ABSTRACT

**Purpose and Study Design:** Recent studies have shown that β-catenin translocated into the cell nucleus functions like an oncogene. Accumulating evidence suggests that activation of the β-catenin oncogenic signaling cascade along with its twin, the K-ras cascade, may exert synergetic or synergistic effects on tumor development and progression. In the study reported here, we analyzed oncogenic β-catenin activation on the basis of its nuclear accumulation (NA) and compared the results with those of mutational activation of K-ras in 74 patients with colorectal cancer to determine whether the two oncogene-mediated signaling cascades interact.

**Results:** We found two distinct patterns of β-catenin activation, i.e., diffuse NA in 20 cases (27%) and selective NA at the tumor invasion front (NAinv) in 19 cases (26%). The presence of the NAinv pattern was significantly correlated with advanced Dukes’ stage tumor (P = 0.0005) and the presence of distant metastases (P = 0.0064). K-ras proto-oncogene was mutated in the tumors of 31 cases (42%). Activated β-catenin or K-ras was detected in most (78%) colorectal cancers analyzed, although a weak inverse correlation was found between the activities of the two oncogenes in the tumors. Importantly, most (7 of 8) patients with tumor showing both K-ras activation and the NAinv pattern of β-catenin activation were in Dukes’ stage C at surgery, and half of them developed distant metastases to the liver and lungs.

**Conclusion:** The results suggest that although oncogenic activation of β-catenin and K-ras is independent in the process of clinical cancer development, combined analysis of the two major oncogenes can detect most colorectal cancers and identify a subset of patients with poorer outcomes. Consequently, activation of either or both of these oncogenes may serve as a genetic marker for molecular diagnosis.
cancers reported to date (18). In particular, we found that selective oncogenic activation of $\beta$-catenin in the tumor invasion front, as represented by its unique expression pattern, is a reliable independent factor that can detect a subset of colon cancer patients highly susceptible to tumor recurrence and thus likely have a less favorable survival rate (18).

In human colorectal cancers, $K$-ras and $\beta$-catenin ($\beta$-catenin) are considered the twin major oncogenes. Common to them is activation of transducing oncogenic signaling cascades. Activation of either pathway results in cell proliferation and inhibition of apoptosis, thereby promoting tumor development and progression (15, 17). Like multidirectional cross-talking among different signaling networks in the cell (7), the two oncogenic cascades’ signaling may have syngeneic or synergistic effects on tumor development and progression (19). The study reported here was undertaken to compare mutational activation of $K$-ras with distinct patterns of $\beta$-catenin activation in the primary tumors of colorectal cancer patients to determine whether the signaling activities of the two oncogenes are related and the influence of such a relationship on clinical and histopathological characteristics of the patients’ tumors, as well as on clinical outcomes.

PATIENTS, MATERIALS, AND METHODS

Patients. The subjects of the study were 74 patients with colorectal cancer who underwent surgical treatment in the Department of Surgical Oncology at the Cancer Research Institute Hospital and later at Kanazawa University Hospital between 1998 and 2002. The patients included 41 men and 33 women ranging in age from 33 to 93 years (mean, 68.3 years). The primary tumors analyzed here were distributed as follows: 27 tumors in the cecum and ascending segment, 5 in the transverse segment, 2 in the descending segment, 20 in the sigmoid segment, and 20 in the rectum. The Japanese Classification of Colorectal Carcinoma (20) was used to describe the gross findings and histopathological characteristics of the primary tumors (20). Gross observation showed a protruding appearance (type 1) in 12 tumors, localized and ulcerating growth with ulceration (type 2) in 60 tumors, and infiltrative proliferation (type 3) in 2 tumors. Histological types of the primary adenocarcinomas were determined as well differentiated in 35 cases, moderately differentiated in 34 cases, and poorly differentiated (including mucinous adenocarcinoma) in 5 cases. Determination of the tumors’ Dukes’ stage at surgery by routine clinical examinations and pathology diagnosis gave the following results: (a) 4 patients in Dukes’ stage A; (b) 36 in Dukes’ B; and (c) 34 in Dukes’ C (Table 1). Distant metastases (i.e., liver and lung) were found in 4 patients at surgery. Postoperative surveillance revealed that 9 patients developed recurrent tumors in distant organs after surgery.

