Sporadic Fundic Gland Polyps with Epithelial Dysplasia

Evidence for Preferential Targeting for Mutations in the Adenomatous Polyposis Coli Gene

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Gastric fundic gland polyps (FGPs) occur in two distinct clinicopathological scenarios: sporadic and familial adenomatous polyposis (FAP) associated. FAP-associated FGPs arise through somatic second hit alterations of the *adenomatous polyposis coli* (*APC*) gene and frequently demonstrate epithelial dysplasia (*Am J Pathol* 2000, 157:747–754). Sporadic FGPs, in contrast, tend to contain *β-catenin* gene mutations and only infrequently show dysplasia (*Am J Pathol* 2001, 158:1005–1010). However, sporadic FGPs with dysplasia have not been previously investigated. We studied 13 sporadic FGPs with surface/foveolar low-grade dysplasia or changes indefinite for dysplasia for alterations in the *APC/β-catenin* pathway, using chromosome 5q allelic loss assays and direct DNA sequencing of the mutation cluster region in exon 15 of *APC* and the phosphorylation region in exon 3 of *β-catenin*. In addition, to evaluate for possible additional genetic alterations in FGPs, all cases were evaluated for microsatellite instability using fluorescent-based amplification of a standard panel of five microsatellite markers. Alterations in *APC* were present in seven (53.8%) FGPs, including two cases with bi-allelic *APC* inactivation (truncating intragenic mutation plus 5q allelic loss), two cases with *APC* mutation only, and three cases with 5q allelic loss only. In contrast, only two (15.4%) FGPs contained stabilizing *β-catenin* mutations. All 13 FGPs were microsatellite stable. These results indicate that sporadic FGPs with dysplasia/indefinite for dysplasia are molecularly similar to FAP-associated FGPs, and are dissimilar to the more common sporadic nondysplastic FGPs. Mutations in *APC* and *β-catenin*, despite occurring in the same genetic pathway, show differing biological properties, a phenomenon that has previously been demonstrated in colorectal neoplasms. The lack of microsatellite instability in FGPs in this study and of *K-ras* mutations in a previous study suggests that secondary genetic alterations are rare in both dysplastic and nondysplastic FGPs. (*Am J Pathol 2002, 161:1735–1742*)

Fundic gland polyps (FGPs) constitute the most common gastric polyps.[1] FGPs arise in two distinct clinicopathological scenarios: sporadic and syndromic. Sporadic FGPs are encountered in 0.9 to 1.9% of patients who undergo upper endoscopic evaluation, and are most frequent in middle-aged women.[2–4] Syndromic FGPs are found in patients with familial adenomatous polyposis (FAP), a setting in which the polyps are frequently multiple and can occur even at young ages.[3–6] Histopathologically, in both settings, these distinctive corpus- or fundic-based polyps are composed of cystically dilated oxyntic glands containing attenuated parietal cells, chief cells, and mucous neck cells (Figure 1). Most commonly, the surface epithelium of FGPs closely resembles the normal gastric surface epithelium, with a single layer of nondysplastic gastric mucin cells, each containing an apical cap of neutral mucin (Figure 1).

In some FGPs, dysplasia of the foveolar epithelium is encountered. Dysplasia in FGPs is characterized by crowding of surface and neck gastric mucin cells, which display enlarged hyperchromatic nuclei and loss of cytoplasmic mucin.[7,8] However, the underlying cystically dilated oxyntic glands in these polyps do not demonstrate epithelial dysplasia.[7,8] The frequency of dysplasia in FGPs is closely associated with the setting in which they arise. Dysplasia in FAP-associated FGPs
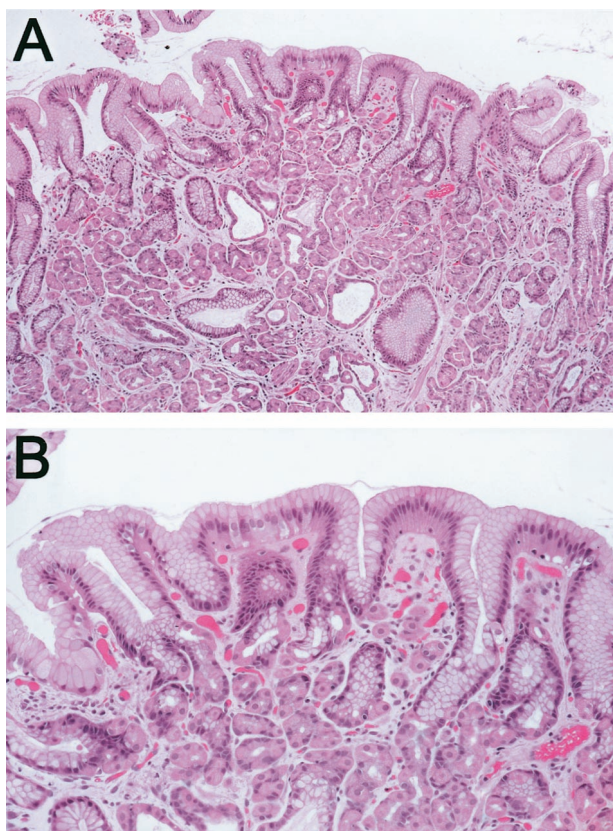
Figure 1. Histological appearance of a nondysplastic FGP. **A:** At low power, the FGP shows characteristic, cystically dilated, and budded oxyntic glands. **B:** At higher power, the surface and foveolar epithelium overlying the dilated glandular compartment is composed of nondysplastic gastric mucin cells, with small, basally oriented nuclei and abundant apical mucin.

