Human Pathology (2018) 74, 64–72

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Human PATHOLOGY

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Original contribution

Histologic and immunohistochemical differences between hereditary and sporadic diffuse gastric carcinoma☆

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Received 16 October 2017; revised 15 December 2017; accepted 20 December 2017

Keywords:
Gastric carcinoma;
Hereditary diffuse gastric carcinoma;
CDH1 mutation;
p16;
CDX2

Summary We aimed to identify histopathologic features unique in hereditary diffuse gastric cancer (HDGC) by comparing with its sporadic counterpart (SDGC). 11 patients with confirmed *CDH1* mutation who were found to have HDGC in a prophylactic total gastrectomy were collected. Median age of HDGC patients was 39 years (range 24-57). All HDGC cases had intramucosal signet ring cell carcinoma. Twenty-three invasive tumor foci from 7 patients with HDGC were available for ancillary studies, and we evaluated each focus separately. Almost all foci (20/23) showed two distinct tumor cell populations, namely, large signet ring cells and small signet ring cells. The large cells were located just beneath the surface epithelium and were positive for mucicarmine and pCEA, while the small cells were found in the deeper lamina propria and were mostly negative for mucicarmine and pCEA. A subset of small cells (6 foci from two resected stomachs) showed poorly differentiated morphology with p16 positivity. All other tumor cells with well-differentiated signet ring cell morphology were negative for p16. In contrast, 18 of 20 SDGCs were positive for p16. In addition, all HDGCs were negative for CDX2, while 19 of 20 SDGCs were positive. We propose that there are three distinct tumor cell populations in HDGC: well-differentiated large cells, well-differentiated small cells, and poorly differentiated small cells, and that the latter group with aberrant p16 expression may represents a more aggressive phenotype. The absence of CDX2 in HDGC suggests that it may develop along a carcinogenetic pathway different from that of SDGC.

1. Introduction

Gastric cancer is the 5th most common cancer worldwide and the 3rd leading cause of cancer-related death [1]. Approximately 10% of gastric cancers have familial segregation and 1%-3% are linked to an inherited cancer predisposition syndrome, with hereditary diffuse gastric cancer (HDGC), familial intestinal gastric cancer (FIGC), and gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) being the best defined [2-5]. Other hereditary syndromes with increased risk for gastric cancer include Lynch syndrome, Li-Faumeni syndrome, familial adenomatous syndrome, Peutz-Jeghers syndrome, hereditary breast and ovarian cancer, MUTYH-associated adenomatous polyposis, juvenile polyposis syndrome, and PTEN hamartoma tumor syndrome (Cowden syndrome) [3-5].

☆ Disclosures: The authors have no conflict of interest or funding to disclose.

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https://doi.org/10.1016/j.humpath.2017.12.023
0046-8177/

HDGC is an autosomal-dominant cancer-susceptibility syndrome characterized by signet ring cell (diffuse) gastric carcinoma and invasive lobular breast carcinoma [6]. *CDH1* germline mutation was first identified as a cause of HDGC in a Maori kindred in 1998 [7], and now it is believed that 30%-40% of HDGC cases are linked to *CDH1* germline mutations. (*CTNNA1* was recently identified as another gene involved in HDGC predisposition in 2013) [8,9].

The International Gastric Cancer Linkage Consortium (IGCLC) proposed clinical criteria for investigating the possibility of *CDH1* germline mutations: (1) two or more documented cases of gastric cancer in first/second degree relatives regardless of age, with at least one confirmed diffuse gastric cancer; (2) diffuse gastric cancer before the age of 40 years without a family history; or (3) families with diagnoses of both diffuse gastric cancer and lobular breast carcinoma, at least one before the age of 50 years [5]. A germline *CDH1* mutation is known to be highly penetrant. The cumulative risk of diffuse gastric cancer for *CDH1* mutation carriers by age 80 is estimated to be 70% for men and 56% for woman [4,9]. Given the high penetrance, comprehensive gastric cancer screening protocols for known *CDH1* carriers, including annual endoscopic surveillance, are recommended by the IGCLC. Even these intensive programs, however, fail to identify the intramucosal carcinomas discovered in many *CDH1* carriers submitted to prophylactic gastrectomy, meaning that prophylactic gastrectomy remains a valid consideration in this setting [3,10-13]. Understandably, some of these generally young and asymptomatic mutation carriers elect to delay or defer surgery [5].

