Local Recurrence in Mismatch Repair – Proficient Colon Cancer Predicted by an Infiltrative Tumor Border and Lack of $CD8^+$ Tumor-Infiltrating Lymphocytes

Inti Zlobec, Luigi M. Terracciano, and Alessandro Lugli

Abstract

Purpose: The identification of colon cancer patients at high risk of local recurrence is necessary to improve the selection of patients for more tailored treatment protocols. The aim of this study was to develop a predictive model of local recurrence by assessing the independent predictive effect of 7 clinicopathologic features, 24 protein markers of tumor progression, and their multifeature combinations in mismatch repair – proficient colon cancers.

Experimental Design: Immunohistochemistry for 24 protein markers was done on 269 patients with complete clinicopathologic data. After univariate and multivariable analyses, independent predictors of local recurrence were identified and their multifeature combinations were analyzed. Kaplan-Meier and Cox proportional hazards regression were done for survival analysis.

Results: Local recurrence was observed in 119 patients (55.8%). Independent predictors of tumor recurrence were lymph node involvement ($P = 0.006$), absence of $CD8^+$ tumor-infiltrating lymphocytes (TIL; $P < 0.001$), and infiltrative tumor margin ($P < 0.001$). This independent effect persisted after adjusting for adjuvant therapy. Risk of recurrence was 0.75 and the 5-year survival rate was 8.8% in patients with these three adverse features. Node-negative patients with an infiltrative tumor margin and absence of $CD8^+$ TILs were identified as high risk with a probability of 0.55 for recurrence and a 60% 5-year survival rate. The remaining node-negative cases fared significantly better with risks ranging from 8% to 26% and 5-year survival rates reaching 97.6%.

Conclusions: An infiltrative tumor margin and absence of $CD8^+$ TILs are highly predictive of local recurrence in node-negative mismatch repair – proficient colon cancer and may help to identify high-risk patients who may benefit from adjuvant chemotherapy.

Despite major advances in the treatment of patients with colorectal cancer, local recurrence rates are reported in approximately 30% to 40% of cases with stage II and III disease (1, 2). In addition to American Joint Committee on Cancer/International Union Against Cancer tumor-node-metastasis stage, several pathologic and clinical features have been identified as risk factors for locoregional relapse including poorly differentiated histology, lymphovascular and perineural invasion, clinical bowel obstruction or perforation, and elevated preoperative plasma levels of carcinoembryonic antigen (3).

Rectal tumors, comprising approximately 30% of colorectal cancer cases, differ significantly from those of the colon not only in terms of their increased rate of local recurrence and decreased overall survival but also by their more frequent treatment with preoperative modalities and choice of surgical techniques (3–7). Several predictive factors of local failure in rectal cancer have been recognized including post-treatment tumor regression grade and positivity of the circumferential resection margin (8, 9).

In colon cancer, postoperative adjuvant chemotherapy has been shown to improve overall and disease-free survival (10–12). Although node positivity is one of the most important predictors of local failure and an indicator for adjuvant chemotherapy, approximately 25% of patients with node-negative colorectal cancer develop a recurrent disease (13). Evidence suggests that adjuvant chemotherapy may confer a survival benefit in a subgroup of node-negative patients; however, no standardized criteria are yet in place for identifying these high-risk patients (14, 15). Treatment of stage II colon cancer with adjuvant chemotherapy is not currently recommended (16).

Sargent et al. recently analyzed disease-free and overall survival from 18 randomized phase III clinical trials comprising more than 20,000 patients (17). They determined that more than 85% of recurrences occurred within the first 3 years and 91% of deaths in patients with local failure arose within the first 5 years. A strong positive correlation between disease-free and overall survival in stage II and III colon cancer was identified. Based on these findings, disease-free survival is currently considered an acceptable surrogate endpoint to

Authors' Affiliation: Institute of Pathology, University Hospital of Basel, Basel, Switzerland

Received 1/5/08; revised 3/4/08; accepted 3/5/08.

Grant support: Novartis Foundation (formerly Ciba-Geigy-Jubilee Foundation; I. Zlobec).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Inti Zlobec, Institute of Pathology, University Hospital of Basel, Schönbeinstrasse 40, CH-4031 Basel, Switzerland. Phone: 41-61-265-28-95; Fax: 41-61-265-29-66; E-mail: izlobec@uhbs.ch.

doi:10.1158/1078-0432.CCR-08-0048

overall survival and a primary endpoint for trials testing adjuvant treatments for colon cancer (18).

