Sporadic Fundic Gland Polyps

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

Susan C. Abraham,* Bunsei Nobukawa,*
Francis M. Giardiello,[†] Stanley R. Hamilton,[‡] and
Tsong-Teh Wu*

From the Department of Pathology,* The Division of
Gastrointestinal/Liver Pathology, and the Department of Internal
Medicine,[†] Division of Gastroenterology, The Johns Hopkins
University School of Medicine, Baltimore, Maryland; and the
Division of Pathology and Laboratory Medicine,[‡] University of
Texas M. D. Anderson Cancer Center, Houston, Texas

Fundic gland polyps (FGPs) are the most common gastric polyps. FGPs 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-3 β phosphorylation region for β -catenin was used with confirmation by *HinfI* 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 gastroin-

testinal 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.¹⁻⁵ 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, nonatrophic gastric mucosa.⁴⁻⁶

FGPs 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.^{4,7,8} In patients with familial adenomatous polyposis (FAP), FGPs are increased in prevalence, are more frequently multiple, occur at younger ages, and show a more equal gender distribution than sporadic FGPs.^{3,7-9} In addition, up to 25% of FAP-associated FGPs show foveolar epithelial dysplasia.¹⁰⁻¹² FAP-associated FGPs have been reported in 12.5 to 84% of patients.^{9,13-23}

The pathogenesis of FGPs remains uncertain. FGPs have generally been regarded as nonneoplastic lesions, either hamartomatous or hyperplastic/functional in nature.^{4,24,25} However, we have recently demonstrated a high frequency of somatic, "second hit" alterations in the adenomatous polyposis coli (APC) gene on chromosome 5q in FGPs associated with FAP, but not in sporadic FGPs.²⁶ 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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Address reprint requests to Susan C. Abraham, M.D., Division of Gastrointestinal/Liver Pathology, Department of Pathology, Ross Building, Room 632, The Johns Hopkins University School of Medicine, 720 Rutland Ave., Baltimore, MD 21205-2196. E-mail: sabraham@jhmi.edu.

Figure 1. Histopathological appearance of FGPs. **A:** Polyps are composed of cystically dilated fundic glands. **B:** The dilated fundic glands are lined by attenuated parietal cells, chief cells, and mucous neck cells. The overlying surface/foveolar epithelium (arrowheads) is typically nondysplastic. H&E stain; original magnifications: $\times 40$ (A); $\times 200$ (B).

signaling pathway.^{27,28} APC tumor suppressor protein, in cooperation with glycogen synthase kinase-3 β (GSK-3 β), 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-3 β phosphorylation sites.^{30,33,34} A majority of colorectal adenomas and carcinomas can be demonstrated to contain either bi-allelic inactivation of the APC gene 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 gastroin-

testinal endoscopy at The Johns Hopkins Hospital between 1998 and 1999. For comparison, we included 19 FGPs 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 ($\times 4$) 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'-ATGGAAC-CAGACAGAGGGGC-3' and reverse, 5'-GCTACTTGT-TCTGAGTGAAG-3'. These amplified a 200-bp fragment of exon 3 of the β -catenin gene encompassing the region for GSK-3 β phosphorylation. PCR reaction was performed under standard conditions in a 25- μ L volume using PCR Master (Boehringer Mannheim, Mannheim, Germany) and 1 μ mol/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'-AAAGCGGCTGTTAGTCACTFF-3' and reverse, 5'-GACTTGGGAGGTATCCACATCC-3'). Oligonucleotides were end-labeled with (γ - 32 P)-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 *HinfI* restriction endonuclease assay (Life Technologies, Inc., Rockville, MD). The 200-bp PCR product for β -catenin contains two *HinfI* 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 *HinfI* site. Mutations in the second position of codon 34 yield 55-bp and 145-bp fragments because of ablation of the other *HinfI* 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-3 β 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 *HinfI* restriction endonuclease digestion, 30 cases demonstrated the expected ablation of the *HinfI* 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 $\rightarrow$ TGT substitution that was not detected by sequencing of the surface epithelium. However, *HinfI* restriction endonuclease

Figure 2. Representative somatic β -catenin gene mutations in sporadic FGPs. **A:** DNA sequencing autoradiograph of a TCT (serine) $\rightarrow$ TGT (cysteine) mutation (arrow) at codon 37, present in both the surface/foveolar epithelium and the epithelium of the dilated fundic glands from an FGP (patient 17). No β -catenin mutation is present in the corresponding normal, nonpolypoid gastric mucosa from the same patient. **B:** DNA sequencing autoradiograph of a TCT (serine) $\rightarrow$ TGT (cysteine) mutation (arrow) at codon 33 in both the surface/foveolar epithelium and the epithelium of the dilated fundic glands from an FGP (patient 13). **C:** *HinfI* restriction endonuclease assay to verify the presence of a point mutation in this case and in other representative cases with codon 32 and 33 mutations. DNA samples are from sporadic FGPs from patients 11, 12, 13, and 2 (dilated fundic glands, lanes 3, 6, 9, and 11; overlying surface/foveolar epithelium, lanes 2, 5, and 8) and normal tissue from the respective patients (lanes 1, 4, 7, and 10). The normal 200-bp PCR product for β -catenin contains two *HinfI* restriction endonuclease sites, yielding 7-bp, 55-bp, and 138-bp DNA fragments after digestion of the wild-type allele (the 7-bp fragment is too small to be visualized on the gel). β -catenin mutations in codons 32 and 33 yield 62-bp and 138-bp fragments after digestion because of ablation of the first *HinfI* site. A molecular weight marker of 50-bp ladder is in lane M.

ase analysis was positive in both compartments, suggesting that *HinfI* digestion was more sensitive for the detection of mutations than was DNA sequencing.

