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## Development and Applications of a $\beta$ -Catenin Oligonucleotide Microarray: $\beta$ -Catenin Mutations Are Dominantly Found in the Proximal Colon Cancers with Microsatellite Instability<sup>1</sup>

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### Abstract

$\beta$ -catenin mutations have been identified in a variety of human malignancies; most of these are missense mutations restricted at hot-spot areas in exon 3.  $\beta$ -catenin mutations are known to be highly associated with colorectal cancers with microsatellite instability (MSI). More than 70  $\beta$ -catenin mutations have been reported in colorectal cancers, and ~90% of  $\beta$ -catenin mutations have been found in 11 codons (codons 29, 31, 32, 33, 34, 35, 37, 38, 41, 45, and 48) as missense mutations or in-frame deletions. We have developed an oligonucleotide microarray for detecting  $\beta$ -catenin mutations at these 11 codons. The developed oligonucleotide microarray can detect a total of 110 types of  $\beta$ -catenin mutations, including the 60 mutations reported previously. Nine  $\beta$ -catenin mutations were identified in this study by five different methods, i.e., PCR- single-strand conformational polymorphism, denaturing high performance liquid chromatography, direct sequencing, cloning-sequencing, and with an oligonucleotide microarray. All nine of the mutations were identified by denaturing high performance liquid chromatography, cloning-sequencing, and by the oligonucleotide microarray. However, PCR-single-strand conformational polymorphism missed 1  $\beta$ -catenin mutation and direct sequencing missed 2. Five  $\beta$ -catenin mutations from 74 colorectal carcinomas (34 proximal colon cancers

and 40 distal colorectal cancers) and 4  $\beta$ -catenin mutations from 31 colorectal cancer cell lines (7 from the proximal colon, 6 from the distal colorectum, and 18 unknown) were identified. In colorectal carcinomas, all 5 of the  $\beta$ -catenin mutations were found in proximal colon tumors. In colorectal cancer cell lines, 2 of 4 cell lines with  $\beta$ -catenin mutations originated from the proximal colon, and the remaining 2 cell lines were simply described as having originated from the colon. Considering the relationships among  $\beta$ -catenin mutations, MSI, and tumor location, the frequency of  $\beta$ -catenin mutations was found to be meaningfully higher in colorectal carcinomas with MSI than in those with microsatellite stability ( $P < 0.001$ ); moreover, MSI was found to be more frequent in proximal colon tumors ( $P < 0.01$ ). In addition,  $\beta$ -catenin mutations were also found to be associated with proximal colon cancer ( $P = 0.017$ ).

### Introduction

Not only does  $\beta$ -catenin function as a downstream transcriptional activator in the Wnt signaling pathway, but it is also as a submembrane component of the cadherin-mediated cell-cell adhesion system (1, 2). APC<sup>4</sup> tumor suppressor protein, along with GSK-3 $\beta$ , promotes the phosphorylation of serine/threonine residues in exon 3 of the  $\beta$ -catenin gene (2). Truncation mutation of the APC gene or the stabilizing of  $\beta$ -catenin gene mutations was found to result in the accumulation of  $\beta$ -catenin protein and the loss of  $\beta$ -catenin regulatory activity (2). The majority of  $\beta$ -catenin mutations have been reported at specific GSK-3 $\beta$  phosphorylation sites, i.e., Ser-33, Ser-37, Thr-41, Ser-45, and other residues (Asp-32 and Gly-34) in many human cancers, including endometrial, gastric, ovarian, hepatoblastomas, and colorectal cancers (3). In colorectal cancers, various frequencies of the  $\beta$ -catenin mutations have been reported, ranging from 0% to 16% (4, 5). Most  $\beta$ -catenin mutations are restricted at some codons in exon 3, and substitution mutations causing amino acid changes predominate in the  $\beta$ -catenin gene (6–9). Although it seems easy to detect  $\beta$ -catenin gene mutations using conventional methods, such as PCR-SSCP and direct sequencing, technical problems associated with the low sensitivity of  $\beta$ -catenin mutation detection have been reported (1). More reliable and faster mutation detection techniques, like that offered by the oligonucleotide microarray for  $\beta$ -catenin gene, may increase the capabilities of cancer studies, especially in

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<sup>4</sup> The abbreviations used are: APC, adenomatous polyposis coli; GSK-3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; SSCP, single-strand conformation polymorphism; MSI-H, high frequency microsatellite instability; MSS, microsatellite stability; DHPLC, denaturing high-performance liquid chromatography; MCR, mutation cluster region; MSI, microsatellite instability; TNM, Tumor-Node-Metastasis.

terms of Wnt signaling related mechanisms. It has also been suggested that MSI-H colorectal cancers are less likely to have *APC* mutations (5) and that  $\beta$ -catenin gene mutations occur frequently in MSI-H colorectal carcinomas (5, 10), although a discrepancy has been reported (11). MSI-H colorectal carcinomas have also been suggested to be involved in tumor location in the case of proximal colon cancers (5, 12). However, the relationship between  $\beta$ -catenin mutations and proximal colon cancers has not been thoroughly studied. Traverso *et al.* (12) used MSI as a marker for the diagnosis of proximal colon cancers in stools, and several other markers, such as *APC*, *p53*, long DNA, and *K-ras*, have also been used for colorectal cancer diagnosis using fecal DNA (12–14). In the present study, we investigated whether  $\beta$ -catenin mutations were prone to occur in proximal colon cancers and whether  $\beta$ -catenin mutations might be used to diagnose proximal colon cancer. We initially developed the oligonucleotide microarray method to robustly detect  $\beta$ -catenin mutations.

