

## Expression of the fructose transporter GLUT5 in human breast cancer

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**ABSTRACT** The primary metabolic characteristic of malignant cells is an increased uptake of glucose and its anaerobic metabolism. We studied the expression and function of the glucose transporters in human breast cancer cell lines and analyzed their expression in normal and neoplastic primary human breast tissue. Hexose uptake assays and immunoblotting experiments revealed that the breast carcinoma cell lines MCF-7 and MDA-468 express the glucose transporters GLUT1 and GLUT2, isoforms expressed in both normal and neoplastic breast tissue. We also found that the breast cancer cell lines transport fructose and express the fructose transporter GLUT5. Immunolocalization studies revealed that GLUT5 is highly expressed *in vivo* in human breast cancer but is absent in normal human breast tissue. These findings indicate that human breast cancer cells have a specialized capacity to transport fructose, a metabolic substrate believed to be used by few human tissues. Identification of a high-affinity fructose transporter on human breast cancer cells opens opportunities to develop novel strategies for early diagnosis and treatment of breast cancer.

Breast cancer is the second leading cause of cancer death in women in the United States and it is estimated that  $\approx 12\%$  of women in the United States will develop breast cancer during their lifetime (1). Breast cancer cells have a high level of glucose uptake and metabolism, a circumstance common to most cancer cells (2). The high rate of glucose uptake in cancer cells is used in the clinic to localize tumors in patients and to assess tumor metabolism and response to therapy by positron emission tomography (PET) scanning with [ $^{18}\text{F}$ ]fluorodeoxyglucose (3–6).

Two systems for the transport of glucose are available in mammalian cells: the  $\text{Na}^+$ /glucose cotransporters (7) expressed primarily in small intestine and kidney, and the facilitative glucose transporter family (8), products of distinct genes that are expressed in all cells in a finely controlled and tissue-specific manner. Six different facilitative glucose transporter isoforms have been molecularly cloned: GLUT1, expressed in all tissues and especially abundant in erythrocytes and brain (9); GLUT2, present in liver, pancreatic islet  $\beta$  cells, kidney, and at the basolateral surface of the adsorptive cells of the small intestine (10); GLUT3, abundant in brain (11); GLUT4, restricted to adipose and skeletal tissues (12); GLUT5, expressed in small intestine and sperm cells (13, 14); and GLUT7, restricted to microsomes of liver cells (15). Available evidence indicates that the mechanism by which cancer cells increase their ability to take up glucose involves the selective overexpression of GLUT1 (16–19).

It is known that GLUT1 is responsible for glucose transport in breast tissue (19–21) and GLUT2 has also been detected in

normal breast tissue (19). Immunohistochemical evidence has been obtained indicating that GLUT1 is highly expressed in breast cancer cells compared to their normal counterparts, but no apparent changes in the expression of GLUT2 were detected (19). We analyzed the expression and function of glucose transporters in the human breast cancer cell lines MCF-7 and MDA-468 and in normal and neoplastic human breast tissue and found that in addition to overexpressing GLUT1, human breast cancer tissue selectively expresses the high-affinity fructose transporter GLUT5.

