used as a guide for macrodissection. Areas with greater than 50% tumor volume were used for examination. In brief, total nucleic acid was isolated from FFPE tumor tissue using a RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Ambion, Austin, TX, USA), according to the manufacturer's specifications. The samples were analyzed using the Oncomine Comprehensive Assay Cancer Panel (Ion Torrent S5 XL, Thermo Fisher Scientific, MA, USA) which covers 2,737 amplicons (2530 DNA + 207 RNA) within 143 cancer-related genes.

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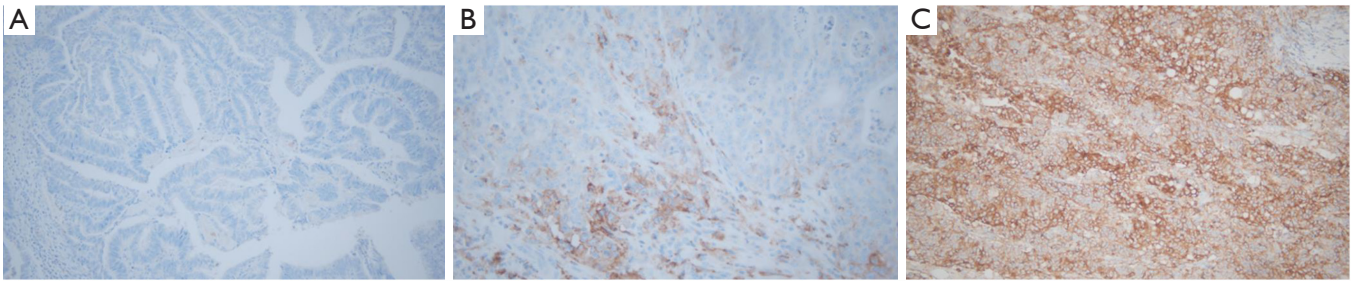

Figure 1 Expression of PD-L1 (SP263) in colorectal cancer. Only membranous staining of tumor cells was evaluated using immunohistochemical staining. (A) TPS 0: <1% of tumor cells; (B) TPS 1: ≥1% to 49% of tumor cells; (C) TPS 2: ≥50% of tumor cells. Magnification: 400×. PD-L1, programmed death ligand-1; TPS, tumor proportion score.

The percentage of amplicons covered was 95%. Reads were aligned to the hg19 reference genome, and variants with allele frequencies less than 3% were excluded. The Oncomine Comprehensive Assay Cancer Panel included 15 amplicons located in *PIK3CA* exons 1, 2, 3, 4, 6, 7, 9, 13, 18, 19, and 20.

Evaluation of PD-L1 expression

Immunohistochemical (IHC) staining was performed on tissue microarray (TMA) sections. FFPE tissue blocks containing representative samples of the tumors were selected by reviewing all H&E-stained slides. The diameter of each TMA core was 4 mm, and two cores per case were constructed. IHC staining with the primary antibody for PD-L1 (clone: SP263; dilution: ready-to-use; Roche) was performed using an automated immunostainer (BenchMark ULTRA, Roche Diagnostics, Mannheim, Germany) following the manufacturer's protocol. The slides stained for PD-L1 were evaluated by two pathologists (MJ Chung and AR Ahn) who had no clinicopathologic information on the patients. Only membranous staining of tumor cells was evaluated; nuclear and cytoplasmic staining was excluded from scoring (*Figure 1*). PD-L1 expression was defined as the percentage of tumor cells with membranous staining at any intensity. The percentage of PD-L1-positive tumor cells in the two TMA cores was averaged. The estimation of PD-L1 expression was performed using the tumor proportion score (TPS), which is applied routinely in diagnostic settings (16). It is a three-point evaluation scale (0 points for <1%, 1 point for ≥1% to 49%, and 2 points ≥50%). The cut-off for positivity of PD-L1 was ≥1% of tumor cells of any intensity (17).

