EXPRESSION OF P16 AND CYCLIN D1 IN THE COURSE OF CARCINOGENESIS OF THE STOMACH

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Abstract

Objective: To determine p16 and cyclin D1 expression in the specimen of gastric carcinoma, atypic hyperplasia, atrophic gastritis, superficial gastritis and normal gastric mucosa. Methods: Using immunohistochemical method (ABC), the samples of 58 adenocarcinomas, 22 atypic hyperplasias, 28 atrophic gastritis, 27 superficial gastritis and 15 gastric epitheliums were analyzed. Results: Positive immunostaining rate for p16 protein was the highest in normal gastric mucosa and decreased with the lesions progressing from superficial gastritis to atrophic gastritis to atypital hyperplasia and to adenocarcinoma (85%, 78.6%, 31.8%, 48.3% respectively); Positive immunostaining of cyclin D1 can observed in atrophic gastritis. With the lesions progressing from atrophic gastritis to atypical hyperplasia to adenocarcinoma, its expression rate increased (17.9%, 36.4%, 53.4% respectively), and there was a significant difference between adenocarcinoma and atrophic gastritis group ($P<0.05$). An interesting observation was that inverse expression between p16 and cyclin D1, was shown in most of gastric cancer detected. Conclusion: It is indicated that p16 and cyclin D1 play an important role in the gastric carcinogenesis, the inverse expression between p16 and cyclin D1 suggested that there is a suppression trend in them.

Key words: p16, Cyclin D1, Gastric carcinoma, Carcinogenesis.

Carcinogenesis may be involved in many different genetic changes, which have an effect on cell proliferation and differentiation.[1] The oncogenes' activation (amplification, overexpression, etc.) and the suppressor genes' inactivation (mutation, etc.) may lead to the normal epithelia cell transformation of malignance. The epidemiology and laboratory study suggested that gastric carcinogenesis is a multistage progressive progress,[2] which often process from superficial gastritis, atrophic gastritis, intestine metaplasia, dysplasia to adenocarcinoma. The intestine metaplasia and heavy hyperplasia are regarded as the precancerous lesions of gastric carcinoma. To further understand the molecular basis for lesions, and the changes of p16 and cyclin D1, in protein level in the normal gastric epithelia and the epithelia with different severities of lesions were detected with the avidin- biotin- peroxidase complex (ABC) method in the present study. This study not only can provide important insights into gastritic carcinogenesis, but also contribute to ascertain the high-risk population and biomark for early diagnosis.

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MATERIALS AND METHODS

Specimens

All the subjects involved in this study were from Henan Province. The precancerous lesions were collected at random by biopsy. The cancerous tissues were surgically resected specimens which were collected from 1992 to 1995, of which there were some archive specimens for 1992 and 1993. For non-cancer patients, every subject underwent a routine gastroscope examination, 1–2 bioptic tissue, was taken, at the antrum and the site that there was lesion seen by naked-eye. All the specimens were fixed with 10% formalin, embedded with paraffin and serially sectioned at 5 μm. The sections were collected for histopathological analysis (hematoxylin) and for immunohistochemical staining. In the same way, the

surgically-resected specimens with different severities of lesions were processed and analyzed. None of all cancer patients received the treatment of chemotherapy of radiotherapy before the operation.

Histopathology Analysis

Histopathological diagnosis was made according to cellular morphological changes and tissue architecture using previously established criteria. The samples analyzed included: adenocarcinoma-58 cases, atypic hyperplasia-22 cases, atrophic gastritis-28 cases superficial gastritis-27 cases and normal gastric epithelium-15 cases.

Immuohistochemical Staining for p16 and Cyclin D1

The avidin-biotin-peroxidase complex (ABC) method was used for p16 and cyclin D1 detection. After dewaxing, inactivating endogenous, peroxidase activity and blocking cross-reactivity with normal serum, the sections were incubated overnight at 4 C with a diluted solution of the primary antibodies (1:100 for p16, from DAKO LTD, CANADA, 1:400 for cyclin D1, from DAKO LTD, AMERICA). Location of the primary antibodies was achieved by subsequent application of a biotiuylated anti-primary antibody, an avidin-biotin complex conjugated to horseradish peroxidase, and diaminobenzidine. Normal serum blocking and omission of the primary antibody were used as negative controls.

Statistical Analysis

The x^2 test was used for the percentage of samples with positive stain ($P<0.05$, was considered significant).

