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Down regulation of gastric and intestinal phenotypic expression in Epstein-Barr virus-associated stomach cancers

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Summary. Aims: We have previously demonstrated the importance of gastric and intestinal phenotypic expression for stomach carcinogenesis. In this study, we focused on Epstein-Barr virus (EBV)-associated stomach cancers, with special attention to Cdx2.

Methods and Results: We evaluated the expression of gastric and intestinal phenotypic markers by immunohistochemistry in 35 EBV-positive [EBV (+)] and 75 EBV-negative [EBV (-)] stomach cancers in Colombia. The lesions were divided phenotypically into gastric (G), gastric-and-intestinal mixed (GI), intestinal (I), and null (N) phenotypes. In the EBV (+) cases, the lesions were divided phenotypically into 9 G (25.7%), 1 GI (2.9%), 3 I (8.6%), and 22 N (62.9%) types. Similarly, the EBV (-) lesions were also classified phenotypically as 15 G (20.0%), 19 GI (25.3%), 24 I (32.0%), and 17 N (22.7%) types. The proportion of N type EBV (+) lesions was higher than for their EBV (-) counterparts (P<0.0001). The expression of Cdx2 and MUC2 was also found to be significantly lower in EBV (+) than in EBV (-) stomach cancers (P=0.0001; P<0.0001). Cdx2 expression in the intestinal metaplastic glands present in non-neoplastic mucosa surrounding EBV (+) lesions was also significantly lower than in EBV (-) tumors (P=0.016) despite no evidence of EBV infection.

Conclusions. EBV (+) stomach cancers are characterized by low expression of intestinal phenotype markers, including Cdx2, and only occasional gastric phenotypic expression.

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Key words: Stomach cancer, Epstein-Barr virus, N type, Cdx2, MUC2

Introduction

Epstein-Barr virus (EBV) is a ubiquitous human herpes virus implicated in the etiology of many human malignancies, such as Burkitt's lymphoma (zur Hausen et al., 1970), nasopharyngeal carcinoma (Raab-Traub, 1992), Hodgkin's disease (Weiss et al., 1989), lymphoproliferative disorders in immunodeficiency patients (Hanto et al., 1981), and stomach cancer (Fukayama et al., 1998). EBV-associated stomach cancer account for about 10% of all gastric neoplasms (Shibata and Weiss, 1992; Tokunaga et al., 1993), although *Helicobacter pylori* (*H. pylor*i) infection is a more important factor for stomach carcinogenesis. There are differences in the proportions of EBV-associated stomach cancers from country to country (Takada, 2000), and the rate in Colombia is significantly higher than in places with heavy gastric cancer burdens, such as Japan, China and Korea (Carrascal et al., 2003). The lesions due to EBV infection resemble nasopharyngeal lymphoepitheliomas and are named lymphoepithelioma-like carcinomas, and specific antigens such as EBV-determined nuclear antigen-1 (EBNA-1) and EBV-encoded small RNA-1 (EBER-1) point to the presence of the virus (Burke et al., 1990; Yanai et al., 1997a,b). Stomach cancers associated with EBV infection were more common in the upper stomach (cardia and fundus), and histologically are most often of undifferentiated type (Yanai et al., 1997). Each EBV-associated stomach cancer appears of monoclonal origin arising from a single EBV-infected cell (Imai et al., 1994). However, there are many obscure points with regard to the

relations between EBV infection and stomach carcinogenesis.

Gastric and intestinal phenotypic expression is important for the histogenesis of stomach cancer (Tatematsu et al., 2003). Several reports have indicated that it is possible to analyze the phenotypic expression of each gastric cancer cell using gastric and intestinal epithelial cell markers (Egashira et al., 1999; Kawachi et al., 2003; Mizoshita et al., 2003; Tsukamoto et al., 2005). Thus, division into gastric (G), gastric-and-intestinal mixed (GI), intestinal (I), and null (N) phenotypes is possible, independent of the histological classification (Tajima et al., 2001; Tatematsu et al., 2003; Inada et al., 2004; Mizoshita et al., 2004a). However, the relation between EBV infection and phenotypic expression has yet to be clarified in detail in stomach cancers associated with the virus. Several authors have demonstrated a correlation between EBV infection and phenotypic marker expression (Lee et al., 2004; Nakamura et al., 2005), but concrete conclusions have yet to be drawn.

