Expression and Clinical Significance of CD44v6 and MVD in Human Colorectal Carcinoma

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Abstract **Objective** To study the expression and Clinical Significance of CD44V6 and MVD in Human Colorectal Carcinoma. **Methods** The expression of CD44V6 and MVD was detected and analyzed by immunohistochemical staining S-P in 57 cases of colorectal adenocarcinoma, 78 cases of colorectal adenoma and 17 cases of normal mucosa. **Results** The positive expression rate of CD44V6 was 45.6%(26/57) in colorectal adenocarcinoma, 58.0%(36/62) in colorectal adenoma, 75.0%(12/16) in colorectal adenoma with malignant change, and 5.9%(1/17) in normal mucosa; MVD was 24.65±7.84 in colorectal adenocarcinoma. The expression of CD44V6 was associated with MVD(P<0.01) in colorectal adenocarcinoma. The expression of CD44V6 and MVD was closely related with tumor size, lymph node metastases, infiltrating depth and differentiation of tumor cells. **Conclusions** The results of this experiment indicates that the expression of CD44V6 and MVD is related to tumor growth, local invasion and metastasis. The combind detection of CD44V6 and MVD may play an important role in the determination of infiltration and metastasis in human colorectal carcinoma. It may be helpful in treatment and prognosis.

Key Words Human colorectal carcinoma; CD44V6 protein; Microvessel density (MVD); Immunohistochemical staining

Recurrence, local relapse and distal metastasis after colorectal carcinoma operation are still the clinical problems. We adopt immunohistochemical method to examine the expression of CD44V6 and MVD in colorectal tumor tissue and discuss their clinical significance.

MATERIALS AND METHODS

Clinical materials

A total of 125 operative excised colorectal tumor samples which were diagnosed by pathological examination were collected from Liaoning People´s Hospital and Shenyang anus-intestine hospital from Mar. 1995 to Jul. 2001 According to ″United standard of National colorectal tumor pathological cooperative study″, which include 57 colorectal adenocarcinoma and 78 colorectal adenoma (in which 16 accompany with canceration). All 57 patients had not been carried out radiation or chemical therapy before operation. Take 17 normal colorectal mucous for control-experiment.

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According to Histology Classification Standard of WHO(1981), the 57 colorectal cancer include 13 adenocarcinomas of nipple, 38 tubular gland carcinomas (14 high differentiation, 10 middle differentiation, and 14 low differentiation), 2 mucous adenocarcinomas and 4 undifferentiated adenocarcinomas. In addition, the adenocarcinoma of nipple, high and middle differentiation tubular gland carcinoma are defined as well differentiated types (37 cases); low differentiated tubular gland carcinoma, mucous adenocarcinoma and undifferentiated adenocarcinoma are defined poor differentiated types (20 cases). Dukes A stage are 24 cases, B 14 cases and C 19 cases.

Agents and methods

The primary antibody CD44V6, CD34 and hypersensitivity agent box are bought from Maxin Biotechnology Corporation of Fujian. All samples were applied 10% neutral Formalin fixation, routine tissue dehydration, paraffin imbedding, 4μm continuous section-cutting, HE and immunohistochemical staining. Use SP method to detect expression of CD44V6 and CD34 according to directions with procedure of high temperature antigen renovation, calf serum protein obstruction, rabbit-anti-human CD34 biotin marked mouse-anti-rabbit IgG, peroxi-

dase strain-affinity reaction, DAB substrate coloration, hematoxylin restain, routine dehydration, and hyaline blocking. Use PBS instead of primary-antibody as negative control, while positive CD44V6 and CD34 samples as positive control.

Results judgments and statistics analysis

Positive expression of CD44V6 showed as brown granules which are located in cell membrane and (or) cytoplasm. If positive cell number less than 5% or had not been stained, which was defined as negative, while they were more than 5%, defined as positive; positive CD34 products are located in vascular endothelial cytoplasm, through whose condition of staining we can sum up the MVD. According to the standard of Weidner etc[1], choose the highest density areas of MVD in sections, count 5 visual fields, and calculate their mean. Single brown endothelium or cluster of endothelia is treated as a vessel, an endothelium without lumen is treated as a vessel, while those with lumen and less than 8 red blood cells in it can be counted; mean of every groups is expressed as X± S. Adopt X^2 and F test to analyze relationships between those indexes and clinical pathological factors.

