Mutations in β-Catenin and APC Genes are Uncommon in Esophageal and Esophagogastric Junction Adenocarcinomas

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β-catenin plays important roles in both intercellular adhesion and signal transduction. Mutations in the *β-catenin* or *adenomatous polyposis coli* (*APC*) gene can alter the degradation of β-catenin and cause aberrant accumulation of β-catenin result in increased transcription of target genes. The dysregulated APC/β-catenin pathway has been recently discovered as an important mechanism of tumorigenesis in various cancers, but its role in esophageal adenocarcinomas is not clear. Therefore, we studied the *β-catenin* gene mutation, allelic loss of chromosome 5q, and *APC* gene mutation in esophageal and esophagogastric junction adenocarcinomas. Two (2%) somatic mutations in exon 3 of the *β-catenin* gene, encompassing the region for glycogen synthase kinase-3β phosphorylation, were detected from 109 adenocarcinomas. Chromosomal allelic loss on 5q was frequent in 45.3% (44/97) of tumors. Only one missense mutation in the mutation cluster region of the *APC* gene was detected from 38 esophageal and esophagogastric junction adenocarcinomas with the 5q allelic loss. Our results based on partial screening mutational analyses indicate that mutations of APC/β-catenin pathway, unlike in colorectal carcinoma, involve only a small subset of esophageal and esophagogastric junction adenocarcinoma.

KEY WORDS: APC, β-Catenin, Esophageal adenocarcinoma, Mutation.

Mod Pathol 2000;13(10):1055–1059

VOL. 13, NO. 10, P. 1055, 2000 Printed in the U.S.A.
Date of acceptance: April 17, 2000.
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The incidence of esophageal adenocarcinoma in Western countries has been rapidly increasing (1–2). Barrett esophagus developing as a complication in approximately 10% to 12% of patients with chronic gastroesophageal reflux is the predisposing condition for developing esophageal adenocarcinoma (3–8). These patients have a greater than 100-fold higher risk than the general population of developing adenocarcinoma of the esophagus (8–9).

Esophageal adenocarcinoma can develop in Barrett esophagus through a multistep process in which the metaplastic epithelium progresses to dysplasia and eventually to adenocarcinoma (8, 10–11). Studies of genetic abnormalities in esophageal adenocarcinoma and its precursor lesion have revealed alterations in *p53* tumor suppressor gene and frequent allelic losses of chromosomal arms containing known tumor suppressor genes such as 17p (*p53* gene), 18q (*DCC*, *DPC4*, and *JV18–1*), 5q (*APC*), and 9p (*p16*) (12–18). However, the molecular pathogenesis of esophageal cancers has not been completely elucidated.

β-catenin is a ubiquitous intracellular protein that plays an important role in the APC/β-catenin/Tcf pathway (19–20). The APC protein can complex with glycogen synthase kinase 3β (GSK-3β) to control degradation of β-catenin by phosphorylation of serine and threonine in the NH2 terminus of β-catenin (21–24). Mutations in the *APC* gene or β-catenin gene, especially in the GSK-3β phosphorylation region, can cause nuclear accumulation of β-catenin (25–26), as well as interact with the transcription factor (Tcf/Lef family) to activate target genes (19–20). This interaction contributes to loss of cell growth control and promotes tumorigenesis in colorectal carcinomas and other tumors (27–29). Mutation of the *APC* tumor suppressor gene has only rarely been reported in esophageal adenocarcinoma despite frequent chromosomal loss in 5q and abnormal β-catenin expression has been re-

ported in esophageal adenocarcinoma (30–33), but their importance to the neoplastic process remains unclear. Therefore, we examined APC and β-catenin mutations and studied their roles in tumorigenesis of esophageal adenocarcinoma.

MATERIALS AND METHODS

Specimens

A total of 109 esophageal and esophagogastric junction (EGJ) adenocarcinomas (103 primary tumors and six xenograft tumors) from patients receiving esophagectomy identified from the surgical pathology files of The Johns Hopkins Hospital from 1988 to 1998 were included in this study. Among the 109 adenocarcinomas, 13 (12%) were stage I, 35 (32%) were stage II, 46 (43%) were stage III, and 14 (13%) were stage IV. Seventy-three (67%) tumors were moderately differentiated, 35 (32%) were poorly differentiated, and 1 (1%) was well differentiated – all adenocarcinomas. Barrett mucosa was identified in 51% (55/109) of the tumors.

