

**β-Catenin Mutations Are More Frequent in Small Colorectal Adenomas Than in Larger Adenomas and Invasive Carcinomas**

Wade S. Samowitz, Michael D. Powers, Lisa N. Spirio, Friedel Nollet, Frans van Roy, and Martha L. Slattery

Departments of Pathology [W. S. S.] and Oncological Sciences [M. D. P., M. L. S.], University of Utah Health Sciences Center, Salt Lake City, Utah 84132; The Whitehead Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142 [L. N. S.]; V.I.B. Department of Molecular Biology, Molecular Cell Biology Unit, University of Ghent, B-9000 Ghent, Belgium [F. N. F. v. R.]

**Abstract**

Loss of serine or threonine phosphorylation sites from exon 3 of β-catenin has been identified in approximately half of colorectal tumors which lack adenomatous polyposis coli (APC) mutations, but the overall contribution of β-catenin mutations to sporadic colorectal tumorigenesis is unclear. We therefore used PCR to amplify and sequence exon 3 of β-catenin from 202 sporadic colorectal tumors. Exon 3 β-catenin mutations were identified in 6 of 48 small (<1 cm) adenomas, 2 of 82 large (≥1 cm) adenomas, and 1 of 72 invasive carcinomas. Eight of the nine mutations, including all of those in the small adenomas and the invasive cancer, involved loss of serine or threonine phosphorylation sites. The percentage of β-catenin mutations in small adenomas (12.5%) was significantly greater than that in large adenomas (2.4%) and invasive cancers (1.4%; P = 0.05 and P = 0.02, respectively). We conclude that mutation of β-catenin can be an early, perhaps initiating, event in colorectal tumorigenesis. Small adenomas with β-catenin mutations do not appear to be as likely to progress to larger adenomas and invasive carcinomas as other adenomas, however, with the result that β-catenin mutations are only rarely seen in invasive cancers. This suggests that APC and β-catenin mutations are not functionally equivalent, and that the APC gene may have other tumor suppressor functions besides the degradation of β-catenin.

**Introduction**

Increased transcriptional activation by β-catenin and Tcf-4 is important in colorectal tumorigenesis (1). In most colorectal tumors, this occurs through mutation in the APC gene (2) and decreased APC-associated degradation of β-catenin (3); in approximately one-half of the remainder of tumors with wild-type APC, it is due to mutations in β-catenin itself (4, 5). The specific β-catenin mutations involve the loss of serines or threonines from exon 3 which are normally phosphorylated by GSK-3β; phosphorylation of these sites is apparently necessary for APC-induced degradation (4). The overall contribution of β-catenin mutations to sporadic colorectal tumorigenesis is unclear, however. The only study to date that was not enriched for tumors with wild-type APC—and is, therefore, somewhat representative of sporadic colon tumors at the population level—found only two β-catenin mutations in 92 cancers, and both of these occurred in individuals younger than 40 years of age (6). Such a young age is more typical of inherited than of sporadic colon cancer, and, indeed, one of these individuals had a family history consistent with hereditary nonpolyposis colon cancer. In addition, it has also been suggested that β-catenin mutations occur early in colorectal tumorigenesis, but adenomas, especially very small adenomas, have not been extensively studied (5). In the present study, we evaluate the contribution to tumorigenesis and the timing of β-catenin mutations in 202 sporadic colorectal tumors, including 48 small (<1 cm) adenomas, 82 large adenomas, and 72 invasive cancers.

**Materials and Methods**

Exon 3 of β-catenin was PCR-amplified from the following sporadic colonic cancers: 48 small (<1 cm) adenomatous polyps, 82 large (≥1 cm) adenomatous polyps, and 72 invasive adenocarcinomas. Microdissection and DNA extraction had been performed on these tumors for previous studies on microsatellite instability (7–9). Exon 3 was amplified in two PCR reactions using intron-based nested primers. The outside primers for the first PCR reaction were 5′-TCAATGCGTCATACAGATCT-3′ and 5′-CTA-

**Results**

Mutations in exon 3 of β-catenin were detected in 6 of 48 small (<1 cm) adenomatous polyps, 2 of 82 large (≥1 cm) adenomas, and 1 of 72 invasive cancers of the colon. The bp changes, predicted amino acid change, size (for the small adenomas), and anatomical site of the respective tumors, as well as the age of the individual, are indicated in Table 1. Eight of the nine mutations involved loss of serines or threonines from the GSK-3β phosphorylation region. The remaining mutation resulted in a substitution of glutamic acid for glycine; this also occurred in the phosphorylation region. Representative chromatograms of the mutations are shown in Fig. 1. All of the mutations appeared to be heterozygous, and none were detected in the corresponding germline DNA. An A to G change in the second intron of β-catenin was detected in one small adenoma and two invasive cancers. This indicates that the mutations were inherited.

