Original Article

Over-expression of Metastasis-associated in Colon Cancer-1 (MACC1) Associates with Better Prognosis of Gastric Cancer Patients

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ABSTRACT

Objective: The aim of this study was to detect metastasis-associated in colon cancer-1 (MACC1) expression in Chinese gastric cancer and analyze the relationship between MACC1 expression and postoperative survival.

Methods: The expression of MACC1 and c-MET protein in a sample of 128 gastric cancer tissues was detected by immunohistochemistry. A retrospective cohort study on the prognosis was carried out and data were collected from medical records.

Results: The positive rate of MACC1 protein expression in gastric cancer was 47.66%, higher than that in adjacent noncancerous mucosa (P<0.001). MACC1 protein expression was not related to the clinicopathological variables involved. Kaplan-Meier analysis revealed that the survival of MACC1 positive group tended to be better than that of MACC1 negative group, particularly in patients with stage III carcinoma (P=0.032). Cox regression analysis revealed that MACC1 protein over-expression in gastric cancer tended to be a protective factor with hazard ratio of 0.621 (P=0.057). Immunohistochemical analysis showed that the positive rate of c-MET protein expression was much higher in cases with positive MACC1 expression in gastric cancer (P=0.002), but P53 expression was not associated with MACC1 expression.

Conclusion: MACC1 over-expression implies better survival and may be an independent prognostic factor for gastric cancer in Chinese patients.

Key words: MACC1; Gastric cancer; Prognosis

INTRODUCTION

Gastric cancer is one of the most common carcinomas and one of the leading causes of cancer death in China. Because of the heterogeneity in gastric cancer cells, the type of cells is of great importance in the prognosis of patients with the same stage of carcinoma and who are receiving similar treatment. And it is difficult to give proper personalized treatment to each patient and to identify the patients with cancer relapse and metastasis at the earliest possible time. There are many factors that may affect the prognosis[1], and serum biomarkers CEA[2-4], CA19-9[5-7], CA72-4[8-11], CA242[12] and a combination of the all[13,14] are widely used prognostic factors. When anastomotic recurrence or distant metastasis is revealed by assistance of tumor markers or medical imaging, gastric cancer can not usually be cured by further surgery. Subsequently, it is of great importance to find new markers that will be helpful in gastric cancer monitoring and prognosis evaluation.

Metastasis-associated in colon cancer-1 (MACC1) gene was identified by differential display real-time polymerase chain reaction (RT-PCR) in primary colon cancer by Stein et al[15]. As for MACC1 translation, the predicted MACC1 consensus coding sequence consists of 2,559 nucleotides encoding a protein with 852 amino acids[16]. MACC1 protein contains several functional motifs, starting with ZU5 domain, Src-Homology (SH3) binding motif followed by a variant SH3 domain and two death domains from the N-terminal region[17]. MACC1 gene is located on 7p21.1, mapped on the same chromosomes as c-MET and hepatocyte growth factor (HGF) involved in the HGF-MET signal pathway. Functional study revealed that the HGF receptor c-MET was the transcriptional target of MACC1. In MACC1-transfected SW480 colon cancer cells, MACC1

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controls c-MET expression via a specific consensus sequence described as transcription factors specificity protein 1 (Sp1)[18]. However, the mechanism of how MACC1 binding to the Sp1 site is not yet clear. Putative factors found by PROMO software include Sp1, transcription factors ETF, E2F-1, p53 and Pax-5. As a direct interaction of Sp1 and p53 has been reported previously[19], tumor suppressor gene p53 may, therefore, play a role in the function of MACC1.

It was reported by Stein that increased MACC1 mRNA expression in primary colon cancer was related to metastasis-free survival in patients with stage I-III carcinoma[15]. Data from our microarray also suggested that MACC1 mRNA expression in gastric cancer cells is much higher than that of adjacent noncancerous mucosa[20]. On the basis of these researches, our study was the first attempt to investigate the relationship of MACC1 expression and gastric cancer prognosis. MACC1 protein expression was analyzed and the relationship between MACC1 expression and survivals was studied. To elucidate the molecular mechanism of MACC1 involved in gastric cancer, the expression of c-MET and p53 in gastric cancer cells were also analyzed.

