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Wide Expression of the Human Erythrocyte Glucose Transporter Glut1 in Human Cancers

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ABSTRACT

Glucose uptake has been found to be increased in cancer cells. Previous work has shown increased expression of the human erythrocyte glucose transporter (Glut1) mRNA in some human cancers, indicating that Glut1 may play a significant role in glucose uptake by these tumors. The distribution of Glut1 protein in normal and malignant human tissues is still largely unknown. Using immunohistochemistry, we found that Glut1 is largely undetectable in normal epithelial tissues and benign epithelial tumors but is expressed in a significant proportion of a variety of human carcinomas. We hypothesize that Glut1 expression by human carcinomas indicates an increased glucose uptake, and probably increased utilization of energy, which may correlate with an aggressive behavior. The biological significance of Glut1 expression needs to be determined.

INTRODUCTION

Malignant cells show an increased glucose uptake and utilization when compared to their benign/normal counterparts (1, 2). This uptake is mediated by glucose transporters, the expression and activity of which have been found to be regulated by oncogenes and growth factors (3–7). Understanding the distribution and function of these transporters in malignant and normal cells and tissues may lead to the development of new diagnostic and prognostic markers, as well as the development of specific monoclonal antibodies that could be used in tumor localization and treatment.

The human erythrocyte glucose transporter (Glut1) is a member of an expanding family of transmembrane proteins that currently has six members (8). Glut1 mRNA[2] and protein have been found in rat brain, endothelia of the human blood-brain barrier and of the liver, human erythrocytes, HepG2 hepatic carcinoma cell line, rat kidney, rat mammary gland, and placenta, including fetal membranes (9–15).

Earlier studies demonstrated the presence of mRNA from different Gluts in human tumors (9, 16–18) and a significant increase in the abundance of mRNA for Glut1 in cancers of the esophagus, colon, and pancreas (19). The apparent overexpression of certain types of glucose transporters in human cancers suggests an important role for these transporters in tumor biology. Yet, Glut1 protein has been characterized only in human head and neck tumors (20), breast cancer (21), insulinomas (22), and renal cell carcinomas (23). GLUT1 expression in normal human tissues and in most tumors is still largely unknown. This study was undertaken to determine by immunohistochemistry: (*a*) the distribution of Glut1 in normal human tissues and malignant tumors; and (*b*) whether the extent of Glut1 expression in malignant human tumors can account for the reported increase in glucose uptake by these tumors.

MATERIALS AND METHODS

Antibody. MYM is a rabbit polyclonal antibody (serum) that we have generated to a 12-amino acid synthetic peptide corresponding to the COOH terminus of human Glut1.

Western Blot Analysis. Human RBC membranes were prepared and solubilized in sample buffer, the protein contents was determined, and equal amounts (30 μg) were loaded on 12% SDS-polyacrylamide gel. After electrophoretic separation, the proteins were transferred onto a nitrocellulose membrane and blocked with 2% normal goat serum in 1% BSA in PBS for 1 h at room temperature. Membranes were incubated for 1 h at room temperature with either 1:10,000 dilution of MYM or with 1:10,000 preimmune rabbit serum from the same rabbit in which the antibody was generated (negative control). The bound antibody was detected using Vectastain ABC Elite rabbit kit (Vector Laboratories, Burlingame, CA), using DAB[3] as chromogen.

Immunohistochemistry. Normal tissues in this study included grossly and microscopically unremarkable tissues removed during operations for benign or malignant tumors involving the same organs. Sections of 251 formalin-fixed and paraffin-embedded normal human tissues, benign tumors, and malignant neoplasms (Table 1) were cut and mounted on Fisher Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA) and heated at 58°C for 4 h. Sections were deparaffinized in xylene, rehydrated through decreasing concentrations of alcohol ending in PBS, and microwaved in 10 mM citrate buffer (pH 6.0) for 15 min. Then the sections were incubated with 2% normal goat serum in 1% BSA/PBS for 30 min at room temperature, washed in PBS, and incubated with MYM antibody diluted 1:3000 in 0.1% BSA/PBS for 60 min at room temperature. Finally, sections were washed in PBS, and the bound antibody was detected using Vectastain Elite ABC rabbit kit (Vector) with DAB as chromogen. Sections were counterstained with hematoxylin, dehydrated, and mounted. Negative controls were sections immunostained as above, but instead of incubation with MYM, the sections were incubated with 1:3000 dilution of the preimmune serum or with the same concentration of MYM, which was preincubated overnight at 4°C with 0.3 mg/ml of the immunizing peptide.

