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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 peri-cryptal 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 Pirc rat models of CRC was studied. FASN mRNA expression from endoscopically normal rectal mucosa was evaluated and correlated with colonoscopic findings (pathological 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 to 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 to those with no adenomas. These effects were accentuated in male (~2 fold) and obese patients (1.4 fold compared to those with BMI < 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.

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Keywords

Colon Cancer; Biomarker; Fatty Acid Synthase; Obesity; Gender

Introduction

Colorectal cancer (CRC) ranks as the second leading cause of death from malignancy among Americans [1]. Screening of the entire at-risk population (all patients over age 50) can decrease the incidence of CRC through identification and endoscopic removal of precursor lesion, the adenomatous polyp. However, this “one-size-fits-all” strategy is inefficient since 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 CRC preventive benefit [2]. Thus more precise strategy to identify patients likely to harbor concurrent neoplasia is urgently needed in order to personalize screening recommendations

Developing predictive models for CRC 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 CRC risk is attributable to lifestyle factors [4]. Therefore, the NIH risk score, which utilizes family history along with obesity, cigarette smoking, diet, exercise, has been developed. Importantly, given the gender differences in CRC behavior, these models are specifically established for both men and women [5]. Unfortunately, the overall performance of NIH risk score has been found to be modest with an area under receiver operator curve (ROC) of 0.61.

One potential challenge in designing CRC prediction models is that genetic and environmental etio-pathogenic factors may not simply be additive, but rather 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 which 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 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 CRC biology has 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 regards to different cancer risk factors, the role of obesity is increasingly becoming evident. However, recent data indicates 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 CRC 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 epidemiological data on obesity and CRC risk with regards 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. These animals were kept in climate controlled housing (ambient temperature of 25°C, humidity of 60% and a light/dark cycle of 12h).

Azoxymethane (AOM)-treated rat model

Twenty four male rats (Fisher F-344; 150-200g) were procured from Harlan, Indianapolis, IN 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 AOM (15mg/kg body weight; 16 rats) or saline (8 rats). The AOM-treated rat has a well-defined time line with adenomas and carcinomas appearing after 15-20 weeks and 35-40 weeks, respectively after the carcinogen initiation. These rats were euthanized after 20 or 40 weeks post injection. Their colons were isolated, flushed clean with PBS, and small distal sections were formalin fixed for immunohistochemical (IHC) processing.

Pirc (polyposis in rat colon) rat Model

Pirc and wildtype rats (8 each) were procured from Taconic (Hudson, NY). Pirc rats possess a germ-line mutation in the adenomatous polyposis coli (APC) tumor suppressor at codon 1137 leading to the development of multiple colonic neoplasms at 3-4 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 inwards [15]. This allows histological evaluation and immunostaining of a large longitudinal colonic tissue within a single field of view.

Immunohistochemical (IHC) Studies

Formalin fixed rodent colonic segments and Swiss roll tissue samples were embedded in paraffin-wax blocks, sectioned (4µm 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, de-

paraffinized 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 min. Endoperoxidase activity was quenched by 10 min wash in 3% H₂O₂ and non-specific binding blocked in 5% horse serum for 2-3h. Sections were incubated with the anti-FASN (1:200; mouse monoclonal; Cell Signaling) or anti-PCNA (proliferating nuclear cell antigen) antibody (1:400; rabbit polyclonal; Santa Cruz) for 4-6h at 4°C. After washing in PBS, the sections were incubated with universal biotinylated secondary antibodies (1:2000) for 30 min 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/colon; n=8). In the pirc model, the random crypts away from any dysplastic or adenomatous regions were selected for FASN scoring.

Humans Studies

The human samples were acquired under an approved Institutional Review Board protocol from the NorthShore University HealthSystem Evanston, IL 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 on anticoagulation, or other confounding factors such as inflammatory bowel disease. Two biopsies using cold forceps were taken from the endoscopically normal appearing rectal mucosa during colonoscope withdrawal. Two rectal biopsies from each patient were subjected to RT-PCR analysis to detect FASN mRNA expression. Similarly for obesity studies we obtained 2 rectal biopsies from subjects that were dichotomized as obese (BMI ≥ 30) or non-obese 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, Grand Island, NY) 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, Foster City, CA) 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^{-Ct}) method. The fold change was calculated as log₁₀ RQ (RQ= 2^{-Ct}) and the real time 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's t-tests performed as a function in Microsoft Excel.

