Overexpression of Fatty Acid Synthase in Ulcerative Colitis

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Abstract

Fatty acid synthase (FAS) is an enzyme that catalyzes the synthesis of long-chain fatty acids. The enzyme expression is minimal in adult tissues and very high in many cancers. Ulcerative colitis is a chronic inflammatory bowel disease that, when long-standing, is associated with an increased risk of colon cancer. The aim of the present study was to establish whether FAS levels in the mucosa without dysplasia of patients with long-standing ulcerative colitis were higher than in control subjects.

Three groups of patients were selected: 30 with active ulcerative colitis, 30 with ulcerative colitis in remission, and 30 undergoing colonoscopy for colorectal cancer screening, as healthy control subjects. FAS expression was evaluated with immunohistochemical procedures. The enzyme was detected in all patients with active colitis, in most patients with quiescent disease, in both pathologic and normal mucosa, but in only 3 healthy control subjects. Our results suggest that extension of ulcerative colitis is greater than that revealed by common diagnostic techniques.

Fatty acid synthase (FAS) is a multifunctional enzyme with 2 identical subunits of 260,000 kd containing 7 catalytic domains and a 4'-phosphopantetheine prosthetic group. FAS catalyzes the synthesis of long-chain fatty acids from acetyl-coenzyme A (CoA), malonyl-CoA, NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate), and adenosine triphosphate.[1] The main product of FAS is palmitate (80%); the enzyme also produces stearate (10%) and myristate (10%). FAS expression in lipogenic tissues is mainly regulated by sterol regulatory element-binding proteins at the transcriptional level.[2] Long-chain fatty acids are essential constituents of membrane lipids and are important substrates for energy metabolism in the cells.[1]

In adult tissues, high FAS expression is found in cells with active lipid metabolism and/or hormone sensitivity such as hepatocytes, adipocytes, type II alveolar lung cells, and cells of the mammary and endometrial glands during the proliferative phase; FAS has specific physiologic functions such as energy storage and production of surfactant in lung and lipids in lactating breast tissue.[3] FAS expression in differentiated tissues is regulated by hormones such as insulin, glucagon, glucocorticoids, and thyroxine and by nutrients (glucose and fatty acids).[4]

FAS levels in most normal tissues are minimal owing to their down-regulation by dietary lipids, but the enzyme is expressed highly in many human cancers, including breast, prostate, colon, lung, and stomach, insensitive to circulating lipids and producing high levels of fatty acids.[5-13] Insensitivity to nutritional signals facilitates disease progression in malnourished patients.[14] Ulcerative colitis (UC) is an inflammatory disease that is associated with an increased risk of colon cancer when the duration is more than 8 years, defined as

long-standing.[15,16] Rashid et al[9] showed that FAS is increased in the dysplastic epithelium arising in UC and in the adjacent mucosa with chronic inflammatory changes. The aim of the present study was to establish whether FAS levels in the mucosa without dysplasia in patients with long-standing UC are higher than in control subjects.

Materials and Methods

Patients

All consecutive patients with long-standing UC, left-sided or diffuse, attending the outpatient department of inflammatory bowel diseases were eligible for the study. Two groups of 30 patients with UC, active and in remission, were selected. Exclusion criteria were dysplasia and current therapy with steroids, thyroid hormone, or insulin. The presence of dysplasia was excluded with endoscopic and histologic surveillance performed annually after 8 years from the onset of UC. We also included 30 healthy volunteers, screened for colorectal neoplasia, as control subjects.

According to the study design, 1 biopsy specimen was obtained from the mucosa involved by UC and from the normal mucosa in all patients. One biopsy specimen was obtained from the sigmoid colon of control subjects. The classification of Truelove et al[17] was used in the histologic diagnosis of biopsy specimens from patients with UC. Informed written consent was obtained from all patients and control subjects, and the study protocol was approved by the University Ethics Committee.

Immunohistochemical Procedures and Evaluation of FAS Expression

Immunohistochemical procedures were performed on formalin-fixed, paraffin-embedded biopsy tissue samples. Briefly, sections 3 μm thick were cut from paraffin blocks and mounted on Super-Frost/Plus microscope slides (BDH Laboratory Supplies, Menzel, Germany). All slides were deparaffinized in xylol and rehydrated with a graduated sequence of ethanol. Endogenous peroxidase activity was blocked with methanol-hydrogen peroxide for 15 minutes. Slides then were incubated with the primary FAS antibody (gift from F.P. Kuhajda, MD, Department of Pathology, JHMI, Baltimore, MD) overnight at a concentration of 1:3,000. Thereafter, slides were reacted with the avidin-biotin system and 3,3'-diaminobenzidine as the chromogen. Nuclear counterstaining was obtained using Meyer hematoxylin. Slides were considered to express FAS when strong granular cytoplasmic staining was observed in the cells of the surface epithelium (SE) and glandular epithelium (GE).

