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Reduction in membranous immunohistochemical staining for the intracellular domain of epithelial cell adhesion molecule correlates with poor patient outcome in primary colorectal adenocarcinoma

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ABSTRACT

Background Epithelial cell adhesion molecule (EPCAM) is a multifunctional transmembrane glycoprotein expressed on both normal epithelium and epithelial neoplasms such as gastric, breast, and renal carcinomas. Recent studies have proposed that the proteolytic cleavage of the intracellular domain of EPCAM (EPCAM-ICD) can trigger signalling cascades leading to aggressive tumour behavior. The expression profile of EPCAM-ICD has not been elucidated for primary colorectal carcinoma. In the present study, we examined EPCAM-ICD immunohistochemical staining in a large cohort of patients with primary colorectal adenocarcinoma and assessed its performance as a potential prognostic marker.

Methods Immunohistochemical staining for EPCAM-ICD was assessed on tissue microarrays consisting of 137 primary colorectal adenocarcinoma samples. Intensity of staining for each core was scored by 3 independent pathologists. The membranous EPCAM-ICD staining score was calculated as a weighted average from 3 core samples per tumour. Univariate analysis of the average scores and clinical outcome measures was performed.

Results The level of membranous EPCAM-ICD staining was positively associated with well-differentiated tumours ($p = 0.01$); low preoperative carcinoembryonic antigen ($p = 0.001$); and several measures of survival, including 2-year ($p = 0.02$) and 5-year survival ($p = 0.05$), and length of time post-diagnosis ($p = 0.03$). A number of other variables—including stage, grade, and lymph node status—showed correlations with EPCAM staining and markers of poor outcome, but did not reach statistical significance.

Conclusions Low membranous EPCAM-ICD staining might be a useful marker to identify tumours with aggressive clinical behavior and potential poor prognosis and might help to select candidates who could potentially benefit from treatment targeting EPCAM.

Key Words Colon cancer, EPCAM, biomarkers

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INTRODUCTION

Epithelial cell adhesion molecule (EPCAM) is a 40-kDa transmembrane glycoprotein expressed in both normal epithelium and epithelial neoplasms. It is thought to be involved in calcium-independent cell adhesion, signalling, migration, proliferation, and differentiation[1]. This glycosylated type 1 transmembrane protein contains an

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extracellular domain (EX) with both epidermal growth factor and thyroglobulin repeat-like domains, a transmembrane domain, and a relatively short intracellular domain (ICD)[2]. Proteolytic cleavage of EPCAM leads to the creation of an extracellular domain (EPCAM-EX) and an intracellular domain (EPCAM-ICD) that consists of a short 26-amino-acid fragment that has been shown to trigger activation of the Wnt/beta-catenin pathway and aggressive tumour behavior[2,3]. In addition, formation of an EPCAM-ICD–beta-catenin complex with other proteins has been shown to lead to transcription and upregulation of several genes, including *c-Myc* and *CCND1*, which might promote tumour growth[4].

Functionally, EPCAM is a cell adhesion molecule that permits tight junction formation between epithelial cells, which can negatively affect cadherin/catenin complex formation[5]. Alterations in EPCAM expression have been identified in several epithelial neoplasms, including lung, breast, prostate, hepatocellular, and renal cell carcinoma[6,7]. In breast cancer cell lines, silencing of EPCAM by RNA interference assays has been found to reduce cell proliferation and invasion[8]. Altered EPCAM expression correlates with aggressive biologic behavior in stomach, breast, kidney, and thyroid carcinomas[9–12].

Although EPCAM has been identified both as a cell adhesion molecule and a mitogenic signalling molecule, relatively little work has been done on the expression profile of EPCAM-ICD and its potential correlation with patient prognosis in colorectal cancer. The loss of membranous EPCAM has been observed in budding colorectal carcinoma cells, as has increased cytoplasmic staining for EPCAM and nuclear staining for beta-catenin[6]. In the same study, reduced EPCAM staining was also shown to correlate with tumour grade and increased risk of local recurrence. In another study, a lower intensity of intracellular EPCAM staining was shown in colorectal adenocarcinoma samples compared with dysplastic samples, though staining for EPCAM-EX remained high in most samples[13]. Finally, serum EPCAM levels were demonstrated to be higher in a group of colorectal cancer patients than in a control group[14]. In the present study, we examined EPCAM-ICD immunohistochemical staining in a large cohort of patients with primary colorectal carcinoma, and we assessed the potential for EPCAM-ICD to be a prognostic marker and therapeutic target.

