5) of 10 tumors with End-cell marker expression, the staining of the markers were observed uniformly all over the whole tumor tissues, while 8 cases had heterogeneity of both Exo-cell and End-cell marker expression within a whole tumor. Therefore, we evaluated the relation between Exo-cell and End-cell phenotypes in the 23 small cancerous areas covering more than 500 μm in diameter with CgA cytoplasmic expression in these 8 cases (Cases 1, 2, 3, 6, 7, 8, 9, and 10).

With regard to the gastric End-cell markers, expression of gastrin was detected in 11 cancerous areas with CgA expression in 5 cases, while somatostatin

Table 2. Relations between exocrine and endocrine cell markers with reference to phenotypic classification in 25 cancerous areas.

Cases	Regions	Phenotype 1[a] (Exocrine cell differentiation)	MUC5AC	MUC6	MUC2	Villin	Cdx2[b]	Phenotype 2 (Exocrine cell differentiation)	Gastrin	Somatostatin	GLP-1	GIP	Glicerin
Cancer 1	a	G	■	■				e-G	■				
Cancer 1	d	G	■	■				e-G	■				
Cancer 1	e	G	■					e-G	■				
Cancer 2	a	G	■					e-G	■				
Cancer 2	b	G		■			■	e-I					■
Cancer 3	a	GI	■		■	■	■	e-GI	■		■	■	
Cancer 3	b	GI	■			■	■	e-GI	■	■		■	
Cancer 3	c	GI	■		■	■	■	e-GI	■			■	
Cancer 3	d	GI	■			■	■	e-GI		■		■	
Cancer 3	e	GI	■		■	■	■	e-GI	■			■	
Cancer 4	whole	GI	■		■	■	■	e-GI		■	■	■	
Cancer 5	whole	GI	■	■	■		■	e-GI		■	■	■	
Cancer 1	b	GI	■		■		■	e-I				■	
Cancer 6	a	I				■	■	e-G	■				
Cancer 1	c	I			■		■	e-I					■
Cancer 6	f	I				■	■	e-I				■	■
Cancer 6	e	I				■	■	e-I					■
Cancer 7	a	I			■		■	e-I			■		
Cancer 8	a	N						e-G	■				
Cancer 6	b	N						e-GI	■		■		
Cancer 8	b	N						e-I			■	■	
Cancer 9	a	N					■	e-I					■
Cancer 6	d	N						e-I				■	
Cancer 6	c	N					■	e-I			■		
Cancer 10	a	N					■	e-I				■	

a: P=0.0004, comapared with Phenotype 2 by c2 test; b: P=0.006, comapared with Phenotype 2 by χ^2 test. Positive gastric and intestinal markers are filled with red or blue, respecitvely; e-G type, gastric endocrine cell phenotype; e-GI type, gastric-and-intestinal endocrine cell phenotype; e-I type, intestinal endocrine phenotype; G type, gastric exocrine cell phenotype; GI type, gastric-and-intestinal exocrine cell phenotype; I type, intestinal exocrine cell phenotype; N type, null exocrine phenotype.

expression was observed in 4 cancerous areas of 4 cases. Regarding the intestinal End-cell markers, expression of glicentin was detected in 5 areas of 4 cases. Expression of GIP was observed in 12 cancerous areas of 7 cases, while GLP-1 expression was detected in 7 lesions in 6 cases. The total of 25 cancerous areas (10 cancer cases) were divided phenotypically into 6 e-G, 8 e-GI, and 11 e-I types. In 15 (60.0%) cancerous areas (areas 1a, 1c, 1d, 1e, 2a, 3a, 3b, 3c, 3d, 3e, 4 whole, 5 whole, 6e, 6f, and 7a), the phenotypes of End-cell markers were in line with those of the Exo-cell counterparts, and strong association was observed between the Exo-cell and End-cell markers from the viewpoint of phenotypic expression in the remainder (Fig. 1, Table 2, P=0.0004). Cdx2 expression was also strongly associated with the presence of intestinal End-cell markers such as glicentin, GLP-1, and GIP (Table 2, P=0.006). When the multiple areas within tumors were compared, the phenotypes of both Exo- and End-cell markers of Cancer Case 3 coincided well among areas a-e. However, those of areas b and c were different from areas a, d, and e in Case 1. This discrepancy was also observed in Case 6 (areas a, f, and e vs. areas b, d, and c). In 4 cancerous areas (Cases 6a, 6b, 6d, and 8b), Cdx2 expression was not in line with the intestinal End-cell marker expression. However, regarding Case 6a, Cdx2 nuclear staining was observed in the cancerous area of e-G type exhibiting villin expression.

Table 3. Relations between exocrine and endocrine cell markers with reference to phenotypic classification in 14 CgA-positive adenomas.

Cases	Phenotype 1[a] (Exocrine cell differentiation)	MUC5AC	MUC6	MUC2	Villin	Cdx2	Phenotype 2 (Endocrine cell differentiation)	Gastrin	Somatostatin	GLP-1	GIP	Glicentin
Adenoma 1	GI		■	■	■	■	e-G		■			
Adenoma 2	GI		■	■	■	■	e-G		■			
Adenoma 3	GI	■	■	■	■	■	e-GI		■	■	■	
Adenoma 4	GI		■		■	■	e-GI		■		■	
Adenoma 5	GI	■	■		■	■	e-I			■	■	
Adenoma 6	I			■	■	■	e-GI		■	■	■	
Adenoma 7	I			■	■	■	e-GI		■	■	■	
Adenoma 8	I			■	■	■	e-I			■	■	
Adenoma 9	I				■	■	e-I			■		
Adenoma 10	I			■	■	■	e-I			■		
Adenoma 11	I			■	■	■	e-I			■		
Adenoma 12	I			■	■	■	e-I			■	■	
Adenoma 13	I			■	■	■	e-I			■	■	
Adenoma 14	I				■	■	e-I			■	■	

[a]: P=0.031, comapared with Phenotype 2 by χ^2 test; Positive gastric and intestinal markers are filled with red or blue, respecitvely; e-G type, gastric endocrine cell phenotype; e-GI type, gastric-and-intestinal endocrine cell phenotype; e-I type, intestinal endocrine phenotype; GI type, gastric-and-intestinal exocrine cell phenotype; I type, intestinal exocrine cell phenotype.

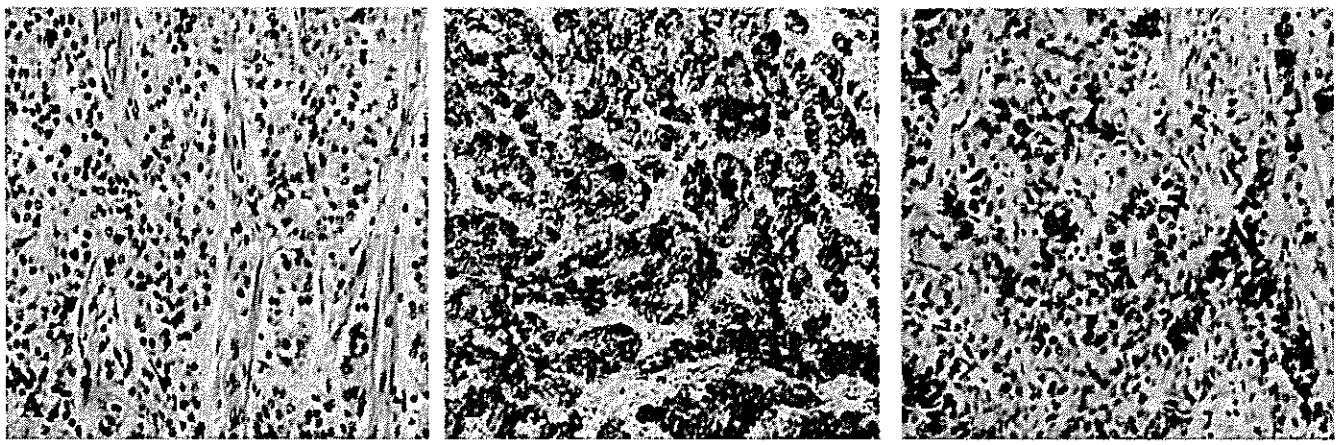

Fig. 3. A stomach carcinoid tumor with expression of gastrin (Carcinoid case No.4). A. H&E staining. B. CgA expression is apparent in the cytoplasm of tumor cells. C. Gastrin is present in the cytoplasm of carcinoid cells. CgA, chromogranin A. x 200

