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Prognostic Role of *PIK3CA* Mutation in Colorectal Cancer: Cohort Study and Literature Review

Xiaoyun Liao[1], **Teppei Morikawa**[1], **Paul Lochhead**[1], **Yu Imamura**[1], **Aya Kuchiba**[1], **Mai Yamauchi**[1], **Katsuhiko Nosho**[2], **Zhi Rong Qian**[1], **Reiko Nishihara**[1], **Jeffrey A Meyerhardt**[1], **Charles S Fuchs**[1,3], and **Shuji Ogino**[1,4]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA

[2]First Department of Internal Medicine, Sapporo Medical University, Sapporo, Japan

[3]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA

[4]Department of Pathology, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA

Abstract

Purpose—Mutations in *PIK3CA* (the gene encoding the p110α catalytic subunit of phosphatidylinositide-3-kinase, PI3K) play an important role in colorectal carcinogenesis. Experimental evidence suggests that *PIK3CA* exon 9 and exon 20 mutations trigger different biological effects, and that concomitant mutations in both exons 9 and 20 synergistically enhance tumorigenic effects. Thus, we hypothesized that *PIK3CA* exon 9 and exon 20 mutations might have differential effects on clinical outcome in colorectal cancer, and that concomitant *PIK3CA* exon 9 and 20 mutations might confer aggressive tumor behavior.

Experimental Design—We sequenced *PIK3CA* by pyrosequencing in 1170 rectal and colon cancers in two prospective cohort studies, and found 189 (16%) *PIK3CA*-mutated tumors. Mortality hazard ratio (HR) according to *PIK3CA* status was computed using Cox proportional hazards model, adjusting for clinical and molecular features including microsatellite instability, CpG island methylator phenotype, LINE-1 methylation, and *BRAF* and *KRAS* mutations.

Results—Compared to *PIK3CA* wild-type cases, patients with concomitant *PIK3CA* mutations in exons 9 and 20 experienced significantly worse cancer-specific survival [log-rank P=0.031; multivariate HR=3.51; 95% confidence interval (CI), 1.28–9.62] and overall survival (log-rank P=0.0008 ; multivariate HR=2.68; 95% CI, 1.24–5.77). *PIK3CA* mutation in either exon 9 or 20 alone was not significantly associated with patient survival. No significant interaction of *PIK3CA* mutation with *BRAF* or *KRAS* mutation was observed in survival analysis.

Conclusion—Co-existence of *PIK3CA* (the PI3K p110α subunit) exon 9 and 20 mutations, but not *PIK3CA* mutation in either exon 9 or 20 alone, is associated with poor prognosis of colorectal cancer patients.

Correspondence to: Shuji Ogino, MD, PhD, MS, (Epidemiology), Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School, 450 Brookline Ave., Room JF-215C, Boston, MA 02215, Tel: 617-632-1972; Fax: 617-582-8558, shuji_ogino@dfci.harvard.edu.
XL, TM, PL and YI contributed equally.

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Keywords

colon cancer; PI3K; RAF; RAS; biomarker

INTRODUCTION

Phosphatidylinositide-3-kinases (PI3K) are lipid kinases that promote various biological processes including cellular proliferation and survival (1). Mutations in the *PIK3CA* gene, which encodes the p110α catalytic subunit of PI3K, have been identified in many human solid tumors, including colon, breast, brain, ovarian, liver, and lung cancers (1). In colorectal cancers, *PIK3CA* mutations, which are found in 10–20% of tumors, have been reported to be associated with specific clinicopathological features and molecular events, such as proximal tumor location, microsatellite instability (MSI), and *KRAS* mutation (2–10).

The prognostic significance of *PIK3CA* mutation in colorectal cancer remains unclear (Table 1) (2, 4, 7, 8, 11–16). The majority of activating *PIK3CA* mutations map to three sites: exon 9, codons 542 and 545 in the helical domain, and exon 20, codon 1047 in the kinase domain. Mutation at any one of these sites has been shown to result in a gain of enzymatic function and to promote oncogenic transformation in vitro and in vivo (17–19). Interestingly, the mechanisms through which helical and kinase domain mutations augment enzyme function differ (20). Furthermore, the coexistence of mutations in both exons 9 and 20 of the same p110α molecule (PIK3CA) leads to a synergistic gain of function, with a potent transforming capacity in vitro (20). Thus, we hypothesized that *PIK3CA* exon 9 and exon 20 mutations might have differential effects on tumor behavior, and that the coexistence of mutations in both exons 9 and 20 might result in more aggressive tumor behavior compared to cancers with wild-type *PIK3CA,* or a single mutation in either exon 9 or exon 20.

The interaction of EGF with EGFR triggers two main signaling pathways, RAS-RAF-MAPK and PI3K-AKT. Activation of these pathways by mutations in *KRAS*, *BRAF* and/or

PIK3CA is an established mechanism that drives colorectal carcinogenesis (21). In thyroid cancers, the coexistence of *BRAF* and *PIK3CA* mutations is associated with aggressive tumor behavior (22, 23). Based on these findings, our third hypothesis was that *PIK3CA* and *BRAF* mutations might interact synergistically to confer a more aggressive colorectal cancer phenotype.

In order to test these hypotheses, we utilized our molecular pathological epidemiology (24–26) database based on two ongoing U.S. nationwide prospective cohort studies. We assessed various additional molecular features, including *KRAS* mutation, CpG island methylator phenotype (CIMP), microsatellite instability (MSI), TP53 negativity, and LINE-1 hypomethylation, and could therefore control for confounding by these potential predictors of outcome.