The identification of both node-negative and node-positive patients at high risk of postoperative tumor recurrence is therefore of primary importance to improve the selection of patients for more tailored treatment protocols. The aim of this study was to develop a predictive model of local recurrence by assessing the independent predictive effect of 7 clinicopathologic features, 24 protein markers, and their multifeature combinations on a large cohort of 269 mismatch repair (MMR)–proficient colon cancers. The immunohistochemical protein markers were included into the study for their well-established involvement in (*a*) Wnt signaling (APC, β-catenin, and E-cadherin); (*b*) cell cycle arrest and apoptosis (Bcl-2, p21, p27, p53, mammalian sterile20-like kinase 1, and apoptosis protease activating factor-1); (*c*) angiogenesis and metastasis [vascular endothelial growth factor, cyclooxygenase-2, ephrin B2 receptor, urokinase plasminogen activator (uPA) and its receptor, and raf-1 kinase inhibitor protein]; (*d*) RAS-mitogen-activated protein kinase, AKT, and transforming growth factor-β signaling (epidermal growth factor receptor, phosphorylated extracellular signal-regulated kinase, phosphorylated AKT, SMAD4, and receptor for hyaluronic acid–mediated motility); and (*e*) for their previously reported prognostic significance including proliferation marker Ki-67, mucins MUC1 and MUC2, and $CD8^+$ tumor-infiltrating lymphocytes (TIL).

Materials and Methods

Tissue microarray and immunohistochemistry. A tissue microarray of 1,420 colorectal cancer tissues was constructed as described previously (19). Immunohistochemistry was done for all 23 tumor markers (APC, β-catenin, E-cadherin, Bcl-2, p21, p27, p53, mammalian sterile20-like kinase 1, apoptosis protease activating factor-1, vascular endothelial growth factor, cyclooxygenase-2, ephrin B2 receptor, uPA, uPA receptor, raf-1 kinase inhibitor protein, epidermal growth factor receptor, phosphorylated extracellular signal-regulated kinase, phosphorylated AKT, SMAD4, receptor for hyaluronic acid–mediated motility, Ki-67, MUC1, and MUC2) as well as for the protein CD8 used to assess the number of TILs. MMR status was established by evaluating proteins MLH1, MSH2, and MSH6. Positivity for all three markers was required for the tumor to be considered MMR proficient (20). Immunostaining protocols have been detailed elsewhere (21, 22). Evaluation of immunoreactivity for all tumor markers was done semiquantitatively by assessing the proportion of immunoreactive tumor cells over the total number of tumor cells per tissue microarray punch resulting in scores from 0% to 100%. This scoring system has been found to lead to strong interobserver agreement between independent pathologists for a variety of tumor markers in colorectal cancer using the tissue microarray approach. Staining intensity was not evaluated (23).

Clinicopathologic data. Of the 1,420 colorectal cancer tissues, 482 were located in the rectum and excluded from further analyses. Seven hundred and forty-six colon cancers were found to be MMR proficient. Of these, information on local recurrence was available for 269 patients. The clinicopathologic features for these patients included gender, tumor location, pT and pN stage, tumor grade, vascular invasion, tumor border configuration, and presence of peritumoral lymphocytes (PTL). The majority of patients (n = 205; 76.2%) did not receive postoperative treatment. Of the 66 patients undergoing adjuvant therapy, 61 received chemotherapy, 1 received only radiotherapy, and 4 patients received both chemotherapy and radiotherapy.

Table 1. Clinicopathologic features of MMR-proficient colon cancers (N = 269)

Clinicopathologic feature	Frequency, *n* (%)
Gender	
Female	126 (46.8)
Male	143 (51.2)
Tumor location	
Left-sided	132 (51.2)
Right-sided	126 (48.8)
pT stage	
pT_1	8 (3.0)
pT_2	38 (14.4)
pT_3	159 (60.2)
pT_4	59 (22.4)
pN stage	
pN_0	129 (50.0)
pN_1	71 (27.5)
pN_2	58 (22.5)
Tumor grade	
1	3 (1.1)
2	195 (73.3)
3	68 (25.6)
Vascular invasion	
Absence	188 (70.7)
Presence	78 (29.3)
Tumor border configuration	
Infiltrating margin	132 (49.6)
Pushing margin	134 (50.4)
Peritumoral lymphocytic infiltration	
Absence	217 (81.6)
Presence	49 (18.4)
Local recurrence	
Absence	150 (55.8)
Presence	119 (44.2)