DNA Extraction

Primary adenocarcinoma and paired normal mucosa were microdissected from hematoxylin and eosin (H&E)-stained slides using either a razor blade or tuberculin needle directly under a microscope for DNA extraction. Genomic DNA was extracted as described by Moskaluk *et al.* (34). For xenograft tumors and paired frozen normal esophageal mucosa, genomic DNA was extracted using Dneasy tissue kit (QIAGEN, Valencia, CA).

Mutational Analyses of the β-Catenin Gene

Genomic DNA from each tumor sample was amplified using the following primer pairs: 5′-ATGGAACCAGACAGAGGGGC-3′ and 5′-GCTACTTGTTCTGAGTGAAG-3′. These amplified a 200-bp fragment of exon 3 of the *β-catenin* encompassing the region for GSK-3β phosphorylation site. Polymerase chain reaction (PCR) was performed under standard conditions in a 50-μL volume using PCR Master (Boehringer Mannheim, Mannheim, Germany) and 1 μM of both 5′ and 3′ oligonucleotides with 40 cycles (94° C for 1 min, 58° C for 1min, and 72° C for 2 min). PCR products were purified using shrimp alkaline phosphatase and exonuclease I (USB Corporation, Cleveland, Ohio) and sequenced directly with internal primers (5′-AAAGCGGCTGTTAGTCACTFF-3′ and 5′-GACTTGGGAGGTATCCACATCC-3′) using SequiTherm EXCEL™ II DNA Sequencing Kit (Epicentre, Madison, WI). The oligonucleotides used for sequencing were end-labeled with (γ-^{32}P)-ATP (NEN DuPont, Boston, MA) using T4 polynucleotide kinase. Each mutation was verified in both the sense and antisense directions. To detect deletion mutations in exon 3, PCR amplification was performed using the following primer pair (5′-CCAGCGTGGACAATGGCTAC-3′ and 5′-TGAGCTCGAGTCATTGCATAC-3′ corresponding to parts of the DNA sequence of exons 2 and 4, respectively) (28) on genomic DNA extracted from 24 frozen primary tumors and six xenograft tumors.

Allelic Loss on Chromosome 5q

Loss of heterozygosity (LOH) on the long arm of chromosome 5 (5q) was assessed by microsatellite assays using PCR amplification of three microsatellite markers (D5S299, D5S346, and D5S82) as described previously (11). Allelic loss of a marker was considered to be present when the microsatellite demonstrated absence or at least 50% decrease in intensity of a heterozygous band from the tumor as compared to the control esophageal mucosa. Loss of 5q was considered positive when at least one of the three microsatellite markers showed loss. The results of allelic loss on 5q for 79 adenocarcinomas from paraffin embedded tissue were published previously (11).

Mutational Analysis of APC Gene

Four sets of oligonucleotide primers (A1: 5′-CAGACTTATTGTGTAGAAGA-3′ and A2: 5′-CTCCTGAAGAAAATTCAACA-3′ for codons 1260 to 1359; B1: 5′-AGGGTTCTAGTTTATCTTCA-3′ and B2: 5′-TCTGCTTGGTGGCATGGTTT-3′ for codons 1339 to 1436; C1: 5′-GGCATTATAAGCCCCAGTGA-3′ and C2: 5′-AAATGGCTCATCGAGGCTCA-3′ for codons 1417 to 1516; D1: 5′-ACTCCAGATGGATTTTCTTG-3′ and D2: 5′-GGCTGGCTTTTTTGCTTTAC-3′ for codons 1497 to 1596) were used to amplify the mutation cluster region of the APC gene for primary tumors showing LOH or the LOH status was undetermined on chromosome 5q and all six xenograft tumors (35). Genomic DNA was amplified by PCR reactions with appropriate annealing temperature for each primer set and the conditions used for β-catenin gene amplification. The PCR products were sequenced directly with the same primers used for genomic DNA amplification.

