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Tumor TP53 expression status, body mass index, and prognosis in colorectal cancer

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Abstract

Inactivation of the TP53 (p53) pathway by *TP53* mutations is one of key steps in colorectal carcinogenesis. TP53 also plays an important role in cellular energy metabolism. We hypothesized that TP53-altered tumor cells might behave aggressively independent of energy balance, while progression of TP53-intact cells might depend on excess energy balance. Utilizing a database of 1060 colon and rectal cancer patients in two prospective cohort studies, we evaluated TP53 expression by immunohistochemistry. Among 1060 colorectal cancers, 457 (43%) tumors were positive for TP53. Cox proportional hazards model was used to compute mortality hazard ratio (HR), adjusting for clinical and tumoral features, including microsatellite instability, the CpG island methylator phenotype, LINE-1 methylation, *KRAS*, *BRAF*, and *PIK3CA*. TP53 positivity was not significantly associated with cancer-specific survival in univariate analysis with HR of 1.16 [95% confidence interval (CI), 0.92–1.45], which became significant after stage adjustment (multivariate HR, 1.30; 95% CI, 1.02–1.65). Notably, we found a possible modifying effect of patient's body mass index (BMI) on tumor TP53. In nonobese patients (BMI <30 kg/m²), TP53 positivity was associated with shorter cancer-specific survival (multivariate HR=1.53; 95% CI, 1.17–2.00), while TP53 positivity was not significantly associated with survival among obese patients (BMI ≥ 30 kg/m²). Effect of TP53 positivity on cancer-specific survival significantly differed by BMI ($P_{\text{interaction}}=0.0051$). The adverse effect of obesity on patient mortality was limited to TP53-negative patients. These molecular pathological epidemiology data may support a dual role of TP53 alterations in cell cycle deregulation and cell autonomy with respect to energy balance status.

Keywords

colon cancer; metabolism; molecular pathological epidemiology; tumor suppressor; weight

INTRODUCTION

Inactivation of the TP53 (p53) pathway by *TP53* mutations is one of the key genetic steps in colorectal carcinogenesis.^{1–3} Wild-type TP53 mediates cell-cycle arrest and cell-death checkpoint, which can be activated by multiple cellular stress signals.⁴ In most tumors, both

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No conflict of interest is present.

TP53 alleles are inactivated, usually by a combination of a missense mutation, and 17p deletion that eliminates a second *TP53* allele.^{1, 2} *TP53* missense mutations frequently lead to accumulation of abnormal TP53 protein with a prolonged half-life, which can be detected by immunohistochemistry.⁵

Accumulating evidence indicates important roles of TP53 in cellular metabolism.^{6–8} TP53 is induced in response to reduced nutrient or energy levels, and prevent cell proliferation under conditions of nutrient deprivation.⁹ TP53 can activate PRKA (AMP-activated protein kinase, AMPK), and then inhibit MTOR signaling through the activation of the PRKA pathway, leading to cell growth inhibition.¹⁰ Epidemiologic studies suggest causal effects of obesity or excess energy balance on colon cancer incidence^{11, 12} and mortality.^{13, 14} Thus, considering evidence, we hypothesized that the influence of host energy balance status on tumor behavior might be stronger in tumors without TP53 alterations than tumors with TP53 alterations.

Despite the well-established carcinogenic role of TP53, previous prognostic studies on immunohistochemical expression of TP53 in colorectal cancer yielded inconsistent results.¹⁵ TP53 expression was associated with poor prognosis in some studies,^{16–19} whereas others reported good prognosis^{20–23} or no prognostic value associated with TP53.^{24–27} Notably, none of these^{16–27} studies examined a potential modifying effect of patient energy balance status on TP53 and tumor cell behavior.

We therefore examined interactive effects of tumor TP53 expression and body mass index (BMI) in a database of 1060 colorectal cancer patients in two prospective cohort studies. As patient characteristics as well as major tumor molecular features such as microsatellite instability (MSI), the CpG island methylator phenotype (CIMP), LINE-1 methylation, and *KRAS*, *BRAF* and *PIK3CA* mutations have been accumulated in our database, we were able to test the hypothesis that prognostic effect of host energy balance status might be stronger in cases without TP53 alterations than TP53-altered tumor cases.

