Glycoprotein CD44 variant 4 expression in tumour epithelial cells of patients with colorectal cancer

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Abstract

The aim of this study was to check if the expression of CD44v4 in epithelial cells of the colorectal cancer correlates with the pTN stage and the histopathological grade of malignancy - G. Samples of tumour tissue (TT), as well as those of healthy tissue (HT) and of tumour adjacent tissue (TAT) were obtained from 25 patients. An evaluation of the expression of CD44v4 was performed in a flow cytometer. The mean value of the percentage of epithelial cells with co-expression of CD44v4 was lower in pT2 stage than that in pT3 only in HT. The expression of CD44v4 in epithelial cells was higher in cases without lymph node metastases only in TAT. The expression of CD44v4 in epithelial cells was higher in G2 than in G3 degree only in TAT as well.. According to the obtained results, it is difficult to state if CD44v4 can influence the progress of colorectal cancer.

Key words: colorectal cancer, carcinogenesis, adhesion molecules, CD44.

Introduction

CD44 is a proteoglycan molecule, distributed on the surface of many types of epithelial cells [1]. It serves as a molecule, binding mononuclear and epithelial cells with extracellular ligands, as it acts as a receptor for hyaluronan, fibronectin and a lot of other substances [2]. Its role in inflammatory process and as adhesion molecule in intercellular communication during carcinogenesis and metastatic processes was described by many authors. However, its role in these processes is still unclear [2, 3, 4].

The aim of this study was to examine, whether the expression of CD44v4 isoform in epithelial cells of tumour, tumour adjacent tissues and healthy tissues of the large bowel in patients with colorectal cancer correlates with the pathomorphological stage of the tumour, acc. to WHO classification (pT), lymph nodes metastases (N) and the histopathological grade of malignancy (G).

Materials and methods

Twenty-five (25) patients were operated on sigmorectal adenocarcinomas in G2-G3 grade of malignancy and pT2- pT3 stage, acc. to WHO score, at our Department in 2002. There were 12 (48%) women and 13 (52%) men. The median age was 62.3 years (the age range: 46 - 84 years). Neoadjuvant radio- or chemotherapy had not been applied to any of the patients. The preoperative diagnosis was based on clinical symptoms and confirmed preoperatively by histopathological examination of biopsy specimens, obtained endoscopically. There were 13 (52%) tumours of the sigmoid colon and 12 (48%) rectal tumours. Other types of cancer and polyps, including inoperable tumours, were excluded from the investigation. There were 12 patients with tumours in G2 grade of malignancy and 13 with G3. Tumour tissue samples were obtained during operations. 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. There were 12 tumours with G2 and 13 with G3 grade of malignancy. pT2 stage was found in 8 tumours, whereas pT3 in 17. Metastases to lymph nodes were found in 11 patients. The second part of the specimens, consisting of three samples, obtained from healthy tissue (HT), tumour adjacent

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Table 1. The mean value of the percentage of epithelial cells with co-expression of CD44v4 in different pT stages.

	CD44v4 (EMA) – medium values ± SD
pT2 / HT	5,2 ± 3,0
pT2 / TAT	12,2 ± 10,9
pT2 / TT	16,8 ± 15,4
pT3 / HT	22,2 ± 11,1
pT3 / TAT	13,7 ± 12,1
pT3 / TT	21,6 ± 13,3
Statistical analysis	pT2/HT v. pT3/HT p<0,009

Table 2. The mean value of the percentage of epithelial cells with co-expression of CD44v4 in different pN stages.

	CD44v4 (EMA) – medium values ± SD
pN0 / HT	20,1 ± 15,1
pN0 / TAT	24,0 ± 12,7
pN0 / TT	18,5 ± 12,2
pN1 / HT	20,0 ± 6,0
pN1 / TAT	8,5 ± 9,1
pN1 / TT	24,7 ± 15,4
Statistical analysis	pN0 / TAT v. pN1 / TAT p<0,006

Table 3. The mean value of the percentage of epithelial cells with co expression of CD44v4 in different grades of malignancy.

	CD44v4 (EMA) – medium values ± SD
G2 / HT	23,9 ± 14,6
G2 / TAT	23,3 ± 9,0
G2 / TT	19,4 ± 10,8
G3 / HT	16,3 ± 8,9
G3 /TAT	14,1 ± 2,5
G3 / TT	25,3 ± 18,8
Statistical analysis	G2 / TAT v. G3 / TAT p<0,05

tissue (TAT) and tumour tissue (TT) was placed in a sterile container with RPMI -1640. Immediately after collection (max. 2h), respective fragments of tissues were minced to receive a homogenous cells suspension. To each 100 µl of the cell suspension, 10 µl of monoclonal antibodies EMA-FITC (Epithelial Membrane Antigen - DAKO) and CD44v4-PE (Immunotech) were added. After 20 minutes of incubation at room temperature in the dark, an automatic lysis was performed (EPICS IMMUNOLOGY WORK STATION - Coulter). Analyses of 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 Mann-Whitney tests were used for a statistical comparison of flow cytometric results between the evaluated groups. The values of $p<0.05$ were accepted as statistically significant.

Results

The mean value of the percentage of epithelial cells with co-expression of CD44v4 was significantly lower in pT2 stage than in pT3 only in healthy tissues (Table 1). The expression of CD44v4 in epithelial cells was significantly higher in cases without lymph node metastases only in tumour adjacent tissues (Table 2). The expression of CD44v4 in epithelial cells was significantly higher in G2 than in G3 degree of malignancy only in tumour adjacent tissues too (Table 3).

Discussion

Colorectal cancer occupies the third position, regarding morbidity and mortality, just after the breast and lung and bronchus cancer in women and prostate and lung and bronchus cancer in men. The mortality is still high and depends on the progression of the disease and neoplasmatic metastases to lymph nodes and distal organs [5]. The relationships between the antigens of tumour cells and the possibility of the immunological system to recognize them play an important role in the progression of the disease. Isoforms of CD44 molecule reveal the co-expression in tumour cells, as well as in the leukocytes infiltrating the tumour [6].

CD44 molecule was described as a one of the leukocyte antigens in 1980 [7]. Different isoforms of CD44 and their role in neoplastic hyperplasia (breast, gastric, liver and others) were described in later years. [8, 9, 10]. However, there are only a few articles about role of CD44v4 in colorectal cancer [11]. Some authors have recently reported on a possible role of CD44 and its variants in the therapy [12, 13]. We have proved that the levels of CD44v5 and v6 variants in serum do not correlate either with pT stage or with the histopathological grade of malignancy [14].

This study evaluated the expression of CD44v4 isoform on the surface of epithelial cells in tumour tissues, tumour adjacent tissues and healthy tissues of the large bowel in patients with colorectal cancer. The statistically significant correlations, as demonstrated in the examinations, do not reveal anything about the influence of this variant on the process of carcinogenesis and the

progression of the disease. There have not been many studies about the expression of CD44v4 in colorectal cancer. Therefore, it is difficult to recognize if it either stimulates, or rather inhibits, the progression of the disease, while it is likely not to play any role in these processes.

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