A Cohort Study of p27 Localization in Colon Cancer, Body Mass Index, and Patient Survival

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Abstract

Energy balance and the AKT pathway are important in colorectal cancer development and regulate p27 (cyclin-dependent kinase inhibitor-1B/*CDKN1B*/KIP1), which plays a role in preventing cell cycle progression. However, little is known on the clinical outcome or prognostic significance of p27 alterations in relation to patient body mass index (BMI). Among 630 colon cancers (stage I-IV) in two prospective cohort studies, we detected p27 alterations (cytoplasmic p27 localization or p27 loss) in 500 tumors (79%) by immunohistochemistry. The remaining 130 (21%) tumors were "p27-nuclear+." Cox proportional hazard models computed hazard ratios (HR) of deaths, adjusted for patient and tumoral characteristics, including p53, p21, cyclin D1, *KRAS*, *BRAF*, *PIK3CA*, cyclooxygenase-2, fatty acid synthase (FASN), β-catenin, microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and long interspersed nucleotide element-1 (LINE-1) hypomethylation. Compared with p27-nuclear+ patients, p27-altered patients experienced low colon cancer–specific [adjusted HR, 0.63; 95% confidence interval (95% CI), 0.42-0.94] and overall mortality (adjusted HR, 0.70; 95% CI, 0.51-0.95), independent of FASN, MSI, CIMP, LINE-1 methylation, and other potential confounders. The effect of p27 alteration on overall mortality significantly differed by BMI ($P_{interaction}$ = 0.013); adjusted HR (p27-altered versus p27-nuclear+ tumors) was 0.28 (95% CI, 0.13-0.59) for BMI ≥30 kg/m^2, 0.67 (95% CI, 0.40-1.14) for BMI 25 to 29 kg/m^2, and 0.91 (95% CI, 0.57-1.46) for BMI <25 kg/m^2. Obesity was associated with inferior overall survival among p27-nuclear+ cases (adjusted HR, 3.07; 95% CI, 1.49-6.32; versus nonobese cases), but not among p27-altered cases (adjusted HR, 1.08). In conclusion, p27 alterations in colon cancer are associated with superior prognosis. Adverse prognostic effect of obesity seems limited to patients with nuclear p27 expression, suggesting a host-tumor interaction. (Cancer Epidemiol Biomarkers Prev 2009;18(6):1849–58)

Introduction

Energy balance, or the ability to maintain body weight by balancing energy intake with energy expenditure, seems to be important in the pathogenesis of human cancers, including colon cancer. Animal studies have found that restricting energy intake reduces tumor development (1). In humans, prospective observational studies show that obesity increases the risk of colon cancer, whereas regular physical activity is associated with a reduced risk (2). Studies of colon cancer patients indicate that sedentary lifestyle and obesity can significantly increase the risk of cancer recurrence and mortality (3, 4).

p27 (*CDKN1B* or cyclin-dependent kinase inhibitor 1B/KIP1) plays a key role in preventing progression into the S phase of the cell cycle (5). Obesity, insulin, and insulin-like growth factor-I (IGF-I) influence cellular proliferation and growth through the phosphatidylinositol 3-kinase/AKT1 signaling pathway (6). Activated AKT1 inhibits transcription of p27 and promotes its degradation (7), and *in vitro* studies have shown that insulin and IGF-I result in down-regulation of p27 (8). In animals, p27 expression increases in a dose-dependent manner in response to energy restriction and/or physical activity (9). Thus, it is of interest to examine a biological effect of p27 alteration in relation to energy balance status.

Prognostic significance of p27 alterations in colorectal cancer has been examined in previous studies (10-31). However, none of these studies (11-31) has examined patient energy balance status or consider the potential confounding effect of MSI and the CpG island methylator phenotype (CIMP), both of which are independently related with p27 alterations (32, 33) as well as patient survival (34).

In this study using a large number (n = 647) of stage I-IV colon cancers in two independent cohort studies, we have examined the prognostic significance of p27 alterations in relation to patient body mass index (BMI). Because we concurrently assessed other related

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molecular variables, including p53, p21, cyclin D1, microsatellite instability (MSI), and CIMP, we could evaluate the effect of p27 alterations, independent of these related molecular events.

Materials and Methods

Study Population. We used the databases of two independent prospective cohort studies; the Nurses' Health Study (n = 121,700 women followed since 1976) and the Health Professionals Follow-up Study (n = 51,500 men followed since 1986; ref. 35). Every 2 y, participants were sent follow-up questionnaires to update information on potential risk factors and to identify newly diagnosed cancer and other diseases in themselves and their first-degree relatives. We calculated BMI (kg/m^2) by using self-reported height from the baseline questionnaire and weight from the biennial questionnaire that immediately preceded the diagnosis of colorectal cancer. In validation studies in both cohorts, self-reported anthropometric measures were well correlated with measurements by trained technicians (r > 0.96). When a participant (or next of kin for decedents) reported colorectal cancer, we sought permission to obtain medical records. Study physicians unaware of exposure data reviewed all records related to colorectal cancer and recorded American Joint Committee on Cancer tumor stage and tumor location. For nonresponders, we searched the National Death Index to discover deaths and ascertain any diagnosis of colorectal cancer that contributed to death or was a secondary diagnosis. Approximately 96% of all incident colorectal cancer cases were identified through these methods. We collected paraffin-embedded tissue blocks from hospitals where patients underwent tumor resections (35). Tissue sections from all colorectal cancer cases were reviewed and confirmed by a pathologist (S.O.). We excluded cases preoperatively treated with radiation and/or chemotherapy. Tumor grade was categorized as high (≤50% glandular area) or low (>50% glandular area). Based on availability of tissue samples, we included a total of 630 stage I-IV colorectal cancer cases diagnosed up to 2002. Informed consent was obtained from all study subjects. This study was approved by the Human Subjects Committees at Brigham and Women's Hospital and the Harvard School of Public Health.

