Original Article

Tissue expression of CD10 protein in colorectal carcinoma: correlation with the anatomopathological features of the tumor and with lymph node and liver metastases

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ABSTRACT: **Background**: The reduced expression of CD10 may be related to unfavorable prognosis of patients with colorectal carcinoma. The authors analyzed the tissue immunostaining of CD10 protein in colorectal carcinoma and its relationship to clinicopathologic features. **Method**: In 130 patients submitted to colorectal carcinoma surgery, a ***tissue microarray*** block was obtained from the tumor and adjacent non-neoplastic mucosa and submitted to immunohistochemistry with monoclonal antibody CD10. The immunostaining was evaluated by semi-quantitative method, with stained cell count in percentage. The results were related to the location, anatomopathological features, presence of lymph node and hepatic metastases and TNM staging of the colorectal neoplasm. The statistical analysis was performed with the Mann-Whitney, Kruskal-Wallis and Fisher exact tests. **Results**: The expression of CD10 marker was higher in colorectal tumor tissue than in adjacent non-neoplastic mucosa ($p<0.0001$) and was higher than in exophytic lesions ($p=0.04$). The expression of CD10 protein was not associated with other clinical and pathological aspects of colorectal neoplasm. **Conclusions**: The expression of CD10 protein was more intense in tumor tissue of colorectal carcinoma than in adjacent non-neoplastic mucosa and was related to the exophytic appearance of the tumor.

Keywords: neoplasm antigens; colorectal neoplasms; prognosis; neoplasm metastasis; CD10 antigens.

RESUMO: **Introdução**: A expressão reduzida de CD10 pode estar relacionada com prognóstico desfavorável de doentes com carcinoma colorretal. Analisou-se a imunoexpressão tecidual da proteína CD10 no carcinoma colorretal e sua relação com os aspectos clinicopatológicos. **Método**: Em 130 doentes operados por carcinoma colorretal, um bloco de *tissue microarray* foi obtido do tecido neoplásico e da mucosa não neoplásica adjacente e submetido ao estudo imuno-histoquímico com anticorpo monoclonal CD10. Avaliou-se a imunoexpressão por método semiquantitativo, com contagem do percentual de células coradas. Os resultados foram relacionados com a localização, aspectos anatomopatológicos, presença de metástases linfonodais e hepáticas e estadiamento TNM da neoplasia. O estudo estatístico foi realizado com os testes de Mann-Whitney, Kruskal-Wallis e exato de Fisher. **Resultados**: A expressão do marcador CD10 foi maior no tecido do carcinoma colorretal do que na mucosa não neoplásica adjacente ($p<0,0001$) e foi maior nas lesões exofíticas ($p=0,04$). A expressão da proteína CD10 não apresentou relação com os demais aspetos clínicos e patológicos da neoplasia colorretal. **Conclusões**: A expressão da proteína CD10 foi mais intensa no tecido neoplásico do carcinoma colorretal do que na mucosa não neoplásica adjacente e relacionou-se com o aspecto exofítico do tumor.

Palavras-chave: antígenos de neoplasia; neoplasias colorretais; prognóstico; metástase neoplásica; antígenos CD10.

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INTRODUCTION

The CD10 tissue marker was originally described in neoplastic cells of patients with leukemia and was used as a marker in acute lymphblastic leukemia. Later, it was identified in liver cell, breast and neoplasm cell of the large bowel[1,2]. It is a 100 kDa glycoprotein, with strong expression in the brush border of the intestinal epithelium, germinal centers of lymph node follicles and liver microvilli. Studies[1-3] have associated this marker with the appearance of liver metastasis, venous invasion and progression of these tumors to more advanced stages in patients with colorectal neoplasm.

Considering the high incidence of colorectal neoplasm worldwide and the difficult prognosis of patients with this disease, specially in stages II and III of the TNM classification, we studied the expression of CD10 tumor marker and its relations with the anatomopathological aspects of the neoplasm, the presence of lymph node and liver metastases and the survival of patients submitted to colorectal carcinoma surgery.