Each patient included in this study signed a written informed consent, and the Institutional Review Board of Kanazawa University Hospital approved the study.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>K-ras</th>
<th>β-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild</td>
<td>Mutant</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&lt;60$</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>$\geq60$</td>
<td>33</td>
<td>24</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Male</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right side</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Left side</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Rectum</td>
<td>12</td>
<td>8</td>
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<tr>
<td>Gross type$^b$</td>
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<td></td>
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<tr>
<td>Type 1</td>
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<td>Type 2</td>
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<td>Type 3</td>
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<td>0</td>
</tr>
<tr>
<td>Histology$^c$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WD</td>
<td>23</td>
<td>12</td>
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<tr>
<td>MD</td>
<td>17</td>
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<tr>
<td>PD, Muc</td>
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<td>2</td>
</tr>
<tr>
<td>Tumor stage$^d$</td>
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<td></td>
</tr>
<tr>
<td>Dukes’ A</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dukes’ B</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Dukes’ C</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Metastasis$^e$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>34</td>
<td>24</td>
</tr>
<tr>
<td>Present</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

$^a$ WD, well-differentiated adenocarcinoma; MD, moderately differentiated adenocarcinoma; PD, poorly differentiated adenocarcinoma; Muc, mucinous adenocarcinoma; M, membranous expression; NS, not significant.

$^b,c$ Gross and histological types of the primary tumors were described according to the Japanese classification of colorectal carcinoma (20). Type 1, protruding tumor; Type 2, localized and ulcerating tumor; Type 3, diffusely infiltrating and ulcerating tumor.

$^d$ Dukes’ stage at surgery was determined by routine clinical examinations and pathology diagnosis of the surgical materials.

$^e$ Distant metastasis.
nazawa University Graduate School of Medical Science approved all protocols.

**Preparation of Samples.** After routine gross observation of the surgical materials, fresh and multiple sets of paired normal and tumor tissues were sampled, immediately snap frozen in liquid nitrogen, and stored at \(-80{\text{°C}}\) until examination. Surgical materials were then fixed with neutral-buffered formalin. In each case, paraffin-embedded tissue sections representative of the histopathological characteristics of the tumors were prepared on silica-coated slide glasses for immunohistochemical analysis of \(\beta\)-catenin activation after routine histological examination to determine tumor stage. From each of paired, fresh normal, and tumor samples, genomic DNA was extracted by proteinase K digestion and purified by serial treatments with phenol and chloroform, as described elsewhere (21).

**Detection of \(\beta\)-catenin Activation.** It is well documented that \(\beta\)-catenin translocated in the nucleus functions as an oncogene through transcriptional activity of its target genes and that its transcriptional activity in complex with members of the Tcf/Lef family depends on the abundant presence of particular members of this family (i.e., TCF-4 and LEF-1) and the presence of other proteins (i.e., groucho proteins) in the nucleus (12, 14–16). Accordingly, we determined activation of \(\beta\)-catenin in the tumor cells on the basis of its NA.4

For each case, the most representative section reflecting the major features of the primary colorectal tumor (i.e., histological type and depth of invasion) were selected for immunohistochemical examination to determine the expression of \(\beta\)-catenin, using a mouse monoclonal antibody to \(\beta\)-catenin (Transduction Laboratories, Lexington, KY) for the standard avidin-biotin-peroxidase complex method as described in our previous study (18). As described in the supplier’s instructions, the antibody epitope that binds to \(\beta\)-catenin is the COOH terminus (amino acids 571–781 of mouse \(\beta\)-catenin), which contains a transcription domain, and this antibody is known to cross-react with the human homologue. Working dilutions of the primary antibody to \(\beta\)-catenin and the biotinylated second antibody to mouse IgG (Vector Laboratories, Burlingame, CA) were 1:100 and 1:1000, respectively. The immunoreactive signal was developed using 3’-diaminobenzidine-tetrahydrochloride catalyzed by the avidin-biotin-peroxidase complex (DAKO, Glostrup, Denmark). As a negative control, the primary antibody was replaced by nonimmune IgG1 (DAKO). We immunostained paraffin sections of normal esophageal mucosa as a positive control for membranous expression of \(\beta\)-catenin and of normal colorectal mucosa adjacent to the tumor as an internal positive control.