is common, whereas it is rare in sporadic FGPs. In a large study, Wu and colleagues[7] found low-grade dysplasia in 25% of FAP-associated FGPs, but in only 3% of sporadic FGPs; furthermore, immunohistochemical markers of epithelial proliferation were dysregulated in both those FGPs with dysplasia and those considered indefinite for dysplasia.

We have recently studied the genetic alterations in FGPs and have found evidence for dysregulation of the *adenomatous polyposis coli* (*APC*)/*β-catenin* pathway similar to that previously demonstrated in colorectal adenomas.[8,9] Interestingly, these studies and others have suggested that divergent genetic alterations are present in FAP-associated and sporadic FGPs. FAP-associated FGPs, with their attendant risk of epithelial dysplasia, arise through second-hit inactivating *APC* gene mutations or chromosome 5q allelic loss,[9,10] whereas sporadic FGPs that are negative for dysplasia instead show a high rate of activating *β-catenin* mutations.[8,11,12] However, sporadic FGPs with dysplasia have not yet been evaluated. In this study we examine the role of the *APC/β-catenin* pathway and microsatellite instability in a series of sporadic FGPs with epithelial dysplasia or cellular changes indefinite for dysplasia.

Materials and Methods

Specimen Selection and Histological Evaluation

The study population consisted of 13 sporadic FGPs (designated SD1 to SD13) that demonstrated either low-grade epithelial dysplasia (eight cases) or surface/foveolar changes indefinite for dysplasia (five cases). Cases were accrued both by prospective evaluation of FGPs seen at routine sign-out between March 2001 to March 2002, as well as by a retrospective search of the computerized Surgical Pathology files at The Johns Hopkins Hospital for FGPs in non-FAP patients that contained "dysplasia" or "indefinite for dysplasia" in the diagnostic report between January 1995 to March 2002. On rereview of the formalin-fixed, hematoxylin and eosin (H&E)-stained histological sections, FGPs were graded as either low-grade dysplasia or indefinite for dysplasia according to previously published criteria.[7] Briefly, FGPs with low-grade dysplasia showed nuclear enlargement, hyperchromatism, crowding, and decreased intracellular mucin content in the surface/foveolar epithelium (Figure 2). FGPs graded as indefinite for dysplasia had milder cytological changes that were considered insufficient for a diagnosis of low-grade dysplasia (Figure 2).

DNA Extraction

Microdissection of H&E-stained slides for DNA extraction was performed from formalin-fixed, paraffin-embedded specimens using a 27½-guage tuberculin needle. In one FGP (SD1), the surface/foveolar epithelium and the underlying dilated oxyntic glands were separately microdissected and sequenced. We did not separately microdissect the glandular and surface/foveolar compartments in the remaining 12 FGPs, because we have previously shown that there is excellent correlation between the genetic status of the two compartments.[8,9] Normal control DNA was microdissected from nonpolypoid gastric mucosa (10 cases), duodenum (two cases), and liver (one case). Genomic DNA was extracted as described previously.[13]

