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CD10 Expression in Colorectal Carcinoma and Premalignant Lesions

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Abstract: CD10 is a zinc-dependent peptidase (metalloproteinase) that has been detected in variety of tumors. The aim of this work is to study the immunohistochemical expression of CD10 in colorectal carcinoma (CRC) and premalignant lesions (colorectal adenomas).Material and Methods:CD10 immunohistochemical expression was studied in tumor cells and stromal cells in 30 cases of colorectal adenocarcinoma and 20 cases of colorectal adenoma. The immunohistochemical demonstration of CD10 was accomplished by using immunoperoxidase method on paraffin-embedded tissue sections with a monoclonal antibody to CD10. Results*:* Significant progression of CD10immunohistochemical expression in tumor cells from 20% in low grade adenoma to 50% in high grade adenoma reaching 80% in invasive CRC cases was reported in this study ($p<0.05$). In addition, inverse significant correlation was detected between CD10 expression in tumor cells and depth of tumor invasion as 100 % and 83% of T2 and T3 cases respectively showed positive CD10 expression and 100 % of T4cases showed negative CD10 expression ($p<0.05$). Stromal CD10 expression was higher in invasive CRC (57%) and high grade adenomas (60%) compared to low grade adenomas (30%), although didn't reach statistical significance ($p>0.05$).Conclusions*:* This study suggests that CD10 expression in tumor cells may serve as a potential biomarker in colorectal carcinogenesis along the adenoma carcinoma sequence and supports the involvement of tumor CD10 in invasiveness potential in early colorectal cancer. Analysis combining CD10 expression in tumor and stromal cells in CRC and premalignant lesions may be one of the quite useful predictors of development and early progression of CRC.

Key words: *Colorectal carcinoma · Premalignant lesions · CD10 · Immunohistochemical*

INTRODUCTION

Colorectal cancer (CRC) is the third most common diagnosed cancer among men and women and the second leading cause of cancer death in USA [1]. Worldwide, more than 1 million people get colorectal cancer annually [2].

At the time of diagnosis, over 20% of patients present with metastatic (stage IV) colorectal cancer and up to 25% of these patients will have isolated liver metastasis that is potentially resectable. Five year survival outcomes are now exceeding 50% in the lesions which undergo curative resection [3]. Survival rates for early detected stages are about 5 times that of late stage Cancer. Colorectal cancer arises from adenomatous polyps in 80% of cases making this cancer suitable for screening [4].

The continuous and bilateral molecular interaction between normal epithelial cells and stromal cells is disrupted by several factors secreted by tumor cells or by stromal cells under the influence of tumor cells [5-9]. One such important factor is the matrix metalloproteinase (MMPs). MMPs have an important role in tumor progression and in defining the role of stromal microenvironment in invasiveness and metastatic potential [10].

CD10 is a matrix metallopeptidases (MMPs) involved in carcinogenesis via the release of bioactive molecules that stimulate invasion, extracellular matrix (ECM) degradation, inhibition of apoptosis and promotion of angiogenesis and immune response modulation [11].

CD10 is a zinc-dependent peptidase (metalloproteinase) that is commonly expressed in bone marrow lymphoid stem cells, pro-B lymphoblasts, mature neutrophils, various subtypes of lymphoma, renal cell carcinoma and endometrial stromal sarcoma cells. CD10-positive cells have also been reported in the stroma of prostate [12], breast[13,14], colorectal[15] and lung carcinomas [16].

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In colorectal cancer tissue, CD 10 has been detected in tumor cells, tumor associated fibroblasts and infiltrating inflammatory cells [17]. Several investigators have reported association between CD 10 expression in CRC and increased invasiveness, lymph node involvement, liver metastasis and poor prognosis [17-19]. However, only few studies have been carried out to determine the potential role of CD10 in colon cancer and its precursor lesions (colorectal adenomas) [20].