RESULTS

Mutations in the Exon 3 of the β-Catenin Gene

Two of 109 (2%) primary and xenograft esophageal and EGJ adenocarcinomas had mutations in exon 3 of the β-catenin gene. Both tumors had a missense mutation (TCT → TTT) at codon 37, replacing serine to phenylanine at the GSK-3β phosphorylation site (Fig. 1). One of the two adenocarcinomas with *β-catenin* gene mutation arose in a background of Barrett esophagus with low-grade columnar epithelial dysplasia. No mutations in the

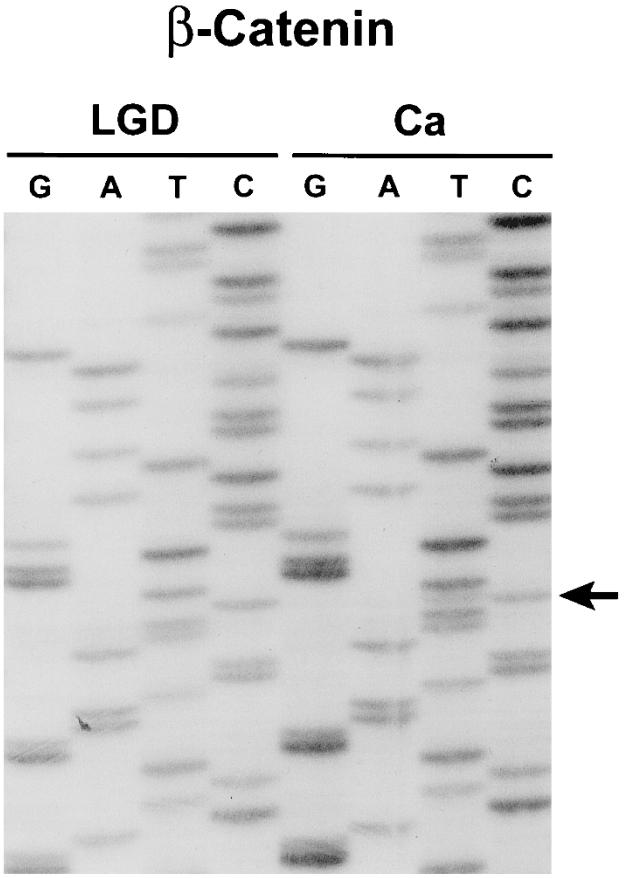

FIGURE 1. β-catenin gene mutation present in adenocarcinoma (Ca), but not in the Barrett mucosa with low grade columnar epithelial dysplasia (LGD). The mutation (***arrow***) was a missense mutation (C → T) at codon 37 changing a serine (TCT) to a phenylanine (TTT) at the GSK-3β phosphorylation site.

β-catenin gene were identified in nondysplastic or dysplastic Barrett mucosa from the same patient (Fig. 1). There was no deletion mutation in exon 3 of *β-catenin* gene in 30 (24 primary tumors and six xenograft tumors) esophageal and EGJ adenocarcinomas.

Allelic Loss on Chromosome 5q and Mutation of the APC Gene

Allelic loss on chromosome 5q was identified in 45.3% (44 out of 97) of the adenocarcinomas (Fig. 2A). In six adenocarcinomas, the status of allelic loss could not be determined (three showed microsatellite instability and three were homozygous for all three microsatellite markers).

There were no nonsense mutation but one (2.6%) missense mutation (AGT → AAT, Ser to Asn) in codon 1398 of the *APC* gene was detected in 38 adenocarcinomas with allelic loss on 5q (Fig. 2B). No mutation was identified in six xenograft tumors and five adenocarcinomas with indeterminate LOH status on 5q.

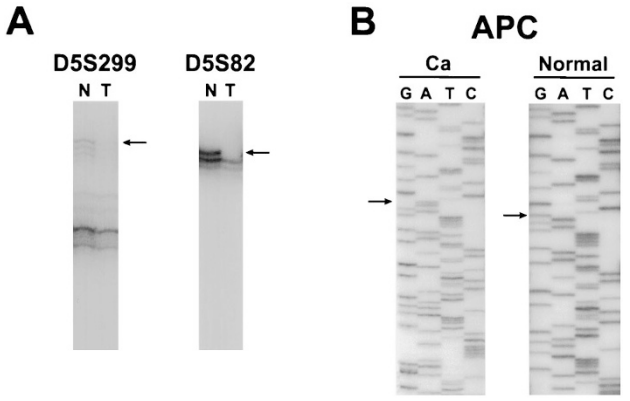

FIGURE 2. **A,** allelic loss of chromosome 5q in adenocarcinoma (T) as compared with control nonneoplastic esophageal mucosa (N). Allelic loss (***arrow***) is evidenced by the absence of one heterozygous band in two microsatellite markers (D5S299 and D5S82). **B**, a missense mutation (AGT → AAT, Ser to Asn) of APC gene at codon 1398 was detected in the same adenocarcinoma (Ca) shown in ***panel A***, but not in the control normal esophageal squamous mucosa. ***Arrow*** indicates the nucleotide change form G to A.