Mutation Analysis of the β-Catenin and APC Genes

Direct DNA sequencing of DNA was used to evaluate for mutations in the *β-catenin* and *APC* genes. For *β-catenin*, genomic DNA was amplified by polymerase chain reaction (PCR) using the primer pair: 5′-ATGGAACCAGACA-GAAAAGC-3′ (forward) and 5′-GCTACTTGTTCTGAGT-GAAG-3′ (reverse). These amplified a 200-bp fragment in exon 3 of the *β-catenin* gene that encompasses the region for glycogen synthase kinase-3β (GSK-3β) phosphorylation and subsequent ubiquitin-mediated degradation of the β-catenin protein. PCR reactions were performed under standard conditions in a 50-μl volume containing 2 μl of genomic DNA, 10 mmol/L dNTP mix, 2.25 U AmpliTaq Gold (Applied Biosystems, Foster City, CA), 0.125 U PFU DNA Polymerase (Stratagene, La Jolla,
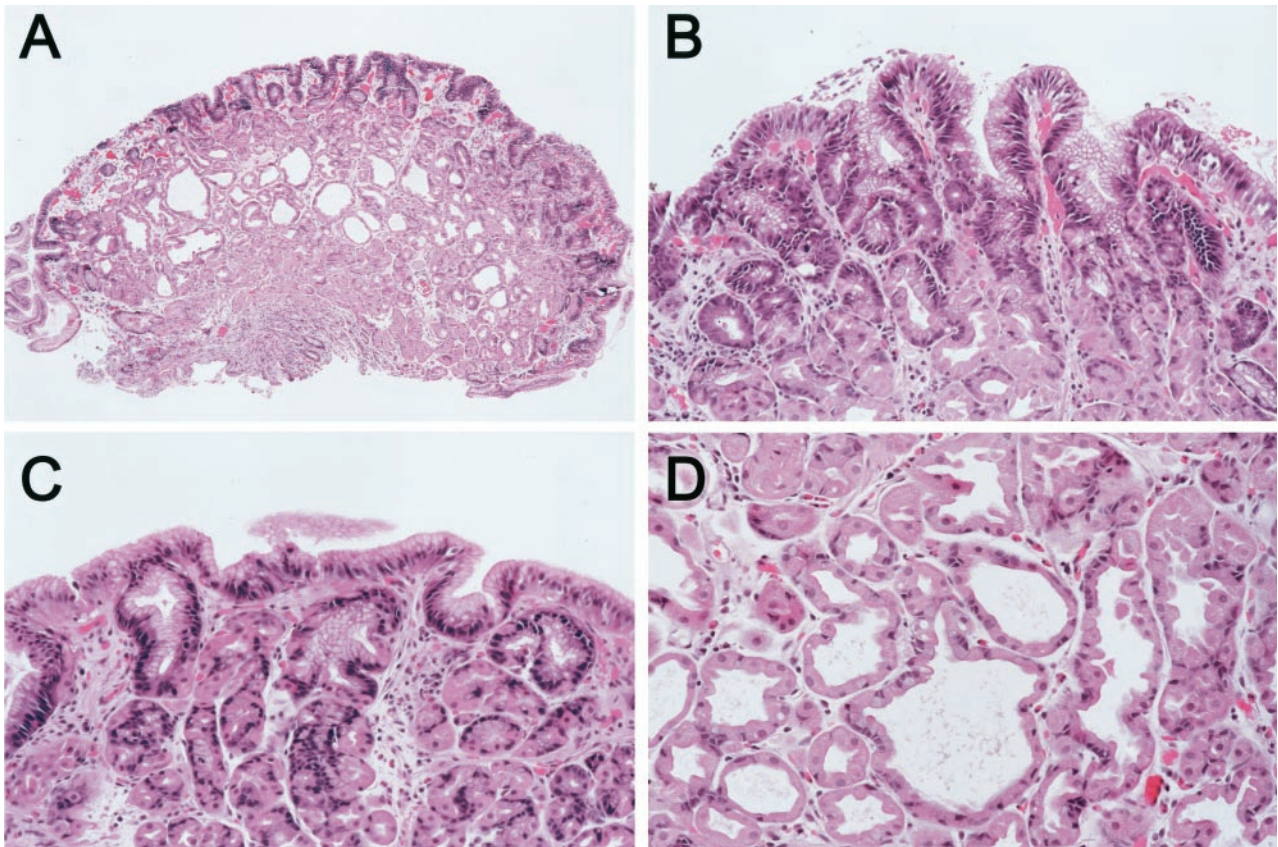
Figure 2. Histological features of sporadic FGPs with dysplasia. **A:** At low power, the characteristic architecture of a FGP is seen. However, the surface appears hyperchromatic in comparison with the FGP in Figure 1. **B:** Low-grade dysplasia of the surface/foveolar epithelium. The nuclei are enlarged, hyperchromatic, and crowded, and there is decreased cytoplasmic mucin content. **C:** Epithelial changes indefinite for dysplasia. The cytological features are intermediate between those of the nondysplastic FGP in Figure 1 and the FGP with low-grade dysplasia in **B**. **D:** The attenuated parietal cells and chief cells lining the dilated oxyntic glands do not demonstrate dysplasia.