We aimed in this study to investigate the immunohistochemical expression of CD10 in tumor and stromal cells of colorectal cancer and premalignant lesions (colorectal adenomas). In addition, the correlation between CD10 expression in colorectal cancer and various clinicopathologic factors is studied.

MATERIALS AND METHODS

Study Group: This work included 50 cases of archived paraffin blocks of colorectal adenomas (n=20) and colorectal carcinomas (n=30) obtained through colonoscopic biopsies and colectomy specimens respectively during the period from January 2013 till February 2015, from the Pathology department, Faculty of medicine, Cairo University.

Histopathological Evaluation:

- Formalin fixed and paraffin embedded blocks were sectioned at 5µm thickness and examined microscopically using H&E stain.
- According to the grade of dysplasia, adenomas were classified into two groups, low grade (mild-moderate dysplasia) and high grade adenomas (marked dysplasia). Low-grade dysplasia consists of stratified dysplastic epithelium that retains its columnar shape. The nuclei are spindle or oval shaped. The stratified nuclei tend to remain in the basal epithelium extending no more than three quarters of the height of the epithelium. There is minimal nuclear hyperchromasia. High-grade dysplasia is present when the nuclei consistently come to the surface of the epithelium, loss of the columnar shape with cellular rounding, increasing nuclear:cytoplasmic ratios, nuclear irregularity, loss of polarity, development of cellular pleomorphism and heaping up of cells [21].
- Colorectal carcinomas were examined microscopically to evaluate the histopathologic type, grade, stage and, nodal metastasis. All colorectal carcinomas (n=30) were adenocarcinoma. Mucinous adenocarcinoma was excluded from the study. The tumors were staged according to American Joint Committee on Cancer (AJCC) Tumor Node Metastasis (TNM) system (2010*)* and graded according to the World Health Organization criteria [22, 23].

Immunohistochemicalstaining: Each paraffin block was cut at 5µm thickand taken for the study. The slides were deparaffinized in xylene, then were rehydrated through a series of graded alcohols then were twicelymicrowave-treated for 4 and 8 minutes in 10 mm sodium citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% H2O2 for 15 min, followed by washing withTris-buffered saline (TBS).The sections were then incubated with monoclonal mouse CD10 antibody clone 56C6 (Genemed, South San Francisco, CA) at 1:50 dilution for one hour at room temperature.

Sections were again washed in TBS and incubated with avidin-biotin-peroxidase system (DAKO) for 30 minutes. Finally the diaminobenzidine was used as a chromogen and hematoxylin as a counterstain.Positive control was obtained from tonsils, which exhibited strong intensity of CD10 immunostaining.

Evaluation of Immunohistochemical Expression of CD10: The immunostaining of CD10 was evaluated in tumor cells (tCD10)and stromal cells (sCD10). The immunostaining of tCD10 was expressed as fine to coarse cytoplasmic granules. Positive tCD10 was considered if more than 10% of tumor cell stained positive for CD10.The extensity of sCD10 was graded according to a 4-point scale based on the percentage of positively stained area: 0 (< 10 % positive tumor cells), +1 (10-25% positive tumor cells), +2(25 to 50% positive tumor cells), +3(>50% positive tumor cells). For statistical analysis purpose, the cases were divided into two groups: negative group (0, 1) and the positive group (2, 3) [20].

Statistical Analysis: SPSS version 18.0 (Statistical Product for services solutions) was used for data analysis and the probability value (P value) of less than 0.05 was chosen to represent statistical significance. The chi-square test was used for evaluation of p value.

RESULTS

A total of 50 tissue samples from colorectal adenomas (n=20) and, colorectal carcinomas (n=30) were studied. The mean age of the studied patients with colorectal carcinoma was 54.87 years, ranging from 29 to 78 years with median age 50 years. The age of colorectal adenoma patients ranged from 25 to 82 years.