### MATERIALS AND METHODS

The human breast cancer cell lines MCF-7 and MDA-468 were obtained from American Type Culture Collection and grown in a mixture of Dulbecco's modified Eagle's medium containing high glucose (17.5 mM) and F-12 medium (1:1; vol/vol) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. For uptake assays, the cells were grown as monolayer cultures in six-well plates to a density of  $\approx 1 \times 10^6$  cells per well. Cultures were carefully selected under the microscope to ensure that only plates showing uniformly growing cells were used. Two wells in each plate were used to determine the number of cells, and the four companion wells were used for the uptake assays. The cells were washed with incubation buffer (22) (15 mM Hepes/135 mM NaCl/5 mM KCl/1.8 mM  $\text{CaCl}_2$ /0.8 mM  $\text{MgCl}_2$ ) and incubated in the same medium for 30 min at 37°C. Uptake assays were performed at room temperature in 1 ml of incubation buffer containing 0.2 mM deoxyglucose and 2–4  $\mu\text{Ci}$  (1 Ci = 37 GBq) of 2-deoxy-D-[1,2-( $\text{N}^3\text{H}$ )]glucose (30.6 Ci per mmol; DuPont/NEN). Uptake was stopped by washing the cells with ice-cold phosphate-buffered saline (PBS). Cells were dissolved in 0.5 ml of lysis buffer (10 mM Tris-HCl, pH 8.0/0.2% SDS), and the incorporated radioactivity was assayed by liquid scintillation spectrometry. Where appropriate, competitors and inhibitors were added to the uptake assays or preincubated with the cells. Fructose uptake assays were performed in incubation buffer containing 1 mM fructose and 0.8  $\mu\text{Ci}$  of D-[ $^{14}\text{C}$ ]fructose per ml (285 mCi/mmol; Amersham). Samples were processed as indicated for deoxyglucose uptake. Data represent means  $\pm$  SD of four samples.

Immunoblotting was performed as described (23) using anti-GLUT antibodies (East Acres Biologicals, Southbridge, MA) and horseradish peroxidase goat anti-rabbit IgG and enhanced chemiluminescence (Amersham). For immunocytochemistry, cells were grown on eight-well microscope slides, fixed with buffered formaldehyde/acetone, washed with PBS, and incubated in PBS containing 5% bovine serum albumin (BSA) followed by incubation for 1 h at room temperature in the same buffer containing 1% BSA, 0.3% Triton X-100, and

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Abbreviation: PET, positron emission tomography.  
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anti-GLUT antibodies (1:100) or rabbit preimmune serum. Cells were then incubated with fluorescein isothiocyanate goat anti-rabbit IgG (Life Technologies; 1:40) for 1 h, mounted, and analyzed by fluorescence microscopy. Breast tissue expression of GLUT5 was determined by immunohistochemical analysis of a set of thin sections prepared from archived paraffin tissue blocks. Paraffin was removed by incubating the sections in xylene followed by absolute alcohol and then the sections were hydrated by immersion in graded alcohol solutions. Sections were incubated in PBS containing 5% skim milk, washed with PBS, and incubated with the anti-GLUT antibodies (1:100) for 2 h. After extensive washing with PBS, sections were incubated for 1 h with alkaline phosphatase goat anti-rabbit IgG (1:500) and color developed with 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate.

## RESULTS

We measured the uptake of deoxyglucose, an analog of glucose transported only by the facilitative glucose transporters (8), in MCF-7 and MDA-468 cells. These cell lines are widely used to characterize the behavior of human breast cancer *in vitro* and *in vivo* (24). Both cell lines showed a notable capacity to take up deoxyglucose (Fig. 1A). Uptake was approximately linear for the first 20 min of incubation, with the MDA-468 cells taking up at least 4-fold more deoxyglucose than the MCF-7 cells. Methylglucose, an analog of glucose that enters the cells but is not metabolized, was used to estimate the intracellular volume available for exchange with the external medium (8). MDA-468 cells had an approximate intracellular exchange volume of 3  $\mu$ l per 10<sup>6</sup> cells, while in MCF-7 cells the value was 5  $\mu$ l per 10<sup>6</sup> cells. The volume estimates were used to express

