

Human Erythrocyte Glucose Transporter (Glut1) Is Immunohistochemically Detected as a Late Event during Malignant Progression in Barrett's Metaplasia¹

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Abstract

We have previously demonstrated that the human erythrocyte glucose transporter (Glut1) is expressed in adenocarcinoma arising in Barrett's metaplasia (BM). We have also shown that Glut1 is expressed as a late event during colorectal carcinogenesis. The aim of this work was to determine the chronology of Glut1 expression during the neoplastic progression in Barrett's metaplasia. Formalin-fixed, paraffin-embedded tissue sections of 251 biopsies from 97 patients with BM were immunostained with the anti-Glut1 antibody MYM, after microwave-aided antigen retrieval, using the standard avidin-biotin complex immunoperoxidase technique. Dysplasia was graded as negative (ND), low grade (LGD)/indefinite or high grade (HGD). None of the 181 biopsies with ND (0%) or 51 biopsies with LGD (0%) showed Glut1 immunoreactivity. More importantly, although 0 of 6 biopsies with HGD (0%) expressed Glut1, 9 of 13 biopsies with adenocarcinoma (69%) were Glut1 positive ($P = 0.0108$, Fisher's exact test). Our results indicate that Glut1 is expressed as a late event during the neoplastic progression in BM. Prospective studies are needed to determine the clinical utility of Glut1 immunoreactivity as a marker of carcinoma in patients with BM.

Introduction

BM³ is a condition in which the normal squamous lining of the esophagus is replaced by columnar epithelium and is mainly caused by chronic gastroesophageal reflux (1). The association of this condition with esophageal adenocarcinoma (carcinoma) has been well established (2-4). Whereas esophageal carcinoma resected at an early stage has a good prognosis, the majority of carcinomas arising in BM present clinically at an

advanced stage and have a poor prognosis (5, 6). Understanding the molecular events leading to the development of carcinoma from BM will lead to improved strategies for follow-up, early detection, and treatment.

Malignant cells show an increased glucose uptake *in vitro* and *in vivo* (7, 8). This process is thought to be mediated by Gluts, the expression and activity of which is regulated by oncogenes and growth factors (9-13). Glut1, the human erythrocyte Glut, is a member of an expanding family of transmembrane proteins known as the facilitative glucose transporters which currently has six members (14). Earlier studies have demonstrated the presence of mRNA from different Gluts in human tumors (15-17) and a significant increase in the amount of mRNA for Glut1 in cancers of the esophagus, colon, and pancreas (18). Glut1 protein expression has been reported in human head and neck tumors (19), breast cancer (20, 21), insulinomas (22), renal cell carcinomas (23), and human brain hemangioblastoma (24). We have recently shown that Glut1 can be immunohistochemically detected in only a few normal human tissues, but can be detected in a variety of malignant neoplasms, including adenocarcinoma arising in BM (25). More recently, we have shown that Glut1 expression, as detected by immunohistochemistry, occurs as a late event during carcinogenesis in the human colon (26). The aim of this work was to determine the chronology of Glut1 expression during the neoplastic progression in BM.

Materials and Methods

Tissues. The material used in this study consisted of 251 biopsies from 97 consecutive patients with BM, with or without associated dysplasia or carcinoma. Only the specialized ("intestinal") type of metaplasia is considered as BM. For the purpose of this study, all biopsy tissue obtained during a single endoscopic procedure is considered as one biopsy, regardless of the number of sites biopsied or the number of tissue pieces.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue sections of all biopsies were immunostained with the anti-Glut1 antibody MYM, a rabbit polyclonal antibody (serum) that we have generated against a 12-amino acid synthetic peptide corresponding to the COOH terminus of human Glut1 (25).

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 avidin-biotin complex rabbit kit (Vector) with 3,3'-diaminobenzidine as chromogen. Sections

Received 12/11/96; revised 1/28/97; accepted 2/6/97.

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¹ Supported in part by the Methodist Hospital Foundation. This work has been presented in part at the 85th Annual Meeting of the United States and Canadian Academy of Pathology, March 23-29, 1996, Washington, DC.

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³ The abbreviations used are: BM, Barrett's metaplasia; Glut, glucose transporter; LGD, low grade; HGD, high grade; IND, indefinite; ND, negative for dysplasia.

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. Staining was considered positive only when strong membrane-associated immunoreactivity was observed.

Morphologic Grading of Dysplasia. Dysplasia was graded as negative (ND), low grade/indefinite (LGD/IND), or high grade (HGD) using published criteria recently reviewed by Haggitt (27).

Evaluation of immunohistochemical staining and dysplasia was carried out in a blinded fashion.

Statistical Analysis. Statistical analysis was performed with Fisher's exact test using StatView 4.5 for the Macintosh.

Results

Morphologic evaluation of dysplasia was performed on H&E-stained tissue sections of the 251 biopsies. One hundred eighty-one were classified as ND, 51 as LGD/IND, 6 as HGD, and 13 contained carcinoma. The 6 biopsies with HGD came from 6 different patients, and the 13 biopsies with carcinoma came from 13 different patients.

