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Estrogens Reduce and Withdrawal of Estrogens Increase Risk of Microsatellite Instability-positive Colon Cancer[1]

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ABSTRACT

There are sex differences in the occurrence of microsatellite instability (MSI) in colon tumors. Taken together with the epidemiological evidence that hormone replacement therapy (HRT) and, less consistently, parity, are inversely associated with colon cancer, it has been hypothesized that estrogens are associated with MSI. The purpose of this study was to evaluate sex-specific differences in the prevalence of MSI in colon tumors and to determine whether reproductive history and hormonal exposures are associated with MSI.

Using data from a population-based case-control study of 1836 cases with MSI data and 2410 population-based controls, we evaluated sex, reproductive factors, and hormone exposure in relation to the presence or absence of MSI in tumors. MSI was evaluated by a panel of 10 tetranucleotide repeats, the noncoding mononucleotide repeat BAT-26, and the coding mononucleotide repeat in transforming growth factor β receptor type II (TGFβRII). Exposure data on reproduction, hormone use, obesity, and physical activity were obtained from an interviewer-administered questionnaire.

Women were less likely then men to have MSI+ tumors at a young age and more likely to have unstable tumors at an older age; we observed a significant interaction ($P < 0.01$) between age, sex, and MSI. Evaluation of reproductive factors showed that women who had ever been pregnant had half the risk of MSI+ tumors compared with women who had never been pregnant. In complementary fashion, total ovulatory months were associated with an increased risk of MSI+ tumors [odds ratio (OR), 2.1; 95% confidence interval (CI), 1.1–4.0 comparing MSI+ *versus* MSI− tumors]. Age at first and last pregnancy did not influence the association. The observed associations were strongest among women <60 years of age at the time of diagnosis. Having used oral contraceptives was associated with a lower risk of MSI+ tumors (OR, 0.7; 95% CI, 0.4–1.2); recent users of HRT were at a reduced risk of MSI+ tumors (OR, 0.8; 95% CI, 0.5–1.4); and women who were former HRT users were at an increased risk of MSI+ tumors (OR, 1.8; 95% CI, 1.1–3.0). Obesity and lack of physical activity were associated with an elevated risk of both MSI+ (OR, 1.7; 95% CI, 0.7–3.3) and MSI− (OR, 2.2; 95% CI, 1.7–3.) tumors in men, but only with MSI− (OR, 1.5; 95% CI, 1.1–2.2) tumors in women.

The excess of MSI+ tumors in women is explained by the excess of MSI+ tumors at older ages. Our data suggest that estrogen exposure in women protects against MSI, whereas the lack of estrogen in older women increases risk of instability. HRT in these older women may, again, reduce the risk of unstable tumors. A model for the way in which estrogens (endogenous, exogenous, and obesity-associated) modify the risk of MSI+ tumors is proposed.

INTRODUCTION

Age-adjusted colon cancer incidence rates are somewhat higher for men than for women (1). Other sex-specific differences in colon cancer include women having more proximal tumors than men (2) and women having significantly more MSI[3] than men, independent of tumor site.[4] These sex-specific differences in colon cancer have been attributed to estrogen (3). Some epidemiological studies support a hormonal basis for the etiology of colon cancer: parity has been inversely associated with colon cancer; subgroups of the population with low parity, such as nuns, have higher rates of colon cancer than populations where parity is high; and HRT use has been shown to decrease the risk of developing colon cancer (4–7). It has been hypothesized that estrogen is associated with MSI on the basis of the observation that women are more likely to have MSI+ tumors than men (8). We have previously shown that obesity and lack of physical activity increase the risk of colon cancer (9) and adenomatous polyps[5] in men but not women. In this study, we explored the sex differences in MSI observed in colon tumors and evaluated the contribution of estrogen-related factors to these differences. We evaluated the role of obesity in postmenopausal women, a source of endogenous estrogen, in influencing the risk of MSI+ tumors in men and women. Our hypothesis was that, in women, obesity and lack of physical activity would be associated with an elevated risk of MSI− tumors but not MSI+ tumors, whereas in men these factors would be associated with both MSI− and MSI+ tumors.

