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Postmenopausal hormone therapy is associated with a reduced risk of colorectal cancer lacking CDKN1A expression

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Abstract

Experimental studies have shown that estrogen- or progesterone-activated signaling leads to growth inhibition effects on colon cancer cells through the upregulation of several cell cycle regulators. However, epidemiologic studies evaluating hormone therapy (HT) use and colorectal cancer risk by the status of cell cycle regulators are lacking. In this study, we used data from the prospective Nurses' Health Study to evaluate whether the association between HT use and colorectal cancer risk differs by the molecular pathological status of microsatellite instability (MSI) and expression of cell cycle-related tumor biomarkers, including CDKN1A (p21, CIP1), CDKN1B (p27, KIP1), and TP53 (p53) by immunohistochemistry. Duplication Cox regression analysis was used to determine an association between HT use, cancer risk, and specific tumor biomarkers in 581 incident colon and rectal cancer cases that occurred during 26 years of follow-up among 105520 postmenopausal women. We found a difference between HT use and colorectal cancer risk according to CDKN1A expression (p-value for heterogeneity=0.01). Current HT use was associated with a reduced risk for CDKN1A-nonexpressed (multivariate relative risk (RR)=0.61, 95% confidence interval (CI), 0.46–0.82), but not for CDKN1A-expressed (RR=1.32, 95% CI, 0.76–2.31) tumors. The lower risk for CDKN1A-nonexpressed, but not for CDKN1A-expressed cancers was also present among current users of estrogen-alone therapy. We found no significant difference in the relations between HT use and cancer risk according to MSI, CDKN1B, or TP53 status. Together, our molecular epidemiology findings suggest a preventive

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effect of HT against colorectal carcinogenesis which depends, in part, on loss of cyclin-dependent kinase inhibitor CDKN1A.

Introduction

Numerous observational studies have reported an inverse association between use of postmenopausal hormone therapy (HT) and colorectal cancer risk. Three meta-analysis reviews pooling at least 15 observational studies have concluded ≥15% reduction in risk for colorectal cancer among ever users of HT (1–3), with the risk reduction being more pronounced for more recent use (1–3) and duration of use exceeding 5 years (1). In support of these findings, the recent Women's Health Initiative (WHI) randomized trial of estrogen plus progestin (E+P) reported a 40% lower risk for colorectal cancer in the treatment group as compared with the placebo group (4, 5). The WHI estrogen-alone (E-alone) trial among hysterectomized women did not observe a lower risk of colorectal cancer in the treatment group, although the association was modified by age (6). Observational data on types of HT formulation have shown a lower risk for colorectal cancer with use of E-alone (7–10) and/or E+P (9–20) therapies.

The potential mechanisms by which HT use reduces risk for colorectal cancer development remain unclear. Experimental studies on mice and cell lines have shown that estrogen- or progesterone-activated signaling leads to growth inhibition effects on colon cancer cells through the upregulation of several cell cycle regulators including CDKN1A (p21) (21–23), CDKN1B (p27) (22, 24), and TP53 (p53) (25–27). It has also been suggested that estrogen treatment helps maintain genomic stability in colonic epithelial cells through upregulation of mismatch repair genes (28, 29). Observational data, however, on HT use and risk for colorectal cancer by microsatellite instability (MSI) status are very limited (16, 18, 29, 30). No data have been reported on HT use and colorectal cancer risk by cell cycle regulator status. We hypothesized that the lower risk for colorectal cancer with HT use might result from its protection against high MSI and/or aberrant expression of cell cycle regulators in cells.

We, therefore, tested the association of HT use with colorectal cancer risk according to MSI status and the expression of cell cycle regulators including CDKN1A, CDKN1B, and TP53 in the Nurses' Health Study (NHS), a large prospective cohort study with 26 years of follow-up. We additionally examined whether types of formulation and duration might contribute to differential risks associated with HT use.