MATERIALS AND METHODS

Study population

We utilized a database of two prospective cohort studies, the Nurses' Health Study (N=121,701 women followed since 1976) and the Health Professionals Follow-up Study (N=51,529 men followed since 1986).²⁸ Every two years, cohort participants have been sent follow-up questionnaires to update information on dietary and lifestyle factors (including BMI), and to identify newly diagnosed cancers in themselves and their first degree relatives. We collected paraffin-embedded tissue blocks from hospitals where patients underwent colorectal cancer resections.²⁸ Hematoxylin and eosin stained tissue sections from all colorectal cancer cases were reviewed by a pathologist (S.O.) unaware of other data. Tumor differentiation was categorized as well-moderate vs. poor (>50% vs. 50% gland formation). We excluded cases which were preoperatively treated. Based on the availability of tumor tissue and data, 1060 stage I-IV colorectal cancer cases diagnosed up to 2004 were included in this study (Table 1). Patients were observed until death or January 1, 2011, whichever came first. Death of participants was confirmed by the National Death Index. Informed consent was obtained from all study subjects. This study was approved by the Human Subjects Committees at Harvard School of Public Health and Brigham and Women's Hospital.

DNA extraction, Pyrosequencing of *KRAS*, *BRAF* and *PIK3CA*, and microsatellite instability (MSI) analysis

DNA was extracted from paraffin embedded tissue, and PCR and Pyrosequencing targeted for *KRAS* (codons 12 and 13),²⁹ *BRAF* (codon 600)³⁰ and *PIK3CA* (exons 9 and 20) were performed.³¹ MSI analysis was carried out using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67 and D18S487).³² MSI-high was defined as instability in ≥30% of the markers, and MSI-low/microsatellite stability (MSS) as instability in <30% of the markers.

Methylation analyses for CpG islands and LINE-1

Using bisulfite DNA treatment and real-time PCR (MethyLight), we quantified DNA methylation in eight CIMP-specific promoters (*CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3* and *SOCS1*).^{33, 34} CIMP-high was defined as the presence of ≥6/8 methylated promoters, and CIMP-low/0 as 0/8–5/8 methylated promoters, according to the previously established criteria.³⁴ In order to accurately quantify relatively high LINE-1 methylation levels, we used Pyrosequencing as previously described.^{35, 36}

Immunohistochemical analysis

Tissue microarrays were constructed as previously described.³⁷ For TP53 immunohistochemistry, antigen retrieval was performed using deparaffinized tissue sections in citrate buffer (BioGenex, San Ramon, CA) that were treated in a microwave for 15 minutes. Tissue sections were incubated for 10 minutes with 3% H₂O₂ (to block endogenous peroxidase), with 10% normal goat serum (Vector Laboratories, Burlingame, CA) in phosphate-buffered saline for 10 minutes. The primary antibody against TP53 (p53, clone DO-1, 1:50 dilution; EMD chemicals, Gibbstown, NJ) was applied for 30 minutes at room temperature. Later, the secondary antibody (BioGenex) was applied for 20 minutes and then streptavidin peroxidase conjugate (BioGenex) was applied for 20 minutes. Sections were visualized with diaminobenzidine for 5 minutes and by methyl green counterstain. Appropriate positive and negative controls were included in each run of immunohistochemistry.

We visually estimated the fraction of tumor cells with unequivocal moderate/strong nuclear staining for TP53 by examining at least two tissue cores in tissue microarrays, or the whole tissue section in each case for which there was not enough tissue for tissue microarrays or results were equivocal in tissue microarrays. TP53 positivity was defined as 50% or more of tumor cells with unequivocal moderate/strong nuclear staining, as recommended for improved specificity.^{38, 39} TP53 negativity was defined as either at most absent/weak staining or <50% of tumor cells with moderate/strong staining. The categorization we used in this report has previously been shown to correlate sufficiently well with TP53 sequencing analyses (sensitivity, 0.85; specificity, 0.77 with a chosen cut point of 50% or more positive cells).^{38, 40} All immunohistochemically stained slides were interpreted by a pathologist (S.O.) blinded to any other clinical or laboratory data. A random sample of 126 tumors was reexamined by a second pathologist (T.M.) who was unaware of other data. The concordance between the two observers was 0.89 ($\kappa=0.78$; $P<0.0001$), indicating substantial agreement.