RESULTS

Western blot analysis showed that the antibody MYM recognizes the M_r 55,000 Glut1 protein in a preparation of human RBC membranes (Fig. 1). This is similar to what has been reported previously by Haspel *et al.* (24), who generated a polyclonal antibody to a similar synthetic peptide.

Immunostaining of Glut1 was always intense and membranous (Fig. 2*A*), whereas negative controls showed no Glut1 staining (Fig. 2*B*). Occasionally, especially in gastrointestinal tissues, there was nonspecific granular cytoplasmic staining, mainly localized in the Golgi area, and also present in the negative control slides. When benign tissues were positive, approximately 100% of the cells were positive. Glut1 expression in carcinomas, when present, was more variable, ranging from a few to almost 100% of the cells. For the purpose of this study, cases in which 100% of the cells were Glut1 negative by immunohistochemistry were considered Glut1 negative; otherwise, they were considered Glut1 positive. The results of the Glut1 immunostaining are summarized in Table 1.

RBC in tissue sections were always positive and served as internal positive control. Other normal structures that were positive for Glut1 were perineurium, microvessels in the brain (blood-brain barrier),

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[2] The gene symbol for Glut1 is *SLC2A1*.

[3] The abbreviation used is: DAB, 3,3′-diaminobenzidine.

Table 1 *Glut1 expression in benign and malignant tissues*

Tissue	Diagnosis	Positive cases/Total
Bladder	Transitional cell carcinoma	3/3
Brain	Normal (excluding vessels)	0/2
Breast	Normal	0/5
	Carcinoma	5/11
Colon	Normal	0/8
	Hyperplastic polyp	0/3
	Adenoma	0/2
	Adenocarcinoma	7/9
Esophagus	Normal	2/4[a]
	Adenocarcinoma	4/7[b]
Heart (myocardium)	Normal	0/2
	Rejection	0/4
Kidney	Normal	1/4
	Renal cell carcinoma	2/4
Liver	Normal	0/3
	Cirrhosis	0/4
	Adenoma	0/2
	Hepatocellular carcinoma	0/5
Lung	Normal	0/6
	Carcinoma	11/14[b]
Lymphoid	Lymphoma	0/7
	Reactive follicular hyperplasia	0/5
Ovary	Normal	0/2
	Carcinoma	4/7[b]
Pancreas	Normal	0/5
	Adenocarcinoma	10/26[b]
	Neuroendocrine tumors	0/3
Parathyroid	Hyperplasia	0/2
Placenta	Normal	2/2[b]
Prostate	Hyperplasia	0/4
	Carcinoma	1/4
Skeletal	Muscle	0/2
Skin	Normal	0/3
	Squamous cell carcinoma	3/4[b]
	Melanoma	0/4
Small bowel and ampulla	Normal	0/6
	Adenoma	0/3
	Adenocarcinoma	7/24
Stomach	Normal	0/4
	Chronic inflammation	0/2
	Adenocarcinoma	3/6[b]
Testis	Seminoma	2/3[b]
	Mixed germ cell tumor	3/3[b]
Thyroid	Benign	0/5
	Papillary carcinoma	0/4
Uterus	Normal	0/4
	Leiomyoma	0/2
	Adenocarcinoma	2/3[b]

[a] Only the basal cell layer adjacent to Barrett's epithelium was positive.
[b] Tumors (or normal tissues) in which 50% or more of the positive cases showed Glut1 immunoreactivity in at least 25% of the cells.

germinal centers of reactive lymphoid tissues, placental trophoblastic cells, renal tubules, and the basal cell layer of the esophageal mucosa in the area adjacent to Barrett's metaplasia, when present. Smooth muscle, skeletal muscle, myocardium, endothelium (other than in brain vessels and reactive lymphoid follicles), neurons, melanocytes, fibroblasts, mesothelial cells, tissue lymphocytes, and macrophages were negative. Epithelial cells of the stomach, small intestine, colon, breast, liver, lung, endometrium, pancreas, prostate, skin, parathyroid, and thyroid were also negative.

