# *CEACAM-7*: A predictive marker for rectal cancer recurrence

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***Background.*** *The identification of rectal cancer patients predisposed to developing recurrent disease could allow directed adjuvant therapy to improve outcomes while decreasing unnecessary morbidity. This study evaluates carcinoembryonic antigen cellular adhesion molecule-7 (CEACAM-7) expression in rectal cancer as a predictive recurrence factor.*

***Methods.*** *A single-institution colorectal cancer database and a frozen tissue biobank were queried for rectal cancer patients. CEACAM-7 messenger RNA (mRNA) expression from normal rectal mucosa and rectal cancers was analyzed using quantitative real-time polymerase chain reaction (PCR). Expression-level differences among normal tissue, disease-free survivors, and those that developed recurrence were analyzed.*

***Results.*** *Eighty-four patients were included in the study, which consisted of 37 patients with nonrecurrent disease (median follow-up, 170 months), 29 patients with recurrent disease, and 18 patients with stage IV disease. CEACAM-7 expression was decreased 21-fold in rectal cancers compared with normal mucosa (*P = *.002). The expression levels of CEACAM-7 were relatively decreased in tumors that developed recurrence compared with nonrecurrence, significantly for stage II patients (14-fold relative decrease,* P = *.002). For stages I–III, disease-free survival segregates were based on relative CEACAM-7 expression values (*P = *.036), specifically for stage II (*P = *.018).*

***Conclusion.*** *CEACAM-7 expression is significantly decreased in rectal cancer. Expression differences between long-term survivors and those with recurrent disease introduce a potential tumor marker to define a subset of patients who benefit most from adjuvant therapy. (Surgery 2010;147:713-9.)*

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Despite a multimodality approach to rectal cancer treatment, reported recurrence rates vary from 11–40% and outcomes depend mainly on the disease stage.[1,2] Determining which patients are at the greatest risk of recurrence and thus offering them the most aggressive therapy remains a challenge. Accurate patient selection for neoadjuvant therapy would improve outcomes for those lymph node-negative cancers that might not otherwise receive chemoradiation, while sparing others the unnecessary morbidity of treatments that may not affect the outcome.[3] The ability to predict rectal cancer recurrence may lie in specific gene expression patterns within each individual tumor.

The carcinoembryonic antigen (CEA) family of genes is a potential biomarker for colorectal neoplasia, as it has been shown to be expressed in a variety of epithelial derived neoplasms, including colorectal cancer.[4] In general, the 12-member CEA gene family exhibits homophilic and heterophilic adhesion properties,[5] and their functional deregulation has been linked to cancer and has been shown to promote metastases in animal models.[6] One particular CEA gene family member, the CEA cellular adhesion molecule-7 (*CEACAM-7*), regulates normal cellular differentiation.[4] Normal CEA expression, which is found only in fully differentiated epithelial cells,[7] occurs in the lower two thirds of the colonic crypts where cell–cell adhesion is typically observed but not in the upper one third of crypts.[5] Because of its regulation of normal cellular differentiation and expression patterns, it has since been suggested that CEA downregulation, specifically *CEACAM-7*, inhibits cellular differentiation, which leads to less well-differentiated tumors with worse prognosis.[7]

Although most members of the CEA family are expressed in a variety of tissues and exhibit differential expression, *CEACAM-7* (formerly CGM2) is expressed only in pancreatic and colorectal epithelium.[4] The deregulation of *CEACAM-7* has been

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shown to occur early in oncogenesis, exhibiting decreased expression in adenomas, hyperplastic polyps, and even aberrant crypt foci.[4,8] Despite the strong association with tumorigenesis and colorectal neoplasia, the impact of *CEACAM-7* on rectal cancer and recurrence has not been established. This study evaluates *CEACAM-7* expression in rectal adenocarcinomas and determines its ability to predict disease recurrence.

