TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2013) 43: 946-950
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doi:10.3906/sag-1211-60

The expression of cell adhesion molecules in colorectal cancer tissue and its clinical values

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Received: 17.11.2012 • **Accepted:** 07.01.2013 • **Published Online:** 02.10.2013 • **Printed:** 01.11.2013

Aim: To investigate the role of cell adhesion molecules CD44v6 and CEA in the occurrence, development, and metastasis of colorectal cancer (CRC).

Materials and methods: The expression of CD44v6 and CEA in 76 CRC tissues and 43 normal colon tissues was detected using an immunohistological technique. The relationship between the 2 molecules' expression and their pathologic characteristics, especially for metastasis, was investigated. Furthermore, the relevance between the expression of CD44v6 and of CEA was also assessed.

Results: Expression of CD44v6 and CEA protein in CRC tissues was higher than in paracarcinoma tissue and normal colorectal mucosa (both $P < 0.001$). Positive expression of CD44v6 in CRC was associated with lymph node metastasis, Dukes' stage, and histological differentiation. Higher expression intensity of CEA in CRC was related to lymph node involvement, distant metastasis, and invasion depth. Combining analysis of CD44v6 and CEA can improve the sensitivity and specificity in evaluating the metastatic potential of CRC ($P < 0.05$). There was no relevance between the expression of CD44v6 and CEA in CRC ($P > 0.05$).

Conclusion: CD44v6 is associated with the occurrence and lymph node metastasis of CRC. The expression of CEA is related to distant metastasis of CRC positively. Combined analysis of CD44v6 and CEA can be used to evaluate metastasis of CRC.

Key words: Colorectal cancer, immunohistochemistry, cell adhesion molecule

1. Introduction

Colorectal cancer (CRC) has traditionally been one of the commonest disorders in western populations, while cancers of the upper gastrointestinal tract (such as the esophagus and stomach) and liver have predominated in eastern populations (1). However, during the past few decades, there have been remarkable changes in the incidence of CRC in China, which is becoming higher and higher. Metastasis and recurrence of CRC are the key causes of death for the patients, so it is very important to evaluate the potential for metastasis and recurrence in CRC.

Both CD44v6 and CEA are cell adhesion molecules; they can enhance metastasis by mediating the interaction between cancer cells or between cancer cells and the extracellular matrix (2). Our study was designed to detect the different expression of CD44v6 and CEA in CRC tissues, paracarcinoma tissue, and normal colorectal mucosa by using an immunohistological technique, and to observe the relationship between their expression and clinical pathological features, especially metastasis. We also studied the expressional dependability of CD44v6 and CEA in CRC, and their functions and interactions during the stages of initiation, development, and metastasis of CRC.

2. Materials and methods

2.1. Patients and samples

A total of 76 samples of colorectal cancer, paracarcinoma, and normal colorectal mucosa were collected from patients operated on between March and November of 2011 in the Colorectal Surgical Department of the First Affiliated Hospital of Zhejiang University. The 76 CRC patients (average age: 58.9 years; 30 women and 46 men; 25 colonic cancer and 51 rectal cancer patients; 31 with lymphoid invasion and 13 with distant metastasis) were retrospectively identified by pathological diagnosis and samples were collected from the tissues within 30 min after being resected from the body. None of the patients had been given chemical or radical therapies before

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the operation. Paracarcinoma tissue was defined as the colorectal mucosa 1–2 cm from the edge of the cancer, and the normal colorectal mucosa was defined as that more than 10 cm from the edge of the cancer.

2.2. Immunohistochemistry

The samples were fixed in 10% formaldehyde, dehydrated with ethanol, and then embedded in paraffin. Serial sections were cut at 2-μm intervals and were collected sequentially onto glass slides coated with poly-L-lysine. The sections were deparaffinized with xylene and rehydrated in graded ethanol, and then they were stained using the EnVision method. The primary antibodies used consisted of rat monoclonal antihuman CD44v6 and CEA (1:50, Zhongshan, P.R. China), and the secondary antibodies were purchased from the DAKO company. Diagnosis standard (3): the numbers of positive cells were expressed as the percentage of the total number of epithelial cells and assigned to 1 of 4 categories: (-), 0%; (+), <25%; (++), 25%–50%; (+++), >50%.

