Immunohistochemical Expression of Human Erythrocyte Glucose Transporter and Fatty Acid Synthase in Infiltrating Breast Carcinomas and Adjacent Typical/Atypical Hyperplastic or Normal Breast Tissue

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Abstract

To evaluate the immunohistochemical expression of GLUT1, human erythrocyte glucose transporter 1, and fatty acid synthase (FAS), 66 human breast carcinomas and adjacent peritumoral tissue were studied. GLUT1 and FAS were expressed in 53 and 61 carcinomas, in 17 and 14 typical/atypical hyperplastic tissues, and in 16 and 13 tissues adjacent to tumor normal breast tissue, respectively. Statistical analysis revealed association between invasive carcinomas, invasive carcinomas with in situ component and GLUT1 immunostaining. GLUT1 staining was associated with tumor grade, FAS with tumor stage, and GLUT1 and FAS coexpression with tumor grade. Controls expressed no immunostaining. GLUT1 and FAS are new markers involved in the biologic activities of cancer cells. GLUT1 and FAS coexpression may indicate increased use of energy by the neoplastic cells correlated with poorly differentiated features and aggressive behavior. The innovative finding that GLUT1 and FAS are observed in mammary carcinoma adjacent nonneoplastic tissues may suggest a role in detecting initial phases of breast carcinogenesis.

Malignant cells reveal an increased glucose uptake and use compared with their benign and normal counterparts.1 This uptake is mediated by glucose transporters, the expression and activity of which are regulated by oncogenes and growth factors.2 One of the glucose transporters, the human erythrocyte glucose transporter (GLUT1) belongs to an expanding family of transmembrane proteins that currently has 6 members.3 GLUT1 protein has been found in the endothelia of the human blood-brain barrier, in human liver and erythrocytes, in the HepG2 hepatic carcinoma cell line, in rat kidney and mammary gland, and in the placenta, including fetal membranes.4-8 Aberrant GLUT1 protein expression also has been described in human tumors including head and neck carcinomas,9 breast cancers,10 insulinomas,11 renal cell carcinomas,12 and lung carcinomas.13

Fatty acid synthase (FAS) is a recently isolated marker of aggressive tumors in humans. FAS is the primary enzyme responsible for the synthesis of fatty acids.14 It is a multifunctional enzyme that catalyzes the biosynthesis of long chain fatty acids as palmitate from acetyl coenzyme A and malonyl coenzyme A. FAS normally is expressed at low levels in some human tissues due to down-regulation by dietary lipids.15,16 Alò et al17 and others18,19 have observed that FAS is overexpressed in nonneoplastic, highly proliferative lesions and in aggressive carcinomas with poor outcome. Since both markers are involved in the cellular metabolic activities of many normal tissues, the aim of the present study was to evaluate, by means of immunohistochemical analysis, whether GLUT1 and FAS were expressed abnormally in infiltrating and in situ breast carcinomas and in the adjacent typical/atypical hyperplastic or normal breast tissues and to reveal statistical associations in their expression, neoplastic progression, and histologic features, type, grade, and stage of the tumors.
Materials and Methods

Patient Specimens

We studied 66 female patients surgically treated for breast disease at the University of Rome “La Sapienza” from June 1995 to December 1999. Clinical information was obtained from the medical records. No patient received adjuvant therapy. Clinical data included race, menstrual status at the time of cancer diagnosis, family and patient history, and type of surgery. Histopathologic data included tumor size, histologic subtype and grade, degree of inflammation, evidence of necrosis and lymphatic vessel invasion, desmoplastic reaction, involvement of perineural spaces, pathology of the surrounding breast, and stage of the disease according to the TNM classification. Pathologic data for in situ carcinoma included size of the lesion and histologic subtype. All specimens were reviewed by two of us (P.L.A. and T.A.) using only the surgical accession numbers as identifiers, and surgical pathology diagnoses were made independently according to the Page classification of breast diseases. Evaluation of normal and typical/atypical hyperplastic peritumoral tissue, when available on the slides, and carcinomas with and without in situ features was done after review of all histologic sections. Control specimens were obtained from 10 patients surgically treated for fibrocystic disease and who did not have cancer.

