ORIGINAL ARTICLE

Gut and Liver, Vol. 6, No. 1, January 2012, pp. 71-77

The Role of CDX2 in Intestinal Metaplasia Evaluated Using Immunohistochemistry

Byoung Hwan Lee*, Nayoung Kim*,†, Hye Seung Lee‡, Jung Mook Kang†, Hyun Kyung Park*, Hyun Jun Jo*, Cheol Min Shin*, Sang Hyub Lee*, Young Soo Park*,†, Jin Hyeok Hwang*,†, Jin-Wook Kim*,†, Sook-Hyang Jeong*,†, Dong Ho Lee*,†, Hyun Chae Jung†, and In Sung Song†

Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, †Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, and ‡Department of Pathology, Seoul National University Bundang Hospital, Seongnam, Korea

Background/Aims: Intestinal metaplasia (IM) has been regarded as a premalignant condition. This study evaluated the role of the transforming factor CDX2 according to the severity and type of IM. **Methods:** This analysis was performed on 383 subjects with IM in the antrum and/or body, with diagnoses that were categorized as controls, dysplasias, and gastric cancers. The IM grades were classified into four groups as negative, mild, moderate or severe using the updated Sydney scoring system. The IM subtypes were categorized as type I, type II, and type III using high iron diamine and alcian blue (pH 2.5) staining. The CDX2 expression in the IM foci was evaluated using immunohistochemistry in specimens from the antrum and/or body. **Results:** CDX2 expression increased according to IM severity (p=0.001) but was not associated with the IM subtype (p=0.881) in the antrum specimens. Similarly, CDX2 expression increased according to the IM grade (p=0.001) but was not associated with the IM subtype (p=0.755) in the body specimens. CDX2 expression was also increased according to baseline disease in the antrum, especially dysplastic and GC group (p=0.003), but not in the body (p=0.582). However, status of *Helicobacter pylori* infection was not associated with CDX2 expression in the antrum (p=0.692) and body (p=0.271). **Conclusions:** These results show that CDX2 expression is associated with the IM grade regardless of the IM subtype and that it was more frequent in the dysplasia group. These results suggest that CDX2 expression might play an important role in the progression of IM in various environments that can affect neoplastic change. **(Gut Liver 2012;6:71-77)**

Key Words: *Helicobacter pylori*; Intestinal metaplasia; CDX2; Grade; Subtype

INTRODUCTION

Gastric cancer (GC) is one of the most common malignancies in Korea. The risk factors for GC are known to be *Helicobacter pylori* infection, atrophic gastritis (AG), and intestinal metaplasia (IM). IM is defined as the replacement of gastric columnar epithelial cells by cells with intestinal morphology, which appears in the multi-step progression of GC, especially in the intestinal type GC as proposed by Correa.[1] According to Correa hypothesis, *H. pylori* was thought to play a crucial role in the formation and progression of IM. That was generally accepted in the epidemiologic and population-based study,[2,3] though arguing data were existed.[4] In addition, there was not common consensus about the effect of *H. pylori* on the subtype or progression of IM in these epidemiologic studies. Furthermore, IM was considered to be irreversible change in spite of *H. pylori* eradication.[5] Therefore, the beneficial effect of *H. pylori* eradication was not clear in the area of already formed IM.

In regard to the property of IM itself, it is important to search the high risk of IM in the gastric carcinogenesis and frequent surveillance may be needed in this IM. Many investigations have researched the molecular and genetic mechanisms of IM, and have attempted to classify IM according to the risk for developing GC. Jass and Filipe[6] suggested a classification of IM based on morphology and classic mucin staining, and the incomplete type (type II and III) was considered to have a higher risk for GC than the complete type (type I).[7,8] Another classification of IM was suggested according to the gastric phenotype as well as the intestinal phenotype such as gastric (G) type, gastric-