Statistical analysis. Cutoff scores for all protein markers were assessed by receiver operating characteristic (ROC) curve analysis (24). Briefly, at each protein expression score, the sensitivity and specificity of the marker for local recurrence was evaluated. The (0, 1) criterion was used to select the point on the ROC curve leading to the greatest correct classification of patients with or without local recurrence. The association of protein expression with local recurrence was determined by univariate and multiple regression analyses using a stepwise selection procedure. Odds ratios (OR), 95% confidence intervals (95% CI), and P values were used to interpret the effect of each marker on local recurrence. A Bonferroni correction for multiple comparisons was done. To maintain an overall type I $\alpha = 0.05$, only ROC curve analysis was used to evaluate the diagnostic accuracy of the combinations of independent predictors of recurrence. Kaplan-Meier curves for these multifeature combinations were compared by the log-rank test and multiple Cox regression analysis was carried out for multivariable survival analysis. In addition, a retrospective power calculation was done based on the OR for each of the independent predictors of local recurrence in univariate analysis (%power3 MACRO; The SAS Institute). The general guidelines for assessing the adequacy of sample size in multivariable analysis state that at least 10 recurrences must occur for each independent predictor.

Results

Univariate analysis of local recurrence. The clinicopathologic features are described in Table 1 and included 119 (44.2%) patients with local recurrence and 150 (55.8%) without this outcome. Of the protein markers evaluated, only absence of

Table 2. Association of protein marker expression and local recurrence

Protein marker	*n*	Cutoff score (%)	*P*	OR (95% CI)
Apoptosis protease activating factor-1 (c)	224	95	0.829	1.07 (0.6-2.0)
APC (c)	212	70	0.803	0.93 (0.5-1.6)
β-catenin (n)	231	5	0.043	2.03 (1.0-4.0)
Bcl-2 (c)	238	5	0.155	0.69 (0.4-1.2)
CD8 (TILs)	237	5 TILs	<0.001*	0.19 (0.1-0.4)
Cyclooxygenase-2 (c)	247	95	0.137	0.62 (0.3-1.2)
E-cadherin (m)	237	95	0.84	0.92 (0.4-2.1)
Epidermal growth factor receptor (c/m)	242	90	0.489	0.83 (0.5-1.4)
Ephrin B2 receptor (c/m)	184	60	0.022	0.5 (0.3-0.9)
Ki-67 (n)	232	40	0.467	0.435 (0.6-3.0)
Mammalian sterile20-like kinase 1 (c)	198	70	<0.001*	0.38 (0.2-0.7)
MUC1 (c)	225	10	0.337	0.77 (0.5-1.3)
MUC2 (c)	223	5	0.499	1.2 (0.7-2.0)
p21 (n)	244	0	0.321	0.77 (0.5-1.3)
p27 (n)	208	75	0.122	0.63 (0.4-1.1)
p53 (n)	223	40	0.316	0.32 (0.8-2.3)
Phosphorylated AKT (c)	245	5	0.308	0.75 (0.4-1.3)
Phosphorylated extracellular signal-regulated kinase (n)	243	0	0.546	0.82 (0.4-1.6)
SMAD4 (c/m)	207	10	0.051	0.5 (0.2-1.0)
Receptor for hyaluronic acid–mediated motility (c)	201	80	0.532	0.83 (0.5-1.5)
raf-1 kinase inhibitor protein (c)	146	70	0.027	0.41 (0.2-0.9)
uPA (c)	196	10	0.318	1.35 (0.7-2.5)
uPA receptor (c/m)	200	60	0.814	1.07 (0.6-1.9)
Vascular endothelial growth factor (c)	230	80	0.271	0.74 (0.4-1.3)

Abbreviations: c, cytoplasmic; n, nuclear; m, membranous.
*Significant after adjustment for multiple comparisons.

$CD8^+$ TILs [$P < 0.001$; OR (95% CI), 0.19 (0.1-0.4)] and negative mammalian sterile20-like kinase 1 expression [$P < 0.001$; OR (95% CI), 0.38 (0.2-0.7)] were significantly associated with the presence of local recurrence (Table 2). Late T stage ($P = 0.004$), lymph node involvement ($P < 0.001$), infiltrative tumor border ($P < 0.001$), and presence of vascular invasion ($P < 0.001$) were predictive of tumor recurrence in univariate analysis (Table 3) after adjustment for multiple comparisons.

