Published OnlineFirst August 25, 2014; DOI: 10.1158/1055-9965.EPI-14-0026

Cancer Epidemiology, Biomarkers & Prevention

Research Article

Colonic Mucosal Fatty Acid Synthase as an Early Biomarker for Colorectal Neoplasia: Modulation by Obesity and Gender

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Abstract

Background: We have previously reported that colonic pericryptal microvascular blood flow is augmented in the premalignant colonic epithelium, highlighting the increased metabolic demand of the proliferative epithelium as a marker of field carcinogenesis. However, its molecular basis is unexplored. In this study, we assessed the expression of a regulator of the "lipogenic switch," fatty acid synthase (FASN), in early colon carcinogenesis for its potential biomarker utility for concurrent neoplasia.

Methods: FASN expression (IHC) in the colonic epithelium from azoxymethane and polyposis in rat colon (Pirc) models of colorectal cancer was studied. FASN mRNA expression from endoscopically normal rectal mucosa was evaluated and correlated with colonoscopic findings (pathologic confirmation of neoplasia).

Results: FASN expression progressively increased from premalignant to malignant stage in the azoxymethane model (1.9- to 2.5-fold; $P < 0.0001$) and was also higher in the adenomas compared with adjacent uninvolved mucosa (1.8- to 3.4-fold; $P < 0.001$) in the Pirc model. Furthermore, FASN was significantly overexpressed in rectal biopsies from patients harboring adenomas compared with those with no adenomas. These effects were accentuated in male (~2-fold) and obese patients (1.4-fold compared with those with body mass index < 30). Overall, the performance of rectal FASN was excellent (AUROC of 0.81).

Conclusions: FASN is altered in the premalignant colonic mucosa and may serve as a marker for colonic neoplasia present elsewhere. The enhanced effects in men and obesity may have implications for identifying patient subgroups at risk for early-onset neoplasia.

Impact: These findings support the role of rectal FASN expression as a reliable biomarker of colonic neoplasia. *Cancer Epidemiol Biomarkers Prev; 23(11); 2413–21. ©2014 AACR.*

Introduction

Colorectal cancer ranks as the second leading cause of death from malignancy among Americans (1). Screening of the entire at-risk population (all patients older than 50) can decrease the incidence of colorectal cancer through the identification and endoscopic removal of precursor lesion, the adenomatous polyp. However, this "one-size-fits-all" strategy is inefficient as only about 7% of patients have screen relevant neoplasia, and thus the vast majority of patients are subjected to the discomfort, expense, and potential complications without deriving any significant colorectal cancer–preventive benefit (2). Thus, a more precise strategy to identify patients likely to harbor concurrent neoplasia is urgently needed to personalize screening recommendations.

Developing predictive models for colorectal cancer is complex as carcinogenesis is determined by both genetic and exogenous factors. While the genetic risk factors (e.g., germline mutations, polymorphisms) are well established, the predictive ability is suboptimal because it does not incorporate environmental risk factors (3), where it is estimated that approximately 70% of colorectal cancer risk is attributable to lifestyle factors (4). Therefore, the NIH risk score, which utilizes family history along with obesity, cigarette smoking, diet, and exercise, has been developed. Importantly, given the gender differences in colorectal cancer behavior, these models are specifically established for both men and women (5). Unfortunately, the overall performance of the NIH risk score has been found to be modest with an area under receiver operating characteristics curves (ROC) of 0.61.

One potential challenge in designing colorectal cancer prediction models is that genetic and environmental etiopathogeneic factors may not simply be additive, but rather

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doi: 10.1158/1055-9965.EPI-14-0026

Published OnlineFirst August 25, 2014; DOI: 10.1158/1055-9965.EPI-14-0026

interact in a complex unpredictable manner. For these reasons, assessing the colonic mucosa may be a more robust way to determine the neoplastic consequences of gene–environment interactions. The study of colonic field carcinogenesis (also known as field effect, field defect, field of injury) may provide unique insights into neoplastic transformation. Field effect posits that neoplastic insults lead to diffuse molecular events that provide the permissive environment for the stochastic mutations to take place in tumor suppressor gene/proto-oncogene, leading to focal tumors. The concept of field effect forms the underpinnings of our current clinical practice in which adenomas are used as biomarkers for determination of colonoscopic surveillance intervals. Previous work focusing on biomarkers in the endoscopically normal epithelium have shown that microvascular blood content was altered diffusely, suggesting that early metabolic changes may be a powerful marker of field carcinogenesis (6, 7). In many cancers, especially those driven by obesity, there is an increasing realization of a "lipogenic switch" as being one of the fundamental events. For instance, in breast and prostate cancer, fatty acid synthase (FASN) is overexpressed and may drive these changes (8). However, while incontrovertible in other organs, the data on FASN in colorectal cancer biology have been equivocal (9, 10). While FASN has not been explored in field carcinogenesis, there are intriguing reports on the abundance of lipid droplets (downstream fingerprint of FASN) in the premalignant colonic mucosa (11, 12). With regard to different cancer risk factors, the role of obesity is increasingly becoming evident. However, recent data indicate that men appear to be impacted disproportionately by obesity-related cancers than females (13). Therefore, based on these emerging trends, identifying susceptibility of obese subjects to elevated risk of developing colorectal cancer is very critical.