Correspondence to: Nayoung Kim
Department of Internal Medicine, Seoul National University Bundang Hospital, 300 Gumi-dong, Bundang-gu, Seongnam 463-707, Korea
Tel: +82-31-787-7008, Fax: +82-31-787-4051, E-mail: nayoungkim49@empal.com
Received on June 10, 2011. Accepted on September 13, 2011.
pISSN 1976-2283 eISSN 2005-1212 http://dx.doi.org/10.5009/gnl.2012.6.1.71
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

and-intestinal (GI) mixed type, and intestinal (I) type.[9] Many arguments still existed in the classification of IM for the cancer risk in spite of these efforts.[10] Furthermore, the extensive IM was thought to be more important as a gastric carcinogenesis than subtype.[11]

CDX2, a member of the caudal-related homeobox gene family and intestinal-specific transcriptional factor, plays an important role in the development of small and large intestine.[12] Aberrant expression of CDX2 was observed in the gastric IM and various site of adenocarcinoma.[13,14] The crucial role of CDX2 in the formation of IM has been identified in the transgenic mice and gastric carcinoma was observed in the CDX2 transgenic mice.[15] In our previous study, CDX1 and CDX2 were found to play an important role in the formation of IM and in the progression to dysplasia and GC in human gastric specimens using real-time polymerase chain reaction (RT-PCR) method.[16] However, this is only gene level that it is necessary to be proved by protein level. Nevertheless, there have not been well-known about the roles of CDX2 transcriptional factor in the subtype or progression of IM until now. In addition, there were few data regarding the role of CDX2 in the progression of gastric carcinogenesis related with *H. pylori* infection by the immunohistochemistry (IHC) method. From this background, we tried to investigate the role of the CDX2 transcriptional factor in the formation and progression of IM among the diverse gastric disorders such as gastritis, dysplasia, and GC using IHC method.

MATERIALS AND METHODS

1. Study subjects

Three hundred and eighty-three patients were enrolled from 2003 to 2007 in Seoul National University Bundang Hospital. Approximately 50% of the patients had gastrointestinal symptoms within 3 months, but most of them received gastroscopy for the GC screening. The subjects were categorized into three groups, mainly depending on the gastroscopy findings and histological diagnosis; a control group and two different disease groups (dysplasia and GC). As CDX2 expression usually appears in the presence of IM, the study pool for IHC of CDX2 was selected when there was microscopic IM finding in either antrum or body after H&E staining. In GC group, poorly differentiated or signet ring cell type GC was excluded because these types of GC are usually thought to be not associated with multistep progression of GC. Subjects with a history of gastric surgery, *H. pylori* eradication therapy or systemic diseases requiring chronic medication were excluded. The Institutional Review Board at Seoul National University Bundang Hospital approved this study, and written informed consent was obtained from all of the participants.

2. *H. pylori* testing and histology

To determine the presence of current *H. pylori* infection, 10 biopsy specimens were obtained for the three types of *H. pylori* testing (histology, *Campylobacter* like organism [CLO] test [Delta West, Bentley, Australia], and culture). Among them, two biopsy specimens (one each from the greater curvature and lesser curvature) of the antrum and body were fixed in formalin, respectively, and used for evaluation of the presence of gastric atrophy (loss of appropriate glands including both metaplastic and non-metaplstic atrophy) and IM (by H&E staining). The presence of atrophy on any of two specimens in the antrum and body were averaged regarding gastric atrophy, and the same method was applied to IM. For determination of *H. pylori,* modified Giemsa staining was performed for two specimens from each of the antrum and body, and histological features of gastric mucosa were recorded using the updated Sydney scoring system. All biopsies were examined independently by two experienced pathologists (H.S.L. and Hee Eun Lee), who were unaware of the patient details. In the event of disagreement, the biopsies were reexamined by these two pathologists until agreement was reached. One specimen from each of the lesser curvature of the antrum and body was used for CLO test. Two specimens from each of the antrum and body were used for culture. If any one of these three *H. pylori* tests were positive, then the host was regarded as having an ongoing *H. pylori* infection. The anti-*H. pylori* immunoglobulin G (Genedia *H. pylori* ELISA; Green Cross Medical Science Co., Eumsung, Korea) was also determined when the three above-mentioned *H. pylori* tests were negative.[17] Korean strain was used as antigen in this *H. pylori* antibody test. When the *H. pylori* serology was positive, these subjects were regarded as having past *H. pylori* infection and classified as *H. pylori*-positive.