Independent predictors of local recurrence. Lymph node involvement [$P = 0.006$; OR (95% CI), 2.46 (1.3-4.6)], absence of $CD8^+$ TILs [$P < 0.001$; OR (95% CI), 3.93 (1.9-8.0)], and infiltrative tumor margin [$P < 0.001$; OR (95% CI), 3.5 (1.8-8.6)] were identified as adverse independent predictive factors of local recurrence in multivariable analysis. This independent effect was maintained after adjusting for postoperative therapy (lymph node positivity, $P = 0.043$; infiltrative margin, $P < 0.001$; absence of TILs, $P < 0.001$). In addition, a retrospective power calculation was done. The strong associations of each of these features with local recurrence in univariate analysis led to a power for each exceeding 99%. Moreover, considering that 119 recurrences occurred and that the minimum number of recurrences acceptable for this type of multivariable analysis is approximately 10 per independent feature, the prognostic effects of N stage, tumor border configuration, and TILs have been established using an appropriate sample size.

Combinations of these three features were evaluated (Table 4). The risk of local recurrence was the highest in patients with lymph node positivity, with absence of TILs, and with an infiltrative tumor margin (probability = 0.75). whereas patients with the opposite characteristics (node-negativity, presence of TILs and a pushing tumor border) showed the lowest likelihood of developing recurrent disease with a risk of only 0.08.

The second most adverse subgroup of high-risk patients included lymph node–negative cases with absence of TILs and an infiltrative tumor margin. These patients had a probability of 0.55 for local recurrence, whereas the remaining node-negative cases benefited from a significantly lower likelihood of recurrent disease ranging from 8% to 26%. In addition, a subgroup of node-positive patients with a low risk of recurrence was also identified, that is, those with presence of TILs and a pushing tumor border.

ROC curve analysis. The diagnostic accuracy of the combination of lymph node status, $CD8^+$ TILs, and invasive tumor border was determined to be 0.78. By evaluating the ROC curve for this predictive model (Fig. 1), two clusters of patients could

Table 3. Association of clinicopathologic features and local recurrence

Clinicopathologic feature	*P*	OR (95% CI)
T stage	0.004*	2.96 (1.4-6.1)
N stage	<0.001*	4.27 (2.5-7.2)
PTL infiltration	0.083	0.47 (0.2-1.1)
Tumor grade	0.046	1.76 (1.0-3.1)
Invasive margin	<0.001*	5.77 (3.4-9.8)
Vascular invasion	<0.001*	4.46 (2.5-7.9)
Tumor location	0.652	1.12 (0.7-1.8)

*Significant after adjustment for multiple comparisons.

Table 4. Multifeature combinations of independent predictors and probability of local recurrence

Lymph node status	CD8+ TILs	Invasive margin	Probability	Local recurrence, *n* (%)	
				Absence	Presence
Present	Absent	Infiltrative	0.75	19 (31.2)	42 (68.8)
Absent	Absent	Infiltrative	0.55	10 (38.5)	16 (61.5)
Present	Absent	Pushing	0.47	10 (43.5)	13 (56.5)
Present	Present	Infiltrative	0.44	6 (40.0)	9 (60.0)
Absent	Absent	Pushing	0.26	28 (73.7)	10 (26.3)
Absent	Present	Infiltrative	0.24	7 (77.8)	2 (22.2)
Present	Present	Pushing	0.18	9 (90.0)	1 (10.0)
Absent	Present	Pushing	0.08	40 (95.2)	2 (4.8)
			Total	129	95

be observed. The first group considered high risk included (*a*) node-positive cases with absence of TILs, (*b*) node-positive cases with presence of TILs and infiltrative margin, and (*c*) node-negative tumors with absence of TILs and an infiltrative tumor margin. These groupings correspond to the subgroups described in Table 4 with the highest probabilities of local recurrence. The remaining patients were considered low risk and included mostly node-negative tumors as well as a subgroup of node-positive patients with TILs and a pushing tumor border.

Survival analysis. Survival times were compared for the eight combinations of features (Fig. 2). Five-year survival rates were the lowest (8.8%) in patients with the most adverse combination of the three predictors, that is, in cases with lymph node involvement, absence of TILs, and infiltrative tumor margin. Patients with the opposite combination of features benefited from a significantly improved 5-year survival rate of 97.6%. The high-risk node-negative subgroup of patients with infiltrative tumor border and lack of $CD8^+$ TILs had a significantly worsened survival time compared with the remaining node-negative tumors and a 5-year survival rate of 60%. Contrarily, the low-risk node-positive subgroup with a pushing border and presence of $CD8^+$ TILs experienced a favorable outcome and survival rate of 87.5%.

