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Colorectal Cancer Risk Associated with Hormone Use Varies by Expression of Estrogen Receptor-β

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Abstract

The risk of colorectal cancer is reduced among users of oral contraceptives or menopausal hormone therapy, but associations with reproductive characteristics that are markers of a woman's endogenous hormone milieu have not been consistently observed. To help understand possible mechanisms through which exogenous and endogenous hormonal exposures are involved in colorectal cancer, we assessed the risk of these malignancies according to tumor expression of estrogen receptor-β (ESR2). In a population-based study of postmenopausal women (503 cases and 721 controls matched for sex and age), immunohistochemical expression of ESR2 was determined in 445 cases of incident colorectal cancer. Unconditional logistic regression was used in case–case analyses to assess heterogeneity between risk associations according to ESR2 status and in case–control analyses to estimate associations separately for ESR2-negative and ESR2-positive tumors. For ESR2-positive tumors but not ESR2-negative tumors, colorectal cancer risk significantly decreased with duration of oral contraceptive use [per five-year increments OR ESR2-positive, 0.87, 95% confidence interval (CI), 0.77–0.99; OR ESR2-negative, 1.02, 95% CI, 0.91–1.15; $P_{heterogeneity} = 0.07$] and with duration of menopausal hormone therapy use (per five-year increments OR ESR2-positive, 0.84, 95% CI, 0.74–0.95; OR ESR2-negative, 0.94, 95% CI 0.84–1.05; $P_{heterogeneity} = 0.06$). Significant heterogeneity according to ESR2 expression was found for the association with current use of menopausal hormone therapy (<0.5 years ago; $P_{heterogeneity} = 0.023$) but not for associations with reproductive factors. In conclusion, our results suggest that hormone use decreases risk for ESR2-positive but not ESR2-negative colorectal cancer. *Cancer Res; 73(11); 3306–15. ©2013 AACR.*

Introduction

Evidence has accumulated that sex hormones play a potential role in the development of colorectal cancer (1, 2). In a recent meta-analysis of 4 randomized controlled trials, 8 cohorts and 8 case–control studies, ever use of a combined estrogen–progestagen therapy was associated with a 26% decreased risk for colorectal cancer [OR, 0.74; 95% confidence interval (CI), 0.68–0.81] and a similar result was observed with ever use of estrogen monotherapy (OR, 0.79; 95% CI, 0.69–0.91; ref. 3). The results of a meta-analysis investigating the association between ever use of oral contraceptives and colorectal cancer risk indicated also an inverse relationship, with a risk reduction of 19% (OR, 0.81; 95% CI, 0.72–0.92; ref. 4).

The associations observed with reproductive factors such as age at menarche, parity, and age at menopause have been conflicting and the majority of studies reported null associations (5, 6). The inconsistent results could be caused by differing associations of reproductive factors with colorectal cancer risk depending on the molecular characteristics of the tumor. A potential mediator of estrogen effects is the estrogen receptor-β (ESR2), which is the primarily expressed estrogen receptor in the large intestine (1). Loss of ESR2 expression in tumor tissue of patients with colorectal cancer has been associated with poorer differentiation of tumors and more advanced cancer stages (7–10). However, it has also been postulated that endogenous and exogenous sex hormones may have differential effects on the development of colorectal cancer (6), as high levels of endogenous estrogens have been found to be associated with an increased risk for colorectal cancer (11, 12).

Colorectal cancer risk associated with exogenous hormone use as well as with reproductive factors may differ depending on the presence of ESR2 expression in the tumor. We hypothesize that the reduced risk associated with use of exogenous estrogens varies by ESR2 expression and is greater for tumors expressing ESR2. This has not been investigated so far and is addressed in the present study.

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Materials and Methods

Study population

Colorectal cancer cases and controls were drawn from the DACHS (Darmkrebs: Chancen der Verhütung durch Screening) study, a population-based case–control study conducted in the southwest of Germany. Details of the study design have been reported elsewhere (13, 14). Briefly, cases were patients with ages of at least 30 years, with a first histologically confirmed diagnosis of primary invasive colorectal cancer (ICD-10 codes C18-C20) between January 2003 and December 2007, and recruited during first hospitalization due to cancer treatment or shortly afterward at their homes. The controls were selected randomly from lists of population registries and frequency matched according to age, sex, and county of residence. Additional inclusion criteria for both cases and controls were proficiency in the German language, mental and physical ability to participate in a personal interview of about 1 hour, no previous history of colorectal cancer, and residency in the study region.

Written informed consent was obtained from every participant. The study was approved by the ethical committees of the University of Heidelberg (Heidelberg, Germany) and the Medical Chambers of Baden-Württemberg and Rhineland-Palatinate.

Data collection

Trained interviewers used a standardized questionnaire to collect information on demographic data, education, the medical history, medications, anthropometric data, lifestyle factors as well as reproductive factors in face-to-face interviews with the participants. In addition, discharge letters and pathology reports were obtained. About exogenous hormones, information collected on menopausal hormone therapy use included start and end of therapy, hormone preparation, dose, regimen, and mode and route of application. The standardized questionnaire asked for up to 8 sequential phases of menopausal hormone therapy use, and a change in medication was recorded as starting a new phase. The interviewers presented a medication list as memory aid to identify the preparations. Information collected on use of oral contraceptives included age at initial use and total duration. Self-reported menopausal hormone therapy use was validated by medical records requested from the women's physicians for every woman recruited between 2003 and 2006 (15). On the basis of statistics of patients with colorectal cancer treated in the hospitals, the participating patients accounted for 50% of the expected number of eligible cases in the study region. The response rate among eligible control individuals was likewise slightly more than 50%.