Statistical analysis

All statistical analyses were performed by SAS software (Version 9.1, SAS Institute, Cary, NC). All p values were two-sided. When we performed multiple hypothesis testing, a p value for significance was adjusted by Bonferroni correction to $p=0.0036$ ($=0.05/14$). For

categorical data, the chi-square test was performed. The t test was done to compare mean age and mean LINE-1 methylation level.

Kaplan-Meier method and log-rank test were used for survival analyses. For analyses of colorectal cancer-specific mortality, deaths as a result of other causes were censored. To control for confounding, we used multivariate Cox proportional hazards regression models. The multivariate model initially included sex, age at diagnosis (continuous), year of diagnosis (continuous), BMI (<30 vs. ≥ 30 kg/m²), family history of colorectal cancer in any first-degree relative (present vs. absent), tumor location (proximal vs. distal), tumor differentiation (well-moderate vs. poor), MSI (high vs. low/MSS), CIMP (high vs. low/0), LINE-1 methylation (continuous), *KRAS*, *BRAF*, and *PIK3CA* mutation. Disease stage (I, IIA, IIB, IIIA, IIIB, IIIC, IV, unknown) was used as a stratifying variable using the “strata” option in the SAS “proc phreg” command, to avoid overfitting and residual confounding. A backward elimination was performed with $p=0.20$ as a threshold to avoid overfitting. For cases with missing information in any of the covariates [BMI (0.5% missing), tumor location (0.3%), tumor differentiation (1.2%), MSI (7.5%), CIMP (5.9%), *KRAS* (5.8%), *BRAF* (6.0%), and *PIK3CA* (15%)], we included those cases in a majority category of a given covariate to avoid overfitting. We confirmed that excluding cases with missing information in any of the covariates did not substantially alter results (data not shown). The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross-product of the TP53 variable and survival time ($p>0.50$). An interaction was assessed by the Wald test on the cross product of TP53 and another variable of interest (without data-missing cases) in a multivariate Cox model.

A multivariate logistic regression analysis was performed to examine an independent relationship of each of the covariates with TP53 positivity (as an outcome variable). The multivariate model initially included a similar, but not the same set of the covariates as the initial Cox model, as we considered possible cause-effect relations with TP53 positivity. Specifically, disease stage and tumor differentiation were likely consequences (rather than causes) of TP53 positivity. Thus, those variables were not included in the logistic regression model. A backward elimination with a threshold of $p=0.20$ was used to select variables in the final models, to avoid overfitting.

RESULTS

TP53 status in colorectal cancer

Among the database of 1060 stage I–IV colorectal cancers in the two prospective cohort studies, 457 (43%) showed TP53 positivity (defined as 50% or more of tumor cells with unequivocal moderate/strong nuclear staining) (Figure 1). As in Table 1, proximal tumor location, MSI-high, CIMP-high, *BRAF* mutation and *PIK3CA* mutation were associated inversely with TP53 positivity (all $p<0.002$). These characteristics appeared to be consistent regardless of patients’ pre-diagnosis BMI (Supplementary Table 1).

Multivariate analysis to assess relations with TP53 positivity

Since several interrelated variables were associated with TP53 positivity, we performed multivariate logistic regression analysis to assess independent relations of those variables with TP53 positivity (Table 2). MSI-high and proximal tumor location were inversely associated with TP53 positivity (both $p<0.0009$). CIMP-high or *BRAF* mutation was not independently associated with TP53.