CA), and 20 pmol of forward and reverse primers. PCR conditions consisted of an initial denaturation at 95°C for 10 minutes, 40 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 2 minutes, and a final extension at 72°C for 10 minutes on a GeneAmp PCR System 9700 (Applied Biosystems). For *APC*, 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 in exon 15 of the *APC* gene.[14] PCR reactions were performed in a reaction mixture as described above. PCR conditions for *APC* consisted of an initial denaturation step of 95°C for 10 minutes, 40 cycles (94°C for 1 minute, 55°C for 1 minute, and 68°C for 1.5 minutes for APC-B, -C, and -D primer pairs and 94°C for 1 minute, 52°C for 1 minute, and 68°C for 1.5 minutes for APC-A), followed by a final extension at 72°C for 10 minutes.

PCR products were purified using shrimp alkaline phosphatase and exonuclease I (Amersham, Buckinghamshire, UK). The purified PCR products were se-

quenced on an ABI Prism 3700 DNA Analyzer (Applied Biosystems) using the ABI Prism Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems). The same primers were used for both amplification and sequencing. The resulting sequencing data were analyzed using GeneScan Analysis software (Applied Biosystems) in accordance with the manufacturer's protocol. All mutations were verified in both forward and reverse directions.

Allelic Loss on Chromosome 5q

Allelic loss on 5q was evaluated by microsatellite assays using three microsatellite markers (D5S82, D5S299, and D5S346). Assays were performed by fluorescent-labeled PCR amplification using fluorescent dye-labeled forward primer and unlabeled reverse primer. The forward primer was end-labeled with 6-FAM (Applied Biosystems). PCR reactions were performed in 15-μl reaction volumes that contained 40 ng of genomic DNA, 9 μl of ABI Prism True Allele PCR Premix (Applied Biosystems), 5 pmol of 6-FAM-labeled forward primer, and 10 pmol of unlabeled reverse primer. The following cycling conditions were used: denaturation at 95°C for 6 minutes; 45 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1 minute; and a final extension at 72°C for 30 minutes.

The resulting PCR products were diluted with 30 μl of H$_2$O, and a 1.0-μl aliquot of each diluted fluorescent-labeled PCR product was combined with 12 μl of form-amide and 0.5 μl of GeneScan 400HD (ROX) size standard (Applied Biosystems). The samples were then capillary electrophoresed on an ABI 3700 DNA Analyzer and analyzed using GeneScan Analysis software. Allelic loss was considered to be present when there was at least a 50% reduction in the height of a heterozygous peak as compared with normal control DNA in at least one informative microsatellite marker.

Microsatellite Instability Analysis

MSI testing was performed using the five microsatellite loci (D2S123, D5S346, D17S250, Bat-25, and Bat-26) recommended by the 1997 National Cancer Institute (NCI)-sponsored consensus conference.[15] Assays were performed as described above for 5q allelic loss. Chromatograms were interpreted according to the criteria described in detail by Berg and colleagues.[16] As per NCI criteria, high-level microsatellite instability (MSI-H) is defined as shifting in at least two of five microsatellite loci, low-level microsatellite instability (MSI-L) as shifting in only one locus, and microsatellite stable (MSS) as stability in all five loci.[15]

Statistical Analysis

Fisher's exact test was used to compare gene mutation frequencies in the sporadic FGPs in the current study with those of previously published FAP-associated FGPs[8] and sporadic nondysplastic FGPs.[9,11] Two-tailed P values of <0.05 were considered statistically significant.

