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Prognostic Value of E-cadherin-, CD44-, and MSH2-associated Nomograms in Patients With Stage II and III Colorectal Cancer

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Abstract

BACKGROUND: To evaluate the prognostic value of E-cadherin, CD44, and MSH2 expression for colorectal cancer (CRC) and construct nomograms that can predict prognosis. *METHODS:* We retrospectively analyzed the expression of E-cadherin, CD44, and MSH2 in 223 paraffin-embedded stage II and III CRC specimens using immunohistochemistry in the training cohort. Their prognostic values were assessed using Kaplan–Meier curves and univariate and multivariate COX regression models. Moreover, a number of risk factors were used to form nomograms to evaluate survival, and Harrell's concordance index (C-index) was used to evaluate the predictive accuracy. Further validation of the nomograms was performed in an independent cohort of 115 cases. *RESULTS:* Low E-cadherin expression and low CD44 expression were significantly associated with diminished overall survival (OS) and disease-free survival (DFS) in stage II and III CRC patients and patients with negative MSH2 expression had better clinical outcomes. Moreover, the multivariate COX analysis identified E-cadherin, CD44 and MSH2 expression as independent prognostic factors for DFS and OS. Using these three markers and three clinicopathological risk variables, two nomograms were constructed and externally validated for predicting OS and DFS (C-index: training cohort, 0.779 (95% CI 0.722–0.835) and 0.771 (0.720–0.822), respectively; validation cohort, 0.773 (0.709–0.837) and 0.670 (0.594–0.747), respectively). *CONCLUSION:* The expression levels of E-cadherin, CD44 and MSH2 were independent prognostic factors for stage II and III CRC patients. By incorporating clinicopathological features and these biomarkers, we have established two nomograms that could be used to make individualized predictions for OS and DFS.

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second in women worldwide [1], and surgery remains the mainstay of curative treatment. The application of regular screening as well as adjuvant and neoadjuvant therapeutic regimens have contributed to the improved prognosis of colorectal cancer

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patients [2–4]. However, a subset of patients will develop local recurrences and metachronous metastases after resection of the primary tumor. The current American Joint Committee on Cancer (AJCC) TNM staging system is widely used as a guideline for staging and survival estimates [5]. However, patients within the same AJCC stage vary considerably in prognosis. Although the seventh edition of AJCC staging provided improved prognostic prediction within each stage, the clear rank ordering between stages was lost [5,6]. The survival of stage IIIA and IIIB patients is superior compared with stage IIC colon cancer patients [5,6], and there have been many 'prognostic marker' studies that have aimed to improve the prognostic prediction of the TNM system. However, most proposed biomarkers for CRC are not clinically implemented due to the lack of reproducibility and/or standardization [7].

Adjuvant chemotherapy significantly improves survival in stage III CRC and is accepted as standard treatment for these patients [8]. The majority of patients with stage II CRC are cured by surgery alone. However, perforation of the tumor and an insufficient number of examined lymph nodes are associated with reduced survival, so adjuvant chemotherapy is usually considered for these patients [9]. A proportion of stage II and III patients without an increased risk of relapse based on current clinical factors still do relapse. One could consider treating all patients with stage II CRC with adjuvant chemotherapy, but the effects of this treatment plan have not been conclusive [9,10]. This highlights the need for new biomarkers that allow for the more precise prediction of high-risk patients with stage II and III CRC, especially stage II CRC, and the consequent improvement of individualized cancer care.

Currently, several molecular markers are being evaluated and established for a wide variety of tumors, including CRC. These markers have potential diagnostic, prognostic, and even therapeutic implications. It is widely known that the tumor cell adhesion molecules involved in cell–cell and cell-extracellular matrix (ECM) adhesion contribute to the development of invasive and metastatic phenotypes. E-cadherin (ECAD), a member of the calcium dependent adhesion molecule (CAM) family, mediates homophilic cell–cell adhesion in epithelial tissues and is localized to adjacent cell membranes. Its abrogation has been linked to increased invasiveness and poor prognosis in several malignancies, including CRCs [11]. Reduced expression of E-cadherin has also been reported in a variety of epithelial cancers, including CRCs [12,13]. Low levels or a lack of ECAD expression is associated with dedifferentiation and metastasis [11,13]. CD44 is a member of the immunoglobulin family, which increases the metastatic potential of tumor cells [14]. The incubation of cells with a CD44 antibody inhibits the binding of Osteopontin and reduces migration [15]. It is also possible that CD44 isoforms interact with ECM materials (e.g., Hyaluronan), resulting in the abnormal mitogenesis and migration of epithelial cells [16]. This could be one of the critical steps of tumor invasion and metastasis. Recent studies have shown that CD44 is also expressed in tumor stem cells which have the unique ability to initiate tumor cell-specific properties [17]. Studies involving ECAD and CD44 have indicated that these molecules are responsible for tumor progression and metastasis [13,18], but the prognostic importance of these markers in CRC remains controversial.

Colorectal carcinogenesis is characterized by three major mechanisms: chromosomal instability (CIN), microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP). MSI occurs in approximately 15% of all CRCs, and in recent years, growing evidence has accumulated indicating that the assessment of MSI status in CRC provides useful prognostic information [19,20]. MSI is a manifestation of a defect in one of several DNA mismatch repair (MMR) genes, including MLH1 and MSH2. Several studies have shown that the immunohistochemical (IHC) assessment of MLH1 and MSH2 protein expression has a high degree of correlation with the MSI phenotype, as determined by PCR [21,22]. The sensitivity of IHC for the detection of MSI tumors is 80–95% and the specificity has reached 100% in most reports [23]. The relationship between MSI tumors and improved survival has also been confirmed in a systematic review [19]. Retrospective studies have demonstrated improved survival in patients with MMR-competent tumors after receiving 5-FU based chemotherapy [24–26]. A large multicenter AGEO study supports the use of adjuvant chemotherapy with fluoropyrimidine plus oxaliplatin for stage III-deficient MMR (dMMR) colon cancer [27]. Therefore, MSH2 expression may be a promising marker for prognosis as well as a predictive factor for the benefit of adjuvant chemotherapy [28,29].

