Genetic Alterations in Gastric Adenomas of Intestinal and Foveolar Phenotypes

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Gastric adenomas are unusual neoplasms that can constitute one of the direct precursors to gastric adenocarcinoma. Most gastric adenomas are comprised of polypoid projections of dysplastic epithelium with at least focal intestinal-type differentiation (containing goblet cells and/or Paneth cells), whereas adenomas comprised entirely of dysplastic foveolar-type epithelium are rare. It has been shown that nearly all intestinal-type adenomas arise in association with background intestinal metaplasia and gastric atrophy, approximately 40% harbor high-grade dysplasia, and nearly one fourth progress to adenocarcinoma. In contrast, foveolar-type adenomas tend to occur in otherwise normal, nonatrophic gastric mucosa and rarely harbor high-grade dysplasia or carcinoma. Potential differences in the genetic alterations between intestinal-type and foveolar-type gastric adenomas have not been systematically studied. We investigated 11 intestinal-type and 7 foveolar-type gastric adenomas (all from patients without familial adenomatous polyposis) for alterations in *APC* (using 5q allelic loss assays and direct DNA sequencing of the mutation cluster region), *β-catenin* (using direct DNA sequencing of the phosphorylation region in exon 3), *K-ras* (using direct DNA sequencing of codons 12 and 13), and microsatellite instability (MSI; using fluorescent-based PCR amplification of a standard panel of 5 microsatellite markers). Overall, 10 of 11 (91%) intestinal-type adenomas harbored at least one detectable genetic alteration, whereas only 3 of 7 (43%) of foveolar-type adenomas did ($P = .047$). However, no statistically significant differences in any particular genetic alteration were found. Among intestinal-type adenomas, *APC* alterations were present in seven (64%), high-level MSI in three (27%), and *K-ras* mutations in two (18%). Among foveolar-type adenomas, *APC* alterations were present in three (43%) and a *K-ras* mutation in one of six amplifiable polyps (17%). Neither *APC* nor MSI correlated with the size of the adenoma, but *K-ras* mutations were found only in lesions of ≥1 cm. *β-catenin* mutations were not present in any gastric adenoma, irrespective of the presence or absence of *APC* alterations. These results suggest that the types and frequencies of genetic alterations occurring in gastric and colorectal adenomas are similar. Although intestinal-type and foveolar-type gastric adenomas display divergent biologic behavior, the specific genetic events accounting for these differences in morphology and biologic behavior are unclear.

KEY WORDS: Adenoma, *APC*, β-catenin, *K-ras*, Microsatellite instability, Stomach.

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Gastric adenomas are relatively infrequent neoplasms which constitute at most approximately 10% of polyps arising in the stomach in Western populations (1). Despite this, adenomas hold importance because they can serve both as a direct precursor to gastric adenocarcinoma and as a marker for increased neoplastic risk elsewhere in the stomach (2–7). Previous series have reported rates of carcinoma arising within adenomas that range from 2.5% to >50% (2–7); in a 16-year retrospective review of gastric adenomas examined at The Johns Hopkins Hospital, we recently found that 15% of the polyps harbored carcinoma, and 10% of patients with adenomas had carcinomas elsewhere in the stomach (8).

Although all gastric adenomas are definitionally composed of polypoid, dysplastic epithelium, the morphologic features of adenomas vary. Most are formed from dysplastic intestinal-type epithelium that is comprised of a mixture of goblet cells with intestinal mucin and gastric (foveolar-type) cells

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with neutral mucin (Fig. 1; 2, 8–10). Much less commonly, adenomas are formed entirely or predominantly from dysplastic epithelial cells that resemble gastric (foveolar-type) cells both morphologically and by mucin immunohistochemistry (Fig. 2; 8, 9, 11). We found that intestinal-type gastric adenomas were more likely than those of foveolar-type to show high-grade dysplasia, to harbor intramucosal or invasive adenocarcinoma, and to arise in a background of extensive mucosal atrophy and intestinal metaplasia (8). A few other reports have also suggested that an intestinal *versus* foveolar phenotype of gastric adenomas correlates with greater malignant potential (2, 12).

Genetic alterations contributing to the development and neoplastic progression of gastric adenomas have been studied with respect to the *adenomatous polyposis coli* (*APC*; 13–15), *K-ras* (6, 13, 15–18), and *p53* (6, 11, 13, 19–24) genes and with respect to MSI (15, 18, 23–28). However, only one study has separately evaluated intestinal-type and foveolar-type adenomas. In that series, Kushima *et al.* (11) found that p53 immunolabeling was significantly more common in intestinal-type than in gastric-type adenomas, suggesting that there might be genetic differences that underlie the phenotypic differences between these two types of lesions. In this study, we evaluate 18 gastric adenomas for differences in MSI and in *APC*, *β-catenin,* and *K-ras* gene mutations between those of intestinal and foveolar phenotypes.