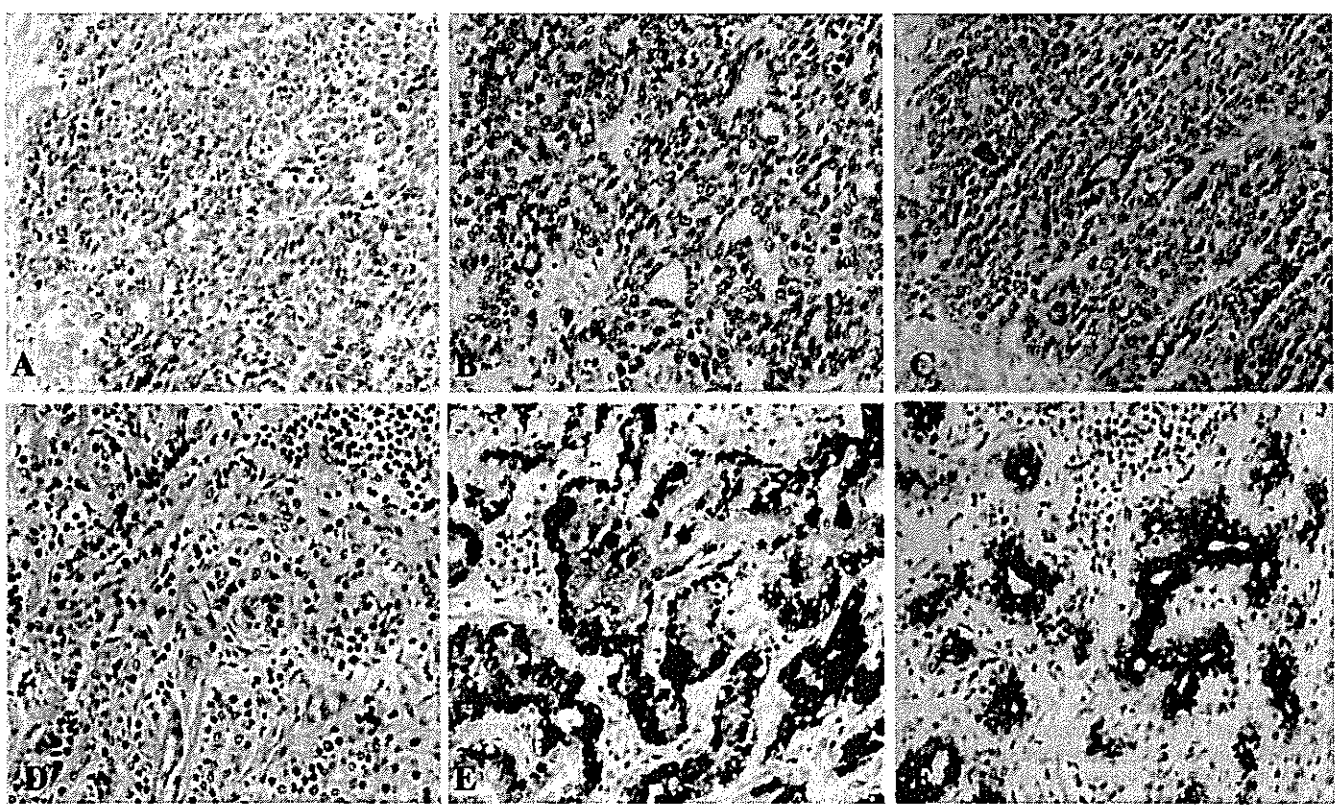

Fig. 4. Two cases of ECCs featuring expression of Exo-cell markers. A-C. ECC1 in Table 4. A. H&E staining. B. Lack of CgA cytoplasmic staining in the cancer cells. Positive for synaptophysin and CD56 (data not shown). C. Villin is positive on the luminal surfaces of cancer cells. D-F. ECC2 in Table 4. D. H&E staining. E. CgA expression is evident in the cytoplasm of cancer cells. F. MUC6 is positive in the cytoplasm of cancer cells. ECC, endocrine cell carcinoma; CgA, chromogranin A; Exo-cell, exocrine cell. x 200

Relations between expression of Exo-cell and End-cell markers in 14 CgA-positive stomach adenomas

Totals of 15 (50%) and 15 (50%) stomach adenomas were judged to be CgA-positive and CgA-negative, respectively. We examined the expression of End-cell markers in the 15 CgA-positive adenomas. Of the 15 cases, 14 CgA-positive cases had the expression of at least one End-cell marker, while 1 case exhibited no End-cell marker expression. Therefore, we also analyzed expression of Exo-cell markers in the above-mentioned 14 cases. The lesions were divided by the End-cell marker expression into 2 e-G, 4 e-GI, and 8 e-I types (Table 3). They were also classified by the Exo-cell marker expression as 5 GI and 9 I types. The phenotypes of End-cell markers in 2 e-GI and 7 e-I types were in line with those of the Exo-cell counterparts. Strong association was observed between the Exo-cell and End-cell markers from the viewpoint of phenotypic expression in adenoma cases (Fig. 2, Table 3, P=0.031). Cdx2 expression was present in all stomach adenoma cases exhibiting the intestinal Exo-cell phenotypic expression.

Expression of CgA, Exo-cell and End-cell markers in 8 carcinoid tumors and 4 ECCs of the stomach

Data for expression of Exo-cell and End-cell markers in the End-cell tumors are summarized in Table 4. Eight carcinoid tumors (100%) and 1 ECCs (25%)