Statistical analysis

The association between molecular characteristics and clinicopathological factors was evaluated using the Chi-square test or Fisher's exact test. For each patient, the date of the last follow-up was that of the last contact or of death of patient up to April 2020. The median follow-up period was 16.1 months (range, 4–23 months). We evaluated the prognosis of CRC patients by analyzing overall survival (OS). Patient death from CRC was considered an event for OS analysis. Relapse-free survival (RFS) was defined as the time from primary surgical treatment to detection of relapse or death. Survival curves were plotted using the Kaplan-Meier method and analyzed using the log-rank test. We performed univariate and multivariate Cox proportional hazards regression analyses, Kaplan-Meier survival analysis, and Pearson's Chi-square tests using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA), with $P<0.05$ indicating a significant difference.

Results

Genetic alterations of PIK3CA and their correlation with clinicopathological parameters in CRC patients

A total of 224 patients with primary CRC was enrolled in this study. The mean age was 69.2 years (range, 35–91 years). Overall, 127 (56.7%) patients were male and 97 (43.3%) were female (*Table 1*). Analysis of *PIK3CA* mutations was performed using targeted NGS, which examined 160 hotspots and included single-nucleotide variants and deletions. *PIK3CA* mutations were observed in 21.4% (48/224) of the patients. A total of 27 cases (56.3%) of *PIK3CA* mutations was detected in exon 9, and 12 cases (25.0%) in exon 20. In two cases, two types of *PIK3CA*

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Table 2 Association between site of *PIK3CA* mutation and PD-L1 expression

PIK3CA mutation	No.	PD-L1	
		Positive, n (%)	P
Exon			<0.001
9	27	11 (40.7)	
20	12	3 (25.0)	
Other	11	3 (27.3)	
Wild type	176	8 (4.5)	
Domain			<0.001
Helical D	20	9 (45.0)	
Kinase D	9	1 (11.1)	
Other	21	7 (33.3)	
Wild type	176	8 (4.5)	

PD-L1, programmed death ligand-1.

mutations were detected. Of all *PIK3CA* mutations, 56.3% were identified in three major hotspots: E545K (22.9%) and E542K (16.7%) in exon 9 and H1047R/L (16.7%) in exon 20 (Table S1). *PIK3CA* mutations were more significantly associated with cancer in the right colon than in the left colon (P=0.011). *PIK3CA* mutations were inversely correlated with lymph node metastasis (P=0.026), distant metastasis (P=0.047), and high TNM stage (P=0.036) (*Table 1*).

Expression of PD-L1 and its correlation with PIK3CA mutations in CRC patients

PD-L1 expression was found in 23 of 224 cases (10.3%): 19 cases with TPS 1 and 4 cases with TPS 2. The results revealed that PD-L1 expression was more significantly associated with right-sided colon cancer (P=0.034) and poorly differentiated carcinoma (P<0.001). PD-L1 expression was correlated inversely with distant metastasis (P=0.024) (*Table 1*).

Additionally, PD-L1 expression was associated significantly with *PIK3CA* mutations (P<0.001). In particular, the exon 9 (helical domain) mutations of *PIK3CA* were significantly associated with PD-L1 expression (P<0.001) (*Table 2*). However, there was no relationship between the TPS level of PD-L1 expression and *PIK3CA* mutations. PD-L1 expression was found in 15 of 48 (31.3%) cases of *PIK3CA* mutation: 12 cases with TPS 1 and three cases with TPS 2. In *PIK3CA* non-mutated cases, 4.5% (8/176) showed PD-L1 expression: seven cases with TPS 1 and one case with TPS 2.

