Enzymes of the Fatty Acid Synthesis Pathway Are Highly Expressed in *in Situ* Breast Carcinoma[1]

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ABSTRACT

Expression of high levels of fatty acid synthase (FAS), an important enzyme in fatty acid synthesis, has been identified in a wide variety of human carcinomas. In breast and prostate carcinoma, FAS expression appears to be associated with aggressive disease. Recent biochemical studies have demonstrated that FAS expression in cancer cells connotes activation of the entire fatty acid synthesis pathway leading to the production of palmitic acid. Here, we explore the immunohistochemical expression of FAS and human acetyl-CoA carboxylase (HACC), the rate-limiting enzyme in fatty acid synthesis, in breast cancer progression from histologically normal breast through the development of *in situ* duct and lobular carcinoma to infiltrating carcinoma. Both FAS and the M_r 275,000 isoform of HACC are expressed in a small subset of cells in normal breast lobules and terminal ducts. Upon development of either *in situ* duct or lobular carcinoma, FAS and both isoforms of HACC are expressed at higher levels and in a majority of the cells. These findings suggest that expression of the enzymes of fatty acid synthesis are frequently altered early in the progression of human breast carcinoma.

INTRODUCTION

Alterations in gene expression and mutation have been frequently identified in invasive breast carcinoma (1, 2), and several recent studies have sought these genetic changes in *in situ* breast carcinoma to determine their relationship to tumor progression, as was done for colon carcinoma (3). Both c-erb-B2 amplification and p53 mutations have been described in high-grade (comedo-type) DCIS[3] (4, 5). This association is not surprising because high-grade DCIS is most often associated with foci of invasive disease and thus represents a preinvasive stage of tumor progression. In contrast, c-erb-B2 amplification and p53 mutations are uncommon in low-grade DCIS (4–6) and have not yet been identified in LCIS. Thus, the genetic alterations associated with human breast carcinoma thus far appear to occur at or just prior to the development of invasive carcinoma.

High levels of FAS expression have been recently identified in invasive human breast carcinoma and other human solid tumors (7–12) compared to normal human tissues. FAS is a M_r 270,000 multisite enzyme that is responsible for the terminal catalytic step in the *de novo* synthesis of saturated fatty acid. Biochemical analysis of human breast carcinoma cell lines has demonstrated that FAS is active in cancer cells and a surrogate marker of activation of the fatty acid synthesis pathway (13). Moreover, recent *in vitro* studies demonstrate that, in addition to FAS, both isoforms of HACC, the rate-limiting enzyme in the fatty acid synthesis pathway, are also highly expressed in human breast carcinoma cell lines (14).

This study uses immunohistochemistry to examine the expression of both FAS and HACC in histologically normal breast from patients with and without cancer, in DCIS and LCIS alone and in association with infiltrating carcinoma. Our results indicate that both FAS and HACC are normally expressed in a minority of cells comprising the breast lobule and lining the terminal duct of histologically normal breast tissue. Upon development of *in situ* carcinoma, FAS and HACC expression patterns change to high levels of expression in the majority of the cells in subsets of both LCIS and DCIS. These data suggest activation of the fatty acid synthesis genetic program may occur early in breast cancer tumor progression and present a strategy for early intervention or disease prevention.

MATERIALS AND METHODS

Case Selection. Sixty cases of formalin-fixed, paraffin-embedded breast and tumor tissues were selected from files of the Division of Surgical Pathology of The Johns Hopkins Hospital, encompassing the years 1990–1994. The following categories of breast diseases were represented: 8 cases of normal breast from reduction mammoplasties from patients

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[3] The abbreviations used are: DCIS, ductal carcinoma *in situ*; LCIS, lobular carcinoma *in situ*; FAS, fatty acid synthase; HACC, human acetyl-CoA carboxylase; HACC 275, M_r 275,000 isoform of HACC; HACC 265, M_r 265,000 isoform of HACC.