3. HID-AB2.5 staining for metaplasia subtyping

Sulfated (brown) and acidic non-sulfated (blue) mucosubstances were simultaneously stained with high iron diamine and alcian blue (pH 2.5) (HID-AB2.5). The IM was classified as follows: type I, mature absorptive cells and goblet cells, the latter secreting sialomucins; type II, few or absent absorptive cells, presence of columnar 'intermediate' cells in various stages of differentiation secreting sialomucins or, occasionally, sulfomucins, or both; type III, columnar intermediate cells secreting predominantly sulfomucins and goblet cells secreting sialomucins or sulfomucins, or both.[18] If more than one HID subtype of IM was present, it was classified as the dominant IM phenotype. In addition, the pathologists (H.S.L. and H.E.L) were blinded to the clinical information of the patients.

4. IHC for CDX2

Tissues from the antrum and body were fixed in 10% neutral buffered formalin, paraffin-embedded, and then cut into sections (4 μm). IHC was performed by the standard avidin-biotin complex method. A monoclonal antibody for CDX2 (BioGenex; San Ramon, CA, USA; dilution 1:200) was used as a primary

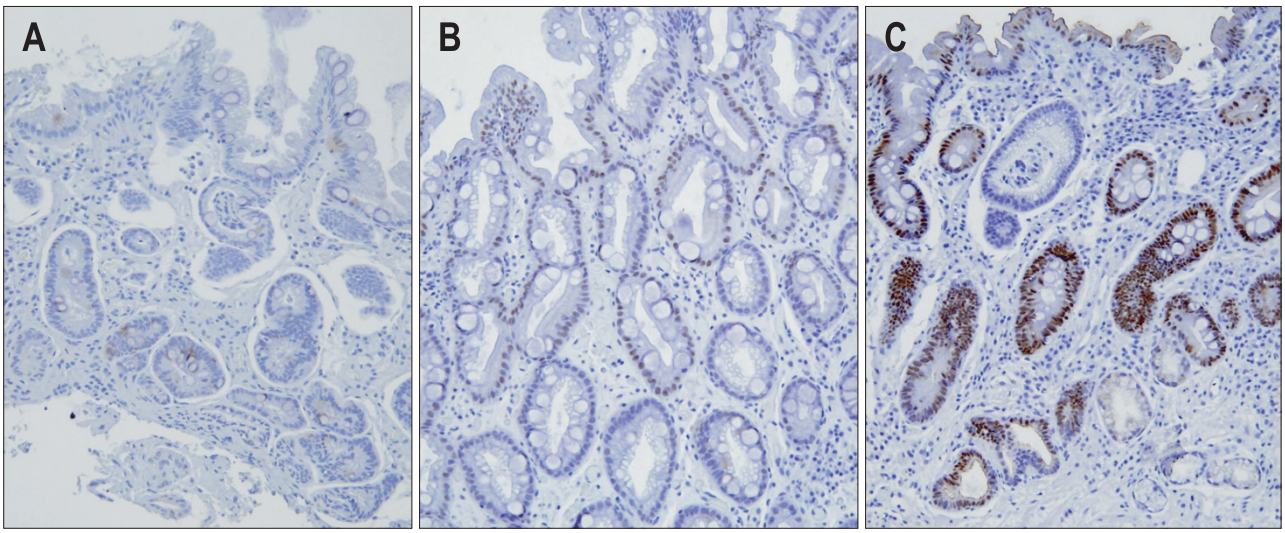

Fig. 1. Immunohistochemistry of CDX2. (A) Negative, (B) weak positive, and (C) strong positive (×200).

antibody and anti-mouse immunoglobulin G (Sigma, St Louis, MO, USA) labeled with biotin was used as a secondary antibody. Negative controls were treated similarly with the exception of the primary antibodies. The whole areas of slides were evaluated and positive CDX2 IHC was defined by at least more than 10% staining of the IM foci (Fig. 1).[19] Among 383 subjects, the specimen of 42 in the antrum and that of 155 in the body were excluded from CDX2 staining due to no observable foci of IM, respectively. In addition, 49 and 24 subjects with IM foci could not be used in CDX2 IHC, mainly due to loss of paraffin block. Finally, the specimen of the antrum (293 subjects) and body (204 subjects) were used in CDX2 IHC.

