Chin J Clin Oncol (2008) 5: 429~432
DOI 10.1007/s11805-008-0429-0

Relationship between Expression of CEA, E-cadherin and Liver Metastasis in Colorectal Cancer

Ning Li[1]
Xiaobing Chen[1,2]
Xiangbin Wan[3]
Suxia Luo[1]
Lili Han[1]
Mengqiang Zhou[1]
Dengwen Yin[1]
Li Zhang[4]

[1] Department of Oncologic Medicine, Henan Tumor Hospital, Zhengzhou 450003, Henan Province, China.
[2] Zhengzhou University Cancer Center, Zhengzhou 450000, Henan Province, China.
[3] Department of Surgery, Henan Tumor Hospital, Zhengzhou 450003, Henan Province, China.
[4] Department of Oncologic Medicine, Sun Yat-Sen University Cancer Center, Guangzhou 510000, Guangdong Province, China.

Correspondence to: Li Zhang
E-mail: zhangli6@mail.sysu.edu.cn

Received May 27, 2008; accepted September 19, 2008.

CJCO http://www.cjco.cn
E-mail: 2008cocr@gmail.com
Tel (Fax): 86-22-2352 2919

OBJECTIVE To investigate the expression of E-cadherin and CEA in serum in colorectal carcinoma and their relationship with liver metastasis.
METHODS CEA level was measured post-operatively by radioimmunoassay of 60 patients with colorectal cancer. Immunohistochemical analysis was used to evaluate the expression of E-cadherin.
RESULTS In liver metastasis group, 24 patients (24/26, 92.3%) were high level of CEA, but only 9 patients in non-liver metastasis group. The difference is significant (P = 0.004). Expression of E-cadherin significantly correlated with differentiation, but was not associated with T stage or N stage. Liver metastatic rate in negative expression was higher than that in positive expression. And the survival analysis showed that time of liver metastasis was significant different in two groups ($P < 0.05$).
CONCLUSION The expression of CEA in serum can be used to predict liver mestatasis of colorectal cancer after operation. E-cadherin, associated with tumor differentiation, is also a hopeful indicator for the prediction of liver metastasis in patients with colorectal cancer.

KEY WORDS: colorectal cancer, liver metastasis, E-cadherin, CEA.

Introduction

Colorectal cancer (CRC) is the most common malignancy in China. The rates of incidence are increasing rather rapidly. Because liver metastasis is a leading cause of death, early diagnosis of colorectal cancer with liver metastasis is becoming an important clinical issue[1]. The metastatic cascade starts with a breakdown of the epithelial integrity, which enables tumor cells to invade the surrounding stroma. Epithelial differentiation is critically dependent on maintenance of intact intercellular junctions by cell-cell adhesion molecules. There are several cell-cell adhesion molecules, including cadherins (cadherin, E-, P-cadherin), catenins, and the CD44 family[2]. E-cadherin is normal transmembrane protein and is necessary for epithelial integrity[3,4]. In the present study, the down regulation of E-cadherin is proven to be a significant independent predictor of infiltration, lymphatic metastasis, and poor survival time. However, the study of E-cadherin in colorectal cancer is not enough. In addition, the elevation of CEA in serum is believed to be indicative of a poor prognosis. But the relationship between CEA level and liver metastasis after surgery is not clear.

So, we hope to examine the levels of CEA in serum and E-cadherin expression in primary tumors from a series of 60 CRC patients and assess the relationship with liver metastasis.

Materials and Methods

Clinical data

The study was conducted at Henan Tumor Hospital from January 2003 to December 2003. In this study 60 patients (31 males, 29 females) had been diagnosed as CRC, received surgery or lymphoid node biopsy. Thirty-four patients were more than sixty years old. Liver metastasis was not found before surgery and during surgery. All patients were treated with 5-FU-based chemotherapy for 6 cycles. Every patient was assessed by CEA level determination, ultrasound B of abdomen and chest X-ray. CT or MRI should be examined when something abnormal is found in ultrasound B.

Methods

Serum CEA level was measured post-operatively by radioimmunoassay of 60 patients with colorectal cancer. The normal is 0~5 ng/ml.