Statistical Analysis

Statistical analysis was performed using the Fisher exact test with Graph Software. The odds ratio and 95% confidence interval were considered as the value of statistical significance of comparisons of FAS expression as follows: (1) in patients with UC for active, quiescent, and normal mucosa; (2) in patients with UC between active and quiescent mucosa; and (3) between active and quiescent mucosa of patients with UC and control subjects. All P values are 2-sided. A P value of less than .05 was considered significant.

Results

Demographic and clinical characteristics of the study population are shown in **Table 1**. No significant differences were found in the characteristics of patients with active and quiescent UC.

In active UC, FAS was observed in all patients (100%) in both SE and GE of the pathologic **Image 1** and **Image 2** and normal mucosa **Image 3**. In quiescent UC, FAS in the pathologic mucosa was found in SE in 17 patients (57%) and in GE in 28 (93%) **Image 4**; in the normal mucosa, FAS was found in SE in 13 patients (43%) and in GE in 17 (57%) **Image 5**. In control subjects, FAS was observed in 3 patients (10%) in both SE and GE **Image 6**.

Results of the statistical comparisons of FAS in the mucosa for the 2 groups of patients are shown in **Table 2**. FAS was significantly higher in active than in quiescent mucosa if SE, and it was comparable in GE. Moreover, in patients with UC in remission, FAS expression in SE was comparable in pathologic and normal mucosa but was significantly higher in GE. Finally, FAS expression in the pathologic and normal mucosa of patients with UC was significantly greater than in the healthy mucosa of control subjects **Table 3**.

Table 1
Demographic and Clinical Characteristics of Patients and Control Subjects

	Patients		
	Active UC (n = 30)	Quiescent UC (n = 30)	Control Subjects (n = 30)
Mean age ± SD (y)	58.0 ± 9.9	56.9 ± 8.9	63 ± 15.0
Sex (M/F)	17/13	15/15	14/16
Mean duration of UC ± SD (y)	16.2 ± 8.6	16.3 ± 5.6	—
Mean age at onset ± SD (y)	35.0 ± 9.3	40.0 ± 12.0	—
Extension of disease			
Left-sided	9	12	—
Diffuse	21	18	—

UC, ulcerative colitis.

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Discussion

FAS expression was very high in our patients with UC in pathologic and normal mucosa. These results are surprising and stimulate further research that should explain whether the increase of FAS in UC mucosa is a cause or an effect of the disease and, in the latter case, whether it is as favorable for epithelial cells of the colon as it is for cancer cells or is harmful. At the moment, it is possible only to advance hypotheses.

First, it is necessary to assess in mucosa affected by UC the activity of FAS and of acetyl-CoA carboxylase, the enzyme that synthesizes malonyl-CoA, the predominant substrate for FAS. However, the increase in intracellular levels of FAS is associated with increased production of fatty acids in many physiologic and pathologic conditions, as shown by many investigators; therefore, we can reasonably assume that the same also occurs in UC.

Treatment of human breast and prostate cancer xenografts with FAS inhibitors in athymic mice has shown a significant antitumor effect without toxic effects on normal proliferating tissues such as bone marrow, skin, liver, and gastrointestinal tract.[18] These results seem to suggest that

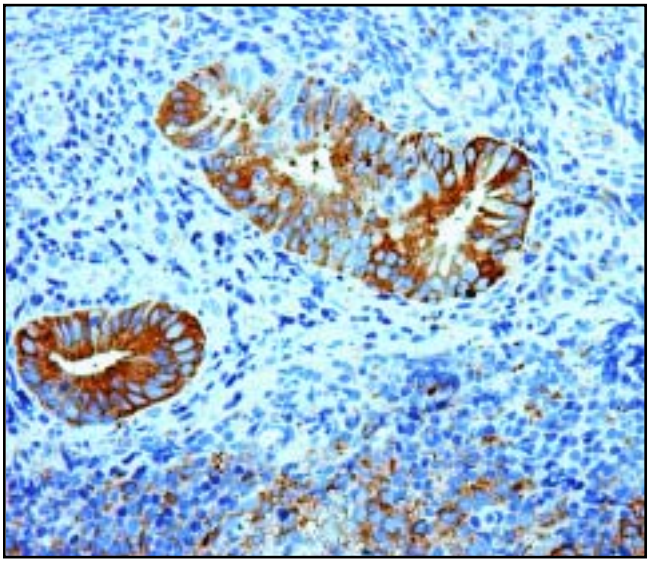

Image 1 Fatty acid synthase expression in diseased mucosa of colon in patient with active ulcerative colitis (original magnification ×100).

