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Mutational analysis by next generation sequencing of gastric type dysplasia occurring in hyperplastic polyps of the stomach Mutations in gastric hyperplastic polyps

Marcela Salomao *, Aesis M. Luna, Jorge L. Sepulveda, Antonia R. Sepulveda

Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY, United States

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ABSTRACT

Gastric hyperplastic polyps (GHP) are the most common type of polyps occurring in the stomach. Although GHP are broadly interpreted as benign lesions, they may progress to dysplasia and adenocarcinoma. Objective: In this study, we aimed to identify genomic mutations that characterize and may drive malignant transformation in GHP by using next-generation sequencing. Eight GHP (2 with dysplasia, 1 indefinite for dysplasia and 5 without dysplasia) were studied. Only large polyps (>1 cm) with gastric differentiation were included in this study, while adenomatous polyps (intestinal-type) were excluded. Immunohistochemistry for MUC2, MUC5A, MUC6, CDX2, p53, and Ki67 was performed. DNA was extracted from formalin-fixed paraffin-embedded sections and sequenced for the detection of somatic mutations. Multiplex sequencing was done with the TrueSeq Amplicon Cancer Panel in the MiSeq platform. Variant annotation and visualization were performed using NextGENe (SoftGenetics) software. No pathogenic mutations were detected in GHP without dysplasia. *TP53* gene mutations were the most common alteration in dysplastic GHP (2 of 2 dysplastic cases). *PIK3CA* mutation was identified in a GHP with pyloric-type dysplasia, whereas foveolar-type dysplasia carried *TP53* mutations. In conclusion, *TP53* gene mutations are a common alteration in the early dysplastic stage during malignant transformation of GHP. GHP with dysplasia may show dual differentiation. In our study, pyloric-type dysplasia was associated with a *PIK3CA* alteration whereas foveolar dysplasia carried *TP53* mutations. The identification of carcinoma-associated mutations in large GHP provides additional evidence of their neoplastic potential and emphasizes the need for their complete resection and follow-up.

1. Introduction

Gastric hyperplastic polyps (GHP) are the most common type of polyps occurring in the stomach, typically occurring in the setting of mucosal injury, most commonly chronic gastritis associated with *Helicobacter pylori* infection and autoimmune gastritis (Abraham et al., 2001; Abraham et al., 2002). GHP are broadly interpreted as benign lesions, however a minority of cases may progress to dysplasia (0.2 to 10%) and adenocarcinoma (0.6%–3%) (Daibo et al., 1987; Orlowska et al., 1995; Zea-Iriarte et al., 1996; Abraham et al., 2001; Terada, 2011). Polyp size of >1 cm is considered a risk factor for malignant transformation (Daibo et al., 1987; Hizawa et al., 1995; Yao et al., 2002).

To date, most studies evaluating the malignant potential of GHP have focused on the morphologic and immunohistochemical features of GHP. Increased p53 immunoreactivity has been reported in dysplasia and adenocarcinoma, but not in the non-dysplastic hyperplastic areas of the polyp (Zea-Iriarte et al., 1996; Terada, 2011; Yao et al., 2002; Murakami et al., 2001; Dijkhuizen et al., 1997). Data from earlier studies showed that chromosomal alterations and rare cases of *KRAS* mutations have been implicated in the development of dysplasia in GHP, while alterations in the *TP53* gene were the most common abnormality and a late event in GHP transformation (Weiss et al., 2003; Murakami et al., 2001; Dijkhuizen et al., 1997). However, thorough characterization of genomic alterations driving the dysplasia–carcinoma sequence in GHP is lacking.

The present study is a comprehensive evaluation of GHP, including histopathologic and genetic profiling by using immunohistochemistry and highly sensitive next-generation targeted sequencing with a large panel of genes frequently altered in cancer.

2. Materials and methods

GHP were retrieved through a search of our surgical pathology archives. Only large polyps (>1 cm) with primarily gastric differentiation were included in this study. Adenomatous (intestinal-type) polyps were excluded. The presence of low-grade (LGD) or high-grade dysplasia (HGD) was assessed as previously described (Lauwers et al., 2010).

* Corresponding author at: Department of Pathology and Cell Biology, Columbia University Medical Center, 630 West 168th Street, VC14-238C, New York, NY 10032, United States.
E-mail address: mas2300@cumc.columbia.edu (M. Salomao).

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Table 1
Clinical data.

Case	Age	Sex	Clinical presentation	Size/location	Other endoscopic findings	*H. pylori*
1	80	M	Nausea, abdominal pain	2.5 cm, body	None	No
2	89	F	Anemia	2.5 cm, antrum	Duodenal diverticulosis	No
3	80	M	Melena	2 cm, body	Reactive gastropathy	No
4	61	F	Abdominal pain, gallstones	5 cm, body	Choledocholithiasis	No
5	57	F	Occult blood in stool	1.5 cm, antrum	Chronic active gastritis	Yes
6	65	F	Abdominal pain	2 cm, antrum	Chronic active gastritis	Yes
7	53	M	Anemia	6 cm, antrum	Multiple antral polyps	No
8	56	M	Heartburn	2 cm, antrum	Mild diffuse gastritis	No

All cases were reviewed by 2 gastrointestinal pathologists (M.S. and A.R.S.).

Immunohistochemical studies for MUC2 (clone MRQ-18, pre-diluted, Ventana Systems, Tucson, AZ), MUC5AC (clone MRQ-19, pre-diluted, Ventana Systems, Tucson, AZ), MUC6 (clone MRQ-20, pre-diluted, Ventana Systems, Tucson, AZ), CDX2 (clone EPR2764Y, pre-diluted, Ventana Systems, Tucson, AZ), p53 (Clone DO-7, Dako, Carpenteria, CA, dilution 1:200), and Ki67 (clone MIB-1, Dako, Carpenteria, CA, dilution 1:100) were performed on Ventana Ultra instruments(Ventana Systems, Tucson, AZ).