We hypothesized that FASN may be a marker of colonic field carcinogenesis. Therefore, this study sought to evaluate early expression of FASN in two experimental models of colon cancer (a carcinogenic and a genetic), as well as from human rectal tissue (assessing presence of dysplasia elsewhere in the colon). We were particularly interested in whether FASN expression recapitulated the epidemiologic data on obesity and colorectal cancer risk with regard to gender predilection for concurrent neoplastic transformation.

Materials and Methods

Animal studies

All the animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Northshore University HealthSystem (Evanston, IL). These animals were kept in climate-controlled housing (ambient temperature of 25°C, humidity of 60%, and a light/dark cycle of 12 hours).

Azoxymethane-treated rat model

Twenty-four male rats (Fisher F-344; 150–200 g) were procured from Harlan and maintained on AIN-76A diet (Harlan Teklad) with *ad libitum* access to drinking water. The rodents were randomized into two groups and given 2 weekly intraperitoneal injections of either azoxymethane (15 mg/kg body weight; 16 rats) or saline (8 rats). The azoxymethane-treated rat has a well-defined timeline with adenomas and carcinomas appearing after 15 to 20 weeks and 35 to 40 weeks, respectively, after the carcinogen initiation. These rats were euthanized after 20 or 40 weeks after injection. Their colons were isolated, flushed clean with PBS, and small distal sections were formalin fixed for immunohistochemical (IHC) processing.

Pirc rat model

Polyposis in rat colon (Pirc) and wild-type rats (8 each) were procured from Taconic. Pirc rats possess a germline mutation in the adenomatous polyposis coli (APC) tumor suppressor at codon 1137 leading to the development of multiple colonic neoplasms at 3 to 4 months of age (14). The animals were euthanized and the isolated colons longitudinally opened in entirety and rolled lengthwise into "Swiss rolls" with mucosa facing inward (15). This allows histologic evaluation and immunostaining of a large longitudinal colonic tissue within a single field of view.

IHC studies

Formalin-fixed rodent colonic segments and Swiss roll tissue samples were embedded in paraffin-wax blocks, sectioned (4 μm thick) along the vertical axis of the crypt, and mounted on Vectabond-coated Superfrost^{+} glass slides. Slides were baked for an hour at 70°C, deparaffinized in two washes of xylene, and rehydrated via graded ethanol washes. Antigen-epitope retrieval was achieved by pressure (cooker) microwaving (Nordic-Ware) in antigen-unmasking solution (Vector Laboratories) at a high-power setting for 2 × 9 minutes. Endoperoxidase activity was quenched by a 10-minute wash in 3% H_2O_2 and nonspecific binding blocked in 5% horse serum for 2 to 3 hours. Sections were incubated with the anti-FASN (1:200; mouse monoclonal; Cell Signaling Technology) or anti-proliferating nuclear cell antigen (PCNA) antibody (1:400; rabbit polyclonal; Santa Cruz Biotechnology) for 4 to 6 hours at 4°C. After washing in PBS, the sections were incubated with universal biotinylated secondary antibodies (1:2,000) for 30 minutes followed by complexing with avidin–biotin peroxidase using Vectastatin Elite ABC Reagent Kit (Vector Laboratories). For stain development 3,3′-diaminobenzidine (DAB) was used as chromagen substrate. For negative controls, sections were processed in the absence of primary antibodies. Complete longitudinal crypts extending from the muscularis mucosa to colonic lumen were counted for FASN-positive epithelial cells (8–10 random crypts per colon; $n = 8$). In the Pirc model, the random crypts away from any dysplastic or adenomatous regions were selected for FASN scoring.

Human studies

The human samples were acquired under an approved Institutional Review Board protocol from the NorthShore

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University HealthSystem with informed consent. For these studies, patients undergoing colonoscopy for screening or surveillance were included. The exclusion criteria included incomplete colonoscopy (poor preparation, inability to intubate cecum, or failure to recover polyps for pathology), patients taking anticoagulants, or other confounding factors such as inflammatory bowel disease. Two biopsies using cold forceps were taken from the endoscopically normal appearing rectal mucosa during colonoscopic withdrawal. Two rectal biopsies from each patient were subjected to real-time polymerase chain reaction (RT-PCR) analysis to detect FASN mRNA expression. Similarly, for obesity studies, we obtained 2 rectal biopsies from subjects that were dichotomized as obese [body mass index; (BMI) $\geq$ 30] or nonobese BMI (BMI $<$ 30), with each group equally distributed between subjects with (8) or without adenomas (8). These biopsies were subjected to FASN RT-PCR as described below.

