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Expression of CD44s in Human Colorectal Cancer

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CD44s is a cell adhesion molecule, which belongs to the family of hyaluronan binding proteins. Antibody to CD44s is used to establish the association of its expression with the clinicopathological characteristics of colorectal cancer using immunohistochemical methods. The aim of this study is to investigate the expression of the standard form of CD44 (CD44s) in colorectal cancer tissues as compared to adjacent normal colonic tissues. Furthermore, the level of expression of CD44s in colorectal cancer tissues was correlated with the degree of histological differentiation, Duke's classification, sex, size and site of the tumor. Immunohistochemical analysis for CD44s was carried out in 49 paraffin-fixed sections of neoplastic colorectal tissues and non-neoplastic ones adjacent to the lesion, by the standard peroxidase-antiperoxidase method. Expression of these antigens were compared in normal and malignant epithelium and stromal cells. The results show that the level of CD44s in the epithelial and stromal cells was significantly higher in the colorectal cancer tissues than the normal ones. However, there was no association between the percentages of expressions of CD44s and the degree of histological differentiation, Duke's classification, sex or size of the tumor. There was however, a significantly higher expression of CD44s in the epithelium of rectal cancer than that of colonic cancer. This study indicates that the expression of CD44s is significantly higher in colorectal cancer tissues. However, further studies are required to understand its role in tumor progression and metastasis of this disease. (Pathology Oncology Research Vol 8, No 3, 170–174)

Keywords: cell adhesion, CD44, colorectal cancer, immunohistochemistry

Introduction

Metastasis is responsible for the majority of failures in cancer treatment. Some of the key molecules that have been implicated with this process are the cell adhesion molecules (CAM). Deregulation of adhesion mechanisms play a major role in the metastasis of various tumors.[1] A better understanding of the role of CAM and their modulators in tumor progression and metastasis could lead to the development of novel therapeutic strategies to prevent tumor progression and metastasis.

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CD44 is a family of glycoprotein adhesion molecules that is implicated as major contributors to tumor progression and metastasis. They are the product of a single gene, located on the short arm of human chromosome 11 and encoded by at least 20 exons. The first five (s1-s5) as well as exons 16 to 20 (s6-s10) are expressed by a large number of non-epithelial cells, including those which are hematopoietic. Their product is referred to as "standard" or "hematopoietic" form of CD44.[2] Exons 6 to 15, more often referred to as v1 to v10 are alternatively spliced.[2,3] Generally most epithelial and non-epithelial tissues express the standard form of CD44 (CD44s), whereas positive reactions with antibodies against exon v6, v9, and v4 were far more restricted. Hyaluronan, a glycosaminoglyamine, is the main ligand of CD44. It is found in the extracelullar matrix. The standard form of CD44 binds to hyaluronan.[4] The binding potential and tumorigenecity depend on whether CD44 remains

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attached to the plasma membrane or be shed in the extracellular space.[5]

The role of CD44 in tumor progression and metastasis was first documented in an animal tumor model.[6] Furthermore, the expression of CD44 v6 molecule in transfected non-metastasizing cells was reported to confer the ability to form distant metastases. Antibodies specific for this CD44 variant inhibited formation of secondary neoplastic foci when injected with tumor cells.[7] It was shown that human colonic and breast carcinomas were associated with the generation of a number of large CD44 splicing isoforms. Later observations have also shown that more than one CD44 isoform is involved in tumor progression depending on the type of neoplasm.

The role of CD44 isoforms in human tumor progression is still a matter of controversy.[8,9] The current study is aimed at determining the expression of CD44s in colorectal carcinoma using immunohistochemical methods. The results should enhance our understanding of the prevalence as well as the diagnostic and prognostic value of these molecules in human colorectal cancer.

Materials and Methods

Patients

Tissue samples from 49 patients who underwent resection of colorectal carcinoma in Mubarak Al-Kabeer hospital, Kuwait, from 1998 to 2000 were used for this study. A piece of non-necrotic tumor and a piece of unaffected colon mucosa at a standard distance of 10 cm away from the tumor were taken and fixed in 10% formaldehyde. The fixed normal and neoplastic tissues were then processed for paraffin sectioning. Tissue sections of 5 µm thick were taken on slides stained and fixed with hematoxylin and eosin (HE) stain. The slides were then examined for the degree of histological differentiation and the Duke's classification of the disease for each patient.