MATERIALS AND METHODS

Study Population

The study population consisted of 11 intestinal-type gastric adenomas and 7 foveolar-type adenomas removed at The Johns Hopkins Hospital between 1993 and 2001. All were endoscopically polypoid lesions projecting above the adjoining gastric mucosa (*i.e.*, no "flat" or "depressed adenomas" were included). As previously described (8), we classified adenomas with the aid of periodic acid-Schiff/Alcian blue at pH 2.5 staining as intestinal type if there were Paneth cells or goblet cells (even focally) and as foveolar type if they were composed entirely of dysplastic foveolar cells with an apical cap of neutral mucin (Figs. 1, 2). The degree of epithelial dysplasia was categorized as either low grade or high grade according to previously published criteria (29–31). Adenoma size was

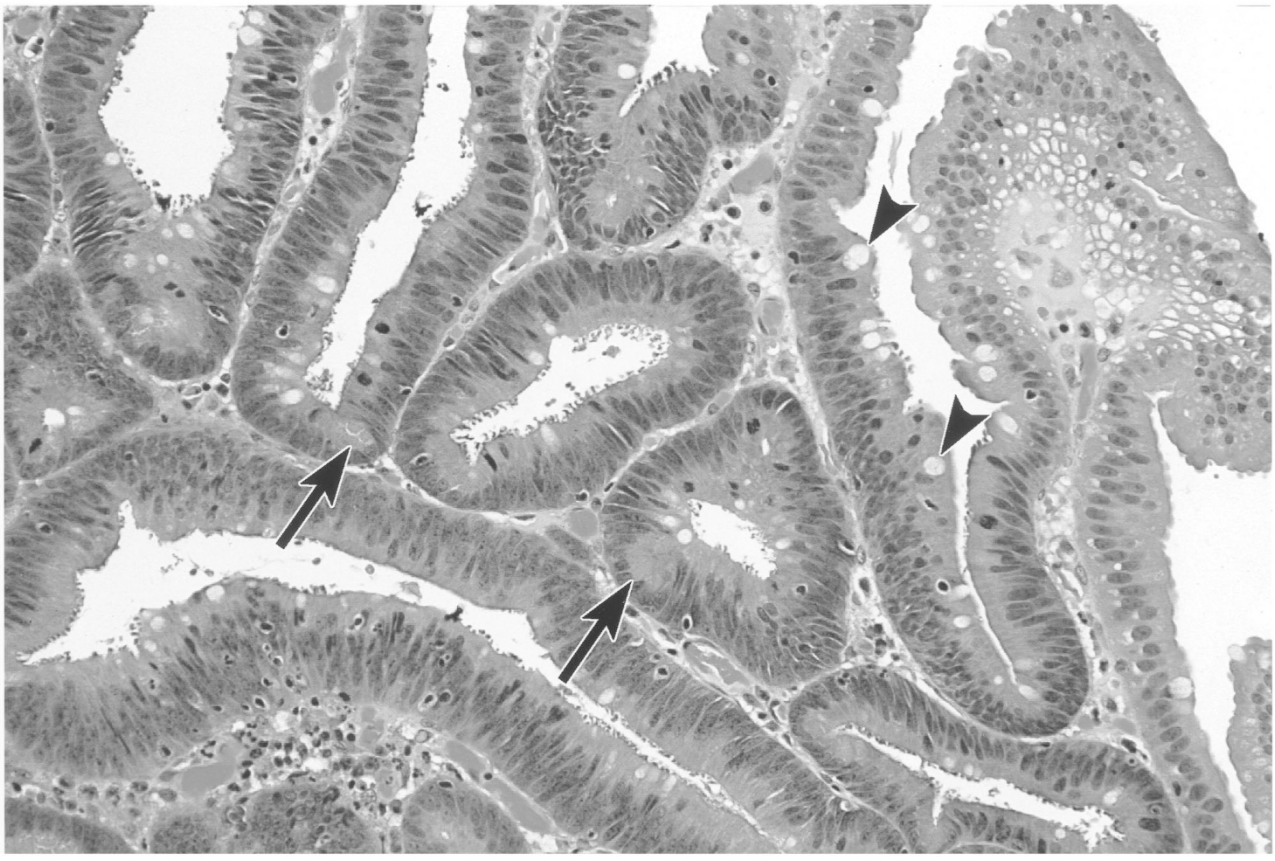

FIGURE 1. Histologic appearance of an intestinal-type gastric adenoma. Scattered goblet cells (**arrowheads**) and Paneth cells (**arrows**) are present. Epithelial dysplasia is classified as low grade in this adenoma (hematoxylin and eosin stain).

