

Lack of CD44 variant 6 expression in rectal cancer invasive front associates with early recurrence

Suvi Tuulia Avoranta, Eija Annika Korkeila, Kari Juhani Syrjänen, Seppo Olavi Pyrhönen, Jari Toivo Tapio Sundström

Suvi Tuulia Avoranta, Eija Annika Korkeila, Kari Juhani Syrjänen, Seppo Olavi Pyrhönen, Department of Oncology and Radiotherapy, University of Turku and Turku University Hospital, FIN-20521 Turku, Finland

Jari Toivo Tapio Sundström, Department of Pathology, University of Turku and Turku University Hospital, FIN-20520 Turku, Finland

Author contributions: All authors designed the research; Avoranta ST and Korkeila EA collected the clinicopathological data; Avoranta ST and Sundström JTT analyzed the immunohistochemical staining; Avoranta ST and Syrjänen KJ performed the statistical work; Avoranta ST wrote the paper; Korkeila EA, Syrjänen KJ, Pyrhönen SO and Sundström JTT gave comments and suggested revisions.

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Correspondence to: Tuulia Avoranta, MD, Department of Oncology and Radiotherapy, University of Turku and Turku University Hospital, Hämeentie 11, FIN-20521 Turku, Finland. stavor@utu.fi

Telephone: +35-82-3131834 Fax: +35-82-3132809

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Abstract

AIM: To investigate the prognostic value of CD44 variant 6 (CD44v6), a membranous adhesion molecule, in rectal cancer.

METHODS: Altogether, 210 rectal cancer samples from 214 patients treated with short-course radiotherapy (RT, $n = 90$), long-course (chemo) RT ($n = 53$) or surgery alone ($n = 71$) were studied with immunohistochemistry for CD44v6. The extent and intensity of membranous and cytoplasmic CD44v6 staining, and the intratumoral membranous staining pattern, were analyzed.

RESULTS: Membranous CD44v6 expression was seen in 84% and cytoplasmic expression in 81% of the cases. In 59% of the tumors with membranous CD44v6 expression, the staining pattern in the invasive front was determined as "front-positive" and in 41% as "front-negative". The latter pattern was associated with narrower circumferential margin ($P = 0.01$), infiltrative growth pattern ($P < 0.001$), and shorter disease-free survival in univariate survival analysis ($P = 0.022$) when compared to the "front-positive" tumors.

CONCLUSION: The lack of membranous CD44v6 in the rectal cancer invasive front could be used as a method to identify patients at increased risk for recurrent disease.

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Key words: CD44 variant 6; Rectal cancer; Invasive front; Disease-free survival; Disease-specific survival

Peer reviewer: Dr. Cem Onal, Department of Radiation Oncology, Baskent University Faculty of Medicine, Adana Research and Treatment Center, 01120 Adana, Turkey

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INTRODUCTION

In 2008, approximately 1.2 million new cases of colorectal cancer (CRC) were diagnosed globally^[1]. Preoperative radiotherapy (RT) for rectal cancer has improved local control rates^[2], but patients may still present with fairly differing clinical responses and prognoses^[3]. To improve

disease predictability, strong expectations have been placed on tumor markers.

CD44 is a family of transmembrane glycoproteins serving as a major receptor for hyaluronate, an important component of the extracellular matrix (ECM)^[4]. *CD44* has been suggested to act both as a tumor-suppressing cofactor and as a growth- and invasiveness-promoting molecule^[5] through its participation in many important cellular processes, including adhesion, growth regulation, survival, differentiation and motility^[6]. Of the several isoforms produced by alternative splicing of the *CD44* gene^[7], variant 6 (CD44v6) has been intensely studied in relation to CRC progression and outcome^[8].

Induction in CD44v6 expression is suggested to represent an early event in colorectal carcinogenesis^[9], and has in some studies been related to disease progression, metastatic potential^[7,9,10], and poor disease outcome^[11]. Instead, in some other studies, stronger CD44v6 expression has been reported in adenomas than in carcinomas, as well as in primary carcinomas compared to metastatic tumors^[12], and has been shown to have favorable^[8,13] or no^[14] effect on CRC outcome. Furthermore, strong expression has been shown to indicate more favorable response to chemotherapy^[15].