Methods

Study population

The NHS was established in 1976 when 121700 female registered nurses aged 30 to 55 years old from 11 states were enrolled into the study. Every 2 years, participants have been sent follow-up questionnaires to update information on HT use, lifestyle factors, medical history, and occurrence of diseases including colorectal cancer. Dietary information was first collected in 1980 and updated in 1984, 1986 and every 4 years thereafter through a semi-quantitative validated food frequency questionnaire asking participants their average intakes of foods and beverages during the past year.(31, 32) Follow-up for this cohort through 2006 was greater than 90%. This study was approved by the Human Subjects Committee at Brigham and Women's Hospital in Boston, MA.

The present analysis was limited to postmenopausal women only. Women were considered as postmenopausal if they reported no menstrual periods from the time of natural menopause or hysterectomy with bilateral oophorectomy. Women who underwent hysterectomy without

bilateral oophorectomy were considered postmenopausal when they reached the age at which natural menopause had occurred in 90% of the cohort (ie, 54 years for smokers and 56 years for nonsmokers). Self-report of natural menopause and extent of ovarian surgery has been shown to be highly accurate and reproducible in this cohort (33). In 1980, there were 31959 postmenopausal women included in the study, and 73561 women were added over follow-up as they became postmenopausal.

Ascertainment of colorectal cancer

On each biennial questionnaire, participants were asked whether they had been diagnosed with colon or rectal cancer in the prior two years. We sought permission to obtain medical records and pathology reports for those who reported a diagnosis of colorectal cancer and for those who were deceased. Study physicians who were blinded to exposure data reviewed and extracted information on histopathology, anatomic location, and stage of cancer. We included cases confirmed after the return of the 1980 questionnaire and before June 2006.

Assessment of hormone use

HT information was updated every 2 years on biennial questionnaires where respondents were asked whether they had been using HT since the previous 2-year follow-up cycle. Women were considered current HT users if they reported current use of HT during the follow-up cycle. Past users were those who reported HT use at any time before but not at the follow-up cycle. Respondents were also asked about the number of months they had used since the previous follow-up cycle. Duration of HT use was estimated through the summation of HT use across questionnaire cycles. Information on HT formulation was consolidated into 2 types: E-alone and E+P therapies. E-alone therapy contained the categories of oral conjugated or other estrogen except vaginal estrogen, and E+P therapy was comprised by oral or other estrogen combined with progestin.

Collection of primary colorectal tumor tissue specimens

We have sought to retrieve archived primary colorectal tumor specimens from a total of 1035 postmenopausal women with a confirmed diagnosis of colorectal cancer between 1980 and 2006. We have obtained tumor blocks from 651 women. We were not able to retrieve material from the remaining 384 women due to the following reasons: (a) tissue samples were either discarded or lost by the hospitals (N=200); (b) hospitals did not respond (N=64); (c) the participants were deceased and no further information was available (N=58); (d) hospitals refused to give us the samples or charged high processing fees (N=50); (e) the medical records were unavailable (N=12). Characteristics among cases whom we had tissue samples for molecular and genetic analyses were largely similar to those whom we had no samples. Briefly, both groups were not different in means of age at diagnosis (66 and 68 years) and other risk factors for colorectal cancer prior to diagnosis including means of BMI (26.3 and 26.9 kg/m^2), proportion of current smoking (15% and 14%), proportion of aspirin use (42% and 46%), proportion of multivitamin use (42% and 45%), proportion of current HT use (25% and 28%), and proportion of former HT use (31% and 34%).

Microsatellite instability (MSI) analysis

Genomic DNA was first extracted from dissected tumor tissue sections and MSI status was determined using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487) as previously described (34). MSI-high was defined when ≥ 30% of the markers in tumor cells were unstable and tumors with <30% unstable markers were considered as MSI-low/microsatellite stable (MSS).