METHODS

Clinical Data Collection

After obtaining approval from the Queen's University Health Sciences Research Ethics Board (HSREB 6007275), we undertook a comprehensive retrospective patient chart review for the 149 patients diagnosed with colorectal carcinoma between 2004 and 2008 whose tissue samples were housed at Kingston General Hospital. Clinical parameters including patient demographics, comorbidities, colorectal cancer risk factors, and tumour characteristics were collected, as were referral dates, treatment outcomes, and survival data. Tumours were staged according to the 7th edition of the American Joint Committee on Cancer's *Cancer Staging Manual*. Overall survival was calculated from the date of diagnosis to the date of death. The final update of the clinical dataset with respect to progression and survival was completed in October 2013.

Tissue Microarray and Immunohistochemistry

Expression of EPCAM-ICD was assessed by immunohistochemistry, using tissue microarrays developed from the patient population already described. Figure 1 presents detailed images of representative colonic tissue samples used in validating EPCAM-ICD immunohistochemistry before the tissue microarray analysis. All samples were obtained from resected tissue and were reviewed by a pathologist and marked before coring. Four 0.6-mm tissue cores were collected for tissue microarray creation. For each patient, 3 tumour cores and 1 core from normal (non-neoplastic) colonic epithelium were arrayed using a Beecher MTA-2 manual tissue microarrayer (Beecher Instruments, Sun Prairie, WI, U.S.A.).

The EPCAM-ICD monoclonal antibody (catalogue no. 4A7: KalGene Pharmaceuticals, Toronto, ON) was used at a 1:500 dilution. Staining was performed using a Ventana Discovery XT automated staining system (Ventana Medical Systems, Tucson, AZ, U.S.A.) under the CCI protocol using the higher-pH EDTA buffer solution. Samples were incubated for 1 hour with the primary antibody and for 16 minutes with the secondary antibody. The presence and intensity of EPCAM-ICD membranous and cytoplasmic staining were independently scored by 3 pathologists blinded to the clinical parameters. The percentage of tumour cells stained and the staining intensity (strong, 3; moderate, 2; weak, 1; none, 0) were recorded for each sample. Tissue cores with fewer than 50 viable colonic epithelial cells were excluded. Of 149 samples initially included in the experiments, 128 met the viability criterion and were included in the results.

Statistical Analyses

Membranous EPCAM-ICD staining was calculated as a weighted average of EPCAM score (0–3) adjusted for the percentage of cells stained at the reported intensity. The median weighted average score from 9 observations (3 cores, each scored by 3 pathologists) was used for further analysis. Median scores were used to minimize the effects of outlier samples; however, those effects were small because a parallel analysis of mean scores led to comparable results. Cytoplasmic EPCAM-ICD staining was calculated as the difference between tumour and matched normal tissue, using the weighted averages of the intensity scores assigned by the 3 pathologists. Statistical analyses included Pearson correlations and the 2-tailed t-test, calculated using the StatPlus software application for Macintosh (version 5: AnalystSoft, Walnut, CA, U.S.A.) and Microsoft Excel.

RESULTS

Clinical and Pathologic Characteristics of the Sample Set

Table I summarizes the clinicopathologic characteristics of the sample set. Samples were obtained from patients with an age range of 45–98 years (median: 76 years). The sample set came from approximately equal numbers of men (48%) and women (52%). The study population had a large number of comorbidities, with cardiac diseases, diabetes, and respiratory illnesses being the most common. A history of smoking or alcohol consumption was common in the study population. In this cohort, 75% had no family