Antibodies to GLUT1 and FAS

The antibodies used were monoclonal mouse purified antibodies specific to GLUT1 and FAS. GLUT1 is a transmembrane glycoprotein, and FAS is a cytoplasm enzyme, so their specific immunohistochemical expressions are, respectively, membrane staining for GLUT1 and cytoplasmic staining for FAS.

Scoring of GLUT1 and FAS Immunoreactivity

GLUT1 immunoreactivity was scored blindly in tissue sections identified only by the surgical accession number, by the primary author (P.L.A.), using the following criteria: (1) adequacy of immunohistochemical technique, as judged by the presence and intensity of immunoreaction in positive internal controls (perineurium and erythrocytes); (2) intensity of the breast epithelium immunoreaction in comparison with that of the internal controls: 0, no staining; 1, weak staining (less than that observed in control specimens); and 2, strong staining (equal to that seen in control specimens); and (3) approximate percentage of normal, typical/atypical hyperplastic, and neoplastic breast epithelium showing positive immunoreactivity. Breast stroma was excluded because it was negative in all cases. Negative control specimens, prepared for each specimen in the absence of the primary antibody, confirmed the specificity of the breast epithelial immunoreaction.

All specimens were scored independently by two authors (E.S.P. and P.V.), with subsequent reconciliation of scored values. All examined lesions were characterized adequately using a 3-grade composite scoring system, encompassing intensity and extent of lesional immunoreaction: 0, no immunoreaction; 1, weak immunoreaction (focal immunoreaction less than that present in control specimens in the same tissue section); or 2, strong immunoreaction (equal in intensity to control specimens with more than 50% of breast epithelial cells positive). Analysis of FAS was performed using a combined scoring system based on the fraction of positive tumor cells and the predominant staining intensity in the tumor. The fraction of positive tumor cells was estimated using a 4-tiered scale (10% = 1; 11%-50% = 2; 51%-80% = 3; >80% = 4). The staining intensity also was scored on a 4-tiered scale (negative, 0; low-intensity positive staining, 1; moderate-intensity positive staining, 2; strong-intensity positive staining, 3). The overall score in each case was scored as the product of the staining intensity and the positive fraction score. Each case was scored twice, independently by each of 2 pathologists (U.D.T. and V.S.), with subsequent reconciliation of scored values. For purposes of discussion, scores of 3 or less were considered negative.

Statistical Analysis

The Fisher exact test was used to study the difference in prevalence of GLUT1 and FAS immunoreactivity between groups. All evaluations were performed by means of
Results

GLUT1 and FAS Immunoreactivity in Breast Carcinomas

GLUT1 immunostaining was expressed in 31 (74%) of 42 infiltrating carcinomas, in 4 (80%) of 5 in situ carcinomas, and in 18 (95%) of 19 infiltrating carcinomas with an in situ component [Image 1A]. In these 18 cases, both in situ and infiltrating components showed strong GLUT1 overexpression. For statistical purposes, lobular and duct infiltrating carcinomas were grouped together. All in situ carcinomas were intraductal. Statistical analysis revealed association among pure infiltrating carcinomas, infiltrating carcinomas with an in situ component, and GLUT1 immunostaining ($P = .04$). No association was revealed between GLUT1 immunostaining and in situ carcinomas. FAS immunostaining was observed in 39 (93%) of 42 infiltrating carcinomas, in all 5 in situ carcinomas (100%), and in 17 (89%) of 19 infiltrating carcinomas with an in situ component [Image 1B]. In these 17 cases, only 5 showed strong FAS overexpression. No statistical association was revealed between FAS immunoreactivity and the histologic pattern. Coexpression of GLUT1 and FAS was detected in 30 (71%) of 42 infiltrating carcinomas, in 16 (84%) of 19 infiltrating carcinomas with an in situ component, and in 4 (80%) of 5 in situ carcinomas. No statistical association was revealed between GLUT1 and FAS coexpression and the histologic pattern.

