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Mutations in β-Catenin Are Uncommon in Colorectal Cancer Occurring in Occasional Replication Error-positive Tumors

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Abstract

β-Catenin has been identified as an oncogene in colon cancer and melanoma. Phosphorylation of sites in exon 3 of β-catenin leads to degradation of this protein. These sites are primary targets for activating mutations. The frequency with which oncogenic mutations at these sites are found in colorectal cancer is unknown, as is the frequency of their occurrence in other malignancies. We analyzed 92 colorectal cancers (CRCs) and 57 cancer cell lines (representing a diversity of tumor types) to determine the frequency of activating mutations in this gene. Mutations in exon 3 of β-catenin were found in 2 of 92 CRCs and in the colorectal cancer cell line HCT 116. Both tumors with β-catenin mutations exhibited widespread microsatellite instability, which is indicative of a replication error phenotype, a phenotype known to be present in HCT 116. This suggests that mutations in β-catenin are infrequent in CRC and miscellaneous cancer cell lines and may occur in association with a replication error phenotype.

Introduction

Recent evidence suggests that the β-catenin gene is an oncogene in colorectal and other tissues (1–3). β-Catenin is a multifunctional protein with important functions in cell-cell interaction. It may also effect gene expression by supplying an activation domain when it is bound to the (Tcf/Lef) family of DNA-binding proteins (3–6). β-Catenin levels are low in the normal colon, where wild-type APC[2] protein binds to β-catenin, promoting its degradation and inactivation (3, 7, 8). APC cooperates with a serine/threonine kinase (GSK-3β) to regulate β-catenin via multiple phosphorylation sites on exon 3 (9). Inactivation of the tumor suppressor APC occurs early in the initiation of colon cancer and is associated with a rise in free β-catenin levels (3, 7, 8). Approximately 15% of colon cancers express normal APC protein (10). Three of five colorectal cancers with normal APC function, as well as two cancer cell lines with normal APC function, were found to have activating mutations in β-catenin that altered functionally significant phosphorylation sites in exon 3 (2). Abnormal β-catenin function and similar mutations were also found in 6 of 26 melanoma cell lines (1). Deletion of the NH_2 terminus of β-catenin and mutations in serine/threonine sites in exon 3 of β-catenin can both constitutively activate the gene and increase free β-catenin levels (2, 11). *In vitro* data indicate that, in colon cancers, inactivation of APC and activation of β-catenin appear to have the same tumorigenic effect (2, 3). The frequency of mutations in β-catenin in colon cancer and other tumors is unknown at this time. Given the incidence of normal APC in CRC (15%) and the detection of mutations in β-catenin in three of five cancers with normal APC, a frequency of β-catenin mutations of approximately 10% in CRC is suggested (2). We analyzed 92 CRCs (7 stage I, 16 stage II, 44 stage III, and 25 stage IV) to determine the frequency of activating mutations in exon 3 of the β-catenin gene in this population. We also analyzed a diverse panel of 57 well-characterized cancer cell lines from the National Cancer Institute drug screen (12).

Materials and Methods

Samples. After being reviewed by a pathologist, tumor tissue from colorectal paraffin blocks was microdissected from areas with at least 70% tumor cells. Normal tissue was microdissected from the same blocks. The tissues were digested with lysis buffer [50 mM Tris (pH 8.5), 1 mM EDTA, 0.5% Tween 20, and 200 μg/ml proteinase K), extracted with phenol-chloroform, and precipitated with ethanol using standard techniques. DNA from harvested cell lines was similarly extracted and purified.

PCR and Sequencing of β-Catenin. Exon 3 of β-catenin was amplified in a PCR (primers: Forward, 5′-atttgatggagttggacatggc-3′; reverse, 5′-ccagctacttgttcttgagtgaagg-3′). Fifty ng of DNA were amplified in each reaction using AmpliTaq (Perkin-Elmer) with the following reaction conditions: 1 cycle of 95°C for 3 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and 1 cycle of 72°C for 10 min. PCR products were purified using a QIAquick PCR purification kit (Qiagen), and the purified PCR products were sequenced using dye terminator cycle sequencing chemistry with AmpliTaq polymerase FS (Perkin-Elmer), all according to the manufacturers' instructions. Sequencing was performed in both directions using the forward primer from the PCR and a reverse primer, 5′-tgagtgaaggactgagaaaatccc-3′. The sequence reactions were run on an Applied Biosystems 377 DNA sequencer. The data were collected and analyzed using Applied Biosystems sequencing analysis software, all according to the manufacturer's protocols. The sequence data were visually confirmed by two investigators.