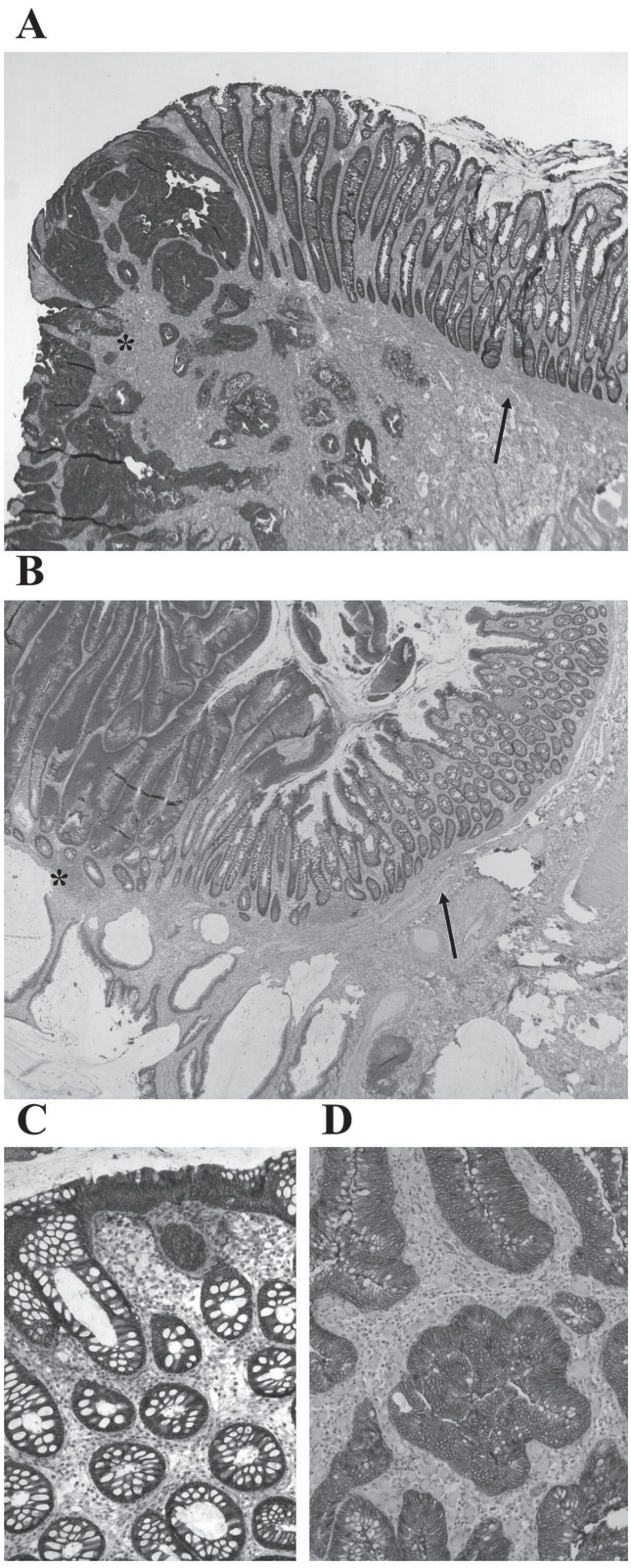

FIGURE 1 Detailed images of staining of the intracellular domain (ICD) of epithelial cell adhesion molecule (EpCAM) in representative colonic tissue samples used to validate EpCAM-ICD immunohistochemistry. Positive membrane and cytoplasmic staining is seen both in normal colonic epithelium (arrow in A, B, and C) and in colon carcinoma (asterisk in A, B, and D).

history of colorectal cancer, and 40% (49 individuals) had a history of some other form of cancer (Table I provides a breakdown of the subtypes reported for 2 or more patients).

Table II summarizes the pathologic features for the dataset. Tumours were distributed 28:102 between rectum and colon, and spanned the entire colon. Histologically, there were approximately twice as many non-mucinous as mucinous adenocarcinomas. Most samples fell into the moderate differentiation category, with some examples of both poorly- and well-differentiated tumours. The set contained examples of T stages 1 through 4, with more than half the samples being T stage 3. Invasiveness was present in just more than half the samples, with lymph

TABLE I Clinicopathologic characteristics of the 128 study patients

Characteristic	Value
Age (years)	
Range	45–98
Median	76
Sex [*n* (%)]	
Men	62 (48)
Women	66 (52)
Comorbidities [*n* (%)][a]	
Cardiac	78 (65)
Diabetes	27 (22)
Respiratory	25 (20)
Renal	5 (4)
Hepatic	3 (2)
Smoking history [*n* (%)][b]	
Active smoker	20 (17)
Ex-smoker	43 (36)
Non-smoker	58 (48)
Alcohol history [*n* (%)][b]	
Yes	77 (64)
No	44 (36)
Family history of ... [*n* (%)]	
Colorectal cancer[b]	
Maternal	22 (18)
Paternal	12 (10)
None	91 (75)
Other cancer[b,c]	
Overall	49 (40)
Lung	7 (6)
Breast	4 (3)
Prostate	4 (3)
Bladder	4 (3)
Lymphoma	3 (2)
Melanoma	3 (2)
Liver	2 (2)
Pancreas	2 (2)
Ovarian	2 (2)

[a] For 6 patients, no data were available; percentages were calculated based on 122 patients.
[b] For 7 patients, no data for history measures were available; percentages were calculated based on 121 patients.
[c] Subtypes are presented only when 2 or more patients had the relevant history.

