RESEARCH COMMUNICATION

Expression of CDX2 and Villin in Gastric Cardiac Intestinal Metaplasia and the Relation with Gastric Cardiac Carcinogenesis

Zhong-Yue Xiao[1], Yi Ru[1], Jiang-Tao Sun[1], She-Gan Gao[1], Yu-Feng Wang[1], Li-Dong Wang[2], Xiao-Shan Feng[1]*

Abstract

Objective: To determine whether CDX2 and villin protein expression are associated with intestinal metaplasia (IM) in gastric cardiac mucosa and to explore the relationship with evolution of gastric cardiac adenocarcinoma (GCA). Methods: We studied 143 gastric cardiac biopsy or resection specimens from Henan province China, including 25 cardiac gastritis specimens with IM, 65 dysplasia specimens with IM and 35 gastric cardiac adenocarcinoma specimens and stained them for CDX2 and villin by the immunohistochemical SP method. 15 normal gastric cardiac biopsy specimens were also collected as control. Results: (1) Normal gastric mucosa presented no CDX2 and villin expression. The positive rates of CDX2 protein in cardiac gastritis with IM, dysplasia with IM, and carcinoma tissues were 84.0% (21/25), 66.7% (32/48) and 36.4% (20/55), respectively. While the positive rates of villin protein in cardiac gastritis with IM, dysplasia with IM, and carcinoma tissues were 76.0% (19/25), 70.8% (34/48) and 45.5% (25/55), respectively.There were significant differences among the three groups for both CDX2 and villin (P<0.01). Spearman's rank correlation coefficient(rho) showed a close correlation between the two proteins (r=0.843, P<0.01) and both were positively related with tumor differentiation (both P<0.05), but not associated with age, sex, invasion and metastasis of lymph node (P>0.05). Conclusion: Our results suggest that ectopic expression of CDX2 and villin may be involved in early-stage IM and tumorigenesis in gastric cardia and the expression of villin may be regulated by CDX2.

Keywords: CDX2 - villin - gastric cardiac - intestinal metaplasia - adenocarcinoma - protein expression

Asian Pacific J Cancer Prev, **13**, 247-250

Introduction

The gastric cardia is the most proximal part of the stomach adjoining the oesophagus. Interestly, over the past 50 years, adenocarcinoma at this region has increased remarkably due to the recognition that it is epidemiologically distinct from adenocarcinoma of the rest of the stomach (Botterweck et al., 2000; McColl, 2006). Adenocarcinoma of gastric cardia (GCA) tract is associated with several different underlying risk factors and the presence of intestinal metaplasia (IM) is an important one. Studies in the IM in gastric cardia are very important to identify the relationship between IM and GCA. Also very important in finding useful markers to help the earlier diagnosis of GCA (Palli et al., 1992). The caudal relate homologues genes 2 (CDX2) are important during the early differentiation and maintenance of intestinal epithelium but also necessary for the expression of numbers of intestine-specific genes. The CDX2 expression is physiological throughout the small and large intestine, with the proximal limit occurring at the gastroduodenal junction. Ectopic CDX2 expression is considered to involve in the development of intestinal metaplasia and play significant roles in oncogenesis in gastrointestinal tract and its expression has been demonstrated in intestinal metaplasia of the stomach, and Barrett's metaplasia in human beings. So, CDX2 has concerned recently as an early marker for IM epithelium (Eda et al., 2003; Guo et al., 2004; Tsukamoto et al., 2004; Shi et al., 2008). Villin is a tissue-specific actin-binding cytoskeletal protein that is associated with actin filaments in the microvilli and terminal web of epithelial cells. Its expression tends to be correlated with the development of differentiated polarized cells that contain a brush border and microvillus inclusions and can be induced by long-term acid exposure as an end-differentiated marker of intestinal cells, Villin needs to be of a sufficient quantity to result in a mature brush border. Immunohistochemical analyses of gastric intestinal metaplasia and cancer revealed that villin and Cdx2 expression are tightly coupled. Both of them are demonstrated to be useful maker for the diagnosis of Barrett's esophagus and intestinal metastasis in stomach

[1]*Department of Oncology, Cancer Institute, the First Affiliated Hospital of Henan University of Science and Technology, Luoyang,* [2]*Henan Key Laboratory for Esophageal Cancer Research, College of Medicine, Zhengzhou University, Zhengzhou, China *For correspondence: samfeng137@hotmail.com*

(Dudouet et al., 1987; Fitzgerald et al., 1997; Athman et al., 2002; Niwa et al., 2005; Shi et al., 2008; Yamamichi et al., 2009).

