Original Article

Reduction of CAII Expression in Gastric Cancer: Correlation with Invasion and Metastasis

Xiao-jie Li[1, 2], Hai-long Xie[2*], San-ju Lei[3], Hui-qiu Cao[1], Tian-Yun Meng[1], Yu-lin Hu[1]

[1]*Department of Pathology, 1st People's Hospital of Chenzhou, Chenzhou 423000, China*
[2]*Institute of Cancer Research, University of South China, Hengyang 421001, China*
[3]*Department of Otorhinolaryngology, 1st People's Hospital of Chenzhou, Chenzhou 423000, China*

10.1007/s11670-012-0196-6

ABSTRACT

Objective: Human carbonic anhydrases II (*CAII*) gene plays an important role in different cancer. However, its relevance to gastric cancer (GC) remains unclear. In the present study, we aimed to investigate the expression of CAII in GC and explore its correlation with some clinicopathologic characteristics of GC.

Methods: The expression of CAII in 20 specimens of normal gastric mucosa, 38 specimens of intraepithelial neoplasia and 112 specimens of gastric carcinoma were detected by immunohistochemical techniques. Survival in GC with CAII expression was studied.

Results: The positive rate of CAII protein in normal gastric mucosa was significantly higher than that in intraepithelial neoplasia and gastric carcinoma (100% vs. 63.16% and 28.57%, $P<0.001$). The positive rate of CAII protein was significantly higher in gastric carcinoma at early stages than that at advanced stages (70.0% vs. 19.57%, $P<0.001$). The positive rate of CAII protein was significantly lower in gastric carcinoma with lymph node metastases than that without lymph node metastases (10.81% vs. 37.33%, $P<0.05$). Furthermore, the positive rate of CAII protein was significantly lower in poorly-differentiated gastric carcinoma than in moderately- or well-differentiated gastric carcinoma (15.94% vs. 31.03% or 60.00%, $P<0.05$). Moreover, CAII expression was not related with sex, age and tumor size. The patients with CAII-positive tumors showed a better survival rate than those with CAII-negative tumors ($P=0.024$, log-rank test).

Conclusion: CAII expression was related with stages and lymph node metastases in gastric carcinoma. The reduction of CAII expression in GC might promote tumor cell motility and contribute to tumor growth and metastasis.

Key words: CAII; Gastric Carcinoma; Immunohistochemistry; Invasion; Metastasis

INTRODUCTION

Gastric cancer (GC) is the most common cancer in Eastern Asia and South America. Although the incidence in western countries is much lower than that in Asia, it is still a significant worldwide health burden, only second to lung tumors as a leading cause of cancer death[1]. The occurrence and development of gastric carcinoma is a complex multi-step process controlled by multiple factors. The activation of oncogenes (such as *myc, K-ras, c-erbB-2* and *K-sam*) and deactivation of anti-oncogenes [such as *p53*, adenomatous polyposis coli (*APC*) and *p16*] play important roles. Therefore, it is important to identify new oncogenes involved in occurrence and development of gastric carcinoma for prognosis and clinical treatment of GC[2].

Carbonic anhydrase (CA) is a type of zinc-containing metalloenzyme. At least 16 types of CA isozymes have been reported in mammals with different tissue distribution and catalytic properties[3]. CAI, CAII, CAIX and CAXII are highly tumor-related[4, 5], and among them, CAIX and CAXII are related to soft tissue sarcoma, breast cancer and non-small cell lung cancer[6-8]. Human *CAII* gene, which locates at chromosome 8q22, codes a cytoplasmic protein that has the highest turnover rate and widest tissue distribution among the known human CA isozymes[3]. CAII is correlated with several types of tumors[9-12]. In our previous study, the expression of CAII was down-regulated in GC[2]. However, the

Received 2011–09–04; **Accepted** 2012–03–14
This work was supported by the National Natural Science Foundation of China (No.81172576) and by a grant from Technology Division of Chenzhou, China (No. 2008gl15).
*Corresponding author.
E-mail: xhl0078@sina.com.cn

relationship between CAII expression and tumor invasion as well as metastasis in gastric carcinoma has not been examined. In the present study, we aimed to investigate the expression of CAII in GC and explore its correlation with some clinicopathologic characteristics of GC.