## Materials and Methods

**Samples.** Specimens of 74 colorectal carcinomas were collected from Seoul National University Hospital, and 31 colorectal cancer cell lines were obtained from the Korean Cell Line Bank. Of the 74 colorectal carcinomas, 34 were from the proximal colon (cecum to splenic flexure), and 40 were from the distal colorectum (splenic flexure to rectum). Of 31 colorectal cancer cell lines, 7 originated from the proximal colon and 6 from the distal colorectum. The origin of the remaining 18 colorectal cancer cell lines was unknown. The gastric cancer cell lines SNU-638 and SNU-719 were used as positive controls for  $\beta$ -catenin mutations (15). SNU-638 has  $\beta$ -catenin mutation at codon 41 (ACC→GCC, Thr→Ala) and SNU-719 mutation at codon 34 (GGA→GTA, Gly→Val). Genomic DNA was extracted from frozen specimens using TRI reagent (Molecular Research Center, Cincinnati, OH) or the automatic magnetic bead-based system (KingFisher; ThermoLab-systems, Vantaa, Finland).

**Oligonucleotides for the  $\beta$ -Catenin Microarray.** All of the oligonucleotides for this work were 21-bp long, and the mismatch sequence was located in the middle of oligonucleotides (after 10<sup>th</sup> base sequence). Oligonucleotides were modified with 5'-amino residues for chemical binding with the slides (Metabion, Martinsried, Germany). Twelve carbon spacers were used to increase the efficiency of the hybridization and to make the target sample (labeled with fluorescent dye) approach the spotted oligonucleotides more easily. Nine oligonucleotides were designed to cover all of the possible substitutions at each codon, and 1 oligonucleotide for the wild type. Thus, a total of 10 oligonucleotides were designed for 1 codon. One hundred and ten oligonucleotides were designed for codons 29, 31, 32, 33, 34, 35, 37, 38, 41, 45, and 48, and 11 oligonucleotides were designed to detect in-frame deletions (3-bp deletion) of the 11 codons. In total, the 121 designed oligonucleotides covered all of the substitutions and in-frame deletions in the above 11 codons of exon 3. The concentrations of oligonucleotides for each codon were determined by preliminary experimentation. Twenty pmol/ $\mu$ l of oligonucleotides were spotted for codons 37, 41, and 45, and 40 pmol/ $\mu$ l for the remaining 8 codons. Se-

quence information for all of the oligonucleotides is available on request.

**$\beta$ -Catenin Oligonucleotide Microarray Manufacture.** Manufacturing steps were conducted in a dust-free clean room. The manufacturing processes used were similar to that described for our previous microarray (16). Micro spotting solution (TeleChem International Inc., Sunnyvale, CA), aldehyde-coated glass slides (26 × 76 × 1 mm; CEL Associates Inc., Houston, TX), and a pin microarrayer (Cartesian Microsys 5100; Cartesian Technologies Inc., Irvine, CA) were used to create the  $\beta$ -catenin oligonucleotide microarray.

**Sample Preparations, Hybridization, and Washing.** Cy5-labeled dCTP was incorporated directly into DNA templates during PCR. After PCR amplification, Cy5-labeled PCR product was purified using a purification kit (Qiagen Inc., Valencia, CA), digested with 0.25 units of DNase I (Takara, Shiga, Japan) at 25° for 10 min, and the enzyme was inactivated at 85° for 10 min. PCR-prepared products were then resuspended in prewarmed 5× HybIt Solution (TeleChem International Inc.) at 3.5  $\mu$ l, and hybridized in a saturated vapor tube or automatic hybridization system (GeneTAC; Genomic Solutions, Ann Arbor, MI) at 56° for 3 h. The microarray was washed in 0.2% SDS solution and sodium borohydride solution (Sigma Chemical Co., St. Louis, MO) before it was hybridized with the prepared samples. The hybridized microarray was then rinsed three times at room temperature in a buffer of 2× SSC and 0.2% SDS in the dark.

**Analysis of Signals.** The  $\beta$ -catenin oligonucleotide microarray was scanned using a ScanArray Lite (Packard Instrument Co., Meriden, CT), and analyzed using Imagen (Biodiscovery, version 4.2) and quantitative microarray analysis software (QuantArray, version 2.0). Eleven wild-type signals were compared with each other and adjusted to be equal by signal normalization. The remaining 110 signals at each codon were also adjusted in the same way as the wild-type signals. After signal normalization, all of the signals were reanalyzed as described previously (16). The mean (BA) and the SD (BSD) of the background signals were calculated, and the cutoff level was established to be BA + 2.58 BSD. BA + 2.58 × BSD indicated the upper limit of the 99% confidence interval; signals over this value were identified as specific signals. All of the data analysis was carried out using a SigmaPlot (SPSS Inc., San Rafael, CA), and means and SDs were calculated.

