Supplementary Method

Immunohistochemistry (IHC) of fatty acid synthase (FASN)

For IHC analysis of FASN expression, antigen retrieval was performed by incubating deparaffinized tissue sections in 10 mM citrate buffer (BioGenex, San Ramon, CA) in a microwave for 15 minutes. Tissue sections were incubated with 3% H_2O_2 for 20 minutes to block endogenous peroxidase, and then incubated with 10% normal goat serum in phosphated buffered saline for 10 minutes at room temperature. Incubation with mouse monoclonal anti-human FASN antibody (clone 23, BD Biosciences, Mississauga, ON, Canada) (dilution 1:100) was carried out for 60 minutes at room temperature, followed by incubation with Multilink secondary antibody (BioGenex) for 20 minutes and incubation with streptavidin horse radish peroxidase (BioGenex) for another 20 minutes. Sections were visualized by diaminobenzidine (DAB) (5 minutes) and methyl-green counterstain. Colorectal cancer with known FASN-positive status as a positive control and colorectal cancer with known FASN-negative status as a negative control were included in each run of immunohistochemistry as positive and negative controls. A pathologist (Shuji Ogino) blinded to other data interpreted FASN expression in all sections. At least two tissue cores in tissue microarrays (1) were examined for each patient. If cores did not yield the same results, scoring in a core with the strongest FASN expression was recorded. FASN overexpression was categorized as negative (no or weak expression) or positive (moderate or strong expression), based on consistency and reproducibility in an interobserver agreement study, as described previously (2). For the agreement study, a random selection of 123 cancers was evaluated by a second pathologist (Teppei Morikawa) unaware of other data. When the cutoff of no-to-weak expression vs moderate-to-strong expression was used, concordance between the two pathologists was 0.90 (κ = 0.80, 95% confidence interval [CI] =

0.69 to 0.91, P <.001), indicating substantial agreement. When a cutoff of no expression vs weak-to-strong expression was used, a κ value of 0.44 (95% CI = 0.24 to 0.63) was obtained, indicating only fair agreement. When a cutoff of no-to-moderate expression vs strong expression was used, a κ value of 0.50 (95% CI = 0.27 to 0.72) was obtained, indicating only fair agreement. Thus, we used the most reproducible cutoff defined as above for further analyses. In most tissue sections, FASN expression was not detectable or was very weak in the normal colon.

Reference

1. Chan AT, Ogino S, Fuchs CS. Aspirin and the Risk of Colorectal Cancer in Relation to the Expression of COX-2. *New Engl J Med.* 2007;356(21):2131-2142.

2. Ogino S, Brahmandam M, Cantor M, et al. Distinct molecular features of colorectal carcinoma with signet ring cell component and colorectal carcinoma with mucinous component. *Mod Pathol.* 2006;19(1):59-68.

Supplementary Table 1. Body mass index and colon and rectal cancer risk according to FASN expression status*

	Cumulative mean body mass index†, kg/m^2					P_{trend}‡	$P_{heterogeneity}$§
	<18.5	**18.5–22.9**	**23.0–24.9**	**25.0–29.9**	**≥30**		
All colon cancers (n = 409)							
No. of cancers/person-years	5/23128	114/686843	79/387146	141/521052	70/230149		
Age-adjusted incidence rate‖ (95% CI)	12.6	11.1	14.2	14.9	13.4		
Age-adjusted HR (95% CI)	1.32 (0.54 to 3.23)	1.00 (referent)	1.06 (0.80 to 1.42)	1.34 (1.04 to 1.71)	1.53 (1.13 to 2.06)	.002	
Multivariable HR (95% CI)	1.37 (0.56 to 3.37)	1.00 (referent)	1.02 (0.77 to 1.37)	1.28 (1.00 to 1.65)	1.47 (1.08 to 2.02)	.007	
FASN-negative colon cancers (n = 170)							
No. of cancers/person-years	2/23132	41/686905	26/387193	66/521112	35/230176		
Age-adjusted incidence rate‖ (95% CI)	4.0	3.8	5.1	7.2	6.7		
Age-adjusted HR (95% CI)	1.51 (0.36 to 6.26)	1.00 (referent)	0.96 (0.59 to 1.57)	1.73 (1.17 to 2.56)	2.13 (1.36 to 3.35)	<.001	
Multivariable HR (95% CI)	1.59 (0.38 to 6.59)	1.00 (referent)	0.92 (0.56 to 1.51)	1.65 (1.11 to 2.45)	2.06 (1.29 to 3.27)	<.001	
FASN-positive colon cancers (n = 239)							.047
No. of cancers /person-years	3/23128	73/686879	53/387167	75/521113	35/230181		
Age-adjusted incidence rate‖ (95% CI)	8.6	7.2	9.1	7.8	6.7		
Age-adjusted HR (95% CI)	1.21 (0.38 to 3.86)	1.00 (referent)	1.12 (0.79 to 1.60)	1.11 (0.81 to 1.54)	1.19 (0.79 to 1.78)	.30	
Multivariable HR (95% CI)	1.25 (0.39 to 4.00)	1.00 (referent)	1.08 (0.76 to 1.55)	1.07 (0.77 to 1.49)	1.15 (0.76 to 1.74)	.44	
All proximal colon cancers (n = 255)							
No. of cancers /person-years	4/23130	77/686877	49/387174	92/521088	33/230180		
Age-adjusted incidence rate‖ (95% CI)	10.6	7.3	8.3	10.0	5.4		
Age-adjusted HR (95%	1.52 (0.55 to	1.00 (referent)	0.96 (0.67 to	1.27 (0.94 to	1.05 (0.70 to	.47	