RER Phenotype. Polymorphic microsatellite regions, including mononucleotides (BAT 25, BAT 26, BAT 40, and the transforming growth factor β receptor II), dinucleotides (D2s123, D10s197, D5s107, and D8s87), a trinucleotide (AR), and tetranucleotides (FGA, ACTBP2, and UT574), were amplified using published primer sets to determine microsatellite instability. Some samples and markers were amplified using ^{32}P-labeled primers and processed according to standard protocols (13). The majority of normal tumor pairs were amplified using fluorescence-labeled primers. These PCR products were separated using 6% polyacrylamide gels on an Applied Biosystems 377 fluorescent DNA sequencer. The microsatellite data were analyzed with Genescan and Genotyper (Applied Biosystems) software.

An RER-positive phenotype, characteristic of an underlying defect in mismatch repair, was defined by instability that precedes transformation and involves widespread alterations in multiple microsatellite markers. In practice, this was defined as the presence of new alleles in two or more markers, and for at least one marker, the new alleles had to predominate and represent a shift away from the germ-line allele. Tumors with instability present in only one marker were considered RER-negative and interpreted as representing the evolution of clonal changes in a microsatellite region rather than an underlying

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[2] The abbreviations used are: APC, adenomatous polyposis coli; CRC, colorectal cancer; RER, replication error; HNPCC, hereditary nonpolyposis CRC.

defect in mismatch repair. No normal control DNA was available for assessment of the cancer cell lines. A recent study demonstrated that BAT 26, a mononucleotide marker, has a quasimonomorphic pattern, with only minor size variations in the population (14). Here, a method with a 99.4% efficiency for determining RER status was described, in which RER was defined as a >4-bp shift in the monomorphic pattern of this marker (14). The RER statuses of the 57 cell lines were assessed using this method.

Results

Two of 92 primary tumors tested had mutations in β-catenin (2%). One patient had a mutation at codon 45 (TCT→CCT), changing a serine to a proline at a site that had already been previously shown to be mutated in a number of cancers. A second patient's mutation at codon 41 (ACC→GCC) changed a threonine to alanine and is identical to a previously described mutation (Fig. 1). Both patients had right-sided stage III cancers. The first patient was 39 years old at diagnosis and had no family history of cancer, whereas the second patient was 34 years old at diagnosis and had a family history consistent with the HNCC syndrome. Both were alive and free of disease 1 and 5 years, respectively, postdiagnosis. In an analysis of these same tumor blocks for the RER-positive phenotype, both of these tumors exhibited widespread instability in the majority of microsatellite regions tested. Fourteen of 92 tumors were RER positive, and among the RER-positive tumors, 8 were stage III at diagnosis (Table 1). Microsatellite instability was detected in seven or more markers in 11 tumors, with instability in four, five, and six markers in each of the other 3 tumors, respectively. Two RER-positive adenomatous polyps and three other RER-positive tumors (two endometrial and one skin), all from different patients, showed no evidence of β-catenin mutation.

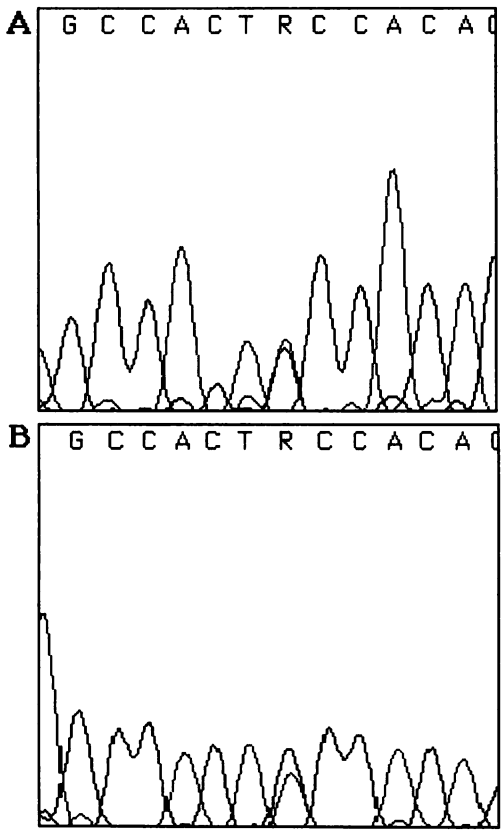

Fig. 1. Electropherograms of a patient's β-catenin sequence, identifying the mutation at codon 41 (ACC→GCC), changing a threonine to alanine. The forward sequence is represented in *panel A*, and the reverse is represented in *panel B*. *A*, adenosine; *C*, cytidine; *G*, guanosine; *T*, thymidine; *R* represents A or G, according to IUB codes.

