BIOCHEMICAL PHARMACOLOGY 71 (2006) 1581–1588

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The transcription factor Cdx2 regulates the intestine-specific expression of human peptide transporter 1 through functional interaction with Sp1

Jin Shimakura [a], *Tomohiro Terada* [a], *Yutaka Shimada* [b], *Toshiya Katsura* [a], *Ken-ichi Inui* [a,*]

[a] *Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan*
[b] *Surgery and Surgical Basic Science, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

ARTICLE INFO

Article history:
Received 13 February 2006
Accepted 2 March 2006

Keywords:
PEPT1
Cdx2
Sp1
Transporter
Caco-2
Intestinal metaplasia

Abbreviations:
Cdx2, caudal-related homeobox protein 2
ChIP, chromatin immunoprecipitation
GAPDH, glyceraldehydes-3-phosphate dehydrogenase
HNF, hepatocyte nuclear factor
PEPT1, H+/peptide cotransporter 1
TBS, Tris-buffered saline

ABSTRACT

H^+/peptide cotransporter 1 (PEPT1, SLC15A1) localized at the brush-border membranes of intestinal epithelial cells plays important roles in the intestinal absorption of small peptides and a variety of peptidemimetic drugs. We previously demonstrated that transcription factor Sp1 functions as a basal transcriptional regulator of human PEPT1. However, the factor responsible for the intestine-specific expression of PEPT1 remains unknown. In the present study, we investigated the effect of the intestinal transcription factors on the transcription of the PEPT1 gene and found that only Cdx2 markedly trans-activated the PEPT1 promoter. However, the promoter region responsible for this effect lacked a typical Cdx2-binding sequence, but instead, possessed some Sp1-binding sites. In vitro experiments using Caco-2 cells showed that (1) mutation of the Sp1-binding site diminished the effect of Cdx2, (2) co-expression of Cdx2 and Sp1 synergistically trans-activated the PEPT1 promoter and (3) Sp1 protein was immunoprecipitated with Cdx2 protein. These results raise the possibility that Cdx2 modulates the PEPT1 promoter by interaction with Sp1. The significance of Cdx2 in vivo for PEPT1 regulation was shown by the determination of mRNA levels of Cdx2 and PEPT1 in human tissue. In gastric samples, some with intestinal metaplasia, the levels of PEPT1 and Cdx2 mRNA were highly correlated. Taken together, the present study suggests that Cdx2 plays a key role in the transcriptional regulation of the intestine-specific expression of PEPT1, possibly through interaction with Sp1.

1. Introduction

Di- and tripeptides are taken up into the intestinal and renal epithelial cells by H^+-coupled peptide transporters (PEPT1/SLC15A1 and PEPT2/SLC15A2). Many functional studies using heterologous expression systems have demonstrated molecular natures in their transport characteristics. For example, despite having similar substrate specificity, PEPT1 and PEPT2

* *Corresponding author*. Tel.: +81 75 751 3577; fax: +81 75 751 4207.
E-mail address: inui@kuhp.kyoto-u.ac.jp (K.-i. Inui).
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doi:10.1016/j.bcp.2006.03.001

were characterized as low- and high-affinity type transporters, respectively [1,2]. In addition, the two transporters differ in their tissue distribution and play distinct physiological roles. PEPT1 is expressed predominantly in the small intestine and slightly in the kidney [1,2]. On the other hand, PEPT2 is expressed mainly in the kidney, but it is also expressed in various tissues such as lung [3], choroid plexus [4] and mammary gland [5], and plays tissue-specific roles. As PEPT1 has broad substrate specificity, the intestinal absorption of several pharmacologically active drugs such as oral β-lactam antibiotics and the anti-viral agent valacyclovir are mediated by this transporter, and therefore, PEPT1 also plays important roles not only as a nutrient transporter but also as a drug transporter [2].

Previously, we isolated the promoter region of PEPT1 and demonstrated that the transcription factor Sp1 plays an important role in the basal transcriptional regulation of PEPT1 [6]. But, as Sp1 is expressed ubiquitously, the intestine-specific expression of PEPT1 cannot be controlled only by Sp1; thus, an intestine-restricted transcription factor is assumed to be involved.

