

# 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

計畫編號： NSC 88 — 2314 — B038 — 123

執行期間： 87年 8月 1日至 88年 7月 31日

計畫主持人： 謝茂志

共同主持人：

余家利，雷永耀，吳秋文

處理方式：可立即對外提供參考

(請打✓) 一年後可對外提供參考

兩年後可對外提供參考

(必要時，本會得展延發表時限)

執行單位： 台北醫學院 醫學系

中華民國 88 年 10 月 31 日

## 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 ( $\alpha$ -,  $\beta$ -,  $\gamma$ -) 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  $\gamma$ -catenin 在腫瘤組織較正常粘膜表現減少之現象。另外,  $\gamma$ -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,  $\gamma$ -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 understand the role  $\alpha$ -,  $\beta$ - and  $\gamma$ -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 ( $\alpha$ -,  $\beta$ - and  $\gamma$ -) 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  $\gamma$ -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  $\gamma$ -catenin in consistence with normal gastric mucosa. Since the E-cadherin-catenin complex is a functional unit, the decreased expression of  $\gamma$ -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( $\alpha$ -,  $\beta$ -,  $\gamma$ -) 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.  $\beta$ -catenin was reported to alter the cancer prognosis, but  $\gamma$ -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  $-70^{\circ}\text{C}$  until use. These samples were treated by Laemmli sample buffer, then heated under  $95^{\circ}\text{C}$  for 5 min., and stored at  $-20^{\circ}\text{C}$ . Determination of the protein concentration of the samples were done by BioRad Protein Assay Kit.

### *Detection of catenin ( $\alpha$ -, $\beta$ -, $\gamma$ -) 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 nitrocellulose 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 ( $\alpha$ -,  $\beta$ -,  $\gamma$ -) were similar to the expression of E-cadherin, but not parallel to it (Figure 1 to 8).

### *Clinicopathological relationships*

For each catenin (for example,  $\alpha$ -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  $\gamma$ -catenin from gastric cancer patients and their clinicopathological data were studied 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  $\gamma$ -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  $\gamma$ -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.

## References

1. Reynolds AB, Daniel J, McCrea PD, Wheelock MJ, Wu J, Zhang Z. Identification of a new catenin: the tyrosine kinase substrate p120cas associates with E-cadherin complex. *Mol Cell Biol* 1994; 14: 8333-42.
2. Shibamoto S, Hayakawa M, Taceuchi K et al. Association of p120, a tyrosine kinase substrate, with E-cadherin/catenin complexes. *J Cell Biol* 1995; 128:949-57.
3. Aghib DF, McCrea PD. The E-cadherin complex contains the src substrate p120. *Exp Cell Res* 1995; 218: 359-69.
4. Shapiro L, Fannon AM, Kwong PD, et al. Structural basis of cell-cell adhesion by cadherins. *Nature* 1995; 374: 327-37.
5. Hinck L, Nathke IS, Papkoff J, Nelson WJ. Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. *J Cell Sci* 1994; 107: 3655-63.
6. Hart IR and Saini A. Biology of tumor metastasis. *Current Surg Nov/Dec: 577-583, 1992.*
7. Zetter BR. Adhesion molecules in tumor metastasis. *Seminars Cancer Biol* 4:219-229, 1993.
8. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 251: 1451-1455, 1991.
9. Vleminckx K, Vakaet L, Mareel M, et al. Genetic manipulation of E-Cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell* 66: 107-119, 1991.
10. Shimoyama Y, Hirohashi S. Cadherin intercellular adhesion molecule in hepatocellular carcinoma: loss of E-Cadherin expression in an undifferentiated carcinoma. *Cancer Lett* 57:131-135, 1991.
11. Oka H, Shiozaki H, Kobayashi K, et al. Expression of E-Cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res* 53:1696-1701, 1993.
12. Oka H, Shiozaki H, Kobayashi K, et al. Immunohistochemical evaluation of E-Cadherin adhesion molecule expression in human gastric cancer. *Virchows Arch [A]* 421:149-156, 1992.

