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Assessment of Prognostic Significance of E-Cadherin and ERCC1 Expression in Colorectal Cancer

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ABSTRACT

The aim of the study is to study the relationship between the expression of the adhesion protein and the marker of epithelial cells of E-cadherin and the repair enzyme ERCC1 associated with drug resistance and the evaluation of their prognostic value in patients with colorectal cancer (CRC). A tumor material from 50 patients with stage III-IV CRC was studied, the phenotype of tumor cells was characterized by the presence of E-cadherin and ERCC1 expression in the histological sections of the primary tumors. Based on the results of the correlation analysis of the antigens expression with survival, it was established that the presence of E-cadherin in the tumor material evidences on positive prognosis for the survival of patients, whereas the presence of ERCC1 expression significantly worsens the prognosis of the CRC course.

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INTRODUCTION

The search for informative markers of the sensitivity of intestinal cancer cells to cytotoxic drugs is an actual trend since the selection of an effective chemotherapy regimen for the treatment of CRC patients remains a serious problem and often the use of modern therapeutic regimens for patients with this pathology is not accompanied by a pronounced effect. Currently, in the medicinal treatment of CRC traditional anticancer therapeutic schemes are used. FOLFOX and FOLFIRI, which include platinum preparations [1, 2, 3], irinotecan and fluorouracil, with a positive response to treatment with these drugs not exceeding 50%, and sometimes completely absent [1]. This determines the relevance of the search for new predictive markers of such resistance of tumor cells to cytostatics since in the presence of the latter high-dose chemotherapy with the presence of serious side effects becomes inexpedient. This calls for a search for new ways to improve the effectiveness of chemotherapy in CRC patients, one of which is a more detailed study of the phenotype of tumor cells, which forms both their behavioral and metastatic characteristics.

A promising area in the study of CRC is the detection of the presence and activity of enzymes associated with the tumor drug resistance and involved in DNA repair in tumor cells after damage by anticancer agents, namely: ERCC1, ERCC2, XRCC1 and their analogues [4, 5, 6]. Today, the literature describes the relationship between a low level of their expression and a tumor response to the cytostatic: carboplatin or oxaliplatin in malignant tumors of the ovary, esophagus, stomach and colon [7,8]. It has been established that the mutation of the XRCC1 gene, which inactivates its functional activity, is accompanied by a decrease in the intensity of repair processes in DNA and, as a consequence, the realization of the antitumor effect of oxaliplatin and other cytotoxic drugs [9,10]. In addition to evaluating the proteins directly associated with drug sensitivity, we consider it promising to introduce into this complex a prognostic evaluation of antigens that are closely related to the malignancy of the transformed cells, namely, proteins associated with the epithelial-mesenchymal transition (EMT). With a tumor progression, the presence of EMT the cells of benign tumors acquire the ability to invade and migrate, which in turn leads to activation of tumor metastasis and development of the malignant process [11,12,13,14]. Therefore, EMT becomes a target for the development of antitumor drugs [15, 16]. In addition, there is evidence that tumor cells that are characterized by the properties of stem cells and possess resistant phenotype, also undergo the EMT process, the main stages of which are the change in the dominance of certain markers: a decrease in the number of E-cadherin-positive cells along with an increase in the amount of Vimentin and N-cadherin-positive cells, a change in the

activity of transcription factors Slug, Twist, etc. [17, 18].

In our opinion, the evaluation of the expression of the above markers in tumor cells will allow for prediction of the effectiveness of certain chemotherapeutical regimens in the appropriate category of patients.

MATERIALS AND METHODS

The study included (according to patient consent) the clinical data of 50 patients with stage III-IV CRC (T3N0M0, T4N0M0), of them patients with rectal cancer (upper and middle-ampullar department) - 39 patients, colorectal cancer — 11 patients. The average age of patients was 57.2 years with individual fluctuations from 37 to 81 years. Histologically, tumors were represented by adenocarcinoma (10% - low-grade tumors, 63% - moderately differentiated, 27% - highly differentiated). The observation time in the retrospective group was 4 years, all patients of the group were treated according to scheme: in the neoadjuvant mode - the course of telegamma therapy, in adjuvant mode - from 4 to 6 courses of polychemotherapy (PCT) according to the FOLFOX scheme. The tumor cell phenotype was characterized by the presence of E-cadherin and ERCC1 in the histological sections of the primary tumors assessed by immunohistochemical analysis with the use of monoclonal antibodies (anti-E-cadherin (ThermoScientific), ERCC1 (Abcam)) using the Poly Vue (ThermoScientific) imaging system. Evaluation of the results was carried out by a semiquantitative method according to the percentage of positively stained tumor cells throughout the histological section. Cells were considered positive for marker expression in the case of cell membrane staining (in the study of E-cadherin), cytoplasm and nucleus (ERCC1), including that found in isolated clusters or cell complexes. The analysis of the results was carried out on the basis of the calculation of positive (+) cells using an AxioStarPlus microscope (Carl Zeiss, Germany) with magnification x400-1000, and evaluated using the classical H-Score method: S = 1xA + 2xB + 3xC, where S is the "H-Score", the value of which is in the range from 0 (protein is not expressed) to 300 (strong expression in 100% of cells); A - % of weakly "stained" cells, B - % of moderately "stained" cells, C - % of intensely "stained" cells.