The objectives of this study were to determine whether CDX2 and Villin expression is associated with intestinal differentiation and to evaluate the clinic pathologic patterns of expression in different gastric cardiac lesions before GCA, include cardiac gastritis, dysplasia and adenocarcinoma.

Materials and Methods

Tumor samples

A total of 143 gastric cardiac biopsy or resection specimens was selected from high risk section of GCA of HeNan province, China. The mean patient age was 54.9 years (minimum, 31; maximum, 75; standard deviation, 7.94 years), 26 were female and 117 were male. Clinical data and pathologic slides were reviewed in all cases. These specimens including 25 cardiac gastritis specimens with IM, 65 dysplasia specimens with IM and 35 gastric cardiac adenocarcinoma specimens and stained them for CDX2 and villin by Immunohistochemical SP method. 15 normal gastric cardiac biopsy specimens were also collected as control. The diagnoses were based on the histomorphology according to the criteria of the WHO classification of tumors 2000.

Immunohistochemistry

Sections of formalin-fixed, paraffin-embedded tissues were used for immunohistochemistry. Immunohistochemical staining was performed using CDX2 (clone AMT28, dilution 1:100; ZSJQ Medical technology Co., BeiJing, China) and villin (clone CWWB1, dilution 1:100; ZSJQ Medical tecnology Co., BeiJing, China), PV9000 SP kit (ZSJQ Medical technology Co., BeiJing, China) was used for the standard biotin-avidin method. To retrieve antigen conditions, the slides were treated in 0.01 mol/L EDTA, pH 8.0, in a microwave oven at 98°C for 20 minutes both for CDX2 and Villin. Known positive tissue sections served as positive control samples. All immunohistochemical stains were separately evalu¬ated by 2 pathologists. Semiquantitative evaluation was performed as follows: 0, less than 10% positive cells (-); 1, 10% to 50% positive cells (+); and 2, 50% to 75% positive cells (++); 3, more than 75% (+++) displaying nuclear staining (CDX2) and cytoplasmic and membrane-associated immunoreactivity (Villin). Scores of 1, 2 and 3 were interpreted as positive (+) staining in all tissues.

Statistical methods

All the data was analysed by Spass 17.0 statistical package. The χ^2 test or a Fisher exact test was used for comparison of the posi¬tive rates of CDX2 and Villin, and P values of less than 0.05 were regarded as significant. The relation between CDX2 and Villin expression and clinic pathology details was tested by χ^2 test or Fisher exact test, with additional use of the Spearman's rank correlation coefficient (rho) to analyse the correlation between CDX2 and Villin.

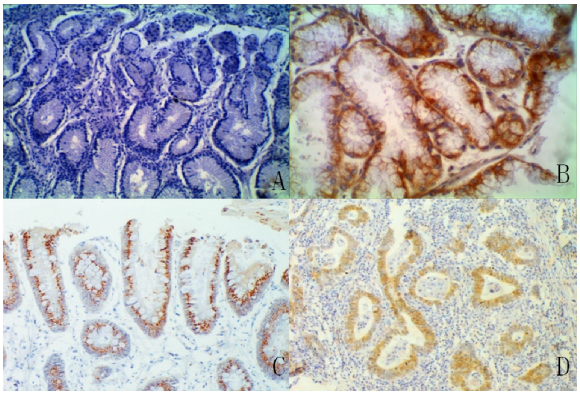

Figure 1. The Expression of CDX2 Protins in Precancerous Lesion with IM and GCA. A. negative expression of CDX2 in normal cardiac mucosa (SP×200) B. positive expression of CDX2 in cardiac gastritis with IM (SP×400) C. positive expression of CDX2 in dysplasia with IM (SP×200) D. positive expression of CDX2 in GCA (SP×200)

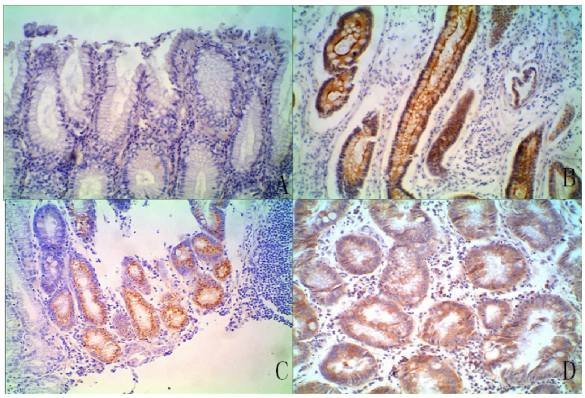

Figure 2. The Expression of Villin Protins in Precancerous Lesion with IM and GCA. A. negative expression of Villin in normal cardiac mucosa (SP×200) B. positive expression of Villin in cardiac gastritis with IM (SP×400) C. positive expression of Villin in dysplasia with IM(SP×200) D. positive expression of Villin in GCA (SP×200)