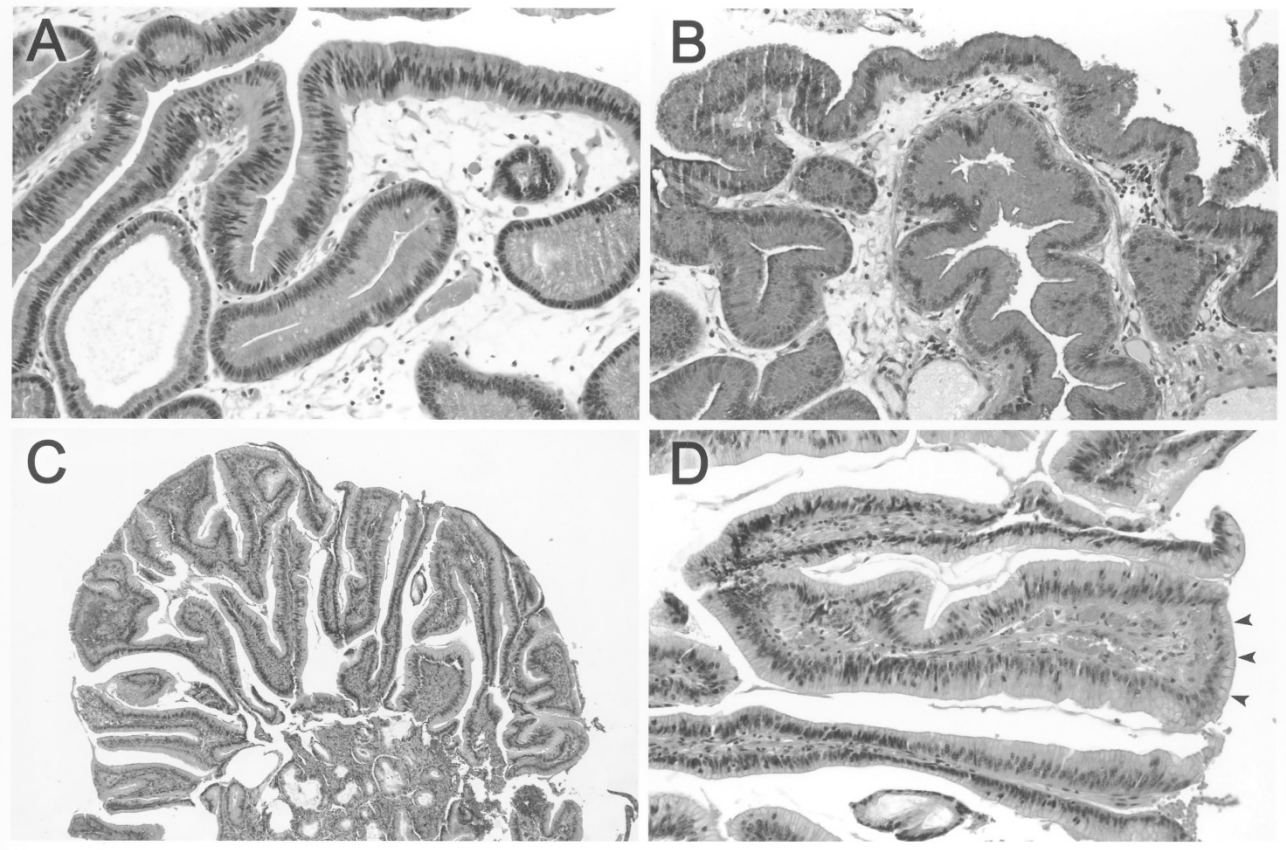

FIGURE 2. Histologic appearance of foveolar-type gastric adenomas. **A,** foveolar-type adenoma with low-grade dysplasia in a patient with familial adenomatous polyposis (FAP; hematoxylin and eosin stain). **B,** periodic acid-Schiff/Alcian blue stain at pH 2.5 highlights the apical neutral mucin cap of the epithelial cells in this adenoma and its lack of goblet cells. **C,** the sporadic, non–FAP-associated foveolar-type adenomas analyzed in this study are histologically similar to FAP-associated adenomas (hematoxylin and eosin stain). **D,** higher power view demonstrating the presence of apical mucin caps in the lining epithelium (**arrowheads**). The nuclei are enlarged and crowded but show only mild pseudostratification, characteristic of low-grade dysplasia (hematoxylin and eosin stain).

obtained from either the endoscopic or gross pathology reports.

Although we have previously noted that most foveolar-type adenomas occur in patients with familial adenomatous polyposis (FAP; 8), the seven foveolar-type adenomas analyzed here were all sporadic adenomas removed from patients with no clinical indication of FAP.

DNA Extraction

Genomic DNA was obtained by microdissection of H&E-stained slides prepared from routinely processed, formalin-fixed, paraffin-embedded specimens. A 27.5-gauge needle tip was used for microdissection under a low-power, 4× objective. DNA was extracted according to protocol described elsewhere (32). Gastric adenomas were microdissected as well as normal control tissue that was obtained from nonpolypoid stomach mucosa (in 16 cases), duodenum (in 1 case), or esophagus (in 1 case).

Mutational Analysis of the *APC*, *β-Catenin,* and *K-ras* Genes

Direct DNA sequencing was used to evaluate for mutations in *APC*, *β-catenin,* and *K-ras*. For *APC*, genomic DNA was amplified by polymerase chain reaction (PCR) using four sets of overlapping oligonucleotide primers that span the mutation cluster region in exon 15 (A1: 5'-CAGACTTATTGTGTAGAAGA-3' and A2: 5'-CTCCTGAAGAAAATTCAACA-3' for codons 1260–1359; B1: 5'-AGGGTTCTAGTTTATCTTCA-3' and B2: 5'-TCTGCTTGGTGGCATGGTTT-3' for codons 1339–1436; C1: 5'-GGCATTATAAGCCCCA-GTGA-3' and C2: 5'-AAATGGCTCATCGAGGCTCA-3' for codons 1417–1516; D1: 5'-ACTCCAGATGGATTTT-CTTG-3' and D2: 5'-GGCTGGCTTTTTTGCTTTAC-3' for codons 1497–1596). For *β-catenin,* PCR was performed using a primer pair (forward: 5'-ATGGAACCAGACAGAAAAGC-3' and reverse: 5'-GCTACTTGTTCTGAGTGAAG-3') that amplified a 200-bp fragment in exon 3 encompassing the region for glycogen synthase kinase-3β (GSK-3β) phosphorylation and ubiquitin-mediated degradation of β-catenin protein. For *K-ras*, PCR was performed with

a primer pair (forward: 5'-GAGAATTCATGACTGAATATAAACTTGT-3' and reverse: 5'-TCGAATTCCTCTATTGTTGGATCATATTCG-3') that amplified a region in exon 1 spanning codons 12 and 13. All PCR reactions were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) in 50-μL volumes that contained 2 μL of genomic DNA, 10 mM dNTP mix, 2.25 U AmpliTaq Gold (Applied Biosystems), 0.125 U Pfu DNA Polymerase (Stratagene, La Jolla, CA), and 20 pmol of forward and reverse primers. 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. For *β-catenin* amplification, 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. For *K-ras*, PCR reactions were performed with an initial denaturation at 94° C for 3 minutes, 40 cycles of 94° C for 1 minute, 50° C for 1 minute, and 72° C for 1 minute, and a final extension at 72° C for 7 minutes.

