The expression of CD44 in colorectal cancer and association with clinicopathological features.

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Abstract

Background: Cluster of differentiation number 44 (CD 44) is a family of cell-surface adhesion molecules which exist in several isoforms arising from mRNA alternative and encoded by using gene located at chromosome 11 on the short arm p13. The CD44 gene is composed of 10 constitutively spliced exons and 10 variable exons, residing between constitutive exons 5 and 6.In colorectal carcinoma, the relationship between the expression of variant exons and tumor progression is controversial. Some studies have suggested that the expression of certain variant exons resulted in increased tumor progression, while other has no correlation.

Objectives: Investigate CD44 immunoreactivity in colorectal carcinoma and determine the association with clinicopathological features.

Material and methods: Paraffin- embedded tumor specimens from (70) patients with colorectal adenocarcinoma and (70) healthy control were assessed by immunohistochemisrty for the expression of CD44.

Result: Statistical analysis of CD44 expression revealed highly significant difference in colorectal carcinoma patients than in control group. Also there were a relationship between CD44 expression and range of clinicopathological features.

 Conclusion: CD44 is robust marker for colorectal carcinoma.

INTRODUCTION

The CD44 is a polymorphic family of immunologically related cell surface proteoglycans and glycoproteins which normally take part in cell- cell and cell matrix adhesion interactions lymphocyte activation and homing, and cell migration [1,2,3]. It participates in many cellular processers, including growth survival differentiation and motility [4,5].Its a unique molecule plays a role in cancer cell migration and matrix adhesion in response to cellular microenvironmemt, thus enhancing cellular aggregation and tumor cell growth[6].

 Recent studies have identified CD44 glycoprotein as a potentially components of tumor progression and metastatic cascade, and also showed a causal role of specific CD44 isoforms in metastasis formation and deregulated CD44 expression in human cancer [7,8]. Many studies have shown that expression of CD44 is up regulated in malignant tumors, such as gastric cancer, pancreatic cancer, lung cancer and renal cell cancer [8,9].

 In colorectal carcinoma, which results from a series of genetic events that disorder the normal mechanisms controlling cell growth [7,9],the characterization of molecular changes in recent years has been the focus of great interest for both researchers and clinicians because it may lead to the identification of new prognostic markers more closely resembling the biological nature of the disease[2,10].

Some studies have suggested that a high level of expression of the variant form of CD44 has been linked to the development and spread of malignancies[11,12,13], on the other hand, there are studies in which down-regulation or absence of CD44 expression indicate aggressiveness[7]. The aim of this study was to investigate CD44 immunoreactivity in colorectal carcinoma and to detect its association with clinicopathological features.

PATIENTS AND METHODS

Seventy patients with colorectal adenocarcinoma (mean age 46.12 years and range 20- 82), the control group include 70 colorectal normal mucosa with mean age 48.2 and range between 20 - 75 were involved in this study.Biopsy specimens were collected from the archive of the department of histopathology of teaching laboratories of Al-Yarmook Teaching Hospital and Baghdad medical city for the period between 2009 and 2012 and informed consent was obtained from all patients. Biopsies were fixed in 10% formal buffer saline for histological examination. The biopsies were used for histological evaluation and immunological staining for CD44 monoclonal antibody. Tissue sections cut into 5µm thickness, put on Fisherbrand positively charged slides.

Mucosal biopsies were immune staining with primary antibody: anti-CD44 monoclonal antibody (clone DF 1485, Dakocytomation, Denmark. Dillution 1:50). The use of universal DakoCytomation streptavidin- biotin system purchased from DakoCytomation (USA) Immuno-histochemistry detection kit. The primary antibody reacts with antigen in the tissue, and then a biotin labeled secondary antibody (link antibody) binds to the primary antibody. When the conjugate is added, the biotinylated secondary anti-body will form a complex with the peroxidase-conjugated streptavidin and by adding the substrate, which contains 3,3diaminobenzidine (DAB) in a chromogen solution, a brown-colored precipitate will form at the antigen site. In the peroxidase secondary detection system, the presence of a brown reaction product at the site of the target antigen is indicative of positive reactivity. Counter stain will be pale to dark blue coloration of the cell.

Evaluation of the immunostaining was done with the assistance of a histopathologist. The observer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observer positive or negative cases, positive immunostaining gave dark brown granules.

In the peroxidase secondary detection system, the presence of a brown reaction product at the site of the target antigen is indicative of positive reactivity. Counter stain will be pale to dark blue coloration of the cell.

. **Scoring:**

Counting the number of positive cells which give brown staining system under light microscope.The extent of the IHC signal was determined in 10 fields (X100 magnification). In each field the total number of cells was counted.

The total staining score was divided by the number of the whole cells per fields in 10 fields, so the percentage of positivity stained cells in the 10 fields was calculated for each case by taking the mean of the percentage of the positively stained cell in the 10 fields.

The expression of CD44 <10% of positive neoplastic cells was used to define low expression, 10-50% to define moderate expression and >50% to define extensive expression.

Statistical analysis:

Statistical analysis was performed and Student test (t-test) was used for the quantitative data i.e. CD44- , CD44+ and controls.Categorical variables were assessed by Pearson chi- squared analysis test with ANOVA test wherever required.

RESULT

The relationship between CD44 expression and a range of clinicopathological variables is summarized in table (1). There was a significant correlation between CD44 staining and tumor differentiation , as well as tumor stage ($P<0.05$) but there was no significant correlation with tumor site.