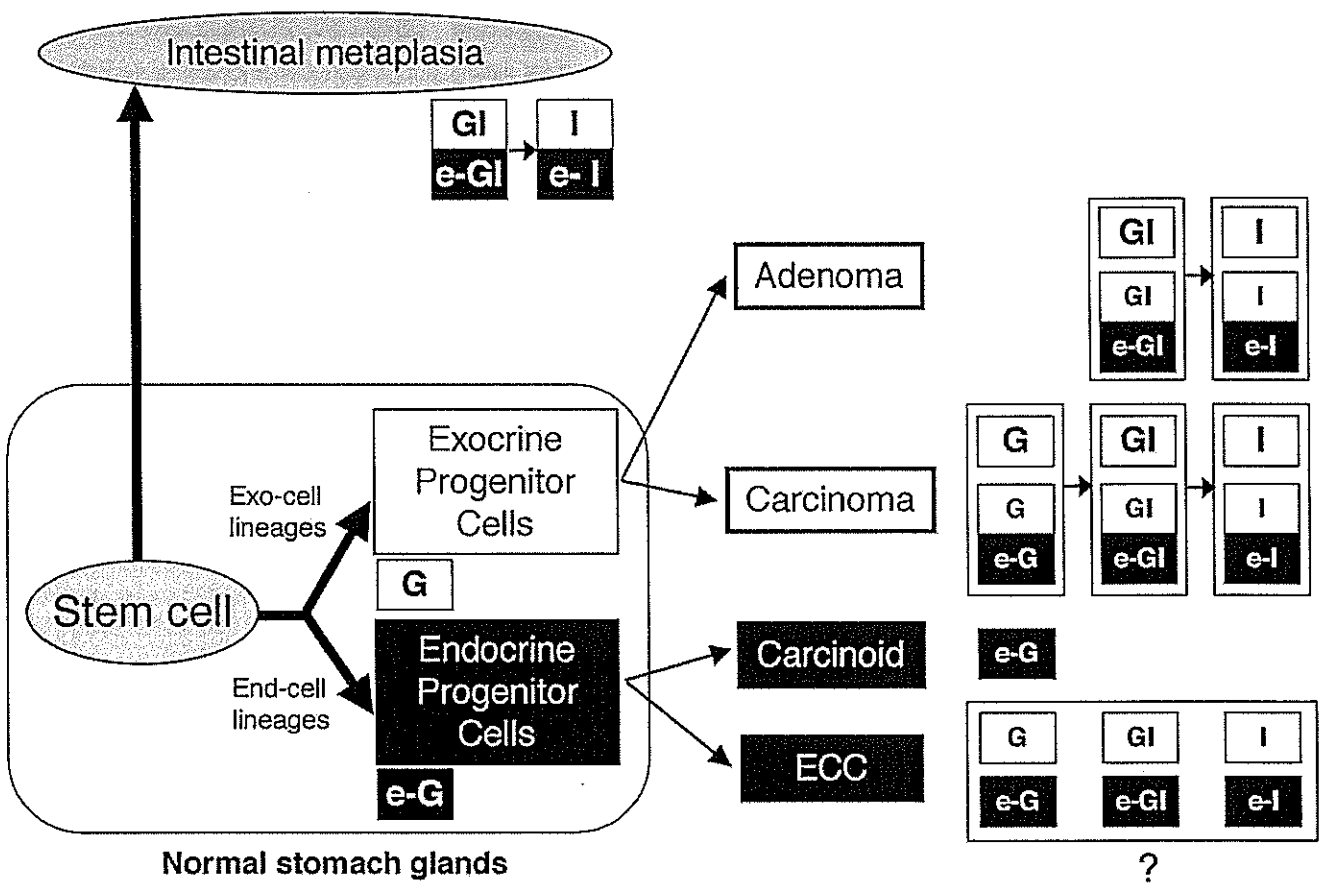

Fig. 5. Schematic illustration of the hypothesis for tumorigenesis pathways in stomach from the viewpoint of exocine and endocrine phenotypic expression. IM is due to abnormal stem cell differentiation in the *Helicobacter pylori* infected stomach. Stomach cancer and adenoma originate from a progenitor cell specializing towards Exo-cell lineages in the gastric mucosa. Most stomach cancers have no phenotypic expression of End-cell. Some of them have End-cell phenotypic expression, preserving the link between End-cell and Exo-cell phenotypes. Almost half of adenomas have no phenotypic expression of End-cell. The remainder of them have End-cell phenotypic expression, again keeping the link between End- and Exo-cell phenotypes. Stomach carcinoid and ECC occur from a progenitor cell specializing towards End-cell lineages in the gastric mucosa, some of the latter may possess Exo-cell lineage. IM, intestinal metaplasia; ECC, endocrine cell carcinoma; Exo-cell, exocrine cell; End-cell, endocrine cell; G, exocrine gastric type; GI, exocrine gastric-and-intestinal-mixed type; I, exocrine intestinal type; e-G, endocrine gastric type; e-GI, endocrine gastric-and-intestinal-mixed type; e-I, endocrine intestinal type.

Table 4. Expression of endocrine and exocrine cell markers in the carcinoids and the endocrine cell carcinomas of the stomach.

Cases	Endocrine cell differentiation					Exocrine cell differentiation			
	CgA	Gastrin	Somatostatin	GIP	GLP-1	MUC5AC	MUC6	MUC2	Villin
Carcinoid 1	■	■							
Carcinoid 2	■								
Carcinoid 3	■	■							
Carcinoid 4	■	■	■						
Carcinoid 5	■	■							
Carcinoid 6	■								
Carcinoid 7	■								
Carcinoid 8	■	■							
ECC 1	*	■		■					■
ECC 2	■						■		
ECC 3	*								
ECC 4	*	■	■						

CgA positive cases are filled in black. Positive gastric and intestinal markers are filled with red or blue, respecitvely; *: ECC cases 1, 3, and 4 are positive for synaptophysin and/or CD56.

were judged to be CgA-positive. Of the 8 carcinoids, 5 and 1 demonstrated expression of gastrin (Carcinoid cases 1, 3, 4, 5, and 8) and somatostatin (Carcinoid case 4) respectively, while none of the intestinal End-cell markers and no Exo-cell markers were observed. Of the 4 ECCs, expression of gastrin, somatostatin, and GIP was found in 2 (ECC cases 1 and 4), 1 (ECC case 4), and 1 (ECC case 1), respectively. Regarding Exo-cell markers, MUC6 and villin were positive in ECC cases 2 and 1, respectively (Figs. 3, 4, Table 4).

Discussion

We summarized with a schematic illustration of the hypothesis for pathways of carcinoma, adenoma, carcinoid, and ECC in the stomach in Fig. 5. In the human stomach, we have documented clear evidence that the phenotypes of End-cells are strongly associated with those of Exo-cells in intestinal metaplasia (IM) (Otsuka et al., 2005). Especially, expression of both gastric and intestinal End-cell markers is observed in the End-cells of gastric-and-intestinal-mixed Exo-cell phenotype IM (GI-IM) at the cellular level, as well as the glandular level (Otsuka et al., 2005). Intestinalization progresses from GI to solely intestinal phenotype IM (I-IM) in the non-cancerous mucosa of human stomach (Tatematsu et al., 2003). Moreover, IM glands have both End-cells and Exo-cell lineages. Thus, it is evident that IM is due to abnormal stem cell differentiation in the Helicobacter pylori infected stomach (Tatematsu et al., 2003).

In the present study, 85.5% (94/110) of the stomach cancer cases had no CgA expression. Therefore, most of the stomach cancers, which had a tendency to differentiate into not End-cells but solely Exo-cells, were thought to be compatible with our hypothesis that stomach cancer originates from a progenitor cell specializing towards Exo-cell lineages (Tatematsu et al., 2005). However, this cannot explain the other cancer cases having the remarkable expression of CgA or the link between Exo-cell and End-cell phenotypes. This result suggests the necessity to consider the concept of cancer stem cells in stomach cancer. The existence of cancer stem cells in human myeloid leukemias (Bonnet and Dick, 1997), breast cancers (Al-Hajj et al., 2003), and brain tumors (Singh et al., 2004) was demonstrated. The similar concept may be introduced into stomach cancer. The cancer stem cells, which possess self-renewal properties and the ability to produce both Exo-cell and End-cell types like normal stem cells, may appear secondarily in some stomach cancer cases. Should stomach cancers have originated from stem cell itself in stomach glands, most stomach cancers could have both End-cell and Exo-cell phenotypic carcinoma cells. However, actually, most stomach cancers have no tendency to differentiate into End-cells in the present study. Furthermore, Cancer cases 1 and 6 showed several areas harboring both Exo- and End-cell markers, which did not coincide with each other in terms of gastric and intestinal phenotypes. Thus, we consider that most stomach cancers occur from a progenitor cell specializing towards Exo-cell lineages, and the cancer stem cells appear secondarily in some of them.

Our data have demonstrated the evidence that all of the adenoma cases had the intestinal Exo-cell phenotypic expression as GI or I types, and no G type adenoma was detected. It is well-known that human stomach cancers at an early stage, independent of the histological type, mainly consist of G type malignant cells, while their advanced counterparts tend to have more I type malignant cells, suggesting a phenotypic shift from gastric to intestinal phenotypic expression during the course of tumor progression (Yamachika et al., 1997; Yoshikawa et al., 1998; Egashira et al., 1999; Bamba et al., 2001; Tatematsu et al., 2003, 2005). Most stomach cancers develop independently of adenomas (Hattori, 1986; Hirohashi and Sugimura, 1991; Ogasawara et al., 1994; Sakurai et al., 1995; Tahara and Yokozaki, 1996),

which is also supported by our present data of the conflict of Exo-cell marker expression between the stomach adenomas and cancers. On the other hand, the origin of adenomas may be from a progenitor cell specializing towards Exo-cell lineages in IM glands, considering the similarity of Exo-cell phenotypes between adenoma and IM. IM is widely thought to be a precancerous lesion for differentiated type stomach cancers. However, previous studies on phenotypic expression and microsatellite instability (MSI) of individual intestinal metaplastic or stomach cancer cells have pointed to several contradictions in the prevailing paradigm (Hattori, 1986; Tatematsu et al., 1990, 2003, 2005; Kushima and Hattori, 1993; Tamura et al., 1995; Endoh et al., 2000; Kawachi et al., 2003; Tatematsu et al., 2003; Mizoshita et al., 2004b, 2005). Therefore, we consider that the pathway of adenoma and IM occurrence may be different from that of stomach carcinogenesis, essentially. In addition, half of the adenoma cases had expression of CgA, and this percentage was much higher than that of the stomach cancers. The link between Exo-cell and End-cell phenotypes was also observed in adenoma cases, being similar to stomach cancers. There may be the possibility that the tumor stem cells appear more easily in the stomach adenomas than in the stomach cancerous tissues.

