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 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 hormone therapy use and colorectal cancer risk differs by the molecular pathologic 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 hormone therapy use, cancer risk, and specific tumor biomarkers in 581 incident colon and rectal cancer cases that occurred during 26 years of follow-up among 105,520 postmenopausal women. We found a difference between hormone therapy use and colorectal cancer risk according to CDKN1A expression (P\(_{\text{heterogeneity}} = 0.01\)). Current hormone therapy 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 hormone therapy use and cancer risk according to MSI, CDKN1B, or TP53 status. Together, our molecular pathological epidemiology findings suggest a preventive effect of hormone therapy against colorectal carcinogenesis that depends, in part, on loss of cyclin-dependent kinase inhibitor CDKN1A.

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Introduction

Numerous observational studies have reported an inverse association between use of postmenopausal hormone therapy 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 hormone therapy (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 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 Women’s Health Initiative 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 hormone therapy 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 hormone therapy 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; refs. 21–23), CDKN1B (p27; refs. 22, 24), and TP53 (p53; refs. 25–27). It has also been suggested that estrogen treatment helps in maintaining genomic stability in colonic epithelial cells through upregulation of mismatch repair genes (28, 29). Observational data, however, on hormone therapy use and risk for colorectal cancer by microsatellite instability (MSI) status
are very limited (16, 18, 29, 30). No data have been reported on hormone therapy use and colorectal cancer risk by cell-cycle regulator status. We hypothesized that the lower risk for colorectal cancer with hormone therapy 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 hormone therapy 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, in addition, examined whether types of formulation and duration might contribute to differential risks associated with hormone therapy use.

Patients and Methods

Study population

The NHS was established in 1976 when 121,700 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 hormone therapy 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 semiquantitative 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 (i.e., 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 31,959 postmenopausal women included in the study, and 73,561 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 2 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

Hormone therapy information was updated every 2 years on biennial questionnaires where respondents were asked whether they had been using hormone therapy since the previous 2-year follow-up cycle. Women were considered current hormone therapy users if they reported current use of hormone therapy during the follow-up cycle. Past users were those who reported hormone therapy 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 hormone therapy use was estimated through the summation of hormone therapy use across questionnaire cycles. Information on hormone therapy 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 1,035 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: (i) tissue samples were either discarded or lost by the hospitals (N = 200); (ii) hospitals did not respond (N = 64); (iii) the participants were deceased and no further information was available (N = 58); (iv) hospitals refused to give us the samples or charged high processing fees (N = 50); and (v) 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 before diagnosis including means of body mass index (BMI; 26.3 and 26.9 kg/m²), proportion of current smoking (15% and 14%), proportion of aspirin use (42% and 46%), proportion of multivitamin use (42% and 45%), proportion of current hormone therapy use (25% and 28%), and proportion of former hormone therapy use (31% and 34%).

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 (TMA) were first constructed for TP53 analysis (35, 36) and CDKN1A and CDKN1B assays were conducted on whole tissue sections (37, 38). Immunohistochemistry for CDKN1A, CDKN1B, and TP53 were carried...
out as previously described (35, 37–39). Appropriate positive and negative controls were included in each run of immunochemistry. All immunohistochemically stained slides were interpreted by a pathologist (to S. Ogino) 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 2 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-positivity 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 value 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. Shima, CDKN1B by K. Shima, TP53 by K. Nosho), and the concordance between readers was 0.83 (k = 0.62, N = 179) for CDKN1A, 0.94 (k = 0.60, N = 114) for CDKN1B, and 0.87 (k = 0.75, N = 108) for TP53 (all P < 0.0001).

