Molecule CD44 variant 10 expression in lymphocytes infiltrating tumour tissues and epithelial cells in patients with colorectal cancer

Zalewski B[1], Stasiak-Barmuta A[2], Guzińska-Ustymowicz K[3], Cepowicz D[1], Gryko M[1]

[1]Department of General and Gastroenterological Surgery, [2]Laboratory of Flow Cytometry, Children Teaching Hospital,
[3]Department of General Patomorphology, Medical University of Bialystok, Poland

Abstract

The aim of this study was to evaluate the expression of CD44v10 in colorectal tumour cells and in lymphocytes infiltrating the tumour (CD45+). Samples of tumour tissue (TT), as well as of healthy tissue (HT) and of tumour adjacent tissue (TAT), were obtained from 20 patients. An evaluation of CD44v10 expression was performed in a flow cytometer. The mean value of the percentage of CD45+ with co-expression of CD44v10 was significantly higher in the lower stage of the tumour (pT). The mean value of the percentage of epithelial cells with CD44v10 co-expression was significantly higher in pN2 than in pN1 stage. Only in TAT the mean value of the percentage of epithelial cells and CD45+ with tCD44v10 co-expression was significantly lower in the higher degree of histological malignancy. It is supposed that CD44v10 takes part in local cancer progression.

Key words: colorectal cancer, CD44v10, leucocytes, tumour tissue.

Introduction

Neoplasmal diseases are the second, or, according to some authors, the third cause of deaths all over the world. Colorectal cancers are, among other neoplasms, placed on the third position, regarding morbidity and mortality rates after the breast and lung and bronchus cancers in women and prostate and lung and bronchus cancers in men [1]. The recurrence of the disease depends on the progression of neoplasmal metastases to lymph nodules and distal organs and is the most common cause of death [2, 3].

The aim of this study was to evaluate, whether the expression of CD44v10 isoform on epithelial cells and leucocytes infiltrating tumour tissues, tumour adjacent tissues and healthy tissues of the large bowel in patients with colorectal cancer, correlates with the pathomorphological stage of the tumour, due to WHO classification (pT), lymph nodes metastases (N) and the histopathological grade of malignancy (G).

Material and methods

Twenty (20) patients were operated on sigmorectal adenocarcinomas in G2-G3 grade of malignancy and pT2- pT4 stage, acc. to WHO score, at our Department in 2003. There were 11 (55 %) women and 9 (45 %) men. The median age was 63.27 years (the age range: 32 - 78 years). Neoadjuvant radio- or chemotherapy had not been applied to any of the patients. Nobody of them had metastases to distal organs. The preoperative diagnosis was based on clinical symptoms and preoperatively confirmed by histopathological examination of a biopsy specimen, obtained endoscopically. Other types of cancer and polyps, including inoperable tumours, were excluded from the investigation. Tumour tissue samples were obtained during the operation. They were divided into two parts. One part of tissues was typically prepared and paraffin embedded sections were examined to estimate pTN stage and the grade of malignancy in G1-G3 score. The second specimen consisted of three samples, obtained from HT, TAT and TT and placed in a sterile container with RPMI - 1640. Immediately after collection (max. 2h), respective fragments of tissues were minced to receive the homogenous cells suspension. The cell suspension, obtained in this way, was twice washed with PBS with 0.5% bovine albumin and 2mM of EDTA. Two parts of 100 µl of cell suspension were conjugated - the first part with 10 µl of CD45-PerCP and the second one with 10 µl

ADDRESS FOR CORRESPONDENCE:
Bogdan Zalewski
Department of General and Gastroenterological Surgery
M. C. Skłodowskiej 24 A; 15-276 Białystok, Poland
Tel. (+48 85) 746 82 87 fax. (+48 85) 746 86 24;
e-mail: bogdan-zalewski@wp.pl

Table 1. The mean value of the percentage of CD45+ and epithelial cells with co-expression of CD44v10 in different pT stages.