uptake data in terms of intracellular concentrations. After 1 h of incubation, the MDA-468 cells accumulated an intracellular concentration of deoxyglucose in excess of 140-fold the external concentration, whereas the MCF-7 cells accumulated intracellularly  $\approx$ 15-fold the external glucose concentration. Data from Lineweaver–Burk plots (Fig. 1B and C) or from Eadie–Hofstee plots (data not shown) revealed the presence of two functional components with separate affinities for uptake of deoxyglucose in both cell lines—a high-affinity component with an apparent  $K_m$  for transport of 2 mM and a second component of lower affinity with an apparent  $K_m$  for transport of 10 mM. When we measured uptake of deoxyglucose at very short intervals, from 5 sec to 2 min, the rate of uptake of deoxyglucose by MDA-468 cells was 3-fold higher than that of MCF-7 cells (Fig. 1D). Therefore, the 5-fold difference in uptake observed in long-term uptake experiments (Fig. 1A) reflects a step secondary to transport, most likely the intracellular trapping of deoxyglucose as deoxyglucose 6-phosphate (8). Kinetic analysis using Lineweaver–Burk (Fig. 1E and F) or Eadie–Hofstee (data not shown) plots confirmed the presence of two functionally distinct glucose transporters in the breast cancer cells. Using the Michaelis expression for a single-substrate reaction under conditions of initial velocity (25), we estimated that at a glucose concentration of 5.5 mM, the high-affinity system comprises three-quarters of the capacity of the breast carcinoma cell lines to take up glucose (Fig. 1G and H).

The apparent  $K_m$  values for transport of deoxyglucose in breast cancer cells were similar to those described for the transport of deoxyglucose mediated by GLUT1 and GLUT2 in other cellular systems. We further tested the expression of GLUT2 in breast cancer cells by measuring the transport of

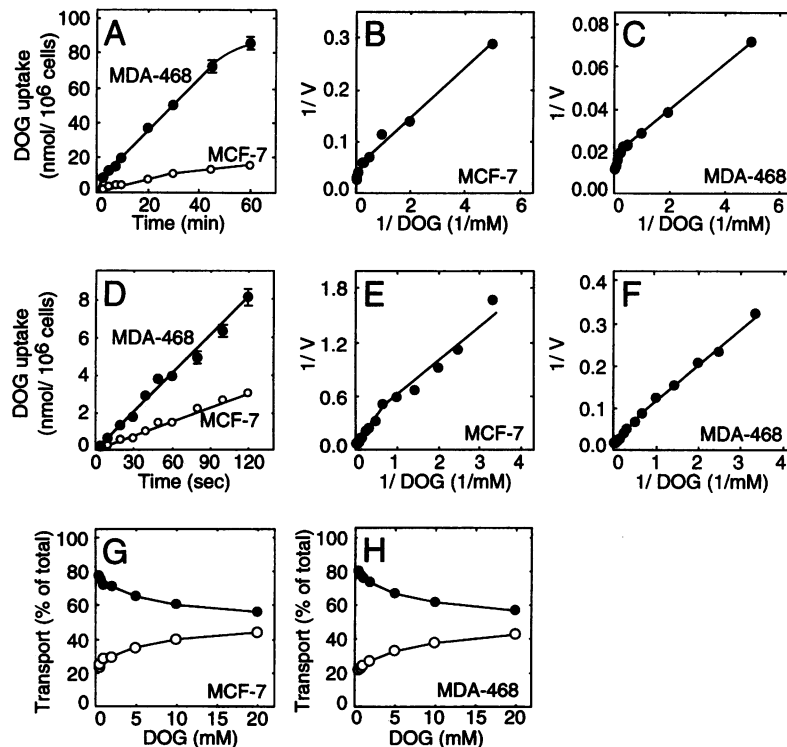


FIG. 1. Human breast cancer cell lines MCF-7 and MDA-468 express two functionally distinct facilitative glucose transporters. (A) Time course of the uptake of deoxyglucose by MCF-7 (○) and MDA-468 (●) cells. (B) Double reciprocal plot of substrate dependence for the uptake of deoxyglucose by MCF-7 cells using 10-min assays. (C) Double reciprocal plot of substrate dependence for the uptake of deoxyglucose by MDA-468 cells using 10-min assays. (D) Time course of transport of deoxyglucose by MCF-7 (○) and MDA-468 (●) cells. (E) Double reciprocal plot of substrate dependence for the transport of deoxyglucose by MCF-7 cells using 40-sec assays. (F) Double reciprocal plot of substrate dependence for the transport of deoxyglucose by MDA-468 cells using 40-sec assays. (G) Uptake of deoxyglucose mediated by the high-affinity (●) and the low-affinity (○) transporter in MCF-7 cells. (H) Uptake of deoxyglucose mediated by the high-affinity (●) and the low-affinity (○) transporter in MDA-468 cells. Data represent means  $\pm$  SD of four samples. DOG, deoxyglucose.