Our study showed that all the examined stomach carcinoid tumors had expression of CgA but no Exo-cell phenotype, and 75% (6/8) of carcinoid cases were classified as e-G type. Thus, we consider that stomach carcinoids originate from a progenitor cell specializing towards End-cell lineages in the stomach glandular ducts (Tahara et al., 1975; Bordi et al., 1991; Tatematsu et al., 2005). Regarding the ECCs cases, there was no clear tendency. However, 50% (2/4) of ECC cases had the Exo-cell phenotypic expression. ECCs of the stomach may arise from endocrine precursor cell clones occurring in preceding adenocarcinoma components as the Exo-cell types (Tahara et al., 1975; Nishikura et al., 2000, 2003). The presence of both Exo-cell and End-cell components in the ECCs may be explained by the hypothesis of cancer stem cells, being similar to stomach cancers.

We demonstrated the clear evidence that Cdx2 nuclear staining is strongly associated with intestinal End-cell phenotypic expression in stomach cancer cases. La Rosa et al. (2004) have previously suggested Cdx2 to be a sensitive and specific marker of midgut End-cells and we have presented evidence that Cdx2 is strongly associated with intestinal Exo-cell phenotypic expression of the alimentary tract (Mizoshita et al., 2001). Tsukamoto et al. (2004) also earlier showed Sox2 and Cdx1/2 to be gastric and intestinal specific transcription factors, respectively. In isolated pyloric and intestinal metaplastic glandular ducts, the phenotypes of Exo-cells were found to be strongly associated with these specific transcription factors (Tsukamoto et al., 2004). The phenotypes of malignant cells in human stomach cancers were also found to be strongly associated with these specific transcription factors, independent of the histological type (Mizoshita et al., 2003, 2004a,b; Tsukamoto et al., 2005). Thus, we consider that Cdx2 is important for expression of intestinal End-cell markers even in stomach cancer cells as well as intestinal Exo-cell phenotypic expression.

In conclusion, most stomach cancers might develop from a progenitor cell specializing towards Exo-cell lineages, but some cases possessed both Exo-cell and End-cell markers with maturely differentiated phenotypes. In such cases, Exo-cell and End-cell phenotypes were found to correlate strongly, suggesting the possibility of histogenesis from "cancer stem cells" occurring secondarily.

Acknowledgements. The authors thank Dr. Malcolm A. Moore for revision of the scientific English language. This study was supported in part by a Grant-in-Aid for the Third-term Comprehensive 10-year Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Al-Hajj M., Wicha M.S., Benito-Hernandez A., Morrison S.J. and Clarke M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. USA 100, 3983-3988.

Bamba M., Sugihara H., Kushima R., Okada K., Tsukashita S., Horinouchi M. and Hattori T. (2001). Time-dependent expression of intestinal phenotype in signet ring cell carcinomas of the human stomach. Virchows Arch. 438, 49-56.

Blumenfeld W., Chandhoke D.K., Sagerman P. and Turi G.K. (1996). Neuroendocrine differentiation in gastric adenocarcinomas. An immunohistochemical study. Arch. Pathol. Lab. Med. 120, 478-481.

Bonnet D. and Dick J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. 3, 730-737.

Bordi C., Yu J.Y., Baggi M.T., Davoli C., Pilato F.P., Baruzzi G., Gardini G., Zamboni G., Franzin G., Papotti M. and Bussolati G. (1991). Gastric carcinoids and their precursor lesions. A histologic and immunohistochemical study of 23 cases. Cancer 67, 663-672.

Egashira Y., Shimoda T. and Ikegami M. (1999). Mucin histochemical analysis of minute gastric differentiated adenocarcinoma. Pathol. Int. 49, 55-61.

Endoh Y., Sakata K., Tamura G., Ohmura K., Ajioka Y., Watanabe H. and Motoyama T. (2000). Cellular phenotypes of differentiated-type adenocarcinomas and precancerous lesions of the stomach are dependent on the genetic pathways. J. Pathol. 191, 257-263.

Hattori T. (1986). Development of adenocarcinomas in the stomach. Cancer 57, 1528-1534.

Hirohashi S. and Sugimura T. (1991). Genetic alterations in human gastric cancer. Cancer Cells 3, 49-52.

Japanese Gastric Cancer Association (1998). Japanese Classification of Gastric Carcinoma - 2nd English Edition. Gastric Cancer 1, 10-24.

Kawachi H., Takizawa T., Eishi Y., Shimizu S., Kumagai J., Funata N. and Koike M. (2003). Absence of either gastric or intestinal

phenotype in microscopic differentiated gastric carcinomas. J. Pathol. 199, 436-446.

Kushima R. and Hattori T. (1993). Histogenesis and characteristics of gastric-type adenocarcinomas in the stomach. J. Cancer Res. Clin. Oncol. 120, 103-111.

La Rosa S., Rigoli E., Uccella S., Chiaravalli A.M. and Capella C. (2004). CDX2 as a marker of intestinal EC-cells and related well-differentiated endocrine tumors. Virchows Arch. 445, 248-254.

Lauren P. (1965). The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol. Microbiol. Scand. 64, 31-49.

Mizoshita T., Inada K., Tsukamoto T., Kodera Y., Yamamura Y., Hirai T., Kato T., Joh T., Itoh M. and Tatematsu M. (2001). Expression of Cdx1 and Cdx2 mRNAs and relevance of this expression to differentiation in human gastrointestinal mucosa--with special emphasis on participation in intestinal metaplasia of the human stomach. Gastric Cancer 4, 185-191.

Mizoshita T., Tsukamoto T., Nakanishi H., Inada K., Ogasawara N., Joh T., Itoh M., Yamamura Y. and Tatematsu M. (2003). Expression of Cdx2 and the phenotype of advanced gastric cancers: relationship with prognosis. J. Cancer Res. Clin. Oncol. 129, 727-734.

Mizoshita T., Inada K., Tsukamoto T., Nozaki K., Joh T., Itoh M., Yamamura Y., Ushijima T., Nakamura S. and Tatematsu M. (2004a). Expression of the intestine-specific transcription factors, Cdx1 and Cdx2, correlates shift to an intestinal phenotype in gastric cancer cells. J. Cancer Res. Clin. Oncol. 130, 29-36.

Mizoshita T., Tsukamoto T., Inada K., Ogasawara N., Hirata A., Kato S., Joh T., Itoh M., Yamamura Y. and Tatematsu M. (2004b). Immunohistochemically detectable Cdx2 is present in intestinal phenotypic elements in early gastric cancers of both differentiated and undifferentiated types, with no correlation to non-neoplastic surrounding mucosa. Pathol. Int. 54, 392-400.

Mizoshita T., Tsukamoto T., Cao X., Otsuka T., Ito S., Takahashi E., Nakamura S., Nakamura T., Yamamura Y. and Tatematsu M. (2005). Microsatellite instability is linked to loss of hMLH1 expression in advanced gastric cancers: lack of a relationship with the histological type and phenotype. Gastric Cancer 8, 164-172.

Nakamura K., Sugano H. and Takagi K. (1968). Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. Gann 59, 251-258.

Naritomi K., Futami K., Arima S. and Iwashita A. (2003). Malignant potential regarding mucin phenotypes and endocrine cell differentiation in gastric adenocarcinoma. Anticancer Res. 23, 4411-4422.

Nishikura K., Watanabe H., Iwafuchi M., Ajioka Y., Hashidate H. and Tanabe T. (2000). Histological overview of the classification of gastric carcinoid. Stomach Intestine 35, 1349-1354. (in Japanese with English summary).

Nishikura K., Watanabe H., Iwafuchi M., Fujiwara T., Kojima K. and Ajioka Y. (2003). Carcinogenesis of gastric endocrine cell carcinoma: analysis of histopathology and p53 gene alteration. Gastric Cancer 6, 203-209.

Ogasawara S., Maesawa C., Tamura G. and Satodate R. (1994). Lack of mutations of the adenomatous polyposis coli gene in oesophageal and gastric carcinomas. Virchows Arch. 424, 607-611.

Otsuka T., Tsukamoto T., Mizoshita T., Inada K., Takenaka Y., Kato S., Yamamura Y., Miki K. and Tatematsu M. (2005). Coexistence of gastric- and intestinal-type endocrine cells in gastric and intestinal mixed intestinal metaplasia of the human stomach. Pathol. Int. 55, 170-179.

Park J.G., Choe G.Y., Helman L.J., Gazdar A.F., Yang H.K., Kim J.P., Park S.H. and Kim Y.I. (1992). Chromogranin-A expression in gastric and colon cancer tissues. Int. J. Cancer 51, 189-194.