5. Statistical analysis

All statistical analyses were performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). The Pearson chi-square test was used to compare pairs of groups and Student's t-test was used to compare the mean value of each group. p-value below 0.05 was considered as a statistically significant.

RESULTS

1. Baseline characteristics

The 383 subjects with histological IM was classified to three groups: control group (n=165), dysplastic group (n=67), and GC group (n=151). The baseline characteristics are shown in Table 1. The mean age and the proportion of male were higher in the dysplastic and GC group, respectively (p<0.001). The severity of AG was higher in dysplasia and GC group than control group in the body specimens (p<0.001), but has no significant difference in the antrum specimens (p=0.769) (Table 1).

2. Pathological features of IM

The distribution and pathologic features of IM that were determined by an updated Sydney classification were shown in Table 2. One antrum specimen was not obtained for histology in control group and one body specimen in GC group. Therefore, 164 antrum specimens were used for the analysis of IM grade and extent in control group and 150 body specimens in GC group (Table 2). More extended distribution of IM was observed in the dysplasia and GC groups than the control group

Table 1. Baseline Characteristics

Characteristic	Control (n=165)	Dysplasia (n=67)	Gastric cancer (n=151)	p-value
Age, yr	58.6±12.6	63.3±8.4	65.0±9.4	**<0.001***
Sex				**<0.001** †
Male	93 (56.4)	52 (78.8)	114 (75.5)	
Female	72 (43.6)	15 (21.2)	37 (24.5)	
H. pylori positivity				0.326 †
H. pylori positive	146 (88.5)	63 (94.0)	128 (84.8)	
H. pylori negative	19 (11.5)	4 (6.0)	23 (15.2)	
Atrophic gastritis				
Antrum				0.769 †
None	34 (31.8)	11 (32.4)	32 (39.0)	
Mild	46 (43.0)	10 (29.4)	25 (30.5)	
Moderate	20 (18.7)	11 (32.4)	15 (18.3)	
Severe	7 (6.5)	2 (5.9)	10 (12.2)	
Body				**<0.001** †
None	63 (64.3)	11 (28.9)	26 (32.1)	
Mild	16 (16.3)	10 (26.3)	19 (23.5)	
Moderate	18 (18.4)	13 (34.2)	29 (35.8)	
Severe	1 (1.0)	4 (10.5)	7 (8.6)	

Data are presented as mean±SD or number (%). Bold indicates statistical significance.
*One-way ANOVA test; † Chi-square test.

(p<0.001). That is, dysplasia and cancer group showed IM in both of antrum and body, 65.7% and 62.7%, respectively, but control group showed 31.1%. In addition, The IM grade determined by an updated Sydney classification was more severe in the dysplastic and GC groups than in the control group, in both antrum (p=0.001) and body (p<0.001). However, there was no significant difference when comparing according to the subtypes of IM in both the antrum and body (Table 2).

3. CDX2 IHC

The specimen of the antrum (293 subjects) and body (204 subjects) were stained by CDX2 IHC. CDX2 expression was demonstrated in 219 (74.7%) and 169 (82.8%), respectively. CDX2 expression was increased with IM grade in the antrum (p=0.001) and body (p=0.001). However, CDX2 expression was not associated with IM subtype in the antrum and body (Table 3). In addition, CDX2 expression was higher in the dysplasia and GC group than control group in the antrum (p=0.003), but not in the body (p=0.582) (Table 3).

