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• CLINICAL RESEARCH •

Levels of v5 and v6 CD44 splice variants in serum of patients with colorectal cancer are not correlated with pT stage, histopathological grade of malignancy and clinical features

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Abstract

AIM: This study was designed to compare the levels of v5 and v6 splice variants of CD44 evaluated using ELISA test in the serum of patients with colorectal cancer in different stages of progression of the disease estimated in pT stage according to WHO score, histopathological grade of malignancy and some clinicopathological features.

METHODS: The serum obtained from 114 persons with colorectal adenocarcinomas was examined using ELISA method. pT stage and grade of malignancy of the tumour were examined in formalin fixed and paraffin embedded materials obtained during operation.

RESULTS: Only the level of CD44 v5 in the serum of patients before operation with G2 pT4 tumour was lower than that in other probes and the difference was statistically significant. We did not find any other correlations between the level of v5 and v6 CD44 variants and other evaluated parameters.

CONCLUSION: The level of CD44 v5 and v6 estimated by ELISA test in the serum can not be used as a prognostic factor in colorectal cancer.

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INTRODUCTION

Neoplasmal diseases are the second, or according to some authors, the third cause of deaths all over the world just after the cardiovascular and infectious diseases, and just before communication accidents. Colorectal cancers are among other neoplasms on the third place in morbidity and mortality after the breast and lung and bronchus cancers in women and prostate and lung and bronchus cancers in men. According to American National Cancer Institute, 105 500 persons had a colon cancer and 42 000 rectum cancer in 2002 in the United States of America. Colorectal cancer is also the third cause of death among all neoplasmal diseases. It is estimated that over 57 000 persons died in 2003 in the United States due to colon cancer[1]. According to World Health Organization data in 1990, over 437 000 persons died and 783 000 became ill due to colorectal cancer. The incidence of this disease has been rising, and is more frequent in developed countries. The survival rate after radical operative treatment and chemo- or radiotherapy is still unsatisfactory. The 5-year survival after radical treatment was about 60% in the Unites States and Western Europe, whereas it was only about 40% in Eastern Europe[2]. The recurrence of the disease is dependent on the progression of neoplasmal metastases to lymph nodules and distal organs and is the most common cause of death[3-6]. The routine examinations of specimens with the estimation of the pT according to WHO score as well as the histopathological grade of malignancy, clinical evaluation and even the changes in CEA levels in the serum evaluation are still insufficient to determine the precise prognosis about recurrence of the disease and survival rate. This is the reason, why a lot of medical investigations are concerned with understanding these problems and determining the prognostic factors in colorectal cancers, *i.e.* mutations of c-Ki-ras, C-Myc, APC, p53, DCC and other genes, CD44 protein in the tumour tissues and CEA and CA19.9 in the serum[6-14]. The aim of this study was to determine if there were any differences between levels of variant 5 and 6 glycoprotein CD44 according to pT stage and histopathological grade of malignancy as well as some clinical features.

MATERIALS AND METHODS

Patients

One hundred and fourteen patients with colorectal adenocarcinoma were operated at the 2nd Department of General and Gastroenterological Surgery of Medical University of Bialystok, Poland in 1997-2001. There were 68 (59.65%) men and 46 (40.35%) women. The median age was 65 years (range: 32-87 years). The diagnosis was made based on clinical symptoms and endoscopic examination as well as histopathological study of tissue samples. Other types of cancer and polyps were excluded from the investigation. Fifty-nine cases (51.75%) of tumours were localized in the rectum and sigmoid colon and the rest, fifty-five cases (48.25%) were localized in other parts of the large bowel. Twelve tumours were inoperable. All the patients were divided into 6 groups according to the grade of malignancy and pT stage (Table 1, Table 2). The patients had not any preoperative chemo- or radiotherapy. All the patients were monitored after operation. Thirty-one (27.19 %) of the controls died due to the recurrence of the disease (Table 3).

Tissue and serum samples

Tumour tissues were obtained during the operation. They were typically preparated and paraffin embedded sections were examined to estimate pT score and malignancy grade in G1-G3 score. The blood was collected from these patients before (sample A) and 10 days after the operation (sample B). The blood was centrifuged at 4℃ and the serum samples were

stored at -80℃ until examination. The level of v5 and v6 variants of CD44 protein in the serum samples was estimated by ELISA test using kits for SCD44var(v5) and SCD44var(v6) ELISA (Bender MedSystem) according to the manufacturer' s protocol. The values of levels obtained in our examination are presented in Table 4.

Statistical analysis

χ^2 Pearson, χ^2 NW, NIR and *U* Mann-Whithney tests were used to analyse the correlation between CD44v5 and CD44v6 expressions before (v5A, v6A) and after the operation (v5B, v6B), and such features as sex, age, location of the tumour in the large bowel (sigmo-rectal or the rest of the colon), the clinical stage according to pT stage and the grade of malignancy as well as the time of survival and the presence of recurrence. Student *t* test was used to analyse correlation between control and tested groups. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

The level of CD44 v5 splice variant in the serum obtained from patients with colorectal adenocarcinoma evaluated by ELISA test before operation was lower in G2 pT4 stage of tumour than those in others probes and the difference was statistically significant. But there were not any other statistically significant correlations with clinicopathological features like sex, age, location of the tumour, pT stage and the grade of malignancy estimated in 3-stage score from G1 to G3. There were not any other differences between levels of that variant before and after operation as well.