Qvigstad G., Sandvik A.K., Brenna E., Aase S. and Waldum H.L. (2000). Detection of chromogranin A in human gastric adenocarcinomas using a sensitive immunohistochemical technique. Histochem. J. 32, 551-556.

Sakurai S., Sano T., Maeshima A., Kashiwabara K., Oyama T., Fukuda T. and Nakajima T. (1995). Gastric adenoma-carcinoma sequence with special reference to p53 and Ki-ras gene alterations. Virchows Arch. 427, 119-124.

Schier S. and Wright N.A. (2005). Stem cell relationships and the origin of gastrointestinal cancer. Oncology 69 Suppl 1, 9-13.

Singh S.K., Hawkins C., Clarke I.D., Squire J.A., Bayani J., Hide T., Henkelman R.M., Cusimano M.D. and Dirks P.B. (2004). Identification of human brain tumour initiating cells. Nature 432, 396-401.

Sugano H., Nakamura K. and Kato Y. (1982). Pathological studies of human gastric cancer. Acta Pathol. Jpn. 32 (Suppl 2), 329-347.

Tahara E. and Yokozaki H. (1996). The sequential accumulation of genetic alterations characteristic of the colorectal adenoma-carcinoma sequence does not occur between gastric adenoma and adenocarcinoma. J. Pathol. 178, 475-476.

Tahara E., Haizuka S., Kodama T. and Yamada A. (1975). The relationship of gastrointestinal endocrine cells to gastric epithelial changes with special reference to gastric cancer. Acta Pathol. Jpn. 25, 161-177.

Tamura G., Sakata K., Maesawa C., Suzuki Y., Terashima M., Satoh K., Sekiyama S., Suzuki A., Eda Y. and Satodate R. (1995). Microsatellite alterations in adenoma and differentiated adenocarcinoma of the stomach. Cancer Res. 55, 1933-1936.

Tatematsu M., Ichinose M., Miki K., Hasegawa R., Kato T. and Ito N. (1990). Gastric and intestinal phenotypic expression of human stomach cancers as revealed by pepsinogen immunohistochemistry and mucin histochemistry. Acta Pathol. Jpn. 40, 494-504.

Tatematsu M., Fukami H., Yamamoto M., Nakanishi H., Masui T., Kusakabe N. and Sakakura T. (1994). Clonal analysis of glandular stomach carcinogenesis in C3H/HeN<==>BALB/c chimeric mice treated with N-methyl-N-nitrosourea. Cancer Lett. 83, 37-42.

Tatematsu M., Masui T., Fukami H., Yamamoto M., Nakanishi H., Inada K., Kusakabe M. and Sakakura T. (1996). Primary monoclonal and secondary polyclonal growth of colon neoplastic lesions in C3H/HeN<-->BALB/c chimeric mice treated with 1,2-dimethylhydrazine immunohistochemical detection of C3H strain-specific antigen and simple sequence length polymorphism analysis of DNA. Int. J. Cancer 66, 234-238.

Tatematsu M., Tsukamoto T. and Inada K. (2003). Stem cells and gastric cancer: role of gastric and intestinal mixed intestinal metaplasia. Cancer Sci. 94, 135-141.

Tatematsu M., Tsukamoto T. and Mizoshita T. (2005). Role of Helicobacter pylori in gastric carcinogenesis: the origin of gastric cancers and heterotopic proliferative glands in Mongolian gerbils. Helicobacter 10, 97-106.

Tsukamoto T., Inada K., Tanaka H., Mizoshita T., Mihara M., Ushijima T., Yamamura Y., Nakamura S. and Tatematsu M. (2004). Down-regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to

complete intestinal metaplasia. J. Cancer Res. Clin. Oncol. 130, 135-145.

Tsukamoto T., Mizoshita T., Mihara M., Tanaka H., Takenaka Y., Yamamura Y., Nakamura S., Ushijima T. and Tatematsu M. (2005). Sox2 expression in human stomach adenocarcinomas with gastric and gastric-and-intestinal-mixed phenotypes. Histopathology 46, 649-658.

Tsukamoto T., Yamamoto M., Fukami H., Yoshikawa A., Sakai H., Hirata A., Kusakabe M. and Tatematsu M. (2006). Susceptibility to colon carcinogenesis in C3H <–> C57BL/6 chimeric mice reflects both tissue microenvironment and genotype. Cancer Lett. (in press).

Tzaneva M.A. (2002). Endocrine cells in gastric carcinoma and adjacent mucosa. An immunohistochemical and ultrastructural study. Histochem. J. 34, 173-180.

Waldum H.L., Aase S., Kvetnoi I., Brenna E., Sandvik A.K., Syversen U., Johnsen G., Vatten L. and Polak J.M. (1998). Neuroendocrine differentiation in human gastric carcinoma. Cancer 83, 435-444.

Yamachika T., Inada K., Fujimitsu Y., Nakamura S., Yamamura Y., Kitou T., Itzkowitz S.H., Werther J.L., Miki K. and Tatematsu M. (1997). Intestinalization of gastric signet ring cell carcinomas with progression. Virchows Arch. 431, 103-110.

Yoshikawa A., Inada Ki K., Yamachika T., Shimizu N., Kaminishi M. and Tatematsu M. (1998). Phenotypic shift in human differentiated gastric cancers from gastric to intestinal epithelial cell type during disease progression. Gastric Cancer 1, 134-141.

Accepted September 22, 2006

Histol Histopathol (2007) 22: 641-649

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

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

N. Hirano[1,2], T. Tsukamoto[1], T. Mizoshita[1], C. Koriyama[3], S. Akiba[3], F. Campos[3], G. Carrasquilla[4], E. Carrascal[5], X. Cao[1], T. Toyoda[1], H. Ban[1], K. Miki[2] and M. Tatematsu[1]

[1]Division of Oncological Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan, [2]Division of Gastroenterology and Hepatology, Department of Internal Medicine (Ohmori) School of Medicine, Faculty of Medicine, Toho Unviersity, Tokyo, Japan, [3]Department of Epidemiology and Preventive Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan, [4]Department of Microbiology, Faculty of Health Sciences, Universidad del Valle, San Fernando, Cali, Colombia and [5]Department of Pathology, Faculty of Health Sciences, Universidad del Valle, San Fernando, Cali, Colombia

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.

Offprint requests to: Tetsuya Tsukamoto, M.D., Ph.D., Division of Oncological Pathology, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. e-mail: ttsukamt@aichi-cc.jp

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. pylori) 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.

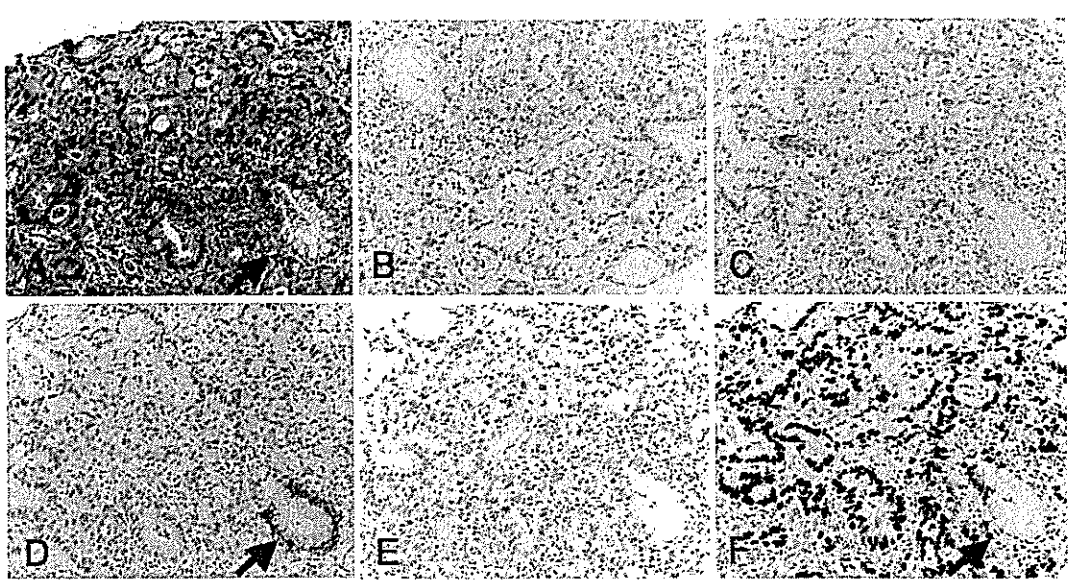

Fig. 1. An EBV (+) stomach cancer. A. HE staining. B. Note the lack of Cdx2 nuclear staining in the cancer cells. C. No MUC2 expression is detected in the cytoplasm of tumor cells. D. MUC5AC is present in the cytoplasm of normal gastric foveolar epithelium (red arrow), but not cancer cells. E. No MUC6 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.