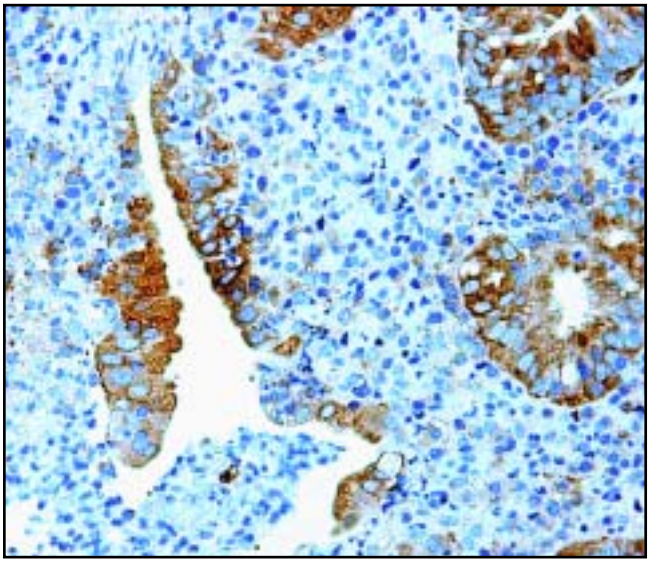

Image 2 Fatty acid synthase expression in mucosa of colon in patient with active disease; longitudinal section of a crypt (original magnification ×100).

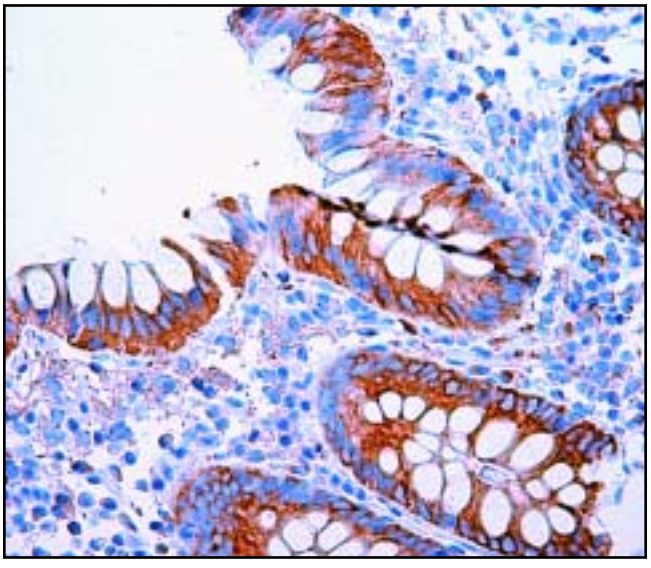

Image 3 Fatty acid synthase expression in normal mucosa of colon in patients affected with active ulcerative colitis (original magnification ×100).

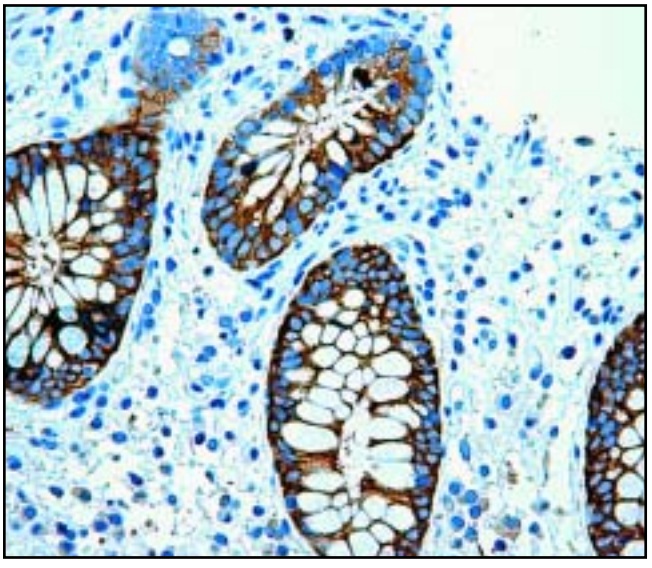

Image 4 Fatty acid synthase expression in diseased mucosa of colon in patients with quiescent ulcerative colitis (original magnification ×100).