PCR products were cleaned using shrimp alkaline phosphatase and exonuclease I (Amersham, Buckinghamshire, United Kingdom). The products were then sequenced on an ABI Prism 3700 DNA Analyzer (Applied Biosystems) using the ABI Prism Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems). For all three genes, the same primers were used for both amplification and sequencing. Sequencing data were analyzed using GeneScan Analysis software (Applied Biosystems) as per the manufacturer's protocol. Mutations were verified in both forward and reverse directions.

Chromosome 5q Allelic Loss

Allelic loss on chromosome 5q was evaluated by microsatellite assays using three microsatellite markers: D5S82, D5S299, and D5S346. Assays were performed by fluorescent-labeled PCR amplification using forward primers labeled with 6-FAM (Applied Biosystems) and unlabeled reverse primers. PCR reactions were performed in 15-μL 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. Cycling conditions consisted of 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 PCR products were subsequently diluted with 30 μL of H_2O, and a 1.0-μL aliquot of each diluted fluorescent-labeled PCR product was combined with 12 μL of formamide 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 defined as a $\geq$50% reduction in the height of a heterozygous peak as compared with normal control DNA in one or more informative microsatellite markers.

MSI Analysis

Evaluation for MSI 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 (33). PCR reaction mixtures and cycling conditions for D2S123, D17S250, Bat-25, and Bat-26 were identical to those described above for 5q allelic loss (already including D5S346). Primer sequences and interpretation of the chromatograms were in accordance with those previously described in detail by Berg *et al.* (34). High-level MSI was defined as shifts in at least two of five microsatellite loci ($\geq$40%); low-level MSI as a shift in only one locus (20%); and microsatellite stable, as when none of the loci were shifted, as per the NCI criteria (33).

Statistical Analysis

Fisher's exact test was used to evaluate differences in the frequencies of genetic alterations among intestinal-type and gastric-type adenomas. Two-tailed P values of $<.05$ were considered statistically significant.

RESULTS

The histopathologic and molecular findings in the 18 gastric adenomas are summarized in Table 1.

Intestinal-Type Adenomas

The 11 intestinal-type adenomas occurred in 6 females and 5 males with a mean age of 75.5 years (range, 62–93 years). As previously described (8), histologic evaluation of the surrounding gastric mucosa revealed that these adenomas arose in the setting of intestinal metaplasia, including extensive antral-predominant intestinal metaplasia in eight patients, extensive corpus-predominant intestinal metaplasia (*i.e.*, autoimmune gastritis) in two patients, and focal intestinal metaplasia only in one patient. In 10 patients, the polyps were endoscopically or surgically resected and examined in their entirety, whereas in the remaining patient the lesion was biopsied only. Polyp size in these 10 re-

TABLE 1. Genetic Alterations in Intestinal-Type and Foveolar-Type Gastric Adenomas

Case	Adenoma Type	Grade of Dysplasia	*β-catenin*	*APC*	5q Allelic loss	*K-ras*	MSI Status	Markers Shifted
IA-1	Intestinal	Low	Wild-type	R1450X	+	Wild-type	Stable	—
IA-2	Intestinal	Low	Wild-type	1554–1556FS (del A)	N/I	Wild-type	Stable	—
IA-3	Intestinal	Low	Wild-type	1554–1556FS (ins A)	N/I	Wild-type	Stable	—
IA-4	Intestinal	Low	Wild-type	1554–1556FS (ins A)	−	G13C	Stable	—
IA-5	Intestinal	High	Wild-type	E1554X	−	Wild-type	Stable	—
IA-6	Intestinal	High	Wild-type	Wild-type	+	G12S	Stable	—
IA-7	Intestinal	High	Wild-type	Wild-type	+	Wild-type	Stable	—
IA-8	Intestinal	High	Wild-type	Wild-type	−	Wild-type	MSI-high	Bat-25, Bat-26, D2S123, D17S250, D5S82
IA-9	Intestinal	High	Wild-type	Wild-type	−	Wild-type	MSI-high	Bat-25, Bat-26, D2S123, D17S250, D5S346, D5S82, D5S299
IA-10	Intestinal	Low	Wild-type	Wild-type	−	Wild-type	MSI-high	Bat-25, Bat-26, D5S346, D5S82, D5S299
IA-11	Intestinal	High	Wild-type	Wild-type	−	Wild-type	Stable	—
FA-1	Foveolar	Low	Wild-type	1463–1465FS (del AG)	−	G12R	Stable	—
FA-2	Foveolar	Low	Wild-type	N/A	+	Wild-type	Stable	—
FA-3	Foveolar	Low	N/A	N/A	+	N/A	Stable	—
FA-4	Foveolar	Low	Wild-type	Wild-type	−	Wild-type	Stable	—
FA-5	Foveolar	Low	Wild-type	Wild-type	−	Wild-type	Stable	—
FA-6	Foveolar	Low	Wild-type	Wild-type	−	Wild-type	Stable	—
FA-7	Foveolar	Low	Wild-type	Wild-type	−	Wild-type	Stable	—

The location of mutations is given by codon.

