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# Colorectal cancer expression of PPARG (peroxisome proliferator-activated receptor-γ) is associated with good prognosis

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## Abstract

**Background & Aims—**The peroxisome proliferator-activated receptor-γ (PPARG) is a nuclear receptor that regulates expression of mediators of lipid metabolism and the inflammatory response. There is controversy over the pro- or anti-oncogenic effects of PPARG and little is known about how its expression correlates with prognosis in patients with colon cancer.

**Methods—**Among 470 colorectal cancer patients (stages I-IV) identified in 2 independent prospective cohorts, PPARG expression was detected in 102 tumors (22%) by immunohistochemistry. Cox proportional hazard models computed hazard ratios (HRs) of colorectal cancer-specific and overall mortalities, unadjusted and adjusted for patient characteristics and tumor molecular features, including cyclooxygenase-2 (COX-2), fatty acid synthase (FASN), *KRAS*, *BRAF*, *PIK3CA*, p53, p21, β-catenin, LINE-1 hypomethylation, microsatellite instability (MSI) and the CpG island methylation phenotype (CIMP).

**Results—**Compared to patients with PPARG-negative tumors, patients with PPARG-positive tumors had significantly lower overall mortality, determined by Kaplan-Meier analysis (p=0.0047), univariate Cox regression (HR 0.55; 95% confidence interval [CI], 0.37-0.84; p=0.0053) and multivariate analysis (adjusted HR 0.43; 95% CI, 0.27-0.69; p=0.0004). Patients with PPARG-positive tumors experienced a lower colorectal cancer-specific mortality (HR 0.65; 95% CI, 0.40-1.07; p=0.092), which became significant in multivariate analysis (adjusted HR 0.44; 95% CI, 0.25-0.79; p=0.0054). The relationship between PPARG and lower mortality did not appear to be significantly modified by tumor stage or the other clinical and molecular variables examined (all $P_{interaction}>0.05$).

**Conclusions—**Tumor expression of PPARG is independently associated with longer survival of patients. PPARG expression appears to mark an indolent subset of colorectal cancers.

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## INTRODUCTION

PPARG (the official symbol for peroxisome proliferator-activated receptor-γ) is a member of the nuclear hormone receptor PPAR superfamily.[1, 2] Ligands for PPARG include naturally occurring fatty acids and the thiazolidinedione (TZD) class of antidiabetic drugs. PPARG plays an important role in adipose cell differentiation, modulation of metabolism and inflammatory response, and cellular apoptosis.[1, 3-5] PPARG interacts with and/or regulates multiple signaling pathways, including those related to p53, p21, BCL2, NF-kappa-β, STAT, cyclin D1 and cyclooxygenase-2 (COX-2).[1-7] Nonetheless, with regard to the role of PPARG in cancer, debate has still continued as to whether PPARG is pro-oncogenic or anti-neoplastic.[2, 8-13] In fact, the effect of PPARG is likely multifaceted and tissue-specific. Experimental studies have suggested the role of PPARG in cell cycle regulation and cellular differentiation in colonic epithelium, supporting its anti-neoplastic effect.[8, 9, 13, 14] PPARG expression has been examined in human colon cancer tissue.[15-17] Although two previous studies (N=86 15 and N=99 [16]) did not demonstrate a prognostic value of tumoral PPARG status, these studies were limited by the small sample sizes. Thus, clinical significance of PPARG expression in human colorectal cancer remains uncertain.

We therefore examined the prognostic role of PPARG expression in a large number (N=470) of stage I-IV colorectal cancer patients identified in two independent, prospective cohort studies. Since we concurrently assessed other related molecular variables [including fatty acid synthase (FASN), COX-2, p53, p21, *KRAS*, *BRAF*, *PIK3CA*, LINE-1 hypomethylation, microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP)], we could evaluate the independent effect of PPARG on patient survival after controlling for these molecular events. In particular, it is important to control for the effect of MSI, CIMP and LINE-1 hypomethylation (and related molecular events such as *KRAS*, *BRAF* and *PIK3CA* mutations), because these molecular characteristics refect genomic and epigenomic status of cancer cells, and have been related with patient survival in colon cancer.[18-25]