\(\beta\)-catenin expression was classified into three patterns we recently determined (18) as follows: (a) membranous expression, similar to that in normal colonic or rectal crypts; (b) NA, defined as cancer cells with \(\beta\)-catenin-positive nuclei distributed throughout the tumor (Fig. 1A); and (c) NAinv showing NA by cancer cells in the deepest parts of the tumor, whereas cancer cells elsewhere in the same tumor showed only membranous expression (Fig. 1B). Using these definitions, two well-trained pathologists (A. O. and K. Y.), without knowledge of the presence of K-\(ras\) mutations in the tumors or of the patients’ clinical and pathologic parameters, independently reviewed expression patterns of \(\beta\)-catenin in each specimen.

**Detection of K-\(ras\) Mutations.** Mutations in codons 12 and 13 of the K-\(ras\) gene were detected by mismatched primer-mediated PCR-RFLP analysis using restriction enzymes MvaI (Takara, Tokyo, Japan) and BglII (TOYOBO, Kyoto, Japan) specific for RFLP in codons 12 and 13, respectively. Briefly, 200 ng of genomic DNA extracted from each tumor were PCR amplified with each set of mismatched primers designed to enable the RFLP analysis of the two codons (21, 22). When PCR products were separated on native PAGE after digestion with the restriction enzymes, PCR products encoding the wild-type and mutant sequences were distinguished as 114- and 143-bp fragments, respectively, in the case of codon 12, and 125- and 157-bp fragments, respectively, in the case of codon 13 (Fig. 2; Refs. 22 and 23). Mutant alleles detected by PCR-RFLP were then selectively amplified by the enriched PCR (21). To

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4 The abbreviations used are: NA, nuclear accumulation; NAinv, nuclear accumulation in the invasion front of the tumor; RFLP, restriction fragment-length polymorphism; NAd, diffuse nuclear accumulation.
determine the types of mutations, the enriched mutant products were sequenced using a Dye Terminator Sequencing Kit and an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer ABI, Foster City, CA), according to the manufacturer’s protocol.

In every run of PCR-RFLP analysis, DNAs for wild-type and mutant controls were amplified in parallel with DNA samples. The wild-type DNA in codons 12 and 13 was obtained from human placenta (Sigma, St. Louis, MO). The DNA for positive control of codon 12 mutant was derived from the human colon adenocarcinoma cell line SW480 (American Type Culture Collection, Rockville, MD) that has been found to harbor a homozygous mutation in codon 12 (24). For a positive control of codon 13 mutant, we used DNA samples obtained from a clinical colon cancer confirmed to have a codon 13 mutation in our previous study (23). In cases in which mutations were found, we repeated the steps in the analysis from PCR amplification to sequencing at least two times.

**Statistical Analysis.** Statistical analysis of all data obtained in this study was based on the $\chi^2$ test. A $P < 0.05$ was used in determining statistically significant differences.

**RESULTS**

In the 74 colorectal cancers available for analysis, membranous expression of $\beta$-catenin similar to its localization in nonneoplastic colorectal crypts was found in 35 cases (47%) and NA in 39 cases (53%). The latter group included 20 cases (27%) showing the NAd pattern and 19 cases (26%) with NAinv pattern of $\beta$-catenin expression (Fig. 1, A and B). Compared with the findings of our previous study (18), although the incidence of tumors showing NA (both NAd and NAinv patterns) was similar, the frequency of tumors with the NAinv expression pattern was greater in the present series. The latter was partly attributed to the difference in tumor site distribution, because rectal cancers were included in the present study but not the earlier one (18). Mutational activation of $K$-ras proto-oncogene was detected in the tumors in 31 (42%) of 74 patients. In these cases, mutations in codons 12 and 13 were detected in 27 and 4 cases, respectively.

The next step was to relate the presence of the $K$-ras mutation and different patterns of $\beta$-catenin activation to clinicopathological characteristics of the patients (Table 1). There was no significant correlation between the presence of mutational activation of $K$-ras and any clinicopathological parameters. However, certain correlations were found between the different patterns of $\beta$-catenin activation and clinicopathological characteristics. Strikingly, there was a significantly higher association of the NAinv activation pattern with advanced tumor stage ($P = 0.0005$) and with the development of distant metastases ($P = 0.0064$). Additionally interesting were statistically significant differences in the incidence of $\beta$-catenin activation according to tumor site in the large bowel. We divided the large bowel anatomically into three segments: (a) the right side of the colon, including cecum, ascending, and transverse colon; (b) the left side of the colon, including descending and sigmoid colon; and (c) the rectum. $\beta$-catenin was more frequently activated in tumors of the left side of the colon and rectum than in those of the right side of the colon. Although no statistical correlation was found between $\beta$-catenin activation patterns and histological types of the primary tumors, none of three mucinous adenocarcinomas showed NA of $\beta$-catenin.