**Acknowledgments**

This work was supported by National Cancer Institute Grants CA101755, CA48998, CA61757, and 5P30 CA42104-10. L. N. S. is funded by the Leukemia Society of America as a special fellow. F. N. and F. v. R. are respectively postdoctoral researcher and research director for the Fund for Scientific Research, Flanders.

1. Received 12/17/98; accepted 2/16/99.

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2. To whom requests for reprints should be addressed, at Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah 84132. Phone: (801) 585-2556; Fax: (801) 585-3831.

3. The abbreviations used are: Tcf, T-cell factor; GSK-3β, glycogen synthase kinase-3β; APC, adenomatous polyposis coli.


Table 1  β-catenin mutations in small (<1 cm) colorectal adenomas, large (≥1 cm) adenomas, and invasive carcinomas

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>Sequence change</th>
<th>Amino acid change</th>
<th>Site</th>
<th>Age (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small adenomas (&lt;1 cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA 34</td>
<td>TCT to TTT</td>
<td>Ser^{41} to Phe</td>
<td>R</td>
<td>70</td>
</tr>
<tr>
<td>SA 16</td>
<td>TCT to TTT</td>
<td>Ser^{41} to Phe</td>
<td>L</td>
<td>69</td>
</tr>
<tr>
<td>SA 22</td>
<td>TCT to TTT</td>
<td>Ser^{41} to Phe</td>
<td>L</td>
<td>62</td>
</tr>
<tr>
<td>SA 18</td>
<td>TCT to TTT</td>
<td>Ser^{41} to Phe</td>
<td>R</td>
<td>57</td>
</tr>
<tr>
<td>SA 33</td>
<td>ACC to GCC</td>
<td>Thr^{41} to Ala</td>
<td>R</td>
<td>86</td>
</tr>
<tr>
<td>SA 36</td>
<td>ACC to GCC</td>
<td>Thr^{41} to Ala</td>
<td>R</td>
<td>64</td>
</tr>
<tr>
<td>Large adenomas (≥1 cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA 24</td>
<td>GGA to GAA</td>
<td>Gly^{34} to Glu</td>
<td>R</td>
<td>72</td>
</tr>
<tr>
<td>LA 26</td>
<td>TCT to TTT</td>
<td>Ser^{41} to Phe</td>
<td>R</td>
<td>79</td>
</tr>
<tr>
<td>Invasive carcinomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA 53</td>
<td>ACC to GCC</td>
<td>Thr^{41} to Ala</td>
<td>R</td>
<td>71</td>
</tr>
</tbody>
</table>

* Mutated exon 3 codon is indicated by subscript.

* R, right-sided; L, left-sided.

at the minus four position of exon 3 in a large adenoma was also present in the germline DNA from that individual and was considered to be a polymorphism.

The percentage of small adenomas with exon 3 mutations (12.5%) was significantly greater than the percentage of large adenomas (2.4%) or cancers (1.4%) with exon 3 mutations (P = 0.05 and P = 0.02, respectively). The one cancer with a β-catenin mutation had been shown to exhibit microsatellite instability in a previous study, but 12 other cancers previously shown to exhibit high degrees of instability (7) did not harbor exon 3 β-catenin mutations. None of the eight adenomatous polyps with exon 3 mutations had exhibited significant microsatellite instability in previous studies (8, 9).