In the present study, we therefore evaluated the expression of gastric and intestinal phenotypic markers by immunohistochemistry in 110 stomach cancers in Colombia, along with adjacent non-neoplastic mucosa. The EBV infection status was also evaluated by in situ hybridization in these lesions.

Materials and methods

Samples and tissue collections

The study subjects were stomach carcinoma patients newly diagnosed during the period between September 2000 and June 2003 in the following four reference hospitals in Colombia: Instituto de los Seguros Sociales "Rafael Uribe Uribe", Hospital Universitario del Valle, Hospital San Juan de Dios in Cali, and Instituto Nacional de Cancerologia in Bogota. We examined EBER-1 expression among formalin-fixed paraffin-embedded blocks of 368 cases with gastric carcinomas, and found that 42 cases were positive (Koriyama et al., manuscript submitted). We selected paraffin-embedded blocks of 35 cases with gastric carcinomas, mainly surgically resected tumors, for the present analysis. Seventy-five EBER-1-negative cases were selected matched for gender, age (5-year category), histology [differentiated (well and moderately differentiated) and undifferentiated (poorly differentiated and signet-ring cell) types in majority area], and area (Bogota or Cali) (Table 1). The Institutional Review Board of the Faculty of Health, Universidad del Valle, Cali, Colombia, approved this study and all subjects gave informed consent.

The patient group comprised 84 men and 26 women, aged 59.0±12.5 years (mean ± standard deviation). All specimens were fixed in 10% buffered formalin. Classification was made according to the Japanese Classification of Gastric Carcinomas (Japanese Gastric Cancer Association, 1998) in spite of widely used Lauren's classification (Lauren, 1965), which is inadequate for the studies of histogenesis of stomach cancers and phenotypic expression at the cellular level, because it confuses intestinal phenotypic cancer cells with "diffuse" structure and gastric phenotypes with the "intestinal" (glandular or tubular) morphology. Carcinomas with adjacent non-neoplastic mucosa were serially cut into 5-mm slices in parallel with the lesser curvature and embedded in paraffin, and then sectioned and stained with hematoxylin-eosin (HE) for histological examination.

In situ hybridization of EBER-1

EBER-1 *in situ* hybridization was performed with a kit according to the manufacturer's instructions (Dako, Glostrup, Denmark). Paraffin sections 4 μm thick were deparaffinized, rehydrated, predigested with proteinase K for 15 min at room temperature and hybridized with a fluorescein-conjugated EBV oligonucleotide probe (EBER PNA Probe/Fluorescein) for 90 min at 55°C. After washing with 0.1M TBS (pH 10) for 25 min at 55°C, hybridization signals were detected by serial incubation with anti-fluorescein isothiocyanate rabbit polyclonal antibody (Anti-FITC/AP), and then with biotinylated Mouse IgG as secondary antibody, followed by the avidin biotinylated horseradish peroxidase complex (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, USA). Finally, immune complexes were visualized by incubation with 0.01% H_2O_2 and 0.05% 3,3'-diaminobenzidine tetrachloride (DAB). Nuclear counterstaining was accomplished with Mayer's hematoxylin. From the results, EBER-positive and EBER-negative lesions were defined as EBV-positive [EBV (+)] and EBV-negative [EBV (-)](Fukayama et al., 2001).

Histological and immunohistochemical examination

Immunohistochemical staining was carried out with monoclonal antibodies against the following antigens:

Table 1. Correlations between clinicopathologic findings and EBV infection in 110 stomach cancers.

Clinicopathologic findings	EBV (+) (n=35)	EBV (-) (n=75)	P-value
Age			
Years (mean±SD)	58.9±13.6	59.1±12.0	P=0.88
Sex			
Male(n=84)	28	56	P=0.63
Female(n=26)	7	19	
Histological classification [a]			
Differentiated type (n=44)	13	31	P=0.835
Undifferentiated type (n=66)	22	44	

SD: standard deviation. [a]: Classified based on structure of elements. "Differentiated type" includes tubular and papillary types, whereas "Undifferentiated type" consists of signet-ring cell and poorly differentiated types.

MUC5AC (CLH2, 1:500; Novocastra Laboratories, Newcastle upon Tyne, UK); MUC6 (CLH5, 1:500; Novocastra Laboratories); MUC2 (Ccp58, 1:500; Novocastra Laboratories); villin (12, 1:20,000; Transduction Laboratories, Lexington, KY, USA); and Cdx2 (Caudal-related homeobox gene 2) (CDX2-88, 1:100; BioGenex, San Ramon, CA, USA).