MATERIALS AND METHODS

Study Population. Study participants were black, white, or Hispanic and were from either the Kaiser Permanente Medical Care Program of Northern California, an eight county area in Utah (Davis, Salt Lake, Utah, Weber, Wasatch, Tooele, Morgan, and Summit counties), or the Twin Cities Metropolitan area in Minnesota. Eligibility criteria for cases included diagnosis with first-primary incident colon cancer (ICD-O, second edition, codes 18.0, 18.2 to 18.9) between October 1, 1991, and September 30, 1994; between 30 and 79 years of age at the time of diagnosis; and mentally competent to complete the interview. Cases with adenocarcinoma or carcinoma of the rectosigmoid junction or rectum (defined as the first 15 cm from the anal opening), with known familial adenomatous polyposis, ulcerative colitis, or Crohn's disease, were not eligible. Of all cases asked to participate, 75.6% cooperated.

Controls, in addition to the eligibility criteria for cases, had no history of colorectal cancer. Controls were selected from eligibility lists for Kaiser Permanente Medical Care Program of Northern California; driver's license lists for Minnesota; and random-digit dialing, driver's license lists, or Health Care Finance Administration lists for Utah. These methods have been described in detail (9). Of all controls selected, 63.7% participated.

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[3] The abbreviations used are: MSI, microsatellite instability; HRT, hormone replacement therapy; BMI, body mass index; ER, estrogen receptor; TGFβRII, transforming growth factor β receptor type II.

[4] W. S. Samowitz, K. Curtin, D. Schaffer, L. Ballard, M. Leppert, and M. L. Slattery. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level, submitted for publication.

[5] J. D. Potter, L. Fosdick, R. Bostick, M. L. Slattery, T. A. Louis, and P. Grambsch. Energy balance and adenomatous polyps: beyond physical activity, submitted for publication.

Questionnaire Data. Data were collected by trained and certified interviewers using laptop computers (10). The referent period for the study was the calendar year, approximately 2 years before the date of diagnosis. Using this referent period, information was collected on dietary intake using a detailed diet history questionnaire (11). Demographic factors; physical activity (7); body size, including usual adult height and weight 2 and 5 years before diagnosis; use of aspirin and/or nonsteroidal anti-inflammatory drugs; cigarette smoking history; medical history; and reproductive history were obtained from a standardized questionnaire. A measure of long-term (past 20 years) levels of vigorous leisure-time physical activity was used because this was shown to be a sensitive predictor of cancer risk in this population (9). BMI of weight/height (2) was used as an indicator of body size.

A detailed reproductive history was obtained from women. The interview included questions on the use of exogenous hormones such as estrogen, progestin, or other female hormones for both contraceptive and noncontraceptive purposes. Dates of first and last use and duration of use of HRT were ascertained. Information also was collected on total number of pregnancies, total amount of time pregnant, number of live births, age at first and last pregnancy, and menstrual history. Total ovulatory months were calculated by subtracting age first menstruated from age at menopause; total months pregnant was subtracted from this number.

Tissue Ascertainment. Methods for ascertaining tumor tissue and extracting DNA have been described in detail (12). Tumor DNA, obtained from paraffin blocks, was amplified, and MSI status was determined. From those on whom we obtained tumor DNA, MSI results were obtained for 98% of tumor DNA samples. Of the 1836 cases with MSI data, 1510 also had valid interview data and are included in these analyses. Of these cases, 689 were women with both MSI status and a complete reproductive history.

