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• ESOPHAGEAL CANCER •

Tumor suppressor gene p16 and Rb expression in gastric cardia precancerouslesions from subjects at a high incidence area in northern China

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Abstract

AIM: **To further understand the molecular basis for gastric cardia carcinogenesis and to provide etiological clues.**

METHODS: **Endoscopic mucosa biopsy and histopathological examinations were made on 37 subjects from a high incidence area for both esophageal and gastric cardia carcinomas in northern China. All the biopsy samples were fixed in 850ml.$^{-1}$ Lalcohol and embedded in paraffin. Each block contained one piece of tissue and was serially section at 5 μm. Immunohistochemistry (ABC) was carried out on these gastric cardia samples to determine the alterations of p16 and Rb.**

RESULTS: **Based on the histopathlogical examination there were 11 cases of chronic superficial gastritis, 12 cases of chronic atrophic gastritis and 14 cases of dysplasia. The immunostaining demonstrated different levels of unclear immunostaining of p16 and Rb in normal gastric cardia tissue and the tissues with different severity of lesions. With the lesions progressing, the positive immunostaining rates for p16 protein had a decreasing tendency. In contrast, the positive immunostaining rate for Rb protein had an increasing tendency. There was a significant negative relationship between the two parameters. Changes of p16 was CSG 11(100%), CAG 7 (58%), DYS 4(29%) and changes of Rb was CSG 2(18%), CAG 8(67%) and DYS 12(86%), (P<0.05).**

CONCLUSION: **The alterations of p16 and Rb protein may play a role in the early stages of gastric cardia carcinogenesis.**

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INTRODUCTION

Gastric cardia cancer is subject being studied. An interesting observation is that gastric cardia cancer and esophageal cancer seem to occur together in many high-incidence areas in China, and both were referred to as esophageal cancer (EC) by the public because of the common syndrome of dysphagia[1,2]. Histologically, esophageal and gastric cardia cancers have been considered as single clinical entity for incidence and mortality calculation in China. The molecular changes in the early stage of gastric cardia carcinogenesis have not been characterized [3-5]. In the present study, we investigated the roles of p16 and Rb alteration in gastric cardia carcinogenesis by measuring the expression rates of p16 and Rb in normal gastric cardia tissues and the tissues with different severity of lesions from the symptom-free subjects at a high incidence area of gastric cardia cancers in Henan, northern China.

MATERIALS AND METHODS

Tissue collection and processing

Gastric cardia biopsies were taken from 37 symptom-free subjects at Huixian County and Linxian County, Henan Province, China, the high-risk areas for esophageal and gastric cardia cancers during the mass survey. All the biopsy specimens were fixed with 850ml·L^{-1} alcohol, embedded with paraffin, and serially sectioned at 5μm. The sections were mounted onto histostick-coated slides. Three or four adjacent ribbons were collected for histopathological analysis (HE stain), and immunohistochemical staining. Histopathological diagnosis for gastric cardia epithelia was made using the previously established criteria. Based on the cellular morphological changes and tissue architecture, the gastric cardia epithelia were graded as chronic superficial gastritis(CSG), chronic atrophic gastritis (CGT) and dysplasia(DYS)[6-9]. The polyclonal p16 antibody is rabbit antiserum against human p16 protein(Dakoco, USA). The polyclonal Rb antibody is rabbit antiserum against man Rb protein (Oncogene Science Inc., USA). After dewaxing, inactivating endogenous peroxidase activity, and blocking cross-reactivity with normal serum, we incubated the sections with a diluted solution of the primary antibodies overnight at 4℃(1:100 for p16, 1:100 for Rb). Location of the primary antibodies was achieved by subsequent application of a biotinylated anti-primary antibody, an avidin biotin complex conjugated to horseradish peroxidase, and diaminobenzidine (Vectastain Elite Kit, Dako, USA). Normal serum blocking and omission of the primary antibody were used as negative controls[10,15].

Statistical analysis

The data were expressed as the mean±SD unless otherwise stated. The χ^2 test was used for histopathological and immunostaining rate evaluation (P<0.05 considered significant).