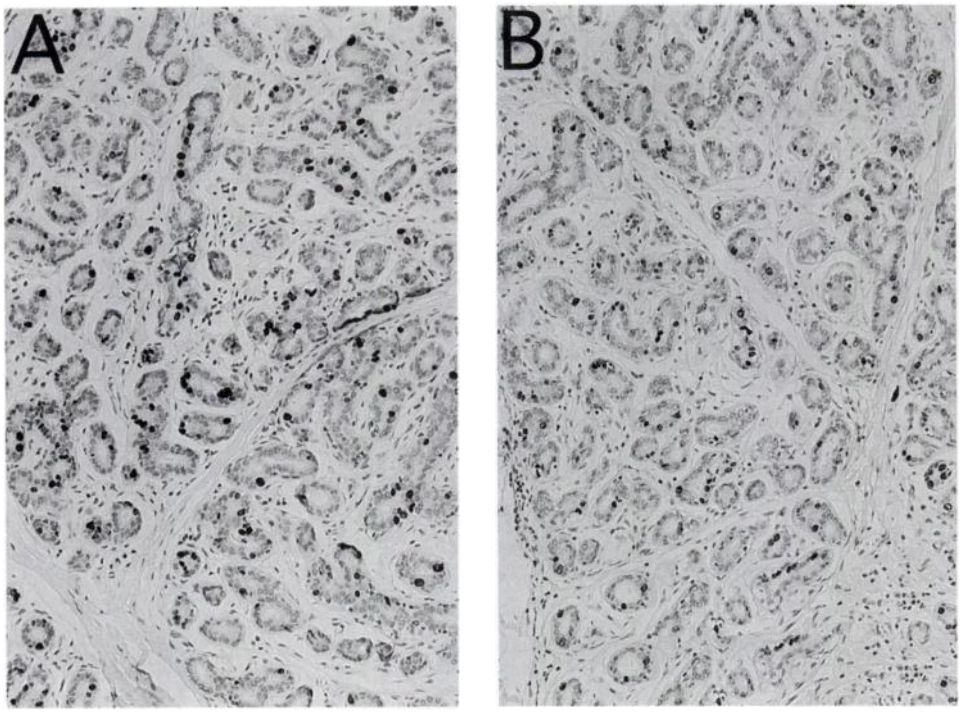

Fig. 1 Immunohistochemical staining for FAS (*A*) and HACC 275 (*B*) in a normal human breast lobule from a reduction mammoplasty. Note the scattered positive cytoplasmic staining for both FAS and HACC 275.

Table 1 FAS and HACC in histologically normal breast lobules and terminal ducts from patients with benign or malignant breast disease

Disease category	FAS	HACC 275	HACC 265
Benign breast	11/12 (91.6%)	3/3 (100%)	0/3 (0%)
In situ cancer	24/25 (96%)	4/4 (100%)	1/3 (33%)
Invasive cancer[a]	17/21 (80.9%)	3/3 (100%)	1/3 (33%)

[a] Includes four cases of invasive cancer in the contralateral breast.

Table 2 FAS expression in situ and associated infiltrating carcinoma

In situ carcinoma	Negative	Low expression	High expression
Low-grade DCIS[a]	4/8 (50.0%)	3/8 (37.5%)	1/8 (12.5%)
High-grade DCIS	0/7 (0%)	1/7 (14.2%)	6/7 (85.7%)
DCIS and invasion	0/8 (0%)	2/8 (25%)	6/8 (75%)
LCIS	0/11 (0%)	5/11 (45.4%)	6/11 (54.4%)
LCIS and invasion	0/10 (0%)	6/10 (60.0%)	4/10 (40.0%)

[a] FAS expression is lower in low-grade DCIS than it is in other groups ($P < 0.003$, χ^2 test).

of ages 25–44; 4 cases of fibrocystic changes from reduction mammoplasties from patients of ages 25–59; 4 cases of normal breast tissue from patients with infiltrating breast cancer in the contralateral breast from patients of ages 35–66; 11 cases of LCIS; 10 cases of *in situ* and infiltrating lobular carcinoma; 7 cases of high-grade DCIS (comedo type); 8 cases of low-grade DCIS; and 8 cases of intraductal and infiltrating duct carcinoma. No patient received any chemotherapy or hormonal therapy prior to biopsy or mastectomy.