The transcription factor Cdx2 is a member of the caudal-related homeobox gene family and expressed mainly in the intestine [7]. Cdx2 plays important roles in the early differentiation, proliferation and maintenance of intestinal epithelial cells [7,8], and in the transcription of intestinal genes, such as the sucrase–isomaltase [9], lactase–phlorizin hydrolase (LPH) [10], claudin-2 [11] and UDP glucuronosyltransferases genes (UGTs) [12] through binding to a TTTAT/C consensus sequence. Over-expression of Cdx2 in undifferentiated rat IEC-6 intestinal epithelial cells leads to the development of a differentiated phenotype [8]. Furthermore, in humans, CDX2 has been reported to be associated with intestinal metaplasia in the stomach [13] in which ectopic expression of CDX2 is speculated to cause the gastric epithelial cells to trans-differentiate and take the intestinal phenotype. In our recent study, PEPT1 was also found to be expressed in the stomach, induced by intestinal metaplasia [14].

Considering the functions of Cdx2 mentioned above and overlapping of its expression with PEPT1, it is possible to suggest a link between these two genes. In the present study, we investigated the role of Cdx2 in the transcriptional regulation of PEPT1 using the human intestinal cell line Caco-2 cells. In addition, the correlation between PEPT1 and CDX2 mRNA expression levels in human gastric tissue samples developing intestinal metaplasia was also assessed.

2. Materials and methods

2.1. Materials

The anti-CDX2 monoclonal antibody was purchased from BioGenex (San Ramon, CA). The polyclonal antibody recognizing human Sp1 was from Upstate (Charlottesville, VA). The anti-FLAG M2 monoclonal antibody and anti-FLAG M2 monoclonal antibody conjugated to agarose gel (anti-FLAG M2 affinity gel) were obtained from Sigma (St. Louis, MO). The mouse Cdx2 expression vector (pRc/CMV-Cdx2) was a gift from Dr. Eun Ran Suh (University of Pennsylvania). The human HNF-1α and HNF-1β expression vectors were kindly supplied by Dr. Marco Pontoglio (Institute Pasteur, Paris, France). The CMV-Sp1 expression vector was kindly provided by Dr. Robert Tjian (University of California, Berkeley). The FLAG-Cdx2 expression plasmid was constructed by cloning the *Hin*dIII fragment of pRc/CMV-Cdx2 into pFLAG-CMV-6a (Sigma) at the *Hin*dIII restriction site. All other chemicals used were of the highest purity available.

2.2. Cloning of the 5′-regulatory region of PEPT1 gene and preparation of deletion reporter constructs

Cloning of the 5′-regulatory region of the PEPT1 gene and preparation of various reporter constructs were carried out as previously described [6]. Briefly, the 2940-bp flanking region upstream of the transcription start site was subcloned into the firefly luciferase reporter vector, pGL3-Basic (Promega, Madison, WI). This full-length reporter plasmid is hereafter referred to as −2940/+60. The 5′-deleted (−1111/+60, −960/+60, −401/+60, −247/+60, −172/+60 and −21/+60) constructs were generated by digestion of the −2940/+60 construct with the restriction enzymes. The −35/+60 construct was generated by PCR. Site-directed mutations in putative Sp1-binding sites were introduced into the −172/+60 construct with a Quik Change XL site-directed mutagenesis kit (Stratagene, La Jolla, CA).

2.3. Cell culture, transfection and reporter gene assay

Caco-2 cells were obtained from the American Type Culture Collection (ATCC CRL-1392) and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% non-essential amino acids. Caco-2 cells were plated into 24-well plates (3×10^5 cells/well) and transfected the following day with the reporter constructs, the expression plasmid for the transcription factor and 2.5 ng of the *Renilla reniformis* vector, pRL-TK (Promega), using Lipofectamine 2000 (Invitrogen Japan KK, Tokyo, Japan) according to the manufacturer's recommendations. The medium was changed after 24 h. The firefly and *Renilla* activities were determined 48 h after the transfection using a dual luciferase assay kit (Promega) and a LB940 luminometer (Berthold, Bad Wildbad, Germany). For the immunoprecipitation and chromatin immunoprecipitation experiments, Caco-2 cells were plated into 60-mm dishes (1.2×10^6 cells/dish) and transfected the following day with the expression plasmid for FLAG-Cdx2 using Lipofectamine 2000.