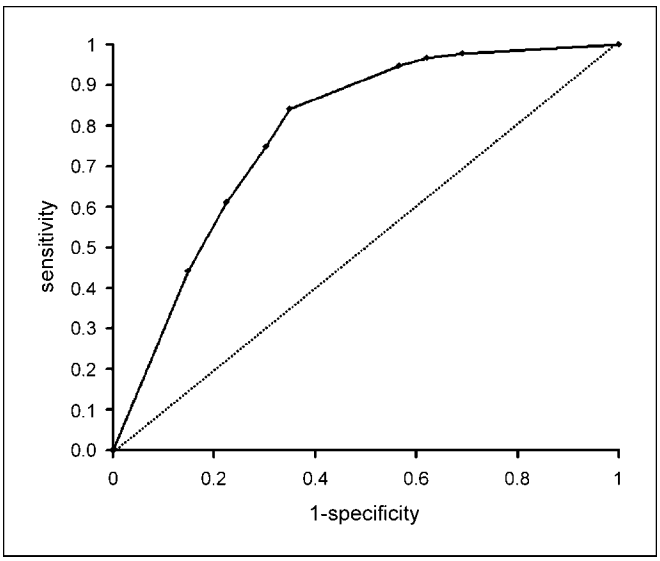

Fig. 1. ROC curve illustrating the diagnostic accuracy of lymph node involvement, absence or presence of $CD8^+$ TILs, and tumor border configuration for predicting local recurrence in colon cancer. Area under the ROC curve (*AUC*) = 0.78.

Discussion

During the first 8 years after surgical resection for colon cancer, 80% of patient deaths are preceded by a tumor recurrence (25). Although node positivity is reported to predict recurrence with a likelihood of 60%, approximately 25% to 30% of patients with node-negative colorectal cancer develop recurrence and die from the disease (13, 26). The results of this study have identified the combined assessment of tumor border configuration (infiltrative versus pushing), lymph node invasion, and $CD8^+$ TILs as highly predictive of local recurrence in MMR-proficient colon cancer. Moreover, an infiltrative tumor margin and simultaneous absence of $CD8^+$ TILs constituted the most adverse combination of features independently of nodal status. We defined MMR proficiency as positivity for three MMR proteins, that is, MLH1, MSH2, and MSH6. Lindor et al. found that immunohistochemistry for MLH1 and MSH2 was highly specific (specificity of 100%) for MMR proficiency, a conclusion reached after assessment of 794 cases (20). By including MSH6, we have further decreased the likelihood of having incorrectly included non-MMR-proficient tumors in this study.

Tumor border configuration has been established as an independent prognostic factor in colorectal cancer and is characterized by widespread dissection of normal tissue structures with loss of a clear boundary between tumor and host tissue (27, 28). In addition, an infiltrative tumor margin diagnosed at low magnification is strongly associated with the presence of tumor budding defined as the transition from glandular structures to single cells or clusters of up to four cells at the invasive front and observed at high magnification (29, 30). Tumor budding itself has been shown to have independent prognostic value in colorectal cancer and may be the most important prognostic indicator after lymph node spread (30). Budding is associated with liver and lung metastasis, lymph node invasion, and tumor recurrence following curative surgery (31, 32). Tumor buds have also been credited with the properties of malignant stem cells including the potential for redifferentiation both locally and at sites of distant metastasis and appear to be the first histologic event of invasion and metastasis in colorectal cancer (33). Overexpression of β-catenin in dedifferentiated cancer cells at the invasive margin has been reported and is strongly

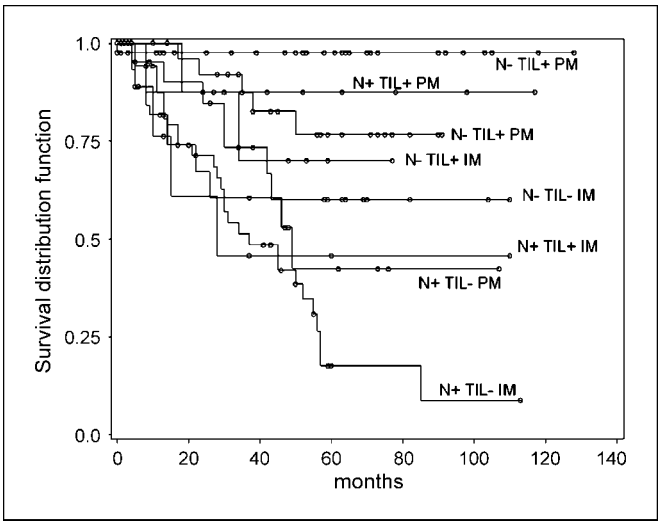

Fig. 2. Kaplan-Meier survival curves for patients with possible combinations of lymph node involvement (*N-*, node-negative; *N+*, node-positive), absence (*TIL-*) or presence (*TIL+*) of $CD8^+$ TILs, and invasive [infiltrative (*IM*) versus pushing (*PM*)] tumor margin.