2.3. Statistical analysis

Statistical analysis was performed by using SPSS 10.0. Fisher's exact test or chi-square test was used in enumeration of data, and the Spearman rank correlation was used in correlation analysis.

3. Results

3.1 The expression of CD44v6 and CEA in CRC tissue, paracarcinoma tissue, and normal colorectal mucosa

The positive CD44v6 expression was located in the cell membrane, and its distribution was a continuous, girdle-shaped buffer around the cell membrane. We noted the expression of CD44v6 in CRC tissue, paracarcinoma tissue, and normal colorectal mucosa. The positive CD44v6 expression was observed in 3/43 (7.0%) and 1/43 (2.3%) samples of paracarcinoma tissue and normal colorectal mucosa, respectively; however, the incidence of CD44v6 expression was significantly higher in CRC tissue (36/76, 47.4%, $P < 0.001$; Table 1).

The positive CEA expression was located in the glandular cavity of epithelial cells of paracarcinoma tissue and normal colorectal mucosa, and the incidence of expression was observed in 6/43(14.0%) and 3/43(7.0%), respectively. In CRC tissue, CEA expression was diffusely located in the cytoplasm, membrane, and the cavity of cancerous cryptae, and even located in the matrix. The positive expression in CRC was observed in 68/75 (90.7%), with the delitescence of heteropolarity. The CEA expression in CRC was significantly higher in CRC tissue ($P < 0.001$; Table 1)

3.2. The expression of CD44v6 and CEA in CRC tissue, and their relationship with clinical pathological features

The positive CD44v6 expression was observed in 67.7% of cases of CRC with lymphoid metastasis. This was significantly higher than in the group without lymphoid metastasis (33.3%, $P < 0.05$). In the Dukes' stage C+D group, positive CD44v6 expression was 59.5%, statistically higher than in the Dukes' stage A+B group (35.9%, $P < 0.05$). In the poorly differentiated group it was 68.8%, significantly higher than in the well and moderately differentiated group (41.7%, $P < 0.05$). However, there was not enough evidence to confirm that positive CD44v6 expression is significantly different in different gross types, histological types, invasion depths, and distant metastasis.

The expression of CEA in CRC with lymphoid invasion was significantly higher than in the group without lymphoid invasion ($P < 0.05$). It was significantly higher in the group with distant metastasis than in the group without distant metastasis ($P < 0.05$). It was also significantly higher in the T4 group than in the T2 group ($P < 0.05$) (Table 2).

3.3. The value of a positive expression of CD44v6 and a high expression of CEA in the diagnosis of CRC metastasis

In our study, the sensitivity and specificity of the expression of these 2 cell adhesion molecules in the diagnosis of CRC metastasis were different. For CD44v6 expression they were 59.5% and 64.1%, and for CEA expression

Table 1. The expression of CD44v6 and CEA in CRC tissue, paracarcinoma tissue, and normal colorectal mucosa.

Lesion	CD44v6				CEA			
	Total	–	+	Positive rate	Total	–	+	Positive rate
NCM	43	42	1	2.3%*	43	40	3	7.0%*
PCT	43	40	3	7.0%*	43	37	6	14.0%*
CRC	76	40	36	47.4%	75	7	68	90.7%

*: Compared with CRC tissue, there was a significant difference ($P < 0.001$).
NCM: normal colorectal mucosa. PCT: paracarcinoma tissue.

Table 2. The expression of CD44v6 and CEA in CRC tissue, and their relationship with clinical pathological features of the patients.