Our analysis of tumor cell lines confirmed the frameshift mutation in β-catenin that had already been identified in HCT 116. None of the other cell lines examined showed evidence of mutations, including nine lung (ERVX, H23, H226, H322M, H460, H522, A549, HOP62, and HOP92), six ovarian (IGROV1, SKOV3, OVCAR 3, OVCAR 4, OVCAR 5, and OVCAR 8), six leukemia (CCRF-CEM, K562, MOLT-4, HL-60, RPMI 8826, and SR), seven renal (UO-31, 498, CAKI-1, RXF393, ACHN, 786-O, and SN12G), seven breast (MCF7, HS578T, MDA-MB-231, MDA-MB-435, MDA-N, BT-549, and T-47D), eight melanoma (LOX, MALME, SK-MEL2, SK-MEL5, SK-MEL 28, UACC62, M14, and UACC 257), two prostate (DU-145 and PC-3), five brain (SF539, SNB 19, SNB 75, U251, and SF295), and six other colon (HT 29, HCC-2998, SW620, COLO 205, HCT-15, and KM12). Four of these cell lines (HCT116, IGROV1, KM12, and DU145) were RER positive, as determined by the BAT 26 microsatellite analysis. HCT 15 was RER positive, as determined by analysis of multiple single-cell clones for instability (15). However, it has a mutation in GT binding protein, which predisposes it to more subtle changes in polymorphic microsatellites (16). SKOV3 was also known to be RER positive. It was not detected by the BAT 26 assay (17). Among 14 RER-positive normal/tumor pairs, BAT 26 was analyzed in 13 and was positive in 11.

Discussion

A current hypothesis suggests that inactivation of APC or activating mutations in β-catenin may have similar functional and tumorigenic consequences in the human colon (2, 3, 18). Consequently, β-catenin can behave as an oncogene, providing a potential alternative mechanism of initiation in the pathway of colorectal carcinogenesis (18). Inactivation of APC has been demonstrated in approximately 85% of colorectal cancers, with the initiating event unknown in the remaining 15% (10). Recently, mutations in exon 3 of β-catenin were found in three of five tumors with normal APC protein, suggesting β-catenin might be mutated in a significant minority (perhaps as high as 10%) of CRCs (2). Here, mutations in the activating region of β-catenin were found in only two tumors, representing 2% of all colon cancers tested. Both mutations alter serine/threonine phosphorylation sites, consistent with the proposed mechanism of activation of β-catenin (18).

Interestingly, both our patients' tumors with mutations in β-catenin were RER positive. Both patients had stage III colon cancer and were under 40 years old at diagnosis, and one patient had a family history consistent with the HNPCC syndrome. To date, published β-catenin mutations have been found in only two CRC cell lines (HCT 116 and SW48), both of which are RER positive and have mutated *hMLH 1* genes (19, 20). The RER status of the three other published CRCs with β-catenin mutations is not stated (2). Here, the majority of RER-positive cancers, including a majority with stage III disease, did not have mutations in β-catenin. Although frameshift mutations are particularly common in RER-positive malignancies, the β-catenin mutations found to date are mainly transitions or transversions. Our data indicate that β-catenin mutations occur rarely, if at all, in RER-negative CRC and in only a minority of RER-positive CRC. Activating mutations in β-catenin that occur in colon tumors may be associated with an underlying defect in mismatch repair.