associated with uPA and uPA receptor (the uPA/uPA receptor system) whose up-regulation is a primary mediator of extracellular matrix degradation, angiogenesis, and metastasis (34, 35). Accumulation of nuclear β-catenin following dysregulation of WNT signaling is a hallmark of MMR-proficient colorectal cancer. Although β-catenin expression was found to be related to local recurrence in univariate analysis, it was not selected as an independent predictor of this outcome. We have shown previously that β-catenin is associated with an infiltrating tumor margin, whereas this margin itself is significantly more common in budding versus nonbudding tumors (21, 29). In addition, Brabletz et al. found an increasing expression of this protein from the tumor center to the tumor margin (36).The lack of independence of β-catenin on recurrence is therefore likely due to its association with features that themselves are stronger predictors of recurrence. KRAS mutations have been linked to tumor budding as well and are more frequently found in MMR-proficient compared with microsatellite instability-high patients (37, 38).

Shinto et al. described cytoplasmic pseudofragments in the stroma surrounding budding cells and absence of PTLs in tumors with this feature (39). PTL infiltration at the invasive margin has also been strongly correlated with the lack of tumor budding, with the presence of an infiltrative tumor border and itself has been found previously to hold important prognostic value (21, 39). We have reported previously the strong association of $CD8^+$ TILs with PTL infiltration in both tissue microarray punches and whole tissue sections as well as a sensitivity of 90% for tumor budding when analyzed in conjunction with E-cadherin and apoptosis protease activating factor-1 (21). An immune response responsible for destruction of tumor buds, a so-called nipping in the bud phenomenon, is underlined by the absence of this feature and the presence of both PTLs and $CD8^+$ TILs. Tumor immunity may therefore be a possible mechanism underlying the improved prognosis in nonbudding patients and also in tumors lacking the infiltrative tumor border associated with this feature.

$CD8^+$ TILs have also been described as having independent prognostic value in MMR-proficient but not MMR-deficient colorectal cancers (40). The inherent abundance of TILs in microsatellite instability-high colorectal cancers as well as the less frequent occurrence of tumor budding in these patients again underlines the possible role of tumor immunity in conferring an improved prognosis in colorectal cancer. We have also highlighted previously the importance of $CD8^+$ TILs in improved survival in patients with MMR-proficient node-negative colorectal cancer (22). In this study, an infiltrative tumor margin and absence of $CD8^+$ TILs were highly predictive of local recurrence in patients with MMR-proficient colon cancer. Moreover, node-negative patients with lack of $CD8^+$ TILs and infiltrative margin were identified as high risk of tumor recurrence. This subgroup showed a significantly worsened survival time and greater probability of local recurrence compared with the remaining node-negative patients, that is, those with presence of $CD8^+$ TILs or with an expansive, pushing tumor margin.

Immunohistochemical or molecular profiling of local recurrence has been done by several study groups. Galizia et al. identified p27, Dukes' staging, and epidermal growth factor receptor as independent prognostic indicators of disease recurrence in 149 patients undergoing curative surgery (41). Rosati et al. evaluated thymidylate synthase, Ki-67, p53, p27, and Bcl-2 and found that only pathologic stage was an independent prognostic factor for disease-free survival (42). Cascinu et al. identified vascular endothelial growth factor and S-phase fraction as significant predictors of local recurrence (43). The predictive ability of these immunohistochemical findings was not reproduced in our study for several possible reasons. First, rather than evaluating disease-free survival over time, we analyzed absence or presence of tumor recurrence. Second, whereas Rosati et al. evaluated colorectal cancers selected for Dukes' B and C disease, our study focused on MMR-proficient colon cancers of all tumor stages. Our study involved a greater number of patients (n = 269) with more than 50% of these positive for recurrence. Moreover, we used a systematic method for evaluating immunoreactivity and selecting optimal cutoff scores for our protein markers using ROC curve analysis. This approach allowed us to avoid a composite scoring system using the degree of staining intensity, which we have found previously to lack reproducibility for several markers of tumor progression in colorectal cancer (23). Gene expression profiling was done by Wang et al. to predict local recurrence in Dukes' B colon cancer (26). They described a 23-gene signature validated on 36 independent patients with an overall predictive performance of 74%. In our study using only three features, the diagnostic accuracy was 78% for local recurrence.