RNA isolation

Total RNA from the human rectal biopsies was isolated using Ribopure RNA kit (Ambion; Life Technologies) and its concentration and purity established by spectrophotometric analysis (OD 260/280). The RNA was reverse transcribed with human FASN-specific TaqMan probes (Applied Biosystems) according to the manufacturer's instructions. Samples were assayed on Step-One Plus Real Time Thermocycler (Life Technologies) using RT kit and Universal PCR Master Mix. The relative concentration of FASN was calculated using the comparative ($2^{-\Delta\Delta C_t}$) method. The fold change was calculated as $\log_{10}$ RQ (RQ $= 2^{-\Delta\Delta C_t}$), and the RT-PCR data analysis was done using the RQ Manager 1.2.1 (Life Technologies).

Statistical analysis

Statistical significance (i.e., *P* value) of the observed differences between groups was determined by two-tailed Student *t* tests performed as a function in Microsoft Excel. Calculations of the ROC curves were performed in Matlab using the "roc" command.

Results

Modulation of FASN expression in uninvolved colonic mucosa of carcinogen-induced rat model of colon cancer

To demonstrate the applicability of FASN as a marker of field carcinogenesis, we utilized a well-characterized azoxymethane-treated rat model of colorectal cancer. This is a widely used multistep progression model of colorectal cancer that recapitulates a number of morphologic and molecular features typical of human sporadic colon cancer. For these studies, we performed IHC expression analyses of FASN from uninvolved colonic tissue (cancer field) collected from rats at 20 weeks (premalignant stage) and 40 weeks (malignant stage) of azoxymethane treatment. As demonstrated in Fig. 1, FASN expression (mean $\pm$ SD) in the uninvolved mucosa was augmented in azoxymethane-treated rats at both 20-week premalignant adenoma stage (11.05 ± 2.40) and 40-week malignant stage (14.53 ± 3.96) compared with saline control group (5.78 ± 1.75). These results demonstrate a progressive increase of FASN expression from premalignant (1.9-fold compared with saline-treated group; $P < 0.0001$) to malignant (2.5-fold compared with saline-treated group; $P < 0.0001$) stages of colorectal cancer development. FASN was mostly localized in the nuclear compartment (Fig 1C, inset) of colonic epithelial cells and abundantly expressed in the hyperproliferative zone of the crypt base.

Modulation of FASN expression in uninvolved colonic mucosa of genetic colon cancer model (Pirc rat)

To further establish that the overexpression of FASN in colonic tissues is not just a model-specific effect to carcinogen-initiated rats, we also utilized Pirc rats that possess a germline mutation in the APC tumor suppressor leading to early postnatal onset of multiple colonic adenomas (14). This model replicates the initiating genetic events in patients with sporadic colorectal cancer as well as familial adenomatous polyposis. As can be seen in the composite Fig. 2, hematoxylin and eosin (H&E)-stained colon (Swiss roll) demonstrates the presence of adenomas, microadenomas, and adenocarcinomas (Fig. 2B) in the Pirc rat. Pirc rat colon also demonstrates a hyperproliferative state of the mucosa illustrated by increased expression of a well-defined proliferation marker, PCNA, as compared with wild-type rat colons (Fig. 2D vs. C; $\sim$2.2-fold increase in average epithelial cell PCNA-positivity per crypt). Furthermore, higher expression of FASN was also observed in Pirc rat colon as compared with wild-type, with even higher expression in adenomas compared with adjacent histologically normal tissue (Fig. 2F). For these studies, complete longitudinal crypts extending from the muscularis mucosa to colonic lumen were scored for FASN-positive epithelial cells (8–10 random crypts per colon; $n = 8$) by two independent observers. FASN was overexpressed in the colonic uninvolved tissue compared with wild-type rats (1.71 ± 0.59 vs. 0.96 ± 0.77; $P \leq 0.05$) and in colonic adenomas (3.28 ± 0.88 vs. 0.96 ± 0.77; $P \leq 0.001$). These results demonstrate that the expression of FASN rises early during the carcinogenic process.

Human studies

In these studies, we compared the mRNA expression of FASN in random rectal biopsies collected from subjects diagnosed with (80 patients) or without (24 patients) the adenomas detected elsewhere in the colon. Demographically the population sets were comparable with 80% Caucasians, mean age (59 ± 7) and 51% males in the no-adenoma group and 83% Caucasians mean age (61 ± 9) and 58% males in the adenoma group. The patient samples selected from a larger repository of banked rectal biopsies were matched by gender.