Clinical pathological features	CD44v6			CEA				
	n	–	+△ (%)	n	-	+	++	+++ (%)
Gross types								
Protruding type	10	4	6 (60.0)	10	1	8	0	1 (10.0)
Localized ulceration	51	25	26 (51.0)	50	5	25	9	11 (22.0)
Invasive ulceration	15	11	4 (26.7)	15	1	8	4	2 (13.3)
Histological types								
Tubular adenocarcinoma	68	37	31 (45.6)	68	4	40	12	12 (17.6)
Mucinous adenocarcinoma + signet-ring cell carcinoma	8	3	5 (62.5)	7	3	1	1	2 (28.6)
Differentiation								
Well and moderately differentiated	60	35	25 (41.7)	60	4	36	9	11 (18.3)
Poorly differentiated	16	5	11 (68.8)*	15	3	5	4	3 (20.0)
Depth of invasion								
T2	17	9	8 (47.1)	17	3	11	2	1 (5.9)
T3	41	22	19 (46.3)	40	3	24	7	6 (15.0)
T4	18	9	9 (50.0)	18	1	6	4	7 (38.9)**
Lymph node metastasis								
+	31	10	21 (67.7)*	30	3	9	9	9 (30.0)*
-	45	30	15 (33.3)	45	4	32	4	5 (11.1)
Distant metastasis								
+	13	6	7 (53.8)	13	1	4	2	6 (46.2)*
-	63	34	29 (46.0)	62	6	37	11	8 (12.9)
Dukes' stages								
A+B	39	25	14 (35.9)	39	3	28	3	5 (12.8)
C+D	37	15	22 (59.5)*	36	4	13	10	9 (25.0)

△: Positive or strong positive expressions.
*: Have significant difference compared to the control group ($P < 0.05$). **: Significantly higher than the T2 group ($P < 0.05$).

they were 25% and 87.2%, respectively. If compounded in parallel, the sensitivity of CRC metastasis was 66.7%, significantly higher than that by CEA expression alone ($P < 0.05$). If compounded in series, the specificity was 92.3%, significantly higher than that by CD44v6 expression alone ($P < 0.05$) (Table 3).

3.4. The dependability of CD44v6 and CEA in CRC tissues

In our study, the expression of CD44v6 was not correlated with CEA in CRC tissues (Table 4).

4. Discussion

Isoforms of CD44 are generated by the insertion of alternative exons (V1–V11) at a single site within the membrane-proximal portion of the extracellular domain (4). Some research has shown that the different expression style of CD44, and especially the variants, may play an important role in the metastasis of various carcinomas (5–9). CD44v6 has been proven in cell cultivation and animal studies to be a metastasis-related gene. Recently, CD44v6 was found to be closely related to the invasion and

Table 3. The values of positive expression of CD44v6 and high expression of CEA in the diagnosis of CRC metastasis.

	Nonmetastasis group		Metastasis group$^{\triangle}$		Sensitivity	Specificity
	–	+	–	+		
CD44v6	39	14	37	22	59.5%	64.1%
CEA	39	5$^{\#}$	36	9$^{\#}$	25.0%	87.2%
Compounding in parallel	39	16$^{\#\#}$	36	24$^{\#\#}$	66.7%*	59.0%
Compounding in series	39	3$^{\#\#\#}$	36	7$^{\#\#\#}$	19.4%	92.3%**

$^{\triangle}$: Both lymph node metastasis and distant metastasis. *: Compared with the CEA group, $P < 0.05$. **: Compared with the CD44v6 group, $P < 0.05$. #: The expression of CEA > ++. ##: CD44v6 (+) or CEA > ++. ###: CD44v6 (+) and CEA > ++.

Table 4. The values of positive expression of CD44v6 and high expression of CEA in the diagnosis of CRC metastasis.

CD44v6	CEA					r_s	P
	n	–	+	++	+++		
+	36	3	17	6	10		
–	39	4	24	7	4	0.183	>0.05

metastasis of CRC (10). Chun et al. (11) found that a high level of CD44v6 mRNA was transcripted in CRC tissues compared with that in normal colorectal mucosa. In the present study, the expression of CD44v6 in CRC tissues (47.4%) was significantly higher than in paracarcinoma tissue and normal colorectal mucosa (7.0% and 2.3%, respectively) ($P < 0.001$). A recent study (12) also showed that the positive expression of CD44v6 in CRC tissues can even increase to 77.5%. Our and other teams' results hint that the high expression of CD44v6 was possibly related to the initiation of CRC.