Calculations of the receiver operating characteristic (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 AOM-treated rat model of CRC. This is a widely used multi-step progression model of CRC that recapitulates a number of morphological and molecular features typical of human sporadic colon cancer. For these studies, we performed immunohistochemical expression analyses of FASN from uninvolved colonic tissue (cancer field) collected from rats at 20 week (pre-malignant stage) and 40 week (malignant stage) of AOM treatment. As demonstrated in Figure 1, FASN expression (mean $\pm$ S.D) in the uninvolved mucosa was augmented in AOM-treated rats at both 20 week pre-malignant adenoma stage (11.05 ± 2.40) and 40 week malignant stage (14.53 ± 3.96) compared to saline control group (5.78 ± 1.75). These results demonstrate a progressive increase of FASN expression at pre-malignant (1.9 fold compared to saline-treated group; $p < 0.0001$) to malignant (2.5 fold compared to saline-treated group; $p < 0.0001$) stages of CRC development. FASN was mostly localized in the nuclear compartment (inset Fig 1, panel C) 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 adenomatous polyposis coli (APC) tumor suppressor leading to early postnatal onset of multiple colonic adenomas [14]. This model replicates the initiating genetic events in patients with sporadic CRC as well as familial adenomatous polyposis (FAP). As can be seen in the composite Figure 2, hematoxylin-eosin (H&E) stained colon (Swiss roll) demonstrates the presence of adenomas, micro adenomas and adenocarcinomas (panel B) in the Pirc rat. Pirc rat colon also demonstrates a hyper-proliferative state of the mucosa illustrated by increased expression of a well-defined proliferation marker, PCNA, as compared to wild-type rat colons (panel D vs C; ~ 2.2 fold increase in average epithelial cell PCNA-positivity/crypt). Furthermore, higher expression of FASN was also observed in Pirc rat colon as compared to wild-type, with even higher expression in adenomas compared to adjacent histologically normal tissue (inset panel F). 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/colon; $n=8$) by two independent observers. FASN was overexpressed in the colonic uninvolved tissue compared to wildtype rats (1.71 ± 0.59 vs 0.96 ± 0.77 ; $p < 0.05$) and in colonic adenomas (3.28 ± 0.88 vs 0.96 ± 0.77 ; $p < 0.001$). 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.

Increased Expression of FASN in Rectal Biopsies from Patients harboring Colonic Adenomas - Gender-Related Implications

As demonstrated in Fig 3a, compared to 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 while 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 to 0.68 and 0.83 in females. These results are consistent with the greater increase in FASN expression observed in males. This may offer biological underpinning for the differences in the CRC 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

Fig 4a shows that even though FASN was higher in obese than non-obese subjects with no adenomas, it was significantly overexpressed in patients harboring lesions in both obese and non-obese subjects. The extent of overexpression however was higher (~1.4 fold) in adenoma-harboring obese subjects than in non-obese subjects with adenomas. This effect was found to be higher in males than females but statistically non-significant (data not shown). The ROC curves generated for FASN expression in obese and non-obese 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.

Based on 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 AOM-treated rats, where FASN was also found to be overexpressed in males compared to females (compared to 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 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 prior to 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 epidemiological data on the obesity→CRC relationship. In this regard, when the literature is viewed in toto, obesity more reliably increases CRC 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 carcinogen animal models of colonic cancer as well as in human CRC. This is consistent with previous reports that have demonstrated increased FASN expression in the serum of CRC patients [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 biological precedence for molecular markers of field carcinogenesis including methylation, microRNA, proteomic, gene expression (TNF α , MSH2, MLH1) and immunohistochemical (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 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 CRC 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.