Measurement of Mortality. Patients were observed until death or June 2006, whichever came first. Ascertainment of deaths included reporting by the family or postal authorities. In addition, the names of persistent nonresponders were searched in the National Death Index. More than 98% of deaths in the cohorts were identified by these methods. The cause of death was assigned by physicians unaware of information on lifestyle exposures and tumoral molecular changes.

Pyrosequencing of *KRAS*, *BRAF*, and *PIK3CA*, and MSI Analysis. Genomic DNA from paraffin-embedded tissue was extracted, and whole-genome amplification was done (36). PCR and Pyrosequencing targeted for *KRAS* codons 12 and 13 (36), *BRAF* codon 600 (37), and *PIK3CA* exons 9 and 20 were done as previously described (38). MSI status was determined using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487; ref. 39). MSI-high was defined as the presence of instability in ≥30% of the markers, MSI-low as the presence of instability in <30% of the markers, and microsatellite stability (MSS) as no unstable marker.

Real-time PCR for CpG Island Methylation and Pyrosequencing to Measure Long Interspersed Nucleotide Element-1 Methylation. Sodium bisulfite treatment on tumor DNA and subsequent real-time PCR (MethyLight) assays were validated and done as previously described (40). We quantified promoter methylation in eight CIMP-specific genes (*CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*; refs. 41-43). CIMP-high was defined as ≥6/8 methylated promoters using the eight-marker CIMP panel, CIMP-low/0 as 0 to 5 methylated promoters, according to the previously established criteria (42, 44). To accurately quantify relatively high long interspersed nucleotide element-1 (LINE-1) methylation levels, we used Pyrosequencing as previously described (45, 46).

Immunohistochemistry for p27, Cyclin D1, p53, p21, β-Catenin, FASN, and Cyclooxygenase-2. Methods of immunohistochemical procedures and representative images were previously reported as follows: cyclin D1 (47), β-catenin (48), and FASN (39, 49); p53, p21, and p27 (Fig. 1; refs. 32, 33, 50); and cyclooxygenase-2 (COX-2; refs. 35, 39). "Cytoplasmic p27 localization" was defined as the presence of at least weak cytoplasmic staining in ≥10% of tumor cells. "Loss of p27 expression" was defined as the absence of nuclear staining or the presence of nuclear p27 staining in <20% of tumor cells. The remaining tumors, which showed nuclear p27 expression in ≥20% of tumor cells and no cytoplasmic p27 localization, were categorized as "p27-nuclear-positive" (or "p27-nuclear+") and used as a reference group in survival analysis. Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically stained slides for p27 were interpreted by one of the investigators (S.O.) unaware of other data. A random sample of 114 tumors was reexamined by a second observer (K.S.) unaware of other data. The concordance between the two observers was 0.94 (κ = 0.60, P < 0.0001), indicating substantial agreement. For other markers, a random selection of 108 to 402 cases was reexamined for each marker by a second pathologist (p53 and FASN by K.N.; p21 and cyclin D1 by K.S.; β-catenin by S.O.; COX-2 by R. Dehari, Kanagawa Cancer Center, Japan) unaware of other data, and concordance rates and κ coefficients between the two pathologists were as follows: 0.87 (κ = 0.75; n = 118) for p53; 0.93 (κ = 0.57; n = 246) for FASN; 0.83 (κ = 0.62; n = 179) for p21; 0.83 (κ = 0.64; n = 160) for cyclin D1; 0.83 (κ = 0.65; n = 402) for β-catenin; 0.92 (κ = 0.62; n = 108) for COX-2, indicating overall good to substantial agreement.

Statistical Analysis. We used stage-matched (stratified) Cox proportional hazard models to calculate hazard ratios (HR) of death by tumoral p27 status, adjusted for age (continuous), sex, year of diagnosis (continuous), BMI (<30 versus ≥30 kg/m^2), family history of colorectal cancer in any first-degree relative (present versus absent),