Statistical analysis

In the present analysis, a total of 105,520 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 intervals (CI) of colorectal cancer comparing women in various categories of hormone therapy use with those who never used hormone therapy. The multivariate models were adjusted for age (years, continuous) and, in addition, for potential risk factors for colorectal cancer, including BMI (≤25, 25–<30, ≥30 kg/m²), physical activity (METs/wk, in quartiles), family history of colorectal cancer in 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/wk), alcohol consumption (none, ≤15, >15 g/d), vitamin D intake (IU/d, in quartiles), and fiber intake (mg/d, in quartiles). We used the most updated information for all covariates before each 2-year interval. We, in addition, assessed the effects of current hormone therapy use in colorectal cancer risk according to hormone therapy formulation (E-alone and E + P therapies) and duration (≤5, >5 years).

To compare the specific effects of hormone therapy 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 hormone therapy use according to the types of outcome (e.g., cancer with MSI-high vs. cancer with MSS). We then assessed the difference between the risk estimates according to tumor types (e.g., MSI-high, MSS) with a likelihood ratio test comparing the model that allowed for separate associations of hormone therapy use according to tumor types with a model that assumed a common association regardless of tumor types (termed as heterogeneity test). All analyses were conducted with SAS version 9.0 (SAS Institute Inc.). A 2-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 with 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 hormone therapy 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 hormone therapy 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 hormone therapy 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 hormone therapy 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 hormone therapy use and risk for colorectal cancer according to CDKN1A expression status (P heterogeneity = 0.01). Current hormone therapy 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 hormone therapy use according to status of MSI, CDKN1B, or TP53 in colorectal cancers were not statistically significant (P heterogeneity ≥ 0.05; Table 2). The differences in the associations with former hormone therapy use according to tumor marker status were also not significant in either <5 or ≥5 years since last use (P 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 hormone therapy use and colorectal cancer risk, we found that the lower risk with current hormone therapy 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 ($P_{\text{heterogeneity}} = 0.02$; Table 3). There was, however, no difference in the association between current E + P use and colorectal cancer risk according to CDKN1A expression status ($P_{\text{heterogeneity}} = 0.38$). The risk patterns by hormone therapy formulation among past users were largely similar to those among current hormone therapy users (data not shown).

With respect to duration of current hormone therapy use, users taking >5 years were at a lower risk for colorectal cancer (multivariate RR, 0.69; 95% CI, 0.55–0.85, $P_{\text{trend}} < 0.001$; Table 4). Current users with >5 years also had a lower risk for developing CDKN1A-nonexpressed colorectal cancer ($P_{\text{trend}} = 0.002$) but not for CDKN1A-expressed cancer ($P_{\text{trend}} = 0.24$), and test for the difference in the associations with current hormone therapy duration according to CDKN1A status was statistically significant ($P_{\text{heterogeneity}} = 0.002$; Table 4). Although the association of past hormone therapy use with tumor marker status by duration was weaker as compared with that observed in current hormone therapy use, the patterns of risk estimates were largely similar in either hormone therapy groups (data not shown).

Discussion

Little is known about the etiologic mechanisms underlying the inverse association between hormone therapy 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 hormone therapy 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 hormone therapy 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 hormone therapy 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 hormone therapy use and colorectal cancer risk did not differ by MSI status and expression of CDKN1B (p27) and TP53 (p53).

Our finding that current hormone therapy 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 α and/or or estrogen receptor β exhibits growth inhibition effects on colon cancer cells by activating CDKN1A, a key protein that governs cell-cycle progression from $G_1$-S-phase and is responsible for the regulation of cell proliferation, growth arrest, and

Table 1. Selected characteristics according to postmenopausal hormone use in the NHS, 1994