Figure 3. Summary of β -catenin mutations in sporadic FGPs. Schematic of the β -catenin gene, wild-type GSK-3 β binding sequence in humans (with serine/threonine phosphorylation sites in bold type), and β -catenin point mutations present in sporadic FGPs. S, serine; Y, tyrosine; L, leucine; D, aspartic acid; G, glycine; I, isoleucine; H, histidine; A, alanine; T, threonine; P, proline; K, lysine; N, asparagine. In addition to the point mutations shown in the diagram, one FGP contained a 15-bp deletion spanning codons 32 to 37.

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.

Immunohistochemistry did not demonstrate nuclear accumulation of β -catenin in the surface/foveolar epithelium in any of the sporadic FGPs. The dilated fundic glands showed diffuse membranous and cytoplasmic staining of the parietal cells lining the fundic glands in all polyps. However, similar staining was also present in separate fragments of nonpolypoid fundic mucosa present on the same slides (Figure 4).

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-3 β 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.³⁶ 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

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

Patient No.	FGP No.	β -Catenin mutation	
		Codon	Mutation
14	1	33	TCT $\rightarrow$ TGT
	2	37	TCT $\rightarrow$ TGT
16	1	32	GAC $\rightarrow$ CAC
	2	33	TCT $\rightarrow$ TGT
	3	33	TCT $\rightarrow$ TTT
	4	37	TCT $\rightarrow$ TTT
17	1	34	GGA $\rightarrow$ GAA
	2	37	TCT $\rightarrow$ TGT
	3	37	TCT $\rightarrow$ TAT
18	1	34	GGA $\rightarrow$ GTA
	2	37	TCT $\rightarrow$ TTT
21	1	33	TCT $\rightarrow$ TTT
	2	37	TCT $\rightarrow$ TTT
31	1	37	TCT $\rightarrow$ TTT
	2	32-37	15-bp deletion
32	1	33	TCT $\rightarrow$ TGT
	2	33	TCT $\rightarrow$ TGT
	3	37	TCT $\rightarrow$ TAT
	4	37	TCT $\rightarrow$ TAT
34	1	32	GAC $\rightarrow$ GGC
	2	33	TCT $\rightarrow$ TGT
	3	33	TCT $\rightarrow$ TGT
	4	33	TCT $\rightarrow$ CCT
35	5	34	GGA $\rightarrow$ GTA
	1	37	TCT $\rightarrow$ TTT
	2	37	TCT $\rightarrow$ TTT

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%).³⁹⁻⁴⁴ Our rate of 91% of β -catenin gene mutations in sporadic FGPs of the stomach is the highest rate of β -catenin alterations in tumors reported to date.

The high frequency of β -catenin mutations in this series of sporadic FGPs indicates that these lesions arise through activating mutations of the β -catenin gene. In contrast, somatic APC gene alterations are common but β -catenin mutations are absent in FAP-associated FGPs, suggesting that both sporadic and FAP-associated FGPs arise through different routes of an altered APC/ β -catenin/Tcf pathway.²⁶ Despite previous conjectures that FGPs, the most commonly identified polyps of the stomach, represent hamartomatous or hyperplastic polyps, our results provide evidence that FGPs are clonal lesions and arise through genetic alterations similar to those associated with some adenomatous polyps of the gastrointestinal tract.^{4,24,25,35,36}

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.⁴⁵⁻⁴⁷ 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

Figure 4. Immunohistochemical staining for β -catenin in sporadic FGPs. **A:** Membranous and cytoplasmic staining for β -catenin are present in the epithelial cells lining the dilated fundic glands of an FGP, but nuclear accumulation of β -catenin is not seen. **B:** The same pattern of β -catenin staining is seen in the nonpolyloid fundic mucosa from the same patient.

to APC gene mutations in their oncogenic potential.⁴⁷ Our finding of a high rate of β -catenin mutations in sporadic FGPs, in contrast to FAP-associated FGPs that primarily arise in association with second-hit APC alterations,²⁶ supports this hypothesis: FAP-associated FGPs are significantly more likely to show epithelial dysplasia than are sporadic FGPs, in which epithelial dysplasia is uncommon.¹⁰ Furthermore, rare reports of invasive carcinoma arising in association with FGPs have only involved patients with FAP.^{11,48,49}

Our results also demonstrate that β -catenin mutations in FGPs are localized both to the surface/foveolar epithelial cells and to the epithelial cells lining the dilated fundic glands, an expected finding because both compartments are known to derive from the foveolar neck region. We were unable to demonstrate nuclear accumulation of β -catenin protein by immunohistochemistry in either the surface/foveolar epithelial compartment or in the glandular compartment of these FGPs. The reason for the lack of immunohistochemical localization of β -catenin to the nucleus is unclear. However, in a study of the intracellular localization of β -catenin protein in colonic neoplasms, Kobayashi and colleagues⁵⁰ found nuclear immunostaining for β -catenin in only some intramucosal and invasive adenocarcinomas but in none of the colonic adenomas, suggesting that nuclear translocation of β -catenin is seen in invasive neoplasms rather than adenomas, regardless

of APC mutation status. Similarly, Anna and colleagues⁵¹ 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,⁵² FGPs 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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