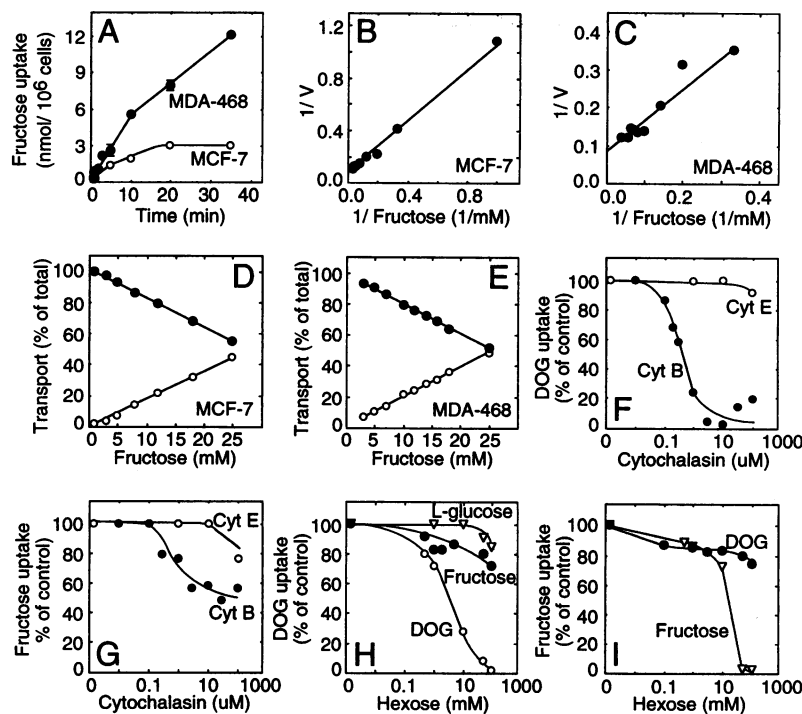


FIG. 2. Human breast cancer cell lines MCF-7 and MDA-468 express a high-affinity fructose transporter distinct from GLUT2. (A) Time course of the uptake of fructose in MCF-7 (○) and MDA-468 (●) cells. (B) Double reciprocal plot of substrate dependence for the transport of fructose by MCF-7 cells using 50-sec assays. (C) Double reciprocal plot of substrate dependence for the transport of fructose by MDA-468 cells using 50-sec assays. (D) Uptake of fructose mediated by the high-affinity (●) and the low-affinity (○) pathway in MCF-7 cells. (E) Uptake of fructose mediated by the high-affinity (●) and the low-affinity (○) pathway in MDA-468 cells. Data represent means  $\pm$  SD of four samples. (F) Effect of cytochalasin B (●) and cytochalasin E (○) on uptake of fructose by MCF-7 cells. (G) Effect of cytochalasin B (●) and cytochalasin E (○) on uptake of deoxyglucose by MCF-7 cells. (H) Effect of fructose (●), deoxyglucose (○), and L-glucose (▽) on uptake of deoxyglucose by MCF-7 cells. (I) Effect of deoxyglucose (●) and fructose (▽) on uptake of fructose by MCF-7 cells. DOG, deoxyglucose.