The following statistical methods were used: standard descriptive, parametric and nonparametric methods (Student's t-test), regression analysis and correlation analysis (by Pearson).

RESULTS

The data on phenotype of the tumor cells were analyzed in comparison with the data on the course of the tumor process in patients with CRC (Figure 1), namely the progression of the

disease or the presence of a disease-free period during the observation of the patient. In addition, the phenotypic characteristics were compared with the overall survival of patients with CRC (Table 1) according to regression analysis data.

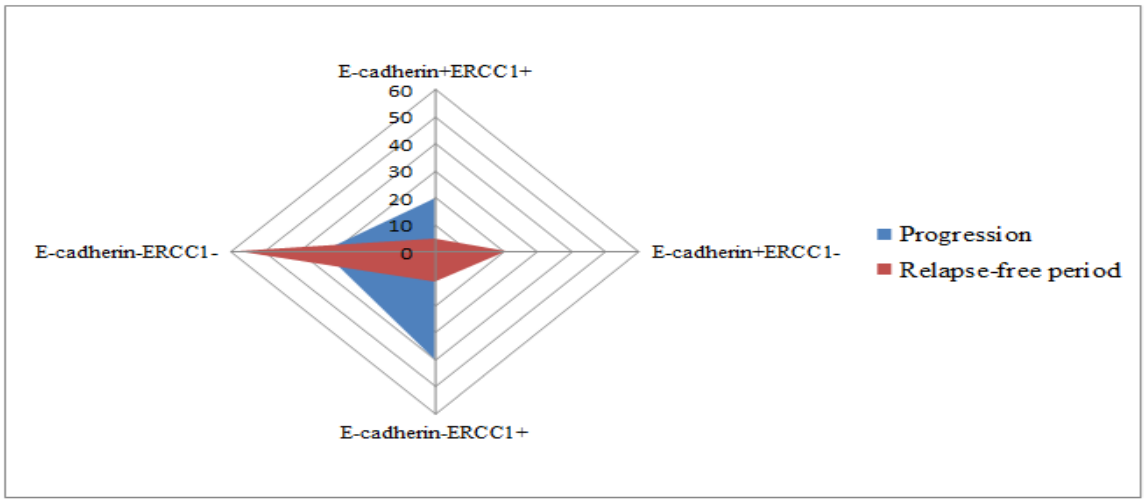
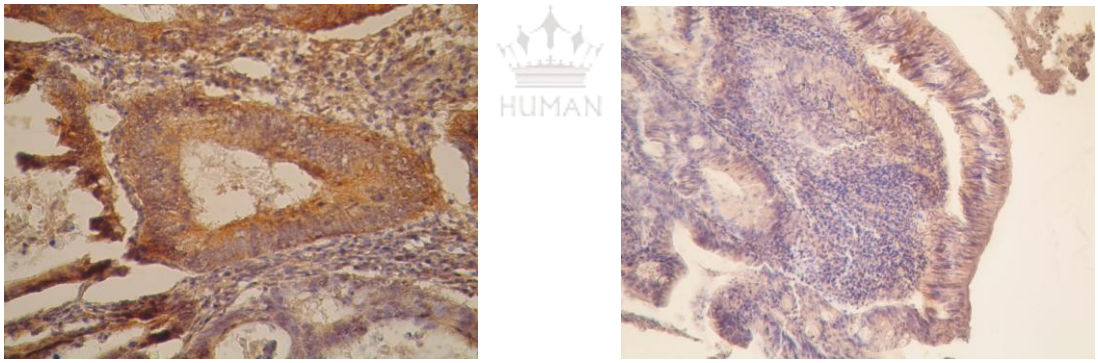
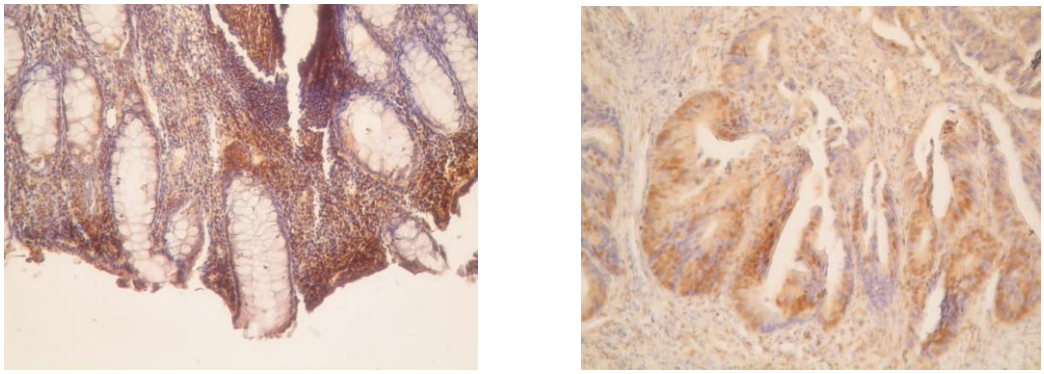


Figure 1. Distribution of E-cadherin / ERCC1 phenotype of tumor cells in CRC patients dependent on the CRC course.



a. b.



c. d.