In 2007, formalin-fixed paraffin-embedded specimens of 1,564 patients were requested from the pathologies of the cooperating clinics and transferred to the tissue bank of the National Center for Tumor Diseases (Heidelberg, Germany). Of 1,329 acquired tumor blocks, 1,262 contained sufficient tumor tissue to construct tissue microarray blocks, which was accomplished in June 2009. The present investigation included 503 postmenopausal female patients with available tissue microarray sample and 721 postmenopausal female controls.

Immunohistochemistry

The tissue microarray blocks contained 4 punched 0.6-mm cores (2 cores each from tumor and adjacent non-neoplastic tissue) from each surgical specimen. The anti-ESR2 antibody (primary mouse monoclonal, 14C8; Abcam) was applied to 5-μm thick sections mounted on superfrost slides at a dilution of 1:50 at room temperature for 30 minutes. After the incubation with the appropriate biotinylated secondary antibody (Dako antimouse, 1:200 dilution; Dako) at room temperature for 15 minutes and incubation with the streptavidin/avidin–biotin complex kit (Dako), antigen retrieval was conducted following endogenous peroxidase blocking. The antibody reactions were revealed using the Dako EnVision+System-HRP. ESR2 expression was visualized with 3,3′-diaminobenzidine (Vector). Cores of adjacent non-neoplastic tissue as well as the lymphocytes in the lamina propria were used as positive control. Sections after the omission of the primary antibody or incubation with the appropriate blocking peptide were used as negative controls. The staining was conducted on an autostainer (Dako). The sections were counterstained with hematoxylin, dehydrated, and coverslipped.

To evaluate the ESR2 expression of the tumor tissue and nontumorous mucosa, a 3-level scoring system was applied [based on Konstantinopoulos and colleagues (7)] that involved staining frequency and intensity. Samples with less than 10% of the cell nuclei showing strong positive staining or with less than 50% of the nuclei showing weak positive staining were regarded as ESR2-negative. ESR2-positivity was defined as weak staining of more than 50% of the cell nuclei or strong positive staining in at least 10% of the cell nuclei. The scoring was conducted independently by 2 pathologists. The preparation of the immunohistochemistry as well as the scoring was conducted blinded to other case characteristics. Results of the scoring were identical for 96.8% of the samples and discordant results were resolved by an additional joint review of the respective sample.

Variable definitions

To define variables, the reported history at the reference date was used (date of interview for controls and date of diagnosis for cases). Women were defined as "ever users" of hormone therapy and oral contraceptives when the respective reported total duration of use was at least 3 months. If the last use of hormone therapy was less than 6 months ago before the reference date, it was defined as "current use."

Statistical analysis

The statistical analyses were conducted using SAS 9.2 (SAS Institute). Two-sided tests were conducted and a P value of less than 0.05 was regarded as significant. Pearson χ^2 test and the Wilcoxon rank-sum test were applied to test differences between ESR2-negative and ESR2-positive cases and between cases and controls.

To assess heterogeneity in colorectal cancer risk by ESR2 status, logistic regression models with tumoral ESR2 status as

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Table 1. Distribution of selected risk determinants for colorectal cancer according to ESR2 status