## MATERIALS AND METHODS

### Study Population

We utilized the databases of two independent prospective cohort studies; the Nurses' Health Study (N = 121,700 women followed since 1976),[26, 27] and the Health Professionals Follow-up Study (N = 51,500 men followed since 1986).[27] Every 2 years, participants have been 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 body mass index (BMI, kg/m$^2$), 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 ($r > 0.96$).[28] On each biennial follow-up questionnaire, participants were asked whether they had a diagnosis of colorectal cancer during the previous 2 years. When a participant (or next of kin for decedents) reported colorectal cancer, we sought permission to obtain medical records. Study physicians, while blinded to exposure data, reviewed all records related to colorectal cancer, and recorded AJCC (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.[27] 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 470 stage I-IV colorectal cancer cases (180 from the men's cohort and 290 from the women's cohort) diagnosed up to 2002. We utilized the well-established colorectal cancer tissue database with long-term follow-up data. PPARG has not been examined in our colorectal cancers. Moreover, our rich tissue database readily enabled us to control for confounding by any of the clinical and tumoral molecular characteristics in survival analyses, and to assess independent effect of PPARG expression on patient outcome after controlling for possible confounders. It is analogous to a novel study that utilizes well-known cancer cell lines or well-characterized animal cancer models. Written 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. The cause of death was assigned by physicians blinded to information on lifestyle exposures and molecular changes in colorectal cancer. In rare patients who died as a result of colorectal cancer not previously reported, we obtained medical records with permission from next of kin. More than 98% of deaths in the cohorts were identified by these methods.

### DNA Extraction, Pyrosequencing of *KRAS, BRAF* and *PIK3CA*, and Microsatellite Instability (MSI) Analysis

Genomic DNA from paraffin-embedded tissue was extracted, and whole genome amplification was performed.[29] PCR and Pyrosequencing targeted for *KRAS* codons 12 and 13, *BRAF* codon 600 and *PIK3CA* exons 9 and 20 were performed as previously described.[30] MSI status was determined using a microsatellite marker panel consisting of D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67 and D18S487 (i.e., 10-marker panel).[29] 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 (MethyLight) to Determine CIMP (CpG Island Methylator Phenotype) Status

Sodium bisulfite treatment on tumor DNA and subsequent real-time PCR (MethyLight)[31] assays were validated and performed.[29] We quantified promoter methylation in 8 CIMP-specific genes (CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3 and *SOCS1*).[29, 32, 33] CIMP-high was defined as ≥6/8 methylated promoters using the 8-marker CIMP panel, CIMP-low/0 as 0 to 5 methylated promoters, according to the previously established criteria.[29]

### Pyrosequencing to Measure LINE-1 Methylation

In order to accurately quantify relatively high LINE-1 methylation levels, we utilized Pyrosequencing.[24] LINE-1 methylation level measured by Pyrosequencing has been shown to correlate well with overall 5-methylcytosine level (i.e., global DNA methylation level) in tumor cells.[34]

### Immunohistochemistry for PPARG, cyclin D1, p53, p21, p27, β-catenin, COX-2 and FASN

Tissue microarrays (TMAs) were constructed as previously described.[27] Methods of immunohistochemical procedures and interpretation were previously described as follows: cyclin D1,[35] β-catenin,[36] p21, p27, p53,[37, 38] fatty acid synthase (FASN),[39] and COX-2.[27]

For PPARG immunohistochemistry (Figure 1), antigen retrieval was performed, and deparaffinized tissue sections in Target Retrieval Solution (pH 9.0, Dako, Glostrup, Denmark) were treated by a microwave for 5 min in a pressure cooker. Tissue sections were incubated with 10% normal goat serum (Vector Laboratories, Burlingame, CA) in phosphate-buffered saline (30 min). Primary antibody against PPARG (mouse monoclonal anti-PPARG, 1:50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) was applied, and the slides were maintained for 2 hours at room temperature. Next, we applied Envision System HRP labeled polymer anti mouse (Dako) for 30 min, followed by visualizing signal with diaminobenzidine (5 min) and methyl-green counterstain. PPARG positivity was defined as the presence of at least weak nuclear staining in ≥20% of tumor cells or moderate/strong staining in any fraction of tumor cells. Either absent staining or weak staining in <20% of tumor cells was interpreted as negative. Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically-stained slides for PPARG were interpreted by a pathologist (K.S.) unaware of other data. A random sample of 145 tumors were re-examined by a second pathologist (Y.B.) unaware of other data. The concordance between the two observers was 0.92 ($\kappa$=0.58, $p<0.0001$), indicating good to substantial agreement.

