Available online at www.sciencedirect.com
ScienceDirect
EJSO 34 (2008) 636–641
EJSO the Journal of Cancer Surgery
www.ejso.com

Tissue inhibitor of metalloproteinase-2 G-418C polymorphism is associated with an increased risk of gastric cancer in a Chinese population

L. Yang [a,b,1], H.-J. Gu [a,1], H.-J. Zhu [a,1], Q.-M. Sun [a], R.-H. Cong [a], B. Zhou [a], N.-P. Tang [a], B. Wang [a,*]

[a] *Key Laboratory of Reproductive Medicine, Department of Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, Jiangsu Province, China*
[b] *Department of General Surgery, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China*

Accepted 4 September 2007
Available online 22 October 2007

Abstract

Aims: To examine the effect of the *TIMP-2* G-418C polymorphism on gastric cancer risk.
Methods: We conducted a hospital-based, case–control study using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method in 412 individuals (206 gastric cancer patients and 206 age, sex matched cancer-free controls).
Results: The genotype and allele frequencies were significantly different ($P = 0.007$ and 0.005, respectively) between cases and controls. Further analysis showed that the variant *TIMP-2* genotypes (CC + GC) had a 51% increased risk of gastric cancer compared with GG [adjusted odds ratio (OR) 1.51, 95% confidence interval (CI) 1.00–2.26, $P = 0.049$]. The elevated gastric cancer risk was especially evident in younger individuals (age < 58 years old) (adjusted OR 2.21, 95% CI 1.18–4.16) and smokers (adjusted OR 2.61, 95% CI 1.01–6.72). However, no significant association was observed between the variant genotypes and clinicopathological features of gastric cancer.
Conclusions: These findings suggest that the *TIMP-2* G-418C polymorphism is a genetic predisposing factor for gastric cancer.

Keywords: Gastric cancer; Tissue inhibitor of metalloproteinase-2; Polymorphism

Introduction

Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in degradation or breakdown of the extracellular matrix (ECM), which plays an important role in carcinogenesis and malignant progression,[1] including gastric cancer. Indeed, MMP-2 is overexpressed in gastric cancer cells and is not identified in adjacent normal gastric epithelium.[2] As a major endogenous inhibitor of MMP-2 and other active MMPs, tissue inhibitor of metalloproteinase-2 (TIMP-2) is particularly interesting due to its important effects on various malignancies.[1,3,4]

Recently, Hirano et al. identified a polymorphism (G to C substitution at position −418) located within the consensus sequence for the Sp1-binding site in the promoter of *TIMP-2* gene.[5] This substitution may downregulate TIMP-2 expression[5,6] and consequently cause an imbalance between the activities of TIMP-2 and MMP-2.[1,4,5]

Several epidemiological studies have investigated the association between *TIMP-2* G-418C polymorphism and various diseases including chronic obstructive pulmonary disease,[5] moyamoya disease,[7] periodontitis[8,9] and human malignancies.[10–14] However, the previous findings on different types of cancer were inconsistent. Therefore, the inconsistent results have raised the important question as to whether *TIMP-2* G-418C polymorphism relates to carcinogenesis and malignant progression.

Moreover, about half of the worldwide gastric cancer cases occur in China.[15] As implied previously by our studies, ethnic diversity, differences in lifestyle and variation of residential environment might account for geographical

* Corresponding author. Department of Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, Jiangsu Province, China. Tel./fax: +86 25 86862884.
E-mail address: binwang@njmu.edu.cn (B. Wang).
[1] These authors have contributed equally to this work.

0748-7983/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejso.2007.09.003

distribution of diseases.[16–18] Therefore, we conducted the present hospital-based, case–control study to assess the role of *TIMP-2* G-418C polymorphism on gastric cancer risk in Chinese population.

Materials and methods

Subjects

This study included 412 individuals (206 cases and 206 controls) collected from Nanjing Medical University First Affiliated Hospital. Gastric cancer was histopathologically confirmed by endoscopic biopsy or surgical specimen. Those with secondary, recurrent malignancies were excluded. Control subjects were randomly recruited during the time of case collection. The selection criteria included non-neoplastic diseases, and matched to gastric cancer cases by gender and age (within 5 years). Control subjects with severe clinical symptoms or previous diagnosis of cancer and genetic disease were excluded. All subjects were unrelated Chinese. Information on smoking status, residence (urban or rural), body weight and personal medical history was collected by questionnaire. Individuals who formerly or currently smoked $\geq$10 cigarettes per day for at least 2 years were defined as smokers. All gastric carcinomas were classified according to the TNM (1992) classification criteria of International Union Against Cancer (UICC).[19] Differentiation-grade was classified according to World Health Organization (WHO) classification. Informed consent was obtained from each subject and the study was approved by the Ethics Committee of Nanjing Medical University First Affiliated Hospital.