RBCs were always positive for Glut1. None of the 181 biopsies with ND (0%) (Fig. 1A) or 51 biopsies with LGD/IND (0%) showed Glut1 immunoreactivity. More importantly, while 0 of 6 biopsies with HGD (0%) expressed Glut1 (Figure 1B), 9 of 13 biopsies with carcinoma (69%) were Glut1 positive (Fig. 1C; $P = 0.0108$, Fisher's exact test). BM with or without dysplasia in biopsies showing Glut1-positive carcinoma was negative for Glut1.

Discussion

Our finding that Glut1 is expressed in 69% of carcinomas and in none of the biopsies with LGD/IND or 6 HGD strongly suggests that Glut1 is expressed as late event during progression of BM to carcinoma.

HGD is often associated with carcinoma, up to 55% of the cases in one series (28). When such diagnosis is rendered, a decision to resect or not resect takes center stage. With the high incidence of carcinoma in patients with HGD, it seems that such a decision should be easy. However, the reported operative mortality for esophagogastrectomy ranges from 5% to as high as 20% in some series, major perioperative complications occur in 20–30% of the patients, and there is significant long-term morbidity. Therefore, it has been suggested that the decision to intervene surgically in patients who have HGD on biopsy must balance the risk of missing a small area of invasive cancer against the risk inherent to surgical resection (27). The conservative approach is advocated by the Seattle group, but requires follow-up with extensive sampling using large biopsy forceps (27, 29). This approach is rarely utilized outside Seattle and does not address the fact that sometimes it is not possible to distinguish HGD from carcinoma (30). Currently, there are no flow cytometric or molecular markers that differentiate HGD from carcinoma in BM (27). Glut1 immunostaining may provide a unique marker that could distinguish between HGD and a well-differentiated carcinoma, when such distinction cannot be made on purely morphological grounds. Prospective studies are needed to determine the sensitivity and specificity of Glut1 immunostaining as a marker of carcinoma in patients with BM.

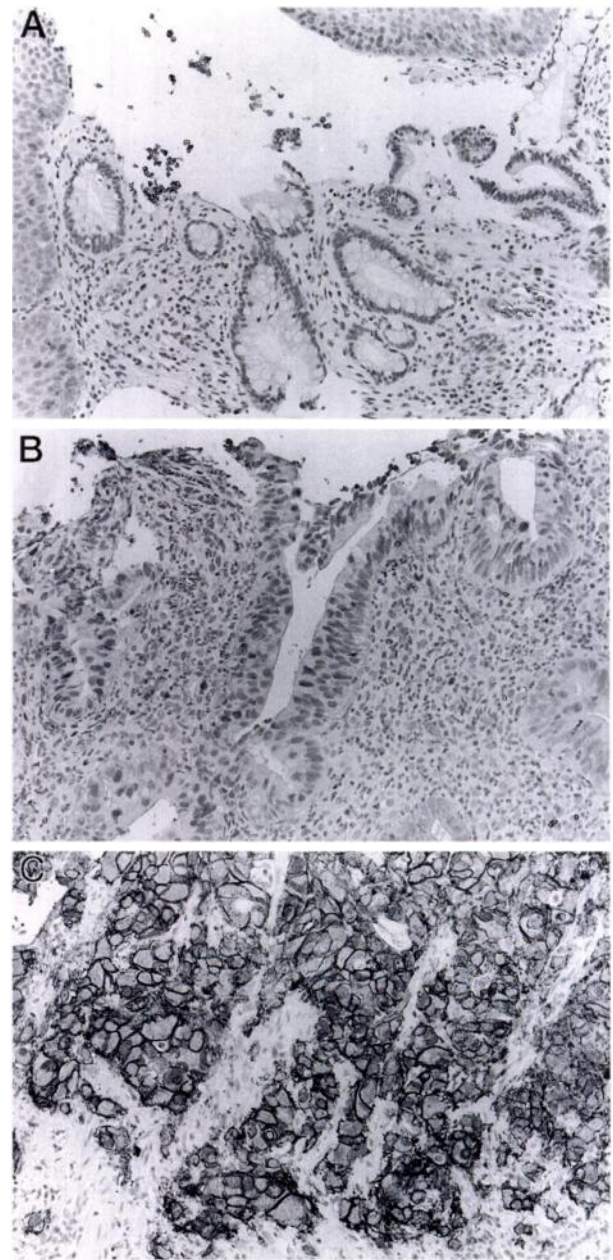


Fig. 1. Glut1 immunoreactivity in biopsies of BM negative for dysplasia (A), with high grade dysplasia (B), and adenocarcinoma (C). Note that although epithelial cells in A and B are negative, RBCs are positive for Glut1. Immunoperoxidase staining with hematoxylin counterstaining. A, $\times 90$; B and C, $\times 180$.

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