行政院國家科學委員會專題研究計畫成果報告

Catenin與E-cadherin的表現與臨床之關係

The Clinical Relationships of Gastric Cancer and the expression of catenin and E-cadherin

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摘　　要

我們對胃癌細胞株（SC-M1, NU-GC-3）及臨床切除之胃癌標本研究其E-cadherin及catenin（α-, β-, γ-）的表現程度及其臨床病理因素之關係。結果發現不論是E-cadherin或catenin，在胃癌細胞株及臨床切除之胃癌標本，都有不同程度之表現。進一步的分析發現，在中部胃癌的病例，大部分有γ-catenin在腫瘤組織較正常粘膜表現減少之現象。另外，γ-catenin在腫瘤部位的表現與正常粘膜表現不一致時，病患的短期存活率較差。因為E-cadherin-catenin複合體乃一功能性之結構單位，γ-catenin在腫瘤部位的表現與正常粘膜表現不一致時，勢必影響到E-cadherin的功能，進而影響到癌細胞的轉移程度及病患的預後關係。

關 鍵 詞：胃癌，Catenin，E-cadherin

ABSTRACT

In order to understain the role α-, β- and γ-catenin and E-cadherin in the gastric cancer, we used two gastric cancer cell lines (SC-M1, NU-GC-3) and twenty-two clinical resected gastric cancer specimens for study. The expression of E-cadherin and catenins (α-, β- and γ-) were well demonstrated by Western blot, with some specimens increased and others decreased than normal gastric mucosa. Further clinicopathological studies disclosed that decreased expression of γ-catenin in gastric cancer tissue than normal gastric mucosa were those cancers mostly located at midbody of the stomach. In Kaplan-Meier cumulative survival analysis revealed that, better short term survival was found in those cancers expressing γ-catenin in consistence with normal gastric mucosa. Since the E-cadherin-catenin complex is a functional unit, the decreased expression of γ-catenin may affect the function of E-cadherin which in turn may affect the cancer metastasis and the prognosis of gastric cancer patients. A longer follow up period of these patients is necessary for further stoudies.

Key words: gastric cancer, E-cadherin, catenin

Background and Purpose

Catenins(α-, β-, γ-) are the binding proteins to the intracellular portion of E-cadherin and this E-cadherin-catenin complex then binds to actin. E-cadherin is widely distributed at the cell surface of epithelial cells of ectodermal, mesodermal and endodermal origins. The expression of E-cadherin is associated to the cell morphology and differentiations, as well as cancer invasion and metastasis. Cancer cells with reduced expression of E-cadherin have higher ability of invasion and metastasis than those with strong expression of E-cadherin. The function of E-cadherin is highly dependent on the functional activity of catenins. β-catenin was reported to alter the cancer prognosis, but γ-catenin was rarely reported. This preliminary study showed its relationship to the gastric cancer patients.

Materials and Methods:

Cells and treatment

Two gastric cancer cell lines, NU-GC-3 and SC-M1, were used for this study. The gastric cancer samples were obtained by surgical resected specimen, including the tumor part and the normal mucosa and stored at -70°C until use. These sampleswere treated by Laemmli sample buffer, then heated under 95°C for 5 min., and stored at -20°C. Determination of the protein concentration of the samples were done by BioRad Protein Assay Kit.

Detection of catenin (α-, β-, γ-) and E-cadherin expression

Using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), the same protein amount of each sample is added. After electrophoresis, the proteins are transferred to nitrocellulose paper. The nitrocellulose paper is blocked by 5% non-fat milk in Tris Buffer Saline for one hour and then washed. Monoclonal antibody was applied and incubated for one hour. The HRP-conjugated secondary antibody is applied and incubated for one hour after washing the nitrpcellulose paper. The nitrocellulose paper was washed and using enhanced chemiluminescence (ECL) method to express on X-ray film. The expression was detected by densitometer.