## Statistical Analysis

We used stage-matched, conditional Cox proportional hazard models to calculate hazard ratios (HRs) of death according to tumoral PPARG status, adjusted for age, sex, year of diagnosis, BMI, family history of colorectal cancer in any first degree relative, tumor location, stage, grade, MSI, CIMP, LINE-1, *KRAS*, *BRAF*, *PIK3CA*, β-catenin, p53, p21, p27, cyclin D1, FASN and COX-2. In addition, we also performed Cox regression analysis to assess the unadjusted, main effect of PPARG expression on mortality. 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 PPARG variable and survival time (p=0.62 for colon cancer-specific mortality; p=0.58 for overall mortality). To adjust for potential confounding, age, year of diagnosis and LINE-1 methylation were used as continuous variables, and all of the other covariates were used as categorical variables. Tumor stage (I, IIA, IIB, IIIA, IIIB, IIIC, IV) was used as a matching variable. We dichotomized family history (present vs. absent), BMI (<30 kg/m$^2$ vs. ≥30 kg/m$^2$), tumor location (rectum vs. colon), grade (high vs. low), CIMP (high vs. low/0), MSI (high vs. low/MSS), p53, p21, p27, cyclin D1, β-catenin, *KRAS*, *PIK3CA*, *BRAF*, FASN and COX-2. For cases with missing information in other covariates [including BMI (2.8% missing), tumor location (0.9% missing), MSI (0.4% missing), *KRAS* (0.2% missing), *BRAF* (2.8% missing), β-catenin (7.4% missing), p53 (0.6% missing), p21 (2.1% missing), p27 (4.5% missing) and cyclin D1 (4.7% missing)], we included those cases in a majority category, in order to minimize the number of "missing" indicator variables and maximize the efficiency of multivariate Cox regression analyses. 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 PPARG variable and another variable of interest in a multivariate Cox model, and the likelihood ratio test was performed. P values were conservatively interpreted, considering multiple hypotheses testing. To assess an interaction of PPARG and stage, we dichotomized tumor stage (I-II vs. III-IV) as well as treated stage as a linear ordinal variable (I to IV).

The Kaplan-Meier method was used to describe the distribution of colon cancer-specific and overall survival time, and the log-rank test was performed. The chi square test was used to examine an association of PPARG with any of the categorical variables. The t-test assuming

unequal variances was performed to compare mean age and mean LINE-1 methylation level. All analyses used SAS version 9.1 (SAS Institute, Cary, NC) and all p values were two-sided.

## RESULTS

### PPARG expression in colorectal cancer and normal mucosa

Among 470 patients with stage I-IV colorectal cancer, PPARG expression was observed in 102 (22%) tumors by immunohistochemistry (Figure 1). We assessed clinical and molecular characteristics of colorectal cancers according to tumoral PPARG status, to assess potential confounders (Table 1). Compared to PPARG-negative cases, PPARG-positive cases were more likely diagnosed in 1995 or after, slightly more like to be located in rectum, and less likely to have a family history of colorectal cancer.

In 264 cases for which we could evaluate PPARG expression in normal colonic mucosa, 134 cases (51%) show at least weak positivity. Tumoral PPARG positivity was significantly more common in cases with PPARG-positive normal mucosa (26%=35/134) than those with PPARG-negative normal mucosa (13%=17/130; p=0.008). This phenomenon could be a result of field effect. An alternative possibility was the presence of some poor quality specimens that showed false negative in either tumor or normal mucosa or both, driving PPARG staining in tumor and normal mucosa towards a concordant pattern. PPARG expression in normal mucosa was not significantly related with patient survival (data not shown).

### PPARG expression and prognosis in colorectal cancer

During follow-up, there were 199 deaths, including 118 colorectal cancer-specific deaths. We assessed the influence of PPARG expression on patient survival. In Kaplan-Meier analysis, PPARG-positive patients experienced significantly longer overall survival (log-rank p=0.0047) (Figure 2). Five-year overall survival was 83% in PPARG-positive patients and 71% in PPARG-negative patients. In univariate Cox regression analysis, compared to patients with PPARG-negative tumors, those with PPARG-positive tumors experienced a significantly lower overall mortality [HR 0.55; 95% confidence interval (CI), 0.37-0.84; p=0.0053] (Table 2). In the multivariate Cox model adjusting for potential predictors of patient outcome, PPARG positivity was associated with a significantly lower overall mortality (multivariate HR 0.43; 95% CI, 0.27-0.69; p=0.0004).

In the analysis using colorectal cancer-specific mortality as the end point, PPARG-positive cases experienced a similar reduction of mortality in univariate and multivariate analyses (multivariate HR 0.44; 95% CI, 0.25-0.79; p=0.0054). The greater effect of PPARG on cancer-specific mortality in multivariate analysis than univariate analysis was simply due to the effect of adjusting for tumor stage. When we simply adjusted for tumor stage, HR for colorectal cancer specific mortality was 0.54 (95% CI, 0.33-0.90; p=0.017) (Table 2). No other major confounder was present.

### Stratified analysis of PPARG and mortality

We examined the influence of PPARG positivity on overall mortality across strata of other potential predictors of patient survival (Figure 3). Accordingly, we assessed whether there was potential modifying effect (on the relation between PPARG and a low mortality) by any of the other variables examined, including sex (cohort), age, BMI, family history of colorectal cancer, year of diagnosis, tumor location, stage, grade, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*,*PIK3CA*, β-catenin, p53, p21, p27, cyclin D1, FASN and COX-2. There was no evidence for significant effect modification by any of the variables (all p for interaction >0.05). Notably, the effect of PPARG did not significantly differ between the two independent cohort studies (p for interaction = 0.43).