Discussion

We identified mutations in exon 3 of the β-catenin gene in 9 of 202 sporadic colorectal adenomas and carcinomas. As in previous studies (4–6), most of the mutations involved the loss of serines or threonines from the GSK-3β phosphorylation region. The loss of these phosphorylation sites is thought to promote tumorigenesis through decreased APC-associated degradation of β-catenin and increased β-catenin/Tcf-4 transcriptional activation (4). One mutation, a glycine to glutamic acid substitution at codon 34, did not involve the loss of a phosphorylation site, although mutation of this codon has also been predicted to interfere with β-catenin degradation (5). In addition, the same codon 34 mutation has been reported in azoxymethane-induced rat colon tumors (11), and other codon 34 mutations have been reported in one colorectal carcinoma (5) and in one hepatocellular carcinoma (12) in humans.

Exon 3 β-catenin mutations were relatively uncommon in colorectal cancers in this study, being seen in only 1 of 72 invasive tumors. These results are similar to those of a previous study of sporadic colon cancers that found only two mutations in 92 cancers (6), and suggests that this genetic alteration is not a large contributor to overall sporadic colon cancer. Both this previous study and our present study represent a limited analysis of β-catenin, however, and it could be argued that other molecular mechanisms may increase levels of the associated gene product. A recent Japanese study, for example, did not identify any exon 3 missense mutations but instead found interstitial deletions of exon 3 in approximately 10% of colon cancers with wild-type APC (13). This would still account for a very small proportion of sporadic colon cancers, however, because it is estimated that only 15% of sporadic colon cancers lack APC mutations (2). Also, exon 3 deletion does not appear to be a common activating mutational event in American populations; in one study, only one such deletion was identified among 13 β-catenin mutations (5).

β-catenin mutations were identified in 6 of 48 adenomatous polyps <1 cm in size; indeed, all of these adenomas measured 0.5 cm or less. As suggested by a previous study (5), this implies that β-catenin mutations can be a very early change in colorectal tumorigenesis, perhaps representing the initiating event in this subset of tumors. An interesting finding in the present study is that β-catenin mutations were significantly more common in very small adenomas than in large adenomas or invasive cancers. A possible explanation for this is that adenomas with β-catenin mutations are not as likely to progress to larger adenomas and invasive cancers as other adenomas, most of which presumably harbor APC mutations (14). Such a finding, if confirmed in other series of sporadic tumors, would suggest that APC and β-catenin mutations, although mutually exclusive (5), are not functionally equivalent, and that APC has other tumor suppressor functions besides helping to degrade β-catenin.

The one invasive cancer with an exon 3 β-catenin mutation also exhibited widespread microsatellite instability, although the other 12 highly unstable cancers did not harbor β-catenin mutations. This result of β-catenin mutations in unstable cancers, but in only a minority of those, is consistent with a previous study in which β-catenin mutations were identified in 2 of 14 unstable tumors and in zero of 78 stable tumors (6). None of the adenomas with β-catenin mutations exhibited microsatellite instability, a not unexpected finding inasmuch as instability is only rarely seen in sporadic colorectal adenomas (8, 9, 15–18). It seems likely, then, that β-catenin mutations may be an early (perhaps initiating) event in a small subset of the colorectal tumors that ultimately demonstrate the microsatellite instability phenotype. The initiating event in the majority of unstable tumors is somewhat unclear at this time, however. Although one study detected APC mutations in a majority of unstable cancers (19), another study found that APC mutations in such tumors were rare (20).

In addition, tumors with wild-type APC do not appear to harbor mutations in genes other than β-catenin in the APC/β-catenin/Tcf pathway. It is possible, then, that other as yet unknown initiating

![Fig. 1. Chromatograms demonstrating β-catenin mutations in three tumors. In SA 16 (small adenoma #16), there is a TCT to TTT change at codon 45. In SA 33 (small adenoma #33) there is an ACC to GCC change at codon 41, and in LA 24 (large adenoma #24) there is a GGA to GAA change at codon 34. (Arrows, mutations. Each mutation is heterozygous with two partially superimposed peaks. The top peak and bottom peak bases [top peak base/bottom peak base] are indicated above the arrow).](image-url)
events are responsible for the development of most tumors with microsatellite instability.

In conclusion, the presence of β-catenin mutations in very small adenomas suggests that mutations in β-catenin, like those in APC, can be early and perhaps initiating events in colorectal neoplasia. The fact that β-catenin mutations appear to be more common in small adenomas than in cancers suggests that adenomas initiated by mutation in β-catenin are not as biologically aggressive as those initiated by APC mutations and that APC may have other tumor suppressor functions besides the degradation of β-catenin.

References
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