<table>
<thead>
<tr>
<th>Postmenopausal hormone use</th>
<th>None</th>
<th>Past</th>
<th>Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (N)</td>
<td>20,856</td>
<td>14,196</td>
<td>29,440</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>61.3</td>
<td>63.7</td>
<td>59.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1</td>
<td>26.6</td>
<td>25.8</td>
</tr>
<tr>
<td>Physical activity, METS/wk</td>
<td>20.0</td>
<td>19.4</td>
<td>20.9</td>
</tr>
<tr>
<td>Family history of colorectal in first-degree relatives, %</td>
<td>2.8</td>
<td>3.7</td>
<td>4.2</td>
</tr>
<tr>
<td>History of colorectal polyps, %</td>
<td>2.4</td>
<td>3.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Sigmoidoscopy or colonoscopy, %</td>
<td>12.0</td>
<td>18.4</td>
<td>18.5</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>15.9</td>
<td>12.4</td>
<td>10.7</td>
</tr>
<tr>
<td>Age at menopause, y</td>
<td>50.4</td>
<td>48.6</td>
<td>48.8</td>
</tr>
<tr>
<td>Hysterectomy, %</td>
<td>15.4</td>
<td>44.8</td>
<td>53.4</td>
</tr>
<tr>
<td>Aspirin use (≥1 tab/wk), %</td>
<td>45.3</td>
<td>48.3</td>
<td>46.9</td>
</tr>
<tr>
<td>Multivitamin use, %</td>
<td>33.8</td>
<td>37.7</td>
<td>41.9</td>
</tr>
<tr>
<td>Alcohol intake, g/d</td>
<td>4.8</td>
<td>5.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Fiber intake, mg/d</td>
<td>18.9</td>
<td>19.6</td>
<td>19.6</td>
</tr>
<tr>
<td>Vitamin D intake, IU/d</td>
<td>382.3</td>
<td>405.3</td>
<td>418.6</td>
</tr>
</tbody>
</table>

aMETS are metabolic equivalents that were calculated on the basis of the frequency of a range of physical activities.
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 antiproliferative 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 a difference in the associations between hormone therapy use and expression status of CDKN1B and TP53. These observations suggest that the risk reduction for CDKN1A-nonexpressed tumors by hormone therapy 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 and colleagues have reported an inverse association between recent hormone therapy 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 and colleagues and other studies are likely attributable to differences in age distribution among study cohorts. For instance, the case subjects in the study by Slattery and colleagues appeared to be younger than in our cohort, which was also seen among cases with MSI-high tumors. In the study by Slattery and colleagues, 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.

Table 2. RR for colorectal cancer associated with use of hormone therapy overall and by tumor marker status in the NHS