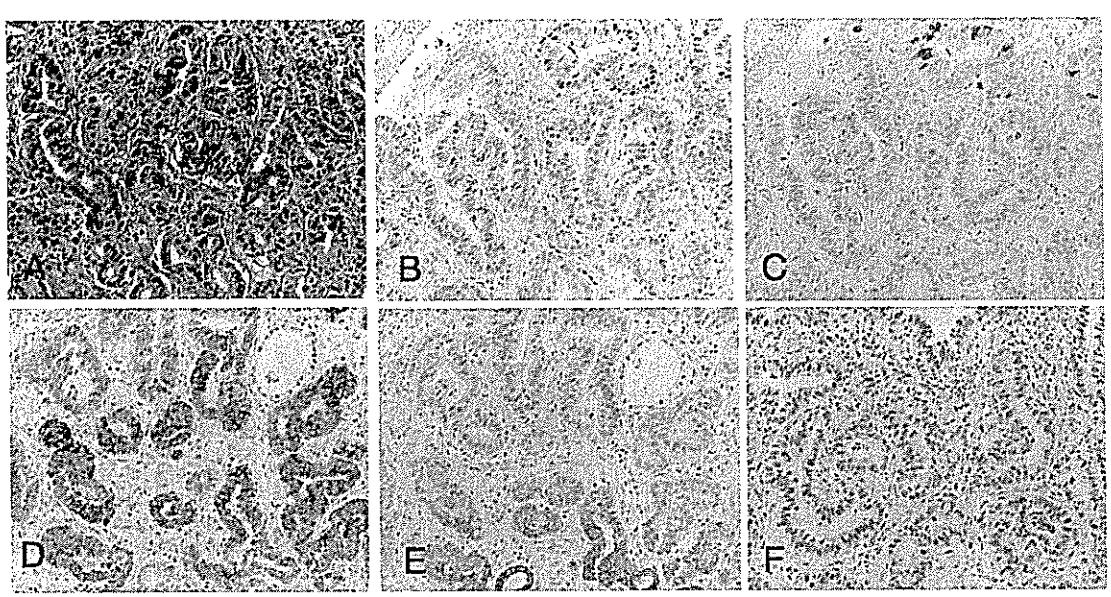

Fig. 2. An EBV (-) stomach cancer. A. HE staining. B. Cdx2 nuclear staining is positive in some cancer cells. C. MUC2 expression is detected in the cytoplasm of some tumor cells. D. MUC5AC is present in the cytoplasm of the cancer cells. E MUC6 is apparent in the cytoplasm of some tumor cells. F. EBER-1 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.

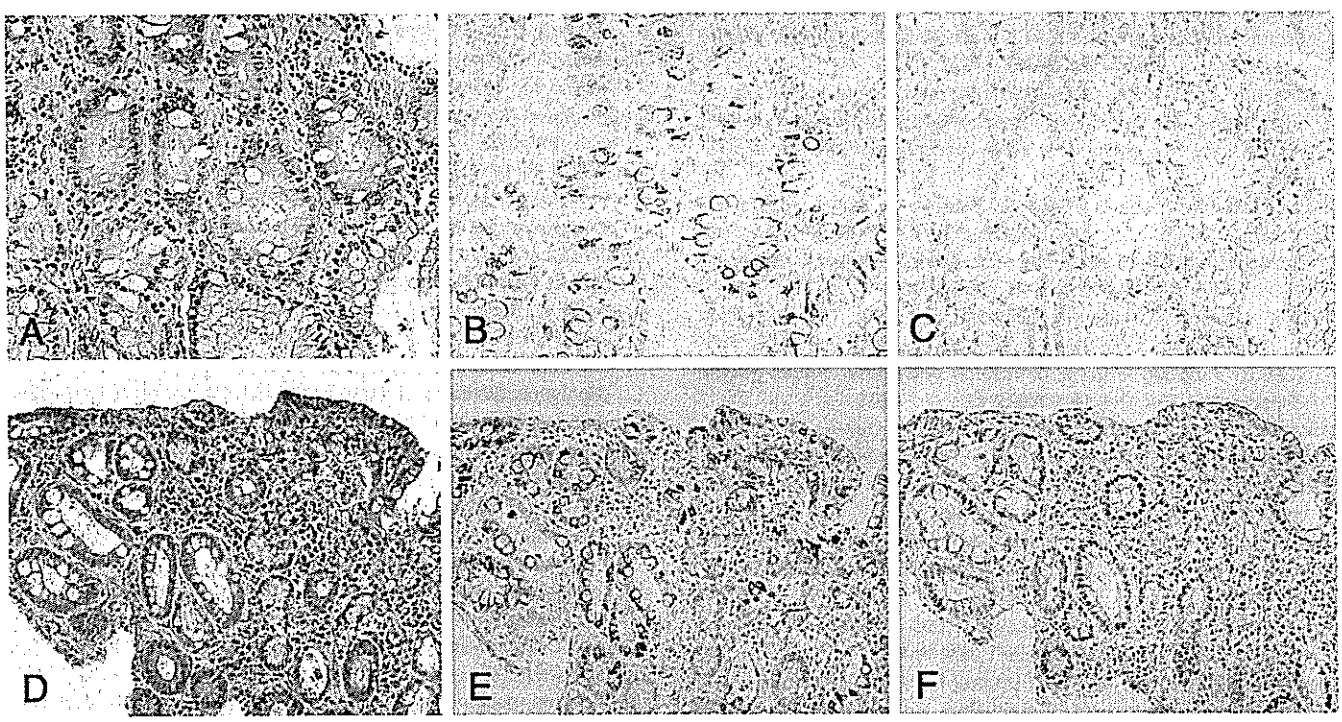

Fig. 3. Expression of MUC2 and Cdx2 in intestinal metaplastic glands in tissue surrounding adenocarcinomas. EBV (+) (A-C) and EBV (-) (D-F) stomach cancers. A and D. HE staining. B and E. MUC2 is detectable in the cytoplasm of intestinal metaplastic glands. C and F. No Cdx2 nuclear staining in intestinal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

Acknowledgements. The authors thank Dr. Malcolm A. Moore for revision of the scientific English language. This study was supported in part by a Grant-in-Aid for the Third-term Comprehensive 10-year Strategy for Cancer Control, a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (12218231 & 17015037).

References

Almeida R., Silva E., Santos-Silva F., Silberg D.G., Wang J., De Bolos C. and David L. (2003). Expression of intestine-specific transcription factors, CDX1 and CDX2, in intestinal metaplasia and gastric carcinomas. J. Pathol. 199, 36-40.

Burke A.P., Yen T.S., Shekitka K.M. and Sobin L.H. (1990). Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. Mod. Pathol. 3, 377-380.

Carrascal E., Koriyama C., Akiba S., Tamayo O., Itoh T., Eizuru Y., Garcia F., Sera M., Carrasquilla G., Piazuelo M.B., Florez L. and Bravo J.C. (2003). Epstein-Barr virus-associated gastric carcinoma in Cali, Colombia. Oncol. Rep. 10, 1059-1062.

Chen X., Leung S.Y., Yuen S.T., Chu K.M., Ji J., Li R., Chan A.S., Law S., Troyanskaya O.G., Wong J., So S., Botstein D. and Brown P.O. (2003). Variation in gene expression patterns in human gastric cancers. Mol. Biol. Cell 14, 3208-3215.

Dixon M.F., Genta R.M., Yardley J.H. and Correa P. (1996). Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am. J. Surg. Pathol. 20, 1161-1181.

Egashira Y., Shimoda T. and Ikegami M. (1999). Mucin histochemical analysis of minute gastric differentiated adenocarcinoma. Pathol. Int. 49, 55-61.

Fukayama M., Chong J.M. and Kaizaki Y. (1998). Epstein-Barr virus and gastric carcinoma. Gastric Cancer 1, 104-114.