Table 1: Clinicopathologic features of colorectal adenoma cases

Colorectal adenoma (n=20)	Number of cases	%
Age		
<50 years	5	25
≥50 years	15	75
Sex	11	55
Male	9	45
Female		
Histologic pattern		
Tubular	3	15
Villous	2	10
Tubulovillous	15	75
Site		
Left colon	9	45
Rectum	6	30
Sigmoid	5	25
Grade of dysplasia		
Low grade	10	50
High grade	10	50

Table 2: Clinicopathologic features of colorectal carcinoma cases.

Clinicopathologic features (n=30)	Number of cases	%
Age		
<50 years	10	35
≥50 years	20	65
Sex		
Male	11	45
Female	19	55
Tumor Site		
Right colon	11	36.7
Transverse colon	4	13.3
Left colon	7	23.3
Rectosigmoid	8	26.7
Gross Picture		
Infiltrating	12	40
Fungating	10	33.3
Ulcerating	8	26.7
Histologic type		
Adenocarcinoma	30	100
Histolgic grade		
Grade II	27	90
Grade III	3	10
Depth of tumor invasion		
T2	4	13.3
T3	24	80
T4	2	6.7
Lymph nodal metastasis		
Negative	16	53.3
Positive	14	46.7
TNM Stage		
I	2	6.7
II	14	46.7
III	14	46.7

The clinicopathologic features of colorectal adenoma and colorectal carcinoma patients are shown in (Table 1) and (Table 2) respectively.

CD 10 immunostaining was observed in colorectal adenomas and carcinomas as brown, fine to coarse granular cytoplasmic staining in both tumor and stromal cells. Immunohistochemical expression of CD10 in tumor cells (tCD10) progressively increased from20% (2 out of 10) in low grade adenomas (Fig 1), to 50% (5 out of 10) in high grade adenomas (Fig 2), reaching 80% (24 out of 30) cases in invasive CRC (Fig 3) and that was statistically significant, (P=0.002). Invasive CRC and high grade adenoma cases showed higher CD10

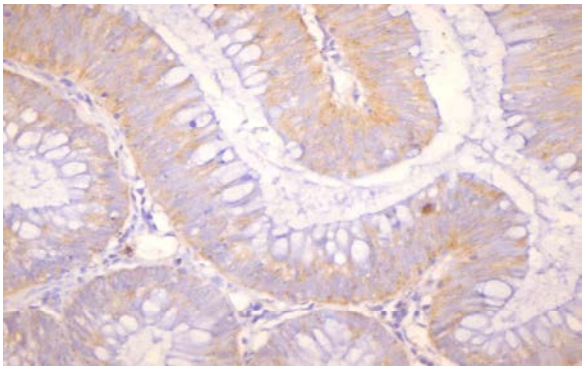

Fig. 1: Faint Cytoplasmic staining of CD10 in tumor cells of low grade adenoma (immunoperoxidase, 400x).

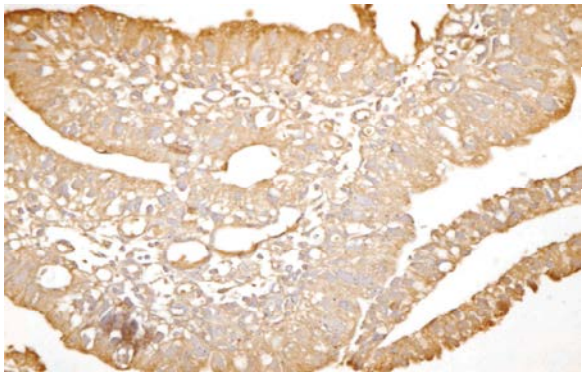

Fig. 2: Strong cytoplasmic staining of CD10 in tumor and stromal cells in high grade adenoma (immunoperoxidase, 400x).