Immunohistochemical analyses for CDKN1A (p21), CDKN1B (p27), and TP53 (p53)

Tissue microarrays (TMAs) were first constructed for TP53 analysis (35, 36) and CDKN1A and CDKN1B assays were performed on whole tissue sections (37, 38). Immunohistochemistry for CDKN1A, CDKN1B, and TP53 were performed as previously described (35, 37–39). Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically stained slides were interpreted by a pathologist (S.O.) unaware of any laboratory and clinical data.

For CDKN1A immunohistochemistry, normal colonic mucosa or rare mesenchymal cells served as internal positive controls. We visually estimated the fraction of tumor cells expressing CDKN1A. Expression of CDKN1A was considered as 'nonexpressed' in a tumor with <20% of cells that were positive and as 'expressed' if ≥20% of the cells were positive (37). The extent of nuclear CDKN1B expression was visually estimated and was interpreted as 'nonexpressed' (no staining, only weakly staining, or <20% of tumor cells positive for moderate/strong staining) and as 'expressed' if moderately or strongly positive in≥20% of the cells (38). For TP53, we visually estimated the fraction of tumor cells with strong and unequivocal nuclear staining by examining at least two TMA tissue cores, or the whole tissue section in each case for which there was not enough tissue for TMAs or results were equivocal in TMAs. TP53-postivity was defined as 50% or more of tumor cells with unequivocal strong nuclear staining, and TP53-negativity (or TP53-wild-type) was as <50% or absent/weak nuclear staining of tumor cells (36); this cutoff has been shown to correlate well with TP53 mutation status (40). In addition, a random selection of more than 100 cases was independently reviewed by a second observer (CDKN1A by K.S.; CDKN1B by K.S.; TP53 by K.N.), and the concordance between readers was 0.83 (κ=0.62, N=179) for CDKN1A, 0.94 (κ=0.60, N=114) for CDKN1B, and 0.87 (κ=0.75; N=108) for TP53 (all p-values<0.0001).

Statistical Analysis

In the present analysis, a total of 105520 postmenopausal women were eligible for analysis who accrued follow-up beginning from the date when women were first classified as being postmenopausal to the date of colorectal cancer diagnosis, death from any cause, or June 2006, whichever occurred first. Cox proportional hazard regression was used to model the relative risks (RR) and 95% confidence interval (CI) of colorectal cancer comparing women in various categories of HT use with those who never used HT. The multivariate models were adjusted for age (years, continuous) and additionally for potential risk factors for colorectal cancer, including body mass index (<25, 25–<30, ≥30 kg/m^2), physical activity (METs/week, in quartiles), family history of colorectal cancer in a first-degree relative (yes, no), previous history of colorectal polyps (yes, no), screening test of sigmoidoscopy or colonoscopy (yes, no), smoking status (never, past, current), multivitamin use (yes, no), aspirin use (none, ≥1 tablets/week), alcohol consumption (none, ≤15, >15 g/day), vitamin D intake (IU/day, in quartiles), and fiber intake (mg/day, in quartiles). We used the most updated information for all covariates before each 2-year interval. We additionally assessed the effects of current HT use in colorectal cancer risk according to HT formulation (E-alone and E+P therapies) and duration (≤5, >5 years).

To compare the specific effects of HT use as well as types of formulation and duration on colorectal cancer risk according to tumor markers, we used a previously described duplication method of Cox regression (41, 42). This method permits estimation of separate regression coefficients for HT use according to the types of outcome (eg, cancer with MSI-high vs. cancer with MSS). We then assessed the difference between the risk estimates according to tumor types (eg, MSI-high, MSS) with a likelihood ratio test comparing the model that allowed for separate associations of HT use according to tumor types with a

model that assumed a common association regardless of tumor types (termed as heterogeneity test). All analyses were performed using SAS version 9.0 (SAS Institute Inc, Cary, NC). A two sided p-value of less than 0.05 was used to determine statistical significance.