The clinical value of this predictive model is further supported by the ease of interpretation of the three features involved. The pathologic assessment of lymph node involvement is part of routine diagnostic pathology. Tumor border configuration is an evaluation that can be made even at low magnification, whereas intraepithelial lymphocytes are highly visible after immunostaining for CD8. Together, these facts suggest that considerable reproducibility can be achieved between pathologists.

In summary, although these results need to be confirmed on a larger number of patients, our findings indicate that

evaluation of the invasive tumor margin and lymph node spread in addition to immunohistochemical staining for $CD8^+$ TILs is predictive of local recurrence in patients with MMR-proficient colon cancer. Moreover, we have identified a subgroup of node-negative patients with an infiltrative tumor margin and absence of $CD8^+$ TILs at high risk of developing local recurrence and should perhaps be considered candidates for adjuvant therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991;253:49–53.
2. Wiggers T, Arends JW, Schutte B, Volovics L, Bosman FT. A multivariate analysis of pathologic prognostic indicators in large bowel cancer. Cancer 1988;61:386–95.
3. Wolpin BM, Meyerhardt JA, Mamon HJ, Mayer RJ. Adjuvant treatment of colorectal cancer. CA Cancer J Clin 2007;57:168–85.
4. Andreoni B, Chiappa A, Bertani E, et al. Surgical outcomes for colon and rectal cancer over a decade: results from a consecutive monocentric experience in 902 unselected patients. World J Surg Oncol 2007;5:73.
5. Heald RJ, Ryall RD. Recurrence and survival after total mesorectal excision for rectal cancer. Lancet 1986;1:1479–82.
6. Peeters KC, Marijnen CA, Nagtegaal ID, et al. The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma. Ann Surg 2007;246:693–701.
7. Wong RK, Tandan V, De Silva S, Figueredo A. Preoperative radiotherapy and curative surgery for the management of localized rectal carcinoma. Cochrane Database Syst Rev 2007: CD002102.
8. Glynne-Jones R, Mawdsley S, Novell JR. The clinical significance of the circumferential resection margin following preoperative pelvic chemo-radiotherapy in rectal cancer: why we need a common language. Colorectal Dis 2006;8:800–7.
9. Rodel C, Martus P, Papadoupolos T, et al. Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. J Clin Oncol 2005;23:8688–96.
10. Laurie JA, Moertel CG, Fleming TR, et al. Surgical adjuvant therapy of large-bowel carcinoma: an evaluation of levamisole and the combination of levamisole and fluorouracil. The North Central Cancer Treatment Group and the Mayo Clinic. J Clin Oncol 1989;7:1447–56.
11. Moertel CG, Fleming TR, Macdonald JS, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 1990;322:352–8.
12. Moertel CG, Fleming TR, Macdonald JS, et al. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. Ann Intern Med 1995;122:321–6.
13. Bilchik AJ, Hoon DS, Saha S, et al. Prognostic impact of micrometastases in colon cancer: interim results of a prospective multicenter trial. Ann Surg 2007;246:568–75; discussion 575–7.
14. Mamounas E, Wieand S, Wolmark N, et al. Comparative efficacy of adjuvant chemotherapy in patients with Dukes' B versus Dukes' C colon cancer: results from four National Surgical Adjuvant Breast and Bowel Project adjuvant studies (C-01, C-02, C-03, and C-04). J Clin Oncol 1999;17:1349–55.
15. Quasar Collaborative G, Gray R, Barnwell J, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet 2007;370:2020–9.
16. Benson AB III, Schrag D, Somerfield MR, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol 2004;22:3408–19.
17. Sargent DJ, Wieand HS, Haller DG, et al. Disease-free survival versus overall survival as a primary end point for adjuvant colon cancer studies: individual patient data from 20,898 patients on 18 randomized trials. J Clin Oncol 2005;23:8664–70.
18. Punt CJ, Buyse M, Kohne CH, et al. Endpoints in adjuvant treatment trials: a systematic review of the literature in colon cancer and proposed definitions for future trials. J Natl Cancer Inst 2007;99:998–1003.
19. Sauter G, Simon R, Hillan K. Tissue microarrays in drug discovery. Nat Rev Drug Discov 2003;2:962–72.
20. Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. J Clin Oncol 2002;20:1043–8.