In most of the previous studies on CD44v6 expression in colorectal tumors, both colonic and rectal carcinomas have been included. There is, however, some evidence that proximal and distal colonic lesions differ in their expression of CD44v6^[16]. In those few studies including solely rectal tumors^[17-19], CD44v6 has not been considered in relation to RT. In the present study, we examined the expression of CD44v6 immunohistochemically in a cohort of 214 primary rectal carcinomas treated with or without preoperative (chemo)radiotherapy. Considering the fundamental role of the tumor invasive front in tumor-host interaction^[20], as well as the discrepant data on the prognostic value of the extent of CD44v6 expression in CRC^[8,11,14], intratumoral staining pattern of this protein was also systematically assessed. We hypothesized that this aspect could offer additional information of the significance of CD44v6 expression in rectal cancer.

MATERIALS AND METHODS

Patients and study material

The material of this study consisted of formalin-fixed, paraffin-embedded tissue samples from 214 patients operated upon for rectal cancer at Turku University Hospital between 2000 and 2009. Operative samples were retrieved from the archives of the Department of Pathology, Turku University Hospital. To ensure a biologically and therapeutically homogeneous study population, only tumors of the middle and lower rectum were included. Superficial tumors treated with excision only, as well as patients with distant metastases at the time of diagnosis, were excluded. The use of archival tissue material was approved by the National Supervisory Au-

thority for Welfare and Health (permission No. Dnro 1709/32/300/02, May 13th 2002).

Tumor staging was done according to the tumor node metastasis classification of malignant tumors, 2002^[21]. Selection of treatment was based on preoperative tumor staging which included computed tomography (CT) or magnetic resonance imaging of the rectum, CT of the abdomen, and X-ray or CT of the chest. According to the common clinical guidelines^[22], patients were treated either with short-course preoperative RT ($n = 90$), long-course preoperative (chemo) RT ($n = 53$), or received no treatment before surgery ($n = 71$). Short-course RT consisted of five 5-Gy fractions during 1 wk, with surgery on the following week. Long-course RT was given in 1.8-Gy fractions to a total dose of 50.4 Gy over a 6-wk period, with ($n = 44$) or without ($n = 9$) concomitant chemotherapy, and operation was performed at 5-7 wk after RT. Chemotherapy regimens were either bolus 5-fluorouracil ($n = 5$) or capecitabine ($n = 39$). Anterior resection was performed in 118 cases (55%), and abdominoperineal resection in 92 cases (43%). In four cases (2%), some other technique, such as low Hartmann's procedure, was used. The presence of vascular invasion was assessed in 159 cases, in which cancer cells could be detected in 47 cases (30%), either in the extramural lymphatic or blood vessels. Postoperative adjuvant chemotherapy was given to patients with lymph node positive or high-risk lymph node negative tumors according to the standard clinical practice^[22]. The median follow-up time was 45.5 mo. In 61 patients (29%), local or distant disease recurrence was seen. Of them, 39 (64%) were treated with chemotherapy with or without biological treatments. Among these 39 cases, progression-free survival (PFS) was retrospectively defined from the medical records, and the median PFS was 20.7 mo. The key demographic and clinical characteristics of the patients are summarized in Table 1.

Immunohistochemistry

Four tumors with T0 after long-course RT were not stained, and accordingly, the actual study material consisted of 210 samples. The most representative blocks were selected and cut into 5- μ m sections. Antigen retrieval was performed by heating in a microwave oven in 10 mmol/L sodium citrate, pH 6.0 two times for 7 min. Endogenous peroxidase activity was blocked by incubating the slides in 0.3% hydrogen peroxide in Tris-buffered saline. The sections were subjected to immunohistochemical staining with monoclonal antibody for CD44v6 (concentration 1:1000; Bender MedSystems, Vienna, Austria) and monoclonal mouse anti-cytokeratin (Ck-Pan) (clone: AE1/AE3, concentration 1:50; Zymed Laboratories, South San Francisco, CA, United States) using EnVision + Dual Link System - HRP (Dako, Denmark).