Most prophylactic total gastrectomies from asymptomatic carriers of *CDH1* mutations often harbor multifocal intramucosal signet ring cell carcinomas [6]. While these tumors are invasive carcinoma by definition, they may remain indolent for a long time, and there has been no report of distant metastasis present at the time of gastrectomy [4,6]. Unanswered questions include how long early lesions of HDGC remain indolent, which tumors progress to an aggressive phenotype, and can that progression be predicted [4,6]. The answers to these questions could help with treatment decisions, especially among patients who elect to delay surgery. The aim of this study was to elucidate molecular mechanisms of underlying disease progression of HDGC by comparing HDGC and sporadic diffuse gastric cancer (SDGC).

2. Materials and methods

2.1. Patients

Twenty-three HDGC patients who underwent prophylactic total gastrectomy were identified in the Surgical Pathology archives (2000-2016) of the Mayo Clinic, Rochester, MN, and 11 patients were found to have HDGC in the resected specimens. All but one underwent genetic testing, which confirmed a germline *CDH1* (pathogenic) mutation, and the remaining patient was an obligate carrier since the mutation was found in her sister as well as her daughter. The specific *CDH1* mutations are listed in Table 1. A control group of 20 patients with SDGC who did not fulfill the criteria of HDGC syndrome [14] included 13 patients with total gastrectomy and 7 with partial gastrectomy. Clinical information was obtained from the medical record and pathology reports. The study was approved by the Mayo Clinic Rochester Institutional Review Board.

2.2. Pathologic examination

The total gastrectomy specimens from 11 HDGC were submitted in their entirety for morphological examination. Hematoxylin and eosin (H&E)–stained slides of stomach were reviewed by three pathologists (H.E.L., T.C.S., and L.Z.). When tumor was present, the number of tumor foci was recorded. Each invasive tumor focus was evaluated separately for presence or absence of "large (signet ring) cells" and/or "small (signet ring) cells" based on morphology of tumor cells in the cases of HDGC. "Large cells" were defined as signet ring cells with abundant mucinous cytoplasm, low N/C ratio, eccentrically located and flattened nuclei with mild atypia. "Small cells" were signet ring cells with less abundant mucin, more round, hyperchromatic and atypical nuclei, and high N/C ratio. Signet ring cell carcinoma in situ was also carefully sought in all sections.

Tumor size, invasion depth of tumor, lymph node metastasis, and distant metastasis, if available, were evaluated in the cases of HDGC and SDGC. pTNM (pathologic Tumor-Node-Metastasis) stage was determined using the 7th UICC/AJCC manual [15]. All available tumor blocks were stained with mucicarmine.

2.3. Immunohistochemistry

Immunohistochemistry (IHC) was performed on paraffin-embedded formalin-fixed tissue, cut at 4 microns, using antibodies against pCEA (Dako Glostrup, Denmark, Polyclonal, catalog #A0115, 1/2000), CDX2 (Cell Marque, clone EPR2764Y, catalog #235R-16, 1/200), and p16 (INK4a/CDKN2A; Ventana Tucson, AZ, clone E6H4TM, catalog #725-4713, predilute). All stains used the following protocol, using a Ventana BenchMark XT stainer. Pretreatment with Cell Conditioner 1 (EDTA) MILD was followed by a primary antibody incubation time of 32 minutes at 37°C and Ventana UltraView detection with Ventana DAB, which was followed by Ventana Hematoxylin II and Ventana Bluing Reagent.

Immunohistochemical staining was evaluated by two pathologists (H.E.L. and L.Z.) independently. Nuclear staining for CDX2 and cytoplasmic staining for pCEA were regarded positive respectively. For p16, it was regarded positive when tumor cells showed positive staining in both nucleus and cytoplasm. In the HDGC group, staining patterns were recorded