Primary Antibodies and Immunohistochemistry. FAS was detected using an affinity-purified rabbit polyclonal antibody to FAS purified from ZR-75-1 human breast cancer cells (15). This antibody is monospecific for FAS on Western blot. HACC 275 was identified using an affinity-purified rabbit polyclonal antibody raised against the M_r 280,000 isoform from rat heart, which cross-reacts with HACC 275 (14). HACC 265 was identified with an affinity-purified polyclonal rabbit antibody raised against the 15-amino acid NH_2-terminal peptide of the rat M_r 265,000 isoform, which cross-reacts with HACC 265 (14). In addition, these antibodies have been validated by Western blot on human breast carcinoma cell lines (14).

A representative tissue block was selected from each case, and 5-μm sections were cut and mounted on ChemMate 228 slides. An infiltrating duct carcinoma served as the positive control for all antibodies. A negative control with the primary antibody omitted was run with each case. Immunohistochemistry was performed on the TechMate 1000 automated stainer (BioTek Solutions Inc., Santa Barbara, CA) using the TechMate detection kit, which uses biotinylated secondary antibodies and avidin-horseradish peroxidase conjugate. Diaminobenzidine was the chromagen, with hematoxylin counterstain. Immunohistochemistry for FAS was performed on all 60 cases. HACC isoform immunohistochemistry was performed on a subset of 17 cases. Staining distribution and intensity were assessed by two investigators independently (F. P. K. and L. Z. M.). Intensity of staining in the *in situ* and infiltrating lesions was graded semiquantitatively as negative, low, or high expression. Because of the scattered staining of cells in normal breast structures, semiquantitative grading was not performed on normal breast structures.

Table 3 HACC 275 and HACC 265 expression in *in situ* and associated infiltrating carcinoma

	Negative		Low expression		High expression	
In situ carcinoma	HACC 275	HACC 265	HACC 275	HACC 265	HACC 275	HACC 265
DCIS with and without invasion	0 (0%)	0 (0%)	2/5 (40%)	2/5 (40%)	3/5 (60%)	3/5 (60%)
LCIS with and without invasion	0 (0%)	0 (0%)	0 (0%)	2/3 (66.6%)	4/4 (100%)	1/3 (33.3%)
Invasive tumor	0 (0%)	0 (0%)	0 (0%)	4/4 (100%)	4/4 (100%)	0 (0%)