Surprisingly, activating mutations in β-catenin were recently found in melanoma cell lines, implying that β-catenin may act as an oncogene in a variety of other cancers, including those without a clear association with APC mutations (1). We analyzed 57 well-characterized cell lines representing a broad spectrum of human cancers for β-catenin mutations (12). Only one cell line (HCT 116) had a frameshift mutation, and no mutations were detected in the eight melanoma

Table 1 *Clinicopathological and molecular characteristics of all 92 patients*[a]

Patient no.	Age at diagnosis (yr)	Sex	HNPCC family history[b]	Site	Pathology	Stage[c]	No. of unstable microsatellite markers (no. tested)	RER[d]	*hMSH 2/hMLH 1* germ-line sequence[e]	β-catenin exon 3 mutations[f]
1	55	M	+	R[g]	PDA[g]	III	9 (9)	+	Exon 12 *hMSH 2* codon 621 (CGA-TGA) ARG-stop	−
2	66	F	+	R	MDA-PDA	II	7 (10)	+		−
3	34	M	+	R	MDA	III	12 (12)	+	*hMLH 1*-negative, *hMSH 2*-negative	+
4	48	M	−	L	MDA	I	4 (14)	+	*hMLH 1*-negative, *hMSH 2*-negative	−
5	49	F	−	L	MDA-PDA	III	7 (14)	+		−
6	38	F	−	L	MDA	III	5 (12)	+		−
7	48	F	+	R	MDA	II	9 (15)	+	Exon 12 *hMLH 1* codon 461 (AAG to TAG) Lys-stop	−
8	36	F	−	R	MDA-PDA	III	12 (12)	+		−
9	47	F	−	L	MDA-PDA	IV	10 (11)	+		−
10	43	M	+	R	WDA	II	11 (11)	+	*hMLH 1* EX14 codon 548 (TAC TAA) Tyr-stop	−
11	38	F	−	L	PDA	III	11 (12)	+		−
12	39	F	−	R	MDA	III	9 (9)	+		+
13	38	F	+	R	PDA	III	6 (8)	+	*hMLH 1*-negative, *hMSH 2*-negative	−
14	30	F	−	L	WDA-MDA	II	9 (11)	+		−
15	38	F	+	L	MDA	III	0 (12)	−	*hMLH 1*-negative, *hMSH 2*-negative	−
16	38	M	−	L	MDA-PDA	IV	0 (11)	−		−
17	39	M	−	R	MDA	III	1 (8)	−		−
18	62	F	−	L	MDA	II	1 (12)	−		−
19	32	M	−	L	WDA-MDA	III	0 (12)	−		−
20	49	M	+	L	MDA	III	1 (12)	−	*hMLH 1*-negative, *hMSH 2*-negative	−
21	31	M	−	NA	WDA	IV	0 (11)	−		−
22	40	F	−	L	MDA	III	0 (5)	−		−
23	39	F	−	NA	MDA	IV	1 (13)	−		−
24	64	M	−	L	MDA	III	0 (11)	−		−
25	66	M	−	L	MDA	I	0 (10)	−		−
26	29	M	−	R	MDA-PDA	II	1 (12)	−		−
27	63	F	−	L	MDA	III	0 (11)	−		−
28	54	M	−	L	MDA	III	0 (10)	−		−
29	62	M	−	R	MDA	III	1 (9)	−		−
30	47	M	−	L	MDA	IV	0 (12)	−		−
31	54	M	−	L	MDA	I	0 (11)	−		−
32	69	F	−	L	MDA	III	0 (11)	−		−
33	64	M	−	L	WDA	III	0 (10)	−		−
34	66	F	−	L	WDA	II	0 (11)	−		−
35	65	M	−	L	PDA	III	0 (11)	−		−
36	71	F	−	L	MDA	III	0 (11)	−		−
37	62	F	−	L	MDA	II	1 (12)	−		−
38	51	F	−	R	MDA	IV	1 (12)	−		−
39	56	F	−	L	MDA	III	0 (8)	−		−
40	29	F	−	R	MDA	II	0 (11)	−		−
41	55	F	−	L	MDA	II	0 (11)	−		−
42	26	F	−	L	MDA	III	0 (9)	−		−
43	70	M	−	R	PDA	II	1 (11)	−		−
44	64	M	−	L	MDA	III	0 (8)	−		−
45	59	F	−	R	MDA	III	1 (13)	−		−
46	63	F	−	L	MDA	II	1 (12)	−		−
47	43	F	−	L	MDA	III	0 (4)	−		−
48	49	M	−	L	MDA-PDA	III	0 (8)	−		−
49	50	M	+	R	WDA	I	1 (14)	−		−
50	57	F	−	L	MDA	II	1 (11)	−		−
51	23	M	−	L	MDA-PDA	IV	1 (9)	−		−
52	63	M	−	R	PDA	III	1 (13)	−		−
53	41	M	−	L	WDA	I	0 (10)	−		−
54	44	M	−	NA	PDA	III	0 (6)	−		−
55	44	M	−	L	MDA	II	0 (11)	−		−
56	62	F	−	L	MDA	III	0 (14)	−		−
57	32	M	−	L	MDA	III	0 (11)	−		−
58	25	F	−	R	WDA	II	0 (10)	−		−
59	59	F	−	L	MDA	III	0 (10)	−		−
60	50	M	−	NA	MDA-WDA	III	1 (9)	−		−
61	35	F	−	R	MDA	III	0 (8)	−		−
62	47	M	−	L	WDA-MDA	IV	0 (10)	−		−
63	48	F	−	L	MDA-PDA	IV	0 (12)	−		−
64	74	M	−	R	MDA	I	1 (11)	−		−
65	70	M	−	L	MDA	I	0 (10)	−		−
66	37	F	−	L	MDA	III	0 (8)	−		−
67	56	M	−	L,R	MDA-WDA	III	0 (8)	−		−
68	47	F	+	R	PDA	III	1 (11)	−		−
69	53	F	−	L	MDA	III	0 (9)	−		−
70	51	F	−	L	MDA	III	0 (7)	−		−
71	26	M	−	L	MDA	III	0 (9)	−		−
72	28	F	−	NA	MDA-WDA	III	0 (10)	−		−
73	57	M	−	R	MDA	IV	0 (11)	−		−
74	47	M	NA	R	PDA	IV	0 (6)	−		−
75	26	M	NA	R	PDA	IV	0 (6)	−		−
76	41	F	NA	NA	MDA	IV	0 (6)	−		−
77	64	M	NA	L	MDA	IV	0 (6)	−		−
78	51	M	NA	R	WDA	IV	0 (6)	−		−
79	39	M	NA	R	MDA	IV	0 (6)	−		−
80	39	F	NA	L	MDA	IV	0 (6)	−		−