Relationship between PIK3CA mutation and the prognosis of patients with CRC

In univariate survival analysis, the factors significantly associated with OS were poor histologic differentiation (P<0.001), elevated preoperative serum level of CA19-9 (P<0.001) and CEA (P=0.015), high T category (P<0.001), lymph node metastasis (P=0.019), presence of distant metastasis (P<0.001), and high tumor stage (P=0.014). The *PIK3CA* mutation were not correlated with OS. Low TILs (P=0.025), CA19-9 elevation (P<0.001), high T category (P<0.001), lymph node metastasis (P<0.001), presence of distant metastasis (P<0.001), high tumor stage (P<0.001), and wild *PIK3CA* (P=0.048) were significantly associated with shorter RFS (*Table 3*). Patients with a *PIK3CA* mutation exhibited better RFS compared to that of the wild-type *PIK3CA* group. To confirm that the correlation between the *PIK3CA* mutations and longer RFS was not caused by sampling bias, the effect of *PIK3CA* mutation on RFS was examined only in CRC patients with a low T category. The CRC patients with a low T category who had *PIK3CA* mutations exhibited longer RFS compared to those with wild-type *PIK3CA* (P=0.028) (*Figure 2*). The factors that had significant or borderline significant (P<0.1) correlation with OS or RFS by univariate analysis were included in the multivariate analysis. In multivariate analysis, CA19-9 elevation (P=0.005) and T category (P=0.045) were independent indicators for OS of patients with CRC. Distant metastasis was an independent indicator of RFS (P<0.001). No variables were significantly correlated with both OS and RFS (*Table 4*).

Discussion

In this study, we investigated *PIK3CA* mutations and IHC expression of PD-L1 in human CRC tissues. Our results show: (I) *PIK3CA* mutations in 21.4% of CRC (48/224) samples; (II) *PIK3CA* mutations correlated with negative lymph node status, low tumor stage, and absence of distant metastasis; (III) *PIK3CA* mutations correlated with longer RFS by univariate analysis; and (IV) *PIK3CA* mutations correlated with PD-L1 expression.

The mutation of *PIK3CA* is the most common alteration in the PI3K-AKT pathway and plays an important role in

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Table 3 Univariate Cox proportional hazards regression analysis for the overall survival and relapse-free survival of CRC patients

Characteristics	No.	OS		RFS	
		HR (95% CI)	P	HR (95% CI)	P
Age, years, ≥70 (*vs.* <70)	121/224	1.935 (0.840–4.454)	0.121	1.680 (0.858–3.291)	0.130
Sex, female (*vs.* male)	97/224	0.734 (0.327–1.650)	0.455	1.170 (0.616–2.222)	0.632
Tumor site, left colon (*vs.* right colon)	151/224	0.647 (0.297–1.410)	0.274	0.794 (0.411–1.536)	0.493
Histologic grade, moderated (*vs.* well)	193/224	0.190 (0.069–0.523)	0.001	0.652 (0.199–2.131)	0.479
High TIL (inter + high) (*vs.* low)	144/224	0.677 (0.312–1.466)	0.322	0.427 (0.225–0.809)	0.009
CEA elevation (*vs.* normal range)	56/224	2.648 (1.209–5.800)	0.015	1.815 (0.907–3.632)	0.092
CA19-9 elevation (*vs.* normal range	31/224	6.890 (3.164–15.006)	<0.001	4.860 (2.456–9.616)	<0.001
pT category, pT4 (*vs.* pT1–pT3)	36/224	4.501 (2.063–9.818)	<0.001	4.331 (2.230–8.413)	<0.001
pN stage, pN1 & pN2 (*vs.* pN0)	97/224	2.623 (1.169–5.886)	0.019	5.152 (2.435–10.899)	<0.001
Distant metastasis, presence (*vs.* absence)	52/224	4.886 (2.241–10.651)	<0.001	30.143 (12.515–72.603)	<0.001
Stage, ≥ III & IV (*vs.* I & II)	95/224	2.758 (1.229–6.190)	0.014	4.025 (1.994–8.124)	<0.001
PD-L1, positive (*vs.* negative	23/224	0.955 (0.310–3.453)	0.955	0.391 (0.094–1.629)	0.197
PIK3CA, mutated (*vs.* wild-type)	48/224	1.402 (0.589–3.337)	0.446	0.304 (0.093–0.988)	0.048

CA19-9, cancer antigen 19-9; CEA, carcinoembryonic antigen; CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; OS, overall survival; PD-L1, programmed death ligand-1; RFS, relapse-free survival; TILs, tumor infiltrating lymphocytes.