CI)	4.15)		1.37)	1.72)	1.58)		
Multivariable HR (95% CI)	1.59 (0.58 to 4.37)	1.00 (referent)	0.93 (0.65 to 1.34)	1.27 (0.93 to 1.73)	1.07 (0.69 to 1.64)	.45	
FASN-negative proximal colon cancers (n = 110)							
No. of cancers /person-years	2/23132	31/686914	16/387200	47/521126	14/230195		
Age-adjusted incidence rate‖ (95% CI)	4.0	3.0	3.1	4.9	2.3		
Age-adjusted HR (95% CI)	1.94 (0.46 to 8.13)	1.00 (referent)	0.77 (0.42 to 1.41)	1.61 (1.02 to 2.55)	1.11 (0.59 to 2.09)	.51	
Multivariable HR (95% CI)	2.03 (0.48 to 8.54)	1.00 (referent)	0.75 (0.41 to 1.37)	1.60 (1.01 to 2.54)	1.13 (0.59 to 2.16)	.49	
FASN-positive proximal colon cancers (n = 145)							.81
No. of cancers /person-years	2/23130	46/686904	33/387188	45/521135	19/230193		
Age-adjusted incidence rate‖ (95% CI)	6.6	4.3	5.1	5.1	3.1		
Age-adjusted HR (95% CI)	1.24 (0.30 to 5.12)	1.00 (referent)	1.09 (0.69 to 1.70)	1.04 (0.69 to 1.58)	1.01 (0.59 to 1.73)	.69	
Multivariable HR (95% CI)	1.31 (0.31 to 5.42)	1.00 (referent)	1.06 (0.68 to 1.67)	1.04 (0.68 to 1.58)	1.03 (0.59 to 1.78)	.67	
All distal colon cancers (n = 154)							
No. of cancers /person-years	1/23130	37/686908	30/387186	49/521137	37/230177		
Age-adjusted incidence rate‖ (95% CI)	2.0	3.8	5.9	4.9	8.0		
Age-adjusted HR (95% CI)	0.86 (0.12 to 6.26)	1.00 (referent)	1.28 (0.79 to 2.08)	1.46 (0.95 to 2.25)	2.55 (1.61 to 4.02)	<.001	
Multivariable HR (95% CI)	0.86 (0.12 to 6.32)	1.00 (referent)	1.22 (0.75 to 1.98)	1.35 (0.87 to 2.08)	2.25 (1.39 to 3.66)	<.001	
FASN-negative distal colon cancers (n = 60)							
No. of cancers /person-years	0/23132	10/686932	10/387207	19/521159	21/230189		
Age-adjusted incidence rate‖ (95% CI)	0	0.8	1.9	2.2	4.4		
Age-adjusted HR (95%	-	1.00 (referent)	1.56 (0.65 to	2.07 (0.96 to	5.38 (2.53 to	<.001	