MATERIALS AND METHODS

Study Group

We used the database of two prospective cohort studies, the Nurses' Health Study (NHS, N = 121,700 women observed since 1976) and the Health Professionals Follow-Up Study (HPFS, N = 51,500 men observed since 1986). Every two years, participants were sent follow-up questionnaires to update information on potential risk factors, and to identify newly diagnosed cancers and other diseases. Paraffin embedded tissue blocks were collected

from hospitals where participants with colorectal cancer underwent resection of their primary tumors. We collected diagnostic biopsy specimens for rectal cancers patients who received per-operative therapy in order to avoid treatment-related artifact or bias. The tissue retrieval rate was approximately 70% when specimens were requested within five years of diagnosis. All colorectal cancer cases were confirmed through review of histology by a pathologist (S.O.) blinded to other data. Tumor grade was categorized as high (≤50% glandular area) or low (>50% glandular area). Based on the availability of DNA (at least some amount of DNA was available in 1267 cases), *PIK3CA* sequencing data, and survival data, a total of 1170 colorectal cancer cases diagnosed up to 2006 were included in this study. Patients were observed until death, or January 2011, whichever came first. Ascertainment of deaths included reporting by family members or, where study correspondence had been returned, by postal authorities. The National Death Index was used to ensure completeness of ascertainment. The cause of death was assigned by study physicians. Written informed consent was obtained from all study subjects. Tissue collection and analyses were approved by the Human Subjects Committees at Harvard School of Public Health and Brigham and Women's Hospital.

Sequencing of *PIK3CA*, *BRAF* and *KRAS*, and Microsatellite Instability Analysis

Genomic DNA was extracted from paraffin-embedded tissue. Methods for PCR and pyrosequencing targeted at *PIK3CA* exons 9 and 20 were adapted from those previously-described (10) with the following modifications: we replaced the sequencing primer *PIK3CA* 9-RS2 with 5′-TTCTCCTT/GCTT/CAGTGATTT-3′, and employed a new nucleotide dispensation order (ATACACATGTCAGTCAGACTAGCTAGCTAGCTAG), which was particularly sensitive for c.1624G>A; sequencing primer *PIK3CA* 9-RS3 was replaced with 5′-TAGAAAATCTTTCTCCTGCT-3′, and a new dispensation order (ATAGCACTGACTGACTGACTACTGACTGACTG) used to detect the most common mutations, c.1633G>A and c.1624G>A.

PCR and pyrosequencing assays targeted at *BRAF* (codon 600) (27) and *KRAS* (codons 12 and 13) mutations (28) were performed as previously described. Microsatellite instability was assessed using a panel of 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67 and D18S487) (29). MSI-high was defined as the presence of instability in ≥30% of the markers, and MSI-low/microsatellite stability (MSS) as 0–29% unstable markers (29).

Analysis of CpG Island Methylation and LINE-1 Hypomethylation

Sodium bisulfite treatment of DNA and real-time PCR assays (MethyLight) were performed as previously described (29, 30). We quantified promoter methylation at eight CIMP-specific loci: *CACNA1G, CDKN2A* (p16), *CRABP1, IGF2, MLH1, NEUROG1, RUNX3,* and *SOCS1* (31–33). CIMP-high was defined as ≥6 (of 8) methylated promoters, and CIMP-low/0 as 0–5 (of 8) methylated promoters (32). To accurately quantify small methylation changes on a background of relatively high methylation, a LINE-1 PCR-pyrosequencing assay was employed (34, 35).

TP53 Immunohistochemistry

Tissue microarray blocks were constructed and immunohistochemistry for TP53 (p53) was performed (36). Positive and negative controls were included in each run of immunohistochemistry. All immunostaining slides were scored by a pathologist (S.O.) blinded to other data. A random sample of 118 tumors was re-examined by a second observer (K.N.) unaware of other data. The concordance between the two observers was 0.87 ($\kappa = 0.75$; $P < 0.0001$), indicating substantial agreement.

Statistical Analysis

All statistical analyses were performed using SAS software (version 9.1; SAS Institute Inc, Cary, North Carolina). For categorical data, the chi-square test or Fisher's exact test was performed. All *P* values were two sided. When multiple hypothesis testing was performed, the *P* value for significance was adjusted to $P = 0.0038$ (= 0.05/13) by Bonferroni correction. To compare mean age and mean LINE-1 methylation levels, a t-test or ANOVA, assuming equal variances, was performed. To assess whether associations between *PIK3CA* mutation and the variables in Table 2 were independent of other variables, a multivariate logistic regression analysis was conducted for cross sectional analyses. Odds ratios (OR) were adjusted for age at diagnosis (continuous), sex, tumor location (proximal vs. distal), CIMP status (high vs. low/0), MSI status (high vs. low/MSS), LINE-1 methylation (continuous), *BRAF* mutation and *KRAS* mutation. A backward stepwise elimination with a threshold of $P = 0.05$ was used to select variables in the final model to avoid overfitting.

The Kaplan-Meier method and the log-rank test were performed for survival analysis. Deaths from causes other than colorectal cancer were censored in colorectal cancer-specific mortality analyses. We performed power calculations. Assuming a total number of patients of 1170, 7 cases with *PIK3CA* mutations in both exons 9 and 20, 50% mortality, and an alpha (type I error rate) of 0.05, there was a 50% power to detect an HR of 4.6. To control for confounding, we used Cox proportional hazards models to calculate HR of death according to tumor *PIK3CA* status, adjusting for age at diagnosis (continuous), sex (NHS vs. HPFS), year of diagnosis (continuous), tumor location (proximal vs. distal colon vs. rectum), tumor grade, MSI (high vs. low/MSS), CIMP (high vs. low/0), LINE-1 methylation (continuous), *KRAS* mutation, and *BRAF* mutation. To minimize residual confounding and overfitting, disease stage (I, II, III, IV, or unknown) was used as a stratifying variable using the "strata" option in the SAS "proc phreg" command. To avoid overfitting, variables in the final model were selected using backward stepwise elimination with a threshold of $P = 0.05$. Interaction was assessed using the Wald test on the cross-product of *PIK3CA* and another variable of interest (excluding cases missing data) in a multivariate Cox model. The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross products of the *PIK3CA* variable and survival time ($P = 0.44$ for colon cancer-specific mortality; $P = 0.95$ for overall mortality).

To avoid overfitting, cases with missing data in any of the categorical variables [tumor location (0.6%), tumor grade (0.6%), CIMP (6.6%), MSI (1.4%), *BRAF* (0.5%), and *KRAS* (0.4%)], were included in the majority category for that variable. We confirmed that excluding cases with missing information in any of the covariates did not substantially alter the results (data not shown).

