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Increased expression of fatty acid synthase in human aberrant crypt foci: possible target for colorectal cancer prevention

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Abstract

Aberrant crypt foci (ACF), the earliest identified monoclonal lesions in the colon, provide insights into changes that promote and/or accompany the transformation of normal colonic epithelial cells to colorectal cancer. Fatty acid synthase (FAS), the primary enzyme involved in *de novo* lipogenesis from carbohydrates, is expressed at low levels in most normal human tissues but is elevated in several human neoplasms including colorectal adenomas and carcinomas. To mdetermine if this pathway is altered even earlier in colorectal tumorigenesis, 35 human ACF from 21 patients were evaluated for the immunohistochemical expression of FAS. Sections of colon cancer served as positive controls, and normal colonic mucosa distant from cancer or ACF served as negative controls. FAS expression was increased in 30 (86%) ACF compared with that in adjacent normal colonic mucosa. The expression of FAS in ACF was not related to the degree of dysplasia or to the number of crypts in the ACF. The over expression of FAS in a high proportion of ACF suggests that this enzyme plays an important role very early in colorectal tumorigenesis and may be a target for chemoprevention.

Keywords

human aberrant crypt foci; colon cancer precursors; biomarkers; chemoprevention; colorectal cancer

Fatty acid synthase (FAS) is the only mammalian enzyme capable of *de novo* long-chain fatty acid synthesis from smaller carbohydrate substrates.1 In adults, it is expressed primarily in hormone-sensitive tissues and those with high lipid metabolism.2 The expression and activity of FAS in normal tissues is highly regulated by diet, hormones and growth factors: carbohydrates, insulin, and transforming growth factor-beta all upregulate the expression and activity of FAS (reviewed in ref 3). In humans on a normal diet in industrialized countries, the expression and activity of FAS is low, even in lipogenic organs like liver and adipose tissue, due to its suppression by small amounts of dietary fatty acids.3,4 Consequently, FAS plays only a minor role in human lipogenesis.4 In contrast, FAS is highly expressed by many human cancers including prostate, breast, ovarian, endometrial, thyroid, colorectal (reviewed in ref 5); bladder,6 lung,7 oral mucosa,8 tongue,9 esophageal,10 and stomach11 carcinomas as well as melanoma,12 retinoblastoma,13 and nephroblastoma.14 Increased expression of FAS has also been reported in some benign or preinvasive lesions of the prostate, colon, breast (reviewed in refs 5,15); lung,7 cutaneous nevi,12 and stomach.11 We evaluated the expression of FAS in aberrant crypt foci (ACF), the earliest microscopically identified monoclonal lesions in the colon.16 Increased

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expression of FAS was seen in 30 of 35 (86%) of ACF from 21 patients with sporadic colorectal cancer or familial adenomatous polyposis (FAP).

Materials and methods

Paraffin-embedded sections of ACF with adjacent normal colonic mucosa or samples of human colorectal cancers were stored at 4°C for several months prior to immunohistochemical analysis as detailed previously.17 These studies on discarded tissues were performed after approval by our Institutional Review Board for Human Subjects and in accord with an assurance filed with and approved by the U.S. Department of Health and Human Services. Sections were heated in a pressure cooker with 0.1 M Tris buffer, pH 8.6 for 5 minutes at full pressure and incubated with affinity purified rabbit anti-human FAS antibody (Assay Designs, Inc, Ann Arbor, MI) diluted 1:100 and 1:200 in 0.1 M Tris buffered saline. This antibody, raised against a conjugate of a synthetic peptide from human FAS, was developed and characterized by Kusakabe et al.2 Colorectal tumor sections provided positive controls for FAS expression; rabbit normal IgG (Dako, Carpinteria, CA) at a similar protein concentration provided negative controls. All 35 ACF were stained twice; their expression of FAS was scored as positive if it appeared increased relative to the surrounding normal crypts by two investigators and negative if the ACF did not stain or stained similarly to the surrounding normal crypts.

Of the ACF evaluated in this study, 33 were from 20 patients with sporadic colorectal cancer; two were from one patient with FAP without colon cancer. The sporadic colorectal cancer patients were 66 ± 10 (range: 42–81) years of age; 8 female, 12 males; 2 black, 16 Caucasian, and 2 unidentified race. The FAP patient was a 31-year-old, black female. One ACF was from the proximal colon, 34 ACF from the distal colon; 11 had mild dysplasia, 24 were without dysplasia; and the ACF had a mean of 64 ± 48 (range: 13–200) crypts per focus.