	Cases			
Characteristic	**ESR2-negative *N* (%)**	**ESR2-positive *N* (%)**	**ESR2 unknown *N* (%)**	**Controls *N* (%)**
Total	219 (100.0)	226 (100.0)	58 (100.0)	721 (100.0)
Age, y				
Mean (SD)	72.3 (9.3)	70.4 (9.7)	71.4 (9.7)	70.5 (8.7)
Body mass index (kg/m^2) ≥5 y before diagnosis/date of interview				
<23	40 (18.3)	51 (22.6)	14 (24.1)	189 (26.2)
23 to <25	41 (18.7)	47 (20.8)	16 (27.6)	157 (21.8)
25 to <27	32 (14.6)	34 (15.0)	7 (12.1)	138 (19.1)
27 to <30	53 (24.2)	38 (16.8)	10 (17.2)	126 (17.5)
>30	42 (19.2)	51 (22.6)	10 (17.2)	106 (14.7)
Unknown	11 (5.0)	5 (2.2)	1 (1.7)	5 (0.7)
Average lifetime of ethanol intake per day, g/d				
None	83 (37.9)	76 (33.6)	20 (34.5)	197 (27.3)
0< to <3.1	39 (17.8)	52 (23.0)	14 (24.1)	127 (17.6)
≥3.1 to <6.0	31 (14.2)	39 (17.3)	7 (12.1)	135 (18.7)
≥6.0 to <10.9	27 (12.3)	25 (11.1)	8 (13.8)	131 (18.2)
≥10.9	38 (17.4)	32 (14.2)	9 (15.5)	131 (18.2)
Unknown	1 (0.5)	2 (0.9)	0 (0.0)	0 (0.0)
Average physical activity in the last 12 months (metabolic equivalent of task h/wk)				
<84.6	79 (36.1)	64 (28.3)	14 (24.1)	183 (25.4)
≥84.6 to <122.5	43 (19.6)	58 (25.7)	14 (24.1)	183 (25.4)
≥122.5 to <183.0	38 (17.4)	49 (21.7)	15 (25.9)	179 (24.8)
≥183.0	39 (17.8)	46 (20.4)	14 (24.1)	171 (23.7)
Unknown	20 (9.1)	9 (4.0)	1 (1.7)	5 (0.7)
Average lifetime pack-years of regular smoking				
Nonsmoker	157 (71.7)	167 (73.9)	42 (72.4)	530 (73.5)
>0 to <10	21 (9.6)	28 (12.4)	5 (8.6)	83 (11.5)
10 to <20	14 (6.4)	14 (6.2)	3 (5.2)	42 (5.8)
20 to <30	13 (5.9)	6 (2.7)	5 (8.6)	34 (4.7)
≥30	12 (5.5)	10 (4.4)	3 (5.2)	28 (3.9)
Unknown	2 (0.9)	1 (0.4)	0 (0.0)	4 (0.6)
Ever been diagnosed with diabetes (through a physician)				
No	163 (74.4)	184 (81.4)	51 (87.9)	633 (87.8)
Yes	52 (23.7)	40 (17.7)	7 (12.1)	87 (12.1)
Unknown	4 (1.8)	2 (0.9)	0 (0.0)	1 (0.1)
Ever had colorectal endoscopy				
No	170 (77.6)	182 (80.5)	48 (82.8)	335 (46.5)
Yes	49 (22.4)	43 (19.0)	10 (17.2)	386 (53.5)
Unknown	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Ever regular use of nonsteroidal anti-inflammatory drugs 2+ times/wk ≥1 y				
No	166 (75.8)	173 (76.5)	44 (75.9)	509 (70.6)
Yes	53 (24.2)	53 (23.5)	13 (22.4)	208 (28.8)
Unknown	0 (0.0)	0 (0.0)	1 (1.7)	4 (0.6)
Number of full-term pregnancies				
None	33 (15.1)	24 (10.6)	5 (8.6)	89 (12.3)
1	61 (27.9)	65 (28.8)	13 (22.4)	169 (23.4)
2	65 (29.7)	79 (35.0)	27 (46.6)	266 (36.9)
3	39 (17.8)	42 (18.6)	7 (12.1)	128 (17.8)
≥4	21 (9.6)	15 (6.6)	6 (10.3)	69 (9.6)
Unknown	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)

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Table 1. Distribution of selected risk determinants for colorectal cancer according to ESR2 status (Cont'd)

	Cases			
Characteristic	**ESR2-negative *N* (%)**	**ESR2-positive *N* (%)**	**ESR2 unknown *N* (%)**	**Controls *N* (%)**
Age at menarche, y				
<13	37 (16.9)	47 (20.8)	13 (22.4)	129 (17.9)
13	48 (21.9)	44 (19.5)	9 (15.5)	152 (21.1)
14	62 (28.3)	48 (21.2)	14 (24.1)	201 (27.9)
≥15	65 (29.7)	78 (34.5)	20 (34.5)	233 (32.3)
Unknown	7 (3.2)	9 (4.0)	2 (3.4)	6 (0.8)
Age at menopause, y				
<45	49 (22.4)	55 (24.3)	19 (32.8)	165 (22.9)
45 to >50	41 (18.7)	57 (25.2)	12 (20.7)	159 (22.1)
50 to >55	74 (33.8)	78 (34.5)	22 (37.9)	257 (35.6)
≥55	33 (15.1)	25 (11.1)	5 (8.6)	115 (16.0)
Unknown	22 (10.0)	11 (4.9)	0 (0.0)	25 (3.5)
Ever use of oral contraceptives				
No	147 (67.1)	146 (64.6)	41 (70.7)	408 (56.6)
Yes	71 (32.4)	79 (35.0)	17 (29.3)	312 (43.3)
Unknown	1 (0.5)	1 (0.4)	0 (0.0)	1 (0.1)
Duration of oral contraceptive use among users, y				
Median (range)	10.0 (0.3–39.0)	7.5 (0.3–28.0)	6.5 (0.5–19.0)	9.0 (0.3–39.0)
Ever use of menopausal hormone therapy				
No	149 (68.0)	159 (70.4)	39 (67.2)	361 (50.1)
Yes	66 (30.1)	61 (27.0)	19 (32.8)	350 (48.5)
Unknown	4 (1.8)	6 (2.7)	0 (0.0)	10 (1.4)
Duration of menopausal hormone therapy use among users, y				
Median (range)	9.4 (0.3–45.5)	8.0 (0.3–31.0)	10.0 (0.3–19.0)	10.0 (0.3–41.4)
Ever use of estrogen monotherapy				
No	207 (94.5)	204 (90.3)	54 (93.1)	624 (86.5)
Yes	8 (3.7)	16 (7.1)	4 (6.9)	87 (12.1)
Unknown	4 (1.8)	6 (2.7)	0 (0.0)	10 (1.4)
Ever use of combined estrogen–progestagen therapy				
No	188 (85.8)	196 (86.7)	51 (87.9)	547 (75.9)
Yes	27 (12.3)	24 (10.6)	7 (12.1)	164 (22.7)
Unknown	4 (1.8)	6 (2.7)	0 (0.0)	10 (1.4)
Recency of ever use of menopausal hormone therapy				
Current	23 (10.5)	14 (6.2)	4 (6.9)	120 (16.6)
Former	40 (18.3)	43 (19.0)	14 (24.1)	222 (30.8)
Never	149 (68.0)	159 (70.4)	39 (67.2)	361 (50.1)
Unknown	7 (3.2)	10 (4.4)	1 (1.7)	18 (2.5)