Results

Clinical Findings

The 13 FGPs with dysplasia/indefinite for dysplasia were present in 12 patients, including 8 (67%) women and 4 (33%) men. Mean patient age at the time of upper endoscopy was 61 years (range, 37 to 71 years). None of the patients had a clinical history of FAP and none was enrolled in the Johns Hopkins Familial Colorectal Cancer Registry. Review of previous and subsequent surgical pathology specimens in all cases revealed that none of the patients had extraintestinal manifestations of FAP such as desmoid tumors. Only four patients had undergone colonoscopy and biopsy at The Johns Hopkins Hospital; of these four patients, two were negative for colorectal adenomas, one had multifocal polypoid and flat dysplasias in the setting of ileocolonic Crohn's disease, and one had several colorectal adenomas. None had duodenal or gastric adenomas.

Single FGPs were biopsied in six patients and several FGPs in six patients; however, none of the patients had fundic gland polyposis (>10 FGPs).[11] In one patient with several FGPs, one polyp showed low-grade dysplasia, one polyp was indefinite for dysplasia, and the others
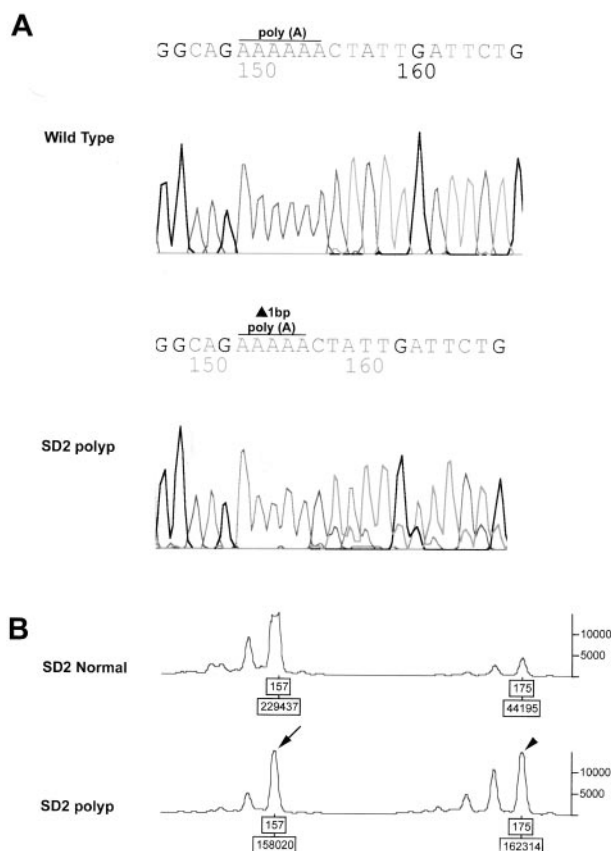

Figure 3. Bi-allelic *APC* inactivation in a sporadic FGP graded as indefinite for dysplasia (case SD2). **A:** DNA sequencing chromatogram showing deletion of base (A) from a 6-base poly(A) tract spanning codons 1554 to 1556. **B:** Allelic loss on chromosome 5q is also present (shown here after amplification with the microsatellite marker D5S299). The ratio of smaller allele (**arrow**) to larger allele (**arrowhead**) is inverted in the FGP as compared to the patient's normal tissue because of loss of smaller allele. Allelic loss can also be appreciated on the sequencing chromatogram in **A**.

were nondysplastic. In the other five patients with more than one FGP, only one of the FGPs demonstrated dysplasia/indefinite for dysplasia whereas the other FGPs were nondysplastic. As has been described in previous reports, the dysplastic changes in all FGPs were limited to the surface/foveolar epithelium and were not seen in the attenuated parietal cells and chief cells lining the dilated oxyntic glands (Figure 2).[7,8]