4. *H. pylori* status

There was no significant difference between *H. pylori*-positive and -negative group in terms of IM grade, IM subtype, and CDX2 expression in both of antrum and body (Table 4).

DISCUSSION

In this study, we demonstrated that CDX2 expression was associated with IM grade, but not subtype. These results suggest that CDX2 plays a role in the formation of IM. The biology of gastric IM has been investigated regarding variant molecular and genetic factors such as Shh, BMP4, CDX1, CDX2, and C-myc *et al*., but the formation and progression of IM is still difficult and undisclosed area.[20] Among the efforts, the transgenic mice model has shown that CDX transcriptional factors play

Table 2. The Relationship between Underlying Disease Groups and IM Extension, Grade, and Subtype

Variable	Control	Dysplasia	Gastric cancer	p-value*
Extension of IM				**<0.001**
One side	113 (58.9)	23 (12.0)	56 (29.2)	
Both side	51 (27.0)	44 (23.3)	94 (49.7)	
Grade of IM				
Antrum				**0.001**
None	23 (56.1)	8 (19.5)	10 (24.4)	
Mild	75 (51.7)	20 (13.8)	50 (34.5)	
Moderate	55 (38.2)	24 (16.7)	65 (46.9)	
Severe	11 (21.2)	15 (28.8)	26 (50.0)	
Body				**<0.001**
None	90 (59.6)	15 (9.9)	46 (30.5)	
Mild	41 (40.6)	21 (20.8)	39 (38.6)	
Moderate	30 (31.3)	21 (21.9)	45 (46.9)	
Severe	4 (11.8)	10 (29.4)	20 (58.8)	
Subtype of IM				
Antrum				0.427
Type I	18 (50.0)	4 (11.1)	14 (38.9)	
Type II	45 (42.1)	20 (18.7)	42 (39.3)	
Type III	39 (38.2)	13 (12.7)	50 (49.0)	
Body				0.150
Type I	21 (39.6)	14 (26.4)	18 (34.0)	
Type II	29 (28.7)	20 (19.8)	52 (51.5)	
Type III	11 (37.9)	3 (10.3)	15 (51.7)	

Data are presented as number (%). Bold indicates statistical significance.
IM, intestinal metaplasia.
*Chi-square test.

Table 3. IM Grade, Subtype, and Disease Status according to CDX2 Expression

Variable	CDX2 negative	CDX2 positive	p-value*
Grade of IM			
Antrum			**0.001**
Mild	43 (34.7)	81 (65.3)	
Moderate	26 (20.3)	102 (79.7)	
Severe	5 (12.2)	36 (87.8)	
Body			**0.001**
Mild	26 (28.6)	65 (71.4)	
Moderate	8 (9.8)	74 (90.2)	
Severe	1 (3.2)	30 (96.8)	
Subtype of IM			
Antrum			0.881
Type I	9 (25.7)	26 (74.3)	
Type II	22 (22.9)	74 (77.1)	
Type III	23 (23.7)	74 (76.3)	
Body			0.755
Type I	7 (13.7)	44 (86.3)	
Type II	9 (9.3)	88 (90.7)	
Type III	5 (18.5)	22 (81.5)	
Disease group			
Antrum			**0.003**
Control	39 (34.8)	73 (65.2)	
Dysplasia+GC	35 (19.3)	146 (80.7)	
Body			0.582
Control	12 (19.4)	50 (80.6)	
Dysplasia+GC	23 (16.2)	119 (83.8)	

Data are presented as number (%). Bold indicates statistical significance.
IM, intestinal metaplasia; GC, gastric carcinoma.
*Chi-square test.