This study was conducted to evaluate the potential prognostic significance of ECAD, CD44 and MSH2 expression in CRC and to determine the relationships with various clinicopathologic variables. We then developed and validated two nomograms that incorporated these biomarkers and clinicopathologic risk factors for the individual prediction of overall survival (OS) and disease-free survival (DFS) in patients with stage II and III CRC.

Methods

Patients and Tissue Specimens

We obtained pathologically proven FFPE specimens from 338 patients who were diagnosed with stage II and stage III disease and admitted for curative CRC surgery between 2007 and 2010. Of these patients, 223 received curative surgery at the First Affiliated Hospital, Wenzhou Medical University (Wenzhou, China), between January 2007 and December 2010, and 115 received curative surgery in the Second Affiliated Hospital, Zhejiang University (Hanzhou, China) between January 2007 and December 2009. Baseline information for each specimen donor, including age, gender, disease location, TNM staging at surgery, and rule-based postoperative chemotherapy, was documented. TNM staging was reclassified according to the AJCC staging manual (seventh edition). All participants were Han Chinese (self-reported). This study was approved by the institutional review board at each participating center. A written informed consent was obtained from each patient.

Antibodies

This study used the following commercially available monoclonal antibodies: an anti-human E-cadherin antibody (clone EP700Y; Neomarkers, Fremont, CA, USA); a CD44 antibody (clone 156-3C11; Neomarkers, Fremont, CA, USA); and an MSH2 antibody (ab52266, Abcam plc, Cambridge, UK) (Figure 1).

Immunohistochemistry

Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections were cut at a thickness of 4 μm, de-waxed in xylene, and rehydrated in decreasing concentrations of ethanol. Prior to staining, the sections were subjected to endogenous peroxidase blocking in 1% of H_2O_2 solution in methanol for 10 min and then heated in a microwave for 30 min in 10 mM citrate buffer, pH 6.0.

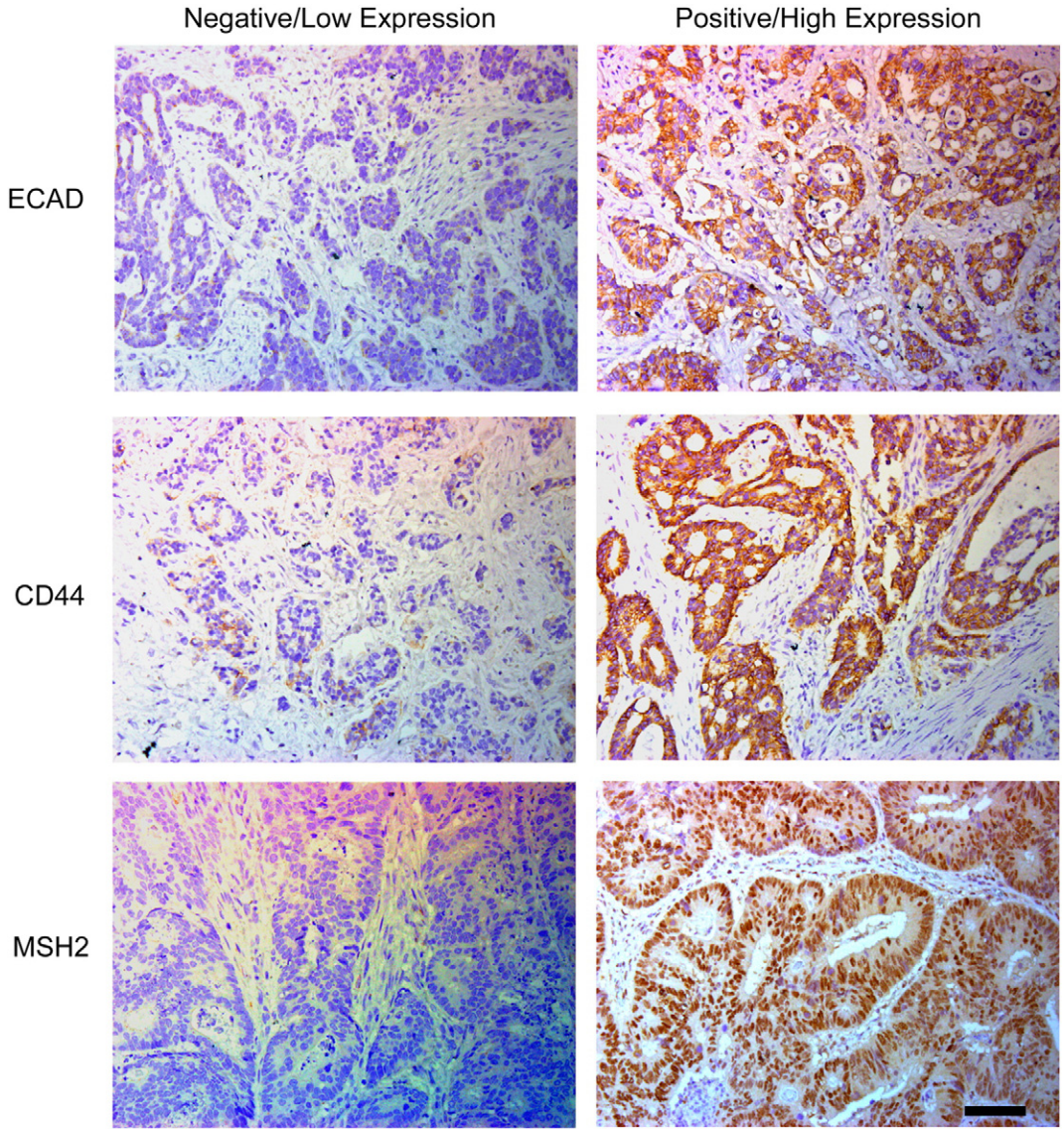

Figure 1. Representative IHC images of the E-cadherin, CD44, and MSH2 expression. Bar, 100 μm.

Serum blocking was performed using 10% normal rabbit serum for 30 min. The slides were incubated with the primary antibody (anti-E-cadherin was used at a 1:250 dilution; anti-CD44 at 1:100; and anti-MSH2 at 1:200) overnight at 4 °C and then incubated with a labeled polymer/HRP amplification system (EnVision™, DakoCytomation, Denmark) for 30 min. To visualize the sites of bound peroxidase, 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB) was used prior to counterstaining with modified Harris hematoxylin.