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FAS is not necessary for survival of normal tissues, mucosa of the colon included. Activation of FAS and fatty acid synthesis are related closely to malignant transformation of normal tissues and, in hormone-sensitive cancers, are more pronounced as the tumor progresses toward a more advanced stage.[19-21] Kuhajda et al[22] demonstrated that inhibition of FAS leads to apoptosis in most populations of cancer cells; thus, FAS is considered a promising new target in antineoplastic therapy, demonstrating that FAS activation is crucial for the survival of cancer cells. However, our data indicate that FAS is not useful for survival of cells in UC because apoptosis is increased.[23]

The present results show that FAS in the mucosa affected by UC, unlike normal mucosa of healthy control subjects, is not down-regulated by dietary lipids; therefore, from this viewpoint, cells of the mucosa with UC act like cancer cells.

In UC, increased permeability of the mucosa of the colon to intraluminal bacteria and their products occurs mainly in the acute phase, leading to an inflammatory reaction. Infection of monolayers of human colon epithelial cells (T84, HT29, Caco-2) with invasive strains of bacteria resulted in the coordinated expression and up-regulation of a specific array of 4 proinflammatory cytokines: interleukin-8, monocyte chemotactic protein-1, granulocyte-macrophage colony stimulating factor, and tumor necrosis factor (TNF)-α.[24] These cytokines trigger marked disorders in metabolism. Feingold and Grunfeld[25] showed that administration of TNF-α increases serum triglycerides in rats by increasing de novo hepatic fatty

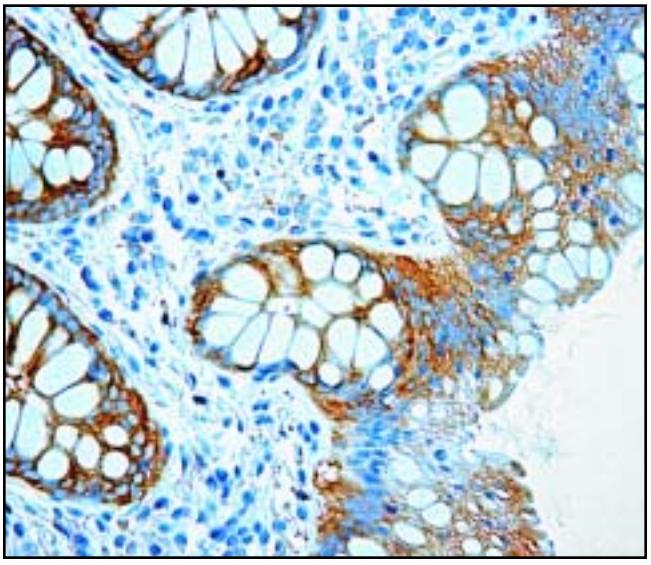

Image 5 Fatty acid synthase expression in normal mucosa of colon in patients with quiescent ulcerative colitis (original magnification ×100).

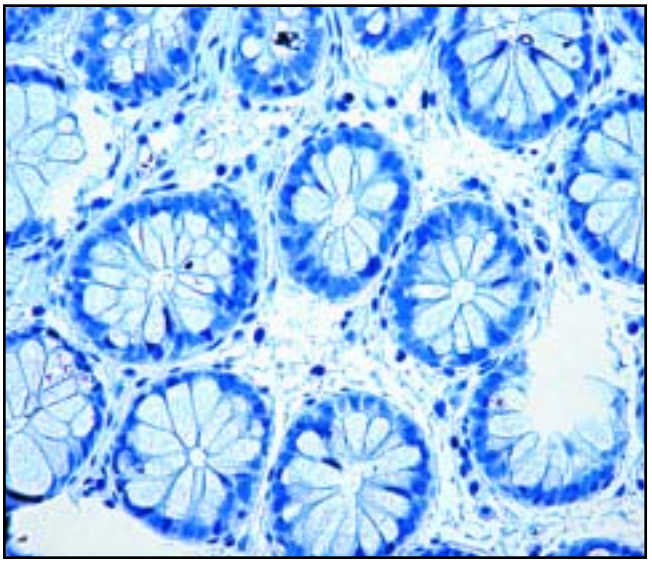

Image 6 Healthy mucosa of control subjects (original magnification ×100).