## DISCUSSION

We conducted this study to examine the relation between expression of PPARG (the official symbol for peroxisome proliferator-activated receptor-γ) and patient survival in 470 patients with stage I-IV colorectal cancer. We have shown that PPARG expression is independently associated with good prognosis in colorectal cancer. We have been able to demonstrate that this relation did not appear to be significantly modified by tumor stage or any of the other clinical features and tumoral molecular characteristics. Furthermore, our resource of a large number of colorectal cancers derived from the two independent, prospective cohort studies has enabled us to precisely estimate the frequency of colorectal cancers with PPARG expression, and provided us with robust statistics in survival analysis. Our results suggest that PPARG expression in colorectal cancer is independently associated with low mortality, and marks colorectal cancer with indolent biological behavior.

Examining molecular alterations is important in cancer research.[40-46] In particular, determining status of microsatellite instability (MSI), the CpG island methylator phenotype (CIMP) and LINE-1 hypomethylation in colorectal cancer is increasingly important, because these molecular characteristics refect genomic and epigenomic status of cancer cells, and have been related with patient survival in colon cancer.[19, 21, 23, 24] In addition to examining status of CIMP, MSI and LINE-1 hypomethylation, we assessed various molecular variables potentially related with PPARG and/or energy balance, including fatty acid synthase (FASN), cyclooxygenase-2 (COX-2), p53, p21, *KRAS*, *BRAF* and *PIK3CA*. Thus, unlike other studies, we were able to evaluate the independent effect of PPARG on patient survival after controlling for those related molecular events.

A role of PPARG in colorectal cancer has been controversial. Animal models generated by different methods show different effects of PPARG. Genetic models with APC mutations suggest that PPARG promote tumorigenesis.[10-12] On the contrary, colon tumors induced by carcinogens can be suppressed by thiozolidinedione (TZD) based PPARG agonists, suggesting a tumor-suppressor role of PPARG.[4, 13] A large epidemiologic study of a diabetic population suggests that thiozolidinedione usage may reduce the risk of a number of cancers including lung, colon and prostate.[47] Various studies have shown the effect of PPARG ligands on normalization of cell cycle progression and cellular differentiation.[8, 9, 14] It is proposed that PPARG could be a conditional tumor suppressor or conditional oncogene that modulates the tumor pathogenesis depending on cellular conditions, tissue types, or genetic background of individuals.[2] Nonetheless, our current data suggest that PPARG expression may mark an indolent subset of colorectal cancers.

Excess energy balance, obesity and lack of exercise have been linked to increased risks of a variety of human cancers including colorectal cancer.[48, 49] PPARG is one of potential molecules that link between energy balance, cellular metabolism and cancer pathogenesis. PPARG has been shown to play an important role in the control of gene expression linked to a variety of cellular processes.[1] Activation of PPARG improves insulin sensitivity through a combination of metabolic actions, including partitioning of lipid stores and the regulation of metabolic and inflammatory mediator adipokines.[1] Thus, we could hypothesize that there might be an interactive effect of PPARG expression and energy balance (or related tumoral molecular events) on tumor aggressiveness. However, we did not show any significant interaction of PPARG with patient body mass index (BMI), FASN expression or *PIK3CA* mutation in terms of patient survival. Nonetheless, it is still possible that energy balance may differentially influence the occurrence of colorectal cancer according to PPARG expression. We await future studies that examine the effect of energy balance on the occurrence of PPARG-positive or negative tumors.

In our dataset, compared to PPARG-negative cases, PPARG-positive cases were more likely to be diagnosed in 1995 or after. This might have been due to poor antigenicity of PPARG in older specimens. However, when we examined the strata of "year of diagnosis" and a potential interaction between PPARG and "year of diagnosis", "year of diagnosis" did not significantly alter the relation between PPARG and patient survival (p for interaction = 0.80; see Figure 3). This suggests that misdiagnosis due to poor antigenicity, if any, did not substantially alter survival analysis results. Moreover, in our multivariate Cox regression analysis, we adjusted hazard ratio (HR) for clinical and tumoral characteristics, including year of diagnosis. Thus, any potential confounding effect of year of diagnosis on patient survival was controlled in our multivariate analysis model.

In our cohorts, data on cancer treatment were limited. Nonetheless, it is unlikely that chemotherapy use differed according to tumoral PPARG status, since 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 approximately 10 to 12 months during much of the time period of this study,[50] colon cancer-specific survival should be a reasonable surrogate for cancer-specific outcomes.