Of the tumors, all lymphomas, central nervous system tumors, papillary thyroid carcinomas, melanomas, and hepatocellular carcinomas were negative. Glut1 was expressed in 3 of 3 transitional cell carcinomas of the urinary bladder (100%), 5 of 11 breast carcinomas (45%), 7 of 9 colon carcinomas (78%), 7 of 24 carcinomas of the ampulla of Vater (29%), 4 of 7 esophageal adenocarcinomas (57%), 2 of 4 renal cell carcinomas (50%), 11 of 14 lung carcinomas (79%), 4 of 7 ovarian carcinomas (57%), 10 of 26 pancreatic carcinomas (38%), 1 of 4 prostatic carcinomas (25%), 3 of 6 gastric carcinomas (50%), 3 of 4 squamous cell carcinomas of the skin (75%), 5 of 6 testicular germ cell tumors (83%), and 2 of 3 endometrial carcinomas (67%) (Table 1).

DISCUSSION

Our results show that, with few exceptions, Glut1 is not detectable in normal human tissues and benign lesions. Glut1 is believed to have a ubiquitous distribution in most cell types, but such belief is based largely on mRNA studies carried out on mouse and rat tissues (8). Failure of our antibody to detect such expression in almost all normal and benign human tissues suggests that this protein may be either rapidly degraded in normal human tissues or that our antibody and/or immunodetection method are not sensitive enough to detect Glut1 in most normal tissues, where it may be expressed at low levels. Moreover, the presence of a given mRNA in certain tissues does not always correlate with expression of the corresponding protein. This has been shown with human Glut3, where mRNA studies showed ubiquitous distribution in most tissues (25), whereas subsequent research re-

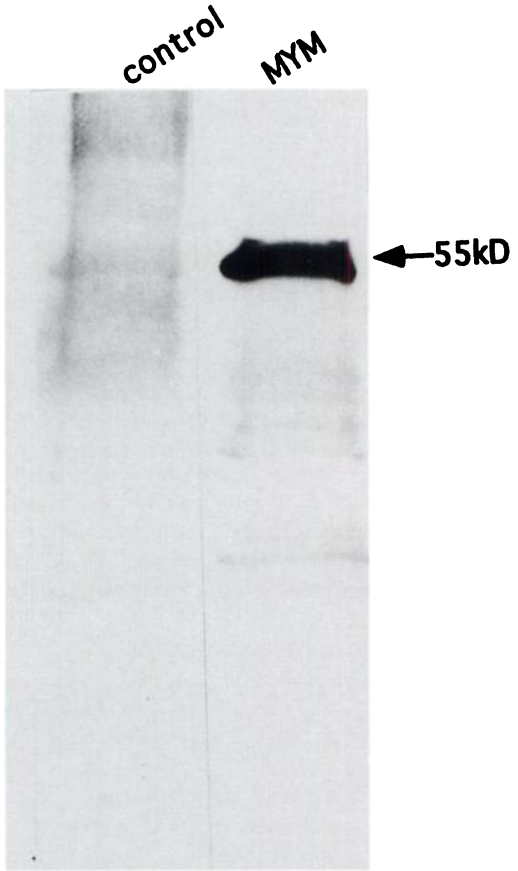

Fig. 1. Identification of Glut1 in human RBC membranes by the polyclonal antibody MYM. Thirty μg of RBC membranes were loaded per lane of 12% SDS-polyacrylamide gels and subjected to electrophoresis. The separated proteins were transferred onto nitrocellulose membranes and incubated with the anti-Glut1 antibody MYM or with preimmune rabbit serum (control), and the bound antibody was then detected by the ABC immunoperoxidase technique, using DAB as chromogen.