Results

Table 1 presents the characteristics of the study population in 1994, the middle follow-up year. Compared to never users, past and current users appeared to be leaner, younger when experiencing menopause, more likely to receive sigmoidoscopy or colonoscopy exams, have their uterus removed, be users of multivitamin and aspirin, and consume more alcohol and vitamin D. There was, however, no difference among the 3 groups of HT use in family history of colorectal cancer, physical activity, and intake of total fiber.

Seventy among the 651 women with available tumor samples had missing HT information and were excluded from the analysis. As a result, there were 581 incident colorectal cancer cases included for analyses of tumor markers over 26 years of follow-up. Among these tumors, 118 (22%), 294 (79%), 277 (76%), and 194 (43%) were found to be MSI-high, CDKN1A-nonexpressed, CDKN1B-nonexpressed, and TP53-positive, respectively.

As in our previous studies (43), current use of HT was associated with a lower risk for colorectal cancer in our cohort, regardless of the status of tumor markers (multivariate RR=0.70, 95% CI, 0.56–0.86) (Table 2). The association between past HT use and colorectal cancer risk was attenuated (multivariate RR=0.90, 95% CI, 0.74–1.09). When evaluating the risk for colorectal cancer according to tumor marker status, we found a difference of statistical significance in the associations between HT use and risk for colorectal cancer according to CDKN1A expression status (p-value for heterogeneity= 0.01). Current HT use was associated with a reduced risk for CDKN1A-nonexpressed (multivariate RR=0.61, 95% CI, 0.46–0.82) but not for CDKN1A-expressed (multivariate RR=1.32, 95% CI, 0.76–2.31) colorectal cancers. The differences in the associations with HT use according to status of MSI, CDKN1B, or TP53 in colorectal cancers were not statistically significant (p-values for heterogeneity were ≥0.05) (Table 2). The differences in the associations with former HT use according to tumor marker status were also not significant in either <5 or ≥5 years since last use (p-values for heterogeneity ≥0.19). Additional adjustment for hysterectomy status (removed, intact, unknown) in the model did not materially change the original results (data not shown).

When evaluating the association between types of current HT use and colorectal cancer risk, we found that the lower risk with current HT use was present among E-alone users (multivariate RR=0.70, 95% CI, 0.55–0.90). In addition, current E-alone use was associated with a lower risk of statistical significance for developing CDKN1A-nonexpressed but not CDKN1A-expressed colorectal cancers (p-value for heterogeneity =0.02) (Table 3). There was, however, no difference in the association between current E+P use and colorectal cancer risk according to CDKN1A expression status (p-value for heterogeneity=0.38). The risk patterns by HT formulation among past users were largely similar to those among current HT users (data not shown).

With respect to duration of current HT use, users taking >5 years were at a lower risk for colorectal cancer (multivariate RR=0.69; 95% CI, 0.55–0.85, p-value for trend <0.001) (Table 4). Current users with >5 years also had a lower risk for developing CDKN1A-nonexpressed colorectal cancer (p-value for trend =0.002) but not for CDKN1A-expressed cancer (p-value for trend =0.24), and test for the difference in the associations with current HT duration according to CDKN1A status was statistically significant (p-value for

heterogeneity =0.002) (Table 4). Although the association of past HT use with tumor marker status by duration was weaker as compared to that observed in current HT use, the patterns of risk estimates were largely similar in either HT groups (data not shown).

Discussion

Little is known about the etiological mechanisms underlying the inverse association between HT use and risk for colorectal cancer development. To address this question, we conducted a molecular epidemiology study hypothesizing that tumors with different molecular features arise from specific risk factors (44–46). We, thus, examined the preventive effects of HT use on colorectal cancer development according to MSI status and expression in cell cycle regulators. This approach may also help in the identification of individuals who are susceptible to the development of tumor subtypes with specific molecular characteristics, which ultimately could lead to the development of novel strategies for prevention and intervention in these individuals (44–46).

