Assessment expression of the adhesion molecules, CD134 and CD137, in patients with colorectal cancer by flow cytometry

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Abstract

The aim of the study was to assess the expression of adhesion molecules, CD134 and CD137, in the peripheral blood in correlation with clinical advancement, the histological grade, the size and type of tumour growth, tissue infiltration, and lymph node and metastases to lymph nodes and liver. The study involved 28 patients with primary colorectal cancer. The expression of both molecules was investigated on the day of the surgery, before the procedure and ten days after the operation by means of flow cytometry. The expression of CD134 was markedly higher, compared to CD137, both on the day of the surgery and ten days after the operation. A significant increase was observed in CD134 expression ten days after the surgery. CD137 expression increased with the higher stage of clinical advancement, but decreased with the enhancement of colon wall infiltration. CD134 showed a similar expression for all the stages of tumour clinical advancement.

Key words: colorectal cancer, adhesion molecules, CD134, CD137, metastases.

Introduction

A threat of metastases in colorectal cancer can be expressed as the percentage of risk in relation to the histological grade of the tumour, the extent of involvement at the primary site and lymph node metastases [1]. Immune defence mechanisms start to intervene at the moment of cancer cell formation, that is, at a later phase, when natural anticancerogenic mechanisms have failed. The aim of the study on neoplastic antigens is to evaluate their usefulness in detection, monitoring and treatment, e.g., with monoclonal antibodies, directed against these antigens or using a variety of vaccines that particularly stimulate cell-type response [2]. Studies on the locoregional expression and correlations in colorectal cancer metastasis formation have recently been directed towards immune mechanisms and forms of immunotherapy. Adhesion molecules (CD-clusters of differentiation) constitute a wide group of molecules that vary in structure and function and which are involved in immune responses in a number of pathologies The most commonly investigated ones include: CD49a, CD11a, CD54, CD44, CD102, CD62L [3]. Lately, the role of CD134 (OX-40) and CD137 (4-1BB)) in the course and therapy of colorectal cancer has been emphasized [2, 5, 6, 7, 8, 9]. CD134 (the OX-40 receptor) and OX-40L (ligand) belong to the TNFR superfamily and act as a pair of costimulatory molecules that facilitate the activation of Th2 lymphocytes and IL-4 (ligand) secretion by these cells. The activation of OX-40 leads to an enhanced secretion of antibodies by B cells. OX-40 is expressed on activated T and B cells, dendritic cells and endothelium [9]. CD137 (4-1BB) molecules are the receptors on the surface of T cells and NK (natural killer) cells, and their ligand 4-1BBL is produced by B cells, macrophages and dendritic cells. Due to costimulation of 4-1BB and 4-1BBL, T cells are induced to produce increased amounts of IL-2. The aim of the present study is to assess the expression of adhesion CD134 and CD137 molecules in the peripheral blood in correlation with clinical advancement, histological grade, the size and type of tumour growth, tissue infiltration and lymph node and liver involvement.

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Material and method

The study involved 28 patients with primary colorectal adenocarcinoma, aged 52-83 years, operated on at the 2nd Depart-

ment of General and Gastroenterological Surgery, Medical University of Białystok. Clinical advancement was evaluated, according to Dukes' scale: Dukes A- 2 (7.14%), Dukes B-13 (46.42%), Dukes C- 8 (28.57%) and Dukes D- 5 (17.86%) patients. The study material included fresh peripheral venous blood, collected on the day of the surgery before the procedure and 10 days after the operation. Lymphocytic cells were obtained by mechanical centrifugation. After a two-fold rinsing they were counted in a Burker's chamber. A 10^6 mononuclear cells/ ml RPU/1640 suspension was made and portioned out to obtain 100ul samples, each supplemented with 10 ul of monoclonal CD 134 and CD 137 antibodies, obtained from manufactured sets: CD134 (OX 40)-FITC-34464 X and CD137 (4-1BB)-PE-34465 X, Pharmingen. Then, the samples were quantitatively analysed in a flow cytometer EPICS XL (Coulter), 10^4 cells each time. The results were statistically analysed, using Fisher's test.

Results

The expression of CD134 was markedly higher, compared to CD137, both on the day of the surgery and ten days after the operation. The mean expression of CD134 in both examinations was 13.57% (SD - 9.95), being significantly higher than the mean expression of CD137-6.38% (SD - 10.86) ($p<0,001$). A significant increase was observed in the expression of CD134 ten days after the surgery. Although the mean activities were comparable, significant differences were noted between the medians: on the examination before surgery-10.45 and on the 10th day after surgery-14.35. Figure 1. CD137 expression before the operation (the mean value-5.14%) did not change significantly after the surgical procedure (the mean value-7.63%). CD134 showed a similar level, irrespectively of the tumour stage. The respective mean expression values were following: Dukes A-13.38%, Dukes B-13.41%, Dukes C-11.68%, Dukes D-17.38%, not differing much between the assays before and after the operation. Details, presented in Figure 2, showed that CD137 expression differed significantly between the respective stages of clinical advancement. The highest expression was noted in most advanced lesions ($p<0,001$). In Dukes D patients, high expression of CD137 was observed in both examinations. The activity of CD137 was significantly increased on day 10 after the surgery. In the remaining stages (Dukes A-C), the examinations revealed a similar, much lower activity. Depth of tumour infiltration did not cause any distinct differences in the expression of CD134 in pT1, pT3 and pT4 tumours. Reduced expression was observed in pT2. Tumour resection had no effect on the expression in the respective groups. A similar comparison of CD137 expression revealed its decrease, accompanied by enhanced colon wall infiltration. The mean CD137 expression was 7.38% for pT1, 8.78% for pT2, 6.59% for pT3, and 2.37% for pT4 ($p<0.01$). No differences were found between the examination before and after the operation. Figure 3.