APC and β-Catenin Alterations

Alterations in *APC* were detected in 7 of 13 (53.8%) FGPs (four demonstrating low-grade dysplasia and three indefinite for dysplasia). These included two FGPs with bi-allelic *APC* inactivation (intragenic mutation coupled with 5q allelic loss) (Figure 3), two FGPs with *APC* gene mutation only, and three FGPs with 5q allelic loss only. The four intragenic *APC* mutations would all be predicted to result in protein truncation. Three were frameshift mutations resulting from insertion (two cases) or deletion (one case) of a base (A) into a 6-base poly(A) tract spanning codons 1554 to 1556, and the other was a stop codon mutation (TTA→TAA, codon 1564). This latter mutation occurred in the one FGP in which we separately micro-
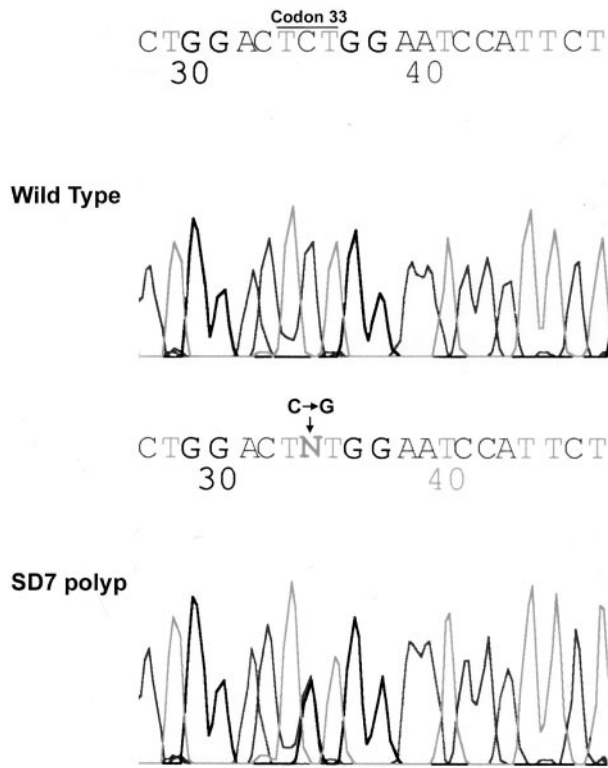
Figure 4. *β-catenin* missense mutation in a sporadic FGP graded as indefinite for dysplasia. DNA sequencing chromatogram shows a codon 33 TCT (serine)→TGT (cysteine) mutation in case SD7. This mutation would be expected to result in stabilization of β-catenin protein because of loss of a serine phosphorylation site. A mixture of the mutant and wild-type is present, reflective of the dominant nature of β-catenin mutations.

dissected the surface/foveolar and dilated glandular compartments; as expected, the mutation was present in both compartments.

β-catenin exon 3 mutations were identified in only 2 of 13 (15.4%) FGPs (both indefinite for dysplasia). Both were 1-bp missense mutations affecting serine/threonine residues for GSK-3β phosphorylation: codon 33, TCT→TGT (one case) (Figure 4) and codon 37, TCT→TTT (one case). Both mutations would therefore be expected to interfere with normal phosphorylation of β-catenin protein by GSK-3β and subsequent ubiquitin-mediated protein degradation.

With the exception of one case, β-catenin mutations and *APC* alterations were mutually exclusive. In this case (SD7, a polyp graded as indefinite for dysplasia), the FGP contained a codon 33 β-catenin mutation as well as 5q allelic loss with the D5S299 marker (D5S82 and D5S346 did not show loss of heterozygosity). If the 5q allelic loss in this FGP is disregarded as a nonspecific genetic abnormality, then overall six (46.2%) FGPs demonstrated APC alterations, two FGPs (15.4%) demonstrated β-catenin mutations, and five (38.5%) did not show identifiable genetic alterations (Table 1).

Microsatellite Instability

All 13 FGPs in this study were microsatellite stable (Table 1).

Discussion

We have identified *APC* alterations in 7 of 13 (53.8%) of sporadic FGPs with epithelial dysplasia or surface/foveolar changes indefinite for dysplasia, including 2 FGPs with bi-allelic *APC* inactivation (truncating *APC* mutations coupled with 5q allelic loss), 2 FGPs with truncating *APC* gene mutations only, and 3 FGPs with 5q allelic loss only. In contrast, in a previous study of 13 sporadic FGPs that were negative for dysplasia, we found only one FGP with 5q allelic loss (7.7%), and no *APC* gene mutations (53.8% *versus* 7.7%, $P = 0.030$).[8] However, this is nearly identical to the frequency of *APC* alterations previously demonstrated in a series of 41 FAP-associated FGPs (51%, $P > 0.999$).[8] In addition, the types of *APC* alterations found in FAP-associated FGPs and sporadic FGPs with dysplasia/indefinite for dysplasia are quite similar; frameshift mutations in a 6-base poly(A) tract spanning codons 1554 to 1556 in exon 15 are the most common alteration in both FAP-associated FGPs and dysplastic/indefinite sporadic FGPs, whereas exon 15 stop codon mutations are less frequent.[8,10]