In this large prospective study with 26 years of follow-up, current use of HT was associated with a lower risk for developing colorectal cancer, and the risk reduction was mostly present in E-alone therapy and with >5 years of use. The lower risk among current HT users was attributable to a reduction in the risk of CDKN1A-nonexpressed but not of CDKN1A (p21)-expressed colorectal cancers. In addition, the lower risk for CDKN1A (p21)-nonexpressed colorectal cancer was present among current users of E-alone therapy and of >5 years of use. In contrast, the inverse association between current HT use and colorectal cancer risk did not differ by MSI status and expression of CDKN1B (p27) and TP53 (p53).

Our finding that current HT use, especially use of E-alone therapy, was associated with a lower risk for developing CDKN1A-nonexpressed colorectal cancer is the first observational evidence of the preventive role of estrogen against colorectal carcinogenesis through cell cycle regulation. Estrogen-activated signaling through estrogen receptor alpha and/or estrogen receptor beta exhibits growth inhibition effects on colon cancer cells by activating CDKN1A, a key protein that governs cell cycle progression from G1 to S phase and is responsible for the regulation of cell proliferation, growth arrest, and apoptosis (21, 22, 24). In addition, we observed an inverse association of borderline significance between current E +P use and risk for developing CDKN1A-nonexpressed colorectal cancer. Cell line studies have also shown that progesterone treatment exerts anti-proliferative effects though modulating cell cycle-related proteins (23).

It is known that, in response to DNA damage, CDKN1A is transactivated by TP53, another cell-cycle checkpoint protein, to inhibit downstream tumor growth proteins (47). In this study population, however, CDKN1A was not significantly associated with either TP53 (r=0.17) or CDKN1B (r=0.15) expression status. In addition, we did not observe difference in the associations between HT use and expression status of CDKN1B and TP53. These observations suggest that the risk reduction for CDKN1A-nonexpressed tumors by HT use is less likely affected by the signaling of these markers. Instead, CDKN1A activation may be through other nuclear receptors such as estrogen and/or progesterone receptors.

Cell line studies have shown that estrogen treatment protects against the development of MSI tumors through upregulation of mismatch repair gene expression in colonic epithelial cells which coordinate the repair of nucleotide base mismatches (28, 29). Slattery et al. have reported an inverse association between recent HT use and risk for MSI-high colorectal cancer (29). However, our study and 3 other studies (16, 18, 30) did not observe such an association. The discrepant findings between the study by Slattery et al. and other studies are likely attributable to difference in age distribution among study cohorts. For instance, the

case subjects in Slattery et al. appeared to be younger than in our cohort, which was also seen among cases with MSI-high tumors. In Slattery et al., 56% of cases (or 67% of cases with MSI-high tumors) were 65 years or older. In our cohort, 66% of cases (or 80% of cases with MSI-high tumors) were ≥65 years. Alternatively, in light of the observations from our study and two other studies (16, 30) showing that HT use was inversely associated with MSS/MSI-low but not with MSI-high colorectal cancers, it is possible that the benefits of HT use may be relevant only when the mismatch repair genes are normally expressed. It has been postulated that the beneficial effects by estrogen against cancer development are lost when the mismatch repair genes are deregulated resulting from factors such as epigenetic changes (48). Future studies are warranted to study the complex relationship between HT use and status of individual mismatch repair genes.

