Gastroenterology Report, 9(4), 2021, 370–373

doi: 10.1093/gastro/goaa061
Advance Access Publication Date: 10 December 2020
Brief Report

BRIEF REPORT

Pyloric-gland metaplasia may be an origin of cancer and intestinal metaplasia with possible CDX2 expression

Kazuyoshi Yagi[1], Atsunori Tsuchiya[2,*], Satoru Hashimoto[2], Taisuke Kato[3], Osamu Onodera[4] and Shuji Terai[2]

[1]Department of Gastroenterology and Hepatology, Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan; [2]Division of Gastroenterology and Hepatology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan; [3]Department of System Pathology for Neurological Disorders, Brain Science Branch, Brain Research Institute, Niigata University, Niigata, Japan; [4]Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan

*Corresponding author. Niigata University, 1-757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan. Tel: +81-25-227-2207; Fax: +81-25-227-0776; Email: atsunori@med.niigata-u.ac.jp

Introduction

The majority of cases of gastric cancer are thought to develop due to chronic inflammation from *Helicobacter pylori* (*H. pylori*) infection leading to intestinal metaplasia, termed the intestinal metaplasia–dysplasia–cancer sequence [1]. Chronic inflammation from *H. pylori* infection causes mucin core protein 6-positive (MUC6+) pyloric-gland metaplasia; however, its origin is controversial. This MUC6+ pyloric-gland metaplasia is similar to spasmolytic polypeptide-expressing metaplasia in mouse studies [2–4]. We hypothesized that intestinal metaplasia and cancer develop from MUC6+ pyloric-gland metaplasia in humans. To test this hypothesis and demonstrate the relationship between pyloric-gland metaplasia, intestinal metaplasia, and cancer, we focused on the expression pattern of caudal-related homeobox protein 2 (CDX2), which is indispensable for MUC6+ cells to develop into intestinal metaplasia [5], and we analysed the relationship between MUC6 and CDX2. Furthermore, we investigated the commonalities and differences in gene variation of pyloric-gland metaplasia cells, intestinal metaplasia, and cancerous cells by hierarchical cluster analysis calculated from the data obtained during analysis of cancer panel genes.

Methods

Immunohistochemistry and microdissection

Forty-two human resected gastric-cancer tissues arising from chronic *H. pylori* infection were used for immunohistochemistry; three were used for DNA analysis after microdissection. Baseline characteristics of the patients are shown in Supplementary Table 1. The tissues were formalin-fixed and cut into 4-μm-thick sections for immunostaining and 10-μm-thick sections for microdissection. The antibodies used are listed in Supplementary Table 2. The pyloric-gland metaplasia, intestinal metaplasia, and cancers were selectively cut using Leica LMD6500 and LMD7000 systems (Leica, Wetzlar, Germany). This study was approved by the Ethical Committee of Niigata University (Japan).