Evaluation of Results of Immunohistochemical Staining Results

Two pathologists who were unaware of the clinical parameters or outcomes for each patient independently reviewed the immunohistochemically stained sections. For the scoring of all molecules, 10 fields in the tumor frontier region were randomly selected and examined with high-power magnification. All discrepancies were resolved by a joint review of the slides in question.

For ECAD expression, the staining was scored as strong when the immunoreactivity in the tumor region showed a similar membranous staining to its normal counterpart in more than 75% of the cells. Discontinuous membranous staining in 25–75% of the cells was scored as moderate staining. The absence of membranous staining or positive immunoreactivity in less than 25% of the cells was graded as weak or none [18,30]. The presence of more than 10% of distinct cells with membrane staining was considered positive for CD44 [18]. Tumors showing a complete loss of nuclear MSH2 expression were classified as MSH2 negative [29]. Nuclear immunostaining of normal epithelial cells, lymphocytes, and stromal cells served as internal positive controls in each case.

Construction of the Nomograms

In the training cohort, survival curves for different variable values were generated using Kaplan–Meier estimates and were compared using the log-rank test. Variables that achieved a significance of $P < .05$ were entered into the multivariable analyses via the Cox regression model. Statistical analyses to identify independent prognostic factors were conducted in SPSS 17.0 for Windows (SPSS, Chicago, IL, USA). On the basis of the results of the multivariable analysis, two nomograms were formulated by R 3.0.1 (http://www.r-project.org) with the survival and rms package.

Validation and Calibration of the Nomograms

The performance of the developed nomograms was tested in an external validation cohort. The model performance for predicting outcomes was evaluated by calculating the concordance index (C-index) [31]. Calibration of the nomogram for 1-, 3-, 5-year OS and DFS was performed by comparing the predicted survival with the observed survival after bias correction.

Clinical Use

Decision curve analysis was conducted to determine the clinical usefulness of the nomograms by quantifying the net benefits at different threshold probabilities [32,33].

Risk Group Stratification Based on the Nomogram

Using the X-title [34], the composite scoring of the nomograms was divided into three risk groups that accurately discriminated patients with good, intermediate, and poor prognosis.

Statistical Analysis

Differences in the distributions between the variables examined were assessed using the χ^2 test or the Fisher's exact test, as appropriate. Survival curves were generated according to the Kaplan–Meier method and compared by the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model. The reported *P* values are two-sided and *P* values of less than 0.05 were considered statistically significance. All data were analyzed using the SPSS statistical software, Version 17.0 (SPSS Inc., Chicago, IL, USA) and R software (version 3.0.1; http://www.Rproject.org).

Results

Clinicopathologic Correlations

Tables 1 and S1–2 list the clinical characteristics of the patients and the clinicopathologic correlations with E-cadherin, CD44, and MSH2 in the training and validation cohorts. Specimens from 338 patients with CRC were obtained for this study. The primary tumor was located in the colon for 188 patients (55.6%) and in the rectum for 150 patients (44.4%) (Table 1). Negative or low ECAD expression levels were observed in 33.2% (74/223) of CRC cases in the training cohort and 32.2% (37/115) of CRC cases in the validation cohort. Low CD44 expression was noted in 29.1% (65/223) of CRC cases in the training cohort and 29.6% (34/115) of CRC cases in the validation cohort. A loss of MSH2 expression was observed in 30 (13.5%) of the patients in the training cohort and 12 (10.4%) of the patients in the validation cohort.

Low ECAD expression was associated with a poor tumor grade, a higher N stage, and a higher TNM stage (Table 1). Low ECAD expression was significantly associated with low CD44 expression, but it was not associated with MSH2 expression. Meanwhile, CD44 and MSH2 expression were not significantly associated with tumor differentiation, T stage or N stage (Table S1–2).

Prognostic Value of E-Cadherin, CD44, and MSH2 Expression

In the training cohort, patients with low expression levels of ECAD and CD44 showed statistically unfavorable OS and DFS (Figure 2). Patients with MSH2-negative tumors had a better clinical outcomes than patients with MSH2-positive carcinomas (Figure 2). Similar

Table 1. Clinical characteristics of patients according to E-cadherin in the training and validation cohorts

Variables	Training cohort (n = 223)				Validation cohort (n = 115)			
	N	low ECAD (%)	high ECAD (%)	p value	N	low ECAD (%)	high ECAD (%)	p value
Gender				0.137				0.171
Male	133	39(29.3%)	94(70.7%)		64	24(37.5%)	40(62.5%)	
Female	90	35(38.9%)	55(61.1%)		51	13(25.5%)	38(74.5%)	
Age(years)				0.525				0.924
<60	75	27(36.0%)	48(64.0%)		49	16(32.7%)	33(67.3%)	
≧60	148	47(31.8%)	101(68.2%)		66	21(31.8%)	45(68.2%)	
Tumor location				0.815				0.961
Colon	120	39(32.5%)	81(67.5%)		68	22(32.4%)	46(67.6%)	
Rectum	103	35(34.0%)	68(66.0%)		47	15(31.9%)	32(68.1%)	
Differentiation status				0.018				0.022
Well	36	10(27.8%)	26(72.2%)		22	5(22.7%)	17(77.3%)	
Moderate	129	36(27.9%)	93(72.1%)		64	17(26.6%)	47(73.4%)	
Poor and undifferentiated	58	28(48.3%)	30(51.7%)		28	15(53.6%)	13(46.4%)	
CEA				0.404				0.371
Elevated	76	28(36.8%)	48(63.2%)		37	14(37.8%)	23(62.2%)	
Normal	147	46(31.3%)	101(68.7%)		78	23(29.5%)	55(70.5%)	
Depth of invasion				0.759				0.545
T1 + T2	24	7(29.2%)	17(70.8%)		11	2(18.2%)	9(81.8%)	
T3	109	35(32.1%)	74(67.9%)		72	25(34.7%)	47(65.3%)	
T4	90	96(35.6%)	94(64.4%)		32	10(31.3%)	22(68.7%)	
Lymph node metastasis				0.015				0.007
N0	126	33(26.2%)	93(73.8%)		50	9(18.0%)	41(82.0%)	
N1	73	28(38.4%)	45(61.6%)		47	18(38.3%)	29(61.7%)	
N2	24	13(54.2%)	11(45.8%)		18	10(55.6%)	8(44.4%)	
TNM stage				0.011				0.004
II	126	33(26.2%)	93(73.8%)		50	9(18.0%)	41(82.0%)	
III	97	41(42.3%)	56(57.7%)		65	28(43.1%)	37(56.9%)	
CD44				<0.0001				<0.0001
low	65	35(53.8%)	30(46.2%)		34	20(58.8%)	14(41.2%)	
high	158	39(24.7%)	119(75.3%)		81	17(21.0%)	64(79.0%)	
MSH2				0.985				0.457
low	30	10(33.3%)	20(66.7%)		12	5(41.7%)	7(58.3%)	
high	193	64(33.2%)	129(66.8%)		103	32(31.1%)	71(68.9%)	