<table>
<thead>
<tr>
<th>Tumor markera</th>
<th>N cases</th>
<th>Model 1</th>
<th>Model 2</th>
<th>N cases</th>
<th>Model 1</th>
<th>Model 2</th>
<th>(P_{\text{heterogeneity}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never use</td>
<td>221</td>
<td>1.00</td>
<td>1.00</td>
<td>212</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Past use</td>
<td>199</td>
<td>0.84 (0.69–1.02)</td>
<td>0.90 (0.74–1.09)</td>
<td>190</td>
<td>0.84 (0.69–1.02)</td>
<td>0.90 (0.74–1.09)</td>
<td></td>
</tr>
<tr>
<td>Current use</td>
<td>161</td>
<td>0.62 (0.50–0.76)</td>
<td>0.70 (0.56–0.86)</td>
<td>152</td>
<td>0.62 (0.50–0.76)</td>
<td>0.70 (0.56–0.86)</td>
<td></td>
</tr>
<tr>
<td>MSI</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never use</td>
<td>34</td>
<td>1.00</td>
<td>1.00</td>
<td>35</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Past use</td>
<td>52</td>
<td>1.25 (0.80–1.95)</td>
<td>1.35 (0.86–2.10)</td>
<td>53</td>
<td>1.25 (0.80–1.95)</td>
<td>1.35 (0.86–2.10)</td>
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<tr>
<td>Current use</td>
<td>32</td>
<td>0.83 (0.51–1.36)</td>
<td>0.94 (0.57–1.54)</td>
<td>33</td>
<td>0.83 (0.51–1.36)</td>
<td>0.94 (0.57–1.54)</td>
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<tr>
<td>CDKN1A (p21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never use</td>
<td>131</td>
<td>1.00</td>
<td>1.00</td>
<td>132</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Past use</td>
<td>83</td>
<td>0.80 (0.60–1.05)</td>
<td>0.83 (0.63–1.10)</td>
<td>84</td>
<td>0.80 (0.60–1.05)</td>
<td>0.83 (0.63–1.10)</td>
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<tr>
<td>Current use</td>
<td>80</td>
<td>0.55 (0.41–0.73)</td>
<td>0.61 (0.46–0.82)</td>
<td>81</td>
<td>0.55 (0.41–0.73)</td>
<td>0.61 (0.46–0.82)</td>
<td></td>
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<tr>
<td>CDKN1B (p27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never use</td>
<td>119</td>
<td>1.00</td>
<td>1.00</td>
<td>120</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Past use</td>
<td>76</td>
<td>0.79 (0.59–1.06)</td>
<td>0.83 (0.62–1.11)</td>
<td>77</td>
<td>0.79 (0.59–1.06)</td>
<td>0.83 (0.62–1.11)</td>
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<tr>
<td>Current use</td>
<td>82</td>
<td>0.62 (0.46–0.82)</td>
<td>0.69 (0.51–0.92)</td>
<td>83</td>
<td>0.62 (0.46–0.82)</td>
<td>0.69 (0.51–0.92)</td>
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<tr>
<td>TP53 (p53)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never use</td>
<td>81</td>
<td>1.00</td>
<td>1.00</td>
<td>82</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Past use</td>
<td>61</td>
<td>0.84 (0.60–1.18)</td>
<td>0.90 (0.64–1.26)</td>
<td>62</td>
<td>0.84 (0.60–1.18)</td>
<td>0.90 (0.64–1.26)</td>
<td></td>
</tr>
<tr>
<td>Current use</td>
<td>52</td>
<td>0.53 (0.37–0.76)</td>
<td>0.61 (0.42–0.87)</td>
<td>53</td>
<td>0.53 (0.37–0.76)</td>
<td>0.61 (0.42–0.87)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Model 1 is adjusted for age, and Model 2 is, in addition, 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 hormone therapy use (e.g., never, current) and colorectal cancer according to tumor types (e.g., MSI-high, MSS/MSI-low) and the model that assumes a common association regardless of tumor types.

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 antiproliferative 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 a difference in the associations between hormone therapy use and expression status of CDKN1B and TP53. These observations suggest that the risk reduction for CDKN1A-nonexpressed tumors by hormone therapy 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 and colleagues have reported an inverse association between recent hormone therapy 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 and colleagues and other studies are likely attributable to differences in age distribution among study cohorts. For instance, the case subjects in the study by Slattery and colleagues appeared to be younger than in our cohort, which was also seen among cases with MSI-high tumors. In the study by Slattery and colleagues, 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 3 other studies (16, 18, 30) did not observe such an association. The discrepant findings between the study by Slattery and colleagues and other studies are likely attributable to differences in age distribution among study cohorts. For instance, the case subjects in the study by Slattery and colleagues appeared to be younger than in our cohort, which was also seen among cases with MSI-high tumors. In the study by Slattery and colleagues, 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 2 other studies (16, 30) showing that hormone therapy use was inversely associated with MSS/MSI-low but not with MSI-high colorectal cancers, it is possible that the beneficial effects of hormone therapy use may be relevant only when the mismatch repair genes are normally expressed. It has been postulated that the beneficial effects of estrogen against cancer development are lost when the mismatch repair genes are normally expressed. It has been postulated that the beneficial effects of estrogen against cancer development are lost when the mismatch repair genes are normally expressed. It has been postulated that the beneficial effects of estrogen against cancer development are lost when the mismatch repair genes are normally expressed. It has been postulated that the beneficial effects of estrogen against cancer development are lost when the mismatch repair genes are normally expressed.
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 hormone therapy use was self-reported, which might be subject to misclassification. Although misclassification of hormone therapy use would not affect disease status as information on hormone therapy use was prospectively collected, the misclassification may be nondifferential and attenuate the true association. In addition, hormone therapy 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 hormone therapy use by dosage and by specific regimens (e.g., 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 hormone therapy 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 hormone therapy information.