METHOD

This study was approved by the Research Ethics Committee of the Universidade Federal de São Paulo (UNIFESP) and registered under 1958/07.

The inclusion criterion of this study was: adult patient with colorectal carcinoma confirmed through an anatomopathological exam. The exclusion criteria were: presence of hereditary colorectal cancer, Crohn's disease, ulcerative rectocolitis, colorectal metachronic cancer or cancer of another origin and another previously treated neoplasm.

The study analyzed 130 patients with colorectal carcinoma, mean age of 64.2±9.6 years (29 to 90 years old), submitted to curative or palliative surgery, between October 2001 and March 2005 at the Hospital São Paulo, by the Coloproctology Group of Surgical Gastroenterology of the Department of Surgery of the Universidade Federal de São Paulo – Escola Paulista de Medicina (UNIFESP – EPM). There were 65 male patients (50%) and 65 female patients (50%). Eighty-two of them (63.1%) had been submitted to curative intervention and 48 (36.9%) to palliative intervention.

The parameters analyzed were: macroscopic (location and aspect) and microscopic characteristics (degree of histological differentiation and lymphatic, vascular and neural invasion) of the lesion, clinical staging, according to the TNM classification developed by the Union for International Cancer Control (UICC), presence of liver metastases and in other locations and tissue immunoexpression of CD10 in the colorectal carcinoma tissue, evaluating the intensity of immunostaining versus the percentage of immunostained cells.

For the production of a *tissue microarray* (TMA) block, all cases of colorectal carcinoma were evaluated by two pathologists through histological sections with hematoxylin-eosin (HE) stain, regarding the histological degree, diagnosis confirmation and selection of core removal sites for the TMA exam. With a Beecher™ device (Beecher Instruments, Silver Spring, MD, USA), TMA blocks were produced, using the standard technique[4]. For the adhesion of the TMA block sections to the blades, a system of adhesive tapes was used (Instrumedics Inc., Hackensak, NJ, USA). The samples were cut with 4 μm thickness, and a small roll was used to pressure the section to the tape. Then, the tape with adhered histological section was placed on the resin-coated blade and pressed with the roll for better adherence. After that, the blades with histological sections adhered to the tapes were placed under ultraviolet (UV) light for 20 min. The blades were dried and the adhesive tapes were removed. The blades were then sent for immunohistochemical analysis. The technique of streptavidin-biotin-peroxidase with monoclonal antibody CD10 (56C6 clone, Neomarkers, USA) was used, applying the 1:40 dilution.

The evaluation of protein immunoexpression was performed in the tumor tissue and non-neoplastic mucosa taken from the surgical margins adjacent to the tumor. Brown color in the cytoplasmic or membranous area of the cell was considered as positive immunoexpression for the studied antibodies. The positive control were sections of the germinal center area in normal tonsil for anti-CD10 antibody. On blades used as negative control, the primary antibody of the reaction was subtracted. The immunoexpression evaluation was performed by two experienced pathologists and in isolated form. Divergences were submitted to a joint analysis.

The sections were analyzed using a blade scanner (Scan Scope CS System, Aperio, United Kingdom), a blade scanning system that produces high-resolution

images. The percentage of cells with positive reaction was analyzed in high enlargement field (400x). The images were captured using a camera (Samsung, South Korea) and WinTV32 connected to a microscope (Bx40, Olympus, Japan). The positive and negative expressions were identified in the neoplastic tissue and the normal mucosa tissue.

The criteria used in the evaluation of CD10 proteins were those established by Ogino et al.[5]. The negative samples were those with less than 10% of stained cells and the positive samples were those with more than 10% of stained cells[6]. The percentage of stained cells was used as indicator of marker expression degree. Score 0 indicated less than 10% of stained cells; score 1 indicated 11 to 25% of stained cells; score 2 indicated 26 to 50% of stained cells; score 3 indicated more than 50% of stained cells. The cells with doubtful stain and those considered as morphologically non-neoplastic cells were excluded from the immunoexpression evaluation of markers.