TP53 positivity and survival of colorectal cancer patients

Among the 1060 patients, there were 538 deaths, including 305 colorectal cancer-specific deaths, during a median follow-up of 162 months (interquartile range, 125–213 months) for those who were censored. We assessed the influence of TP53 positivity on patient survival. Five-year colorectal cancer-specific survival among patients with TP53-positive tumors (74%) was not significantly different from those with TP53-negative tumors (77%; log rank $p=0.21$) (Figure 1). In univariate Cox regression analysis, TP53 positivity was not significantly associated with cancer-specific survival (HR 1.16; 95% CI, 0.92–1.45; compared with TP53-negative patients) (Table 3). In the multivariate Cox model adjusting for potential predictors of patient outcome, TP53 positivity was associated with a significantly higher colorectal cancer-specific mortality (multivariate HR, 1.30; 95% CI, 1.02–1.65). The increase in the HR for TP53-positive cases (vs. TP53-negative cases) in the multivariate analysis was mainly the result of adjusting for tumor stage; when we simply adjusted for tumor stage, the HR for colorectal cancer-specific mortality in TP53-positive cases was 1.40 (95% CI, 1.11–1.77). Since there were less TP53-positive tumors than TP53-negative tumors among stage IV cases, the adverse prognostic effect of TP53 positivity was diminished in the univariate analysis without stage adjustment. No other major confounder was present.

Interactive effect of TP53 and BMI on patient survival

Since the TP53 pathway plays crucial roles in cellular energy metabolism,^{6–8} we examined a potential modifying effect of pre-diagnosis BMI on the relation between TP53 positivity and patient survival (Table 4). Notably, there was a significant modifying effect of BMI ($P_{\text{interaction}}=0.0051$ in multivariate analysis). In nonobese patients (BMI <30 kg/m²), TP53 positivity was associated with significantly increased cancer-specific mortality (multivariate HR, 1.53; 95% CI, 1.17–2.00). In contrast, among obese patients (BMI ≥ 30 kg/m²), TP53 positivity was not significantly associated with patient survival (multivariate HR, 0.64; 95% CI, 0.36–1.11). The P value for interaction in multivariate analysis was lower than that in univariate analysis primarily due to stage adjustment. This implies that adjustment for stage revealed the true association independent of stage. The differential effect of TP53-positivity on cancer-specific survival according to BMI was also observed in Kaplan-Meier analyses (Figure 2).

We also examined whether the effect of BMI on survival was modified by tumor TP53 status (Table 4). Among TP53-negative patients, obesity (BMI ≥ 30 kg/m²) was significantly associated with increased cancer-specific and overall mortality. In contrast, among patients with TP53-positive tumors, obesity was not significantly associated with patient mortality ($P_{\text{interaction}}=0.0051$ in multivariate analysis).

Exploratory analyses of interactions between TP53 and other covariates

We further examined the influence of TP53 positivity on cancer-specific mortality across strata of other covariates, including sex, age, year of diagnosis, family history of colorectal cancer, tumor location, stage, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, and *PIK3CA* mutation status. We found no significant interaction between TP53 and any of the variables (all $P_{\text{interaction}} > 0.05$). Notably, the effect of TP53 positivity did not significantly differ between the two cohort studies ($P_{\text{interaction}}=0.38$).

DISCUSSION

We performed this study to test the hypothesis that influence of host energy balance status (BMI) on tumor behavior might be stronger in tumors without TP53 alterations than in tumors with TP53 alterations. We found a possible modifying effect of patient's BMI on

tumor TP53. Specifically, TP53 positivity, presumably reflecting *TP53* abnormality, was associated with shorter cancer-specific survival in nonobese patients, while TP53 positivity was not significantly associated with survival among obese patients. Moreover, the adverse prognostic effect of obesity appeared to be principally limited to TP53-negative tumors. Our results support the role of tumor TP53 status and its interaction with patient energy balance status in determining behavior of colorectal cancer cells.

Examining molecular changes or prognostic factors is important in cancer research.^{41–46} Previous data on immunohistochemical expression of TP53 in colorectal cancer and patient outcome have been conflicting.¹⁵ TP53 expression has been associated with poor prognosis in some studies,^{16–19} whereas other studies have shown good prognosis,^{20–23} or no prognostic value associated with TP53 expression.^{24–27} There might be a number of reasons for these discrepant results, including a chance variation and differences in patient cohorts, available covariates, statistical methods, and/or methods of immunohistochemical analyses. Our data suggest that differential effects of tumor TP53 according to patient energy balance status might also be one of the reasons.