Submitted: 29 May 2020; **Revised:** 7 August 2020; **Accepted:** 18 August 2020

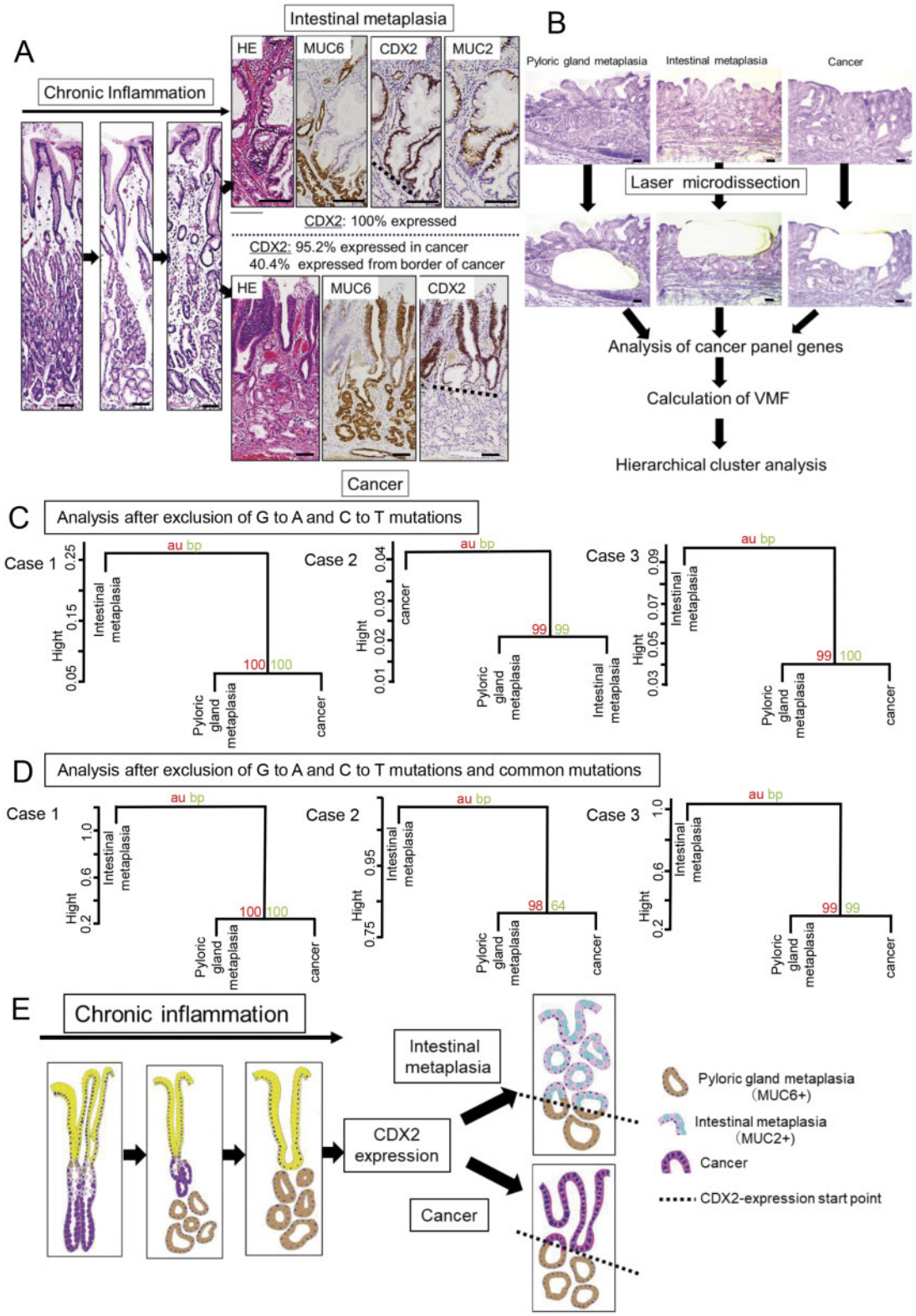

Figure 1. Immunohistochemistry, laser microdissection, hierarchical clustering analysis, and our model of cancer progression. (A) Time course of development for intestinal metaplasia and cancer during chronic inflammation. During the development of intestinal metaplasia, MUC6+ cells develop into MUC2+ cells that express CDX-2. In the cancers, 95.2% of cases express CDX2 and 40.4% of cases have CDX2 expression from the border of the cancer. Dotted lines are the start points of CDX2 expression during the progression of intestinal metaplasia (upper panel) and cancer (lowed panel). Scale bar = 100 μm. (B) Flow chart of DNA collection and analysis from laser microdissection to hierarchical cluster analysis. (C) Hierarchical cluster analysis using VMF data after exclusion of G-to-A and C-to-T mutations and (D) after exclusion of G-to-A and C-to-T mutations and other common mutations. (E) Potential scheme of cancer development seen in some gastric cancers during chronic inflammation caused by *Helicobacter pylori*. CDX2, caudal-related homeobox protein 2; HE, Hematoxylin and Eosin; MUC2, mucin core protein 2; MUC6, mucin core protein 6; VMI, variant unique molecular indexes level allele fraction.

DNA extraction

DNA was extracted from dissected tissues using the QIAamp® DNA FFPE Tissue kit (QIAGEN, Hilden, Germany). The concentration of each DNA sample was assessed using QIAseq DNA QuantiMIZE Kits (QIAGEN).

Cancer panel analysis

DNA libraries were prepared using the QIAseq Targeted DNA Panel Kit (QIAGEN) according to the manufacturer's instructions. Briefly, 250 ng of sample DNA was fragmented, and end-repair and A-addition were performed. A 5′-end adapter, which contains a sample-specific index and UMIs (unique molecular indexes), was ligated. After purification, target enrichment was performed using the 'Human Comprehensive Cancer Panel' (QIAGEN; Cat No. DHS-3501Z, total number of primers: 11,311; panel size: 836,670 bp). Enriched samples were purified and final universal polymerase chain reaction was performed. The libraries were sequenced on a HiSeq sequencer (Illumina, San Diego, CA, USA) to generate 150-nt paired-end reads. Duplicated reads were removed with Clumpify of BBTools (ver. 38.79) and deduplicated reads were analysed at the GeneGlobe Data Analysis Center. Hierarchical cluster analysis of variant patterns of samples was performed with pvclust (ver. 2.0_0) [6] using VMF (variant UMI level allele fraction) values as input data. G-to-A and C-to-T mutations were excluded to remove the bias from formalin fixation. To further select mutations specific to each sample, in a second analysis, common mutations were excluded by removing variants with VMF values in all samples.

Results

CDX2 expression is the branch point of cancer and intestinal metaplasia from pyloric-gland metaplasia in some cancers

In our 42 gastric-cancer cases, the histological background of cancer was pyloric-gland metaplasia rather than intestinal metaplasia (Figure 1A). Therefore, to analyse the relationship between pyloric-gland metaplasia, intestinal metaplasia, and cancers, we performed immunostaining of the tissues for mucin core protein 2 (MUC2), MUC6, and CDX2. Pyloric-gland-metaplasia cells were MUC6+; therefore, the continuity between pyloric-gland metaplasia and intestinal metaplasia, as well as pyloric-gland metaplasia and cancer, was analysed. With intestinal metaplasia, MUC6+ cells were converted into MUC2+ cells. In all cases, CDX2 was expressed from the border between MUC6+ and MUC2+ cells (Figure 1A). Of the 42 cancers, 40 (95.2%) were CDX2+, 31 cancers (73.8%) were MUC6+, and 17 cancers (40.4%) were CDX2+ from the border between cancerous cells and pyloric-gland-metaplasia cells (Figure 1A). This suggests that, in some cancers, CDX2 expression is the branch point of cancer and intestinal metaplasia from pyloric-gland metaplasia.