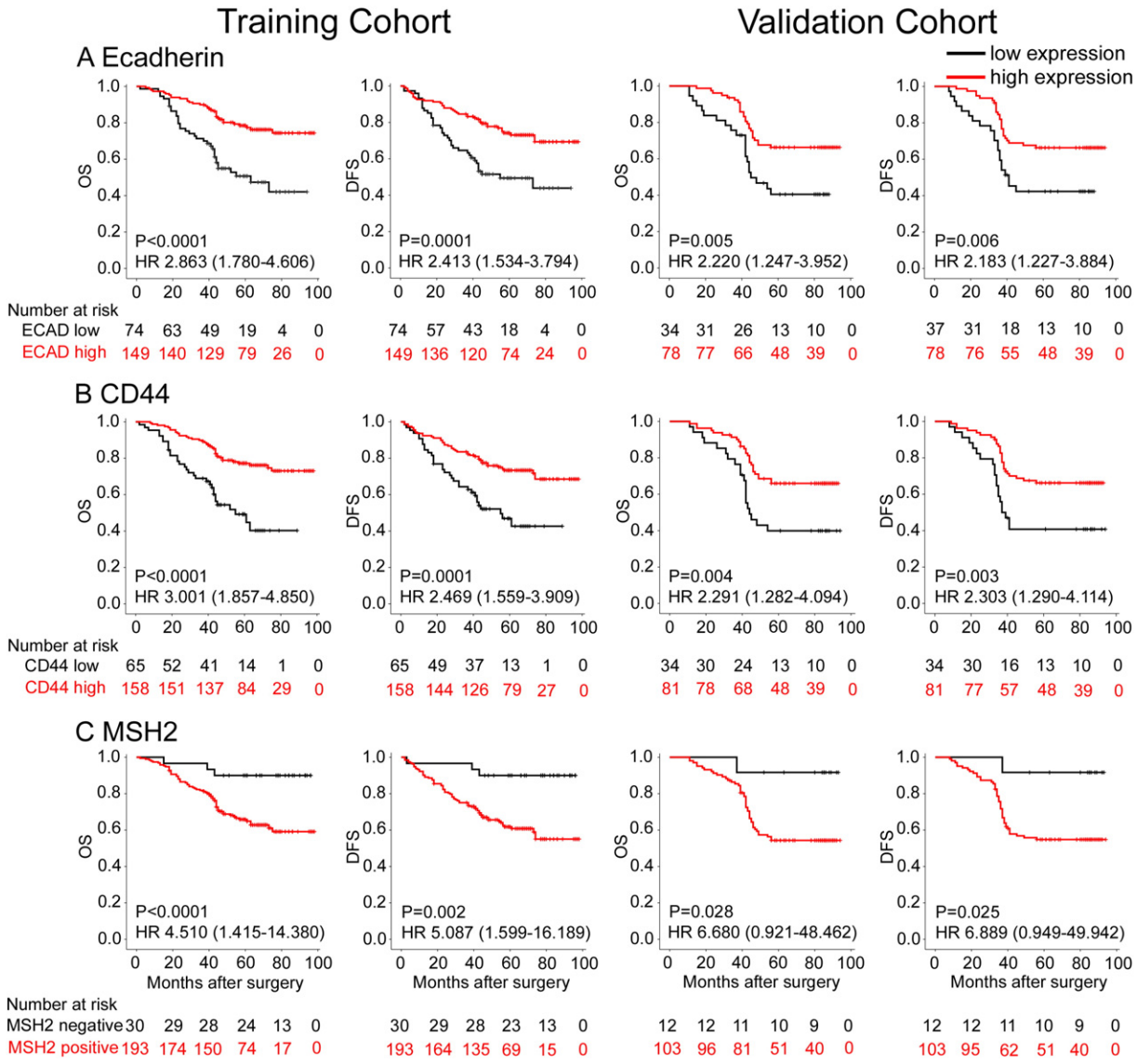

Figure 2. Kaplan-Meier survival analysis of overall survival and disease-free survival according to E-cadherin (A), CD44 (B), and MSH2 (C) expression status of GC patients in the training cohort and validation cohort. The left panel shows the results from the training cohort, and the right panel shows the results from the validation cohort.

results were observed in the validation cohort. The clinicopathological parameters for the prediction of OS and DFS were further investigated by univariate analysis with the Cox regression model. In the univariate analysis, T stage, N stage, the level of CEA, and the expression of ECAD, CD44 and MSH2 were significantly associated with OS and DFS ($P < .05$, Table S3–4). These significantly associated variables were used for the multivariate Cox regression model. In the models of OS and DFS, ECAD, CD44 and MSH2 expression levels remained powerful and independent prognostic factors for patients with stage II and III CRC ($P < .05$) (Table 2).