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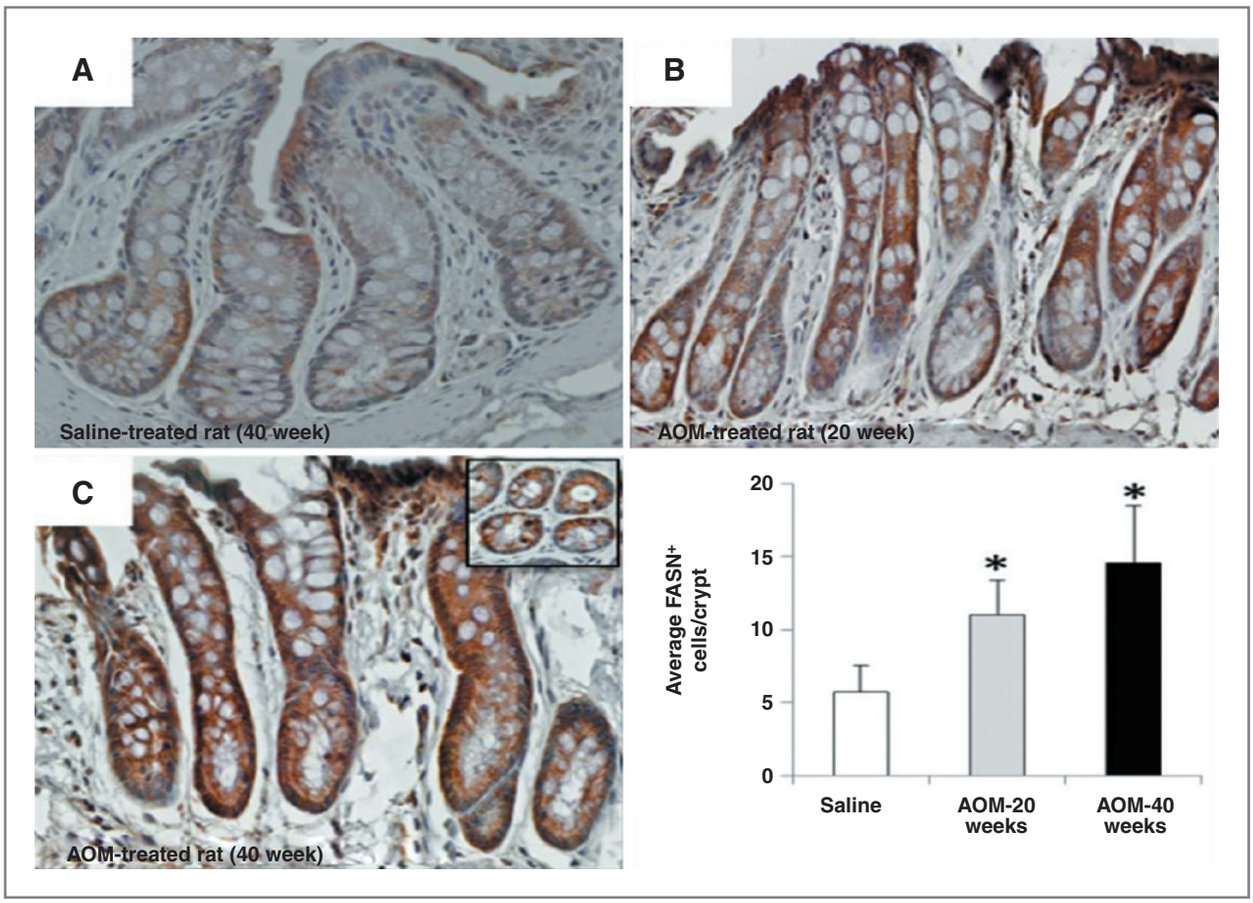

Figure 1. Increased IHC expression of FASN in the uninvolved colonic mucosa of the azoxymethane (AOM)-treated rat (carcinogen model of colorectal cancer). Distal colonic segments were fixed in formalin and sections immunostained for FASN as described in "Materials and Methods." The composite figure displays a collage of representative images of FASN staining (×20 optical and ×10 digital zoom) of the uninvolved colonic tissue collected from saline-treated (A; control; $n = 8$), 20-week azoxymethane-treated (B; premalignant stage; $n = 8$), and 40-week azoxymethane-treated rats (C; malignant stage; $n = 8$). The inset in C (×20 digital magnification) exhibits cross-sectional crypts with discrete cytoplasmic localization of FASN in the epithelial cells. Complete longitudinal crypts extending from the muscularis mucosa to colonic lumen were scored for FASN-positive epithelial cells (8–10 random crypts per colon; $n = 8$) by two independent observers. As shown in the histogram, FASN expression in the uninvolved mucosa progressively augmented in the azoxymethane-treated rats from premalignant (1.9-fold, compared with saline-treated group; $P < 0.0001$) to malignant stage (2.5-fold, compared with saline-treated group; $P < 0.0001$). FASN was highly expressed in the crypt base, and the proliferative zone was greatly extended in the azoxymethane-treated colons.

Increased expression of FASN in rectal biopsies from patients harboring colonic adenomas: gender-related implications

As demonstrated in Fig. 3A, compared with patients with no detected adenomas, FASN was significantly overexpressed in patients with adenomas detected elsewhere in the colon irrespective of gender. However, the increase was found to be greater in males (~5.5-fold) than females (~2.8-fold). The ability of FASN expression to distinguish between the patients with and without adenomas is summarized by the ROC curves in Fig. 3B. For males, the overall accuracy (quantified as the area under the ROC curve AUROC) is 0.87, whereas for females it is 0.77. This was also reflected in the performance characteristics of FASN in a gender-related manner with sensitivity and specificity of 0.78 and 0.92, respectively, in males compared with 0.68 and 0.83 in females. These results are consistent with the greater increase in FASN expression observed in males. This may offer biologic underpinning for the differences in colorectal cancer development between men and women and support our earlier findings suggesting that biomarkers of field carcinogenesis may have a gender selectivity (16).