Table 4. IM Grade, Subtype, and CDX2 Expression according to *H. pylori* Status

Variable	*H. pylori* negative	*H. pylori* positive	p-value*
Grade of IM			
Antrum			0.512
None/Mild/Moderate/Severe	6/14/17/9	35/131/127/43	
Body			0.768
None/Mild/Moderate/Severe	17/11/12/6	134/90/84/28	
Subtype of IM			
Antrum			0.619
Type I/II/III	6/15/11	30/92/91	
Body			0.505
Type I/II/III	7/12/6	46/89/23	
CDX2 expression			
Antrum			0.692
Negative	9 (12.2)	65 (87.8)	
Positive	23 (10.5)	196 (89.5)	
Body			0.271
Negative	6 (17.1)	29 (82.9)	
Positive	20 (11.8)	149 (88.2)	

Data are presented as number (%).
IM, intestinal metaplasia.
*Chi-square test.

a crucial role in the formation of IM.[21] Furthermore, GC was developed in the CDX2 transgenic mice model.[15] In the present study, the CDX2 expression was found to be 74.7% and 82.8% in the antrum and body, which are quite similar to the previous data, about 80%.[22-24] IHC for CDX2 was performed for the positive IM specimen, our data suggest that IM could be present without CDX2 in human stomach, and the CDX transcriptional factors might not be the unique factors in the IM formation. That is, other factors such as bile reflux, genetic or environmental factor might intermediate IM formation, especially in case of CDX-2 negative subjects.

There has been a great diversity of opinions in the role of CDX2 in gastric carcinogenesis. Some reports even claimed that CDX2 transcriptional factor may act as a tumor suppressor.[25,26] In the present study, CDX2 expression was increased in the neoplastic groups (dysplasia and GC) in the antrum, but not in the body, suggesting that CDX2 is not directly with gastric carcinogenesis. Instead, CDX2 looks like to be important in the progression of IM to the more severe and widespread form. There is a high chance of development of dysplasia and intestinal type of GC in this environment. In our previous study, we preformed quantitative analysis using RT-PCR for the evaluation of the role of CDX1 and CDX2 in the formation of IM and the progression to dysplasia and GC.[16] In this study which is not identical pool with the present study, CDX2 transcriptional factor was associated with both IM grade and subtype. Taken together, quantitative study such as RT-PCR might be a more powerful tool regarding the role of CDX2 in the research of IM biology. However, RT-PCR is based on transcriptional level but not on the translational level that IHC research could be very definite in supporting data. In the present study, CDX2 expression by IHC was correlated with IM grade, which is the identical finding of RT-PCR. Autoregulatory effect of CDX2 transcriptional factor has been assumed for this relationship. That is, CDX2 could autoregulate itself by binding CDX2 promotor area.[27] We also tried to perform the IHC of CDX1 but we could not find the adequate antibody for human study in spite of several times of trial.

The direct relationship of CDX2 expression and *H. pylori* infection was not confirmed.[22,28-30] However, disappearance of CDX2 expression was observed by *H. pylori* eradication in the level of gastritis in the absence of definitive IM.[29,30] However, *H. pylori* eradication did not cause any effects on the CDX2 expression when IM was already documented.[22,28] In addition, a recent study has shown that low degree of CDX2 expression was observed more than 50% in subjects with normal and healthy gastric mucosa without evidence of *H. pylori* infection, and bile reflux is one of the assumptive mechanisms in that case.[30,31] In the present study, the prevalence of *H. pylori* was not associated with IM grade, IM subtype and CDX2 expression. However, CDX2 expression was significantly higher in the *H. pylori*-positive group than -negative group when RT-PCR method was used in our previous study.[16] There could be several reasons. First, CDX2 IHC method may have limitation as the interpretation is not simple, which is different from quantitative study. Another reason could be study design. That is, the present study was not age and sex-matched study, which is different from the previous study. In addition, the prevalence of *H. pylori* in the control group of the present study was 88.5%, which was not different from dysplasia or GC group. This prevalence of *H. pylori* in the control group is much higher than 51.4% in comparison to the population-based study during similar periods.[32] The main reason of high prevalence of *H. pylori*, 88.5% is selection criteria of the present study. That is, as CDX2 expression usually appears in the presence of IM the study pool for IHC of CDX2 was selected when there was microscopic IM finding in either antrum or body regardless of control, dysplasia, and GC in the present study. This result suggests that *H. pylori* plays an important role in the formation of IM.