Cases IA-8 and IA-11 also contained intramucosal adenocarcinomas, but only the adenoma components were analyzed.

FA = foveolar-type adenoma; FS = frameshift mutation; IA = intestinal-type gastric adenoma; MSI = microsatelite instability; N/A = insufficient DNA for evaluation or reaction failed; N/I = non-informative for allelic loss; X = stop codon mutation.

corded cases ranged from 0.3–3 cm, with a mean of 1.35 cm. The degree of epithelial dysplasia was classified as low grade in 6 adenomas and as high grade in 5 adenomas. In addition, 2 of the adenomas with high-grade dysplasia contained foci of intramucosal carcinoma, but for the purposes of this study only the adenoma precursors were microdissected and analyzed.

Alterations in the *APC* tumor-suppressor gene were the most common genetic alteration identified in intestinal-type adenomas. *APC* mutations were present in 5 of 11 (45.5%) cases and 5q allelic loss in 3 of 9 (33.3%) informative cases. The *APC* mutations included two stop-codon mutations and three frameshift (insertion or deletion) mutations, and therefore all would be expected to result in APC protein truncation. In one of the adenomas, biallelic inactivation of *APC* (truncating mutation plus loss of the normal allele) could be demonstrated.

MSI was present in 3 of 11 (27.3%) intestinal-type adenomas and constituted high-level MSI in all 3 cases (Fig. 3). Among the adenomas studied here, the presence of MSI and *APC* alterations (mutation and/or allelic loss) appeared to be mutually exclusive. Overall, therefore, 10 of 11 (90.9%) intestinal-type adenomas demonstrated either *APC* alterations or MSI, but not both. Notably, neither MSI nor *APC* alterations correlated with adenoma size or degree of dysplasia (Table 1); the only adenoma in this group that did not demonstrate either MSI or *APC* alteration was a 1.4-cm polyp (thus approximating the mean size among the group of intestinal-type adenomas) with high-grade dysplasia and intramucosal carcinoma.

In contrast to the relative frequency of *APC* alterations in intestinal-type adenomas, *K-ras* oncogene mutations were present in only two (18.2%) cases, representing activating codon 12 and codon 13 missense mutations, respectively (Fig. 4). Both of these *K-ras* mutations occurred in adenomas that also contained *APC* alterations. No *β-catenin* mutations were identified, either among adenomas with or without *APC* mutations.

Foveolar-Type Adenomas

The seven foveolar-type adenomas occurred in four females and three males who had a mean age of 65.9 years (range, 54–80 y). Foveolar-type adenomas were in general smaller lesions than intestinal-type adenomas, with a mean size of 0.5 cm (range, 0.3–1 cm). As was described elsewhere (8), in contrast to intestinal-type adenomas, these foveolar-type adenomas were most frequently associated with a nonatrophic and nonintestinalized mucosal background. In only one case (FA-1) was there any metaplasia or atrophy of the surrounding mucosa, a patient with autoimmune gastritis, extensive associated pseudopyloric metaplasia of the gastric body/fundus, and a 1-cm foveolar-type adenoma in the fundus. Epithelial dysplasia was low grade only in all of the foveolar-type adenomas, and none harbored intramucosal or invasive carcinoma.

Alterations in *APC* were present in three of seven (42.9%) foveolar-type adenomas, including two adenomas with 5q allelic loss (Fig. 5) and one adenoma with an intragenic *APC* mutation (Fig. 6). This latter adenoma, FA-1, was the 1-cm fundus polyp in

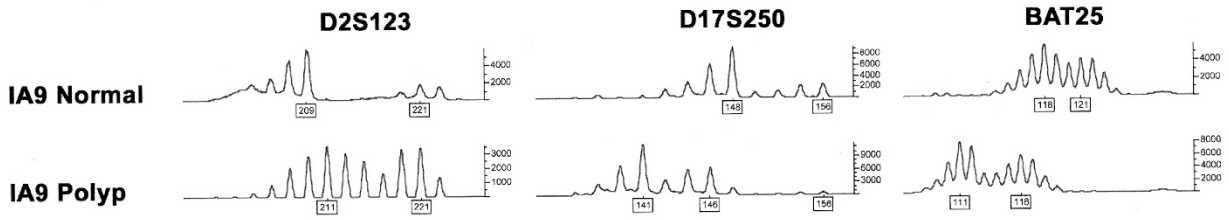

FIGURE 3. High-level microsatellite instability in an intestinal-type adenoma. Representative allelic shifts in D2S123, D17S250, and Bat-25 are illustrated (Case IA-9).