RESULTS

PIK3CA Mutation Status in Colorectal Cancer

PIK3CA pyrosequencing analysis was successful in 95.6% (1212/1267) of colorectal cancers. Pyrosequencing has been shown to be a reproducible, precise and sensitive method of mutation analysis in paraffin-embedded tumor tissues (10, 28). Cases lacking survival data were excluded. Of the remaining 1170 colorectal cancer cases, 536 cases were from HPFS (men) and 634 cases were from NHS (women).

PIK3CA mutation was detected in 189 (16%) out of 1170 cases, among which 109 cases (58%) had mutations in only exon 9, 73 cases (39%) had mutations in only exon 20, and seven cases (3.7%) had mutations in both exons 9 and 20. Supplementary Table 1 shows the frequencies of specific *PIK3CA* mutations. The relationships between *PIK3CA* mutation and clinical, pathological, and molecular features in each cohort (NHS and HPFS) are

presented separately in Supplementary Table 2 and Supplementary Table 3, and demonstrate general consistency between the two cohorts. Table 2 summarizes clinical, pathological and molecular features of colorectal cancer according to *PIK3CA* mutation status.

In contrast to mutations in exon 9, mutations in exon 20 were associated with MSI-high ($P = 0.0007$), CIMP-high ($P = 0.028$), and *BRAF* mutation ($P = 0.030$). Associations between concomitant *PIK3CA* exon 9 and exon 20 mutation status and family history of colorectal cancer ($P = 0.030$), MSI-high ($P = 0.0050$), CIMP-high ($P = 0.025$), and TP53 negativity ($P = 0.0080$) were of borderline significance taking into account multiple hypothesis testing (requiring $P = 0.0038$ as a significance level). Supplementary Table 4 shows the clinical, pathological, and molecular features of colorectal cancers categorized by overall *PIK3CA* mutation status (any mutation vs. no mutation). *PIK3CA* overall mutation status was significantly associated with *KRAS* mutation ($P < 0.0001$), however there was no significant difference in the frequency of *KRAS* mutation between exon 9 and exon 20 mutants ($P = 0.29$).

Detailed information on the seven cases with concomitant *PIK3CA* exon 9 and exon 20 mutations is shown in Table 3. One case had three *PIK3CA* mutations (c.1624G>A, c. 1631C>A and c.3140A>G). Although the number of patients with cancers harboring concomitant *PIK3CA* exon 9 and exon 20 mutations was small, this subgroup appeared to have an association with family history of colorectal cancer. Notably, all seven patients died of colorectal cancer or other causes within the follow-up period.

Independent Association between *PIK3CA* and *KRAS* Mutations

We performed multivariate logistic regression analysis to assess for independent relationships between *KRAS* mutation, and other factors, and *PIK3CA* overall mutation. In multivariate model analysis, *KRAS* mutation remained significantly associated with *PIK3CA* overall mutation [multivariate odds ratio (OR) = 2.65; 95% confidence interval (CI), 1.89–3.73; $P < 0.0001$]. In addition, CIMP-status remained in the final model (multivariate OR = 1.65; 95% CI, 1.07–2.54; $P = 0.024$), with borderline significance given multiple hypothesis testing (Supplementary Table 5).

PIK3CA Mutation in Colorectal Cancer and Patient Survival

We assessed the prognostic role of *PIK3CA* mutation in 1170 colorectal cancers to test the hypotheses that *PIK3CA* exon 9 and exon 20 mutations might have differential effects on tumor behavior, and that the presence of mutations in both exon 9 and exon 20 might result in more aggressive tumor behavior. During a median follow-up period of 141 months for survivors (interquartile range, 105–192), there were 552 deaths, including 328 colorectal cancer-specific deaths. Notably, patients with *PIK3CA* mutations in both exons 9 and 20 (henceforth referred to as “exon 9 and 20 double mutants”) experienced significantly shorter cancer-specific survival (log-rank $P = 0.031$) (Figure 1A) and overall survival than patients with wild-type *PIK3CA* (log-rank $P = 0.0008$) (Figure 1B). In Cox regression analysis, compared to *PIK3CA* wild-type cases, exon 9 and 20 double mutant status was associated with significantly higher colorectal cancer-specific mortality (univariate HR = 2.84; 95% CI, 1.05–7.69; multivariate HR = 3.51; 95% CI, 1.28–9.62) and overall mortality (univariate HR = 3.37; 95% CI, 1.58–7.15; multivariate HR = 2.68; 95% CI, 1.24–5.77) (Table 4).

In contrast, the presence of a single *PIK3CA* mutation, in either exon 9 or 20, was not significantly associated with patient survival (Figure 1A, 1B; Table 4). The prognostic association of *PIK3CA* exon 9 mutations in colorectal cancer (regardless of exon 20 status) was examined. No significant difference was found between exon 9 mutants and wild-type cases in colorectal cancer-specific or overall survival. The HRs for exon 9 mutants were not

influenced by the inclusion of double mutants, even after adjusting for exon 20 mutation status. Likewise, the prognostic association of *PIK3CA* exon 20 mutations (regardless of exon 9 status) was similar to wild-type cases in colorectal cancer-specific and overall survival analysis (Supplementary Table 6). Overall *PIK3CA* mutation status (exon 9 or 20 mutation) was not significantly associated with colorectal cancer-specific or overall survival when compared to wild-type cases (Figure 1C, 1D; Table 4). When each cohort was analyzed separately, overall *PIK3CA* mutation status was not significantly associated with colorectal cancer-specific or overall survival (Supplementary Table 7). HRs were similar for both cohorts and 95% CI were largely overlapping, showing the consistency of results between the two cohorts.

Combined *PIK3CA* and *BRAF*, *KRAS* Mutation Status and Colorectal Cancer Prognosis

To test the third hypothesis, that the presence of both *BRAF* and *PIK3CA* mutations might result in aggressive tumor behavior, we examined combined *BRAF* and *PIK3CA* mutation status and patient prognosis (Supplementary Table 8). Compared to *PIK3CA* wild-type/*BRAF* wild-type cases, the presence of mutations in both *PIK3CA* and *BRAF* was not significantly associated with colorectal cancer-specific mortality in univariate analysis (HR = 1.24; 95% CI, 0.61–2.52). However, in multivariate analysis, the presence of mutations in both *PIK3CA* and *BRAF* was significantly associated with colorectal cancer-specific mortality (multivariate HR = 2.40; 95% CI, 1.12–5.16). We found that MSI and CIMP status were confounders; when we simply adjusted for MSI and CIMP, the adjusted HR (*PIK3CA* mutated/*BRAF* mutated vs. *PIK3CA* wild-type/*BRAF* wild-type) was 3.08 (95% CI, 1.44–6.61).