the outcome were used in case–case analyses. It should be pointed out that for a dichotomous tumor characteristic like the ESR2 status, the OR from the case–case analysis corresponds to the ratio of the 2 subtype specific case–control ORs for association with the risk factor of interest. We conducted case–control analyses using multinomial logistic regression models to evaluate colorectal cancer risk according to disease subtype (ESR2-negative disease, ESR2-positive disease) and calculated ORs and the respective 95% CIs.

In case–control analyses, all models were adjusted for age and county of residence. Additional covariates were considered as potential confounders if they modified the OR associated with ever use of hormone therapy by at least 5%. The models included ever colorectal endoscopy, former body mass index (5–14 years before reference date), ever diagnosis of diabetes, ever general health checks, physical activity, and ever use of oral contraceptives as well as hormone therapy. Although we investigated several exposures, we did not try to incorporate a different set of confounders for each exposure. We regarded most of the covariates that modified the OR associated with ever use of hormone therapy to be also potential confounders for all of the investigated exposure associations, perhaps with the exception of physical activity.

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Table 2. Associations between colorectal cancer risk and use of oral contraceptives, use of menopausal hormone therapy, and reproductive factors according to ESR2 status

		ESR2-negative		ESR2-positive		ESR2-negative vs. ESR2-positive	
Characteristic	**Controls *N* (%)[a]**	**Cases *N* (%)[a]**	**OR (95% CI)**	**Cases *N* (%)[a]**	**OR (95% CI)**	**OR (95% CI)[b]**	***P*[b]**
Use of menopausal hormone therapy[d]							
Never	356 (50.2)	123 (65.8)	1.00 (Ref.)	148 (69.5)	1.00 (Ref.)	1.00 (Ref.)	
Ever	343 (48.4)	62 (33.2)	0.72 (0.49–1.06)	61 (28.6)	0.55 (0.38–0.80)	1.43 (0.89–2.29)	0.14
Duration of use of menopausal hormone therapy, y[d]							
Never	356 (50.2)	123 (65.8)	1.00 (Ref.)	148 (69.5)	1.00 (Ref.)	1.00 (Ref.)	
<5	165 (23.3)	33 (17.7)	0.74 (0.46–1.19)	35 (16.4)	0.62 (0.39–0.98)	1.23 (0.69–2.19)	0.47
≥10	178 (25.1)	29 (15 5)	0.71 (0.43–1.16)	26 (12.2)	0.47 (0.29–0.78)	1.72 (0.91–3.23)	0.09
Per 5 y	699 (98.5)	185 (98.9)	0.94 (0.84–1.05)	209 (98.1)	0.84 (0.74–0.95)	1.15 (0.99–1.33)	0.06
Use of estrogen monotherapy[d]							
Never	612 (86.3)	178 (95.2)	1.00 (Ref.)	193 (90.6)	1.00 (Ref.)	1.00 (Ref.)	
Ever	87 (12.3)	7 (3.7)	0.32 (0.14–0.74)	16 (7.5)	0.62 (0.33–1.15)	0.64 (0.24–1.66)	0.36
Use of combined estrogen–progestagen therapy[d]							
Never	537 (75.7)	158 (84.5)	1.00 (Ref.)	185 (86.9)	1.00 (Ref.)	1.00 (Ref.)	
Ever	162 (22.9)	27 (14.4)	0.64 (0.38–1.09)	24 (11.3)	0.40 (0.24–0.68)	1.68 (0.85–3.29)	0.13
Time since last use of menopausal hormone therapy, y[d]							