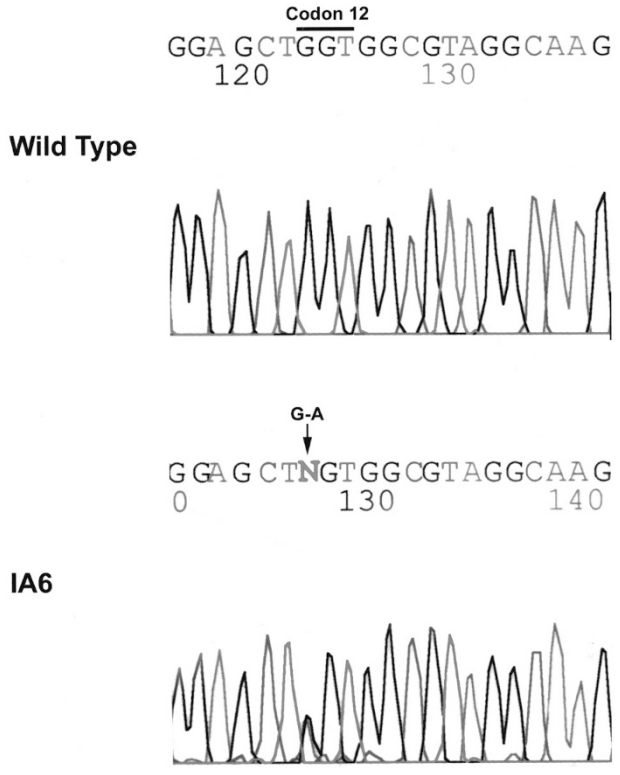

FIGURE 4. K-ras oncogene mutation in an intestinal-type adenoma. A codon 12 GGT(glycine) → AGT(serine) mutation is present (Case IA-6). The wild-type (normal) mucosa at **top** shows only the normal GGT DNA sequence at codon 12 of *K-ras*, whereas the intestinal-type adenoma at **bottom** contains a G (**black peak**) to A (**green peak**) point mutation that will result in the substitution of serine for glycine in the K-ras protein. The sequencing chromatogram for IA-6 shows overlap of the **green** and **black** peaks, reflecting the dominant positive nature of the mutation and its presence in only one of the *K-ras* alleles.

the patient with autoimmune gastritis, representing the only foveolar-type adenoma to arise in association with background mucosal atrophy; it harbored a 2-bp deletion frameshift mutation that would be expected to result in protein truncation. This adenoma also harbored an activating codon 12 *K-ras* mutation; the other five foveolar-type adenomas in which *K-ras* sequencing was completed contained only wild-type sequences. All seven foveolar-type adenomas were microsatellite stable. Only wild-type *β-catenin* sequences were present in six amplifiable cases.

Foveolar-type adenomas demonstrated a lower overall frequency of detectable clonal genetic alterations than did intestinal-type adenomas (*i.e.*, 10 of 11 intestinal-type adenomas contained alterations in *APC, K-ras*, or MSI *versus* only 3 of 7 foveolar-type adenomas; $P = .047$). However, there were no particular statistically significant differences between gastric-type and intestinal-type adenomas with respect to alterations in *APC, K-ras*, or *β-catenin*, or with respect to the presence of MSI.

DISCUSSION

Unlike colorectal carcinogenesis, the adenoma-carcinoma sequence is an uncommon pathway for gastric adenocarcinoma development, and most intestinal-type gastric carcinomas do not appear to arise from adenoma precursors (13, 15, 35, 36). Not only do most intestinal-type gastric carcinomas lack an identifiable adenoma precursor histologically, but divergent patterns of allelic loss (35, 36) and molecular alterations in genes including *APC* (13, 15) exist between most adenomas and most carcinomas. Nevertheless, that gastric adenomas can serve as the direct precursors to adenocarcinoma is well documented. Estimations of the frequency of such neoplastic progression have ranged widely in the literature, from 2.5% to >50% (2–7).

We recently reported that 15% of gastric adenomas examined at The Johns Hopkins Hospital over a 16-year period contained either intramucosal (7%) or infiltrating (8%) carcinoma (8). However, there was a striking difference between adenomas with an intestinal-type phenotype and those with a foveolar phenotype. Whereas 23% of intestinal-type adenomas harbored carcinoma, none of the foveolar-type adenomas did (8). The foveolar-type adenomas in that study occurred in two distinct scenarios: FAP associated (in the majority of cases) and sporadic. The fact that foveolar-type adenomas rarely undergo neoplastic progression is further reinforced by epidemiologic evidence, which has failed to show a statistically significantly increased risk of gastric carcinoma among Western patients with FAP, despite the increased incidence of gastric adenomas in these patients (37). We therefore hypothesized that there might be different genetic alterations in foveolar-type and intestinal-type ad-

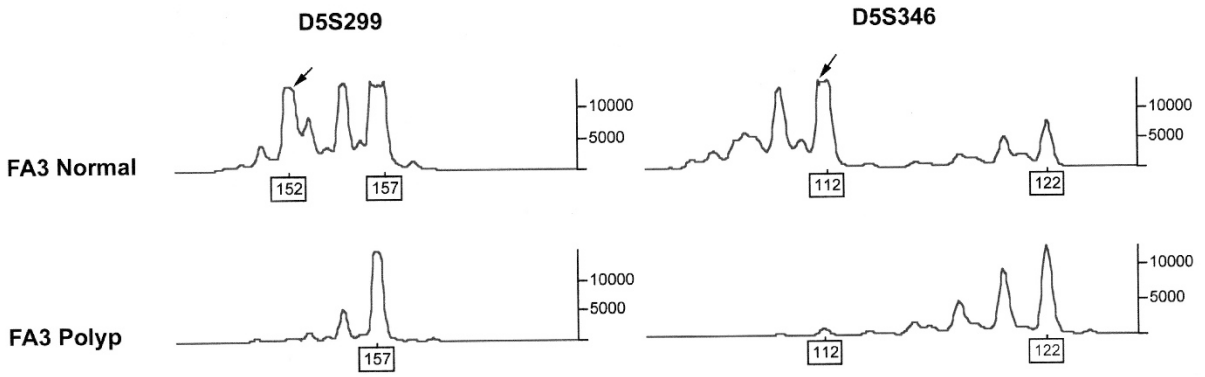

FIGURE 5. Chromosome 5q allelic loss in a foveolar-type adenoma. Loss of the shorter alleles (**arrows**) is seen for both microsatellite markers D5S299 and D5S346 (Case FA-3).