TABLE II Macroscopic and histologic characteristics of colorectal adenocarcinoma in 128 patients

Characteristic	Value
Location[a] [*n* (%)]	
Rectum	28 (22)
Colon (all)	102 (80)
Sigmoid	19 (15)
Descending	14 (11)
Splenic flexure	3 (2)
Transverse	9 (7)
Hepatic flexure	2 (2)
Ascending	39 (30)
Cecum	16 (13)
Histologic subtype [*n* (%)]	
Non-mucinous	82 (64)
Mucinous	46 (36)
Differentiation[b] [*n* (%)]	
Poor	11 (9)
Moderate	110 (87)
Well	6 (5)
Pathologic T stage [*n* (%)]	
T1	5 (4)
T2	22 (17)
T3	77 (60)
T4	24 (19)
Invasiveness [*n* (%)]	
Any	65 (51)
Lymph node–positive	58 (45)
Perineural invasion	9 (7)
Macroperforation	3 (2)
Vascular invasion	26 (20)
Preoperative CEA (ng/mL)	
Median	2.2
Range	0.4–1349

[a] Two tumours were identified as spanning the colon and rectum, and were counted in both categories.
[b] Differentiation was not available for 1 tumour, and thus the percentages were calculated for 127 patients.
CEA = carcinoembryonic antigen.

node positivity being the most common indication; perineural invasion and macroperforation were also observed. Vascular invasion was observed in 20% of samples. Levels of carcinoembryonic antigen (CEA) varied widely between the samples, with preoperative values in the range 0.4–1349 μg/L (median: 2.2 μg/L).

The samples represented every clinical stage at diagnosis: 15 stage I, 43 stage II, 57 stage III, and 13 stage IV. Table III presents survival data by stage. For stage I–III patients, median survival was more than 6.3 years, more than 6.0 years, and more than 6.0 years respectively; each of those values is limited by follow-up time since diagnosis. For stage IV patients, median survival was 3.0 years.

EpCAM Staining and Scoring

Figure 2 presents images of normal colonic tissue and 3 colorectal adenocarcinomas with varying intensities of EPCAM staining. In Figure 2(A), the EPCAM staining is strong and predominantly membranous. In Figure 2(B–D), membranous EPCAM staining moves from strong to weak, with notable cytoplasmic staining in each sample. The illustrated slides are representative of the staining intensities used by the 3 independent pathologists as the baseline for scoring all the samples. Membrane-bound EPCAM staining was assessed as strong [score 3, Figure 2(B)], moderate [score 2, Figure 2(C)], weak [score 1, Figure 2(D)], or none (score 0). The percentage of cells stained at that intensity was also recorded.

After all data had been collected, samples scored discrepantly by the pathologists were re-examined to reach consensus; most discrepancies were attributable to the presence of a low number of non-necrotic cells in the core. Once all discrepancies had been resolved, the scores and frequencies were used to calculate a weighted average staining score. With respect to membranous staining, the median EPCAM intensity value for each tumour was used for further analysis. To reduce sample variability with respect to the cytoplasmic staining, the difference in score between the tumour and a matched normal sample was used for the analysis.

Loss of EpCAM Staining Correlates with Diagnostic Criteria Suggesting Poor Outcome

Tumour samples from patients with high CEA scores showed significantly reduced EPCAM-ICD staining (Table IV). The intensity of membranous EPCAM staining was strongly negatively correlated with the preoperative ($p = 0.001$), highest ($p = 0.001$), and lowest ($p = 0.02$) CEA levels. Cytoplasmic EPCAM-ICD staining was similarly significantly negatively correlated with CEA level—although, in each case, the association was weaker than that with membranous staining (Table IV). Membranous and cytoplasmic staining of EPCAM-ICD both showed a negative correlation with stage at diagnosis, although neither reached statistical significance ($p = 0.2$ and $p = 0.1$ respectively.) In each case, a trend of low EPCAM immunohistochemical staining being characteristic of samples with other diagnostic criteria suggesting a poor outcome was observed.

