Loss of Raf-1 Kinase Inhibitor Protein Expression Is Associated With Tumor Progression and Metastasis in Colorectal Cancer

Parham Minoo, MD, PhD,1 Inti Zlobec, MSc,1 Kristi Baker,1 Luigi Tornillo, MD,2 Luigi Terracciano, MD,2 Jeremy R. Jass, MD, DSc, FRCPATH, FRCPA,1 and Alessandro Lugli, MD1,2

Key Words: Colorectal cancer; Raf-1 kinase inhibitor protein; RKIP; Prognosis; Tissue microarray; Immunohistochemistry; Apoptosis; Metastasis

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Abstract

Raf-1 kinase inhibitor protein (RKIP) is known as a critical down-regulator of the mitogen-activated protein kinase signaling pathway and a potential molecular determinant of malignant metastasis. The aim of this study was to determine the prognostic significance of RKIP expression in colorectal cancer (CRC).

Immunohistochemical staining for RKIP was performed on a tissue microarray comprising 1,197 mismatch repair (MMR)-proficient and 141 MMR-deficient CRCs. The association of RKIP with clinicopathologic features was analyzed. Loss of cytoplasmic RKIP was associated with distant metastasis (P = .038), higher N stage (P = .032), vascular invasion (P = .01), and worse survival (P = .001) in the MMR-proficient group. In MMR-deficient CRCs, loss of cytoplasmic RKIP was associated with distant metastasis (P = .043) and independently predicted worse survival (P = .004). Methylation analysis of 28 cases showed that loss of RKIP expression is unlikely to be due to promoter methylation.

Loss of RKIP expression is a marker of tumor progression and distant metastasis in MMR-proficient and MMR-deficient CRCs.

Raf-1 kinase inhibitor protein (RKIP) is a ubiquitously expressed and highly conserved protein that belongs to the phosphatidylethanolamine-binding protein family.1,2 RKIP is present in the cytoplasm and at the cell membrane3 and appears to have multiple biologic functions that implicate spermatogenesis, neural development, cardiac function, and membrane biogenesis.4-6 RKIP has also been shown to have a role in the regulation of multiple signaling pathways. Originally, RKIP was identified as a phospholipid-binding protein and, subsequently, as an interacting partner of Raf-1 kinase that blocks mitogen-activated protein kinase (MAPK) initiated by Raf-1.7 Initial studies showed that RKIP achieves this role by competitive interference with the binding of MEK to Raf-1.8 Recently, RKIP was shown to inhibit activation of Raf-1 by blocking phosphorylation of Raf-1 by p21-activated kinase and Src family kinases.9 It has also been suggested that RKIP could be involved in regulation of apoptosis by modulating the NF-κB pathway10 and in regulation of the spindle checkpoint via Aurora B.11

RKIP has also been implicated in tumor biology. In breast and prostate cancers, ectopic expression of RKIP sensitized cells to chemotherapeutic-induced apoptosis, and reduced expression of RKIP led to resistance to chemotherapy.12 A link between RKIP and cancer was first established in prostate cancer, with RKIP showing reduced expression in prostate cancer cells and the lowest expression levels in metastatic cells, suggesting that RKIP expression is inversely associated with the invasiveness of prostate cancer.13 Restoration of RKIP expression in metastatic prostate cancer cells inhibited invasiveness of the cells in vitro and in vivo in spontaneous lung metastasis but not the growth of the primary tumor in a murine model.13 These results suggest that RKIP has no major
impact on primary tumorigenesis but functions as a suppressor of metastasis. An association between loss of RKIP expression and metastatic progression has also been documented in patients with melanoma, prostate cancer, and breast cancer. In these studies, low RKIP expression was associated with higher stage and/or recurrence of cancer, suggesting that RKIP has potential as a molecular determinant of tumor metastasis and may, therefore, serve as a prognostic marker.