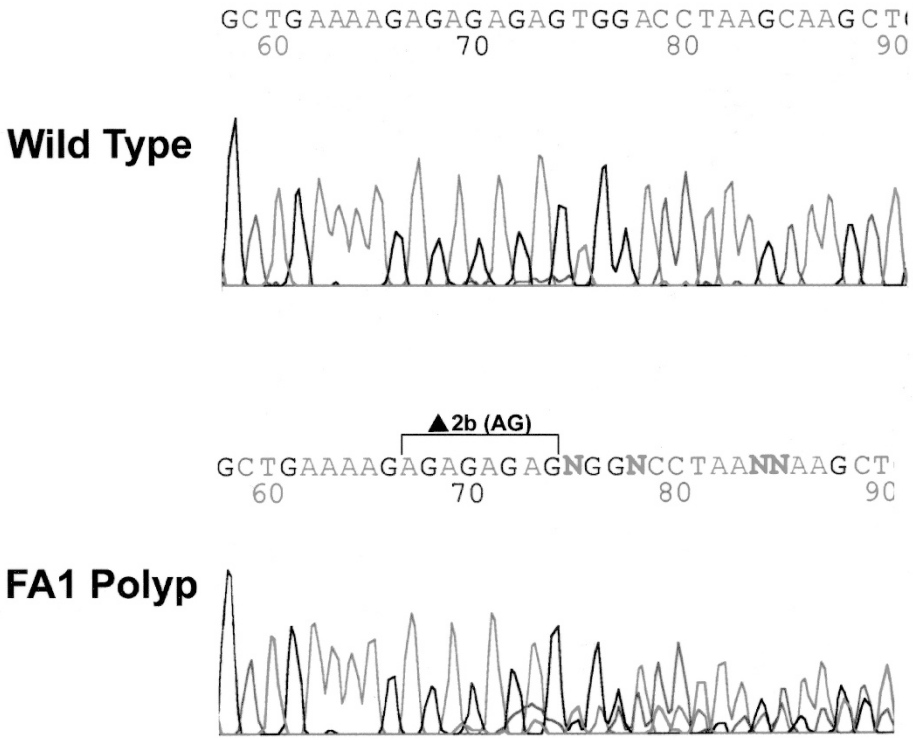

FIGURE 6. Frameshift *APC* gene mutation in a foveolar-type adenoma. Sequencing chromatogram shows a 2-bp (AG) deletion in a tract spanning codons 1463–1465 (Case FA-1). The wild-type (normal) mucosa at **top** shows the expected DNA sequence for this portion of exon 15 of the *APC* gene. In the FA-1 polyp at **bottom**, the DNA sequencing chromatogram shows overlapping bases after the AG deletion because the wild-type and mutated alleles are of different lengths.

enomas to account for this divergent biologic behavior.

We found that intestinal-type adenomas were more likely than foveolar-type adenomas to harbor a detectable clonal genetic alteration in *APC*, MSI, and/or *K-ras* (91% *versus* 43%, respectively). In particular, 5 of 11 intestinal-type adenomas had truncating *APC* mutations, but only 1 of 5 amplifiable foveolar-type adenomas did. In addition, 3 of 11 intestinal-type adenomas showed high-level MSI, but all 7 foveolar-type adenomas were microsatellite stable. Furthermore, differences in adenoma size did not account for the presence or absence of *APC* mutations or MSI. However, despite the higher frequency of detectable genetic alterations in intestinal-type *versus* foveolar-type adenomas, no particular alteration (*i.e.*, truncating *APC* mutation, 5q allelic loss, MSI, or *K-ras* mutation) reached statistical significance. This may be due to the relatively low number of foveolar-type adenomas available for study, because sporadic foveolar-type adenomas are very uncommon lesions.

Previous studies of genetic alterations in *APC*, *K-ras*, and MSI in gastric adenomas have not generally specified the epithelial phenotype of the adenomas; however, because the vast majority of

non–FAP-associated gastric adenomas arise in the setting of background intestinal metaplasia and show at least focal intestinal-type differentiation, it is likely that the genetic changes reported in these previous studies reflect mainly those seen in intestinal-type adenomas. Only two prior investigations of *APC* in polypoid gastric adenomas have been reported and have shown mutation frequencies of 20% (6 of 30 adenomas from Japanese patients; 14) and 78% (25 of 32 adenomas from Korean patients; 15), respectively. In neither study did degree of dysplasia or adenoma size correlate with the presence of *APC* mutations, suggesting that, as in the colorectum, *APC* alterations are early neoplastic events. The lack of correlation that we also observed between *APC* alterations and adenoma size or dysplasia grade in this study also supports this. Interestingly, unlike colorectal adenocarcinomas, which arise predominantly from adenoma precursors and typically harbor truncating *APC* mutations, only a minority of differentiated gastric adenocarcinomas contain *APC* mutations (13, 15, 38), an observation suggesting that most gastric adenocarcinomas do not arise from adenoma precursors. Lee *et al.* (15), for example, found somatic *APC* mutations in only 6% of gastric adenocarcinomas and hypothesized that dysplastic or adenomatous lesions with *APC* mutations were actually *less* likely to progress to carcinoma. In the current study, neither of the two gastric adenomas that harbored adenocarcinoma (intramucosal carcinoma in both) contained detectable *APC* mutations or 5q allelic loss.