We compared $K$-ras mutations and the different patterns of $\beta$-catenin activation in the primary tumors to determine the presence of interaction or cross-talking between the ras- and $\beta$-catenin-mediated signaling cascades in clinical colorectal cancers. Contrary to the in vitro results (12, 15), there was an inverse, although statistically weak ($P = 0.046$), correlation between mutational activation of $K$-ras and oncogenic activation of $\beta$-catenin. The sites or types of $K$-ras mutation did not affect this correlation (data not shown). It is intriguing, however, that most (78%) of the clinical colorectal cancers showed activation of either the $K$-ras or $\beta$-catenin oncogene (Table 2).

An analysis of the influence of mutational activation of $K$-ras and pattern of oncogenic $\beta$-catenin activation on tumor stage is presented in Table 3. Interestingly, of a small number of patients showing both $K$-ras activation and the NAinv pattern of $\beta$-catenin activation, 7 of 8 patients were in Dukes’ stage C at surgery ($P = 0.0727$). As summarized in Table 4, 4 of these 8 patients, including 1 (case 5) with ascending colon cancer in Dukes’ stage B at surgery, developed simultaneous and/or metastatic metastases to the liver and lungs. Another patient (case 4) had a second primary cancer of the right ureter 4 years and 8 months after wide resection of the colon. On the other hand, of 11 patients with tumor showing wild-type $K$-ras and NAinv pattern of $\beta$-catenin activation, 4 developed distant metastases (clinical data not shown in Table 4).

**Table 2** Comparison between the presence of $K$-ras mutations and distinct patterns of $\beta$-catenin activation in the colorectal cancers

<table>
<thead>
<tr>
<th>$\beta$-catenin activation pattern</th>
<th>$K$-ras codons 12, 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Mutant</td>
</tr>
<tr>
<td>M</td>
<td>16</td>
</tr>
<tr>
<td>NAd</td>
<td>16</td>
</tr>
<tr>
<td>NAinv</td>
<td>11</td>
</tr>
</tbody>
</table>

*M, membranous expression (no activation); $P = 0.0463$. 

![Fig. 2 Detection of mutations in $K$-ras codons 12 and 13 by PCR-RFLP analysis.](attachment:image_url)
DISCUSSION

The present study highlights the relationship between the two major oncogenes, K-ras and β-catenin, and their roles in, and influence on, the development and progression of clinical colorectal cancers. Contrary to the suggestion of previous in vitro studies (25–28), our results pointed to a seemingly independent activation of K-ras and β-catenin in human colorectal cancers but also displayed a trend toward a statistically inverse correlation of the frequencies of activation of the two oncogenes. To put it another way, activation of either oncogene could detect most (~80%) of the cancer patients. The latter finding suggests that detecting these oncogenes in clinical materials/samples may have application to molecular diagnosis of clinical cancers. The most striking finding was that synchronous activation of the two oncogenes identified exclusively a small subset of patients with advanced Dukes’ stage tumors, in which metastases to distant organs are frequently seen.

On the basis of accumulating evidence in vitro for interactions of effectors downstream to the respective signal transduction cascade (14–17, 26–28), one may expect the eventual demonstration of a link between ras-mediated and Wnt/β-catenin-signaling pathways. One of the possible mechanisms that could link them was reported to be tyrosine phosphorylation of β-catenin by activated ras (25). It is known that colorectal cancers express several growth factors and their corresponding receptors, including, e.g., epidermal growth factor and epidermal growth factor receptor (or c-erbB-2) and hepatocyte growth factor and hepatocyte growth factor receptor (or c-met; Refs. 29 and 30). Similar to the effects of these growth factors on β-catenin (31, 32), the effect of the activated ras oncogene product promotes tyrosine phosphorylation of β-catenin and disrupts organization of a complex of cell adhesion molecules, thereby leading to shifting subcellular localization of β-catenin (25). Alternatively, a possible mechanism might be ras activation of protein kinase B/Akt, a known inhibitor of glycogen synthase kinase-3β via its phosphorylation (33). This mechanism is attributed to the role of glycogen synthase kinase-3β in targeting β-catenin for ubiquitination and subsequent proteasomal degradation (15, 16). Considering more complex mechanisms integrated in the process of colorectal tumor development and progression (1, 2), an absence of straightforward interaction or transactivation between these signaling pathways is conceivable and no doubt occurs in clinical cancers. However, this does not imply that the two oncogenic signaling pathways never interact or display connections in vivo in human colorectal cancers.