Evaluation of immunohistochemistry for CD44v6

Immunohistochemistry (IHC) staining was individually evaluated by two observers (Avoranta ST and Sundström

Table 1 Clinical characteristics of the patients *n* (%)

Clinical characteristics	Short course RT	Long course RT	Control	<i>P</i> ¹
Study population (<i>n</i> = 214)				
Male	57 (63)	34 (64)	32 (45)	0.03
Female	33 (37)	19 (36)	39 (55)	
Mean age (yr)	65.2	64.7	74.5	< 0.001
Preoperative T				
T1-2	28 (31)	0 (0)	22 (31)	
T3	54 (60)	2 (4)	12 (17)	< 0.001
² T4	1 (1)	50 (94)	3 (4)	
Tx	7 (8)	1 (2)	34 (48)	
Postoperative T				
T1	3 (3)	2 (4)	5 (7)	
T2	34 (38)	6 (11)	26 (37)	
T3	50 (56)	27 (51)	37 (52)	< 0.001
T4	3 (3)	14 (26)	3 (4)	
T0	0	4 (8)	0	
Postoperative N				
N0	53 (59)	36 (68)	41 (58)	
N1	26 (29)	14 (26)	15 (21)	0.33
N2	11 (12)	3 (6)	12 (17)	
Nx	0	0	3 (4)	
Grade				
G1	9 (10)	10 (19)	12 (17)	
G2	58 (64)	33 (62)	48 (68)	0.07
G3	21 (23)	3 (6)	11 (16)	
Gx	2 (2)	7 (13)	0	
³ Crm (mm)				
0	3 (4)	11 (25)	4 (9)	
0 ≤ crm ≤ 2	9 (12)	7 (16)	7 (16)	0.009
> 2	65 (84)	26 (59)	32 (74)	
Disease specific outcome				
Alive without recurrence	64 (71)	25 (47)	39 (55)	
Alive with recurrence	7 (8)	7 (13)	4 (6)	0.07
Died of disease	11 (12)	12 (23)	18 (25)	
Died of other causes	8 (9)	9 (17)	10 (14)	

¹Differences between the three treatment groups using Fisher's exact test; mean age with analysis of variance; ²Includes the T3 tumors with threatened circumferential margin involvement; ³Circumferential margin, data available in 164 cases. RT: Radiotherapy.

JTT) blinded to clinical data. Squamous basal cells of anal epithelium included in some of the cases were used as a positive control for CD44v6. Using 5×, 10× and 20 × objectives, the immunostaining of CD44v6 in every slide was assessed using the following parameters: (1) the extent of expression (membranous and cytoplasmic staining scored separately); (2) the intensity of expression (membranous and cytoplasmic staining scored separately); and (3) the intratumoral pattern of membranous CD44v6 expression. The extent of expression within the whole tumor area was analyzed using four categories: 1 for immunopositivity < 5%, 2 for 5%-20%, 3 for 21%-50% and 4 for > 50%. The intensity of expression was analyzed using four categories: 1 for negative staining; 2 for weak staining (a faint immunopositivity in the membrane or cytoplasm); 3 for moderate staining (a clear immunopositivity in the membrane or cytoplasm); and 4 for strong staining (a pronounced immunostaining in the membrane or cytoplasm equivalent to that of the basal cells of anal squamous epithelium). The intratumoral pattern of mem-

branous CD44v6 expression was classified into three categories: 1 for immunopositivity mainly in the tumor invasive front; 2 for immunopositivity mainly in the tumor central areas; and 3 for heterogenous immunopositivity (no apparent difference in CD44v6 expression between the invasive front and central areas of tumor).

For statistical purposes, categories 1 and 2 were studied as one group and categories 3 and 4 as another group when analyzing the extent and intensity of expression. With regard to the intratumoral pattern of membranous staining, categories 1 and 3 were studied as one group ("front-positive"), and category 2 as in its own group ("front-negative").

Evaluation of tumor growth pattern

In order to evaluate tumor growth pattern, hematoxylin-eosin and Ck-Pan stainings were scrutinized in each operative sample. Using the Jass' classification of the tumor growth pattern, tumours were appointed as "expanding" when the invasive margin was pushing or reasonably well circumscribed, and "infiltrating" when the tumor invaded in a diffuse manner with widespread penetration into adjacent normal tissues^[23].

Evaluation of tumor regression grade

Tumor regression grade (TRG) after long-course RT was defined by a pathologist (Sundström JTT) as poor, moderate or excellent according to the modified Dworak and Rödel scales, as described recently^[24]. Briefly, poor TRG was defined as minimal or no tumor regression after (chemo) RT. In case of poor response, many tumor cells remained after treatment. In tumors with moderate response, there were only some tumor cells or tumor cell groups left in the primary tumor, lymph nodes or perirectal fat. In tumors with excellent response, few or no tumor cells could be detected.