2.4. Immunoprecipitation and Western blotting

Caco-2 cells expressing FLAG-Cdx2 were washed with PBS twice, scraped off, and suspended in lysis buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.5 mM PMSF and 1% protease inhibitor cocktail (Nacalai tesque, Kyoto, Japan)). After an incubation at 4 °C for 15 min, the cells were disrupted by vigorous vortexing and repeated passages through a 24-gauge needle. The homogenate was centrifuged at 4 °C and 20,000 ×*g* for 10 min, and the supernatant was recovered. Immunoprecipitation of FLAG-Cdx2 was performed using anti-FLAG affinity gel at 4 °C overnight. The gel was washed with the lysis buffer five times. The immunoprecipitated FLAG-Cdx2 proteins were solubilized

in SDS sample buffer, separated on a 10% polyacrylamide gel at room temperature, and transferred onto polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, MA) by semidry electroblotting. Blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris, 137 mM NaCl, pH 7.5) with 0.1% Tween 20 (TBS-T) for 3 h at room temperature. The blots were washed in TBS-T and then incubated with the anti-FLAG M2 monoclonal antibody (10 μg/ml, 1 h at room temperature) or anti-Sp1 polyclonal antibody (1 μg/ml, overnight at 4 °C). Blots were washed three times with TBS-T, and the bound antibody was detected on X-ray film by enhanced chemiluminescence with a horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG antibody (Amersham Pharmacia Biotech, Piscataway, NJ).

2.5. Chromatin immunoprecipitation (ChIP)

Caco-2 cells expressing FLAG-Cdx2 were cross-linked with 1% formaldehyde at room temperature for 10 min. Cells then were rinsed with ice-cold PBS twice, scraped off, and suspended in lysis buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.5 mM PMSF and 1% protease inhibitor cocktail). Cells were sonicated three times for 15 s each time at 40% of the maximal setting (VP-5S, TAITEC, Koshigaya, Japan) and centrifuged at 4 °C and 20,000 ×*g* for 10 min. After the supernatants were collected and diluted in lysis buffer, immunoprecipitation was performed overnight at 4 °C with anti-FLAG affinity gel. The gel was then washed five times with lysis buffer and extracted with 1.5% SDS, followed by 0.5% SDS. Eluates were pooled and heated at 68 °C for 6 h to reverse the formaldehyde cross-linking. Chromatin-associated proteins were digested with proteinase K at 55 °C. The DNA was recovered by phenol–chloroform extraction and ethanol precipitation. Pellets were dissolved in 20 μl of TE buffer and used as a template for PCR. Primers used for amplifying the PEPT1 promoter were 5′-GACTGGCTCTCCCCCGGCCTGCCACG-3′ and 5′-CCCGGCCCCGTTGCCCCAGGTACAGC-3′ (−209 to −26 upstream of the transcriptional start site). PCR was performed using Advantage GC Genomic Polymerase Mix (BD Biosciences, Franklin Lakes, NJ) and cycling conditions were as follows: 1 min of denaturation at 95 °C, followed by 30 cycles of 30 s of denaturation at 94 °C, 3 min of primer annealing and extension at 68 °C, and 3 min of final extension at 68 °C.

2.6. Human gastric tissue sample

The gastric mucosal samples from normal stomachs were obtained from cancer patients ($n = 30$) during surgery at the First Department of Surgery, Kyoto University Hospital. Normal mucosal samples were resected at the site most distant from the affected portions. Three samples from different portions were resected in some patients. Pathologists diagnosed intestinal metaplasia in some patients. No patients underwent preoperative chemotherapy and/or radiation therapy. The samples were frozen in liquid nitrogen and stored at −80 °C until RNA extraction. This study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by the Ethics Committee of Kyoto University (G-39). Written informed consent was obtained from all patients for surgery and the use of their resected samples.

2.7. Real-time PCR

Isolation of total RNA from the human stomach samples and real-time PCR were carried out as described previously [14]. The primer–probe set used for CDX2 was pre-developed TaqMan Assay Reagents (Applied Biosystems, Foster, CA). Glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA was also measured as an internal control with GAPDH Control Reagent (Applied Biosystems).

2.8. Data analysis

The results were expressed relative to the result obtained with the pGL3-Basic vector set as 1 and represent the means ± S.E. ($n = 3$). Two or three experiments were conducted, and representative results were shown. In the mutational experiment, statistical analysis was performed with the one-way ANOVA followed by Scheffé *F* post hoc testing.