The clinical relevance is further suggested by the fact that 11% of all CRCs are diagnosed prior to the recommended age of conventional colonoscopic screening (age 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<50 years) with risk factors such as obesity might benefit from an earlier colonoscopy. FASN may be particularly well-suited biomarker, since the levels of expression are higher in obese patients with neoplasia than in non-obese. Thus, while FASN expression is a marker for both non-obese 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 CRC ranks as the third leading cause of deaths in both men and women, there are distinct biological characteristics that may have clinical implications. For instance, women have a higher propensity of developing proximal colonic neoplasia and harbor microsatellite instability [23]. Additionally our group has shown that other modifiable risk factors of CRC 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 biological basis and implication of these findings is unclear, it is intriguing to note that obesity as a CRC 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 is 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 CRC prognosis [26]. Thus, while the data on CRCs and progression is somewhat incongruous, our data on the role of FASN at the premalignant (initiation) phases is 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 CRC 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) [27] as well as from field carcinogenesis data (biophotonically derived or advanced adenomas) [28].

Our finding that FASN may be involved in the earliest stages of colon carcinogenesis may give biological 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) [30]. While unequivocal evidence for 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, 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 pro-neoplastic bio-active lipids (prostaglandins etc.) [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 (prostate, breast etc.) [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 biological consequences of FASN include increased endothelial activity (important given that increased micro-circulation 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 not only well established in CRC [12] but also in premalignant mucosa (as shown in MIN mouse model of intestinal tumorigenesis) [39]. Importantly, pharmacological

inhibition of FASN by C75 not only decreases lipid droplets but also cellular proliferation [11]. Thus, there is clear biological rationale to suggest that FASN overexpression is a potential early driver of neoplastic transformation in the colon. Furthermore, pharmacological 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, a FDA-approved anti-obesity drug which also inhibits thioesterase domain of FASN has been reported to be anti-neoplastic in xenograft models [43].

The strengths of our study includes 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 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 pro-neoplastic changes. Accordingly, 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) has been shown to correlate well with CRC risk in both men and women [44].

In conclusion, our data provides 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 epidemiological data of obesity driven CRC 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 age 50.

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Figure 1.

Increased immunohistochemical expression of FASN in the uninvolved colonic mucosa of the azoxymethane (AOM) treated rat (carcinogen model of CRC). 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 (Panel A; Control; n=8), 20 week AOM-treated (Panel B; premalignant stage; n=8) and 40 week AOM-treated (Panel C; malignant stage; n=8). The inset in Panel 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/colon; n=8) by two independent observers. As shown in the histogram, FASN expression in the uninvolved mucosa progressively augmented in the AOM-treated rats from pre-malignant (1.9 fold compared to saline-treated group; $p < 0.0001$) to malignant stage (2.5 fold compared to saline-treated group; $p < 0.0001$). FASN was highly expressed in the crypt base and the proliferative zone was greatly extended in the AOM-treated colons.

Figure 2.

Increased immunohistochemical expression of FASN in the uninvolved colonic mucosa of the Pirc rat (genetic model of CRC). 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 inwards. 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 (Panels A, C, E and G) and Pirc rats (Panels B, D, F and H). Panels A-B depict H&E staining of the whole colon (Swiss roll; 10× image) showing the presence of adenomas, micro adenomas and adenocarcinomas in the Pirc rat colon. Panels C - D demonstrate the hyper-proliferative state of a section of the mucosa from the pirc rat colon (compared to wild-type). Panels E-F demonstrates the overall staining pattern of FASN in the whole colon (Swiss roll), with higher expression in Pirc rats. Panels G-H demonstrate the high power (40× optical zoom) expression of FASN in the colonic mucosa from wild-type rat (Panel G; n=8) and Pirc-rat colon (Panel 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/colon; n=8) by two independent observers. FASN was overexpressed in the colonic uninvolved tissue (1.8 fold compared to wildtype rat; ^+p 0.05) and in colonic adenomas (3.4 fold compared to wildtype rat; *p 0.001).

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 to 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 ($p < 0.018$) (Panel a). However, the increase was found to be greater in males (~5.5 fold) than females (~2.8 fold). Panel (b) depicts the predictive ability of FASN expression as a biomarker of CRC. The accuracy of the test was higher for males (AUROC=0.87) than for females (AUROC=0.77).

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 (non-obese) 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 0.0001) and non-obese (p 0.0002) subjects (Panel a). The extent of overexpression however was greater in adenoma-harboring obese subjects than non-obese subjects with adenomas (~1.4 fold). Panel (b) depicts the predictive ability of FASN expression as a biomarker of CRC.