RESULTS

Expression of p16 and Cyclin D1 in the Normal Gastric Mucosa and the Precancerous Lesions of Gastric Carcinoma

Positive immunostaining rate for p16 protein was the highest in normal gastric mucosa and decreased with the lesions progressing from superficial gastritis to atrophicgastritis to atypical hyperplasia and to adenocarcinoma (85%, 78.6%, 31.8%, 48.3% respectively); No immuno-reactivity for cyclin D1 was detected in the normal gastric mucosa and superficial gastritis sample. But positive immuno-costaining of cyclin D1 can be observed in atrophic gastritis, with the lesions progressing from atrophic gastritis to atypical hyperplasia to adenocarcinoma, its expression from atrophic gastritis to atypical hyper-plasia to adenocarcinoma its expression rate increased (17.85%, 36.4%, 53.4% respectively), and there was a significant difference between adenocarcinoma and atrophic gastritis group ($P<0.05$) (Table 1).

Table 1. Changes of expression of cyclin D1, p16 in different series of lesions of gastric epithelia

Histology	P16 positive immunostaining n/n (%)	Cyclin D1 positive immunostaining n/n (%)
Normal	13/15 (86.7)	0/15 (0)
Superficial gastritis	23/27 (85)	0/27 (0)
Atrophic gastritis	22/28 (78.6)	5/28 (17.85)
Atypic hyperplasia	7/22 (31.8)*	8/22 (36.4)
Adenocarcinoma	28/58 (48.3)*	31/58 (53.4)**

*$P<0.005$, vs normal ** $P<0.005$, vs atrophic gastritis

Inverse Expression between p16 and Cyclin D1 in Gastric Carcinoma

An interesting observation was that inverse expression between p16 and cyclin D1, was shown in most of gastric cancer detected of 58 gastric cancers detected. Of 58 gastric cancers detected, inverse expression for p16 and cyclin D1, samples amount to 27 cases (46.6%). Samples of co-positive staining for p16 and cyclin D1 were 16 cases (27.6%). Samples of co-negative staining were 15 cases (25.9%). There was significant difference between inverse expression rate and co-positive/co-negative rate ($P<0.05$, χ^2 test).

DISCUSSION

P16 gene is a new type of tumor suppressor gene. It's deletion or mutation plays an important role in carcinogenesis. Oncogene cyclin D1 is a cell-cycle control gene, whose overexpression may lead to the disorder of cell proliferation. It was reported that its amplificating was determined in many tumors.[3]

The probe about oncogene and suppressor gene have been the hot point of carcinogenic and molecular biology. Lots of studies had indicated that carcinogenesis is a multistage progressive process caused by many genes' abnormality. With the development of

the theory and technique of cellular and molecular biology, the new studies on the transformation from normal cell to tumor cell have been done more than those on the characters of tumor cells.

In the early stage of gastric carcinogenesis, the epithelia tissues show cellular morphological and tissue architectural changes different from the normal tissues. It was given more attention whether there was intrinsical relation between these changes and oncogene's activation or suppressor gene's inactivation. The results of the present study shows: The expression of p16 protein in normal gastric epithelia was highest (86.6%, 13/15) and decreased with the lesion's progress. The expression expanded slightly upwards in the denocarcinoma group, but was much lower than the normal epithelia, superficial gastritis and atrophic gastritis groups. It has been reported that the deletion of p16 gene is a later event of gastric carcinogenesis.[4] But the results of this study indicates that deletion of p16 protein occurred in the early stage of gastric carcinogenesis. In comparison with normal epithelia, expression rate of p16 protein showed more or less decrease in the superficial and atrophic gastritis. The decrement was the most obvious in the dysplasia group. It was likely that except homozygous deletion, p16 gene was involved in carcinogenesis through multi-mechanisms,[5] such as point mutation, methylating of promotor domain, etc, which all disabled p16 gene and caused disorder of cells proliferating.

The study shows that the overexpression of oncogene cyclin D1 not only be observed in cancerous tissues, but also in the lesions of atypic hyperplasia and atrophic gastritis. The rate or its expression increased with the lesion's progress. The fact indicates that cyclin D1 gene's amplification may occur in the early stage of gastric carcinogenesis, may be important factor in promoting gastric carcinogenesis. It remained to further probe that cyclin D1 was acted as a biomark for early diagnosis of gastric cancer.

Both p16 and cyclin D1 are important gene for cell cycle regulation and there is a link with tumor suppressor gene Rb. P16 protein has an effect on activity of Rb gene. The combination of Rb and some transcription factors, which be of ability of promoting cell division can suppress activity of transcription. When Rb protein is phosphorylated, it will lose the ability of combination with transcription factors, and the activity of transcription factor will be regained. Combination of cyclin D1 with CDK4 can promote Rb phosphorylated, and p16 can competitively combine with CDK4, which can suppress activity of CDK4 and maintain active condition for Rb by preventing Rb from phosphorylating. We observed that an inverse expression, between p16 and cyclin D1, was show in most of gastric cancer detected, which was consistent with Ailous' report.[6] The results suggested that there was a suppression trend in them.

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