RESULTS

CD44V6 expression

Positive ratio of CD44V6 expression in colorectal carcinoma was 45.6%(26/57), in adenoma was 58.1%(36/62), in adenoma canceration was 75.0% (12/16), while which in normal large intestine mucous was only 5.9% (table)

According to table 1, from adenocarcinoma to adenoma to adenoma canceration, positive expression of CD44V6 gradually strengthen, positive expression ratio of CD44V6 in adenocarcinoma obviously lower than that in adenoma canceration ($p<0.05$); while although positive expression ratio of CD44V6 is lower than that of adenoma, but there is no obvious difference ($p>0.05$). Compared with normal large intestine mucous, adenocarcinoma, adenoma canceration and adenoma have obvious difference separatly ($p<0.01$).

Expression of CD34

CD34 positive products located in vascular endothelial cytoplasm and showed brown color. Micro vessels in normal large intestine mucous was of small quantity, MVD value was 6.00±0.71~12.00± 2.76, which in adenoma was slightly increased, and in colorectal carcinoma unevenly distributed, fissure or sponge lumen shaped, micro vessels concentrate in tissues around cancer nests. MVD value in 57 colorectal carcinoma is 24.65±7.48, showed in table 1.

Relationship between CD44V6 expression, MVD and colorectal carcinoma clinical pathological features

MVD expression in colorectal carcinoma is closely related to tumor´s volume, differentiate degree, infiltrate depth, clinical pathological stage and lymphometastasis; Comparation among MVD value in every Dukes stages showed obvious difference ($p<0.01$); MVD values in stage A and B were obviously lower than that in stage C, which indicate that MVD value increase with state of illness. Positive expression of CD44V6 has no obvious correlation with sex and histologic types, while has obvious correlation with differentiation, infiltration stage and lymph node metastasis of tumor ($p<0.05$). Stage correlation analysis shows that CD44V6 expression in colorectal carcinoma has negative correlation with MVD (r=–0.4378), shows obvious differentiation ($p<0.01$), showed in table 2.

DISCUSSION

Significance of CD44V6 in colorectal carcinoma

Table 1. Expression of CD44V6 and MVD in human colorectal carcinoma

Tissue	number	MVD	CD44V6		positive ratio(%)
		($\bar{x} \pm s$)	+	–	
Colorectal adenocarcinoma	57	4.65±7.48	26	31	45.6
Colorectal adenoma canceration	16	19.11±5.30	12	4	75.0
Colorectal adenoma	62	13.60±4.12	36	26	58.1
Normal large intestine mucous	17	9.04±6.82	1	16	5.9

Fig. 2 Relationship between CD44V6、MVD and colorectal carcinoma clinical pathological feature

clinical pathological feature	number	MVD (x±s)	*P*	CD44V6			
				+	−	positive ratio(%)	*P*
Sex							
male	35	25.28±7.19	>0.05	17	18	48.5	>0.05
female	22	23.64±6.64		9	13	40.9	
Age (years)							
≤60	38	23.90±7.18	>0.05	9	29	23.6	<0.01
>60	19	26.15±6.24		17	2	89.4	
Volume of tumor(cm3)							
≤4	30	20.06±5.73	<0.01	6	24	20.0	<0.01
>4	27	29.75±3.85		20	7	74.9	
Differentiation degree							
High	27	19.80±4.12	<0.01	7	20	25.9	<0.01
Middle	10	24.72±5.76		4	6	40.0	
Low	20	31.17±6.53		15	5	75.0	
Infiltration depth							
Sub mucous	38	21.83±6.14	<0.01	12	26	31.5	<0.01
Muscle layer	19	30.29±4.54		14	5	73.6	
Dukes stage							
A	24	20.72±3.96	<0.01	7	17	29.1	<0.01
B	14	24.27±4.27		6	8	42.8	
C	19	30.08±4.71		13	6	68.4	
Lymph node metastasis							
Positive	19	30.08±4.72	<0.01	13	6	68.4	<0.05
Negative	38	21.93±6.22		13	25	34.2	
Histological types							
Adenocarcinoma of nipple	13	17.78±2.80	>0.05	2	11	15.3	>0.05
Tubular gland carcinoma	24	23.39±4.18		9	15	37.5	
Mucous adenocarcinoma	2	33.20±4.20		0	2	0.0	
Undifferentiated adenocarcinoma	18	30.75±3.21		15	3	83.3	