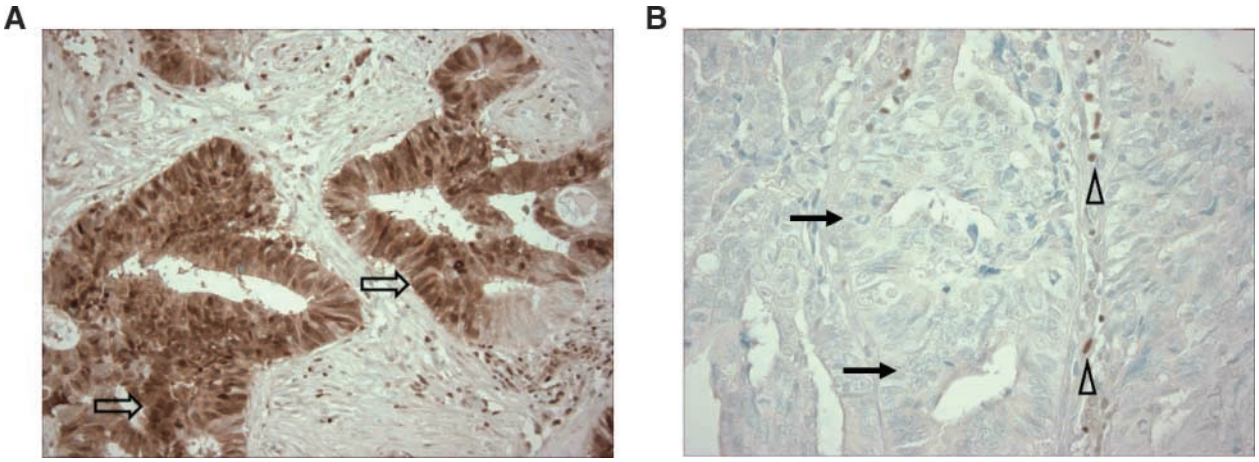

Figure 1. p27 immunohistochemistry in colon cancer. **A.** Colon cancer with cytoplasmic localization of p27 (*block arrows*). Nuclear p27 is also present. **B.** Colon cancer with loss of nuclear p27 expression (*arrows*). Cytoplasmic p27 is absent. Nuclear p27 expression in mesenchymal and inflammatory cells serves as an internal positive control (*arrowheads*).

tumor location (proximal versus distal), grade (high versus low), CIMP (high versus low/0), MSI (high versus low/MSS), LINE-1 methylation (continuous), *KRAS, BRAF, PIK3CA*, β-catenin, p53, p21, cyclin D1, FASN, and COX-2. To adjust for confounding, tumor stage (I, IIA, IIB, IIIA, IIIB, IIIC, IV, missing) was used as a matching (stratifying) variable (using the "strata" option in the SAS "proc phreg" command). For analyses of colorectal cancer–specific mortality, death as a result of colorectal cancer was the primary end point and deaths as a result of other causes were censored. The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross-product of the p27 variable and survival time ($P = 0.62$ for colon cancer–specific mortality; $P = 0.19$ for overall mortality). For cases with missing information in any of the covariates [including BMI (3.8% missing), tumor location (1.3% missing), tumor grade (0.5% missing), MSI (2.2% missing), *KRAS* (1.7% missing), *PIK3CA* (13% missing), *BRAF* (4.1% missing), β-catenin (6.8% missing), p53 (0.6% missing), p21 (2.1% missing), cyclin D1 (13% missing), COX-2 (0.3% missing), and FASN (3.0% missing)], we included those cases in a majority category to minimize the number of indicator variables in multivariate Cox models. We confirmed that excluding cases with missing information in any of the covariates did not substantially alter results (data not shown). An interaction was assessed by including the cross-product of the p27 variable and another variable of interest in a multivariate Cox model, and the likelihood ratio test was done. *P* values were conservatively interpreted, considering multiple hypothesis testing. To assess an interaction of p27 and stage, we used stage as a linear ordinal variable (I-IV). We examined the relationship between BMI (as a continuous variable) and mortality in strata of p27 status, nonparametrically with restricted cubic splines. This method allowed us to evaluate modifying effect of BMI without any predetermined categorization of BMI.

The Kaplan-Meier method was used to describe the distribution of colon cancer–specific and overall survival time and the log-rank test was done. The χ^2 test was used to examine an association of p27 with any of the categorical variables. The ANOVA was done to compare mean age and mean LINE-1 methylation level. All analyses used SAS version 9.1 (SAS Institute) and all *P* values were two-sided.

Results

Tumoral p27 Status in Colon Cancer. Among 630 patients with stage I-IV colon cancer, by immunohistochemistry, cytoplasmic p27 localization was observed in 330 (52%) tumors and loss of p27 expression was observed in 170 (27%) tumors. The remaining 130 (21%) tumors showed nuclear p27 expression and no cytoplasmic p27 localization. Thus, first, we classified tumors into the three categories—cytoplasmic p27 localization, loss of p27 expression, and nuclear p27 expression. We assessed clinical and molecular characteristics of colon cancers, according to tumoral p27 status (Table 1). Tumors with loss of p27 expression were more likely to arise in proximal colon ($P < 0.0001$) and exhibit MSI-high ($P < 0.0001$), CIMP-high ($P < 0.0001$), *BRAF* mutation ($P < 0.0001$), and less likely to exhibit p53 expression ($P = 0.002$) and COX-2 expression ($P < 0.0001$).