Data related to quantitative variables were presented as mean values. The categorical variables were analyzed using the Mann-Whitney test and the correlation between them was performed using the Spearman test. The analysis of variance in the samples from the three groups or more was performed using the Kruskal-Wallis test and the dichotomous variables were analyzed using Fisher's exact test. The significance level adopted was lower than 0.05, or 5%. The statistical program used was Prism 4.0 (GraphPad Software Inc., USA).

RESULTS

The lesions were in the colon in 78 (60%) cases in the rectum in 52 (40%) patients. The expression of CD10 did not show any significant difference (p=0.79) in relation to its location in the colon or rectum.

The mean score of CD10 expression in the tumor tissue (Figure 1) was 1.2 and in the non-neoplastic mucosa was 0.1, and this difference was significant (p<0.0001).

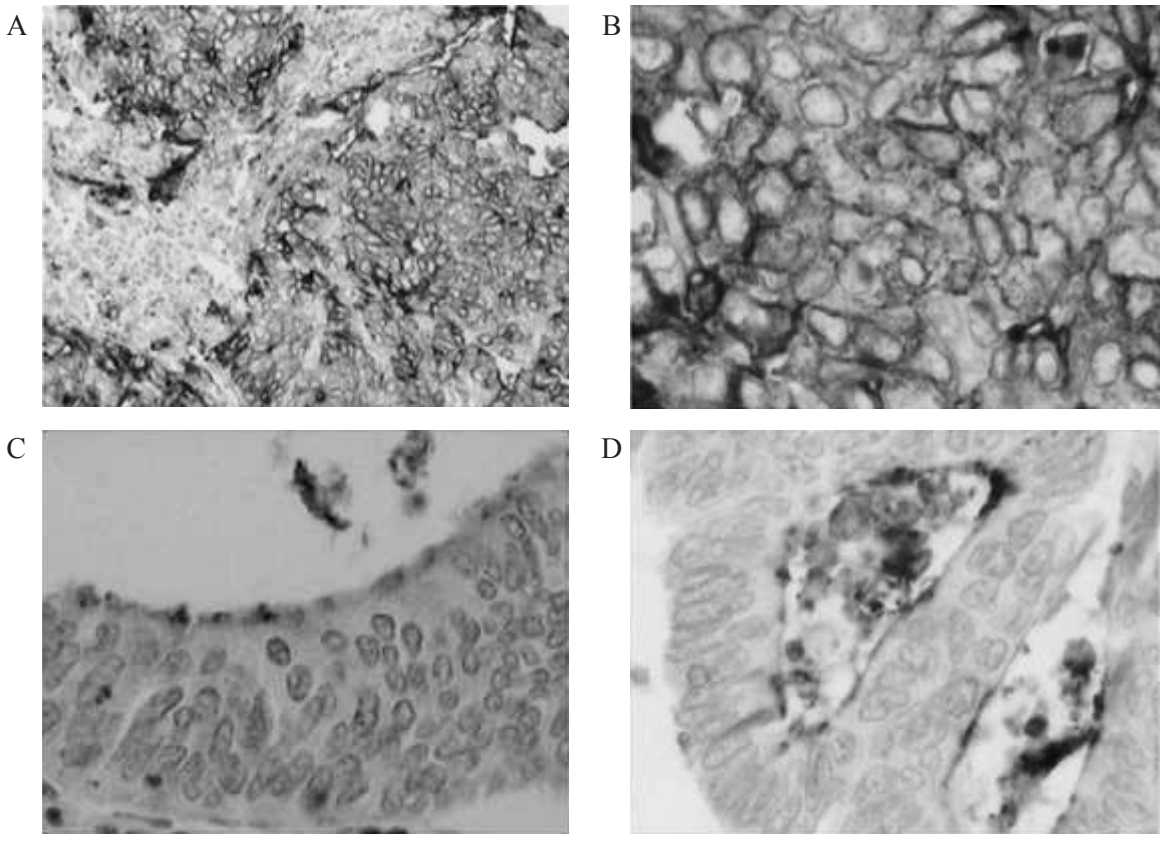

Figure 1. *Colorectal carcinoma with cytoplasmic and membranous positive expression for CD10 marker (immunohistochemical analysis). (A) Intense (100x); (B) Intense (400x); (C) Mild (200x) and (D) Intense, with brush border pattern (200x).*