**PCR Amplification.** The PCR primers for exon 3 were used as described previously (5). For the interstitial deletion, genomic DNA was amplified using a primer set by Udatu *et al.* (7). PCR product of 1115 bp was visualized on 1% agarose gel with ethidium bromide to detect samples with size alterations. PCR conditions consisted of 35 cycles of 94° for 30 s, 56° for 30 s, and 72° for 1 min, with a final elongation of 7 min at 72°. The PCR reactions contained 50  $\mu$ M of each of dATP, dTTP, and dGTP, and 10  $\mu$ M of each of Cy5-dCTP and dCTP, and were performed in thermal cycler (Perkin-Elmer Corp. 9600; Roche Molecular Systems, Inc., Pleasanton, CA).

**Mutational Analysis (PCR-SSCP, DHPLC, protein truncation test, Cloning, and Sequencing).** After all of the samples were first investigated by the  $\beta$ -catenin oligonucleotide microarray, PCR-SSCP and DHPLC analysis were then performed in all of the samples. All of the samples showing abnormal bands or signals in PCR-SSCP, DHPLC, and a  $\beta$ -cate-

Table 1  $\beta$ -Catenin mutations in colorectal carcinomas and colorectal cancer cell lines

Sample			$\beta$ -Catenin mutation			
Name	Type	Location	Codon	Mutation	MSI	APC mutation
207	Tumor	Ascending <sup>a</sup>	32	GAC→AAC	+ <sup>b</sup>	— <sup>c</sup>
395	Tumor	Ascending	45	TCT→TTT	+	—
396	Tumor	Ascending	45	In-frame deletion	+	—
400	Tumor	Ascending	45	TCT→TTT	+	—
435	Tumor	Ascending	41	ACC→GCC	+	—
SNU-407 <sup>d</sup>	Cell line	Transverse <sup>e</sup>	41	ACC→GCC	+	—
SNU-1047 <sup>d</sup>	Cell line	Transverse	45	TCT→TTT	+	4107delC
LS174T <sup>f</sup>	Cell line	Colon <sup>g</sup>	45	TCT→TTT	+	—
HCT 116 <sup>f</sup>	Cell line	Colon <sup>g</sup>	45	In-frame deletion	+	—

<sup>a</sup> Ascending colon.<sup>b</sup> MSI in BAT-26.<sup>c</sup> No APC mutation was found in MCR.<sup>d</sup> These two cell lines were reported in our previous work (26).<sup>e</sup> Transverse colon.<sup>f</sup> These cell lines were reported previously (21).<sup>g</sup> Detailed information on the origin of these cell lines could not be found. It was confirmed that these cell lines originated from human colon adenocarcinomas.

nin oligonucleotide microarray were then investigated by direct sequencing and cloning sequencing. PCR-SSCP and DHPLC analysis were performed as described previously (17, 18). DHPLC analysis was done using WAVE (Transgenomic, Omaha, NE), and running conditions were optimized using WAVE-MAKER software. A protein truncation test was performed for mutation detection of the MCR (codons 1263–1513) of the APC gene, as described previously (19). During the cloning, fresh PCR products were ligated into PCR-TOPO vectors, and subcloned using the TA cloning system (Invitrogen, Carlsbad, CA). Bidirectional sequencing was performed using a Taq dideoxy terminator cycle sequencing kit and an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA).

**MSI Analysis.** Genomic DNA purified from 74 colorectal carcinomas was amplified using BAT-26 to determine the MSI status, as described previously (10, 20).

**Statistical Methods.** Statistical analyses were performed using the  $\chi^2$  or Fisher's exact test to determine the strength of the correlations among the  $\beta$ -catenin mutations, MSI, and tumor location.  $\alpha = 0.05$  was set as the significance level using the STATISTICA software (StatSoft Inc., Tulsa, OK).

## Results

**$\beta$ -Catenin Mutations in Colorectal Carcinomas and Colon Cancer Cell Lines.** Mutations of the  $\beta$ -catenin gene were identified in 5 (tissue samples 207, 395, 396, 400, and 435) of 74 (7%) colorectal carcinomas. Five  $\beta$ -catenin mutations were identified in 34 proximal colon cancers (15%, 5 of 34), and 0 were found in 40 distal colorectal cancers (0%, 0 of 40). Of 34 proximal colon cancers, 5  $\beta$ -catenin mutations were found in 25 right-sided colon cancers, and no mutation was in the 9 transverse colon cancers. All of the  $\beta$ -catenin mutations from cancer tissues were somatic mutations because DNA from the matched normal tissue was proven to be a wild type. According to our data,  $\beta$ -catenin mutations were associated with the tumors in the proximal colon ( $P = 0.017$ ). In 31 colorectal cancer cell lines, 4  $\beta$ -catenin mutations were found in cell lines SNU-407, SNU-1047, LS174T, and HCT 116. Of these 4  $\beta$ -catenin mutations, 2