MSI. Each tumor was evaluated for MSI with a panel of 10 tetranucleotide repeats used by us in previous studies, the mononucleotide repeat BAT-26, and a mononucleotide repeat within the coding region of TGFβRII (13). The primer sequences and PCR conditions for these repeats were as described previously (13–16). Both tumor DNA and normal DNA were PCR amplified with these 12 primer sets. MSI for a given primer set was defined as the appearance of one or more new PCR products either smaller or larger than those produced from normal DNA; for BAT-26, we required a that a smaller PCR product be at least 4 bp smaller than the normal. Results from the tetranucleotide repeat panel were considered to indicate significant MSI if three or more repeats were unstable. Results were considered to indicate stability if fewer than 30% of the repeats were unstable and at least 6 of the 10 repeats were typed. To simplify analyses, we combined these primer sets into a panel of 12 and scored a tumor as unstable if it was unstable with the panel of 10 tetranucleotides (as defined above), or BAT-26 or TGFβRII. Any associations detected in this way were then further defined by considering MSI results with each of these three measures separately. The evaluation of MSI was begun before the development of the Bethesda consensus panel; however, we have evaluated the markers used in this study with the Bethesda consensus panel. We observed that instability in the panel of 10 tetranucleotide markers ($\geq$30% unstable markers) showed 98.4% concordance in classifying instability in a sample of 427 people; the BAT-26 marker showed 98.6% concordance with the Bethesda consensus panel in a sample of 442 people.

Statistical Analyses. The distribution of MSI by population characteristics was determined. Logistic regression models were fit with the dichotomous-dependent variables as either "no disease" or "MSI+" or "no disease" or "MSI−." The case-control comparison was conducted to estimate the relative risk for developing the disease given the exposure with specific genetic mutations. Logistic regression models also were fit with a dichotomous-dependent variable (MSI+ or MSI−). The purpose of the case-case comparison was to evaluate etiological heterogeneity with respect to the risk factor under study. Logistic regression models were used to adjust for other potential confounding variables; these models varied slightly by the test being done. In evaluating age and gender differences in MSI, we adjusted for age, cigarette smoking, and alcohol intake because these factors are associated with gender as well as MSI (17). Models evaluating reproductive effects were adjusted for age and energy intake because these variables were thought to be potentially important confounding variables for these associations. In evaluating the interaction between physical activity and BMI in men and women we adjusted for factors that may influence these variables, such as age, dietary intake, and cigarette smoking.

RESULTS

We evaluated the effects of age and gender on distribution of MSI in colon tumors (Table 1). For men, the effect of age was of borderline statistical significance ($P = 0.07$); for women the difference in MSI by age was statistically significant ($P < 0.01$). Men <50 years of age were more likely to have MSI+ colon tumors than younger women (Table 1). However, older women had more MSI+ tumors relative to younger men whereas older men were less likely to have unstable tumors than younger men. We observed a statistically significant interaction between gender and age and MSI tumors status ($P \leq 0.01$). Although the panel of 12 MSI markers was used in these analyses, results were similar for individual marks (*i.e.*, panel of 10 tetranucleotide repeats, BAT-26, and TGFβRII).

Evaluation of the association between sex and MSI status showed that cases were twice as likely to be women and to have MSI+ tumors for all indicators of MSI+ when compared with population-based controls (*i.e.*, panel of 10, BAT-26, and TGFβRII; Table 2). However, women were at no greater risk of having MSI− tumors than men. The differences in association were confined to proximal tumors.

Pregnancy was associated with a lower frequency of MSI+ tumors (Table 3). Mean age at first and last pregnancy, age at menarche, and age at menopause were not different between those with MSI+ tumors and those with MSI− tumors or controls. Means of total months pregnant and total ovulatory months were greater in those with MSI+ tumors than either MSI− tumors or controls. Those who had used oral contraceptives or were recent users of HRT were less likely to have MSI+ tumors. Results for BAT-26 and TGFβRII were similar to those presented in Table 3 for the panel of 12 markers, although TGFβRII showed slightly stronger associations.