Results

The Expression of CDX2 and Villin proteins in precancerous lesion with IM and GCA

The antigens were localized as follows: CDX2 was nuclear (Figure 1). Villin stained the brush border of the superficial IM epithelium and luminal borders of the glandular epithelium with or without cytoplasmic staining (Figure 2). There was no staining for CDX2 or Villin in the 15 control samples. Both cardiac gastritis with IM, dysplasia with IM, the positive rates of CDX2 protein were 84.0% (21/25), 66.7% (32/48) respectively. The positive rates are significant higher then that in the adenocarcinoma tissues 36.4% (20/55). While the positive rates of Villin protein In cardiac gastritis with IM and dysplasia with IM were 76.0% (19/25), 70.8% (34/48) respectively, and 45.5% (25/55) in adenocarcinoma tissues. Same as the result of CDX2, the positive rates are significant higher in precancerous lesion with IM then that in the adenocarcinoma tissues ($P<0.01$) (Table 1).

The correlation between CDX2 and Villin

Spearman's rank correlation coefficient (rho) showing

Table 1. Expression of CDX2 and Villin in Pathological Tissues of Gastric Cardiac

Groups	n	CDX2 n(%)		Villin n(%)	
		-	+	-	+
Normal cardiac	15	15(100.0)	0(00.0)	15(100.0)	0(00.0)
Cardiac gastritis with IM	25	4(16.0)	21(84.0)	6(24.0)	19(76.0)
Cardiac dysplasia with IM	48	16(33.3)	32(66.7)	14(29.2)	34(70.8)
Cardiac carcinoma	55	35(63.6)	20(36.4)	30(54.5)	25(45.5)

Table 2. The Correlation of CDX2 and Villin

CDX2	Villin				Total	R value	P value
	-	+	++	+++			
-	56	13	1	0	70	R=0.843	P<0.01
+	6	31	3	0	40		
++	0	1	10	1	12		
+++	0	2	1	18	21		
Total	62	47	15	19	143		

a close correlation between CDX2 and Villin (r=0.843, P<0.01) (Table 2).

The relation between CDX2 and Villin expression and clinic pathology details

Either the expression of CDX2 or Villin was correlated with tumor differentiation (both P<0.05), but was not associated with age, sex, invasion and metastasis of lymph node (P>0.05).

Discussion

GCA have shown an increasing incidence in recent years, leading to increased interest in these tumors (Blot et al., 1991). It is recognised that GCA is epidemiologically distinct from adenocarcinoma of the rest of the stomach (Botterweck et al., 2000; McColl, 2006). JR Siewert (Siewert et al., 2006) also believed that GCA is histopathologic different with distal esophagus adenocarcinoma. Kenneth EL McColl consider that GCA probably arise by two different aetiological and pathogenic pathways. One resembles the cancer of the more distal stomach, due to the damage of H. pylori atrophic gastritis or more rarely autoimmune. The other resembles adenocarcinoma of distal esophagus and is likely to be due to the damage of short-segment gastro-esophageal reflux. These two chronic damages contributes to the high incidence of IM and inflammation at the cardiac mucosa (Odze, 2005; McColl, 2006). Our previous research showed that the incidence of IM in tissue adjacent to gastric cardiac adenocarcinoma is much higher than gastric cardiac biopsy tissue from normal population (Gao et al., 2005). The result suggest that IM is an important precancerous lesion. Studies in IM at the cardiac mucosa were important to reveal the evolution of GCA and helpful to find useful markers for early diagnose of GCA.