Development and Validation of Nomograms for Predicting CRC Prognosis

To predict OS and DFS for patients with stage II and III CRC, two nomograms were established using the multivariate Cox regression model according to all the significantly independent factors for OS and DFS (Figure 3, *A* and *B*). Nomograms can be interpreted by summing up the points assigned to each variable, which are indicated at the top of scale. The total points can be converted to predicted 1-, 3-, and 5-year OS and DFS for a patient in the lowest scale [6]. In the training cohort, the C-indexes for the prediction of OS and DFS were 0.779 (95% CI: 0.722–0.835) and 0.771 (95% CI: 0.720–0.822), respectively. Calibration curves for the two nomograms (Figure 4) revealed no deviations from the reference line and no need for recalibration. In the validation cohort, the C-indexes for the prediction of OS and DFS were 0.773 (95% CI: 0.709–0.837) and 0.670 (95% CI: 0.594–0.747), respectively. The calibration curves yielded good agreement between the predicted and observed outcomes for 1-, 3-, and 5-year OS and DFS (Figure S1).

Using the X-title, the composite scoring was divided into three risk groups that accurately discriminated between patients with good, intermediate, and poor prognosis (Figure 5 and S2). Therefore, we further analyzed subgroups of CRC patients in stages II and III. The three risk groups were able to significantly distinguished between CRC patients with different prognoses in stage II or III (Figure S3–4).

Table 2. Multivariable Cox regression analysis in the training cohort

Variables	Overall survival		Disease-free survival	
	HR (95% CI)	p value	HR (95% CI)	p value
CEA(ng/ml) (elevated vs. normal)	1.711 (1.051–2.787)	0.031	NA	NA
Depth of invasion		0.037		0.001
T3 vs. T1+2	2.930 (0.851–10.079)	0.088	5.897 (1.367–25.442)	0.017
T4 vs. T1+2	3.843 (1.128–13.092)	0.031	8.238 (1.930–35.166)	0.004
Lymph node metastasis		0.0002		<0.0001
N1 vs. N0	1.817 (1.034–3.195)	0.0380	2.107 (1.243–3.570)	0.006
N2 vs. N0	4.162 (2.177–7.956)	<0.0001	4.459 (2.357–8.437)	<0.0001
Ecadherin (high vs. low)	2.071 (1.254–3.420)	0.004	1.670 (1.038–2.687)	0.035
CD44 (high vs. low)	1.978 (1.203–3.254)	0.007	1.635 (1.017–2.630)	0.043
MSH2 (positive vs. negative)	3.509 (1.077–11.430)	0.037	3.628 (1.122–11.730)	0.031

CEA: carcino-embryonic antigen.

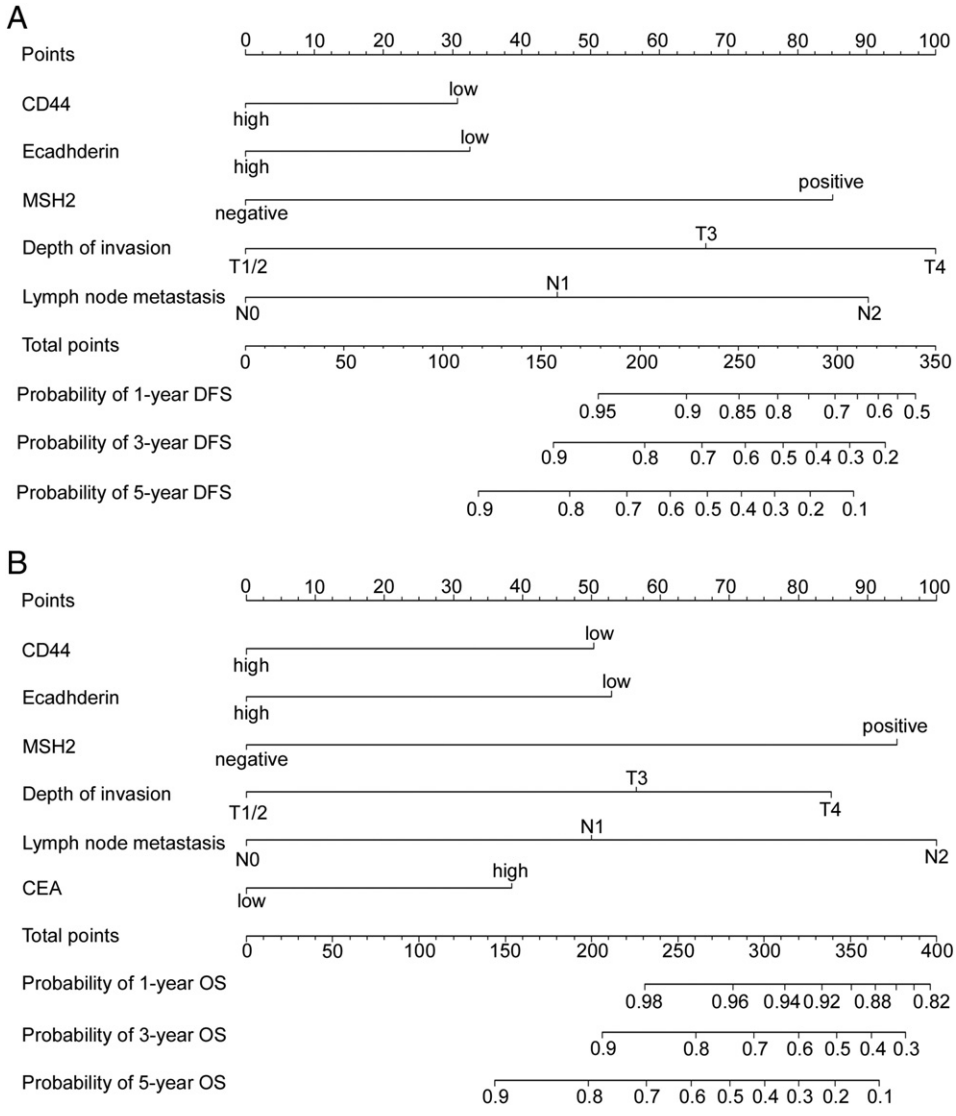

Figure 3. Nomogram for predicting overall survival (OS) and disease-free survival (DFS): Locate the grade of the patient on the grade axis and then draw a straight line upward to the Points axis to determine how many points toward survival the patient receives for her/his grade. Repeat this process for the other axes, each time drawing a straight line upward toward the Points axis. Take the sum of the points received for each predictor and locate this sum on the Total Points axis. Draw a straight line down to the survival-probability axis to find the patient's probability of surviving colorectal cancer.