For gastric and intestinal phenotypic markers, we used normal gastric mucosa and ileum as controls. The precise procedures for immunohistochemical techniques were as previously described (Tatematsu et al., 2003; Mizoshita et al., 2003, 2004b; Tsukamoto et al., 2005). Briefly, 4 μm-thick consecutive sections were deparaffinized and hydrated through a graded series of alcohols. After inhibition of endogenous peroxidase activity by immersion in 3% H_2O_2/methanol solution, antigen retrieval was conducted for detection of binding of the above-mentioned antibodies with 10 mM citrate buffer, pH 6.0, in a microwave oven for 10 min at 98°C. Sections were incubated with primary antibodies, thoroughly washed in phosphate-buffered saline (PBS), then incubated with biotinylated secondary antibody, followed by the avidin biotinylated horseradish peroxidase complex (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, USA). Finally, immune complexes were visualized by incubation with 0.01% H_2O_2 and 0.05% DAB. Nuclear counterstaining was accomplished with Mayer's hematoxylin.

Three independent pathologists (N.H., T.M., and T.T.) judged the histology and immunohistochemical staining for the phenotypic markers and Cdx2. Reactivity for the phenotypic markers and Cdx2 was scored according to the percentage of positively stained tumor cells in the section areas on a 4-point-scale: score 0, <10%; score 1, 10-33%; score 2, 34-66%; score 3, 67-100%. A result was considered positive (+) with a score of 1 or more.

Phenotypic classification of cancers

The phenotypes of stomach cancer cells were determined using two gastric (MUC5AC and MUC6) and two intestinal (villin and MUC2) phenotypic markers. The decisions as to the phenotypes of stomach cancerous areas in which 10% or more of the section area consisted of at least one gastric or intestinal epithelial cell phenotype were classified as gastric (G type) or intestinal (I type) phenotype cancers, respectively. Those which showed both gastric and intestinal phenotypes were classified as gastric and intestinal mixed phenotype (GI type) cancers, while those showing neither gastric nor intestinal phenotype expression were grouped as unclassified (N type) (Tatematsu et al., 2003; Mizoshita et al., 2003; Tsukamoto et al., 2005).

Evaluation of the background gastritis of stomach cancer

Inflammatory response in non-neoplastic surrounding mucosa [of 26 EBV (+) and 57 EBV (-) stomach cancers] were scored according to the Updated Sydney System (Dixon et al., 1996). The degree of gastric mucosal inflammation including mononuclear cell infiltration, neutrophils infiltration, glandular atrophy, and intestinal metaplasia were classified into four grades as follows: 0 = none, 1 = mild, 2 = moderate and 3 = marked.

Expression of gastric and intestinal phenotypic markers and Cdx2 in intestinal metaplastic glands in non-neoplastic surrounding mucosa of EBV (+) and EBV (-) stomach cancers

Intestinal metaplastic glands were observed in non-neoplastic surrounding mucosa of 9 EBV (+) and 26 EBV (-) stomach cancers. The expression of gastric and intestinal phenotypic markers and Cdx2 was also evaluated in intestinal metaplastic glands of both EBV (+) and EBV (-) cases (Mizoshita et al., 2004b, Tatematsu et al., 2005). Reactivity for the phenotypic markers and Cdx2 was scored according to the percentage of positively stained epithelial cells in the intestinal metaplastic glands on a 4-point-scale: score 0, <10%; score 1, 10-33%; score 2, 34-66%; score 3, 67-100%.

Statistical analysis

The data were analyzed by the Fisher's exact test, c2 test or Mann-Whitney U test for differences between EBV (+) and EBV (-) groups. P-values <0.05 were considered as statistically significant.

Results

Relations between EBV infection and expression of gastric and intestinal phenotypic markers, and Cdx2, in stomach cancers

Data for comparisons between EBV (+) and EBV (-) lesions for phenotypic marker and Cdx2 expression in cancerous tissues are summarized in Table 2. The average scores for MUC2 and Cdx2 expression were significantly lower in EBV (+) than in EBV (-) cases ($P<0.0001$ and $P=0.0001$, respectively), independently of whether differentiated ($P<0.005$ and $P<0.02$, respectively) or undifferentiated ($P<0.01$ and $P<0.005$, respectively). Regarding the other phenotypic markers, there were no significant differences between the two groups.