We also examined the influence of *KRAS* and *BRAF* mutation status on the prognostic association of mutations in *PIK3CA*. We classified colorectal cancers into three subtypes according to *KRAS* and *BRAF* status: *BRAF* wild-type/*KRAS* wild-type, *BRAF*-mutated/*KRAS* wild-type, and *BRAF* wild-type/*KRAS*-mutated. No substantial effect modification by *KRAS/BRAF* mutation status was observed in survival analyses (Table 5).

PIK3CA Mutation Status and Mortality in Strata of Other Variables

In further exploratory analyses, the prognostic effect of *PIK3CA* mutation in strata of other variables was evaluated. The effect of *PIK3CA* on cancer-specific mortality did not significantly differ according to disease stage ($P_{\text{interaction}} = 0.93$), tumor location ($P_{\text{interaction}} = 0.099$), or any of the other variables examined (all $P_{\text{interaction}} > 0.05$).

DISCUSSION

We conducted this study to test the hypotheses that *PIK3CA* exon 9 and exon 20 mutations might have differential effects on colorectal cancer behavior, and that the presence of concomitant mutations in both exons 9 and 20 might lead to aggressive tumor behavior. We found no significant association between overall or exon-specific *PIK3CA* mutation status and survival. The concomitant presence of mutations in both exons 9 and 20 was, however, associated with a poorer prognosis for colorectal cancer patients, although a confirmation by other studies would be essential. Our data support the hypothesis that concomitant exon 9 and 20 mutations may have a synergistic effect on tumor behavior, and are consistent with experimental data by Zhao et al (20), that show potent synergistic transforming effects of concomitant *PIK3CA* exons 9 and 20 mutations. Patients with concomitant mutations in exons 9 and 20 of *PIK3CA* were more likely to report a family history of colorectal cancer. At present, the cause of this potential association remains obscure. One could speculate that a family history of colorectal cancer might confer a genetic predisposition to the development of the *PIK3CA* mutations. The number of cases with the concomitant *PIK3CA*

exon 9 and 20 mutations was, however, very small, and we should exercise caution in the interpretation of any apparent clinical, pathological and molecular associations within this subgroup. These findings must be validated by independent datasets.

The assessment of prognostic factors or biomarkers is important in cancer research (37–45). A number of previous studies have examined the prognostic role of *PIK3CA* mutation in colorectal cancer (Table 1) (2, 4, 7, 8, 11–16, 46). Although some studies have shown that *PIK3CA* mutation is associated with shorter survival (2, 11), the statistical power of these studies is quite limited (sample size N < 160). In a larger study (N = 586), the presence of a mutation in any of *PIK3CA, BRAF* or *KRAS* was associated with poor three-year survival, but the effect of *PIK3CA* mutation in isolation was not studied (7). Two additional studies demonstrated that, compared to patients with wild-type *PIK3CA*, patients with exon 20 mutations experienced worse survival while exon 9 mutation was not associated with outcome (14, 16). In one of these two studies, tumor *KRAS* and *BRAF* mutations were not examined (16). In our current study, cancers with *PIK3CA* exon 20 mutation were found to have a higher frequency of *BRAF* mutation. *BRAF* mutation has been associated with a poorer prognosis in colorectal cancer (47), and might therefore confound analyses of *PIK3CA* exon 20 mutation and survival in colorectal cancer. Other groups have reported that *PIK3CA* mutations are not associated with liver metastasis (48) or with overall survival (with or without adjuvant treatment) (4, 8, 15).

In our current study, neither *PIK3CA* overall mutation status, nor *PIK3CA* mutation in exon 9 or 20 alone, was significantly associated with patient survival. This is in contrast to some of the published literature. Notwithstanding the potential for confounding by *BRAF* status in other studies, it is worth bearing in mind that small studies with null results have a higher probability of being unpublished compared to similarly sized datasets with “significant” findings. Large studies with adequate statistical power are less prone to this type of “publication bias”. We should therefore place more emphasis on the results of large-scale studies when we evaluate publications on the prognostic significance of cancer biomarkers. Furthermore, experimental evidence suggests that *PIK3CA* mutation alone has a relatively modest effect on tumor cell growth, and that *PIK3CA* mutations need to cooperate with other PI3K enzyme mutations for effective cellular transformation (17, 49). Because of the power limitations in the previous studies on *PIK3CA* mutation, we feel that there is currently insufficient evidence to support a role for *PIK3CA* exon 9 or 20 mutation alone as a prognostic biomarker in colorectal cancer. Our findings warrant validation in additional large cohort studies.

We previously described an association between *PIK3CA* mutation and shorter cancer-specific survival among 450 stage I-III colon cancer cases (12). All of those 450 cases were included in our current study. Sample size and adequate statistical power are critically important in such exploratory studies (48). Since our previous study was restricted to stage I-III colon cancers, the numbers of adverse events (66 colon cancer-specific deaths and 152 overall deaths) was also much smaller than in our current study (328 colorectal cancer-specific deaths and 552 overall deaths), which included cancers of all stages. As a result, our current findings are more robust than those of our previous study where, as a result of smaller sample size and lower power, there would have been increased risk of finding chance associations. This underscores the critical importance of careful study design, adequate statistical power, and cautious interpretation of data, which are prerequisites for exploratory studies of this nature (50).

Even in our larger dataset of 1170 colorectal cancers, there were only seven patients who harbored *PIK3CA* mutations in both exons 9 and 20. However, given that over 550,000 individuals are diagnosed with colorectal cancer each year in the U.S. and Europe, we

estimate that, in these regions combined, there would be approximately 3,300 colorectal cancer patients every year with *PIK3CA* mutations in both exons 9 and 20. The incidence of this potentially aggressive type of colorectal cancer may in fact be similar to the combined sum of the incidences of Burkitt lymphoma, hairy cell leukemia, ALK-positive large B-cell lymphoma, and angioimmunoblastic T-cell lymphoma in these western countries. Other cancers with a similar incidence include osteosarcoma, medulloblastoma, gestational choriocarcinoma, and ovarian clear cell carcinoma. Thus, those colorectal cancers with *PIK3CA* mutations in both exons 9 and 20 may represent as significant a cancer burden as these other cancer types in our society.

