Sporadic Fundic Gland Polyps

Common Gastric Polyps Arising Through Activating Mutations in the β-Catenin Gene

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Fundic gland polyps (FGPs) are the most common gastric polyps. FGP traditionally have been regarded as nondysplastic hamartomatous or hyperplastic lesions, but their pathogenesis remains unclear. We have recently shown that somatic adenomatous polyposis coli (APC) gene alterations are frequently present in FGPs associated with familial adenomatous polyposis (FAP), raising the possibility that mutations of the β-catenin gene affecting the APC/β-catenin pathway might be involved in the pathogenesis of sporadic FGPs. We analyzed somatic β-catenin gene mutations in 57 sporadic FGPs from 40 patients without FAP and in 19 FGPs from 13 FAP patients. Direct DNA sequencing of exon 3 encompassing the glycogen synthase kinase-3β phosphorylation region for β-catenin was used with confirmation by HindIII restriction endonuclease digestion. The foveolar epithelium and dilated fundic glands of the polyps were separately microdissected and analyzed in 22 of 57 sporadic FGPs. Activating β-catenin gene mutations were present in 91% (52 of 57) of sporadic FGPs. Both the foveolar epithelium and the dilated fundic gland epithelium comprising the polyps were shown to have the same somatic β-catenin mutation in 21 of 22 (95%) sporadic FGPs. In contrast, β-catenin gene mutations were not present in any of the 19 FAP-associated FGPs (P < 0.000001). The high frequency of β-catenin mutations in sporadic FGPs indicates that these lesions arise through activating mutations of the β-catenin gene. β-catenin mutations in gastrointestinal tract polyps have previously only been demonstrated in a subset of adenomatous (dysplastic) or neoplastic polyps. Sporadic FGPs are therefore the only lesions of the gastrointestinal tract to demonstrate β-catenin mutations while lacking dysplastic morphology. (Am J Pathol 2001, 158:1005–1010)

Fundic gland polyps (FGPs) are the most common polyps of the stomach, comprising almost half of benign gastric polyps.1 FGPs are typically small (2 to 5 mm) polyps located in the gastric body and fundus, and may be single or multiple.1-3 Histopathologically, FGPs are characterized by cystically dilated fundic glands lined by flattened parietal cells, chief cells, and variable numbers of mucous neck cells. The overlying surface and foveolar gastric epithelium is typically nondysplastic in morphology (Figure 1). FGPs arise in a background of otherwise normal, nonatrophy gastric mucosa.1-6 FGP occur in both sporadic and syndromic forms. Sporadic FGPs are identified in 0.8 to 1.9% of patients undergoing upper gastrointestinal endoscopy, and are especially prevalent among middle-aged females.4,5 The APC gene product regulates the level of β-catenin protein, which functions both as a submembranous component in cadherin-mediated cell-cell adhesion and as a downstream transcriptional activator in the Wnt

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signaling pathway.27,28 APC tumor suppressor protein, in cooperation with glycogen synthase kinase-3β (GSK-3β), promotes phosphorylation of serine/threonine residues encoded in exon 3 of the β-catenin gene.27,29,30 Phosphorylation is followed by ubiquitin-mediated degradation of β-catenin protein.31,32 Loss of β-catenin regulatory activity resulting in accumulation of β-catenin protein can occur via either truncating APC gene mutations or stabilizing β-catenin gene mutations at GSK-3β phosphorylation sites.30,33,34 A majority of colorectal adenomas and carcinomas can be demonstrated to contain either bi-allelic inactivation of the APC/β-catenin pathway or activating β-catenin gene mutations.35,36

The presence of somatic APC gene alterations in FAP-associated FGPs but not in sporadic FGPs raised the possibility that β-catenin gene mutations affecting the APC/β-catenin pathway might be involved in the pathogenesis of sporadic FGPs. We therefore analyzed for β-catenin mutations in a series of sporadic FGPs from patients without FAP and compared the findings with those of FAP-associated FGPs.