Comparison of phenotypes between EBV (+) and EBV (-) stomach cancers

Data for comparisons between EBV (+) and EBV (-) lesions are summarized in Table 3. In the EBV (+) cases, the lesions were divided phenotypically into 9 G (25.7%), 1 GI (2.9%), 3 I (8.6%), and 22 N (62.9%) types. Similarly, the EBV (-) lesions were also classified phenotypically as 15 G (20.0%), 19 GI (25.3%), 24 I

(32.0%), and 17 N (22.7%) types. There was a significant difference in the proportions of each phenotype between EBV (+) and EBV (-) lesions (P<0.0001).

Comparison of phenotypic markers in differentiated and undifferentiated regions in EBV (+) and EBV (-) stomach cancer cases

To further analyze the expression of gastric and intestinal phenotypic markers, the phenotypes were compared in mixed structure cases containing differentiated and undifferentiated regions (Table 4). Six EBV (+) cases consisted of 2 adenocarcinomas with differentiated predominance and 4 tumors with larger undifferentiated areas. Among them, 3 cases lacked the phenotypic markers in the undifferentiated regions (3/6=50%). For EBV (-) cases, 2 cases were differentiated region dominant and 7 were undifferentiated predominant, none of them lost the

Table 2. Correlations between EBV infection and the expression of the phenotypic markers, and Cdx2 in the stomach cancer cases.

	The average scores of each marker[a]				
	MUC5AC	MUC6	MUC2	villin	Cdx2
EBV (+) (n=35)	0.51±0.16	0.029±0.029	0.057±0.040	0.086±0.063	0.20±0.099
Differentiated (n=13)	0.615±0.266	0.077±0.077	0.077±0.077	0.231±0.166	0.231±0.166
Undifferentiated (n=22)	0.455±0.194	0±0	0.045±0.045	0±0	0.182±0.125
EBV (-) (n=75)	1.013±0.15	0.16±0.063	1.033±0.13	0.23±0.070	1.060±0.13
Differentiated (n=31)	1.000±0.2236	0.226±0.101	0.903±0.169	0.484±0.153	1.355±0.2
Undifferentiated (n=44)	1.023±0.191	0.114±0.081	1.125±0.166	0.045±0.032	0.852±0.156
P-values between EBV (+) and (-) cases [b]	P= 0.098	P= 0.58	P< 0.0001	P= 0.39	P= 0.0001
P-values between EBV (+) and (-) differentiated adenocarcinomas	NS	NS	P< 0.005	NS	P< 0.02
P-values between EBV (+) and (-) undifferentiated adenocarcinomas	NS	NS	P< 0.01	NS	P< 0.005

[a]: Each score is average ± standard error (SE); [b]: Each P-value is analyzed by Mann-Whitney U test. NS, not significant.

Table 3. The phenotype classification in EBV (+) and EBV (-) stomach cancers.

	Phenotypic classification[a]				
	G type	GI type	I type	N type	total
EBV (+) (n=35)	9 (25.7%)	1 (2.9%)	3 (8.6%)	22 (62.9%)	35 (100%)
Differentiated	3	1	2	7	13
Undifferentiated	6	0	1	15	22
EBV (-) (n=75)	15 (20.0%)	19 (25.3%)	24 (32.0%)	17 (22.7%)	75 (100%)
Differentiated	4	10	11	6	31
Undifferentiated	11	9	13	11	44
Total	24 (21.8%)	20 (18.2%)	27 (24.5%)	39 (35.5%)	110 (100%)

[a]: P< 0.0001 among G, GI, I , and N types between EBV (+) and (-) cases (χ^2 test).

Table 4. Correlation between EBV infection and the expression of the phenotypic markers, and Cdx2 in intestinal metaplasia.

	The average scores of each marker[a]				
	MUC5AC	MUC6	MUC2	villin	Cdx2
EBV (+) (n=9)	1.000±0.441	0	2.333±0.441	2.286±0.421	0.556±0.377
EBV (-) (n=26)	1.769±0.256	0.231±0.139	2.808±0.136	2.350±0.244	1.654±0.192
P-value[b]	P=0.15	P=0.61	P=0.50	P=0.80	P=0.016

[a]: Each score is average±standard error (SE); [b]: Each P-value is analyzed by Mann-Whitney U test.

phenotypic markers. Thus, EBV (+) carcinomas appeared to lose phenotypic markers during progression from differentiated to undifferentiated structure (P<0.02).