Table 1. Sporadic Fundic Gland Polyps with Epithelial Dysplasia/Indefinite for Dysplasia

Polyp designation	Age/sex	Epithelial dysplasia	β-catenin mutation	APC mutation	5q allelic loss	MSI
SD1	49/F	Low-grade	Wild-type	1564X	+	Stable
SD2	52/M	Indefinite	Wild-type	1554-1556FS	+	Stable
SD3	67/M	Low-grade	Wild-type	1554-1556FS	−	Stable
SD4	59/F	Indefinite	Wild-type	1554-1556FS	−	Stable
SD5	71/M	Low-grade	Wild-type	Wild-type	+	Stable
SD6	48/F	Low-grade	Wild-type	Wild-type	+	Stable
SD7	69/F	Indefinite	S33C	Wild-type	+	Stable
SD8	50/F	Indefinite	S37F	Wild-type	−	Stable
SD9	59/F	Low-grade	Wild-type	Wild-type	−	Stable
SD10	61/F	Low-grade	Wild-type	Wild-type	−	Stable
SD11	46/M	Indefinite	Wild-type	Wild-type	−	Stable
SD12	37/F	Low-grade	Wild-type	Wild-type	−	Stable
SD13	62/F	Low-grade	Wild-type	Wild-type	−	Stable

Fundic gland polyps SD4 and SD9 were separate fundic gland polyps from the same patient. Locations of somatic mutations in β-catenin and APC are shown by codon.

FS, frameshift mutation; MSI, microsatellite instability.

Three previous studies of sporadic FGPs have shown high prevalences of activating *β-catenin* gene mutations of 91%,[9] 76%,[11] and 64.4%.[12] In particular, the two studies of sporadic FGPs performed in our laboratory looked exclusively at nondysplastic polyps, and demonstrated exon 3 missense mutations or in-frame deletion mutation in 52 of 57 and 47 of 62 FGPs, respectively (total = 99 of 119, 83.2%).[9,11] These mutations would be predicted to result in β-catenin protein stabilization. In contrast, only 2 of 13 (15.4%) sporadic FGPs with dysplasia/indefinite for dysplasia in the current study contained *β-catenin* gene mutations ($P < 0.00001$). These results suggest that whereas nondysplastic sporadic FGPs and FAP-associated FGPs arise through different arms of an altered *APC/β-catenin* pathway, sporadic FGPs with dysplasia/indefinite for dysplasia are molecularly more similar to FAP-associated FGPs.

The role of the *APC/β-catenin* pathway has been best studied in gastrointestinal adenomas. Among patients with FAP, most of the numerous colorectal and duodenal adenomas (and less frequent gastric adenomas) can be demonstrated to harbor bi-allelic inactivation of the *APC* gene on chromosome 5q21-22 (germline *APC* mutation coupled with a somatic, second-hit *APC* alteration).[17–19] A major function of the APC tumor-suppressor protein involves regulation of the level of cellular β-catenin protein. β-catenin functions as both as a submembranous component of the cadherin-mediated cell-cell adhesion system as well as a downstream transcriptional activator in the Wnt signaling pathway.[20,21] APC, in cooperation with AXIN and GSK-3β, promotes phosphorylation of serine/threonine residues encoded in exon 3 of the *β-catenin* gene.[20,22,23] Phosphorylation targets β-catenin for ubiquitin-mediated degradation. Increased levels of cytoplasmic β-catenin can result either from truncating *APC* gene mutations or from stabilizing *β-catenin* gene mutations. Increased cytoplasmic β-catenin subsequently translocates to the nucleus where, as part of a β-catenin/Tcf/Lef complex, it can stimulate transcription of cell-cycle regulatory genes including *cyclin D1* and c-*myc*.[24–26]

In addition to FAP-associated adenomas, a majority of sporadic gastrointestinal adenomas and adenocarcinomas demonstrate bi-allelic *APC* inactivation in the form of truncating mutations and/or 5q allelic loss.[17,27] Approximately half of colorectal adenomas without detectable *APC* alterations instead harbor *β-catenin* mutations.[27] Interestingly, however, there is some evidence for different biological behavior of *APC* and *β-catenin* gene mutations in colorectal neoplasms. *β-catenin* mutations are found in 12.5% of small (<1 cm) adenomas,[28] but are only rarely (1 to 2%) found in large adenomas[28] or invasive adenocarcinomas,[28,29] suggesting that colonic adenomas with *β-catenin* mutations are less likely to progress to carcinoma than are those with *APC* mutations.