Immunoreactivity of GLUT1 and FAS According to Neoplastic Grade and Stage

GLUT1 immunoreactivity was expressed in 8 (57%) of 14 grade 1-2 tumors and in 41 (87%) of 47 grade 3 tumors. Statistical analysis revealed association between grade 1-2 vs grade 3 tumors and GLUT1 immunostaining ($P = .01$). FAS immunoreactivity was expressed in 12 (86%) of 14 grade 1-2 and in 44 (94%) of 47 grade 3 tumors. No statistical association was revealed between neoplastic grade and FAS expression. GLUT1 and FAS coexpression was observed in 7 (50%) of 14 grade 1-2 tumors and in 39 (83%) of 47 grade 3 tumors. Statistical analysis revealed association between grade 1-2 tumors and GLUT1 and FAS coexpression ($P = .01$). GLUT1 was expressed in 20 (80%) of 25 stage I tumors, in 15 (75%) of 20 stage II tumors, and in 18 (86%) of 21 stage III tumors. No statistical association was revealed between neoplastic stage and GLUT1 immunostaining. FAS immunoreactivity was expressed in 20 (80%) of 25 stage I tumors, in all 20 stage II tumors (100%), and in all 21 stage III tumors (100%). FAS immunostaining was associated with stage I vs stage II tumors ($P = .03$) and with stage I vs stage III tumors ($P = .03$). No statistical association was revealed between FAS immunostaining and stage II vs stage III tumors.

GLUT1 Immunoreactivity Pattern in Breast Carcinomas and Adjacent Tissues

As depicted in [Table 1], 10 (36%) of 28 evaluable typical/atypical hyperplastic adjacent tissues showed GLUT1 immunoreactivity, while adjacent normal tissues were positive in 15 (31%) of 48 evaluable cases. In all but 1 case, GLUT1 reactivity was observed in both the nonneoplastic

[Image 1A] Strong human erythrocyte glucose transporter 1 (GLUT1) and fatty acid synthase (FAS) coexpression in a duct infiltrating breast carcinoma with in situ features. A, Note positive GLUT1 erythrocytes in the lower left corner (original magnification $\times$60).

[Image 1B] Strong GLUT1 and FAS coexpression in a duct infiltrating breast carcinoma with in situ features. B, Note positive GLUT1 erythrocytes in the lower left corner (original magnification $\times$60).
tissue and the carcinoma. The pattern of GLUT1 immunoreactivity of adjacent tissue was similar in infiltrating carcinomas and infiltrating carcinomas with an in situ component.

FAS Immunoreactivity Pattern in Breast Carcinomas and Adjacent Tissues

As depicted in Table 2, 14 (52%) of 27 evaluable typical/atypical hyperplastic adjacent tissues showed FAS immunoreactivity, while adjacent normal tissues were positive in 13 (28%) of 47 evaluable cases. All positive cases of adjacent tissue showed FAS positivity in corresponding carcinomas. The pattern of FAS immunostaining of adjacent tissue was similar in infiltrating carcinomas and infiltrating carcinomas with an in situ component.

Specimens from the 10 patients surgically treated for fibrocystic disease and who did not have cancer expressed no FAS and GLUT1 immunostaining. The other histopathologic data considered, including degree of inflammation, evidence of necrosis, lymphatic vessel invasion, desmoplastic reaction, and involvement of perineural spaces, showed no relation to the presence or the absence of GLUT1 or FAS staining.

Discussion

The comprehension of the mechanisms that promote and sustain carcinogenesis is a major challenge worldwide. Many factors have been identified as being involved in these mechanisms, but with controversial results. Among the most promising markers in the uptake and use of energy sources from neoplastic cells are GLUT1 and FAS.