21. Zlobec I, Lugli A, Baker K, et al. Role of APAF-1, E-cadherin and peritumoral lymphocytic infiltration in tumour budding in colorectal cancer. J Pathol 2007;212:260–8.
22. Zlobec I, Minoo P, Baumhoer D, et al. Multi-marker phenotype predicting adverse survival in patients with lymph node-negative colorectal cancer. Cancer 2008;112:495–502.
23. Zlobec I, Terracciano L, Jass JR, Lugli A. Value of staining intensity in the interpretation of immunohistochemistry for tumor markers in colorectal cancer. Virchows Arch 2007;451:763–9.
24. Zlobec I, Steele R, Terracciano L, Jass JR, Lugli A. Selecting immunohistochemical cut-off scores for novel biomarkers of progression and survival in colorectal cancer. J Clin Pathol 2007;60:1112–6.
25. Sargent DJ, Goldberg RM, Jacobson SD, et al. A pooled analysis of adjuvant chemotherapy for resected colon cancer in elderly patients. N Engl J Med 2001;345:1091–7.
26. Wang Y, Jatkoe T, Zhang Y, et al. Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer. J Clin Oncol 2004;22:1564–71.
27. Hase K, Shatney C, Johnson D, Trollope M, Vierra M. Prognostic value of tumor "budding" in patients with colorectal cancer. Dis Colon Rectum 1993;36:627–35.
28. Jass JR, Love SB, Northover JM. A new prognostic classification of rectal cancer. Lancet 1987;1:1303–6.
29. Compton CC. Optimal pathologic staging: defining stage II disease. Clin Cancer Res 2007;13:6862–70s.
30. Ueno H, Murphy J, Jass JR, Mochizuki H, Talbot IC. Tumour "budding" as an index to estimate the potential of aggressiveness in rectal cancer. Histopathology 2002;40:127–32.
31. Nakamura T, Mitomi H, Kikuchi S, Ohtani Y, Sato K. Evaluation of the usefulness of tumor budding on the prediction of metastasis to the lung and liver after curative excision of colorectal cancer. Hepatogastroenterology 2005;52:1432–5.
32. Tanaka M, Hashiguchi Y, Ueno H, Hase K, Mochizuki H. Tumor budding at the invasive margin can predict patients at high risk of recurrence after curative surgery for stage II, T_3 colon cancer. Dis Colon Rectum 2003;46:1054–9.
33. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells—an integrated concept of malignant tumour progression. Nat Rev Cancer 2005;5:744–9.
34. Berger DH. Plasmin/plasminogen system in colorectal cancer. World J Surg 2002;26:767–71.
35. Hiendlmeyer E, Regus S, Wassermann S, et al. β-catenin up-regulates the expression of the urokinase plasminogen activator in human colorectal tumors. Cancer Res 2004;64:1209–14.
36. Brabletz T, Jung A, Hermann K, Gunther K, Hohenberger W, Kirchner T. Nuclear overexpression of the oncoprotein β-catenin in colorectal cancer is localized predominantly at the invasion front. Pathol Res Pract 1998;194:701–4.
37. Prall F, Ostwald C. High-degree tumor budding and podia-formation in sporadic colorectal carcinomas with K-ras gene mutations. Hum Pathol 2007;38:1696–702.
38. Prall F. Tumour budding in colorectal carcinoma. Histopathology 2007;50:151–62.
39. Shinto E, Mochizuki H, Ueno H, Matsubara O, Jass JR. A novel classification of tumour budding in colorectal cancer based on the presence of cytoplasmic pseudo-fragments around budding foci. Histopathology 2005;47:25–31.
40. Baker K, Zlobec I, Tornillo L, Terracciano L, Jass JR, Lugli A. Differential significance of tumour infiltrating lymphocytes in sporadic mismatch repair deficient versus proficient colorectal cancers: a potential role for dysregulation of the transforming growth factor-β pathway. Eur J Cancer 2007;43:624–31.
41. Galizia G, Lieto E, Ferraraccio F, et al. Prognostic significance of epidermal growth factor receptor expression in colon cancer patients undergoing curative surgery. Ann Surg Oncol 2006;13:823–35.
42. Rosati G, Chiacchio R, Reggiardo G, De Sanctis D, Manzione L. Thymidylate synthase expression, p53, bcl-2, Ki-67 and p27 in colorectal cancer: relationships with tumor recurrence and survival. Tumour Biol 2004;25:258–63.
43. Cascinu S, Graziano F, Valentini M, et al. Vascular endothelial growth factor expression, S-phase fraction and thymidylate synthase quantitation in node-positive colon cancer: relationships with tumor recurrence and resistance to adjuvant chemotherapy. Ann Oncol 2001;12:239–44.

Clinical Cancer Research

Local Recurrence in Mismatch Repair–Proficient Colon Cancer Predicted by an Infiltrative Tumor Border and Lack of CD8 + Tumor-Infiltrating Lymphocytes

Inti Zlobec, Luigi M. Terracciano and Alessandro Lugli

Clin Cancer Res 2008;14:3792-3797.