The strength of our study includes that our tumor biomarkers have been assessed in a blinded manner to exposure history and tumor characteristics. There are also several limitations in the study. First, information on HT use was self-reported, which might be subject to misclassification. Although misclassification of HT use would not affect disease status as information on HT use was prospectively collected, the misclassification may be non-differential and attenuate the true association. In addition, HT users were more likely to receive screening exams than never users, which may affect risk estimates for colorectal cancer. However, it is hard to predict whether the differential screening has effects, if any, on the status of tumor markers. Our study is also not powered to study the association with HT use by dosage and by specific regimens (eg, continuous-combined versus sequential E+P use). Although we have adjusted for the potential confounders which showed little effects on our findings, we still could not rule out other potential residual confounding or measurement error in confounders. Finally, we were unable to obtain tumor tissue specimens from all cases of confirmed colorectal cancer and did not have HT use information in all cases. However, we observed no difference in characteristics between those with and without tumor samples nor difference in characteristics between those with and without HT information.

In summary, our study supports a possible role of HT use, in particular E-alone use, in colorectal cancer prevention through CDKN1A signaling. Given recent evidence from the WHI randomized trials suggesting that current HT use may increase risk for several other diseases including cardiovascular disease and breast cancer (4, 6), our findings on the potential benefit of HT use against colorectal cancer development do not warrant the application of HT for primary prevention. Nevertheless, our understanding of the effects of HT use on colorectal cancer development is far from complete. There is a need for continuous investigation of CDKN1A-related pathways for the development of new treatments and the use of potential alternatives to HT (eg, phytoestrogens) in relation to colorectal cancer prevention (49, 50).

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Table 1

Selected characteristics according to postmenopausal hormone use in the Nurses' Health Study, 1994.

	Postmenopausal Hormone Use		
	None	**Past**	**Current**
N participants	20856	14196	29440
Mean age, yr	61.3	63.7	59.9
Body mass index, kg/m^2	27.1	26.6	25.8
physical activity, METS/week *	20.0	19.4	20.9
Family history of colorectal in first degree relatives, %	2.8	3.7	4.2
History of colorectal polyps, %	2.4	3.8	3.2
Sigmoidoscopy or colonoscopy, %	12.0	18.4	18.5
Current smoking, %	15.9	12.4	10.7
Age at menopause, yr	50.4	48.6	48.8
Hysterectomy, %	15.4	44.8	53.4
Aspirin use (≥1 tab/week), %	45.3	48.3	46.9
Multivitamin use, %	33.8	37.7	41.9
Alcohol intake, g/day	4.8	5.0	5.2
Fiber intake, mg/day	18.9	19.6	19.6
Vitamin D intake, IU/day	382.3	405.3	418.6

* METS are metabolic equivalents which were calculated based on the frequency of a range of physical activities.

Table 2

Relative risk for colorectal cancer associated with use of hormone therapy (HT) overall and by tumor marker status in the Nurses Heath Study [*].

ALL	N Cases	Model 1	Model 2
Never use	221	1.00	1.00
Past use	199	0.84 (0.69–1.02)	0.90 (0.74–1.09)
Current use	161	0.62 (0.50–0.76)	0.70 (0.56–0.86)

Tumor marker[†]	N Cases	Model 1	Model 2	N Cases	Model 1	Model 2	$P_{heterogeneity}$[‡]
MSI		MSI-high			MSS/MSI-low		
Never use	34	1.00	1.00	172	1.00	1.00	
Past use	52	1.25 (0.80–1.95)	1.35 (0.86–2.10)	135	0.76 (0.60–0.96)	0.82 (0.65–1.03)	0.05
Current use	32	0.83 (0.51–1.36)	0.94 (0.57–1.54)	118	0.59 (0.46–0.74)	0.66 (0.52–0.85)	0.21
CDKN1A (p21)		Nonexpressed			Expressed		
Never use	131	1.00	1.00	22	1.00	1.00	
Past use	83	0.80 (0.60–1.05)	0.83 (0.63–1.10)	27	1.46 (0.83–2.58)	1.53 (0.87–2.70)	0.06
Current use	80	0.55 (0.41–0.73)	0.61 (0.46–0.82)	31	1.19 (0.68–2.07)	1.32 (0.76–2.31)	0.01
CDKN1B (p27)		Nonexpressed			Expressed		
Never use	119	1.00	1.00	32	1.00	1.00	
Past use	76	0.79 (0.59–1.06)	0.83 (0.62–1.11)	29	1.14 (0.69–1.89)	1.19 (0.72–1.98)	0.22
Current use	82	0.62 (0.46–0.82)	0.69 (0.51–0.92)	26	0.70 (0.41–1.18)	0.78 (0.46–1.32)	0.68
TP53 (p53)		Positive			Negative (wild-type)		
Never use	81	1.00	1.00	121	1.00	1.00	
Past use	61	0.84 (0.60–1.18)	0.90 (0.64–1.26)	89	0.76 (0.58–1.01)	0.82 (0.62–1.08)	0.67
Current use	52	0.53 (0.37–0.76)	0.61 (0.42–0.87)	97	0.67 (0.51–0.88)	0.76 (0.57–1.00)	0.32