In this study, we documented the prognostic significance of cytoplasmic expression of RKIP in 1,197 mismatch repair (MMR)-proficient colorectal cancers (CRCs) randomized into 2 subgroups (n = 599 and n = 598) and in 141 MMR-deficient CRCs using tissue microarray (TMA) technology and a simple and reproducible scoring system. In addition, we studied hypermethylation of the promoter region of RKIP in 1,197 mismatch repair (MMR)-proficient colorectal cancers (CRCs) randomized into 2 subgroups (n = 599 and n = 598) and in 141 MMR-deficient CRCs using tissue microarray (TMA) technology and a simple and reproducible scoring system. In addition, we studied hypermethylation of the promoter region of RKIP as a mechanism for loss of RKIP expression in MMR-proficient and MMR-deficient CRCs.

Material and Methods

TMA Construction

A TMA of 1,420 unselected, nonconsecutive CRCs was constructed. Formalin-fixed, paraffin-embedded tissue blocks of CRC resections were retrieved from the archives of the Institute of Pathology, University Hospital of Basel, Basel, Switzerland; the Institute of Clinical Pathology, Basel; and the Institute of Pathology, Stadtpital Triemli, Zürich, Switzerland. One tissue cylinder with a diameter of 0.6 mm was punched from morphologically representative tissue areas of each donor tissue block and brought into 1 recipient paraffin block (3 × 2.5 cm) using a homemade semiautomated tissue arrayer. The TMA did not contain any duplicate or triplicate samples.

Clinicopathologic Parameters

The clinicopathologic data for 1,420 patients included T stage (T1, T2, T3, and T4), N stage (N0, N1, and N2), tumor grade (G1, G2, and G3), vascular invasion (presence or absence), and survival. The distribution of these features has been described previously. For 478 patients, information on local recurrence and distant metastasis was also available.

Immunohistochemical Analysis of TMA

The 1,420 CRC samples derived from representative areas away from the infiltrating tumor border and 57 normal colonic mucosa samples were transferred to an adhesive-coated slide system (Instrumedics, Hackensack, NJ) to facilitate the transfer of TMA sections to slides and to minimize tissue loss. Standard indirect immunoperoxidase procedures were used for immunohistochemical analysis (DAKO EnVision+, DakoCytomation, Carpinteria, CA). The 1,420 CRCs and 57 normal colonic mucosa samples were immunostained for RKIP (dilution 1:1,000; Upstate, Charlottesville, VA), MLH1 (clone MLH-1, dilution 1:100; BD Biosciences Pharmingen, San Jose, CA), MSH2 (clone MSH-2, dilution 1:200; BD Biosciences Pharmingen), and MSH6 (clone 44, dilution 1:400; BD Biosciences Pharmingen).

After dewaxing and rehydration in distilled water, sections for immunostaining were subjected to heat antigen retrieval in a microwave oven (1,200 W; 15 minutes) in 1 mmol/L of EDTA buffer, pH 8.0, for RKIP, MLH1, and MSH2 and in 0.01 mol/L citrate buffer, pH 6.0, for MSH6. Endogenous peroxidase activity was blocked using 0.5% hydrogen peroxide. After transfer to a humidified chamber, the sections were incubated with 10% normal goat serum (DakoCytomation) for 20 minutes and with primary antibody at 4°C. Subsequently, the sections were incubated with a peroxidase-labeled secondary antibody for 30 minutes at room temperature. For visualization of the antigen, the sections were immersed in 3-amino-9-ethylcarbazole+substrate-chromogen for 30 minutes and counterstained lightly with Gill hematoxylin Image II. The positive immunohistochemical control sample was a normal colonic mucosa section known to express RKIP. The negative immunohistochemical control sample was the same normal colonic mucosa section without the primary antibody.

Evaluation of Immunohistochemical Results

Cytoplasmic RKIP immunoreactivity was evaluated semiquantitatively by scoring the proportion of positive tumor cells to the total number of tumor cells on a scale from 0% to 100% using 5% increments (eg, 0%, 5%, 10%). The 1,420 CRCs were scored by one experienced surgical pathologist (A.L.) blinded to the clinicopathologic features. MLH1, MSH2, and MSH6 were scored as negative (0% staining) or positive (>0% staining).

Interobserver Reliability of the Scoring Method

To determine the reproducibility of the scoring method used for RKIP, a second observer (J.R.J.) evaluated immunoreactivity in 128 tumors semiquantitatively. The interobserver agreement was analyzed by using the intraclass correlation coefficient (ICC). The ICC is defined as the ratio of the between-subject variance to the (between-subject + within-subject variances) and has previously been used to assess agreement of immunohistochemical scores.