In summary, our large cohort study suggests that PPARG expression is independently associated with good prognosis in colorectal cancer. Our findings may have considerable clinical implications, given that PPARG has been used as a drug target. Future studies are needed to confirm this association as well as to elucidate exact mechanisms by which PPARG affects tumor behavior.

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## Abbreviations and the HUGO Gene Nomenclature Committee-approved official gene symbols

AJCC, American Joint Committee on Cancer
BMI, body mass index
CI, confidence interval
CIMP, CpG island methylator phenotype
COX-2, cyclooxygenase-2
FASN, fatty acid synthase
HPFS, Health Professionals Follow-up Study
HR, hazard ratio
LINE-1, long interspersed nucleotide element-1
MSI, microsatellite instability
MSS, microsatellite stable
NHS, Nurses' Health Study

PPARG, peroxisome proliferator-activated receptor gamma.

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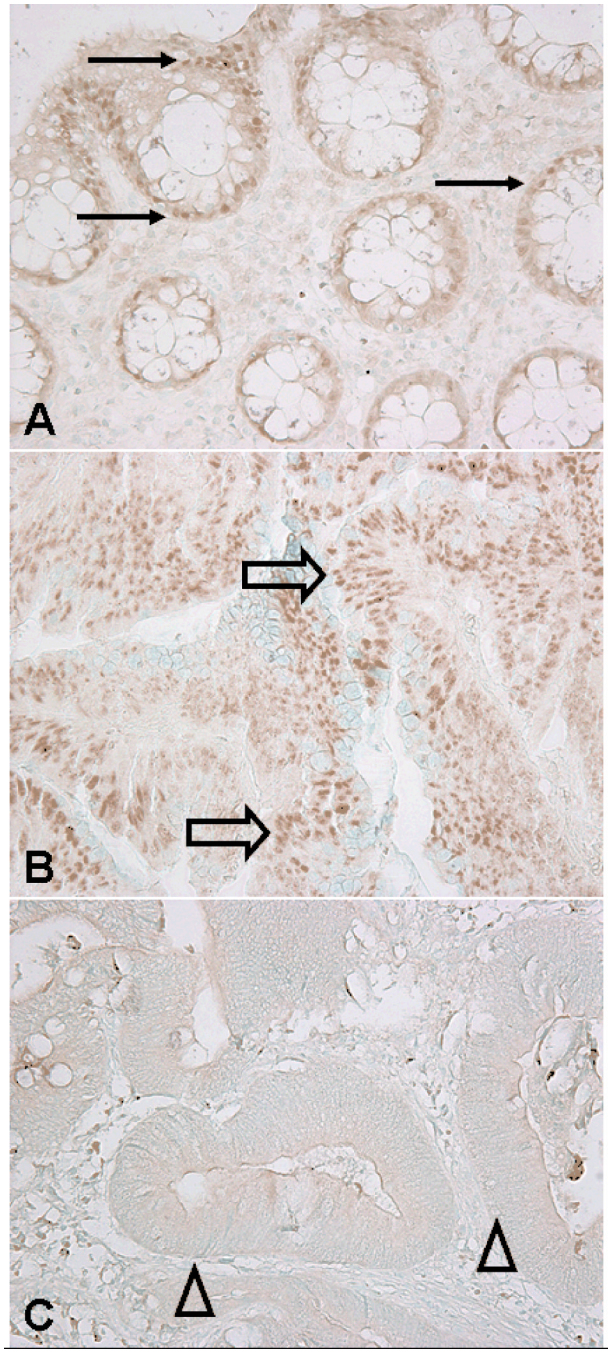
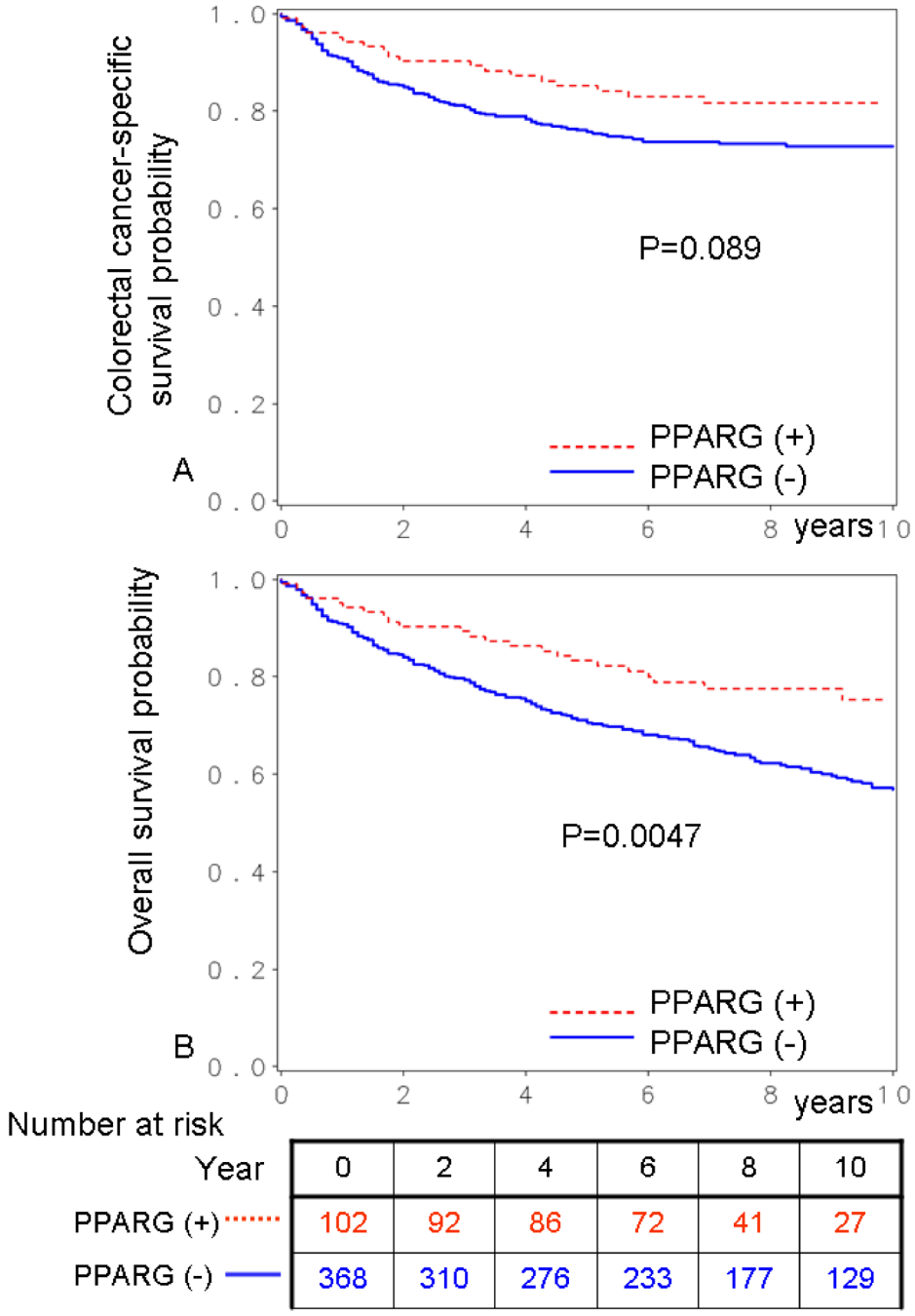