MATERIALS AND METHODS

Clinical Specimens

Retrospective analysis was performed on gastric carcinoma patients who had undergone gastrectomy between August 2004 and March 2007 in the pathological department of the 1st Hospital of Chenzhou. The mean age of the patients was 49 years, ranging from 23 to 79 years. A total of 78 males and 34 females (2.29:1) were included in our study, of which 65 patients were under 55 years old and 47 patients were over 55 years old. In terms of tumor size, 78 specimens were larger than 5 cm, and 36 specimens were smaller than 5 cm. According to tumor differentiation, we investigated 20 highly-differentiated tumors (17.86%), 29 moderately-differentiated tumors (25.89%) and 63 poorly-differentiated tumors (56.25%). In terms of the depth of tumor invasion, 20 (17.86%) and 92 (82.14%) patients were classified as early type and advanced type, respectively. Seventy-five (66.96%) patients were lymph node metastasis negative, whereas 37 (33.04%) were lymph node metastasis positive. No adjuvant radiotherapy or chemotherapy was administered on those patients before surgery. Multiple sections were microscopically examined to confirm the degree of differentiation, the depth of invasion and lymph node metastasis. One representative block was then selected for immunohistochemical study. Twenty normal mucosa and 38 intraepithelial neoplasia paraffin blocks derived from subtotal gastrectomy specimens because of duodenal ulcer. The study was approved by the Ethical Committee for Clinical Research of the Hospital.

Immunohistochemistry

Immunoperoxidase staining of formalin-fixed and paraffin-embedded tissue sections was performed by an ordinary biotin-streptavidin method. Briefly, sections were deparaffinized in xylene, heated with 10 mmol/L citrate buffer (pH 6.0) in a pressure cooker for 5 min and washed with phosphate-buffered saline (PBS, pH 7.2). In order to block endogenous peroxidase activity, sections were immersed in methanol containing 0.3% hydrogen peroxide (H_2O_2) at room temperature for 20 min. Then the sections were blocked with 10% normal calf serum in PBS for 10 min. Subsequently, the sections were incubated with anti-CAII mouse monoclonal antibody (1:200) (Santa Cruz Biotechnology Inc., USA) for 1 h in a humidified chamber. After incubating with secondary antibody and avidin-biotin complex reagent, color reaction was developed in 0.02% H_2O_2 in Tris buffer (pH 8.0). Hematoxylin was used for counterstaining. The positive control was positive slides bought from Santa Cruz Biotechnology Inc., USA. In addition, a parallel negative control without primary antibody was established.

Evaluation of Immunostaining

Yellow particles in the cytoplasm or cytomembrane meant positive staining. The scores were evaluated in terms of staining intensity as follows: 0, no reaction; +, weak reaction; ++, moderate reaction; and +++, strong reaction. In the statistical analyses, the specimens were grouped into two categories based on the staining intensity and positive cells: CAII(+) tumors, including more than 10% of neoplastic cells exhibiting moderate or strong reaction; and CAII(−) tumors, including less than 10% of neoplastic cells with moderate or strong reaction, or weak or negative immunostaining results.

Analysis of Survival Rate

We collected 112 patients' postal address and telephone number from the medical record department of the 1st Hospital of Chenzhou. Then, we contacted patients or their family members, and collected 50 cases until July 2010. Finally, we drew the five-year survival curves.

Statistical Analysis

The correlation between CAII expression of neoplastic cells and clinicopathological factors was evaluated by chi-squared test or Fisher's exact test. $P<0.05$ was considered statistically significant. The log-rank test was used in the survival analysis. All statistical analyses were carried out by using SPSS 13.0 (SPSS Inc., Chicago, IL, USA)

RESULTS

Immunostaining Analysis of CAII Expression in Normal Mucosa, Intraepithelial Neoplasia and GC

Table 1 shows that the positive rate of CAII protein was 28.57% (32/112) in 112 tumor specimens, 63.15% (24/38) in intraepithelial neoplasia, and 100% (20/20) in normal mucosa. Figure 1 shows that CAII protein was expressed in cytoplasm, and CAII expression at the protein level in normal mucosa was stronger than that in intraepithelial neoplasia and GC.

Correlation between CAII Expression and Age or Sex

The expression rates of CAII was 29.48% and 26.47% in male and female GC specimens, respectively, and there was no correlation between CAII expression and age.