MMR Status

The 1,420 CRCs were stratified according to DNA MMR status and consisted of 1,197 MMR-proficient tumors expressing MLH1, MSH2, and MSH6; 141 MLH1-negative tumors; and 82 presumed hereditary nonpolyposis colorectal cancer cases demonstrating loss of MSH2 and/or MSH6 at any age or loss of MLH1 at younger than 55 years.
MMR-proficient tumors were used for the purpose of randomization owing to the overwhelming majority of these cases (1,197 [84.3%]). Presumed hereditary nonpolyposis colorectal cancers were excluded from the study because of the low number of cases and lack of germline mutation status.

**DNA Extraction and Bisulfite Modification**

We selected 14 cases of MMR-proficient and 14 cases of MMR-deficient CRCs with immunohistochemical expression of RKIP of less than 20% for methylation analysis. Corresponding paraffin blocks were retrieved from the archives and manually microdissected from two 8-µm-thick sections. Meticulous care was taken to exclude any normal tissue. Cell lysis and DNA extraction were performed using a QIAamp DNA mini kit (QIAGEN, Mississauga, Canada) according to the manufacturer’s protocol. Extracted genomic DNA was diluted in 40 µL of distilled water and denatured by adding 6 µL of 2N sodium hydroxide and incubation at 75°C for 20 minutes. Next, 500 µL of freshly prepared 4.8-mol/L sodium bisulfite and 28 µL of 10-mmol/L hydroquinone were added to the denatured genomic DNA, and the reaction was carried out overnight in dark at 55°C. DNA then was purified by using Wizard DNA clean-up (Promega, Madison, WI) and then ethanol-precipitated after 5 minutes of alkali treatment with 8.8 µL of 2N sodium hydroxide at room temperature.

**Methylation of RKIP**

Methylation of RKIP promoter was examined by methylation-specific polymerase chain reaction (PCR) using an AmpliTaq Gold kit (Roche, Branchburg, NJ) as described previously.25 The primers for amplification of the unmethylated sequence were 5'-TTTAGTGATATTTTTTGAGATATGA-3' and 3'-CACTCCCTAACCCTCTAATTAACCAA-5' and for the methylated reaction were 5'-TTTAGCGATATTTTTTGAGATACGA-3' and 3'-GCTCCCTAACCCTCTAATTAACCG-5'. The conditions for amplification were 10 minutes at 95°C followed by 39 cycles of denaturing at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and 30 seconds of extension at 72°C. The PCR products were subjected to electrophoresis on 8% acrylamide gels and visualized by SYBR gold nucleic acid gel stain (Molecular Probes, Eugene, OR). CpGenome Universal Methylated DNA (Chemicon, Temecula, CA) was used as a positive control sample for methylation.

**Randomization of MMR-Proficient CRCs**

The 1,197 MMR-proficient CRCs were randomly assigned into 2 groups consisting of 599 (group 1) and 598 (group 2) cases and matched for sex, tumor location, T stage, N stage, tumor grade, vascular invasion, and survival (Table 1). Immunohistochemical cutoff scores for RKIP expression were determined for group 1, and the association of RKIP expression and T stage, N stage, tumor grade, vascular invasion, local recurrence, distant metastasis, and 10-year survival were studied in group 2.

**Receiver Operating Curve Analysis**

To select a relevant immunohistochemical cutoff score to describe RKIP loss in CRC, receiver operating characteristic (ROC) curve analysis was carried out on group 1 of the MMR-proficient CRCs and on all MMR-deficient CRCs. At each immunohistochemical score, the sensitivity and specificity of RKIP for the outcome under study was plotted, generating an
was carried out using all variables significant (intervals (CIs) were obtained. Multivariate survival analysis was performed by using Cox proportional hazards regression. Hazard ratios (HRs) and 95% confidence intervals using the point (0.0, 1.0), was selected as the cutoff score in MMR-proficient tumors were 70% for the presence of metastasis, 80% for T stage and tumor grade, 90% for N stage and vascular invasion, and 100% for survival. In MMR-deficient CRCs, the most clinically relevant cutoff for all clinicopathologic features was greater than 80% except for metastasis, with a cutoff score of 40%.