A possible host-tumor interaction between energy balance status (BMI) and tumor TP53 status which may modify tumor cell behavior is intriguing. Analysis of tumor-host interactions represents the emerging interdisciplinary science of “molecular pathological epidemiology”.^{47, 48} Accumulating evidence suggests that the TP53 pathway plays crucial roles not only in tumor-suppressing effect but also in cellular energy metabolism.^{6–8} TP53 is induced in response to reduced nutrient or energy levels, and prevents cell proliferation under conditions of nutrient deprivation.⁹ TP53 can also activate PRKA (AMPK), and promote the PRKA pathway to inhibit MTOR signaling and reduce cell growth, thereby help cell balance its growth and proliferation.¹⁰ Given the link between energy balance and the TP53 pathway, energy balance may be important in determining biological behavior in TP53-negative tumors. Obesity and excess energy balance have been associated with increased risks of colon cancer^{11, 12} as well as mortality.^{13, 14} Our data support the hypothesis that obesity and excess energy balance are specifically detrimental among patients with TP53-negative tumors. These intriguing findings need to be confirmed by additional studies.

There are limitations in this study. For example, data on cancer treatment were limited. Nonetheless, it is unlikely that chemotherapy use substantially differed according to TP53 status in tumor, since such data were unavailable for treatment decision making. In addition, our multivariate survival analyses finely adjusted for disease stage (I, IIA, IIB, IIIA, IIIB, IIIC, IV, unknown), on which treatment decision making was mostly based. As another limitation, beyond cause of mortality, data on cancer recurrences were not available in these cohorts. Nonetheless, colorectal cancer-specific survival was a reasonable surrogate of colorectal cancer-specific outcome, given adequate median follow-up of over 10 years among survivors.

There are advantages in utilizing the database of the two nationwide prospective cohort studies. Data on anthropometric measurements, cancer staging, and other clinical, pathologic, and tumor molecular variables had been prospectively collected, blinded to patient outcome. Cohort participants who developed cancer were treated at hospitals throughout the U.S., and thus more representative colorectal cancer cases in the general U.S. population than patients in a few academic hospitals. In addition, we assessed the effect of TP53 independent of other critical molecular events such as *BRAF* and *PIK3CA* mutations, LINE-1 hypomethylation, CIMP and MSI, all of which have been associated with colorectal cancer outcome.^{35, 49, 50}

In summary, this large prospective study of patients with colorectal cancer suggests that TP53 positivity is a significant independent predictor of shorter survival among nonobese patients with colorectal cancer, but not among obese patients. These findings may have considerable clinical implications. Future studies are needed to confirm these results as well as to elucidate exact mechanisms by which tumor TP53 and cellular energy balance status interact and affect tumor behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMI	body mass index
CI	confidence interval
CIMP	CpG island methylator phenotype
HR	hazard ratio
MSI	microsatellite instability
MSS	microsatellite stability
OR	odds ratio

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The novelty and impact of this paper

1. We examined interactive effects of tumor TP53 expression and body mass index on patient survival, utilizing the database of 1060 colorectal cancer patients in two prospective cohort studies.
2. Our results support the role of tumor TP53 status and its interaction with patient energy balance status in determining behavior of colorectal cancer cells.

Figure 1.
 (A) TP53-negative colon cancer. (B) TP53-positive colon cancer. (C,D) Kaplan-Meier curves for colorectal cancer-specific survival (C) and overall survival (D).

Number at risk

Year	0	2	4	6	8	10
Negative	496	414	374	347	301	244
Positive	359	303	263	231	213	175

Year	0	2	4	6	8	10
Negative	103	89	77	69	62	40
Positive	97	81	76	70	60	49

Figure 2.
Kaplan-Meier curves for colorectal cancer-specific survival in nonobese (A) and obese (B) patients.