Results

FAS showed immunohistochemically demonstrable increased expression in 30 of 35 (86%) of ACF compared with the very low or absent expression of FAS in the adjacent normal mucosa (Figure 1). As seen in this figure, some ACF (Figure 1C,D) from sporadic colorectal cancer patients had uniformly high expression of FAS throughout the crypts of the ACF while other ACF (Figure 1E,F) showed a gradient of expression from the bottom of the crypt, where it is highest, to the top of the crypt. This type of gradient of expression was seen in 20 of 30 ACF with increased expression of FAS. The two ACF from an FAP patient also showed a gradient of expression, but here the highest expression was at the top of the crypts. Since only a single FAP patient was evaluated, we do not know if this is characteristic of the FAP phenotype.

In our study, increased expression of FAS was seen in ACF with 13–200 crypts per focus and in 21 of 30 (70%) ACF without dysplasia, i.e., the expression of FAS was independent of the number of crypts in the ACF and whether or not the ACF was dysplastic. With a very large proportion of ACF showing increased expression of FAS, it would require a very large sample size to detect any variation with these parameters. Overexpression of FAS in 86% of ACF is second only to the overexpression of carcinoembryonic antigen (CEA) in 93% of human ACF (see ref 18 for review of altered phenotypes in human ACF).

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Discussion

Various mechanisms have been proposed to explain the increase of FAS in a wide range of malignancies and their putative precursors. The requirements of increased proliferation seen in cancer cells and their precursors may lead to up regulation of FAS to satisfy the demands for fatty acids as a source of energy and of phospholipids for membranes.2،3 In addition, the high levels of circulating insulin, associated with increased colon cancer risk,19 may contribute to the stimulation of the expression and activity of FAS in the colon. Since increased levels of insulin are not associated with many other tumors and precancerous lesions, this is unlikely to be the major factor affecting FAS expression in tumors and their precursors. The microenvironments of most tumors include areas of hypoxia and low pH. Another interesting hypothesis (discussed in ref 3) is that the induction of FAS may be a means of producing oxidized intermediates, under limited oxygen conditions, through its utilization of NADPH. For every palmitate generated, the predominant product of FAS, 14 equivalents of NADPH are consumed (see ref 5). The ability of cancer cells to supply oxidizing equivalents under hypoxic conditions may provide a major growth advantage to these cells.

Increased FAS expression in cancer is often associated with poor prognosis (reviewed in refs 5،13،14،20،21). While increased FAS expression was reported in 81%20 to 100%22 of colorectal cancers, neither study found FAS expression to be an independent prognostic indicator of survival. Both studies20،22 found increased expression of FAS in colonic polyps; Visca et al20 found increased FAS expression in 18% of 100 adenomas while Rashid et al22 found increased FAS expression in all 36 adenomas evaluated as well as microadenomas in FAP patients and 3 of 3 hyperplastic polyps. Our studies extend these observations to microscopically identified ACF, the earliest monoclonal lesions identified in the colorectum.

FAS has been proposed as a drug target for cancer due to its limited distribution in humans. Cerulenin, a natural antibiotic and an inhibitor of FAS, inhibited the growth of a wide variety of cancer cell lines, but did not inhibit the growth of normal human fibroblasts in vitro (reviewed in ref 5). In a series of human breast cancer cell lines, demonstration that the cytotoxic effect of cerulenin was proportional to the level of FAS activity provided further evidence that neoplastic growth was dependent on FAS (reviewed in ref 5). Cerulenin and C75, a small molecule inhibitor of FAS, are also selectively cytotoxic for human cancer xenografts in vivo in rodents, but both cause significant weight loss as well (reviewed in refs 1،5). A more recent synthetic FAS inhibitor, C93, is cytotoxic to cancer cell xenografts without causing significant weight loss or cytotoxicity to proliferating cells.23 While there appears to be little toxicity with the inhibition of FAS in normal cells, its inhibition in human cancer cells both *in vitro* and *in vivo* induces apoptosis (reviewed in ref 1).

With the identification of increased FAS expression in early neoplastic lesions, including ACF, FAS inhibitors are also being considered for chemoprevention (reviewed in ref 1). The FAS inhibitor, trisclosan, reduced the incidence of mammary tumors in rats initiated with a single dose of methylnitrosourea.24 More recently, soy protein reduced the colonic expression of FAS and the circulating level of insulin one day after rats were initiated with azoxymethane. This biochemical pathway likely explains the previously demonstrated inhibition of colon cancer by soy protein in this animal model.25

In summary, the increased expression of FAS in 86% human ACF suggests its importance from very early in the development of colonic neoplasms. The selective toxicity of inhibitors of FAS makes this pathway a good therapeutic target for both chemopreventive and chemotherapeutic drugs in the colorectum.