Immunohistochemistry

Paraffin sections from each case were immunostained with monoclonal antibodies against CD44s (DAKO, Denmark). Sections were cut and dried at 37°C, dewaxed in xylene and rehydrated using serial concentrations of ethanol. Sections were washed with Tris buffer and preincubated with normal rabbit serum (10%) for 20 minutes. Slides were incubated with primary antibody (CD44s, 1:100 dilution) and exposed to peroxidase/antiperoxidase. The slides were then counterstained with hematoxylin and reviewed. For every batch a positive control (reactive lymph node) and negative control (no primary antibody) were run simultaneously.

Expression of CD44s was evaluated by two independent observers. Where there was a discrepancy, the slides were reviewed by the two observers at a multi-headed microscope to reach a consensus. CD44s was evaluated in normal and malignant epithelium and stromal cells (fibroblasts, lymphocytes, macrophages, plasma cells). Expression of CD44s was graded both in terms of percentage of staining in each block and intensity of staining. For percentage the following grades were used: negative – no detectable staining; (+) expression in less than one third of cells; (++) expression in one-third to two-thirds of cells; (+++) expression in more than two-thirds of cells. Intensity of staining was graded as: negative, weak, moderate or strong. For all positive cases, localization of staining to cell membrane, cytoplasm or both was performed.

Statistical analysis

Statistical calculations were performed by using Statview programme package. Data were compared by using Chi-square test, and the difference between the means of continuous data were compared by using paired t-test. The percentage of expression of CD44s in both epithelial and stromal cells was used to assess the role of Duke's classification, differentiation, sex, site (colon, rectum) and size of tumor (< or > 4 cm).

Results

The mean age of patients was 55.7 years (22 females and 27 males) ranging from 27 to 84 years. In 12 patients the tumor was in the rectum while in the others the tumor was located in the colon (right, transverse and left). All the tumors were adenocarcinomas, 29 were Dukes B and 20 were Duke's C. The mean size of the tumors was 5.5 cm, ranged from 1.5 cm to 16 cm. Sixteen tumors were well differentiated, 31 were moderately differentiated and 2 were poorly differentiated. None of the patients received chemotherapy or radiotherapy prior to surgery.

Table 1. **Expression of CD44 in normal and malignant colorectal tissues**

*Level of expression**	*Epithelium*		*Stromal Cell*	
	Malignant (%)	*Normal (%)*	*Malignant (%)*	*Normal (%)*
Negative	59	90	14	16
+	8	6	24	14
++	10	4	23	19
+++	23	0	39	51

*(negative) no detectable expression; (+) expression in less than one-third of cells in each compartment (33%); (++) expression in one-third to two-third of cells in each compartment (34% to 66%); (+++) expression greater than 67% of cells.

CD44s expression in malignant and normal tissues

Staining was positive in 41% of malignant epithelial tissue in comparison to 10% of normal tissue (*Table 1*). There was high staining in stromal cells of both malignant (86%) and normal colorectal tissues (84%). CD44s staining was either cytoplasmic, mixed or membranous. CD44s staining was strong to moderate in 39% of epithelial cells and 74% stromal cells. Furthermore, in adjacent normal tissue CD44s staining was moderate to strong in only 4% of epithelial cells and 65% of stromal tissue (*Table 2*). CD44s staining is shown in *Figure 1*.

Table 2. **Intensity of CD44 staining in cells of normal and malignant colorectal tissues**

*Intensity of expression**	*Epithelium*		*Stromal Cell*	
	Malignant (%)	*Normal (%)*	*Malignant (%)*	*Normal (%)*
Negative	59	90	14	16
Weak	2	6	12	19
Moderate	18	2	39	20
Strong	21	2	35	45

Clinical and histopathological features

The percentage of expression in epithelial and stromal cells was compared using Duke's staging, sex, size and degree of differentiation. Statistical significance was not observed (*Table 3*). There was however higher expression of CD44s in epithelial cells of rectal tumors than colon tumors, which was found to be statistically significant ($p = 0.03$) (*Table 3*).