ROC Curve Analysis

With respect to RKIP expression, the most significant cutoff scores in MMR-proficient tumors were 70% for the presence of metastasis, 80% for T stage and tumor grade, 90% for N stage and vascular invasion, and 100% for survival. In MMR-deficient CRCs, the most clinically relevant cutoff for all clinicopathologic features was greater than 80% except for metastasis, with a cutoff score of 40%.

Cytoplasmic RKIP in MMR-Proficient CRC

In univariate analysis, tumors with loss of RKIP expression were associated with the presence of distant metastasis (P = .038), lymph node metastasis (P = .032), vascular invasion (P = .01), and a significantly worse survival time (P < .001; HR [95% CI], 0.55 [0.41-0.75]) ROC Curve Analysis

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Cytoplasmic RKIP in MMR-Deficient CRC

In univariate analysis, tumors with loss of RKIP expression were associated with the presence of distant metastasis (P = .038), lymph node metastasis (P = .032), vascular invasion (P = .01), and a significantly worse survival time (P < .001; HR [95% CI], 0.55 [0.41-0.75]) ROC Curve Analysis

With respect to RKIP expression, the most significant cutoff scores in MMR-proficient tumors were 70% for the presence of metastasis, 80% for T stage and tumor grade, 90% for N stage and vascular invasion, and 100% for survival. In MMR-deficient CRCs, the most clinically relevant cutoff for all clinicopathologic features was greater than 80% except for metastasis, with a cutoff score of 40%.

Cytoplasmic RKIP in MMR-Deficient CRC

In univariate analysis, tumors with loss of RKIP expression were associated with the presence of distant metastasis (P = .038), lymph node metastasis (P = .032), vascular invasion (P = .01), and a significantly worse survival time (P < .001; HR [95% CI], 0.55 [0.41-0.75]) ROC Curve Analysis

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Cytoplasmic RKIP in MMR-Deficient CRC

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Interobserver Agreement

The ICC was 0.75, indicating strong interobserver agreement in the scores from both observers.

Difference in Survival Time by RKIP Status in Patients Receiving Treatment

Information about the treatment of patients was available for only 479 cases that were retrieved from the Triemli Center (Stadtpital Triemli, Zürich). The number of patients receiving surgery alone, surgery along with radiotherapy (adjuvant radiotherapy), surgery along with chemotherapy (adjuvant chemotherapy), or surgery plus radiotherapy and chemotherapy is given in Table 4. Our statistical analysis showed that in the surgery alone and surgery plus adjuvant treatment groups, RKIP expression of 100% was associated with longer survival than in cases with less than 100% (P < .001) Figure 2.

In the MMR-deficient CRC group, there were 80 cases. All had surgery except 6 patients who had chemotherapy

### Table 2
Association of Clinicopathologic Features and Positive and Negative Cytoplasmic Raf-1 Kinase Inhibitor Protein in Mismatch Repair–Proficient Colorectal Cancer Cases (Study Group 3)*

<table>
<thead>
<tr>
<th>Variable/Cutoff (%)</th>
<th>Negative</th>
<th>Positive</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>T stage/80</td>
<td></td>
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</tr>
<tr>
<td>T1</td>
<td>2 (1.8)</td>
<td>22 (7.1)</td>
<td>.084</td>
</tr>
<tr>
<td>T2</td>
<td>13 (11.7)</td>
<td>53 (17.0)</td>
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<tr>
<td>T3</td>
<td>77 (69.4)</td>
<td>193 (61.9)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>19 (17.1)</td>
<td>44 (14.1)</td>
<td></td>
</tr>
<tr>
<td>N stage/90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>66 (44.3)</td>
<td>148 (55.2)</td>
<td>.032</td>
</tr>
<tr>
<td>N1 + N2</td>
<td>83 (55.7)</td>
<td>120 (44.8)</td>
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</tr>
<tr>
<td>Tumor grade/80</td>
<td></td>
<td></td>
<td>.4</td>
</tr>
<tr>
<td>G1 + G2</td>
<td>97 (67.4)</td>
<td>291 (93.6)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>14 (12.6)</td>
<td>20 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion/90</td>
<td></td>
<td></td>
<td>.01</td>
</tr>
<tr>
<td>Presence</td>
<td>52 (35.4)</td>
<td>65 (23.6)</td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>96 (64.6)</td>
<td>210 (76.4)</td>
<td></td>
</tr>
<tr>
<td>Metastasis/70</td>
<td></td>
<td></td>
<td>.038</td>
</tr>
<tr>
<td>Presence</td>
<td>9 (41)</td>
<td>40 (21.2)</td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>13 (59)</td>
<td>149 (78.8)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE survival</td>
<td>60.33 ± 2.9</td>
<td>84.23 ± 4.1</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* Positive refers to a score equal to or higher than the cutoff score and negative to a score less than the cutoff. Data are given as number (percentage) unless otherwise indicated. Data were not available for all cases; percentages are based on the number of cases available for the variable, not the total number of cases in the group.