Caveats of our current study include the limited data on cancer treatment in the cohorts, which prevented the inclusion of treatment as a variable in our analyses. Nonetheless, it is unlikely that chemotherapy use or regimens differed substantially by *PIK3CA* mutation status given that a vast majority of cases were diagnosed prior to 2006, and *PIK3CA* mutation data were unavailable to physicians or patients. In addition, our multivariable Cox regression analysis adjusted for disease stage (I, II, III, or IV), on which treatment decision-making was mostly based. Colorectal cancer-specific survival is therefore a reasonable colorectal cancer-specific outcome.

Our findings relating to survival in patients whose tumors harbored mutations in both exons 9 and 20 of *PIK3CA* are novel. However, given that the number of such cases in our study was small, and statistical power was consequently limited, these findings warrant validation by independent studies.

Our study gains several strengths through utilization of the database of two U.S. nationwide prospective cohort studies. Clinicopathological information, various exposures, and tumor molecular data have been integrated into our molecular pathological epidemiology (24–26) database. Cohort participants with colorectal cancer sought medial attention and were treated at hospitals throughout the U.S. Hence, our sample is more representative of colorectal cancer in the general U.S. population than a convenience sample collected at one or a few hospitals. Moreover, our extensive tumor database enabled us to assess the prognostic association of *PIK3CA* mutations independent of other critical molecular events such as *BRAF* and *KRAS* mutations, LINE-1 hypomethylation, MSI, and CIMP, which have all been associated with colon cancer outcome (29, 34).

In conclusion, in our study of 1170 colorectal cancers, concomitant *PIK3CA* mutation of both exons 9 and 20 was associated with a poorer prognosis, although a statistical power was limited due to only 7 cases with the concomitant mutations. In contrast, neither *PIK3CA* exon 9 nor exon 20 mutation alone appeared to have substantial prognostic influence. The robustness of our findings would be enhanced by replication in other large studies. Our findings might give additional insight into the relevance of the PI3K pathway in colorectal cancer progression, and suggest that detailed genotyping of *PIK3CA* might serve towards personalized medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ANOVA	analysis of variance
CI	confidence interval
CIMP	CpG island methylator phenotype
HR	hazard ratio
MSI	microsatellite instability
MSS	microsatellite stable
OR	odds ratio
PI3K	phosphatidylinositide 3-kinase
SD	standard deviation

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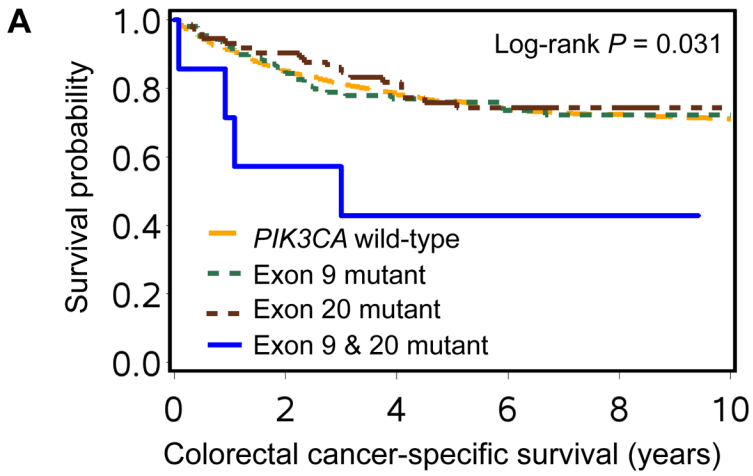
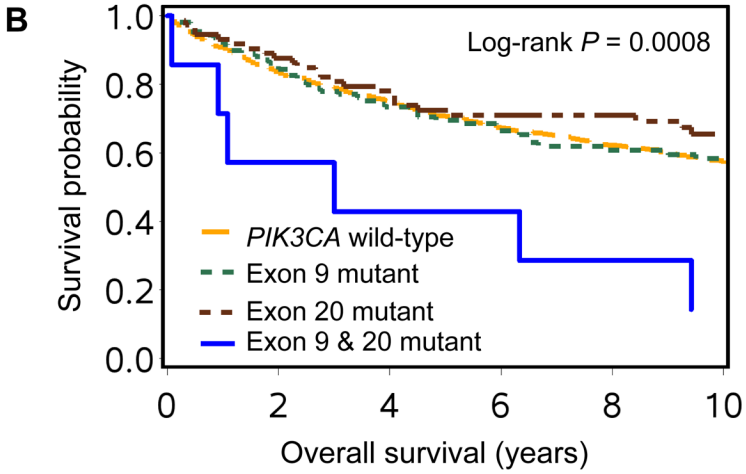
Statement of Translational Relevance

PIK3CA mutation is present in various human cancers, and plays a role in cancer cell proliferation and survival. The relationship between *PIK3CA* mutation in colorectal cancer and patient survival remains controversial. In this study we utilized a database of 1170 colorectal cancers in two prospective cohort studies. Our study benefitted from adequate participant follow-up, and the availability of clinical information and data on additional molecular characteristics that are important in colorectal carcinogenesis. This is, by far, the largest study on the prognostic role of *PIK3CA* mutations in colorectal cancer to date, and suggests that patients with concomitant *PIK3CA* mutations in both exons 9 and 20 might be associated with worse survival. The presence of a single *PIK3CA* mutation in either exon 9 or 20 was not significantly associated with patient survival. Considering the role of the PI3K signaling pathway in cancer, our findings might be relevant towards personalized medicine.