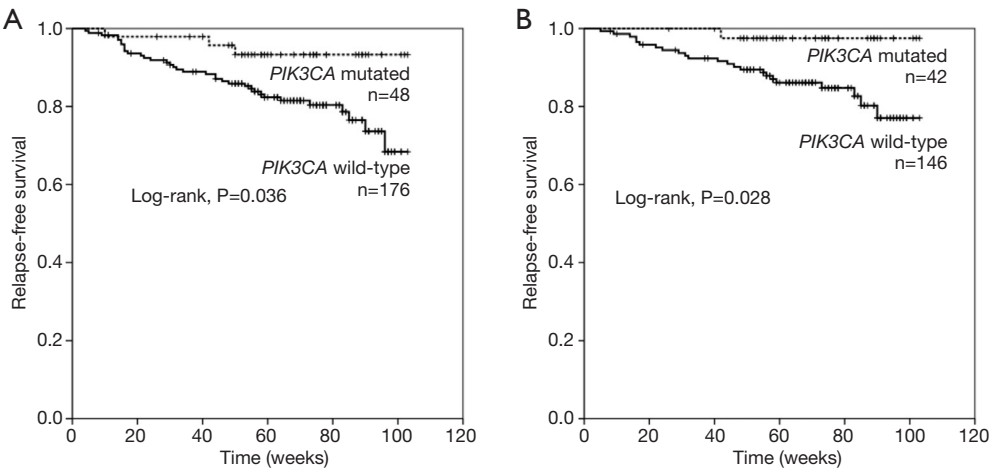

Figure 2 A Kaplan-Meier curve of relapse-free survival according to *PIK3CA* status. (A) Mutation of *PIK3CA* in 224 CRC patients was significantly associated with relapse-free survival; (B) in patients with low T category CRC, *PIK3CA* mutations were significantly associated with relapse-free survival. CRC, colorectal cancer.

the pathogenesis of CRC (18). Previous studies report that 14–32% of patients with CRC have *PIK3CA* mutations (19). Somatic missense mutations of *PIK3CA* were scattered across most of the exons but were found predominantly in the kinase (H1047R) and helical (E542K and E545K) domains of the *PIK3CA* subunit (20). Our results are similar to those of previous studies. *PIK3CA* mutations were observed in 48 (21.4%) of the 224 CRC samples, and the most frequently recurring mutations were E545K (22.9%), E542K (16.7%), and H1047R/L (16.7%).

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Table 4 Multivariate Cox regression analysis for the overall survival and relapse-free survival of CRC patients

Characteristics	OS[†]		RFS[‡]	
	HR (95% CI)	P	HR (95% CI)	P
CA19-9 elevation (*vs.* normal range)	3.853 (1.514–9.809)	0.005	1.459 (0.677–3.144)	0.335
pT category, pT4 (*vs.* pT1–pT3)	2.492 (1.021–6.080)	0.045	1.391 (0.672–2.878)	0.374
Distant metastasis, presence (*vs.* absence)	2.058 (0.667–6.348)	0.209	30.143 (12.515–72.603)	<0.001

[†], variables considered in multivariate analysis for overall survival were CEA elevation, CA19-9 elevation, T stage, lymph node metastasis, distant metastasis, stage. [‡], variables considered in multivariate analysis for relapse-free survival were CEA elevation, CA19-9 elevation, TILs, T stage, lymph node metastasis, distant metastasis, stage, and *PIK3CA* mutation. CA19-9, cancer antigen 19-9; CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; OS, overall survival; RFS, relapse-free survival.