In 85 (65.6%) cases, the macroscopic aspect was exophytic, in 17 (13.1%) cases, it was non-exophytic and 28 (21.5%) cases did not present any reference to the macroscopic aspect of the neoplasm. The CD10 immunoexpression was significantly higher (p=0.04) in carcinomas with exophytic macroscopic than in those with non-exophytic macroscopic aspect (Table 1).

The distribution of cell differentiation degree of tumors and the expression of CD10 tumor marker did not show any significant difference (p=0.48) (Table 2).

Lymph node infiltration was observed in 55 (42.3%) patients, 46 (35.4%) patients had free lymph nodes and 29 (22.3%) patients submitted to palliative surgery had no lymph nodes. No significant difference (p=0.3) was observed between the expression of CD10 and the presence of lymph node infiltration.

Regarding the presence of vascular, lymphatic and perineural invasion, the expression of CD10 did not show any significant difference in relation to lymphatic invasion (p=0.4), vascular invasion (p=0.5) and perineural invasion (p=0.2) (Table 3).

Table 1. *Immunoexpression of CD10 marker according to the exophytic or non-exophytic macroscopic aspect of colorectal carcinoma.*

CD10	Macroscopic Aspect (n=101) p=0.04*	
	Exophytic (n)	Non-Exophytic (n)
Positive	10 (58.82%)	26 (38.29%)
Negative	7 (41.17%)	58 (61.70%)

Fisher's exact test
*n: number of patients; *: Significant.*

Table 2. *Distribution of patients according to the degree of cell differentiation of the colorectal carcinoma.*

Degree of differentiation	n	%
Well differentiated	52	40.0
Moderately differentiated	70	53.8
Poorly differentiated	8	6.2
Total	130	100.0

Fisher's exact test: p=0.4 (N.S.).
n: number of patients; N.S.: Non-Significant.

The TNM classification of colorectal neoplasms did not show any significant difference (p=0.09) in relation to the expression of CD10 (Table 4).

Regarding the presence of liver metastases or the preoperative and/or postoperative distance, no significant difference (p=0.3) was observed in relation to the expression of CD10 in the colorectal tissue (Table 5).

Table 3. *Distribution of patients according to lymphatic, vascular and perineural invasion of the colorectal carcinoma.*

Invasion	No	Yes	p-value
Lymphatic	52 (40.0%)	78 (60.0%)	0.4 (N.S.)
Vascular	103 (79.2%)	27 (20.8%)	0.5 (N.S.)
Perineural	92 (70.8%)	38 (29.2%)	0.2 (N.S.)

Fisher's exact test.
N: number of patients; N.S.: Non-Significant.

Table 4. *Distribution of patients according to the clinical stages of the TNM classification for colorectal carcinoma.*

Clinical Stage	n	%
I	9	6.9
II	44	33.8
III	45	34.6
IV	32	24.6
Total	130	100.0

Fisher's exact test: p=0.09 (N.S.).
n: number of patients; N.S.: Non-Significant.

Table 5. *Distribution of patients according to the occurrence of metastasis in relation to the preoperative and/or postoperative distance.*

Metastasis	n	%
Absent	79	60.8
Other sites, except liver	27	20.8
Only liver	19	14.6
Liver and other sites	5	3.8
Total	130	100.0

Fisher's exact test: p=0.3 (N.S.).
n: number of patients; N.S.: Non-Significant.

DISCUSSION

The colorectal carcinoma tissue analysis used the TM, which employs a small sample of the tumor tissue. The TMA validation for colorectal tumor studies was obtained from a study of cytokeratin expression in colorectal carcinoma[7]. This method enables the utilization of reduced antibodies and tissues and antibody immunoexpression homogenization, as the immunohistochemical reaction is performed at the same time and on the same blade in all cases[8].