Immunohistochemical analysis was used to evaluate the expression of E-cadherin. Four μm sections from the tissue microarray were stained immunohistochemically with monoclonal antibodies directed against E-cadherin (1:100) (NeoMarkers, USA). After deparaffinization and rehydration with graded alcohol, the sections were soaked in 1% hydrogen peroxidase for 15 min. After washing in phosphate-buffered saline (PBS, pH 7.2), the microarray slide was heated in 10 mM citrate buffer at pH 6.0 by microwave (12 min, 700 W), then cooled at room temperature for 30 min. After being washed, slides were incubated with the primary antibody (mouse anti-E-cadherin) overnight at 4 degrees centigrade. Subsequently, the slide was washed with PBS. The secondary step consisted of incubation with rabbit anti-mouse antibody (DAKO, Glostrup, Denmark) and goat anti-rabbit antibody (DAKO, Glostrup, Denmark) at 37°C for 60 min, respectively. After being washed, the specimens were reacted with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) until staining was optimal as determined by light microscopic examination. The sections were washed in PBS and finally counter-staining with hematoxylin as usual. Then, slides were washed in tap water, dehydrated in alcohol, and mounted.

Two pathologists independently reviewed the slide and were in agreement regarding the extent of immunohistochemical staining. Staining for E-cadherin was observed basically in the cell membrane or cytoplasm. Four hundred tumor cells were counted at a 400 × magnification. The minimum number of tumor cells count was 150. For E-cadherin, cases were scored as 0 (no staining), 1+ (staining in 1%~25% of cells), 2+ (staining in 26%~50% of cells), 3+ (staining in 51%~75% of cells), 4+ (staining in 76%~100% of cells); score ≥ 2+ were considered to be indicative of positive staining (Fig.1).

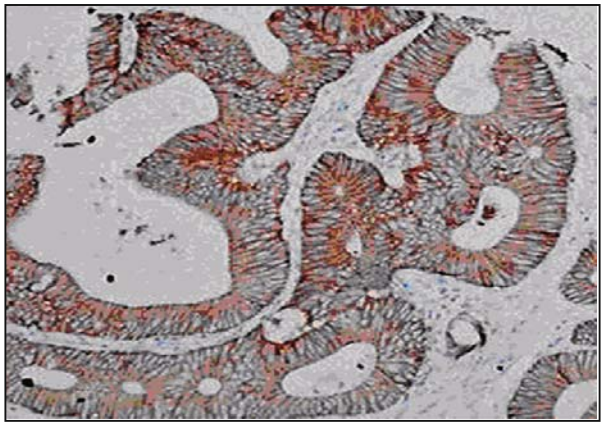

Fig.1. E-cadherin postive expression (10 × 40).

Statistical analysis

All statistical analysis were performed by using the SPSS for windows (version 10.00) (SPSS Inc., Chicago, IL) statistical packages. Associations between categorical groups were evaluated using the χ^2 or the Fisher's exact test when appropriate. Time to liver metastasis was analyzed using Kaplan-Meier method and log-rank test.

Results

Patient characteristics and the relationship with CEA or E-cadherin

All patients could be assessed for CEA in serum as well as for E-cadherin. The patients were prospectively followed up every three months. The median follow-up period was 1.5 years. All patients were divided into two groups by liver metastasis. Twenty-six (43.3%) patients were diagnosed as having liver metastasis.

CEA expression in serum

CEA expression was assessed in all patients. In the liver metastasis group, CEA expression of two patients was normal (0~5 ng/ml). The other 24 patients had elevated levels (mean ± SD: 79 ± 114.02). CEA expressions of nine patients were elevated in non-liver metastasis group (Mean ± SD: 4.9 ± 4.20. And the difference was significant (P = 0.004). CEA expression had no relationship with clinical characteristics (Table 1).

E-cadherin expression

E-cadherin expression in ten cases (41.6%) was assessed as being positive in the liver metastatic group. But 22 patients (64.7%) were positive in the non-metastatic group. E-cadherin expression decreased in patients who developed liver metastasis (P = 0.043). The differentiation of primary tumor had an impact on expression of E-cadherin. T-staging and N-staging had no relationship

Table 1. Relationship between CEA, E-cadherin and clinical characteristics.