PIK3CA mutations and prognosis in human cancers have yielded variable results (10,21,22). Previous studies have indicated that *PIK3CA* mutations are oncogenic and associated with an aggressive CRC phenotype and/or poor prognosis (16,23,24). It has been suggested that *PIK3CA* mutations either increase kinase activity or cause overexpression of these mutant *PIK3CA* proteins, which leads to concomitant phosphorylation of proteins in the AKT pathway. The activated AKT pathway plays an important role in tumor proliferation, survival, invasion, and angiogenesis, through which it exhibits aggressive tumor behavior. However, some studies have reported different results. *PIK3CA* mutations have been associated with a favorable cancer phenotype, and recent reports using meta-analysis have found no difference in *PIK3CA* mutations with relation to prognosis (10). Therefore, there is controversy surrounding the clinical significance of *PIK3CA* mutations. Our study revealed that *PIK3CA* mutations were correlated with favorable clinicopathologic factors and longer RFS in CRC patients.

The relationship between the *PIK3CA* mutations and good prognosis can be explained as follows. First, to obtain malignant properties, AKT inactive tumors (i.e., tumors in which the PI3K-AKT pathway is not activated) must have an abnormality that can be an alternative to AKT activation. These deviations can lead to more aggressive behavior than that with AKT activation (11). Second, in a study examining the clinical significance of *PIK3CA* overexpression in ovarian clear cell carcinoma, carcinomas containing activated *PIK3CA* had a better prognosis compared to tumors without activated *PIK3CA*. It has been asserted that the observed effect of *PIK3CA* on prognosis was not due to the function of downstream effectors but to *PIK3CA* genetic modification (25). A third possible explanation for the relationship between *PIK3CA* mutations and favorable prognosis is "oncogene-induced senescence", an endogenous tumor suppression mediated by replication stress (26). Oncogenes are key factors in carcinogenesis that trigger activation of various signaling pathways and downstream effectors. However, oncogenes also act as tumor suppressors by activating senescence. Activation of the PI3K-AKT pathway by the *PIK3CA* mutations lead to accumulation of reactive oxygen species (ROS) that induce activation of the p53/p21WAF1 pathway and senescence (27). ROS are major factors that induce cell cycle arrest and cellular senescence (28). Unfortunately, there is no satisfactory explanation for these conflicting results. Elucidating how mutations in *PIK3CA* achieve this opposite effect (favorable versus poor prognosis) will be important in understanding the pathogenesis of CRC. In addition, this knowledge could be used to determine therapeutic approaches for patients with *PIK3CA* mutations.

Our results show *PIK3CA* mutations correlated with favorable prognostic factors and longer RFS in the univariate analysis, but this was not supported by the multivariate analysis. Additionally, right-sided colon cancer had a higher rate of *PIK3CA* mutations, and these results are consistent with previous studies. The outcomes of patients with left-sided cancers were better than those with right-sided cancers in instances of metastatic CRC. In relation to prognosis, the contradiction shown by tumor sidedness and *PIK3CA* mutations needs to be confirmed through additional studies. This result is presumed to be affected by the short follow up time (~23 months), a limitation of this study. Further studies are required to confirm our results.

