Human Pathology (2013) **44**, 2438–2448

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Human PATHOLOGY

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Original contribution

Alteration in the Wnt/β-catenin signaling pathway in gastric neoplasias of fundic gland (chief cell predominant) type☆,☆☆

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Received 19 February 2013; revised 4 June 2013; accepted 12 June 2013

Keywords:
Fundic gland/chief cell differentiation;
Wnt/β-Catenin;
AXIN;
APC;
Adenoma;
Adenocarcinoma;
Stomach

Summary Gastric neoplasia of chief cell–predominant type (GN-CCP) has been reported as a new, rare variant of gastric tumor. GN-CCPs were defined as tumors consisting of irregular anastomosing glands of columnar cells mimicking chief cells of fundic gland with nuclear atypia and prolapse-type submucosal involvement. We comparatively evaluated clinicopathologic features between 31 GN-CCPs and 130 cases of conventional gastric adenocarcinoma invading into submucosa (CGA-SM) in additon to nuclear β-catenin immunolabeling and direct sequencing of members of the Wnt/β-catenin pathway, *CTNNB1*, *APC*, and *AXIN*, in a subset of these tumors. GN-CCP presented as small protruded lesions located in the upper third of the stomach, with minimal involvement into the submucosa and rare lymphovascular invasion. None of the lesions have demonstrated a recurrence of disease or metastasis on follow-up. Nuclear β-catenin immunolabeling was higher in GN-CCP (labeling index [LI]: median, 19.3%; high expresser [LI >30%], 7/27 cases [26%]) than CGA-SM (median LI, 14.7%; high expresser, 1/19 cases [6%]). Missense mutation of *APC* was observed in 1 GN-CCP but not CGA-SM. Missense or nonsense mutations of *CTNNB1* and *AXIN1* were higher in GN-CCPs (14.8%, both) than CGA-SMs (5.3%, both). Missense mutations of *AXIN2* were higher in GN-CCPs (25.9%) than in CGA-SMs (10.5%). Overall, 14 (51.9%) of 27 GN-CCPs and 5 (26.3%) of 19 CGA-SM cases harbored at least 1 of these gene mutations. In conclusion, GN-CCPs as a unique variant of nonaggressive tumor are characterized by nuclear β-catenin accumulation and mutation of *CTNNB1* or *AXIN* gene, suggesting activation of the Wnt/β-catenin pathway.

☆ The work was supported, in part, by a Grant-in-Aid from the Japan Society for the Promotion of Science (No. 24590429 to H. Mitomi and No. 23590434 to T. Saito) and by a Grant-in-Aid from the Juntendo University School of Medicine for Project Research (No. 2419 to Y. Hidaka).
☆☆ Disclosure/conflict of interest: The authors declare no conflict of interest.
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http://dx.doi.org/10.1016/j.humpath.2013.06.002

1. Introduction

Recently, our group [1] and others [2-4] described a new histologic variant of gastric cancer termed *gastric adenocarcinoma of chief cell–predominant type*. Most of this type of tumor present as solitary, small (mostly <10 mm), and well-circumscribed lesions extending into the submucosa in the upper third of the stomach. Histologically, they are composed of tightly packed glands and cords of predominantly chief cells with anisonucleosis, lacking chronic gastritis, atrophic change, or intestinal metaplasia in their surrounding mucosa. On immunohistochemical analysis, these tumors have been found to be diffusely positive for MUC6 and pepsinogen-I, sparsely positive for H+/K+-ATPase and negative for MUC2 with low Ki-67 labeling and no p53 overexpression [1-4]. Although submucosal involvement was noted in previous reports [1-4], as thin wisps of muscluaris mucosae with the absence of desmoplasia were identified throughout the lesions, Singhi et al [3] suggested that these changes represent prolapse-type changes into the submucosa rather than more typical submucosal invasion from a malignant neoplasm. In all cases reported to date, none of the lesions have demonstrated a recurrence of disease or metastasis on follow-up [1,3]. Considering their benign outcome and the lack of definitive features of invasive growth or lymphovascular invasion, they termed this lesion as *oxyntic gland polyp/adenoma*. Chen et al [4] reported a case of this type of tumor that extended into the submucosa with mild stromal desmoplasia and suggested the continued use of the original term *gastric adenocarcinoma of chief cell–predominant type*, with a clear understanding that this lesion is quite unlike the usual types of gastric adenocarcinoma and has an excellent prognosis. In fact, at present, it is uncertain whether they are benign (adenoma) or malignant (adenocarcinoma). We therefore tentatively use the term *gastric neoplasia of chief cell–predominant type* (GN-CCP) in the present study.