RESULTS

Histopathology findings

Histopathlogical examination showed that there were 11 cases of chronic superficial gastritis, 12 cases of chronic atrophic gastritis and 14 cases of dysplasia (Table 1). Both p16 and Rb immunostaining-positive cells were observed in different severity of lesions of gastric

cardia epithelia. In CSG, the positive immunostaining rates of p16 was much higher than that of Rb. An interesting observation is that the positive immunostaining rate of p16 was much lower than that of Rb in DYS. As the lesions of gastric cardia epithelia progressed from CSG to DYS, the positive immunostaining rates of p16 decreased significantly ($P<0.05$), especially from CSG to CAG. However, the positive immunostaining rates of Rb increased significantly ($P<0.05$). Correlation analysis showed significantly negative correlation between the decreasing tendency of P16 and the increasing tendency of Rb with the lesions progressing from CSG, CAG to DYS.

Table 1 Changes of p16 and Rb in gastric cardia precancerous lesions

Histological types	Number examined	P16 IHC positive[a] *n* (%)	Rb IHC positive[b] *n* (%)
CSG	11	11(100)	2(18)
CAG	12	7(58)	8(67)
DYS	14	4(29)	12(86)

[a]$P<0.05$, CSC *vs* CAG, CAG *vs* DYS; [b]$P<0.05$, DYS *vs* CAG, CAG *vs*. CSG

DISCUSSION

Gastric cardia carcinoma (GCC) is one of the most frequent digestive malignant diseases in northern China. A remarkable epidemiological characteristic for GCC is the occurring together with esophageal cancer in the same high-incidence area (HIA). In contrast with the strikingly decreasing of incidence rate of distal gastric cancer around the world in the past two decades, especially in America and Europe, the incidence of GCC increased dramatically; the incidence of esophageal-gastric-junction cancer increased to 6 folds with a speed of 4% yearly, which was one of the fastest increasing malignant diseases, the mechanism in unclear. There are several distinct differences between GCC and distal gastric cancer with respect to epidemiology, etiological factors, histogenesis and clinical characteristics, and therefore GCC should be catergorized as a distinct clinical disease. Lacking of sensitive and diagnostic biomarker and technique in early stage of GCC as well as the deficiency of effective and specific reagents for its treatment and prevention leads to its poor prognosis and higher mortality. An interesting observation in this study was that the alterations of tumor suppressor gene p16 and Rb products occurred in the early stage of gastric cardia carcinogenesis, even in CSG. With the lesions progressing from CSG, CAG to DYS, the positive immunostaining rates of p16 decreased significantly, especially from CSG to CAG. However, the positive immunostaining rates of Rb increased significantly. The positive immunostaining rate of p16 was much higher than of Rb In CSG. But, in DYS, the positive immunostaining rates of p16 was much lower than that of Rb. These results suggested that the tumor suppressor gene p16 and Rb may play different roles in the different stages of gastric cardia epithelia carcinogenesis. CAG and GYS have been considered as precancerous lesions of stomach cancer. Although the role of CSG is not clear during the gastric cardia carcinogenesis, it may provide a favorable macroenvironment for gastric carcinogenesis. The significance of CSG in the development of stomach cancer remains to be further characterized.

P16 gene, located at chromosome 9p21, is a new tumor suppressor gene, which was identified by an American molecular geneticist in 1995 and is also called multiple tumor suppressor 1 (MTS1) for its suppressing function to multiple tumors. Recent studies showed that the changes of p16 gene and its products were found in many primary tumors and cell lines[9,11-15]. Rb gene is the first tumor suppressor gene identified by the location cloning method, located at chromosome 13p. The product of Rb is a nuclear phosphoprotein, which is distributed extensively in different kinds of tissues[16-22]. It was considered that the cell-cycle progression normally depends on regulation by cyclins and cyclin inhibiting proteins. The overexpression of cyclins and/or the deletion of inhibiting protein could result in overworking of cell-cycle dependent kinetics (cdk), which makes cells enter into proliferative stage. The p16 could functionally inhibit cdks activity specifically and make Rb unphosphorylated, thus preventing the cell cycle progression from G1 phase to S phase[22-26].

Tam *et al* found that inactive Rb and/or Rb protein exist in all of the p16 over-expression cell lines, and inactive Rb protein could act directly on p16, suggesting that Rb can inhibit p16 protein expression. P16, Rb and cdk may constitute a feedback regulation circle. In the present study, a significant negative relationship between p16 and Rb protein expression was observed, which is consistent with Tam's observation[26-33].

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