Evaluation of reproductive factors and MSI status showed that having been pregnant resulted in a halving of risk of having MSI+ tumors (Table 4) although no linear trend was observed in that increasing number of pregnancies was not associated with a greater risk reduction than having been pregnant. A halving of risk of having MSI+ tumors was observed, regardless of the number of pregnancies,

Table 1 *Distribution of MSI status[a] in colon tumors by age and sex*

Age	Controls *n* (%)	MSI+ *n* (%)	MSI− *n* (%)	OR (95% CI)	χ^2 *P*[b] MSI+ *vs.* MSI−	Interaction *P*[c] for age and sex
Men						
<50	131 (10.2)	16 (13.3)	53 (7.6)	1.0		
51–64	414 (32.1)	34 (28.3)	242 (34.5)	0.5 (0.2–0.9)		
65–79	745 (57.7)	70 (58.3)	406 (57.9)	0.5 (0.3–1.0)	0.07	
Women						
<50	98 (8.7)	10 (6.9)	45 (8.3)	0.8 (0.3–1.9)		
51–64	346 (30.9)	38 (26.0)	211 (38.9)	0.6 (0.3–1.2)		
65–79	676 (60.4)	98 (67.1)	287 (52.8)	1.2 (0.7–2.3)	<0.01	<0.01

[a] MSI determined by 30% or more of 10 tetranucleotide markers unstable, BAT26 unstable, or TGFBRII unstable.
[b] χ^2 *P*s are for difference in proportion of MSI+ *vs.* MSI− tumors by age in men and in women separately.
[c] The *P* shows the interaction between gender and age as a risk factor for an MSI+ tumor.

Table 2 *Sex-specific associations with MSI+ tumors vs. control and MSI− tumors vs. control by tumor site*

		MSI+ *vs.* control			
	Panel 12	Panel 10	Bat-26	TGFβRII	MSI− *vs.* control
Men/women N (%)[a]	119 (14.6)/146 (21.3)	96 (11.8)/111 (16.2)	92 (11.3)/114 (16.6)	81 (9.9)/111 (16.2)	697 (85.4)/541 (78.7)
Men (referent)	1.0	1.0	1.0	1.0	1.0
Women (OR 95% CI)[b]	1.7 (1.3–2.2)	1.5 (1.1–2.1)	1.8 (1.3–2.4)	1.9 (1.4–2.6)	1.0 (0.8–1.1)
Proximal tumors					
Men/women N (%)	92 (22.4)/120 (33.7)	76 (18.5)/94 (26.4)	76 (18.5)/102 (28.7)	67 (16.3)/98 (27.5)	319 (77.6)/236 (66.3)
Men (referent)	1.0	1.0	1.0	1.0	1.0
Women (OR 95% CI)	1.8 (1.4–2.5)	1.7 (1.2–2.4)	1.9 (1.4–2.6)	2.1 (1.5–2.9)	0.9 (0.7–1.1)
Distal tumors					
Men/women N (%)	24 (6.2)/25 (7.9)	18 (4.7)/16 (5.1)	13 (3.4)/12 (3.8)	11 (2.8)/13 (4.1)	363 (93.8)/291 (92.1)
Men (referent)	1.0	1.0	1.0	1.0	1.0
Women (OR 95% CI)	1.4 (0.7–2.5)	1.1 (0.5–2.2)	1.2 (0.5–2.8)	1.5 (0.7–3.6)	1.0 (0.8–1.2)

[a] Number and percentage of cases with MSI+ and MSI− tumors for men and women.
[b] Adjusted for age, cigarette smoking, and alcohol consumption comparing risk MSI+ *vs.* control and MSI− *vs.* control. There are 1288 male controls and 1117 female controls.

the age at first or last pregnancy, or the number of total months pregnant (data not shown). In complementary fashion, total ovulatory months increased the risk of having a MSI+ tumor. All indicators of MSI showed similar results, with the strongest associations being detected for TGFβRII.