Incomplete type (type II and III) of IM was considered to be more important in the carcinogenesis than complete type (type I), but statistical significance was usually marginal.[7,8] Instead, a lot of studies demonstrated that the extension of IM was important risk for the gastric carcinogenesis.[11,33-35] In the present study, IM grade and extension were more important than IM subtype with GC progression in the background IM. This finding is consistent with the results of prior studies demonstrating that the IM subtype was less important than the extension or grade of IM.[11] There have been few data regarding the IM grade as a high risk

factor for the gastric carcinogenesis. However, the present study suggests that IM grade might be an important factor as IM extension for the gastric carcinogenesis.

In conclusion, CDX2 expression was associated with IM grade regardless of IM subtype, and it was frequent in the dysplasia group. These results suggest that CDX2 expression might play an important role in the progression of IM which environments provide neoplastic change.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process: First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 1992;52:6735-6740.
2. Asaka M, Sugiyama T, Nobuta A, Kato M, Takeda H, Graham DY. Atrophic gastritis and intestinal metaplasia in Japan: results of a large multicenter study. Helicobacter 2001;6:294-299.
3. Sonnenberg A, Lash RH, Genta RM. A national study of Helicobactor pylori infection in gastric biopsy specimens. Gastroenterology 2010;139:1894-1901.
4. Yee YK, Wong KW, Hui CK, et al. Prevalence and time trend of intestinal metaplasia in Hong Kong. J Gastroenterol Hepatol 2009;24:896-899.
5. Wang J, Xu L, Shi R, et al. Gastric atrophy and intestinal metaplasia before and after *Helicobacter pylori* eradication: a meta-analysis. Digestion 2011;83:253-260.
6. Jass JR, Filipe MI. A variant of intestinal metaplasia associated with gastric carcinoma: a histochemical study. Histopathology 1979;3:191-199.
7. Filipe MI, Muñoz N, Matko I, et al. Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. Int J Cancer 1994;57:324-329.
8. Kang KP, Lee HS, Kim N, et al. Role of intestinal metaplasia subtyping in the risk of gastric cancer in Korea. J Gastroenterol Hepatol 2009;24:140-148.
9. Inada K, Nakanishi H, Fujimitsu Y, et al. Gastric and intestinal mixed and solely intestinal types of intestinal metaplasia in the human stomach. Pathol Int 1997;47:831-841.
10. El-Zimaity HM, Ramchatesingh J, Saeed MA, Graham DY. Gastric intestinal metaplasia: subtypes and natural history. J Clin Pathol 2001;54:679-683.
11. Tava F, Luinetti O, Ghigna MR, et al. Type or extension of intestinal metaplasia and immature/atypical "indefinite-for-dysplasia" lesions as predictors of gastric neoplasia. Hum Pathol 2006;37:1489-1497.
12. Grainger S, Savory JG, Lohnes D. Cdx2 regulates patterning of the intestinal epithelium. Dev Biol 2010;339:155-165.
13. Lord RV, Brabender J, Wickramasinghe K, et al. Increased CDX2 and decreased PITX1 homeobox gene expression in Barrett's esophagus and Barrett's-associated adenocarcinoma. Surgery 2005;138:924-931.
14. Wu XS, Akiyama Y, Igari T, et al. Expression of homeodomain protein CDX2 in gallbladder carcinomas. J Cancer Res Clin Oncol 2005;131:271-278.
15. Mutoh H, Sakurai S, Satoh K, et al. Development of gastric carcinoma from intestinal metaplasia in Cdx2-transgenic mice. Cancer Res 2004;64:7740-7747.
16. Kang JM, Lee BH, Kim N, et al. CDX1 and CDX2 expression in intestinal metaplasia, dysplasia and gastric cancer. J Korean Med Sci 2011;26:647-653.
17. Kim SY, Ahn JS, Ha YJ, et al. Serodiagnosis of *Helicobacter pylori* infection in Korean patients using enzyme-linked immunosorbent assay. J Immunoassay 1998;19:251-270.
18. Bodger K, Campbell F, Rhodes JM. Detection of sulfated glycoproteins in intestinal metaplasia: a comparison of traditional mucin staining with immunohistochemistry for the sulfo-Lewis(a) carbohydrate epitope. J Clin Pathol 2003;56:703-708.
19. Mizoshita T, Tsukamoto T, Nakanishi H, et al. Expression of Cdx2 and the phenotype of advanced gastric cancers: relationship with prognosis. J Cancer Res Clin Oncol 2003;129:727-734.
20. Gutiérrez-González L, Wright NA. Biology of intestinal metaplasia in 2008: more than a simple phenotypic alteration. Dig Liver Dis 2008;40:510-522.
21. Mutoh H, Hayakawa H, Sakamoto H, Sashikawa M, Sugano K. Transgenic Cdx2 induces endogenous Cdx1 in intestinal metaplasia of Cdx2-transgenic mouse stomach. FEBS J 2009;276:5821-5831.
22. Ahn SY, Lee SY, Hong SN, et al. Endoscopic diagnosis of open-type atrophic gastritis is related to the histological diagnosis of intestinal metaplasia and Cdx2 expression. Dig Dis Sci 2011;56:1119-1126.
23. Bai YQ, Yamamoto H, Akiyama Y, et al. Ectopic expression of homeodomain protein CDX2 in intestinal metaplasia and carcinomas of the stomach. Cancer Lett 2002;176:47-55.
24. Kim HS, Lee JS, Freund JN, et al. CDX-2 homeobox gene expression in human gastric carcinoma and precursor lesions. J Gastroenterol Hepatol 2006;21:438-442.
25. Hayes S, Ahmed S, Clark P. Immunohistochemical assessment for Cdx2 expression in the Barrett metaplasia-dysplasia-adenocarcinoma sequence. J Clin Pathol 2011;64:110-113.
26. Park do Y, Srivastava A, Kim GH, et al. CDX2 expression in the intestinal-type gastric epithelial neoplasia: frequency and significance. Mod Pathol 2010;23:54-61.
27. Barros R, da Costa LT, Pinto-de-Sousa J, et al. CDX2 autoregulation in human intestinal metaplasia of the stomach: impact on the stability of the phenotype. Gut 2011;60:290-298.
28. Satoh K, Mutoh H, Eda A, et al. Aberrant expression of CDX2 in the gastric mucosa with and without intestinal metaplasia: effect