In summary, our study supports a possible role of hormone therapy use, in particular E-alone use, in colorectal cancer prevention through CDKN1A signaling. Given recent evidence from the WHI-randomized trials suggesting that current...
Table 4. RR for colorectal cancer associated with duration of current use of hormone therapy overall and by tumor marker status in the NHS

<table>
<thead>
<tr>
<th>Tumor marker</th>
<th>N cases</th>
<th>Model 1</th>
<th>Model 2</th>
<th>P_trend</th>
<th>N cases</th>
<th>Model 1</th>
<th>Model 2</th>
<th>P_trend</th>
<th>P_heterogeneity</th>
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<tr>
<td>MSI</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never use</td>
<td>172</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td>34</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 y</td>
<td>40</td>
<td>0.83</td>
<td>(0.58–1.18)</td>
<td>0.94</td>
<td>(0.66–1.34)</td>
<td>0.001</td>
<td>0.13</td>
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<tr>
<td>&gt;5 y</td>
<td>78</td>
<td>0.51</td>
<td>(0.39–0.67)</td>
<td>0.58</td>
<td>(0.44–0.76)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDKN1A (p21)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Never use</td>
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<td>1.00</td>
<td>1.00</td>
<td></td>
<td>131</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 y</td>
<td>9</td>
<td>1.21</td>
<td>(0.56–2.64)</td>
<td>1.34</td>
<td>(0.61–2.94)</td>
<td>0.24</td>
<td>0.02</td>
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<tr>
<td>&gt;5 y</td>
<td>22</td>
<td>1.19</td>
<td>(0.65–2.17)</td>
<td>1.32</td>
<td>(0.72–2.42)</td>
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<tr>
<td>CDKN1B (p27)</td>
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<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Never use</td>
<td>32</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td>119</td>
<td>1.00</td>
<td>1.00</td>
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<td></td>
</tr>
<tr>
<td>Duration</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 y</td>
<td>6</td>
<td>0.58</td>
<td>(0.24–1.38)</td>
<td>0.64</td>
<td>(0.26–1.53)</td>
<td>0.52</td>
<td>0.38</td>
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<tr>
<td>&gt;5 y</td>
<td>20</td>
<td>0.76</td>
<td>(0.43–1.33)</td>
<td>0.84</td>
<td>(0.47–1.48)</td>
<td></td>
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<td></td>
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<td>TP53 (p53)</td>
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<tr>
<td>Never use</td>
<td>121</td>
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<td>1.00</td>
<td></td>
<td>81</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
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<td>0.004</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 y</td>
<td>31</td>
<td>0.88</td>
<td>(0.59–1.31)</td>
<td>0.99</td>
<td>(0.66–1.48)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5 y</td>
<td>66</td>
<td>0.60</td>
<td>(0.44–0.82)</td>
<td>0.68</td>
<td>(0.50–0.93)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

NOTE: Model 1 is adjusted for age, and Model 2 is, in addition, 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.

hormone therapy use may increase risk for several other diseases including cardiovascular disease and breast cancer (4, 6), our findings on the potential benefit of hormone therapy use against colorectal cancer development do not warrant the application of hormone therapy for primary prevention. Nevertheless, our understanding of the effects of hormone therapy 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 hormone therapy (e.g., phytoestrogens) in relation to colorectal cancer prevention (49, 50).

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: J.H. Lin, J.E. Manson, E. Giovannucci, C.S. Fuchs, S. Ogino
Development of methodology: J.H. Lin, C.S. Fuchs

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Hormone Therapy Use and Colorectal Cancer Biomarkers

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