The level of CD44 v6 evaluated in the serum obtained from patients did not correlate with any clinical and histopathological features. Also, there was not any statistically significant difference between its levels before and after operation.

The level of both v5 and v6 CD44 variants in the serum did not correlate with the recurrences of cancer and mortality (Table 4).

Table 1 Sex of patients, grade of malignancy (G) and pT stage

	Inoperable tumours	G2 pT2	G2 pT3	G2 pT4	G3 pT3	G3 pT4	All
Women	4	4	23	5	10	0	46
	33.33%	44.44%	40.35%	41.67%	52.63%	0.00%	40.35%
Men	8	5	34	7	9	5	68
	66.67%	55.56%	59.65%	58.33%	47.37%	100.00%	59.65%
All	12	9	57	12	19	5	114
	10.53%	7.89%	50.00%	10.53%	16.67%	4.39%	100.00%

Table 2 Location of tumour, grade of malignancy (G) and pT stage

	Inoperable tumours	G2pT2	G2pT3	G2pT4	G3pT3	G3pT4	All
Sigmoid and	6	4	34	6	7	2	59
rectum	59.00%	44.44%	59.65%	50.00%	36.84%	40.00%	51.75%
Colon	6	5	23	6	12	3	55
	50.00%	55.56%	40.35%	50.00%	63.16%	60.00%	48.25%
All	12	9	57	12	19	5	114
	10.53%	7.89%	50.00%	10.53%	16.67%	4.39%	100.00%

Table 3 Mortality, grade of malignancy (G) and pT stage

	Inoperable tumours	G2pT2	G2pT3	G2pT4	G3pT3	G3pT4	All
Alive	1	8	48	11	12	3	83
	8.33%	88.89%	84.21%	91.67%	63.16%	60.00%	72.81%
Dead	11	1	9	1	7	2	31
	91.67%	11.11%	15.79%	8.38%	36.84%	40.00%	27.19%
All	12	9	57	12	19	5	114
	10.53%	7.89%	50.00%	10.53%	16.67%	4.39%	100.00%

Table 4 Mean values of CD44 v5 and CD44 v6 before and after operation (ng/ml)

		v5 A	v5 B	v6 A	v6 B
Age:	<65	28.38	26.50	185.42	171.52
	>65	25.18	27.61	178.78	183.18
Location:	the sigmoid and the rectum	24.92	30.08	173.26	178.15
	the colon	29.38	25.28	192.17	186.04
Inoperable tumours		29.88	25.92	178.42	189.17
G2 pT2		24.47	25.01	184.49	177.44
G2 pT3		26.98	27.87	181.96	181.78
G2 pT4		32.72	28.85	153.63	165.09
G3 pT3		23.21	23.76	181.74	183.00
G3 pT4		25.14	23.28	142.00	164.80

DISCUSSION

CD44 described at first by Dalchau in 1980 is a molecule which can take part in carcinogenesis and formation of metastases in lymph nodes and distal organs[15]. It is a cell surface transmembrane glycoprotein which occurs in both healthy and neoplasm cells. The gene which codes for CD44 consists of 20 exons, and 10 of them can be combined, so there are a lot of its variants. It can also be modified just after the process of translation. The most common molecule with a molecular weight 85-90 kd is called standard molecule and is the main surface receptor of hyaluronic acid. It also takes an active part in intracellular communication and interactions between the cell and the extracellular matrix. It is responsible for T lymphocyte and natural killer cell activation, aggregation and B and T cell migration. It also induces the tumour necrosis factor and interleukin 1 release. So it is described as a main factor in the formation of metastases, however the precise mechanism of its function is still unknown[16-19]. Examinations of standard CD44 molecule in tumour tissues and in neoplasm lymph nodes showed its higher expression[6,20-23]. The examinations of some isoforms of CD44 in cancer and lymph nodes tissues showed the same results, but the number of examined patients was small[24-26]. Especially v5 and v6 CD44 splice variant expression was higher in tumour tissues and depended on cancer progression[6]. Elevated levels of sCD44v6 in malignant ascites was also described[27]. Some publications about expression of v6 CD44 and v8-10CD44 isoform in the serum of patients with colorectal cancer did not show any correlation with pathological features and metastases[28-30]. The same results of examination concerned with CD44 v5 and v6 were presented in this study. The lack of correlation between expression of v5 and v6 variants of CD44 and clinicopathological features could depend on the absence of these molecules in the soluble form in the serum.

In conclusion, there is no association between CD44v5 and v6 expression estimated in the serum and any clinicopathological features in patients with colorectal adenocarcinomas. Due to the short time of observation it is not implicated as a prognostic factor in colorectal cancer and it demands further investigations.

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