Relations between EBV infection and grading of gastritis surrounding non-neoplastic mucosa

Data for comparisons between EBV (+) and EBV (-) cases regarding the grade of gastritis surrounding non-neoplastic mucosa using Updated Sydney System are summarized in Table 5. The grades of mononuclear cell and neutrophil infiltration, mucosal glandular atrophy, and intestinal metaplasia showed no significant difference between the two groups.

Relations between EBV infection and expression of gastric and intestinal phenotypic markers, and Cdx2 in intestinal metaplastic glands

Data for comparisons between EBV (+) and EBV (-) cases regarding phenotypic marker and Cdx2 expression in intestinal metaplastic glands are summarized in Table 6 (Fig. 3). The average score for Cdx2 expression was significantly lower in EBV (+) than in EBV (-) cases (P=0.016). Regarding the other phenotypic markers, there were no significant differences between the two groups.

Discussion

Cdx2 is important for the maintenance of intestinal phenotypic expression not only in the normal small and large intestine (Silberg et al., 2000), but also in intestinal metaplasia (Mizoshita et al., 2001; Almeida et al., 2003; Tsukamoto et al., 2004) and carcinomas of the stomach (Almeida et al., 2003; Mizoshita et al., 2003). Cdx2 nuclear expression can be detected in approximately half of advanced stomach cancers (Mizoshita et al., 2003) and about 80% of early lesions (Mizoshita et al., 2004a,b). Many stomach cancers have expression of genes associated with induction and maintenance of the differentiation of small and large intestine, such as Cdx2 and Cdx1 (Chen et al., 2003). However, our present data provide clear evidence that Cdx2 expression is less frequent in EBV (+) than in EBV (-) stomach cancers.

Table 5. Correlation between EBV infection and status of surrounding non-neoplastic mucosa.

	The average grades in surrounding mucosa[a]			
	Neutrophils	Mononuclear Cells	Atrophy	Intestinal Metaplasia
EBV (+) stomach cancer (n=26)	1.154±0.107	1.692±0.173	1.154±0.120	0.577±0.173
EBV (-) stomach cancer (n=57)	1.175±0.087	1.474±0.118	1.140±0.088	0.720±0.120
P-values[b]	P=0.914	P=0.324	P=0.879	P=0.504

[a]: Each score is average±standard error (SE) for Updated Sydney System; [b]: Each *P*-value is analyzed by Mann-Whitney U test.