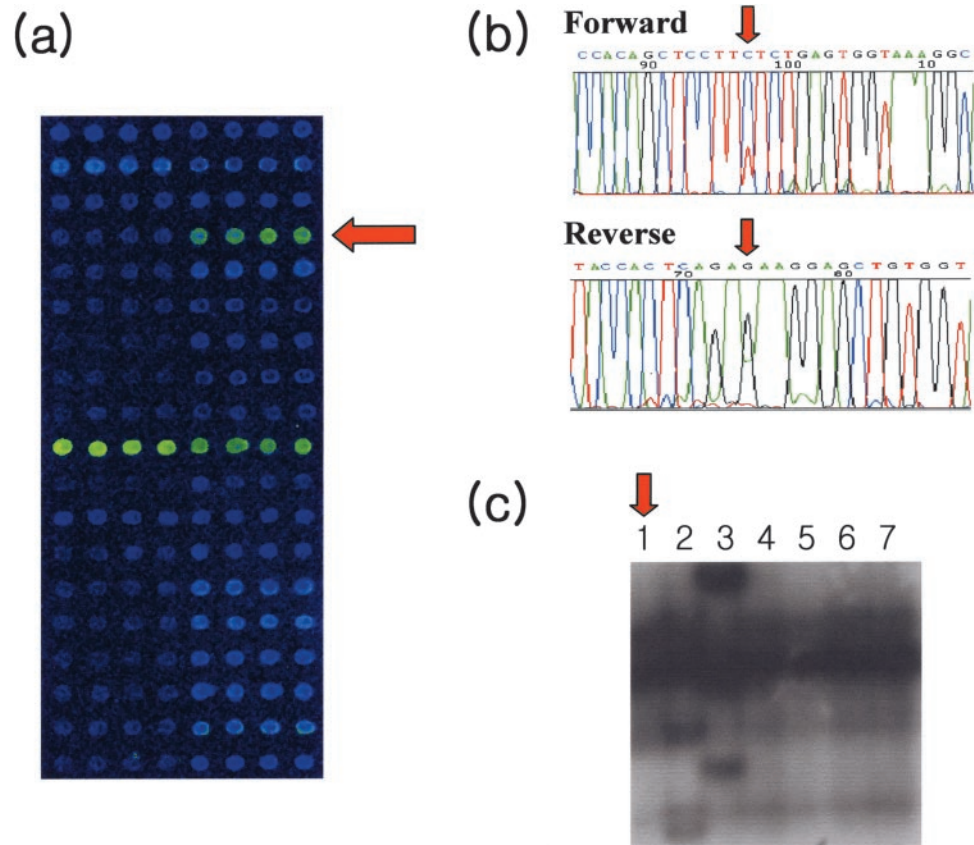
(SNU 407 and SNU 1047) were found in cell lines originating from the proximal colon (transverse colon) as expected. The origins of the other 2 cell lines (LS174T and HCT 116) harboring  $\beta$ -catenin mutations were not determined (Table 1). A total of 9 mutations were found among 74 colorectal cancer tissues and 31 colorectal cancer cell lines. Eight mutations of these 9 mutations were identified in GSK-3 $\beta$  phosphorylation sites (Table 1). Of the 9 mutations, 7 were point mutations and 2 were in-frame deletions at codon 45 in exon 3. All of the point mutations were amino acid substitutions and occurred at codons 32, 41, and 45. Six mutations were concentrated at codon 45, and 4 of these 6 point mutations at codon 45 were the same missense mutations (TCT→TTT, Ser→Phe). No interstitial large deletion of the  $\beta$ -catenin gene was detected in this study. All 9 of the samples with the  $\beta$ -catenin mutations were investigated for APC mutations in the MCR (codons 1263–1513). Only 1 cell line (SNU-1047) harbored an APC truncation mutation at codon 1369 (4107delC). The cell line LS174T, which had been reported not to carry  $\beta$ -catenin mutation, was found to harbor a  $\beta$ -catenin mutation (codon 45, TCT→TTT, Ser→Phe) in the present study (21). A total of 74 colorectal tissues were classified according to the tumor grade and stages. Tumor grade was classified into a low (well-differentiated and moderately differentiated adenocarcinomas) and a high (poorly differentiated, undifferentiated, and mucinous adenocarcinomas) grade. Tumor stage was also classified according to the TNM staging.  $\beta$ -Catenin mutations have no statistical significance with the characteristics (stage and grade) of tumors. Clinical information of 5 colorectal tissues with  $\beta$ -catenin mutations were summarized in Table 2.