of eradication of *Helicobacter pylori*. Helicobacter 2002;7:192-198.
29. Shiotani A, Uedo N, Iishi H, et al. Re-expression of sonic hedgehog and reduction of CDX2 after *Helicobacter pylori* eradication prior to incomplete intestinal metaplasia. Int J Cancer 2007;121:1182-1189.
30. Vauhkonen M, Vauhkonen H, Sipponen P. *Helicobacter pylori* infection induces a reversible expression of the CDX2 transcription factor protein in human gastric epithelium. Scand J Gastroenterol 2008;43:915-921.
31. Xu Y, Watanabe T, Tanigawa T, et al. Bile acids induce cdx2 expression through the farnesoid x receptor in gastric epithelial cells. J Clin Biochem Nutr 2010;46:81-86.
32. Kim JI, Kim SG, Kim N, et al. Changing prevalence of upper gastrointestinal disease in 28 893 Koreans from 1995 to 2005. Eur J Gastroenterol Hepatol 2009;21:787-793.
33. Cassaro M, Rugge M, Gutierrez O, Leandro G, Graham DY, Genta RM. Topographic patterns of intestinal metaplasia and gastric cancer. Am J Gastroenterol 2000;95:1431-1438.
34. Kato I, Tominaga S, Ito Y, et al. A prospective study of atrophic gastritis and stomach cancer risk. Jpn J Cancer Res 1992;83:1137-1142.
35. Stemmermann GN, Hayashi T. Intestinal metaplasia of the gastric mucosa: a gross and microscopic study of its distribution in various disease states. J Natl Cancer Inst 1968;41:627-634.