Pathology – Research and Practice 206 (2010) 24–29

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.de/prp

Original Article

Expression of the GLUT1 glucose transporter and p53 in carcinomas of the pancreatobiliary tract

Ji-Youn Sung, Gou Young Kim, Sung-Jig Lim, Yong-Koo Park, Youn Wha Kim *

Department of Pathology, School of Medicine, Kyung Hee University, #1 Hoiki-dong, Dongdaemun-gu, Seoul 130-701, Republic of Korea

ARTICLE INFO

Article history:
Received 4 June 2009
Received in revised form
10 July 2009
Accepted 30 July 2009

Keywords:
Pancreatobiliary cancer
GLUT1
p53
Immunohistochemistry

ABSTRACT

Only few studies have evaluated the usefulness of the GLUT1 and p53 status of pancreatobiliary tract carcinomas in revealing tumorigenesis. We studied GLUT1 and p53 immunoexpression in a total of 355 cases of the pancreatobiliary carcinoma to determine the biological significance of GLUT1 and p53 expression. Positive expression of GLUT1 was identified in 38 out of 67 (57.7%) ampulla of Vater (AV) carcinomas, in 27 out of 52 (51.8%) pancreatic (PA) carcinomas, in 38 out of 121 (31.4%) extrahepatic bile duct (EBD) carcinomas, and in 53 out of 115 (46.5%) gallbladder (GB) carcinomas. GLUT1 expression in pancreatobiliary carcinomas showed some positive correlation with histological grade, T stage, N stage, TNM stage, and lymphatic invasion. However, p53 expression showed no correlation with any prognostic factors. In the Kaplan–Meier test, positive GLUT1 expression was a poor prognostic factor in the pancreatobiliary tract cancers; however, only GB cancers were statistically significant ($P = 0.002$). Our results suggest that GLUT1 expression in the AV, EBD, and GB carcinomas is associated with some prognostic factors, and GLUT1 expression is associated with a worse prognosis in the patients with GB carcinomas.

Introduction

About 2.0% of all cancer patients in the United States in 2007 had pancreatic cancer, while 0.64% had gallbladder or another type of biliary tract cancer [6]. In Korea, there is a similar incidence of pancreatic cancer (2.6%). However, the incidence of gallbladder and biliary tract cancer (2.9%) is higher than that in the United States [1]. The reason for the high prevalence of these tumors in Korea is still unknown, but it is likely that these tumors are strongly associated with an increased incidence of pigmented stones in the gallbladder and extrahepatic bile ducts. Delayed onset of symptoms and rapid growth of these tumors lead to limited therapeutic efficacy and a high mortality rate.

Intracellular glucose transport is necessary for the survival, proliferation, and function of cells. This process is mediated by a family of 13 related molecules collectively termed glucose transporter (GLUT) proteins. Among these molecules, GLUT1 is the best-known basic, high-affinity glucose transporter, and it is restricted to erythrocytes [12]. It has long been recognized that cancer cells have increased rates of glucose metabolism compared with normal cells [5]. Increased GLUT1 expression has been described in many cancers, including breast, lung, kidney, urinary bladder, stomach, colorectum, endometrium, thyroid, head and neck, liver, ovary, salivary gland, and prostate cancer [2–4,7,9,13–17,20–22]. Some of these studies have shown that GLUT1 expression is correlated with aggressive tumor behavior and poor prognosis [4,10,13,14,22]. To date, a few immunohistochemical studies have been directed to assess GLUT1 expression in carcinomas of the pancreatobiliary tract, including ampulla of Vater (AV), pancreas (PA), and gallbladder (GB) cancers [8,10,11,19]. However, GLUT1 expression has not been evaluated in the setting of extrahepatic bile duct (EBD) carcinoma.

Recently, Schwartzenberg-Bar-Yoseph et al. [18] evaluated the relationship between p53 expression and GLUT1 expression, and reported that wild-type p53 suppresses GLUT1 gene transcription in a tissue-specific manner. Mutations within the DNA-binding domain of p53 were found to impair the ability of p53 to suppress the transcriptional activity of the GLUT1 gene promoters, thereby causing an increase in glucose metabolism and cellular energy supply. This, in turn, is predicted to facilitate tumor growth.

In the present study, we evaluated GLUT1 and p53 immunoexpression in carcinomas of the AV, PA, EBD, and GB in order to determine the biological significance. To our knowledge, no large-scale study of GLUT1 or p53 expression has been reported regarding carcinomas of the pancreatobiliary tract.

* Corresponding author. Tel.: +82 2 961 0543; fax: +82 2 962 5278.
E-mail address: kimyw@khu.ac.kr (Y.W. Kim).

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doi:10.1016/j.prp.2009.07.018

Materials and methods

Patients and tissue samples

We studied 355 cases of pancreatobiliary carcinoma: 67 cases of AV carcinoma, 52 cases of PA carcinoma, 121 cases of EBD carcinoma (42 proximal, 25 middle, and 54 distal EBD carcinomas), and 115 cases of GB carcinoma. All patients underwent surgical resection (Whipple operation, pylorus-preserving pancreaticoduodenectomy, radical cholecystectomy, etc.) at Kyung Hee University Hospital between January 1983 and December 2007. The clinical characteristics of each patient were evaluated through a hospital record review. The authors reviewed all the hematoxylin–eosin slides and used the most representative slides from each case to evaluate immunohistochemical staining. Research protocols for the use of human tissues were approved by the Institutional Review Board at Kyung Hee Medical Hospital and were conducted in accordance with their policies.