MATERIALS AND METHODS

Patients and Tissue Specimens

Tissue specimens were obtained with informed written consent from 128 gastric cancer patients who were treated at the Peking University Beijing Cancer Hospital between January 2000 and December 2002. The investigation was approved by the Ethics Committee of Peking University. All patients (83 males, 45 females, mean age = 57 years, range 26-81 years) were diagnosed as having gastric cancer without preoperative chemotherapy or radiotherapy. A number of clinicopathological variables such as gender, age, tumor size and location, Borrmann classification, histological type, tumor-node-metastasis (TNM) stage, depth of tumor invasion, lymph node metastasis, distant metastasis and vascular invasion were included for survival analysis. p53 protein expression in clinical pathological reports was also included. Postoperative staging of gastric cancer was classified according to the 2002 tumor-node-metastasis (TNM) classification system recommended by the American Joint Committee on Cancer[21]. There were 14 patients with stage I, 20 patients with stage II, 56 patients with stage III and 38 patients with stage IV carcinoma. After gastrectomy, resected specimens of gastric cancer were routinely processed for macroscopic pathological assessment and fixed with 10% formalin in phosphate buffered saline (PBS) for immunohistochemistry. The patients were followed from a period of 1.23 months to 97.47 months (mean: 31.09 months). Follow-up was managed through correspondence, over the telephone or in the clinic every 3 to 6 months for 3 years and half a year thereafter. In the clinic, a complete history, physical examination, complete blood count, chemistry profile, imaging studies and endoscopy were routinely completed. One hundred and twenty-eight gastric cancer patients in our study were followed up regularly and follow-up information is complete. The primary endpoint of the follow-up was death of gastric cancer patients. Patients who did not die as a result of gastric cancer were excluded.

Immunohistochemistry

Formalin-fixed paraffin sections of 4μm thickness were mounted on poly-L-lysine-coated slides. The samples were then deparaffinized in xylene and rehydrated in graded alcohol. After hydration, endogenous peroxidase activity was blocked with 3% (v/v) hydrogen peroxide (H_2O_2) for 20 minutes at room temperature. Standard antigen retrieval was then performed with heat induced epitope retrieval (HIER) by heating the slides immersed in retrieval solution (pH 6.0) in a pressure boiler. After boiling, the slides remained in the pressure boiler for 3 minutes and then gradually cooled at room temperature. After washing with PBS three times, the sections were incubated with primary antibody anti-MACC1 (2.50μg/ml, 5197, ProSci, USA) or anti-c-MET (18-2257, Invitrogen) at 4°C overnight. After rinsing, the slides were incubated with peroxidase-labeled polymer conjugated to poly Peroxidase-anti-Mouse/Rabbit IgG (PV-9000, Zhongshan Biotechnology Company, Beijing, China) at 37°C. Diaminobenzidine (DAB) staining reaction was then performed and followed by Meyer hematoxylin counterstain. The slides were then dehydrated, cleared and mounted as normal. For negative controls, the primary antibody was replaced by non-immune rabbit serum to confirm the specificity. Internal positive control was used for quality assurance.

MACC1 staining was principally evaluated according to the scoring criteria. The information recorded was: subcellular location (nuclear and/or cytoplasmic), intensity of staining (negative, weak, moderate or strong) and percentage of positive immunoreactive cells. The positive group referred to the cases with >20% cells having positive immunoreactivity. The rest were defined as negative. The slide evaluation was performed by two board-certificated pathologists, and both pathologists gave almost identical reports with only minor differences. A consensus regarding controversial cases was reached after discussion.

Statistical Analysis

Regarding MACC1 expression and the clinicopathological variables, data were cross-tabulated and a Chi-square test was performed, except for the age parameter which was assessed by Student's *t* test. Cumulative survival was estimated by the Kaplan-Meier method and comparisons between groups were done with a log-rank test. Postoperative survival was measured from the date of first surgery to the date of death caused by gastric cancer, or the last date of information collection if no end event was documented. A multivariate analysis of Cox proportional hazards regression model (backward, stepwise) was analyzed to assess the influence of each variable on survival. $P<0.05$ was considered statistically significant.