Tumoral p27 Status and Patient Survival. During follow-up, there were 272 deaths, including 160 colon cancer–specific deaths. We assessed the influence of cytoplasmic p27 localization and loss of p27 expression on patient survival. In univariate Cox regression analysis, compared with patients with p27-nuclear+ tumors, patients with cytoplasmic p27 localization experienced a lower cancer-specific mortality [HR, 0.77; 95% confidence interval (95% CI), 0.52-1.12], which became significant in multivariate analysis (adjusted HR, 0.63; 95% CI, 0.41-0.97) after adjusting for potential predictors of patient survival (Table 2). The decrease in the HR for cytoplasmic p27+ tumors (versus p27-nuclear+ tumors) in the multivariate analysis was mainly the result of adjusting for tumor stage; when we simply adjusted for tumor stage, the HR for colon cancer–specific mortality was 0.63 (95% CI, 0.42-0.94; p27 cytoplasmic+

Table 1. Clinical and molecular characteristics according to p27 status in colon cancer

Clinical or molecular feature	All cases	p27			*P*
		Nuclear+ (no cytoplasmic localization)	Cytoplasmic localization	Loss of expression	
Total *n*	630	130	330	170	
Sex					0.004
Male (HPFS)	268 (43%)	42 (32%)	160 (48%)	66 (39%)	
Female (NHS)	362 (57%)	88 (68%)	170 (52%)	104 (61%)	
Mean age ± SD	66.4 ± 8.2	65.9 ± 7.8	66.6 ± 8.7	66.4 ± 7.5	0.71
BMI (kg/m^2)					0.25
<30	502 (83%)	107 (86%)	256 (81%)	139 (84%)	
≥30	104 (17%)	17 (14%)	60 (19%)	27 (16%)	
Family history of colorectal cancer in any first-degree relative					0.80
(−)	467 (74%)	94 (72%)	248 (75%)	125 (74%)	
(+)	163 (26%)	36 (28%)	82 (25%)	45 (26%)	
Year of diagnosis					0.24
Before 1995	276 (44%)	56 (43%)	154 (47%)	66 (39%)	
1995 to 2002	354 (56%)	74 (57%)	176 (53%)	104 (61%)	
Tumor location					<0.0001
Proximal (cecum to transverse colon)	368 (59%)	83 (64%)	167 (51%)	118 (71%)	
Distal (splenic flexure to sigmoid)	254 (41%)	47 (36%)	158 (49%)	49 (29%)	
AJCC tumor stage					0.44
I	127 (20%)	22 (17%)	74 (22%)	31 (18%)	
IIA	194 (31%)	45 (35%)	90 (27%)	59 (35%)	
IIB	18 (2.9%)	5 (3.8%)	10 (3.0%)	3 (1.8%)	
IIIA	21 (3.3%)	8 (6.2%)	9 (2.7%)	4 (2.4%)	
IIIB	87 (14%)	13 (10%)	49 (15%)	25 (15%)	
IIIC	54 (8.6%)	9 (6.9%)	33 (10%)	12 (7.1%)	
IV	85 (13%)	18 (14%)	45 (14%)	22 (13%)	
Unknown	44 (7.0%)	10 (7.7%)	20 (6.1%)	14 (8.2%)	
Tumor grade					<0.0001
Low	556 (89%)	103 (80%)	314 (95%)	139 (82%)	
High	71 (11%)	26 (20%)	15 (4.6%)	30 (18%)	
MSI					<0.0001
MSI-low/MSS	502 (81%)	94 (74%)	298 (92%)	110 (66%)	
MSI-high	114 (19%)	33 (26%)	25 (7.7%)	56 (34%)	
Mean LINE-1 methylation (%) ± SD	61.0 ± 9.4	60.7 ± 10.3	61.0 ± 9.2	61.3 ± 9.3	0.74
CIMP					<0.0001
CIMP-low/0	509 (81%)	99 (76%)	295 (89%)	115 (68%)	
CIMP-high	121 (19%)	31 (24%)	35 (11%)	55 (32%)	
p53 expression					0.002
(−)	381 (61%)	80 (63%)	181 (55%)	120 (71%)	
(+)	245 (39%)	48 (37%)	148 (45%)	49 (29%)	
p21 (CDKN1A)					<0.0001
Expressed	132 (21%)	40 (32%)	40 (12%)	52 (31%)	
Lost	485 (79%)	85 (68%)	283 (88%)	117 (69%)	
Cyclin D1 expression					0.22
(−)	131 (23%)	20 (17%)	76 (25%)	35 (23%)	
(+)	444 (77%)	97 (83%)	228 (75%)	119 (77%)	
BRAF mutation					<0.0001
(−)	507 (84%)	97 (79%)	291 (91%)	119 (73%)	
(+)	97 (16%)	26 (21%)	28 (8.8%)	43 (27%)	
KRAS mutation					0.060
(−)	389 (63%)	88 (69%)	191 (59%)	110 (66%)	
(+)	230 (37%)	39 (31%)	135 (41%)	56 (34%)	
PIK3CA mutation					0.43
(−)	449 (82%)	91 (84%)	234 (80%)	124 (85%)	
(+)	96 (18%)	17 (16%)	57 (20%)	22 (15%)	
COX-2 expression					<0.0001
(−)	107 (17%)	19 (15%)	39 (12%)	49 (29%)	
(+)	521 (83%)	110 (85%)	290 (88%)	121 (71%)	
β-Catenin activation					0.003
(−)	369 (67%)	74 (67%)	187 (62%)	108 (78%)	
(+)	182 (33%)	37 (33%)	115 (38%)	30 (22%)	
FASN expression					0.37
(−)	535 (88%)	104 (84%)	284 (89%)	147 (88%)	
(+)	76 (12%)	20 (16%)	36 (11%)	20 (12%)	

NOTE: (%) indicates the proportion of tumors with a specific clinical or molecular feature in tumors with specific p27 status.
Abbreviations: HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study.

versus p27 nuclear+). No other major confounder was present.