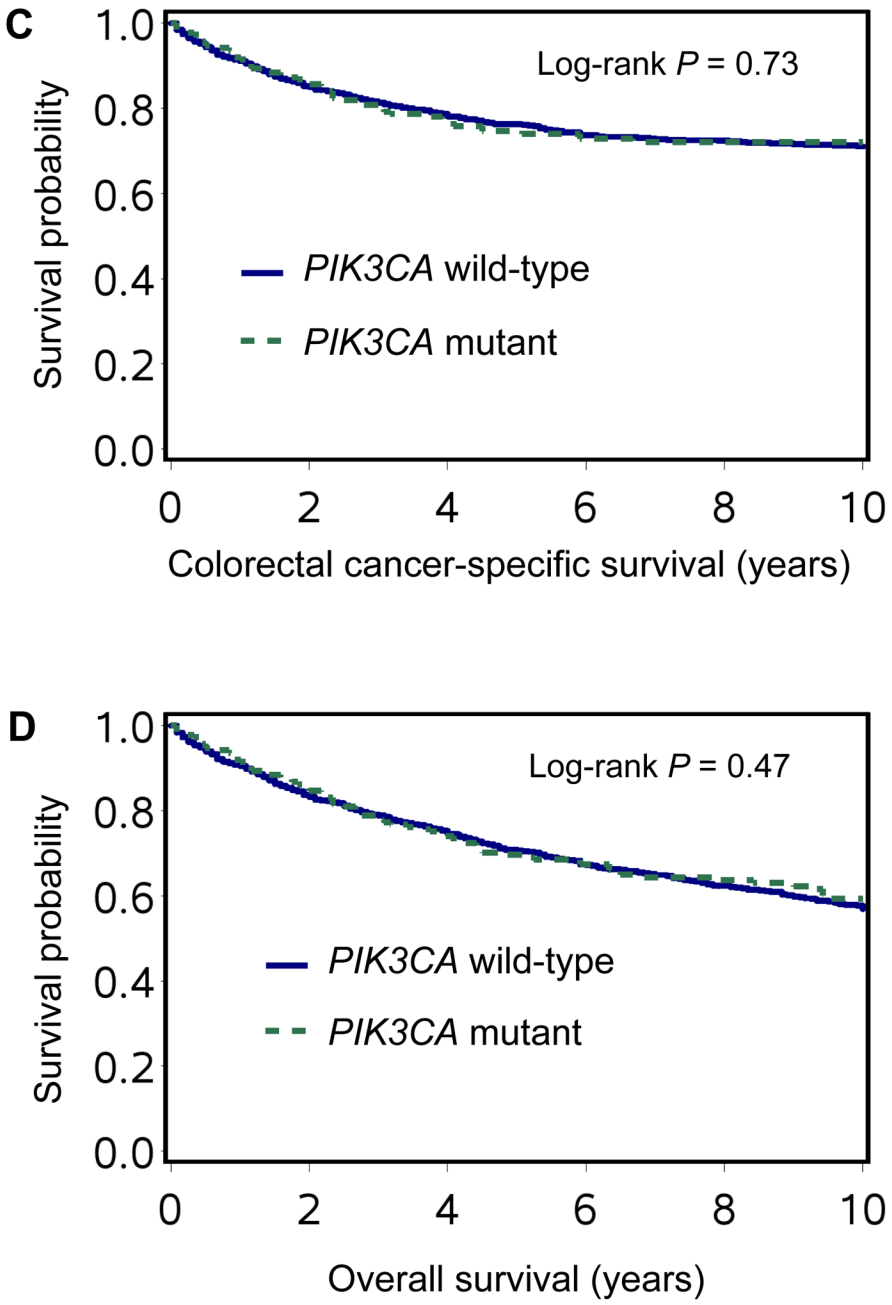


Figure 1.
Kaplan-Meier curves for colorectal cancer-specific (A) and overall survival (B) according to *PIK3CA* exon-specific mutation status in colorectal cancer. Kaplan-Meier curves for colorectal cancer-specific (C) and overall survival (D) according to overall *PIK3CA* mutation status in colorectal cancer.

Table 1

Studies on prognostic significance of *PIK3CA* exon 9 and 20 mutations in colorectal cancer

Ref.	Authors (year)	No. of hospitals	Total no. of events*	Sample size	Tumor location	Disease stage	No. of *PIK3CA* mutants		*BRAF* data	*KRAS* data	CS, OS, DFS, RFS or PFS log-rank *P* value	Multivariate HR (95% CI), *P* value	Notes and/or a list of variables examined in multivariate analysis
							Exon 9	Exon 20					
4	Kato et al. (2007)	1	32	158	Colon & rectum	II/III	11	7	No	Yes	*P* = 0.022 (RFS) *P* = 0.036 (CS)	2.48 (1.03 – 5.97) *P* = 0.043 (RFS) Exon 9 or 20	Lymph node metastasis, CEA level, tumor size and lymphatic invasion.
6	Abubaker et al. (2008)	1	N/A	418	Colon & rectum	I–IV	38	13	No	No	NS (OS)	Exon 9 or 20	
9	Barault et al. (2008)	3	197	586	Colon	I–IV	46	29	Yes	Yes	N/A	N/A Exon 1, 2, 9 or 20	*PIK3CA*, *KRAS* and *BRAF* mutations were not evaluated separately.
10	Souglakos et al. (2009)	2	43	92	Colon & rectum	I–IV	18	8	Yes	Yes	NS (OS)	2.1 (1.2 – 3.9) *P* = 0.01 (PFS) Exon 9 or 20	Age, tumor grade, metastatectomy, tumor location and number of treatment lines (1 vs >3). Patients with metastatic colorectal cancer after cetuximab treatment.
13	Sartore-Bianchi et al. (2009)	2	88	110	Colon & rectum	III–IV	4	11	No	Yes	*P* =0.0035 (PFS)	Exon 9 or 20	
14	Ogino et al. (2009)	Many	152	450	Colon	I–III	82		Yes	Yes	*P* = 0.075 (CS)	2.23 (1.21–4.11) (CS) Exon 9 or 20	Age, sex, body mass index, year of diagnosis, tumor location, stage, grade and status of MSI, CIMP, *KRAS*, *BRAF*, LINE-1 methylation and TP53.
15	He et al. (2009)	Many	84	240	Rectum	I–III	12	7	Yes	Yes	*P* = 0.008 (LR) NS (OS)	3.4 (1.2–9.2) *P* = 0.017 (LR) Exon 9 or 20	TNM stage, circumferential margin.