† Associations were determined on study group 2 (n = 598). The χ² test was used for T stage, N stage, tumor grade, and vascular invasion and the log-rank test for univariate survival analysis.

### Table 3
Association of Clinicopathologic Features With Positive and Negative Cytoplasmic Raf-1 Kinase Inhibitor Protein in Mismatch Repair–Deficient Colorectal Cancer Cases*

<table>
<thead>
<tr>
<th>Variable/Cutoff (%)</th>
<th>Negative</th>
<th>Positive</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>T stage/80</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>.418</td>
</tr>
<tr>
<td>T2</td>
<td>2 (4)</td>
<td>3 (4)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>30 (65)</td>
<td>60 (76)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>14 (30)</td>
<td>16 (20)</td>
<td></td>
</tr>
<tr>
<td>N stage/80</td>
<td></td>
<td></td>
<td>.185</td>
</tr>
<tr>
<td>N0</td>
<td>23 (51)</td>
<td>50 (63)</td>
<td></td>
</tr>
<tr>
<td>N1 + N2</td>
<td>22 (49)</td>
<td>29 (37)</td>
<td></td>
</tr>
<tr>
<td>Tumor grade/80</td>
<td></td>
<td></td>
<td>.416</td>
</tr>
<tr>
<td>G1 + G2</td>
<td>37 (82)</td>
<td>60 (76)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>8 (18)</td>
<td>19 (24)</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion/80</td>
<td></td>
<td></td>
<td>.079</td>
</tr>
<tr>
<td>Presence</td>
<td>15 (33)</td>
<td>15 (19)</td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>30 (67)</td>
<td>63 (81)</td>
<td></td>
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<tr>
<td>Metastasis/40</td>
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<td></td>
<td>.043</td>
</tr>
<tr>
<td>Presence</td>
<td>3 (38)</td>
<td>7 (11)</td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>5 (63)</td>
<td>56 (89)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE survival</td>
<td>46.33 ± 5.5</td>
<td>69.19 ± 4.4</td>
<td>.004</td>
</tr>
</tbody>
</table>

* Positive refers to a score equal to or higher than the cutoff score and negative to a score less than the cutoff. Data are given as number (percentage) unless otherwise indicated. Data were not available for all cases; percentages are based on the number of cases available for the variable, not the total number of cases in the group.

† The χ² test was used for T stage, N stage, tumor grade, and vascular invasion and the log-rank test for univariate survival analysis.

Figure 1 Kaplan-Meier overall survival curve for loss of Raf-1 kinase inhibitor protein (RKIP) expression in mismatch repair (MMR)-proficient (<100%) (A) and MMR-deficient (<80%) (B) colorectal cancer cases. A, P < .001. B, P = .004.
Methylation Analysis of **RKIP** Promoter

To determine whether loss of RKIP expression is a result of promoter methylation, we carried out methylation-specific PCR studies on 14 cases with near complete or complete loss of RKIP expression in the MMR-proficient group and 14 cases in the MMR-deficient group. The results showed no methylation in the selected promoter region of **RKIP** in either group (data not shown).