Fundic gland polyps, small, benign mucosal polyps arising in the gastric body and fundus, are considered a benign counterpart of GN-CCP. They are composed of dilated glands lined by mixtures of chief, parietal, and mucous neck cells without cytologic atypia. Albeit rare, dysplasia or microadenocarcinoma in surface areas can occur within fundic gland polyps [5,6], but their histologic features are quite different from GN-CCP [1-4]. Fundic gland polyps have activating mutations of cadherin-associated protein, beta 1 (*CTNNB1*; the gene that encodes for β-catenin) [7,8], and those with dysplasia are characterized by nuclear β-catenin accumulation [5] and adenomatous polyposis coli (*APC*) alterations [9,10]. These facts suggest that activation of the Wnt/β-catenin signaling pathway is involved in the pathogenesis and malignant transformation of these polyps.

β-Catenin in a resting state is degraded by proteasomes resulting from its phosphorylation by a multiprotein complex containing APC, axis inhibition protein (AXIN), glycogen synthase kinase 3β (GSK3β), and protein phosphatase 2A (PP2A). When Wnt binds to the cell surface receptor Frizzled and activates disheveled, GSK3β is dissociated from their complex. As a result, free β-catenin accumulates and translocates into the nucleus and subsequently binds to T-cell factor, initiating transcription of its target genes such as myelocytomatosis viral oncogene homolog (*c-myc*) and *cyclin D1*, which may be relevant for tumor development [11,12]. Associated with this signal pathway, *CTNNB1* mutations linked with nuclear β-catenin accumulation have been investigated in gastric cancer and reported to be less frequent than expected from its nuclear expression [13-16]. Mutations of *APC* occurred more commonly in gastric adenoma as compared with well-differentiated type of adenocarcinoma, but this mutation is rare in poorly differentiated types [17-20]. However, *AXIN1* and *AXIN2*, other key components in the Wnt/β-catenin signal pathway, have not been fully investigated in gastric cancer [21,22].

Herein we first report a series of 31 cases (10 cases from our previous report [1] and an additional 21 cases) of GN-CCP, compared with 130 cases of conventional gastric adenocarcinoma invading into submucosa (CGA-SM), focusing on clinicopathologic features. We next comparatively evaluated immunoreactivity of nuclear β-catenin and subsequently performed a mutational analysis of the members of the Wnt/β-catenin signal pathway, *CTNNB1*, *APC*, and *AXIN*, in subsets of GN-CCP and CGA-SM. This article documents for the first time a molecular analysis of GN-CCPs.

2. Materials and methods

2.1. Patients and materials

The material for our study was provided by 31 GN-CCPs (from 31 patients) resected endoscopically (n = 24) or surgically (n = 7) at Juntendo University Hospital and our affiliated hospitals between 2004 and 2011. In Juntendo University Hospital, there were 6 GN-CCPs (1.6%) of 373 cases of gastric cancer between 2009 and 2011. In accordance with our previous report [1], GN-CCPs were defined as tumors consisting of irregular anastomosing glands or cords of columnar cells mimicking fundic gland cells (mainly chief cells) with mild nuclear atypia and submucosal involvement (Figs. 1A and B and 2A and B). Without exception, these tumors were positive for MUC6 (Fig. 1C) and pepsinogen-I (Fig. 1D) immunostaining (in some cases, also focally positive for the H+/K+-ATPase α subunit) and negative for MUC2 and CD10. For comparison, 130 cases (from 130 patients) of CGA-SM (differentiated type of adenocarcinoma invading into the submucosa) were randomly selected from pathologic files of Juntendo