Ref.	Authors (year)	No. of hospitals	Total no. of events*	Sample size	Tumor location	Disease stage	No. of *PIK3CA* mutants		*BRAF* data	*KRAS* data	CS, OS, DFS, RFS or PFS log-rank *P* value	Multivariate HR (95% CI), *P* value	Notes and/or a list of variables examined in multivariate analysis
							Exon 9	Exon 20					
16	De Roock et al. (2010)	11	N/A	743	Colon & rectum	IV	74	22	Yes	Yes	*P* = 0.013 (PFS) *P* =0.0057 (OS)	2.27 (1.10 – 4.66) *P* = 0.042 (PFS) 3.30 (1.46 – 7.45) *P* = 0.012 (OS) Exon 20	Age, sex, number of previous chemotherapy lines, center, mutation status of *KRAS*, *BRAF* and *NRAS*. Exon 9 mutation was not associated with outcome.
17	Tol et al. (2010)	Many	N/A	436	Colon & rectum	IV	32	11	Yes	Yes	NS (PFS, OS)	Exon 9 or 20	Serum LDH, number of affected organs and previous adjuvant therapy.
18	Farina Sarasqueta et al. (2011)	?	?	685	Colon	I–III	66	17	No	No	*P* = 0.04 (DFS) *P* = 0.03 (CS)	Exon 20	Exon 9 mutation was not associated with outcome.
	Liao et al. (current study)	Many	552	1170	Colon & rectum	I–IV	116	80	Yes	Yes	*P* = 0.031 (CS) *P* =0.0008 (OS)	3.51 (1.28–9.62) (CS) 2.68 (1.24 – 5.77) (OS) Cases with mutations in both exons 9 and 20 1.05 (0.71–1.56) (CS) 0.90 (0.67–1.22) (OS) Cases with exon 9 mutation alone 0.96 (0.59–1.55) (CS) 0.80 (0.55–1.16) (OS) Cases with exon 20 mutation alone 1.07 (0.79–1.45) (CS)	Age, sex, year of diagnosis, tumor location, stage, grade and status of MSI, CIMP, *KRAS*, *BRAF* and LINE- 1 methylation.

Ref.	Authors (year)	No. of hospitals	Total no. of events*	Sample size	Tumor location	Disease stage	No. of *PIK3CA* mutants		*BRAF* data	*KRAS* data	CS, OS, DFS, RFS or PFS log-rank *P* value	Multivariate HR (95% CI), *P* value	Notes and/or a list of variables examined in multivariate analysis
							Exon 9	Exon 20					
												0.91 (0.72–1.15) (OS) Cases with mutations in either or both of exons 9 and 20	

CI, confidence interval; CIMP, CpG island methylator phenotype; CS, cancer specific survival; DFS; disease free survival; HR, hazard ratio; LR, local recurrence; MSI, microsatellite instability; N/A, not available; NS, not significant; OS, overall survival; PFS, progression free survival, RFS, relapse free survival.

* relapses or deaths.

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Table 2

Clinical, pathological and molecular features of colorectal cancer according to *PIK3CA* mutation status

					PIK3CA mutation present								
	Total		*PIK3CA* wild-type		Only in exon 9		*P* (exon 9 vs. exon 20)	Only in exon 20		In both exon 9 and exon 20		*P* (across all categories)
Feature	**No.**		**No.**		**No.**			**No.**		**No.**		
Total No.	1170		981		109			73		7		
Sex							0.88					0.26
Male, (HPFS)	536	46%	439	45%	56	51%		36	49%	5	71%	
Female, (NHS)	634	54%	542	55%	53	49%		37	51%	2	29%	
Mean age at diagnosis (years) ± SD	68.7 ± 8.7		68.6 ± 8.7		69.4 ± 8.9		0.94	68.3 ± 9.0		75.6 ± 10.0		0.95
Year of diagnosis							0.76					0.92
Prior to 1997	501	43%	424	43%	43	39%		31	43%	3	43%	
1997 or after	669	57%	557	57%	66	61%		42	57%	4	57%	
Family history of colorectal cancer in first degree relatives							0.19					0.030
Absent	951	81%	804	82%	90	83%		54	74%	3	43%	
Present	219	19%	177	18%	19	17%		19	26%	4	57%	
Tumor location							0.80					0.19
Rectum	258	22%	230	24%	17	16%		10	14%	1	14%	
Distal colon	359	31%	300	31%	32	29%		25	34%	2	29%	
Proximal colon	546	47%	444	45%	60	55%		38	52%	4	57%	
Disease stage							0.093					0.23
I	282	24%	231	24%	33	30%		15	21%	3	43%	
II	327	28%	270	28%	28	26%		28	38%	1	14%	
III	308	26%	264	27%	24	22%		19	26%	1	14%	
IV	151	13%	124	13%	15	14%		10	14%	2	29%	
Unknown	102	9%	92	9%	9	8%		1	1%	0	0	
Tumor grade							0.067					0.27
Low	1052	91%	880	90%	102	94%		63	86%	7	100%	
High	111	9%	95	10%	6	6%		10	14%	0	0	
MSI status							0.0007					0.0050
MSI-low/MSS	978	85%	820	85%	100	92%		52	72%	6	86%	

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Feature	Total		*PIK3CA* wild-type		*PIK3CA* mutation present						*P* (across all categories)
					Only in exon 9		*P* (exon 9 vs. exon 20)	Only in exon 20		In both exon 9 and exon 20	
	No.		No.		No.			No.		No.	
MSI-high	176	15%	146	15%	9	8%		20	28%	1	14%	
CIMP status							0.028				0.025
CIMP-low/0	906	83%	762	83%	88	87%		52	73%	4	57%	
CIMP-high	187	17%	152	17%	13	13%		19	27%	3	43%	
BRAF status							0.030				0.15
Wild-type	993	85%	831	85%	99	91%		57	79%	6	86%	
Mutant	171	15%	145	15%	10	9%		15	21%	1	14%	
KRAS status							0.29				0.0001
Wild-type	747	64%	659	67%	48	44%		38	53%	2	29%	
Mutant	418	36%	318	33%	61	56%		34	47%	5	71%	
Mean LINE-1 methylation level (%) ± SD	62.8 ± 9.5		62.5 ±9.6		64.3±9.6		0.53	63.9±8.9		62.3±7.5	0.79
TP53 expression							0.71				0.0080
Negative	520	57%	422	55%	56	72%		39	68%	3	60%	
Positive	385	43%	343	44%	22	28%		18	32%	2	40%	

The % number indicates the proportion of patients with a specific clinical, pathological or molecular feature among all patients, or patients with specific *PIK3CA* mutation status. CIMP, CpG island methylator phenotype; HPFS, Health Professionals Follow-Up Study; MSI, microsatellite instability; MSS, microsatellite stable; NHS, Nurses' Health Study; SD, standard deviation.