Table 1

Clinical, pathologic or molecular characteristics according to TP53 status in colorectal cancer

Clinical, pathologic or molecular feature	Total N	TP53		P value
		Negative	Positive	
All cases	1060	603	457	
Sex				0.19
Male	417 (39%)	227 (38%)	190 (42%)	
Female	643 (61%)	376 (62%)	267 (58%)	
Mean age $\pm$ SD	66.9 $\pm$ 8.4	67.1 $\pm$ 8.3	66.8 $\pm$ 8.5	0.60
Body mass index (kg/m ²)				0.094
<30	855 (81%)	496 (83%)	359 (79%)	
30	200 (19%)	103 (17%)	97 (21%)	
Family history of colorectal cancer in any first degree relative				0.52
(–)	858 (81%)	484 (80%)	374 (82%)	
(+)	202 (19%)	119 (20%)	83 (18%)	
Year of diagnosis				0.24
Prior to 1997	574 (54%)	317 (53%)	257 (56%)	
1997–2004	486 (46%)	286 (47%)	200 (44%)	
Tumor location				<0.0001
Proximal colon (cecum to transverse)	487 (46%)	325 (54%)	162 (36%)	
Distal colon (splenic flexure to sigmoid)	326 (31%)	172 (29%)	154 (34%)	
Rectum	244 (23%)	104 (17%)	140 (31%)	
Disease stage				0.0002
I	240 (23%)	122 (20%)	118 (26%)	
II	313 (30%)	201 (33%)	112 (25%)	
III	274 (26%)	138 (23%)	136 (30%)	
IV	140 (13%)	94 (16%)	46 (10%)	
Unknown	93 (8.8%)	48 (8.0%)	45 (10%)	
Tumor differentiation				0.13
Well to moderate	944 (90%)	531 (89%)	413 (92%)	
Poor	103 (9.8%)	66 (11%)	37 (8.2%)	
MSI status				<0.0001
MSI-low/MSS	833 (85%)	437 (77%)	396 (95%)	
MSI-high	148 (15%)	127 (23%)	21 (5.0%)	
CIMP status				<0.0001
CIMP-low/0	839 (84%)	451 (79%)	388 (92%)	
CIMP-high	158 (16%)	123 (21%)	35 (8.3%)	
KRAS mutation				0.19
(–)	641 (64%)	357 (63%)	284 (67%)	
(+)	357 (36%)	214 (37%)	143 (33%)	
BRAF mutation				0.0002
(–)	854 (86%)	467 (82%)	387 (90%)	

Clinical, pathologic or molecular feature	Total N	TP53		P value
		Negative	Positive	
(+)	142 (14%)	101 (18%)	41 (9.6%)	0.0011
<i>PIK3CA</i> mutation				
(-)	762 (84%)	420 (81%)	342 (89%)	
(+)	140 (16%)	98 (19%)	42 (11%)	0.0050
LINE-1 methylation level (Mean $\pm$ SD)	61.8 $\pm$ 9.4	62.5 $\pm$ 9.6	60.8 $\pm$ 9.0	

(%) indicates the proportion of cases with a specific clinical, pathologic or molecular feature among each expression group. CIMP, CpG island methylator phenotype; MSI, microsatellite instability; MSS, microsatellite stable; SD, standard deviation.

Table 2

Multivariate logistic regression analysis to assess relations with TP53 positivity in colorectal cancer

Variables in the final model to predict TP53 positivity (as an outcome variable)	Multivariate OR (95% CI)	P value
MSI-high (vs. MSI-low/MSS)	0.22 (0.13–0.36)	<0.0001
Proximal colon (vs. distal colorectum)	0.63 (0.49–0.83)	0.0008
<i>PIK3CA</i> mutation (vs. wild-type)	0.59 (0.39–0.88)	0.010
<i>KRAS</i> mutation (vs. wild-type)	0.76 (0.57–0.99)	0.046
BMI ≥ 30 kg/m ² (vs. <30 kg/m ²)	1.35 (0.97–1.87)	0.077
Female (vs. male)	0.83 (0.64–1.08)	0.17
LINE-1 hypomethylation (for a 30% decrease)	1.35 (0.88–2.08)	0.17

Multivariate logistic regression analysis assessing the relationship with TP53 positivity (as outcome variables) initially included age, sex, year of diagnosis, body mass index, tumor location, family history of colorectal cancer, microsatellite instability, CpG island methylator phenotype, *KRAS*, *BRAF*, *PIK3CA*, and LINE-1 methylation. A backward elimination with a threshold of $p=0.20$ was used to select variables in the final models. Because of multiple hypothesis testing, a p value for significance was adjusted by Bonferroni correction to $p=0.0036$. BMI, body mass index; CI, confidence interval; MSI, microsatellite instability; MSS, microsatellite stable; OR, odds ratio.