Table 3 Combined analysis of influences of K-ras mutation and β-catenin activation patterns on tumor stage of colorectal cancer patients

<table>
<thead>
<tr>
<th>K-ras codons 12, 13</th>
<th>Wild type</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-catenin activation</td>
<td>M&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NAd</td>
</tr>
<tr>
<td>Dukes’ stage A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup> P = 0.0727.

<sup>b</sup> M, membranous expression.

<sup>c</sup> Dukes’ stage at surgery was determined by routine clinical examinations and pathology diagnosis of the surgical materials.

Table 4 Patients with colorectal cancer showing mutant K-ras and NAinv activation of β-catenin

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/sex</th>
<th>Site of primary tumor</th>
<th>Gross appearance&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Size of tumor (cm)</th>
<th>Histological type&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Depth of tumor invasion&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Metastasis at surgery</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73/M</td>
<td>Sigmoid</td>
<td>Type 2</td>
<td>3.5 × 3.5 cm</td>
<td>W/D</td>
<td>ss (T2)</td>
<td>LN</td>
<td>Alive, 4 yr and 7 mo after surgery</td>
</tr>
<tr>
<td>2</td>
<td>64/M</td>
<td>Rectum</td>
<td>Type 2</td>
<td>3.7 × 3.0 cm</td>
<td>M/D</td>
<td>mp (T2)</td>
<td>LN</td>
<td>Alive, 2 yr and 4 mo after surgery</td>
</tr>
<tr>
<td>3</td>
<td>74/M</td>
<td>Transverse</td>
<td>Type 2</td>
<td>3.5 × 5.0 cm</td>
<td>M/D</td>
<td>ss (T2)</td>
<td>LN</td>
<td>Alive, 1 yr and 4 mo after surgery</td>
</tr>
<tr>
<td>4</td>
<td>33/M</td>
<td>Ascending</td>
<td>Type 2</td>
<td>6.0 × 6.0 cm</td>
<td>M/D</td>
<td>sm (T1)</td>
<td>LN</td>
<td>Right ureter cancer developed 4 yr and 8 mo after colon resection</td>
</tr>
<tr>
<td>5</td>
<td>30/M</td>
<td>Ascending</td>
<td>Type 2</td>
<td>2.0 × 1.8 cm</td>
<td>W/D</td>
<td>ss (T2)</td>
<td>None</td>
<td>Liver metastasis developed 2 yr and 9 mo after right hemicolectomy</td>
</tr>
<tr>
<td>6</td>
<td>89/F</td>
<td>Sigmoid</td>
<td>Type 1</td>
<td>9.5 × 5.0 cm</td>
<td>W/D</td>
<td>ss (T2)</td>
<td>Liver</td>
<td>Alive with disease, 11 mo after surgery</td>
</tr>
<tr>
<td>7</td>
<td>69/M</td>
<td>Descending</td>
<td>Type 2</td>
<td>6.0 × 5.0 cm</td>
<td>M/D</td>
<td>ss (T2)</td>
<td>LN, liver, peritoneum</td>
<td>Bilateral lung metastasis developed 1 yr and 3 mo after anterior resection</td>
</tr>
<tr>
<td>8</td>
<td>30/M</td>
<td>Rectum</td>
<td>Type 2</td>
<td>4.0 × 2.5 cm</td>
<td>M/D + muc</td>
<td>ss (T2)</td>
<td>LN</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> F, female; M, male; W/D, well-differentiated adenocarcinoma; M/D, moderately differentiated adenocarcinoma; Muc, mucinous adenocarcinoma; sm, submucosal layer; mp, muscularis propria layer; ss, subserosal layer; LN, lymph node(s).

<sup>b</sup> Gross types of the primary tumors.

<sup>c</sup> Histological types of the primary tumors.