TT /pT	CD45 / CD44 v10	EMA /CD44v10
2	76.6 ± 12.1	49.6 ± 9.6
3	48.7 ± 11.8	31.6 ± 17.1
4	43.8 ± 7.8	53.5 ± 10.1
Statistical analysis	2 v. 3; p<0.01 2 v. 4; p<0.04	Not significant
TAT /pT		
2	85.2 ± 13.2	36.9 ± 16.9
3	49.3 ± 10.2	29.2 ± 13.6
4	61.0 ± 12.1	46.3 ± 7.6
Statistical analysis	2 v. 3; p<0.001 2 v. 4; p<0.05	Not significant
HT /pT		
2	74.9 ± 19.5	32.4 ± 18.8
3	50.8 ± 14.1	19.2 ± 14.2
4	51.4 ± 10.4	31.5 ± 11.3
Statistical analysis	2 v. 3; p<0.05 2 v. 4; p<0.05	Not significant

Table 2. The mean value of the percentage of CD45+ and epithelial cells with co-expression of CD44v10 in different pN stages.

TT /pN	CD45 / CD44 v10	EMA / CD44 v10
0	52.0 ± 11.2	37.9 ± 15.6
1	53.1 ± 35.4	31.4 ± 16.1
2	53.8 ± 14.1	43.8 ± 13.7
Statistical analysis	Not significant	Not significant
TAT /pN		
0	57.7 ± 16.6	33.6 ± 14.4
1	61.0 ± 31.0	22.0 ± 4.2
2	57.6 ± 4.9	46.8 ± 9.8
Statistical analysis	Not significant	1 v. 2; p<0.05
HT /pN		
0	55.4 ± 16.0	26.5 ± 12.3
1	47.6 ± 19.1	31.4 ± 16.1
2	58.3 ± 9.7	40.9 ± 13.5
Statistical analysis	Not significant	Not significant

of Anti-EpCAM-PerCP-Cy 5.5 antibodies (Bencton Dickinson). After 20 minutes of incubation in room temperature, 10µl of CD44v10 antibodies (AB2082 Chemicon) were added. After 30 minutes of incubation in 4°C in the dark, the cells were washed three times in PBS solution. Next, IgG (Fab2 - Mouse antiRabbit IgG AP 1889 - Chemicon), conjugated with EMA-FITC (DAKO), was added and the suspension was again incubated during 30 minutes in 4°C. The cells were washed again three times. PBS and 1% solution of paraformaldehyde was added. Analyses of the cells were performed, using a Coulter EPICS XL flow cytometer. A minimum of 10^4 cells were counted. Conforming isotypic negative controls were used. The non-parametric ranking Mann-Whithney test was used for statistical analysis of flow cytometric results between the evaluated groups. Values of $p<0.05$ were accepted as statistically significant.

Table 3. The mean value of the percentage of CD45+ and epithelial cells with co-expression of CD44v10 in different grades of malignancy.

TT /G	CD45 / CD44 v10	EMA /CD44 v10
0	52.3 ± 10.4	35.1 ± 11.9
2	52.9 ± 16.7	41.2 ± 17.3
3	55.5 ± 9.2	20.0 ± 8.5
Statistical analysis	Not significant	Not significant
TAT /G		
0	59.7 ± 15.0	37.9 ± 14.4
2	63.0 ± 15.2	38.3 ± 12.5
3	40.7 ± 7.6	20.0 ± 9.7
Statistical analysis	0 v. 3; p<0.05 2 v. 3; p<0.03	0 v. 3; p<0.05 2 v. 3; p<0.03
HT /G		
0	49.9 ± 18.2	38.9 ± 13.6
2	60.5 ± 13.8	29.2 ± 20.4
3	41,3 ± 12,4	18,7 ± 12,6
Statistical analysis	Not significant	Not significant

Results

The mean value of the percentage of lymphocytes with co-expression of CD44v10, infiltrating all kinds of tissues, was significantly higher in the lower stage of the tumour, acc. to WHO classification (pT), whereas there were no correlations in this parameter, evaluated on the epithelial cells (Table 1). The mean value of the percentage of epithelial cells with co-expression of CD44v10 was significantly higher in pN2 than in pN1 stage. There were no significant correlations in this parameter, estimated on the leucocytes infiltrating tissues (Table 2). The mean values of the percentage of epithelial cells, as well as of leucocytes infiltrating tissues with co-expression of CD44v10, were significantly lower in the higher degree of histological malignancy (G) only inTAT (Table 3).