13. Hsieh MC, Yu JL, Lui WY, Wu CW. The adhesive interaction between gastric cancer cell and endothelial cell. NSC83-0412-B-075-041.
14. Hsieh MC, Yu JL, Lui WY, Wu CW. Effects of hyperthermia to the adhesion between gastric cancer cells and endothelial cells. NSC84-2331-B075-080.
15. Hsieh MC, Wu CW, Lui WY, P'eng FK and Yu JL. Heat shock and cytokines modulate the expression of adhesion molecules on different human gastric cancer cell lines. *Int J Cancer* 67: 690-694, 1996.
16. Huang YC, Tsai SF, Lee SS, et al. Epidemiologic characteristics of malignant neoplasms in Taiwan: III. Stomach cancer. *J Natl Public Health Assoc (ROC)* 8:176-188, 1988.
17. Fink AS, Longmire WP. Carcinoma of the stomach. in *Textbook of surgery*, pp 881-893, W.B.Saunders, 1986.
18. Kodama Y, Sugimachi K, Soejima K, et al. Evaluation of extensive lymph node dissection for carcinoma of the stomach. *World J Surg* 5: 241-248, 1981.
19. Shiu MH, Fortner JG. Intraperitoneal hyperthermic treatment of implanted peritoneal cancer in rats. *Cancer Res* 40: 4081-4084, 1980.
20. Fujimoto S, Shrestha RD, Kokobun M, et al. Clinical trial with surgery and intraperitoneal hyperthermic perfusion for peritoneal recurrence of gastrointestinal cancer. *Cancer* 64: 154- 160, 1989.
21. Fujimura T, Yonemura Y, Fushida S, et al. Continuous hyperthermic peritoneal perfusion for the treatment of peritoneal dissemination in gastric cancers and subsequent second-look operation. *Cancer* 65: 65-71, 1990.
22. Koga S, Maeta M. Hyperthermochemotherapy for peritoneal dissemination of gastric cancer. *消化器外科* 6(8): 1189-1194, 1983.
23. Teicher, B. A., Kowal, C. D., Kennedy, K. A., and Sartorelli, A. C. Enhancement by hyperthermia of the in vitro cytotoxicity of mitomycin C toward hypoxic tumor cells. *Cancer Res.*, 41: 1096-1099, 1981.
24. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature(Lond.)*, 227: 680-685, 1970.
25. Bradford M. *Annal Biochem* 72: 248, 1976.
26. Dunn MJ. *Gel Electrophoresis: protein*. Bios Scientific Publisher Limited, Oxford, UK. 1993.
27. 謝茂志, 吳秋文, 雷永耀, 戚謹文. 胃癌病患電腦資料庫之建立. *中華醫學雜誌*, Vol. 45 (1): 26-33, 1990.

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

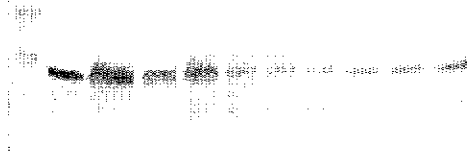


Figure 2. The expression of E-cadherin in clinically resected gastric cancer specimens and the paired normal gastric mucosa from a same patient.

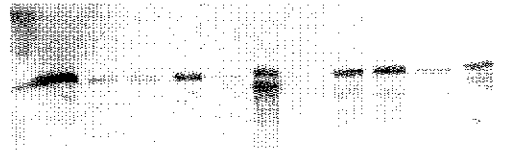


Figure 3. The expression of  $\alpha$ -catenin in gastric cancer cell lines.



Figure 4. The expression of  $\alpha$ -catenin in clinically resected gastric cancer specimens and the paired normal gastric mucosa from a same patient.



Figure 5. The expression of  $\beta$ -catenin in gastric cancer cell lines.

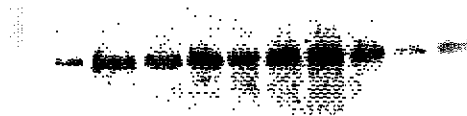


Figure 6. The expression of  $\beta$ -catenin in clinically resected gastric cancer specimens and the paired normal gastric mucosa from a same patient.



Figure 7. The expression of  $\gamma$ -catenin in gastric cancer cell lines.





Figure 8. The expression of  $\gamma$ -catenin in clinically resected gastric cancer specimens and the paired normal gastric mucosa from a same patient.

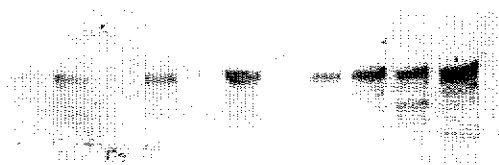


Figure 9. The Kaplan-Meier cumulative survival analysis. The expression of  $\gamma$ -catenin in cancerous tissue is A: increased than the normal gastric mucosa; B: equal to the normal gastric mucosa; and C: decreased than normal gastric mucosa.