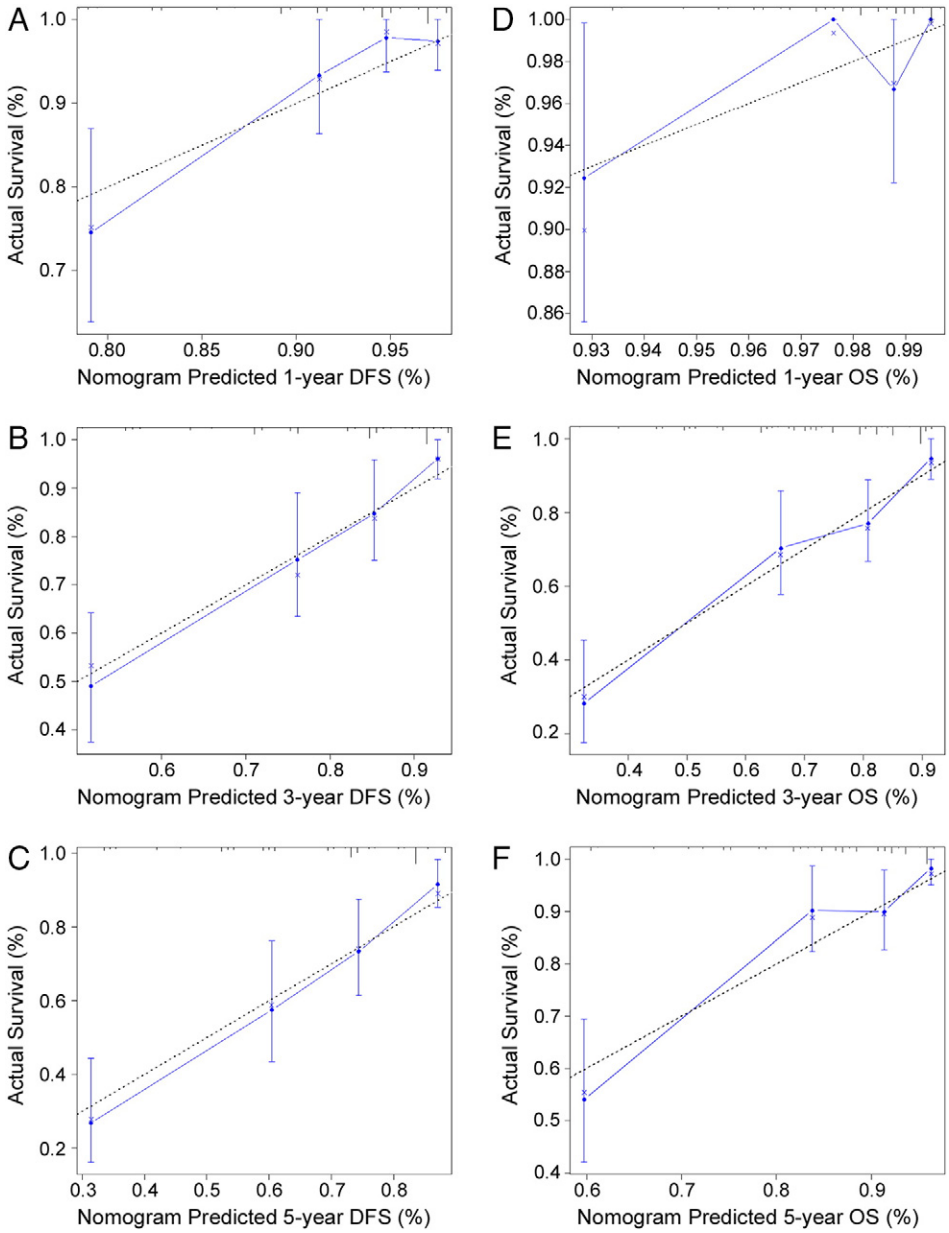

Figure 4. Calibration curves for the nomogram. The calibration curve for predicting patient OS and DFS at (A, D) 1 year, (B, E) 3 years, and (C, F) 5 years in the training cohort. Nomogram-predicted OS and DFS are plotted on the x-axis, and actual OS and DFS are plotted on the y-axis. The dotted line represents an ideal nomogram, and the solid blue line represents the current nomogram. The vertical bars are 95% CIs, and the ×'s are bootstrap-corrected estimates.

Clinical use

The decision curve analysis for the two nomograms is presented in Figures. 6 and S5. The decision curve showed that if the threshold probability of a patient or doctor was >10%, using the two nomograms to predict 5-year OS and DFS added more benefit than either the treat-all-patients scheme or the treat-none scheme. Within this range, the net benefit was comparable with several overlaps on the basis of the nomograms.

Discussion

We evaluated the prognostic value of ECAD, CD44, and MSH2 expression in stage II and III CRC. The results indicated that low ECAD expression and low CD44 expression were associated with poor prognosis and patients with negative MSH2 expression had better clinical outcomes. Using these three markers and three clinicopathological risk variables, two nomograms were constructed and externally validated for predicting 1-, 3-, and 5-year OS and DFS probabilities after curative resection. The nomograms performed well with good discrimination and calibration, identifying this model as a simple and easy tool for estimating the survival of individual Chinese patients with stage II and III CRC.