[*] Model 1 is adjusted for age, and Model 2 is additionally adjusted for age, BMI, physical activity, family history of colorectal cancer, history of polyps, screening exam, smoking, alcohol intake, vitamin D intake, fiber intake, aspirin use, and multivitamin use.

[†] The number of cases does not add up to the total due to missing values in the tumor biomarkers.

[‡] Test for heterogeneity between the model that allows for separate associations between HT use (eg. never, current) and colorectal cancer according to tumor types (eg, MSI-high, MSS/MSI-low) and the model that assumes a common association regardless of tumor types.

Table 3

Relative risk for colorectal cancer associated with formulation of current use of hormone therapy (HT) overall and by tumor marker status in the Nurses Heath Study*.

ALL	N Cases	Model 1	Model 2
Never use	221	1.00	1.00
Formulation			
E-alone	85	0.67 (0.53–0.85)	0.72 (0.57–0.91)
E+P	60	0.81 (0.61–1.07)	0.88 (0.66–1.16)

Tumor marker†	N Cases	Model 1	Model 2	N Cases	Model 1	Model 2	$P_{heterogeneity}$‡
MSI		MSI-high			MSS/MSI-low		
Never use	34	1.00	1.00	172	1.00	1.00	
Formulation							
E-alone	17	0.80 (0.44–1.45)	0.90 (0.50–1.63)	62	0.56 (0.42–0.76)	0.64 (0.47–0.86)	0.30
E+P	10	0.80 (0.39–1.67)	0.91 (0.44–1.89)	45	0.74 (0.52–1.04)	0.83 (0.59–1.18)	0.83
CDKN1A (p21)		Nonexpressed			Expressed		
Never use	131	1.00	1.00	22	1.00	1.00	
Formulation							
E-alone	45	0.56 (0.40–0.79)	0.62 (0.44–0.88)	19	1.35 (0.72–2.51)	1.49 (0.80–2.78)	0.02
E+P	27	0.59 (0.38–0.91)	0.64 (0.41–0.99)	8	0.90 (0.39–2.05)	0.97 (0.43–2.23)	0.38
CDKN1B (p27)		Nonexpressed			Expressed		
Never use	119	1.00	1.00	32	1.00	1.00	
Formulation							
E-alone	46	0.63 (0.45–0.89)	0.69 (0.49–0.99)	17	0.85 (0.47–1.54)	0.94 (0.51–1.70)	0.40
E+P	26	0.62 (0.40–0.96)	0.67 (0.43–1.05)	7	0.58 (0.25–1.33)	0.63 (0.27–1.45)	0.89
TP53 (p53)		Positive			Negative (wild-type)		
Never use	81	1.00	1.00	121	1.00	1.00	
Formulation							
E-alone	28	0.54 (0.35–0.83)	0.61 (0.39–0.94)	51	0.64 (0.46–0.89)	0.72 (0.52–1.01)	0.54
E+P	22	0.73 (0.45–1.19)	0.82 (0.50–1.35)	33	0.71 (0.47–1.05)	0.79 (0.53–1.19)	0.91

*E-alone=estrogen-only; E+P=combined estrogen with progestin; Model 1 is adjusted for age, and Model 2 is additionally adjusted for age, BMI, physical activity, family history of colorectal cancer, history of polyps, screening exam, smoking, alcohol intake, vitamin D intake, fiber intake, aspirin use, and multivitamin use.