Immunohistochemistry

Immunohistochemical staining was performed using a Bond Polymer Intense Detection System (VisionBioSystems, VIC, Australia), according to the manufacturer's instructions. In brief, 4-μm sections of formalin-fixed, paraffin-embedded tissues were deparaffinized by Bond Dewax Solution, and antigen retrieval was done using Bond ER solution for 30 min at 100 °C. Endogenous peroxidase was quenched by incubation with hydrogen peroxide for 5 min. Sections were incubated for 15 min at ambient temperature with primary polyclonal rabbit anti-human GLUT1 (1:200; DAKO, Carpenteria, CA, USA) and primary monoclonal rabbit anti-human p53 protein (1:200; DAKO, Carpenteria, CA, USA) using a biotin-free polymeric horseradish peroxidase-linker antibody conjugate system in a Bond-max automatic slide stainer. Red blood cells present in each section served as positive controls for GLUT1. As a negative control, normal horse serum was substituted for the primary antibody.

Three examiners who were blinded to the pathological findings evaluated the slides independently. GLUT1 immunostaining was quantified by grading the proportion of cells that were GLUT1 positive. Cells showing strong and distinctive membranous immunoreactivity for GLUT1 were considered positive. Cytoplasmic staining, including a supranuclear dot pattern or nuclear staining, was regarded as negative. The grading system was as follows: absence or less than 5% immunoreactive tumor cells = 0; more than 5% to less than one-third immunoreactive cells = 1+; one-third to two-third immunoreactive cells = 2+; and more than two-third immunoreactive cells = 3+. Cells showing strong, distinctive nuclear immunoreactivity for p53 were considered positive. Cytoplasmic or membranous staining was considered negative. This grading system was also used for p53. For statistical analysis, grade 0 was considered negative, and anything above grade 1 was considered positive.

Statistical analysis

The SPSS 13 statistical program (SPSS, Chicago, IL, USA) was used for statistical evaluation. We compared prognosis and intensity or GLUT1 and p53 immunoreactivity. GLUT1 expression in pancreatobiliary carcinomas was compared with clinical and histological features, including age, sex, tumor size, histological grade, T stage, N stage, TNM stage, lymphatic invasion, vascular invasion, neural invasion, and p53 expression. A follow-up of patients whose tumors were examined in this study is currently in progress. Follow-up data were available for 325 patients. However, we lost track of 25 patients during the observation period, and another 5 patients died of other causes. The follow-up time ranged from 1 to 264 months (mean, 73 months) for AV carcinomas; from 2 to 244 months (mean, 28 months) for PA carcinomas; from 1 to 235 months (mean, 45 months) for EBD carcinomas; and from 1 to 160 months (mean, 36 months) for GB carcinomas. We used the χ^2 test and Fisher's exact test to compare the expression of GLUT1 according to variables. The Cox proportional hazards model was done for univariate analysis of the factors said to affect prognosis in patients with pancreatobiliary carcinomas. The median survival curves of patients with positive and negative GLUT1 expression were evaluated using the Kaplan–Meier method. The statistical significance of the differences in survival was calculated using the log rank test. A *P*-value below 0.05 was considered statistically significant.

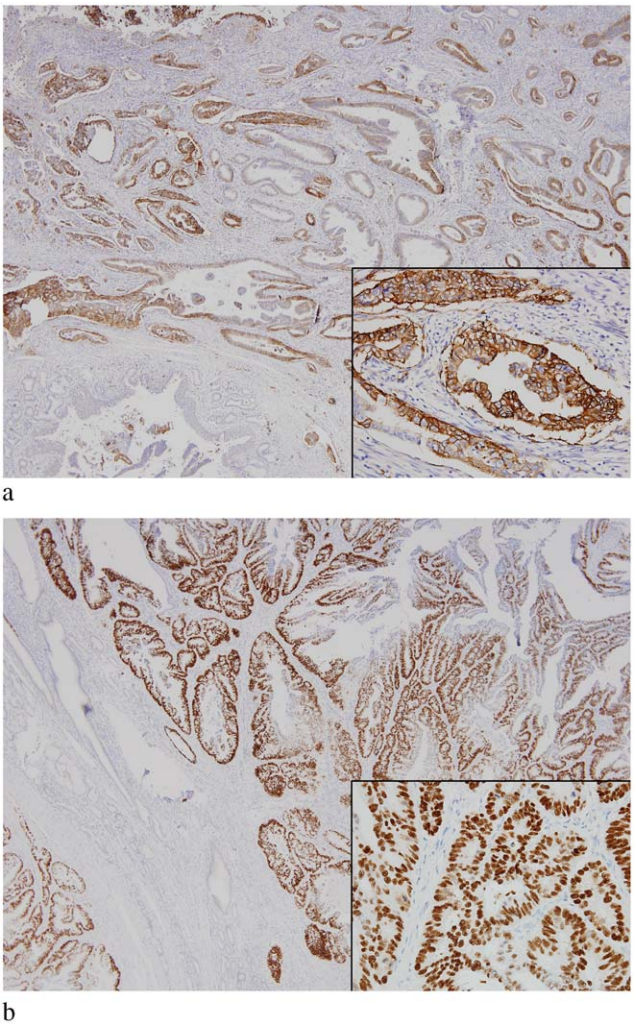

Fig. 1. Membranous GLUT1 staining (a – 40 × and 400 × (inlet)) and nuclear p53 staining (b – 40 × and 400 × (inlet)) in ampulla of Vater carcinoma.

Results

The normal pancreatobiliary tract mucosa did not express GLUT1 protein. Also regenerative, reactive, or dysplastic epithelium of the pancreatobiliary tract did not express GLUT1 immunoreactivity. GLUT1 expression in cancer tissue differed based on the patient and extent of the disease. Staining is usually strongly positive in the center of the necrotic and infiltrative areas