Analysis of the clinicopathological factors with the relationships to the expression of catenins and E-cadherin

The clinical data of the gastric cancer patients were collected by our previous database for gastric cancer patients The expression of catenins and E-cadherin of these patients were recorded. The clinicopathological factors (depth of invasion, size, differentiations, cell type, vessel invasion, lymph node metastasis, peritoneal seeding and survival time) will be analysed for their relationships. Pearson chi-square and logistic regression were used for analysis. Kaplan-Meier survival was applied too. $P<0.05$ was considered statistical significance.

Results

Twenty-two randomized specimens from gastric cancer patients who received radical gastrectomy were studied as well as two gastric cancer cell lines. All were prepared for studies.

Expression of E-cadherin and catenins

Expression of E-cadherin was seen in these two gastric cancer cell lines. It is also expressed by some resected gastric cancer samples but weak or no expression was seen in some specimens. The expression of catenins(α-, β-, γ-) were similar to the expression of E-cadherin, but not parallel to it (Figure 1 to 8).

Clinicopathological relationships

For each catenin (for example, α-catenin), its expression from gastric cancer specimen was compared to its expression from the normal gastric mucosa in a same patient. These results could be categorized into two groups according to whether its expression is weaker than the normal mucosa or not. After grouping, further studies from the clinical data were made.

The expression of γ-catenin from gastric cancer patients and their clinicopathological data were studies and showed it is related to the tumor location ($p=0.017$) and most of the decreased expression was located at midbody of the stomach.

However, when survival function was applied, The expression of γ-catenin was also related to the sort term survival, $p=0.0055$ (Figure 9).

Conclusions

Though there were only 22 cases in this study and the the patient follow

up is not long enough, a tendency of good survival was seen from those patients with a unique expression of γ-catenin in normal mucosa and in cancerous tissue. Since the E-cadherin-catenin complex form a functional unit, the expression of catenins also affect the function of E-cadherin and its effects need further studies.

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Figure 1. The expression of E-cadherin in gastric cancer cell lines.

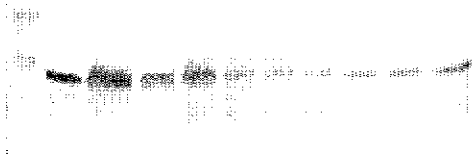

Figure 2. The expression of E-cadherin in clinically resected gastric cancer specimens and the paired normal gastric mucosa from a same patien.

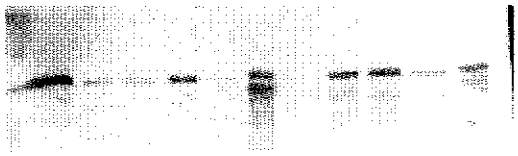

Figure 3. The expression of α-catenin in gastric cancer cell lines.

Figure 4. The expression of α-catenin in clinically resected gastric cancer specimens and the paired normal gastric mucosa from a same patien.

Figure 5. The expression of β-catenin in gastric cancer cell lines.

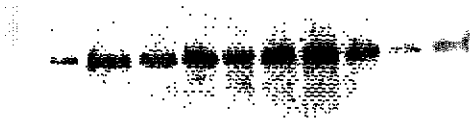

Figure 6. The expression of β-catenin in clinically resected gastric cancer specimens and the paired normal gastric mucosa from a same patien.

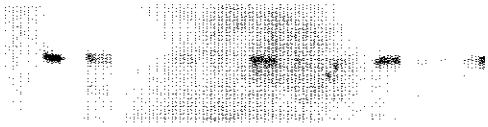

Figure 7. The expression of γ-catenin in gastric cancer cell lines.

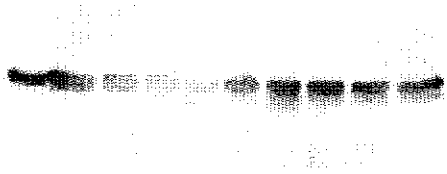

Figure 8. The expression of γ-catenin in clinically resected gastric cancer specimens and the paired normal gastric mucosa from a same patien.