Increased expression of FASN in rectal biopsies from patients with colonic adenomas: obesity-related implications

Figure 4A shows that even though FASN was higher in obese than nonobese subjects with no adenomas, it was significantly overexpressed in patients harboring lesions in both obese and nonobese subjects. The extent of overexpression, however, was higher (~1.4-fold) in adenoma-harboring obese subjects than in nonobese subjects with adenomas. This effect was found to be higher in males than females but statistically nonsignificant (data not

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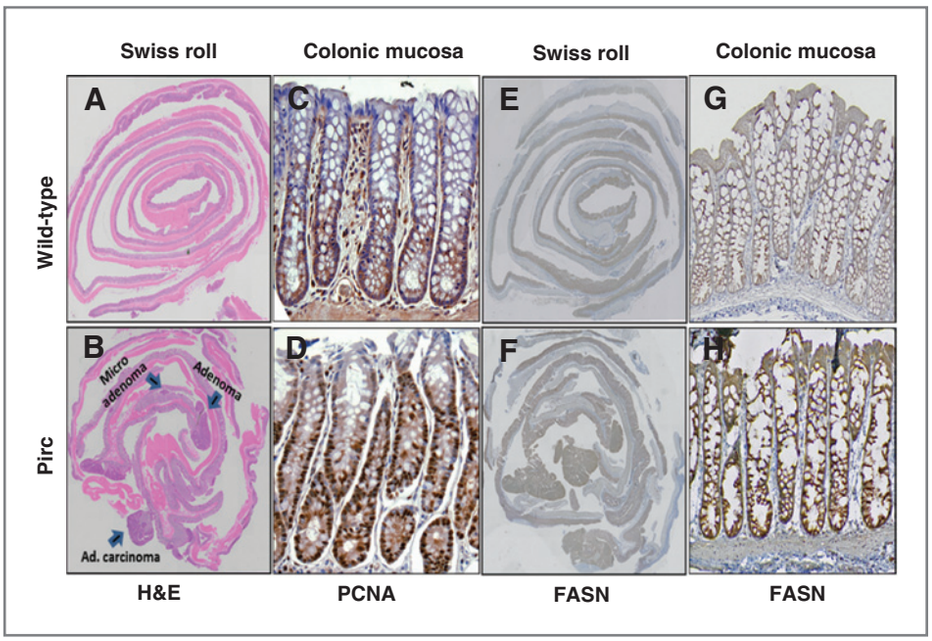

Figure 2. Increased IHC expression of FASN in the uninvolved colonic mucosa of the Pirc rat (genetic model of colorectal cancer). Sixteen Pirc and 8 wild-type rats were euthanized at 12 weeks of age (a stage when these animals express large number of intestinal polyps). The colons were longitudinally opened in entirety and rolled lengthwise into "Swiss rolls" with polyp-laden mucosa facing inward. The whole colonic preparation was then carefully placed in a formalin fixative and immunostained for proliferation marker PCNA as well as FASN as described in "Materials and Methods." The composite figure exhibits representative tissue sections from wild-type (A, C, E, and G) and Pirc rats (B, D, F, and H). A and B, H&E staining of the whole colon (Swiss roll; 10× image) showing the presence of adenomas, microadenomas, and adenocarcinomas in the Pirc rat colon. C and D, hyperproliferative state of a section of the mucosa from the Pirc rat colon (compared with wild-type). E and F, overall staining pattern of FASN in the whole colon (Swiss roll), with higher expression in Pirc rats. G and H, high-power (40× optical zoom) expression of FASN in the colonic mucosa from wild-type rat (G; $n = 8$) and Pirc rat colon (H; $n = 8$). Complete longitudinal crypts extending from the muscularis mucosa to colonic lumen were scored for FASN-positive epithelial cells (8–10 random crypts per colon; $n = 8$) by two independent observers. FASN was overexpressed in the colonic uninvolved tissue (1.8-fold, compared with wild-type rat; $P \leq 0.05$) and in colonic adenomas (3.4-fold, compared with wild-type rat; $P \leq 0.001$).

shown). The ROC curves generated for FASN expression in obese and nonobese subjects are shown in Fig. 4B. The performance characteristics of rectal FASN, for the presence of any sized adenomas, was good with a sensitivity and specificity of 0.75 and 0.79 for BMI > 30 and BMI < 30, respectively.

On the basis of our results, alterations in FASN expression in the rectal mucosa was a reliable biomarker for the presence of colonic neoplasia elsewhere in the colon. The improved discrimination in men versus women was mirrored in the data from azoxymethane-treated rats, where FASN was also found to be overexpressed in males compared with females (compared with saline-treated rats a fold increase of 2.5 in males vs. 0.5 in females, respectively $P < 0.05$).