Women who used oral contraceptives or who were recent users of HRT were at a reduced risk of MSI+ tumors (Table 5). Former users of HRT were at a greater risk of MSI+ tumors, although not of MSI− tumors, than women who never used HRT. Further evaluation of these data by age showed that the increase in risk of MSI+ tumors was confined to women <60 years of age who had taken HRT for <5 years. All indicators of MSI showed similar results, with the strongest associations being detected for TGFβRII.

Because peripheral adipose tissue is a source of estrogens in postmenopausal women, we evaluated MSI in conjunction with the combined effects of obesity and physical activity. Among men, having a high BMI and low levels of activity increased the risk of cancer for both those MSI+ and MSI− when compared with controls (Table 6). However, for women, high BMI was associated with a reduced risk of having an MSI+ tumor. Among women, the combined effects of low physical activity and high BMI increased risk only of MSI− colon cancer.

DISCUSSION

These data suggest that aspects of estrogen metabolism contribute directly to sex differences in colon cancer through a mechanism involving MSI. Although women have more MSI+ tumors overall, at a younger age, they are less likely to have a MSI+ tumor compared with young men. As men age, they become less likely to have a MSI+ tumor relative to younger men; as women age, they become more likely to have a MSI+ tumor relative to younger men. HRT use results in a lower risk of MSI+ tumors. This is consistent with a model in which estrogen protects against instability and the lack of estrogen increases the risk of instability, an increased risk that can be modified by HRT use. These findings have implications for previously identified risk factors and provide information on the MSI disease pathway.

It has been previously hypothesized that estrogens could inhibit the

Table 3 *MSI as determined by the panel of 12 markers, reproduction, and hormone use*

Reproductive factors	Controls *n* (%)	MSI+ *n* (%)	MSI− *n* (%)	*P* MSI+ *vs.* MSI−
Number of pregnancies				
None	93 (8.3)	24 (16.4)	41 (7.6)	<0.01
1–2	288 (25.7)	26 (17.8)	147 (27.1)	
3+	739 (66.0)	96 (65.8)	355 (65.4)	
Oral contraceptive use (% ever for ≥3 months)	280 (25.0)	24 (16.4)	133 (24.5)	0.04
HRT				
Ever for ≥3 months	354 (36.1)	46 (35.7)	161 (33.1)	0.58
Recent	259 (73.5)	23 (50.0)	115 (71.4)	<0.01
Former	94 (26.5)	23 (50.0)	46 (28.6)	<0.01
	Mean (SD)	Mean (SD)	Mean (SD)	
Age at first pregnancy (yr)	23.6 (4.5)	23.4 (4.1)	23.8 (4.7)	0.42
Age at last pregnancy (yr)	31.8 (5.7)	31.6 (5.6)	31.5 (5.9)	0.84
Total months pregnant	31.2 (16.8)	32.8 (16.5)	29.6 (16.3)	0.05
Age menarche (yr)	12.9 (1.6)	12.9 (1.4)	13.1 (4.0)	0.30
Total ovulatory months	376.0 (93.7)	391.0 (81.8)	375.1 (94.0)	0.05
Age at menopause	47.4 (7.3)	48.3 (6.9)	47.3 (6.8)	0.11

Table 4 *Associations between MSI as determined by the panel of 12 markers and reproductive factors*

	OR (referent)[a]	OR (95% CI)	OR (95% CI)	OR (95% CI)	*P* trend
Number of pregnancies	None	1–2	3–4	5+	
MSI+/MSI−/control no.	24/41/93	26/147/288	53/210/416	43/145/323	
MSI+ *vs.* control	1.0	0.4 (0.2–0.7)	0.5 (0.3–0.9)	0.5 (0.3–0.9)	0.36
MSI− *vs.* control	1.0	1.2 (0.8–1.8)	1.2 (0.8–1.7)	1.0 (0.7–1.6)	0.68
MSI+ *vs.* MSI−	1.0	0.3 (0.2–0.6)	0.4 (0.2–0.7)	0.5 (0.3–0.9)	0.63
Total ovulatory months	<294	294–369	370–430	431+	
MSI+/MSI−/control no.	14/92/196	35/124/257	41/156/309	49/144/302	
MSI+ *vs.* control	1.0	1.8 (0.9–3.5)	1.8 (0.9–3.3)	2.2 (1.2–4.1)	0.12
MSI− *vs.* control	1.0	1.1 (0.8–1.5)	1.1 (0.8–1.5)	1.1 (0.8–1.5)	0.89
MSI+ *vs.* MSI−	1.0	1.7 (0.9–3.4)	1.5 (0.8–3.0)	2.1 (1.1–4.0)	0.14