CI)			3.75)	4.47)	11.46)		
Multivariable HR (95% CI)	-	1.00 (referent)	1.47 (0.61 to 3.53)	1.88 (0.87 to 4.08)	4.74 (2.19 to 10.26)	<.001	.005
FASN-positive distal colon cancers (n = 94)							
No. of cancers /person-years	1/23130	27/686916	20/387193	30/521151	16/230196		
Age-adjusted incidence rate‖ (95% CI)	2.0	3.0	4.0	2.7	3.6		
Age-adjusted HR (95% CI)	1.16 (0.16 to 8.53)	1.00 (referent)	1.18 (0.66 to 2.11)	1.24 (0.73 to 2.08)	1.50 (0.80 to 2.79)	.24	
Multivariable HR (95% CI)	1.13 (0.15 to 8.44)	1.00 (referent)	1.13 (0.63 to 2.02)	1.14 (0.67 to 1.94)	1.33 (0.70 to 2.53)	.52	
All rectal cancers (n = 127)							
No. of cancers /person-years	0/23132	29/686917	28/387189	48/521129	22/230190		
Age-adjusted incidence rate‖ (95% CI)	0	3.3	5.1	5.1	9.6		
Age-adjusted HR (95% CI)	-	1.00 (referent)	1.59 (0.94 to 2.67)	1.97 (1.24 to 3.13)	2.05 (1.17 to 3.57)	.013	
Multivariable HR (95% CI)	-	1.00 (referent)	1.55 (0.92 to 2.62)	2.01 (1.25 to 3.23)	2.22 (1.24 to 3.99)	.01	
FASN-negative rectal cancers (n=47)							
No. of cancers /person-years	0/23132	9/686933	10/387202	18/521155	10/230198		
Age-adjusted incidence rate‖ (95% CI)	0	1.1	1.2	1.8	3.9		
Age-adjusted HR (95% CI)	-	1.00 (referent)	1.69 (0.69 to 4.18)	2.29 (1.03 to 5.12)	2.88 (1.17 to 7.13)	.024	.42
Multivariable HR (95% CI)	-	1.00 (referent)	1.67 (0.67 to 4.13)	2.39 (1.06 to 5.37)	3.14 (1.25 to 7.93)	.018	
FASN-positive rectal cancers (n = 80)							
No. of cancers /person-years	0/23132	20/686925	18/387201	30/521147	12/230200		
Age-adjusted incidence rate‖ (95% CI)	0	2.1	3.9	3.3	5.8		
Age-adjusted HR (95% CI)	-	1.00 (referent)	1.54 (0.81 to 2.93)	1.82 (1.03 to 3.21)	1.66 (0.81 to 3.39)	.17	

Multivariable HR (95% CI)	-	1.00 (referent)	1.51 (0.79 to 2.86)	1.84 (1.03 to 3.28)	1.80 (0.86 to 3.77)	.13

* Subanalyses were done by stratifying cases according to tumor location and FASN expression status. To compare the influence of BMI on FASN-positive colorectal cancer risk with that on FASN-negative colorectal cancer risk, we used a data duplication method in Cox proportional hazards model. We assessed the difference between the HR estimates according to tumor FASN status by likelihood ratio test that compared the model that allowed for separate associations of BMI by FASN status with a model that assumed a common association. The analysis was stratified by 1-year age and adjusted for age. In multivariate analyses, we adjusted for potential confounders, including age, physical activity (MET score/week in quintiles), energy-adjusted folate, vitamin D and calcium intake (in quintiles), total calorie (continuous), red meat intake (in quintiles), current smoking status (current, past, never), pack year of smoking before 30 years of age (0, 1-4, 5-10, ≥11), alcohol intake (0, 0.1-4.9, 5-14.9, ≥15 g/d), current multivitamin use (yes, no), current aspirin use (yes, no), previous sigmoidoscopy (never, ever), family history of colorectal cancer in any first-degree relative (yes, no), and menopausal status/postmenopausal hormone-replacement therapy use (premenopausal, postmenopausal never use, postmenopausal past use, postmenopausal current use). Colorectal cancers without FASN expression data were censored observations at the date of diagnosis. FASN = fatty acid synthase; BMI = body mass index; HR = hazard ratio; CI = confidence interval.

†Cumulative mean BMI was calculated using baseline height and cumulative mean weight which was the mean of all available weight data up to the start of each 2-year follow-up period.

‡*P* value for a linear trend by two-sided Wald test in women with cumulative mean body mass index greater than or equal to 18.5 kg/m^2.

§*P* value for heterogeneity by two-sided likelihood ratio test (for a multivariate linear trend) between FASN-positive and FASN-negative cancer risks in women with cumulative mean body mass index greater than or equal to 18.5 kg/m^2.

|| Incidence rate per 100,000 person-years. Age-adjusted incidence rates were standardized to the age distribution of the study population.