Discussion

We demonstrated, for the first time, that FASN expression in histologically normal colorectal mucosa accurately mirrored the risk of colonic neoplasia, suggesting its role as a biomarker. Our data spans the gamut of preneoplastic and neoplastic time points in two animal models demonstrating that the expression of FASN increased even before the development of any neoplasia. The human case–control data also showed a marked increase in rectal FASN mRNA in patients harboring neoplasia elsewhere in colon. The effect was accentuated in obese patients, especially males, which is consonant with the epidemiologic data on the obesity → colorectal cancer relationship. In this regard, when the literature is viewed *in toto*, obesity more reliably increases colorectal cancer risk especially in men (17) but not in women (18).

This work suggests that FASN may be a robust marker of field carcinogenesis in both the genetic and carcinogenic animal models of colonic cancer as well as in human colorectal cancer. This is consistent with previous reports that have demonstrated increased FASN expression in the serum of patients with colorectal cancer (19). Furthermore, the observation that FASN was elevated at premalignant time points supports its potential application of predicting future neoplasia, albeit this needs to be corroborated with human data. There is considerable biologic precedence for molecular markers of field carcinogenesis, including methylation, miRNA, proteomic, gene expression (TNFα, MSH2, MLH1), and immunohistochemistry (crypt restricted cytochrome C oxidase). While the performance characteristics of FASN versus other biomarkers is difficult to compare given differences in study design and analysis (as many did not specify

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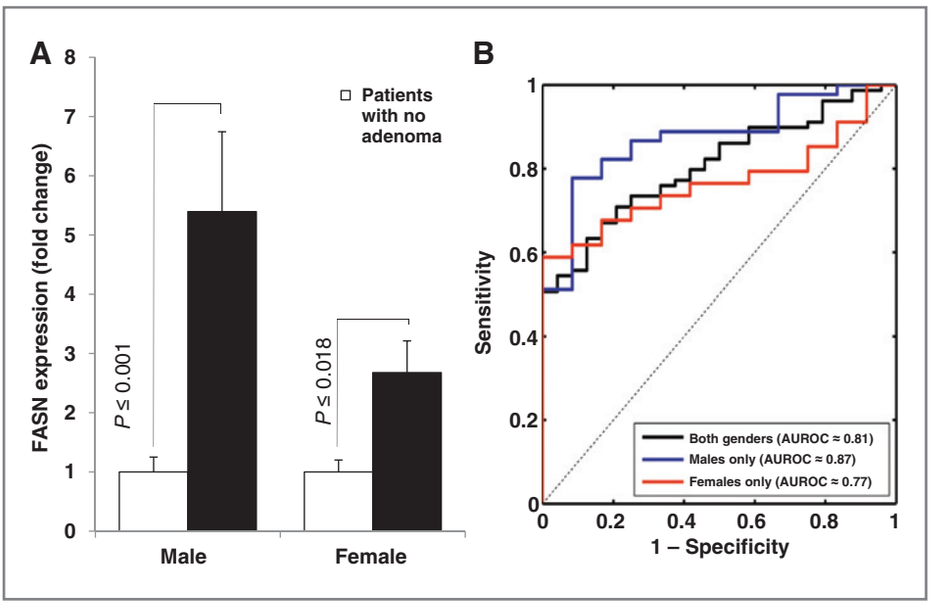

Figure 3. Increased mRNA expression of FASN in the rectal biopsies from patients with presence of colonic adenomas —gender-related implications. For these studies, two random rectal biopsies were collected from endoscopically normal appearing rectal mucosa from subjects diagnosed with (24 patients) or without (24 patients) any adenomas detected elsewhere in the colon. The patient samples selected were equally randomized between males and females. Total RNA from these biopsies was isolated using Ribopure RNA Kit (Ambion) and reverse transcribed with human FASN-specific TaqMan probes as described in the "Materials and Methods." Compared with patients with no detected adenomas, the FASN was significantly overexpressed in patients with adenomas detected elsewhere in the colon in both males ($P < 0.001$) and females ($P < 0.018$; A). However, the increase was found to be greater in males (~5.5-fold) than in females (~2.8-fold). B, the predictive ability of FASN expression as a biomarker of colorectal cancer. The accuracy of the test was higher for males (AUROC = 0.87) than for females (AUROC = 0.77).

performance characteristics), for those that did such as methylation, FASN did appear to be a superior biomarker (20). Furthermore, rectal FASN appeared to outperform high-profile reports of several standard fecal tests for colorectal cancer testing. For instance, at 95% specificity, the sensitivity of fecal DNA and fecal immunohistochemical test was only 17.2 and 7.6%, respectively (21). Again, comparisons among reports are fraught with peril, but the data does strongly support the potential utility of rectal FASN assessment.