Variable	CD44 immunohistochemistry			p-value
	< 10%	10%-50%	> 50%	
Sex				
Female	3(23.07%)	10(38.46%)	11(35.48%)	0.623
Male	10(76.92%)	16(61.53%)	20(64.51%)	
Age (years)				
Median	50	45	53	
Tumor stage				
Stage B	10(76.92%)	20(76.92%)	22(70.96%)	0.852
Stage C	3(23.07%)	6(23.07%)	9(29.03%)	
Tumor site				
Rectum	9(69.23%)	13(50%)	20(64.51%)	0.720
Right colon	2(15.38%)	10(38.46%)	9(29.03%)	
Left colon	2(15.38%)	3(11.53%)	2(6.45%)	
Tumor grade				
Well/moderately differentiated	10(76.92%)	20(76.92%)	23(74.19%)	0.966
Poorly differentiated	3(23.07%)	6(23,07%)	8(25.8%)	

*Significant using Pearson Chi-square test at 0.05 level

As shown in table (2) there were significant difference ($p<0.0001$) in the mean percentage of CD44 proteins expression in tissue of colorectal carcinoma (CRC) and colorectal normal tissue (CRN). The expression of CD44 was heterogeneous dark brown staining in the tissue shown in figure(1).

Table (2): Comparison of mean percentage of CD44 protein among studied group.

Studied groups	N	Mean ± Std. Error	p-value
Controls (CRN)	70	18.1 ± 1.7	0.0001*
Patients (CRC)	70	75.2 ± 2.2	
Total	90		

*Significant using Student-t-test for difference between two independent means at 0.05 level
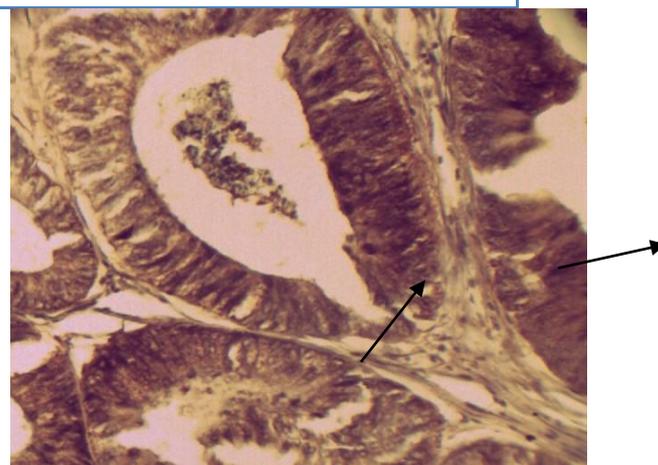

Figure (1): Immunohistochemical staining (IHC) of CD44 proteins in tissue of colorectal carcinoma (CRC). Staining by DAB chromogen (dark brown) counterstained with nuclear fast red. Positive CD44 immunostaining (X400).

The intensity scoring ranged from <10 - >50, (Table-3) was conducted to examine the association between CD44 staining pattern of normal colonic mucosa and CRC cases.It was found that significant association (*P* value 0.0001) between them in the three scoring levels. The results showed that percentage of medium and extensive CD44 was elevated in the colorectal adenocarcinoma group than in normal control

Table (3): Scoring of CD44 expression in normal control and patients with colorectal adenocarcinoma (CRC).

Groups	N	Low (<10%)	Medium (10%-50%)	Extensive(> 50%)	p-value
Control	70	48(68.6%)	13(18.6%)	9(12.8%)	0.0001*
CRC	70	13(18.57%)	26(37.14%)	31(44.28%)	

*Significant using Pearson Chi-square test at 0.05 level

The present study shows about 38.77 of cases were younger than 50 years.

DISCUSSION

This study demonstrates that the incidence of CRC in young age group in Iraq is much higher than western countries which showed that the mean age is 60 -65 years. This increase in CRC may be related to multiple factors like exposure to the carcinogenic substances during the last years due to the war, westernization of diet (low fiber diet and high fat content) and improved diagnostic procedures[14].

Also, the study show highly significant difference in the mean percentage of CD44 protein expression in tissue of colorectal carcinoma (CRC) and that was agreed with several studies [15, 16, 17] that show over expression of the adhesion molecule CD44 and its splice variants is associated with poor prognosis and metastasis.

The current study shows as well correlation between CD44 expression and range of clinicopathological variables including differentiation, tumor stage but no correlation with tumor site, patient sex, and age (table 3) and that was in agreement with many studies [6, 12 ,13].

Tumor progression is characterized by a stepwise accumulation of specific molecular alternations and involving multiple modificationof cell surface components, intracellular alterations and genetic molecules, ultimately resulting in invasive and metastatic cancer [4].

Some studies suggest that alterations in CD44 alternative splicing associated with transformation of colonic mucosa to carcinoma result in up- regulation of variant CD44 isoforms that have been implicated in tumor metastases, on the other hand, malignant transformation is associated with down- regulation of CD44 [15 ,18, 19].

A number of studies indicate gain of chromosome11 has been observed in human colon adenocarcinoma and may play apart in tumourigenesis and metastasis, also CD44 v8-10 isoforms are over expressed in malignant and distant metastasis[20] .Recent evidence suggests that nuclear CD44/ acetylated- STAT3

performs by an unexpected tumor-progressing function by enhancing cell outgrowth into structures where cells with properties of cancer stem cells can be generated from differentiated somatic cells and then exhibit attributes of cells that have undergone an epithelial-mesenchymal transition leading to tumor metastasis and a resulting worse prognosis[21].We conclude that CD44 is a robust marker for colorectal carcinoma .

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