TIMP-2 genotyping

As our previous study described,[16] blood samples were collected and the genomic DNA was extracted by standard phenol–chloroform extraction. Identification of a genetic variant (*TIMP-2* G-418C) in the promoter of *TIMP-2* gene is shown in Fig. 1. The polymorphism is located 418 bp upstream from the *TIMP-2* translation start site.[5] Molecular detection of the *TIMP-2* G-418C polymorphism was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay, as reported previously.[12]

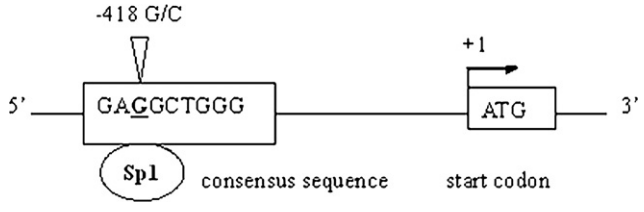

Figure 1. Identification of a genetic variant (*TIMP-2* G-418C) in the promoter of *TIMP-2* gene. The polymorphism is located 418 bp upstream from the *TIMP-2* translation start site.

The PCR was carried out in a total volume of 20 μl containing 2 μl 10× PCR buffer, 1.5 mM $MgCl_2$, 0.5 μM each primer (forward 5′-CGTCTCTTGTTGGCTGGTCA-3′, reverse 5′-CCTTCAGCTCGACTCTGGAG-3′), 0.2 mM *d*NTP, 1.2 U *Taq* polymerase (MBI fermentas) and 200 ng of genomic DNA. The 304 bp PCR products including the polymorphic site were digested with restriction enzyme *BsoB* I (New England BioLabs, Waltham, MA) at 37 °C overnight and separated on a 2.5% agarose gel. The G allele had two *BsoB* I restriction sites and produced 230, 51 and 23 bp bands whereas the C allele lacked one *BsoB* I restriction site and thus produced 253 and 51 bp bands (Fig. 2). All assays were conducted blindly without the knowledge of case or control status. Additionally, about 10% of the samples were randomly selected and retested, and the results were 100% concordant.

Statistics

Statistical analyzes were performed using Stata Version 8.0 (STATA Corporation, College Station, TX). Quantitative variables departing from the normal distribution including age and weight were summarized as median and analyzed by the Mann–Whitney rank sum test. Distributions of categorical variables, and genotype and allele frequencies between cases and controls were compared by the Pearson χ^2 test. Hardy–Weinberg equilibrium was assessed for controls by a goodness-of-fit χ^2 test. Odds ratio (OR) and 95% confidence interval (CI) were used to estimate the association between the polymorphism and the risk of gastric cancer. Crude OR was calculated using the

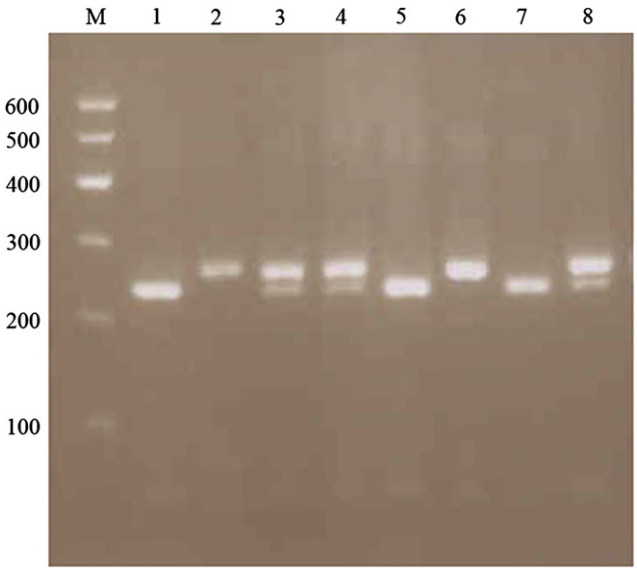

Figure 2. The typical PCR figures of G homozygous, C homozygous, GC heterozygous. PCR products were digested with restriction enzyme *BsoB* I and analyzed on a 2.5% agarose gel. M, DNA marker; lanes 1, 5 and 7, GG homozygous; lanes 3, 4 and 8, GC heterozygous; and lanes 2 and 6, CC homozygous.