Discussion

Studies on the locoregional tumour expression and correlations in metastasis formation in colorectal cancer have recently been directed towards immune mechanisms and forms of imunotherapy. In patients with cancer, immune reactions, especially of cell type, are attenuated. Moreover, certain immune processes, observed in the patients, may even promote tumour growth [1]. Our studies showed increased CD137 expression with a higher stage of tumour clinical advancement and decreased CD137 expression with enhanced infiltration of the colon wall. We also proved that tumour clinical stage advancement does not affect CD134 expression and its expression increases in the early postoperative period. No results have been found in the available world literature that could be compared with the present findings. Studies, conducted on animals in the USA on the OX-40 molecule and OX-40L ligand, indicate that the molecule is activated by CD4 on T cell surface. Observations on human and animal models revealed an improved therapy outcome in cases of colorectal cancer. In patients with high OX-40 expression, 20% lymphocytes were infiltrated, in approximately 40% of infiltrated cells along tumour margins, in 50% of tumour cells and in all the samples of mesenteric lymph nodes. The levels of OX-40 were not high in the healthy tissue margin. High expression of OX-40 in the primary tumour correlated with 2-55% longer survival time. It thus seems that CD134 molecules can be employed in colorectal cancer immunotherapy [5, 6]. Until now, studies on CD137 (4-1 BB) in colorectal cancer have been performed on animal models. The authors, who investigated mouse liver with metastatic tumours, due to colorectal cancer, reported an optimum outcome of the combined application of IL-12 and CD137 (4-1BB) [7, 8]. The results have indicated that

Figure 1. CD 134 expression before surgery and ten days after surgery.

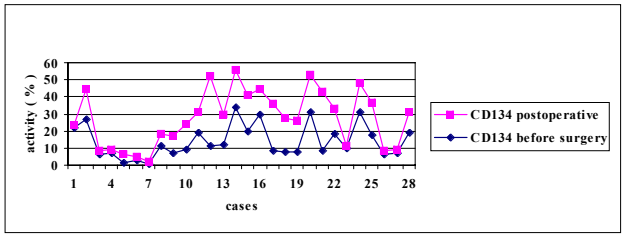


Figure 2. Mean values of CD 137 expression in relation to tumour clinical advancement.

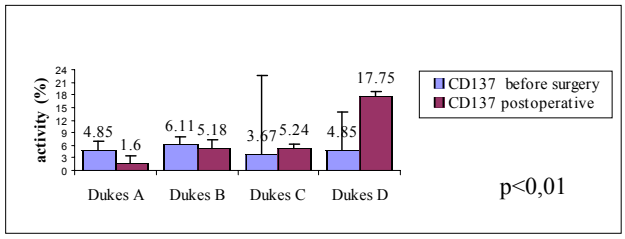
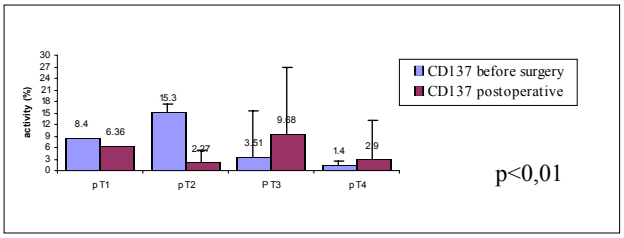


Figure 3. Mean values of CD 137 expression in relation to tumour infiltration depth.



a synergistic action between congenital and acquired immunity can contribute to the treatment of metastatic colorectal cancer, mainly to the liver. However, no comparative studies of this adhesion molecule, conducted either on animal models or in humans, have been available so far [7, 8]. Very few trials have been described of its application in the immunotherapy of chosen neoplasms, including colorectal adenocarcinoma [2]. The two described above adhesion molecules (CD134 and CD137) may create a chance for a positive prognosis of colorectal cancer course and treatment. CD134 (OX-40) can affect the immunotherapy of the tumour itself and prevention of metastasis formation. CD137 seems to have a beneficial effect on the immunotherapy of metastases (mainly to the liver), the fact that inspired the present study. The determination of the level of chosen molecules in the tumour, border tissues, lesion-free margins of the colon and peripheral blood can help analyse the therapy outcome, as well as the risk of metastases and prognosis. It is necessary to perform further investigations of the CD134 and CD137 during 3-6 month postoperative control periods.

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