**Figure 1.**
PPARG expression in normal colonic mucosa and colorectal cancer.
A. Normal colonic epithelial cells with nuclear PPARG expression (arrows). B. Colorectal cancer cells with nuclear PPARG expression in (block arrows). C. Colorectal cancer cells with no nuclear PPARG expression (arrowheads).




Number at risk

| Year | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|
| PPARG (+) | 102 | 92 | 86 | 72 | 41 | 27 |
| PPARG (-) | 368 | 310 | 276 | 233 | 177 | 129 |

**Figure 2.**
Kaplan-Meier survival curves in colorectal cancer according to PPARG status. A. Colorectal cancer-specific survival. B. Overall survival.

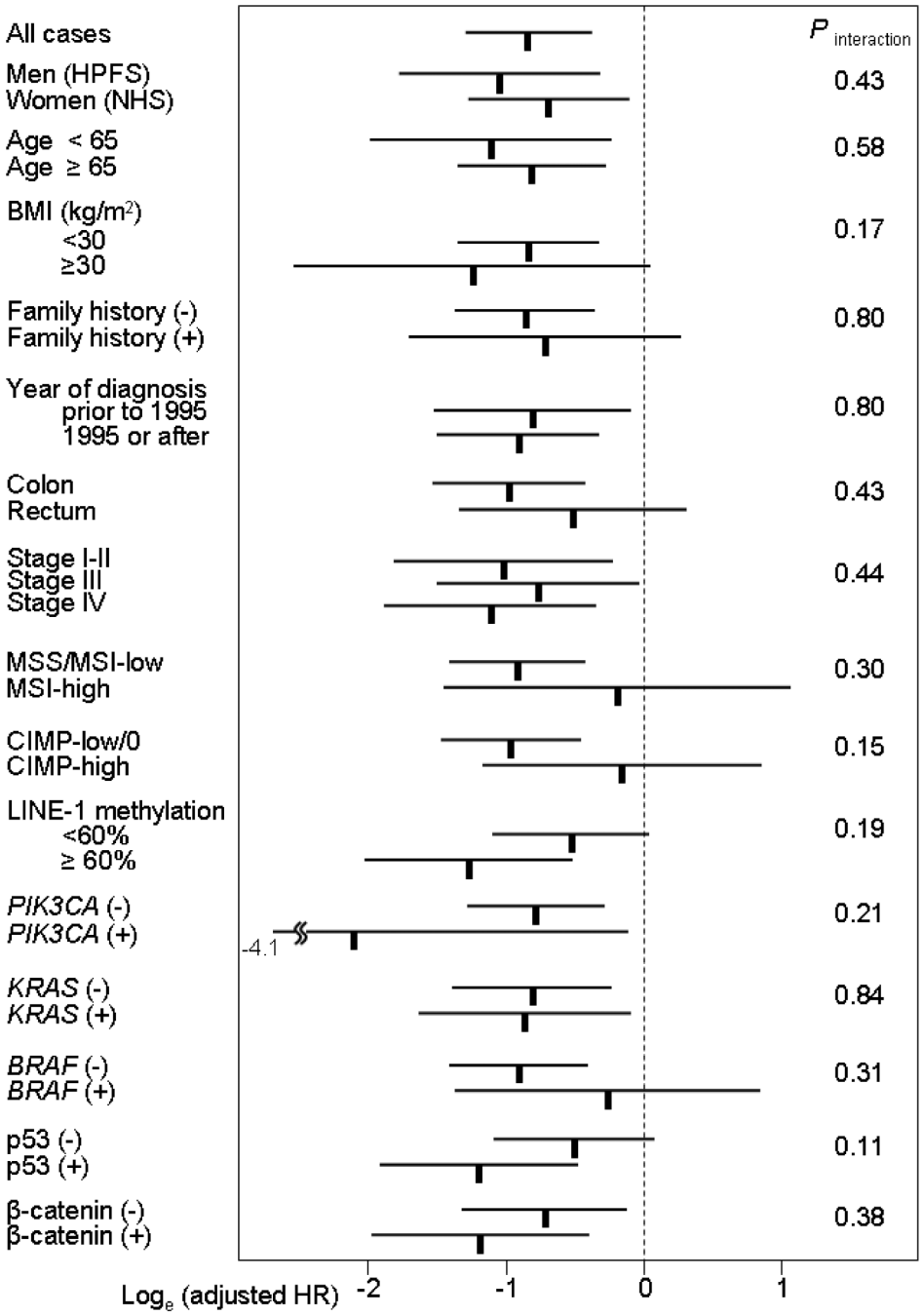



**Figure 3.**

PPARG status and overall mortality in various strata.

$Log_e$(adjusted HRs) with 95% CI for overall mortality in PPARG+ tumors (vs. PPARG- tumors) are shown.

BMI, body mass index; CI, confidence interval; CIMP, CpG island methylator phenotype; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; NHS, Nurses' Health Study.

**Table 1**

Clinical and molecular characteristics according to PPARG status in colorectal cancer

| Clinical or molecular feature | No. of cases | PPARG | |
|---|---|---|---|
| | | Negative | Positive |
| Total N | 470 | 368 | 102 |
| Sex | | | |
| Male (HPFS) | 180 (38%) | 148 (40%) | 32 (31%) |
| Female (NHS) | 290 (62%) | 220 (60%) | 70 (69%) |
| Mean age ± SD | 66.0 ± 8.6 | 65.8 ± 8.9 | 66.5 ± 7.6 |