3. Results

3.1. Cdx2 activates transcription of the PEPT1 promoter–reporter construct

To investigate whether Cdx2 activates the PEPT1 promoter, the −2940/+60 reporter construct was transiently transfected into Caco-2 cells simultaneously with Cdx2 expression plasmids. Besides Cdx2, the transcription factor hepatocyte nuclear factor (HNF)-1α is also expressed in the intestine and involved in the expression of some intestinal genes although it was first discovered in the liver [15,16]. In the regulation of LPH expression, Cdx2 is reported to directly interact with HNF-1α [10]. Thus, the effect of HNF-1α and a related transcription factor, HNF-1β, on the PEPT1 promoter was also assessed. Cdx2 over-expression resulted in a four-fold increase in promoter activity (Fig. 1). However, HNF-1α could neither activate the PEPT1 promoter nor enhance its activity driven by Cdx2. HNF-1β could activate it only a little as compared to Cdx2. Thus, we focused on Cdx2 as a possible regulator of PEPT1 expression and further investigated the Cdx2-responsive region in the PEPT1 promoter.

3.2. Cdx2-responsive region located near the Sp1-binding sites

To determine the elements contributing to the expression of PEPT1, we carried out a promoter 5′-deletion analysis (Fig. 2). The promoter activity in the absence of Cdx2 was strongest with the −401/+60 construct and gradually decreased by the deletion between −401 and −35, consistent with our previous result [6]. The promoter activity in the presence of Cdx2 increased three- to four-fold as compared to that in the absence of Cdx2 with the −2940 to −172 constructs whereas it was completely diminished with the −35/+60 construct, suggesting that the Cdx2-responsive region is located between −172 and −35. Unexpectedly, this region lacked a consensus Cdx2-binding site but contained multiple Sp1-binding sites as reported previously [6]. We could not find any transcription factor-binding sites which are likely to be responsible for the

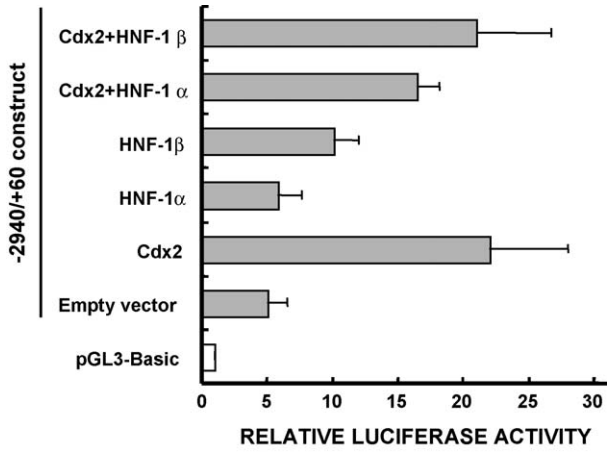

Fig. 1 – Effects of Cdx2, HNF-1α and HNF-1β overexpression on the PEPT1 promoter activity. Caco-2 cells were transiently transfected with 250 ng of the −2940/+60 construct and 250 ng of the expression vector for Cdx2, HNF-1α or HNF-1β. The total amount of transfected DNA (750 ng) was kept constant by adding empty vector. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Data are reported as the relative fold-increase compared with pGL3-Basic and represent the mean ± S.E. (n = 3).

effect of Cdx2 except for Sp1 in this region. We next carried out a mutational analysis to determine whether the effect of Cdx2 was mediated through these Sp1-binding sites. The promoter activity in the absence of Cdx2 was reduced with the mutation of Sp-A, Sp-B or Sp-C sites, consistent with our prior study [6]. Trans-activation by Cdx2 was markedly decreased with the construct possessing the mutation of Sp-A or Sp-C sites (Fig. 3). These deletion and mutational analyses collectively suggest that Cdx2 may functions via interaction with Sp1 on the PEPT1 promoter.

3.3. *PEPT1 promoter was synergistically activated by Cdx2 and Sp1*

Sp1 has been shown to trans-activate the PEPT1 promoter [6]. We therefore determined whether Cdx2 enhances the promoter activity in cooperation with Sp1. Cdx2 or Sp1 alone caused a 1.5–2-fold increase of the promoter activity, whereas co-expression of Cdx2 and Sp1 resulted in a four-fold increase in the promoter activity (Fig. 4), suggesting a synergistic effect.

3.4. *Protein–protein interaction of Cdx2 and Sp1*

The synergistic effect of Cdx2 and Sp1 observed in the co-expression experiment, together with the observations from the mutational analysis, raise the possibility that these two proteins interact physically to regulate PEPT1 expression. We therefore investigated the interaction of Cdx2 and Sp1 within the cell using co-immunoprecipitation. Whole-cell extracts of Caco-2 cells transfected with the expression vector for FLAG-Cdx2 or empty vector were subjected to immunoprecipitation followed by Western blotting. Detection with anti-FLAG antibody confirmed that FLAG-Cdx2 protein was expressed in the cells transfected with FLAG-Cdx2 and appropriately immunoprecipitated (Fig. 5, upper panel). In the input samples, as expected, the band of Sp1 protein was detected both in the cells transfected with FLAG-Cdx2 and empty vector, while in the immunoprecipitated sample, it was detected only in the FLAG-Cdx2 transfected cells (Fig. 5, lower panel). These findings show that endogenous Sp1 protein was co-immunoprecipitated with FLAG-Cdx2 and suggest that Cdx2 and Sp1 were associated in a protein complex in Caco-2 cells.