Targeted multiplex sequencing was performed in all cases. Briefly, DNA was extracted from 10 sections of formalin-fixed paraffin-embedded tissue cut at 5-μm thickness. When limited lesional tissue was available, areas of interest were isolated with a PALM Zeiss laser-capture microdissection. DNA was purified using QIAamp DSP DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany) according to manufacturer specifications. DNA concentration was measured by Qubit fluorometric quantitation (Life Technologies, Grand Island, NY).

Following DNA purification, samples were sequenced for the detection of somatic mutations using a multiplexed sequencing assay (MiSeq system, Illumina, San Diego, CA). Briefly, we used pre-designed, optimized oligonucleotide probes provided by the manufacturer to generate an amplicon library using the Illumina TruSeq Amplicon Cancer Panel (Illumina, San Diego, CA). The panel covers 48 genes with 212 amplicons containing known mutational 'hotspots': *ABL1*, *AKT1*, *ALK*, *APC*, *ATM*, *BRAF*, *CDH1*, *CDKN2A*, *CSF1R*, *CTNNB1*, *EGFR*, *ERBB2*, *ERBB4*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *FLT3*, *GNA11*, *GNAQ*, *GNFAS*, *HNF1A*, *HRAS*, *IDH1*, *JAK2*, *JAK3*, *KDR*, *KIT*, *KRAS*, *MET*, *MLH1*, *MPL*, *NOTCH1*, *NPM1*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *PTPN11*, *RB1*, *RET*, *SMAD4*, *SMARCB1*, *SMO*, *SRC*, *STK11*, *TP53*, and *VHL* genes. Data deconvolution was performed using MiSeq Reporter v1.3+ software (Illumina). Alignment, variant call, annotation and visualization were performed using NextGENe® (SoftGenetics) software. We used reasonably stringent filtering criteria to avoid false negatives while capturing as many single nucleotide variants (SNV) as possible. The impact of SNV on protein function was assessed and ranked by using mutation assessor, PROVEAN, SIFT, and TransFIC prediction tools (Reva et al., 2011; Choi et al., 2012; Kumar et al., 2009; Gonzalez-Perez et al., 2012). Finally, the resulting list of alterations was compared to the previously reported somatic mutations in the Catalogue of Somatic Mutations in Cancer (COSMIC) database.

3. Results

3.1. Clinical and histopathological characteristics

Our search identified 67 gastric polypectomy specimens with a pathological diagnosis of GHP diagnosed between 2000 and 2013 (25 male:42 female; mean age 62.2 ± 14.5 years). Of these, 2 cases showed high-grade dysplasia (2.99%). One case originally classified as having focal low-grade dysplasia was re-categorized in this study as indefinite for dysplasia, favor reactive.

Eight large GHP were selected for immunohistochemical evaluation and next-generation sequencing, including the 3 cases with dysplasia. These eight selected polyps ranged between 1.5 and 6 cm in largest dimension (mean size 2.94 ± 1.6 cm) and were most frequently located in the gastric antrum (n = 5, 62.5%). *H. pylori* gastritis was identified in 2 cases (25%) (Table 1).

Review of H&E-stained sections demonstrated the classical features of gastric hyperplastic polyps, including elongated, dilated and tortuous glands lined by foveolar-type epithelium with intervening edematous stroma and variable amounts of acute and chronic inflammation (Fig. 1).Two polyps (cases 1 and 3) harbored high-grade dysplasia. Polyp 1 had two morphologically separate areas of dysplasia, each with distinct immunohistochemical features (Figs. 2 and 3), and is described below. Polyp 2 showed mildly altered architecture with budding and branching of antral glands, and focal (<5%) surface epithelial changes (hyperchromasia, elongation and pseudostratification of nuclei) originally classified as low-grade dysplasia. Since these changes were associated with extensive surface epithelial erosion and acute inflammation, for the purpose of this study, they were re-classified as 'indefinite for dysplasia, favor reactive' (Fig. 4). Polyp 3 showed the most

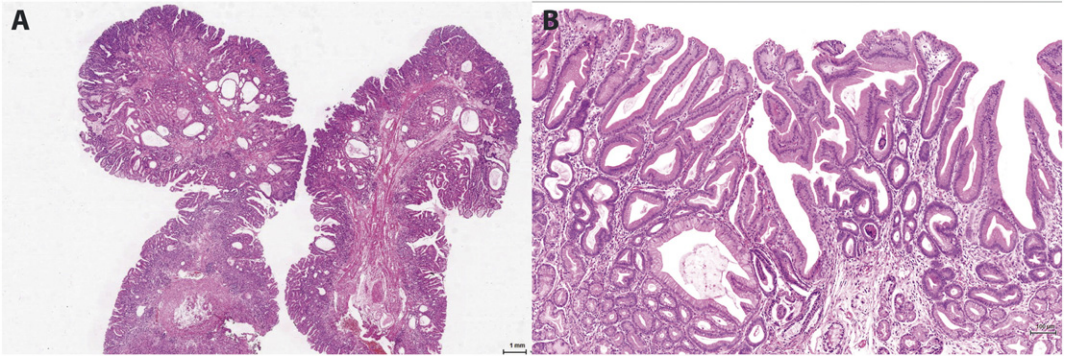

Fig. 1. Example of gastric hyperplastic polyp. On low magnification, elongated, dilated and tortuous glands are seen (A). Higher magnification demonstrates the lining foveolar-type epithelium with intervening edematous stroma and mild inflammation (B) (hematoxilin–eosin, original magnification ×10 [A], original magnification ×100 [B]).