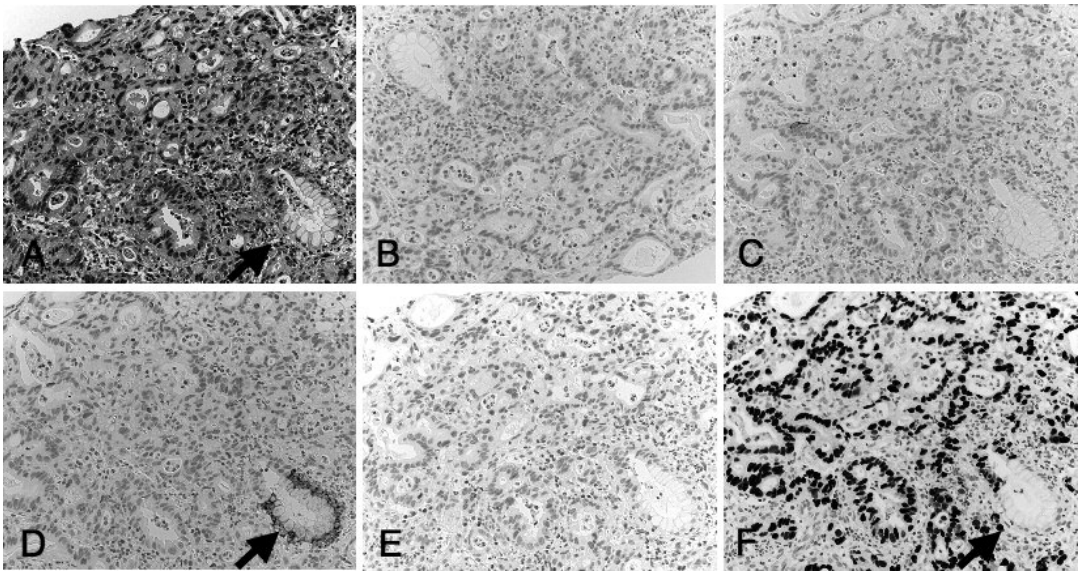

Fig. 1. An EBV (+) stomach cancer. **A.** HE staining. **B.** Note the lack of Cdx2 nuclear staining in the cancer cells. **C.** No MUC2 expression is detected in the cytoplasm of tumor cells. **D.** MUC5AC is present in the cytoplasm of normal gastric foveolar epithelium (red arrow), but not cancer cells. **E.** No MUC6 expression is apparent in the cytoplasm of tumor cells. **F.** EBER-1 is positive in the nuclei of cancer cells, but not normal gastric foveolar epithelium (arrow). x 200; EBER-1, EBV-encoded small RNA-1.

Chen et al. (2003) similarly found expression of intestinal specific genes to be lower in EBV (+) stomach cancers, as compared with EBV (-) lesions. Regarding the regulation of MUC2 expression, Yamamoto et al. (2003) have demonstrated that Cdx2 interacts with the MUC2 promoter and activates MUC2 transcription. Lee et al. (2004) have previously shown that there is negative association between EBV infection and expression of MUC2 in stomach cancers, again in line with the our present data (Table 2). Therefore, we consider that the absence of Cdx2 and MUC2 is linked in EBV (+) stomach cancers.

We also here demonstrated that stomach cancers are more likely to be of N type in the EBV (+) group, in line with the previous report that EBV (+) stomach cancers have lower MUC5AC and MUC2 expression than their EBV (-) counterparts (Lee et al., 2004). EBV associated stomach carcinomas are reported to lack intestinal phenotypic expression (Chen et al., 2003) and most EBV (+) stomach cancers were here classified phenotypically as N or G types (Table 3). Nakamura et al. (2005) also previously showed the G type to be more common in EBV (+) cases.

Several reports have shown that EBV (+) stomach

Table 6. Comparison of phenotypic markers in differentiated and undifferentiated regions in EBV (+) and EBV (-) stomach cancer cases.

Case No.	EBER-ISH	Histology	Phenotypes in total area	Phenotypical marker expression in each region		Ratio of N types in U region[a]
				D region	U region	
1	+	D>U	G	G	N	N=3/6 (50%)
2	+	D>U	I	I	I	
3	+	U>D	G	G	G	
4	+	U>D	G	G	G	
5	+	U>D	G	G	N	
6	+	U>D	I	I	N	
1	–	D>U	GI	GI	GI	N=0/9 (0%)
2	–	D>U	I	I	I	
3	–	U>D	G	G	G	
4	–	U>D	G	G	G	
5	–	U>D	G	G	G	
6	–	U>D	GI	GI	GI	
7	–	U>D	GI	GI	I	
8	–	U>D	GI	GI	I	
9	–	U>D	I	I	I	

[a]: P<0.02 (Fisher's exact test). Abbr.: D, differentiated; U, undifferentiated; G, gastric; I, intestinal; GI, gastric-and-intestinal-mixed; N, null.