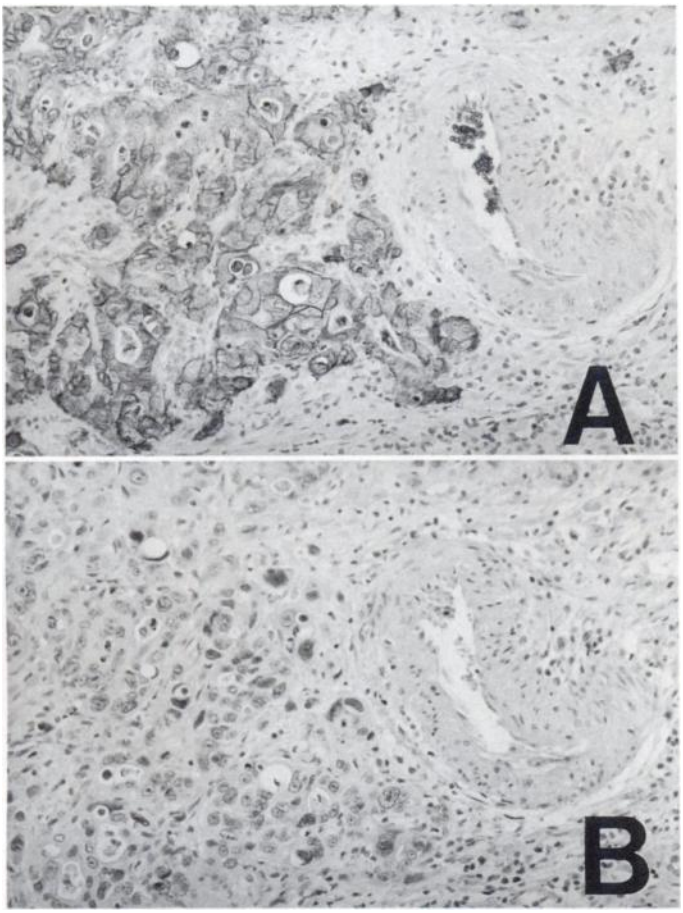

Fig. 2. Immunohistochemical localization of Glut1 in a section of infiltrating ductal carcinoma of the breast with the MYM antibody. Note the membranous staining of cancer cells and RBC in the adjacent blood vessel in the section immunostained with 1:3000 dilution of the antibody (*A*) and the absence of such staining in a section immunostained with the same concentration of MYM that was preincubated overnight at 4°C with 0.3 mg/ml of the immunizing peptide (*B*; negative control). Stromal cells were negative. Immunoperoxidase staining with hematoxylin counterstaining, ×200.

vealed restricted protein distribution (26, 27). Studies of Glut1 localization in sections of normal human tissues are few and are limited to few organs, such as the brain, placenta, breast, and kidney, in addition to RBC. The lack of detectable Glut1 in most of our normal kidney specimens, despite consistent demonstration by others of Glut1 mRNA and protein in rat kidney, is consistent with the findings of Nagase *et al.* (23), who were unable to detect Glut1 in sections of 71 of 75 histologically normal human kidney samples. We speculate that the occasional detection of Glut1 in human kidney [in one kidney in this study and in 4 in the study by Nagase *et al.* (23)] could be the result of the effect of autocrine growth factors secreted by the adjacent tumor, since all our tissues were from histologically normal samples, collected next to renal cell carcinomas. Although none of the benign tumors expressed Glut1 in our study, because of the relatively small number of cases, it is possible that additional studies may show occasional benign tumors with Glut1 expression.

Positron emission tomography has demonstrated increased glucose uptake by human cancers and their metastasis *in vivo* (28–31). This uptake is unlikely to be mediated by Glut1 alone. The fact that 87 of 154 malignant human neoplasms did not show detectable Glut1 in our study indicates that other Gluts may mediate glucose uptake in these tumors. Furthermore, expression of Glut1 in only a portion of the cancer cells in some of the positive tumors suggests that glucose uptake in these tumors may be mediated by other Gluts in addition to Glut1. This would be consistent with the findings by Yamamoto *et al.* (19), who showed that some carcinomas of the digestive system overexpress mRNA for more than one type of glucose transporter.

Finally, the expression of Glut1 by a significant number of carcinomas in a variety of organs, and its relatively undetectable expression in corresponding normal and benign tissues, indicates that this transporter probably plays an important role in the uptake of glucose by malignant neoplasms. Glucose is a major source of energy for many human cancers, and increased Glut1 expression may indicate an increased utilization of energy, which in turn may correlate with aggressive behavior. Using our antibody and immunodetection method, we found recently that Glut1 expression by tumor cells in colorectal adenocarcinomas is associated with a high incidence of lymph node metastasis.[4] The full biological significance of Glut1 expression in human cancers needs to be explored further.

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