fructose because GLUT2 is able to transport fructose in addition to glucose (26). Both cell lines were able to take up fructose (Fig. 2A), and uptake was linear for approximately the first 60 sec. In long-term uptake studies, we observed that fructose uptake by MDA-468 cells was  $\approx$ 5-fold greater than in MCF-7 cells. At short uptake times the difference was only 2-fold, similar to that observed for transport of deoxyglucose. Lineweaver–Burk analysis of the transport of fructose revealed two fructose transport activities—a high-affinity component with an apparent  $K_m$  of  $\approx$ 10 mM for fructose transport in both MCF-7 and MDA-468 cells (Fig. 2B and C) and a low-affinity pathway that showed no saturation at concentrations of fructose as high as 50 mM (data not shown). The value of 10 mM is 1 order of magnitude lower than the values previously described for transport of fructose by GLUT2 (26). At concentrations of fructose lower than 10 mM, the high-affinity pathway contributed  $>$ 90% of the capacity of the MCF-7 (Fig. 2D) and MDA-468 (Fig. 2E) cells to transport fructose.

These data suggested the presence of the fructose transporter GLUT5 in MCF-7 cells. GLUT5 has a  $K_m$  for the transport of fructose of 6 mM, is not inhibited by cytochalasin B, a specific inhibitor of the facilitative hexose transporters, and does not transport deoxyglucose (14). In MCF-7 cells, transport of deoxyglucose was completely inhibited by cytochalasin B but not by cytochalasin E, an analog of cytochalasin B that does not interact with the glucose transporters (8) (Fig. 2F). Fifty percent inhibition was observed at 0.4  $\mu$ M cytochalasin B, a value that falls between the value of the  $K_i$  for GLUT1 ( $\approx$ 0.2  $\mu$ M) and the  $K_i$  for GLUT2 ( $\approx$ 2  $\mu$ M) (27). On the other hand, 100  $\mu$ M cytochalasin B inhibited  $<$ 40% of fructose uptake, and  $<$ 20% inhibition was observed with 1  $\mu$ M cytochalasin B (Fig. 2G). Cytochalasin E did not interfere with the transport of fructose. It is known that fructose can completely inhibit the uptake of deoxyglucose mediated by

GLUT2 (26). At 10 mM fructose, the transport of deoxyglucose was not substantially affected, and at 100 mM fructose there was only  $\approx$ 30% inhibition of deoxyglucose uptake (Fig. 2H). These results suggest that GLUT2 likely corresponded to the low-affinity pathway. As controls we used L-glucose (8), a sugar that is not transported by the facilitative glucose transporters and did not inhibit the transport of deoxyglucose by the MCF-7 cells, and deoxyglucose, which competed for

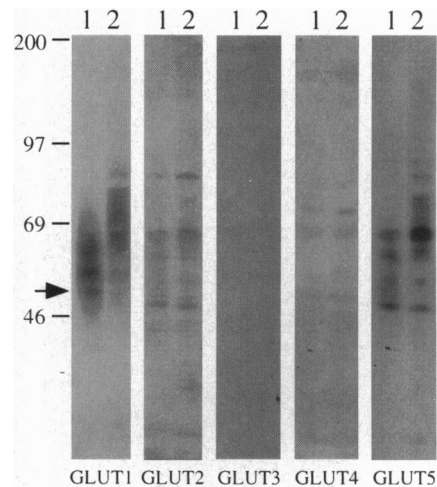


FIG. 3. Hexose transporters GLUT1, GLUT2, and GLUT5 are expressed in human breast cancer cell lines MCF-7 (lanes 1) and MDA-468 (lanes 2). Results of the immunoblots with anti-GLUT1, anti-GLUT2, anti-GLUT3, anti-GLUT4, and anti-GLUT5 antibodies are shown. Sizes on left are kDa. Arrow indicates the migration of the human erythrocyte glucose transporter.