Clinical characteristics	Cases	CEA in serum		E-cadherin expression	
		Positive (%)	*P*	Positive (%)	*P*
Differentiation					
Well-differented	35	20 (57.1)	0.693	23 (65.7)	0.023
Poor-differented	25	13 (52)		9 (36)	
T staging					
T_1/T_2	33	16 (48.5)	0.586	21 (63.6)	0.077
T_3/T_4	27	15 (55.6)		11 (40.7)	
N staging (Positive lymphoid nodes)					
< 4	42	18	0.079	20 (47.6)	0.175
≥ 4	18	15		12 (66.7)	
Liver metastasis					
Yes	26	24 (92.3)	0.004	10 (41.6)	0.043
No	34	9 (26.5)		22 (64.7)	

with E-cadherin (Table 1).

Co-evluation of CEA and E-cadherin in tissue

Statistical analysis showed that co-evaluation of CEA and E-cadherin could not be used to indicate or to predict liver metastasis ($P > 0.05$) (Table 2).

Relationship between E-cadherin expression and time to liver metastasis

Time to liver metastasis was censored and the rate of metastasis in the group with positive expression was 31.2% (10/32). The median time to liver lesions was 14.5 months, and that in the group of negative expression was 57.1% (16/28). The median time to metastasis there was 13.5 months. Survival analysis showed that the difference was significant ($P < 0.05$) (Fig.2).

Table 2. Co-evluation of CEA and E-cadhrein in tissue.

	Both negative	Any positive	Both positive
Liver metastasis	8	21	5
Non-liver metastasis	2	14	10
P	0.058		

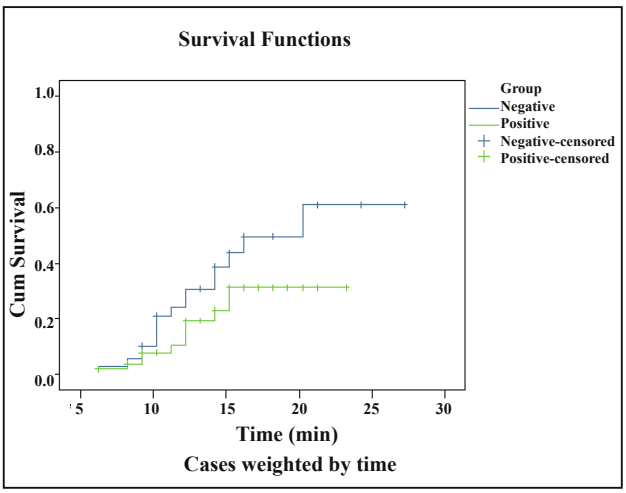

Fig.2. Relationship between E-cadherin expression and time to liver metastasis.

Discussion

Liver metastasis is one of the important prognostic factors after colorectal operation. At present, markers to predict liver metastasis are unclear. In some studies it was suggested that CEA in serum, especially the level after operation, be used to predict metastasis and survival time[5,6]. Others draw the opposite conclusion so the relationship between CEA and liver metastatsis is not clear[7]. In this prospective study, sixty patients with colorectal cancer had the level of their serum CEA assessed. CEA was higher in the liver metastatic group than in the non-metastatic group ($P = 0.004$). CEA may be the predictor of liver metastasis after operation.

A recent study demonstrates that the invasion or metastasis of tumors is a continuous process: a reduced cell-cell adhesion, alterations in the interaction of the tumor cell with the extracellular matrix, and invasion into surrounding tissues including blood vessels and lymph ducts. The first and most critical step is tumor cell detachment from the primary focus and re-adhering to the metastatic position. E-cadherin is a transmembrane glycoprotein which maintains normal epithelial polarity and intercellular adhesion. Catenins, including E-cadherin and P-cadherin, have been recognized as important suppression genes[8,9]. E-cadherin expression was reported in some studies in the whole epithelial cell, while P-cadherin only in the basal cell[10]. Basic research has suggested an inverse correlation between E-cadherin expression and dedifferentiation, tumor aggressivity, metastasis or a poor survival time in several tumor types including lung[11], gastric cancer[12], breast[13] and colorectal cancer[14]. How-

ever, it is not clear the relationship between E-cadherin expression and liver metastasis after surgery. In this study, E-cadherin expression was significantly higher in the well-differentiated tumor cells. But T-stage and N-stage had no impact on E-cadherin expression. Patients with liver metastasis showed significantly higher expression of E-cadherin compared with those without metastasis. The time to liver metastasis in the positive expression group was longer than in negative group. So, E-cadherin is an important predictor of liver metastasis after surgery.