3.5. *Cdx2 associates with the PEPT1 promoter*

As mentioned above, the Cdx2-responsive region lacked a consensus Cdx2-binding site, and an electrophoretic mobility shift assay failed to demonstrate the binding of Cdx2 with the PEPT1 promoter (data not shown). Thus we adopted an

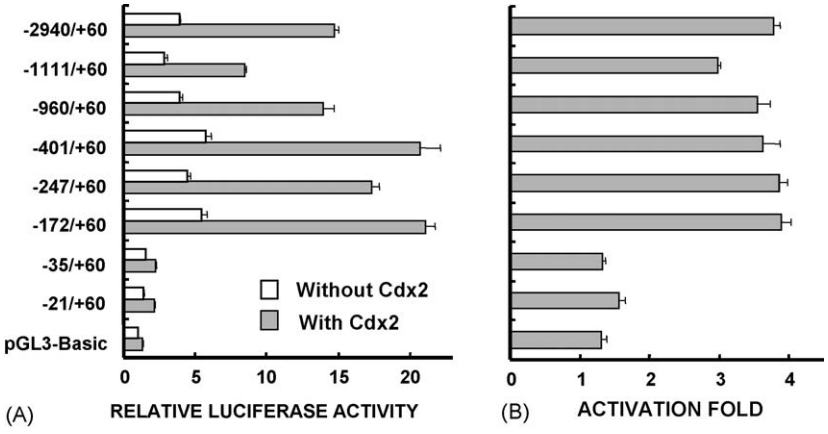

Fig. 2 – Identification of the Cdx2-responsive region in the PEPT1 promoter. A series of deleted promoter constructs (equimolar amounts of the −2940/+60 construct (500 ng)) and 500 ng of the Cdx2 expression vector or empty vector were transiently transfected into Caco-2 cells for luciferase assays. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Data are reported as the relative fold-increase compared with the pGL3-Basic vector (A) or as the ratio of Cdx2-expressing vector to empty vector (B) and represent the mean ± S.E. (n = 3).