## METHODS

**Tissue collection and patient information.** The Institutional Review Board at the Cleveland Clinic approved this study. Patients with rectal cancer were identified from a prospectively maintained colorectal cancer database and frozen tissue biobank. Patients with follow-up of at least 36 months or with earlier recurrence were included. Patients with local recurrence were excluded to allow for a more uniform population of recurrent patients that were less likely to be affected by operative technique. No patient included in this study received neoadjuvant chemotherapy or radiation. All patients were staged by pathology according to TNM classification after formal cancer resection. Under vascular occlusion, a total mesorectal excision was performed for all patients by colorectal surgeons. Benign rectal mucosa samples were used as a normal control group for the expression analysis.

After confirmation of the cancer stage, an independent gastrointestinal pathologist confirmed all rectal cancer tissues on hematoxylin-and-eosin-stained slides included in this study. Cancer tissue that contained at least 60% adenocarcinoma and benign rectal mucosa without any signs of dysplasia were used for cancer and normal samples, respectively. Patient demographics, tumor characteristics, and clinical follow-up were reviewed.

**RNA isolation and relative gene expression assay.** The total RNA was isolated using the RNAqueous kit (Ambion, Austin, TX) according to the manufacturer's guidelines and then subject to DNAse treatment using TURBO DNA-free (Ambion). A 1-step quantitative real-time polymerase chain reaction (PCR) protocol was performed on malignant tissue and normal controls using the TaqMan Gene Expression Assay (Applied Biosystems, Foster City, CA) as described previously.[9] Human 18S ribosomal RNA (Applied Biosystems) was used as an endogenous control for all samples.

Briefly, all reactions were performed in triplicate in a total volume of 10 $\mu$L: 5 $\mu$L master mix (5Prime, Gaithersburg, MD), 0.5 $\mu$L primer and probe set (Applied Biosystems), 0.0625 $\mu$L multiscribe reverse transcriptase (Invitrogen, Carlsbad, CA), 0.4375 $\mu$L water, and 4 $\mu$L RNA at 2 ng/$\mu$L. The following thermal cycling specifications were performed on an ABI Prism 79000HT sequence detection system (Applied Biosystems): 30 min at 48°C, for reverse transcription, followed by 40 cycles of 1 min at 95°C, 15 s at 95°C, and 1 min at 60°C.

A relative quantity (RQ) expression value of *CEACAM-7* for all samples was determined using 1 normal, nonmalignant sample as the calibrator. The calibrator was chosen as the normal sample that resulted in the mean normal control expression value of all normal samples used being closest to an arbitrarily set value of 1.0, as previously described in Applied Biosystems User Bulletin No. 2 (P/N 4303859).[10] An analysis was performed on the software program SDS.2.2.2 (Applied Biosystems) using the $2^{-\Delta\Delta CT}$ method to generate RQ values.[10] The RQ values were then expressed as the mean of the $Log_{10}$ for each group representing the geometric means (95% confidence interval [CI] of the means). The differences in values between groups were then measured as fold differences.

**Statistical analysis.** The differences in categoric variables among normal, nonrecurrent, and distal-recurrent cancer groups, and stage IV cancers were analyzed using the Chi-square and Fisher exact tests. For characteristics that are quantitative in nature, the Wilcoxon rank sum (for 2-way comparison) and Kruskal-Wallis (for 3-way comparison) tests were used to compare categories with respect to distributions of expression, described by means and selected percentiles. Associations between gene expression and clinical outcomes such as survival were assessed using Cox proportional hazards models and Kaplan-Meier estimation. Normally distributed quantitative variables were reported as mean ± standard deviation, and non-normally distributed quantitative variables were reported as median, minimum, and maximum. Overall significance levels were set at $P < .05$.

## RESULTS

**Patient demographics and survival.** In all, 84 patients were included. Demographics are shown in Table I. Thirty-seven patients were disease free at a median follow-up of 170 months (range, 53–261) and were defined as nonrecurrent. Twenty-nine patients developed recurrent rectal cancer at a median follow-up of 21.1 months (range, 3–79). Eighteen patients with stage IV rectal cancer were also studied. Seven patients with

**Table I.** Rectal cancer patient demographics

| | *Nonrecurrent* (n) | *Recurrent* (n) | *Stage IV* (n = *18*) |
|---|---|---|---|
| Male/female | 24/13 | 18/11 | 12/6 |
| Deceased | 10 | 26 | 18 |
| Median age at resection, years | 63 (30–82) | 65 (43–85) | 65 (42–87) |
| Median follow-up, months | 169.5 (53.1–261.5) | 21.1 (3.3–79.1) | 12.6 (3.0–88.5) |