**MSI and  $\beta$ -Catenin Mutations.** Twelve of 74 colorectal cancer tissues (16%) showed MSI in the BAT-26 marker. Ten of 34 proximal colon cancers (29%) were found to carry MSI and only 2 of 40 distal colorectal cancers (5%) were found to harbor MSI. MSI was statistically correlated with a proximal location ( $P < 0.01$ ). All 5 of the  $\beta$ -catenin mutations were found in 12 colorectal cancers with MSI (42%, 5 of 12), and none were found in 62 colorectal cancers with MSS.  $\beta$ -Catenin mutations

Table 2 Clinical information of 5 colorectal tissues with  $\beta$ -catenin mutations

Sample	Sex	Age	Location	Grade	T-stage	N-stage	M-stage	TNM
207	M	59	Proximal	Low	T <sub>3</sub>	N <sub>0</sub>	M <sub>0</sub>	II
395	M	42	Proximal	Low	T <sub>3</sub>	N <sub>0</sub>	M <sub>0</sub>	II
396	M	28	Proximal	High	T <sub>3</sub>	N <sub>0</sub>	M <sub>0</sub>	II
400	M	35	Proximal	Low	T <sub>3</sub>	N <sub>1</sub>	M <sub>0</sub>	III
435	M	34	Proximal	Low	T <sub>3</sub>	N <sub>1</sub>	M <sub>0</sub>	III

**Fig. 1 a**,  $\beta$ -catenin mutation using an oligonucleotide microarray in colon cancer tissue 400. The abnormal signal (marked with an *arrow*) along with the wild-type signals indicates a missense mutation at codon 45 (TCT→TTT). Oligonucleotides were spotted four times horizontally to ensure accuracy. Mutations were confirmed by cloning and sequencing. **b**, direct sequencing result of colon cancer tissue 400 in both directions. A lower T mutation peak occurred under the C peak (marked with an *arrow*) in a forward direction, and A mutation peak also occurred under the wild-type G peak in the reverse chromatogram. **c**, PCR-SSCP result of colon cancer tissue 400. Lane 1, 400; Lane 2, 395; Lane 3, 396; Lanes 4–7, normal controls. The positive controls (395 and 396; Lanes 2 and 3) showed abnormal band patterns. However, 400 (Lane 1) showed no aberrant band in duplicate experiments. This mutation was not detected by PCR-SSCP.



were more common in colorectal carcinomas with MSI than in those with MSS ( $P < 0.001$ ).

**$\beta$ -Catenin Oligonucleotide Microarray.** The  $\beta$ -catenin oligonucleotide microarray was developed to cover all of the point mutations and in-frame deletions at the hypermutable 11 codons. The mutations identified in the  $\beta$ -catenin oligonucleotide microarray were also confirmed by the DHPLC and cloning-sequencing analysis. In the  $\beta$ -catenin oligonucleotide microarray, the abnormal signal, along with the wild-type signals, which indicated a  $\beta$ -catenin mutation, was shown in all 9 of the samples. In the case of tissue 400, an additional signal in combination with wild-type signals was shown, which indicated a missense mutation at codon 45 (TCT→TTT, Ser→Phe; Fig. 1). Eight of the 9 samples with  $\beta$ -catenin mutations showed both wild-type signals at each codon and an aberrant signal, which indicated the presence of heterozygous mutations, whereas LS174T showed only an abnormal signal in the absence of a

wild-type signal at codon 45. This meant that LS174T had a homozygous  $\beta$ -catenin mutation. The screening results obtained using 5 different techniques are shown in Table 3. After the mutational analysis, a total of 60 samples from 74 colorectal cancers and 31 colorectal cancer cell lines including the 9  $\beta$ -catenin mutation-positive samples were investigated to confirm the validity of the  $\beta$ -catenin oligonucleotide microarray in a blind manner. All 9 of the  $\beta$ -catenin mutations were detected by the oligonucleotide microarray, and 1 mutation-negative sample showed an ambiguous signal, which was regarded as false positive by the oligonucleotide microarray.

## Discussion

It has been well established that tumors in the proximal colon are significantly more likely to show MSI than those in the distal colorectum (5). In addition,  $\beta$ -catenin mutations have

Table 3 Comparisons of the different methods examined for detecting  $\beta$ -catenin mutations

Sample	SSCP	DHPLC	Direct sequencing	Cloning-sequencing	Microarray
207	+ <sup>a</sup>	+	ND	+	+
395	+	+	+	+	+
396	+	+	+	+	+
400	ND <sup>b</sup>	+	ND	+	+
435	+	+	+	+	+
SNU-407	+	+	+	+	+
SNU-1047	+	+	+	+	+
LS174T	+	+	+	+	+
HCT 116	+	+	+	+	+

<sup>a</sup> Detected.

<sup>b</sup> Not detected.