In both univariate and multivariate analyses, compared with p27-nuclear+ tumors, tumors with loss of p27 expression exhibited a low mortality (adjusted HR, 0.62; 95% CI, 0.39-1.02; Table 2). In terms of patient survival, tumors with cytoplasmic p27 localization were very similar to tumors with loss of p27 expression.

Table 2. p27 alterations in colon cancer and patient mortality

Tumoral p27 status	Total *n*	Colon cancer-specific mortality				Overall mortality			
		Deaths/ person-years	Univariate HR (95% CI)	Stage-matched HR (95% CI)	Multivariate HR (95% CI)	Deaths/ person-years	Univariate HR (95% CI)	Stage-matched HR (95% CI)	Multivariate HR (95% CI)
Nuclear+ (no cytoplasmic expression)	130 (21%)	40/989	1 (reference)	1 (reference)	1 (reference)	61/989	1 (reference)	1 (reference)	1 (reference)
All p27-altered tumors*	500 (79%)	120/4157	0.74 (0.51-1.05)	0.65 (0.44-0.95)	0.63 (0.42-0.94)	211/4157	0.83 (0.62-1.10)	0.77 (0.57-1.04)	0.70 (0.51-0.95)
Cytoplasmic localization	330 (52%)	82/2787	0.77 (0.52-1.12)	0.63 (0.42-0.94)	0.63 (0.41-0.97)	140/2787	0.82 (0.61-1.11)	0.75 (0.54-1.02)	0.70 (0.50-0.97)
Loss of expression	170 (27%)	38/1370	0.68 (0.43-1.06)	0.68 (0.43-1.08)	0.62 (0.39-1.02)	71/1370	0.84 (0.59-1.18)	0.83 (0.58-1.18)	0.71 (0.49-1.02)

NOTE: The multivariate, stage-matched conditional Cox regression model included age, year of diagnosis, sex, family history of colorectal cancer, tumor location, stage, grade, *KRAS*, *BRAF*, *PIK3CA*, p53, p21, cyclin D1, β-catenin, COX-2, FASN, LINE-1 methylation, MSI, and CIMP.
*p27-altered tumors included tumors with cytoplasmic p27 localization and tumors with loss of p27 expression.

Thus, we combined tumors with cytoplasmic p27 localization (n = 330) and tumors with loss of p27 expression (n = 170) into a category called p27-altered tumors (n = 500). Compared with patients with p27-nuclear+ tumors, patients with p27-altered tumors experienced a significantly low cancer-specific mortality (adjusted HR, 0.63; 95% CI, 0.42-0.94) and overall mortality (adjusted HR, 0.70; 95% CI, 0.51-0.95) in multivariate analysis (Table 2).

We further compared colon cancer–specific and overall survival according to tumoral p27 status by the Kaplan-Meier analysis (Fig. 2A). The 5-year colon cancer– specific survival was 73% among patients with p27-nuclear+ tumors and 83% among patients with p27-altered tumors (log-rank P = 0.093). The 5-year overall survival was 69% among patients with p27-nuclear+ tumors and 77% among patients with p27-altered tumors (log-rank P = 0.20).

Modifying Effect of BMI on the Relation between p27 Alteration and Mortality. In light of the potential link between energy balance and p27 regulation (7-9), we examined whether BMI modified the effect of p27 on mortality. We found a significant modifying effect of BMI on the relation between p27 alteration and overall mortality ($P_{interaction}$ = 0.013). Among patients with a BMI ≥30 kg/m^2, compared with p27-nuclear+ tumors, p27-altered tumors were associated with a significantly low overall mortality (multivariate HR, 0.28; 95% CI, 0.13-0.59; Table 3). In contrast, among patients with BMI <30 kg/m^2, the improved mortality associated with p27-altered tumors (versus p27-nuclear+ tumors) was attenuated or absent. Similar results were obtained for colon cancer–specific mortality; however, due to limited power, statistical significance (for interaction between p27 and BMI) was not reached ($P_{interaction}$ = 0.30; Table 3).

Effect of BMI on Mortality in Strata of p27 Status. Because of the significant interaction between p27 and BMI ($P_{interaction}$ = 0.013) in the overall mortality analysis, we examined the effect of obesity (BMI ≥30 kg/m^2) on mortality among colon cancer patients in strata of p27 status (Table 4). Notably, obesity was significantly associated with inferior overall survival among p27-nuclear+ cases (adjusted HR, 3.07; 95% CI, 1.49-6.32), whereas obesity was not associated with overall survival among p27-altered cases (adjusted HR, 1.08; 95% CI, 0.71-1.65). We also examined the relation between BMI (as a continuous variable) and overall mortality in strata of p27 status, nonparametrically with restricted cubic splines (Fig. 2B). This flexible method allowed us to evaluate the effect of BMI without any predetermined categorization of BMI or assumption of a linear relation between BMI and mortality. The relation between BMI and overall mortality seemed to differ according to p27 status.