[†]The number of cases does not add up to the total due to missing values in the tumor biomarkers.

[≠]Test for heterogeneity between the model that allows for separate associations between HT use (eg. never, current E-alone) and colorectal cancer according to tumor types (eg, MSI-high, MSS) and the model that assumes a common association regardless of tumor types.

Table 4

Relative risk for colorectal cancer associated with duration of current use of hormone therapy (HT) overall and by tumor marker status in the Nurses Heath Study*.

ALL	N Cases	Model 1	Model 2	P_{trend}
Never use	221	1.00	1.00	
Duration				<.001
≤5 years	48	0.81 (0.59–1.10)	0.89 (0.65–1.21)	
> 5 years	113	0.62 (0.51–0.77)	0.68 (0.55–0.85)	

Tumor marker†	N Cases	Model 1	Model 2	P_{trend}	N Cases	Model 1	Model 2	P_{trend}	$P_{heterogeneity}$‡
MSI		MSI-high				MSS/MSI-low			
Never use	34	1.00	1.00		172	1.00	1.00		
Duration				0.62				<.001	0.13
≤5 years	5	0.55 (0.22–1.43)	0.62 (0.24–1.61)		40	0.83 (0.58–1.18)	0.94 (0.66–1.34)		
> 5 years	27	0.92 (0.55–1.55)	1.04 (0.62–1.76)		78	0.51 (0.39–0.67)	0.58 (0.44–0.76)		
CDKN1A (p21)		Nonexpressed				Expressed			
Never use	131	1.00	1.00		22	1.00	1.00		
Duration				<.001				0.24	0.002
≤5 years	31	0.75 (0.50–1.12)	0.84 (0.56–1.25)		9	1.21 (0.56–2.64)	1.34 (0.61–2.94)		
> 5 years	49	0.47 (0.34–0.66)	0.52 (0.37–0.73)		22	1.19 (0.65–2.17)	1.32 (0.72–2.42)		
CDKN1B (p27)		Nonexpressed				Expressed			
Never use	119	1.00	1.00		32	1.00	1.00		
Duration				0.007				0.52	0.38
≤5 years	33	0.87 (0.59–1.29)	0.97 (0.65–1.44)		6	0.58 (0.24–1.38)	0.64 (0.26–1.53)		
> 5 years	49	0.52 (0.37–0.72)	0.57 (0.40–0.81)		20	0.76 (0.43–1.33)	0.84 (0.47–1.48)		
TP53 (p53)		Positive				Negative (wild-type)			
Never use	81	1.00	1.00		121	1.00	1.00		
Duration				0.02				0.004	0.83
≤5 years	16	0.66 (0.38–1.13)	0.74 (0.43–1.28)		31	0.88 (0.59–1.31)	0.99 (0.66–1.48)		
> 5 years	36	0.50 (0.33–0.74)	0.56 (0.37–0.84)		66	0.60 (0.44–0.82)	0.68 (0.50–0.93)		

* Model 1 is adjusted for age, and Model 2 is additionally adjusted for age, BMI, physical activity, family history of colorectal cancer, history of polyps, screening exam, smoking, alcohol intake, vitamin D intake, fiber intake, aspirin use, and multivitamin use.

[†]The number of cases does not add up to the total due to missing values in the tumor biomarkers.

[‡]Test for heterogeneity between the model that allows for separate associations between HT use (never, years of current use) and colorectal cancer according to tumor types (eg, MSI-high, MSS) and the model that assumes a common association regardless of tumor types.