DISCUSSION

The Wnt signaling transduction pathway, a homologue of the Wingless (Wg) signaling pathway in Drosophila, plays an important role in mammalian tumorigenesis (36–37). Both APC and β-catenin are involved in the Wnt signaling transduction pathway (36–37). APC can bind to GSK-3β to control phosphorylation and degradation of β-catenin (21–24). The *APC* gene functions as a gatekeeper and plays an important role in the early stage of colorectal carcinogenesis (37–38). The majority (more than 80%) of the sporadic colorectal carcinomas have somatic *APC* mutations (39–40), and mutations of *β-catenin* are frequently identified in colorectal carcinomas without *APC* gene mutation (19, 27–28). In esophageal adenocarcinoma, in contrast to colorectal carcinoma, *p53* tumor suppressor gene appears to play an important role in the early stage of carcinogenesis (41–43). Chromosomal allelic loss on 17p has been detected in the diploid cell populations and precedes the development of aneuploidy and often precedes allelic loss on 5q during the neoplastic progression of Barrett adenocarcinoma (41–43).

In this study, 45.3% of the esophageal and EGJ adenocarcinomas had 5q allelic loss, but only one tumor with missense mutation at codon 1398 was detected. No DNA polymorphism of *APC* gene has been mapped to this codon. The presence of normal DNA sequence in the normal mucosa again supports that this is a missense mutation rather than just a DNA polymorphism. The significance of this missense mutation is not clear. The absence of nonsense mutations of *APC* gene in this series might be attributed to the fact that we sequenced only the mutation cluster region of *APC* gene (35).

However, this result is similar to the previously published report that nonsense mutation of *APC* gene is identified in only 5.5% (1/18) esophageal adenocarcinoma (30). Therefore, in esophageal adenocarcinoma, unlike colorectal carcinoma, mutations of *APC* gene are uncommon in spite of frequent chromosomal 5q allelic loss. The presence of frequent 5q allelic loss but not *APC* gene mutations suggest that other candidate tumor suppressor gene in the chromosome 5q may play an important role in the esophageal carcinogenesis.

β-catenin is involved in two major functions: cell adhesion and the transmission of the proliferation signal of the Wnt pathway (36). Activation of the *β-catenin* gene occurring through mutation in the region of GSK-3β phosphorylation site in exon 3 has been frequently reported in sporadic colorectal carcinomas with microsatellite instability phenotype and hereditary nonpolyposis colorectal cancer (29, 44). In addition to colorectal carcinoma, mutations of *β-catenin* gene have also been reported in various tumors including hepatoblastoma, hepatocellular carcinoma, Wilms' tumor, endometrial carcinoma, gastric carcinoma, sporadic medulloblastoma, thyroid carcinoma, and prostate cancer (45–54). We found two (2%) esophageal and EGJ adenocarcinomas with mutations in the *β-catenin* gene. Both mutations were missense mutations at codon 37 representing the phosphorylation site for GSK-3β. No mutations were identified in dysplastic or nondysplastic Barrett mucosa adjacent to one adenocarcinoma with *β-catenin* gene mutation. These findings indicate that mutations in the *β-catenin* gene occur in a small subset of esophageal and EGJ adenocarcinoma and occur in the late stages of carcinogenesis. Abnormal expression of β-catenin/E-cadherin complex, especially reduction of β-catenin/E-cadherin gene expression using immunohistochemical stains, has been reported in Barrett esophagus and esophageal adenocarcinomas (31–33). The reason for the presence of abnormal β-catenin/E-cadherin gene expression is not clear and certainly can not be explained by the presence of *β-catenin* gene mutations, a condition in which accumulation of β-catenin protein is expected (25–26).

In conclusion, we have shown that mutations in the *β-catenin* gene occur in a small subset of esophageal and GEJ adenocarcinomas. The presence of frequent 5q allelic loss but absence of nonsense mutations in the *APC* gene further supports that other candidate tumor suppressor gene(s) in the long arm of chromosome 5 may be responsible for esophageal and EGJ carcinogenesis.

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