Our findings in this current study as well as in previous studies suggest that the functional results of *APC versus β-catenin* mutations may be different in gastric FGPs as well. FAP-associated FGPs, which are associated with second-hit *APC* mutations, frequently show epithelial dysplasia,[7,30–32] and neoplastic progression of FAP-as-

sociated FGPs in the form of large dysplastic polyps[33] or invasive adenocarcinoma[32,34,35] has been reported, albeit rarely. Sporadic FGPs, with their predominance of *β-catenin* mutations, only infrequently show dysplasia[7] and have never been reported to progress to adenocarcinoma, even among patients with numerous polyps or fundic gland polyposis. Here we show that dysplasia/cellular changes indefinite for dysplasia in sporadic FGPs is also more likely to be associated with *APC* alterations than with *β-catenin* mutations. This distinction is not absolute; we could find no discernable differences (either in the histological appearance, size, or presence of other genetic alterations such as microsatellite instability) between the FGPs indefinite for dysplasia that harbored *APC* alterations and those that instead harbored *β-catenin* mutations. However, the high frequency of both *APC* alterations (in FAP-associated FGPs and dysplastic sporadic FGPs) and *β-catenin* mutations (in nondysplastic sporadic FGPs) underscores the fact that FGPs are neoplastic lesions harboring clonal genetic alterations similar to those found in gastrointestinal adenomas.

Among colorectal adenomas, it has been demonstrated that alterations in the *APC/β-catenin* pathway are sufficient for adenoma initiation and early growth.[36] No additional genetic alterations (including *K-ras* mutations, microsatellite instability, or allelic loss at chromosome 1p) are required for growth up to a size of ~1 cm in diameter.[36] Likewise, *APC* and *β-catenin* mutations are the only high-frequency genetic alterations found in gastric FGPs to date.[8,9,11,12] Although a single large FAP-associated FGP was reported to contain an activating codon 12 *K-ras* mutation,[33] we previously found only one *K-ras* mutation among 39 FAP-associated FGPs, and none among 13 sporadic FGPs.[8] In this study, we have additionally shown that microsatellite instability was not present in any of 13 sporadic FGPs. One explanation for this relative lack of secondary genetic alterations among FGPs is that the molecular progression of neoplastic alterations in FGPs could differ from those typically found in colorectal carcinogenesis. However, these results also raise the possibility that alterations in the *APC/β-catenin* pathway may be the sole genetic alteration in most FGPs.

Although FGPs were once considered to represent hamartomatous or hyperplastic/functional lesions[2,37,38] that were poorly linked to proton pump inhibitor ingestion,[39–44] the available genetic evidence supports that FGPs are neoplastic growths with clonal molecular alterations similar to those of colorectal and duodenal adenomas. Interestingly, this study and previous studies of FGPs highlight several unanswered questions with regard to the morphology and biological behavior of FGPs. First, all polyps of the colorectum and small bowel that harbor mutations in the *APC/β-catenin* pathway also concomitantly demonstrate epithelial dysplasia, a phenomenon that does not hold true for most FGPs. The data from the current study suggest that FGPs with dysplasia are more likely to harbor *APC* alterations than *β-catenin* mutations; however, overall the majority of FAP-associated FGPs with *APC* mutations are not dysplastic, and only rare FGPs with *β-catenin* mutations are dysplastic. Furthermore, we have previously demonstrated that identical

mutations in *APC* or *β-catenin* are present in both the surface/foveolar epithelium and the dilated oxyntic glands, despite the fact that the glandular compartment virtually never shows epithelial dysplasia.[8,9] This was again borne out in the current study, in which the one FGP in which the dysplastic surface and nondysplastic glandular epithelia were separately analyzed showed identical *APC* stop codon mutations in both compartments. Finally, the almost uniformly benign behavior of FGPs as compared to colorectal adenomas, despite the presence of *APC/β-catenin* alterations in both types of lesions, is intriguing. It is clear that for colorectal adenomas, it is the accumulation of additional genetic alterations that is associated with neoplastic progression. The results of this study showing no evidence for microsatellite instability in dysplastic FGPs, and a previous study showing only rare *K-ras* mutations in dysplastic and nondysplastic FGPs,[8] would suggest that these additional genetic alterations tend not to occur in the gastric milieu.

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