Table 1 *Continued*

Patient no.	Age at diagnosis (yr)	Sex	HNPCC family history[b]	Site	Pathology	Stage[c]	No. of unstable microsatellite markers (no. tested)	RER[d]	*hMSH 2/hMLH 1* germ-line sequence[e]	β-catenin exon 3 mutations[f]
81	47	M	NA	R	MDA	IV	0 (6)	−		−
82	51	F	NA	L	MDA	IV	0 (6)	−		−
83	38	F	NA	L	MDA-PDA	IV	0 (6)	−		−
84	64	F	NA	L	MDA	IV	0 (6)	−		−
85	58	M	NA	NA	MDA	IV	0 (6)	−		−
86	60	M	NA	NA	MDA	IV	0 (6)	−		−
87	51	F	NA	L	MDA	IV	0 (6)	−		−
88	60	F	NA	L	MDA	IV	0 (6)	−		−
89	74	F	NA	L	MDA	III	0 (6)	−		−
90	65	F	NA	L	MDA	III	0 (6)	−		−
91	60	F	NA	L	MDA	II	0 (6)	−		−
92	53	M	NA	R	MDA	III	0 (6)	−		−

[a] The patients that we specifically discuss in this study are patients 3 and 12 in this table.
[b] +, present; −, absent; *NA*, data not available.
[c] Tumor-node-metastasis staging.
[d] +, widespread microsatellite instability; −, no instability.
[e] Complete genomic sequencing of coding regions.
[f] +, mutation present; −, no mutation.
[g] R, right-sided colon cancer (caecum to splenic flexure); L, left-sided colon cancer; NA, not available; WDA, well-differentiated adenocarcinoma; MDA, moderately differentiated adenocarcinoma; PDA, poorly differentiated adenocarcinoma.

cell lines used in this study. None of the melanoma cell lines in this study were RER positive using the BAT 26 assay, and the RER status of the melanoma cell lines with β-catenin mutations is not published. Although microsatellite instability has been described in a variety of melanomas, the frequency of a true RER phenotype, as evidenced by instability in more than one marker in a melanoma, appears quite low (21, 22). This study suggests that β-catenin activating mutations occur in occasional RER-positive tumors, but they also occur uncommonly in both colon cancer and a variety of other malignant cell lines.

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