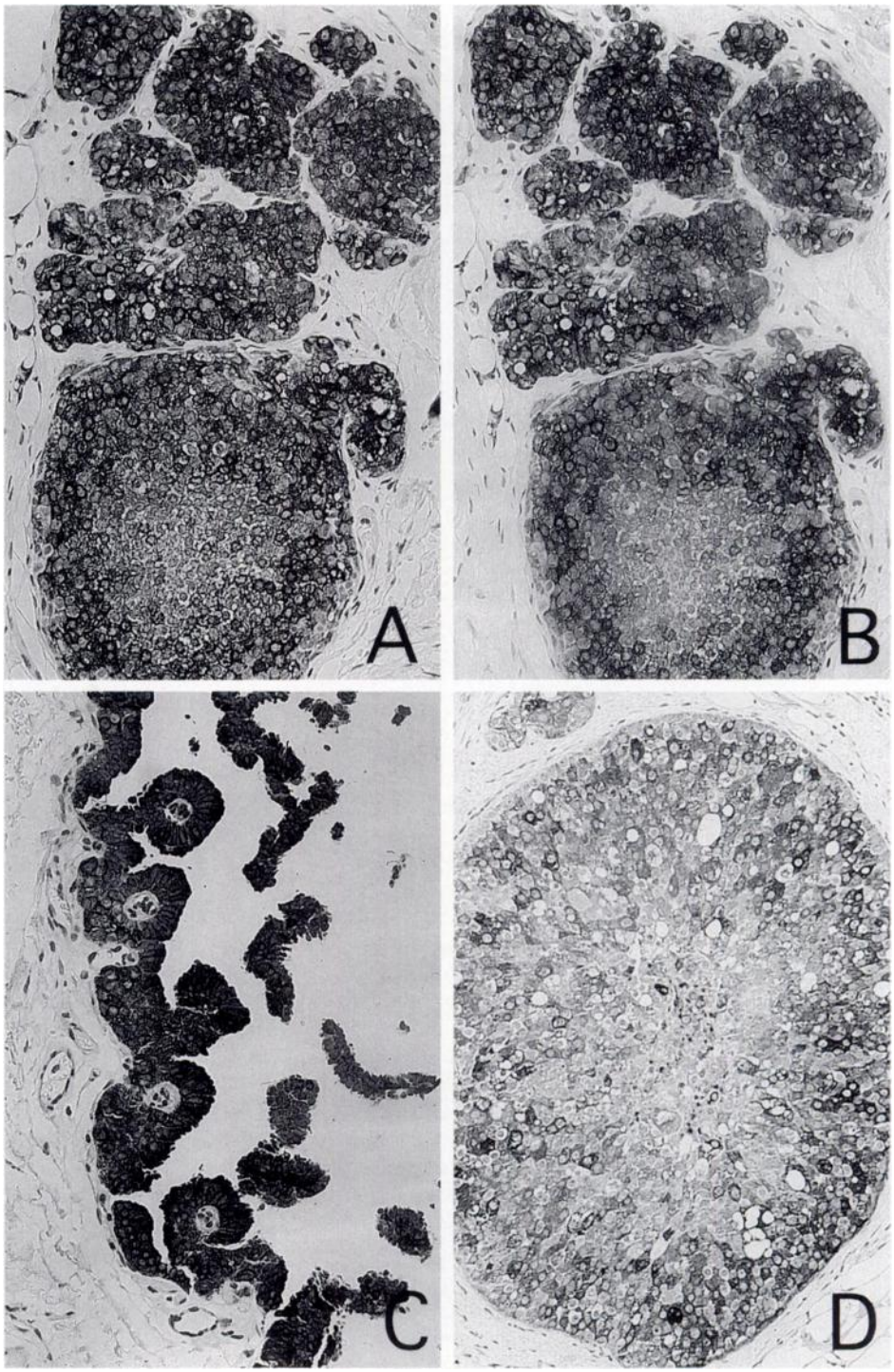

Fig. 2 Immunohistochemical staining for FAS (*A*) and HACC 275 (*B*) in solid DCIS. The distribution and intensity of reactivity for both enzymes are similar. Both micropapillary DCIS (*C*) and high-grade comedo DCIS (*D*) show intense reactivity for FAS.