Statistical analysis

Statistical analysis were run using IBM SPSS® Statistics 19.0.1 for Windows software. Frequency tables were analyzed using the χ^2 test, with the likelihood ratio (LR) or Fisher's exact test for categorical variables. 2 × 2 tables were used to calculate odds ratio and 95% CI using the exact method. Fisher's exact test, Spearman's correlation and LR were used to assess the significance of the correlation between individual variables in univariate analysis. Inter-observer reproducibility of the assessments was tested using weighted κ . It was calculated using the intraclass correlation coefficient (ICC) test, in parallel mode with a two-way random model, using consistency assumption and the average-measures option to interpret the ICC (95% CI). The ICC of assessments was very good with weighted κ ranging from 0.70 to 0.90.

Univariate survival analysis for disease-free survival (DFS) and disease-specific survival (DSS) was based on the Kaplan-Meier method where stratum-specific outcomes were compared using log-rank (Mantel-Cox) statistics. To adjust for the covariates, a Cox proportional

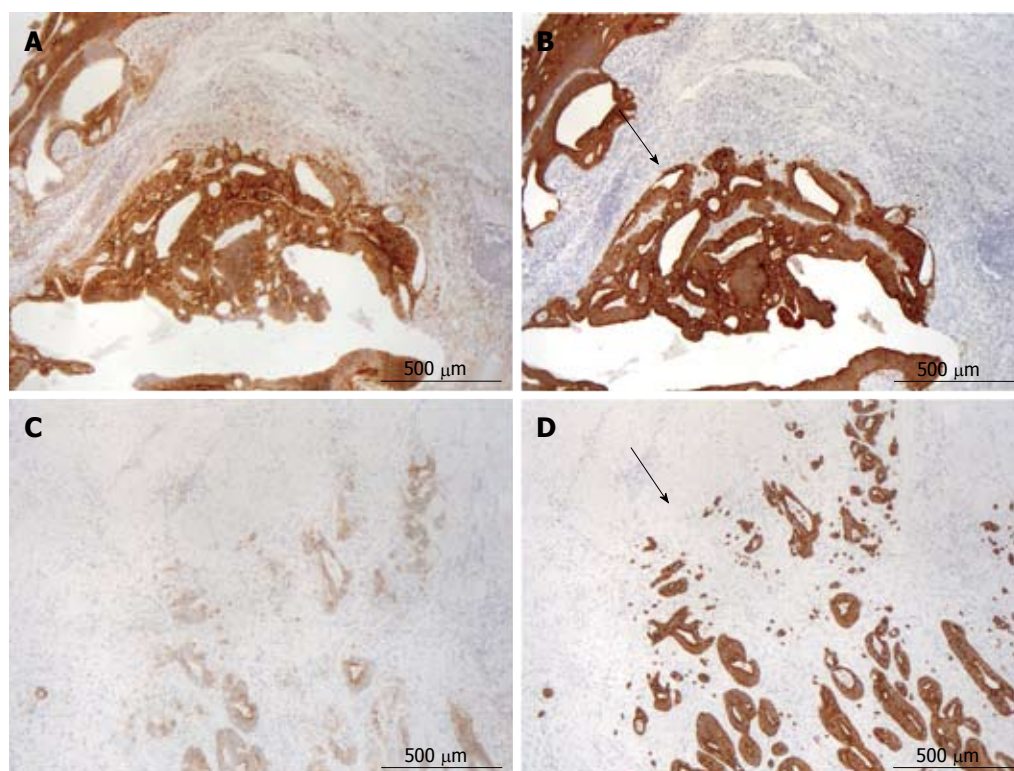


Figure 1 The immunohistochemistry for CD44 variant 6 and Pan-cytokeratin. A: The same tumor with “front-positive” membranous staining of CD44 variant 6 (CD44v6); B: Expanding growth pattern as shown with Pan-cytokeratin (Ck-Pan) staining; C: The same tumor with “front-negative” membranous staining of CD44v6; D: Infiltrating growth pattern as shown with Ck-Pan staining. Invasive front is indicated with arrows.

hazards regression model was used, covariates (as listed separately) being inserted using the enter mode. Of the variables significant in univariate analysis, two (circumferential margin, vascular invasion) were not included in the multivariate model because the data were incomplete. All statistical tests were two-sided and declared significant at a *P* value of < 0.05.