Never	356 (50.2)	123 (65.8)	1.00 (Ref.)	148 (69.5)	1.00 (Ref.)	1.00 (Ref.)	
≥5	102 (14.4)	18 (9.6)	0.69 (0.38–1.23)	18 (8.5)	0.58 (0.32–1.03)	1.25 (0.60–2.59)	0.55
≥0.5 to <5	114 (16.1)	20 (10.7)	0.85 (0.48–1.53)	25 (11.7)	0.74 (0.43–1.27)	1.25 (0.62–2.51)	0.53
<0.5	119 (16.8)	22 (11.8)	0.66 (0.38–1.16)	14 (6.6)	0.30 (0.16–0.56)	2.47 (1.13–5.41)	0.023
Trend	691 (97.5)	183 (97.9)	0.88 (0.74–1.04)	204 (96.2)	0.72 (0.60–0.86)	1.27 (1.02–1.60)	0.036
Use of oral contraceptives[c]							
Never	395 (56.4)	117 (62.9)	1.00 (Ref.)	136 (65.1)	1.00 (Ref.)	1.00 (Ref.)	
Ever	304 (43.4)	68 (36.6)	0.92 (0.60–1.41)	73 (34.9)	0.66 (0.44–1.00)	1.34 (0.80–2.24)	0.27
Duration of oral contraceptives use, y[c]							
Never	395 (56.4)	117 (62.9)	1.00 (Ref.)	136 (65.1)	1.00 (Ref.)	1.00 (Ref.)	
<10	150 (21.4)	32 (17.2)	0.83 (0.50–1.40)	40 (19.1)	0.71 (0.44–1.16)	1.14 (0.62–2.11)	0.67
≥10	151 (21.6)	35 (18.8)	1.01 (0.61–1.67)	33 (15.8)	0.63 (0.38–1.05)	1.51 (0.81–2.84)	0.20
Per 5 y	696 (99.4)	184 (98.9)	1.02 (0.91–1.15)	209 (100)	0.87 (0.77–0.99)	1.15 (0.99–1.34)	0.07
Time since last use of oral contraceptives, y[c]							
Never	395 (56.4)	117 (62.9)	1.00 (Ref.)	136 (65.1)	1.00 (Ref.)	1.00 (Ref.)	
≥30	129 (18.4)	25 (13.4)	0.80 (0.47–1.38)	30 (14.4)	0.68 (0.41–1.13)	1.14 (0.60–2.18)	0.68
≥20 to <30	114 (16.3)	26 (14.0)	1.05 (0.59–1.85)	33 (15.8)	0.85 (0.50–1.44)	1.22 (0.62–2.40)	0.56
<20	52 (7.4)	16 (8.6)	1.19 (0.58–2.45)	10 (4.8)	0.43 (0.19–0.97)	2.42 (0.95–6.17)	0.06
Trend	690 (98.6)	184 (98.9)	1.04 (0.84–1.29)	209 (100)	0.83 (0.67–1.02)	1.23 (0.95–1.60)	0.11
Age at menopause, y[c]							
<45	162 (23.1)	43 (23.1)	1.00 (Ref.)	49 (23.4)	1.00 (Ref.)	1.00 (Ref.)	
45 to <50	155 (22.1)	37 (19.9)	0.74 (0.44–1.26)	54 (25.8)	0.83 (0.51–1.35)	0.92 (0.50–1.71)	0.80
50 to <55	248 (35.4)	66 (35.5)	0.79 (0.49–1.27)	74 (35.4)	0.70 (0.44–1.10)	1.12 (0.64–1.96)	0.69
≥55	113 (16.1)	28 (15.1)	0.89 (0.50–1.59)	25 (12.0)	0.62 (0.35–1.12)	1.41 (0.70–2.84)	0.33
Trend	678 (96.9)	174 (93.5)	0.96 (0.80–1.15)	202 (96.7)	0.85 (0.72–1.01)	1.12 (0.91–1.38)	0.30
Age at menarche, y[c]							
<13	125 (17.9)	31 (16.7)	1.00 (Ref.)	45 (21.5)	1.00 (Ref.)	1.00 (Ref.)	
13	146 (20.9)	42 (22.6)	0.97 (0.56–1.70)	41 (19.6)	0.63 (0.37–1.06)	1.49 (0.78–2.86)	0.23
14	198 (28.3)	56 (30.1)	1.04 (0.61–1.76)	45 (21.5)	0.57 (0.34–0.94)	1.84 (0.97–3.47)	0.06
≥15	225 (32.1)	55 (29.6)	0.96 (0.56–1.63)	73 (34.9)	0.85 (0.53–1.37)	1.09 (0.59–2.02)	0.77
Trend	694 (99.1)	184 (98.9)	0.99 (0.84–1.16)	204 (97.6)	0.97 (0.83–1.13)	1.01 (0.84–1.23)	0.89
Ever full-term pregnancy[c]							
Yes	616 (88.0)	160 (86.0)	1.00 (Ref.)	186 (89.0)	1.00 (Ref.)	1.00 (Ref.)	
No	84 (12.0)	26 (14.0)	1.04 (0.62–1.74)	23 (11.0)	0.84 (0.50–1.42)	1.31 (0.70–2.44)	0.40

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Table 2. Associations between colorectal cancer risk and use of oral contraceptives, use of menopausal hormone therapy, and reproductive factors according to ESR2 status (Cont'd)

