Research Article

Postmenopausal Hormone Therapy and Colorectal Cancer Risk in Relation to Somatic KRAS Mutation Status among Older Women


Abstract

**Background:** Postmenopausal hormone (PMH) therapy represents a controversial colorectal cancer (CRC) preventive intervention. Because colorectal carcinogenesis is a heterogeneous process, we evaluated associations between PMH therapy and incident CRC in relation to KRAS mutation status in a population-based cohort of older women [Iowa Women’s Health Study (IWHS)].

**Methods:** The IWHS enrolled 41,836 randomly selected women, ages 55 to 69 years, in 1986. PMH therapy and other exposure data were recorded at baseline. Tissue samples from prospectively identified CRC cases (n = 507) were analyzed for somatic KRAS mutations (exon 2, codons 12 and 13). Multivariable Cox regression models were fit to estimate relative risks (RR) and 95% confidence intervals (CI).

**Results:** PMH therapy (ever vs. never) was inversely associated with KRAS mutation–negative (RR = 0.83; 95% CI, 0.66–1.06; P = 0.14) and KRAS mutation–positive (RR = 0.82; 95% CI, 0.58–1.16; P = 0.27) tumors, although the observed risk estimates were not statistically significant. When anatomic subsite was additionally considered, the strongest association was found for KRAS mutation–negative, distal colorectal tumors (RR = 0.64; 95% CI, 0.43–0.96; P = 0.03).

**Conclusions:** To our knowledge, we provide the first report of KRAS-defined CRC risks associated with PMH therapy. These data suggest that PMH therapy may reduce CRC risk through mechanisms beyond KRAS mutation status but might provide greater benefits for KRAS mutation–negative than mutation–positive tumors (at least in the distal colorectum).

**Impact:** Findings from this prospective cohort study provide novel insights about the molecular biology of PMH therapy–related CRC risk reduction. Cancer Epidemiol Biomarkers Prev; 21(4); 681–4. ©2012 AACR.

Introduction

On the basis of recent global estimates, colorectal cancer (CRC) ranks third and fourth in terms of incident and fatal malignancies, respectively, in both sexes combined (1). Yet, rather than representing a uniform condition, CRC is becoming increasingly recognized as a heterogeneous disease (2), with distinct tumor subtypes resulting from different combinations of genetic and epigenetic events (3, 4). Despite growing awareness of the need to consider molecular pathogenesis in CRC early detection and adjuvant therapy settings (5, 6), relatively few studies have evaluated CRC risk/protective factors in relation to molecularly distinct phenotypes (7).

Postmenopausal hormone (PMH) therapy, which represents a potential CRC preventive intervention (8), may influence carcinogenesis through cellular pathways involving the KRAS oncogene (9). However, to date, no prior studies have reported associations between PMH therapy and CRC subtypes stratified by somatic KRAS mutation status. Using data and tissue resources from the prospective, population-based Iowa Women’s Health Study (IWHS), we examined associations between PMH therapy and KRAS-defined CRC risks in a well-characterized cohort of older women. The current study complements another recent report from our investigative team that describes PMH therapy–related risks for CRC subtypes defined by microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and somatic BRAF mutation status (10).

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Materials and Methods

Approvals for this study were obtained from the University of Iowa (Iowa City, IA), University of Minnesota (Minneapolis, MN), and Mayo Clinic (Rochester, MN) Institutional Review Boards for human research. Recruitment methods for the parent I淮S cohort have been reported elsewhere (11). In brief, 41,836 women (age, 55–69 years) completed the baseline evaluation, which included a comprehensive cancer risk factor questionnaire, in 1986. For the present study, women who were premenopausal, had a history of malignancy other than skin cancer, follow-up time of <1 day, or provided incomplete PMH therapy data were excluded, leaving 37,285 women in the analytic cohort. PMH therapy at baseline was assessed and analyzed with respect to exposure status (ever, never), with ever-users further categorized by recency (current, former) and duration (>5, ≤5 years) of exposure. Data about PMH formulation and dose were not obtained. Vital status was updated through follow-up questionnaires (1987, 1989, 1992, 1997, and 2004) and by checking nonrespondents against the U.S. National Death Index. Incident CRC cases were identified through the Iowa Cancer Registry, which participates in the Surveillance, Epidemiology, and End Results (SEER) program (12), using International Classification for Diseases in Oncology (ICD-O) codes of 18.0, 18.2–18.9, 19.9, and 20.9. Tumors were further classified as proximal (cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure) or distal (descending colon, sigmoid colon, rectosigmoid junction, and rectum) in origin, unless anatomic subsite was ambiguous or not specified.