Discussion

CD44, described for the first time by Dalchau in 1980, is a molecule, which can take part in carcinogenesis and formation of metastases in lymph nodes and in distal organs [4]. It also plays an important role in intracellular communication and interactions between the cell and the extracellular matrix. It is responsible for lymphocyte T and natural killer activation, aggregation and cell B and T migration. It also induces the Tumour Necrosis Factor and interleukin 1 release. So, it is described as one of the main factors in the formation of metastases, however, the precise mechanism of its function is still unknown [5, 6]. Examinations of standard CD44 molecule in tumour tissues and in neoplasmatically changed lymph nodes show its higher expression [7, 8, 9, 10]. Examinations of some isoforms of CD44 in cancer and lymph node tissues show the same results but the number of examined patients is small [11, 12, 13]. We have recently reported that the levels of CD44v5 and v6 in serum of patients with colorectal cancer are not correlated with the progression of the disease [14].

This study evaluated the expression of CD44v10 isoform on the surface of epithelial cells and leucocytes infiltrating

tumour tissues, tumour adjacent tissues and healthy tissues of the large bowel in patients with colorectal cancer. The statistically significant correlations, found in results, inform that this variant of CD44 can play an important role in the local progression of the cancer and that it probably stimulates the growth of the tumour. However, it is rather of no importance in the development of neoplasmal metastases to lymph nodes. Due to a small number of examined patients, the role of CD44v10 demands further investigations.

References

1. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thurn MJ. Cancer Statistics, 2004. CA Cancer J Clin, 2004; 54: 8-29.

2. Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A, Muschel RJ. Intravascular origin of metastasis from the proliferation of endothelium - attached tumor cells: a new model for metastasis. Nature Med, 2000; 6: 100-2.

3. Wielenga VJM, van der Voort R, Taher TEI, Smit L, Beuling E, van Krimpen C, Spaargaren M, Pals ST. Expression of c-Met and Heparan-Sulfate proteoglycan forms of CD44 in colorectal cancer. Am J Path, 2000; 157: 1563-73.

4. Dalchau R, Kirkley J, Fahre JW. Monoclonal antibody to a human leukocyte-specific membrane glycoprotein probably homologous to the leukocyte-common (L-C) antigen of the rat. Eur J Immunol, 1980; 10: 737-44.

5. Isacke C, Yarwood H. The hyaluronan receptor, CD44. Int J Biochem Cell Biol, 2002; 34: 718-21.

6. Lindblom A, Liljegren A. Tumour markers in malignancies. BMJ, serial online, Feb 2000; 320: 424-7.

7. Liu PF, Wu MC, Cheng H, Qian GX, Fu JL. Clinical significance of expression of metastasisa2associated splice variants of CD44 mRNA in early primary liver cancer. China Natl J New Gastroenterol, 1996; 2: 112-4.

8. Fujisaki T, Tanaka Y, Fujii K, Mine S, Saito K, Yamada S, Yamashita U, Irimura T, Eto S. CD44 stimulation induces integrin-mediated adhesion of colon cancer cell lines to endothelial cells by up-regulation of integrins and c-Met and activation of integrins. Cancer Res, 1999; 59: 4427-34.

9. Llaneza A, Gonzales A, Andicoechea A, Fernandez JC, Allende MT, Garcia-Muniz JL, Vizoso F. CD44s, CD44v5 and CD44v6 protein contents in colorectal cancer and surrounding mucosa. Int J Biol Marker, 2000; 15: 192-4.

10. Zalewski B, Famulski W, Sulkowska M, Sobaniec-Lotowska M, Piotrowski Z, Kisielewski W, Sulkowski S. CD44 expression in colorectal cancer. An immunohistochemical study including correlation with cathepsin D immunoreactivity and some tumour clinicopathological features. Folia Histochem Cytobiol, 2001; 39: 152-3.

11. Harada N, Mizoi T, Kinouchi M, Hoshi K, Ishii S, Sasaki I, Matsuno S. Introduction of antisense CD44s CDNA down-regulates expression of overall CD44 isoforms and inhibits tumor growth and metastasis in highly metastatic colon carcinoma cells. Int J Cancer, 2001; 91: 67-75.

12. Wong LS, Cantrill JE, Morris AG, Fraser IA. Expression of CD44 splice variants in colorectal cancer. Br J Surg, 1997; 84: 363-7.

13. Chun SJ, Bae OS, Kim JB. The significance of CD44 variants expression in colorectal cancer and its regional lymph nodes. J Korean Med Sci, 2000; 15: 696-700.

14. Zalewski B. 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. World J Gastroenterol, 2004; 10: 583-5.