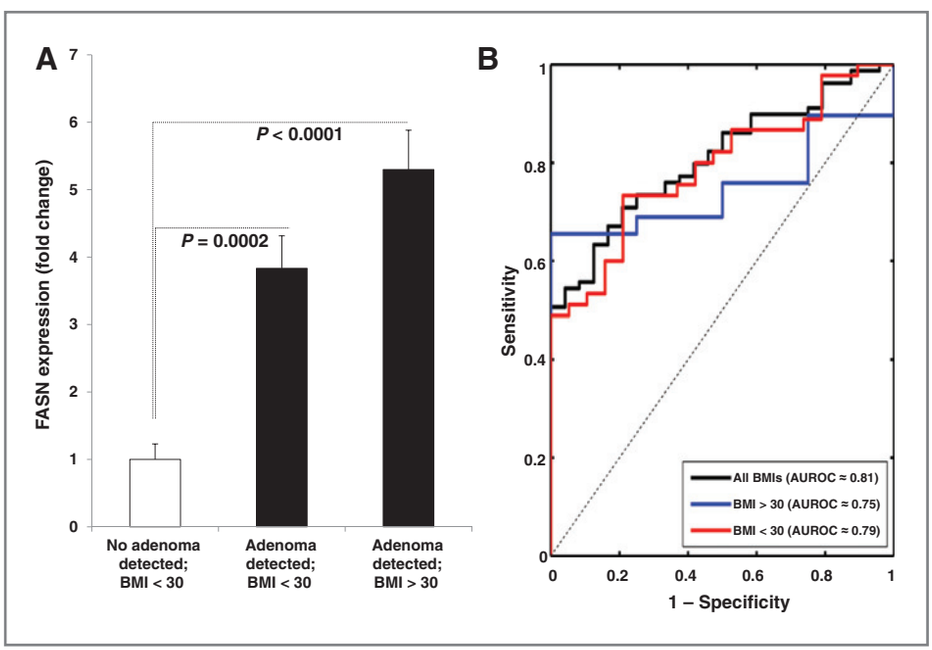

Figure 4. Increased mRNA expression of FASN in rectal biopsies from patients with presence of colonic adenomas —obesity-related implications. For these studies, we collected 2 rectal biopsies from subjects separated on the basis of their BMI of <30 (nonobese) or >30 (obese) with each group equally distributed between subjects with (8) or without adenomas (8). The biopsies were subjected to FASN RT-PCR as described in the "Materials and Methods." FASN was significantly overexpressed in patients harboring adenomas in both obese ($P \leq 0.0001$) and nonobese ($P \leq 0.0002$) subjects (A). The extent of overexpression, however, was greater in adenoma-harboring obese subjects than in nonobese subjects with adenomas (~1.4-fold). B, the predictive ability of FASN expression as a biomarker of colorectal cancer.

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The clinical relevance is further suggested by the fact that 11% of all colorectal cancers are diagnosed before the recommended age of conventional colonoscopic screening (age $\geq$ 50). Thus, one could speculate that inexpensive, minimally intrusive, and highly accurate biomarkers could be used to screen patients at an earlier age (22). A biomarker such as FASN could potentially be used to determine which younger patient (age $\leq$ 50 years) with risk factors such as obesity might benefit from an earlier colonoscopy. FASN may be particularly well-suited biomarker, as the levels of expression are higher in obese patients with neoplasia than in nonobese. Thus, while FASN expression is a marker for both nonobese and obese subjects, it may be particularly clinically valuable in obesity-related colonic neoplasia, which disproportionately occurs in young patients.

The superior performance of rectal FASN in men versus women may reflect well-established differences in biology. Indeed, while colorectal cancer ranks as the third leading cause of death in both men and women, there are distinct biologic characteristics that may have clinical implications. For instance, women have a higher propensity of developing proximal colonic neoplasia and harbor microsatellite instability (23). In addition, our group has shown that other modifiable risk factors of colorectal cancer such as smoking disproportionately target women (16, 23). Even though our results demonstrate that FASN was significantly overexpressed in colonic mucosa of patients with adenomas of both genders, the increase was more dramatic in males than females. While the biologic basis and implication of these findings are unclear, it is intriguing to note that obesity as a colorectal cancer risk factor has a penchant for men, mirroring our FASN expression data. There is precedence for biomarkers of colonic field carcinogenesis having a gender predilection (16). In colorectal cancer tissues, FASN has been reported to have gender specificity (24).

The data on presence of FASN and its implications in tumors *per se* are discordant with the survival effect appearing to be modified by obesity (25). On the other hand, FASN in the blood appears to be a more robust marker for poor colorectal cancer prognosis (26). Thus, while the data on colorectal cancers and progression are somewhat incongruous, our data on the role of FASN at the premalignant (initiation) phases are unequivocal. Indeed, the performance characteristics of FASN from the histologically normal mucosa to predict concurrent adenomas was excellent (AUROC = 0.81). The accentuation of FASN with obesity suggests its potential utility for colorectal cancer in younger patients (which can be driven by obesity). The increased FASN in males versus females suggests that this colonic neoplasia biomarker may have a significant gender predilection. There is precedence for significant gender-based biomarker specificity, as can be seen in serum (C-reactive protein; ref. 27) as well as from field carcinogenesis data (biophotonically derived or advanced adenomas; ref. 28).