Figure 2. Cells from the tumor material with different levels of marker expression: a, b - E-cadherin; c, d - ERCC1.

Table 1. Overall survival of CRC patients with a certain phenotype of the tumor material (regression analysis).

Coefficient	Phenotype of tumor material			
	E-cadherin+ ERCC1+	E-cadherin+ ERCC1-	E-cadherin- ERCC1+	E-cadherin- ERCC1-
β	0.09	0.25	-0.5*	0.8*

*p<0.05

According to the results of the data on relation between phenotypic characteristics of tumor material from the patients and their overall survival and CRC course, it has been established that the E-cadherin-ERCC1 antigenic complex is significant informative index for prediction of relapse-free survival and overall survival of patients with CRC: in the presence of E-cadherin-ERCC1 + tumor cell phenotype the prognosis of the disease worsens, a significant decrease in disease-free cases and overall survival rate was observed, while more favorable prognosis of survival of the CRC patients and increased relapse-free period of the disease was observed for E-cadherin + ERCC1- antigenic characteristics of tumor material (Fig. 1).

After receiving the data on the informativity of the phenotypic complex we proposed, the contribution of each individual marker to the prognosis of the CRC course was assessed, namely, the relationship between positive expression of the investigated antigens (E-cadherin, ERCC1 (Fig. 2)) and survival of CRC patients (by correlation coefficient): - 0.537* for E-cadherin and 0,359* for ERCC1 (*p<0.05). According to the results of the correlation analysis of the antigens expression with survival, it was found that the presence of E-cadherin-positive cells in the tumor material is associated with an improvement in the prognosis of the tumor process. It should be remembered that E-cadherin is a marker of epithelial cells and its presence in malignantly transformed cells most often indicates a decrease in their metastatic potential.

DISCUSSION

Summarizing the data of the retrospective analysis, it can be concluded that in the treatment of CRC of the ERCC1 + phenotype, the effectiveness of oxaliplatin in the therapeutic regimens is significantly reduced and the use of such platinum-containing regimens is highly questionable. Such data have been shown before, but more often in correlation with the transcription factor

Twist and with the study of topoisomerase II alpha, which is also associated with the drug sensitivity of cells to certain antitumor drugs and their proliferative potential. In this case, the use of additional approaches to the characterization of the phenotypic profile of tumor cells, in particular markers associated with the EMT process, as a result of clarifying the nature of the spread of the tumor process and the aggressiveness of tumor cells, their behavioral features and their potential for metastasis directly affects both the prognostic evaluation of the course of the tumor process in general, and the results of PCT in particular. According to the researchers, colorectal cancer can become one of the most common types of malignant tumors, in which the evaluation of EMT markers will reveal additional clinically relevant data[19]. There is already the data on the association between CD44, EMT activation and invasive properties of CRC [20]. It is also shown that Vimentin is much more expressed in the stromal component of CRC compared to normal intestinal tissue, but not directly in tumor cells. At the same time, a large number of Vimentin-positive cells in the CRC stroma correlates with poor prognosis of the course of the disease [21].

In this study, it was shown that there is no effect of chemotherapy on the CRC cells under conditions when E-cadherin is not identified. In the case of E-cadherin-negative phenotype, the number of tumor cells expressing ERCC1 was high, and we conclude about the potential predictive role of this complex of markers. Such association of E-cadherin, ERCC1, tumor progression and the effectiveness of specific treatment is considered by some authors from the position of regulation of the transcription factor Snail: with a large number of Snail- and ERCC1-positive cells, a decrease in E-cadherin-positive cells is observed [22]. A decrease in E-cadherin expression is also observed in cases of progression of the tumor process in lung cancer and ovarian cancer, which is also accompanied by low sensitivity to platinum preparations [23, 24], from which it can be assumed that the use of anticancer drugs of platinum-containing groups is ineffective against tumor cells, in particular, CRC without expression of E-cadherin. The results of this study confirm the significant role of EMT during the CRC development and importance of including additional antigens in the study of the molecular profile of this cancer type.

CONCLUSION

Based on the results of the correlation analysis of the studied antigens with survival, it was established that the presence of E-cadherin expression in the tumor material evidences on a positive prognosis of the patient's survival, whereas the presence of ERCC1 expression significantly worsens the prognosis of the CRC course. It is shown that combined assessment of

markers associated with EMT of tumor cells and ERCC1 is an informative and effective complex in predicting the CRC course.

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