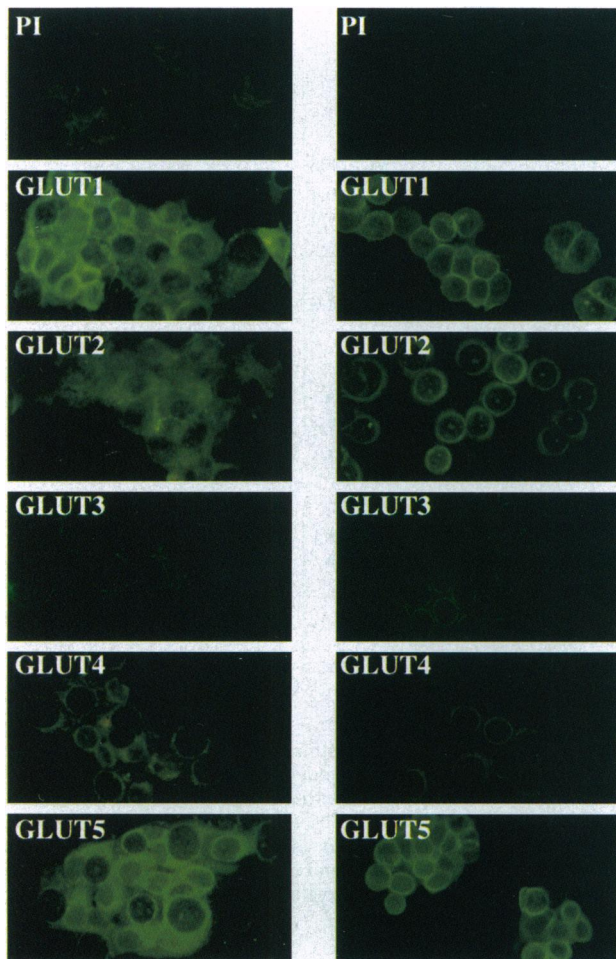


FIG. 4. Immunolocalization of hexose transporters expressed in human breast cancer cell lines MCF-7 and MDA-468. For immunohistochemistry, cells were incubated with the different anti-GLUT antibodies followed by incubation with a secondary antibody coupled to fluorescein.

$^3\text{H}$ ]deoxyglucose uptake completely at 100 mM (Fig. 2H). Previous data indicated that deoxyglucose is able to inhibit

completely the uptake of fructose mediated by GLUT2 but does not affect fructose uptake mediated by GLUT5 (14). Deoxyglucose (100 mM) inhibited <20% of fructose uptake (Fig. 2J), contrasted with the effect of 100 mM fructose, which inhibited  $^3\text{H}$ ]fructose uptake completely (Fig. 2J).

The presence of GLUT1, GLUT2, and GLUT5 in the human breast cancer cell lines was confirmed by immunoblotting and immunolocalization with anti-glucose transporter antibodies. Immunoblotting experiments revealed the presence of several overlapping anti-GLUT1 immunoreactive bands in total cell homogenates from both cell lines (Fig. 3). Bands of 45–80 kDa were labeled in the MCF-7 cells, compared to bands of 60–90 kDa in the MDA-468 cells. The anti-GLUT1 antibody reacted with a band of  $\approx 45$  kDa in an immunoblot of human erythrocyte proteins (data not shown). GLUT2 was also expressed in both breast cancer cell lines (Fig. 3). Anti-GLUT2 antibodies reacted with several bands of 46–90 kDa and differences in the intensities of the various bands were observed depending on the cell line. The anti-GLUT2 antibody reacted with a unique band of  $\approx 50$  kDa in an immunoblot of total liver proteins (data not shown). GLUT5 was also expressed in the MCF-7 and MDA-468 cell lines (Fig. 3). Bands of 50–70 kDa were labeled in the MCF-7 cells, compared to bands of 50–85 kDa in the MDA-468 cells. Proteins from human testis showed a broad band of 40–60 kDa when immunoblotted with the anti-GLUT5 antibody (data not shown). No reactivity was observed when the blots were incubated with an anti-GLUT3 antibody, whereas GLUT4 was present in very small amounts in the two breast cancer cell lines (Fig. 3). GLUT3 reacted with a unique 50-kDa band present in human sperm proteins and GLUT4 reacted with a 40- to 50-kDa band from human adipose tissue (data not shown). Immunolocalization studies using immunofluorescence confirmed the presence of the transporters GLUT1, GLUT2, and GLUT5 in the breast cancer cell lines (Fig. 4). Most of the fluorescence was associated with the cytoplasm adjacent to the nucleus and the plasma membrane, and the staining was most intense in cells probed with the anti-GLUT1 and anti-GLUT5 antibodies. Cellular staining with anti-GLUT2 antibodies was weaker than with anti-GLUT1 or anti-GLUT5 antibodies and was clearly stronger in MDA-468 than in MCF-7 cells (Fig. 4). A low level of fluorescence was observed when both cell lines were probed with anti-GLUT4 antibodies, and no fluorescence