| Body mass index (BMI, kg/m$^2$) | | | |
| <30 | 200 (44%) | 159 (45%) | 41 (41%) |
| 25-30 | 174 (38%) | 130 (36%) | 44 (44%) |
| ≥30 | 83 (18%) | 68 (19%) | 15 (15%) |
| Family history of colorectal cancer | | | |
| Absent | 353 (75%) | 270 (73%) | 83 (81%) |
| Present | 117 (25%) | 98 (27%) | 19 (19%) |
| Year of diagnosis | | | |
| Prior to 1995 | 193 (41%) | 167 (45%) | 26 (25%) |
| 1995 to 2002 | 277 (59%) | 201 (55%) | 76 (75%) |
| Tumor location | | | |
| Right (cecum to transverse colon) | 230 (49%) | 176 (48%) | 54 (54%) |
| Left colon (splenic flexure to sigmoid colon) | 150 (32%) | 128 (35%) | 22 (22%) |
| Rectum | 86 (18%) | 62 (17%) | 24 (24%) |
| Tumor stage | | | |
| I | 106 (23%) | 79 (21%) | 27 (26%) |
| II | 163 (35%) | 130 (35%) | 33 (32%) |
| III | 140 (30%) | 110 (30%) | 30 (29%) |
| IV | 61 (13%) | 49 (13%) | 12 (12%) |
| Tumor grade | | | |
| Low | 428 (91%) | 333 (90%) | 95 (93%) |
| High | 42 (8.9%) | 35 (9.5%) | 7 (6.9%) |
| MSI | | | |
| MSI-low/MSS | 385 (82%) | 299 (82%) | 86 (84%) |
| MSI-high | 83 (18%) | 67 (18%) | 16 (16%) |
| CIMP | | | |
| CIMP-low/0 | 395 (84%) | 309 (84%) | 86 (84%) |
| CIMP-high | 75 (16%) | 59 (16%) | 16 (16%) |
| Mean LINE-1 methylation (%) | 60.3 ± 9.4 | 60.1 ± 9.7 | 61.1 ± 8.2 |
| *BRAF* mutation | | | |
| (-) | 392 (86%) | 307 (86%) | 85 (84%) |
| (+) | 65 (14%) | 49 (14%) | 16 (16%) |
| *KRAS* mutation | | | |
| (-) | 295 (63%) | 231 (62%) | 64 (63%) |