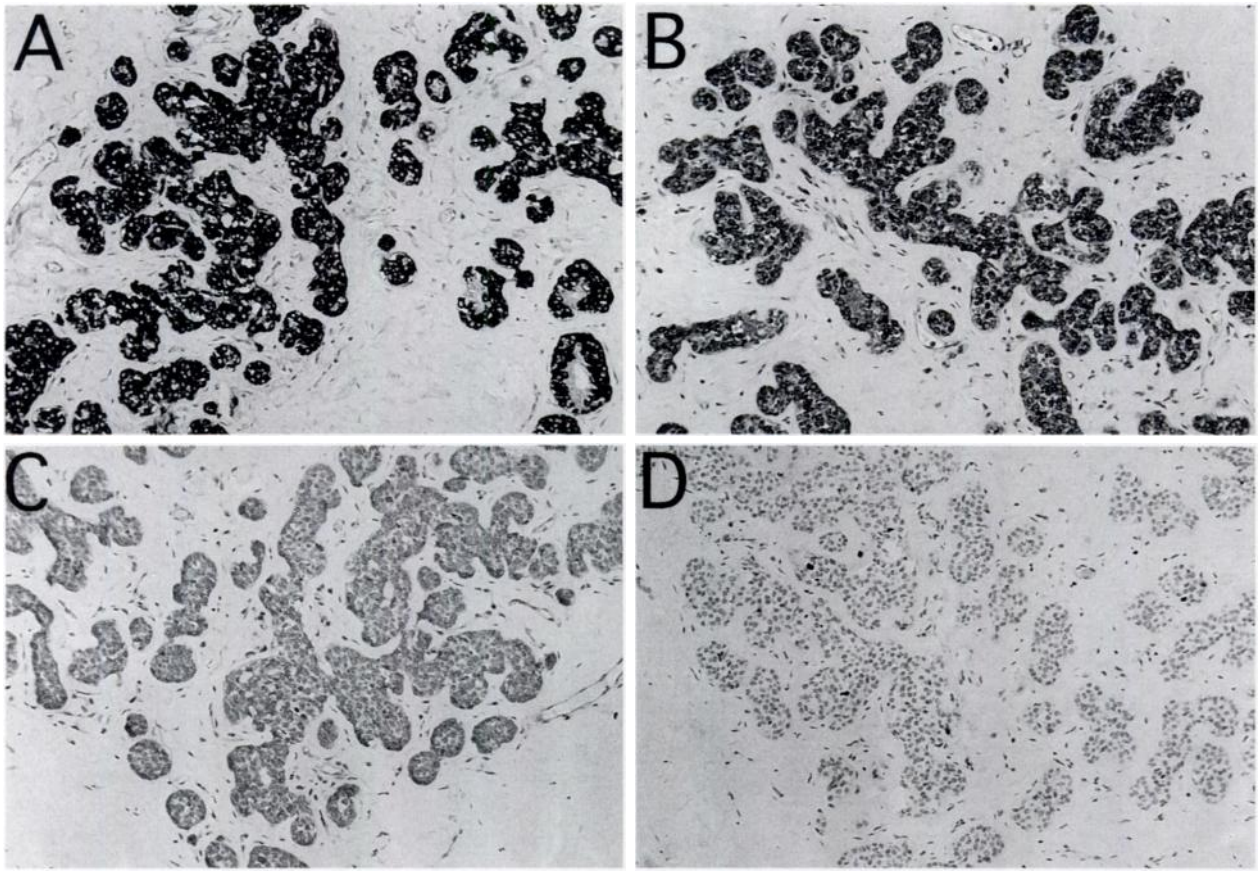

Fig. 3 Immunohistochemical staining for FAS (*A*), HACC 275 (*B*), HACC 265 (*C*), and negative control (*D*) in LCIS. FAS and both isozymes of HACC are expressed in this lesion. Note the homogeneous staining pattern for these enzymes in this lesion compared to the distribution seen in normal breast lobules (Fig. 1).

RESULTS

FAS and HACC Expression in Histologically Normal Breast Epithelium. In normal breast structures, reactivity for FAS and HACC isoforms was restricted to the cytoplasm of epithelial cells confined to the breast lobule and terminal duct. No staining of epithelial cells was noted in larger ducts. Fig. 1 illustrates the distribution of FAS and HACC 275 reactivity in cells scattered in the normal breast lobule and terminal duct. These immunoreactive cells were histologically indistinguishable from adjacent negative cells. There was no identifiable pattern to their distribution in the lobule or terminal duct, nor was there any association between expression and the presence of intracellular lipid droplets. In contrast to the intense reactivity of scattered cells for HACC 275, HACC 265 reactivity was less intense and was distributed in many epithelial cells of the breast, including the cells lining the larger ducts (data not shown).

Table 1 demonstrates that the pattern of reactivity in normal breast for FAS and HACC was independent of the presence *in situ* or infiltrating carcinoma in the same or opposite breast. FAS was identified in scattered cells of lobules and terminal ducts in 11 of 12 (91.6%) cases from benign breast reduction mammoplasties, which is comparable to 41 of 46 (89.1%) from cases with either *in situ* or malignant disease. HACC 275 had a similar distribution.

FAS and HACC Expression in Mesenchymal Cells of the Breast. FAS and both HACC isoforms were variably expressed in adipocytes. Both isoforms of HACC are identified in smooth muscle in the nipple and skin appendages, consistent with the reported HACC expression in muscle (16).