A reduction in the expression of ECAD has frequently been observed in CRC as well as prostate, bladder, and renal cell cancer as tumors progress [13,18,30]. Several previous studies of CRC have reported relationships between lost or reduced expression of ECAD and clinicopathologic factors, such as tumor grade, tumor stage, metastasis, and patient survival [11,18,35]. Dorudi et al. reported that ECAD expression was significantly related to the stage and grade of

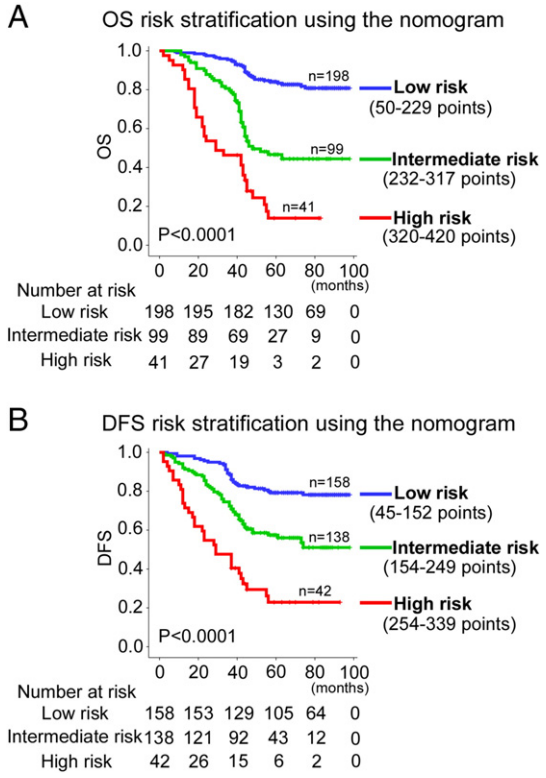

Figure 5. Kaplan-Meier survival analysis of OS and DFS according to three risk groups. The entire population was divided in 3 subgroups according to the total number of points given by the nomograms. (A): OS nomogram; and (B): DFS nomogram.

the tumor, and showed that more aggressive cancers exhibited an obvious reduction in ECAD expression [12]. Ghadimi et al. revealed a close relationship between reduced ECAD expression and lower tumor grade, but they did not observe a clear correlation between the loss of ECAD expression and the depth of tumor invasion [36]. In a multivariate study involving 84 CRC cases, Roca et al. showed that ECAD expression was not related to pathologic parameters, such as tumor stage, tumor grade, or lymph node metastasis, but the loss of ECAD was found to be an independent adverse prognostic factor [37]. A study of 1420 CRC cases indicated that a loss expression of ECAD was associated with a higher T stage, a higher N stage, vascular invasion, and worse survival in MMR-proficient CRC and with a higher N stage and worse survival in MLH1-negative CRC [35]. Two recent studies reported that ECAD expression was inversely associated with tumor differentiation and showed that lost or low expression of ECAD was an independent predictor of CRC [18,30]. In the present study, lost or low expression of ECAD was associated with a higher pT stage, a higher pN stage, and less tumor differentiation. Therefore, ECAD expression can be viewed as an independent prognostic factor for DFS and OS in stage II and III CRC.

CD44 is a transmembrane glycoprotein that is involved in cell-to-cell and cell-to-matrix interactions [14]. CD44 is located on chromosome 11p13 and the human CD44 gene consists of at least 20 exons. There are conflicting conclusions regarding the potential relationship between variations in CD44 expression and the prognosis of CRC patients [38]. Asao et al. has reported that the loss of CD44s expression was a sensitive marker for lymph node metastasis in CRC [38]. A multivariate analysis of 74 CRC cases revealed that CD44s expression was an independent prognostic factor for OS [39]. Another study involving 72 CRC cases that used an immunoenzymatic assay reported that CD44s expression was not associated with patient outcome [40]. In the present study, lost or low expression of CD44 was not significantly associated with a higher T stage, a higher N stage, or more differentiation. Patients with reduced expression of CD44 patients had better DFS and OS according to univariate and multivariate COX analyses.

This previous study has demonstrated the prognostic value of MMR protein expression in stage II and III CRC [28,29]. The majority of previous studies evaluating the prognostic or predictive value of MMR status in colorectal cancer have been performed using microsatellite analysis to assess tumor phenotype. However, the genetic analysis of MSI status is time consuming, expensive, and requires specialized equipment. The immunohistochemical analysis of MLH1 and MSH2 expression has recently been shown to be a rapid, cost-effective, and accurate method for the assessment of MMR status in CRC [29]. In this investigation, we evaluated the prognostic significance of the immunohistochemical expression of MSH2 in a large series of stage II and stage III colorectal cancer patients. In our study, patients with stage II and III CRC whose tumors demonstrated loss of MSH2 protein expression (MSH2 negative) had a better clinical outcome than patients with MSH2-positive tumors. Moreover, in the multivariate analysis, the survival advantage for patients with MSH2-negative carcinomas was independent of several clinical and pathologic parameters.

In vitro studies have shown that CRC cells with MSI are less responsive to 5-FU [41]. In a randomized retrospective study, Ribic et al. reported a survival advantage in 5-FU-treated CRC patients with MSI-L and MSS cancers but not in patients with MSI-H tumors [24]. Two other non-randomized retrospective studies involving 204 and 1263 CRC patients also reported a benefit of 5-FU treatment in patients without MSI [25,26]. In a randomized trial of 491 CRC patients who received adjuvant chemotherapy, MMR protein expression did not have predictive value for response to 5-FU treatment with respect to OS [28]. In a pooled molecular reanalysis of randomized chemotherapy trials (n = 341), MMR deficiency was shown to be a predictive marker for a lack of benefit from 5-FU-based chemotherapy in stage II and III colon cancer [42]. Even if the use of MMR expression to predict the outcomes of adjuvant chemotherapy was controversial, the results from the previous trials are very promising and indicate that 5-FU is beneficial for CRC patients with MSI tumors [43]. A large multicenter AGEO study reported that high-risk stage II dMMR colon cancer tended to have better outcomes with oxaliplatin-based adjuvant chemotherapy than with surgery alone [27]. However, before MMR status can be implemented as a prognostic and predictive marker in clinical practice, its value must be proven in large, high-powered prospective trials.