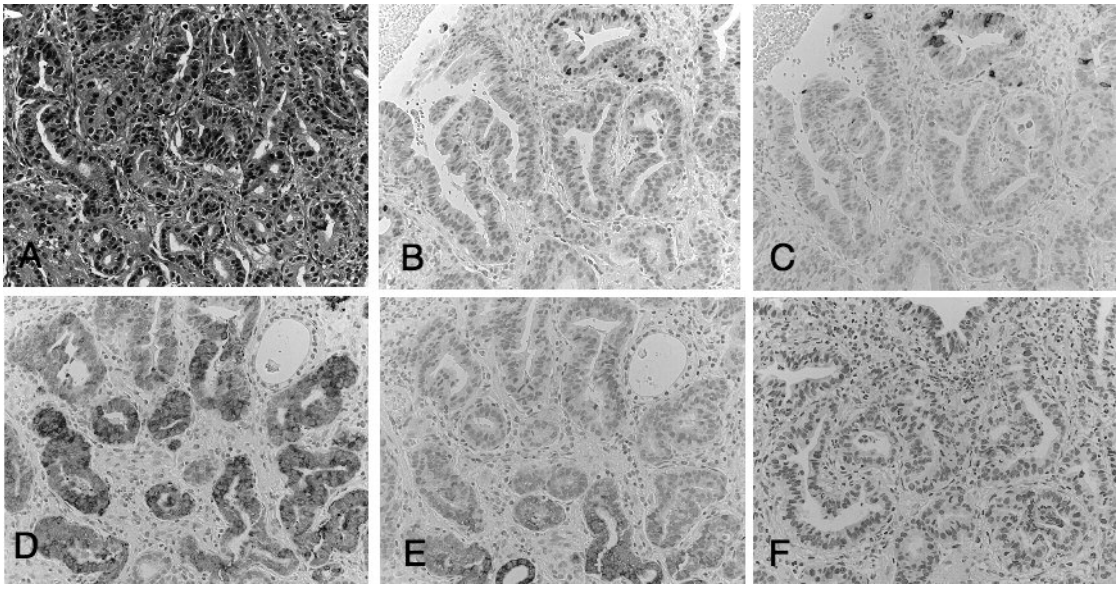

Fig. 2. An EBV (-) stomach cancer. **A.** HE staining. **B.** Cdx2 nuclear staining is positive in some cancer cells. **C.** MUC2 expression is detected in the cytoplasm of some tumor cells. **D.** MUC5AC is present in the cytoplasm of the cancer cells. **E** MUC6 is apparent in the cytoplasm of some tumor cells. **F.** EBER-1 is negative in the nuclei of the cancer cells. x 200; EBER-1, EBV-encoded small RNA-1.

cancers are most often undifferentiated histopathologically, according to the Japanese Classification of Gastric Carcinomas (Yanai et al., 1997; Wu et al., 2000; Lee et al., 2004). EBV (+) stomach cancers are more frequently moderately differentiated tubular adenocarcinomas (tub2), and solid poorly differentiated adenocarcinomas (por1) as compared with other histological types (Carrascal et al., 2003). To avoid bias, phenotypic expression was here evaluated in morphologically matched samples for EBV (+) and EBV (-) cases.

Regarding the histogenesis of EBV associated stomach cancers, Fukayama et al. (2001) previously suggested the hypothesis that they develop by clonal expansion of rare EBV-infected epithelial cells within stomach mucosa. EBV infection of intestinal metaplastic cells is unlikely (Fukayama et al., 2001). We have argued that the origin of stomach cancers is from progenitor cells specializing towards mucous differentiation in the fundic/pyloric glands, rather than intestinal metaplastic glands (Tatematsu et al., 2005). With EBV infection the histogenesis may be from cells that are specialized towards mucous differentiation in the fundic/pyloric glands, harboring neither typical gastric nor intestinal phenotypic expression.

In the present study, inflammatory response in the surrounding non-neoplastic mucosa was not statistically different between EBV (+) and EBV (-) cases. So EBV may not have significantly induced inflammatory cell infiltration in our Columbia cases. The Cdx2 expression in the intestinal metaplastic glands was also lower in non-neoplastic mucosa of EBV (+) cases, despite no EBV infection being observed by in situ hybridization. However, the presence of EBV in non-carcinomatous surrounding mucosa of EBV (+) stomach cancers has been detected by immunostaining of EBNA-1 and latent membrane protein 1 (LMP-1) (Yanai et al., 1997a,b). Hayashi et al. (1996) detected EBV in gastric glands with IM. Yanai et al. (1999) reported the evidence that all eight lesions of EBER-1-positive gastric carcinomas had intestinal metaplasia in the background among 8 EBER-1-positive stomach carcinomas. In contrast, Kaizaki et al. (1999) reported that only 13% of EBV (+) stomach cancers were surrounded by intestinal metaplasia, in contrast to 41% of EBV (-) ones. Zur Hausen et al. (2004) concluded that EBER-1/2 transcripts were restricted to the carcinoma cells in accordance with exclusive positivity of EBNA-1 immunohistochemistry (IHC) to the tumor cells. Negative LMP-1 IHC in all cases tested and absence of EBER-1/2 transcripts in preneoplastic gastric lesions (intestinal metaplasia and dysplasia) strongly suggested that EBV could only infect neoplastic gastric cells, indicating it as a late event in gastric carcinogenesis.