The cadual homeobox genes 2 (CDX2) is a transcription factor involved in the early differentiation and maintenance of the intestinal epithelial cell during gastrointestinal development (Silberg et al., 2000). Ectopic CDX2 expression was believed related with intestinal metaplasia of the stomach, and Barrett's esophagus(BE) in human beings (Eda et al., 2003; Guo et al., 2004; Shi et al., 2008; Tsukamoto et al., 2004). Silberg DG found that the expression of CDX2 in transgenic mice can lead occurrence of IM in gastric mucosa (Silberg et al., 2002). Analogous results have been found in human beings too (Mesquita et al., 2003). Further study by Mutoh showed that ectopic CDX2 expression not only involved in the appearance of IM in gastric but also involved in the early in the progress of gastric cancer (Mutoh et al., 2004). There are also some reports suggested ectopic CDX2 expression was an important originate event for BE and the expression is involved in the evolution of esophagus adenocarcinoma (Villanacci et al., 2007). In this study, normal gastric cardiac mucosa presented no CDX2 expression. The expression of CDX2 was remarkableness in IM in gastric cardiac mucosa. But the positive rates was significant lower in GCA tissues than that in IM tissues. This suggest that ectopic CDX2 expression was involved in the occurrence of IM in gastric cardia and GCA.

Villin is an tissue-specific actin-binding cytoskeletal protein in the microvillus core of the brush border. As an end-differentiated marker of intestinal cells, its expression can be induced by long-term acid exposure and correlated with the development of differentiated polarized cells that contain a brush border and microvillus inclusions (Fitzgerald et al., 1997). The fre¬quency of villin expression was reported varies from 73% (22/30) to 100% (21/21) in BE and 85% (17/20) in gastric IM (Regaledo et al., 1998; Kerkhof et al., 2006; Zhao et al., 2009). We found villin expression to be present 53 cases in all 73 cases of gastric cardiac IM tissues. There are 25 cases present Villin expression in all 55 GCA tissues, but the positive rate is obviously lower than that in gastric cardiac IM. None of the gastric cardiac biopsy specimens from normal population showed labeling with villin. Besides luminal borders of glands containing goblet cells, villin was also expressed in the cytoplasm of cardiac gastritis and dysplasia tissues. This observation suggests that Villin play an important role in the procedure of gastric cardiac IM to GCA, meanwhile cells expressing villin in the cytoplasmic expression alone may indicate an earlier stage of intestinal differentiation.

Villin was thought to be a less specific marker of IM than CDX2 by some investigators. However, in our hands, the villin and CDX2 expression are tightly coupled. Similar result was reported in BE too (Shi et al., 2008). A recent study found that knockdown of CDX2 in SW480 cells caused a clear down regulation of villin. Indicated that villin is regulated by CDX2 (Yamamichi et al., 2009). Therefore, some investigators suggest to use both CDX2 and Villin as markers for the detection of early IM and supplements for the histologic diagnosis of BE.

The expression of CDX2 and Villin were also found to be correlated with tumor differentiation, and showed down positive rates accompany the differentiation becoming poorer. Whereas no correlate were significant with age, sex, invasion and metastasis of lymph node. Functional studies have already shown CDX2 could regulate many intestine-specific gene transcription in vivo. Such like intestinal-type alkaline phosphatase (ALP); the well

characterized brushborder enzyme, sucrase-isomaltase (SI), human defensin-5 (HD), and mucus-secreting goblet cell-mucin marker (MUC2) (Eda et al., 2002; Guo et al., 2004; Weimann et al., 2010). These genes control the differentiation of the IM of gastrointestinal tract. And an in vitro study reported the rate of villin expression to be dramatically increased in the course of enterocyte dif¬ferentiation (Dudouet et al., 1987). Summarise these consequences we found that CDX2 and Villin play a key roles in the differentiation of GCA.In conclusion, Our result suggest that ectopic expression of CDX2 and Villin were involved in early-stage IM at gastric cardia and the expression of Villin was regulate by CDX2. The expression of CDX2 and Villin may play an important role in the procedure of GCA.

Acknowledgements

Supported by National Outstanding Young Scientist Award of China, No. 30025016 and National Natural Science Foundation of China, No. 30670956.

References

Athman R, Louvard D, Robine S (2002). The epithelial cell cytoskeleton and intracellular trafficking. III. How is villin involved in the actin cytoskeleton dynamics in intestinal cells? *Am J Physiol Gastrointest Liver Physiol*, **283**, G496-502.

Blot WJ, Devesa SS, Kneller RW, et al (1991). Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *JAMA*, **265**, 1287-9.

Botterweck AA, Schouten LJ, Volovics A, et al (2000). Trends in incidence of adenocarcinoma of the oesophagus and gastric cardia in ten European countries. *Int J Epidemiol*, **29**, 645-54.

Dudouet B, Robine S, Huet C, et al (1987). Changes in villin synthesis and subcellular distribution during intestinal differentiation of HT29-18 clones. *J Cell Biol*, **105**, 359-69.