Among colorectal neoplasms that lack detectable *APC* mutations, two alternative genetic alterations may be found—*β-catenin* oncogene mutations and MSI. Mutations that disrupt phosphorylation sites involved in regulating normal β-catenin protein degradation can substitute for *APC* mutations and theoretically should stimulate similar downstream targets of the *APC/β-catenin* pathway, including transcriptional activation of *cyclin D1* and *c-myc* (39–41). Approximately half of colonic adenomas lacking *APC* alterations instead harbor stabilizing *β-catenin* mutations (42). Notably, *β-catenin* mutations are more prevalent among small adenomas than larger adenomas or colonic adenocarcinomas (43, 44). This latter observation led us to hypothesize that *β-catenin* mutations might be present in gastric adenomas without *APC* alterations, particularly the small, foveolar-type adenomas. However, none of the 17 gastric adenomas sequenced here for *β-catenin* contained mutations. Only one previous study has evaluated for *β-catenin* mutations in gastric adenomas, and similarly reported no *β-catenin* mutations among 12 (intestinal-type) adenomas from Korean patients (15). *β-catenin* also does not appear to be a frequent mutational target in gastric carcinomas, having been reported in only 0–7.5% of cases studied (45–50).

A second pathway, MSI, was present in 3 of 11 (27.2%) intestinal-type adenomas, all of which were MSI high. Several previous studies have reported prevalences of MSI in gastric adenomas ranging from 8% to 28.6% (15, 23, 24, 26, 27), with the majority of MSI-high cases associated with decreased hMLH1 expression due to hypermethylation of the *hMLH1* gene promoter, as occurs in sporadic microsatellite-unstable colorectal neoplasms (26). One recent study of gastric carcinomas identified MSI by Bat-25/Bat-26 analysis in only 8% of intestinal-type carcinomas but in 57% of carcinomas with a foveolar phenotype (foveolar-type carcinomas expressing gastric mucins; 51). We therefore hypothesized that foveolar-type adenomas might also have a high frequency of MSI. However, all seven foveolar-type adenomas evaluated here were microsatellite stable, further suggesting a different molecular pathogenesis between foveolar-type adenomas and foveolar-type adenocarcinomas.

Among colorectal neoplasms, both *APC/β-catenin* pathway alterations and MSI represent early neoplastic events, whereas *K-ras* oncogene mutations and *p53* tumor suppressor gene mutations are secondary, subsequent alterations in the neoplastic pathway. We found codon 12 and 13 *K-ras* mutations in only 3 of 18 gastric adenomas (16.7%), a prevalence not significantly different from the range (0–16%) in previously studied gastric adenomas (6, 13, 15–18). Isogaki *et al.* (18) found that the *K-ras* mutational frequency increased with higher degrees of dysplasia in their set of 50 adenomas. Similarly, Tsuchiya *et al.* 16) found that the *K-ras* mutations present in 10.5% of their set of 38 adenomas were not homogeneously distributed (*i.e.*, could be present only focally within different sites from the same adenoma). The three *K-ras* mutations detected in the current study were present only in adenomas of $\geq$ 1 cm (1 cm, 1.5 cm, and 2 cm), supporting that these are secondary genetic events in neoplastic progression.

In summary, the findings in this study suggest that gastric adenomas (particularly those of intestinal phenotype), like colorectal adenomas, arise predominantly through mutational inactivation of the *APC* tumor suppressor gene, with MSI representing a less frequent pathway. Adenomas of pure foveolar phenotype are encountered only rarely. They were less likely to harbor detectable clonal genetic alterations than those of intestinal type, although no particular genetic alteration reached statistical significance.

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Book Review

Gray W, McKee G: Diagnostic Cytopathology, Second Edition, 1100 pp, city, Churchill Livingstone, 2002 ($295.00).

Diagnostic Cytopathology, Second Edition is aimed at practicing and training pathologists as well as screening personnel. The book is easy to read and well organized. Color-coded main topic sections serve as thumb indexes and are coordinated to match the table of contents, making the sections very quick and easy to find. Also useful are the topic and subtopic headings within each chapter. The organization of each chapter remains constant throughout the book, making each subtopic exceptionally easy to find.

There are summaries of "Cytological Findings" at the beginning of each diagnostic entity in a bulleted format for a quick glimpse of the "nitty-gritty" of what one needs to know, followed by an extensive explanation and expansion of each finding. These sections give brief and to-the-point explanations of nuclear features, grouping patterns, cytoplasmic features, and even staining patterns where appropriate. The expansions that follow give more detail and essentially everything one needs to know about a given sample. There are "Cytological Findings" sections not only for each diagnostic entity, but also for many given techniques for that entity. For example, findings in washings are in a separate section from findings in brushings. This makes for much more specific information and is extremely useful.

The "Cytological Findings" sections are followed by "Diagnostic Pitfalls" sections for each case. These sections are also quite useful, not only for those in training, but for experienced pathologists as well. These sections cover "why it isn't" another entity and answer many questions before they are asked. The "Diagnostic Pitfalls" also give helpful information on the alternative diagnoses as well as suggestions for further testing.

The photomicrographs are plentiful and of excellent quality, with helpful legends and appropriate referencing within the text. The tables throughout the book are invaluable for organizing large amounts of information, and we found them exceptionally easy to follow.

In general, this reviewer is impressed by the *Diagnostic Cytopathology, Second Edition* and would highly recommend it for practicing pathologists, fellows, and residents as well as screening personnel as a bench reference.

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