Discussion

CD44, was first identified in 1982 in lymphocytes.[10] It is an adhesion molecule involved in cell-to-cell and cell-to-matrix interactions. CD44 is normally weakly expressed in the colon, as the "s" or standard form in few cells at the bases of the crypts.[11] As reported by others[12-15] we have seen topographic progression of CD44s expressions from peripheral mucosa with normal appearance to the adjacent carcinoma.[12-15] Ninety percent of normal colonic epithelial cells did not stain for CD44s. However, 84% of stromal cells were stained for CD44s in normal tissues. Furthermore, in tumor tissues 41% were staining for CD44s in epithelial cells while 86% of stromal cells were staining for CD44s. It is possible that only minimal amounts of protein are necessary to anchor the epithelial cells of a normal mucosa to the extracellular matrix. This amount may be too small to be detected by immunohistochemistery. The higher percentage of CD44s in tumor tissue is probably essential to anchor the abnormally proliferating cells in place. Sugino et al[16] reported that the loss of CD44 seems to play a role in the development of invasion, which may facilitate loss of cell-cell cohesion, detachment of the basement membrane, and subsequent infilteration of the underlying tissue. In addition, out of 20 patients with positive staining in tumor tissues, 95% of the staining was moderate to strong, while only 40% of staining in normal tissues was moderate to strong. These findings support previous reports that CD44s is normally weakly expressed in the colon.[11]

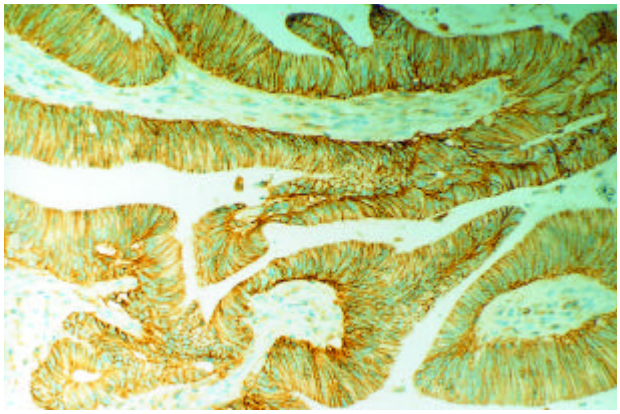

Figure 1. CD44s staining in colorectal cancer tissues.

It has been found that the role of CD44 isoforms in human tumor progression is a matter of controversy.[8,9] Some studies associated the expression of CD44v6 with tumor progression and metastatic potential.[17-20] It has also been suggested that a differential expression of CD44 splice variants could occur during neoplastic transformation and cancer progression.[11,17,21] CD44v6 was also reported to be strongly associated with the spread of metastatic tumors.[6,17,18,22,23] This was associated with Duke's C and D[17,24] and adversely with prognosis.[9,11,21] Other studies questioned the role of CD44v6 in colon cancer progression.[25] Abbasi et al[26] found that the expression of CD44v6 is not restricted to metastatic tumors, as it occurs in the normal, adenomatous, and carcinomatous colonic epithelium. Mazeki et al[27] have shown that the expression of CD44 variants in adenomatous colorectal polyps was as strong as in primary and metastatic tumor tissues. Gotley et al[25] have shown using both immunohistochemistery and reverse transcription polymerase chain reaction analysis of CD44 variant mRNA, that none of the CD44 variants epitopes correlated with tumor progression or with colorectal tumor metastasis to the liver.

In this study we found no correlation between the level of CD44s expression and certain clinical parameters such as Dukes classification, degree of histological differentiation, sex and size of the tumor. There was however a significantly higher expression of CD44s in the epithelium of

Table 3. **Expression of CD44 in relation to Duke's staging, degree of differentiation, sex, size and site**

		Epithelium		*Stromal Cell*	
		(%)	*p value*	*(%)*	*p value*
Duke's staging	B	31.8	0.3	49	0.9
	C	21.8		48	
Degree of differentiation	Well	33.7	0.3	55.6	0.3
	Moderate	23.3		45.8	
Sex	Female	18.6	0.12	40	0.08
	Male	35.2		55.5	
Size	Size	22.6	0.3	48.5	0.9
	Site	32.3		48.6	
Site	Rectum	48	0.03	58.4	0.2
	Colon	21.2		45.4	

rectal cancer (48%) than colonic cancer (*Table 3*). Furthermore, we evaluated CD44s expression not only on the basis of staining intensity and percentage of expression but also staining sites. When the CD44s expression sites were located at the cell-cell bounderies, they were classified as membranous and if not they were classified as cytoplasmic. The present study demonstrated that CD44s expression in the adjacent normal tissue was predominantly cytoplasmic, while it is cytoplasmic and membranous in tumor tissues. This could be explained by the fact that the linkage of transmembranous CD44s and cytoplasmic proteins is necessary for the formation of strong cell-cell adhesion and may reflect biological behavior related to different pathways of tumorigenesis.[12]

This study indicates that CD44s is expressed significantly higher in colorectal cancer tissues. However, it's role in tumor progression and metastasis in colorectal cancer require further studies.

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