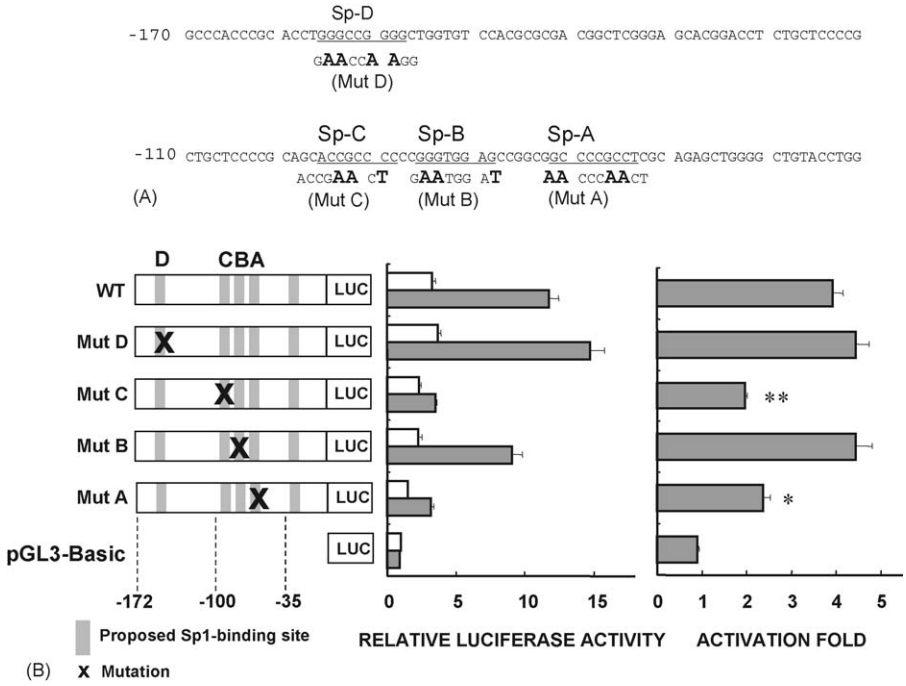

Fig. 3 – Mutational analysis of Sp1-binding sites in the Cdx2-responsive region in the PEPT1 promoter. (A) The nucleotide sequence of the promoter region from −170 to −41 is shown with the putative Sp1-binding elements (Sp-A, Sp-B, Sp-C, Sp-D, underlined). Site-directed mutations that destroy Sp1-binding elements were introduced individually and designated mut A, mut B, mut C and mut D. The nucleotides altered for mutational analysis are shown in bold letters under the wild-type sequence. (B) The mutated −172/+60 constructs (500 ng) and 500 ng of the Cdx2 expression vector or empty vector were transiently expressed in Caco-2 cells for luciferase assays. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Data are reported as the relative fold-increase compared with the pGL3-Basic vector and as the ratio of Cdx2-expressing vector to empty vector, and represent the mean ± S.E. ($n = 3$). (* and **) Significantly different from wild type (WT), $^{*}p < 0.05$, $^{**}p < 0.01$.

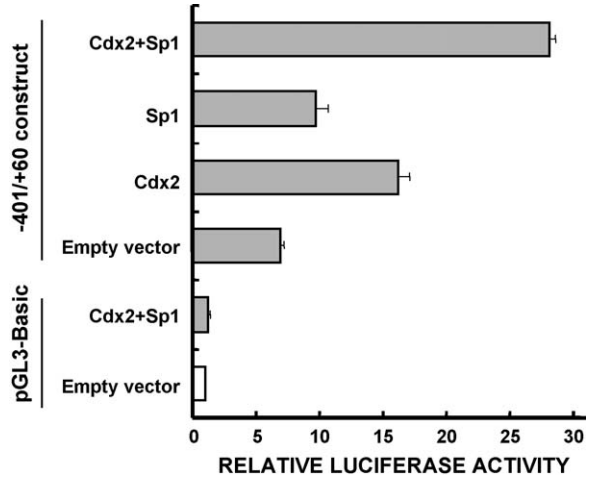

Fig. 4 – Synergistic activation of the PEPT1 promoter by Cdx2 and Sp1. Caco-2 cells were transiently transfected with 150 ng of the −401/+60 construct and the expression vector for Cdx2 (500 ng) or Sp1 (1000 ng). The total amount of transfected DNA (1650 ng) was kept constant by adding empty vectors. Data are reported as the relative fold-increase compared with pGL3-Basic and represent the mean ± S.E. ($n = 3$).

alternative methodology, the ChIP assay, to investigate the association of Cdx2 with the PEPT1 promoter. An approximately 200-bp fragment of the PEPT1 promoter covering the Cdx2-responsive region, which had the Sp1-binding sites, was recovered by immunoprecipitation of FLAG-Cdx2 from the transfected cells, whereas only a trace amount of the fragment was recovered from the mock-transfected cells (Fig. 6).

3.6. *The level of PEPT1 mRNA is correlated with that of CDX2 in the gastric samples with the intestinal metaplasia*

mRNA levels of PEPT1 and CDX2 in the human gastric samples, some with intestinal metaplasia, were determined using quantitative real-time PCR (Fig. 7). PEPT1 and CDX2 mRNA levels differed by more than 100-fold between the samples. The mRNA level of PEPT1 was highly correlated with that of CDX2. Furthermore, both PEPT1 and CDX2 were expressed at apparently higher levels in the samples diagnosed pathologically with intestinal metaplasia as compared to the normal tissue.

4. Discussion

The molecular mechanisms responsible for the intestine-specific expression of PEPT1 are largely unknown. In the

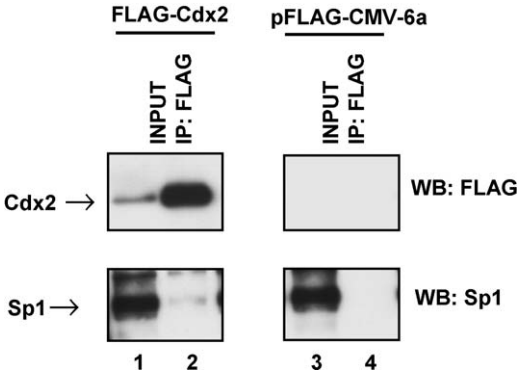

Fig. 5 – Physical interaction of Cdx2 with Sp1 protein. Whole-cell extracts of Caco-2 cells transfected with FLAG-Cdx2 or pFLAG-CMV-6a (empty vector) were subjected to immunoprecipitation followed by Western blotting. Proteins were immunoprecipitated with anti-FLAG M2 affinity gel (lanes 2 and 4, indicated as IP:FLAG). Whole-cell extracts before immunoprecipitation were also analyzed (lanes 1 and 3, indicated as INPUT). FLAG-Cdx2 and Sp1 protein were detected with anti-FLAG M2 monoclonal antibody (upper panels) and anti-Sp1 polyclonal antibody (lower panels), respectively.