[a] Adjusted for age and energy intake.

Table 5 *Associations between MSI as determined by the panel of 12 markers and exogenous hormone*

	OR (referent)[a]	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use	Never	Ever		
MSI+/MSI−/control	121/392/817	24/133/280		
MSI+ *vs.* control	1.0	0.6 (0.4–1.1)		
MSI− *vs.* control	1.0	0.9 (0.7–1.2)		
MSI+ *vs.* MSI−	1.0	0.7 (0.4–1.2)		
HRT[b]	Never	Ever[c]	Recent[d]	Former[e]
MSI+/MSI−/control	83/326/628	46/161/354	23/115/260	23/46/94
MSI+ *vs.* control	1.0	1.0 (0.7–1.5)	0.7 (0.4–1.1)	1.8 (1.1–3.1)
MSI− *vs.* control	1.0	0.9 (0.7–1.1)	0.9 (0.7–1.1)	1.0 (0.7–1.4)
MSI+ *vs.* MSI−	1.0	1.2 (0.8–1.8)	0.8 (0.5–1.4)	2.0 (1.1–3.5)

[a] Adjusted for age, energy intake.
[b] Analyses restricted to postmenopausal women.
[c] Ever used for 3 months or longer.
[d] In the year prior to referent date.
[e] Having stopped 2 years prior to the referent date.

Table 6 *MSI as determined by TGFβRII and BMI/activity interactions in men and women*

	Men BMI		Women BMI	
	Intermediate/ low OR (95% CI)[a]	High OR (95% CI)	Intermediate/ low OR (95% CI)	High OR (95% CI)
MSI+ *vs.* control				
Physical activity				
High	1.0	1.2 (0.4–3.6)	1.0	0.3 (0.1–1.1)
Inter/low	2.1 (1.0–4.5)	1.7 (0.7–3.7)	0.6 (0.3–1.1)	0.9 (0.5–1.7)
MSI− *vs.* control				
High	1.0	1.3 (0.9–1.9)	1.0	0.9 (0.5–1.5)
Inter/low	1.4 (1.0–1.8)	2.2 (1.7–3.0)	1.2 (0.8–1.6)	1.5 (1.1–2.2)

[a] Adjusted for age, energy intake, dietary fiber, calcium, and usual number of cigarettes smoked.

pathway to colon cancer involving mismatch repair deficiency and MSI (8). This hypothesis was based on the observation that younger women had a low prevalence of MSI in colon tumors whereas, among older women, the prevalence of MSI in colon tumors was high (8). As pointed out by Breivik *et al.* (8), hereditary nonpolyposis colon cancer, an inherited colon cancer syndrome in which MSI is seen in nearly all colon cancers, women with hereditary nonpolyposis colon cancer have half the risk of developing colorectal adenomas as their male relatives (18).

Our data provide further support for the involvement of estrogens in reducing the risk of colon cancer via a MSI-related pathway. We observed a reduction in risk of MSI in tumors with ever having been pregnant and the reverse with total ovulatory months. Having taken oral contraceptives and being a recent user of HRT also were associated with a lower likelihood of having an unstable tumor. Consistent with our previous observations of the association between HRT and colon cancer risk (7), former users of HRT were, if anything, at an elevated risk of MSI+ tumors. Pregnancy, a state of steady elevated estrogen levels, was associated with reduced risk of MSI+ tumors. During pregnancy different forms of estrogen are present than in the nonpregnant state (19). However, variation in other factors, such as growth hormones and prostaglandins, also exist during pregnancy and could contribute to observed associations (19, 20). Given that neither numbers of pregnancies nor age at first or last pregnancy altered the observed association, it is likely that a mechanism involving homeostasis is involved.