RESULTS

CD44v6 expression in tumors

Altogether, 177 out of 210 tumors (84%) showed membranous and 170 (81%) cytoplasmic CD44v6 expression. Both the extent and intensity of membranous and cytoplasmic staining were closely correlated ($r = 0.4$; $r = 0.31$, $P < 0.001$ for both). Of the 177 tumors with membranous CD44v6 positivity, 105 (59%) were “front-positive” (Figure 1A), usually showing an expanding growth pattern (Figure 1B), and 72 (41%) were “front-negative” (Figure 1C), usually showing an infiltrating growth pattern (Figure 1D).

Tumor growth pattern

In 206 tumors, a proper invasive front could be detected to evaluate tumor growth pattern. Altogether, 99 (48%) showed an expanding (Figure 1B) and 107 (52%) an infiltrating growth pattern (Figure 1D).

CD44v6 related to treatment groups and TRG

Only the extent of cytoplasmic staining was related to

treatment group. Patients in the long-course RT group had less cytoplasmic CD44v6 expression in their tumors as compared to patients in the control group ($P = 0.002$). Within the long-course RT group, no differences in CD44v6 expression were seen according to concomitant chemotherapy. TRG after long-course RT, as analyzed also from the four tumors without IHC for CD44v6, was poor in 27 cases (51%), moderate in 14 cases (26%) and excellent in 12 cases (23%). No significant differences were seen in CD44v6 expression, as related to TRG.

CD44v6 related to clinicopathological variables

No significant differences were seen in the extent or intensity of CD44v6 expression concerning the variables listed in Table 1. With regard to the intratumoral pattern of membranous staining, “front-negative” was associated with narrower circumferential margin ($P = 0.01$), infiltrative tumor growth pattern ($P < 0.001$), and a greater risk for disease recurrence ($P = 0.01$ overall) as demonstrated in Table 2. No difference was seen in the number of patients receiving postoperative adjuvant chemotherapy between “front-positive” and “front-negative” groups ($P = 0.65$). Of the “front-positive” tumors, 37% were diagnosed before and 63% after 2005; the corresponding proportions for “front-negative” tumors being 22% and 78%, respectively ($P = 0.035$). No other significant correlations were seen between the staining pattern and clinicopathological variables, including PFS.

Table 2 Intratumoral staining pattern of membranous CD44 variant 6 expression related to treatment group and some clinicopathological variables *n* (%)

Variable (<i>n</i> = 177)	"Front-positive" (<i>n</i> = 105)	"Front-negative" (<i>n</i> = 72)	<i>P</i> ¹
Treatment group			
Short-course RT	46 (44)	32 (44)	0.87
Long-course RT	24 (23)	14 (19)	
Control	35 (33)	26 (36)	
Postoperative T			
T1-2	43 (41)	21 (29)	0.19
T3	55 (52)	42 (58)	
T4	7 (7)	9 (13)	
Postoperative N			
N0	68 (65)	40 (56)	0.37
N1-2	36 (34)	31 (43)	
Nx	1 (1)	1 (1)	
² Crm			
≤ 2 mm	14 (18)	22 (38)	0.01
> 2 mm	64 (82)	36 (62)	
Tumor growth pattern			
Expanding	63 (60)	23 (32)	< 0.001
Infiltrating	42 (40)	49 (68)	
Disease-specific outcome			
Alive without recurrence	68 (65)	39 (54)	0.01
Alive with recurrence	3 (3)	12 (17)	
Died of disease	21 (20)	15 (21)	
Died of other causes	13 (12)	6 (8)	

¹Pearson χ^2 ; *P* value for difference between "front-positive" and "front-negative" groups; ²Circumferential margin, data available in 136 out of 177 cases. RT: Radiotherapy.