**Table II.** Rectal tumor characteristics

| | *Nonrecurrent* (n) | *Recurrent* (n) | *Total* (n) |
|---|---|---|---|
| Stage I rectal cancer | 12 | 5 | 17 |
| Stage II rectal cancer | 13 | 12 | 25 |
| Stage III rectal cancer | 12 | 12 | 24 |
| | Nonrecurrent | Recurrent | Stage IV |
| Median tumor size, range (cm) | 4.0 (2.7–9.0) | 4.4 (2.0–8.0) | 5.0 (3.2–10.7) |
| Median lymph nodes examined | 20 (5–180) | 19 (3–52) | 20 (2–41) |
| Median lymph nodes involved | 1 (0–10) | 2 (0–19) | 4 (0–27) |
| Median distance from anal verge (cm) | 9.0 (2.0–15.0) | 9.0 (3.0–22.0) | 11.5 (4.0–17.0) |
| Degree of differentiation | Nonrecurrent | Recurrent | Stage IV |
| Well | 7 | 1 | 1 |
| Moderate | 25 | 24 | 12 |
| Poor | 5 | 4 | 5 |

normal rectal mucosa frozen tissue were used as a control group. The median age at resection was similar among cancer groups. Ten (27%) patients with nonrecurrent rectal cancer died from events unrelated to rectal cancer. The median survival time for those with recurrent rectal cancer was 48 months (range, 13–197) and only 10% (3/29) remained alive ($P < .001$). All deaths in patients with recurrent disease were cancer related.

**Tumor characteristics.** Tumor characteristics and stage distribution are shown in Table II. The tumor size was similar between recurrent and nonrecurrent disease at 4.4 cm (range, 2.0–8.0) and 4.0 cm (range, 2.7–9.0), respectively ($P = .9$). Between recurrent and nonrecurrent cancers, similarities were also observed in the degree of tumor differentiation ($P = .2$), median number of lymph nodes examined ($P = .5$), number of involved lymph nodes ($P = .5$), and median distance from the tumor to the anal verge ($P = .9$). All resected rectal cancer specimens were staged by pathology, had an R0 resection with a tumor resection margin >2.0 mm, and the median number of examined lymph nodes was at least 19 in all stages for recurrent and nonrecurrent cancers.

**Relative *CEACAM-7* expression values.** *All rectal cancer patients*: Compared with normal rectal mucosa, *CEACAM-7* expression was decreased 21-fold in rectal cancer ($P = .002$). This decrease was found across all stages of rectal cancer; stage IV patients had the greatest decrease in expression at 59-fold ($P < .003$). A relative decrease in *CEACAM-7* expression was also significant between stages I and II ($P = .047$), stages II and III ($P = .005$), and stages III and IV ($P = .031$).

*Recurrent versus nonrecurrent rectal cancer*: Stage I–III rectal cancers were analyzed according to recurrence status. A 31-fold relative decrease in *CEACAM-7* expression was found in rectal cancer specimens ($P < .003$) for patients who subsequently developed a recurrence, and a 9-fold decrease in nonrecurrent rectal cancers ($P = .01$) was found when compared with normal rectal mucosa. Comparing nonrecurrent neoplasms with those that subsequently developed a recurrence, a three-fold relative decrease in *CEACAM-7* expression in the recurrent group ($P = .1$) was observed. When recurrent and nonrecurrent rectal cancers were broken down by stage, differences for stage II patients were the most prominent with a 14-fold relative decrease in *CEACAM-7* expression for those who developed recurrence compared with disease-free survivors, $P = .002$ (Fig 1). No significant differences in *CEACAM-7* expression were observed for stage I ($P = .8$) and stage III ($P = 1.0$) cancers.