Table 3

Clinical, pathological and molecular data of colorectal cancers with concomitant *PIK3CA* mutations in both exons 9 and 20

								Exon 9	Exon 20							
Case ID	Age	Sex	No. of first-degree relatives with colorectal cancer	Tumor location	TNM stage	Size (cm)	Tumor grade	Nucleotide change	Nucleotide change	*KRAS*	*BRAF*	LINE-1 methylation (%)	MSI	CIMP [a]	TP53 expression	Follow-up (months)	Clinical outcome
1	83	Male	0	A	T2N0M0	4.2	Low	c.1633G>A	c.3140A>T	c.35G>T	WT	59	MSS	L	(−)	162	DFO
2	69	Male	1	S	T3N2M1	3.5	High	c.1633G>A	c.3129G>T	c.35G>A	WT	63	MSS	L	(+)	11	DOD
3	68	Female	1	SF	T3N0M0	4.5	Low	c.1624G>A	c.3137C>A	WT	WT	49	MSS	L	(−)	113	DFO
4	60	Female	0	T	T3N2M0	4.5	High	c.1633G>A	c.3136G>A	c.35G>A	WT	67	MSS	H	(+)	36	DOD
5	82	Male	0	R	T3N1M1	3.0	High	c.1624G>A c.1631C>A	c.3140A>G	c.38G>A	WT	59	MSS	L	(−)	13	DOD
6	77	Male	1	A	T2N0M0	2.0	Low	c.1633G>A	c.3140A>G	WT	c.1799T>A	64	H	H	NA	10	DOD
7	88	Male	1	A	T2N0M0	4.3	Low	c.1633G>A	c.3140A>G	c.34G>T	WT	74	MSS	H	NA	76	DFO

A, ascending colon; CIMP, CpG island methylator phenotype; DFO, died from other causes; DOD, died of disease (colorectal cancer); H, MSI-high; MSI, microsatellite instability; MSS, microsatellite-stable; NA, not available; R, rectum; S, sigmoid; SF, splenic flexure; T, transverse colon; WT, wild-type.

[a]CIMP status; H, CIMP-high; L, CIMP-low.

Table 4

PIK3CA mutation in colorectal cancer and patient mortality

PIK3CA status	Total No.	No. of events	Colorectal cancer-specific mortality			Overall mortality			
			Univariate HR (95% CI)	Stage- stratified HR (95% CI)	Multivariate stage- stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage- stratified HR (95% CI)	Multivariate stage- stratified HR (95% CI)
Wild-type	981	277	1 (referent)	1 (referent)	1 (referent)	467	1 (referent)	1 (referent)	1 (referent)
Mutant (exon 9 or 20)	189	51	0.95 (0.70–1.28)	1.03 (0.77–1.40)	1.07 (0.79–1.45)	85	0.92 (0.73–1.16)	0.97 (0.76–1.22)	0.91 (0.72–1.15)
Mutation in only exon 9	109	29	0.94 (0.64–1.38)	1.05 (0.72–1.55)	1.05 (0.71–1.56)	48	0.93 (0.69–1.25)	0.99 (0.73–1.33)	0.90 (0.67–1.22)
Mutation in only exon 20	73	18	0.83 (0.52–1.34)	0.87 (0.4–1.40)	0.96 (0.59–1.55)	30	0.77 (0.53–1.12)	0.80 (0.55–1.15)	0.80 (0.55–1.16)
Mutations in both exon 9 and exon 20	7	4	2.84 (1.05–7.69)	3.61 (1.32–9.87)	3.51 (1.28–9.62)	7	3.37 (1.58–7.15)	3.91 (1.83–8.36)	2.68 (1.24–5.77)

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, tumor location, tumor grade, microsatellite instability, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation and LINE-1 methylation. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

CI, confidence interval; HR, hazard ratio.

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Table 5

PIK3CA mutation in colorectal cancer and patient mortality according to *KRAS* and *BRAF* mutation status

		Colorectal cancer-specific mortality				Overall mortality			
	Total No.	No. of events	Univariate HR (95% CI)	Stage- stratified HR (95% CI)	Multivariate stage- stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage- stratified HR (95% CI)	Multivariate stage- stratified HR (95% CI)
BRAF wild-type and *KRAS* wild-type									
PIK3CA (−)	516	124	1 (referent)	1 (referent)	1 (referent)	224	1 (referent)	1 (referent)	1 (referent)
PIK3CA (+)	62	13	0.88 (0.49–1.55)	0.91 (0.51–1.62)	0.97 (0.54–1.74)	23	0.83 (0.54–1.28)	0.88 (0.57–1.36)	0.83 (0.54–1.28)
BRAF mutant and *KRAS* wild-type									
PIK3CA (−)	140	41	1 (referent)	1 (referent)	1 (referent)	68	1 (referent)	1 (referent)	1 (referent)
PIK3CA (+)	26	8	1.08 (0.51–2.32)	1.04 (0.48–2.24)	1.67 (0.76–3.69)	12	0.90 (0.49–1.67)	0.88 (0.47–1.64)	1.09 (0.59–2.04)
BRAF wild-type and *KRAS* mutant									
PIK3CA (−)	311	109	1 (referent)	1 (referent)	1 (referent)	165	1 (referent)	1 (referent)	1 (referent)
PIK3CA (+)	100	30	0.81 (0.54–1.21)	0.72 (0.48–1.08)	0.69 (0.46–1.04)	50	0.87 (0.64–1.20)	0.82 (0.59–1.13)	0.75 (0.54–1.04)

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, tumor location, tumor grade, microsatellite instability, CpG island methylator phenotype and LINE-1 methylation. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

CI, confidence interval; HR, hazard ratio.