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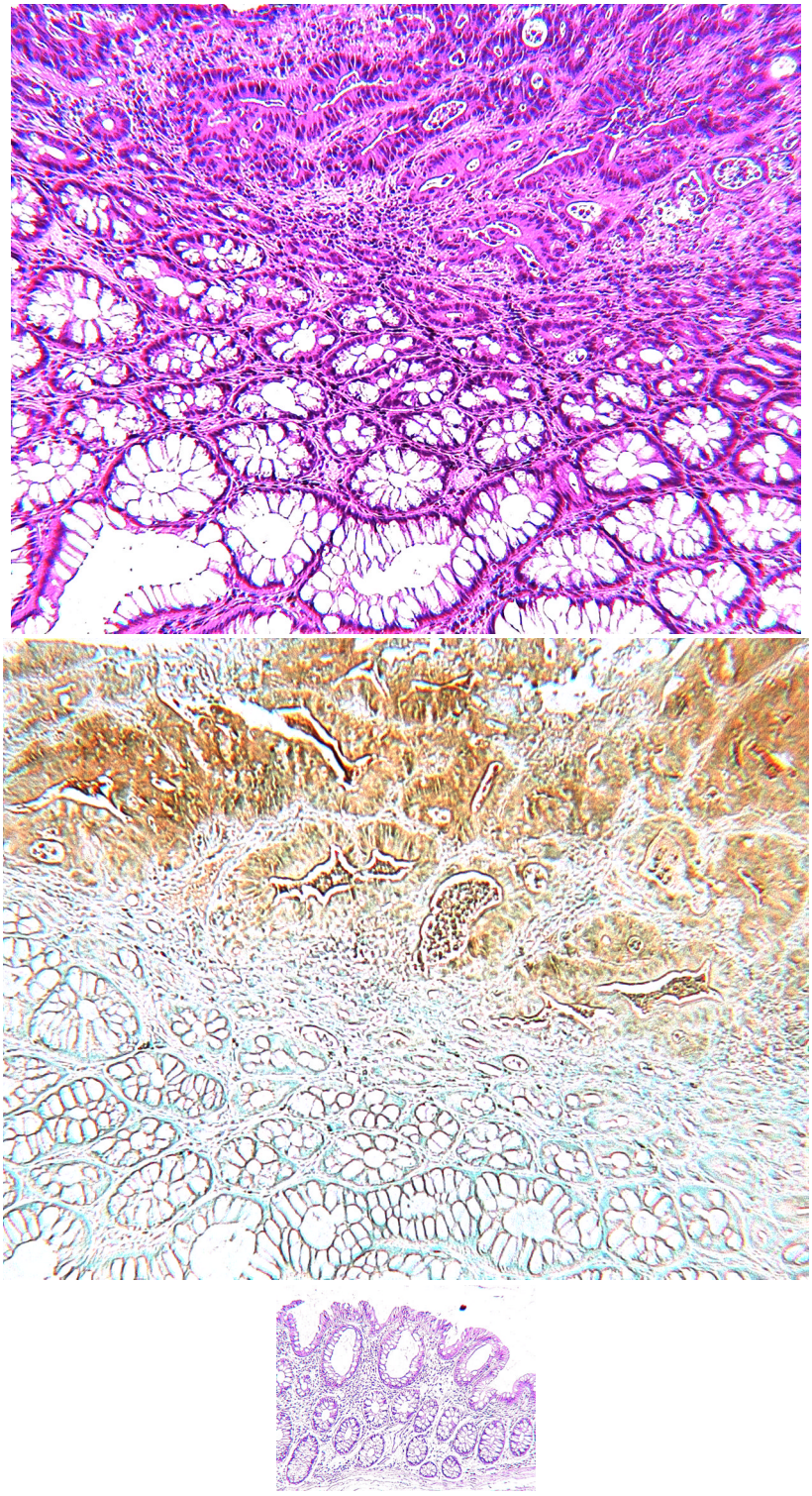