Loss of EpCAM Staining Correlates with a Decrease in Differentiation and an Increase in Invasiveness

The intensity of membranous EPCAM staining was strongly positively correlated with differentiation of the tumour samples, meaning that, as tumours were observed to be less differentiated, EPCAM levels dropped ($p = 0.01$, Table V). Significant negative correlations were also observed between samples showing perineural invasion or macroperforation and level of membranous EPCAM staining—that is, low levels of EPCAM were associated with an increase in those categories of tumour invasiveness ($p = 0.04$ and $p = 0.002$ respectively). Note that those results are based on a very low number of samples showing those invasiveness types ($n = 7$ and $n = 3$ respectively), indicating that further study of the associations is warranted. The EPCAM staining was also negatively correlated with lymph node metastasis (scored as "yes" or "no"), percentage of examined lymph nodes involved, and vascular invasion, but in each case, the correlation did not reach the level of statistical significance ($p = 0.3$, $p = 0.2$, and $p = 0.2$ respectively).

TABLE III Survival for 128 patients by stage at diagnosis

Stage at diagnosis	Pts (*n*)	Deaths (*n*)	Survival [% (*n/N*)]			Median survival duration
			1-Year	2-Year	5-Year	
1	15	4	87 (13/15)	80 (12/15)	73 (11/15)	>6.3 Years
2	43	10	91 (39/43)	91 (39/43)	77 (33/43)	>6.0 Years
3	57	22	86 (49/57)	79 (45/57)	61 (35/57)	>6.0 Years
4	13	8	69 (9/13)	62 (8/13)	38 (5/13)	3.0 Years

Pts = patient.

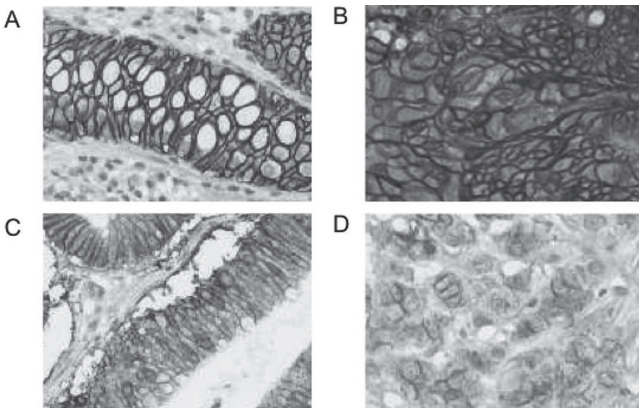

FIGURE 2 Representative images of membranous staining of the intracellular domain (ICD) of epithelial cell adhesion molecule (EpCAM) in (A) normal colonic tissue and in (B–D) colorectal adenocarcinoma (strong, moderate, and weak staining). The subcellular localization of the EpCAM-ICD antibody is both membranous and cytoplasmic in tumour and predominantly membranous in normal colonic mucosa.

Cytoplasmic EPCAM-ICD staining paralleled membranous staining in almost all of the foregoing categories, with correlations of similar magnitude and *p* value (Table V). The only exception was vascular invasion, in which the negative correlation with cytoplasmic EPCAM-ICD staining was far greater (–0.26, $p = 0.003$) than that with membranous staining (–0.12, $p = 0.2$). In all cases, the data suggest that EPCAM-ICD tends to be reduced in samples showing increased levels of various markers of invasion.

Loss of EpCAM Staining Is Associated with Lesser Minimum, Two-Year, and Five-Year Survival

Membranous EPCAM-ICD was correlated with minimum survival time (time to death or time from diagnosis to last follow-up for surviving patients) and with 2- and 5-year survival (Table VI). In each case, survival and EPCAM status were positively correlated, suggesting that high levels of EPCAM are a good indicator of various measures of survival. Each measure reached the level of statistical significance, at $p = 0.03$, $p = 0.02$, and $p = 0.05$ respectively. As with the membranous staining, cytoplasmic EPCAM-ICD staining showed correlations with survival measures in the same direction, but the correlations were weaker for both minimum survival time and 5-year survival. Of the correlations with the 3 survival measures, only the correlation with 2-year survival reached the level of statistical significance ($p = 0.2$, $p = 0.03$, and $p = 0.9$ respectively).