		ESR2-negative		ESR2-positive		ESR2-negative vs. ESR2-positive	
Characteristic	**Controls *N* (%)[a]**	**Cases *N* (%)[a]**	**OR (95% CI)**	**Cases *N* (%)[a]**	**OR (95% CI)**	**OR (95% CI)[b]**	***P*[b]**
Number of full-term pregnancies[c,e]							
1	166 (26.9)	51 (31.9)	1.00 (Ref.)	63 (33.9)	1.00 (Ref.)	1.00 (Ref.)	
2	257 (41.7)	57 (35.6)	0.78 (0.49–1.24)	71 (38.2)	0.68 (0.44–1.05)	1.13 (0.66–1.92)	0.67
3	125 (20.3)	36 (22.5)	0.96 (0.57–1.64)	38 (20.4)	0.74 (0.44–1.23)	1.19 (0.64–2.19)	0.59
≥4	68 (11.0)	16 (10.0)	0.72 (0.36–1.42)	14 (7.5)	0.47 (0.23–0.95)	1.39 (0.60–3.22)	0.44
Per pregnancy	616 (100)	160 (100)	0.94 (0.79–1.12)	186 (100)	0.86 (0.72–1.02)	1.05 (0.86–1.29)	0.64
Age at last pregnancy, y[c,e]							
<25	114 (18.5)	36 (22.5)	1.00 (Ref.)	47 (25.3)	1.00 (Ref.)	1.00 (Ref.)	
25 to <30	221 (35.9)	48 (30.0)	0.74 (0.44–1.26)	53 (28.5)	0.64 (0.39–1.05)	1.18 (0.64–2.17)	0.61
30 to <35	174 (28.2)	46 (28.8)	0.79 (0.46–1.35)	54 (29.0)	0.66 (0.40–1.10)	1.13 (0.62–2.08)	0.69
≥35	107 (17.4)	29 (18.1)	0.82 (0.45–1.50)	32 (17.2)	0.66 (0.37–1.16)	1.26 (0.63–2.52)	0.51
Trend	616 (100)	159 (99.4)	0.95 (0.79–1.15)	186 (100)	0.89 (0.74–1.06)	1.06 (0.86–1.32)	0.57
Breastfeeding[c,e]							
Never	161 (26.1)	42 (26.3)	1.00 (Ref.)	59 (31.7)	1.00 (Ref.)	1.00 (Ref.)	
Ever	455 (73.9)	118 (73.8)	1.03 (0.67–1.58)	127 (68.3)	0.77 (0.52–1.14)	1.33 (0.82–2.16)	0.25

[a]Numbers do not add up to 100% due to individuals with missing data on the exposure of interest.
[b]From case–case analysis using unconditional logistic regression models with tumor subtype as the outcome.
[c]Models adjusted for age, county of residence, former colorectal endoscopy, body mass index, history of diabetes diagnosis, former general health checks, physical activity, and ever use of menopausal hormone therapy.
[d]Models adjusted for age, county of residence, former colorectal endoscopy, body mass index, history of diabetes diagnosis, former general health checks, physical activity, and ever use of oral contraceptives and additional ever use of unknown/other type of menopausal hormone therapy and estrogen monotherapy or combined estrogen–progestagen therapy in analyses assessing risk specific for type of therapy.
[e]Women with full-term pregnancy.

The following variables were considered as confounders, but they did not change the OR associated with ever use of hormone therapy substantially and were therefore not included in the model: having a first-degree relative diagnosed with colorectal cancer, ever regular use of nonsteroidal anti-inflammatory drugs (2+ times/week, ≥1 year), education (3 categories), ever breastfed, number of pregnancies, pack-years of smoking (in categories of 10 pack-years), and average alcohol consumption in the last year before diagnosis (g/d in quartiles). For assessing associations with hormone therapy by type, we used never use of any hormone therapy as the reference category and therefore adjusted for other types of hormone therapy, as appropriate. Models used in case–case analyses included the same covariates as the models used in case–control analyses, except for the matching factor county of residence. Participants with missing values in the explanatory variables or the response variable were excluded from analyses.

For the duration of hormone therapy use as well as oral contraceptives use, we evaluated nonlinear risk relationships by using fractional polynomials (16). Because transformations did not significantly improve the model fit of the linear variables, the untransformed variables were used.

Results

The immunohistochemical analysis of ESR2 expression was successful in 88.5% of the 503 cases samples. Reasons for unsuccessful measurements were an uninformative positive control and loss of cores. Of the 445 samples with successful measurement, 219 (49.2%) were ESR2-negative and 226 (50.8%) were ESR2-positive according to defined thresholds.

The distributions of selected characteristics of the study population are shown in Table 1. Cases were more likely than controls to have a higher body mass index, higher alcohol intake, less physical activity, a history of diabetes, no history of colorectal endoscopies, less regular intake of nonsteroidal anti-inflammatory drugs, less use of hormone therapy as well as of oral contraceptives. The distributions of selected variables did not differ significantly between ESR2-negative cases and ESR2-positive cases or between cases with known ESR2 status and unknown ESR2 status.

As previously reported for the DACHS study (15), ever use of hormone therapy was associated with a reduced risk for colorectal cancer (OR, 0.63; 95% CI, 0.46–0.85). There was no significant association between ever use of oral contraceptives and colorectal cancer risk (OR, 0.74; 95% CI, 0.54–1.03).

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The P values for effect heterogeneity according to ESR2 status and the respective OR estimates and CIs for colorectal cancer risk associated with hormone therapy use, use of oral contraceptives as well as reproductive factors are presented in Table 2. The duration of oral contraceptive use was inversely associated with risk for ESR2-positive tumors (5-year increments OR, 0.87; 95% CI, 0.77–0.99), but not for ESR2-negative tumors (5-year increments OR, 1.02; 95% CI, 0.91–1.15; $P_{\text{heterogeneity}} = 0.07$). Ever use of oral contraceptives was associated with a reduced risk only for ESR2-positive tumors, but there was no significant heterogeneity by ESR2 status ($P_{\text{heterogeneity}} = 0.27$). A more recent use of oral contraceptives (<20 years ago) was associated with a significantly lower risk for ESR2-positive tumors (OR, 0.43; 95% CI, 0.19–0.97) and not ESR2-negative tumors (OR, 1.19; 95% CI, 0.58–2.45). Again, the difference in association was not significantly heterogeneous ($P_{\text{heterogeneity}} = 0.06$).