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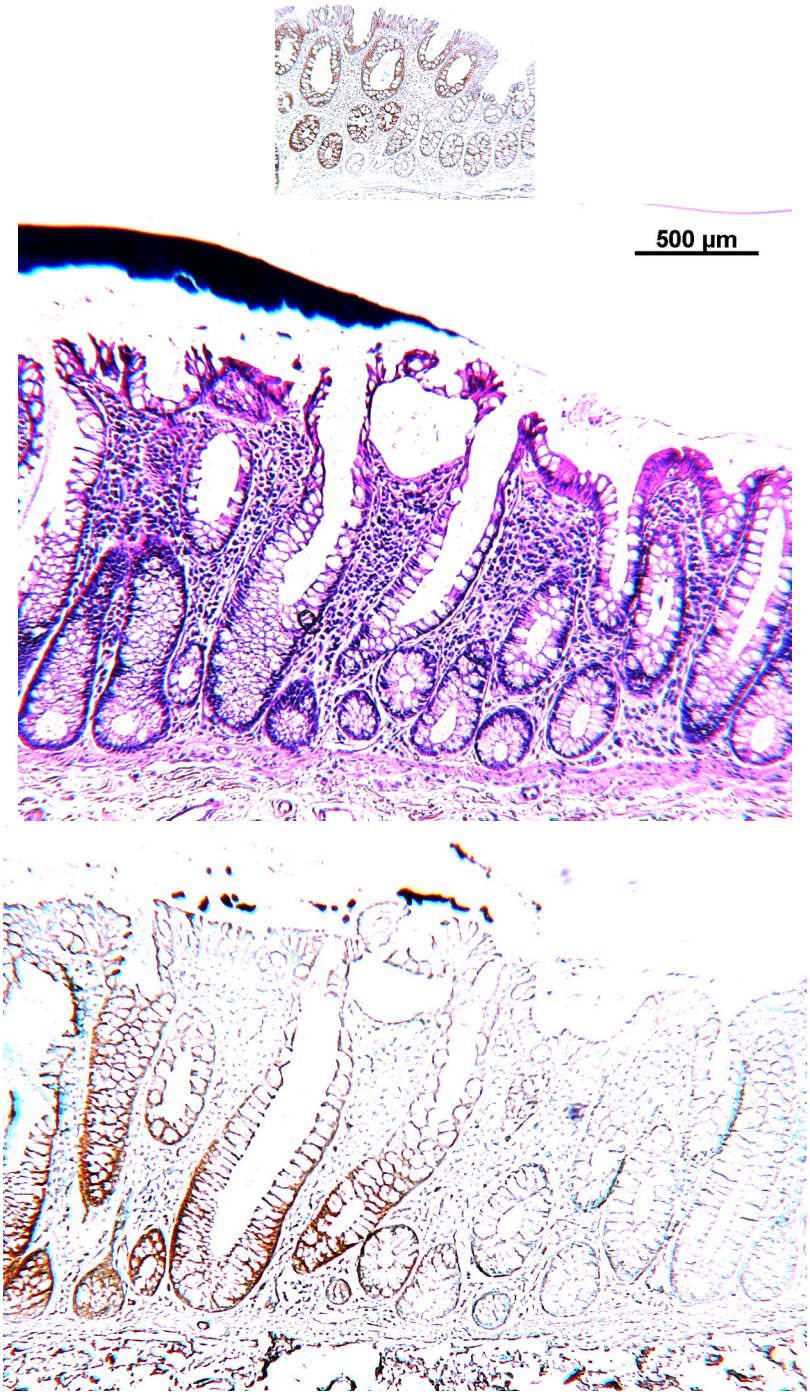

Figure 1.

Photomicrographs of a sigmoid colon cancer (Figures 1A and B) from an 89-year-old female and two aberrant crypt foci (ACF) without dysplasia from two different patients with sporadic colorectal cancer in the distal colon all taken at the same magnification. The top row (Figures 1A, C, E) after staining with hematoxylin and eosin; the bottom row (Figures 1B, D, F), nearby sections from the same tissues as above, after antibody to human FAS with diaminobenzidine as substrate. Note the strong, though somewhat heterogeneous, expression of FAS throughout the cancer (top portion of Figure 1B) compared to the normal

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glands (bottom portion of Figure 1B). Figures 1C and D show a portion of an ACF with 180 crypts, Figures 1E and F, an ACF with 63 crypts. The immunohistochemical expression of FAS in Figures 1D and F clearly delineates the ACF (left portion of the Figures) from the normal adjacent glands (right portion of the Figures). Some ink, used to mark ACF in the intact tissue, is visible above the ACF in Figures 1E and F.