been recognized to be common in colorectal cancers with MSI and in up to 50% of colorectal carcinomas with wild-type *APC* (5). As  $\beta$ -catenin mutations are significantly correlated with cancers with MSI, and because MSI is associated with proximal colon cancers, it seemed likely that  $\beta$ -catenin mutations are linked with tumors located in the proximal colon. However, this relationship has not been thoroughly investigated and remained unclear. We investigated 74 colorectal carcinomas and 31 colorectal cancer cell lines for the presence of  $\beta$ -catenin mutations. In 74 colorectal carcinomas, all 5 of the  $\beta$ -catenin mutations were identified in proximal colon cancers ( $n = 34$ ), but  $\beta$ -catenin mutations were absent from 40 distal colorectal cancers. In 31 colorectal cancer cell lines, 4  $\beta$ -catenin mutations were identified. In total, 9 mutations were identified in the 74 colorectal carcinomas and 31 colorectal cancer cell lines. Six of the 9 were found at codon 45, and 2 were at codon 41. Because codons 41 and 45 are known as GSK-3 $\beta$  phosphorylation sites (3), along with codons 33 and 37, 8 mutations at these sites might block the degradation of  $\beta$ -catenin and cause nuclear  $\beta$ -catenin accumulation. Of the 6 mutations at codon 45 (3 mutations in colorectal carcinomas and 3 in cell lines), 4 were the identical missense mutations (TCT $\rightarrow$ TTT, Ser $\rightarrow$ Phe; in samples 395, 400, SNU-1047, and LS174T), and 2 were the same in-frame deletions in samples 396 and HCT 116. The in-frame deletion at codon 45 was reported previously in a colorectal cancer cell line and in colorectal carcinoma, but not in other types of cancer (21, 22). The in-frame deletion at codon 45 may result in the loss of highly conserved serine residues in a region of the protein that serves as a target for the enzyme GSK-3 $\beta$  (21). In many types of cancer, most of the  $\beta$ -catenin mutations occur at codons 32, 33, 34, 37, and 41 (1–3, 6). It had been reported that a specific codon 45 mutation (Ser45Phe) was frequent in colorectal carcinomas, and that codon 41 mutations, which predominate in hepatoblastomas, are rare in colorectal carcinomas (8). In this study, our results indicate that codon 45 mutations, including the in-frame deletion, are common in colorectal carcinomas but are not common in other types of cancer. The remaining  $\beta$ -catenin mutation occurred at codon 32 in colon tissue 207. It has been proposed that codon 32 is important for  $\beta$ -catenin ubiquitination and proteasome-dependent degradation (23). Mutations at codons 32 might influence serine 33 accessibility by GSK-3 $\beta$  kinase, thus preventing its phosphorylation (8). In the MSI study using the BAT-26 marker, our results

show that MSI is highly associated with proximal colon cancer, which agrees with previous reports ( $P < 0.01$ ; Refs. 5, 12). We used BAT-26 for the MSI analysis, because it has been found that BAT-26 sufficiently represents MSI status in colorectal cancers (24). In terms of the correlation between MSI and  $\beta$ -catenin mutations, 5 of the 12 (42%) colorectal carcinomas with MSI had  $\beta$ -catenin mutations, but 0 of the 62 (0%) MSS colorectal carcinomas was found to harbor  $\beta$ -catenin mutations. All 5 of the colon carcinomas with  $\beta$ -catenin mutations had MSI, and all 5 of the  $\beta$ -catenin mutations were found in proximal colon cancers. These results confirm that MSI is significantly involved in  $\beta$ -catenin mutations and demonstrate that  $\beta$ -catenin mutations are highly associated with proximal colon cancer. It has been suggested previously that  $\beta$ -catenin mutations could uniquely substitute for *APC* mutations, and that  $\beta$ -catenin mutations account for approximately half of colorectal cancers with intact *APC* (25). In the present study, only 1 colorectal cancer cell line (SNU-1047) of the 9 samples with  $\beta$ -catenin mutations had *APC* mutations in the MCR, as expected.

Several groups have tried recently to diagnose colorectal cancers by using molecular markers (12–14). Three to five markers have been used for colorectal cancer diagnosis using fecal DNA (13, 14). In addition, MSI has been used for the diagnosis of proximal colon cancers, which should be the most difficult to detect, because among colorectal cancers, they are located furthest from the anus (12).  $\beta$ -Catenin may be a diagnostic marker for proximal colon cancer if  $\beta$ -catenin mutations correlate with the location of the tumor in the proximal colon. The results of the present study show MSI in 29% of proximal colon cancers and  $\beta$ -catenin mutations in 15%. Although all of the samples with  $\beta$ -catenin mutations exhibited MSI,  $\beta$ -catenin alone or with MSI may be used for the diagnosis of proximal colon cancer.

In the present study, we developed an oligonucleotide microarray for the fast and reliable mutation detection of the  $\beta$ -catenin gene. This oligonucleotide microarray can easily detect point and in-frame deletion mutations of the  $\beta$ -catenin gene at its 11 hypermutable codons. Eleven codons were selected based on previous reports on  $\beta$ -catenin mutations, and we expected to observe  $\sim 90\%$  of  $\beta$ -catenin mutations at these 11 codons (1–11, 21–23). All of the experimental work from DNA extraction to microarray scanning can be completed within a day (10–11 h) using fast DNA extraction. Although frameshift mutations and novel point mutations at the other sites will not be detected, most of the  $\beta$ -catenin mutations may be restricted at these hypermutable 11 codons in colorectal cancer. We compared several techniques with the oligonucleotide microarray using duplicate experiments (Table 3). Automatic direct sequencing, which has been widely used for mutational analysis, clearly detected 7 of the 9  $\beta$ -catenin mutations. However, in the remaining 2 cases (tissues 207 and 400) chromatogram was not clearly recognized as a heterozygous mutation in both directions in the repetitive experiments (Fig. 1*b*). Although this ambiguous peak requires additional experiments for confirming mutation, it is prone to be misunderstood as a background one. It was also reported that DNA sequencing is less sensitive than enzyme restriction for the detection of  $\beta$ -catenin mutations in cancer tissues (1). PCR-SSCP also missed 1  $\beta$ -catenin mutation. These