Similarly, ever use hormone therapy was significantly associated with a decreased risk for ESR2-positive tumors (OR ESR2-positive, 0.55, 95% CI, 0.38–0.80; OR ESR2-negative, 0.72, 95% CI, 0.49–1.06) but effect heterogeneity according to ESR2 status was not statistically significant. Duration of hormone therapy use also showed a significant inverse association with risk for ESR2-positive (5-year increments; OR, 0.84; 95% CI, 0.74–0.95) and not ESR2-negative tumors (5-year increments; OR, 0.94; 95% CI, 0.84–1.05), even though the differential association by ESR2 status did not reach statistical significance ($P_{\text{heterogeneity}} = 0.06$).

Significant heterogeneity according to ESR2 expression was found for the association with time since last use of hormone therapy ($P_{\text{heterogeneity}} = 0.023$) such that current use (<0.5 years ago) was associated with a stronger decreased risk for ESR2-positive tumors (OR, 0.30; 95% CI, 0.16–0.56) than for ESR2-negative tumors (OR, 0.66; 95% CI, 0.38–1.16).

When considering specific types of hormone therapy, the inverse risk associations with ever use of estrogen–progestagen therapy by ESR2 status were comparable with that observed for any hormone therapy, being nonsignificantly stronger for ESR2-positive tumors. The opposite pattern by ESR2 status was observed with ever use of estrogen monotherapy. Here, a nonsignificantly stronger inverse association with colorectal cancer risk was observed for ESR2-negative tumors (OR, 0.32; 95% CI, 0.14–0.74) compared with ESR2-positive tumors (OR, 0.62; 95% CI, 0.33–1.15; $P_{\text{heterogeneity}} = 0.36$).

The associations between reproductive factors and colorectal cancer risk did not differ significantly according to ESR2 expression. However, having 4 or more full-term pregnancies compared with 1 full-term pregnancy was significantly inversely associated with risk for developing ESR2-positive tumors (OR, 0.47; 95% CI, 0.23–0.95) as well as menarche at age of 14 years compared with menarche at age of 12 years or younger (OR, 57; 95% CI, 0.34–0.94).

Discussion

The present study provides first evidence that the association between exogenous hormone use and colorectal cancer risk may be differential according to ESR2 expression in the tumor. Duration of exposure to exogenous hormones, either in the form of oral contraceptives or menopausal hormone therapy, was significantly associated with a decreased risk to develop ESR2-positive tumors but not ESR2-negative tumors. For menopausal hormone therapy, this relationship with ESR2-positive tumors was significantly stronger with greater recency of the exposure.

Several other studies investigated the association between use of menopausal hormone therapy and risk for molecularly defined subtypes of colorectal cancer (17–21). Three studies observed an inverse association between menopausal hormone therapy use and microsatellite stable colorectal cancer (18–20), whereas another study reported differing associations (21). Whether ESR2 expression in colorectal cancer is associated with microsatellite status remains to be clarified, as Wong and colleagues found that ESR2 isoform 1 expression was decreased in microsatellite stable colorectal cancer compared with microsatellite-unstable colorectal cancer, but no differences in expression were observed for isoform 2 and 5 (22). We also did not observe differences in ESR2 expression according to microsatellite status in the DACHS study population (10). One study reported significantly different associations between current use of menopausal hormone therapy and colorectal cancer risk according to CDKN1A expression (19). Current use of hormone therapy was associated with a significantly reduced risk for CDKN1A-negative colorectal cancer but not with CDKN1A-positive colorectal cancer. These results were not consistent with findings from experimental studies, where CDKN1A-expression was found to be upregulated in presence of ESR2, suggesting that *CDKN1A* is a target gene of ESR2-signaling (23, 24).

In experimental cell line and animal studies, protective effects of female sex hormones about colorectal carcinogenesis were found to be mediated by ESR2 (24–29). Therefore, it is biologically plausible that exogenous hormones interact with ESR2 and downregulate the growth of neoplastic cells in the colorectal mucosa. Once the cells lose the expression of ESR2, for example, by acquired mutations or aberrant methylation, the protective effect of exogenous hormones may be attenuated.

In our study, users of oral contraceptives had a higher prevalence of hormone therapy use than nonusers of oral contraceptives (57.2% ever hormone therapy users among ever users of oral contraceptives compared with 29.9% among never users of oral contraceptives). Thus, the reduced risk for ESR2-positive colorectal cancer associated with duration of oral contraceptive use might be in part attributable to a subsequent use of hormone therapy. However, when duration of oral contraceptive use and duration of hormone therapy use were simultaneously included in a respective case–case analysis, the risk estimates according to ESR2 expression were similar for duration of oral contraceptive use (ESR2-negative vs. ESR2 positive; OR, 1.15; 95% CI, 0.99–1.34; $P_{\text{heterogeneity}} = 0.064$) and duration of hormone therapy use (ESR2-negative vs. ESR2 positive; OR, 1.15; 95% CI, 0.99–1.34; $P_{\text{heterogeneity}} = 0.057$). This suggests that the use of oral contraceptives contributes independently to the observed risk differences. Keeping in mind that the development of a sporadic colorectal tumor is thought

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to take years to decades (30), the first steps toward tumorigenesis in a large number of female cases may occur before menopause and during the climacteric period. Our results imply that the development of ESR2-positive tumors at that stage may be prevented by the exposure to exogenous female sex hormones via oral contraceptives use. At a later period in life, the use of oral contraceptives as the source of exogenous hormones is then replaced by the use of menopausal hormone therapy, conferring similarly protective effects toward ESR2-positive tumors.