Using the Cox proportional hazards model, the relative risk of developing rectal cancer recurrence was estimated using the actual *CEACAM-7* expression $Log_{10}$ RQ values. Hazard ratios of recurrence-free survival were then generated for all

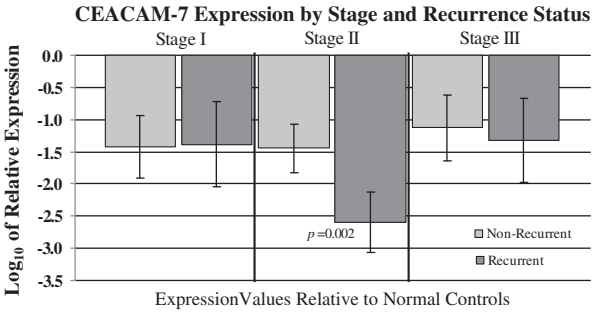


**Fig 1.** This figure shows the relative *CEACAM-7* expression was significantly decreased in patients with stage II recurrent disease compared with those with nonrecurrent disease but not in stages I and III.

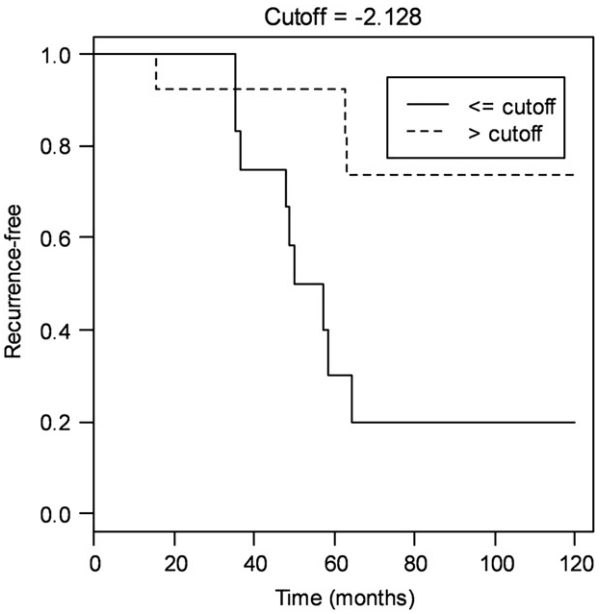


**Fig 2.** Recurrence-free survival based on *CEACAM-7* expression for stage II (HR, 0.55; 95% CI 0.35–0.88; $P = .018$).

stages of rectal cancer, and for stages I–III rectal cancers combined; stages I and II combined; stages II and III combined; and stages I, II, and III individually. Hazard ratios (HRs) are interpreted as the multiplicative decrease in risk of recurrence-free survival corresponding to a 1-unit increase in *CEACAM-7*. To create Kaplan-Meier curves to display the associations, we chose an imposed cutoff value that reflected most closely the mean *CEACAM-7* expression values for the patients with stage II recurrent rectal cancer. The HRs show that decreased recurrence-free survival was associated with lower actual *CEACAM-7* expression values for combined stages I–III (HR, 0.68; 95% CI, 0.48–0.95; $P = .032$), combined stages I and II (HR, 0.56; 95% CI, 0.37–0.86; $P = .007$), and for combined stages II and III (HR, 0.68; 95% CI, 0.48–0.96; $P = .035$). In contrast, when stages I, II, and III were analyzed separately, significance was found for stage II cancers (HR, 0.55; 95% CI, 0.35–0.88; $P = .018$; Fig 2) and not for stage I (HR, 0.93; 95% CI, 0.30–2.92; $P = .91$) or stage III cancers alone (HR, 0.77; 95% CI, 0.44–1.34; $P = .38$; Fig 3).

As *CEACAM-7* expression was associated with decreased recurrence-free survival for stage II rectal cancers, we performed a multivariate Cox regression analysis with covariate adjustments to evaluate its independent prognostic potential. Analyzed covariates included the T-stage, patient age, degree of tumor differentiation, and number of harvested lymph nodes. *CEACAM-7* remained an independent variable for these analyses. The results from the multivariate Cox analysis with covariate adjustments are shown in Table III. After accounting for these covariates, the significance in *CEACAM-7* expression remained, revealing its independent prognostic capability to predict recurrence-free survival for stage II rectal cancers.