false-negative results might have been caused by excessive wild-type DNA in cancer tissues or by the low sensitivity of these two methods. DHPLC, cloning-sequencing, and the oligonucleotide microarray detected all of the  $\beta$ -catenin mutations. To confirm the sensitivity and specificity of the  $\beta$ -catenin oligonucleotide microarray, a total of 60 samples including 9 mutation-positive samples were investigated blindly. All 9 of the samples harboring the  $\beta$ -catenin mutations were identified as mutation positive by the oligonucleotide microarray, and only 1 sample without  $\beta$ -catenin mutation showed nonspecific signals indicating a false positive result. However, this false-positive result was not repeated in repeat microarray experiments. Such false positive results might have been caused by the poor quality of the amplified target DNA or by insufficient slide washing. In our experience, nonspecific results are rarely produced by deviations from the expected experimental conditions. Thus, standardized experimental conditions should be established to reduce nonspecific results to a minimum, and optimal data analysis systems for the different microarrays should be designed.

In this work, we found that  $\beta$ -catenin mutations are prone to occur in proximal colon cancer. This study also resulted in the development of a  $\beta$ -catenin oligonucleotide microarray, which we hope will be widely used for  $\beta$ -catenin mutational analysis.

## References

- Abraham, S. C., Nobukawa, B., Giardiello, F. M., Hamilton, S. R., and Wu, T. T. Sporadic fundic gland polyps: common gastric polyps arising through activating mutations in the  $\beta$ -catenin gene. *Am. J. Pathol.*, 158: 1005–1010, 2001.
- Abraham, S. C., Montgomery, E. A., Giardiello, F. M., and Wu, T. T. Frequent  $\beta$ -catenin mutations in juvenile nasopharyngeal angiofibromas. *Am. J. Pathol.*, 158: 1073–1078, 2001.
- Saegusa, M., and Okayasu, I. Frequent nuclear  $\beta$ -catenin accumulation and associated mutations in endometrioid-type endometrial and ovarian carcinomas with squamous differentiation. *J. Pathol.*, 194: 59–67, 2001.
- Nilbert, M., and Rambeck, E.  $\beta$ -Catenin activation through mutation is rare in rectal cancer. *Cancer Genet. Cytogenet.*, 128: 43–45, 2001.
- Mirabelli-Primdahl, L., Gryfe, R., Kim, H., Millar, A., Luceri, C., Dale, D., Holowaty, E., Bapat, B., Gallinger, S., and Redston, M.  $\beta$ -Catenin mutations are specific for colorectal carcinomas with microsatellite instability but occur in endometrial carcinomas irrespective of mutator pathway. *Cancer Res.*, 59: 3346–3351, 1999.
- Devereux, T. R., Stern, M. C., Flake, G. P., Yu, M. C., Zhang, Z. Q., London, S. J., and Taylor, J. A. CTNNB1 mutations and  $\beta$ -catenin protein accumulation in human hepatocellular carcinomas associated with high exposure to aflatoxin B1. *Mol. Carcinog.*, 31: 68–73, 2001.
- Udatsu, Y., Kusafuka, T., Kuroda, S., Miao, J., and Okada, A. High frequency of  $\beta$ -catenin mutations in hepatoblastoma. *Pediatr. Surg. Int.*, 17: 508–512, 2001.
- Koch, A., Denkhau, D., Albrecht, S., Leuschner, I., von Schweinitz, D., and Pietsch, T. Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the  $\beta$ -catenin gene. *Cancer Res.*, 59: 269–273, 1999.
- de La Coste, A., Romagnolo, B., Billuart, P., Renard, C. A., Buendia, M. A., Soubrane, O., Fabre, M., Chelly, J., Beldjord, C., Kahn, A., and Perret, C. Somatic mutations of the  $\beta$ -catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc. Natl. Acad. Sci. USA*, 95: 8847–8851, 1998.
- Shitoh, K., Furukawa, T., Kojima, M., Konishi, F., Miyaki, M., Tsukamoto, T., and Nagai, H. Frequent activation of the  $\beta$ -catenin-Tcf signaling pathway in nonfamilial colorectal carcinomas with microsatellite instability. *Genes Chromosomes Cancer*, 30: 32–37, 2001.
- Samowitz, W. S., Powers, M. D., Spirio, L. N., Nollet, F., van Roy, F., and Slattery, M. L.  $\beta$ -Catenin mutations are more frequent in small colorectal adenomas than in larger adenomas and invasive carcinomas. *Cancer Res.*, 59: 1442–1444, 1999.