CD44 is a kind of wide spread transmenbrane glucoprotein, as adhensive molecule, whose major function is specific adhension from cell to cell and from cell to matrix. Human CD44 gene locates in short arm of number 11chromosome about 60kb, includes 20 exogens, forms standard CD44 protein (CD44S)and selectively cut variant protein (CD44V). Many researches indicate that in CD44V1-10, expression of CD44V6 exogen may induce infiltrate and metastasis in many types of tumor cells[2,3]. Results of our research indicate that although CD44V6 expression ratio in adenocarcinoma is lower than that in adenoma, but there is no obvious difference; while there is obvious difference between adenocarcinoma and adenoma canceration. Other reports indicate abnormal expression of variant transcript substance composing of CD44V6 exogen is related to tumor´s malignant phenotype and infiltration and metastasis[4−7]. At the same moment positive expression ratio of CD44V6 (75.0%) is higher than that of adenoma (58.0%) and adenocarcinoma (45.6%), which indicates that CD44V6 variation may happens in the early stage of colorectal tumor canceration, in other word, in early stage of adenoma canceration or early carcinoma, with only high expression of CD44V6 that can make cancer cells have more invasiveness. High expression of CD44V6 means that it plays an important role in normal intestine mucous canceration.

Significance of MVD in colorectal carcinoma

It is confirmed when solid tumors grow into certain volume (2~3mm^3) and with 10^7 cells, they need vessels to supply oxygen and nutrition, and also formation of new vessels give chance to tumor cells to go into blood, which transport tumor cells to host and promote malignant growth and metastasis of tumor. According to standard of Weidner, we

use CD34 marked vascular endothelia to count MVD, in normal intestine mucous, colorectal adenoma, adenoma canceration to colorectal carcinoma. Our research found that MVD value increases with the promotion of colorectal tumor malignant degrees, and there are obvious differences between normal and adenocarcinoma and between adenoma and adenocarcinoma. MVD value in adenocarcinoma slightly higher than that in adenoma indicates that tumor cells proliferate actively and which need more blood supply for metabolism. Thus, both CD44V6 and MVD are closely related to growth, infiltration, metastasis and prognosis of colorectal carcinoma. But there is no many report about co -expression and the relationship between CD44V6 and MVD in colorectal carcinoma. Our research indicates that co expressiong of CD44V6 and MVD is related closely to colorectal carcinoma´s growth, infiltration, and metastasis, which is of negative correlation, with malignant degree, MVD value increase while CD44V6 decrease. The mechanism maybe that adhensive molecule CD44 is as an integral glucoprotein, as lymphocyte´s nest-back receptor and as hyaluronic acid receptor in matrix, which can connect fibronectin, laminin , type I collagen and hyaluronic acid molecules and combine with skeketon protein, take part in formation of cell psudofeet and increase flowing changes. Extracellular matrix (ECM) is the first barrier in infiltration and metastasis of tumor, thus in the 3 steps of malignant tumor infiltration and metastasis, adhension, degradation and migration, tumor cells adhensive to EMC by CD44V6, to degradate hyaluronidase, to dissolute matrix and to enlarge tissue gap, which is profitable to deadhensiveness between tumor cells and original matrix, and decrease progressively expression of CD44V6 after carcinoma formation. In addition, as the lymphocyte´s nest-back receptor, CD44V6 can make the metastatic carcinoma cells steadily parasite, colony growth, avoid being recognized and killed by human immune system, produce immunoescape and effectively form metastatic tumor. This was confirmed in the research of Arch et al[8]. So we consider that the cofunction of CD44V6 and MVD increase the chance of rumor infiltration and metastasis, and according to this, the expression of CD44V6 may be of an important biological index of colorectal carcinoma canceration and poor prognosis, combines inspect of CD44V6 and MVD may help to predict inclination of tumor infiltration, relapse and metastasis.

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