 Archived, paraffin-embedded tissue specimens were collected and processed for molecular epidemiologic research from 732 incident CRC cases diagnosed between 1986 and 2002, as previously described (13). General subject demographics and tumor characteristics have been previously compared between incident CRC cases with versus without retrieved tissue specimens, without evidence of selection bias (10). Tumor and normal tissues were scraped from unstained slides and placed into separate tubes for DNA extraction using the QIAamp Tissue Kit (Qiagen), according to the manufacturer’s instructions. KRAS mutation testing (exon 2, codons 12 and 13) was conducted using established laboratory methods, with results reported as mutation-negative or mutation-positive. Two hundred and twenty-five incident CRC cases were subsequently excluded because of inadequate/unusable tissue, multiple primary cancers at the time of initial CRC diagnosis, or noninformative KRAS mutation testing. Thus, complete PMH therapy and molecular marker data were available for 507 incident CRC cases.

Follow-up time was determined from the date of completing the baseline evaluation until incident CRC diagnosis or censorship (defined by a move from Iowa or death); otherwise, subjects were assumed to be alive, CRC-free, and living in Iowa through December 31, 2002. Cox proportional hazards regression models were fit to estimate relative risks (RR) and 95% confidence intervals (CI) for associations between PMH therapy and KRAS-defined CRC subtypes. Multivariable models were adjusted for baseline age, body mass index (quartiles), waist to hip ratio (quartiles), smoking status (current, former, never), age at menopause (<44, 44–49, 50–54, >55 years), age at menarche (8–12, 13–14, ≥15 years), oral contraceptive use (ever, never), physical activity level (low, moderate, high), alcohol consumption (0, 0–3.4, or >3.4 g/d based on cohort median split), history of diabetes mellitus (ever, never), and daily intake (quartiles) of total energy (kcal/d), total fat (g/d), sucrose (g/d), red meat (g/d), calcium (mg/d), folate (µg/d), methionine (g/d), and vitamin E (mg/d). Family history of CRC, nonsteroidal anti-inflammatory drug use, and exposure to CRC screening were not systematically recorded at baseline and were not included in the current analyses. All eligible I淮S subjects were included in the Cox regression analyses, regardless of eventual cancer status. CRC incidence was modeled as a function of age (14). PMH therapy never-users were defined as the reference group for all risk associations. Tests for trend were conducted by ordering the categorized values from lowest to highest and including the resulting variable as a one degree-of-freedom linear term in a Cox proportional hazards model. Separate analyses were conducted for KRAS mutation-negative and KRAS mutation-positive tumors. For each, the outcome variable was defined as incident CRC with KRAS mutation status of interest; all other incident CRCs (including those with the missing or unknown KRAS mutation status) were considered censored observations at the date of diagnosis. Risk ratios for the PMH therapy–related exposures were compared between KRAS mutation statuses using a competing risk form of Cox proportional hazards analysis (15). This approach allowed us to model and test the interaction between PMH therapy exposure (modeled as a covariate) and CRC subtype (modeled as a stratum variable). All statistical tests were 2-sided, and all analyses were conducted using the SAS (SAS Institute, Inc.) and R (R Foundation for Statistical Computing) software systems.

Results

Baseline PMH therapy was categorized as never use for 22,986 (62%) women and ever use for 14,299 (38%) women (including 4,062 (11%) current and 10,237 (27%) former users), respectively. Among PMH therapy users, duration of use at baseline was reported as ≤5 years by 10,017 and >5 years by 4,017 women, respectively. Comparisons of other subject characteristics by PMH therapy exposure status have been previously reported (10). Results of KRAS mutation testing revealed 343 mutation-negative (201 proximal colon, 136 distal colorectum, and 6 site not specified) and 164 mutation-positive (85 proximal colon and 79 distal colorectum) tumors. Multivariable-adjusted risk estimates for associations between PMH therapy
(exposure, recency, and duration) and incident CRC subtypes (KRAS mutation–negative or KRAS mutation–positive) are provided in Table 1. As shown, each of the PMH therapy–related variables was inversely associated with both KRAS-defined CRC subtypes. However, none of the risk estimates were statistically significant, and the observed associations with PMH therapy were not statistically different for KRAS mutation–negative versus KRAS mutation–positive CRCs (P > 0.05 for each comparison). When stratified by anatomic subsite, PMH ever use versus never use was not significantly associated with KRAS-defined tumor subtypes located in the proximal colon (RR = 0.93; 95% CI, 0.69–1.26; P = 0.65 for mutation-negative and RR = 0.71; 95% CI, 0.44–1.15; P = 0.17 for mutation-positive tumors); in the distal colorectum, an inverse association was found for mutation-negative (RR = 0.64; 95% CI, 0.43–0.96; P = 0.03), but not mutation-positive (RR = 0.94; 95% CI, 0.57–1.56; P = 0.82) tumors.