To validate our findings for *CEACAM-7* using reverse transcription (RT)-PCR, we analyzed the expression of a different gene (*CEACAM-6*) with the same study population of rectal cancers used in this study using the same RT-PCR technique as described in the Methods section. *CEACAM-6* is also an adhesion molecule in the same family as *CEACAM-7*, but its expression has been reported to be upregulated in cancer compared with normal colonic mucosa.[11] Our results confirmed that *CEACAM-6* relative expression is upregulated throughout cancer stages compared with normal controls, as previously reported[4,11]; a 1.2-fold increased relative expression in stage I and stage II was found versus normal controls and 1.7-fold increased relative expression was found in stage III rectal cancer.

## DISCUSSION

This study explores the relationship between *CEACAM-7* expression levels and rectal cancer. We have shown that *CEACAM-7* expression is significantly downregulated in rectal cancer compared with normal rectal tissue and is an early event in the neoplastic process. Its decreased expression is evident in stage I tumors and is maintained throughout all stages of disease. Furthermore, relative *CEACAM-7* expression levels were able to distinguish between primary rectal cancers that were prone to develop recurrence compared to nonrecurrent disease, thus suggesting its role as a potential biomarker.

Colorectal cancer remains a substantive problem with more than 1,000,000 new cases globally

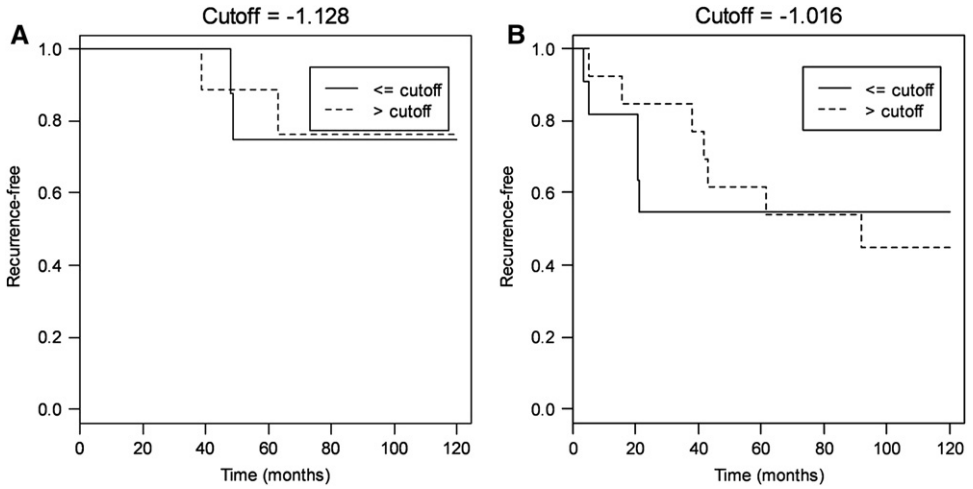


**Fig 3.** Recurrence-free survival based on *CEACAM-7* expression for: (*A*) stage I rectal cancers (HR, 0.93; 95% CI, 0.30–2.92; $P = .91$); (*B*) stage III (HR, 0.77; 95% CI, 0.44–1.3604; $P = .38$).

**Table III.** Cox model covariate

| | *HR (95% CI) for the association between CEACAM-7 and recurrence-free survival* | *Likelihood ratio* P *value* |
|---|---|---|
| *CEACAM-7* (without a covariate) | 0.55 (0.35–0.88) | .018 |
| T-stage | 0.55 (0.35–0.88) | .018 |
| Age | 0.53 (0.32–0.86) | .014 |
| Tumor differentiation | 0.56 (0.34–0.91) | .025 |
| Lymph node harvest | 0.47 (0.28–0.81) | .008 |