Immune checkpoint blockades directed against PD(L)-1 have recently shown excellent activity with response rates to single-agent therapy of 55% in preliminary studies involving patients with stage IV dMMR disease [44]. According to a phase II study, dMMR renders different solid tumors highly sensitive to immune checkpoint blockades in patients treated with the PD-1 inhibitor pembrolizumab [45]. With the ability to fix DNA replication errors compromised, dMMR tumors accumulate hundreds to thousands of somatic mutations, any of which could produce neoantigens capable of triggering a potent antitumor immune response in the presence of the PD-1 blockade [45]. Le et al. found pembrolizumab to be more

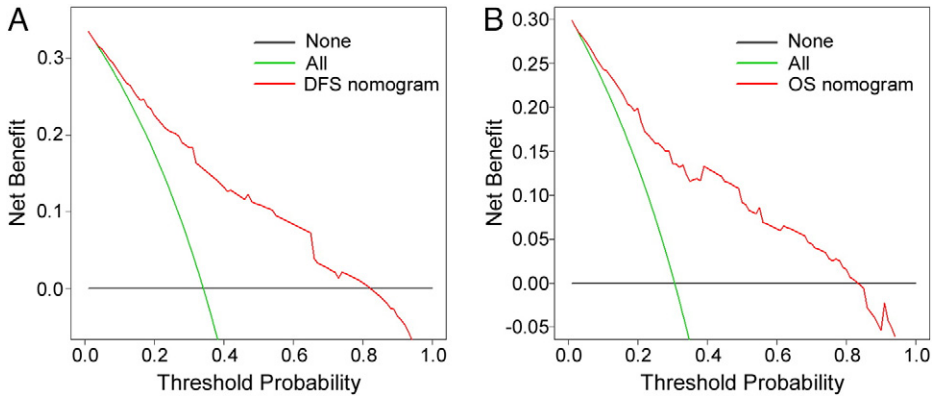

Figure 6. Decision curve analysis for the two nomograms in the training cohort. The y-axis measures the net benefit. The red line represents the nomogram. The blue line represents the assumption that all patients have 5-year survival. Thin black line represents the assumption that no patients have 5-year survival. The net benefit was calculated by subtracting the proportion of all patients who are false positive from the proportion who are true positive, weighting by the relative harm of forgoing treatment compared with the negative consequences of an unnecessary treatment [32,33]. Here, the relative harm was calculated by [pt/(1 – pt)]. "pt" (threshold probability) is where the expected benefit of treatment is equal to the expected benefit of avoiding treatment; at which time a patient will opt for treatment informs us of how a patient weighs the relative harms of false-positive results and false-negative results ([a – c]/[b – d] = [1 – pt]/pt); a – c is the harm from a false-negative result; b – d is the harm from a false-positive result. a, b, c, and d give, respectively, the value of true positive, false positive, false negative, and true negative [32,33]. The decision curve showed that if the threshold probability of a patient or doctor is >10%, using the nomogram in the current study to predict 5-year survival adds more benefit than the treat-all-patients scheme or the treat-none scheme.

effective against MMR–deficient tumors than against MMR–proficient tumors [44]. If these observations are confirmed in randomized trials of stage II and III CRC, MMR status will become relevant as a predictive marker for patients of all disease stages, and adjuvant treatment for dMMR stage II and III CRC patients through immuno- rather than chemotherapy may re-emerge.

For patients who have undergone curative resection for CRC, AJCC stage is the most commonly used system to predict prognosis. However, CRC patients within the same stage have different genetic, cellular, and clinicopathological characteristics, and their survival is not uniform [4]. To provide a more individualized staging system, nomograms have been developed to evaluate a large number of significant clinicopathologic predictors to better predict the prognosis of individual patients. Improved prediction of individual outcomes would be useful for counseling patients, personalizing treatment, and scheduling patients' follow-ups [46]. Although there are several CRC nomograms available, no particular nomogram has been used widely in clinical settings [6,46]. In this study, we developed and validated two nomograms including IHC expression of ECAD, CD44 and MSH2, T stage, N stage, and the level of CEA to improve the accuracy of prognosis prediction for CRC patients. These nomograms can be used to better predict an individual patient's probability of 1-, 3-, and 5-year OS and DFS. Validation of the nomograms was performed using calibration plots and the C-index. The nomograms performed well with a good calibration. Furthermore, the C-index for OS and DFS was satisfactory (0.779 (95% CI 0.722–0.835), 0.771 (0.720–0.822), respectively in the training cohort). Compared to previous studies, our two nomograms included three prognosis biomarkers (E-cadherin, CD44, and MSH2) that highly improved the accuracy.

In addition, the improved survival estimates calculated using the nomograms may assist in identifying patients with a high risk of poor clinical outcome within known AJCC stages, as well as in facilitating the choice of treatment regimen. Current NCCN guidelines recommend adjuvant chemotherapy for high-risk patients with stage II disease. The risk of a poor outcome or recurrence in stage II disease has been clinically identified based on the following: fewer than 12 lymph nodes analyzed after surgery; poorly differentiated histology (exclusive to those that are MSI-H); lymphatic/vascular invasion; bowel obstruction; perineural invasion; localized perforation; and close, indeterminate, or positive margins [47]. However, these clinicopathological risk variables do not clearly identify the high-risk patients who are likely to benefit from additional treatments after surgery [5]. Accordingly, the two nomograms, which incorporate multiple prognostic parameters into the current staging system, might help to identify patients with poor odds of survival who could benefit from adjuvant chemotherapy.

However, there are some limitations of our study. The nomograms were developed and externally validated using two retrospective data sets from two Chinese institutions. Validation by other cohorts is required for the generalized use of the nomograms as the basis for postoperative treatment recommendations. Moreover, the application of the nomograms requires the results of several IHC analyses and pathologic variables that are only available after surgery, i.e., the depth of tumor invasion and the pN stage. Other prognostic and predictive biomarkers may be included to improve the accuracy of the nomograms. Thus, it is difficult to make a precise evaluation of these factors preoperatively. Therefore, the nomograms will have limited impact on alternative treatments prior to surgery, including the use of neoadjuvant chemotherapy.

Conclusions

In summary, low ECAD expression and low CD44 expression are associated with poor prognosis in stage II and III CRC; whereas patients with a negative MSH2 expression have better clinical outcomes. The two nomograms were constructed and externally validated for predicting the probability of 1-, 3-, and 5-year OS and DFS after curative resection. The nomograms performed well with good discrimination and calibration, which suggests that this model is

a simple and easy tool for estimating the individualized survival of Chinese patients with stage II and III CRC. The model may be useful to both clinicians and patients for counseling and decision-making regarding individualized adjuvant treatments as well as follow-up scheduling.

Disclosure Statement

The authors declare no potential conflicts of interest regarding this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.tranon.2016.12.005.

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