Our finding that FASN may be involved in the earliest stages of colon carcinogenesis may give biologic insights into early metabolic events in colon carcinogenesis. Several lines of evidence (increased proliferation and microvascular blood flow) suggest that there is increased metabolic demand in the premalignant (histologically normal) colonic mucosa (7, 29). On the other hand, metabolic efficiency is known to be reduced in colorectal cancers as a consequence of the Warburg effect (preferential utilization of the less efficient glycolysis over oxidative phosphorylation in the normoxic environments; ref. 30). While unequivocal evidence for the Warburg effect in premalignant epithelium is lacking, it is intriguing to note that loss of crypt restrictive cytochrome C oxidase, a marker of mitochondrial status, is decreased in field carcinogenesis (31). Teleologically, the Warburg effect is believed to provide the necessary metabolites for rapid cellular growth. Recently, pronounced changes in lipid synthesis have been noted during carcinogenesis, which have been postulated to be necessary for creating plasma membranes, along with providing proneoplastic bioactive lipids (prostaglandins etc.; ref. 32). The change in fatty acids synthesis, the "lipogenic switch" (shift from lipolysis to lipogenesis) is increasingly well recognized (8). FASN is known to be overexpressed in colonic carcinomas along with a variety of other cancers (e.g., prostate and breast cancer; refs. 13, 33, 34). While this is the first report (to our knowledge) to demonstrate overexpression of FASN in the uninvolved mucosa, its expression has been reported to be elevated in aberrant crypt foci (35, 36), adenomas (37), and carcinomas (25). The biologic consequences of FASN include increased endothelial activity (important given that increased microcirculation is one of the earliest events in colorectal carcinogenesis) as well as proliferation (38). Whether these are related to direct signaling effects of FASN or through accumulation of bioactive mediators in lipid droplets is unclear. Indeed, lipid droplets are well established not only in colorectal cancer (12) but also in premalignant mucosa (as shown in MIN mouse model of intestinal tumorigenesis; ref. 39). Importantly, pharmacologic inhibition of FASN by C75 not only decreases lipid droplets but also cellular proliferation (11). Thus, there is a clear biologic rationale to suggest that FASN overexpression is a potential early driver of neoplastic transformation in the colon. Furthermore, pharmacologic inhibition of FASN has been shown to be a novel therapeutic approach for several cancers (40), including breast cancer chemoprevention (41). Several approaches inhibiting FASN have also been utilized effectively for clinical treatment of cancers, including non–small cell lung cancer (42). Parenthetically, orlistat, an FDA-approved antiobesity drug that also inhibits thioesterase domain of FASN, has been reported to be antineoplastic in xenograft models (43).

The strengths of our study include its novelty as a field effect biomarker, comprehensive approach using data from two animal models, clinical samples with subset analysis of gender and obesity. Nonetheless, we acknowledge several limitations of the study design. The clinical sample size was modest, and given these constraints only RT-PCR

Published OnlineFirst August 25, 2014; DOI: 10.1158/1055-9965.EPI-14-0026

measurements (message) was conducted. Fortunately, the animal data displayed similar trends with FASN protein expression. Moreover, just because obese patients developed, neoplasia does not mean that obesity *per se* may have instigated these proneoplastic changes. Hence, the FASN biomarker may just be true—true unrelated as for a robust estimate of performance is concerned. This necessitates analyzing independent validation sets in future studies. Finally, whereas BMI is a generalized measure of adiposity, anthropomorphic measures (hip/waist ratio) have been shown to correlate well with colorectal cancer risk in both men and women (44).

In conclusion, our data provide the first compelling evidence that FASN expression is altered in the premalignant (histologically normal) colonic mucosa. This has clear implications in the use of FASN as a biomarker for field carcinogenesis and hence in risk stratification. This appears to be true for both colon carcinogenesis in general and more dramatically in obesity related disease in men, which parallels epidemiologic data of obesity-driven colorectal cancer in men > women. Biologically, this work provides potential insights into the role of the lipogenic switch in early colon carcinogenesis. From a clinical perspective, one could envision assessment of FASN expression with a simple rectal swab to identify patients at risk for concurrent neoplasia and thus requiring colonoscopy. Approaches like this could lead to the ability to minimally intrusively identify patients with risk factors (e.g., obesity) who may benefit from screening before the age of 50 years.

Disclosure of Potential Conflicts of Interest

R.K. Wali has ownership interest (including patents) in Pegasus Biosolutions. H.K. Roy has ownership interest (including patents) in Nanocytomics, American Bio-optics, and Pegasus BioSolutons. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors thank Dr. Dhananjay Kunte for the technical help and Beth Parker for excellent work on article preparation.

Grant Support

This work was supported by NIH grants U01CA111257, R01CA156186, RO1CA165309, and R42CA168055 (to H.K. Roy, V. Backman).

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Received January 16, 2014; revised July 28, 2014; accepted August 13, 2014; published OnlineFirst August 25, 2014.

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Published OnlineFirst August 25, 2014; DOI: 10.1158/1055-9965.EPI-14-0026

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Cancer Epidemiol Biomarkers Prev 2014;23:2413-2421. Published OnlineFirst August 25, 2014.