It seems possible that both long-term and short-term fluctuations in estrogen exposure may determine risk and that this results in either or both of the following: variation in lifetime cumulative estrogen exposure (above a certain level is sufficient to prevent MSI+ tumors, irrespective of source) or a homeostasis maintained by the mean lifetime level of estrogen (higher peaks and deeper troughs are associated with elevated risk of MSI+ tumors). The fact that there is an excess of MSI+ tumors in women compared with men, but that this excess is explained wholly by the excess at older ages (indeed, there are fewer MSI+ tumors in young women than young men) and the elevated risk after cessation of HRT use, add weight to the homeostasis argument.

The model we propose to explain these observations is shown in Fig. 1. Issa *et al.* (21) have shown that hypermethylation, and, thus, reduced expression, of the ER in the colon is a concomitant of aging. Furthermore, they have shown that colon tumors almost universally arise from cells that have lost ER expression (21). On the basis of our findings, one could hypothesize that HRT may reduce the risk of colon cancer in women by reducing the likelihood of ER methylation and, thus, the pool of cells that give rise to colon tumors (22).

We have further hypothesized that one role that obesity, and the consequent elevation of estrogens, may play in postmenopausal women is similar to that of HRT, namely lowering risk of loss of ER expression (9).[5] In men, obesity and reduced physical activity will result in an elevated risk of colon cancer, but in women this will be, in part, offset by the reduced risk associated with the elevation of estrogen levels (22).

What we have shown here is that this yin/yang pattern of risk in women (but not men) is confined to MSI+ tumors. It is the absence of this group of tumors that accounts for male/female differences that we have previously shown to exist in relation to obesity/physical activity and colon cancer (9) and colorectal polyps.[5]

What remains to be determined is why estrogen levels are associated with MSI+ tumors. It is not likely to be due just to the already established association between MSI and hypermethylation of specific genes (*e.g.*, *hMLH1*) because the primary issue here is the role of endogenous (reproductive status), exogenous (HRT), and metabolic (obesity-associated) estrogens and their plausible association with preventing ER methylation specifically, not with the methylation process *per se*. It seems reasonable to hypothesize that at least one of the major DNA mismatch repair genes is estrogen responsive and that loss of estrogen results in loss of DNA mismatch repair capacity. Our data do not exclude, however, an effect of estrogens on hypermethylation, in general, or on the specific hypermethylation on *hMLH1*.

In summary, these data provide support for the hypothesis that estrogens prevent MSI+ tumors, whether endogenous, exogenous, or obesity associated. These findings increase the likelihood that the colon neoplasia/HRT association is causal and that HRT use is protective against colon cancer—not just a marker for a low-risk lifestyle. Furthermore, they provide additional details on the long-standing observation that hormones are important in the etiology of colon cancer. Finally, they raise some questions about mechanisms, including the possibility that one or more mismatch repair genes are estrogen responsive.

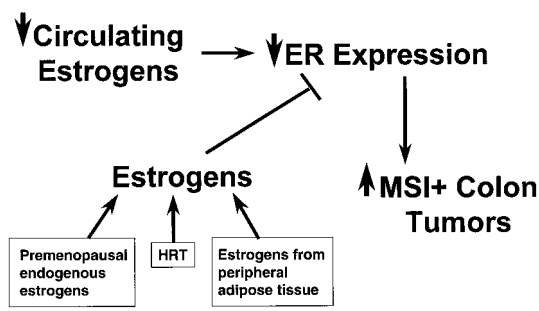

Fig. 1. Estrogens, the ER, and the prevention of colon tumors.

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