Materials and Methods

Case Selection

The study population consisted of 57 sporadic FGPs from 40 patients without FAP who underwent upper gastrointestinal endoscopy at The Johns Hopkins Hospital between 1998 and 1999. For comparison, we included 19 FAPs from 13 patients with FAP who underwent endoscopic biopsy between 1991 and 1999. We had previously analyzed the 19 FAP-associated FGPs for somatic APC gene alterations and had been unable to detect either 5q allelic loss or APC gene mutations on sequencing of polymerase chain reaction (PCR) products in the APC mutation cluster region for gastroduodenal polyps.26 Surface/foveolar epithelial dysplasia in FGPs was graded on histological examination of hematoxylin and eosin (H&E)-stained histological sections according to previously published criteria10: negative for dysplasia (all 57 sporadic FGPs and seven FAP-associated FGPs), indefinite for dysplasia (two FAP-associated FGPs), and dysplastic (10 FAP-associated FGPs).

Immunohistochemistry for β-Catenin

Immunohistochemistry for β-catenin was performed on the 57 sporadic FGPs. Immunoperoxidase stain using diaminobenzidine as the chromogen was performed on the Techmate 1000 automatic staining system (BioTek Solutions, Tucson, AZ). Deparaffinized sections of formalin-fixed tissue were stained with β-catenin antibody (mouse monoclonal antibody; Becton Dickinson Transduction Laboratories, Lexington, KY) at 1:500 dilution after heat-induced antigen retrieval.37

DNA Extraction

Microdissection of slides for DNA extraction was performed from formalin-fixed, paraffin-embedded specimens. A 27½-gauge needle tip was used for microdissection of the H&E-stained tissue under a low-power (×4) objective, and needles and gloves were routinely changed for each dissection. In 22 of 57 sporadic FGPs, the surface/foveolar epithelium and dilated fundic glands were microdissected and analyzed separately. In the remaining sporadic FGPs and in all 19 of the FAP-associated FGPs, only the dilated fundic glands were microdissected. Genomic DNA was extracted as described previously.38 Corresponding control DNA was extracted from nonneoplastic gastric or duodenal epithelium in 37 of 40 non-FAP patients.

Mutation Analysis of the β-Catenin Gene

Genomic DNA from each sample was amplified by PCR using the following primer pair: forward, 5′-ATGGACACAGACAGAGGGGC-3′ and reverse, 5′-GCTACTTGTTCTGAGTGAAG-3′. These amplified a 200-bp fragment of exon 3 of the β-catenin gene encompassing the region for GSK-3β phosphorylation. PCR reaction was performed under standard conditions in a 25-μL volume using PCR Master (Boehringer Mannheim, Mannheim, Germany) and 1 μmol/L of both 5′- and 3′-oligonucleotides with 40 cycles (94°C for 1 minute, 58°C for 1 minute, and 72°C for 2 minutes). PCR products were treated using shrimp alkaline phosphatase and exonuclease I (Amer-
sham, Buckinghamshire, UK) before sequencing. Treated PCR products were sequenced directly with SequiTherm Excel II DNA sequencing kit (Epicentre, Madison, WI) using internal primers (forward, 5′-AAACGGCGCTTATGACTCFF-3′ and reverse, 5′-GACTTGGAGGTACCACTCC-3′). Oligonucleotides were end-labeled with (γ32P)-ATP (New England Nuclear–DuPont, Boston, MA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). All mutations were verified in both sense and antisense directions. Base substitutions in codons 32, 33, and the second position of codon 34 were further confirmed by HindIII restriction endonuclease assay (Life Technologies, Inc., Rockville, MD). The 200-bp PCR product for β-catenin contains two HindIII restriction endonuclease sites, yielding 7-bp, 55-bp, and 138-bp DNA fragments after digestion of the wild-type allele. β-catenin mutations in codons 32 and 33 yield only 62-bp and 138-bp fragments after digestion because of ablation of the first HindIII site. Mutations in the second position of codon 34 yield 55-bp and 145-bp fragments because of ablation of the other HindIII site.

Statistical Analysis

Chi-square test was used to compare frequencies of β-catenin gene mutations between sporadic FGPs and FAP-associated FGPs. A P value of <0.05 was considered statistically significant.