- Traverso, G., Shuber, A., Olsson, L., Levin, B., Johnson, C., Hamilton, S. R., Boynton, K., Kinzler, K. W., and Vogelstein, B. Detection of proximal colorectal cancers through analysis of faecal DNA. *Lancet*, 359: 403–404, 2002.
- Ahlquist, D. A., Skoletsy, J. E., Boynton, K. A., Harrington, J. J., Mahoney, D. W., Pierceall, W. E., Thibodeau, S. N., and Shuber, A. P. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology*, 119: 1219–1227, 2000.
- Dong, S. M., Traverso, G., Johnson, C., Geng, L., Favis, R., Boynton, K., Hibi, K., Goodman, S. N., D'Allesio, M., Paty, P., Hamilton, S. R., Sidransky, D., Barany, F., Levin, B., Shuber, A., Kinzler, K. W., Vogelstein, B., and Jen, J. Detecting colorectal cancer in stool with the use of multiple genetic targets. *J. Natl. Cancer Inst.*, 93: 858–865, 2001.
- Woo, D. K., Kim, H. S., Lee, H. S., Kang, Y. H., Yang, H. K., and Kim, W. H. Altered expression and mutation of  $\beta$ -catenin gene in gastric carcinomas and cell lines. *Int. J. Cancer*, 95: 108–113, 2001.
- Kim, I. J., Kang, H. C., Park, J. H., Ku, J. L., Lee, J. S., Kwon, H. J., Yoon, K. A., Heo, S. C., Yang, H. Y., Cho, B. Y., Kim, S. Y., Oh, S. K., Youn, Y. K., Park, D. J., Lee, M. S., Lee, K. W., and Park, J. G. RET oligonucleotide microarray for the detection of RET mutations in multiple endocrine neoplasia type 2 syndromes. *Clin. Cancer Res.*, 8: 457–463, 2002.
- Kim, I. J., Ku, J. L., Yoon, K. A., Heo, S. C., Jeong, S. Y., Choi, H. S., Hong, K. H., Yang, S. K., and Park, J. G. Germline mutations of the *dpc4* gene in Korean juvenile polyposis patients. *Int. J. Cancer*, 86: 529–532, 2000.
- Wagner, T., Stoppa-Lyonnet, D., Fleischmann, E., Muhr, D., Pages, S., Sandberg, T., Caux, V., Moeslinger, R., Langbauer, G., Borg, A., and Oefner, P. Denaturing high-performance liquid chromatography detects reliably BRCA1 and BRCA2 mutations. *Genomics*, 62: 369–376, 1999.
- Won, Y. J., Park, K. J., Kwon, H. J., Lee, J. H., Kim, J. H., Kim, Y. J., Chun, S. H., Han, H. J., and Park, J. G. Germline mutations of the *APC* gene in Korean familial adenomatous polyposis patients. *J. Hum. Genet.*, 44: 103–108, 1999.
- Samowitz, W. S., Holden, J. A., Curtin, K., Edwards, S. L., Walker, A. R., Lin, H. A., Robertson, M. A., Nichols, M. F., Gruenthal, K. M., Lynch, B. J., Leppert, M. F., and Slattery, M. L. Inverse relationship between microsatellite instability and *K-ras* and *p53* gene alterations in colon cancer. *Am. J. Pathol.*, 158: 1517–1524, 2001.
- Ilyas, M., Tomlinson, I. P., Rowan, A., Pignatelli, M., and Bodmer, W. F.  $\beta$ -Catenin mutations in cell lines established from human colorectal cancers. *Proc. Natl. Acad. Sci. USA*, 94: 10330–10334, 1997.
- Muller, O., Nimmrich, I., Finke, U., Friedl, W., and Hoffmann, I. A  $\beta$ -catenin mutation in a sporadic colorectal tumor of the RER phenotype and absence of  $\beta$ -catenin germline mutations in FAP patients. *Genes Chromosomes Cancer*, 22: 37–41, 1998.
- Tong, J. H., To, K. F., Ng, E. K., Lau, J. Y., Lee, T. L., Lo, K. W., Leung, W. K., Tang, N. L., Chan, F. K., Sung, J. J., and Chung, S. C. Somatic  $\beta$ -catenin mutation in gastric carcinoma—an infrequent event that is not specific for microsatellite instability. *Cancer Lett.*, 163: 125–130, 2001.
- Hoang, J. M., Cottu, P. H., Thuille, B., Salmon, R. J., Thomas, G., and Hamelin, R. BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. *Cancer Res.*, 57: 300–303, 1997.
- Sparks, A. B., Morin, P. J., Vogelstein, B., and Kinzler, K. W. Mutational analysis of the APC/ $\beta$ -catenin/Tcf pathway in colorectal cancer. *Cancer Res.*, 58: 1130–1134, 1998.
- Oh, J. H., Ku, J. L., Yoon, K. A., Kwon, H. J., Kim, W. H., Park, H. S., Yeo, K. S., Song, S. Y., Chung, J. K., and Park, J. G. Establishment and characterization of 12 human colorectal-carcinoma cell lines. *Int. J. Cancer*, 81: 902–910, 1999.

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## Development and Applications of a $\beta$ -Catenin Oligonucleotide Microarray: $\beta$ -Catenin Mutations Are Dominantly Found in the Proximal Colon Cancers with Microsatellite Instability

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