Correlation Between Membranous and Cytoplasmic EpCAM Staining

In all of the analysis so far presented, membranous and cytoplasmic staining for EPCAM showed correlations in the same direction, but with differing magnitudes in some cases. The correlation between the levels of EPCAM membranous and cytoplasmic staining as used in the study was 0.42 ($p = 8\times10^{-7}$)—clearly very strong, although far from perfect. Whether the differences between the two EPCAM staining methods represent a biologic difference in EPCAM function or whether they are attributable to a moderate sample size remains to be determined.

DISCUSSION

The results presented here support the hypothesis that loss of membranous EPCAM in colorectal cancers is associated with decreased tumour differentiation and increased tumour invasiveness, and with poor prognosis and lesser patient survival. A number of other variables—including stage, grade, and lymph node status—showed correlations for EPCAM level with markers of poor outcome, but in those cases, the correlations did not reach statistical significance. Together, the data suggest that EPCAM might be an important diagnostic marker in the context of colorectal cancer.

Since the early 2000s, the prognostic and diagnostic potential of EPCAM has been demonstrated in multiple tumour types[15,16]. It has been found to abrogate E-cadherin–mediated cell–cell adhesion by disruption of the cadherin/catenin/actin complex, which might play an important role in tumour progression by promoting invasion and metastasis[5]. Other functions attributed to EPCAM include regulation of cell proliferation, differentiation, and apoptosis, with the suggestion that the molecule is a key regulator of critical processes involved in tumorigenesis and progression[17].

Although the transcriptional regulation of EPCAM is not well understood, several studies have shown that it can be transcriptionally regulated by tumour necrosis factor α and by demethylation of CpG islands in the promoter region[17,18]. Expression of EPCAM has been observed in various epithelial neoplasms, including gastrointestinal, thyroid, kidney, prostate, breast, and lung carcinomas[6,7], leading to its selection as a target for immunotherapy. The human anti-EPCAM antibody adecatumumab and the murine monoclonal antibody edrecolomab were developed in hope that they could be used in targeted cancer therapy. In colorectal carcinoma specifically, edrecolomab, which binds to epidermal growth factor domain I[40] (located in the extracellular domain of EPCAM), has been tested in both

TABLE IV Epithelial cell adhesion molecule status for 128 patients by diagnostic criteria

Criterion	Pts (*n*)[a]	Staining[b]			
		Membranous		Cytoplasmic	
		Correlation	*p* Value	Correlation	*p* Value
Preoperative CEA	76	–0.40	0.001	–0.25	0.03
Highest CEA	96	–0.39	0.001	–0.26	0.03
Lowest CEA	96	–0.27	0.02	–0.24	0.04
Stage at diagnosis[c]	128	–0.12	0.2	–0.13	0.1

[a] Clinical information was lacking for some patients, and so actual patient numbers are presented for each criterion.
[b] Each type was correlated with the indicated diagnostic criteria, with 2-sided t-tests being used to determine the *p* values.
[c] Stage distribution at diagnosis: I, *n*=15; II, *n*=43; III, *n*=57; IV, *n*=13.
Pts = patients; CEA = carcinoembryonic antigen.

TABLE V Epithelial cell adhesion molecule status for 128 patients by differentiation and markers of invasiveness

Criterion	Pts (*n*)[a]	Staining[b]			
		Membranous		Cytoplasmic	
		Correlation	*p* Value	Correlation	*p* Value
Differentiation (poor, *n*=11; moderate, *n*=110; well, *n*=6)	127	+0.24	0.01	+0.22	0.01
Perineural invasion (yes, *n*=7; no, *n*=109)	127	–0.19	0.04	–0.21	0.02
Macroperforation (yes, *n*=3; no, *n*=125)	127	–0.27	0.002	–0.25	0.004
Lymph node metastasis (yes, *n*=58; no, *n*=70)	128	–0.10	0.3	–0.05	0.5
Percentage of involved lymph nodes	126	–0.11	0.2	–0.13	0.1
Vascular invasion (yes, *n*=26; no, *n*=102)	128	–0.12	0.2	–0.26	0.003

[a] Clinical information was lacking for some patients, and so actual patient numbers are presented for each criterion.
[b] Each type was correlated with the indicated diagnostic criteria, with 2-sided t-tests being used to determine the *p* values.
Pts = patients.