The standard treatment for advanced CRC is surgical resection and adjuvant chemotherapy. Conventional therapy has a limited therapeutic effect in a subset of patients with CRC (2). Immunotherapy for cancer patients has several

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advantages, such as high accuracy and fewer side effects compared to conventional adjuvant chemotherapy (29). There are several immune checkpoint inhibitors (ICIs) approved by the Food and Drug Administration that target different pathways, including PD-1, PD-L1, and the cytotoxic T lymphocyte antigen 4 pathway. PD-L1 expression is a well-known predictive biomarker for PD-1/PD-L1 inhibitors in advanced cancer patients. The PI3K signaling pathway is involved in the immune response of tumors, including the anti-cancer immune response, and the acquisition of leukocyte-like properties by cancer cells (30). Studies have shown that PD-L1 expression is regulated by transcription factors, signaling pathways, and epigenetic factors. The PI3K/AKT signaling pathway has been shown to affect tumoral PD-L1 expression. However, the association between *PIK3CA* mutation and PD-L1 expression in human CRC has not been established. Nusrat *et al.* have shown that PD-L1 expression is significantly correlated with *PIK3CA* mutations (31). Interaction between the helical domain mutation of *PIK3CA* and Ras-GTP can increase PD-L1 mRNA stability, which increases PD-L1 surface expression (32). Others have found no correlation between *PIK3CA* and PD-L1 (27). In the current study, PD-L1 expression was found in 23 of 224 cases (10.3%) and was significantly associated with *PIK3CA* mutations (P<0.001). In particular, the exon 9 (helical domain) mutations of *PIK3CA* were associated significantly with PD-L1 expression (P<0.001). A previous study has shown that *PIK3CA* mutations are associated with greater clinical benefit from immunotherapy (30). Based on these results, it would be meaningful to further investigate the mechanism of the relationship between the *PIK3CA* mutation and PD-L1 expression. Future research into the mechanism is expected to assist in selection of more effective candidates for immunotherapy.

In the current study, we investigated *PIK3CA* mutations in CRC and the relationship between *PIK3CA* mutations and patient clinicopathological characteristics as well as PD-L1 expression. *PIK3CA* mutations were observed in 21.4% of CRCs and were associated with favorable prognostic factors and longer RFS. We discussed possible mechanisms that can explain the association between *PIK3CA* mutations and favorable prognosis in cancer. In addition, we showed that *PIK3CA* mutations had a significant correlation with expression of PD-L1, which suggests that cancer with both *PIK3CA* mutations and PD-L1 expression is a good candidate for immunotherapy. Further investigation is required to identify whether *PIK3CA* mutations are a good prognostic factor. In addition, further studies are needed to understand the mechanisms of the association between *PIK3CA* mutations and favorable prognosis of cancer and the overall correlation between *PIK3CA* mutations and PD-L1 expression.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Jeonbuk National University Hospital (IRB No. 2020-06-013-002) and individual consent for this retrospective analysis was waived.

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Supplementary

Table S1 Summary of *PIK3CA* mutation in colorectal cancers

Exon	Nucleotide	AA mutation	Domain	Cases
9	c.1633G>A	p.E545K	Helical	11
9	c.1624G>A	p.E542K	Helical	8
9	c.1636C>G	p.Q546E	Helical	3
9	c.1637A>G	p.Q546R	Helical	3
9	c.1635G>C	p.E545D	Helical	1
9	c.1637A>C	p.Q546P	Helical	1
20	c.3140A>G	p.H1047R	Kinase	6
20	c.3140A>T	p.H1047L	Kinase	2
20	c.3139C>T	p.H1047Y	Kinase	1
20	c.3061T>C	p.Y1021H	Kinase	1
20	c.3085G>T	p.D1029Y	Kinase	1
20	c.3129G>A	p.M1043I	Kinase	1
4	c.1031T>G	p.V344G	C2	2
4	c.1049A>G	p.D350G	C2	1
4	c.1030G>A	p.V344M	C2	1
1	c.277C>T	p.R93W	ABD	1
1	c.263G>A	p.R88Q	ABD	1
1	c.112C>T	p.R38C	ABD	1
1	c.328_330delGAA	p.E109del	N/S	1
7	c.1258T>C	p.C420R	C2	1
13	c.2176G>A	p.E726K	N/S	1
5	c.1090G>A	p.G364R	C2	1

AA, amino acid; ABD, adapter binding domain; N/S, not specified.

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