GLUT1, 1 of the 6 isoforms of the facilitative cell-surface glucose transporter family, is expressed physiologically and is detectable immunohistochemically in RBC membranes, brain capillary endothelium (blood-brain barrier), and the perineurium of peripheral nerves, all interfaces with cells or tissue with glucose-driven metabolism. GLUT1 favors the transport of glucose into the Golgi complex in lactating mouse mammary gland,22 regulates the myocardial glucose uptake and transport during ischemia and energetic stress,23 and is controlled by the concentration of glucose in the blood during pregnancy. In fact, Hahn et al24 have shown that hyperglycemia down-regulates the GLUT1 glucose transport system of term placental trophoblasts. Aberrant expression of the GLUT1 isoform has been demonstrated in human choriocarcinoma,25 gastric tumors,26 cutaneous neoplasms,27 and malignant thyroid nodules.28 GLUT1 overexpression in colon cancers seems to occur as a late event in carcinogenesis and seems to be associated with an increased incidence of lymph node metastases.29

A recent study of GLUT1 in benign, hyperplastic, and malignant endometrial epithelium has demonstrated that aberrant overexpression of GLUT1 is a constant feature of endometrial carcinoma; its immunostaining may be useful for distinguishing benign hyperplasias from hyperplasias that are associated strongly with malignancy, and some or all cases of atypical hyperplasia may be neoplastic rather than hyperplastic.30 Our data revealed that GLUT1 expression in breast carcinomas is associated with tumor grade. These data are not surprising, as in previous work, Younes et al31 demonstrated that GLUT1 expression was increased in poorly differentiated human breast carcinoma with high proliferative activity.

The other marker used in the present study, FAS, has been related to human cancer since 1989 when Kuhajda et al32 demonstrated that FAS, the main synthetic enzyme that catalyzes the NADPH (reduced form of nicotinamide adenine dinucleotide phosphate)-dependent condensation of malonyl coenzyme A and acetyl coenzyme A to produce the 16-carbon saturated free fatty acid palmitate, was overexpressed in highly proliferating and neoplastic cells. Since
then FAS expression has been studied in breast, prostate, and endometrial carcinomas. It has been associated with a poor outcome and with common prognostic factors of relapse. In accordance with all of these studies it is clear that FAS expression is associated with stage of disease, recurrence, and survival. In fact, patients with tumors with low FAS expression had a better outcome. Our data revealed statistical association between FAS and stage of disease. These data confirm recent findings of Alò et al in the assessment of the importance of FAS as a predictor of recurrence in some human carcinomas. The innovative finding concerns the coexpression of GLUT1 and FAS in breast carcinomas and their association with histologic grade and stage of disease. GLUT1 seems to be expressed more in breast carcinomas with aggressive morphologic features. FAS seems to be more involved with stage of disease, suggesting a role of this enzyme in favoring recurrence and poor outcome.

We found that the sequence normal mammary gland, typical/atypical hyperplastic tissue, in situ carcinoma, and infiltrating carcinoma expressed increased levels of both GLUT1 and FAS staining. In addition, normal and typical/atypical hyperplastic breast tissues adjacent to in situ carcinoma, infiltrating carcinoma, or carcinomas with both components expressed the two markers more than did the negative control specimens. This expression may depend on biochemical changes of apparently normal cells or tissues near neoplastic elements. For this reason, we decided to differentiate all steps of breast carcinogenesis.

GLUT1, FAS, and other markers should be used for patients with a high risk for development of breast carcinoma. An overexpression of GLUT1 and FAS in cytologic samples or in typical/atypical hyperplastic tissue may suggest different therapeutic approaches or the need for closer follow-up. The study of the mechanisms that stimulate a higher uptake and use of energy sources in preneoplastic and neoplastic tissues is an important goal for the understanding of carcinogenesis. Further steps are necessary to determine pharmacologic inhibitors of FAS and aberrant GLUT1 expression to evaluate their antineoplastic effects.

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References