Fukayama M., Chong J.M. and Uozaki H. (2001). Pathology and molecular pathology of Epstein-Barr virus-associated gastric carcinoma. Curr. Top. Microbiol. Immunol. 258, 91-102.

Hanto D.W., Frizzera G., Purtilo D.T., Sakamoto K., Sullivan J.L., Saemundsen A.K., Klein G., Simmons R.L. and Najarian J.S. (1981). Clinical spectrum of lymphoproliferative disorders in renal transplant recipients and evidence for the role of Epstein-Barr virus. Cancer Res. 41, 4253-4261.

Hayashi K., Teramoto N., Akagi T., Sasaki Y. and Suzuki T. (1996). *In situ* detection of Epstein-Barr virus in the gastric glands with intestinal metaplasia. Am. J. Gastroenterol. 91, 1481.

Imai S., Koizumi S., Sugiura M., Tokunaga M., Uemura Y., Yamamoto N., Tanaka S., Sato E. and Osato T. (1994). Gastric carcinoma: monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein. Proc. Natl. Acad. Sci. USA 91, 9131-9135.

Japanese Gastric Cancer Association. (1998). Japanese Classification of Gastric Carcinoma - 2nd English Edition. Gastric Cancer 1, 10-24.

Kaizaki Y., Sakurai S., Chong J.M. and Fukayama M. (1999). Atrophic gastritis, Epstein-Barr virus infection, and Epstein-Barr virus-associated gastric carcinoma. Gastric Cancer 2, 101-108.

Kawachi H., Takizawa T., Eishi Y., Shimizu S., Kumagai J., Funata N. and Koike M. (2003). Absence of either gastric or intestinal phenotype in microscopic differentiated gastric carcinomas. J. Pathol. 199, 436-446.

Lauren P. (1965). The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma: An attempt at a histo-clinical classification. Acta Pathol. Microbiol. Scand. 64, 31-49.

Lee H.S., Chang M.S., Yang H.K., Lee B.L. and Kim W.H. (2004). Epstein-barr virus-positive gastric carcinoma has a distinct protein expression profile in comparison with epstein-barr virus-negative carcinoma. Clin. Cancer Res. 10, 1698-1705.

Mizoshita T., Inada K., Tsukamoto T., Kodera Y., Yamamura Y., Hirai T., Kato T., Joh T., Itoh M. and Tatematsu M. (2001). Expression of Cdx1 and Cdx2 mRNAs and relevance of this expression to differentiation in human gastrointestinal mucosa--with special emphasis on participation in intestinal metaplasia of the human stomach. Gastric Cancer 4, 185-191.

Mizoshita T., Tsukamoto T., Nakanishi H., Inada K., Ogasawara N., Joh T., Itoh M., Yamamura Y. and Tatematsu M. (2003). Expression of Cdx2 and the phenotype of advanced gastric cancers: relationship with prognosis. J. Cancer Res. Clin. Oncol. 129, 727-734.

Mizoshita T., Inada K., Tsukamoto T., Nozaki K., Joh T., Itoh M., Yamamura Y., Ushijima T., Nakamura S. and Tatematsu M. (2004a). Expression of the intestine-specific transcription factors, Cdx1 and Cdx2, correlates shift to an intestinal phenotype in gastric cancer cells. J. Cancer Res. Clin. Oncol. 130, 29-36.

Mizoshita T., Tsukamoto T., Inada K., Ogasawara N., Hirata A., Kato S., Joh T., Itoh M., Yamamura Y. and Tatematsu M. (2004b). Immunohistochemically detectable Cdx2 is present in intestinal phenotypic elements in early gastric cancers of both differentiated and undifferentiated types, with no correlation to non-neoplastic surrounding mucosa. Pathol. Int. 54, 392-400.

Nakamura Y., Yanai H., Kitou T., Matsubara Y., Hirano A., Okamoto T., Yoshida T., Okita K. and Matsusaki K. (2005). Mucin and differentiation in Epstein-Barr virus-associated gastric carcinoma. Hepatogastroenterology 52, 1066-1070.

Raab-Traub N. (1992). Epstein-Barr virus and nasopharyngeal carcinoma. Semin. Cancer Biol. 3, 297-307.

Shibata D. and Weiss L.M. (1992). Epstein-Barr virus-associated gastric adenocarcinoma. Am. J. Pathol. 140, 769-774.

Silberg D.G., Swain G.P., Suh E.R. and Traber P.G. (2000). Cdx1 and cdx2 expression during intestinal development. Gastroenterology 119, 961-971.

Tajima Y., Shimoda T., Nakanishi Y., Yokoyama N., Tanaka T., Shimizu

K., Saito T., Kawamura M., Kusano M. and Kumagai K. (2001). Gastric and intestinal phenotypic marker expression in gastric carcinomas and its prognostic significance: immunohistochemical analysis of 136 lesions. Oncology 61, 212-220.

Takada K. (2000). Epstein-Barr virus and gastric carcinoma. Mol. Pathol. 53, 255-261.

Tatematsu M., Tsukamoto T. and Inada K. (2003). Stem cells and gastric cancer - Role of gastric and intestinal mixed intestinal metaplasia. Cancer Sci. 94, 135-141.

Tatematsu M., Tsukamoto T. and Mizoshita T. (2005). Role of Helicobacter pylori in gastric carcinogenesis: the origin of gastric cancers and heterotopic proliferative glands in Mongolian gerbils. Helicobacter 10, 97-106.

Tokunaga M., Land C.E., Uemura Y., Tokudome T., Tanaka S. and Sato E. (1993). Epstein-Barr virus in gastric carcinoma. Am. J. Pathol. 143, 1250-1254.

Tsukamoto T., Inada K., Tanaka H., Mizoshita T., Mihara M., Ushijima T., Yamamura Y., Nakamura S. and Tatematsu M. (2004). Down-regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to complete intestinal metaplasia. J. Cancer. Res. Clin. Oncol. 130, 135-145.

Tsukamoto T., Mizoshita T., Mihara M., Tanaka H., Takenaka Y., Yamamura Y., Nakamura S., Ushijima T. and Tatematsu M. (2005). Sox2 expression in human stomach adenocarcinomas with gastric and gastric-and-intestinal-mixed phenotypes. Histopathology 46, 649-658.

Weiss L.M., Movahed L.A., Warnke R.A. and Sklar J. (1989). Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. N. Engl. J. Med. 320, 502-506.

Wu M.S., Shun C.T., Wu C.C., Hsu T.Y., Lin M.T., Chang M.C., Wang H.P. and Lin J.T. (2000). Epstein-Barr virus-associated gastric carcinomas: relation to H. pylori infection and genetic alterations. Gastroenterology 118, 1031-1038.

Yamamoto H., Bai Y.Q. and Yuasa Y. (2003). Homeodomain protein CDX2 regulates goblet-specific MUC2 gene expression. Biochem. Biophys. Res. Commun. 300, 813-818.

Yanai H., Murakami T., Yoshiyama H., Takeuchi H., Nishikawa J., Nakamura H., Okita K., Miura O., Shimizu N. and Takada K. (1999). Epstein-Barr virus-associated gastric carcinoma and atrophic gastritis. J. Clin. Gastroenterol. 29, 39-43.

Yanai H., Nishikawa J., Mizugaki Y., Shimizu N., Takada K., Matsusaki K., Toda T., Matsumoto Y., Tada M. and Okita K. (1997a). Endoscopic and pathologic features of Epstein-Barr virus-associated gastric carcinoma. Gastrointest. Endosc. 45, 236-242.

Yanai H., Takada K., Shimizu N., Mizugaki Y., Tada M. and Okita K. (1997b). Epstein-Barr virus infection in non-carcinomatous gastric epithelium. J. Pathol. 183, 293-298.

Zur Hausen A., van Rees B.P., van Beek J., Craanen M.E., Bloemena E., Offerhaus G.J., Meijer C.J. and van den Brule A.J. (2004). Epstein-Barr virus in gastric carcinomas and gastric stump carcinomas: a late event in gastric carcinogenesis. J. Clin. Pathol. 57, 487-491.

zur Hausen H., Schulte-Holthausen H., Klein G., Henle W., Henle G., Clifford P. and Santesson L. (1970). EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. Nature 228, 1056-1058.