Most scientists (13–17) agree that the high expression of CD44v6 is closely related to the development, invasion, metastasis, and prognosis of CRC. Our results also show that the positive expression of CD44v6 in CRC tissue with lymph node metastasis was obviously higher than in the group without lymph node metastasis ($P < 0.05$). The expression in the Dukes' stage C+D group was significantly higher than in the Dukes' stage A+B group ($P < 0.05$), and in the poorly differentiated group it was significantly higher than in the well and moderately differentiated group ($P < 0.05$). The above results indicate that the high expression of CD44v6 was related to the lymph node metastasis of CRC, which made it useful to evaluate the metastasis, Dukes' staging, and differentiation of CRC.

CEA is an important biomarker for treatment and prognosis evaluation and a cell adhesion molecule for CRC expression, which plays important roles in the recurrence and metastasis of CRC. Using immunohistochemistry, we can not only investigate the expression of CEA in CRC, but we can also see the intact morphous cells and locate the distribution of CEA more accurately than by investigating the CEA level in the serum. We observed that in the tissue beside the cancer tissue and normal colonic mucosa, the expressions of CEA were low (14.0% and 7.0%, respectively), but in cancer tissue the expression of CEA was observed at 90.7%. In CRC, CEA is distributed in the cytoplasm, membrane, and lumina, and even in the stroma, but in normal tissue CEA is mostly located in the lumina.

In our study, the expression of CEA in CRC tissue was enhanced when accompanied with a greater depth of invasion. CEA expression in the T4 group was significantly higher than in the T2 group ($P < 0.05$), which hints that the expression of CEA was concerned with the invasion potential of CRC. Our results are consistent with a previous study (18). Additionally, the results show that positive CEA expression was significantly higher in the group with lymph node metastasis and in the group with distant metastasis. The data suggest that CRC cells with high CEA expression enter metastasis more easily. Furthermore, Jessup et al. (19) found that a great number of CRC cells come together with the help of the cell adhesion. We speculate that CEA could enhance the life expectancy of CRC cells and make the CRC cells have more success in nidation in the target tissue, which ultimately increases the invasion potential of CRC cells.

In our study, the sensitivity of these 2 cell adhesion molecules (CD44v6 and CEA) in the diagnosis of CRC metastasis was 59.5% and 25%, respectively. The specificity was 64.1% and 87.2%, respectively. If compounded in parallel, the sensitivity of CRC metastasis was 66.7%, and it was significantly higher than that just for CEA ($P < 0.05$). If compounded in series, the specificity was 92.3%, significantly higher than that just for CD44v6 ($P < 0.05$). We can therefore make our judgment as to whether the metastasis has already occurred more accurately, and this will provide useful reference for the follow-up visit, adjunctive therapy, and prognosis of CRC.

Both CD44v6 and CEA belong to the cell adhesion molecule family, mediating the interaction between cancer cells, or cancer cells and the extracellular matrix, so we presume that both of them may have compounding functions in the process of invasion and metastasis of CRC, which was already supported by the extraorgan experiment. Ishii et al. (20) found that the adhesion mediated by CEA between CRC cells can be blocked by the CD44 monoclonal antibody, which indicated that CEA and CD44 may share the same antibody determinant group and cooperate in the adhesion process. In our study, we did not show that the expression of CD44v6 and CEA have significant dependability ($P > 0.05$). Whether the action processes of CEA and CD44v6 are identical needs further research.

In conclusion, CD44v6 is associated with the occurrence and lymph node metastasis of CRC. The expression of CEA is related to distant metastasis of CRC positively. Combined analysis of CD44v6 and CEA can be used to evaluate the metastasis of CRC.

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