Stratified Analysis of p27 Alteration and Mortality. We further examined the influence of p27 alteration on colon cancer–specific mortality across strata of other potential predictors of patient survival, including age, sex, tumor location, stage, grade, family history of colorectal cancer, MSI, CIMP, LINE-1 hypomethylation, *KRAS*, *BRAF*, *PIK3CA*, p53, p21, cyclin D1, FASN, and COX-2 (Fig. 2C; not all strata were shown). The survival

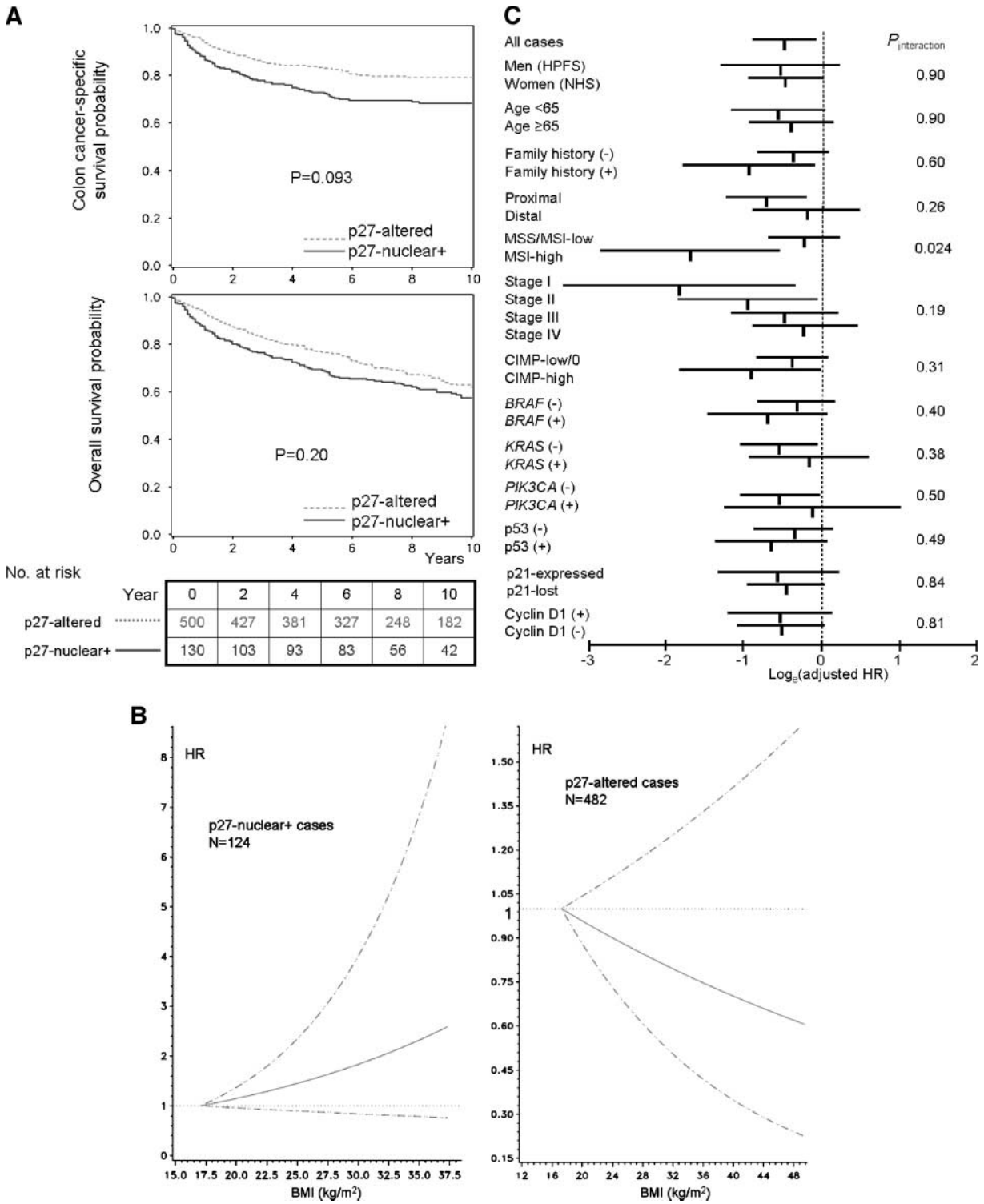

Figure 2. p27 in colon cancer and patient survival. **A.** Kaplan-Meier curves for colon cancer–specific survival (*top*) and overall survival (*bottom*) according to p27 status in colon cancer. p27-altered tumors include tumors with cytoplasmic p27 localization and tumors with loss of p27 expression. **B.** Smoothing spline plot of unadjusted HRs for overall mortality according to BMI (with low BMI as a reference), among p27-nuclear+ cases (*left*) and among p27-altered cases (*right*). *Hatched lines,* 95% CIs. **C.** p27 status and colon cancer–specific mortality in various strata. Log_e(adjusted HRs) with 95% CI for colon cancer–specific mortality in p27-altered tumors (vs p27-nuclear+ tumors). *HPFS,* Health Professionals Follow-up Study; *NHS,* Nurses' Health Study.