Gastric cancer may not follow the metaplasia–dysplasia–cancer sequence

DNA extracted from the pyloric-gland metaplasia, intestinal metaplasia, and cancers was analysed; 270 genes were examined and we obtained VMF data for hierarchical cluster analysis (Figure 1B). We performed the analysis in two ways. First, we excluded G-to-A and C-to-T mutations and analysed data using VMF to form a dendrogram. In two of three cases, the pyloric-gland metaplasia and cancer had a close relationship, whereas the intestinal metaplasia and cancer were distantly related. In one of three cases, the pyloric-gland metaplasia and the intestinal metaplasia were closely related, suggesting that this cancer followed the metaplasia–dysplasia–cancer sequence (Figure 1C). Then, we excluded other common mutations between all samples. The three dendrograms showed that the pyloric-gland metaplasia and cancer were closely related genetically, and the intestinal metaplasia and cancer were distantly related, suggesting that these cancers did not follow the metaplasia–dysplasia–cancer sequence (Figure 1D).

Discussion

Findings of research on MUC6, MUC5AC, and MUC2 in gastric cancer have been reported; however, most of these studies are on the prognosis [7], not occurrence, of gastric cancer. Goldenring suggested the possibility of spasmolytic polypeptide-expressing metaplasia being involved in the occurrence of gastric cancer together with intestinal metaplasia [2]. Our results are identical to theirs.

Although the mechanism driving CDX2 expression is unknown, in some gastric cancers, CDX2 expression is the branch point of the cancer and intestinal metaplasia from the pyloric-gland metaplasia (Figure 1E). Cancer panel analysis also supported this idea: in two of three cases, the intestinal metaplasia and the cancer were distantly related, suggesting that the cancers did not follow the metaplasia–dysplasia–cancer sequence. CDX2 is an essential transcription factor for intestinal cells and for the activation of stemness genes. Interestingly, genomic binding sites for CDX2 include 5′-flanking regions of SALL4 and KLF5 [8].

It is difficult to determine the deviation from the classic metaplasia–dysplasia–cancer sequence without detailed analysis such as hierarchical cluster analysis in humans. Our concept, by which cancer and intestinal metaplasia develop through the expression of CDX2, is consistent with intestinal metaplasia being associated with a high risk of developing cancer in the stomach [9]. However, some cancers develop following the intestinal metaplasia–dysplasia–cancer sequence; therefore, the development of gastric cancer is not uniform.

Supplementary Data

Supplementary data is available at *Gastroenterology Report* online.

Author's contributions

K.Y., A.T., and S.H.: conception and design of the study; generation, collection, analysis, and interpretation of data; and writing the manuscript. T.K., O.O., and S.T.: conception, design, and supervision of the study. All authors read and approved the final paper.

Funding

This research was supported by a Grant-in-Aid for Scientific Research (C) [19K08389] from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Conflicts of interest

None declared.

References

1. Correa P. A human model of gastric carcinogenesis. *Cancer Res* 1988;**48**:3554–60.
2. Goldenring JR, Nam KT, Wang TC *et al*. Spasmolytic polypeptide-expressing metaplasia and intestinal metaplasia: time for reevaluation of metaplasias and the origins of gastric cancer. *Gastroenterology* 2010;**138**:2207–10, 221. e1.
3. Yoshizawa N, Takenaka Y, Yamaguchi H *et al*. Emergence of spasmolytic polypeptide-expressing metaplasia in Mongolian gerbils infected with Helicobacter pylori. *Lab Invest* 2007;**87**:1265–76.
4. Hoffmann W. TFF2, a MUC6-binding lectin stabilizing the gastric mucus barrier and more [Review]. *Int J Oncol* 2015;**47**: 806–16.
5. Mutoh H, Hakamata Y, Sato K *et al*. Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice. *Biochem Biophys Res Commun* 2002;**294**:470–9.
6. Suzuki R, Shimodaira H. Pvclust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* 2006; **22**:1540–2.
7. Kim DH, Shin N, Kim GH *et al*. Mucin expression in gastric cancer: reappraisal of its of its clinicopathologic and prognostic significance. *Arch Pathol Lab Med* 2013;**137**:1047–53.
8. Fujii Y, Yoshihashi K, Suzuki H *et al*. CDX1 confers intestinal phenotype on gastric epithelial cells via induction of stemness-associated reprogramming factors SALL4 and KLF5. *Proc Natl Acad Sci USA* 2012;**109**:20584–9.
9. Gupta S, Li D, El Serag HB *et al*. AGA Clinical Practice Guidelines on Management of Gastric Intestinal Metaplasia. *Gastroenterology* 2020;**158**:693–702.