**Discussion**

Signaling through the RAS-MAPK pathway has emerged as a critical regulator of diverse cellular biologic functions, including growth and differentiation. Lugli et al have shown that the activated Raf/MEK/ERK signaling pathway—as a subfamily of the RAS-MAPK pathway—is involved in tumor progression and has prognostic significance in colorectal cancer. The MEK/ERK signaling pathway is under tight regulatory control by several inhibitory proteins, including Sprouty, SPRED, and RKIP. In the present study, we analyzed the prognostic value of RKIP expression in 1,197 MMR-proficient and 141 MMR-deficient CRCs using TMA technology and immunohistochemical analysis. The TMA technique is an accepted tool of investigation, especially when a large number of samples is analyzed. In addition, the use of a semiquantitative scoring system, ROC curves, and randomization of the study population into 2 subgroups allowed us to introduce a simple approach to analyzing a potentially novel tumor marker and to avoid a scoring system that includes an arbitrarily selected cutoff score or a complex composite scoring system. The reliability of this scoring system for RKIP was further evaluated by a second pathologist and shown to be reproducible.

Univariate analysis in the MMR-proficient group showed that loss of cytoplasmic RKIP is associated with the presence of distant metastasis, lymph node involvement (N stage), vascular invasion, and worse survival. These results are in line with the findings of previous studies that identified loss of RKIP as a key feature in tumor progression and metastasis. The effect of RKIP expression on survival of CRC patients was further confirmed in patients receiving treatment (Table 4; Figure 2).

In the MMR-deficient group, we found that 80% is the best cutoff for different clinicopathologic variables except distant metastasis, with a cutoff of 40%. Univariate analysis in this group showed that the loss of cytoplasmic RKIP is only associated with the presence of distant metastasis and worse survival. Recent studies have revealed that the activating mutation of B-Raf kinase (B-Raf-V600E) is common in this subtype of CRCs. The ability of RKIP to inhibit wild-type B-Raf kinase in addition to Raf-1 kinase is a matter of controversy, and there is no evidence that RKIP is capable of blocking the constitutive signal generation by oncogenic B-Raf. Nevertheless, RKIP was still significantly associated with distant metastasis and worse survival in this group of CRCs.

In multivariate analysis of survival in the MMR-proficient group including T stage, N stage, tumor grade, vascular invasion, and age, loss of RKIP was not an independent prognostic factor (P = .099), suggesting that the correlation between RKIP and worse survival in univariate analysis is secondary to its association with advanced clinicopathologic features. However, multivariate analysis using the same criteria (except age and tumor grade) demonstrated that loss of RKIP was an independent adverse prognostic factor in MMR-deficient CRCs (P = .005). In sporadic microsatellite instability-high CRCs, the underlying pathogenesis is MLH1 silencing that generally results from promoter methylation rather than germline mutation. Loss of MLH1 expression is likely to be highly deleterious, triggering apoptosis due to the rapid accumulation of DNA mismatches. Thus, loss of MLH1
expression is tolerated only when the mechanisms leading to apoptosis are extensively disrupted. In fact inhibition of apoptosis has been regarded as a central pathogenic mechanism in development of the likely precursor lesion, the sessile serrated adenoma. 40,41 Besides its antimetastatic properties, RKIP also has a proapoptotic role in tumor cells. 5,12 This might explain why, in the multivariate analysis, loss of cytoplasmic RKIP showed a significant association with survival only in the MMR-deficient group.

CpG island methylation is an epigenetic mechanism commonly involved in the silencing of gene transcription, leading to development of cancer. 42 Methylation in the promoter region of RKIP has been reported in normal colonic mucosa in patients with hyperplastic polyposis. 25 Our methylation analysis showed that methylation of the gene promoter is not a likely mechanism for explaining the loss of RKIP expression in MMR-proficient or MMR-deficient CRCs. We, therefore, suggest that other mechanisms (eg, mutation or loss of heterozygosity) may be responsible for the down-regulation of RKIP expression in CRCs.

The results of this study indicate that loss of cytoplasmic RKIP is associated with tumor progression, distant metastasis, and poor survival in patients with CRC. Loss of RKIP expression is not likely to be explained by promoter hypermethylation in colorectal tumors.

From the 1Department of Pathology, McGill University, Montreal, Canada; and 2Institute of Pathology, University Hospital of Basel, Basel, Switzerland.

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Address reprint requests to Dr Minoo: Dept of Pathology, McGill University, Duff Medical Bldg, 3775 University St, H3A 2B4 Montreal, Canada.

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