FAS and HACC Expression in Carcinoma *in Situ*. Although FAS and HACC 275 showed distinctive pattern of expression in normal breast, this distribution was altered in malignant transformation. In DCIS, FAS expression was related to the differentiation of the lesion. Table 2 demonstrates that 87.5% of the low-grade DCISs were either negative or exhibited low-level expression for FAS, compared to high levels of FAS expression in 85.7% of high-grade DCISs and 75% of DCISs associated with invasive tumor. For HACC (Table 3), the association of the HACC 275 isozyme with FAS expression seen in normal breast was absent in DCIS. Both isozymes were expressed in low- and high-grade DCIS in a distribution similar to FAS, but no relationship to histological grade was noted. Fig. 2, *A* and *B*, illustrates the immunohistochemical expression of FAS and HACC 275 in a case of solid DCIS showing similar expression levels of both enzymes. Fig. 2, *C* and *D*, demonstrates

the distribution and intensity of FAS expression in micropapillary DCIS and high-grade comedo DCIS.

In LCIS, FAS was consistently expressed, as were both isozymes of HACC. Fig. 3 demonstrates that, for both FAS and HACC, the pattern of scattered positive cells seen in normal breast was absent in LCIS. Instead, either entire lobular units expressed FAS and HACC or staining varied in intensity throughout the lobular unit. The infiltrating tumor associated with *in situ* carcinoma was studied in nine cases; FAS and both isoforms of HACC were expressed in all cases (data not shown).

DISCUSSION

Increased levels of FAS and HACC expression accompany the development of *in situ* carcinoma of the breast, involving either the lobules or ducts. These data suggest the activation of a complex multigene program for fatty acid synthesis early in tumor progression. Furthermore, the restricted expression of these molecules in the lobules and terminal ducts of normal breast tissue coincides with the regions of normal breast, where the earliest precursor lesions are thought to arise.

FAS and the HACC 275 isoform share a similar expression pattern in normal breast: scattered cells are intensely positive in lobules and terminal ducts. In contrast, both the expression pattern and the HACC isoform type change dramatically in *in situ* carcinoma. Either one or both isoforms of HACC may be expressed along with FAS in *in situ* carcinoma. This finding is similar to what we reported for human breast carcinoma cell lines (14). Furthermore, the pattern of scattered positive cells in lobules and terminal ducts changes to either diffuse, intense staining of a majority of the cancer cells or to patchy staining of varying intensity, reflecting the tumor heterogeneity that is common to infiltrating breast carcinoma. Thus, there appears to be significant dysregulation of both the level of expression of these enzymes and the HACC isoform type early in breast cancer progression. It is likely that the genetic program restricting FAS and HACC 275 expression in resting normal breast is altered early in breast cancer progression in concert with these early histological changes.

The appearance of intense FAS and HACC expression in LCIS suggests a potential link between increased expression and increased risk of the development of breast carcinoma. Patients with LCIS are at a 12-fold increased risk to develop infiltrating carcinoma in either breast over a span of 25–30 years (17). All patients with LCIS showed increased expression of both FAS and HACC in the involved lobules. Although any relationship of endogenous fatty acid synthesis to breast cancer risk is as yet unknown, activation of the fatty acid synthesis program could lead to the production of biologically active lipids, some of which are known to be active in signal transduction (18, 19).

In contrast to its anabolic function in normal breast cells, fatty acid synthesis may be directly involved in maintenance or enhancement of the malignant phenotype. Human tissue studies from our laboratory and others have demonstrated that infiltrating carcinomas of the breast, colon, prostate, ovary, and endometrium express high levels of FAS, compared to nontransformed human epithelial and mesenchymal tissues (7–12). Furthermore, in infiltrating breast and prostate cancer, expression of FAS has been associated with aggressive disease (10–12). Taken together, these studies of FAS expression demonstrate a likely functional association with the malignant phenotype.

Identification of FAS and HACC in *in situ* breast carcinoma also provides potential targets for disease prevention and intervention. Inhibition of fatty acid synthesis *in vitro* is selectively cytotoxic to human breast cancer cells compared to human fibroblasts (13). *In vivo* treatment of a xenograft model of human ovarian carcinoma with a fatty acid synthesis inhibitor has led to increased survival and a striking reduction in the development of malignant ascites (20). Hence, high levels of FAS or HACC in cancer cells represent the activation of not a single gene but a complex genetic program to initiate synthesis of fatty acids, which appear to be required for the survival and growth of some malignant cells. Ultimately, expression of these enzymes in both DCIS and LCIS may lead to strategies to eliminate these lesions using either small molecule enzyme inhibitors or novel molecular approaches.

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