Discussion

Data from this large, population-based cohort study of older women provide modest additional support for PMH therapy as a potential CRC preventive intervention. Our findings suggest that PMH therapy–related CRC risk reduction does not depend on somatic KRAS mutation status, but the putatively protective effects may be greater for mutation-negative than mutation-positive tumors (at least in the distal colorectum). To our knowledge, we report the first molecular epidemiologic study of PMH therapy and KRAS-defined CRC risks. These data add to the paucity of existing literature in this area and should serve to inform further investigation of the molecular mechanisms involved in hormone-based CRC chemoprevention.

According to a recently proposed model of colorectal carcinogenesis (4), large bowel tumors appear to arise from 3 predominant pathways, which can be characterized, in large part, by different combinations of MSI, CIMP, and somatic BRAF or KRAS mutation status. Previously, our group reported that PMH therapy was more strongly associated with the microsatellite-stable (MSS)/MSI-L, CIMP-negative, and BRAF mutation–negative CRC subtypes in the IWHS cohort (10), prompting speculation that exogenous estrogens and/or progestins may differentially inhibit the traditional pathway to a greater degree than the serrated or alternate pathways of colorectal carcinogenesis. Because traditional pathway CRCs are also characterized by KRAS mutation–negative status, data from the current study remain consistent with our proposed mechanistic hypothesis.

Overall strengths of our study include the prospective, population-based study design; extensive baseline exposure data with prolonged follow-up time; and large number of samples from incident CRC cases for molecular analyses, without evidence of selection bias based on tissue retrieval status (10). Conversely, the absence of data about PMH formulation and dose limited our

### Table 1. PMH therapy and incident CRC, by KRAS mutation status

| PMH therapy | Person-years | Events, N | RR (95% CI)
<table>
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<tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>KRAS mutation-negative</td>
<td></td>
</tr>
<tr>
<td>Never-user</td>
<td>341,377</td>
<td>224</td>
<td>1.00 (ref.)</td>
</tr>
<tr>
<td>Ever-user</td>
<td>212,696</td>
<td>119</td>
<td>0.83 (0.66–1.06)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Former user</td>
<td>151,535</td>
<td>89</td>
<td>0.81 (0.63–1.06)</td>
</tr>
<tr>
<td>Current user</td>
<td>61,161</td>
<td>30</td>
<td>0.90 (0.61–1.34)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
</tr>
<tr>
<td>Duration ≤5 y</td>
<td>148,704</td>
<td>90</td>
<td>0.88 (0.67–1.14)</td>
</tr>
<tr>
<td>Duration &gt;5 y</td>
<td>60,064</td>
<td>29</td>
<td>0.78 (0.52–1.16)</td>
</tr>
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|              |              | KRAS mutation-positive |                 |
|              |              | Events, N | RR (95% CI)
|              |              |            | 1.00 (ref.)     |
|              |              |            | 0.82 (0.58–1.16) |
|              |              |            | 0.27             |
|              |              |            | 0.80 (0.54–1.17) |
|              |              |            | 0.90 (0.51–1.58) |
|              |              |            | 0.38             |
|              |              |            | 0.84 (0.57–1.24) |
|              |              |            | 0.70 (0.38–1.28) |

*Adjusted for age, body mass index, waist to hip ratio, smoking status, age at menopause, age at menarche, oral contraceptive use, physical activity level, alcohol consumption, self-reported diabetes mellitus, and daily intake of total energy, total fat, sucrose, red meat, calcium, folate, methionine, and vitamin E, as described in the Materials and Methods. Event rates for KRAS mutation–positive cases differ slightly across exposure categories due to missing data.

*P* values comparing KRAS-specific CRC risk in PMH ever-users to PMH never-users.

*Test for trend* *P* value assessing dose–response association of the ordered variable PMH never versus former versus current use with risk of KRAS-specific CRC risk.

*Test for trend* *P* value assessing dose–response association of the ordered variable PMH never versus ≤5 years total use versus >5 years total use with risk of KRAS-specific CRC risk.
ability to more rigorously evaluate the exposure-related, subtype-specific CRC risks. Analyses of additional CRC molecular markers are ongoing in the IWHS cohort, which should allow us to further define the spectrum of molecular events and intracellular pathways that may be affected by PMH therapy among older women.

Disclosure of Potential Conflicts of Interest
P.J. Limburg served as a consultant for Genomic Health, Inc. from August 12, 2008 to April 19, 2010. Mayo Clinic has licensed the intellectual property of P.J. Limburg to Exact Sciences and he and Mayo Clinic have contractual rights to receive royalties through this agreement. No potential conflicts of interests were disclosed by the other authors.

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