Table 3. Patient mortality in p27-altered colon cancer (compared with p27-nuclear+ tumor) in strata of BMI

BMI (kg/m^2)	Colon cancer-specific mortality in p27-altered tumors (vs p27-nuclear+ tumors as a reference)			Overall mortality in p27-altered tumors (vs p27-nuclear+ tumors as a reference)		
	No. deaths/cases (p27-altered vs p27-nuclear+)	Stage-matched HR (95% CI)	Multivariate HR (95% CI)	No. deaths/cases (p27-altered vs p27-nuclear+)	Stage-matched HR (95% CI)	Multivariate HR (95% CI)
<25	47/188 (25%) vs 18/67 (27%)	0.73 (0.42-1.29)	0.72 (0.40-1.32)	78/188 (41%) vs 27/67 (40%)	0.93 (0.59-1.47)	0.91 (0.57-1.46)
25-29	48/207 (23%) vs 14/40 (35%)	0.54 (0.29-1.01)	0.50 (0.26-0.99)	94/207 (45%) vs 20/40 (50%)	0.77 (0.47-1.27)	0.67 (0.40-1.14)
≥30	18/87 (21%) vs 6/17 (35%)	0.40 (0.15-1.06)	0.34 (0.12-0.93)	29/87 (33%) vs 11/17 (65%)	0.31 (0.15-0.65)	0.28 (0.13-0.59)
P for interaction (p27 and BMI)*		0.40	0.30		0.015	0.013

NOTE: p27-altered tumors include tumors with cytoplasmic p27 localization and tumors with loss of p27 expression. The multivariate, stage-matched conditional Cox model included the p27 variable stratified by BMI, age, year of diagnosis, sex, BMI, family history of colorectal cancer, tumor location, stage, grade, *KRAS, BRAF, PIK3CA*, p53, p21, cyclin D1, β-catenin, COX-2, FASN, LINE-1 methylation, MSI, and CIMP.
*BMI is used as a binary variable (<30 versus ≥30 kg/m^2).

advantage associated with p27-altered tumors seemed to be stronger among MSI-high tumors compared with MSS/MSI-low cases ($P_{interaction}$ = 0.024). Otherwise, the effect of p27 alteration was not significantly modified by any other predictors of patient outcome ($P_{interaction}$ ≥0.05). Notably, the effect of p27 alteration did not significantly differ across all stages ($P_{interaction}$ = 0.19) and between the two independent cohort studies ($P_{interaction}$ = 0.90).

Discussion

In this study, we examined the prognostic significance of p27 alterations in stage I-IV colon cancer, particularly in relation to patient BMI. We have found that tumoral p27 alterations (regardless of cytoplasmic localization or loss of expression) were associated with longer survival, independent of other clinical, pathologic, or molecular characteristics. We have also found that the effect of p27 alteration was most evident among obese patients (BMI ≥30 kg/m^2), whereas the effect of p27 is largely absent among normal-weight subjects (BMI <25 kg/m^2). Moreover, the adverse effect of obesity on patient survival was principally limited to tumors with nuclear p27 expression. Our results support the role of tumoral p27 status and its interaction with patient BMI (i.e., a tumor-host interaction) in determining clinical outcome in colon cancer patients.

A possible host-tumor interaction between obesity (BMI ≥30 kg/m^2) and tumoral p27 alteration is intriguing. Cellular proliferation, senescence, and apoptosis have been known to be influenced by cellular energy balance status. Indeed, obesity and physical inactivity have been consistently shown to be risk factors for colon cancer development and mortality (3, 4). One of the possible mechanisms of energy balance–mediated carcinogenesis is through growth factor receptors and their downstream signaling pathways (6). Indeed, obesity, insulin, and IGF-I influence cellular proliferation and growth through the phosphatidylinositol 3-kinase/AKT1 signaling pathway (6). Activated AKT1 inhibits transcription of p27 and promotes its degradation (7), and *in vitro* studies have shown that insulin and IGF-I result in down-regulation of p27 (8). In animal experiments, p27 expression increases in a dose-dependent manner in response to energy restriction and/or physical activity (9). Our current study suggests that obesity confers an increase in patient mortality if tumor cells express intact nuclear p27. Specifically, tumors with functional p27 would be most susceptible to the tumor-promoting effects of excess energy balance through phosphatidylinositol 3-kinase/AKT1 signaling and subsequent p27 degradation. This hypothesis and our findings on the potential host-tumor interaction need to be tested and confirmed by additional studies, because we recognize a limitation of sample size in our subanalysis of BMI strata.

Examining molecular changes or prognostic factors is important in colon cancer research (51-54). Previous studies have examined the relationship between tumoral p27 loss and clinical outcome in colon cancer (10-31). However, none of the previous studies (11-31) have examined the modifying effect of patient BMI. In addition, none of the previous studies (11-31) has comprehensively examined the confounding effects of other related molecular events, including p53, p21, cyclin D1, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, and *PIK3CA*. In colon cancer, loss of p27 expression and cytoplasmic localization of p27 have been reported as aberrant p27 expression patterns (15, 29, 33). In addition, loss of nuclear p27 in colon cancer has been associated with MSI and the CIMP (32), whereas cytoplasmic p27 localization has been inversely associated with MSI and CIMP (33). However, most previous studies (11, 12, 16-19, 21, 23, 26, 27, 30, 31) have simply classified tumors according to nuclear p27 status, without consideration of its cytoplasmic localization. Furthermore, with the presence of cytoplasmic p27 localization, it was often difficult to precisely measure how much fraction of tumor cells expressing nuclear p27 due to obscuring effect of cytoplasmic p27 expression. Only one study (29) examined the prognostic role of cytoplasmic p27 localization in 418 colorectal cancers and showed that cytoplasmic p27 localization was associated with good prognosis, which is consistent with our current results. Nonetheless, that study (29) did not examine the prognostic role of cytoplasmic p27 localization, separately from p27 nuclear expression and loss of p27 expression.