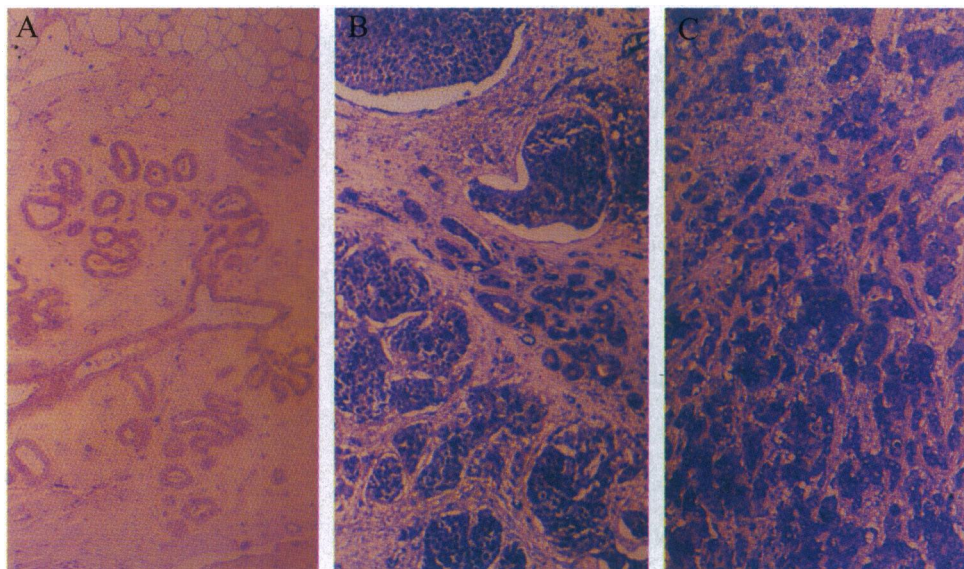


FIG. 5. Immunolocalization of fructose transporter GLUT5 in human breast tissue. (A) Immunohistochemistry of normal mammary tissue. Mammary epithelial cells do not express GLUT5 as revealed by basal anti-GLUT5 reactivity. (B and C) Immunohistochemical localization of GLUT5 in human breast cancer tissue. High-expression of GLUT5 is evidenced by strong anti-GLUT5 immunoreactivity. ( $\times 25$ )

was detected in cells reacted with anti-GLUT3 antibodies or preimmune serum (Fig. 4).

The results of immunoblotting and immunofluorescence experiments were concordant with the results of the transport studies and indicate that the breast cancer cell lines MCF-7 and MDA-468 express high levels of the transporters GLUT1, GLUT2, and GLUT5. The presence of the high-affinity fructose transporter GLUT5 in breast cancer cell lines was surprising since this transporter is believed to have a restricted cell and tissue distribution (13, 14, 28). The finding suggested that the expression of GLUT5 in these cells could be related to the neoplastic state. We therefore tested for expression of GLUT5 in normal and neoplastic primary human breast tissue. All 20 primary breast cancer tissues tested were positive for expression of GLUT5 (Fig. 5 B and C; data not shown). Strong staining was seen in the perinuclear region, cytoplasm, and cell membrane of tumor cells. Staining was also seen in malignant cells invading the fibroadipose tissue. There was no staining of normal mammary tissue, indicating that normal mammary epithelium does not express GLUT5 (Fig. 5A; data not shown). No staining was observed in breast cancer tissue probed with preimmune serum. In control experiments, anti-GLUT1 staining was stronger in breast cancer tissue compared to normal breast tissue, consistent with overexpression of GLUT1 in the neoplastic cells (data not shown). Thus, while GLUT1 is present in normal breast and is overexpressed in breast cancer, GLUT5 is absent in normal breast tissue and is expressed at high levels in human breast cancer.