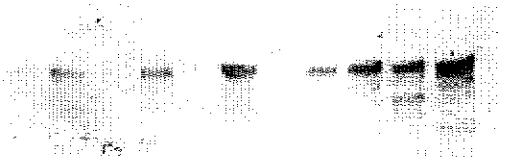
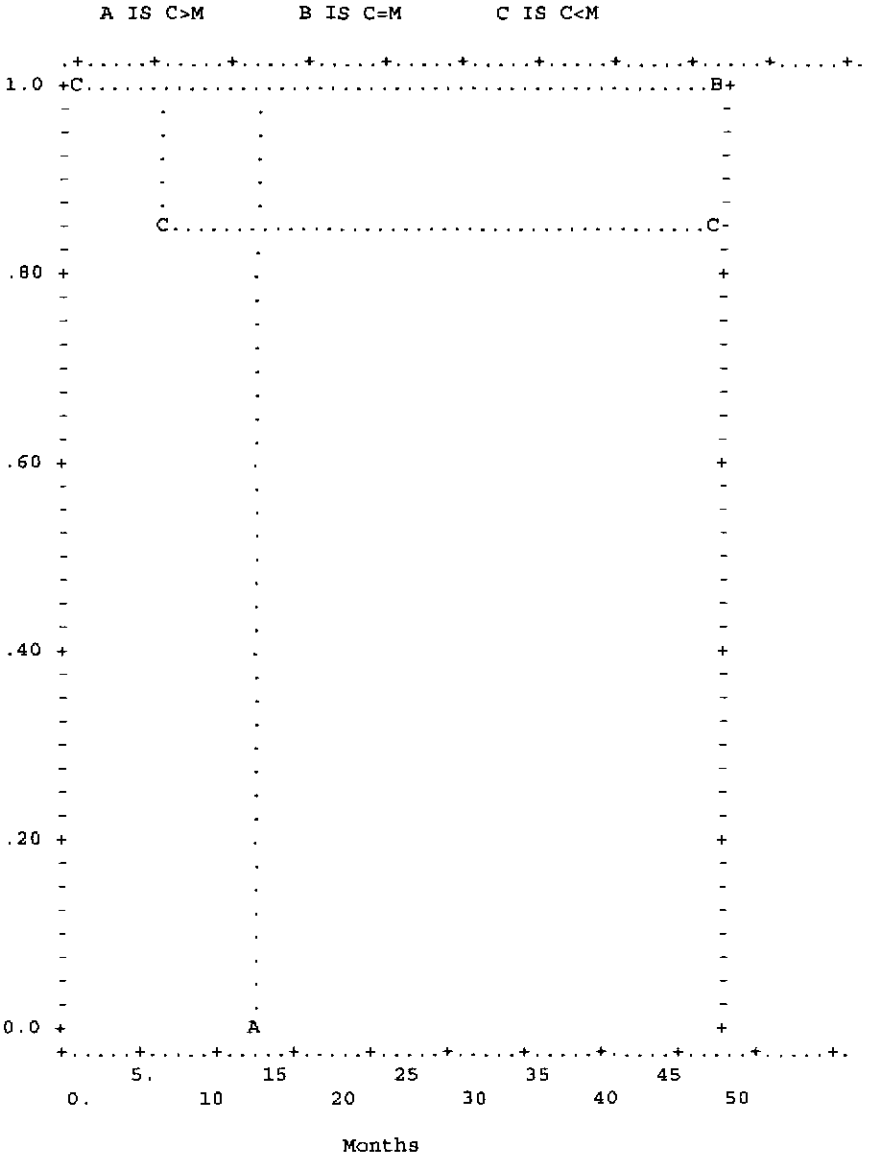
Figure 9. The Kaplan-Meier cumulative survival analysis. The expression of γ-catenin in cancerous tissue is A: increased than the normal gastric mucosa; B: equal to the normal gastric mucosa; and C: decreased than normal gastric mucosa.