Recurrence and survival analysis

In univariate survival (Kaplan-Meier) analysis, no differences in DFS or DSS were seen according to the extent or intensity of CD44v6 expression, neither membranous nor cytoplasmic (data not shown). DFS (*P* = 0.022), but not DSS (*P* = 0.68), in patients with "front-negative" tumors was significantly shorter than in patients with "front-positive" tumors (Figure 2A and B). The same trend/difference was also seen in the short-course RT and control groups (*P* = 0.058 and *P* = 0.024 for difference in DFS, respectively) when analyzing treatment subgroups separately. With regard to tumor growth pattern, infiltrating tumors had shorter DFS (*P* = 0.015), and a tendency towards a shorter DSS (*P* = 0.14), as compared to expanding tumors.

The results of multivariate analysis are summarized in Table 3. The independent adverse prognostic factors for DFS were male sex, high postoperative T and the presence of positive lymph nodes, and those for DSS were patient age, postoperative T4, poor differentiation grade and disease recurrence.

DISCUSSION

Loss and gain of adhesive functions play an essential role in the progression of epithelial neoplasms^[25]. In the present study, the expression of CD44v6, a cell surface mediator of cell-to-ECM adhesion^[4], was systematically stud-

Table 3 Results of multivariate analysis

Variable	DFS			DSS		
	Adjusted HR	95% CI	<i>P</i>	Adjusted HR	95% CI	<i>P</i>
Sex						
Female (ref)	1.0			1.0		
Male	1.9	1.0-3.5	0.04	1.5	0.7-3.0	0.3
Age						
< 70 yr (ref)	1.0			1.0		
≥ 70 yr	1.4	0.8-2.4	0.3	4.7	2.1-10.5	< 0.001
Postoperative T						
T1-2 (ref)	1.0			1.0		
T3	3.2	1.4-7.2	0.005	2.0	0.8-5.1	0.1
T4	5.9	2.0-17.0	0.001	4.8	1.4-17.0	0.02
Postoperative N						
N0 (ref)	1.0			1.0		
N1-2, Nx	2.0	1.1-3.7	0.02	0.8	0.4-1.7	0.6
¹ Postoperative grade						
G1 (ref)				1.0		
G2				1.3	0.4-3.9	0.7
G3				5.2	1.6-17.4	0.007
Growth pattern						
Expanding (ref)	1.0			1.0		
Infiltrating	1.0	0.5-1.8	0.9	0.8	0.4-1.7	0.6
¹ CD44v6 staining pattern						
"Front-positive" (ref)	1.0					
"Front-negative"	1.4	0.8-2.6	0.2			
Recurrence						
No (ref)				1.0		
Yes				74.3	17.0-326.7	< 0.001

¹Not significant in univariate analysis and thus not included in multivariate model. Ref: Reference category; HR: Hazard ratio; DFS: Disease-free survival; DSS: Disease-specific survival; CD44v6: CD44 variant 6.

ied in a cohort of 214 primary rectal carcinomas treated with or without preoperative RT. The actual extent of CD44v6 expression was not associated with disease progression or outcome, but the analysis of its intratumoral staining pattern showed that patients with "front-negative" staining pattern had a significantly shorter DFS.

In our series, both membranous and cytoplasmic staining of CD44v6 was present in most cases, with a strong mutual correlation as reported earlier^[8]. We found that preoperative RT affected cytoplasmic but not membranous CD44v6 expression. Although membranous CD44v6 is known to bind extracellular hyaluronate and growth factors^[5], the biological significance of cytoplasmic CD44v6 is rather unknown^[8]. In some studies it has been considered to have no functional roles^[8], whereas in others, it has been related to neoplastic transformation^[12,26] suggesting a role in loss of differentiation^[26]. As preoperative RT has been shown to cause remarkable histological alterations^[27], the reason for a decrease in cytoplasmic CD44v6 expression after RT could reflect these histological changes. However, further studies are required to elucidate this suggestion.

The prognostic value of CD44v6 expression, regarding the extent of expression, has been studied with inconclusive results in CRC^[8,11,14]. In accordance with Morrin *et al*^[14], we found no differences in disease progression indicators and patient outcome according to the