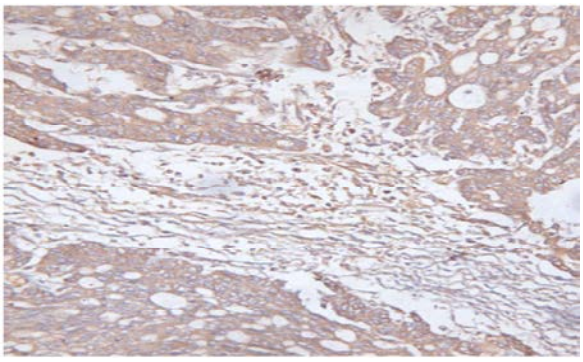

Fig. 3: Strong cytoplasmic staining of CD10 in tumor and stromal cells of invasive colorectal carcinoma(immunoperoxidase, 200x).

Table 3: Correlation between tumor CD 10 and stromal CD 10 immunohistochemical expression in colorectal adenoma and carcinomas

Variables	Number	tCD10	tCD10	P value	sCD10	sCD10	P value
	n	negative(%)	positive(%)		negative(%)	positive(%)	
Low grade adenoma	10	8(80)	2(20)		7(70)	3(30)	
High grade adenoma	10	5(50)	5(50)	0.02	4(40)	6(60)	NS
Invasive CRC	30	6(20)	24(80)		13(43)	17(57)	

Table 4: Relationship of tumor CD 10 and stromal CD 10 immunohistochemicalexpression in CRCandclinicopathologic variables

Variables	Number N	tCD10 negative(%)	tCD10 positive(%)	P value	sCD10 negative(%)	sCD10 positive(%)	P value
Age							
<50 years	10	3(30)	7(10)	NS	4(40)	6(60)	NS
≥50 years	20	3(15)	17(85)		9(45)	11(55)	
Sex				NS			NS
Male	11	2 (19)	9(81)		4(36)	7(64)	
Female	19	4 (21)	15(79)		9(47)	10(53)	
Tumor Grade				NS			NS
Grade II	27	5(18.5)	22(81.5)		13(48)	14(52)	
Grade III	3	1(33.3)	2(66.7)		0(0)	3(100)	
Depth of invasion							NS
T2	4	0(0)	4(100)		2(50)	2(50)	
T3	24	4(17)	20(83)	0.01	9(37.5)	15(62.5)	
T4	2	2(100)	0(0)		2(100)	0(0)	
LN metastasis				NS			NS
Negative	16	3(19)	13(81)		8(50)	8(50)	
Positive	14	3(21)	11(79)		5(36)	9(64)	
TNM stage				NS			NS
Stage I	2	0(0)	2(100)		2(100)	0(0)	
Stage II	14	3(21.4)	11(78.6)		6(43)	8(57)	
Stage III	14	3(21.4)	11(78.6)		5(35.7)	9(64.3)	

NS: not significant (p value<0.05)
LN: lymph node
tCD10: tumor CD10
sCD10: stromal CD10

immunohistochemical expression in stromal cells 57% (17 out of 30) and 60% (6 out of 10)cases respectively, than low grade adenoma cases 30% (3 out of 10), however no statistically significant correlation was detected. The correlation between tCD10 and sCD10 immunohistochemical expression in colorectal adenomas and carcinomas are summarized in (Table 3).

The tCD10 and sCD10immunohistochemical expression in 30 cases of invasive colorectal carcinoma were studied and correlated with clinicopathologic variables as shown in (Table 4). CD10 immunohistochemical expression in tumor cells negatively correlated with the depth of tumor invasion as 100% of T2 cases (4 out of 4) and 83% of T3 cases (20 out of 24) showed positive tCD10 expression and 100 % of T4 cases (2 out of 2) showed negative tCD10 immunohistochemical expression, (p=0.01).

No significant correlation was detected between tCD10 and sCD10 expression in invasive CRC and other clinicopathologic variables.

DISCUSSION

CD10 is an important molecule involved in integrating signals from either the cell environment or the intracellular compartment by cleaving peptides through enzymatic activity and through intracellular signaling pathways that interfere with other major signaling pathways. It is thus obvious that CD10 expression derangement is associated with the development or progression of different tumort ypes[24].