Table 2
Fatty Acid Synthase Expression in Patients With Active and Quiescent UC in SE and GE*

	Odds Ratio	95% Confidence Interval	*P*
Active UC: AM vs NM			
SE	—	—	—
GE	—	—	—
Quiescent UC: QM vs NM			
SE	1.7	0.6-4.7	.43
GE	10.7	2.1-53.3	.002
Active vs quiescent UC: AM vs QM			
SE	47.05	2.6-841.5	<.0001
GE	5.3	0.24-116.4	.49

AM, active mucosa; GE, glandular epithelium; NM, normal mucosa; QM, quiescent mucosa; SE, surface epithelium; UC, ulcerative colitis.
* Not applicable (—) owing to FAS expression in all patients in SE and GE of active and normal mucosa.

Table 3
Fatty Acid Synthase Expression in SE and GE From Patients With UC vs Control Subjects

	Odds Ratio	95% Confidence Interval	*P*
AM vs HM			
SE	479.2	23.6-9,708.1	<.0001
GE	479.2	23.6-9,708.1	<.0001
QM vs HM			
SE	11.7	2.9-47.4	.0003
GE	126	19.4-814.3	<.0001
NM of active UC vs HM			
SE	479.2	23.6-9,708.1	<.0001
GE	479.2	23.6-9,708.1	<.0001
NM of quiescent UC vs HM			
SE	6.8	1.7-27.7	.0074
GE	11.7	2.9-47.4	.0003

AM, active mucosa; GE, glandular epithelium; NM, normal mucosa; QM, quiescent mucosa; SE, surface epithelium; UC, ulcerative colitis.

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acid synthesis and very-low-density lipoprotein production; therefore, the increase of FAS expression in mucosa affected by UC could be caused by TNF-α.

Long-chain fatty acids also are essential constituents of membrane phospholipids, and FAS messenger RNA is increased immediately in culture of fibroblasts when growth factor is added to the medium.[26] Pizer et al[27] showed parallel expression of FAS and Ki-67, a proliferation antigen, in human tissues with rapid growth rates. Several observations support the hypothesis that fatty acid synthesis of tumor cells by increased FAS expression may be linked functionally to proliferation. Therefore, the expression of FAS by cells of the mucosa affected by UC possibly is oriented to the production of membrane phospholipids for the increased cell turnover of the disease. The present results do not confirm this hypothesis because FAS is increased not only in glands, where the proliferative compartment is located, but also in the SE and even in normal mucosa uninvolved by UC.

Malonyl-CoA at high levels is the endogenous inhibitor of carnitine palmitoyl-transferase-1 that catalyzes the esterification of long-chain acyl-CoA to L-carnitine for the transport of fatty acids into the mitochondria, where oxidation and energy production occur.[28] Therefore, in tissues physiologically expressing FAS, high levels of malonyl-CoA prevent useless oxidation of newly formed fatty acids,[29,30] whereas in normal tissues, FAS expression and fatty acid synthesis usually are low and malonyl-CoA does not inhibit energy production.[18] In the mucosa of patients with UC with high levels of FAS, fatty acid synthesis probably is high and the elevated levels of malonyl-CoA could reduce fatty acid oxidation and energy production, making them more vulnerable. Moreover, high production of fatty acids independently by dietary control could be dangerous for the cells affected by UC because several molecules of adenosine triphosphate are consumed for each molecule of fatty acid generated[31]; this energy expenditure could be lethal in the absence of an appropriate energy substrate. High FAS levels in cells could be harmful also because FAS produces palmitate and stearate, 2 fatty acids that induce de novo synthesis of ceramide, a key component of intracellular stress response pathways that, when in an excessive amount, is often toxic and results in apoptosis. Therefore, a delicate balance in ceramide levels is required to maintain normal cell functions.[32-34]

Wood et al[35] studied FAS in the skin after cutaneous barrier disruption. This event elicits TNF-α secretion and a metabolic response rapidly restoring normal cutaneous permeability by an increased epidermal fatty acid and membrane phospholipid synthesis with the respective lipogenic enzymes, among which is FAS.[33,34,36] Epidermis and gastrointestinal mucosa have the same barrier function, and it is tempting to hypothesize that in the mucosa affected by UC, the disruption of the barrier produces a response similar to that of the epidermis.[36]

Our results show that FAS is overexpressed significantly in pathologic and normal mucosa of patients with UC, mainly in the acute phase. FAS expression in the normal mucosa of patients included in the study suggests that the extension of the disease is greater than the extension diagnosed by common investigative techniques or that clinical extension of UC is impending and, in this case, FAS could be a useful signal for early detection of this condition.

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