## DISCUSSION

Our data indicate that the breast carcinoma cell lines MCF-7 and MDA-468 express three members of the facilitative hexose transporter family, the glucose transporters GLUT1 and GLUT2, and the high-affinity fructose transporter GLUT5. Deoxyglucose uptake and competition experiments in the cell lines indicated the presence of two functionally distinct glucose transport systems with the characteristics expected for GLUT1 and GLUT2, with apparent  $K_m$  values of 2 and 10 mM for the transport of deoxyglucose, respectively. Fructose uptake and competition experiments indicated the presence in breast carcinoma cell lines of a high-affinity transporter of fructose functionally similar to GLUT5 (apparent  $K_m$ , 8 mM) and a low-affinity pathway that failed to saturate at fructose concentrations as high as 50 mM functionally similar to GLUT2 ( $K_m > 50$  mM). The presence of GLUT1, GLUT2, and GLUT5 in the breast carcinoma cell lines was confirmed by immunoblotting and immunolocalization experiments, which also revealed a low level of expression of GLUT4 and no expression of GLUT3.

We found that GLUT1 and GLUT2 were present in normal and neoplastic breast tissue but that GLUT5 was expressed in human breast cancer and was absent from normal human breast tissue. Enhanced uptake and metabolism of glucose is a common characteristic of cancer cells and involves increased expression of GLUT1 in neoplasia (2, 16–19). Enhanced glucose uptake in tumor cells is used to detect tumors and to follow their response to treatment in a noninvasive manner by PET imaging with [ $^{18}$ F]fluorodeoxyglucose (3–6), a substrate that enters cells through the glucose transporters. GLUT2 is present in normal and neoplastic human breast tissue, although no changes in expression in the neoplastic state have been detected (19).

Our finding that GLUT5 is expressed in human breast cancer and is absent in normal breast tissue may have clinical application. GLUT2 and GLUT5 can mediate the transport of fructose (14, 26) and GLUT2 is also capable of transporting glucose (26, 27, 29). GLUT2 is expressed in liver, pancreatic  $\beta$  cells, the basolateral surface of kidney and small intestine epithelia (10, 30), and in normal breast tissue (19). GLUT5

does not transport glucose (14) and is expressed in small intestine, sperm cells, and brain (31, 32), with very low level expression in adipose tissue and muscle (13, 14, 28). GLUT5 is also expressed in Caco-2 cells, a human colon cancer cell line that differentiates in culture into cells with the properties of small intestine enterocytes (33). GLUT5 transports fructose with high affinity (14), whereas GLUT2 transports this sugar with very low affinity (26). At the low concentrations of fructose present *in vivo*, GLUT5 is likely to mediate a high fraction (>90%) of the uptake of fructose due to the order of magnitude difference in the respective  $K_m$  for transport of fructose by GLUT2 and GLUT5. These findings suggest an important role for GLUT5 in cellular uptake of fructose by breast cancer cells compared to normal breast tissue in which transport of fructose is mediated by GLUT2. Our results indicate that neoplastic transformation of breast epithelial cells leads to expression of a high-affinity fructose transporter permitting enhanced uptake of fructose, a substrate apparently used by few human tissues. Based on the Warburg theory, we can speculate that fructose may be a good substrate for energy generation in malignant cells that prefer the glycolytic pathway since lactic acid generation through fructolysis may not be subjected to the regulatory steps that control glycolysis. The fructolysis pathway may provide the neoplastic breast cancer cells with a metabolic advantage. These results suggest that fructose uptake could represent a useful target for PET imaging and possibly the development of novel therapeutic agents in breast cancer.

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