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Original contribution

Increased expression of glycinamide ribonucleotide transformylase is associated with a poor prognosis in hepatocellular carcinoma, and it promotes liver cancer cell proliferation☆,☆☆

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Summary Glycinamide ribonucleotide transformylase (GART) is a folate-dependent enzyme in the de novo purine pathway that has been the target of antineoplastic intervention for almost 2 decades. Until now, its expression and functional significance in hepatocellular carcinoma (HCC) have been unclear. We demonstrated by Western blotting that the expression of GART was markedly up-regulated in HCC patients. Immunohistochemistry staining was used to determine the expression of GART in HCC and adjacent nontumor tissues from 96 patients. Increased expression of GART correlated positively with the histologic grade (P = .001), tumor size (P = .043), number of tumorous nodes (P = .020), and intrahepatic metastases (P = .031), suggesting a role for GART in the progression of HCC. Patients with higher GART expression had a much worse overall survival rate than those with low expression (P = .002). Furthermore, multivariate analysis showed that GART expression was an independent predictor of overall survival (hazard ratio, 2.265; 95% confidence interval, 1.335-3.842; P = .002). Depletion of GART by small interfering RNA inhibited cell proliferation and blocked S-phase and mitotic entry in cultured HepG2 and BEL-7404 cells. Western blot analyses showed that GART depletion decreased the proliferating cell nuclear antigen concentration. Collectively, our clinical and in vitro data indicate that GART expression may be one of the causative factors for a poor prognosis in HCC.

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1. Introduction

Globally, hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third leading cause of cancer death because of late diagnosis and lack of effective treatment options [1]. The major risk factors for initiation and development of HCC are nonalcoholic fatty liver disease; exposure to aflatoxin B1; hemochromatosis; obesity [2]; cirrhosis; chronic infection by hepatitis viruses B, C, or D; alcohol abuse; hereditary metabolic liver diseases; and iron overload [3,4]. These cancers are highly resistant to radiotherapy and chemotherapeutic agents [3]. Despite a variety of treatment options, including surgical resection, percutaneous injection of ethanol, radiofrequency thermal ablation, chemoembolization, and liver transplantation, the prognosis for patients with HCC is poor [5,6].

Purine biosynthesis is essential in a number of cellular processes such as signaling, energy metabolism, and cell reproduction [7,8]. Nucleotides can be obtained by 2 routes: synthesis via the de novo pathway or salvage from breakdown of nucleic acids. Most normal cells (except liver and T cells) rely on the salvage of purines, whereas most tumor cells have elevated activity of the de novo pathway and decreased activity of the salvage pathway [9-12]. Because purines play a critical role as required components of DNA and RNA, inhibition of enzymes in the purine biosynthetic pathway has been proposed as an approach for antineoplastic intervention [13,14].

One enzyme that is of particular interest in de novo purine nucleotide biosynthesis is glycinamide ribonucleotide transformylase (GART; also named glycinamide ribonucleotide formyltransferase), which catalyzes the first of 2 folate-dependent transformylation reactions in the pathway [15-20]. Because of its pivotal role in the purine biosynthetic pathway, GART has been the target of antineoplastic intervention for almost 2 decades [18,21]. In addition, inhibition of GART by anticancer drugs was effective against the aggressive phenotype of several cultured tumor cell lines [21,22]. Little is known about its role in human HCC. We screened GART as a potential tumor-promoting gene in HCC.

Reviewing the gene expression in some tissues from Gene Cards, the expression of GART was found to be increased in some tumor cells. Although a considerable amount of information is available about GART, no report has been found on the relation between GART expression and clinicopathological factors in HCC. We found up-regulation of GART in HCC and investigated its involvement in hepatocellular carcinogenesis.

2. Materials and methods

2.1. Patients and tumor tissue samples

Specimens of surgically resected primary HCC and corresponding nontumorous liver tissues were collected from 96 patients at the Affiliated Hospital of Nantong University between the years 2004 and 2005. None of the patients was given preoperative chemotherapy, radiation, or immunotherapy. The diagnosis was confirmed histologically in all cases by at least 2 pathologists, based mainly on examination of sections stained with hematoxylin and eosin (Supplementary Figure 1). The 96 HCC cases consisted of 74 men and 22 women whose ages ranged from 31 to 78 years, with an average age of 49.5 years. Follow-up by telephone and letter was carried out to obtain information on the patients' outcomes. Overall survival was calculated from the time of surgery to the time of death or to the date of the most recent follow-up. The follow-up time ranged from 1 to 107 months from the date of surgery (mean, 37.7 months). According to the International Union against Cancer TNM classification system and the Edmondson grading system [23,24], histologic grades were classified as well (grade I; n = 23), moderately (grade II; n = 58), and poorly (grade III; n = 15) differentiated. The main clinicopathological variables analyzed were age, sex, histologic grade, tumor size, number of tumor-invaded nodes, vascular invasion, intrahepatic metastasis, serum α-fetoprotein (AFP) concentration, presence of hepatitis B virus infection, and liver cirrhosis. All HCC tissues were collected using protocols approved by the Ethics Committee of the Cancer Hospital of Nantong University, and the clinical data were collected after patients gave informed consent. Tissue specimens were processed immediately after surgical removal. For histologic examination, all tumorous and surrounding nontumorous tissue portions were processed into 10% buffered formalin-fixed, paraffin-embedded blocks.

2.2. Immunohistochemistry staining

Paraffin-embedded tissue blocks were sectioned for immunohistochemistry staining. These tissue sections were deparaffinized and rehydrated using xylene and ethanol, respectively. Sections were boiled at a controlled final temperature of 121°C for 20 minutes in 10 mM citrate buffer (pH 6.0) for antigen retrieval. Endogenous peroxidase activity was blocked by immersion in 0.3% hydrogen peroxide for 10 minutes. After rinsing in phosphate-buffered saline (PBS; pH 7.2), 10% goat serum was applied for 1 hour at room temperature to block any nonspecific reactions. The sections were then incubated overnight at 4°C with anti-GART rabbit polyclonal antibody (diluted 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), and anti–Ki-67 mouse monoclonal antibody (diluted 1:400; clone 7B11; Zymed Laboratories, San Francisco, CA). After that, the sections were washed 3 times with PBS, incubated with horseradish peroxidase–conjugated secondary antibody (Envision TM Detection Kit, cat. no. GK500705; Gene Tech Laboratories, Las Vegas, NV) at 37°C for 30 minutes, and then washed 3 more times with PBS. Finally, the sections were incubated with 3,3′-diaminobenzidine in Tris buffer 0.05 mol/L (pH 7.6) containing 0.03% H_2O_2 for signal development, and the sections were counterstained with 20% hematoxylin. The slides were dehydrated, cleared, cover slipped, and evaluated. Each sample was incubated with