TABLE VI Epithelial cell adhesion molecule status for 128 patients by parameters of survival

Criterion	Pts (*n*)	Staining[a]			
		Membranous		Cytoplasmic	
		Correlation	*p* Value	Correlation	*p* Value
Minimum survival time	128	+0.19	0.03	+0.12	0.4
2-Year survival (yes, *n*=104; no, *n*=24)	128	+0.21	0.02	+0.20	0.03
5-Year survival (yes, *n*=88; no, *n*=44)	128	+0.17	0.05	+0.01	0.9

[a] Each type was correlated with the indicated diagnostic criteria, with 2-sided t-tests being used to determine the *p* values.
Pts = patients.

phase II and phase III studies[15,19,20]. In a 7-year randomized prospective trial involving 189 patients with Dukes C colorectal cancer, 99 patients who received edrecolomab as adjuvant therapy (compared with the 90 patients in the observation group) experienced a 32% reduction in overall mortality, a 23% reduction in recurrence rate, and a statistically significant reduction in distant metastasis and disease-free survival[21]. However, that survival benefit was not observed in a larger study that included both stage II and stage III colon cancer[22–24].

The staining pattern for EPCAM-ICD in our study was both membranous and cytoplasmic in tumour and predominantly membranous in normal colonic mucosa. That pattern differs from the expression pattern reported in other studies using EPCAM antibodies not specific for the ICD, which showed mainly membranous staining with basolateral localization in normal colonic epithelium and circumferential distribution in colon cancer[1]. Those differences highlight the advantage of using an ICD epitope, as in the present study, because it allows for recognition and identification of intracellular EPCAM fragments present in the cytoplasm after cleaving of the membranous form. However, we observed that the cytoplasmic and membranous immunohistochemical staining for EPCAM were positively correlated, suggesting that absolute EPCAM levels are seen to drive both the membranous and the cytoplasmic levels, rather than a transition from membranous to cytoplasmic staining because of protein processing—at least under the static conditions of the present work.

In the present study, loss of membranous EPCAM staining was associated with poorly differentiated tumours and poor survival. Those findings are similar to results from other studies[6]. Recent studies have proposed that the proteolytic cleavage of EPCAM-ICD triggers a signalling cascade leading to the activation of the Wnt/beta-catenin pathway[3]. Cleaved EPCAM-ICD binds to adaptor protein FHL-2 and beta-catenin in the cytoplasm, and the resulting complex translocates to the nucleus, where it upregulates c-Myc and cyclins A and E gene transcription, leading to tumorigenesis and progression[25]—a process that is supported by our finding that loss of membranous EPCAM-ICD immunohistochemical staining is associated with lesser 5-year survival, high preoperative CEA, and poor tumour differentiation.

One hypothesis for the association between reduced membranous EPCAM expression and poor prognosis is that EPCAM plays a regulatory role in the budding of colorectal carcinoma. In a study by Gosens *et al.*[6], increased cytoplasmic expression of EPCAM and increased nuclear localization of beta-catenin were observed in budding colorectal carcinoma cells. The same study identified a patient subpopulation with tumours whose loss of membranous EPCAM was associated with an elevated risk of local recurrence compared with the risk for patients with tumours having no decreased membranous EPCAM expression.

Other studies have proposed that the poor prognosis associated with loss of membranous EPCAM expression could be related to increased disseminated tumour cells in lymph node metastasis. In a study by Dhayat *et al.*[26], EPCAM was used to highlight the disseminated tumour cells in peritumoural lymph nodes from rectal cancer patients with stage I disease ($n = 44$). After 59 months of follow-up, EPCAM-positive disseminated tumour cells were found to be significantly associated with overall survival and recurrence-free survival, and with a high density of peritumoural lymphatic vessels. In another study of 40 patients (30 primary, 10 metastatic), lymph node metastases were found to be associated with a trend toward decreased expression of EPCAM ($p = 0.06$)[27].

CONCLUSIONS

Our study describes the immunohistochemical staining profile of EPCAM-ICD in normal colonic mucosa and colorectal carcinoma. We provide further evidence that decreased membranous immunohistochemical staining for EPCAM is associated with poor prognosis in colorectal carcinoma, which could in turn affect its effectiveness as a therapeutic target in the adjuvant or metastatic setting. Results of cytoplasmic staining for EPCAM-ICD were similar. The levels of membranous and cytoplasmic staining for EPCAM-ICD are highly correlated, and yet they show some differences in their correlations with clinical parameters; additional studies are needed to determine whether a biologic mechanism underlies that observation.

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CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

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