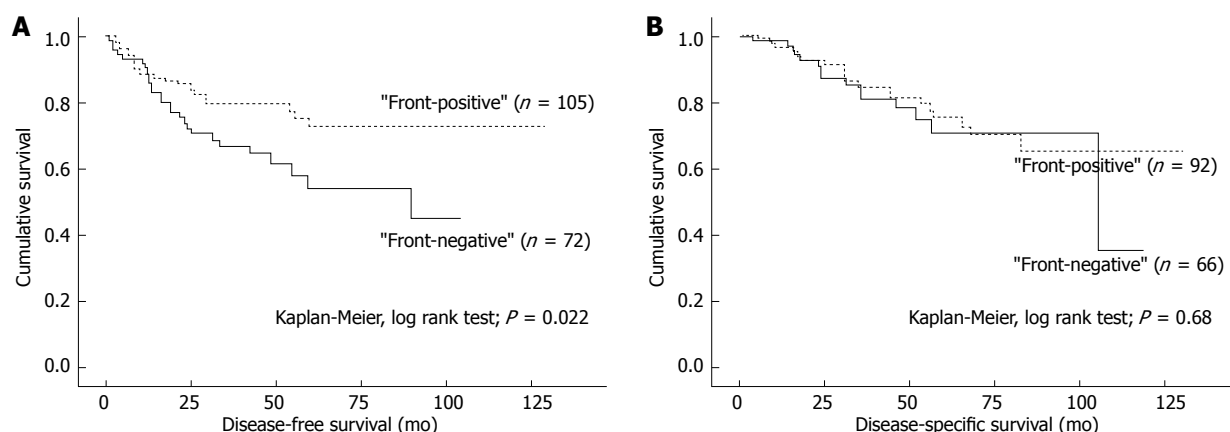


Figure 2 CD44 variant 6 related to disease-free and disease-specific survival in univariate analysis. Disease-free survival (A) and disease-specific survival (B) according to “front-positive” and “front-negative” membranous staining pattern of CD44 variant 6.

extent of CD44v6 expression. This finding is in contrast with some recent studies on rectal tumors, in which high membranous CD44v6 expression was shown to be associated with local recurrence^[19] as well as shorter DFS^[17-19] and DSS^[17,18]. In contrast to these studies, we also included preoperatively treated tumors in our study to assess the possible relation of CD44v6 expression to TRG. This dissimilar study design may explain some of the discrepancy even though we found no differences in membranous CD44v6 expression parameters between the treatment or TRG-groups. In addition, the discrepancy may originate from differences in antibodies, cut-off points as well as disease subtypes in different studies as earlier discussed by Naor *et al.*^[28]

In addition to the extent of CD44v6 expression, we also assessed the intratumoral staining pattern of membranous expression. Given that tumor-host interaction at the invasive CRC front is thought to represent a dynamic interface between pro- and antitumor factors^[20], it is not surprising that abnormalities in cell adhesion functions are seen in this compartment^[29,30]. Although loss of membranous CD44v6 expression towards the CRC invasive front has been reported^[8,12], our study is, to our knowledge, the first to assess the relation of membranous CD44v6 staining pattern to rectal cancer outcome. Furthermore, some of the previous studies are based on tissue microarray analyses, questioning the reliability of invasive front detection. Interestingly, “front-negative” tumors were related to more narrow postoperative circumferential margin and infiltrative tumor growth pattern as compared to “front-positive tumors” with apparent CD44v6 expression in the invasive front. Previously, Ishida^[31] and Zlobec *et al.*^[8] with their co-workers have shown weak/negative CD44v6 expression to be related to infiltrating growth pattern, as assessed by its overall extent irrespective of its localisation. Our results may indicate that more important than the actual quantity of CD44v6 expression is, indeed, its localisation within the tumor. It is possible that “front-negative” tumors are more difficult for surgeon to resect with wide margins as they grow in a more diffuse manner, explaining the more

narrow margins seen in these lesions.

Our observations lend support to the view of Zlobec *et al.*^[8], and Coppola *et al.*^[12], suggesting that the loss of membranous CD44v6 at the advancing edge of the tumor results in defective binding of the tumor cells to ECM, increasing their mobility and metastatic potential^[8,12]. Further supporting this view, treatment with hyaluronic acid has recently been shown to delay the growth of residual colon carcinoma cells after chemotherapy^[32]. Indeed, CD44v6 is known to have a higher affinity for hyaluronate, as compared to standard CD44^[33], and, CD44v6-expressing tumors cells could be more effectively entrapped within the hyaluronic acid at the primary site^[12]. In addition to binding with extracellular hyaluronate, membranous CD44v6 expression in the invasive front may also be important due to its intercellular adhesion properties, because loss of CD44v6 expression has been correlated to the loss of E-cadherin expression^[8].