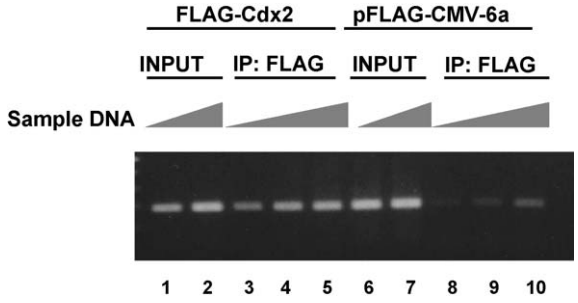

Fig. 6 – Association of Cdx2 with the PEPT1 promoter. Chromatin immunoprecipitation analysis of the endogenous PEPT1 promoter in Caco-2 cells transfected with FLAG-Cdx2 or pFLAG-CMV-6a (empty vector) was performed. The immunoprecipitated DNA fragments were purified and amplified by PCR with primers spanning the Cdx2-responsive region, and subjected to agarose gel electrophoresis. Serially diluted samples of DNA were used for PCR amplification. Lanes 1, 2 and 6, 7 indicate INPUT DNA. Lanes 3–5 and 8–10 show immunoprecipitated DNA.

present study, we provide the first evidence that Cdx2 regulates the transcription of PEPT1 using Caco-2 cells. Unlike other intestinal genes, such as the genes for LPH [10], claudin-2 [11] and UGTs [12], neither HNF-1α nor HNF-1β could trans-activate the PEPT1 promoter, although Cdx2 markedly enhanced the activity of the PEPT1 promoter. Deletion analysis revealed that the Cdx2-responsive region was located between bases −172 and −35 relative to the transcription start site. Computational analysis showed the lack of a canonical Cdx2-binding site in this region, but the presence of several GC-boxes which we previously identified as Sp1-binding sites. Sp1 has been reported to interact with various transcription factors or co-factors, such as estrogen receptor [17], p300/CREB-binding protein [18] and homeobox protein, Hox proteins [19]. Thus we tried to elucidate whether Cdx2 interacts with Sp1 to regulate the PEPT1 promoter.

Introducing mutations into Sp1-binding sites reduced the effect of Cdx2. Furthermore, co-expression of Cdx2 and Sp1 synergistically activated the PEPT promoter. These results suggest that the trans-activating effect of Cdx2 might be mediated via a Sp1-dependent mechanism. Among Sp1-binding sites located in Cdx2-responsive region, Sp-A, Sp-B and Sp-C were involved in the basal promoter activity, whereas only Sp-A and Sp-C appeared to be critical for Cdx2 effect, indicating that the regulatory effect of Cdx2 is site-dependent.

Co-immunoprecipitation of FLAG-tagged Cdx2 precipitated the endogenous Sp1 protein, suggesting the formation of a transcriptional complex involving Cdx2 and Sp1. In addition, ChIP assays indicated that Cdx2 protein was present on the

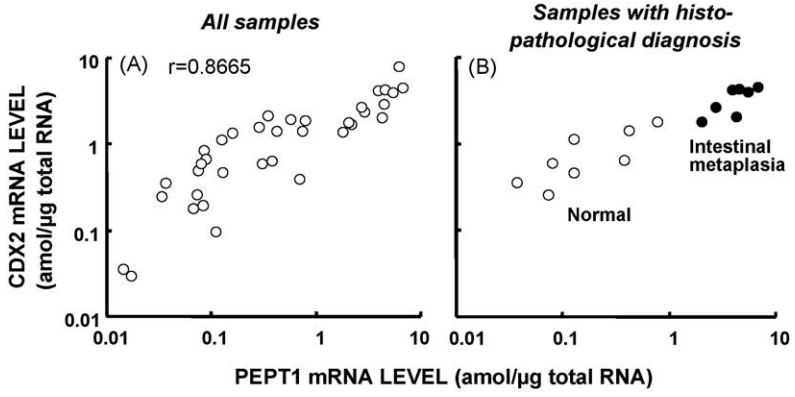

Fig. 7 – Correlation between PEPT1 and CDX2 mRNA levels in the human gastric tissue samples. The mRNA levels of PEPT1 and CDX2 were quantified with real-time PCR analysis in the gastric mucosal samples. Some of these tissue samples were diagnosed by a pathologist and proved to be intestinal metaplasia. (A) All samples were plotted. (B) The samples of patients diagnosed by a pathologist were plotted. Open and closed symbols indicate the normal samples and the samples proved to be intestinal metaplasia, respectively.