Eda A, Osawa H, Satoh K, et al (2003). Aberrant expression of CDX2 in Barrett's epithelium and inflammatory esophageal mucosa. *J Gastroenterol*, **38**, 14-22.

Eda A, Osawa H, Yanaka I, et al (2002). Expression of homeobox gene CDX2 precedes that of CDX1 during the progression of intestinal metaplasia. *J Gastroenterol*, **37**, 94-100.

Fitzgerald RC, Omary MB, Triadafilopoulos G (1997). Acid modulation of HT29 cell growth and differentiation. An in vitro model for Barrett's esophagus. *J Cell Sci*, **110**, 663-71.

Gao SG, Wang LD, Fan ZM, et al (2005). Histochemical studies on intestinal metaplasia adjacent to gastric cardia adenocarcinoma in subjects at high-incidence area in Henan, north China. *World J Gastroenterol*, **11**, 4634-7.

Guo RJ, Suh ER, Lynch JP (2004). The role of Cdx proteins in intestinal development and cancer. *Cancer Biol Ther*, **3**, 593-601.

Kerkhof M, Bax DA, Moons LM, et al (2006). Does CDX2 expression predict Barrett's metaplasia in oesophageal columnar epithelium without goblet cells? *Aliment Pharmacol Ther*, **24**, 1613-21.

McColl KE (2006). Cancer of the gastric cardia. *Best Pract Res Clin Gastroenterol*, **20**, 687-96.

Mesquita P, Jonckheere N, Almeida R, et al (2003). Human MUC2 mucin gene is transcriptionally regulated by Cdx homeodomain proteins in gastrointestinal carcinoma cell lines. *J Biol Chem*, **278**, 51549-56.

Mutoh H, Sakurai S, Satoh K, et al (2004). Development of gastric carcinoma from intestinal metaplasia in Cdx2-transgenic mice. *Cancer Res*, **64**, 7740-7.

Niwa T, Ikehara Y, Nakanishi H, et al (2005). Mixed gastric- and intestinal-type metaplasia is formed by cells with dual intestinal and gastric differentiation. *J Histochem Cytochem*, **53**, 75-85.

Odze RD (2005). Unraveling the mystery of the gastroesophageal junction: a pathologist's perspective. *Am J Gastroenterol*, **100**, 1853-67.

Palli D, Bianchi S, Decarli A, et al (1992). A case-control study of cancers of the gastric cardia in Italy. *Br J Cancer*, **65**, 263-6.

Regalado SP, Nambu Y, Iannettoni MD, et al (1998). Abundant expression of the intestinal protein villin in Barrett's metaplasia and esophageal adenocarcinomas. *Mol Carcinog*, **22**, 182-9.

Shi XY, Bhagwandeen B, Leong AS (2008). CDX2 and villin are useful markers of intestinal metaplasia in the diagnosis of Barrett esophagus. *Am J Clin Pathol*, **129**, 571-7.

Siewert JR, Stein HJ, Feith M (2006). Adenocarcinoma of the esophago-gastric junction. *Scand J Surg*, **95**, 260-9.

Silberg DG, Sullivan J, Kang E, et al (2002). Cdx2 ectopic expression induces gastric intestinal metaplasia in transgenic mice. *Gastroenterology*, **122**, 689-96.

Silberg DG, Swain GP, Suh ER, et al (2000). Cdx1 and cdx2 expression during intestinal development. *Gastroenterology*, **119**, 961-71.

Tsukamoto T, Inada K, Tanaka H, et al (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**, 135-45.

Villanacci V, Rossi E, Zambelli C, et al (2007). COX-2, CDX2, and CDC2 immunohistochemical assessment for dysplasia-carcinoma progression in Barrett's esophagus. *Dig Liver Dis*, **39**, 305-11.

Weimann A, Rieger A, Zimmermann M, et al (2010). Comparison of six immunohistochemical markers for the histologic diagnosis of neoplasia in Barrett's esophagus. *Virchows Arch*, **457**, 537-45.

Yamamichi N, Inada K, Furukawa C, et al (2009). Cdx2 and the Brm-type SWI/SNF complex cooperatively regulate villin expression in gastrointestinal cells. *Exp Cell Res*, **315**, 1779-89.

Zhao CB, Zhou CJ, Guo JQ (2009). Expression of villin in precancerous lesions and gastric carcinoma and its clinical significance. *J ShanDong University Med Sci*, **47**, 46-9