Recent studies have shown that distant metastases also express E-cadherin, often more strongly than the primary tumor. Metastatic lesions derived from E-cadherin-negative tumors remain negative, while those originating from E-cadherin-positive tumors express imcompatible[14]. It is possible that loss of E-cadherin is a transient phenomenon that allows malignant cells to invade vascular channels and tissues. Re-expression of E-cadherin occurs in the circulating tumor cells, enabling the cancer cells to form tumor emboli and to survive[15], or E-cadherin re-expression may be stimulated by the metastatic organ environment. Therefore, treatment, targeted E-cadherin expression in the circulating tumor cells, may prevent liver metastasis after colorectal cancer operation. Study of correlation between E-cadherin expression in primary and liver metastatic lesions is ongoing.

To conclude, it is clear that examining CEA in serum or E-cadherin expression is extremely valuable in predicting disease recurrence. Co-evaluation of CEA and E-cadherin is not the predictor for liver metastasis. The observation of CEA or E-cadherin is obviously of great importance for both the patients and the clinician, and affects significantly decisions concerning the therapy and survival of the patients.

References

1 Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.
2 Elzagheid A, Algars A, Bendardaf R, et al. E-cadherin expression pattern in primary colorectal carcinomas and their metastases reflects disease outcome. World J Gastroenterol 2006; 12: 4304-4309.
3 Ooshio T, Irie K, Morimoto K, et al. Involvement of LMO7 in the association of two cell-cell adhesion molecules, nectin and E-cadherin, through afadin and alpha-actinin in epithelial cells. J Biol Chem 2004; 279: 31365-31373.
4 Mell LK, Meyer JJ, Tretiakova M, et al. Prognostic significance of E-cadherin protein expression in pathological stage I-III endometrial cancer. Clin Cancer Res 2004; 10: 5546-5553.
5 Kijima M, Toqo S, Ichikawa Y, et al. Clinical significance of serum CEA protein and CEA mRNA after resection of colorectal liver metastases. Anticancer Res 2005; 25: 1327-1332
6 Kanellos I, Zacharakis E, Kanellos D, et al. Prognostic significance of CEA levels and detection of CEA mRNA in draining venous blood in patients with colorectal cancer. J Surg Oncol 2005; 94: 3-8.
7 Ishida H, Yoshinaga K, Gonda T, et al. Biliary carcinoembryonic antigen levels can predict metachronous liver metastasis of colorectal cancer. Anticancer Res 2000; 20: 523-526.
8 Li YJ, Ji XR. Relationship between expression of E-cadherin-catenin complex and clinicopathologic characteristics of pancreatic cancer. World J Gastroenterol 2003; 9: 368- 372.
9 Lin YC, Wu MY, Li DR, et al. Prognostic and clinicopathological features of E-cadherin, alpha-catenin, beta-catenin, gamma-catenin and cyclin D1 expression in human esophageal squamous cell carcinoma. World J Gastroenterol 2004; 10: 3235-3239.
10 Furukawa F, Takiqawa M, Matsuvoshi N, et al. Cadherins in cutaneous biology. J Dermatol 1994; 21: 802-813.
11 Qiao GB, Wu YL, Ou W, et al. Expression of E-cadherin in non-small cell lung cancer and it correlation with prognosis. Zhonghua Wai Ke Za Zhi 2005; 43: 913-917 (Chinese).
12 Guzman P, Araya J, Villaseca M, et al. Immunohistochemical expression of the E-cadherin-catenin complex in gastric cancer. Rev Med Chil 2006; 134: 1002-1009.
13 Heimann R, Lan F, McBride R, et al. Separating favorable from unfavorable prognostic markers in breast cancer: the role of E-cadherin. Cancer Res 2000; 60: 298-304.
14 Mayer B, Johnson JP, Leitl F, et al. E-cadherin expression in primary and metastatic gastric cancer: down-regulation correlates with cellular dedifferentiation and glandular disintegration. Cancer Res 1993; 53: 1690-1695.
15 Kowalski PJ, Rubin MA, Kleer CG. E-cadherin expression in primary carcinomas of the breast and its distant metastasis. Breast Cancer Res 2003; 5: 217-222.