<sup>d</sup> Depth of tumor invasion were determined according to the Japanese classification of colorectal carcinoma (20). Type 1, protruding tumor; Type 2, localized and ulcerating tumor; Type 3, diffusely infiltrating and ulcerating tumor.
Interestingly, the present study shows the tumor site-specific prevalence of β-catenin activation, whereas there was no significant difference in the incidence of K-ras mutations in the tumors in different sites of the large bowel. When we divided the large bowel anatomically into three parts, the results indicated more frequent activation of β-catenin—for both NAd and NAinv patterns—in tumors of the recto-sigmoid segments than those of the right side of the colon. This is partly because subjects of the present study had both colon and rectal cancers, unlike the study we reported previously (18). Evidence has been accumulating that molecular mechanisms involved in carcinogenesis and molecular phenotypes differ in the tumors arising in the proximal and distal segments of the large bowel (1). The presence of different molecular pathways to colorectal tumorigenesis is exemplified by the fact that cancers of mutator phenotypes preferentially occur in the proximal (right side) of the colon, whereas the adenoma-carcinoma sequence phenotype represents cancers in the distal (left side) colon and rectum (34–36). A minor but intriguing observation in the current study is infrequent activation of the β-catenin oncogene in poorly differentiated and mucinous adenocarcinomas, although the number of patients analyzed was too small for statistical significance. This may be a consequence of the fact that mucinous adenocarcinoma is more frequently found in the proximal segment of the colon (37). Along with variations in their evolution as well as molecular and morphological phenotypes, clinical characteristics and behaviors of large bowel tumors differ according to whether they arise in the proximal or distal segments (38). From this perspective, differential activation of the β-catenin oncogene is among the molecular alterations that determine tumor phenotypes and malignant potential, as well as clinical outcome.

Regardless of the possible presence of cross-links between the K-ras and β-catenin signaling pathways, combined analysis of the two oncogenes could detect most (78%) colorectal cancers in the present study. This analysis will provide a powerful tool for early diagnosis and screening of colorectal cancers (39, 40) when it is applied to clinical samples that reflect the entirety of the large bowel, i.e., stool, colon washings, or peripheral blood. Mutational activation of K-ras is frequently and effectively documented in colorectal (40–50%), pancreas (70–90%), and lung (25–50%) cancers (10, 41). A line of studies demonstrated that cancers of these types share clinical characteristics of rapid increase in incidence and high mortality because of difficulty in diagnosis at early stages and molecular biological traits of frequent activation by oncogenic signaling, exemplified by K-ras activation, in the process of tumor development. As in a previous study (42), our current preliminary analysis showed no oncogenic activation or loss of β-catenin in pancreas cancers.5 In the case of lung cancer, several studies of β-catenin alteration in the tumor have focused on impairment of its physiological function as a component of the cell adhesion complex cooperating with E-cadherin, other types (α- and γ-) of catenins, and members of the cytoskeleton family (43–46). Thus, the evidence currently available supports the hypothesis that β-catenin acts as an oncogene in colorectal cancer, poses as a bystander in pancreas cancer, but behaves as a tumor suppressor in lung cancer. Given that K-ras and β-catenin have such different roles in different cancers, a combination of these genetic markers in clinical samples could differentiate patients with and potentially individuals who are at risk of developing colorectal, pancreas, or lung cancers.

Clinically, the present study has reproduced earlier findings in our preliminary study (18) as to the importance of the specific pattern (NAinv) of β-catenin activation in the primary tumors in relation to assessment of the malignant potential of colorectal cancers. Most striking in the current study was the ability of synchronous detection of activated K-ras and the specific pattern (NAinv) of β-catenin activation to identify a group of patients with colorectal cancer who are intractable when given standard therapy. Both oncogenes mediate their specific signaling cascades so as to result in increased cell proliferation and inhibition of apoptosis (15, 17). An example of cooperation between the two oncogenes (47) has been demonstrated for Myc; a set of reports shows that ras enhances its stability (48) and that β-catenin transactivates its transcription (49). Therefore, regardless of the possible presence of cross-linking between the two oncogenes, synchronous activation of both may exert syngeneic and/or synergistic effects in enhancing invasion and metastasis of colorectal cancers. In all, comparative and combined analysis of the two major oncogenes is promising in molecular diagnosis and determining the malignant potential of colorectal cancers.

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β-Catenin and ras Oncogenes Detect Most Human Colorectal Cancer


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