Table 3

TP53 positivity in colorectal cancer and patient mortality

	Total N (%)	Colorectal cancer-specific mortality				Overall mortality			
		Number of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)	Number of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)
TP53 (-)	603 (57%)	163	1 (referent)	1 (referent)	1 (referent)	303	1 (referent)	1 (referent)	1 (referent)
TP53 (+)	457 (43%)	142	1.16 (0.92–1.45)	1.40 (1.11–1.77)	1.30 (1.02–1.65)	235	1.03 (0.86–1.22)	1.14 (0.96–1.36)	1.12 (0.93–1.34)
P value			0.21	0.0047	0.035		0.77	0.15	0.23

The multivariate, stage-stratified Cox regression model initially included the TP53 variable (negative or positive), age, sex, year of diagnosis, body mass index, tumor location, tumor differentiation, family history of colorectal cancer in any first degree relative, microsatellite instability, CpG island methylator phenotype, *KRAS*, *BRAF*, *PIK3CA*, and *LINE-1* methylation. A backward elimination with threshold of $p=0.20$ was used to select variables in the final models.

CI, confidence interval; HR, hazard ratio.

Table 4
Colorectal cancer patient mortality according to TP53 status and pre-diagnosis body mass index (BMI)

		Colorectal cancer-specific mortality					Overall mortality		
	Number of cases	Number of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)	Number of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)
Pre-diagnosis BMI <30 kg/m ²									
TP53 (–)	496	129	1 (referent)	1 (referent)	1 (referent)	247	1 (referent)	1 (referent)	1 (referent)
TP53 (+)	359	119	1.29 (1.01–1.66)	1.64 (1.27–2.13)	1.53 (1.17–2.00)	189	1.08 (0.90–1.31)	1.22 (1.00–1.48)	1.19 (0.98–1.46)
P value			0.045	0.0002	0.0017		0.41	0.045	0.087
Pre-diagnosis BMI ≥30 kg/m ²									
TP53 (–)	103	31	1 (referent)	1 (referent)	1 (referent)	53	1 (referent)	1 (referent)	1 (referent)
TP53 (+)	97	23	0.77 (0.45–1.33)	0.73 (0.42–1.26)	0.64 (0.36–1.11)	46	0.83 (0.56–1.24)	0.84 (0.56–1.25)	0.80 (0.53–1.20)
P value			0.35	0.25	0.11		0.36	0.39	0.29
P for interaction *			0.092	0.0087	0.0051		0.24	0.099	0.092
TP53 (–) cases									
Pre-diagnosis BMI <30 kg/m ²	496	129	1 (referent)	1 (referent)	1 (referent)	247	1 (referent)	1 (referent)	1 (referent)
Pre-diagnosis BMI ≥30 kg/m ²	103	31	1.18 (0.80–1.75)	1.58 (1.06–2.36)	1.63 (1.08–2.46)	53	1.16 (0.86–1.56)	1.33 (0.98–1.80)	1.41 (1.03–1.92)
P value			0.41	0.025	0.019		0.33	0.067	0.030
TP53 (+) cases									
Pre-diagnosis BMI <30 kg/m ²	359	119	1 (referent)	1 (referent)	1 (referent)	189	1 (referent)	1 (referent)	1 (referent)
Pre-diagnosis BMI ≥30 kg/m ²	97	23	0.71 (0.45–1.11)	0.70 (0.44–1.10)	0.68 (0.43–1.07)	46	0.89 (0.64–1.23)	0.91 (0.66–1.26)	0.95 (0.68–1.32)
P value			0.13	0.12	0.096		0.48	0.58	0.75

The multivariate stage-stratified Cox regression model included the same set of covariates selected as in Table 3.

* P values for interaction were assessed by the Wald test on the cross product of the TP53 and BMI variables in a Cox model.

BMI, body mass index; CI, confidence interval; HR, hazard ratio.