and approximately 40,000 new rectal cancer cases annually in the United States alone.[12,13] Cancer stage drives the treatment algorithm and clinical outcome. Neoadjuvant chemoradiation is the accepted standard for stage III and selected stage II rectal cancers[14] because of the improved local disease control, but given the relatively good outcome of early stage rectal cancer patients treated by operative resection alone,[15] not all patients will necessarily benefit from adjunctive therapy. Conversely, a substantial number of stage II colorectal cancer patients treated by operative resection alone will have recurrent cancer and die from the disease. Treatment selection must be weighed against the associated morbidity and costs of treatment to the entire group for the benefit of a subset of patients. Unfortunately, no accurate means are available to predict which patients with early stage disease will develop recurrent disease; thus, there is no way to identify which patients should be targeted for adjuvant treatment. Although various molecular markers involved in colorectal cancer development have been identified,[16-19] the process of oncogenesis and cancer metastasis is likely a complex chain of events with multiple intertwined pathways and regulatory mechanisms, most of which remain unknown. This study offers *CEACAM-7* as a contributing factor to help determine rectal cancer recurrence and prognosis for early stage rectal cancers.

A paucity of information is available about *CEACAM-7* in the literature. Work by Thompson et al[8] demonstrated its specificity to the apical surface of highly differentiated colonic and isolated pancreatic duct epithelium. Follow-up studies by the same group determined that its downregulation occurs as an early event in colorectal neoplasia as evident in stage I cancers,[5,8] and it is decreased in hyperplastic polyps and adenomatous polyps compared to normal rectal mucosa.[4] Because of its tissue specificity and potential for early cancer detection, more recent efforts have focused on its detection from blood; however, its benefit has not been shown.[20] Our data are consistent with previous reports regarding down-regulation of *CEACAM-7* in cancer, but the association of decreased *CEACAM-7* expression levels with the predisposition to develop recurrent rectal cancer is a novel finding.

Our data show that decreased *CEACAM-7* expression in early stage patients, specifically those

with stage II disease, can identify those patients who are at increased risk for developing future disease recurrence. Furthermore, *CEACAM-7* expression was able to predict survival differences based on its differential expression using the Cox proportional hazards model and Kaplan-Meier estimation with improved prognosis for stage I–III patients. Subsequent analysis by cancer stage revealed that the large effects seen in stage II disease contributed to the overall trend for the entire population, as only *CEACAM-7* expression in stage II cases proved to predict recurrence statistically.

One potential explanation for the loss of association of *CEACAM-7* expression with stage III rectal cancers relates to variable expression patterns of different genes in different stages of cancer development. For cells to transform into a malignant phenotype, certain genes are required to be silenced and others to be activated, but to develop metastatic potential, the same must happen for a different set of genes.[21,22] This concept implies that the deregulation of genes involved in cancer progression may be turned on or off at different stages of development as they are deemed necessary by selection pressures and fitness of the tumor cells. The loss of expression, or downregulation of *CEACAM-7* and subsequent loss of cellular adhesion capability, is necessary in early stages of oncogenesis to promote the ability of the neoplasm to escape the primary tumor bed, invade the lymphatic system, and become metastatic.[23] The differential expression found in stage III neoplasms may reflect a transient regain of function and actually offer those tumor cells capable of metastasis a selective advantage to adhere in distant tissue. A mechanism theoretically capable of regulating gene expression in this manor involves DNA methylation. Perhaps the best model to support this theory is by Graff et al,[24] who published on the variable expression patterns of this E-cadherin in breast cancer cell lines and throughout varying stages, including metastases.

The prognostic potential of downregulated *CEACAM-7* expression, particularly in stage II disease, has important clinical implications. Realizing that additional study and validation is necessary prior to any potential clinical application, *CEACAM-7* expression levels could be used as additional information in patient discussions. Its ability to distinguish whether a patient is at increased risk of developing recurrent rectal cancer could allow for more individualized treatment decisions. Thus, approximately 80% of patients with stage II disease who would not achieve improved survival by adjunctive chemoradiation could avoid toxicity of these treatments potentially, and those who would receive the greatest benefit could be targeted.

In conclusion, *CEACAM-7* expression is downregulated in rectal cancer tissues compared with normal rectal mucosa. Its downregulation occurs as an early event in the neoplastic process as evident by decreased expression levels in stage I cancers. The relative loss of expression in recurrent stage II cancers with nonrecurrent stage II cancers suggests a potential role for *CEACAM-7* expression as a predictor of recurrent disease. The awareness of potential tumor recurrence in stage II patients could potentially guide postoperative management for these patients, including adjuvant chemotherapy for the prevention of recurrent disease.

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