The inverse association of ever use of estrogen monotherapy with ESR2-negative tumors, although not significantly differential to that with ESR2-positive tumors, was unexpected. However, differing effects between estrogen monotherapy and estrogen–progestagen therapy have been observed for endometrial cancer risk (31) and breast cancer risk (32). Nevertheless, the observed finding could be due to chance in light of the small number of women that used estrogen monotherapy.

Although we observed a significantly reduced risk for ESR2-positive tumors with having 4 or more full-term pregnancies among parous women and menarche at age of 14 years compared with menarche at age of 12 years or younger, our study does not indicate strong associations between reproductive factors and colorectal cancer risk. The inconsistent associations reported by previous studies (5, 6) could not be clarified by investigating colorectal cancer risk according to ESR2 status.

Our results about effect heterogeneity by ESR2 status could have been affected by selection bias if exogenous hormone use in patients differed by the availability of the ESR2 tumor classification. From 811 female patients recruited by the end of 2007, tumor tissue blocks could not be retrieved for 270 patients and immunohistochemistry was unsuccessful in additional 63 patients. Distributions in the relevant risk variables were not significantly different between the patients with and without classifiable tumor tissue (Supplementary Table S1). Thus, availability of ESR2 status is unlikely to have had a major impact on the results. Apart from women without information on ESR2 status, a small proportion (4.4%–9.6%) of individuals did not contribute to the analyses due to missing information on exposures or covariates. Because there is no indication that the respective data are not missing at random, it seems unlikely that excluding participants with missing data biased our results.

Another potential source of selection bias is the incomplete and potentially differential participation of eligible cases and controls. As discussed in detail elsewhere (33), incomplete ascertainment of cases was primarily due to work overload of physicians in charge of case notifications and to lower compliance of home interviews in case of recruitment after discharge, and is unlikely to be related to history of exogenous hormone use. On the other hand, patients with advanced disease were less likely to participate in the study and ESR2-negativity is more prevalent in advanced colorectal cancer (7, 8, 10). However, differences in colorectal cancer risk associated with the use of oral contraceptives and hormone therapy according to disease stage are not established (3–6) and were also not observed in the present study (Supplementary Table S2). Therefore, this particular selection bias in cases is unlikely to strongly affect our results.

Half of the nonparticipating controls provided information by completing a short questionnaire. They were less likely to have undergone preventive health checks and less likely to have used hormone therapy, thus giving some indication for possible overestimation of the protective effect of hormone therapy. This was partly controlled for by adjustment for general health check-ups. The prevalence of ever hormone therapy use among controls was comparable with the prevalence among the general female German population in this age-range, estimated using external prescription data (34). Also recall bias is unlikely to have substantially affected the findings about hormone therapy use, as a former analysis in the DACHS sample found that the agreement between self-reported and the record based duration of hormone therapy use was similarly good in cases and controls (15).

ESR2 expression was independently assessed by 2 pathologists. A common concern raised by using tissue microarrays is whether the punched tumor samples are representative for the whole sample. Validation studies showed that 2 cores of 0.6-mm diameter lead to a sufficient concordance with the whole sample section for various types of tissue, including colorectal cancer (35, 36).

The applied antibody (14C8) has been shown to be useful for the immunohistochemical assessment of ESR2 expression in formalin-fixed paraffin-embedded samples (37–39). It detects most ESR2 isoforms derived from differential splicing variants, including the wild-type ESR2. As variants of ESR2 differ in function from the wild-type ESR2 (40, 41), future studies could potentially gain more detailed insight into how sex steroids influence colorectal carcinogenesis by using variant-specific antibodies.

In conclusion, the present study provides evidence that the use of exogenous hormones is associated with a decreased risk for ESR2-positive and not ESR2-negative colorectal cancer in women. These findings support the hypothesis from cell line and animal studies that the preventive effects of female sex hormones are at least in part mediated by ESR2. Further investigations to delineate the exact mechanisms for loss of ESR2 expression in a large proportion of colorectal tumors are needed to identify potential targets for modulation of ESR2 and chemoprevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Rudolph, H. Brenner, J. Chang-Claude
Development of methodology: A. Rudolph, C. Toth, J. Chang-Claude
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Toth, M. Hoffmeister, W. Roth, E. Herpel, H. Brenner, J. Chang-Claude
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Rudolph, C. Toth, W. Roth, H. Brenner, J. Chang-Claude
Writing, review, and/or revision of the manuscript: A. Rudolph, M. Hoffmeister, E. Herpel, P. Schirmacher, H. Brenner, J. Chang-Claude
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Rudolph, M. Hoffmeister, W. Roth, E. Herpel, P. Schirmacher
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