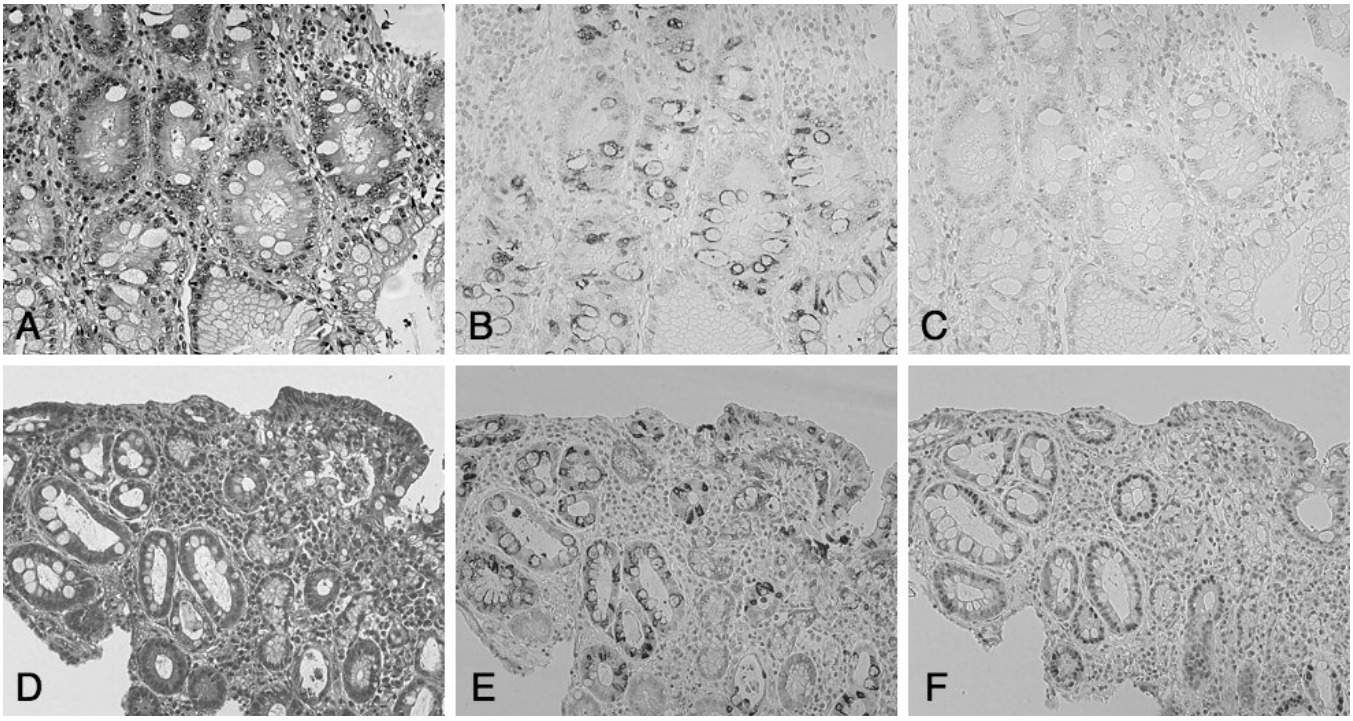

Fig. 3. Expression of MUC2 and Cdx2 in intestinal metaplastic glands in tissue surrounding adenocarcinomas. EBV (+) **(A-C)** and EBV (-) **(D-F)** stomach cancers. **A and D**. HE staining. **B and E.** MUC2 is detectable in the cytoplasm of intestinal metaplastic glands. **C and F.** No Cdx2 nuclear staining in intestinal metaplastic glands in an EBV (+) case (C) in contrast to apparent nuclear staining in an EBV (-) case. x 200.

Thus down regulation of Cdx2 might not be due to infection of EBV to the surrounding mucosa. EBV (+) stomach cancer and surrounding intestinal metaplasia were similar to down regulation of Cdx2. We considered EBV might have infected the progenitor cell or stem cell after late event in gastric carcinogenesis and intestinal metaplasia, and the down regulation of Cdx2 were similar mechanism to EBV (+) stomach cancer and surrounding intestinal metaplasia. Further studies of EBV infection in non-neoplastic stomach epithelia appear warranted.

In conclusion, EBV (+) stomach cancers are characterized by a relative lack of intestinal phenotypic expression, including Cdx2, and only occasional presence of gastric phenotypic expression. The progenitor cell may thus be specialized towards mucous differentiation in the fundic/pyloric glands.

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