| Clinical or molecular feature | No. of cases | PPARG | |
|---|---|---|---|
| | | Negative | Positive |
| (+) | 174 (37%) | 136 (37%) | 38 (37%) |
| *PIK3CA* mutation | | | |
| (-) | 363 (86%) | 277 (85%) | 86 (88%) |
| (+) | 60 (14%) | 48 (15%) | 12 (12%) |
| β-catenin* | | | |
| Inactive (score 0-2) | 274 (63%) | 211 (63%) | 63 (64%) |
| Active (score 3-5) | 161 (37%) | 126 (37%) | 35 (36%) |
| p53 expression | | | |
| (-) | 285 (61%) | 226 (62%) | 59 (59%) |
| (+) | 182 (39%) | 141 (38%) | 41 (41%) |
| p21 (*CDKN1A*) | | | |
| Expressed | 86 (19%) | 70 (19%) | 16 (16%) |
| Lost | 374 (81%) | 291 (81%) | 83 (84%) |
| p27 (*CDKN1B*) | | | |
| Nuclear expression | 94 (21%) | 72 (20%) | 22 (23%) |
| Cytoplasmic expression or loss of expression | 355 (79%) | 280 (80%) | 75 (77%) |
| Cyclin D1 expression | | | |
| (-) | 133 (30%) | 109 (31%) | 24 (24%) |
| (+) | 315 (70%) | 241 (69%) | 74 (76%) |
| Fatty acid synthase (FASN) expression | | | |
| (-) | 322 (83%) | 249 (86%) | 73 (77%) |
| (+) | 64 (17%) | 42 (14%) | 22 (23%) |
| Cyclooxygenase-2 (COX-2) expression | | | |
| (-) | 82 (17%) | 68 (18%) | 14 (14%) |
| (+) | 388 (83%) | 300 (82%) | 88 (86%) |

(%) indicates the proportion of tumors with a specific clinical or molecular feature in PPARG(-) (or PPARG+) tumors.

CIMP, CpG island methylator phenotype; HPFS, Health Professionals Follow-up Study; LINE-1, long interspersed nucleotide element-1; MSI, microsatellite instability; MSS, microsatellite stable; NHS, Nurses' Health Study; SD, standard deviation.

*β-catenin score was calculated as previously described.[36]

**Table 2**

PPARG expression in colorectal cancer and patient mortality

| | Total N | Colorectal 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) |
| PPARG(-) | 368 (78%) | 99/2982 | 1 (referent) | 1 (referent) | 1 (referent) | 173/2982 | 1 (referent) | 1 (referent) | 1 (referent) |
| PPARG(+) | 102 (22%) | 19/789 | 0.65 (0.40-1.07) | 0.54 (0.33-0.90) | 0.44 (0.25-0.79) | 26/789 | 0.55 (0.37-0.84) | 0.52 (0.34-0.79) | 0.43 (0.27-0.69) |
| P value | | | 0.092 | 0.017 | 0.0054 | | 0.0053 | 0.0025 | 0.0004 |

The multivariate, stage-matched conditional Cox regression model included age, year of diagnosis, sex, family history of colorectal cancer, body mass index (BMI), tumor location, stage, grade, *KRAS*, *BRAF*, *PIK3CA*, p53, p21, p27, cyclin D1, β-catenin, COX-2, FASN, LINE-1 methylation, microsatellite instability (MSI), and CpG island methylator phenotype (CIMP).

CI, confidence interval; HR, hazard ratio.