Previous data on p27 status in colorectal cancer and patient outcome are somewhat conflicting. Inconsistent methods of p27 immunohistochemistry and interpretation, a variation in patient cohorts, and inconsistent statistical methods in terms of covariates in multivariate

Table 4. Colon cancer mortality in obese patients (compared with nonobese patients) in strata of p27 status

	Colon cancer–specific mortality			Overall mortality		
	No. deaths/cases	Stage-matched HR (95% CI)	Multivariate HR (95% CI)	No. deaths/cases	Stage-matched HR (95% CI)	Multivariate HR (95% CI)
p27-nuclear+ cases						
BMI <30 kg/m^2	32/107	1 (reference)	1 (reference)	47/107	1 (reference)	1 (reference)
BMI ≥30 kg/m^2	6/17	2.48 (0.98-6.27)	2.25 (0.84-6.01)	11/17	3.15 (1.56-6.35)	3.07 (1.49-6.32)
p27-altered cases						
BMI <30 kg/m^2	95/395	1 (reference)	1 (reference)	172/395	1 (reference)	1 (reference)
BMI ≥30 kg/m^2	18/87	1.58 (0.95-2.63)	1.24 (0.72-2.14)	29/87	1.16 (0.78-1.74)	1.08 (0.71-1.65)
P for interaction (p27 and BMI)		0.40	0.30		0.015	0.013

NOTE: p27-altered tumors include tumors with cytoplasmic p27 localization and tumors with loss of p27 expression. The multivariate, stage-matched conditional Cox model included the BMI variable stratified by p27 status, age, year of diagnosis, sex, family history of colorectal cancer, tumor location, stage, grade, *KRAS, BRAF, PIK3CA*, p53, p21, cyclin D1, β-catenin, COX-2, FASN, LINE-1 methylation, MSI, and CIMP.

analysis models probably contributed to a large variation in patient outcome data. Another concern is publication bias where small studies with null results have higher likelihood of being unpublished than small studies with "significant results" that are consistent with preconceptional hypothesis (e.g., "loss of p27 must be bad for patients"). Loss of p27 expression was associated with poor prognosis in some studies (11-19), whereas other studies showed no significant prognostic role in p27 expression status (20-26), and others showed that p27 loss was associated with superior outcome (27, 28). In one study analyzing 223 MSI-high cancers (30), and another study analyzing 1,274 MSS cancers (21), tumoral p27 status did not show significant prognostic value, whereas another study analyzing 587 stage II MSS cancer, p27 loss was associated with poor prognosis (31). These data collectively support the use of standardized comprehensive assessment of p27 expression in both nuclear and cytoplasmic compartments in clinical research and the examination of other relevant tumoral molecular events (including MSI, CIMP, p53, p21, and cyclin D1), which are potential confounders.

There are currently no standardized methods to assess p27 alterations in colon cancer. Nonuniform methods to evaluate tumoral p27 alterations likely contributed to the somewhat inconsistent results in the previous studies. Nonetheless, our method yielded highly significant associations between p27 loss and other related molecular variables (including p53, MSI, CIMP, and *BRAF* mutation) as well as between cytoplasmic p27 localization and other related molecular variables. Moreover, any random misclassification of tumors in terms of p27 status would drive our results on patient outcome toward the null hypothesis.

There are advantages in using the database of the two independent prospective cohort studies, the Nurses' Health Study and Health Professionals Follow-up Study, to examine tumor-host interactions. Anthropometric measurements and other clinical information were prospectively collected and entered into the database blinded to patient diagnosis, tumoral molecular features, and outcome. Data were updated every 2 years. Cohort participants who developed colon cancer were treated at hospitals throughout the United States. Tumor specimen procurement rate has been ~60%, and there were no demographic differences between cases with tumor tissue analyzed and those without tumor tissue analyzed (35). However, a limitation of this study is that data on cancer treatment were limited. Nonetheless, it is unlikely that chemotherapy use differed according to tumoral p27 status, because such data were not available to patients or treating physicians. In addition, beyond cause of mortality, data on cancer recurrences were not available in these cohorts. Nonetheless, given the median survival for metastatic colon cancer was ~10 to 12 months during much of the time period of this study (46), colon cancer–specific survival should be a reasonable surrogate for cancer-specific outcomes.

In summary, our large cohort study suggests that p27 alterations mark colon cancer with superior prognosis, independent of patient and other tumoral characteristics. The effect of p27 alterations is particularly strong among obese individuals, suggesting the presence of tumor (p27)-host (BMI) interaction in determining clinical behavior of colon cancer. Moreover, the adverse effect of

obesity on patient survival seems to be limited to colon cancer with nuclear p27 expression. These findings may have considerable clinical implications. Future studies are needed to confirm these results as well as to elucidate exact mechanisms by which p27 alterations and cellular energy balance status affect tumor behavior.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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