Importance of the localization of CD44v6 expression was further supported by the fact that “front-negative” tumors more often developed recurrent disease with shorter DFS as compared to patients with “front-positive” tumors. This was also observed in the subgroup analysis for the control group, and with a similar trend in the short-course RT group. No such appearance was seen in the long-course RT group, possibly due to the smaller number of cases available for this assessment. In some of the long-course RT cases, few tumor glands were left after treatment, and for this reason, no evident invasive front could be detected. However, despite this evident difference in DFS between the patients with “front-positive” and “front-negative” tumors, these two groups had practically identical DSS, which, due to the differences in the year of diagnoses between these two groups, could reflect the improvements in the treatment of recurrent rectal cancer.

The staining pattern of CD44v6 lost its significance in multivariate analysis. As in a number of other studies^[34], we also found infiltrating tumor growth pattern to suggest an adverse DFS and a tendency towards a shorter DSS. The close correlation of CD44v6 staining

pattern with this prognostic factor may at least partly explain the lack of CD44v6 as an independent prognostic factor in our study. In addition, the other functions of this protein, such as binding of growth factors^[35], may subvert the advantage of “front-positive” tumors.

Taken together, we found that tumors with “front-negative” CD44v6 membranous staining pattern were strongly related to invasive behavior. Our results substantiate the hypothesis that the lack, rather than overexpression, of membranous CD44v6 in the invasive tumor front contributes to rectal cancer progression. Although associated with a shorter DFS in univariate analysis, “front-negative” phenotype did not prove to be an independent prognostic factor in multivariate analysis, possibly confounded by other prognostic factors, including tumor growth pattern. We propose that analyzing the intratumoral staining pattern of membranous CD44v6 could offer a simple tool to predict the patients at increased risk for disease recurrence and thus in need of more aggressive postoperative treatment approaches and close monitoring.

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COMMENTS

Background

Colorectal cancer is a common malignancy. Radiotherapy alone or in combination with chemotherapy has been used to lower the risk of local recurrence. Still, a significant proportion of patients comes down with disease recurrence that is challenging to treat and causes harmful symptoms. New prognostic markers are constantly being studied to identify better the patients who need more aggressive treatment approaches in addition to surgery. CD44 variant 6 (CD44v6), a cell membrane receptor binding extracellular components, has been demonstrated to associate with rectal cancer progression and prognosis, but with discrepant results.

Research frontiers

In many studies, CD44v6 has been suggested to play a role in tumor progression and metastasis. The important area in the work was to study whether there is a difference in rectal cancer prognosis according to the intratumoral staining pattern of CD44v6. In contrast to previous studies, authors also included patients treated with radiotherapy before surgery.

Innovations and breakthroughs

The previous studies on the percentage and intensity of CD44v6 expression in rectal cancer have not been conclusive with regard to prognosis, because the overexpression of this protein has been associated with both favorable and adverse survival. Although alteration of CD44v6 expression in tumor invasive front has been demonstrated, its association with rectal cancer prognosis has not been studied in detail. Interestingly, the authors found that the patients with tumors showing CD44v6 expression in the invasive front presented with a longer disease-free survival time than did the patients whose tumors expressed protein more centrally. Moreover, the latter “front-negative” type was associated with infiltrating tumor growth and narrow tumor-free margin, both known to be adverse prognostic factors. Instead, the authors did not find the percentage and intensity of CD44v6 staining to be related to disease prognosis. The authors concluded that for rectal cancer progression and prognosis, more important than the actual amount of CD44v6 might be its distribution within the tumor.

Applications

The study suggests that patients with “front-negative” rectal cancer may need

more aggressive treatment and monitoring after surgery.

Terminology

Prognostic factor is a situation or a condition, or a characteristic of a patient, that can be used to estimate the chance to recover from a disease or the risk for the disease recurring. Invasive front is the interface of tumor and host tissue; the deepest rim of cancerous tissue grown in adjacent non-cancerous tissues.

Peer review

In this work, the authors investigated the prognostic value of CD44v6 in patients treated with/without preoperative radiotherapy. The results are interesting and suggest that the lack of membranous CD44v6 in the rectal cancer invasive front could be used as a method to identify patients at increased risk for recurrent disease. The article is generally well written.

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