Accepted December 26, 2006

実績報告書用資料
氏名：

研究結果の概要

*H. pylori*感染は、慢性胃炎や胃がん発生に大きく関わっているが、その予防に安価かつ安全な化学物質の開発が急務である。菜種原油中の酸化ラジカルスカベンジャーであるcanololの抗炎症作用及び胃発がん抑制作用を検討し、炎症所見の改善と抗がん作用が明らかとなった。

研究により得られた成果の今後の活用・提供

キャノロールは、高い抗ラジカル活性を有し、ナタネ原油の抽出物から得られる天然由来の成分であるため、原料面や安全面で有利であると同時に合成も容易である。油脂に溶け易いため、食用油やその他油脂組成物の抗酸化剤として利用し易く、抗炎症剤及び癌予防剤としての有用性が期待される。

研究の実施経過

5週齢スナネズミに、*H. pylori*を強制胃内投与し、実験第2週より、10 ppm MNUを飲水投与した。第8週より、AIN93Gを基礎食に0.1% Canololを混餌投与し、実験52週に屠殺した。組織学的及び血清学的解析を行った。また、胃粘膜における炎症関連因子のmRNAの発現量を定量的に検討した。

平成18年度総括研究報告書用資料
氏名：　　塚本徹哉

1. 研究目的（200字）

H. pylori 感染は、慢性胃炎や胃がん発生に大きく関わっている。*H. pylori* 除菌療法に伴う抗生物質耐性菌の出現や副作用の問題の解決に向けて、胃発がんを予防し得る安価かつ安全な化学物質の開発が急務である。本研究においては、スナネズミ腺胃発がん系を用いて、菜種原油中に含まれる強力な酸化ラジカルスカベンジャーである canolol の抗炎症作用及び胃発がん抑制作用を検討した。

2. 研究方法（200字）

5 週齢スナネズミに、*H. pylori*（ATCC43504株）を強制胃内投与し、実験第2週より、10 ppm MNU を飲水投与した。第8週より、AIN93G を基礎食に 0.1% Canolol を混餌投与し、実験52週に屠殺した。腺胃組織像、Cox-2、iNOS、BrdU 免疫染色、血清 8OH-dG 値、血清抗 H. pylori 抗体価を測定した。また、胃粘膜における炎症関連因子の mRNA の発現量を定量的に検討した。

3. 研究結果（400字）

胃がん発生率は、基礎食のみを摂取した群が 41.7%（15/36）、BHT を添加した対照群が 39.4%（13/33）、0.1% canolol 投与群が 15.0%（6/40）と、後者で有意（$P<0.05$）に発がん率の低下が見られた。

Canolol 投与群、対照群の血清 8OH-dG 値は、0.41±0.04、0.57±0.07 ng/ml（$P<0.01$）、血清抗 *H. pylori* 抗体価は 186.4±74.2、249.5±98.5（$P<0.05$）と前者で低下が認められた。

組織学的にも炎症所見が改善し、Cox-2、iNOS、IL-1β、TNF-α の mRNA の発現量を定量的 RT-PCR 法により解析した結果、Canolol 投与群では、それぞれ、17.5%、53.6%、14.3%、22.3%に有意に低下していた（$P<0.05$）。

4. 考察（400字）

H. pylori 感染 MNU 誘発スナネズミ腺胃発がんモデルにおいて、H. pylori 除菌は、腺胃発がん抑制に非常に有効である。しかし、胃がんの予防に、cagA、cagE、vacA 等の菌由来毒素の消失が必要なのか、あるいは除菌に伴う炎症所見の軽快でも効果があるのか明らかではない。今回、菜種原油中に含まれる強力な酸化ラジカルスカベンジャー、canolol の投与により、組織学的に炎症細胞浸潤、過形成、腸上皮化生の軽快し、胃発がんの抑制に有効であることも明らかにされた。また、血清 8OH-dG 値の低下と血清抗 H. pylori 抗体価の低下が見られた。そのメカニズムとして、Cox-2、iNOS、TNF-α、IL1-β mRNA 発現の低下と Cox-2 や iNOS 陽性細胞数の減少が関与している可能性が示された。

5. 健康危険情報
（情報源となる研究成果、学会発表、論文発表、研究者名）
特になし

6. 結論（400字）

H. pylori の除菌は、胃炎や胃がんの抑制に非常に効果的であるが、経済的問題、耐性菌発生の危険、副作用の発生等、課題が多いのも事実である。多くのヒトを対照として胃がんを予防するには、食品中の安全かつ安価な有効成分を用いる方法がその解決法の一つである。炎症の場において酸化ラジカルの発生が、DNA や細胞に障害性に働くことが知られており、酸化ラジカルの消去が細胞保護の一助となると考える。本研究において、菜種原油中に含まれる強力な酸化ラジカルスカベンジャーである canolol によって、血清 8OH-dG 値、血清抗 *H. pylori* 抗体価の有意な低下が見られた。以上のことから、酸化ラジカルの制御が炎症の抑制に有効であり、ひいては発がん予防にも効果的であることが示唆された。

7. 論文発表（当該年度のもの）

1. Mizoshita, T., Tsukamoto, T., Takenaka, Y., Cao, X., Kato, S., Kaminishi, M., and Tatematsu, M. Gastric and intestinal phenotypes and histogenesis of advanced glandular stomach cancers in carcinogen-treated, Helicobacter pylori-infected Mongolian gerbils. Cancer Sci., 97: 38-44 (2006)

2. Ogasawara, N., Tsukamoto, T., Mizoshita, T., Inada, K., Cao, X., Takenaka, Y., Joh, T., and Tatematsu, M. Mutations and nuclear accumulation of beta-catenin correlate with intestinal phenotypic expression in human gastric cancer. Histopathology, 49: 612-621 (2006)

3. Takenaka, Y., Tsukamoto, T., Mizoshita, T., Cao, X., Ban, H., Ogasawara, N., Kaminishi, M., and Tatematsu, M. Helicobacter pylori infection stimulates intestinalization of endocrine cells in glandular stomach of Mongolian gerbils. Cancer Sci., 97: 1015-1022 (2006)

4. Tsukamoto, T., Mizoshita, T., and Tatematsu, M. Gastric-and-intestinal mixed-type intestinal metaplasia: aberrant expression of transcription factors and stem cell intestinalization. Gastric Cancer, 9: 156-166 (2006)

5. Cao, X., Tsukamoto, T., Nozaki, K., Tanaka, H., Cao, L., Toyoda, T., Takasu, S., Ban, H., Kumagai, T., and Tatematsu, M. Severity of gastritis determines glandular stomach carcinogenesis in Helicobacter pylori-infected Mongolian gerbils. Cancer Sci., (2007)

6. Takenaka, Y., Tsukamoto, T. (equal contributor), Mizoshita, T., Ogasawara, N., Hirano, N., Otsuka, T., Ban, H., Nakamura, T., Yamamura, Y., Kaminishi, M., and Tatematsu, M. Gastric and intestinal phenotypic correlation between exocrine and endocrine components in human stomach tumors. Histol. Histopathol., 22: 273-284 (2007)

7. Hirano, N., Tsukamoto, T., Mizoshita, T., Koriyama, C., Akiba, S., Campos, F., Carrasquilla, G., Carrascal, E., Cao, X., Toyoda, T., Ban, H., Miki, K., and Tatematsu, M. Down regulation of gastric and intestinal phenotypic expression in Epstein-Barr virus-associated stomach cancers. Histol. Histopathol., (in press)

8. 学会発表（当該年度のもの）

1. 曹 雪源、塚本徹哉、田中晴就、溝下 勤、関 孝弘、森村 茂、前田 浩、立松 正衛、スナネズミ動物モデルにおけるナタネ原油由来 4-vinyl-2, 6-dimethoxy-phenol [Canolol]のH. pylori 感染および胃発癌の抑制、第13回日本がん予防学会総会、京都、(2006年7月)
2. 塚本徹哉、曹 雪源、豊田武士、関 孝弘、森村 茂、前田 浩、立松正衛、ナタネ原油由来抗酸化物質 Canolol の *H. pylori* 感染スナネズミ腺胃発癌抑制効果、第23回日本疾患モデル学会総会、伊香保、(2006年11月)

9. 特許取得、実用新案登録

出願中：「キャノロールまたはそのプロドラッグ(PD)を含む抗炎症剤および癌予防剤ならびにこれらを含む医薬、化粧品および食品」