In the current study we examined CD10 immunoreactivity in both tumor cells and stromal cells in thirty cases of colorectal carcinoma and twenty cases of

colorectal adenoma. The correlation between CD10 immunohistochemical expression and different clinicopathologic factors was analyzed.

In this work, CD10immunohistochemical expression in tumor cells (tCD10) progressively increased from 20% (2 cases out of 10 cases) in low grade adenomas to 50% (5 cases out of 10 cases) in high grade adenomas and reached 80% (24 cases out of 30 cases) in invasive CRC and that was statistically significant (P value< 0.05). These findings coincide with the results obtained by other studies. Jang *et al*. [20], who stated that tCD10immunohistochemical expression significantly increased from 14% in low grade adenomas (3 cases out of 22 cases), to 22% in high grade adenomas (6 cases out of 27 cases) and 44% in invasive CRC (14 cases out of 32 cases) and this support the involvement of CD10 in progression and carcinogenesis of colorectal carcinoma. Likewise, Wang *et al*. [25], reported that there was progression in tCD10immunohistochemical expression from 0.8% in low grade adenomas to 9.1% in high grade adenomas and 40% in invasive CRC. Also, this is in agreement with what was reported by Iwase *et al*. [26] and Koga *et al*. [27] and Hirano *et al*. [28], that tCD 10 immunohistochemical expression was reported more frequently in invasive phenotype rather than adenomas.

Stromal cells showed CD10immunohistochemical expression in both adenoma and carcinoma. Fifty-seven percent (17 cases out of 30 cases) of CRC showed positive sCD10immunohistochemical expression while only forty-five percent (9 cases out of 20 cases) of adenoma showed positive sCD10immunohistochemical expression. Invasive CRC and high grade adenoma cases showed higher CD10 immunohistochemical expression in stromal cells 57% and 60% respectively than low grade adenomas which represented only 30%. However, no statistically significant correlation was detected. Ogawa *et al*. [15] similarly reported higher sCD 10 immunohistochemical expression in invasive CRC (79%) and high grade adenoma (70.6%) than low grade adenomas which represented only 21.9%and,also reported that sCD 10 immunohistochemical expression was similar in high grade adenomas and carcinomas and its expression is induced at the step in which low grade adenomas (mild to moderate dysplasia) become high grade adenomas (severe dysplasia). Jang *et al*. [20] also reported progression of expression of sCD10 immunohistochemical expression from low grade adenoma (41%) to high grade adenoma (70%) to invasive CRC (88%) and that was statistically significant.

In the current study, CD10 immunohistochemical expression in tumor cells inversely correlated with the depth of tumor invasion, 100% of cases in T2 and (83%) of cases in T3 showed positive tCD10 immunohistochemical expression and 100% of cases in T4 showed negative tCD10 expression,with statistically significant relationship, these findings suggest that tCD10 is involved in invasiveness potential in early CRC. In contrast, no significant correlation was reported between tCD10 and depth of tumor invasion in the study done by Jang *et al*. [20].

No significant correlation was detected in the current study between tCD10 and sCD10 immunohistochemical expression and clinicopathologic variables as age, sex, histologic grade, lymph node status and, stage in CRC cases.This is most probably due to the low number of cases enrolled in the present study.

CONCLUSIONS

In conclusion, the progression of tCD10 expression from low grade adenoma to high grade adenoma to invasive CRC and the higher percentages of sCD10 expression in CRC and high grade adenomas compared to low grade adenoma as demonstrated in this study supports the role of CD10 as potential biomarker in colorectal carcinogenesis along the adenoma carcinoma sequence. In addition, the significant inverse correlation between tCD10 expression and depth of tumor invasion in CRC suggests that CD10 is involved in invasiveness potential in early colorectal cancer. Thus analysis combining CD10 expression in tumor and stromal cells in CRC and premalignant lesions may be one of the quite useful predictors of development and early progression of CRC and more future studies on larger sample size to validate our results are recommended.

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