PEPT1 promoter at the whole cell level. Considering the lack of Cdx2-binding site in the Cdx2-responsive region and the functional and physical interaction between Cdx2 and Sp1 mentioned above, one explanation for the regulatory mechanism of Cdx2 may be that Cdx2 protein associates with the PEPT1 promoter via the complex formation with Sp1. Although Cdx2 has been reported to interact with the transcription factors such as HNF-1α [10–12] and GATA proteins [20], in all cases, Cdx2 directly binds to its cognate binding site on the promoter of target genes. By contrast, Cdx2 is speculated to exert its effect without direct binding to its cognate binding sequence on the PEPT1 promoter. The nature of the physical interaction between Cdx2 and Sp1 has yet to be determined. One possibility is that Cdx2 directly binds to Sp1 protein. Another possibility is indirect binding mediated by a common cofactor or some adaptor proteins. In both cases, to our knowledge, this is a novel mechanism of transcriptional regulation by Cdx2. Further studies will be needed to obtain the additional proof for supporting and fully characterizing this proposed mechanism.

In order to demonstrate the significance of Cdx2 for PEPT1 expression in vivo, we next investigated the expression profile of Cdx2 and PEPT1 mRNA using human tissue samples. In the gastric samples, some of which had intestinal metaplasia, PEPT1 and Cdx2 mRNA levels were highly correlated. The fact that ectopic expression of Cdx2 accompanied the expression of PEPT1 in human tissues strongly supports the role of Cdx2 demonstrated by in vitro reporter experiments. In addition, a recent study showed that expression of PEPT1 was induced in the gastric epithelium in a transgenic mouse expressing Cdx2 exclusively in the gastric epithelium [21].

The similarities between PEPT1 and Cdx2 in their expression profile are observed not only at the tissue level but also at the cellular level. PEPT1 is localized to brush-border membranes of the absorptive epithelial cells of the small intestine, and this protein is abundant at the tip of the villus and scarce at the crypt base [22]. Cdx2 also has a gradient of expression in the crypt–villus axis being primarily expressed in the villus [7].

It has been reported that intestinal PEPT1 is regulated by various factors [23], such as thyroid hormone [24], dietary conditions [25,26], diurnal rhythm [27] and a selective σ-ligand, pentazocine [28]. In addition, the ectopic induction of PEPT1 expression in the colon, where PEPT1 was not expressed under normal conditions, was observed in cases of functional deficiency of the small intestine such as ulcerative colitis, Crohn's disease and short-bowel syndrome [23]. It is not clear at present whether Cdx2 plays some parts in these regulatory functions. However, Cdx2 exerts physiological roles in the differentiation of intestinal epithelial cells and maintenance of intestinal phenotype. It is possible that Cdx2 helps to regulate colonic PEPT1 expression under such pathological conditions.

In conclusion, we demonstrated that Cdx2 regulated the PEPT1 promoter activity in Caco-2 cells using reporter assays, and confirmed the significance of Cdx2 in vivo in a correlation analysis of mRNA expression in human tissue samples. In addition, it may be possible that Cdx2 physically and functionally interacts with Sp1, and associates with the PEPT1 promoter although no cognate Cdx2-binding site is evident. These results collectively indicate that Cdx2 plays a key role in the transcriptional regulation for the intestine-specific expression of PEPT1, and have implications as a basis for future investigations of efficient enteral nutrition and drug therapy.

Acknowledgments

We are grateful to Dr. Eun Ran Suh (University of Pennsylvania) and Dr. Robert Tjian (University of California, Berkeley) for the generous gift of Cdx2 and Sp1 expression vectors, respectively. We also thank Dr. Marco Pontoglio (Institute Pasteur, Paris, France) for kindly providing Human HNF-1α and HNF-1β expression vectors.

This work was supported by the 21st Century COE Program "Knowledge Information Infrastructure for Genome Science", a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture and Sports of Japan, and a Grant-in-Aid for Research on Advanced Medical Technology from the Ministry of Health, Labor and Welfare of Japan.

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