Results

Fifty-two of 57 sporadic FGPs (91.2%) contained mutations in exon 3 of the β-catenin gene. The somatic nature of the mutations was confirmed by the absence of β-catenin gene mutations in the corresponding normal tissue from these patients. In 51 sporadic FGPs, mutations were 1-bp missense mutations, predominantly in one of the serine/threonine residues at GSK-3β phosphorylation sites: codon 32 (five cases), codon 33 (19 cases), codon 34 (nine cases), and codon 37 (18 cases) (Figures 2 and 3). One additional FGP contained a 15-bp deletion mutation spanning codons 32 to 37. In all cases demonstrating β-catenin gene mutations, a mixture of the wild-type and altered bands was present on sequencing, as expected, because of the dominant-positive nature of β-catenin gene alterations. Of 31 mutations that could theoretically be confirmed by HindIII restriction endonuclease digestion, 30 cases demonstrated the expected ablation of the HindIII recognition site (insufficient DNA was present for analysis in the remaining case) (Figure 2).

Among the 57 sporadic FGPs, we separately microdissected and analyzed both the surface/foveolar epithelium and underlying dilated fundic glands in 22 FGPs. Twenty-one of 22 cases (95%) showed concordant β-catenin mutation analysis, indicating that β-catenin mutations in FGPs are localized both to the glandular and surface epithelial compartments. In the remaining case, the glandular epithelium demonstrated a codon 33 TCT→TGT substitution that was not detected by sequencing of the surface epithelium. However, HindIII restriction endonuclease analysis was positive in both compartments, suggesting that HindIII digestion was more sensitive for the detection of mutations than was DNA sequencing.
In nine patients without FAP, multiple (two to five) FGPs were analyzed. Eight of nine patients had at least two FGPs with different β-catenin gene mutations, emphasizing the somatic and multifocal nature of the genetic alterations. A summary of the findings in these nine patients is shown in Table 1.

In contrast to the high frequency of β-catenin gene mutations in sporadic FGPs, mutations in β-catenin were not present in any of the 19 FAP-associated FGPs, a subset of FAP-associated FGPs in which we had previously failed to detect somatic APC gene alterations (P < 0.000001).

**Discussion**

We identified mutations in exon 3 of the β-catenin gene in 52 (91%) of 57 sporadic FGPs of the stomach. These mutations were predominantly 1-bp missense mutations in codons 33 and 37 (37 cases total) leading to loss of serine or threonine sites for GSK-3β phosphorylation. Other exon 3 mutations in codons 32 or 34 (14 cases total) did not involve loss of a phosphorylation site but may still interfere with degradation of the β-catenin gene product. Only one case showed a deletion mutation, a 15-bp deletion spanning codons 32 to 37 that would lead to loss of multiple phosphorylation sites. β-catenin gene alterations have now been reported in a wide variety of human tumors at low to high frequencies, including, among others, sporadic medulloblastomas (4.3%), prostate carcinomas (5%), endometrial carcinomas (13.2%), childhood hepatoblastomas (48%), anaplastic thyroid carcinoma (61%), and pilomatrixomas (75%).

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**Table 1. Somatic β-Catenin Gene Mutations from Patients with Multiple FGPs**

Among gastrointestinal tract lesions, activating missense or deletion mutations in exon 3 of β-catenin have been identified in 26.9% of intestinal-type gastric cancers and in a subset of colorectal adenomas and carcinomas lacking deletions in the APC gene. Of note, β-catenin mutations have been found to be significantly more frequent in small colorectal adenomas as compared to large adenomas and invasive adenocarcinomas, suggesting that β-catenin gene mutations are not equivalent...
of APC mutation status. Similarly, Anna and colleagues found nuclear immunostaining for β-catenin in most hepatoblastomas with β-catenin gene mutations in rats, but in none of the hepatocellular adenomas and hepatocellular carcinomas that contained β-catenin gene mutations, suggesting that translocation of β-catenin protein from the cell membrane to the nucleus is involved in tumor progression.

The etiology of the characteristic morphology of FGPs remains unclear. In contrast to adenomatous (and by definition dysplastic) gastrointestinal polyps bearing mutations of the APC or β-catenin genes, FGPs are most commonly nondysplastic in morphology. Although epithelial hyperproliferation in FGPs has been demonstrated based on higher proliferating cell nuclear antigen-labeling index in FGPs than in normal fundic mucosa, FGP are now the first gastrointestinal lesion to arise in association with somatic alterations of the APC/β-catenin pathway while typically displaying a nondysplastic morphology. Indeed, neoplastic progression has never been reported in sporadic FGPs. Whether this is because of an intrinsically weaker oncogenic potential of β-catenin than APC gene mutations, or to a reduced exposure of the gastric mucosa to carcinogenic stimuli as compared to that of colorectal mucosa, remains to be elucidated.

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References