The studied used the blade analysis and scanning system, where the cell structure was analyzed without the help of microscopes. In this system, the computer acts as a "robotized microscope". The image moves in high definition on the same blade and, within a few minutes, it is homogeneously scanned. The statistical analysis of the sample showed a direct relation between the quantity of stained cells and the stain intensity for the CD10 marker. Considering this finding, the percentage of stained cells was used as the indicator of marker expression degree, just as other authors have done[9-12].

The expression of CD10 marker in this study was significantly higher in the neoplastic tissue than in normal mucosa. This finding suggests the participation of this protein in the sequence of events for neoplastic alterations. The CD10 maker is a membrane-associated peptidase related to peptide cleavage. This event is believed to be related to neoplastic progression[2]. Studies have identified a difference in marker expression in tumors and non-neoplastic colorectal mucosa adjacent to the tumor[1,13,14]. Ogawa et al.[15] studied the expression of CD10 in tumor tissue of colorectal carcinoma and did not find any expression of this marker in samples of non-neoplastic mucosa adjacent to the tumor.

The literature controversial regarding the expression of this marker and the presence of liver metastasis[11]. Fujita et al.[16] evaluated the expression of CD10 and did not find any relation between the expression of CD10 and the occurrence of liver metastasis. On the other hand, Fujimoto et al.[10] identified a strong expression of CD10 in patients with higher incidence of metastases, which was also observed by Yao et al.[17] and Ohji et al.[2]. This study did not identify any significant relation of the CD10 expression to the occurrence of liver metastases, which can be explained by the fact that this study only recruited patients with synchronic liver metastases.

The expression of CD10 in this study was similar in the various degrees of colorectal carcinoma differentiation. Sato et al.[1] identified a higher expression of CD10 in colorectal carcinomas well or moderately differentiated and a lower expression in poorly differentiated neoplasms. Fujimoto et al.[10] observed that the expression of CD10 was higher in well differentiated tumors when compared to the others. Oshima et al.[18] did not observe any difference in the expression regarding the histological differentiation.

The expression of this marker in this study was similar in tumors that presented or not angiolymphatic and/or perineural invasion. Fujimoto et al.[10] identified a significant association with angiolymphatic and/or perineural invasion, but Fujita et al.[16] did not observe any significant relation. Yao et al.[17] identified greater venous invasion in tumors with positive expression of CD10.

In this sample, the expression of CD10 did not show any significant difference in terms of clinical stage of the colorectal neoplasm, which was also observed by Fujita et al.[16].

A tendency of exophytic tumors with higher expression of CD10 than in non-exophytic tumors, which indicates that neoplasms with CD10 positive lines tend to be significantly more exophytic than CD10 positive lines. Ogawa et al.[15] analyzed the expression of CD10 in the cell stroma in the several stages of carcinogenesis and observed an expression of this marker significantly more positive in protruding lesions than in superficial lesions. These authors also observed that the expression of this marker was higher in more voluminous tumors.

This study did not observe any significant difference in the expression of CD10 markers in terms carcinoma either in the rectum or colon, just as other authors have also observed[16,17,19]. The analysis of CD10 expression did not present any relation to the occurrence of lymph node metastasis, just as observed by Yao et al.[17]. Fujita et al.[16] analyzed the expression of CD10 through polymerase chain reaction (PCR) and identified a higher expression of this market in N1 and N2 tumors than in N0 tumors, but this difference was not significant.

CONCLUSIONS

The expression of CD10 protein was higher in the neoplastic tissue of colorectal carcinoma than in the adjacent non-neoplastic mucosa and presented association with the exophytic aspect of tumor. The immunoexpression of CD10 protein did not show any relation to the neoplasm location in the colon or rectum, presence of lymph node or liver metastases, or in any other site, angiolymphatic and/or perineural invasion, degree of cell differentiation and TNM staging.

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